A pilot study comparing two selenium pour on formulations in cattle

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Introduction

Selenium deficiency and its effects have been widely described in cattle in New Zealand. The effects of selenium deficiency include ill-thrift, diarrhoea and mortalities in growing cattle (Andrews *et. al.* 1968). Increased milk production and fertility following treatment for selenium deficiency has been described in adult cows grazing deficient pastures (Ellison 1992). Jolly (1960) demonstrated a 52% increase in growth rate in Jersey and Jersey-cross heifer calves treated twice orally with selenium on the pumice lands of Rotorua. Pullar *et. al.* (1985) demonstrated a 6.7% increase in growth rate in Hereford beef calves treated once orally with selenium pre-weaning in Te Anau. These two cases confer with Andrews *et. al.* that selenium responsive conditions are widespread on a variety of soils across New Zealand.

Methods of selenium supplementation include top dressing of pasture, oral and injectable formulations, slow release devices and a topical formulation. Many anthelmintic drenches and vaccines also include selenium supplementation (Ellison 2002).

Selpor Selenium Pour-On (A0077040, Boehringer Ingelheim Animal Health NZ Ltd) was a topical formulation for the supplementation of selenium to cattle developed in the early 1990s by Ancare New Zealand Ltd. Being a pour-on made treatment easy and Selpor was a well-received product by both veterinarians and farmers alike. Limited data is available on the efficacy of the product. Only one study has been published with Selpor used at the label dose rate, found in a patent for the application of trace elements to animals (US5543432A Patent). Selpor was removed from the market in 2022.

Due to requests from the market for an easy to use topical selenium supplement Inovata has developed a chemically identical formulation of Selpor. This study completed in Ranfurly, Central Otago in the autumn of 2024, was to support the registration and eventual marketing of the product.

Materials and methods

The primary objective of the study was to assess the absorption of two selenium pour-on formulations. Twenty 18-month Friesian bulls ranging in weight from 516-582kg were blocked by weight and randomly allocated to one of four treatment groups initially containing five animals each. Some untreated animals not included in the study but run with the study animals were blood tested at each time point as well. These animals' results have also been included in this analysis increasing the size of the negative control group to nine. The treatment groups were Inovata Selenium Pour On (Unregistered at the time of the study), Selpor (as mentioned above), Prolaject 2000 B12 + Selenium injection (A006903, Elanco NZ) and a negative control group.

Table 1. Treatment groups, animal numbers and dose rates

Group	n	Treatment	Dose rate	Selenium dose rate
1	5	Inovata Selenium Pour On	1.5 ml per 50kg	0.15mg/kg
2	5	Selpor Selenium Pour-On	1.5 ml per 50kg	0.15mg/kg
3	5	Prolaject B12 2000 Plus Selenium	1 ml per 40kg	0.1mg/kg
4	9*	Negative Control Group	NA	NA

*Increased due to sampling of extra untreated animals running with the trial animals.

Recommendations from Grace *et. al.* suggested that 4-5 animals was a suitable number of animals to sample for assessing the selenium status of a herd. Laven and Nortje's work from 2013 agreed with this recommendation. Given these recommendations and the lack of other published data assessing the efficacy of topical products a treatment group size of five was selected.

The animals were treated once on day 0. The Inovata and Selpor groups were treated according to the label directions, or in the case of Inovata proposed label directions. The Prolaject group was treated at 0.1mg/kg of selenium, greater than the label dose rate of 4-6ml per animal, which would have equated to a dose ranging from 0.03-0.05mg/kg in these animals. 0.1mg/kg was the dose rate recommended by Grace *et. al* for short acting injectable selenium supplementation.

Blood samples were taken pre-treatment and at days 3, 7, 14, 21, 28 and 44. The blood samples were tested for serum selenium at all time points and glutathione peroxidase (GPx) pre-treatment and at days 28 and 44. Analysis of blood samples occurred in batches during the study rather than at the study's conclusion. Blood testing was carried out at Awanui Veterinary Laboratories in Dunedin.

Animals were monitored for general demeanour two hours after treatment and the application site was inspected on day 3.

Due to the low number of animals in this study statistical analysis compared treatment groups separately but also included analysis of both topically treated groups combined. This was justified given that the two formulations are chemically identical with bioequivalence confirmed in results discussed later.

