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Copper deficiency: A review of the economic cost and current constraints to effective management of copper deficiency in southern Australian sheep flocks

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Abstract

As pasture testing has continued to reveal a broader issue of copper deficiency in areas not previously considered at risk, this trial was initiated to investigate the prevalence, effects, identification and treatment options for copper deficiency in sheep flocks. Following an extensive literature review, a series of trials were conducted at six sites in South Australia and Victoria to measure the impact and effect of treatments on copper deficiency. It was found that scouring and lameness in sheep are not directly related to copper deficiency, as is commonly believed. The greatest production effect of copper deficiency was a reduction in ewe and lamb survival rates. A range of methods were tested for identifying the extent of copper deficiency on a property, with pasture mineral analysis proving to be the most useful and cost effective option for producers to implement. A range of treatment options were trialled; each was effective at elevating liver copper concentration in line with their respective copper dose rate. Selection of the most appropriate treatment will depend on the severity of the deficiency and the required treatment length of time. Professional advice should be sought in recommending an appropriate copper dose rate as toxicity can be experienced where excessive amounts of copper are provided.

Executive Summary

As a result of extensive pasture analyses across properties in Victoria and South Australia over the last 10 years it appears that copper deficiency is being detected in areas where it has not been noted previously. Individual property fertiliser and liming practices have changed the available mineral and trace element profile such that it is no longer appropriate to refer to “areas of deficiency” but essential that properties are investigated and deficiencies managed on an individual basis.

Copper deficiency has often been reported to exert the following negative effects on productivity:

- Severe lameness
- Chronic scouring
- Increased lamb mortality
- Infertility
- Reduced wool quality (steely wool and loss of crimp definition) and value

Anecdotal evidence suggests copper supplementation can increase fertility rates by up to 30% in some instances, as well as significantly improving lamb survival and stocking rates.

This project aimed to investigate the true productivity effects of copper deficiency on sheep flocks in Southern Australia, as well as delivering recommendations for appropriate strategies to determine and treat copper deficiency in sheep.

An initial literature review was conducted to investigate the scope of copper deficiency in Southern Australian sheep flocks, review the efficacy of current treatment options and endeavour to determine the most appropriate range of treatment options for producers and industry advisers. This literature review detailed current information on the geographic extent of copper deficiency, changes to farm practices that have predisposed sheep to the effects of copper deficiency, the causes of primary and secondary deficiencies, evidence of the effects of copper deficiency on production and current recommendations for treatment.

Through a series of on-farm trials this project investigated the effects of copper deficiency on sheep flocks, as well as determining the most appropriate method of diagnosis and treatment of copper deficiency.

The relationship between soil, plant, blood and liver copper status was investigated and it was found that the correlation between soil and pasture copper concentration was very low. Similarly, the correlation between blood and liver copper concentration was very low, indicating that blood was a poor indicator of animal copper status. Plant tissue analyses revealed that when the influence of copper and other interacting minerals such as molybdenum in particular were considered they could be used as a good predictor of liver copper concentration.

Observations of scouring, lameness and swayback in lambs were recorded throughout the duration of the trial. No significant effect of copper deficiency could be attributed to either scouring or lameness in sheep. Additionally, very few lambs were observed with symptoms of swayback for the duration of the trial, possibly indicating that severe deficiencies must present for this symptom to be commonly expressed.

A range of copper products were trialled including an injection, water trough block, oral loose lick, ruminal capsule and ruminal bolus. Each product was effective at elevating liver copper concentration and generally in line with the copper dose rate of the product.

Contrary to evidence presented in the literature review, copper supplementation did not have a consistent positive effect on conception rates of ewes, however this most likely due to the limited impact of high molybdenum on ovulation rates (as opposed to low copper) as excessive levels of this mineral are rarely observed in Australia.

Treatments were found to have a positive effect on ewe and lamb survival rates where a severe copper deficiency exists. Supplementation increased lamb marking percentage by up to 15% and in some instances increased the percentage of ewes rearing a lamb by up to 30%. However, where marginal deficiencies exist there was no consistent response to supplementation observed.

Assessing pasture mineral status over a period of 12 months can provide an accurate and relatively simple method of determining whether a potential copper deficiency requires any action. This method is more accurate and often more cost effective than conducting blood or soil tests to assess copper status in sheep.

The use of pasture mineral analyses should be encouraged to identify not only the inherent copper concentration of pasture but also the influence of other minerals such as molybdenum, sulphur and iron which can reduce copper availability and animal copper status.

Selection of the most appropriate copper supplement product will largely depend on the timing and severity of the identified deficiency.

Where a seasonal copper deficiency exists (i.e. it only occurs for a short period of the year) then a short term supplement such as a loose lick would be the most appropriate treatment. The copper dose rate of the product should be formulated to account for the severity of the deficiency identified.

Where a continual and/or severe deficiency exists, a longer term treatment product such as a copper ruminal capsule would be appropriate to deliver a longer treatment effect as well as typically a higher dose rate of copper compared to other products commercially available for sheep. Where a severe deficiency exists, either due to very low copper concentration in pastures and/or high levels of influence from antagonistic minerals, multiple treatments may be required to adequately address a copper deficiency. However, as copper supplementation can be toxic to sheep when excessive levels are provided, professional advice should always be sought to ensure the correct dose rate is supplied.

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1 Background

As a result of extensive pasture analyses across properties in Victoria and South Australia over the last 10 years it has become increasingly apparent that copper deficiency is being detected in areas where it has not been noted previously.

It appears that fertiliser and liming practices within individual farms have changed the available mineral and trace element profile such that it is no longer appropriate to refer to “areas of deficiency” but essential that properties are investigated and deficiencies managed on an individual basis.

Copper deficiency is commonly reported to exert the following negative effects on productivity:

- Severe lameness
- Chronic scouring
- Increased lamb mortality
- Infertility
- Reduced wool quality (steely wool and loss of crimp definition) and value

Anecdotal evidence suggests copper supplementation can increase fertility rates by up to 30% in some instances, as well as significantly improving lamb survival and stocking rates.

This project aimed to investigate the true symptoms and productivity effects of copper deficiency on sheep flocks in Southern Australia through a literature review of previous trial work relating to copper deficiency and a series on on-farm trials to measure the effects of copper supplementation. The project delivers recommendations for critical levels of copper and interacting minerals for predicting copper deficiency in sheep as well appropriate treatment options under each scenario.

2 Project objectives

- Review the literature and consult with industry to:
    - Determine the efficacy of current treatments across different environments
    - Determine the reasons for the apparent variable response to current treatments
    - Quantify the relationship of copper deficiency with scouring, lameness, lamb mortality, infertility and its effect on wool quality
    - Identify the extent of the problem over southern Australian sheep production systems
- Undertake soil, plant and animal measurements to:
    - Determine the correlation between each
    - Determine the potential to use soil and/or plant analyses as an indicator for potential copper deficiency in sheep
    - Determine if the time of year or stage of plant growth influences the copper status of plants
    - Validate the accuracy of blood and liver copper analyses

- Determine the treatment options most likely to be effective in treating copper deficiency at each trial property and implement an efficacy trial
- Improve animal productivity and welfare across southern Australia as a result of more effective treatment of copper deficiency

3 Literature review

3.1 Introduction

Copper deficiency has been reported sporadically in sheep flocks in southern Australia since the 1940's however what remains unclear is whether the incidence is increasing or if it is being more frequently detected as a result of increased pasture and animal testing.

Pasture analyses across properties in SW Victoria, the mid-north of South Australia (SA), the south-east of SA and on Eyre Peninsula over the last 10 years have revealed that copper deficiency is being detected in areas where it has not been noted previously.

It appears that fertiliser practices within individual farms may have changed the available mineral and trace element profile such that it is no longer appropriate to refer to "areas of deficiency" but essential that properties are investigated and deficiencies managed on an individual basis.

As producers endeavour to become more efficient per unit area of land, lime applications have become a common component of pasture management in order to increase dry matter production. Agronomic advice in south-western Victoria often includes the application of lime however, increasing soil pH increases availability of nutrients such as molybdenum which in many areas appears to be reducing the availability of copper for grazing animals.

Copper deficiency is commonly reported to exert the following negative effects on productivity:

- severe lameness
- chronic scouring
- increased lamb mortality
- infertility
- reduced wool quality (steely wool and loss of crimp definition) and value

On several properties anecdotal evidence has reported that the symptoms described above from copper deficiency have resulted in a significant reduction in carrying capacity and up to a 30% reduction in fertility.

Molybdenum, sulphur and iron either singly or in combination effectively reduce the availability of copper.

The current recommendation for copper deficiency in the presence of high levels of molybdenum is the use of slow-release copper intraruminal capsules however the efficacy of these capsules appears to vary markedly across enterprises and regions and the reasons for this remain unclear.

Alternative treatment options such as drenching with copper sulphate, the addition of copper blocks to water troughs and the application of Coppercote to pasture seed or to pastures directly have resulted in differing and inconsistent levels of success.

The contribution of copper deficiency to the winter scouring problem of sheep in western Victoria has been well documented however it appears that there may be an interaction between copper deficiency and high plant levels of molybdenum, nitrogen and neutral detergent fibre (NDF) that further complicate the picture.

Much has been written about mineral deficiencies including copper however the aim of this review is to focus on the research and general information published over the last 15-20 years in the following keys areas of copper nutrition:

- The extent and industry cost of copper deficiency
- Changes in on-farm practice that may have increased the prevalence of copper deficiency
- Bioavailability of copper in two deficiency situations:
    1. Primary - inherent copper deficiency
    2. Secondary - copper deficiency as a result of interactions with other minerals such as molybdenum, iron and sulphur
- Current remedial and prophylactic therapies (including those available in other species that may identify potential options)
- Efficacy of current treatments
- Interaction of copper availability with high protein diets, scouring, lameness and lamb mortality
- Potential of copper deficiency as a precursor to foot scald / footrot

The purpose of the review is to investigate the scope of copper deficiency in Southern Australian sheep flocks, review the efficacy of current treatment options and endeavour to determine the most appropriate range of treatment options for producers and industry advisers.

3.2 Extent and industry cost of copper related disease

3.2.1 Geographic extent

Copper deficiency has been documented and purported to exist in many regions of southern Australia, through surveys of localised areas or via anecdotal information.

During abattoir surveillance conducted 1989-1991 in South Australia where over 2000 liver samples from cattle were analysed for copper concentration, 24% of samples were noted to be indicative of a deficiency (Koh, 1990); unfortunately the origin of these cattle was not published. A similar investigation, conducted by Baumgurtel and Langman (1995) was undertaken on Lower Eyre Peninsula in South Australia where they reported that across seven sub-regions or localities (Table 1) the percentage of cattle with low liver copper concentrations ranged from 12% to 76% with an average of 35%. Unfortunately, with both of these South Australian investigations no record of dissemination of these results to producers is mentioned or published therefore it is unclear if producers were made aware of these findings. Anecdotal evidence from younger producers suggests that their predecessors traditionally put copper sulphate in water troughs to not only minimise algal growth but also to address copper deficiency in livestock; so some awareness and/or education about copper deficiency must have occurred in previous years.

Table 1 - Percentage of animals with low copper concentration at slaughter across seven regions of Lower Eyre Peninsula SA (Baumgurtel and Langman, 1995)

Region (hundred)	% of liver samples low copper (number of samples)
Cummins	20% (10)
Koppio	12% (17)
Lincoln	34% (41)
Mortlock	37% (30)
Shannon	76% (17)
Wanilla	36% (25)
Louth	28% (25)

Unfortunately there is no evidence that either of these surveys have been repeated to monitor any change in prevalence. However if pasture copper concentrations prove to be a reliable indicator of the potential for copper deficiency in sheep and cattle as determined by this project, then pasture tests conducted over the last four years from Lower Eyre Peninsula show that 60% of pasture samples recorded (Productive Nutrition Pty Ltd) were below the recommended minimum copper content for sheep of 7mg/kg DM. This might indicate a potential increase in copper deficiency since the cattle survey taken in 1995 (Baumgurtel and Langman, 1995).

Whilst this comparison must be treated with caution as it does not compare the same measurements and variables such as the timing of measurements will affect the result, it is not unreasonable to suggest that copper deficiency on Eyre Peninsula is at the very least, not decreasing and conservatively has probably increased in incidence by around 10% over the last 10-15 year period.

Edwards et al. (2004) investigated the responses of sheep to copper oxide intra-ruminal capsules grazing saline land in the south-east of South Australia as a determinant of their mineral status. As there were no preliminary investigations of the copper status of the pastures and treated and control animals were run in different paddocks, interpretation of their results is difficult. Further, the copper status of the sheep in this study was determined via blood tests, the reliability about which there exists a diversity of views.

The authors assumed that because the different paddocks had a similar fertiliser history and soil type that their mineral status would be similar; it is the experience of Productive Nutrition researchers that may not be the case as highlighted in Fig. 1. These paddocks adjoin each other and appear very similar however the one on the left has a far greater deficiency of copper than the one on the right.

Fig. 1 - Copper content of two paddocks next to each other with soils very similar in appearance yet with different mineral status.

Publicly accessible evidence of the mineral status of plants and animals across Southern Australia appears to be lacking. Short term projects within state Departments of Agriculture and seemingly random investigations tend to result in anecdotal evidence; however there is little supporting data to be found. The seasonal and sometimes transient nature of mineral deficiencies and the inherent risk to animal health from copper over-supplementation has resulted in an ad-hoc approach to suspected deficiency situations.

Consultation with veterinary officers, advisers, researchers and product salespeople as a component of this project has suggested that copper deficiency remains an issue across various parts of New South Wales, Victoria, South Australia, Tasmania and Western Australia. These opinions have been based on random testing for copper deficiency via blood, liver, pasture, soil as well as positive responses observed in sheep supplemented with copper.

Unfortunately, during the consultation process it became evident that there is a very poor understanding about copper deficiency by professional advisers, researchers and resellers alike.

Research scientists tend to believe that copper deficiency both primary or induced has been alleviated by the introduction of slow-release intra-ruminal capsules however there is anecdotal evidence in the western districts of Victoria that these capsules are not always effective.

Researchers do not agree on the reliability of blood testing as an indicator of copper status, and the general understanding and management of copper responsive disorders appears lacking.

This was highlighted following consultation with a respected industry adviser who cited that pasture copper levels in their area were changing rapidly from severely deficient to toxic within a matter of two weeks. This conclusion was based on the fact that when autopsied, the animals had shown symptoms of copper deficiency such as osteoporosis and low liver copper concentration however blood plasma copper levels were high. As he believed that blood levels react more quickly than liver it was assumed that toxic levels of copper must have been ingested from the pasture.

What was not and possibly should have been considered was that in areas of low copper, high molybdenum and sulphur (which this adviser had described were present) copper is bound in an insoluble complex with thiomolybdates in the blood, often returning normal to high blood copper levels however this copper is not freely available to the animal resulting in symptoms of deficiency. This example highlights that information about the current copper status of southern Australian sheep flocks may vary according to the level of understanding of the informant.

Fig. 2 shows common areas where copper deficiency existed within Australia in 2000; these areas are highlighted in orange. However, the Productive Nutrition database would imply that the deficiency is far more widespread than indicated by Caple and McDonald in Fig. 2 (Buckley, 2000). The areas they depict as being selenium and cobalt deficient also appear to be copper deficient (D Rendell 2012, pers. comm., 29 Jun. M Chirgwin 2012, pers. comm., 29 Jun). Many plant species in the Australian rangeland areas have been found to be copper deficient including in the Lake Cargelligo area of NSW (McNerney & Darwood, 2006).

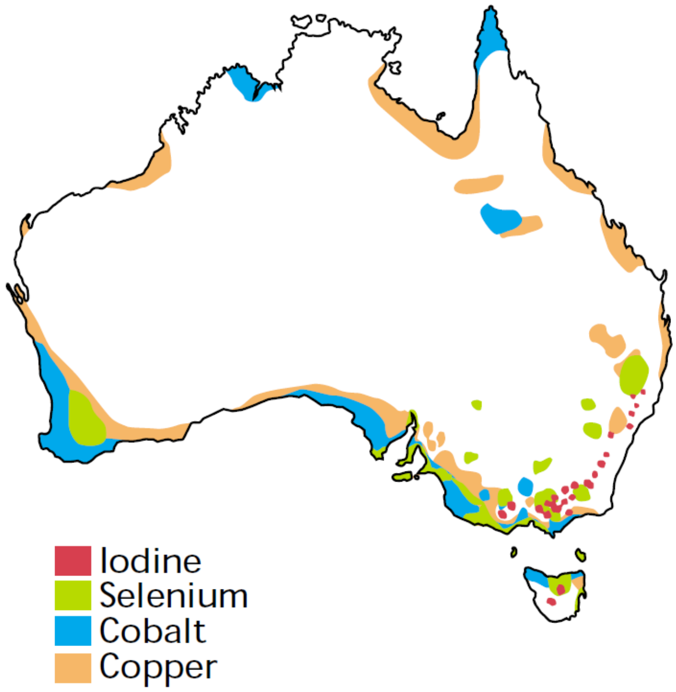

Fig. 2 - Common mineral deficiencies across Australia. Source Ivan Caple and John McDonald; published in Buckley (2000).

Although it is clear that copper deficiency is widespread and in some areas consistently severe, consultation with industry and a review of pasture analyses and animal tests, it appears that the prevalence of copper deficiency across southern Australia is of a sporadic nature and highly variable in its effect year to year. The reasons for this are most likely due to a combination of:

- Increased variation in the application of soil treatments including lime and fertiliser both across and between properties.
- An increased level of testing, albeit randomly, for mineral concentration and status in pasture and animals highlighting the inherent variation that exists in copper status of soil, plants and animals more clearly.
- Many more species and varieties of pastures and crops being provided as feed resources for livestock and the variation between the copper uptake and seasonal change in mineral content of these plants may be contributing to the differences in mineral status of livestock.
- The increased number of mineral supplement products available on the market containing variable amounts of copper and antagonistic minerals has increased the variation in copper status of animals.

Up until 10 years ago producers in some areas used copper sulphate in water troughs predominately as an algaecide. This may have inadvertently treated many unknown occurrences of copper deficiency; however as the next generation have taken over management of the farm this practice seems to have largely ceased.

Evidence and views on whether the incidence of copper deficiency is increasing varies widely. In 1990 Hungerford stated in the renowned animal health reference book Diseases of Livestock, that the “incidence of copper deficiency is becoming more common”. Other advisers and researchers believe that the incidence has not changed and that copper may have simply become a focal point for a period of time (Rendell, 2012, Scammell, 2012).

It is the view of this author that the incidence of copper responsive disorders has increased in the past 20 years as a result of changes in on-farm practices such as long term fertiliser use followed by the application of lime plus or minus molybdenum. However, a more popular opinion is that this is a relatively small increase and that the majority of the perceived increase in copper deficiency is a result of increased levels of understanding, interest and detection of copper related disorders by producers, advisers and researchers alike. The increasing area being sown to canola on mixed farms is also likely to increase copper deficiency in sheep grazing crops and/or stubbles in the future due to the requirement of canola for sulphur and sulphur being a major impediment to availability of copper.

To determine the change in incidence of copper deficiency a broad testing program is required that includes measurement of antagonistic factors to allow comparison of current data sets with those derived over many years. There may be significant amounts of pasture data held in Department of Agriculture and industry program archives (Grain & Graze 1) which if accessible, could provide valuable preliminary or supplementary information to a current and future testing program.

3.2.2 Role of interacting minerals and protein

Just as variation is present in pasture copper concentrations across Australia, there is variation in the levels of interacting or antagonistic minerals which reduce copper absorption and induce symptoms of copper deficiency. Molybdenum is known to have a significant effect on copper availability (Bone, 2010, Lee et al., 1999, Suttle, 1991, Telfer et al., 2004, Vasquez et al., 2001, Underwood and Suttle, 1999) and its concentration in pasture (Table 2) can vary markedly (0.37 – 5.10mg Mo/kg DM). Not only does this vary the effect of molybdenum and subsequent reduction in copper availability but also the copper molybdenum ratio which will be addressed in Section 3.4.

Table 2 - Average copper and molybdenum values of pasture across different regions of Australia during the growing season (May to Oct). Source Productive Nutrition Pty Ltd feed analysis database.

	Copper (mg/kg DM)	Molybdenum (mg/kg DM)
NSW	***5.03***	***1.04***
North East	4.20	0.50
North West	3.70	0.90
North coast	6.00	1.00
New England	5.40	1.00
Western area	3.00	1.10
Southern slopes	6.67	1.27
SA	***8.03***	***1.83***
Adelaide and surrounds	8.00	2.60
Barossa	9.00	1.10
Eyre Peninsula	6.57	1.42
Fleurieu Peninsula	9.50	0.70
Kangaroo Island	8.27	2.13
Lower North	9.04	1.20
Mallee	5.63	1.02
Mid North	10.98	1.06
Pastoral	8.39	3.12
Riverland	5.62	1.20
SE SA	7.60	2.35
Upper EP	7.00	1.10
Upper North	8.80	0.82
Yorke Peninsula	8.67	0.37
TAS	***10.18***	***3.38***
North	9.00	0.99
North West	8.00	1.75
Flinders Island	11.41	5.10
East	4.00	1.50
VIC	***7.07***	***1.56***
Central	11.00	1.50
Gippsland	7.30	2.10
Western districts	6.74	1.57
Wimmera	8.50	1.20
WA	***8.00***	***2.11***
Wheat belt	8.00	2.11

Minerals such as sulphur and iron also influence the availability of copper. Sulphur concentrations in pasture in particular can vary widely not only due to inherent soil variability but also due to differing fertiliser practices across properties and regions.

The crude protein (N x 6.25) content of livestock feed also has the ability to affect copper availability. As proteins are broken down in the abomasum, sulphur is released from the digestion of sulphur amino acids potentially reducing copper absorption, this process is detailed in Section 3.5.

Implementation of the most effective treatment of copper responsive disorders requires a sound determination of the cause of the deficiency. In any investigation to identify the extent of copper deficiency it is also critical to review the status of interacting minerals and feed properties such as protein content.

3.2.3 Industry cost of copper deficiency

Establishment of the cost of copper deficiency on the sheep industry requires reliable information on the current prevalence of the disease as well as its effects on sheep performance; it is important to be able to differentiate these effects from others such as worms and feed quality.

Unfortunately this information is simply not available; while there appears to be an increasing incidence of copper deficiency and/or copper related disorders across southern Australia, at this time we are not able to estimate the numbers of animals affected with any degree of accuracy. A comprehensive survey of the copper status of flocks across southern Australia via methodical testing of animal status (e.g. abattoir surveillance) and the influence of antagonistic factors such as minerals and protein ingestion through pasture analyses may assist in quantification of the extent and hence the cost of the deficiency.

For the purposes of illustrating the potential impact of copper deficiency a relatively simple financial analysis can be conducted to investigate the lost revenue from the effect of this disease as we currently know it.

Copper deficiency affects fertility, lamb survival and wool quality as described in Section 3.5. If these factors are considered in a financial analysis of breeding flocks across southern Australia the potential cost of copper deficiency per year would be approximately **$15,387,833**.

The way in which this figure has been derived is described below.

An economic model was developed which took into account the total number of breeding ewes (both Merino and non-Merino) in southern regions of Australia; currently approximately 30,327,550 (ABS, 2011).

Not all of these ewes will be affected by copper deficiency so for the purposes of this example the assumption was made that only 5% were affected; this resulted in potentially 1.5m ewes displaying the symptoms of copper deficiency.

Given the effect of copper deficiency on fertility and mortality rates of lambs (as described in Section 3.5), if marking percentages were to decrease by 20% across only those ewes affected by copper deficiency then over 227,000 lambs would be lost by the industry at a cost of over $13m (Table 3) where each lamb could potentially return a gross margin of $45 per head.

In addition to the effect of reduced fertility and increased lamb mortality rates, wool quality is often decreased through loss of crimp in the staple. Penalties for this less desirable style of wool can be in the order of 10-30c/kg greasy (AWEX, 2012). Given this effect will be of particular importance to Merino wool assuming a 20c/kg reduction in fleece value for Merino ewes and lambs only affected by copper deficiency may result in negative returns of $1,740,436 pa (Table 4).

Table 3 - Sensitivity analysis of the effect of varying levels of reduction in marking percentage and varying percentage of the ewe flock affected by copper deficiency on lost income.

		Reduction in marking percentage of ewes affected					
		5%	**10%**	**15%**	**20%**	**25%**	**30%**
Percentage of ewes affected	**2.5%**	$1,705,925	$3,411,849	$5,117,774	$6,823,699	$8,529,623	$10,235,548
	5.0%	$3,411,849	$6,823,699	$10,235,548	**$13,647,398**	$17,059,247	$20,471,096
	7.5%	$5,117,774	$10,235,548	$15,353,322	$20,471,096	$25,588,870	$30,706,644
	10.0%	$6,823,699	$13,647,398	$20,471,096	$27,294,795	$34,118,494	$40,942,193
	15.0%	$10,235,548	$20,471,096	$30,706,644	$40,942,193	$51,177,741	$61,413,289
	20.0%	$13,647,398	$27,294,795	$40,942,193	$54,589,590	$68,236,988	$81,884,385

As previously mentioned, further research appears to be required into the prevalence of copper deficiency across southern Australia, however the above estimates may be considered indicative of the potential cost of this disease in terms of lost income for producers.

Table 3 and Table 4 demonstrate the influence of varying the percentage of ewes affected by copper deficiency and the production impacts for marking percentage and discount of wool value. They highlight the fact that the impact of copper deficiency is both variable but quite significant as even in situations where small percentages of ewes are affected, loss of income across the industry can be substantial.

Table 4 - Sensitivity analysis of the effect of varying levels of discount for wool and the percentage of the breeding ewes affected by copper deficiency on the amount of lost income.

		Wool price discount (c/kg greasy)					
		5	**10**	**15**	**20**	**25**	**30**
Percentage of ewes affected	**2.5%**	$217,554	$435,109	$652,663	$870,218	$1,087,772	$1,305,327
	5.0%	$435,109	$870,218	$1,305,327	**$1,740,436**	$2,175,545	$2,610,654
	7.5%	$652,663	$1,305,327	$1,957,990	$2,610,654	$3,263,317	$3,915,980
	10.0%	$870,218	$1,740,436	$2,610,654	$3,480,872	$4,351,089	$5,221,307
	15.0%	$1,305,327	$2,610,654	$3,915,980	$5,221,307	$6,526,634	$7,831,961
	20.0%	$1,740,436	$3,480,872	$5,221,307	$6,961,743	$8,702,179	$10,442,615

As part of the on-farm trials associated with this project, further data will be gained regarding the productivity effects of copper deficiency. This will assist in more accurately defining the industry cost of this mineral deficiency and will also help to further define additional effects of copper deficiency on sheep such as chronic scouring and spontaneous bone fractures.

3.3 Changes to farm practice predisposing sheep to the effects of copper deficiency

In 1986 Hosking et al published the map below (Fig. 3) denoting the areas of Victoria which had been identified as copper deficient by Savage in 1974. Hosking et al. (1986) also stated that “many of the pastures in the most deficient areas have now been treated with copper”.

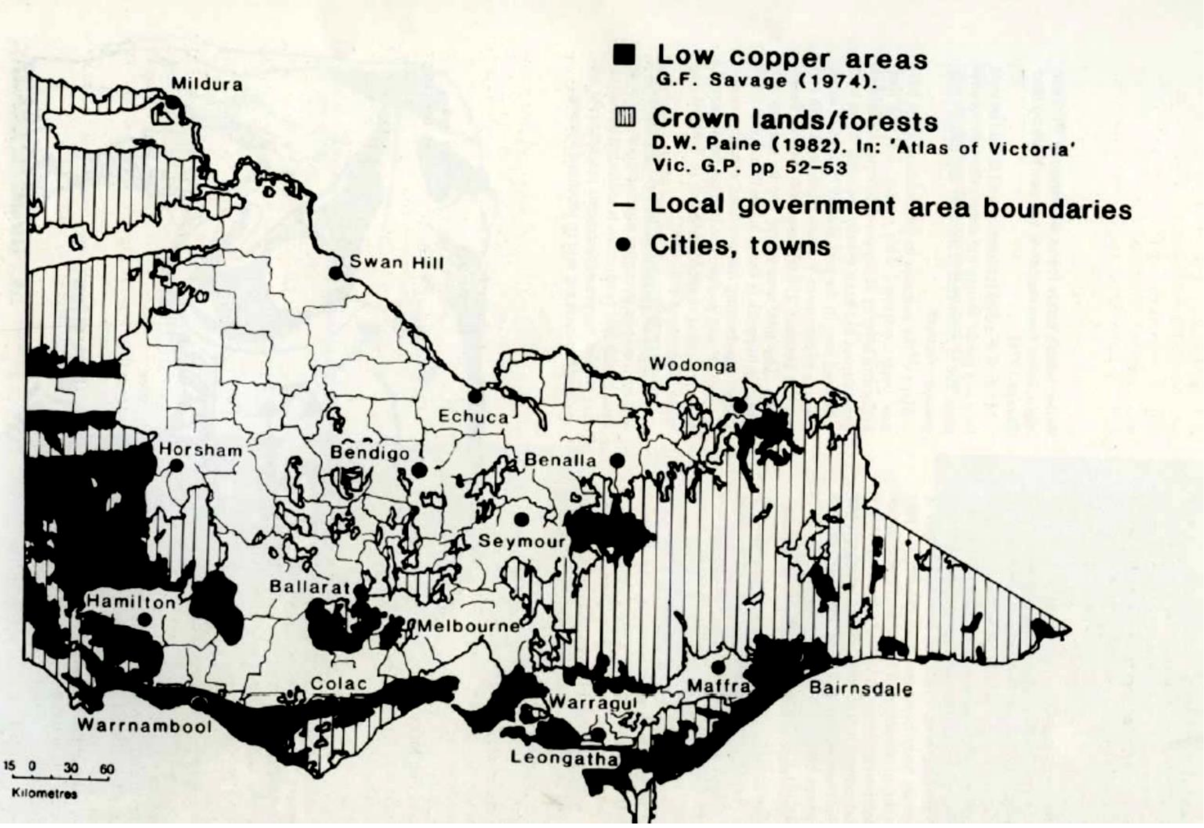

Fig. 3 - Victorian areas where copper deficiency was identified through pasture and livestock testing around 1974. (Hosking et al., 1986)

It is clear from the evidence presented in Section 3.2 that copper deficiency continues to be a problem throughout southern Australia including Victoria; so why is it that despite areas being ‘treated’ for copper there still appears to be on-going evidence of this deficiency (Ryan and Cave, 2011)?

On-farm practices have a significant effect on the availability of copper and practices such as fertiliser application, soil amelioration and improvement of pasture species appear likely to affect the availability of copper to grazing animals (Smith et al., 2006, Cordon and Ashton, 2005, Hosking et al., 1986). There are a number of key on-farm practices that continue to predispose sheep to the effect of copper deficiency including:

- fertiliser application
- liming / gypsum

- clay spreading
- pasture species selection

3.3.1 Fertiliser application

Copper fertiliser applications of 2-6kg/ha have been shown to be effective in maintaining pasture copper levels for over 20 years (Judson and Babidge, 2002). However, despite widespread applications of copper fertiliser many livestock still display the symptoms of copper deficiency throughout southern Australia.

Judson and Babidge (2002) reported that on sandy soils in the south-east of South Australia copper applications of 2kg/ha were able to maintain satisfactory levels of copper for at least 23 years. Their trial was established to measure responses to copper fertiliser and animal treatments. Pastures had been treated with copper fertiliser up to 23 years earlier and the authors measured the effect of reapplication of copper fertiliser (as copper sulphate) on pasture productivity and copper injections on sheep productivity. It was their conclusion that the original copper application was still providing a satisfactory level of copper and that repeated copper dressings were unwarranted. Their conclusions were based on the following observations:

1. land not retreated with copper maintained copper concentration in the pasture generally above 6mg/kg DM which they considered satisfactory
2. land treated with a second copper fertiliser application did not grow significantly more pasture dry matter
3. sheep liveweight, greasy wool production and fibre diameter did not respond to copper injections
4. blood and liver copper levels measured in sheep grazing land treated once up to 23 years earlier were above minimum thresholds

Although copper supplementation did not affect wool growth (although the authors made no mention of wool crimp), fibre diameter or strength and had little effect on liveweight or pasture growth, this research raises interesting and perplexing questions. Molybdenum concentrations in the diet were excessively high (up to 22ppm) and responses to copper supplementation in the form of copper oxide capsules and copper heptonate injections appeared variable.

Whilst the results of this trial suggest that copper fertiliser application may be effective in soil for over 20 years there are conflicting recommendations in the industry regarding the frequency of applications required. Hosking (1986) states that the standard recommendation in Victoria has been 2kg of copper per ha every five to seven years, whilst there is also anecdotal evidence from one property near Portland, Victoria, one copper fertiliser application was effective at raising pasture copper content and repeat treatment has not been necessary. Many authors recognise that applications of copper fertiliser should be varied to suit the individual circumstances of the property and/or region (Hungerford, 1990, Hosking et al., 1986, Underwood and Suttle, 1999). Further research is required in this area of copper nutrition as varying soil types and the influence of other minerals will most likely change the requirement and/or efficacy of this method of treatment.

One of the likely reasons that despite copper fertiliser application a reduction in the prevalence of copper deficiency has not become evident is that in many situations high levels of antagonistic minerals such as molybdenum, sulphur and iron reduce the availability of copper to the animal, despite copper concentration in the pasture increasing (Judson and Babidge, 2002). However interestingly this was not observed in their own research.

Molybdenum deficiencies in pasture have been widely reported over large areas of Victoria (Hosking et al., 1986) as well as parts of south-west Western Australia, South Australia and Tasmania (Incitec Pivot, 2009). The livestock requirement for molybdenum is minimal (0.5ppm) such that small amounts applied to pasture in order to balance the soil profile are likely to reduce the availability of copper to grazing animals. Molybdenum is required by lucerne seed crops to facilitate seed set (S. Oster 2010, pers. comm., 24 Aug.), such that secondary copper deficiency can be found frequently where sheep are grazing lucerne where seed production is a priority. Unfortunately pasture and crop agronomists appear to have limited understanding of animal nutrition and the potential effects of their recommendations. Molybdenum fertilisers have often been applied to pastures with no consideration of the potential impact on copper availability for livestock (Jolly, 2012) and it is highly likely that this farm practice has predisposed many sheep to copper deficiency.

Phosphate fertilisers have been extensively used across cropping and grazing country in southern Australia as large tracts of land are deficient in this important mineral for plant production (Bolland, 2007). Superphosphate is widely used to enhance phosphorus availability from grazing land however this fertiliser also contains significant amounts of **sulphur** (10.5%). While this has increased the sulphur content of many pastures the effects have been variable as not all paddocks are fertilised every year. Sulphur is an antagonistic mineral to copper and reduces the availability of this element to livestock. Both the increased and more variable levels of sulphur in pasture are likely to have contributed to the changes on on-farm practices which have predisposed sheep to copper deficiency.

3.3.2 Liming / gypsum

Soil acidity is a common issue in many farming areas of Australia which may be due to the natural acidity of the soil, nitrate leaching and/or the consistent use of nitrogen fertilisers (Hall, 2008). The optimal pH range for most plants is 6 - 7.5. When soil pH drops below 6 agronomists commonly recommend increasing soil pH by way of application of lime or gypsum if soils sulphur levels appear deficient or they are particularly hard setting (Hall, 2008). As soil pH increases, so too does plant availability of molybdenum (Fig. 4), which can increase the molybdenum content of pasture and result in reduced copper availability for livestock through the antagonistic action of molybdenum.

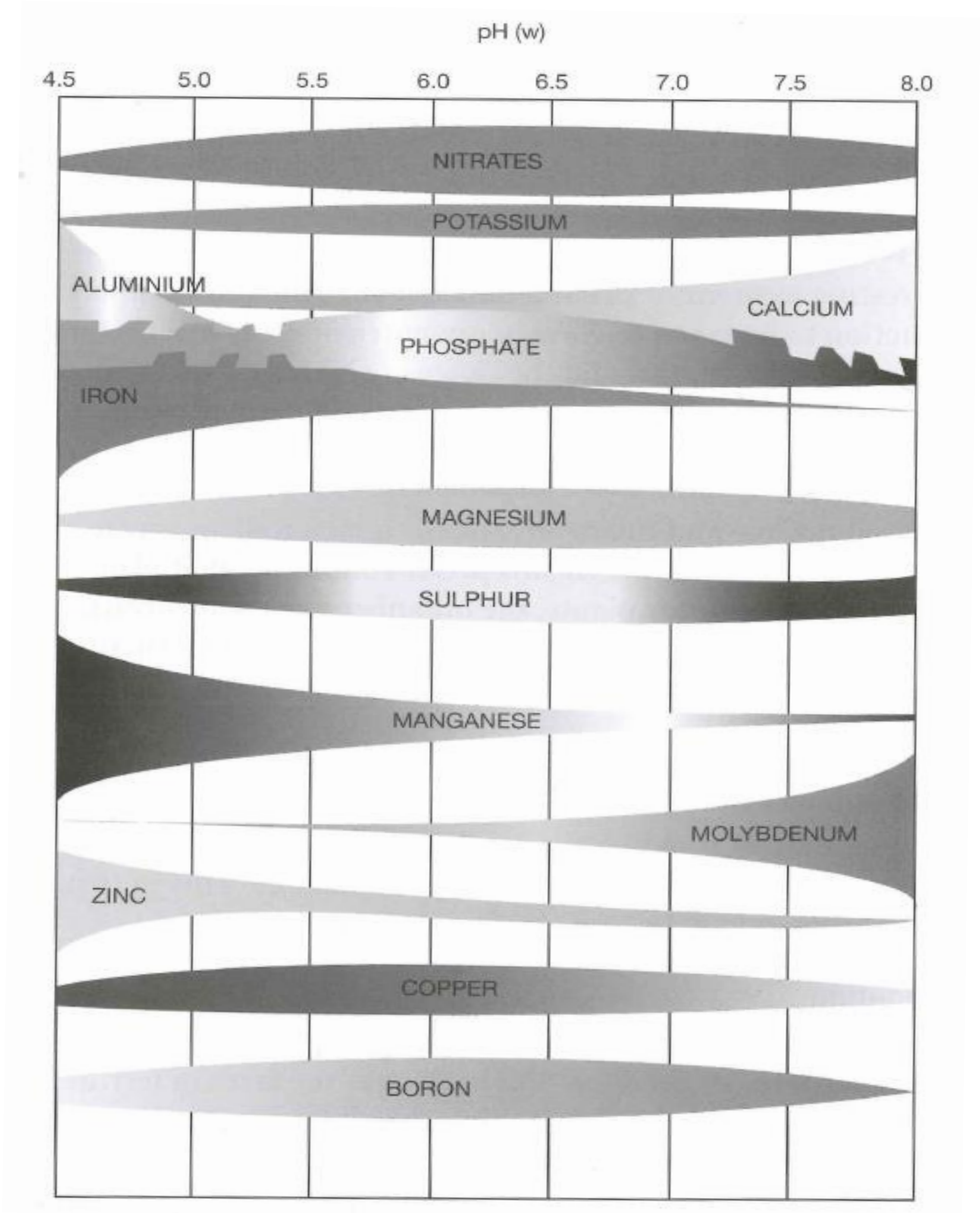

Fig. 4 - Nutrient availability over the pH range most commonly found in soils. (Hall, 2008)

3.3.3 Clay spreading

Clay spreading or delving is becoming more prevalent across areas of Australia with sandy and/or water repellent soils (Carter and Hetherington, 2006) such as Western Australia, the Mallee and south-east regions of South Australia and Victorian mallee. The clay used is normally of neutral or alkaline pH and significantly increases the pH of the soil where it is spread and or incorporated (Hughes, 2001). Increasing soil pH has the same effect as liming soil in that molybdenum is released in increasing amounts which results in a reduction in copper availability for sheep through the antagonistic properties of molybdenum. Producers appear to be becoming more aware of the effect of clay spreading on soil pH and the resultant implications for inducing copper deficiency in livestock. The topic was raised by producers during a consultation meeting conducted by Productive Nutrition in March 2012 in the South Australian Mallee. Producers at this meeting were surprised to realise the impact of clay spreading however they had suspected this might have been contributing to livestock issues with copper deficiency in their area.

