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Understanding photosensitisation in sheep grazing the pasture legume *Biserrula pelecinus L.*

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Executive summary

Biserrula pelecinus L. is an annual legume native to the southern Mediterranean (Howieson et al., 1995). It was first introduced to Australia in 1991 as a potentially valuable rotational pasture species for livestock production. It produces large quantities of biomass, exhibits drought tolerance and is effective for weed suppression in pasture rotations. However, despite proving to be a valuable addition to the pasture toolbox, producers in NSW and WA have reported a limiting factor to uptake: incidence of severe photosensitisation when grazing sheep on biserrula pastures. Biserrula photosensitivity, anecdotally, appeared to be associated with a particular phase of crop maturity and showed an increased severity of clinical signs in young animals, however, the pathogenesis of this photosensitisation and the compound responsible were unknown.

This project aimed to determine the nature of the photosensitisation (PS) observed with animals grazing biserrula, provide mitigation strategies to prevent livestock photosensitivity when grazing this useful pasture species in the future, and to define the critical growth stage of phototoxicity. It examined the aetiology and pathogenesis of biserrula PS, defining the disorder as a primary photosensitivity, and surveyed producers known to have implemented this pasture species in New South Wales and Western Australia, the major zones of utilisation. Both meat sheep and wool producers were identified in the cohort, and a single cattle producer. In all bar one case the cultivar sown was biserulla 'Casbah' as part of a crop rotation. Results showed that 55% of producers grazing livestock on biserrula pasture had experienced outbreaks of photosensitisation. All ages and sexes of animals were found to be affected. A higher incidence of outbreaks of PS was observed in WA compared to NSW. In most cases the outbreaks were identified to be either mild (47%) or moderate (40%). Only a small proportion of producers contacted a veterinarian in the advent of these outbreaks, the vast majority managing them themselves without veterinary involvement.

As part of this project, plant samples were collected from a number of sites in the Riverina, for future analysis, as well as from the grazing trials identified below. These samples ranged in nature from early vegetative, through to senescent. Outbreaks of PS were associated with samples from vegetative through late flowering stages of plant growth, but not with senescent material.

A clinical case study identified a mob of 120 meat lambs grazing biserulla 'Casbah'. This study showed histopathological features of clinical PS in ears and facial tissues of affected lambs without underlying hepathopathy. This was the first reported case study identifying primary PS in lambs grazing *Biserrula pelecinus* cv. 'Cashbah' and is the first reported case study identifying primary photosensitisation in animals grazing this pasture species .

Further supporting evidence that PS subsequent to ingestion of commercially-available biserrula cultivars exerted no hepatic damage was confirmed in a subsequent series of grazing trials which examined prevalence and severity of PS in sheep of various ages and breed characteristics. A PS scoring system was established to quantitate clinical signs of PS in affected animals and this was applied to animals entering controlled grazing trials on pastures of various compositions during 2013 and 2014.

In 2013 a controlled grazing trial was undertaken with cross-bred and merino lambs (n = 72) on various composition of biserrula pastures, from pure stands of biserrula cultivar 'Mauro' comprised of <88% biserrula by composition, split plots of biserrula: subterranean clover, or subterranean clover alone. Animals on biserrula only pastures presented with clinical signs of mild to moderate PS within 4 days on pasture. After 15 days on pasture all animals were removed from pasture and 12 animals were submitted for routine necropsy. No clinopathological changes indicative of hepatophathy were identified either by venous sampling and clinical pathological analysis or at necropsy.

A second controlled grazing trial was undertaken in spring 2014, comparing prevalence and severity of PS on two commercially available cultivars of *Biserrula pelecinus* – 'Mauro' and 'Casbah'. Differential grazing patterns were noted between the two cultivars, which was confirmed by pasture composition analysis. Animals grazing the cultivar 'Mauro' showed less selectivity, with the pasture composition of biserrula versus 'other' species showing equal decline over grazing days, whilst animals grazing biserulla 'Casbah' selectively avoided ingestion of this cultivar in the initial grazing period, only showing increased consumption once other plant species present had diminished. This supports anecdotal evidence that animals grazing biserrula 'Casbah' show a selective preference for other pasture species and / or weeds over this cultivar on grazing. Supporting this evidence of selective intake, animals grazing biserrula 'Casbah' showed a slightly later onset of clinical signs of PS (day 7 on pasture) than their counterparts grazing 'Mauro' (day 3 on pasture).