Comparisons were also made between day 0 results for all animals and the results for treatment groups at subsequent time points during the study. This comparison is relevant to veterinarians who are monitoring selenium supplementation but may not have a negative control group for comparison. This comparison is not affected by exposure to selenium through allo-licking of untreated control animals during the study.

Results

Table 2. Pre-treatment selenium levels all animals

	Serum selenium (nmol/L)	Glutathione peroxidase (KU/L)
Mean	134	2.1
Range	104-176	0.9-3.2
Adequate range[a]	>140	>2.0
Number low	17*	12*

*Out of 24 total animals presented for the study. [a] Grace *et. al.*

No safety issues were reported for the test product or any of the positive control products. One animal from the negative control group was euthanised due to sudden weight loss and ill-thrift believed to be associated with previous rumen acidosis from summer crop feeding.

One animal, No. 3015, Inovata Selenium Pour On, had two EDTA tubes sampled at day 44 as opposed to one serum tube and one EDTA tube, so no sample was available for serum selenium analysis at this time point.

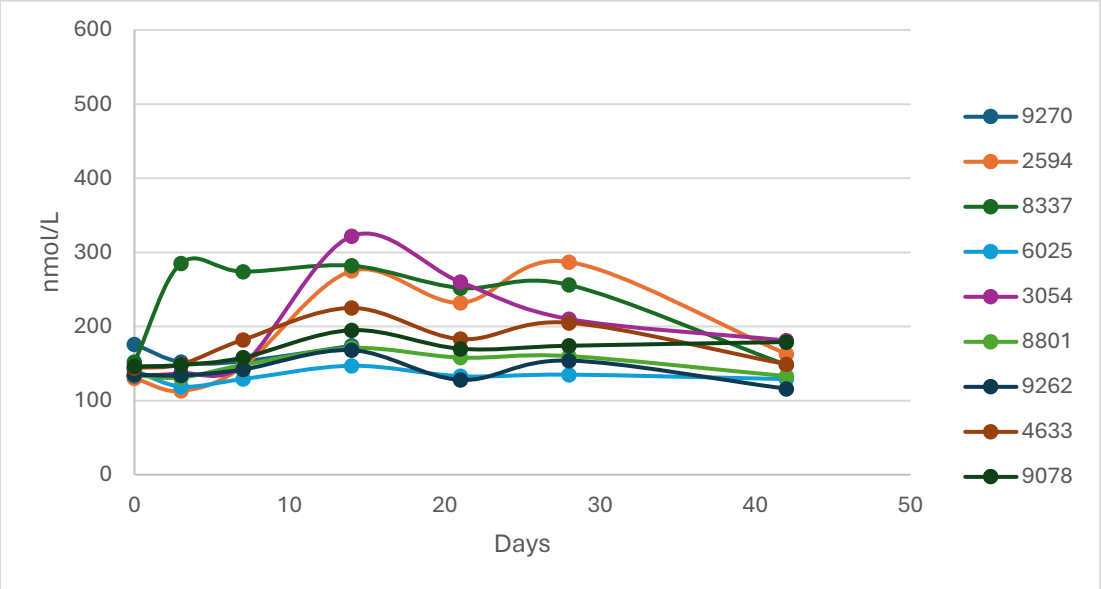
Table 3. Mean Serum Selenium Levels

		GPX KU/L				Serum Selenium nmol/L					
Study day		0	28	44	0	3	7	14	21	28	44
Treatment group	1. Inovata	2.2	3.0	2.6	139	268	300	296	252	248	179
	2. Selpor	2.1	2.2	2.1	124	241	230	255	218	231	177
	3. Prolaject	1.7	3.5	3.2	128	541	445	374	326	312	229
	4. Control	2.1	1.9	1.6	143	152	164	218	190	198	150

For the negative control group blood GPx levels decreased while serum selenium levels increased through the study. For GPx the difference between day 0 and day 44 levels was statistically significant ($p=0.01$) while for serum selenium the difference compared with day 0 was statistically significant for days 14, 21 and 28 only ($p<0.05$).