This raises an important issue about the consultants providing agronomic advice having limited understanding about the implications of that advice on animal health and production. If at the completion of this project more certainty exists in our knowledge of the mechanisms of copper deficiency it may be appropriate to include agronomists in any education programs that might be developed.

3.3.4 Pasture species

Pasture selection in Australia has traditionally been driven by meeting the demands of the dairy industry which predominately target high protein pastures. As dairy cows have access to low protein complementary feeds such as hay and grain at least twice a day, management of high protein pasture does not present the challenges that it does to the health of extensively grazing ruminants such as sheep, goats and beef cattle. This has resulted in pasture and crop species often delivering over 30% crude protein during their active growth phase. This high protein content can reduce copper availability through the release of sulphides from sulphur amino acids as protein complexes are broken down. Further detail on this interaction is provided in section 3.5.1.

As more sheep producers have sought out the latest pasture varieties in the search of higher levels of pasture productivity they have unknowingly increased the risk of predisposing their sheep to copper deficiency. Pasture breeding in the future needs to include some focus on consideration of the needs of grazing sheep – selection of pasture varieties that maintain adequate but not excessive levels of protein will not only reduce the risk of copper deficiency but also a host of other nutritionally related disorders such as lameness, scouring and dystocia.

3.4 Bioavailability of copper for sheep

Bioavailability of copper to the ruminant is dependent on a number of factors such as:

- breed
- age
- rumen environment, and
- other dietary factors and antagonists (Underwood and Suttle, 1999).

A copper deficiency can occur commonly under two different scenarios:

1. Inadequate dietary copper intake resulting in insufficient circulating and stored copper levels – typically referred to as a ‘primary copper deficiency’
2. Reduced copper availability as a result of interaction of copper with other dietary factors and antagonists (Nederbragt et al., 1984) – typically referred to as a ‘secondary copper deficiency’

The purpose of this review is to investigate both scenarios, the likely risk of each to Australian production systems and key thresholds that can be used as an indication for the potential for copper related disease.

3.4.1 Primary copper deficiency

A primary or simple copper deficiency refers to the lack of sufficient levels of copper in the diet to meet daily animal requirements.

To diagnose an animal with primary copper deficiency requires evidence of basic animal requirements or net requirements (as illustrated in Fig. 5) for copper.

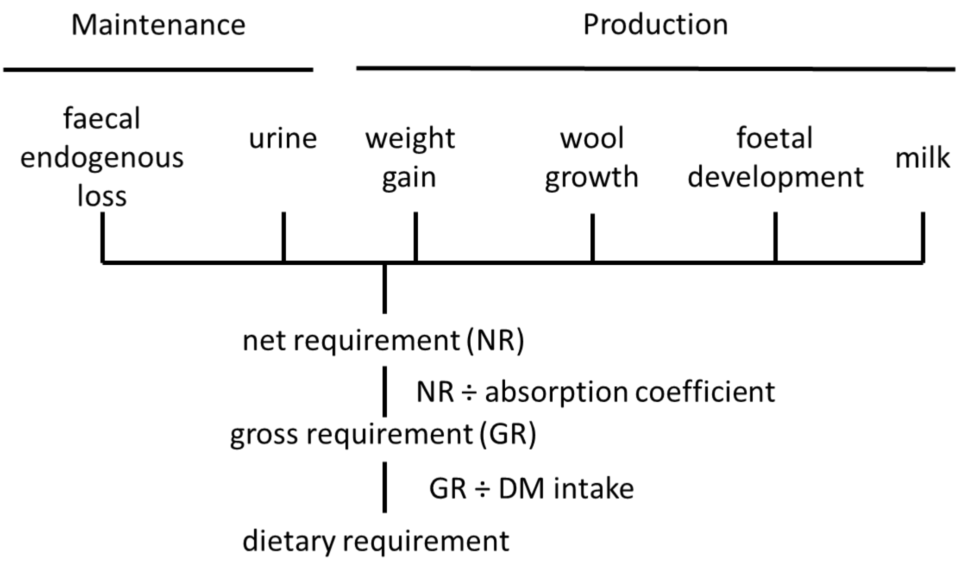

Fig. 5 - The factorial model for determining dietary requirements of minerals (Grace, 2002).

Unfortunately sources of recommendations regarding net copper requirements of ruminants are not in complete agreement. Research has shown that the net copper requirement for sheep is somewhere between 0.5 and 2mg Cu/kg DM (Bone, 2010, Gould and Kendall, 2011). In practice the copper content of pasture, roughage or grain would most often be greater than 2mg/kg DM; in fact more than 95% of feed samples within Productive Nutrition’s database which contains over 3500 samples, exceed 2mg/kg DM copper. However, from Section 3.2 above which describes the extent of copper deficiency in southern Australia it is clear that despite more than 95% of feed samples containing more than adequate copper levels, a deficiency still exists.

The sheep ‘requirements’ stated above describe the necessary amount of copper required to meet the animal’s metabolic demand for maintenance and production, however in reality only a small percentage of elemental copper ingested is absorbed, estimated to be between 3-10% (Lee et al., 1999). It is for this reason that copper requirements are commonly reported to be between 7-11mg/ kg DM (Underwood and Suttle, 1999, Puls, 1989, Hungerford, 1990) which allows for reduced absorption capacity of copper due to antagonists such as molybdenum and sulphur being present in the diet (Suttle, 2010).

In fact, Underwood and Suttle (1999) state that “[ruminant] requirements are so powerfully influenced by interactions with iron, molybdenum and sulphur that fourfold variation in the ability of feeds to provide absorbable copper must be allowed for.”

For this reason it may be argued that within Australian production systems a simple copper deficiency is unlikely to arise, such that to varying extents, interacting factors are more likely to be present and to have a role in the absorption of copper. In this respect copper deficiency should always be described as somewhere along the continuum of secondary (or complex) deficiency.

3.4.2 Secondary copper deficiency

Secondary copper deficiency (also known as complex or conditioned copper deficiency) is a copper responsive disorder caused by the action of antagonistic trace elements or complexes.

These antagonists can include other minerals such as sulphur, molybdenum as previously mentioned as well as iron which will be discussed further in this Section. Importantly the high protein content of pastures as well as high worm burdens (Judson and McFarlane, 1998) can also induce or exacerbate a copper deficiency.

3.4.2.1 Effect of sulphur

Dietary sulphur is able to act both independently and in combination with molybdenum to affect copper availability. When acting independently of molybdenum it binds with copper to form copper monosulphide which is unavailable to the animal as it is almost completely insoluble and therefore not absorbed (Ward, 1978, Van Ryssen et al., 2000). Some authors suggest this interaction can be more powerful in rendering copper unavailable to the animal than the thiomolybdate pathway which will be addressed in Section 3.4.2.2 below.

Van Ryssen et al. (2000) conducted a trial where sheep were fed a diet containing normal concentrations of molybdenum (0.52mg/kg DM); as dietary sulphur concentrations were increased from 0.22% to 0.40% (normal daily requirement 0.14% to 0.26%) copper accumulation in the liver decreased by 55%. This is a significant reduction in copper level; however it is also a relatively large increase in sulphur intake, but indicative of a reasonable cross section of pasture tests (Productive Nutrition, 2012). This may be partly due to the fact that reductions in copper accumulation due to high dietary concentrations of sulphur are greater where diets contain relatively low levels of molybdenum compared to diets high in molybdenum content (Underwood and Suttle, 1999).

However in contrast to the work of Van Ryssen et al. (2000), predictive equations developed by Neville Suttle and published in ‘The Mineral Nutrition of Livestock’ (Underwood and Suttle, 1999) demonstrated the individual influence of molybdenum and sulphur on copper absorption. The equation (Equation 1) for pastures (based on grass species) showed that molybdenum, rather than sulphur, was the primary factor influencing copper availability (Fig. 6).

Equation 1 - The effect of molybdenum and sulphur on copper absorbability for grass pastures.

$$Absorbable\ Cu\ \% = 5.7 - 1.3S - 2.785\log_e Mo + 0.227(Mo\ x\ S)$$

Applying the above equation, if the concentration of either sulphur or molybdenum were increased from the lower to upper recommended normal range for sheep (1.4 – 2.6g S/kg DM; 0.5-1.0mg Mo/kg DM) copper absorbability would be decreased by 1.4% for an increase in sulphur concentration, or 1.7% for an increase in molybdenum concentration in pasture. This shows that whilst sulphur has a significant effect on copper availability where mineral levels vary by what would be more commonly seen in the field, it does not have the same potential to reduce copper availability as molybdenum.

The reason for these differing points of view is not clear and it is difficult to directly compare the two as Van Ryssen et al. (2000) had tested only a single concentration of molybdenum and therefore could not directly compare the effect of changes in sulphur versus molybdenum.

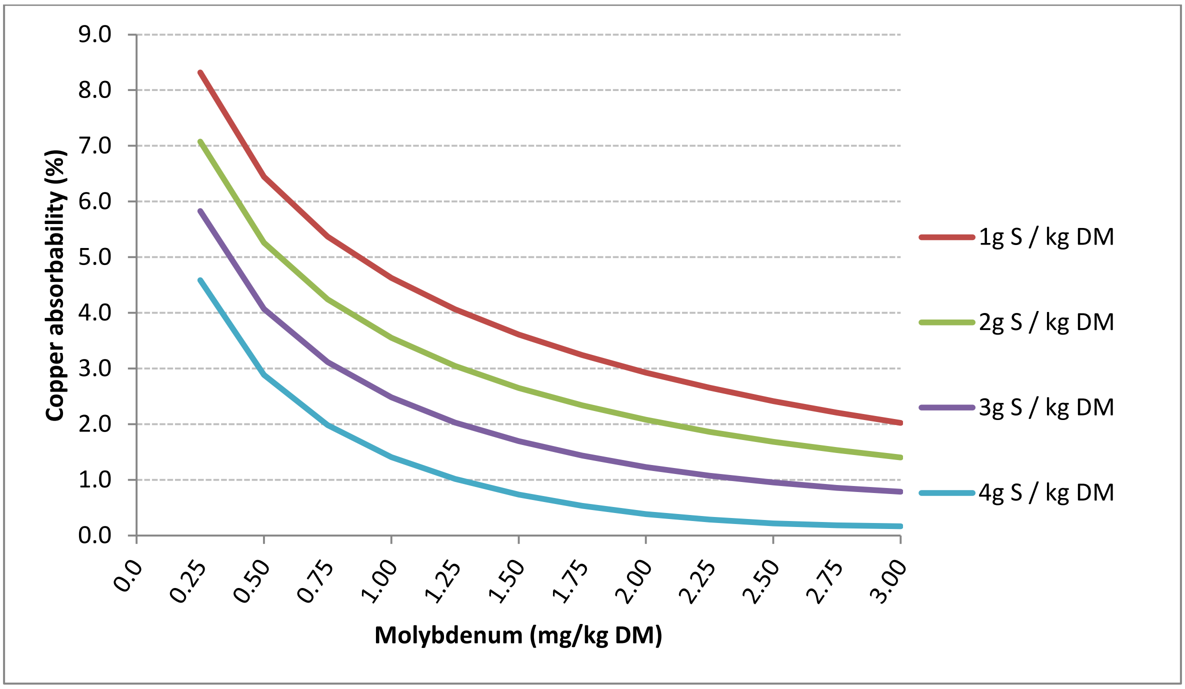

Fig. 6 – The effect of molybdenum and sulphur concentration of grass pasture on copper absorption. Adapted from Underwood and Suttle (1999).

Grace et al. (1997, 1998) investigated the effect of increasing sulphur intake on the copper status of lambs; these authors found that as sulphur levels increased in a pasture containing less than 0.5mg/kg DM molybdenum, no detrimental effect was observed in liver copper levels (Table 5). These authors concluded that the impact of the copper / molybdenum / sulphur interaction was greater than the copper / sulphur interaction.

Table 5 - Effects of increasing intake of sulphur and copper on copper concentrations in the liver and plasma of lambs. (Grace et al., 1998)

Treatment[1]		Cu concentration[2]	
S	**Cu**	**Plasma**	**Liver**
g/day	**mg/day**	**mg/l**	**mg/kg fresh wt.**
0	0	0.85 ± 0.11	43 ± 15^{A}
2	0	0.83 ± 0.03	66 ± 15^{A}
4	0	0.90 ± 0.14	69 ± 15^{A}
0	15	0.84 ± 0.05	168 ± 37^{B}
4	15	0.87 ± 0.05	175 ± 19^{B}

[1] *Treatments are supplemental to the dietary intake from pasture: 3.9 g S/day, 9.3 mg Cu/day.*

[2] *Within-column means with different superscripts are significantly different:* ^{A} *vs* ^{B}*,* $P<0.01$*.*

Based on the evidence of Underwood and Suttle (1999) copper absorption should have decreased from 5.1% to 2.0% resulting in reduced plasma and liver copper concentrations, however this reduction was not reported. One possible explanation for this result might be that the molybdenum concentration of the diet was less than 0.5mg/kg DM. As the authors suggested, this low molybdenum level could have limited the effect that a copper / molybdenum / sulphur interaction might have had on blood or liver copper concentrations.

The effect of additional sulphur on the copper absorption capacity of sheep appears greater with low sulphur diets compared to high sulphur diets (Grace et al., 1998). The sulphur content of the pasture these lambs were grazing was 2.6g/kg DM which is at the upper end of the required range for sheep (1.4 – 2.6g S/kg DM). Therefore, as sulphur levels increased it is possible that the influence of additional sulphur on copper absorption capacity may be limited, explaining the insignificant change in plasma or liver status.

Interestingly, when additional copper was supplemented to the animal a significant increase in liver status was observed, indicating that additional copper was available for absorption.

This may suggest that even in high sulphur situations not all copper can be bound solely through the sulphur / copper interaction and adequate animal performance can be achieved. This is contradictory to the results of Van Ryssen et al. (2000) however one possible explanation is that molybdenum levels in the diet were higher in the trial conducted by Van Ryssen et al. (2000) and that sufficient molybdenum was present in the diet to bind with the additional sulphur and reduce copper availability through the thiomolybdate pathway which will be further detailed in the next section. In the trial of Grace et al. (1998) it is difficult to precisely estimate the influence of molybdenum as the pasture/diet content is only described as less than 0.5mg/kg DM. However, had molybdenum levels been higher it is quite likely that liver copper concentrations would have been significantly reduced as sulphur level increased and production of thiomolybdates reduced copper availability. Still, this is merely supposition.

One factor that was not addressed in the study of Grace et al. (1998) was the high iron content of the pasture at 1417mg/kg DM (daily sheep requirement – 30-50 mg/kg DM). This level of iron in the diet would have significantly affected copper availability. The role of iron in copper deficiency will be discussed in more detail in Section 3.4.2.3 below.

3.4.2.2 Effect of molybdenum (and sulphur)

Molybdenum and sulphur

Whilst the individual effect of changes in molybdenum concentration on copper absorption is often greater than that of sulphur; a key point that must be made is that for molybdenum to reduce copper availability sulphur must be present. This was evidenced by a trial conducted by Bremner and Young (1978) where molybdenum supplementation of lambs in the absence of sulphur had little effect on liver copper concentration compared to molybdenum and sulphur supplementation. The authors reported a small reduction in liver copper concentration with the addition of molybdenum however it appears likely that this reduction was a result of interaction of the molybdenum with the intrinsic sulphur content of the base feed ration.

Dietary copper to molybdenum ratios (Cu:Mo) have been reported (Costello, 2012, Hungerford, 1990) as a key identifier of the potential for copper deficiency. Unfortunately, the “ideal” ratio varies across the literature, predominantly as a result of the different situations under which trials have been conducted. It appears that a ratio of less than 5:1 copper to molybdenum is indicative of a copper deficiency whereas a ratio of less than 2:1 copper to molybdenum (Cu:Mo) is a highly significant indicator. Suttle (2010) recommends that the marginal band for copper to molybdenum ratio in fresh herbage should be between 0.5-2.0. Ratios above 2:1 appear less likely to result in copper responsive disorders. Judson (1998) stated that “in practice Cu:Mo ratios above 3:1 have been used widely as a guide as to when livestock are not at risk of copper deficiency”. However earlier work from the United Kingdom (Suttle, 1991) has suggested that this ratio may be variable and dependent on the relative dietary concentrations of each mineral.

Judson and McFarlane, (1998) reported that the acceptable Cu:Mo ratio progressively declined from 5:1 to 2:1 as pasture molybdenum concentration increased from 2 to 10mg/kg DM. Pasture molybdenum concentrations in excess of 10mg/kg DM are rarely reported in Australia therefore it is unrealistic to assume that a 2:1 ratio could be used an acceptable measure of when sheep are not a risk of symptoms of copper deficiency. With the majority of Australian pastures in the range of 0.5 – 1.5mg/kg DM molybdenum (see Fig. 7) it may be assumed that a more appropriate recommendation for Australian production systems would be 5:1, particularly where concentrations of sulphur and/or iron exceed animal requirements.

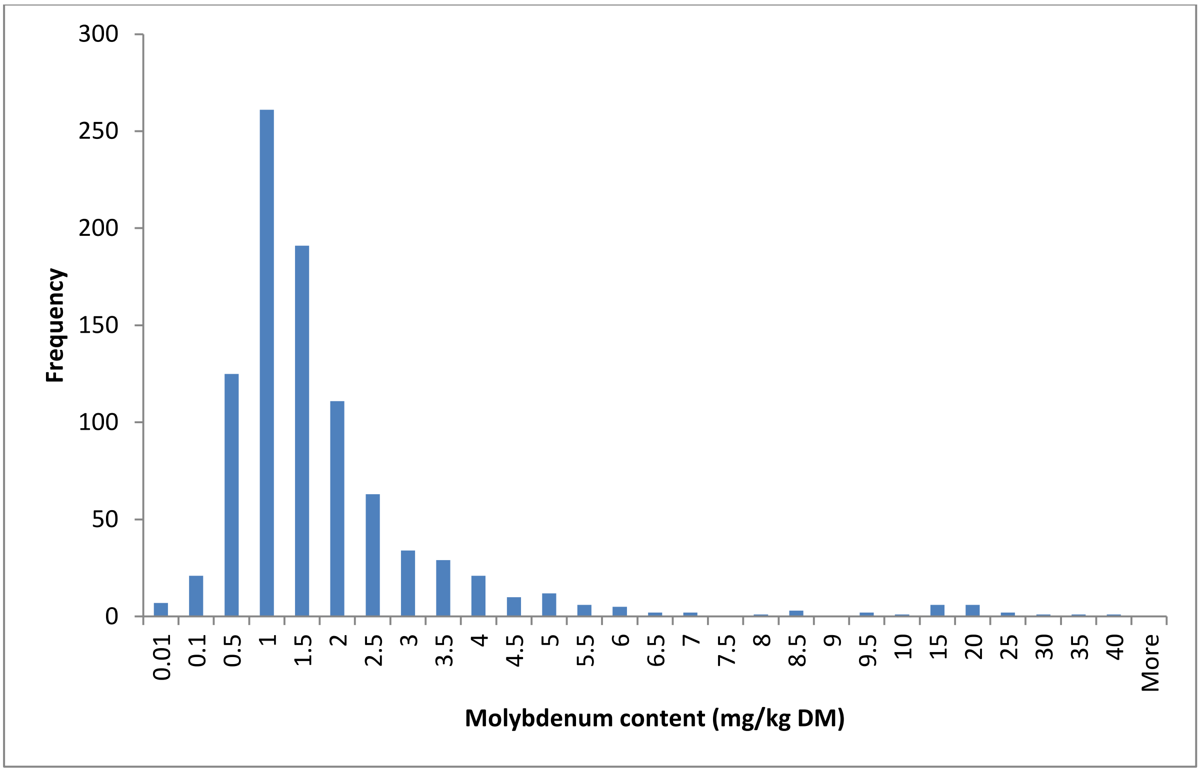

Fig. 7 - Distribution of molybdenum content of 930 pastures tested across southern Australia (Productive Nutrition Pty Ltd).

Thiomolybdates

In the rumen, dietary sulphur is reduced into sulphides, these sulphides combine with molybdates to form thiomolybdate complexes which readily bind to copper (Lee et al., 1999, Bremner and Young, 1978, Vasquez et al., 2001). Thiomolybdates seek out available copper in the rumen to form copper thiomolybdates which renders the copper unavailable for absorption and as such, it is excreted.

The concomitant reduction in copper availability may induce copper deficiency in the animal. Additionally, where insufficient quantities of copper are available in the rumen to satisfy thiomolybdate requirements, thiomolybdates will pass through the rumen and/or intestinal wall into the bloodstream to react with copper in body tissues and affect the activity of copper metalloenzymes such as luteinising hormone, follicle stimulating hormone, caeruloplasmin (copper transport protein), tyrosinase (melanin production) and superoxide dismutase (immune and antioxidant function) (Bone, 2010, Telsol, 2012).

Where situations of high molybdenum concentrations exist (>8mg Mo / kg DM) or low Cu:Mo ratios (<1:1), thiomolybdates leave the rumen to interact with copper (Underwood and Suttle, 1999). Unlike other countries, in Australia dietary intake of molybdenum is unlikely to commonly reach levels of >8mg Mo / kg DM; however this does occur. In addition, the Cu: Mo ratio of pastures grazed in southern Australia is commonly greater than 1:1 (Fig. 8) however low ratios do exist. Using the indicators published by Underwood and Suttle (1999) it is unlikely that in many situations, thiomolybdates would be in sufficient quantities as to exert effects beyond the rumen.

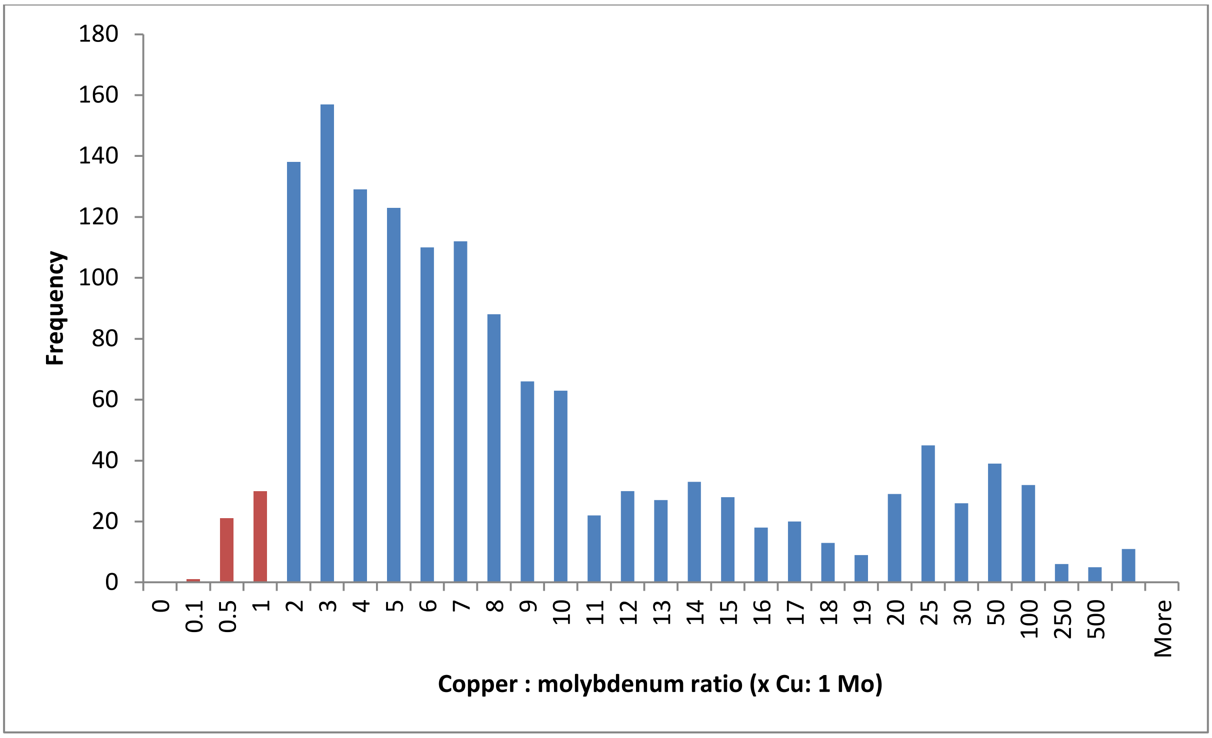

Fig. 8 - Histogram showing the distribution of copper: molybdenum ratios for feeds tested by Productive Nutrition Pty Ltd.

Bone (2010) argued that in order to correctly rectify symptoms of copper deficiency displayed in animals that there is a need to treat 'copper deficiency' caused by high thiomolybdates as thiomolybdate toxicity rather than a secondary copper deficiency. Defining thiomolybdate toxicity as a clinical condition in its own right should change the way treatment for this problem is considered. Effective treatment of thiomolybdate toxicity requires supplying the correct chemical source of copper to deactivate thiomolybdates in the rumen rather than simply providing more copper to meet metabolic demand (Bone, 2010, Telfer et al., 2004). This topic will be addressed in more detail in Section 0.

Forms of thiomolybdate

Copper, molybdenum and sulphur may combine to form either mono-, di-, tri- or tetra-thiomolybdate. Which form predominates is dependent on the rumen environment; however in rumen environments containing high concentrations of molybdenum and sulphur, higher level thiomolybdates (tri and tetra) tend to be produced. Suttle (1991) reported that tri and tetra thiomolybdates represented 34% and 41% respectively of total thiomolybdates formed when sheep were fed a pelleted grass ration containing a Cu:Mo ratio of 1:1. Higher level thiomolybdates appear to have the potential to cause thiomolybdate toxicity or symptoms of copper deficiency (Vasquez et al., 2001).

The rumen environment

Several trials have established a link between copper availability and rumen pH (Gould and Kendall, 2011, Crosby et al., 2004). Rumen protozoa are a key source of sulphur in the ruminant diet. Protozoal sulphur may act as an antagonist to copper, reducing copper availability as copper and sulphur combine to be excreted as copper sulphide or where sulphur combines with molybdenum to form thiomolybdates (Crosby et al., 2004).

As rumen pH decreases ruminal protozoa populations decline. High starch diets favour the proliferation of lactic acid-producing bacteria which results in a reduction in rumen pH; these bacteria ingest protozoa and so contribute to the decline in protozoal populations. Hence the importance of gradual introduction of ruminants to grain based diets to allow adaptation of rumen bacterial populations and avoidance of sudden changes in rumen pH. A decline in pH reduces supply of the copper antagonist sulphur (McDonald et al., 2002). The converse is also true in that increased rumen pH will result in increased protozoal populations, hence increased rumen sulphur production which can affect copper availability negatively (Crosby et al., 2004). In this regard a marginal reduction of rumen pH could be beneficial to copper availability.

However, separate research has also shown that at rumen pH levels of less than 6.5 the dominate form of thiomolybdates produced are tri and tetra thiomolybdates (Gould and Kendall, 2011). Higher thiomolybdates (i.e. tri and tetra) cause copper to be irreversibly bound to protein and reduce copper absorption (Suttle, 1991). According to Suttle (1991) mono and di thiomolybdates tend to be produced under low sulphur situations and do not as greatly impair copper absorption.

Combining these pieces of work would suggest that as rumen pH increases the influence of sulphur, supplied by rumen protozoa may have a significantly negative effect on copper availability. However, as rumen pH decreases whilst protozoal supply of sulphur may be reduced, higher level thiomolybdates are produced which can potentially reduce copper availability.

3.4.2.3 Effect of iron

Ingestion of iron is often overlooked in reviewing copper availability; however this mineral can have a significant effect. Although research has shown a negative correlation between iron ingestion and liver copper concentrations (Lee et al., 2002, Underwood and Suttle, 1999), the exact mechanism of influence of iron on copper availability and absorption remains unclear. Two pathways of activity are possible but these hypotheses may not necessarily be mutually exclusive:

1. Iron reacts with sulphide and copper in the rumen to form an iron/copper/sulphur complex which cannot be absorbed and is therefore excreted (Gould and Kendall, 2011).
2. Iron and sulphur react to form iron sulphide (FeS) in the rumen; when FeS reaches the acidic environment of the abomasum, sulphur is released and able to bind with copper forming copper sulphide which is then excreted (Suttle, 1991).

Whilst it is clear that there is a relationship between copper, iron and sulphur there is little evidence to determine the exact mechanism of iron effect on copper. The mechanism of both pathways involve sulphur which highlights the fact that the influence of iron on copper availability is dependent on sulphur and diets low in sulphur can limit the influence of iron as seen in Fig. 9 (Underwood and Suttle, 1999).

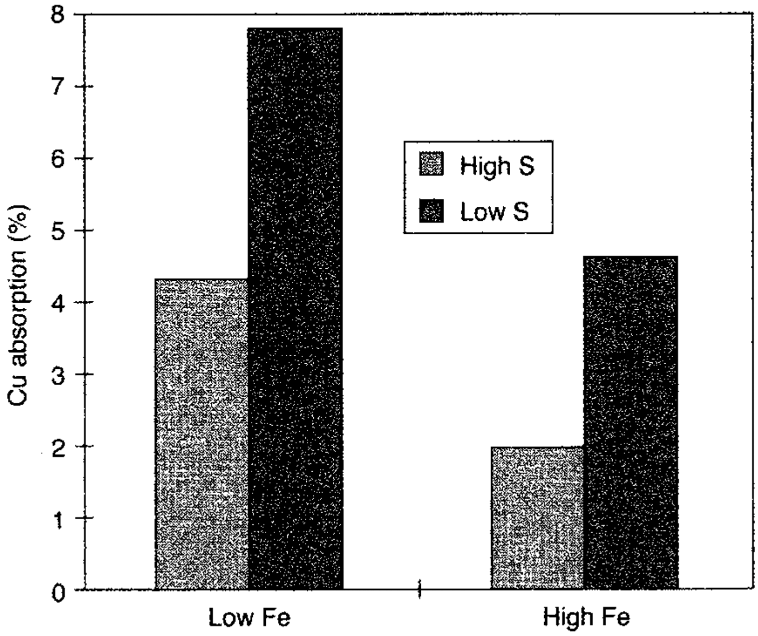

Fig. 9 - The absorbability of copper given to varying levels of iron and sulphur. Source: Underwood and Suttle (1999).

An important consideration is not only the direct impact of iron on copper availability but also that when high dietary concentrations of iron are present and bound with copper, not only do they reduce copper availability in the rumen but they also reduce the copper available to bind with thiomolybdates. If there is sufficient sulphur and molybdenum in the rumen to form thiomolybdates, lack of copper due to its affinity to iron will allow these thiomolybdates to then be absorbed through the rumen or small intestine into the body where they can bind to any available copper (Telsol, 2012). In this manner iron can exacerbate a thiomolybdate toxicity (Gould and Kendall, 2011).

There has been significantly less research conducted into the relationship between copper and iron than with other antagonists however several published recommendations regarding desirable ranges for livestock exist. Suttle (2010) published a recommended copper: iron ratio as an indication of the potential influence of iron on copper availability. The marginal band quoted is 50-100:1 with ratios closer to 100:1 more likely to reduce copper availability. In a review of copper deficiency by Bone (2010) he stated that “the role of iron must not be underestimated” and that diets containing over 150mg Fe / kg DM will have an adverse effect on copper availability.

Published research into the precise relationship between iron, copper and other interacting minerals such as sulphur and molybdenum has not delivered predictive formulae to assess the potential availability of copper under these more complex situations. Further research that combines the effect of all interacting minerals may be helpful in accounting for the individual effects of each.

Ingestion of soil can be a significant component of mineral intake for ruminants, particularly sheep, with commonly 100g/hd/day of soil ingested in paddock grazing situations (Grace et al., 1996). Variance in the level of an individual animal's soil intake and changing soil composition provide significant challenges in accurately predicting the effect of soil ingestion on copper availability. Studies investigating the effect of soil ingestion on liver copper concentrations have had varying results from significant to no reduction in liver copper concentration (Grace et al., 1996). This area of copper nutrition requires further research to fully investigate the effects of soil ingestion at different intake levels, for different forms of iron and in relation to different levels of interacting minerals such as sulphur and molybdenum. This is of particular relevance where sheep are grazing stubbles over summer and autumn.

3.4.2.4 Effect of other mineral interactions

Whilst there are not large numbers of trials to conclusively provide evidence of the effect of other minerals on copper absorption the potential influence of zinc and cadmium is worth mentioning.

Dietary intake of zinc in excess of daily requirements may be associated with a reduction in copper availability in ruminants due to the interaction with the copper binding protein metallothionein (Nederbragt et al., 1984). Excess zinc reduces absorption of copper and has been shown to reduce plasma and liver copper concentrations (Lee et al., 2002, Grace, 1983). Although the pathway by which zinc influences copper availability appears to be well understood, critical levels for zinc and copper at which a deficiency of copper might occur have not been determined and the appropriate level or source of supplementation have yet to be clearly defined.

Jankovska (2011) conducted a trial investigating the effect of cadmium on copper levels in the liver of 6 month old weaner lambs. It was found that the administration of 20g of cadmium per head per day significantly reduced liver copper concentration from 87.6mg/kg to 68.5mg/kg. This is contrary to a statement by Suttle (1991) that minerals such as cadmium that produce acid insoluble sulphides (and hence reduce sulphur availability for binding with copper) "may be protective in restricting both thiomolybdate and copper sulphide formation". The influence of this heavy metal in Australian production systems is difficult to quantify as significantly more information is required on the 'normal' cadmium concentration of pastures as it is not routinely requested nor provided on feed test analyses. This may be an area worthy of further research.

3.5 Interacting factors

3.5.1 Protein content of feed

Anecdotally high protein pastures have long been associated with copper responsive disorders however the degree of influence of nitrogen intake and protein metabolism has not been clearly defined. Several authors such as Grace et al. (1997), Lee et al. (2002) and Ward (1978) have highlighted the association between copper deficiency and high protein intake.

Protein is broken down in the digestive system of ruminants into a range of amino acids including sulphur amino acids (McDonald et al., 2002). It is these sulphur amino acids that appear to be the true cause of the copper disorders related to high protein pastures. As sulphur amino acids are hydrolysed, sulphides are released which combine with copper in the rumen to produce insoluble copper sulphide (Ward, 1978) or copper thiomolybdate complexes where sufficient molybdenum is present (Grace et al., 1997, Gould and Kendall, 2011, Vasquez et al., 2001, Ivan et al., 1985).

Ward (1978) described a copper responsive disorder in ruminants where "copper content of feed was normal (6-12mg/kg DM) and molybdenum was low (below 3 and commonly 1mg/kg DM or less)" but still symptoms of copper deficiency were observed. Ward further suggested that fresh pasture of 25-30% crude protein was the likely cause as when the same pasture was fed as hay no signs of copper deficiency were observed. The lower limit at which the crude protein concentration of the diet will not cause symptoms of copper deficiency is currently unknown and requires further investigation. It is not unusual for pastures through the winter and spring period in Australia to exceed 30% crude protein for extended periods of time.

3.5.2 Copper and scouring

Scouring is often incorrectly described as a symptom of copper deficiency. Whilst the two can be indirectly aligned through molybdenum intake, the exact cause of scouring should be more clearly identified as in most situations scouring can be a result of a number of different causes which will be highlighted below.

3.5.2.1 Role of copper (and molybdenum)

Although scouring is not directly a symptom of molybdenum intake in excess of daily requirements (Ward, 1978; Suttle, 1991; Hosking et al., 1986) it can sometimes be associated with copper deficiency. Ward (1978) reported that cattle consuming high molybdenum forage for only one to three days developed severe diarrhoea.

The physiological basis for the cause of "molybdenum scours" is not well defined, however Suttle (1991) proposes that when molybdenum is ingested and combined with sulphur to form thiomolybdates these thiomolybdates may be absorbed in the rumen and intestine. Thiomolybdates that are absorbed across the intestinal epithelium may remove copper from superoxide dismutase and cytochrome oxidase resulting in impaired cell function and mitochondrial integrity that manifests as diarrhoea.