In addition to comparing clinical presentation of PS on the two cultivars of biserrula, this trial compared severity of PS in pigmented versus non-pigmented meat lambs, and in merino ewes and lambs grazing the same pastures. 'Mauro' pastures showed a slightly lower prevalence of PS overall in the non-pigmented animals (71%) compared to 'Casbah' (100%) but overall the incidence in both cultivars was high. By comparison, pigmented animals showed a lower incidence on both varieties suggesting that pigmentation conferred some protection to expression of clinical signs. Merinos (both ewes and lambs) showed a lower incidence than their meat-breed counterparts indicating that the greater proportion of fleece coverage also offered some physical protection as might be expected although exposed facial areas still showed mild-moderate clinical signs. As before, clinical pathology of affected non-pigmented animals showed no evidence of hepatopahy, even in the most severely affected cases.

Live weight gain was monitored over time during the spring 2014 grazing trial. All animals (meat lambs and Merinos) showed increased weight gain over the period of the trial. There was a suggestion that lack of pigmentation exerted a negative effect on weight gain in the meat lambs after 14 days on pasture but this finding was not significant. Lactating merino ewes grazing both cultivars were compared to their breed counterparts grazing regional unimproved pastures, and growth rates were found to be significantly higher. A similar trend was observed in the Merino sucker lambs. In both cases, cultivar did not exert a significant effect suggesting that weight gain on both biserrula 'Mauro' and 'Casbah' were equivalent in these trials.The findings of these studies have been amalgamated into a 'Tips and Tools' brochure to be disseminated to producers.

This project demonstrated that the clinical presentation of photosensitisation in sheep grazing both commercially available cultivars of *Biserrula pelecinus* was one of a primary photosensitisation in nature. Survey analysis and controlled grazing trials showed that all breed and ages of animals can show clinical signs of PS, but that the severity of these signs could partially be mitigated by skin pigmentation or fleece covering or ingestion of other pasture species in combination with biserrula varieties. Outbreaks of PS were identified on all stages of plant growth from vegetative to late flowering suggesting that there is no ‘safe’ grazing window for this species when the plant is green, actively growing or flowering. Together these studies provide some mitigation strategies for producers wishing to use biserrula as a grazing crop but suggest that careful management is required for all sheep presented with this pasture species.

A bioassay-directed approach was taken to determine the active constituent(s) in biserrula, that examined photocytoactivity across the full growth cycle of the plant, from early vegetative to late senescent stages. Plant material was sourced from producer sites, or from the controlled grazing studies described above, to ensure that material tested for photobioactive compounds was known to be associated with toxicity. Both cultivars were bioactive, and photocytotoxic activity was associated with extracts from field-grown biserrula at all stages of plant growth until senescence. These experiements identified that all fresh green extracts possessed photocytotoxic activity, and this activity diminished greatly with drying of plant tissue by any means (freeze drying or air drying). Bioactivity was associated with the concentration of chlorophyll a and b and related photosynthetic pigments.

To identify the causal photocytotoxic compounds, Biochemical analysis using fractionated extracts, bioactivity-guided metabolic profiling using liquid chromatography mass spectroscopy and quadripole time-of-flight (UPLC/MS-QToF) analysis resulted in identification of multiple novel molecular features, with causal compounds present in both the complex crude extract and the purified bioactive fraction(s).

Gaining a better understanding of the aetiology of biserrula photosensitisation in livestock allows informed grazing management strategies to mitigate future phototoxic outbreaks. Elucidation of secondary plant metabolites which cause photosensitsation 1) identify key targets in the plant’s biosynthetic machinery that could be altered to generate safer cultivars, and 2) allow future screening for bioactive constituents in biserrula selections that might be commercially released.

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1 Background

1.1 General information

***Biserrula pelecinus* L.** denotes a plant species native to the southern Mediterranean and introduced to Australia in 1991 that is commonly referred to as **biserrula**. It has two cultivars of note referred to in this report as **'Casbah'** and **'Mauro'**.