Some of the animal's individual serum selenium levels in the untreated controls showed elevations at different time points within the group and also from what was seen in the other treatment groups. Some untreated control animals didn't show any elevation in serum selenium levels during the study. This suggested that these elevations are due to some other exposure to selenium rather than mistreatment on day 0 or dietary intake which would have been consistent across the group. This exposure was potentially from allo-licking between animals.

Figure 1. Individual serum selenium levels all untreated animals



Using the reference ranges of Grace *et. al.* there was one animal in the Inovata group with a low serum selenium level and one animal from both the Inovata and Selpor groups low for GPx at day 44. Three out of the eight non-treated animals blood sampled at day 44 had low selenium levels for both GPx and serum selenium.

Table 4. Blood GPx day 44 (KU/L)

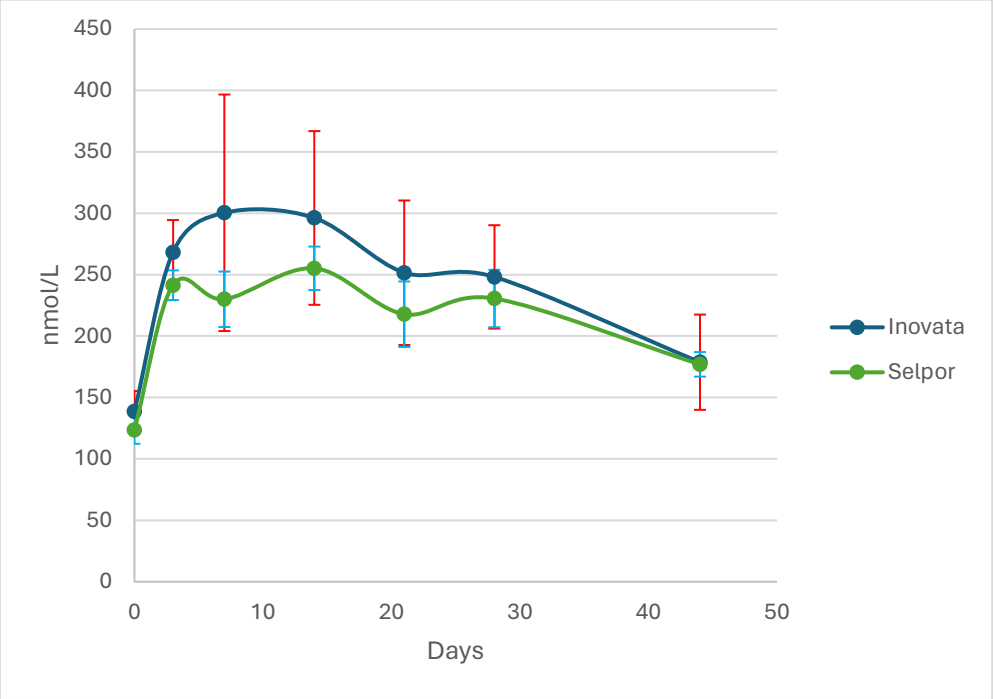
	Inovata	Selpor	Injectable	Control
Mean	2.6	2.1	3.2	1.5
Range	1.5-3.3	1.3-2.7	2.1-3.8	1.2-2.1
Number Low	1/5	1/5	0/5	3/8

Table 4. Serum selenium day 44 (nmol/L)

	Inovata	Selpor	Injectable	Control
Mean	179	177	229	150
Range	125-247	154-190	217-257	129-181
Number Low	1/4	0/5	0/5	3/8