Scouring caused by high molybdenum intake is much more commonly seen in cattle than sheep (Ward, 1978). Suttle (1991) suggests that scouring can be induced in sheep grazing pastures containing high concentrations of molybdenum however no reference is made to the molybdenum concentration at which this might occur. According to Hosking et al. (1986) pastures of over 5mg Mo/kg DM are commonly associated with scouring, although the species of animal is not defined. It is apparent that sheep may be susceptible to scouring as a result of excess molybdenum intake as copper availability is limited however the scouring should not necessarily be attributed to copper deficiency without other signs of copper deficiency being present.

3.5.2.2 Role of internal parasites

Whenever scouring is observed in sheep the potential role of worms should be considered firstly due to the significant risk of mortality and morbidity associated with internal parasites. The relationship between worms and copper deficiency is interesting such that copper sulphate has long been promoted as an anthelmintic but also high worm burdens can reduce copper absorption.

Research has shown that administration of non-copper containing anthelmintics have dramatically increased the concentration of copper in blood plasma (Lee et al., 1999). Additionally, research by Jankovska et al. (2011) showed that sheep infected with tapeworm had significantly lower liver copper levels than those not infected. This demonstrates that parasite burden reduces copper availability and can create, or exacerbate, an existing copper deficiency.

The mechanism by which parasites reduce the availability of copper has been traditionally considered a combination of reduced absorption and increased endogenous loss (Lee et al., 2002, Jacobson, 2006). However Adogwa et al. (2005) investigated the effect of parasitism on blood copper levels of infected and non-infected animals dosed with copper which was administered intramuscularly. They found that parasitic infection significantly reduced blood copper levels indicating that reduced absorption of copper in the gastrointestinal tract may not be the only mechanism by which parasites reduce copper availability, as copper supplementation in this instance was administered parenterally. Further research in this area is required to fully investigate the mechanism by which parasites affect copper metabolism and whether this mechanism is the same for all parasites.

3.5.2.3 Other factors that may cause scouring

Scouring is a complex condition that can be multifactorial. Where scouring is observed alternative influences to copper deficiency (or what is truly molybdenum toxicity) should be considered such as high protein feed, low dry matter content, deficient dietary fibre or possibly nitrate toxicity. These other causes are not the focus of this review however they are highlighted to illustrate the range of causes of scouring that should be considered before conclusions are drawn regarding copper status of affected animals.

3.5.3 Copper and lameness

Questions have been raised about the relationship between copper deficiency and lameness in grazing sheep seemingly because both copper deficiency and lameness are common symptoms of winter grazing, pasture-based sheep. As for scouring, lameness has a number of potential causes which may be unrelated to copper status; these should be considered in addition to any influence copper may be having. There is no evidence in the literature to support lameness occurring as a direct result of copper deficiency. Copper plays a critical role in the cross linking of bone collagen and hence can affect bone strength (VEIN, 2002) however unless lameness is observed as a result of fractures or bone deformities it is unlikely that lameness can be attributed directly to copper deficiency.

The role of copper as copper sulphate in foot baths as a treatment of footrot has been well documented. Alkan and Yavru (2001) investigated copper deficiency as a potential precursor to footrot. Through measuring blood and tissue (hoof) levels of copper (and zinc) in infected and non-infected animals they were able to determine that infected sheep had significantly lower levels of copper (and zinc) than non-infected sheep. However, as blood copper levels of both groups of animals were not below the normal range, the authors concluded that copper was not a predisposing factor in the aetiology of footrot. However, this author believes that although blood copper levels were within the normal range, further investigation into this potentially important link is required. It is the experience of AgriPartner Consulting that flocks with suspected footrot, severe benign footrot or foot abscess in high rainfall areas commonly also have copper deficiency.

3.5.4 Copper and lamb mortality

Lamb mortality is a significant industry issue and as well a concern for individual producers with sheep at risk of copper deficiency. Copper deficiency as such does not result in mortality but rather the symptoms of this disease can result in mortalities. Ataxia or ‘swayback” (lack of muscle coordination) in lambs is a key symptom of copper deficiency and can commonly affect 5-10% of affected flocks but there has been one report of mortality due to ataxia of 100% (Santos et al., 2006). Neonatal ataxia as a result of copper deficiency is an irreversible condition and may inhibit the lamb’s ability to drink and gain sustenance which inevitably leads to death. Santos et al. (2006) found that on one property with a history of enzootic ataxia only 2% of lambs with enzootic ataxia at birth survived.

Copper deficiency may result in reduced immune function in sheep (Minatel and Carfagnini, 2000). This can increase the susceptibility of sheep to bacterial infections, parasites infestation and other diseases which if not addressed have been shown to increase mortality rates (Berger, 2007, McClure, 2003). This is an additional mechanism by which copper deficiency can result in mortality across sheep flocks.

The precise effect of copper deficiency on mortality cannot be singularly defined as the individual circumstances of each flock, the level of copper deficiency and differential factors such as worms, vaccination history and environmental conditions will all have an effect on mortality. Suffice to say copper deficiency can result in symptoms that increase the likelihood of mortality and morbidity, as not all affected lambs may die at birth; potential mortality and morbidity is dependent on many factors in addition to copper status.

3.5.5 Copper and infertility

Reduced reproductive performance has been observed in sheep flocks across southern Australia (Productive Nutrition, 2004) and is commonly linked to copper deficiency as once copper supplementation is implemented significant productivity gains have been achieved. In some instances marking percentages of lambs in response to treatment have increased by 30% over comparable mobs without supplementation (Prime, 2012 pers. comm.). The mechanism by which copper deficiency affects fertility does not appear to be well understood as to whether the dysfunction is caused by copper deficiency, high concentrations of antagonists or both (Hidiroglou, 1979).

It appears that copper deficiency, possibly in combination with molybdenum toxicity, may reduce the synthesis of luteinising hormone which is required for ovulation. Xin et al. (1993) measured pituitary gland release of luteinising hormone and found that a reduction in copper status had little effect on the ability of the pituitary gland to secrete luteinising hormone. They suggested the mechanism by which copper deficiency may reduce fertility is through reduced synthesis of luteinising hormone (as opposed to reduced secretion) and that high molybdenum is most likely to be the causal agent. This view is supported by that of Telfer et al. (2004) who reported that molybdenum supplementation of heifers reduced fertility through reduction in the pre-ovulatory surge of luteinising hormone. Further, molybdenum supplementation delayed puberty (Telfer et al., 2004). However, it should be noted that liveweight gain was reduced in response to an increase in molybdenum intake and as sexual maturity is partially influenced by liveweight it might be argued that molybdenum intake may not have been the direct cause of delayed puberty.

It appears that intakes of molybdenum above daily requirements may have a depressant effect on fertility in sheep however it is difficult to state with any degree of certainty whether copper deficiency is the key prerequisite (Underwood & Suttle, 1999) or if excess intake of molybdenum is the more immediate factor (Hosking et al., 1986). Further research into copper related infertility is recommended to determine the individual influence of copper and molybdenum and to quantify the level to which copper related disorders can reduce fertility.

3.5.6 Copper and wool quality

The loss of crimp definition in the fleece of wool producing sheep appears to be an early symptom of copper deficiency both as a result of low copper intake or induced copper deficiency secondary to thiomolybdate interactivity (VEIN, 2002, Suttle, 1991). Crimp definition flattens out as depicted in Fig. 10, and may be 3 to 4 times the normal width. The period where copper deficiency occurred can often be clearly defined by examination of the wool staple at shearing.

Fig. 10 - Image of wool displaying normal crimp (left) and 'flattened' crimp (right) as a result of copper deficiency. (Source: http://www.sardi.sa.gov.au/livestock/meat_wool/xtreme_sheep/examples)

Lack of crimp definition is believed to occur as a result of delayed keratinisation of the fibre. Crimping occurs in response to the asymmetric cell division in the wool follicle which causes curvature of the fibres such that after wool fibres are produced in the follicle they undergo a period of hardening through keratinisation. Where copper is lacking in the diet the keratinisation process can be delayed, leaving the fibre pliable for longer and increasing the opportunity for it to straighten and lose crimp (Hynd et al., 2009).

Copper deficiency can also result in depigmentation of black wool; however it is rarely seen. The copper dependent enzyme tyrosinase is crucial in the synthesis of black pigment melanin. Copper deficiency results in a reduction in the production of this pigment hence the formation of bands of grey or white wool in the staple can occur (Telsol, 2012, VEIN, 2002).

3.6 Prevention and treatment

A number of commercial products exist within the market place to treat and/or prevent copper deficiency in sheep. As this review clearly demonstrates, effective treatment of copper deficiency requires a thorough understanding of both primary and the apparently more common secondary deficiency. It is also important that any persons recommending copper supplementation have a clear understanding of the amount of copper available in the diet, the potential for interactions and the risks associated with copper toxicity. Copper toxicity is more likely to occur in response to supplementation where the concentrations of known antagonists such as molybdenum, iron and sulphur are deficient.

It should also be mentioned that there are breed effects in relation to copper storage and metabolism such that Merino and Suffolk sheep are known to be less susceptible to copper toxicity than Texel sheep (Lombardini et al, 2008); the reasons for these differences remain unclear.

As management of copper deficiency depends on the underlying cause, treatment options will be reviewed under the following dietary scenarios:

1. Primary copper deficiency; low degree of secondary influence
2. Marginal copper deficiency; high degree of secondary influence
3. No primary copper deficiency; excessive dietary crude protein

In each of the above categories of copper deficiency various products will be reviewed for their efficacy and recommendations will be made where possible as to the most appropriate treatment option(s).

Deficiency type

DIET SCENARIO 1: Primary copper deficiency; low degree of secondary influence

Simple supplementation of adequate levels of dietary copper is likely to address a copper deficiency in the animal. The following treatments are available and likely to be effective in addressing this form of copper deficiency:

- continuous supplementation
    - licks (copper sulphate)
    - blocks (copper sulphate)
    - water medications (copper sulphate)
    - feed additives (copper sulphate)
    - slow release rumen boluses (copper oxide, copper sulphate)
    - soil fertilisers (copper sulphate)
    - foliar sprays (copper sulphate)
- discontinuous methods
    - licks (copper sulphate)
    - drenches (copper sulphate)
    - Injectables (copper heptonate) - not currently registered for use in sheep in Australia.

The administration rates of the above options will vary according to the level of dietary copper available and the daily requirements of each class of sheep. Treatments that provide continuous rates of copper supplementation should be treated with caution to ensure that copper is only delivered at times of year and at correct dose rates for the animals concerned.

Copper sulphate is the most commonly used supplemental form of copper for sheep in Australia and is the major compound in supplements including blocks and drenches.

Its effectiveness has been well proven (Judson, 2002, Yuan et al., 2011, Eckert et al., 1999) and is considered the standard against which other copper supplements are compared (Spears, 2003). However, it should be noted that the efficacy of copper sulphate drenches is short term such that they need to be administered every 2-6 weeks (Judson, 2002).

Copper oxide in the form of slow release rumen boluses is commonly recommended as an effective treatment option for copper deficiency (VEIN, 2002, Hosking et al., 1986). However it appears that the physical form in which copper oxide is provided is a major determinant of its efficacy.

Langlands et al. (1989) conducted a series of trials with sheep investigating the effectiveness of copper oxide administered as either particles or powder. They reported that copper oxide particles were retained in the abomasum for significantly longer than copper oxide powder and hence copper oxide particles were more effective in increasing liver copper concentration.

The proportion of each product that was excreted in the days following administration of copper oxide powder and particles is depicted in Fig. 11. Excretion of copper oxide in particle form was significantly slower than in powder form such that there was an increased opportunity for absorption.

So whilst copper oxide powder may be effective in increasing liver copper concentrations, copper oxide particles are the preferred form as up to 94% of powder form can be excreted within 4 days compared to 7% with copper oxide particles.

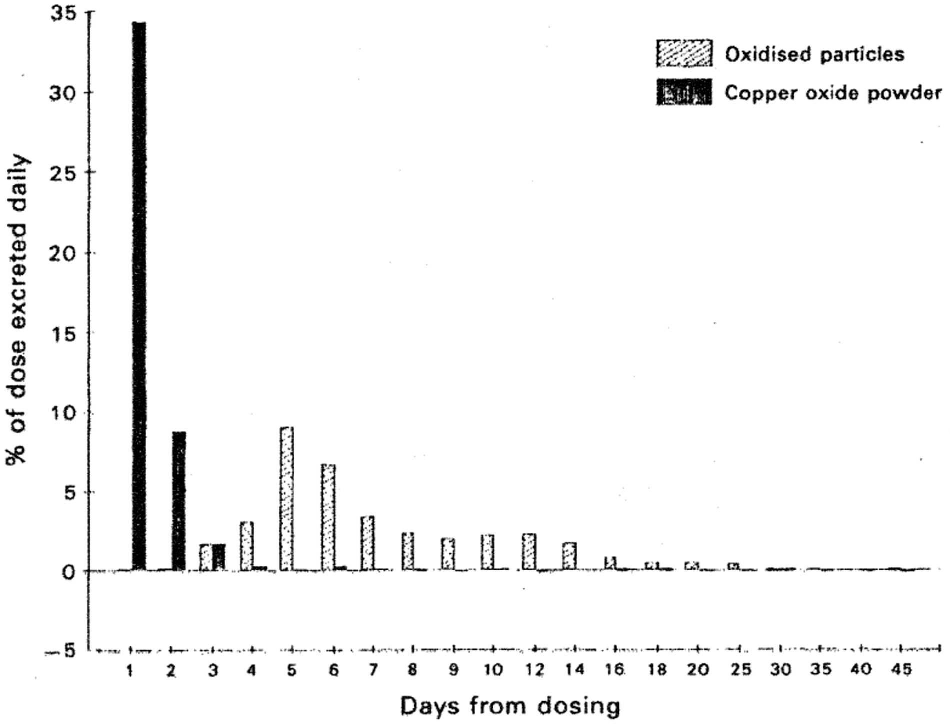

Fig. 11 - Proportion of copper oxide particles and copper oxide powder excreted in faeces. (Langlands et al., 1989)

Increasingly, ‘natural’ liquid mineral products are being promoted in the market for supplementing stock with copper and other minerals; unfortunately there is a dearth of credible information regarding the effectiveness of these products. The form of copper in these products is often not published such that caution should be exercised in recommending these products unless supporting data as to their efficacy can be provided.

Both continuous and discontinuous methods of supplementation aim to deliver sufficient amounts of supplemental copper to meet daily sheep requirements. However as antagonistic minerals are not a factor in limiting availability of copper in primary copper deficiency, the risk of copper toxicity should be considered.

As most treatment options are effective in treating primary copper deficiency the main factors that influence the form and treatment of choice should relate to:

- cost
- longevity of treatment
- ease of administration
- risk of toxicity

DIET SCENARIO 2: Marginal copper deficiency; high degree of secondary influence

Where sheep are grazing pastures of low to adequate copper concentration (3.6-9.7mg/kg), high molybdenum (2.3-22.0mg/kg), low to adequate sulphur (1.5-4.2g/kg) and adequate to high iron (111-204mg/kg), Judson and Babidge (2002) demonstrated that both **copper oxide** particles and **copper heptonate** injection significantly (P=0.05) increased liver copper concentration above that of control animals. Copper oxide particles (2.5g) provided 6-9 months' protection for adults and 5-6 months protection for weaners whereas the authors suggested that copper heptonate injections of up to 1mg/kg liveweight should provide six months protection for sheep without risk of toxicity.

In a subsequent trial Judson and Babidge (2004) investigated the toxicity risk of copper heptonate intramuscular injections in 9 month old wethers fed a daily ration containing what they considered to be adequate levels of copper (6.6mg/kg), molybdenum (0.4mg/kg), sulphur (1.4g/kg) and high levels of iron (351mg/kg). They found that injections of copper heptonate at 1 or 2 mg/kg liveweight did not pose a significant toxicity risk to these animals. This is important work as it shows that even for animals with satisfactory copper status prior to treatment, injections with copper heptonate at the above levels could be considered safe. This treatment option is not currently available to Australian sheep producers as copper injections are not currently registered for sheep, however they were investigated as a treatment option in Year 2 of this project.

Additional treatment options for sheep grazing pastures containing low to adequate levels of copper but high levels of molybdenum, sulphur and/or iron, were investigated by McFarlane et al. (1991). These researchers found that **copper calcium EDTA** injection (50mg Cu), 1 capsule of copper oxide particles (2.5g Cu) or 1 **soluble glass rumen bolus** (134g/kg Cu) were effective in maintaining copper status of Merino wethers.

Soluble glass boluses have been designed to be retained in the reticulo-rumen (McFarlane et al., 1991). In situations of high dietary molybdenum and sulphur, the copper delivered from this product has been designed to act as sacrificial copper for interaction and binding with thiomolybdates. The intention of this treatment option is to limit thiomolybdate absorption through the rumen and intestine and thereby limit interference with body copper stores.

Kendall et al. (2001) investigated the effect of a multi-mineral bolus which included elemental copper on the copper status of sheep. Unfortunately the mineral concentration of the base diet was not deficient as control animals blood plasma levels (normal ≥9.4µM) were consistently around 18µM (Gould and Kendall, 2011); therefore it could be assumed that copper status of the animals prior to treatment was satisfactory. Following treatment, blood plasma remained at satisfactory levels and liver copper levels increased, indicating that this form of treatment may be effective in raising liver copper stores.

Copper sulphate is commonly used in Australia for supplementing sheep with copper deficiency (D. Scammell, 2012 pers. comm.). In situations where high levels of antagonistic minerals are present in the diet (4.9mg Mo/kg, 4.3g S/kg, 686mg Fe/kg), copper sulphate has been shown to be an effective method of treatment where oral doses of 5g of copper sulphate have been administered on a weekly basis. This view is supported by Underwood and Suttle (1999) and Judson (2002) who recommend that where concentrations of antagonistic minerals are high oral dosing may be required on weekly and sometimes daily basis to be effective. However in reality the ability of most sheep producers to implement such a course of action would be questionable.

Copper sulphate has long been used in water troughs as an algaecide however it is also an effective method of supplementing sheep with copper. Whilst this method of supplementation may be effective where stock have access to water troughs, it has limitations for properties watering stock from dams. Copper sulphate can be added to troughs at a rate of 4g / 1000 litre water (Judson, 2002). Precise control over the amount of copper sulphate delivered per animal is impossible due to:

- dilution effects of trough refill
- variation in water intake of different classes of sheep
- variation in water intake between forages
- variation in water intake at different times of year

Where copper deficiency may be considered mild and labour resources limited, water supplementation may be the treatment method of choice.

Despite trials showing that copper pasture applications of between 2 to 6 kg Cu/ha can be effective at maintaining adequate copper levels in pasture for up to 20 years (Judson and Babidge, 2002) livestock in areas such as the south east of South Australia and western districts of Victoria where elemental copper has been applied to pastures, signs of copper deficiency in grazing sheep are still evident (Jolly, 2012) . This may be attributable to high concentrations of antagonistic minerals being present in pastures (Jolly, 2012).

Under these circumstances whilst copper application to pastures may be effective in increasing pasture copper content, it is unlikely to correct a secondary copper deficiency in livestock as high molybdenum, sulphur and/or iron levels will interfere with copper absorption as described in Section 3.4. In this situation it is recommended that ruminal boluses or injectable products (although not currently registered for use in Australia) may be a more effective treatment option (Suttle, 1991).

Alternatively, a different approach might be to determine a method of reducing uptake of antagonistic minerals by plants which may warrant further research. Research is required to determine whether there are strategies that can be employed to reduce the antagonistic mineral content of pastures whilst still meeting both animal and plant requirements for optimal productivity.

Additional forms of injectable copper that are no longer commonly used to treat copper deficiency in sheep include copper glycinate and copper hydroxyquinoline sulphonate.

Copper glycinate is more commonly used in cattle and anecdotally has been used “off label” in sheep albeit at reduced rates, quite successfully. Literature regarding its effectiveness under the influence of antagonistic minerals is limited and suggests that while it is effective in treating copper deficiency its efficacy appears less effective in cases of secondary deficiency (Singh et al., 2006, Khodary et al., 2003).

Copper hydroxyquinoline sulphonate reportedly produces ‘uniform, long lived protection [from copper deficiency] and negligible tissue reaction” at the injection site (Mason et al., 1984); however because it provides a rapidly available source of copper the potential for toxicity may be high (Underwood and Suttle, 1999). Trial results from Australia have had varying levels of success. A commercial product of the supplement called Cujec® was investigated in an experiment conducted on South Australia’s Eyre Peninsula by Judson et al. (1984) where it was compared with copper oxide particles. The injectable form was not as effective at raising liver copper concentration as copper oxide particles and only produced a “small, transient increase in liver copper concentrations”. However, the potential influence of antagonistic minerals was not mentioned which made it difficult to assess the contribution of potential antagonists to this result.

Similarly Allen and Mallinson (1984) reported that copper oxyquinoline sulphonate injections resulted in a short period of protection against copper deficiency, largely because the recommended treatment rates remained relatively low to protect animals from the risk of toxicity. It appears that this form of treatment may not be suitable for sheep as sufficient levels of copper cannot be administered to provide extended periods of protection from copper deficiency due to the risk of toxicity.

To effectively manage secondary deficiency it appears that the treatment aim should be to negate or limit the effect of antagonistic minerals either in the pasture or in the rumen environment prior to absorption beyond the reticulo-rumen.

To achieve this would require supplementation with products able to provide a source of sacrificial copper primarily in the rumen.

Injectable forms act by providing a source of elemental copper which is absorbed from the injection site and then redistributed to the liver for storage; unfortunately this process allows thiomolybdates to be absorbed into the circulatory system as no copper is provided in the rumen to bind with thiomolybdates and be excreted. Whilst it has been shown that some copper injections such as copper heptonate can be effective in correcting secondary copper deficiency it would seem more efficient to limit post-ruminal thiomolybdate absorption in the first instance.

It appears that the most effective treatment of secondary copper deficiency is likely to be continuous slow release reticulo-rumen boluses. These will provide both sacrificial copper to bind with thiomolybdates and supplementary dietary copper over up to a 12 month period. However, it should be noted that the work of Judson and Babidge (2002) demonstrated the effective life of the capsules to be half that which is recommended.

The reluctance of producers to insert rumen boluses should not be underestimated and is currently a considerable constraint to successful uptake of this recommendation if it does become the industry method of choice. It is an onerous task to undertake in a commercial flock and many producers lack the confidence to do it. The development and provision of hands-on training courses for producers may offer significant benefits at the completion of this project.

Further research is also suggested based on the report of one producer in the Western Districts of Victoria who has stated that the boluses became calcified and in his opinion did not appear to be reducing the incidence of swayback in his flock.

DIET OPTION 3: No primary copper deficiency; excessive dietary crude protein

Where the primary cause of copper deficiency is thought to be from sheep grazing high protein pastures with a resultant reduction in availability of copper, copper availability is thought to be bound with sulphides which are released during the breakdown of sulphur amino acids, the building blocks of proteins. No research has been undertaken to determine effective treatment options for protein-induced copper deficiency nor has any threshold levels of protein intake been established. However, Ivan et al (1985) postulated that the addition of dietary zinc or oil may assist in decreasing the rumen populations of ciliate protozoa and hence decrease the formation of insoluble copper sulphide complexes. Clearly this is an area that requires further investigation however dietary intake of polyunsaturated oils at a level in excess of 1% of dietary dry matter will reduce rumen nutrient absorptive capacity.

In the absence of any solution to the hypothesis of protein-induced copper deficiency the following interim management strategies may be of some benefit:

1. Reduce or limit protein intake
2. Supplement with dietary copper

Where the copper content of the feed is meeting the requirements of grazing animals, reducing crude protein and hence sulphur intake may assist in the prevention of copper deficiency.

Protein content of pastures during winter and spring during active vegetative growth can often exceed 30% and practically, little can be done to reduce this. Opportunities exist in not trying to change the protein content of pasture but in changing the protein content of the animals' diet. Supplementing stock with highly palatable low protein feed can be effective in reducing the overall dietary protein intake and may potentially increase copper availability however this is yet to be determined.

The copper concentration and available energy of supplementary feeds provided to reduce pasture intake and hence nitrogen intake must be considered to ensure that introducing significant amounts of supplementary feed does not adversely affect the copper or energy status of the animal.

Where supplementation with low protein feeds is either not possible or cost-effective it may be necessary to supplement with additional copper; the principles of treatment of secondary copper deficiency would apply. Research is required to determine the level of dietary nitrogen intake tolerated by sheep before protein-induced copper deficiency becomes apparent across seasons and between pasture species.

4 On farm trials

A series of on-farm trials were conducted to investigate the effect of copper deficiency on sheep as well as to determine the most appropriate method of diagnosis and treatment of copper deficiency.

The methodology of each trial is detailed in Section 4.1.1, 4.2.1 and 4.3.1 below, however these trials were designed to incrementally build information that would direct the chosen methodology of each successive trial. Broadly, the purposes of each trial were as follows:

1. Year 1: Identify and measure the scope of copper deficiency at six trial sites to be utilised for the duration of the project. Additionally, measure the relationship between soil, plant, liver and blood copper status. No copper supplements were provided in this first year.
2. Year 2: Investigate the effects of copper supplementation on commonly reported signs and symptoms of deficiency at each site using control and treated groups of animals. Assess the effectiveness of a range of copper treatments. Measure the relationship between plant, liver and blood copper status.
3. Year 3: Investigate the effects of copper deficiency on productivity measures such as conception rate, marking rate and lamb weaning weights using control and treated groups of animals. Measure the effectiveness of a range of copper treatment options.

For the purposes of this report the individual properties were given an identifying acronym to signify their location. These correspond to the producers as follows:

- SWVIC1 = Richard Beggs, South-west Victoria
- SWVIC2 = Richard Weatherly, South-west Victoria
- MALLEE1 = Michael and Dianne Anderson, SA Mallee
- MALLEE2 = Kim McMahon, SA Mallee
- EP1 = Roslyn Pope, SA Eyre Peninsula
- EP2 = Chris Prime, SA Eyre Peninsula

4.1 Year 1 (2012)

4.1.1 Methodology

On-farm measurements for plant nutritive value and mineral content, liver and blood copper levels and flock observations including scouring, lameness and mortalities were measured.

These measurements were analysed to determine:

1. Copper and other interacting minerals levels across each trial property
2. Production levels of all unsupplemented trial mobs
3. The recommended treatment strategy for each trial mob in 2012/2013

Plant tissue samples were collected from each trial property throughout 2012. Samples were collected from 1-7 paddocks at each property depending on the number of paddocks the trial mob grazed. Plant sub-samples were collected from at least 6 representative locations across each paddock and combined to form the paddock sample for analysis. Samples were collected by either cleanly tearing or cutting a sample with shears. Care was taken to ensure that no dirt, roots or contamination from the shears occurred which could affect the test results. Plant species composition in the sample was reflective of the plant species composition in the paddock. The only exception to this was where unpalatable weed species were present in the paddock their inclusion rate in the sample for analysis was similar to that estimated of animal intake (generally minimal). Samples were scheduled to be collected in February, May, July, September and November from each property. This schedule was achieved at each property with the exception of:

1. February sample collection at EP1 which was collected in March as their entry in the project was not until the start of March.

2. May sample collections from MALLEE1, EP1, EP2, SWVIC1 and SWVIC2 were conducted in June (July at SWVIC1) due to the late break of the season and little feed on offer in May
3. November sample collection at EP2 delayed until December as labour limitations on-farm during harvest prevented the producer being able to collect the samples. The results of these samples have not been included in this report as results were not available at the time of writing. They will be detailed in the next milestone report.

4.1.2 Results

Fig. 12 to Fig. 17 show that pasture copper content from samples collected across most properties in summer as senescent feed (February 2012) were lower than those collected in winter and spring. Plant mineral content then declined again approaching summer (November 2012). Winter and spring periods also showed an increase in molybdenum, sulphur and iron concentrations potentially reducing copper availability. These results contradict current understanding of mineral trends which suggests plant mineral concentrations are generally lower in winter and spring when feed is lush and in early growth stages. This will be an interesting area for further monitoring in 2013.

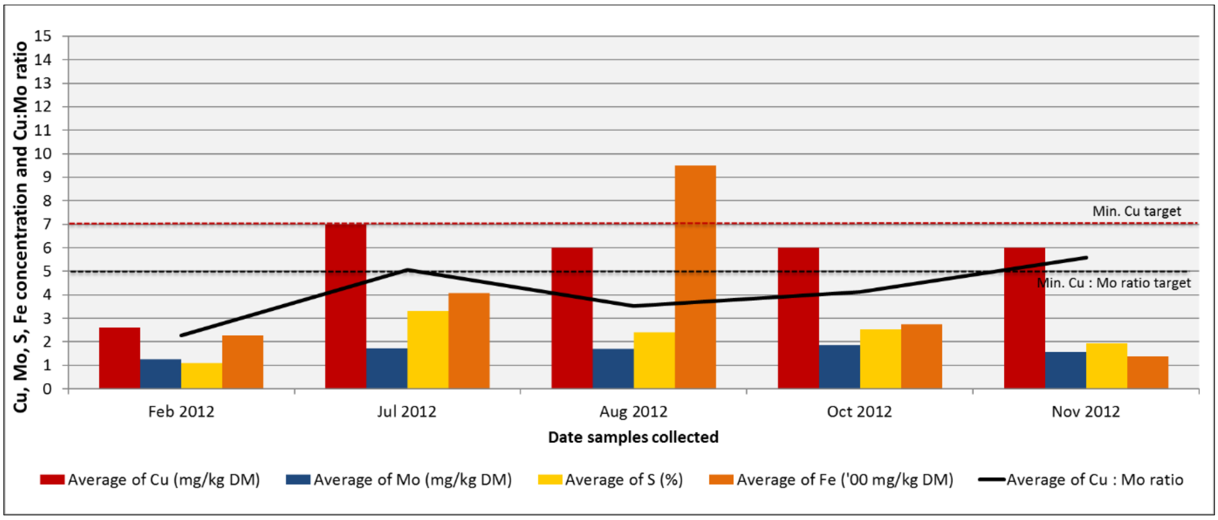

Fig. 12 - Average plant mineral content across all paddocks tested at SWVIC1 in 2012.

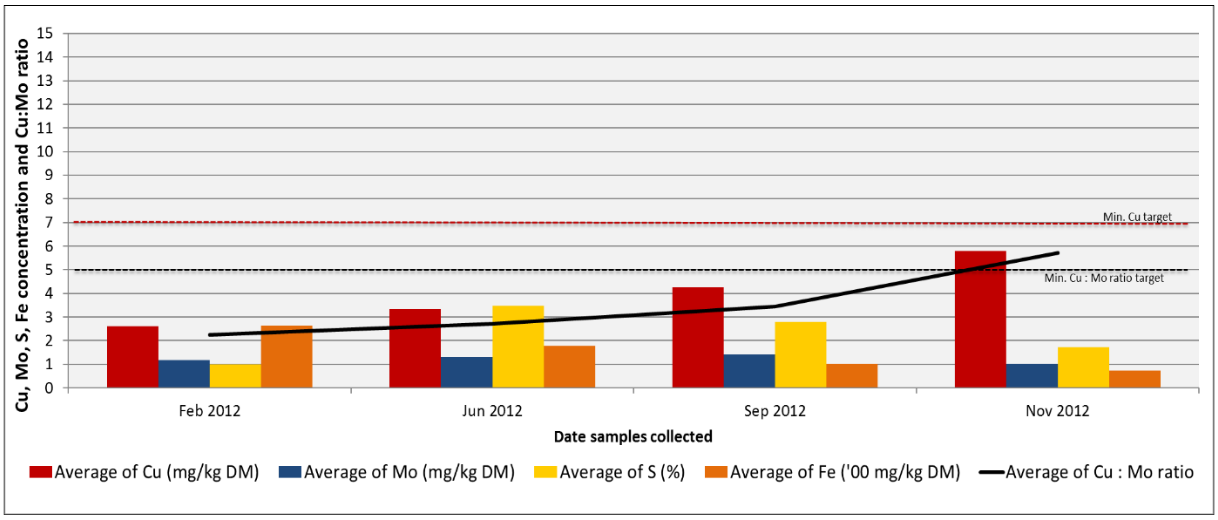

Fig. 13 - Average plant mineral content across all paddocks tested at SWVIC2 in 2012.

Copper levels at the Victorian western districts properties increased or were maintained at SWVIC2 and SWVIC1 respectively during the November testing period. This was contrary to the trend of other properties. This may be due to the fact that pasture growth in this area was not as far advanced as the South Australian properties at the time of testing.

Additional months of testing appear in Fig. 15 when compared with Fig. 14 as not all paddocks were able to be tested at the one time at MALLEE2 and testing for each round occurred over two months as feed became available.

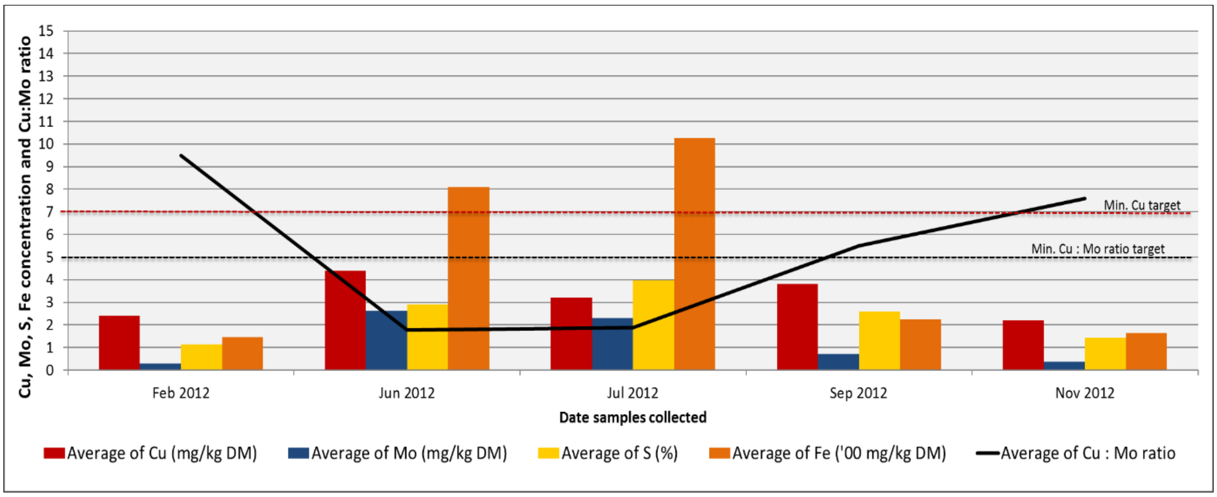

Fig. 14 - Average plant mineral content across all paddocks tested at MALLEE1 in 2012.

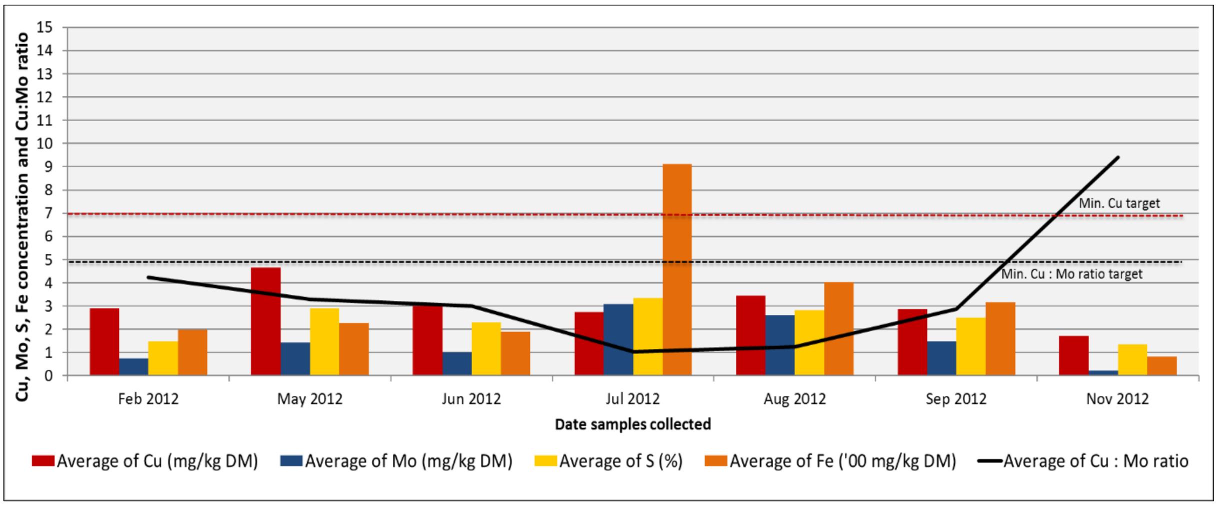

Fig. 15 - Average plant mineral content across all paddocks tested at MALLEE2 property in 2012.

There was a sudden increase in molybdenum levels at EP2 in June where molybdenum levels increased in Pump paddock from 0.4mg/kg DM to 17mg/kg DM increasing the overall average displayed in Fig. 17. This result was queried with the laboratory to ensure it was not a reporting error and with EP2 to determine whether any molybdenum had been applied however neither was the case. Similar spikes in molybdenum concentrations have been observed at this property previously such that this will be further investigated in 2013 through a higher level of testing during May to July when these increases have been observed.

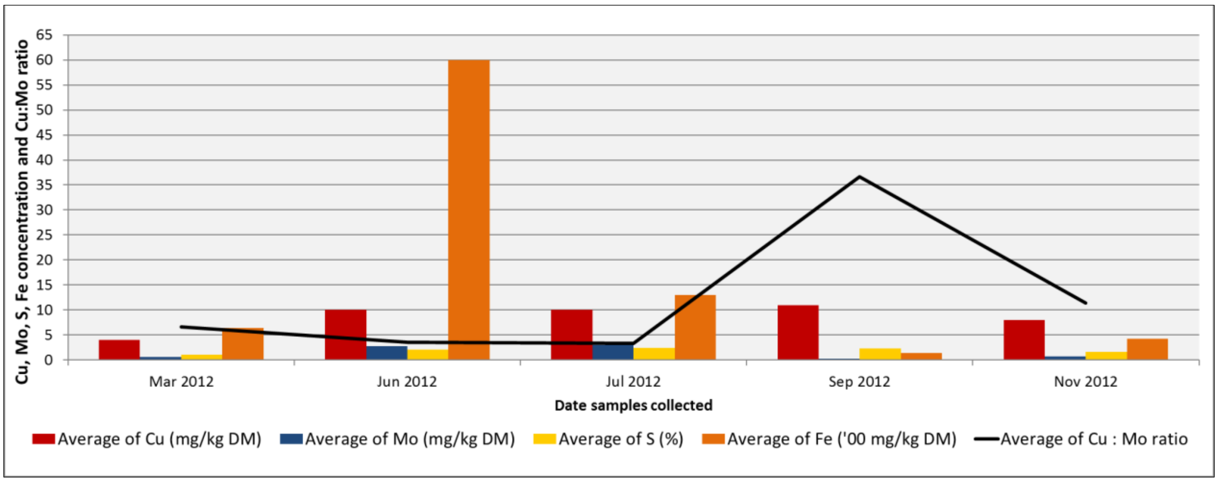

Fig. 16 - Average plant mineral content across all paddocks tested at EP1 in 2012.