1.2 Project background

Biserrula pelecinus is a semi-erect, self-regenerating annual legume originating from the southern Mediterranean (Howieson et al., 1995). It has a range of desirable characteristics, including high seed production, adaptation to a broad range of soil types, a deep root system and grazing tolerance, that lead to a highly productive pasture that is more drought resistant and shows significant self regeneration when compared to other pasture legumes (Howieson et al., 1995, Loi et al., 2006, Vicente et al., 2012, Loi, 2005). *Biserrula* was first developed for agricultural use in Western Australia during the 1990s, and recently improved cultivars have since been introduced across NSW and VIC; anecdotal evidence suggests that *Biserrula* especially 'Casbah', has been positively received by the Australian farming community (Loi, 2005, Loi, 2010, Hackney, 2012). Several years after commercialisation of the first cultivar 'Casbah' in 1997, a small but growing number of cases of photosensitisation have anecdotally been noted in ewes and lambs grazing *Biserrula* dominant pastures in spring (Hackney, 2012, Loi, 2010), although the overall incidence remains unclear. This photosensitisation has been investigated on an individual level, but there are currently no case reports in the peer reviewed literature (Hogg, 2010), nor has the type of photosensitivity been confirmed. In this report we present an outbreak of photosensitivity in lambs on a pasture of pure *Biserrula pelecinus* L. *cv. Casbah*, and record the clinicopathological and histopathological changes that confirm a Type I photosensitivity.

Prior to this study, there had been anecdotal reports of outbreaks of photosensitisation occurring in livestock grazing biserrula presented in producer fact sheets and other informal publications; however, the first published case report of an outbreak in 20% of a 120 head meat lamb flock grazing pasture consisting of a monoculture of the pasture legume *Biserrula pelecinus* L. *cv.* Casbah was presented in the literature in 2015 (Kessell et al., 2015). In this outbreak, a number of animals with moderate to severe clinical lesions, as well as animals showing no clinical abnormalities, were sampled for a complete blood count and biochemistry, and affected animals subjected to a full necropsy. Histopathology of affected tissues noted lesions consistent with photosensitisation of the exposed skin of the face and ears. No abnormalities in histopathology or serum biochemistry indicative of significant hepatopathy were observed in any clinical or subclinical cases. This case study confirmed photosensitisation associated with ingestion of *Biserrula pelecinus L.* to be primary (non-hepatogenous) in nature. This study is included in this report as it was was funded in part by the Charles Sturt University Graham Centre for Agricultural Innovation in a Research Initiative Award and an Australia Wool Education Trust Scholarship Award to CSU honours student G. Ladmore and occurred prior to the controlled grazing trials reported herein.

Apart from this case report, no formal investigation of the incidence, prevalence or pathogenesis of photosensitisation subsequent to ingestion of biserrula pastures had been undertaken, despite this pasture having been adopted for use by several hundred producers across Australia. Some of these producers utilised biserrula as a pasture within their mixed farming system, but were reliant on producer-generated anecdotal information, or advice from DPI's or veterinary professionals in managing this species in terms of its effects on their livestock. Many had discovered preventative strategies by trial and error.

In 2013, when this project was initiated, general knowledge within producer communities was that biserrula could cause, in some cases, severe outbreaks of photosensitisation resulting in both production losses and significant animal welfare and management issues. Although absolute prevalence was not known, this phenomenon was preventing widespread update of biserrula as a pasture species. Limited to no information was available about the biochemistry of *B pelecinus,* or the plant factors associated with these sporadic but often serious outbreaks. This study therefore aimed to address this shortfall by defining an experimental approach to identify the photocytotoxic bioconstituents present in this species.

Together, the aims of the study reported in this document were:

1) To determine the aetiology and pathogenesis of photosensitisation in livestock ingesting biserrula as a pasture;
2) To identify if a particular phase of the plant's life cycle or phenology was associated with these outbreaks;
3) To better understand the extent of the problem for producers, and,
4) To define the phototoxic constituents present in biserrula that were causing photosensitisation in grazing livestock.