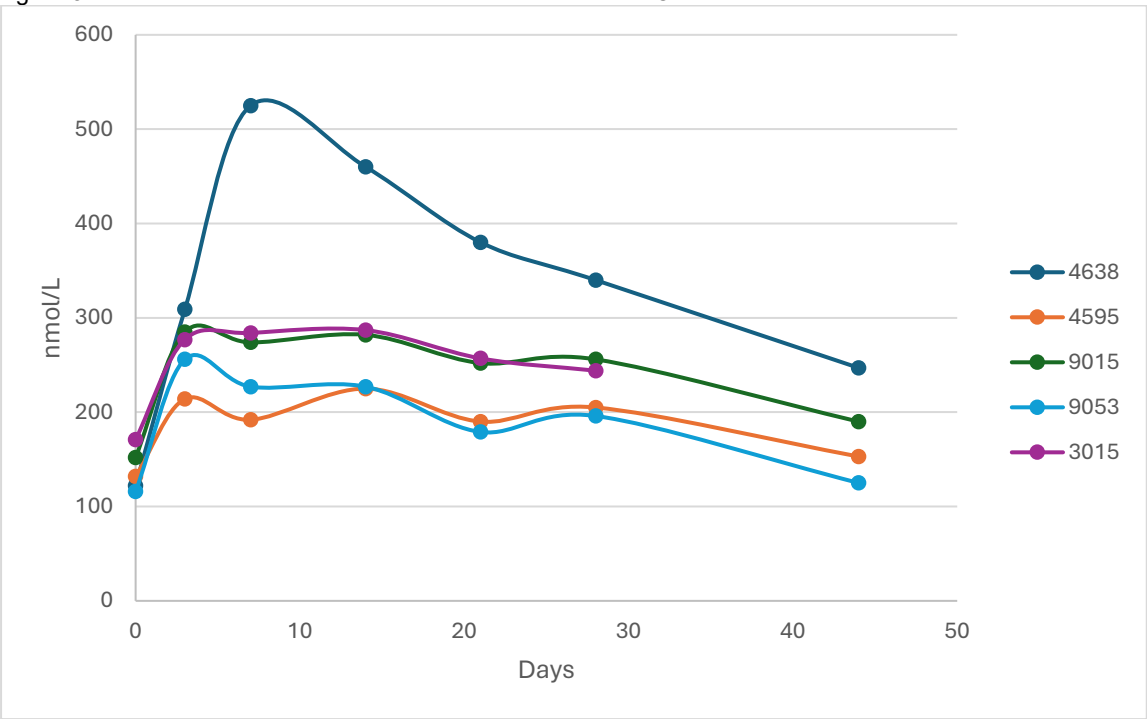
The serum selenium levels of both the Inovata and the Selpor products trended similarly during the study with the 90% Confidence Intervals overlapped at all time points. This was not the case with the blood GPx levels.

Figure 2. Mean serum selenium Inovata selenium PO and Selpor with 90% confidence intervals



The small size of the study contributed to the numerical differences between the mean serum selenium levels over time in Inovata and Selpor group. One animal in the Inovata group (animal 4638) had considerably higher levels of selenium compared to the rest of the group and this had a significant impact on the means and variance for this group.

Figure 3. Individual serum selenium levels Inovata selenium PO



Given that the two topically treated groups results are so similar and that the formulations to treat each were chemically identical the two groups were combined for some analysis to assess the efficacy of topical selenium supplementation.

Differences in serum selenium levels in the combined topical treatment group compared to negative controls were statistically significant ($p<0.05$) at days 3 and 7 and only numerical at all other time points. Differences in blood GPX levels between the same two groupings were statistically significant at day 44 ($p=0.004$) but only numerical at day 28 ($p=0.07$).

Table 5. Mean GPx and serum selenium levels of all topically treated groups combined vs. negative controls

	GPX KU/L			**Serum selenium nmol/L**						
	0	28	44	0	3	7	14	21	28	44
All Topical	2.2	2.6	2.3	131	255	265	276	235	239	178
Controls	2.1	1.9	1.5	146	161	170	240	219	222	155
p value*	0.9794	0.0680	0.0042	0.1354	0.0002	0.0111	0.0707	0.1105	0.0956	0.0641

*T.test, Microsoft Excel.