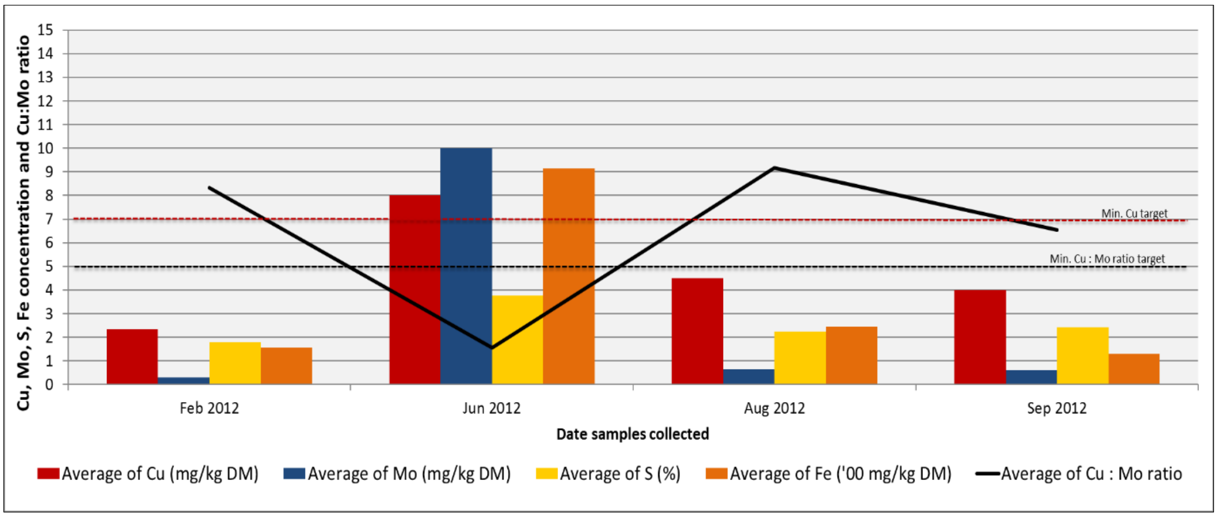

Fig. 17 - Average plant mineral content across all paddocks tested at EP2 in 2012.

Crude protein, dry matter and neutral detergent fibre concentrations of pasture

Crude protein (CP), dry matter (DM) and neutral detergent fibre (NDF) were analysed in pasture samples to determine the influence these attributes had on the incidence of scouring and lameness.

Fig. 18 to Fig. 23 show that across all trial properties pasture CP was highest during winter and lowest during summer. In contrast NDF and DM were lowest during winter and highest during summer which follows expected trends of southern Australian pasture systems.

The contribution of these feed attributes to scouring and lameness are discussed further in this report.

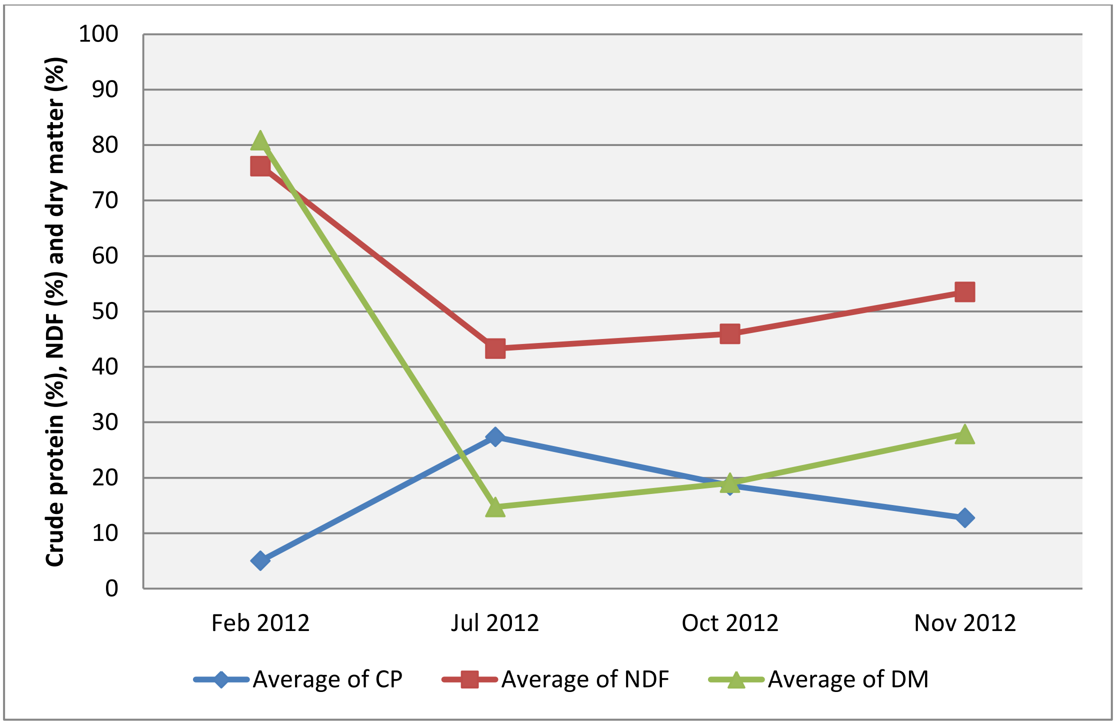

Fig. 18 - Average plant nutritive value across all paddocks tested at SWVIC1 in 2012.

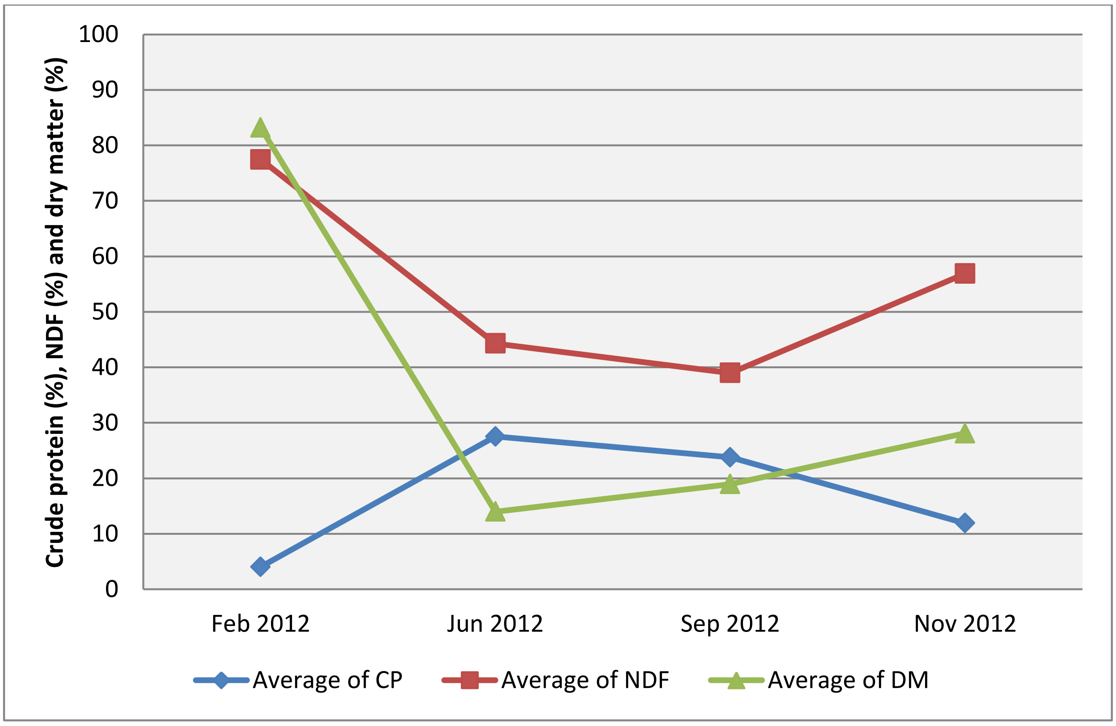

Fig. 19 - Average plant nutritive value across all paddocks tested at SWVIC2 in 2012.

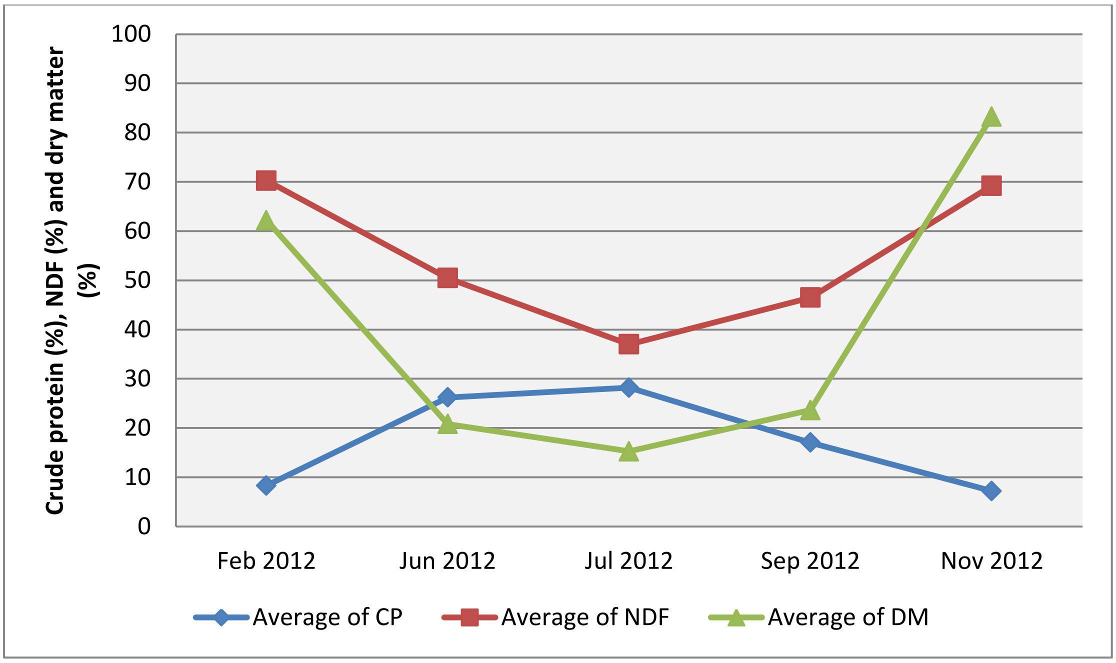

Fig. 20 - Average plant nutritive value across all paddocks tested at MALLEE1 in 2012.

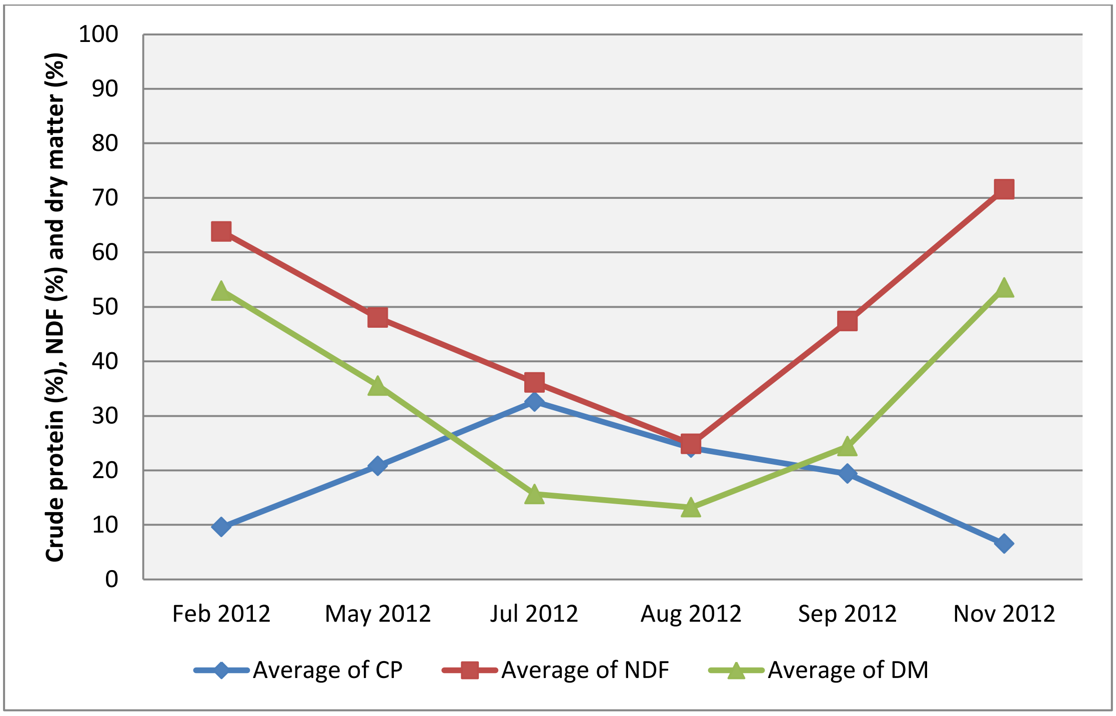

Fig. 21 - Average plant nutritive value across all paddocks tested at MALLEE2 in 2012.

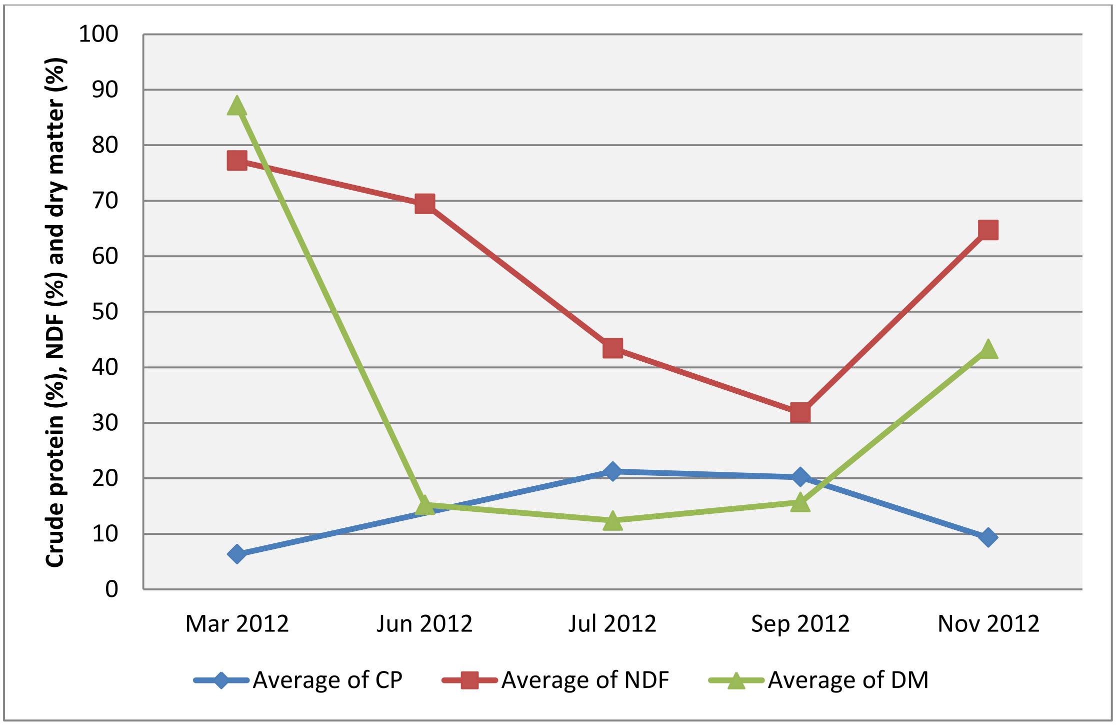

Fig. 22 - Average plant nutritive value across all paddocks tested at EP1 in 2012.

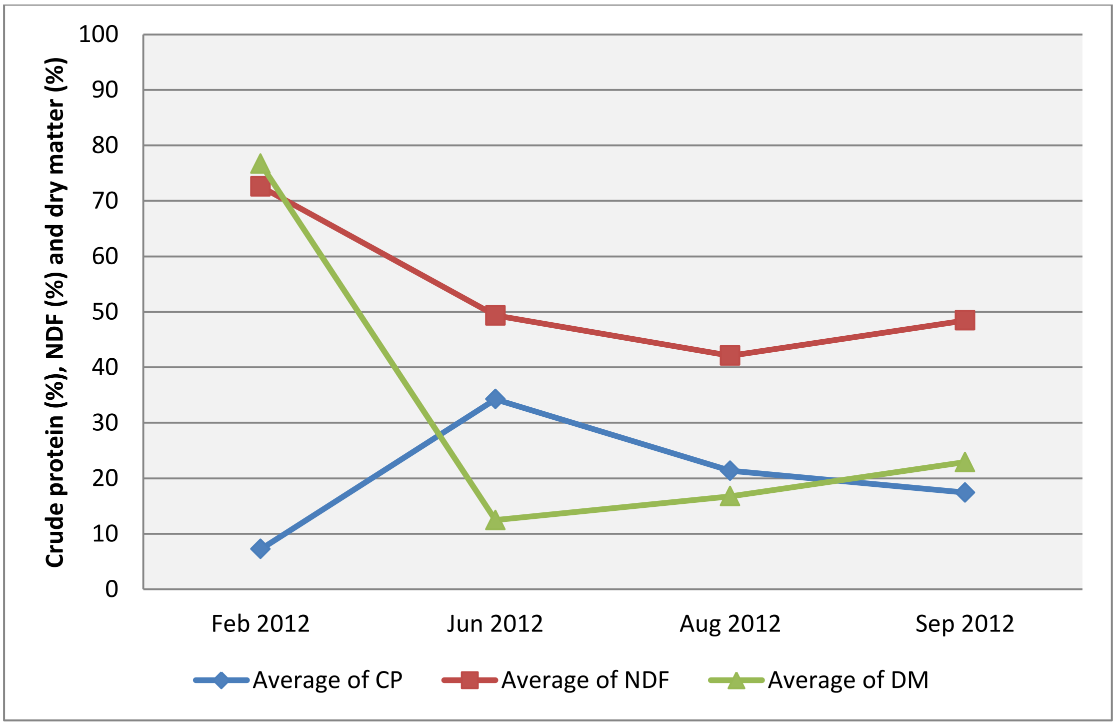

Fig. 23 - Average plant nutritive value across all paddocks tested at EP2 in 2012.

Lameness and scouring

Overall there was minimal lameness and scouring observed in 2012 with most mobs reporting less than 1% of the mob lame or scouring in any month.

Fig. 25 to Fig. 30 show the incidences of scouring and lameness across each trial site during 2012.

The most notable exceptions were South-west Victorian sheep where scouring in the ewes was observed at up to 10% at SWVIC1 and 5% at SWVIC2. Also, scouring at EP2 in July and August reached levels of 10% and 20% respectively.

Scouring observed at SWVIC1 increased slightly after ewes were treated with long acting drench capsules which should have controlled scouring as a result of worms unless resistance issues are present.

A faecal egg count conducted in May revealed 150 worm eggs per gram which was surprising. It is most likely that the scouring observed was a combination of a low level of worm infestation and feed attributes such as low fibre measured as NDF and dry matter (Fig. 24).

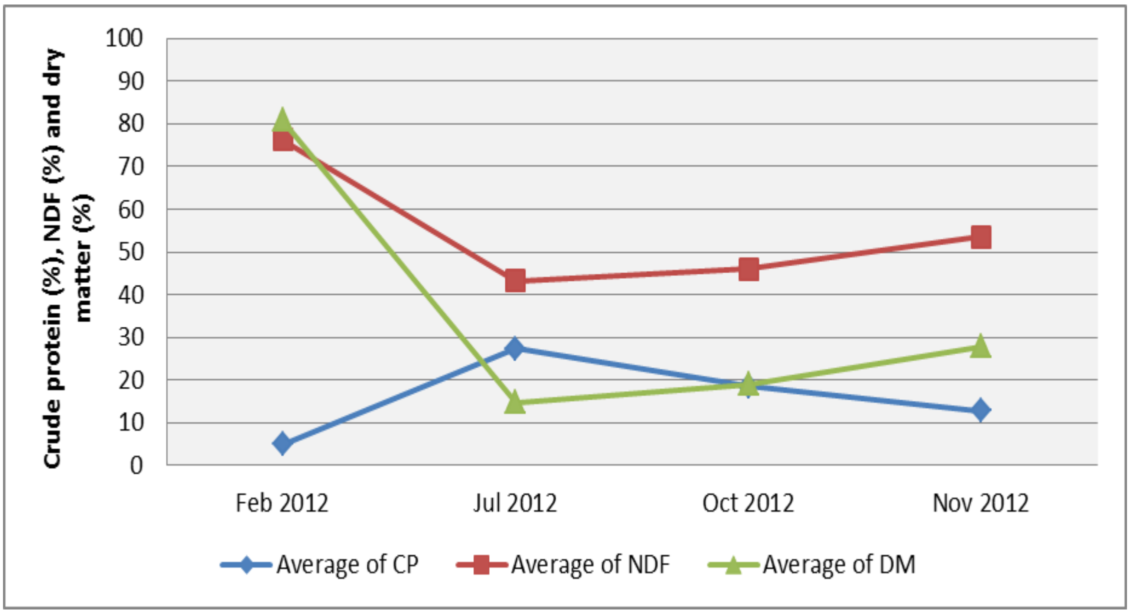

Fig. 24 - Average plant nutritive value across all paddocks tested at SWVIC1 in 2012.

Whilst elevated molybdenum levels are known to induce scouring in cattle, the literature review conducted as part of this project showed these effects have not been as strongly observed in sheep and additionally, the increase in molybdenum level between the February and July sampling period was only modest.

Interestingly, in comparing the nutritive value of feed at SWVIC1 from February to July (Fig. 24) it can be seen that plant crude protein increases to nearly 30% and dry matter and NDF% reduce significantly. This change in feed quality could be the cause for this increase in lameness more so than any problems associated with copper deficiency as copper levels in the pasture actually increased from February to July.

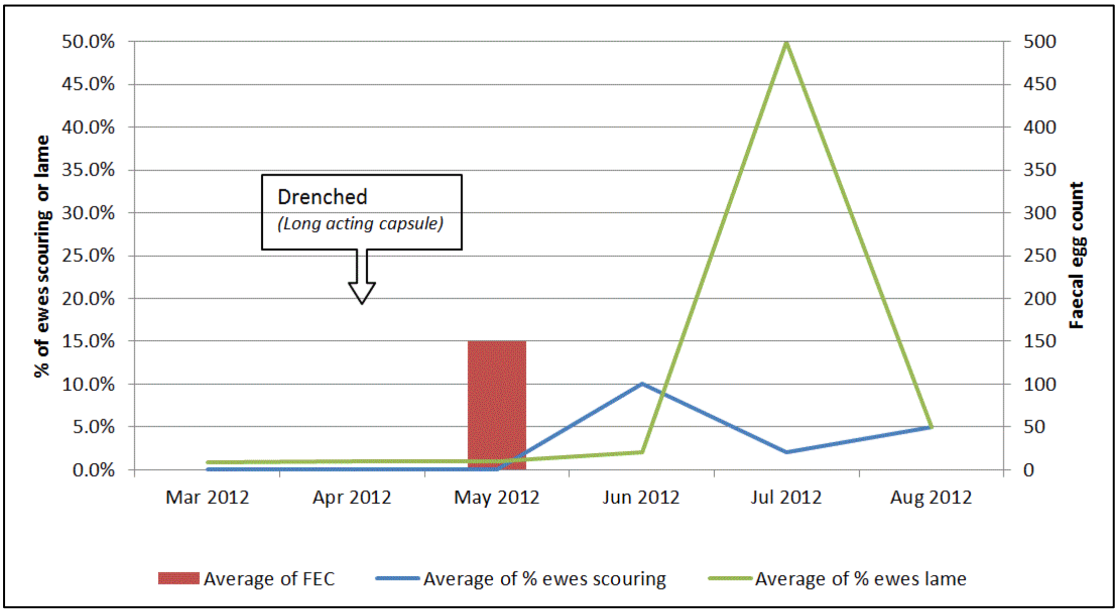

Fig. 25 - Average percentage of ewes scouring or lame and faecal egg count (when collected) at SWVIC1 in 2012.

Lameness was relatively low across all mobs; a notable exception to this was SWVIC1 in July where 50% of ewes (Fig. 25) and 30% of lambs were considered lame. This sudden onset of lameness has been reported historically at this property with virulent footrot being excluded as the cause through veterinary testing.

Foot scald has also been implicated in sudden onsets of lameness, particularly throughout the western districts of Victoria. This was further investigated as an additional cause of lameness in this mob in 2013.

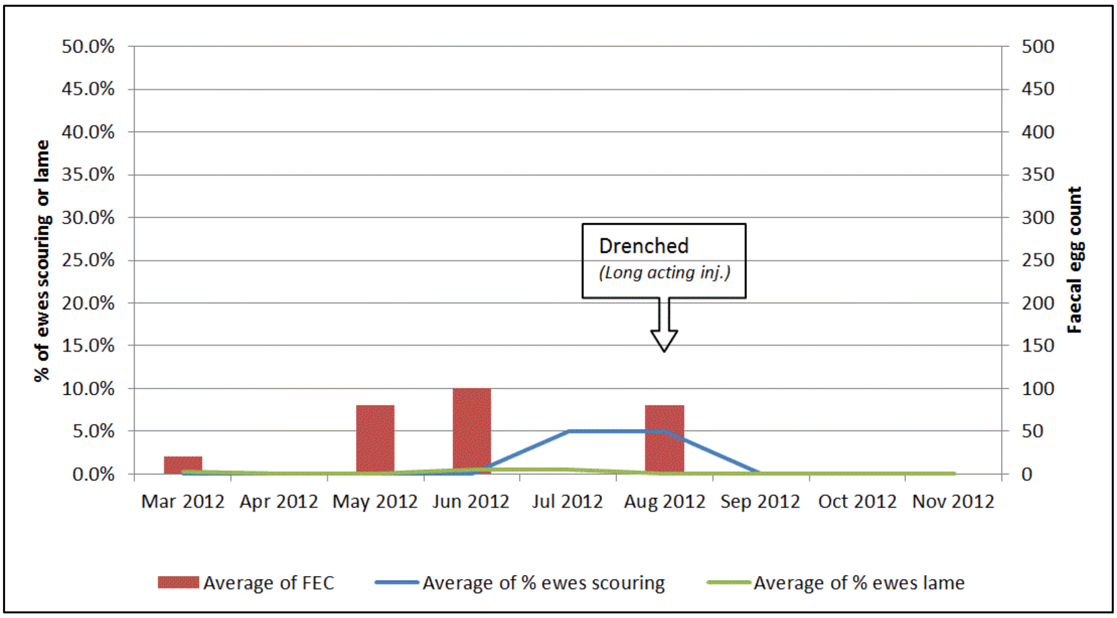

Fig. 26 - Average percentage of ewes scouring or lame and faecal egg count (when collected) at SWVIC2 in 2012.

5% of ewes were observed to be scouring at SWVIC2 (Fig. 26) during July and August, however it appears that this was controlled with a drench as it can be seen that the incidence of scouring declined following this treatment.

Minimal lameness was observed at MALLEE2 (Fig. 28), however scouring rose to 2% in ewes and 3% in lambs in July. This scouring was most likely attributable to worm burden as a faecal egg count in July resulted in 250 worm eggs per gram (*epg*).

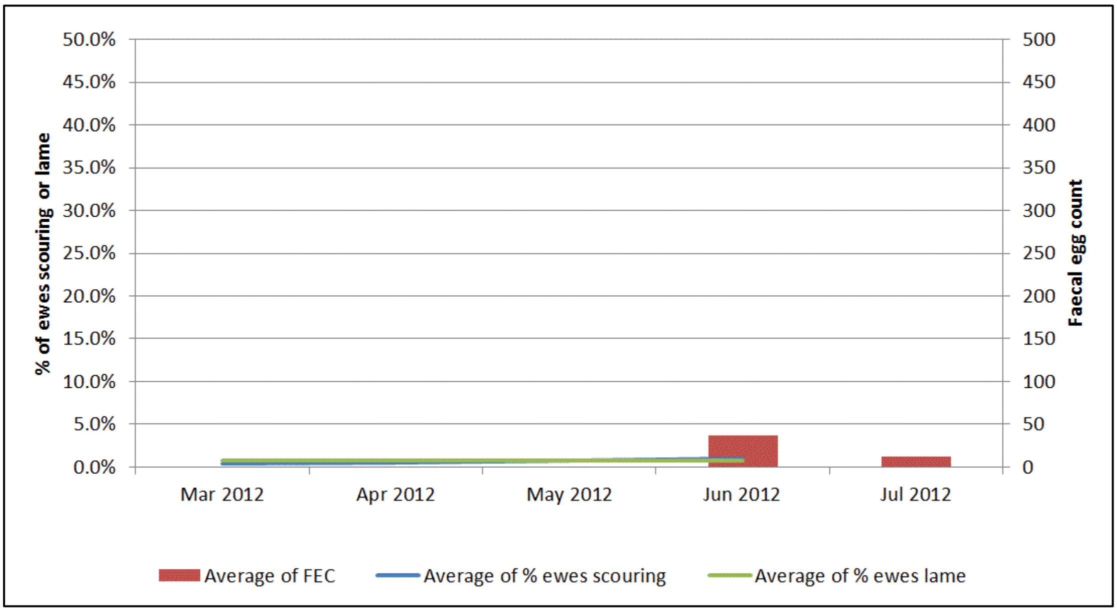

Fig. 27 - Average percentage of ewes scouring or lame and faecal egg count (when collected) at MALLEE1 in 2012.

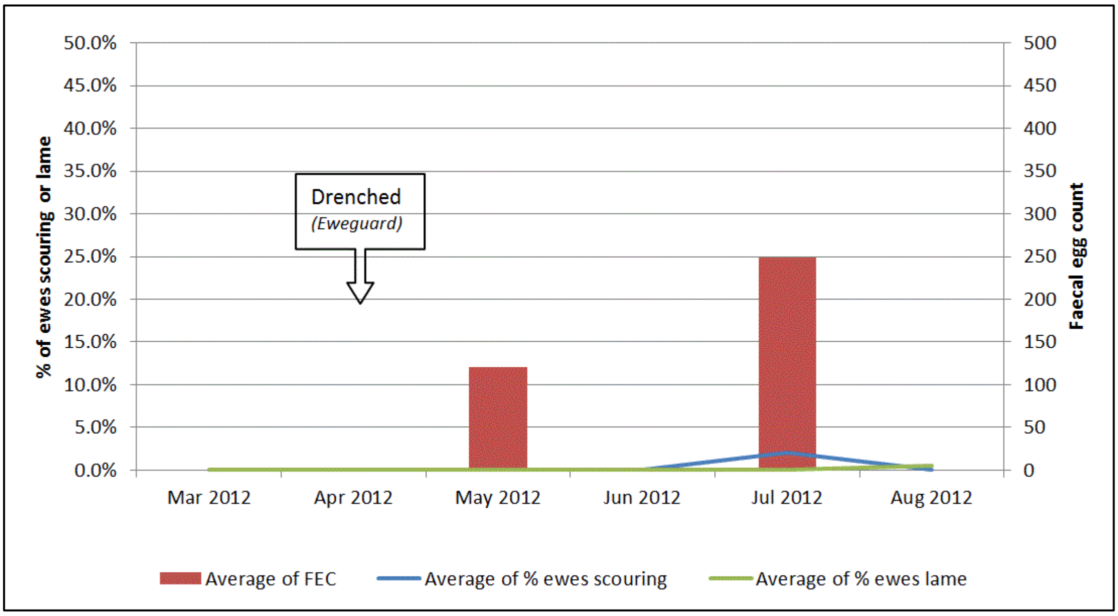

Fig. 28 - Average percentage of ewes scouring or lame and faecal egg count (when collected) at MALLEE2 in 2012.

Scouring at EP2 increased in July to 10% and rose further to 20% in August. A faecal egg count conducted in June showed 350 *epg* and while this would normally warrant drenching, as the ewes were in the middle of lambing it was not done until August when they were next mustered. At that time a faecal sample was collected for analysis and it showed the faecal egg count had reduced to 62 *epg* so it is unlikely that worms were the only contributing factor to the high level of scouring.

During August feed quality was high in moisture content (83%) which may have also contributed to the scouring.

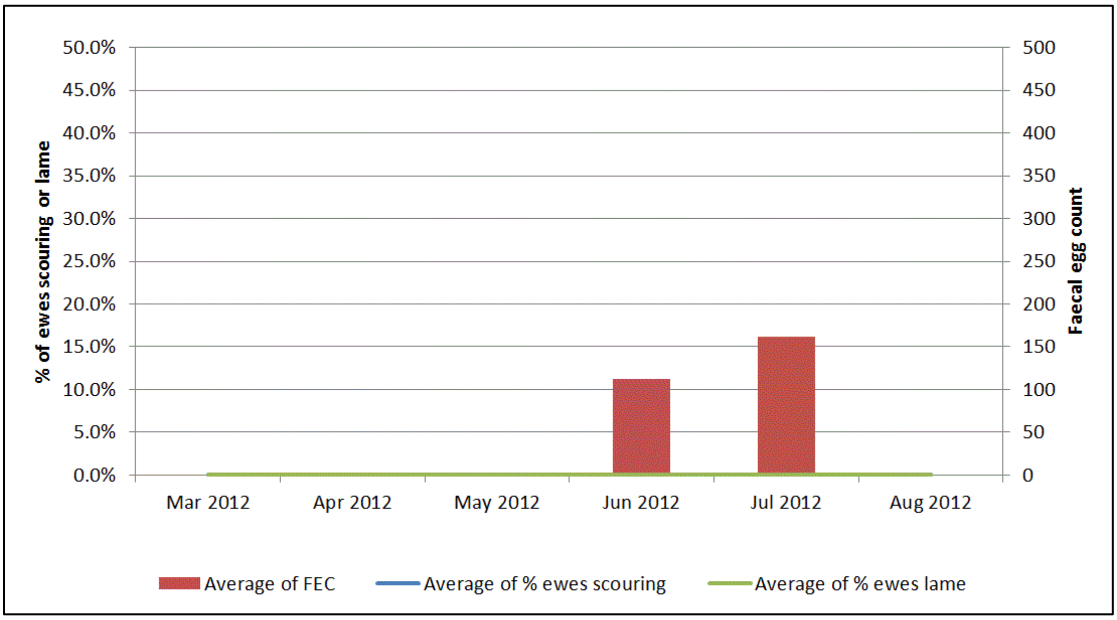

Fig. 29 - Average percentage of ewes scouring or lame and faecal egg count (when collected) at EP1 in 2012.

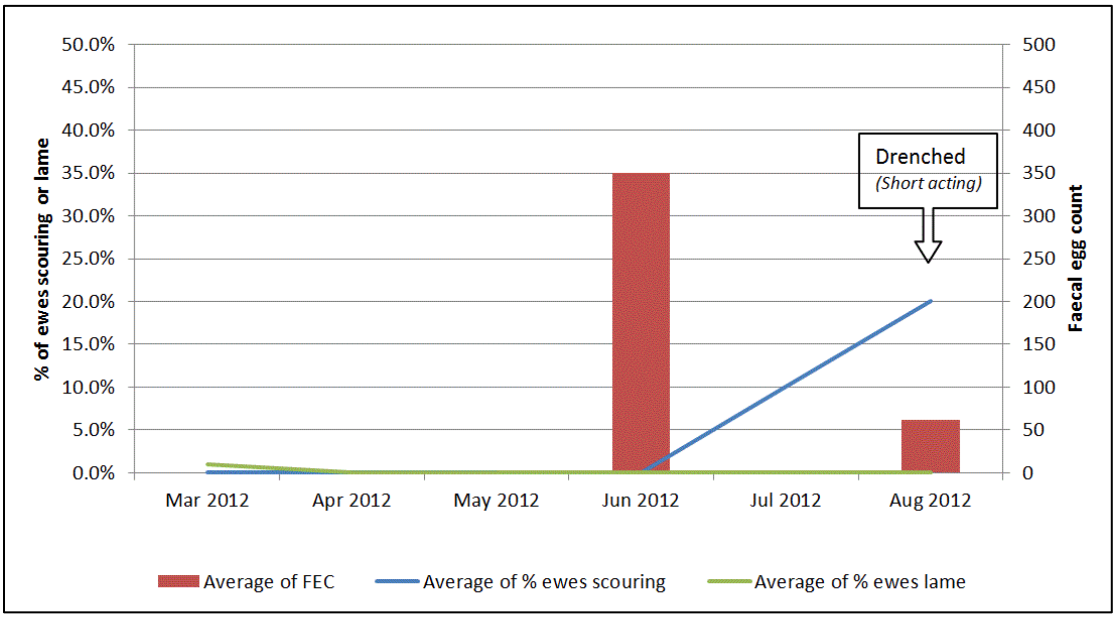

Fig. 30 - Average percentage of ewes scouring or lame and faecal egg count (when collected) at EP2 in 2012.

Nitrate content of feed can induce scouring in sheep and this will be investigated to eliminate this as a potential cause as part of the testing program in 2013, particularly during the winter and spring when feed is lush.

Mortality rate

Mortality across the trial mobs was variable and is highlighted in Fig. 31. The most significant incidence of mortality was observed at MALLEE1 which also showed the most severe copper deficiency in blood and liver results which are summarised in Table 7. Deaths were observed throughout the duration of the trial this year until copper supplementation was resumed after blood and liver sample collection. Although mortality rates were less than 1% each month, in a mob of 550 ewes this equated to nearly 4 ewes per month or 1 per week.