If successful, this project would give: 1) producers a better understading of the factors influencing outbreaks of photosensitisation in animals grazing *Biserrula pelecinus L.* cultivars, 2) identify mitigation strategies to prevent or minimise it's effects on grazing livestock whilst still allowing use as a productive pasture and 3) definition of the photoactive constituents within the plant causing disease outbreaks, thereby providing the possibility of development of safer biserrula cultivars.

2 Project objectives

2.1.1 Define the critical growth stage window of phototoxicity associated with *Biserrula pelecinus*.

2.1.2 Determine the aetiology and pathogenesis of *Biserrula* photosensitisation, defining this disorder as either a primary or secondary hepatotoxic photosensitivity.

2.1.3 Survey producers who have implemented this pasture species on their experience with *Biserrula* photosensitivity.

2.1.4 Identify the compound present in the plant causing *Biserrula* photosensitisation.

3 Methodology

3.1 Abbreviations

^{o}C, degrees Celcius
µg, microgram
ACN, acetonitrile
AST, aspartate transaminase
CBC, complete blood count
CK, creatinine kinase
cm, centimetre
CSU, Charles Sturt University
DM, dry matter
DMEM, Dulbecco's Modified Eagle Medium
DMSO, dimethyl sulfoxide
DSE, dry sheep equivalent
EDTA, ethylenediamine tetra-acetic acid
EtOAc, ethyl acetate
FOO, feed on offer
g, gram
GLDH, glutamate dehydrogenase
GGT, gamma-glutamyltransferase
HPLC, high performance liquid chromatography
Ha, hectare
Kg, kilogram
L, litre
LC, liquid column
LW, live weight
MeOH, methanol
Ml, millilitre
Mm, millimetre
MS, mass spectrometry
MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium
PDA, photodiode array
N, nitrogen
Nm, nanometres
NMR, nuclear magnetic resonance (spectroscopy)
NSW, New South Wales
QToF, Quadrupole Time of Flight
QQQ, Triple Quadrupole (spectrometry)
s.e.m. Standard Error of Means
TFA, trifluoroacetic acid
UV, ultraviolet
VIS, visual spectrum
WA, Western Australia

3.2 Producer survey

A producer survey was designed and delivered by online survey (SurveyMonkey™) or by paper format. The survey was conducted with approval from the Charles Sturt University School of Animal and Veterinary Science Ethics in Human Research Committee (Protocol number 416/2013/01) and was delivered between October 2013 and January 2014.

The questionnaire was developed based on historical and anecdotal factors suspected to contribute to biserrula photosensitization, including farm, paddock and animal effects. See Appendix 1 for survey questions and format.

Participants were recruited *via* contact details supplied by Ballard Seeds Pty for producers known to have purchased biserrula seed and thought to be currently utilising biserrula as a grazing legume in Western Australia or New South Wales. Other producers known personally to the research team to be grazing biserrula cultivars were also targeted. Potential participants (n=103) were contacted *via* mail, phone and/or email with instructions of how to complete the survey. Participants were variably followed-up *via* mail, phone and/or email if no acknowledgement of survey receipt was noted. Participants acknowledged consent by completing the survey. Farmers without experience grazing biserrula were excluded from the survey. The total target population is estimated to be approximately 200 producers who are known to have planted commercially available biserrula cultivars in WA and NSW (B. Hackney and N. Ballard, personal communication). Survey results were collated in SurveyMonkey™, converted to Excel format and analysed using SPSS™.

3.3 Controlled grazing trials 1 - 2013 Spring grazing trials using crossbred and merino lambs

3.3.1 Experimental design of controlled field trials

Two controlled field trials were undertaken to a) confirm the mechanism underlying outbreaks of photosensitisation as primary versus secondary in nature, b) to provide comparison and confirmation of data observed in on-farm outbreaks, c) to compare to incidence and severity data identified in the producer survey, and d) to examine possible mitigation strategies to reduce the incidence and/or prevalence.

These trials compared photosensitisation potential of the two commercially available cultivars biserrula 'Mauro' vs 'Casbah" and the effect of breed (Merino vs Sussex x Dorper), age (lambs vs ewes); and pigment (pigmented stock vs unpigmented stock) on prevalence and severity of photosentisation.