Figure 4. Mean serum selenium levels day 44

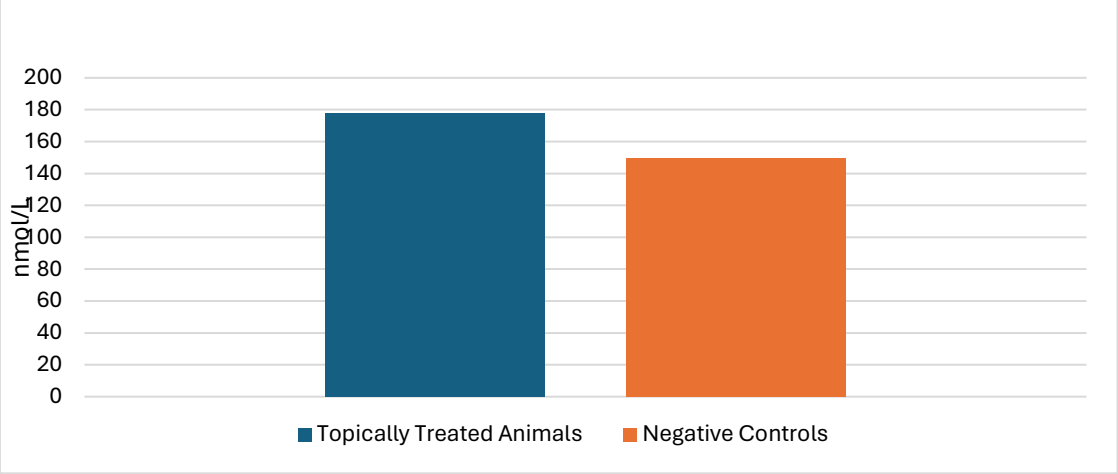


Figure 5. Mean blood GPx levels day 44

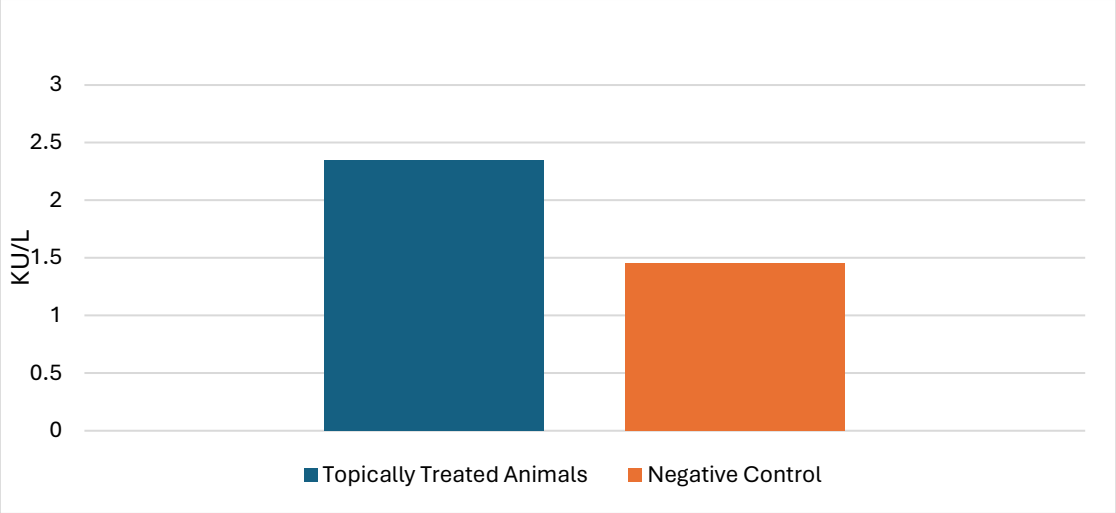
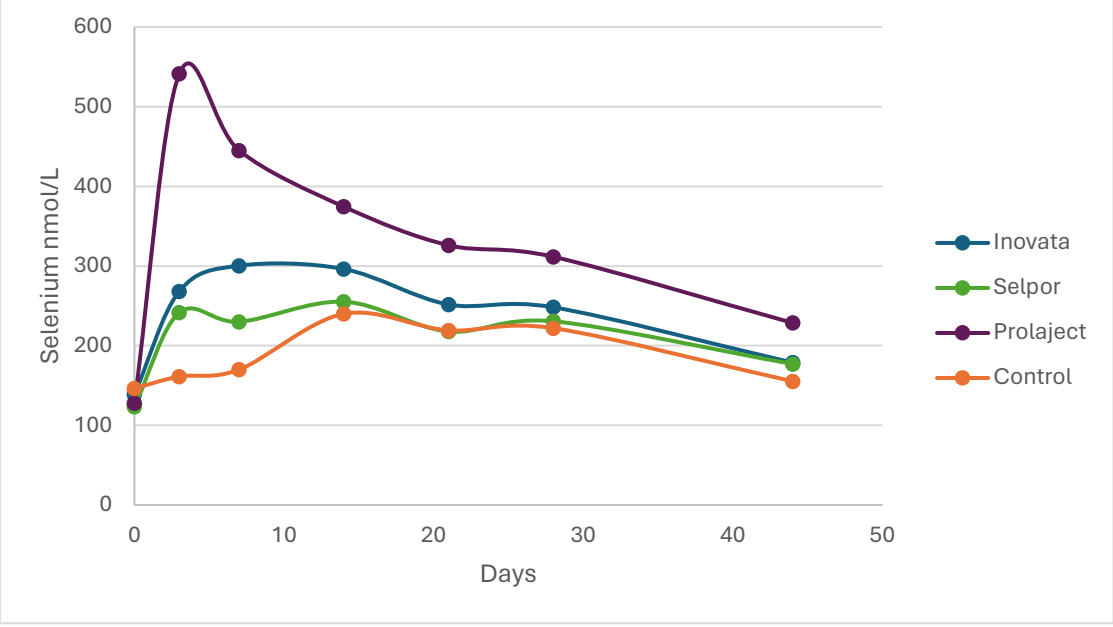