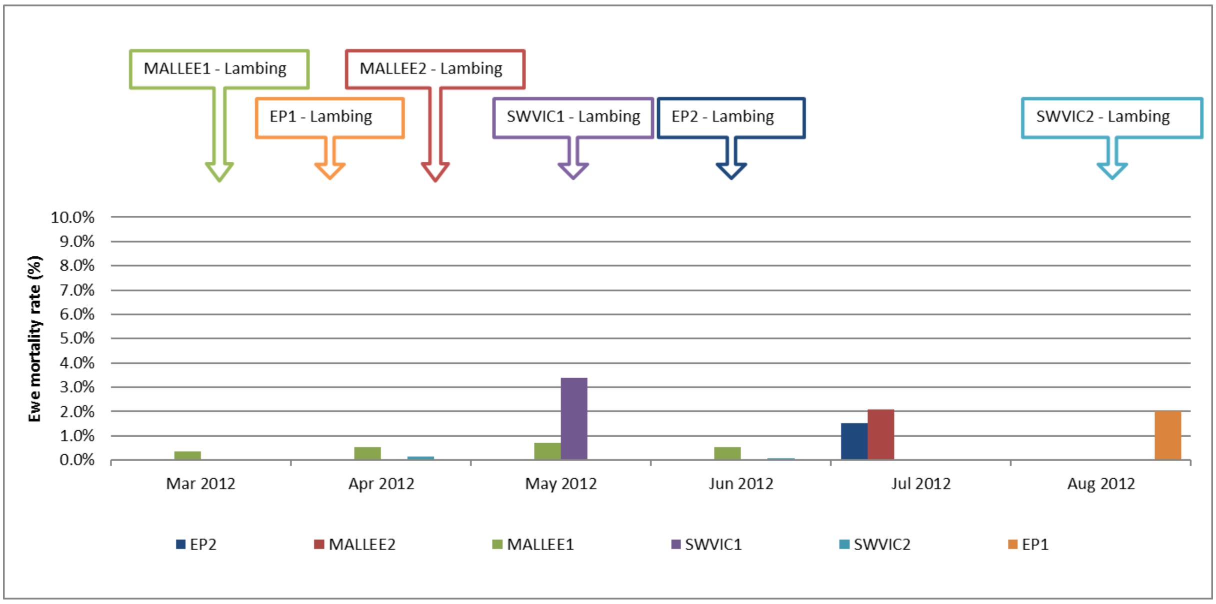

Fig. 31 - Ewe mortality rate up to and during lambing. Note: mortalities were recorded up to weaning.

Whilst some of the early mortalities were attributed to grain poisoning as a result of rapid introduction to barley, the mortalities continued after grain supplementation was ceased leading to questions around the true cause of their death. An autopsy conducted by Craig Wood (Veterinarian, Terang and Mortlake Veterinary Clinic) did not reveal the cause of death other than secondary pneumonia. This may indicate the copper deficiency experienced by the ewes reduced immunity levels and made them more susceptible to infection. In this way copper deficiency was not the primary cause of death but was a contributing factor. This depressive effect on immunity was highlighted in the literature review.

The highest incidence of mortality at one time was observed at SWVIC1 due to lambing difficulties. Mortalities observed at EP2 were due to lambing difficulties and misadventure. Mortalities observed at SWVIC2 were due to misadventure.

Fertility and marking rates

Table 6 provides the scanning and marking percentages across each trial mob in 2012. Overall lamb losses between scanning and marking were high, with on average 23.8% of lambs conceived, either lost in-utero or between birth and marking. This data will provide an interesting comparison to 2013 results following the implementation of copper supplementation.

A scanning percentage was unavailable at EP1 property this year as scanning was only conducted as wet/dry rather than for multiples (as this is their usual practice) and scanning had been completed before the trial commenced.

Dry ewes were not included in this trial mob so it may be assumed that the scanning percentage was not lower than 100%. Scanning in 2013 across all properties will be for multiples which may assist in providing some comparison to EP1 2012 results.

Table 6 - Scanning and marking percentages across the trial mobs in 2012.

Region	Scanning %	Marking %	% lambs lost between scanning and marking
SWVIC1	147%	107%	27%
SWVIC2	102%	80%	22%
MALLEE1	100%	77%	23%
MALLEE2	100%	83%	17%
EP1		100%	
EP2	200%	141%	30%

Swayback

Only one incidence of swayback was reported throughout the trial this year. A lamb was observed with hind leg paralysis at SWVIC1 property in June. Observations for swayback were recorded again in 2013 for comparison.

Blood and liver copper

Blood and liver samples were collected from each trial mob at weaning (approx. 14 weeks post lambing). 20 ewes from each were randomly selected for sampling with blood and liver biopsy samples sent to Regional Laboratory Services (Benalla) for analysis. A summary of the results showing the mean and range from each mob is shown in Table 7.

Table 7 - Blood and liver results from each property showing the mean and range.

		Blood Cu (µmol/L)	Liver Cu (mmol/kg wwt)
	Target range	7.5 - 20.0	0.23 - 3.67
SWVIC1	**Mean** Range	**14.6** (11.5 - 21.7)	**0.59** (0.14 - 1.47)
SWVIC2	**Mean** Range	**12.2** (4.7 - 22.5)	**0.18** (0.02 - 1.33)
MALLEE1	**Mean** Range	**6.1** (2.4 - 10.8)	**0.07** (0.03 - 0.19)
MALLEE2	**Mean** Range	**9.6** (4.4 - 15.7)	**0.12** (0.04 - 0.42)
EP1	**Mean** Range	**11.7** (9.7 - 15.2)	**0.69** (0.09 - 1.94)
EP2	**Mean** Range	**11.4** (8.4 - 14.2)	**0.42** (0.1 - 1.34)

These results showed that half of the trial mobs (SWVIC1, EP1 and EP2) were satisfactory for blood copper as all animals were above the minimum desired copper level. Two of the six properties (MALLEE2 and SWVIC2) were on average, satisfactory for blood copper; however individual animals within each of these mobs had low blood copper concentrations. MALLEE1 trial mob were considered overall to be deficient as the average blood result was below the required copper level and only 40% of animals tested were considered to have satisfactory blood copper levels.

Reviewing the trends between blood and liver results it can be seen that:

1. Where both the mean and minimum blood levels were deficient, all animals had low liver concentrations of copper
2. Where the mean blood copper levels were satisfactory but minimum was deficient, the majority of animals liver copper concentrations were deficient
3. Where all blood results were satisfactory for copper; the majority of animals liver samples were satisfactory, however several animals were still considered deficient.

Overall more animals were found to have copper deficiency based on liver analyses than would have been expected from blood testing.

Results from this year indicated that of the areas tested, the most severe deficiencies were found in the South Australian Mallee, with the least deficiencies determined on Eyre Peninsula in South Australia.

Analysis of results

<u>*Correlation between blood and liver copper*</u>

There was not a strong correlation between blood and liver copper concentration at any site which is depicted in Fig. 32 below.

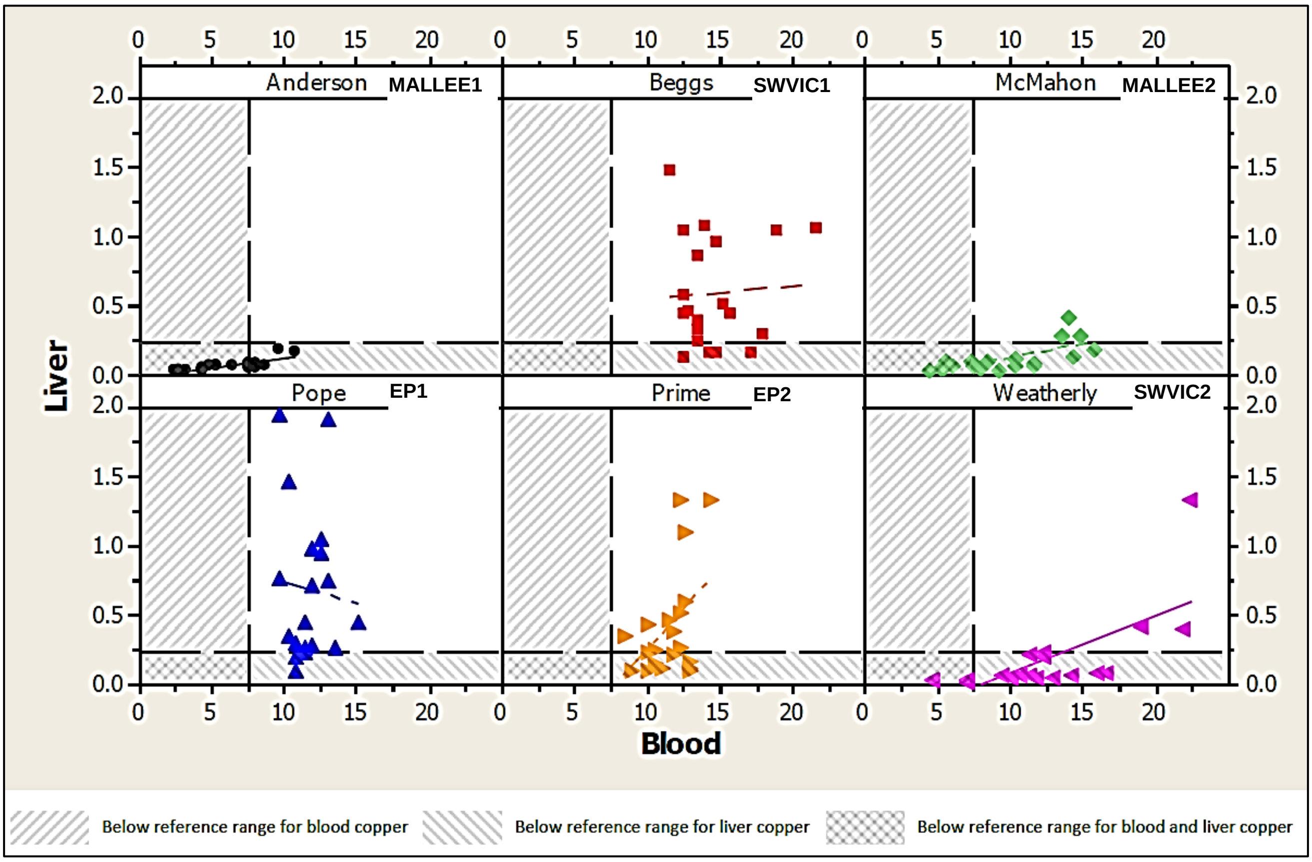

Fig. 32 - Scatterplot of liver and blood copper across each property. Blood results in µmol/L, liver results in mmol/kg wet weight.

Table 8 provides the correlation coefficients between blood and liver copper concentrations for each trial mob. Overall the correlation coefficient between blood and liver copper was only 0.43 (R^2=18.7%) suggesting that 81.3% of the variation in liver copper concentration is due to factors other than blood copper levels.

However, for three of the trial mobs the correlation between blood and liver copper was moderate. Whilst two of these mobs were in the SA Mallee region, one was located in Victoria and further investigation in 2013 will be required to determine the influence of region.

Table 8 – Correlation coefficients between blood and liver results for each trial mob and across all mobs tested in 2012.

	Correlation coefficient
SWVIC1	0.06
SWVIC2	0.70
MALLEE1	0.76
MALLEE2	0.70
EP1	-0.08
EP2	0.45
Overall	0.43

An important finding was that in mobs where blood copper levels were considered adequate, individual animals were found with low liver copper concentrations, suggesting that blood sampling alone may not be a reliable determinant of the copper status of the animal. However further investigation was conducted in 2013 to determine whether significant production increases can be achieved through increasing liver copper concentrations or whether the low liver concentrations observed in 2012 were practically and hence financially irrelevant.

Trials in 2013 compared the blood and liver concentrations between treated and untreated animals, or compared results between seasons where two trial mobs cannot be run, to investigate the effect of treatment and its result on productivity.

Correlation between plant and soil mineral concentration

Often producers have historic soil tests available more so than plant tissue analyses and prefer to base mineral supplementation strategies on the use of existing tests rather than investing in new plant tissue tests. The use of plant tissue tests is regarded as a more accurate indication of animal status as they are a more direct measure of what the animals are ingesting. Correlation coefficients between soil and plant copper concentrations were only moderate (0.62), with correlations for molybdenum (0.47), sulphur (-0.33) and iron (0.17), the key nutrients thought to interfere with copper availability, showing low correlations.

Whilst the correlations show that soil results cannot be used to accurately predict mineral content of plants (or vice versa), the results did show that soil copper concentrations may be used as an indicator only of plant copper status. In instances where soil copper was classified as low, plant mineral content was also low. However, this relationship was not apparent for molybdenum and was inconsistent for sulphur and iron.

Efficacy trial recommendations and plans for 2013

A range of treatments were planned to be trialled across the 6 properties involved in the project to test the effectiveness of different treatments. The treatment for each property was purposefully chosen to suit each situation given the animal testing results and the nature of the copper deficiency from pasture testing i.e. short term deficiency, long term deficiency, and influences from antagonistic minerals.

SWVIC1 (Beggs)

Liver tests showed a deficiency in the ewes at SWVIC1 property. Plant tissue samples were consistently low for copper and high for molybdenum, sulphur and iron.

Given the likely high level of interaction from antagonistic minerals resulting in a secondary copper deficiency, it was expected that a copper bolus would be the most effective treatment method to test.

Both control and treated animals would be tested in this mob to provide a direct assessment of the effectiveness of this form of treatment.

SWVIC2 (Weatherly)

Copper deficiency was shown in many blood and liver tests. Plant tissue tests revealed low copper levels and high molybdenum, sulphur and iron levels resulting in a secondary copper deficiency.

Due to the secondary nature of this deficiency a copper bolus should provide the most effective treatment to trial in 2013.

Both control and treated animals would be tested in this mob to provide a direct assessment of the effectiveness of this form of treatment.

Copper bolus treatment at SWVIC1 (Beggs) and SWVIC2 (Weatherly).

Copper boluses currently available in Australia provide copper in the form of copper oxide needles contained in a gelatine capsule which are released in the rumen as the capsule breaks down following administration. These copper oxide needles pass gradually into the abomasum where they are absorbed.

This may be an effective treatment option for primary copper deficiencies however their effectiveness is questionable under the influence from minerals such as molybdenum, sulphur and iron. In these situations, a higher level of success has been achieved overseas through the use of soluble glass boluses containing copper. This type of bolus is designed to stay in the reticulum/rumen and provide copper to interact and deactivate the influence of antagonistic minerals. These boluses contain supplemental copper, cobalt, selenium and iodine.

These boluses were not available in Australia however Productive Nutrition negotiated for a trial batch to be manufactured in the UK without selenium and iodine for use in this trial. The cobalt content unfortunately could not be removed as it forms an integral part of the product function. An successful application was submitted to the APVMA for a small scale trial permit to import the product for trial purposes.

MALLEE1 (Anderson)

From the results it appeared that a severe deficiency existed on this property. Pasture testing showed very low copper levels and relatively low levels of sulphur, iron and molybdenum during summer, suggesting a primary copper deficiency (little influence from antagonistic minerals). Summer would be the most critical time for supplementation of this mob as this is when the ewes are pregnant and then lambing from April onwards.

Due to the primary deficiency observed in 2012, straight copper supplementation in any form was expected to be effective in increasing animal copper status. These ewes would be provided with both a copper sulphate lick and copper sulphate in their water.

Due to the high number of mortalities experienced in 2012, no control mob would be run at this property and treatment will be compared across seasons to measure the effectiveness of this supplementation strategy.

MALLEE2 (McMahon)

Blood and liver testing at this property revealed a copper deficiency in many of the ewes tested. Plant copper levels were consistently low during 2012 and influence from antagonistic minerals were predominately greater during July and August.

At this property we would trial the use of copper sulphate in water troughs as a form of supplementation to test the effectiveness of this method for treating secondary copper deficiency.

Due to the logistical and labour limitations on farm we would only run a treatment group and results would be compared between seasons. This property has sandy soils and grazing the trial area with two smaller mobs would risk environmental damage as a result of grazing pressure.

EP1 (Pope)

Animal copper status was only shown to be low in several liver samples. Plant tissue testing showed that copper levels were deficient in February and increased steadily to satisfactory levels in June to September. Testing in November showed that copper levels, whilst still satisfactory, declined from September. Until copper levels reduced to deficient levels, supplementation would not be implemented. To more accurately determine when this occurs, we implemented monthly pasture testing. Once copper levels were considered low enough to warrant supplementation copper injections would be implemented immediately.

A low level of interaction from antagonistic minerals suggested that supplementation in the form of an injection should be effective in correcting this most likely, primary deficiency. This form of treatment may also suit the short term nature of the deficiency at this property.

Half of the trial mob would be treated with the remaining animals left untreated as a control mob for comparison.

EP2 (Prime)

Blood sampling from this property showed satisfactory levels of copper however liver testing revealed deficiency in some. Pasture testing across the season showed a primary copper deficiency.

Given the long term nature of the deficiency and previous positive results of supplementation from copper boluses at this property, copper oxide boluses would be trialled in 2013. Two types of copper bolus (Permatrace® and Tracerite®) would be used in the trial mob with remaining animals left untreated for comparison. The two types of bolus have different levels of copper released and would be compared to see if this is seen in blood and liver results.

On farm measurements in 2013

All testing conducted as part of this project in 2012 would be repeated in 2013 as part of the treatment efficacy trial. Additional blood and liver testing would be conducted where treatment and control groups exist.

4.1.3 Conclusion

The data collected in this trial confirmed the existence of primary and secondary copper deficiencies on these properties and provided a basis upon which to recommend treatments to be investigated in 2013. It also confirmed that copper deficiency is a complex issue that appears to have different forms supporting many of the findings of the literature review.

4.2 Year 2 (2013)

4.2.1 Methodology

Following the analysis of 2012 results, each property was allocated one or more copper treatments to test during 2013. The treatments tested at each property are shown in Table 9.

Table 9 - Summary of treatments tested at each property

	EP1	EP2	MALLEE1	MALLEE2	SWVIC1	SWVIC2
Treatment(s)	Coppernate injection	1. Coopers Permatrace capsule 2. Animal Health Supplies Tracerite capsule	Copper trough blocks plus copper sulphate lick	Copper trough blocks	1. Coopers Permatrace capsule 2. Cutemco UK Bolus	1. Coopers Permatrace capsule 2. Cutemco UK Bolus
Control mob	Yes	Yes	No	No	Yes	Yes
Dose rate	60mg	Permatrace = 2.5g Cu (6.8mg/hd/d) Tracerite = 2.1g Cu (5.8mg/hd/d)	Trough block = 3.27mg/hd/d (4L) Lick = 2.52mg/hd/d	Trough block = 3.27mg/hd/d (4L)	Permatrace = 2.5g Cu (6.8mg/hd/d) Cutemco = 4.5g Cu (16.4mg/hd/d)	Permatrace = 2.5g Cu (6.8mg/hd/d) Cutemco = 4.5g Cu (16.4mg/hd/d)
Treatment date	Jan, 15mg Feb, 30mg Jun, 15mg	3rd Jan 2013 (Joining 9th Jan 2013)	6th Aug 2012 (Joining 29th Oct 2012)	15th Nov 2012 (Joining 23rd Nov 2012)	30th May 2013 Supp. groups given trough blocks from 26th Feb 2013 (Joining 1st Mar 2013)	14th March 2013 (Joining 21st Mar 2013)
Expected sustained activity	6mths	Permatrace = 12mths Tracerite = 12mths	As delivered	As delivered	Permatrace = 12mths Cutemco = 9mths	Permatrace = 12mths Cutemco = 9mths

A minimum of 25 mature ewes were allocated to each treatment group at each property. At EP2, MALLEE2 and SWVIC2 treatments were implemented one week prior to joining. At EP1 copper injections were given in January, February and early June. Copper supplementation was provided to ewes at MALLEE1 from August 2012 following blood and liver sampling (July 2012). At SWVIC2 as copper capsules and boluses could not administered prior to joining, copper sulphate trough blocks were used for mobs intended to receive the copper capsules and boluses. One mob was not provided copper trough blocks during this time to act as the control mob. Following joining, trough blocks were no longer used and boluses were administered to ewes that had received copper from the trough blocks. These treated animals were then grouped together with the control mob that had not received any copper supplementation to form the trial group. At EP1, EP2, SWVIC1 and SWVIC2 control and treatment groups were run together as a single mob.

The following on-farm measurements were collected during the period of July to November 2013:

- Pasture nutritive value and mineral content
- Flock observations including scouring, lameness, mortality and incidences of swayback in lambs.
- Liver and blood copper concentration
- Body condition score of each treatment group (at liver and blood sampling)
- Pregnancy scanning and marking percentage

Pasture mineral analyses

Pasture samples were collected from each property throughout 2013. Samples were collected from 1 – 8 paddocks at each property depending on the number of paddocks the trial mob grazed. Plant samples were collected from 5-6 representative locations across each paddock and combined to form the paddock sample for analysis. Samples were collected by either cleanly tearing or cutting a sample with shears. Care was taken to ensure that no dirt, roots or contamination from the shears occurred which could affect the test results. Plant species composition in the sample was reflective of the plant species composition in the paddock. The only exception to this was where unpalatable weed species were present in the paddock their inclusion rate in the sample for analysis was similar to that estimated of animal intake (generally minimal). Pasture samples were sent to SGS laboratory in Queensland for analysis.

Table 10 shows the month in which pasture samples were collected at each property.

Table 10 - Pasture sample collection months for 2013

Property	Round 1	Round 2	Round 3	Round 4	Round 5
EP1	Jan/Feb	May	July	October	
EP2	February	May	August	September	December
MALLEE1	February		August	October	
MALLEE2	February	May	Jul/Aug	October	
SWVIC1	March		July	October	November
SWVIC2	March		August	October	November

Five rounds of pasture sampling were scheduled be collected from each property, however at MALLEE1, SWVIC1 and SWVIC2 the round 2 collection was not completed due to the late break of season resulting in insufficient pasture available to collect. At EP2 and MALLEE2 this also delayed the collection of round 3 to August. At EP1, MALLEE1 and MALLEE2 a fifth round of pasture samples was not collected as sampling for round 4 was conducted relatively closely (October), pasture conditions had not changed significantly and trial animals were no longer grazing these paddocks.

Plant nutritive value

Pasture samples collected for mineral analysis were also tested for nutritive value. Results for crude protein (CP), dry matter (DM) and neutral detergent fibre (NDF) were obtained to determine the influence that these attributes had on scouring and lameness observations within the trial mobs.

Mortality rate

Producers recorded ewe or lamb deaths from the trial mob at each property.

Lameness and scouring

Producers at each property visually assessed the percentage of ewes in the trial mob that were lame or scouring each month. However, as the assessment was an average of the entire mob, where more than one treatment group was present it was not possible to determine whether differences existed between treatment groups.

Swayback

Trial mobs were observed for any incidence of swayback in lambs and the number of lambs observed was recorded.

Reproductive rates

Reproductive rates were recorded at pregnancy scanning and lamb marking. At EP1, EP2, SWVIC1 and SWVIC2 treatment and control groups were run together as a single mob under the same environmental conditions. As a result of this, with the exception of EP2, marking rates from each individual treatment group were not able to be recorded as individual ewes and their progeny were not able to be identified. Ewes and their progeny at EP2 were identified to allow an accurate marking percentage from each treatment group within the mob. An overall marking percentage from SWVIC1 was not obtained due to the following data collection issue related to the use of Pedigree Matchmaker equipment and software; time and date stamps were not recorded by the scanner against each RFID tag.

At EP1, EP2 and SWVIC2 an additional measure of ewe wet/dry status was recorded for each animal at the same time blood and liver samples were collected.

Body condition score

At blood and liver sampling, the body condition score of ewes was estimated and recorded individually. This was done at approximately 14 weeks after the start of lambing.

Blood and liver copper concentration

Blood and liver samples were collected from 20 randomly selected ewes in each control and treatment group at five of the six properties. At the sixth property (EP1) uneven numbers of animals (19 treated and 21 untreated) were sampled due to a tag reading error. Blood and liver biopsy samples were collected at weaning (approx. 14 weeks post lambing) and sent to Regional Laboratory Services for analysis.

4.2.2 Results

Pasture mineral analyses

Pasture mineral testing at EP1 showed that pasture copper concentration varied considerably throughout the year. Low pasture copper levels were identified from testing conducted in January, February and May 2013 (Fig. 33). Pasture copper concentration increased from May to July 2013 to reach adequate levels for livestock during winter and spring.

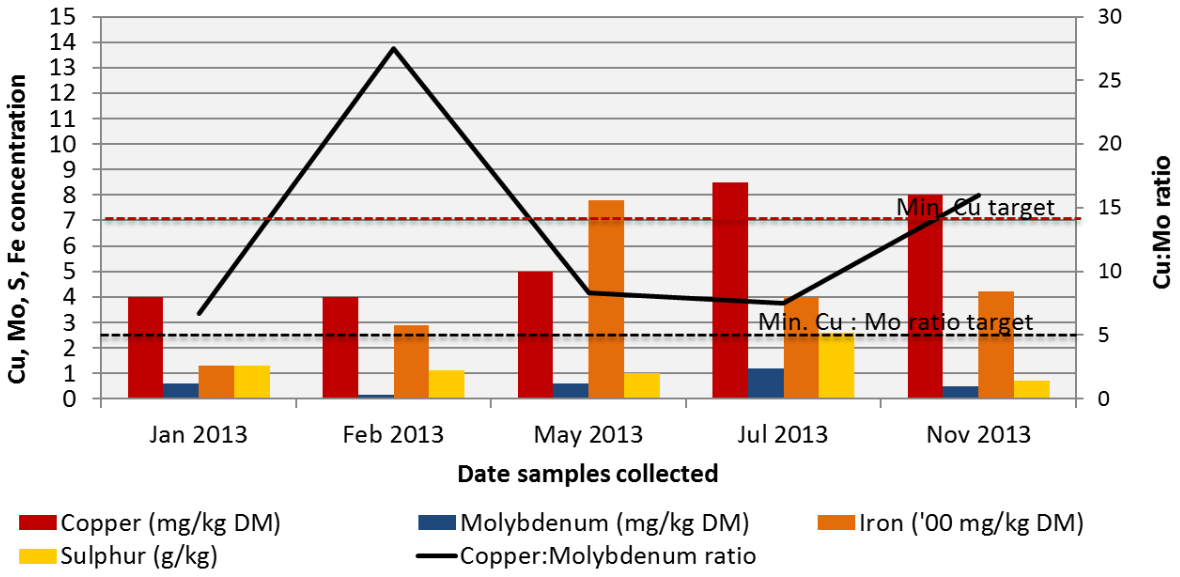

Fig. 33 - Mean pasture mineral concentration across all paddocks tested on property EP1 in 2013.

Whilst molybdenum concentration increased slightly from summer to winter it was not at a high enough level to reduce the copper to molybdenum ratio below 5:1; this is the ratio below which an induced or secondary copper deficiency is likely to occur

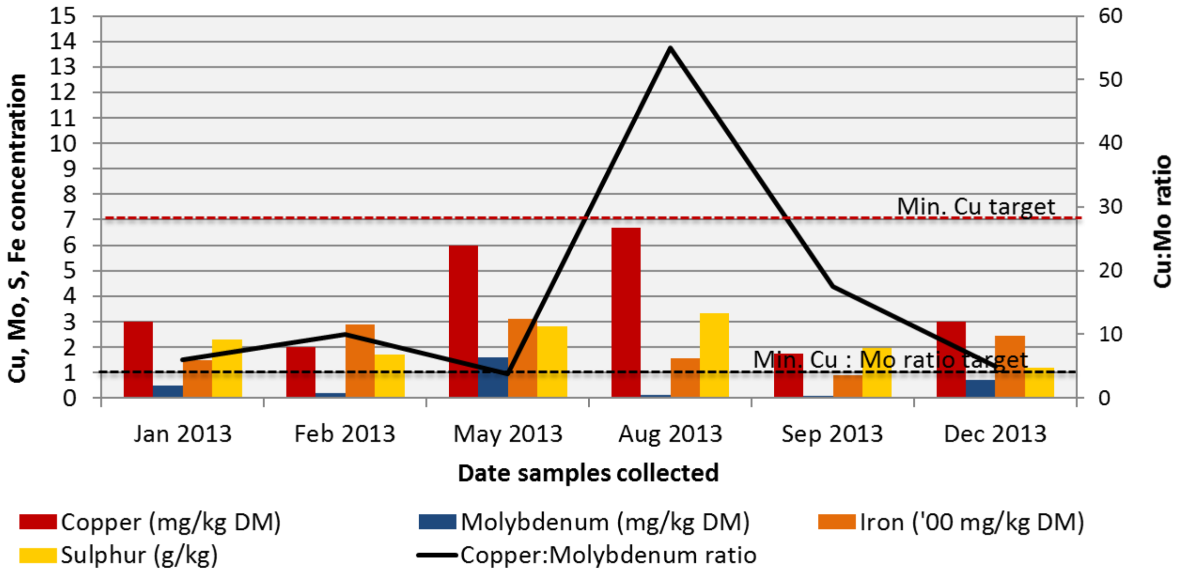

Fig. 34 - Mean pasture mineral concentration across all paddocks tested at EP2 in 2013.

Pasture tissue copper concentration was on average below 7 mg/kg DM throughout the entire year at EP2 (Fig. 34). Molybdenum levels were generally low with the exception of results from May 2013 showing an average concentration of 1.6mg/kg DM resulting in a low copper to molybdenum ratio of 3.8:1. Sulphur and iron concentrations in pasture tissue remained relatively stable throughout the year however sulphur levels increased during May and August in contrast to the findings at EP1. This highlights the need for caution in assuming that deficiencies in one district of a region is representative of an entire region.

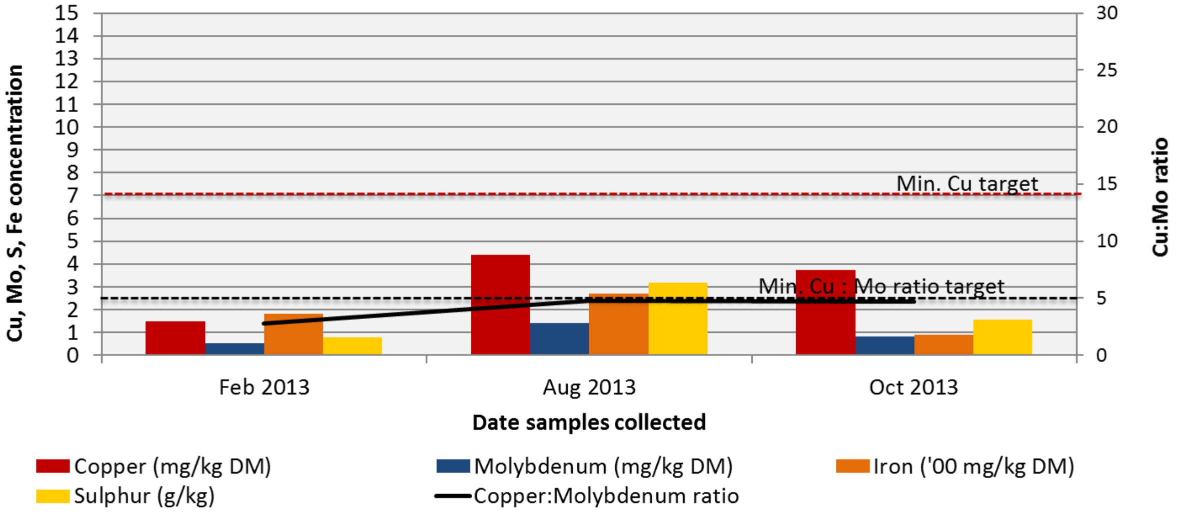

Fig. 35 - Mean pasture mineral concentration across all paddocks tested at MALLEE1 in 2013.

Copper concentration in pasture tissue tests collected from MALLEE 1 (Fig. 35) during 2013 were well below the minimum copper requirements of livestock of 7mg/kg DM. Molybdenum concentration was generally low with a slight increase observed in August 2013. Across the three tests collected in 2013 the molybdenum to copper ratio was below the minimum target of 5:1 however this was only slight during August and October at 4.7:1 in both instances. These low molybdenum to copper ratios were primarily influenced by the low copper concentration observed in pasture throughout the year. Other minerals including sulphur and iron were relatively low during February and October however sulphur concentration reached high levels at the August sampling of 3.18 g/kg DM.

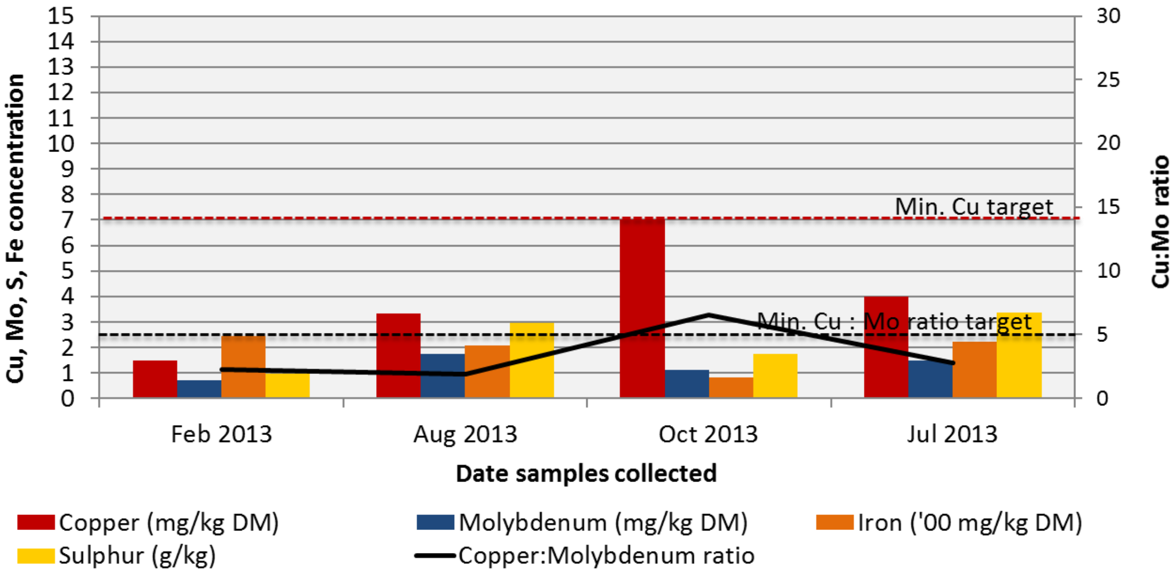

Fig. 36 - Mean pasture mineral concentration across all paddocks tested at MALLEE2 in 2013.

Pasture copper concentration at MALLEE2 was below the minimum target (7mg/kg DM) at each testing period except for October 2013 where it reached 7 mg/kg DM. Molybdenum levels were relatively high during July and August reaching an average of 1.46 and 1.73 mg/kg DM respectively. In combination with the low copper concentration this resulted in the copper to molybdenum ratio being well below 5:1 indicating copper availability was compromised.

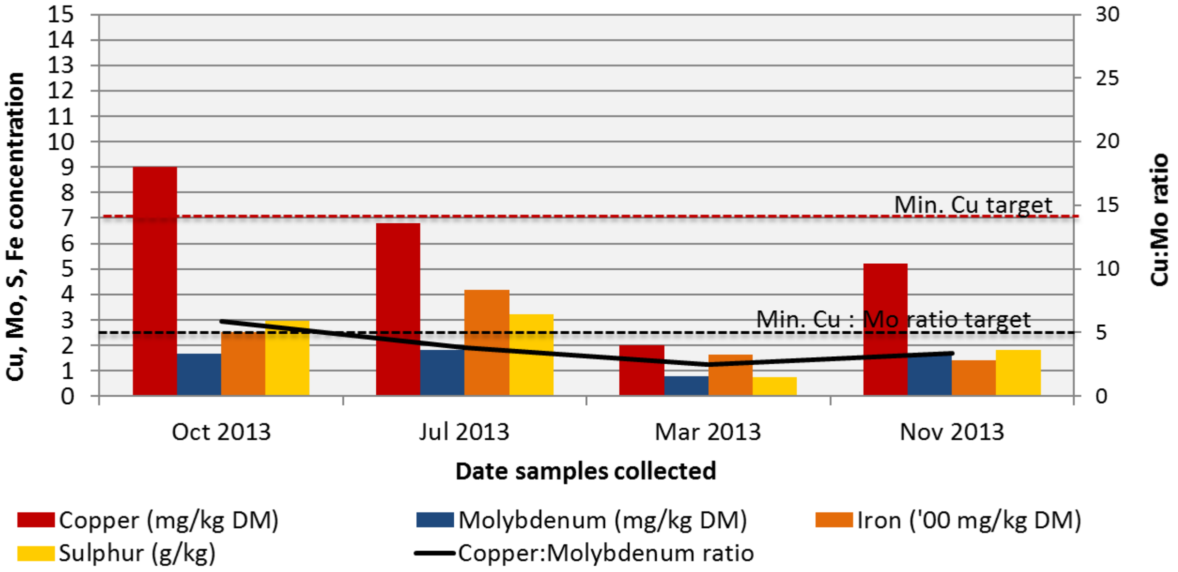

Fig. 37 - Mean pasture mineral concentration across all paddocks tested at SWVIC1 in 2013.

Pasture copper concentration at both SWVIC1 and SWVIC2 showed an increasing trend from the first sample collected in March 2013 to October, which then declined in late November as the pasture matured.

Molybdenum levels at SWVIC1 were particularly high during winter and spring resulting in low copper to molybdenum ratios with the exception of October where a high copper concentration counteracted this effect (Fig. 37).