A visual representation of the principles of design and analysis of these trials is shown in Fig. 1.

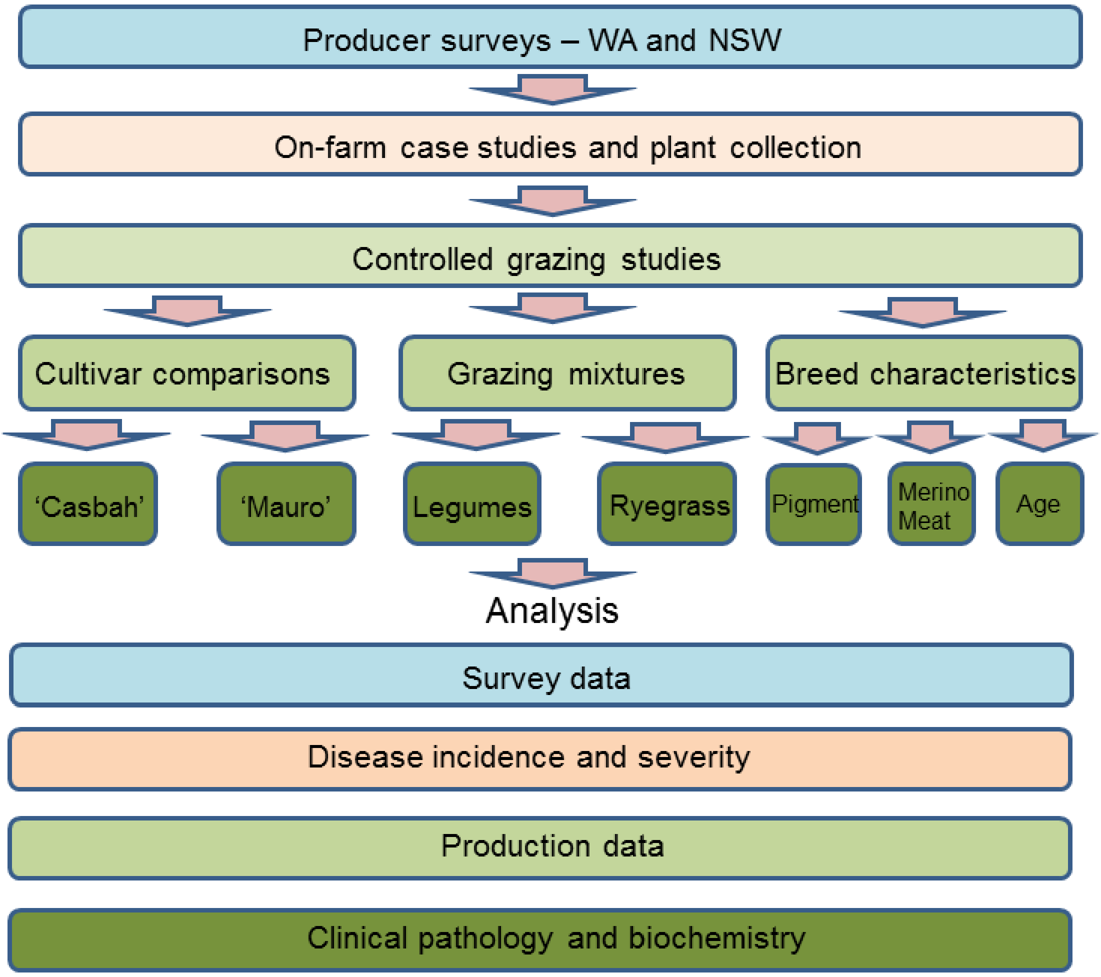

Figure.1. Experimental design of producer data collection, on-farm outbreak analysis and controlled grazing trials.

3.3.2 Location and season

The first study in this series was carried out during October - November 2013 at the Department of Primary Industries Wagga Wagga research farm, Gobbagombalin, New South Wales, Australia (35˚ 03' S, 147˚ 18' E).