Figure 6. Mean serum selenium levels



There was a statistically significant difference between the day 44 serum selenium levels in the topically treated group compared to all animals' day 0 results (p=0.004). This difference was only numerical for GPx (p=0.26).

Figure 7. Mean serum selenium levels day 44 topically treated vs. day 0 all animals

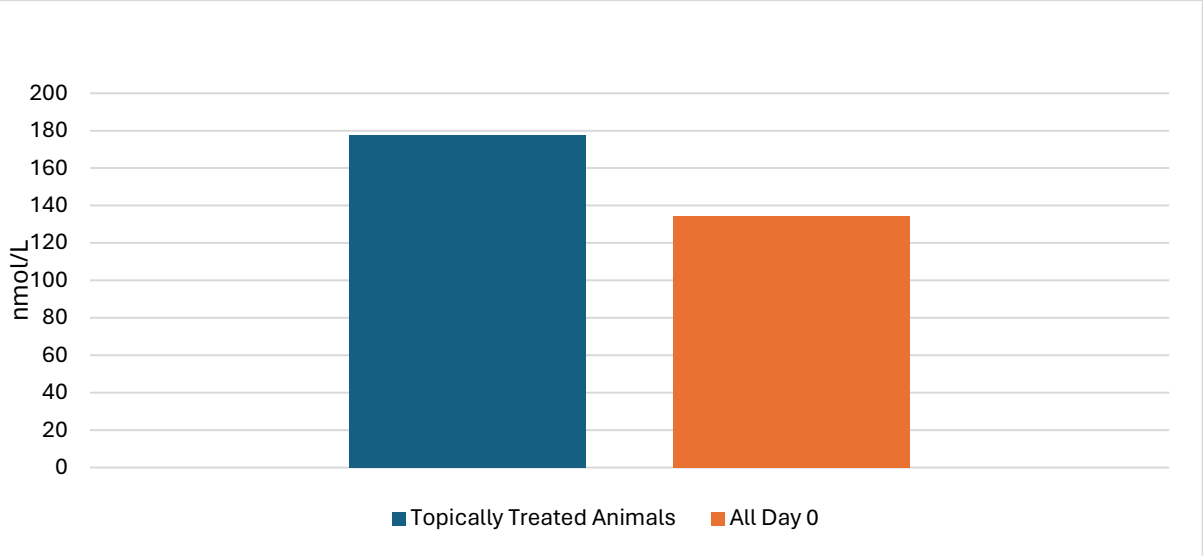
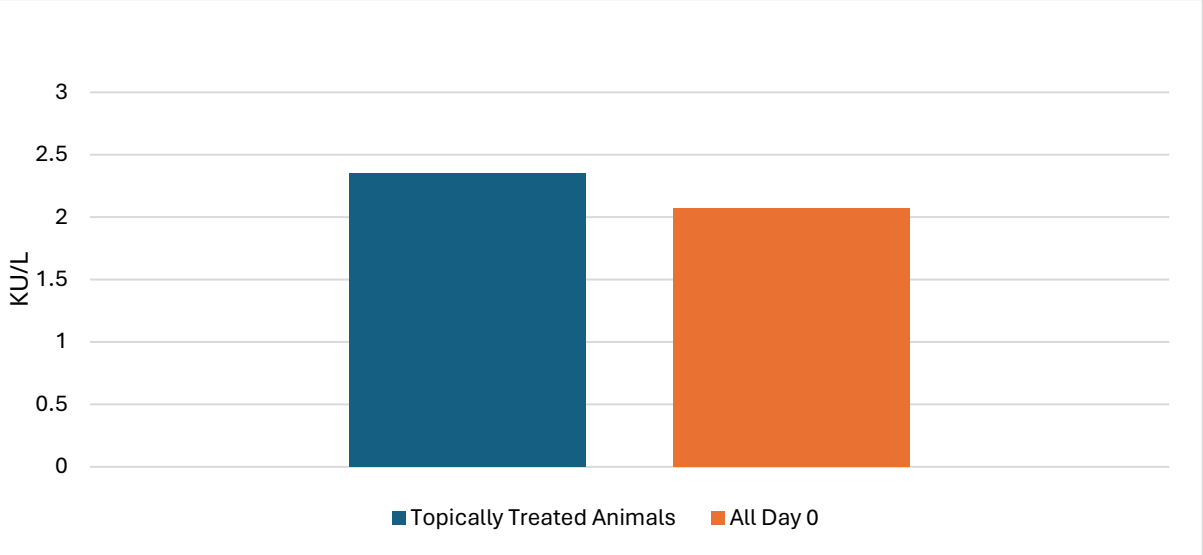