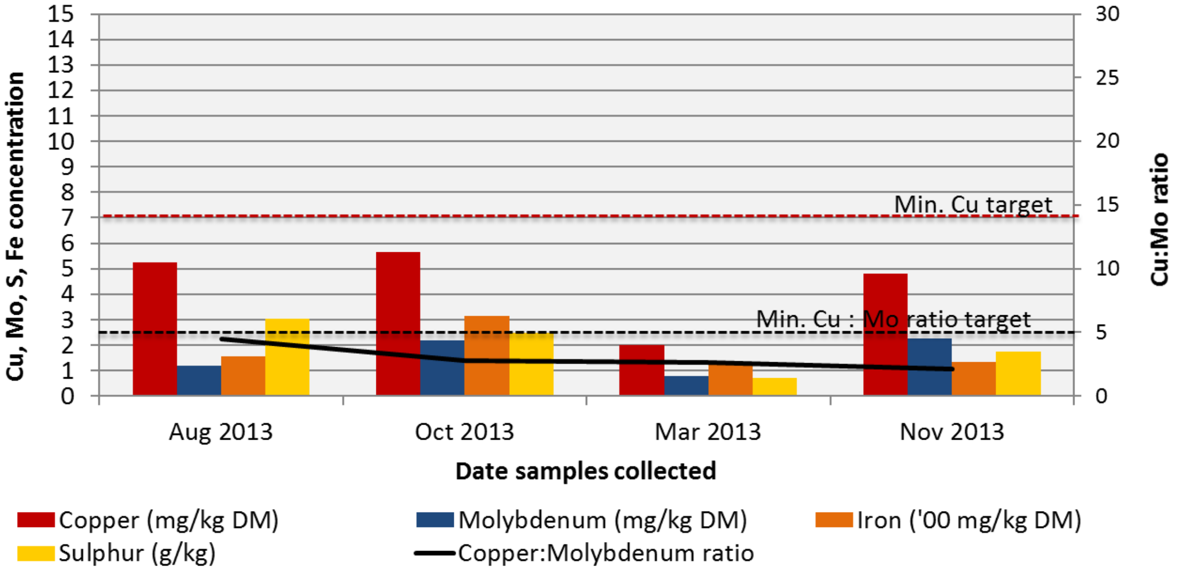

Fig. 38 - Mean pasture mineral concentration across all paddocks tested at SWVIC2 in 2013.

Molybdenum levels at SWVIC2 were high during October and November resulting in low copper to molybdenum ratios indicating a significant influence from molybdenum on copper availability (Fig. 38).

Pasture nutritive value

Fig. 39 to Fig. 44 show the average CP, NDF and DM from paddocks tested at each of the trial properties. Crude protein peaked in winter following early germination of pasture and gradually declined with pasture maturity. In contrast, dry matter and neutral detergent fibre both increased as pastures matured.

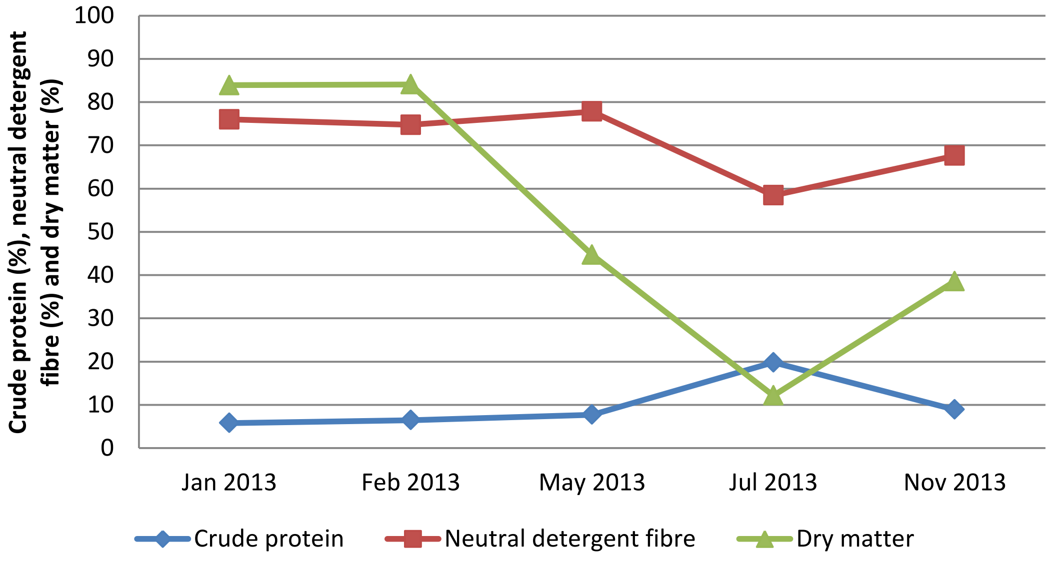

Fig. 39 – Mean pasture nutritive value across all paddocks tested at EP1 in 2013.

Pasture crude protein was found to increase from summer to winter at EP1 however not to the same level as that observed at other properties, only reaching a maximum of 19.8%.

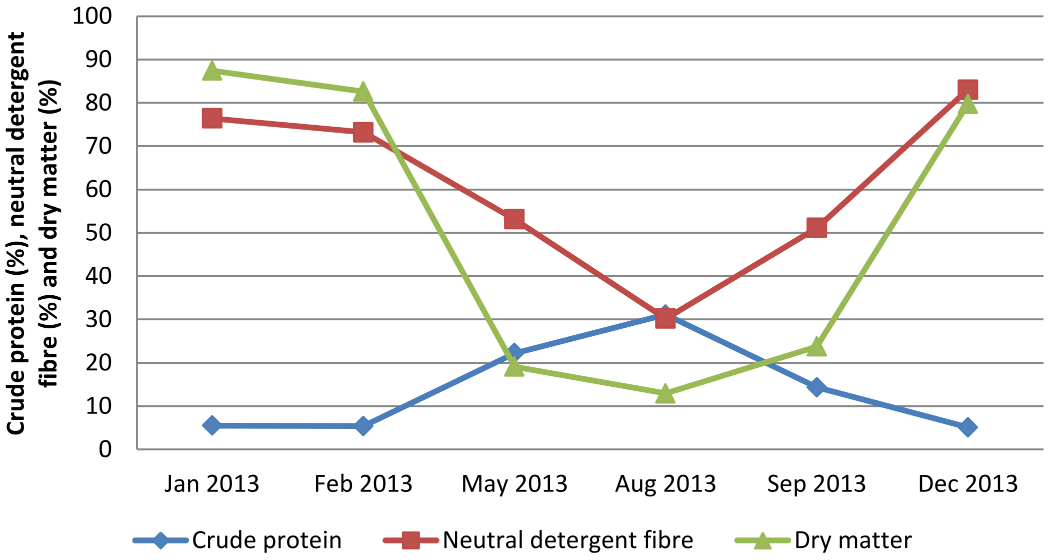

Fig. 40 - Mean pasture nutritive value across all paddocks tested at EP2 in 2013.

Fig. 40 shows that at EP2 neutral detergent fibre declined in August 2013 to the lowest concentration (30%) found at any property; this is most likely to be a reflection of the lack of carryover dry standing pasture from the previous year.

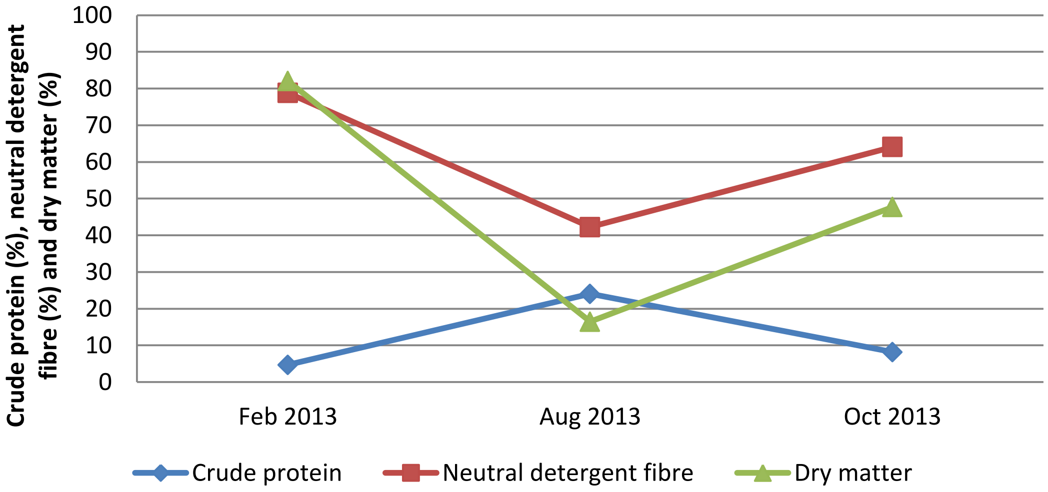

Fig. 41 - Mean pasture nutritive value across all paddocks tested at MALLEE1 in 2013.

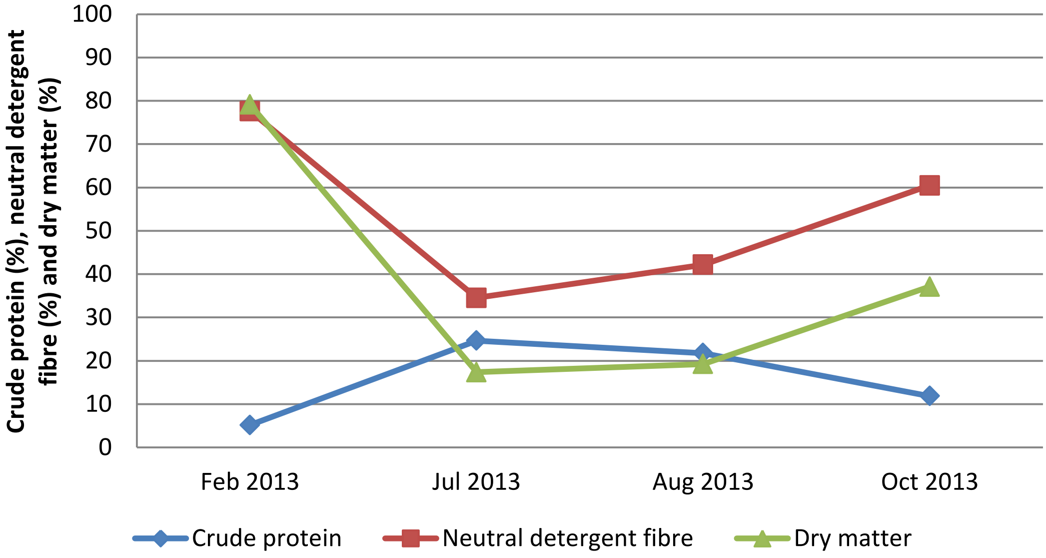

Fig. 42 - Mean pasture nutritive value across all paddocks tested at MALLEE2 in 2013.

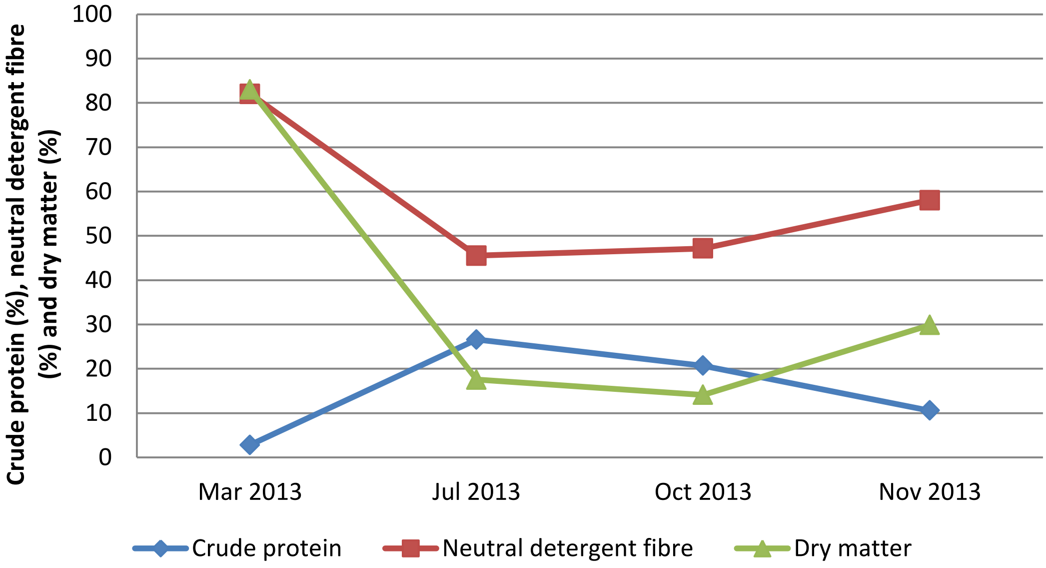

Fig. 43 - Mean pasture nutritive value across all paddocks tested at SWVIC1 in 2013.

Fig. 42 and Fig. 43 show the change in pasture feed value across two different regions. It can be seen that at MALLEE2 pasture protein declined to approximately 10% in October, whereas this equivalent decline at SWVIC1 did not occur until November. This is representative of the different environment of these two properties.

Pasture protein content at SWVIC2 increased sharply from March to August 2013 whilst dry matter and NDF decreased. Interestingly as the pasture matured, testing conducted in November showed that protein content had declined considerably to 12% however dry matter content had not increased at the same rate, but was far below that observed in March 2013, the rapid decline having occurred from March to August.

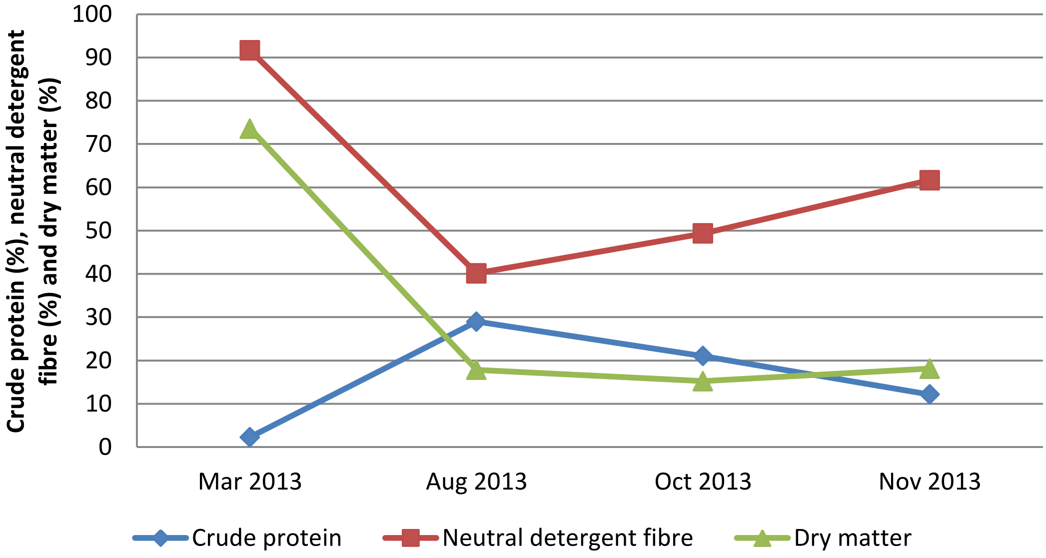

Fig. 44 - Mean pasture nutritive value across all paddocks tested at SWVIC2 in 2013.

Mortality rate

The mortality rate at each property (Table 11) during 2013 was relatively low in comparison to industry standards with the exception of ewe mortality at EP1. The causes of mortality at this property were not determined however it was suspected that pregnancy toxaemia was the most likely cause as the deaths occurred mainly during late pregnancy and lambing.

Table 11 - Mortalities observed at each property during 2013

	Mortality rate (% of ewes)	Number of ewe mortalities
EP1	6.0%	3
EP2	1.7%	5
MALLEE1	1.7%	9
MALLEE2	0%	0
SWVIC1	1.7%	5
SWVIC2	0.9%	8

Lameness and scouring

Fig. 45 to Fig. 50 show the average percentage of ewes at each property that were reportedly lame or scouring up until weaning when assessments ceased. Faecal egg count results are presented as well.

Trial mobs located on Eyre Peninsula and in the Mallee showed very little scouring or lameness with typically less than 1% observed to be either lame or scouring. The maximum scouring score assessed from these four properties was 7% of the mob at MALLEE1 in January 2013. At this time a faecal egg count was conducted and resulted 75epg indicating there may have been some influence from worms.

Greater numbers of ewes on the south-west Victorian properties were observed to be lame and with higher incidence of scouring than the ewes on Eyre Peninsula or in the South Australian Mallee.

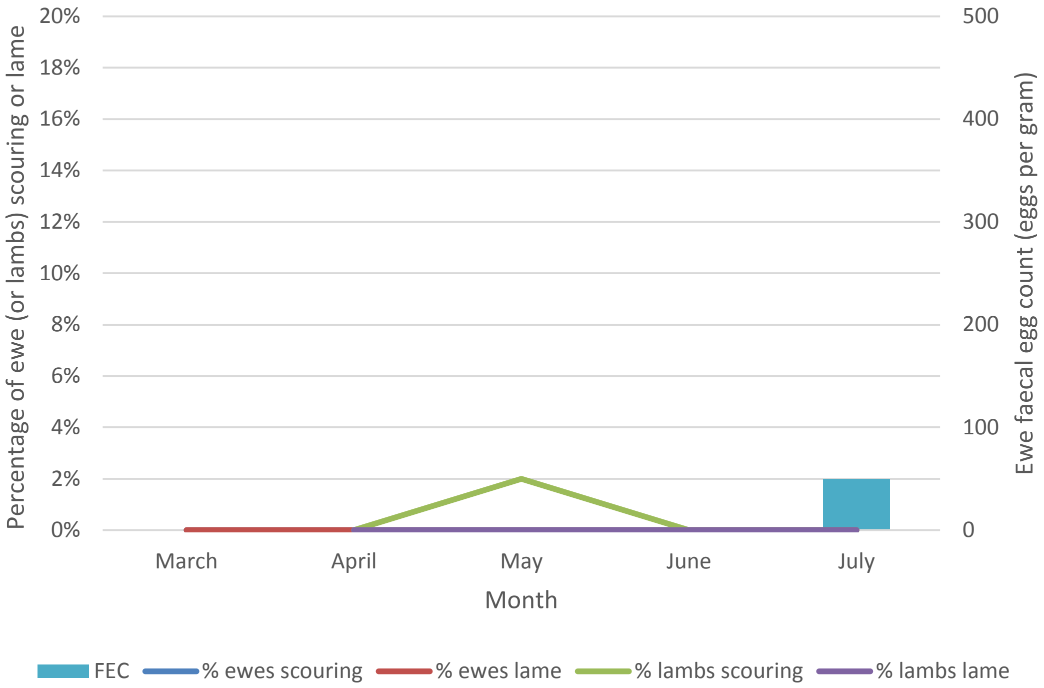

Fig. 45 - Scouring, lameness and faecal egg counts from EP1 during 2013.

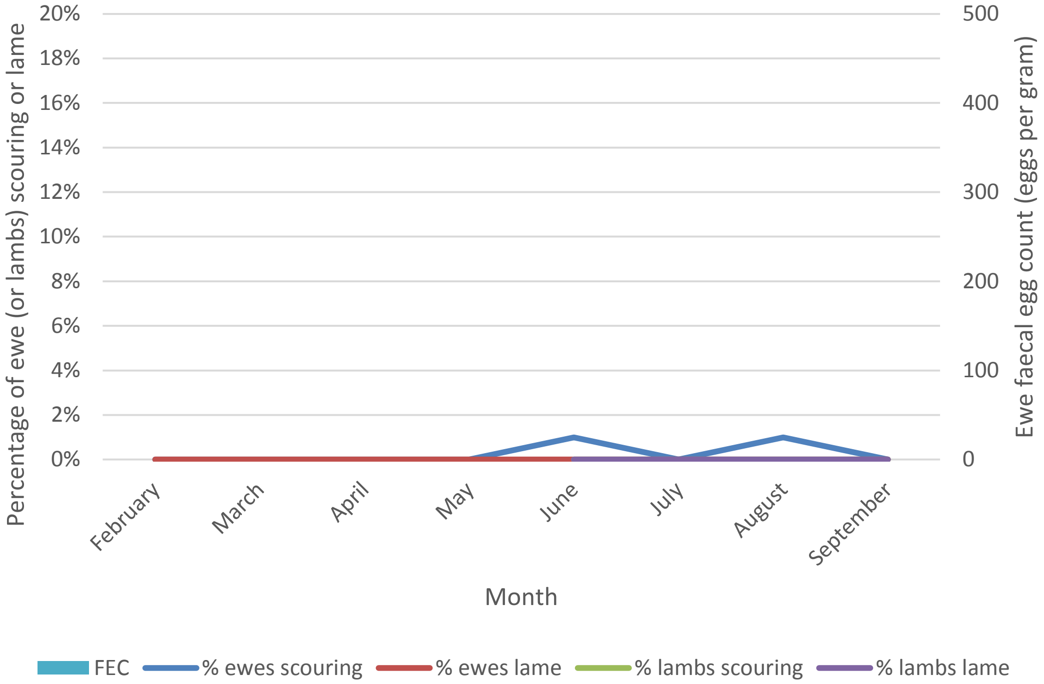

Fig. 46 - Scouring, lameness and faecal egg counts from EP2 during 2013.

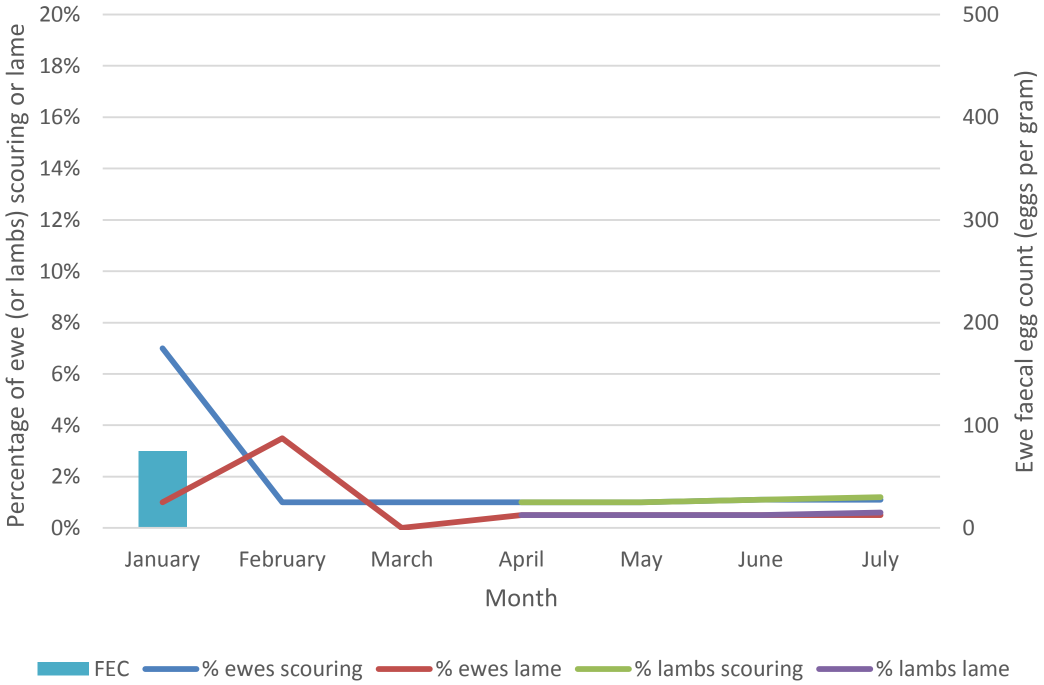

Fig. 47 - Scouring, lameness and faecal egg counts from MALLEE1 during 2013.

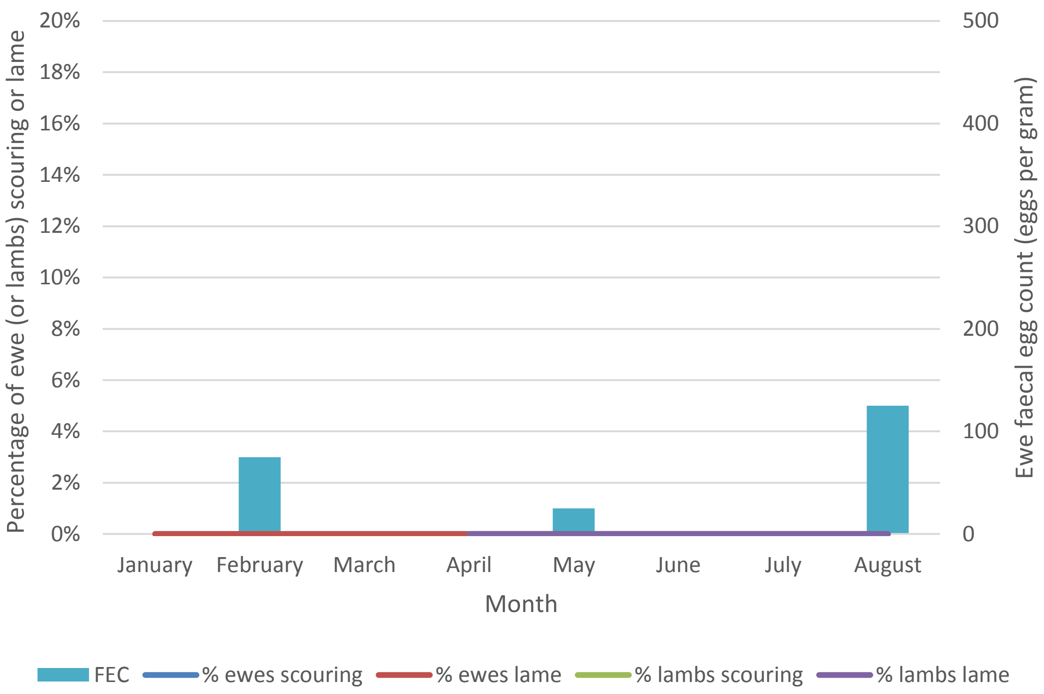

Fig. 48 - Scouring, lameness and faecal egg counts from MALLEE2 during 2013.

The number of ewes scouring at SWVIC1 was relatively low with a maximum of 2% during September and October 2013. However, lameness at this property increased to reach 10% of the mob lame during September and October 2013; this was the highest incidence of lameness observed at any of the properties.

A high incidence of scouring was observed at SWVIC2 during June 2013 however a faecal egg count conducted at this time showed 340epg indicating that worm burden was the major contributing factor.

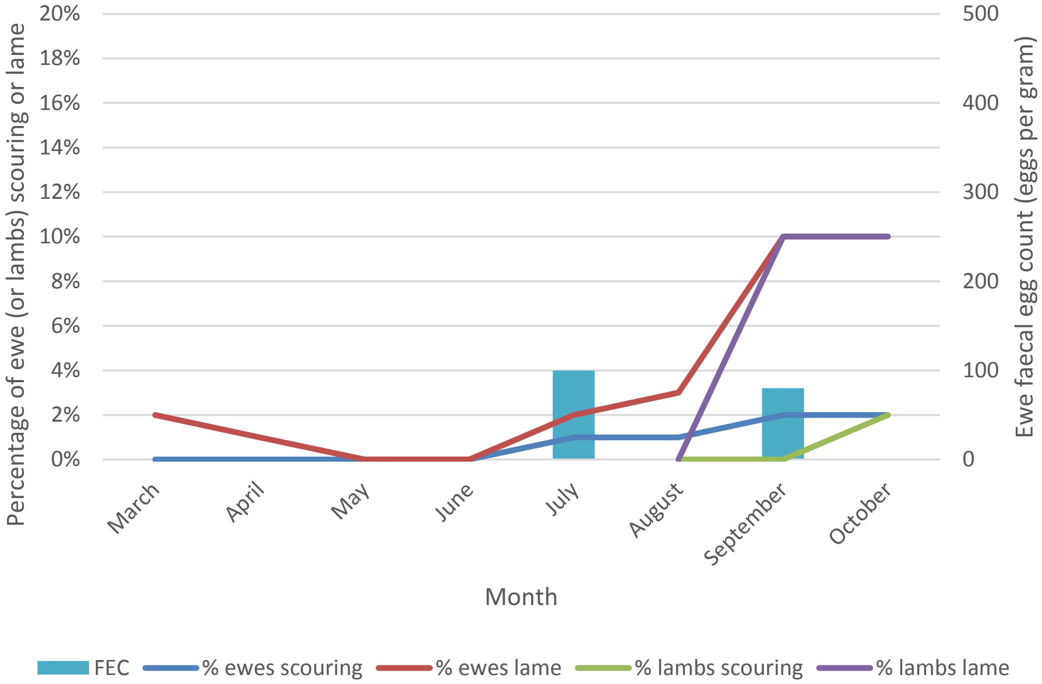

Fig. 49 - Scouring, lameness and faecal egg counts from SWVIC1 during 2013.

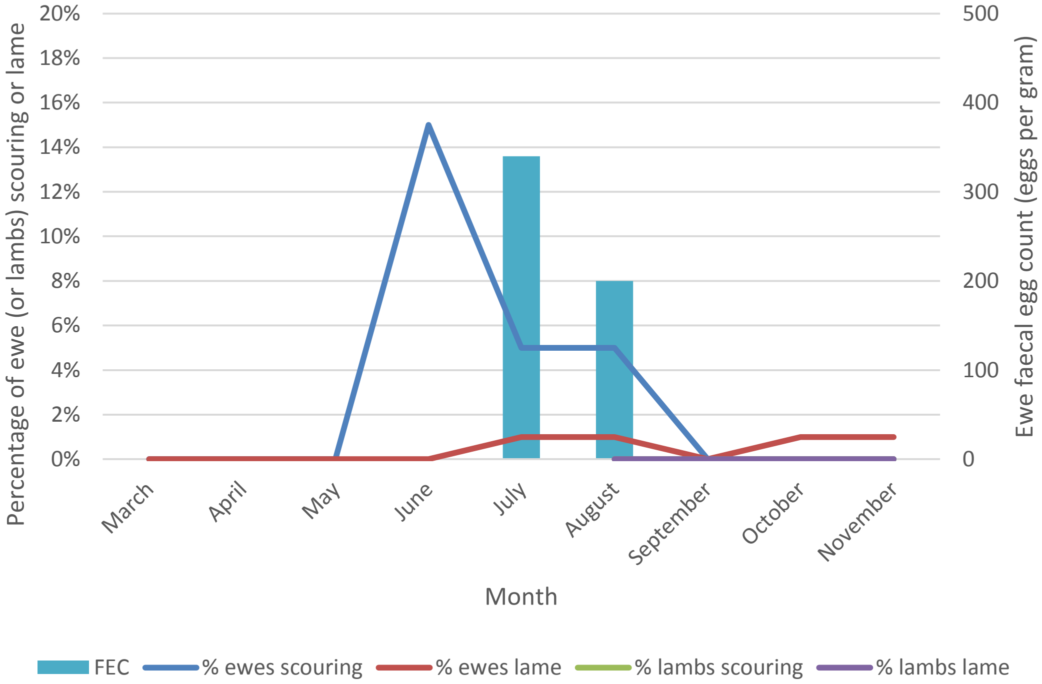

Fig. 50 - Scouring, lameness and faecal egg counts from SWVIC2 during 2013.

Swayback

No lambs were observed with swayback during 2013.

Reproductive rates

As copper treatment occurred after joining at EP1 (as copper deficiency was not observed during this time), the scanning percentage between treated and control animals was assumed to be the same (Table 12).

Scanning percentage at SWVIC1 was far below normal rates achieved at this property due to a suspected ram failure.

Scanning rates between treated and control animals were not considerably different with the greatest variance observed at SWVIC2 of 7% (excluding property SWVIC1 as the data were confounded by the ram failure mentioned previously).

Marking rates observed at EP2 showed ewes treated with the Tracerite capsule marked slightly more lambs than those that were left untreated. Those treated with the Permatrace capsule marked a higher percentage than those treated with the Tracerite capsule and control animals as well as having the least number of lambs lost between scanning and marking.

Table 12 - Pregnancy scanning and marking results for trial mobs during 2013

Property	Treatment	Scanning %	Marking %	% lambs lost between scanning and marking
EP1	Control	152%	*96%*	37%
EP1	Coppernate injection	152%	*96%*	37%
EP2	Control	144%	106%	26%
EP2	Permatrace capsule	141%	113%	20%
EP2	Tracerite capsule	147%	108%	27%
MALLEE1	$CuSO_4$ lick and water	119%	86%	28%
MALLEE2	$CuSO_4$ water	153%	110%	28%
SWVIC1	Control	56%	Unknown	-
SWVIC1	UK Cutemco bolus	70%	Unknown	-
SWVIC1	Permatrace capsule	68%	Unknown	-
SWVIC2	Control	116%	*69%*	40%
SWVIC2	UK Cutemco bolus	121%	*69%*	43%
SWVIC2	Permatrace capsule	123%	*69%*	44%

Note: *marking % results in italics indicate figure is a mob average.*

The percentage of ewes in each treatment and control group at EP1, EP2 and SWVIC2 that reared a lamb is shown in Table 13. At EP1 and SWVIC2 ewes that received copper supplementation were more likely to rear a lamb than those that were untreated. There was up to a 30% difference (SWVIC2) in the number of ewes that reared a lamb when comparing untreated to treated animals.

Table 13 - Percentage of ewes that reared a lamb from each treatment group

Property	Treatment	% ewes reared lamb
EP1	Control	82%
EP1	Coppernate injection	100%
EP2	Control	100%
EP2	Permatrace capsule	100%
EP2	Tracerite capsule	100%
SWVIC2	Control	70%
SWVIC2	Permatrace capsule	85%
SWVIC2	UK Cutemco bolus	100%

Body condition score

The average and range of body condition scores of ewes in each treatment group across the trial properties are presented in Table 14. On average, ewes that received copper treatment at EP1 were half a condition score lower than the controls. Treated ewes at SWVIC1 were generally in better condition than those that were untreated. Ewes at SWVIC2 had similar body condition scores across all treatment groups although those that were treated had a higher minimum condition score compared to untreated animals.

Table 14 - Body condition score of ewes from each treatment group at weaning

Property	Treatment	Average	Range
EP1	Control	3.0	2.0 - 5.0
EP1	Coppernate injection	2.5	1.5 - 4.5
EP2	Control	3.0	2.0 - 4.5
EP2	Permatrace capsule	2.8	2.0 - 3.5
EP2	Tracerite capsule	2.9	1.5 - 4.0
MALLEE1	Copper trough block plus lick	2.2	1.5 - 3.5
MALLEE2	Copper trough block	2.7	1.5 - 4.0
SWVIC1	Control	2.1	1.5 - 3.0
SWVIC1	Permatrace capsule	2.5	2.0 - 3.0
SWVIC1	UK Cutemco bolus	2.5	2.0 - 3.0
SWVIC2	Control	2.1	1.0 - 3.0
SWVIC2	Permatrace capsule	2.2	1.5 - 3.5
SWVIC2	UK Cutemco bolus	2.1	1.5 - 3.0

Blood and liver copper concentration

A summary of the results showing the mean and range of liver and blood copper concentrations from each mob and treatment group is shown in Table 15. Across all properties where more than one treatment was implemented, treated animals consistently had a higher liver copper concentration than untreated animals.

The blood results however did not support this trend as at EP2 and SWVIC1 treated animals had on average a lower blood copper concentration than untreated animals.

Table 15 - Range and mean blood and liver results from each property and treatment group.

			Blood Cu (µmol/L)	**Liver Cu (mmol/kg wwt)**
		Target range	*7.5 - 20.0*	*0.23 - 3.67*
EP1	**Control**	**Mean**	**12.5**	**1.14**^{a}
		Range	(9.8 - 16.2)	(0.05 - 2.5)
EP1	**Coppernate injection**	**Mean**	**13.7**	**1.41**^{a}
		Range	(11.3 - 23.7)	(0.05 - 3.56)
EP2	**Control**	**Mean**	**17.6**	**0.85**^{a}
		Range	(14.1 - 21.9)	(0.15 - 1.66)
EP2	**Coopers Permatrace**	**Mean**	**17.2**	**1.08**^{a}
		Range	(10.5 - 33.3)	(0.22 - 2.09)
EP2	**Tracerite**	**Mean**	**16.5**	**1.09**^{a}
		Range	(12.8 - 20.1)	(0.31 - 2.05)
MALLEE1	**Copper trough block plus lick**	**Mean**	**9.3**	**0.20**
		Range	(2.9 - 14.2)	(0.04 - 0.47)
MALLEE2	**Copper trough block**	**Mean**	**13.1**	**0.09**
		Range	(5.7 - 22.2)	(0.04 - 0.16)
SWVIC1	**Control**	**Mean**	**17.5**	**0.40**^{a}
		Range	(12.9 - 21.9)	(0.06 - 1.36)
SWVIC1	**Permatrace Capsule**	**Mean**	**16.6**	**0.76**^{b}
		Range	(13.9 - 18.9)	(0.15 - 1.78)
SWVIC1	**UK Cutemco Bolus**	**Mean**	**16.7**	**1.03**^{b}
		Range	(10.6 - 20.3)	(0.12 - 1.78)
SWVIC2	**Control**	**Mean**	**14.0**	**0.13**^{a}
		Range	(5.8 - 22.6)	(0.04 - 0.45)
SWVIC2	**Permatrace Capsule**	**Mean**	**16.8**	**0.52**^{b}
		Range	(8.2 - 22.2)	(0.03 - 1.7)
SWVIC2	**UK Cutemco Bolus**	**Mean**	**19.0**	**1.18**^{c}
		Range	(12 - 26)	(0.09 - 2.83)

Note: letters signify statistical differences in means. Liver results only were statistically analysed.

Whilst all properties and treatment groups (with the exception of animals treated with Tracerite at EP2) had some animals considered deficient based on liver status, only three treatment groups across all properties had any animals considered deficient based on blood results. This is an important point of difference in these two methods of determining copper status as based on blood results only three groups (23%) of animals would require supplementation compared to 12 groups (92%) based on liver samples.

Table 16 shows the percentage of animals in each treatment group that were considered copper deficient based on their liver copper concentration. It can be seen that in groups where more than one treatment was tested during 2013 that the percentage of animals considered deficient in treated groups was lower than those that were untreated. At EP2 relatively few animals were considered deficient in untreated or treated groups with only three animals below the minimum requirement. 100% of animals tested at MALLEE2 had deficient levels of liver copper indicating that the copper trough blocks were ineffective.