3.3.3 Meteorological monitoring

Minimum / maximum and mean annual temperature data were reported from the Bureau of Meteorology Wagga Wagga Agricultural Institute weather station (073127). Mean daily global solar exposure was 21.12 MJ/m*m, with a minimum of 3.6 MJ/m*m and maximum of 27.1 MJ/m*m for the duration of the trial. Approximately 391 mm of rain was recorded at the Wagga Wagga Agricultural Institute weather station (35˚ 03' S, 147˚ 35' E) in 2013 (2014).

3.3.4 Pastures

Plots were sown in early June with either subterranean clover or *Biserrula pelecinus* L. cultivar ‘Mauro’. Subterranean clover was sown as a 50:50 mixture of cultivars ‘Riverina’ (Upper Murray Seeds Pty Ltd, Australia) and ‘Bundoon’ (PG Wrightsons Seeds Pty Ltd, Australia). The *Biserrula* cultivar sown on all plots was *‘Mauro’* (Upper Murray Seeds Pty Ltd, Australia) at standard commercial rates (10kg/ha) plus 8kg/ha Alosca granular innoculant (Alosca Technologies Pty Ltd, Australia).

At sowing, 32kg/ha of phosphorus was applied, and 12kg/ha of urea to maximise plant growth. After sowing, plots were sprayed with Astral plus Imidan at a rate of 300ml/ha (Crop Care Australasia Pty Ltd) to protect against red-legged earth mite and lucerne flea. Plots were irrigated to extend the growing season, but only after the grazing experiment described in this report was completed.

Pastures were fenced into plots of 0.7ha in size in a randomized block design with pastures present in triplicate. Water was freely available at all times via automatic drinkers. No shade was available in any of the treatment plots.

Pasture combinations available as fodder were: 1) subterranean clover dominant; 2) 50:50 biserrula : subterranean clover dominant; 3) biserrula dominant. Split plots were established by fencing once pastures were established, In all cases, plots contained some volunteer species contamination. Proportions of pasture species were analysed by performing Botanal analysis.

3.3.5 Animals

This experiment was approved by the Charles Sturt University Animal Care and Ethics Committee, protocol number 13/018.

A total of 74 (male = 36, female = 38) 6 month old lambs were included in the experiment and were stratified by empty weight across the three treatments. Inclusion criteria were a healthy appearance at yarding with no visible pathology. Three sheep, selected as those of highest body weight of each genotype: Merino (n = 36, live weight 23.5 ± 3.5 kg) or White Sussex x Merino first cross lambs: (n=38, live weight 37.7 ± 4.7 kg) were examined from each of the control plots (1 & 2) and 6 sheep of each genotype were examined from each of the biserrula and subterranean clover plots.

3.3.6 Sampling and analysis – pasture

Samples of biserrula plant material were collected on date of entry to the trial and weekly during the trial period. A further set of samples was collected 2 weeks after animals had been removed from the trial. All samples were collected fresh and placed on ice immediately. They were transported to the laboratory and flash frozen before storage at -80^{o}C.

Crude replicated extractions were performed using 5g of fresh chopped plant material in methanol using a Buchi Extractor. Crude extracts were then rotary evaporated to 50% of original volume at 35 ^{o}C and stored in the dark at 4 ^{o}C for further analysis (see 3.7.1 below).

3.3.7 Biological sampling and analysis – animals

A venous blood sample was taken from all animals on entry to the trial. Blood was collected from either the right or left jugular vein as determined by convenience and sampler preference. Needle hubs and sterile, single-use 21G needles and 10 mL Vacutainer® tubes were used for blood collection. Two Vacutainer® tube types were used for each animal: one containing a clot activator for serum collection, the other contained ethylenediamine tetra-acetic acid (EDTA) to prevent clotting. Tubes were inverted repetitively immediately after collection prior to storage on ice for transport to the laboratory. EDTA tubes were kept chilled until processing. Serum blood tubes were allowed to clot for at least 30 minutes before serum separation by centrifugation. All samples were transported and processed within 60 minutes of collection.