Figure 8. Mean blood GPx levels day 44 topically treated vs. day 0 all animals



The group treated with injectable selenium also had elevated serum selenium and blood GPx levels at day 44 compared the non-treated controls (p=0.00002 and p=0.002). When compared with the day 0 levels of all animals in the study the differences were also statistically significant (p=0.00004 and p=0.02 respectively).

Discussion

Selpor was a well-received product in New Zealand for the topical supplementation of selenium in cattle. The ease of application of Selpor and the fact that many oral cattle drenches have selenium in them meant that this product was often used in older, larger cattle, run in more extensive farm systems. A common question from farmers has been, how often should they be treating with the product to maintain adequate selenium levels in their cattle?

The results of this study showed that the topical treatment with selenium elevated both serum selenium and blood GPx levels in cattle. These levels were declining by day 44 but still elevated compared to day 0 levels. The differences between treatment groups and in comparisons between day 44 with day 0 levels for all animals was not always statistically significant in this study.

The size of the treatment groups in this study was selected based on the recommendation of Grace *et al.* and Laven and Nortje for sample size for the detection of selenium deficiency in a herd, not for the comparison of different treatments or showing bioequivalence. The lack of statistical significance between the different treatment groups at different time points through the study is most likely due to inadequate sample size rather than inefficacy of treatment. This statement does not apply to comparisons between Selpor and the Inovata Selenium product who's levels should be very similar given that both formulations are chemically identical. The data from this study could be used to better design future studies for comparing treatments if required.

The difference between topically treated and untreaded controls would likely not have been statistically significant if the extra untreaded animals outside of the allocated study animals that were sampled during the study were not included in the analysis.

There was an indication during the study that allo-licking was causing the transfer of selenium between animals. This was demonstrated graphically looking at the individual animal serum selenium levels in the untreated controls (Figure 1). This exposure to selenium during the study meant that comparisons between the treated and untreated control groups underestimates the efficacy of the treatments. For this reason comparisons between day 0 and day 44 selenium levels were also included in the analysis.

The comparison between day 0 and day 44 results are useful to veterinarians monitoring selenium levels in the field as all animals in the herd would commonly be treated and there would be no negative control group for comparison.

At day 44 the appearance of individuals with low serum selenium and GPx levels when compared with Grace *et. al.* reference ranges would suggest that the requirement for retreatment was imminent.

Grace *et. al.* recommended a period of efficacy of 4-7 weeks for short acting supplements such as oral drenching or injection with sodium selenate at 0.1mg/kg. This study would suggest that topical selenium supplementation is at least as effective as these recommendations for short acting products.