Table 16 - Summary of number of copper deficient animals in each treatment group based on liver copper status

Property	Treatment	% of treatment group deficient. Number of animals in ().	
EP1	Control	24%	(5)
EP1	Coppernate injection	16%	(3)
EP2	Control	10%	(2)
EP2	Permatrace capsule	5%	(1)
EP2	Tracerite capsule	0%	(0)
MALLEE1	Copper trough block plus lick	60%	(12)
MALLEE2	Copper trough block	100%	(20)
SWVIC1	Control	40%	(8)
SWVIC1	Permatrace capsule	10%	(2)
SWVIC1	UK Cutemco bolus	10%	(2)
SWVIC2	Control	85%	(17)
SWVIC2	Permatrace capsule	30%	(6)
SWVIC2	UK Cutemco bolus	10%	(2)

Correlation between blood and liver copper concentration

Overall there was a very low correlation between blood and liver copper concentration ($R^2 = 0.03$).

Table 17 highlights the individual correlations between blood and liver samples for each treatment and control group as well as when all animals at each property are analysed together. It shows that the correlation between blood and liver samples is still relatively low reaching a maximum of $R^2 = 0.568$ for MALLEE1 and the majority are less than $R^2=0.1$.

Table 17 - Correlation (R2) between blood and liver copper concentration for all animals tested at each property and each control and treatment group within the trial properties

Property	Treatment	R^2
EP1	All trial animals combined	0.08
EP1	Control	0.14
EP1	Coppernate injection	0.10
EP2	All trial animals combined	0.00001
EP2	Control	0.13
EP2	Permatrace capsule	0.0002
EP2	Tracerite capsule	0.15
MALLEE1	Copper trough block plus lick	0.57
MALLEE2	Copper trough block	0.45
SWVIC1	All trial animals combined	0.06
SWVIC1	Control	0.004
SWVIC1	Permatrace capsule	0.02
SWVIC1	UK Cutemco bolus	0.26
SWVIC2	All trial animals combined	0.24
SWVIC2	Control	0.22
SWVIC2	Permatrace capsule	0.10
SWVIC2	UK Cutemco bolus	0.14

Correlation between soil and pasture mineral concentration

Correlation between soil and pasture mineral concentration of samples collected during February/March 2013 was assessed. The mean correlation between the mineral status of soil and that of pastures was low (R^2 = 0.1) (Table 18). The correlations for individual minerals ranged from R^2 = 0.003 (molybdenum) to R^2 = 0.36 (manganese). Correlations between soil and pasture copper concentration was low (R^2 = 0.13).

Table 18 - Correlation (R2) between pasture and soil mineral concentration

Mineral	Correlation (R^2)
Phosphorus	0.02
Calcium	0.02
Magnesium	0.22
Potassium	0.18
Sodium	0.07
Boron	0.11
Copper	0.13
Molybdenum	0.003
Zinc	0.05
Manganese	0.36
Iron	0.01
Cobalt	0.01

These correlations indicate that on the properties tested soil mineral concentration was not an accurate predictor of the mineral concentration of pastures. However, where a particular mineral was found to be ‘deficient’ based on soil tests, the corresponding pasture tissue sample was also generally categorised as ‘deficient’. This indicates that whilst soil samples cannot be used to accurately predict the exact pasture mineral concentration, they can be used as an indication that a deficiency may exist in the pasture.

Key findings per property

EP1

- Treatment with a copper injection (Coppernate) was effective at raising liver copper concentration and reducing the number of deficient ewes in the mob when compared to untreated animals
- 100% of treated ewes reared a lamb compared to only 82% of untreated ewes.
- Ewes with low liver copper concentration were still present in the treated group warranting further investigation to determine the appropriate dose rate

EP2

- Treatment with both Permatrace and Tracerite capsules were effective at elevating liver copper concentration above that of untreated animals
- Fewer treated animals were found to be deficient compared to untreated animals
- No substantial effect was observed on the reproductive performance of treated and untreated animals

MALLEE1

- Treatment with a loose mineral lick in combination with copper sulphate trough blocks, increased liver copper status in 2013 compared with 2012 results; however there was no control mob in 2013 for direct comparison. Questions over the appropriate dose rate remain, as 60% of supplemented animals were still found to be deficient.

MALLEE2

- The use of copper sulphate water trough blocks alone were not effective in elevating liver copper status and did not prevent any tested animals from becoming deficient

SWVIC1

- Treatment with the Permatrace capsule or UK Cutemco bolus resulted in a higher liver copper concentration compared to untreated animals
- The UK Cutemco bolus resulted in a higher liver copper concentration than the Permatrace capsule, however the mode of action for this is unknown. It may be a result of either the higher copper dose rate of the UK Cutemco bolus or its different action in supplying copper to the rumen compared to the abomasum.
- Both treatments were effective at decreasing the number of ewes that were considered deficient from 40% in untreated animals to 10% in treated animals

however in a flock of 10,000 ewes 10% is still a significant number of unresponsive animals.

SWVIC2

- Treatment with the Permatrace capsule or UK Cutemco bolus resulted in significantly higher liver copper concentration compared to untreated animals
- Treatment with the Cutemco bolus resulted in significantly higher liver copper concentration than treatment with the Permatrace capsule
- Both treatments increased the number of ewes that reared a lamb compared to untreated animals. Ewes receiving the UK Cutemco bolus reared more lambs than those treated with the Permatrace bolus (100% vs 85%).

4.2.3 Discussion

The concentration of copper in the pasture increased during winter and declined during summer at all participating properties with the exception of MALLEE2. This trend is contrary to the generally accepted view that copper concentration is lowest during the winter and early spring months when pasture growth accelerates. Pasture copper concentration at MALLEE2 was low during summer and did not increase greatly through autumn and winter until spring testing conducted in October. Seasonal pasture growth at this property during 2013 was delayed with a relatively late break of the season and low levels of pasture growth during winter. This later increase in pasture copper concentration may have been a result of the delayed pasture growth and high grazing pressure resulting in pasture availability not increasing until the October sampling.

Pasture copper concentrations at SWVIC1 were below the minimum requirements for sheep (7mg/kg DM) at all testing times except for October 2013. Liver sampling at this property was conducted during November 2013 and resulted in the control mob having on average satisfactory levels of liver copper. However, 40% of ewes tested in the control group were still deficient for copper and it is expected that had copper intake in the month before sampling been lower, an increased number of ewes would have been deficient at sampling.

Molybdenum concentration in the pasture appeared to have a strong influence on liver copper concentration at MALLEE2 and SWVIC2. At both of these properties the copper:molybdenum (Cu:Mo) ratio was below 5:1 for up to 8 months before liver sampling. As a result, liver copper concentration at MALLEE2 and SWVIC2 was lower than the liver copper concentration of control animals at properties such as SWVIC1 where the Cu:Mo ratio was higher.

Other minerals known to reduce the availability of copper include sulphur and iron. Pasture concentration of these minerals varied throughout the year though not to the same extent as copper. Where deficiency was observed it was not possible to differentiate the effects of each mineral and its interference with copper availability.

The highest molybdenum pasture concentrations were observed at SWVIC2 where molybdenum exceeded 2mg/kg DM during October and November. The literature review conducted as part of this project highlighted that research has shown high levels of molybdenum intake can increase scouring in cattle however this effect is not readily observed in sheep. Scouring scores from the trial mob at SWVIC2 during October and

November showed no scouring was observed. This supports the literature that molybdenum induced scouring in sheep is not readily seen, particularly at the relatively low molybdenum concentrations present in Australia compared to countries such as the UK.

Incidences of scouring and lameness at EP1, EP2, MALLEE1 and MALLEE2 were very low throughout the year. The only exception to this was scouring observed at MALLEE1 during January which affected 7% of the mob. A worm egg count conducted in February resulted in 75 eggs per gram indicating that some influence from worms could be attributed to the scouring. Interestingly both NDF and DM fell to very low levels, 30% and 13% respectively, during late winter at EP2 and yet only 1% of ewes were observed scouring. This may have been a result of selective grazing of ewes of residual dry feed in the paddock to counteract the effects of low NDF and DM.

During June 2013 15% of ewes were observed to be scouring at SWVIC2. A worm egg count conducted in July 2013 resulted in 340 eggs per gram indicating worms were the most likely cause of this scouring. Additionally, following this worm egg count the ewes were drenched with Virbamec LV (abamectin) and scouring was reduced to 5% of the mob. A worm egg count conducted in the following month (August) returned 200 eggs per gram signalling the likelihood of drench resistance to abamectin, at this property. Ewes were given a Eweguard (moxidectin) injection in August and scouring was reduced to 0% in September indicating that this treatment had been fully effective.

Lameness in ewes and lambs at SWVIC1 had increased to 10% of the mob by August and September 2013. Pasture testing conducted in July and October showed that copper concentration during this period increased from 6.8 to 9.0mg/kg DM indicating that low copper levels were unlikely to be a cause of the lameness. High protein pastures have been linked to symptoms of lameness observed in sheep and cattle. However, whilst the pasture protein content during August and September was approximately 23% based on samples collected in July and October, many pastures at other properties exceeded this level of protein and did not induce the same level of lameness in ewes. Foot scald was not assessed as part of this project however given the time of year and previous reporting of the condition at this property it is possible to have been a major cause of the lameness observed.

As no incidences of swayback in lambs was observed, conclusions regarding the conditions under which it may or may not be observed cannot be made definitively. However, given that during both mid and late pregnancy, which is when this condition is induced, ewes at all trial sites were subjected to low copper intake it is reasonable to conclude that a severe deficiency in copper must be present before significant levels of swayback will be observed.

The negative effect of copper deficiency on fertility is recognised throughout the literature as a key impact on productivity and profitability. Pregnancy scanning was carried out at all properties to determine to the effect of copper deficiency on conception rates. To gain a true indication of the effect on conception rates it is more accurate to review results from those properties that have both control and treatment(s) groups (i.e. EP1, EP2, SWVIC1 and SWVIC2). Due to the seasonal nature of the copper deficiency at EP1, copper treatment was implemented after joining, therefore no differences in conception rates could be attributable to copper supplementation. Pregnancy scanning results from SWVIC1 were far below normal rates achieved at this property and were assumed to be a result of a ram failure. As such, conclusions between the effect of copper on treatment and control groups cannot be drawn. At both EP2 and SWVIC2 copper supplementation was provided approximately one week prior to joining. At EP2 as minimal differences between conception rates were observed it can be concluded that supplementation had little effect. Further research is required to determine the optimal time and level of supplementation to obtain a reproduction benefit. Whilst a small trend of increased conception rates for treated animals compared to untreated at SWVIC2 was apparent, whether significant differences would be observed if supplementation was provided for a longer period prior to joining requires further investigation.

As treatment (and control groups where applicable) were run as one mob to ensure environmental conditions were identical and that blood and liver analysis results could be compared, marking rates of each group were not able to be collected. The exception to this was at EP2 where ewes and lambs from each treatment group were identified as part of their stud operation, allowing individual marking rates to be collected. Unfortunately, no clear trend was apparent at this property. Further work utilising RFID tags to allow lamb maternal pedigrees to be determined is necessary to assess whether copper deficiency has a significant effect on lamb survival.

Whilst relatively little information was able to be concluded from the marking results, the percentage of ewes that reared a lamb (via wet/dry assessment of ewes that were liver sampled) at EP1, EP2 and SWVIC2 showed some interesting results. At both EP1 and SWVIC2 a much higher percentage of ewes that were treated reared a lamb compared to those that were untreated. This indicates that copper deficiency could have a significant effect on lamb survival as such large differences between groups were not present at pregnancy scanning. No difference was observed in the percentage of ewes that reared a lamb at EP2, most likely as a result of the fact that relatively low levels of copper deficiency was observed in these ewes.

Body condition score of treated and untreated animals was inconsistent. At EP2 and SWVIC2 average condition score of each group was similar whilst at EP1 and SWVIC1 up to half a condition score difference was observed between treated and untreated groups. At EP1 treated animals were half a condition score lower than untreated animals. This may have been a result of the increased number of ewes that reared a lamb and therefore increased level of nutritional demand. In contrast, treated ewes at SWVIC1 were 0.4 of a condition score higher than untreated animals. Unfortunately, without marking data at this property it is difficult to draw conclusions as to the reason for this difference. Further research is recommended to collect individual reproductive performance of each treatment and control group which will facilitate direct comparison to body condition of ewes at weaning.

Based on the liver copper analyses it can be seen that copper accumulation was relatively well explained by both the pasture mineral concentration and the treatment dose rates. At all properties where treatment and control groups existed, treated animals had higher liver copper concentrations than untreated animals; this was correlated to dose rates of the treatments used. For example, at EP2 the average liver copper concentration of both the treated groups was similar which should be expected as the treatments used had similar daily copper delivery rates. In contrast, at SWVIC1 and SWVIC2 the two treated groups were different. Those treated with the Permatrace capsule had lower liver copper than those treated with the UK Cutemco bolus which may be a result of the higher copper dose rate of the UK Cutemco bolus or the different mode of action. The UK Cutemco bolus is a soluble glass bolus designed to remain in the rumen/reticulum and release copper to both be available and absorbed by the animal as well as binding with antagonistic minerals such as molybdenum and negate their ill health effects. This is different to the Permatrace capsule (and Tracerite capsule) which following administration, will release copper oxide needles in the rumen which will then flow into the abomasum for absorption. In this way it does not provide any copper in the rumen for an extended period to counteract the effect of antagonists.

Supplementation with oral forms of copper sulphate via a lick and/or addition to water troughs appears to have been relatively ineffective at commercially available rates. At both MALLEE1 and MALLEE2 a significant number of treated ewes were still deficient suggesting that the required dose rate may need further investigation. An additional complicating factor at these properties was that little supplementation may have been received from the use of copper sulphate water trough blocks during winter and spring months as water intake from pasture would have been high, reducing water and hence copper intake from troughs. This is likely to have been a significant factor at MALLEE2 where ewes were solely supplemented with water trough blocks and 100% of ewes were still deficient based on liver samples.

The range in liver copper concentration whilst all animals were on the same pasture supports the literature that there is genetic variance in the ability of individual ewes to absorb and accumulate copper. Some of this variance may also be attributed to selective grazing of animals and variance in the copper concentration of plants across a single paddock.

Blood and liver copper results highlighted the inconsistency between using these two methods of measuring copper status. Whilst it was clear that blood and liver results were not well correlated, it is interesting to note that at properties where some deficiency existed based on blood samples (MALLEE1, MALLEE2 and SWVIC2), the liver results were significantly lower than that of other properties. This may infer that if any deficiency was determined via blood sampling then it is likely a relatively severe deficiency may exist based on liver results. In contrast, at many properties a deficiency was diagnosed based on liver samples yet blood tests showed no problem, therefore if blood testing were to return all results within the required reference range it may still be likely a deficiency could exist based on liver results.

This is an important point of difference in these two methods of determining copper status as based on the blood results only three groups (23%) of animals in this project would require supplementation compared to 12 groups (92%) based on liver samples. It is important that those assessing the need for supplementation strategies at properties be aware of the differences in the testing methods or mobs may be incorrectly assessed as having satisfactory levels of copper based on blood results alone.

The correlation between blood and liver copper concentration was higher for both MALLEE1 (R^2 = 0.57) and MALLEE2 (R^2 = 0.45) compared to the other properties ($R^2 \leq 0.24$). However, the reasons for this remain unclear. Animals at both these properties experienced severe deficiencies with 60% and 100% of animals deficient at MALLEE 1 and MALLEE2 respectively. However, animals at SWVIC2 displayed similar levels of deficiency both in terms of actual liver copper concentration and percentage of ewes in the control mob that were considered deficient, yet the correlation between blood and liver for this group was far lower. The influence of antagonistic minerals such as molybdenum and sulphur does not explain the differences in correlation either as at all of these properties antagonistic minerals and the Cu:Mo ratio were not dissimilar. Pasture copper concentration and the correlation between blood and liver copper at MALLEE1, MALLEE2 and SWVIC2 followed a trend where sheep grazing pastures of lower copper concentration (MALLEE1) had a higher correlation between blood and liver compared to those on higher copper pastures (SWVIC2). It would be reasonable to assume that the influence from antagonistic minerals would have to be similar for this trend to remain consistent. Further research is required to validate this hypothesis.

The correlation between soil and plant mineral concentration was low as expected. Whilst the mineral concentration in soil and plants were analysed at the same time of year, the rate at which plants take up minerals from the soil will vary depending on their stage of growth and soil conditions making it difficult to predict with any degree of accuracy the mineral concentration of plants based on soil results.

Correlations between plant and animal status of individual nutrients cannot be accurately predicted as the interaction from other minerals may have a significant effect on the copper status of grazing ruminants. However, in reviewing the results from 2013 it can be seen that the current recommendations for the minimum pasture copper content of 7mg/kg DM appears correct as where pasture concentrations fell below this level in the months preceding testing, animal status was generally deficient. However, in assessing the potential copper status of a flock the influence of antagonistic minerals must also be accounted for. From the trial conducted this year there is no evidence to refute the current recommendation of a minimum Cu:Mo ratio of 5:1. Unfortunately it was difficult to separate the effect of antagonistic minerals from that of simply low copper levels as in all instances where the Cu:Mo ratio was below 5:1, pasture copper concentration was below 7mg/kg DM.

4.3 Year 3 (2015)

4.3.1 Methodology

Trial sites

Five properties (sites) across Victoria and South Australia were included in the 2015 trial activities. Site EP1 (Roslyn Pope, SA Eyre Peninsula) which was involved in earlier trials for this project was not required for the 2015 trial activities and therefore is not referenced in the results of this part of the report.

Treatments

Following analysis of results from the trials conducted in 2012 and 2013 under this project, treatments were allocated to each site for testing during 2015. Approximately 100 ewes were allocated to each treatment group at each site. Treatment and control groups were run as a single mob at each site to ensure environmental conditions were identical for each group. The treatments tested at each site and key activity dates are shown in Table 19.

Table 19 - Summary of treatments and key dates at each trial site.

		EP2	MALLEE1	MALLEE2	SWVIC1	SWVIC2
Treatment(s)		1. Cutemco UK Bolus	Copper sulphate lick	Tracerite capsule (x1 dose)	1. Cutemco UK Bolus	1. Cutemco UK Bolus
		2. Tracerite capsule (x2 doses)			2. Tracerite capsule (x2 doses)	2. Tracerite capsule (x2 doses)
Control mob		Yes	No	Yes	Yes	Yes
Dose rate		Cutemco = 4.5g Cu (16.4mg Cu/hd/day)	Lick = 3.1mg Cu/hd/day *Lick intake 7.7g/hd/day x 400mg Cu/kg lick*	Tracerite = 2.1g Cu (5.8mg Cu/hd/day)	Cutemco = 4.5g Cu (16.4mg Cu/hd/day)	Cutemco = 4.5g Cu (16.4mg Cu/hd/day)
		Tracerite = 4.2g Cu (11.6mg Cu/hd/day)			Tracerite = 4.2g Cu (11.6mg Cu/hd/day)	Tracerite = 4.2g Cu (11.6mg Cu/hd/day)
Key dates	**Treatment**	Cutemco; 24 Sep 2014	28 Aug 2014 to 24 Jun 2015	20 Oct 2014	Cutemco; 17 Jan 2015	Cutemco; 22 Oct 2014
		Tracerite; 24 Sep 2014 & 21 Jan 2015			Tracerite; 17 Jan 2015 & 12 May 2015	Tracerite; 22 Oct 2014 & 06 Mar 2015
	Mating	01 Nov 2014	15 Oct 2014	01 Dec 2014	28 Feb 2015	04 Dec 2014
	Lambing	01 Apr 2015	15 Mar 2015	01 May 2015	29 Jul 2015	04 May 2015
	Weaning	16 Jul 2015	24 Jun 2015	13 Aug 2015	10 Nov 2015	11 Aug 2015
Expected sustained activity		Cutemco = 9mths	As delivered	Tracerite = 12mths	Cutemco = 9mths	Cutemco = 9mths
		Tracerite = 12mths			Tracerite = 12mths	Tracerite = 12mths

Treatment descriptions

Cutemco UK Bolus

This product is a soluble glass bolus containing 4.5g of copper for oral administration. Due to its weight it is designed to remain in the rumen/reticulum to supply a sustained release of copper for the animal.

Fig. 51 - Cutemco bolus.

Tracerite

This product is a soluble gelatine capsule which contains 2.5g of copper oxide needles (2.1g Cu) for oral administration. The capsule dissolves in the rumen, releasing the copper oxide needles to be dissolved and absorbed by the animal. This product is similar in nature to the Coopers Permatrace product.

Fig. 52 - Tracerite capsule.

Copper sulphate ($CuSO_4$) lick

This product is a granular powder provided to stock for voluntary consumption. The lick is comprised of copper sulphate (400mg Cu/kg lick) plus lime to act as a bulking agent and salt, sugar and molasses to increase palatability.

Fig. 53 - Copper sulphate lick.

Measurements and analysis

Pasture mineral content

Pasture samples were regularly collected from paddocks grazed by the trial mob to monitor the mineral intake by the ewes. The number of samples collected at each property varied depending on seasonal growth and frequency of rotation through paddocks.

Table 20 shows the months in which pasture samples were collected at each site during the trial.

Table 20 - Month of pasture sample collection for analysis at each trial site.

	EP2	MALLEE1	MALLEE2	SWVIC1	SWVIC2
2014					
Sep	✓				
Oct		✓			
2015					
Jan	✓				✓
Feb		✓	✓		
Mar					
Apr					
May			✓		
Jun		✓			✓
Jul	✓				
Aug			✓	✓	✓
Sep					
Oct					
Nov				✓	

Plant samples were collected from 5-6 representative locations across each paddock and combined to form the paddock sample for analysis. Samples were collected by either cleanly tearing or cutting a sample with shears. Care was taken to ensure that no dirt, roots or contamination from the shears occurred which could affect the test results. Plant species composition in the sample was reflective of the overall composition of the paddock. The only exception to this was where unpalatable weed species were present in the paddock their inclusion rate in the sample for analysis was similar to that estimated of animal intake (generally minimal). Pasture samples were sent to SGS laboratory in Queensland for analysis.

Ewe mortality rate

Ewe mortality rate was measured at EP2, SWVIC1 and SWVIC2 via successive stock counts during trial activities.

Observations of scouring

Visual assessments of the percentage of ewes in the trial mob that were scouring were noted by producers EP2, SWVIC1 and SWVIC2. Although a faecal sample was to be collected for faecal egg count testing and to determine mineral content where scouring was observed, this did not occur as virtually no scouring [<1%] was observed in the trial groups.

Reproductive rates

Conception rate for each individual ewe in each treatment group was recorded at EP2, SWVIC1 and SWVIC2 via pregnancy scanning. Mob conception rate was recorded at sites MALLEE1 and MALLEE2.

Marking percentage for each treatment group was recorded at EP2, SWVIC1, and SWVIC2. As multiple treatment groups were present in the group of animals at these sites, data were collected via RFID tags in both ewes and lambs and the Pedigree Matchmaker process. This process utilises an RFID panel reader placed near a source of attraction such as a grain feeder and/or water trough to record to the RFID tag sequence as ewes and lambs walk past. As lambs typically follow their mothers, this association is used to determine the maternal pedigree of lambs and calculate the marking percentage for each animal and treatment group.

At the time that liver samples were collected from ewes, an assessment of the ewe wet/dry status was recorded for each animal that was sampled. In addition, producers at EP2 and SWVIC1 conducted a wet/dry assessment of all ewes in the trial mob at lamb marking.

Ewe body condition score and live weight

Individual body condition score and live weight was recorded at weaning (Table 19) for each ewe at EP2, SWVIC1 and SWVIC2.

Lamb weaning weight

Individual lamb weaning weight was recorded at EP2, SWVIC1 and SWVIC2 at weaning (Table 19).

Ewe liver copper concentration

Liver samples were collected via biopsy from 20 randomly selected ewes in each treatment group at each site. Liver samples were collected from ewes at weaning (Tabl3 19) and sent to Regional Laboratory Services for analysis.

4.3.2 Results

Pasture mineral content

Pasture mineral analysis at each site showed that the mean copper content of the pasture throughout the duration of the trial was below the recommended minimum copper content of 7mg/kg DM at all sites (Fig. 54).

On average, at MALLEE1 and MALLEE2 the relatively high molybdenum content reduced the copper:molybdenum ratio to below 5:1 indicating that molybdenum may have a negative effect on copper availability. At EP2, SWVIC 1 and SWVIC2 comparatively higher copper and lower molybdenum concentration resulted in a higher mean Cu:Mo ratio indicating that the influence of molybdenum on copper availability would be lower than experienced at MALLEE1 and MALLEE2.

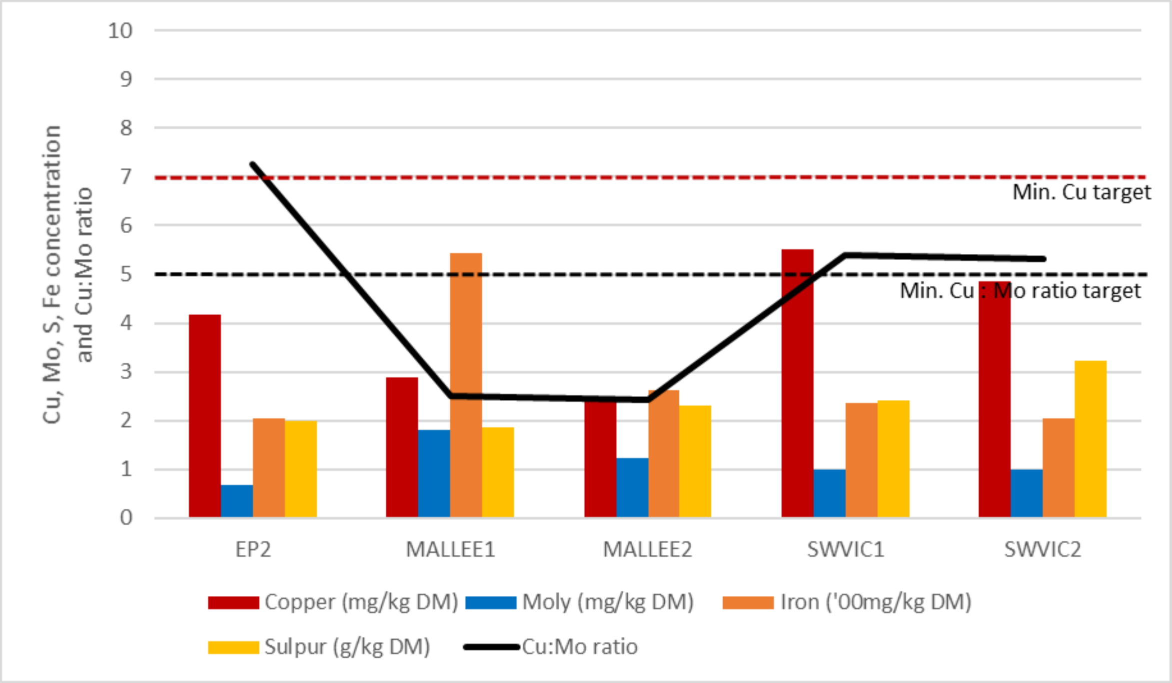

Fig. 54 - Mean pasture mineral concentration for paddocks grazed by the trial mob at each site.

Pasture mineral analysis at EP2 showed that copper concentration reached the minimum level observed during summer and then increased to reach 7mg/kg DM in July when liver samples were collected (Fig. 55).

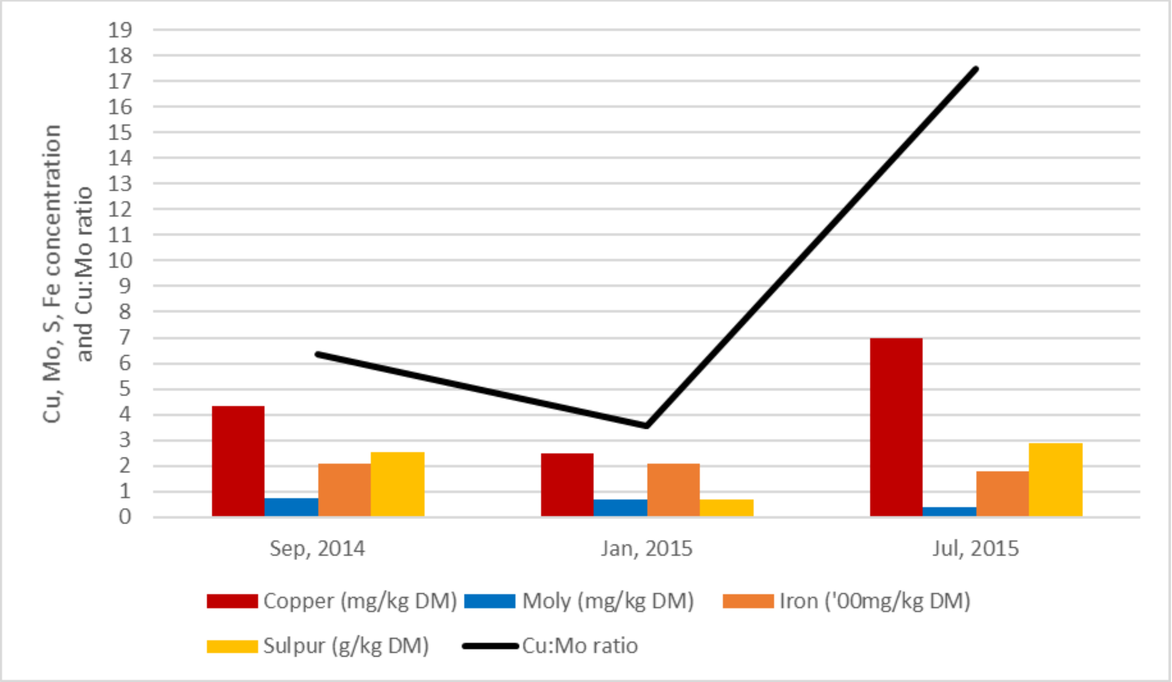

Fig. 55 - Mean pasture mineral concentration for paddocks grazed by the trial mob at EP2.

Pasture copper content was consistently below sheep requirements at MALLEE1, with increasing influence from interacting minerals such as molybdenum and iron between October and June (Fig. 56).

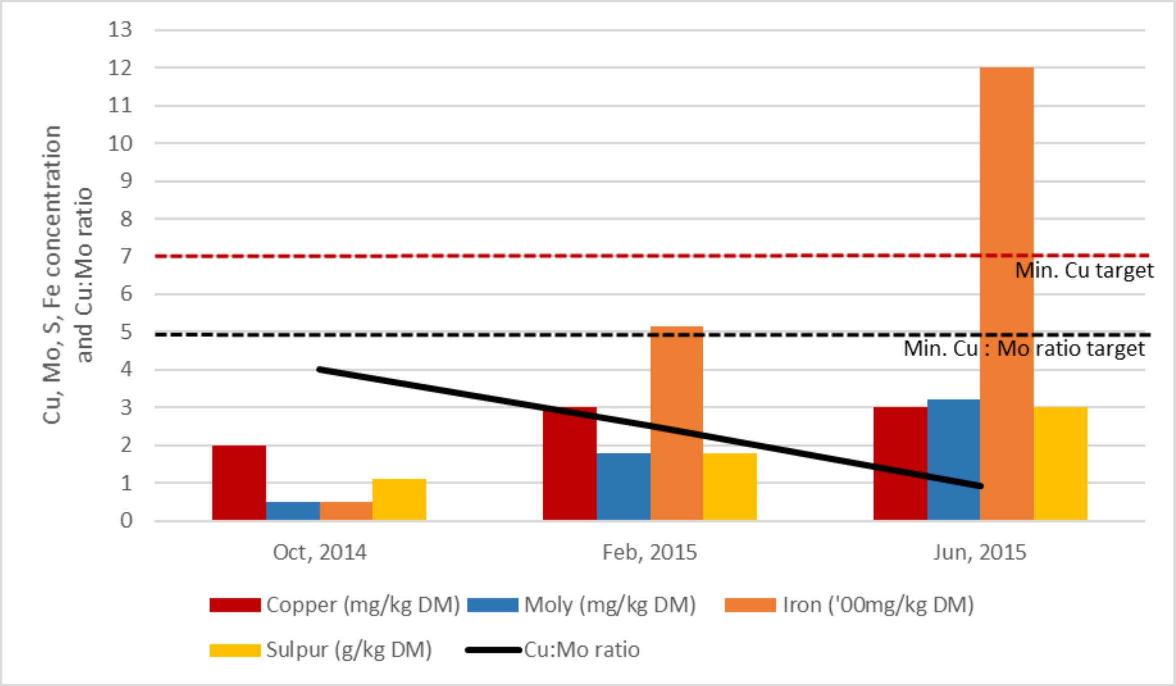

Fig. 56 - Mean pasture mineral concentration for paddocks grazed by the trial mob at MALLEE1.

Pasture copper content at MALLEE2 was consistently below sheep requirements (Fig. 57). The copper to molybdenum ratio was below 5:1 at each test indicating that molybdenum would have been influencing copper availability.

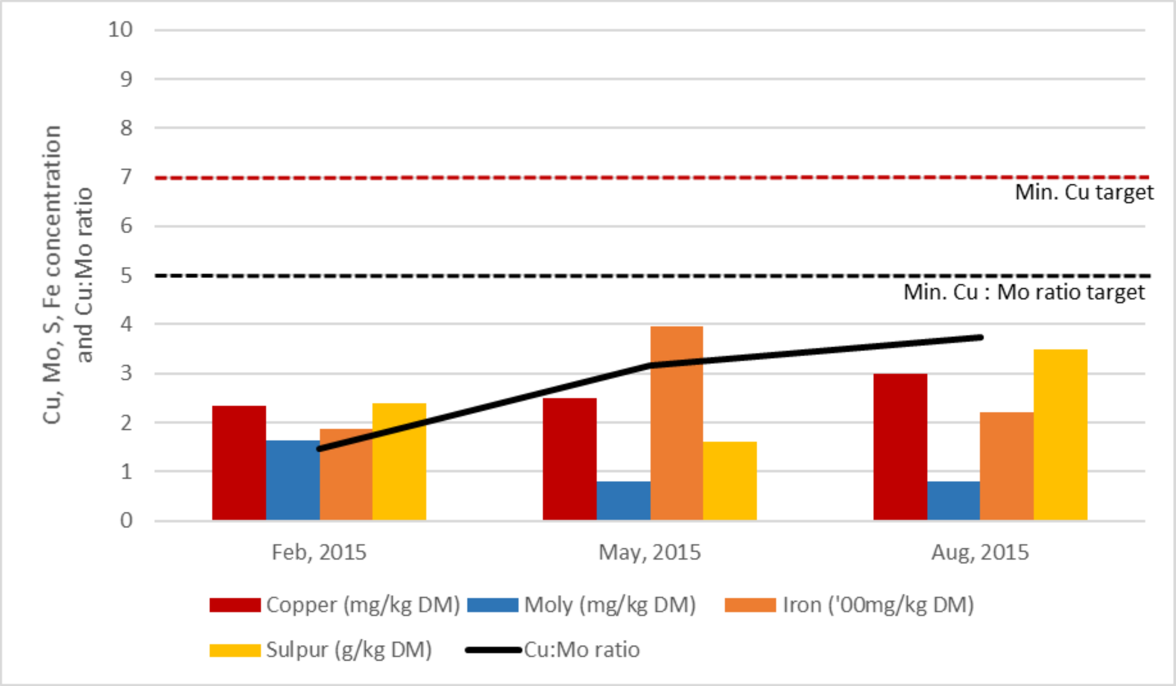

Fig. 57 - Mean pasture mineral concentration for paddocks grazed by the trial mob at MALLEE2.

Pasture copper content decreased from spring to early summer for samples collected at SWVIC1, which also resulted in the copper to molybdenum ratio falling below the minimum target of 5:1.

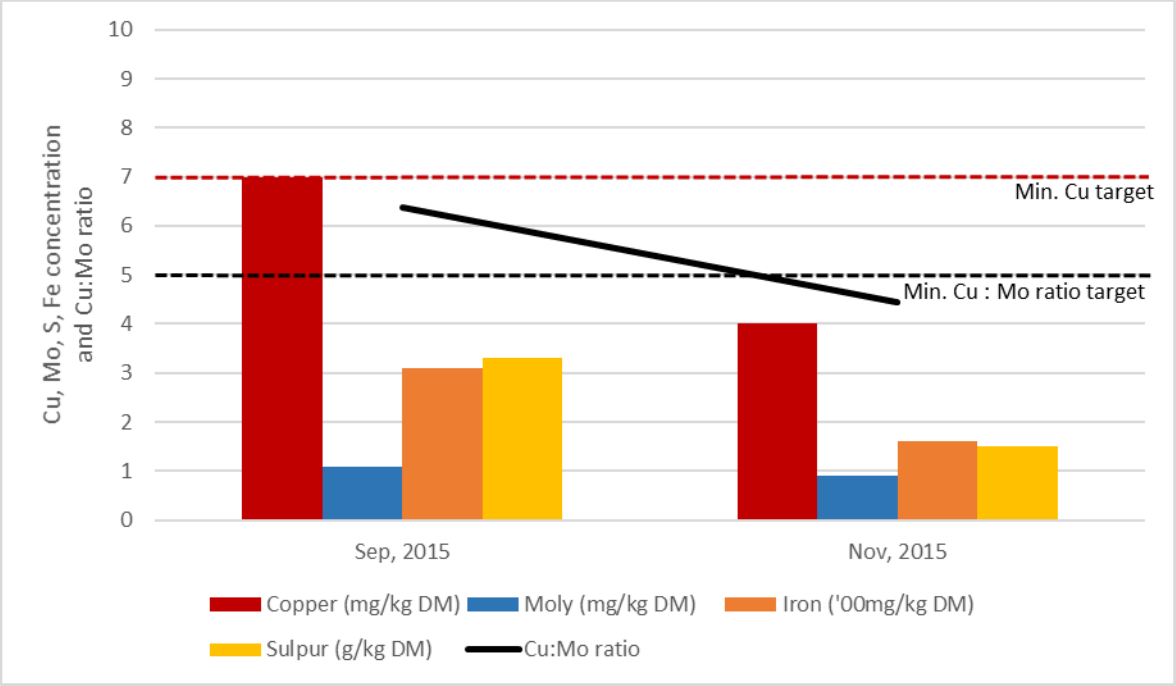

Fig. 58 - Mean pasture mineral concentration for paddocks grazed by the trial mob at SWVIC1.