Haematological and biochemical analysis was performed by the Veterinary Diagnostic Laboratory (Charles Sturt University, Wagga Wagga, NSW Australia) within 60 min of blood collection. Leukocyte differentials were reported using manual differentials of blood smears. Other haematological analytes were measured using a CellDyn 3700 Haematology System (Abbott Diagnostics, Abbott Park, Illinois, USA). Biochemical analysis was performed using a Konelab 30i clinical chemistry analyser (Thermo Electro Corp., Vantaa, Finland), using reagents from Thermo Scientific. See Appendix 2 for parameters and analytes measured. A second blood sample was taken on exit from the trial and processed as described. Lamb weight and body score were also recorded on entry to and exit from the trial.

Animals were scored for clinical signs of photosensitisation using a standardized scoring system (see Appendix 3) after 96 h on pasture and then routinely every 48 h until removal from the trial. Grazing behaviour was also noted at similar intervals.

Animals were either removed from the trial on the basis of clinical signs of moderate to severe photosensitisation, or at the end of the trial period (15 days).

At the conclusion of the trial all animals were subjected to a tissue biopsy taken from the lateral margin of the ear (from one ear for treatments 1 & 2, or both ears for treatment 3 above) and samples processed for histopathological analysis through the diagnostic laboratory. In addition, one animal of each genotype was removed from pasture in duplicate and subjected to routine necropsy, including collection of samples for histopathology and sampling.

Necropsies were performed at the Veterinary Diagnostic Laboratory, Charles Sturt University, Wagga Wagga. Prior to euthanasia, a full clinical examination was performed. Animals were euthanased using intravenous Lethabarb into the jugular vein (20mL/40kg live weight).

3.3.8 Punch biopsy

Sheep were restrained individually as for venipuncture. An appropriate biopsy site was located on each ear, at the lateral margin, away from blood vessels or thick cartilage, and circled as a landmark using a pen. Using a 25G needle, 1 ml of Ilium Lignocaine 20 (Troy Laboratories) (20 mg/mL) was applied subcutaneously. A biopsy was cut using a 6mm sterile single-use biopsy punch. Scissors were used to detach subcutaneous tissues as required. Tissue samples were immersed in 10% fixed formalin within individually labelled sample pots and submitted for histopathology. Topical antiseptic and fly repellent (0.1% cetrimide, Di-N-propylisoncinchomeronate, diethyl toluamide -Cetrigen, Virbac Animal Health Pty) was applied liberally to the wound.

3.3.9 Statistical analysis of biochemical parameters

Reference intervals were defined for this experiment based on samples taken from 62 healthy 6 month old crossbred lambs as the interval containing the central 95% of data obtained for each analyte after the exclusion of outliers. Outliers were excluded based on the method proposed by Dixon (Dixon, 1953), and modified by Reed, Henry & Mason (1971). Distributions of the outlier-excluded values were tested for normality using the Kolmogorov-Smirnov test using IBM SPSS™, Version 20.0.0. A Kolmogorov-Smirnov value > 0.050 was the criterion for describing the data as a normal distribution. For normally distributed data, the mean and standard deviation values are stated. For non-parametrically distributed data, the median values are given. All data were subsequently tabulated to decimal places that were measurable given the laboratory techniques used.

For trial animals, haematological and biochemical analytes were as groups compared using student t-tests as two-tailed datasets, using Microsoft Excel. Pre- and post-experimental bloods were compared using SPSS™. Significance was defined as $p < 0.05$.

3.4 Controlled grazing trial 2 - 2014 Spring grazing trials using crossbred and merino lambs: comparison of two cultivars of biserrula ‘Casbah’ and Mauro’ and effect of pigmentation on expression of photosensitisation in meat lambs

3.4.1 Location and season

Two cultivars of *Biserrula pelecinus* L*., cv.* ‘Casbah’ and ‘Mauro’ were produced in a formerly established long-term cropping paddock at CSU Commercial Farm for use as a pasture for the 2014 grazing season. Planting occurred in a paddock that was previously sown with dual purpose wheat in 2013. The site contained up to 10% infestation of annual ryegrass (*Lolium rigidum*) that potentially exhibited resistance to multiple classes of post-emergent herbicides and also a very low infestation of other weedy species (<5%). All trial plots were sown according to standard commercial practices on 16 May 2014. Prior to sowing, plots were treated with a pre-emergent herbicide and then the stubble burnt and light tillage performed. Trial sites were sown with 10kg/ha of commercially available scarified biserrula cultivars ‘Casbah’ or ‘Mauro’ with 8kg/ha of Alosca ‘biserrula special’ inoculant. Plots were treated post-sowing with Talstar insecticide (bifenthrin) at 100ml/ha on 21 May 2014 to prevent Lucerne flea. Four replications of paired sub-plots were established for each cultivar, with 8 plots in total in a block design for plant biomass and biochemical parameters. Sub-plots were approximately 0.7Ha in size.