Over the course of the study the serum selenium levels in the untreated controls increased while blood GPx levels decreased. GPx activity is indicative of selenium uptake over 3-4 months while serum selenium is used to estimate more recent selenium uptake (Grace *et. al*). It could be surmised that the mean serum selenium levels were more sensitive to selenium uptake via allo-licking than blood GPx, so blood GPx is therefore a more accurate measure of the selenium status in the untreated controls in this study and selenium intakes outside of treatment were deficient during the study. Grace et. al state, that the interpretation of serum selenium versus blood GPx needs to take into account recent selenium supplementation in the interpretation of results.

Serum levels in the patent study (US5543432A) were higher than the Ranfurly study and also increased between the one-month and three-month time point, unlike in the Ranfurly study where the levels were declining from a Tmax of 7-14 days. The injectable selenium group in the

patent study's serum selenium levels were also increasing between the one- and three-month timepoints. This would suggest that there was exposure to selenium, outside of treatment, during the patent study. This reduces the studies usefulness for deciding on a re-treatment interval for the product and one might argue that selenium supplementation in the patent study was not indicated at all.

Table 6. Mean serum selenium levels

	Mean serum selenium levels nmol/L		
	Day 0	**Day 28/1 month (% increase)**	**Three month (Patent Study)**
Patent Study Selpor	643	1,166 (181%)	1,305
SP-BEQ-24-01 Selpor	124	218 (176%)	-
SP-BEQ-24-01 Inovata	139	252 (181%)	-

As previously stated the evidence of allo-licking could be seen in the individual untreated control blood levels. It would be harder to see the impact of allo-licking in treated animals due to the effect of prescribed treatment concealing the effect of the comparatively small amount of selenium absorbed orally. There was one animal in the Inovata group (No. 4638) that had a grossly elevated selenium level at day seven compared with herd mates. This elevation was much larger than any of the elevations seen in the untreated controls and was more akin to the elevation seen with the injectable group. This elevation was at day seven as opposed to the Tmaxs seen in the injectable group individual samples seen at day three making the possibility of mistreatment causing this unlikely. Could elevations this high in a treated animal be from allo-licking or is it from variation in the absorption of the treatment through the skin?

To eliminate the effect of allo-licking this study could be repeated with the untreated control group separated from the treated groups. This does increase the chances of systematic error with treatment groups and untreated controls potentially being run under different conditions and having a different exposure to selenium sources in feed or water.

This study does demonstrate that allo-licking and the transfer of topical products between animals orally does occur in friesian bulls. In practice the effect of allo-licking in treated animals is a moot point with no significant negative effect on efficacy, safety or residues for a mineral product.

Although there is no negative effect from allo-licking in the field if only a proportion of the herd were to be treated this would be different for an anthelmintic treatment. With an anthelmintic treatment under dosing via allo-licking may contribute to the potential development of drench resistance (Bousquet-Melou *et. al.* 2013) In the field instances where only proportions of the herd were treated with a mineral supplement would be rare.

The serum selenium and GPx levels in the injectable group were higher than the topically treated groups right through the study. It should be noted that these animals were treated at a much higher dose rate of selenium than the label directions for the product. This dose rate was the same as the standard dose rate for oral anthelmintic drenches containing selenium. To treat with the injectable product, also containing vitamin B12, at this elevated dose rate would be expensive compared to other forms of selenium supplementation and the dose volume was quite large making injection more difficult (approximately 14 mls per head for these animals).

The dose rate for the topical selenium product was only a 50% increase in selenium from the recommendations for short acting injectable and oral formulations. For macrocyclic lactone anthelmintic products the increase in dose rate from injectable or oral formulation to topical in

cattle is 250%. The results from this study suggest that there could be benefits in increasing the dose rate for selenium in the topical product to give blood levels similar to what was seen with an injectable product given at the recommended dose rate.

A topical selenium product provides ease of use advantages over both oral and injectable products.

Ease of use is an important consideration for treatment of cattle, in particular larger cattle run in extensive situations.

This study showed that treatment with Inovata Selenium Pour On was safe and efficacious elevating selenium levels in treated cattle for approximately 44 days and was bioequivalent to the previously registered Selpor.

References

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This study was approved by the Lincoln University Animal Ethics Committee (AEC2023-04).