Pasture copper content at SWVIC2 was severely deficient in January and then increased to a moderately deficient level in June and August (Fig. 59). The copper to molybdenum ratio was below 5:1 during January and June before reaching a satisfactory level in August.

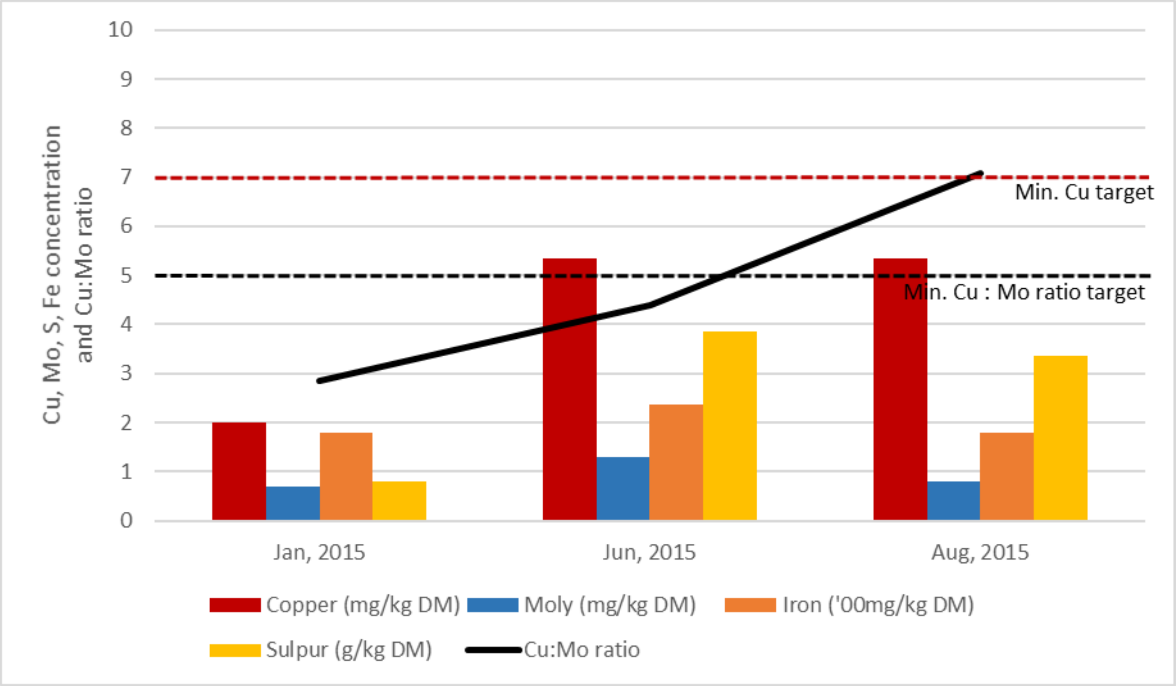

Fig. 59 - Mean pasture mineral concentration for paddocks grazed by the trial mob at SWVIC2.

Ewe mortality rate

Ewe mortality rate between pregnancy scanning and weaning was quite high for some treatment groups such as the control group at site SWVIC2 and the Cutemco treatment group at EP2. Whilst both of the Victorian sites showed that treated groups had a lower mortality rate than the control group, this same trend was not present at EP2.

Table 21 - ewe mortality rate between pregnancy scanning and weaning at EP2, SWVIC1 and SWVIC2.

	EP2	SWVIC1	SWVIC2
Control	7%	5%	10%
Cutemco	9%	2%	7%
Tracerite	4%	3%	4%

Observations of scouring

Scouring was monitored in the trial mob to assess the potential influence of copper deficiency on scouring in sheep. During the winter months worm burden was controlled through the use of anthelmintic products (where required) and no significant scouring was observed at any of the sites. As such, no faecal egg counts or faecal mineral analyses were deemed necessary or conducted.

Reproductive rates

Conception rates at EP2, SWVIC1 and SWVIC2 did not show a consistent trend across treatments. Control animals conceived a slightly higher number of lambs than treated animals at EP2, however there was only a range of 4% between treatments. Treated animals conceived up 9% higher lambs than the control group at SWVIC1, however the control group at SWVIC2 conceived the highest percentage of lambs compared to either treatment group (Table 22).

Table 22 - Conception rate of ewes in each treatment group at EP2, SWVIC1 and SWVIC2.

	EP2	SWVIC1	SWVIC2
Control	160%	166%	127%
Cutemco	157%	171%	108%
Tracerite	156%	175%	113%

Conception rate of ewes at MALLEE1 was 144%. Conception rate of ewes at MALLEE2 was 117%.

The percentage of ewes that did not conceive (dry), as measured at pregnancy scanning, similarly to overall conception rates did not show a consistent trend across treatments (Table 23). At SWVIC1 treated groups had on average 6.5% lower number of dry ewes compared to the untreated group, however at EP2 and SWVIC2 the percentage of dry ewes in treated groups were either similar or higher than the control group.

Table 23 - The percentage of dry ewes in each treatment group at pregnancy scanning at EP2, SWVIC1 and SWVIC2.

	EP2	SWVIC1	SWVIC2
Control	10.1%	9.6%	6.0%
Cutemco	12.0%	3.2%	10.4%
Tracerite	9.9%	3.0%	13.5%

The percentage of dry ewes in the trial group at MALLEE1 was 5%. The percentage of dry ewes in the trial groups at MALLEE2 was 6%.

The effect of treatment on marking percentage at EP2, SWVIC1 and SWVIC2 was not consistent across sites and in some instances copper treatment resulted in a reduced marking percentage compared to untreated animals (Table 24). At SWVIC1 Cutemco treated ewes marked up to 7% more lambs than untreated ewes, however at EP2 Tracerite treated ewes marked nearly 22% less lambs than untreated ewes.

Table 24 - The marking percentage (lambs marked to ewes joined) for each treatment group at EP2, SWVIC1 and SWVIC2.

	EP2	SWVIC1	SWVIC2
Control	137.9%	116.1%	95.6%
Cutemco	131.1%	123.0%	99.0%
Tracerite	116.2%	112.3%	90.0%

The percentage of ewes that did not raise a lamb, as measured across the entire trial mob showed that a higher percentage of ewes treated with Cutemco raised lambs compared to those that were not treated. The effect of treatment with Tracerite was not as consistent (Table 25).

Table 25 - The percentage of dry ewes in each treatment group at weaning at EP2, SWVIC1 and SWVIC2.

	EP2	SWVIC1	SWVIC2
Control	3.0%	5.3%	N/A
Cutemco	1.9%	2.2%	N/A
Tracerite	3.7%	3.0%	N/A

Results from the wet/dry status of ewes at all sites that were sampled for liver copper concentration showed that the percentage of ewes raising a lamb was the same or up to 5% higher for ewes that were treated compared to those that were not.

Table 26 - The percentage of ewes that reared a lamb in each group of 20 animals sampled for liver copper concentration at EP2, SWVIC1 and SWVIC2.

	EP2	SWVIC1	SWVIC2
Control	90%	100%	95%
Cutemco	95%	100%	100%
Tracerite	95%	100%	95%

Table 27 - The percentage of ewes that reared a lamb in each group of 20 animals sampled for liver copper concentration at MALLEE1 and MALLEE2.

Site	Treatment	% of ewes rearing a lamb
MALLEE1	$CuSO_4$	80%
MALLEE2	Control	95%
MALLEE2	Tracerite	95%

Lamb survival from pregnancy scanning to lamb marking showed that survival rates varied from 64% to 91% across EP2, SWVIC1 and SWVIC2 (Table 28). At EP2 and SWVIC1 survival rates of lambs born from ewes that were treated were similar or lower than those born from ewes that were not treated. At SWVIC2, lambs born from ewes that were treated were 4.4% and 15.8% higher for Tracerite and Cutemco treated animals compared to untreated animals.

Table 28 - Lamb survival rate (pregnancy scanning to weaning) for each treatment group at EP2, SWVIC1 and SWVIC2.

	EP2	SWVIC1	SWVIC2
Control	86.4%	70.1%	75.6%
Cutemco	83.5%	71.9%	91.4%
Tracerite	74.3%	64.3%	80.0%

Ewe body condition score and liveweight at weaning

Body condition score of ewes is presented in Table 29 and shows relatively minimal variation across treatment groups of ewes at each site, only varying by a maximum of 0.22 of a body condition score.

Table 29 - Mean and range in body condition score of ewes in each treatment group at EP2, SWVIC1 and SWVIC2.

Site	Treatment	Average	Range
EP2	Control	2.83	1.75 - 3.25
EP2	Cutemco	2.76	1.75 - 3.50
EP2	Tracerite (x2)	2.71	2.25 - 4.00
MALLEE1	$CuSO_4$ Lick	2.09	1.50 - 2.75
MALLEE2	Control	2.44	1.75 - 3.50
MALLEE2	Tracerite	2.54	2.00 - 3.50
SWVIC1	Control	2.48	2.00 - 3.25
SWVIC1	Cutemco	2.53	2.00 - 3.50
SWVIC1	Tracerite (x2)	2.70	2.00 - 3.25
SWVIC2	Control	2.21	1.25 - 3.50
SWVIC2	Cutemco	2.02	1.50 - 3.00
SWVIC2	Tracerite (x2)	2.26	1.50 - 3.75

Similarly to body condition score, the liveweight of ewes at weaning at EP2, SWVIC1 and SWVIC2 varied by very little across treatment groups (Table 30). The maximum range in liveweight of ewes at either site was only 1.9kg at EP2.

Table 30 - Mean ewe liveweight at weaning for each treatment group at EP2, SWVIC1 and SWVIC2.

	EP2	SWVIC1	SWVIC2
Control	73.2	68.1	61.0
Cutemco	74.8	68.9	62.1
Tracerite	72.9	68.7	61.5

Lamb weaning weight

The mean lamb weaning weight for each treatment group at EP2, SWVIC1 and SWVIC2 is shown in Table 31. Lamb weaning weight did not vary consistently across treatments at each site. Lambs from ewes treated with Tracerite at EP2 and SWVIC2 were heavier than both the control and Cutemco groups. Conversely, lambs born from ewes treated with Cutemco were heavier than the control and Tracerite groups at SWVIC1.

Table 31 - Mean lamb weaning weight for each treatment group at EP2, SWVIC1 and SWVIC2.

	EP2	SWVIC1	SWVIC2
Control	31.8	28.7	24.0
Cutemco	31.2	29.7	24.7
Tracerite	32.5	28.5	25.6

Results in Table 32 show that ewes treated with Cutemco on average produced 3kg more lamb at weaning compared to those treated with Tracerite across EP2, SWVIC1 and SWVIC2. However, at EP2, control ewes reared the highest kilograms of lamb per ewe compared to either of the treated groups.

Table 32 - Mean total kilograms of lamb weaned per ewe for each treatment group at EP2, SWVIC1 and SWVIC2.

	EP2	SWVIC1	SWVIC2
Control	43.9	33.3	22.9
Cutemco	40.9	36.5	24.4
Tracerite	37.8	32.0	23.1

Ewe liver copper concentration

The mean and range in liver copper concentration of ewes sampled across each site and treatment group is shown in Table 33. At EP2 and SWVIC2 both the Tracerite and Cutemco treatments resulted in significantly higher liver copper concentration when compared to the control group. At SWVIC1 only the Cutemco treatment resulted in a significantly higher liver copper concentration when compared to the control group. The Tracerite treatment at MALLEE2 did not significantly increase the liver copper concentration compared to the control group. Whilst all control groups included animals that were considered deficient for copper, only the mean of the control group at MALLEE2 was below the recommended minimum copper concentration.

Table 33 - Mean and range in liver copper concentration for each treatment group at EP2, MALLEE1, MALLEE2, SWVIC1 and SWVIC2.

			Liver Cu (mmol/kg wwt)
		Target range	*0.23 - 3.67*
EP2	**Control**	**Mean**	**0.62^a**
		Range	(0.12 - 1.57)
EP2	**Cutemco**	**Mean**	**2.07^b**
		Range	(0.84 - 5.6)
EP2	**Tracerite (x2)**	**Mean**	**1.22^b**
		Range	(0.6 - 2.22)
MALLEE1	**CuSO4 Lick**	**Mean**	**0.34**
		Range	(0.08 - 0.86)
MALLEE2	**Control**	**Mean**	**0.13^a**
		Range	(0.02 - 0.56)
MALLEE2	**Tracerite**	**Mean**	**0.18^a**
		Range	(0.04 - 0.46)
SWVIC1	**Control**	**Mean**	**0.50^a**
		Range	(0.14 - 1.19)
SWVIC1	**Cutemco**	**Mean**	**1.01^b**
		Range	(0.28 - 2.05)
SWVIC1	**Tracerite (x2)**	**Mean**	**0.85^{ab}**
		Range	(0.19 - 2.18)
SWVIC2	**Control**	**Mean**	**0.35^a**
		Range	(0.02 - 3.08)
SWVIC2	**Cutemco**	**Mean**	**1.14^b**
		Range	(0.26 - 2.84)
SWVIC2	**Tracerite (x2)**	**Mean**	**0.71^b**
		Range	(0.15 - 1.82)

The boxplot below shows the mean and median liver copper concentration across all treatments at each site. The mean of the control group at SWVIC2 has been skewed due to several outlier points and the boxplot shows that the majority of animals in this group are considered deficient. This is further confirmed in Table 34 which shows that 65% of ewes in the control group are considered copper deficient.

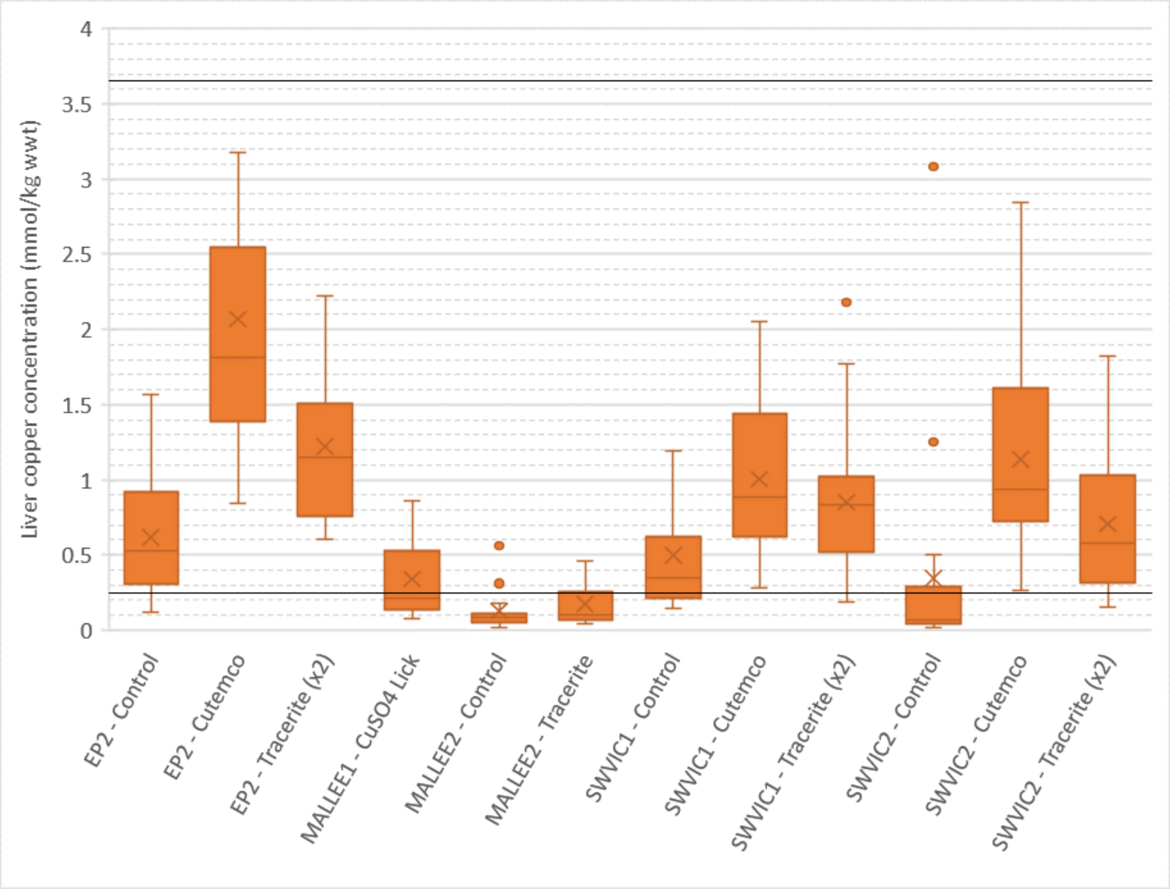

Fig. 60 - Boxplot graph for each treatment group at EP2, MALLEE1, MALLEE2, SWVIC1 and SWVIC2. (Mean shown as 'X'. Lower and upper normal range shown by black lines)

Table 34 shows that treatments were effective at reducing the number of animals considered copper deficient at all sites when compared to the control group.

Table 34 - Percentage and number of animals in each treatment group that were considered copper deficient based on liver copper concentration.

Site	Treatment	% of treatment group deficient. Number of animals in ().	
EP2	Control	15%	(3)
EP2	Cutemco	0%	(0)
EP2	Tracerite (x2)	0%	(0)
MALLEE1	$CuSO_4$ Lick	55%	(11)
MALLEE2	Control	85%	(17)
MALLEE2	Tracerite	70%	(14)
SWVIC1	Control	30%	(6)
SWVIC1	Cutemco	0%	(0)
SWVIC1	Tracerite (x2)	5%	(1)
SWVIC2	Control	65%	(13)
SWVIC2	Cutemco	0%	(0)
SWVIC2	Tracerite (x2)	15%	(3)

Key findings at each site

EP2

Pasture mineral content showed a moderate copper deficiency with minimal interaction from antagonistic minerals such as molybdenum.

Treatment with Cutemco and Tracerite products were effective at elevating liver copper concentration compared to untreated animals.

No consistent effects of copper treatment were observed in relation to production factors such as mortality rates, reproductive rates or growth rates. The greatest effect observed was a reduction in marking percentage with treatment of the Tracerite product.

MALLEE1

Pasture mineral content showed a significant copper deficiency with strong interaction from antagonistic minerals such as molybdenum and iron.

Treatment with a loose mineral lick containing copper sulphate was not fully effective at preventing deficiency in the mob as 55% of the group were still considered deficient.

MALLEE2

Pasture mineral content showed a significant copper deficiency with strong interaction from antagonistic minerals such as molybdenum.

Treatment with a single dose of the Tracerite product marginally increased the liver copper concentration of treated animals and decreased the proportion of animals considered deficient by 15% when compared to the control group. However, 70% of treated animals were still considered deficient based on liver copper concentration.

SWVIC1

Pasture mineral content showed a moderate copper deficiency with limited interaction from antagonistic minerals such as molybdenum.

Treatment with Cutemco and Tracerite (double dose) products were effective at elevating liver copper concentration compared to the control group and reducing the proportion of animals considered deficient in comparison to the control group.

Treatment with Cutemco resulted in 3% less ewe mortalities, 6.4% fewer dry ewes at pregnancy scanning, 7% higher marking rate and 3.2kg more lamb weaned compared to untreated animals.

Treatment with the Tracerite capsule resulted in 3.3% less ewe mortalities, 9% higher conception rate, similar marking rate, similar kilograms of lamb weaned per ewe and 5.8% lower lamb survival rate compared to untreated animals.

SWVIC2

Pasture mineral content showed a moderate copper deficiency with limited interaction from antagonistic minerals such as molybdenum.

Treatment with Cutemco and Tracerite (double dose) products were effective at significantly elevating liver copper concentration compared to the control group and reducing the proportion of animals considered deficient in comparison to the control group.

Treatment with Cutemco resulted in 3% lower ewe mortality, 19% lower conception rate, 4.4% more dry ewes at pregnancy scanning, a similar marking percentage and kilograms of lamb weaned and 15.8% higher lamb survival compared to untreated animals.

Treatment with Tracerite resulted in 6% lower mortality, 14% lower conception rate, 7.5% more dry ewes at pregnancy scanning, 5.6% lower marking percentage, similar kilograms of lamb weaned and 4.4% higher lamb survival compared to untreated animals.

4.3.3 Discussion

Pasture copper content was on average higher during winter and spring compared to summer and autumn analyses. The same trend was observed in the 2013 pasture testing conducted as part of this trial. This result is contrary to the generally accepted view that the concentration of copper (and other minerals) in pasture is lowest during active pasture growth in winter and spring.

Pasture copper content at EP2 had elevated to 7mg/kg DM at the time of liver sampling which was the highest of all sites at the time of liver sampling. This corresponded to the control group of animals with the least number of ewes considered deficient for copper, indicating that liver copper concentration is relatively quick to react to increased availability of copper. This may also indicate that current guidelines for the correlation between pasture and liver minimum concentrations are well aligned, at least where minimal influence from antagonistic minerals is observed, as was the situation at EP2.

Molybdenum concentration of pasture appeared to have the strongest influence on liver copper concentration at MALLEE1 and MALLEE2. As no control group was present at MALLEE1, no conclusions can be drawn on the relative severity of this effect. However, in comparing the treatments at each property it can be seen that the CuSO4 lick resulted in a higher average liver copper concentration and a greater proportion of ewes with adequate liver copper levels despite a lower daily copper dose delivered from the lick compared to the Tracerite capsule. This result would indicate that providing a regular (daily) supply of copper to the animal is more advantageous than providing a large initial dose to be absorbed and subsequently used by the body, particularly where antagonistic minerals such as molybdenum and iron are involved.

Ewe mortality rates were generally lower for treated groups with the exception of the Cutemco treated animals at EP2. At this site treated animals had a 2% higher mortality rate than the control group which is unlikely to be significant. Mortality rate for control group animals was relatively high in industry terms with 5%, 7% and 10% mortalities observed at EP2, SWVIC 1 and SWVIC2 respectively. No direct effect of copper deficiency on mortality has been identified through this project, however the initial literature review detailed the effect of copper deficiency on reducing general immune function. It is expected that copper deficiency was not the primary cause of death in these cases but was a contributing factor.

Given the lack of scouring observed in trial mobs during 2015 the commonly held belief that scouring can be a result of copper deficiency appears to be false. This is further confirmed by data from previous trials under this project in 2012 and 2013 where scouring observed was a result of worm burden rather than copper deficiency. Evidence detailed in the literature review of this project showed that high molybdenum intake can cause scouring in particular for cattle more so than sheep. Additionally, ‘high’ in these trials was considered as greater than 5mg/kg DM molybdenum, a level that was not reached at any of the trial sites for this project, nor observed commonly in Australia.

The negative effect of copper deficiency on fertility is recognised throughout the literature as a key impact on productivity and profitability. In particular, the effect of copper deficiency on conception rate is often discussed however poorly understood. Evidence to date actually suggests that the effect is more directly driven by excess molybdenum, rather than a copper deficiency. Given the relatively low molybdenum levels observed in many pastures analyses in Australia compared to other countries it can be expected that this effect on conception rate would be far lower. Unfortunately, at MALLEE1 and MALLEE2 where the highest influence from molybdenum was observed through pasture testing, no production data were measured between groups as the initial trial design was to simply measure the resulting change in liver copper content from amended treatment programs compared to previous years’ trials. At EP2 minimal difference was observed in the conception rate of treated and untreated ewes; this may be attributed to the low molybdenum content of the pastures observed at this property. Inconsistent conception rate results were observed between SWVIC1 and SWVIC2 and given the evidence presented from the literature review it would be erroneous to attempt to draw conclusions on the effect of copper deficiency on this. Unfortunately pasture samples were not able to be collected at SWVIC1 earlier than September 2015 due to the limited pasture available where the trial group was grazing. However, these results may have helped to explain why a clear positive effect of copper treatment was observed at SWVIC1 when compared to the control group. Previous testing at this site has shown that pasture copper concentration is typically at the lowest point in March which aligns with the mating date of this group. As little effect would have expected from high pasture molybdenum content as previous testing at this site has shown that excessive levels are not typically present, it could be concluded that the copper supplementation assisted in overall animal immune function and health resulting a higher percentage of ewes in lamb compared to those that were not treated, however this is purely speculative.

Results from this project during both 2015 and 2013 have not shown a consistent effect of copper supplementation on conception rates. Given the currently understood mechanism of high molybdenum levels negatively influencing fertility rates it can be expected that under most Australian conditions, where molybdenum concentration is typically less than 1mg/kg DM, copper supplementation to counteract the effect of excessively high concentration of molybdenum will have little effect. It could reasonably be expected that where copper concentration of pasture falls below 3mg/kg DM and/or the Cu:Mo ratio is less than 2:1 for greater than 3 months that a positive effect of supplementation on animal health would be experienced and if this timing coincided with mating that some positive effect may be observed on conception rates.

There was little positive effect of treatment on lamb survival rates from conception (pregnancy scanning) to marking. At EP2 treatment with either the Cutemco bolus or Tracerite capsule resulted in similar or lower lamb survival rates to the control group. The same trend was observed at SWVIC1. However, at SWVIC2 lamb survival from treated ewes was up to 15.8% higher than the control group. In reviewing the pasture analyses around the time of lambing and weaning it can be seen that at SWVIC2 a consistent low level of copper intake was observed which potentially lead to the greater result observed at this site. In comparison, EP2 and SWVIC1 both had a period of time around lambing where pasture copper concentration was meeting requirements. This is supported by the liver copper results which show the average liver copper concentration of control animals at EP2 and SWVIC1 is higher than those at SWVIC2. Interestingly, at all three sites the Tracerite treatment resulted in 7.2% to 11.4% lower lamb survival compared to the Cutemco treatment. Additionally, at SWVIC2 the lamb survival from ewes treated with Tracerite was higher than that of control animals, however at EP2 and SWVIC1 lamb survival from ewes treated with Tracerite was lower than that of control animals. At SWVIC2 the lamb survival rates align well with liver copper concentration, however this correlation does not hold for EP2 and SWVIC1 as the control animals had lower liver copper concentration but higher lamb survival.

Variable results have been demonstrated throughout this trial for the effect of copper supplementation on the marking percentage or lamb survival rate of trial groups. Results from EP2 (the only site with data available to compare the effect treatment on marking percentage) in 2013 showed that treated ewes marked up to 7% more lambs that untreated animals. However, during 2015 results from all sites with data available showed that of those ewes pregnancy tested in lamb, treated animals marked from 24% less lambs to 9% more lambs. Interestingly, the percentage of ewes that reared a lamb (as measured by wet/dry status at marking or weaning) was never less for treated animals compared to untreated animals. During 2013 this effect was particularly pronounced at SWVIC2 where there was up to a 30% difference in the number of ewes that reared a lamb when comparing untreated to treated animals. This effect was not as pronounced in 2015 where there was only up to a 5% improvement in treated animals. This may be reflective of the greater deficiency experienced by sheep in 2013 where 85% of the control group were considered deficient in 2013 compared to 65% in 2015.

In reviewing the effect of copper supplementation on lamb production is was observed that the average weaning weight of lambs from ewes treated with Cutemco was within one kilogram of untreated animals at EP1, SWVIC1 and SWVIC2. Lambs from Tracerite treated animals were generally similar to untreated animals, however at SWVIC2 Tracerite treated lambs were 1.6kg heavier than control animals. To assess the overall effect of copper supplementation on lamb production requires consideration of the lambing percentage as well. This will account for the fact that ewes that rear more lambs will typically rear multiple lighter lambs that in combination produce a greater amount than a single heavier lamb. However, given the relatively small level of variation in lamb weaning weight it can be concluded that the largest effect of copper supplementation on lamb production is the effect on lamb survival (marking rate).

The liver copper concentration across all sites showed that copper accumulation was relatively well explained by both the pasture mineral concentration and the treatment dose rate. An exception to this was observed at MALLEE1 and MALLEE2 where the $CuSO_4$ lick produced higher liver copper concentration compared to the Tracerite treated animals despite the lick delivering a lower daily dose of copper. However, it is important to note that this comparison is between two sites and as no control group was present at MALLEE1 it cannot be stated with certainty that the relative increase in liver copper concentration due to the $CuSO_4$ lick was greater than the Tracerite product.

At all sites the relative increase in liver copper concentration due to treatment followed closely with the daily copper dose of the treatment. One aim of this trial was to determine whether the different mode of action of the Cutemco product provided a more effective method of supplementation than a copper oxide needle product such as Tracerite where high levels of antagonistic minerals are present. Unfortunately, high levels of interaction from molybdenum and other minerals was only observed for relatively short periods of time and a definitive conclusion cannot be drawn. It is this author’s view that a soluble glass bolus product will have an advantage over other supplement products only in limited situations where molybdenum levels are excessively high and the Cu:Mo ratio indicates a significant interaction from antagonistic minerals (i.e. less than 2:1 Cu:Mo) and for extended periods of time.

The range in liver copper concentration whilst all animals were on the same pasture supports the literature that there is genetic variance in the ability of individual ewes to absorb and accumulate copper. Some of this variance may also be attributed to selective grazing of animals and variance in the copper concentration of plants across a single paddock.

Results of this trial have demonstrated that the timing of the deficiency can have a significant effect on the impact on production. For example, it was demonstrated at SWVIC2 that low pasture copper concentration around the time of lambing resulted in a reduced lamb survival rate. In contrast, at EP2 and SWVIC1 where pasture copper concentration at the time of lambing appeared only marginally deficient there was little to no effect on lamb survival.

Due to the highly variable and interactive nature of minerals that influence copper availability precise analysis to determine the exact critical thresholds of where pasture mineral content affected animal production cannot be drawn. However, it has been observed that in reviewing the current recommendations for minimum pasture copper content of 7mg/kg DM and a minimum copper:molybdenum ratio of 5:1 that where these critical amounts have not been met the liver copper concentration has typically been considered deficient as well. This does not necessarily mean that negative production effects have been observed, simply that the current recommended minimum levels in pasture and liver concentration are well aligned.

Overall, results from this project would indicate that the current recommendations for the minimum pasture copper concentration of 7mg/kg DM and the minimum Cu:Mo ratio of 5:1 should be considered the marginal levels where the potential for effects of copper deficiency should be monitored but is unlikely to require significant treatment. In reviewing all of the results of this project it is concluded that copper supplementation strategies should be considered in the manner detailed in Table 36.

Table 35 - Recommended reference ranges for the influence of pasture mineral content on copper deficiency in sheep.

Deficiency state	Pasture Cu concentration	Pasture Cu:Mo ratio	Action required
Adequate	>7 mg/kg DM	>5:1	N/A
Marginal	7 mg/kg DM	5:1	Continue monitoring pasture mineral concentration and observe for any levels of reduced productivity in the flock.
Moderate	5 mg/kg DM	3:1	Consider supplementation to coincide with timing of deficiency, particularly where signs of reduced productivity are observed in the flock.
Severe	3 mg/kg DM	2:1	Supplementation with copper is required to ensure adequate animal health and productivity.

5 Conclusions

5.1 Project conclusions

The results of this project have provided many key learnings that will improve the identification and management of copper deficiency in sheep flocks. These key findings are as follows:

- Soil test results are not an accurate assessment of pasture or animal copper status.
- Blood and liver copper concentration are poorly correlated and as such, blood tests should not be the primary method of measuring the copper status of sheep.
- Pasture tissue analysis can provide an accurate indication of animal copper status where repeat measurements are taken over a period of time, however consideration of the influence of interacting minerals such as molybdenum, sulphur and iron must be included.
- Time of year/stage of growth affects copper concentration in pastures and is not consistent across properties or regions.
- Each treatment tested was effective at increasing liver copper concentration and was generally in line with the daily copper dose rate.
- Ruminal boluses and capsules typically have a higher daily copper dose rate and have the greatest effect on increasing liver copper concentration.
- The soluble glass bolus was most effective at increasing animal copper status due to its high copper dose rate.
- Copper oxide capsules are effective at increasing animal copper status however not to the same extent as the soluble glass bolus product.

- Copper injections are effective at raising animal copper status however the effective dose rate requires further investigation.
- Copper sulphate licks are effective at raising animal copper status however custom formulated high dose rate products will typically be required to deliver sufficient levels to elevate animal copper status significantly.
- Copper supplementation does not appear to have a consistent effect on conception rates of ewes, most likely due to the limited impact of molybdenum on ovulation rates as excessive levels of this mineral are rarely observed in Australia.
- Copper deficiency was not directly related to scouring in sheep in this study.
- Copper deficiency was not directly related to lameness in sheep in this study.
- Where severe copper deficiency exists, supplementation with copper can significantly improve ewe and lamb survival rates.

5.2 Recommendations

The results of this project provide producers with a variety of key actions they can implement on farm to ensure that copper deficiency does not affect the productivity of their sheep flock. To extend this information it is recommended that the media release detailed in the appendix is distributed amongst known industry networks of both the author of this report and MLA. Further communication to industry stakeholders can be conducted in consultation with MLA.

6 Key messages

This project has found that severe copper deficiency can result in a significant increase in ewe and lamb mortality rates. This is likely a result of the importance of copper for proper immune function in sheep.

Where severe copper deficiencies have been observed, marking percentage has been reduced by up to 15% and in some instances up to 30% of ewes have not reared a lamb.

Treatment with a copper supplement product can be effective at elevating the copper status of animals and reducing the effects of deficiency on productivity. A range of copper products were trialled including an injection, water trough block, oral loose lick, ruminal capsule and ruminal bolus. Each product was effective at elevating liver copper concentration and generally in line with the copper dose rate of the product.

Assessing pasture mineral status over a period of 12 months can provide an accurate and relatively simple method of determining whether a potential copper deficiency requires any action. This method is more accurate and often more cost effective than conducting blood or soil testing programs to assess copper status in sheep.

The use of pasture mineral analyses should be encouraged to identify not only the inherent copper concentration of pasture but also the influence of other minerals such as molybdenum, sulphur and iron which can reduce copper availability and animal copper status.

Producers can use table 35 above to review pasture analysis results against the need for action to be taken to correct a potential copper deficiency in sheep.

Selection of the most appropriate copper supplement product will largely depend on the timing and severity of the identified deficiency.

Where a seasonal copper deficiency exists (i.e. it only occurs for a short period of the year) then a short term supplement such as a loose lick would be the most appropriate treatment. The copper dose rate of the product should be formulated to account for the severity of the deficiency identified.

Where a continual and/or severe deficiency exists, a longer term treatment product such as a copper ruminal capsule would be appropriate to deliver a longer treatment effect as well as typically a higher dose rate of copper compared to other products commercially available for sheep. Where a severe deficiency exists, either due to very low copper concentration in pastures and/or high levels of influence from antagonistic minerals, multiple treatments may be required to adequately address a copper deficiency. However, as copper supplementation can be toxic to sheep when excessive levels are provided, professional advice should always be sought to ensure the correct dose rate is supplied.

7 Appendix

7.1 References

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7.3 Media release

A recently completed project funded by Meat and Livestock Australia has investigated whether copper deficiency in Australian sheep flocks has a significant impact on production.

Copper deficiency has been reported sporadically in sheep flocks in southern Australia since the 1940's however its effect on productivity are often inconsistent and difficult to measure. Additionally, there are a range of views on the correct way to diagnose a potential copper deficiency in sheep and clear recommendations are needed.

The project was conducted across six properties in South Australia and Victoria that were identified with low copper levels. AgriPartner Consulting conducted the project and Principal Consultant, Hamish Dickson said "the trial was important for the industry to clearly determine the effect of copper deficiency on Australian sheep properties. Pasture testing over the last 10 years has revealed deficiencies in regions not traditionally thought to be problematic."

Poor conception rates have often been described as a symptom of copper deficiency, however this conclusion has not been supported by the results of this project. Hamish said, "we found that that there was no consistent effect of copper supplementation on conception rates of sheep. The history of this misconception is driven by the relationship between molybdenum and copper. Many minerals including molybdenum interact with copper and can reduce its availability to animals. High molybdenum has been shown to reduce ovulation rates and it is likely that this incorrect diagnosis that has caused 'copper deficiency' to often be blamed as the cause."

The trial found that severe copper deficiency can result in a significant increase in ewe and lamb mortality rates. This is likely a result of the importance of copper for proper immune function in sheep. Where severe copper deficiencies were observed, marking percentage was reduced by up to 15% and in some instances up to 30% of ewes did not rear a lamb.

Treatment with a copper supplement was effective at elevating the copper status of animals and reducing the effects of deficiency on productivity. A range of copper products were trialled including an injection, water trough block, oral loose lick, ruminal capsule and ruminal bolus. Each product was effective at elevating liver copper concentration and generally in line with the copper dose rate of the product.

"Selection of the most appropriate copper supplement product will largely depend on the timing and severity of the identified deficiency" Hamish said.

Where a seasonal copper deficiency exists (i.e. it only occurs for a short period of the year) then a short term supplement such as a loose lick would be the most appropriate treatment. The copper dose rate of the product should be formulated to account for the severity of the deficiency identified. Hamish said, "Many off the shelf mineral licks do not contain sufficient levels of copper to address a significant deficiency."

Where a continual and/or severe deficiency exists, a longer term treatment product such as a copper ruminal capsule would be appropriate to deliver a longer treatment effect as well as typically a higher dose rate of copper compared to other products commercially available for sheep. Where a severe deficiency exists, either due to very low copper concentration in pastures and/or high levels of influence from antagonistic minerals, multiple treatments may be required to adequately address a copper deficiency. However, Hamish warned “copper supplementation can be toxic to sheep when excessive levels are provided; professional advice should always be sought to ensure the correct dose rate is supplied.”

The correlation between soil, pasture, blood and liver copper concentration was investigated in the trial to assist in determining the most effective strategy to identity any deficiencies. Poor correlations were found between soil or blood copper concentration and the liver copper concentration. Hamish said that pasture tissue testing was the most reliable and cost effective strategy to determine whether a potential copper deficiency needs addressing. This was due to the fact that pasture testing allows for the full range of minerals to be tested as an assessment of not only the inherent copper concentration but also the influence from any antagonistic minerals such as molybdenum. As mineral concentration in pasture will change with stage of growth, it is important to repeat the testing several times over the course of a year to gain a full understanding of the situation.

For more information on this project contact:

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