3.4.2 Animals

Sixty-four first cross lambs (Dorper x Suffolk) were included in the trial. Animals were either pigmented (33 Dorper x Black Suffolk) or white (31 Dorper x White Suffolk) with comparable numbers of ewes and whether lambs in each group. Pigmentation ranged from light (fawn face and ears), through moderate (patchy dark pigmented areas on face and ears) to full (dark brown face and ears). Crossbred lambs were purchased from a commercial flock and born in early June 2014. Animals sorted into plots by restricted randomisation by gender and pigmentation, giving equal numbers of all representative characteristics to each treatment plot (8 in total, of equal sex and pigment ratios where possible). A mob of Merino ewes with late drop lambs at foot (July drop) were also recruited to the trial. Five ewes were allocated to each trial plot and with their lambs. A maximum of 6 lambs were present on any plot with plots receiving 5 ewes and between 3-6 lambs. Overall stocking densities were between 15.5 - 17 DSE/ha.

3.4.3 Sampling and analysis – pasture

Pasture samples were collected from mid- September until trial termination; parameters monitored included kg DM/ha, plant height, digestibility, and number of flowering heads/25 cm^2. Plant samples were collected at bi-weekly intervals during the grazing season for later photochemical analysis.

3.4.4 Sampling and analysis – animals

On trial initiation, venous blood was collected by jugular venepuncture, and bodyweight recorded. EDTA preserved blood and serum were placed on ice and transported to the laboratory for complete blood count (CBC) and serum biochemical analyses to be performed within 4 hours of collection. Blood was also taken from clinically affected animals as well as unaffected pigmented animals in similar plots when first signs of photosensitisation were observed.

Body weight of each animal was recorded initially at time 0, at 14 days on pasture, and every 7 days thereafter, weather permitting, until trial completion. Animals were also scored individually for clinical signs of photosensitisation (See Appendix 3). Faecal samples were collected on entry and exit from the trial for analysis of worm egg count and larval culture. Exit from the trial was determined by limitation of Feed on Offer (FOO). Total days grazing on pasture for each group was 37 days for crossbred lambs and 28 days for Merino ewes and lambs at foot.

3.4.5 Meteorological monitoring

Minimum / maximum and mean annual temperature data were reported from the Bureau of Meteorology Wagga Wagga Agricultural Institute weather station (073127). Mean daily global solar exposure was 17.72 MJ/m*m, with a minimum of 5 MJ/m*m and maximum of 23.6 MJ/m*m for the duration of the trial.

3.4.6 3.4.6. Statistical analysis

All data were collated and analysed as described in section 3.3.7.

3.5 On-farm photosensitisation outbreak investigation, NSW 2013

3.5.1 Location and season

This case study was sampled in September 2013 at a commercial farm near Mirrool, New South Wales, Australia (34˚ 14’ S, 147˚ 6’ E). Approximately 278 mm of rain was recorded at nearby Beckom weather station in 2013, including 33 mm for the month of September (2014).

3.5.2 Animals

The mob consisisted of a mixed-sex 120 head flock of 20 week old South African Mutton Merino (SAMM) x Dorset lambs, born in March / April 2013. Lambs had been grazing paddocks which contained mixture swards of *Biserrula pelecinus* L. *var.* 'Casbah', perennial ryegrass and Mediterranean subclover since lambing. Two weeks prior to sampling, the flock was transferred to a monoculture of biserrula, and observed every 2-3 days.

Clinical signs included swelling (oedema) and drooping of the ears, accompanied by a patchy alopecia in sun exposed skin of the face and external ears in approximately 25% of the flock. Five days later animals were moved to a pasture in which the biserrula stand was less dense (approximately 90%), and at an earlier stage of growth (late vegetative stage as opposed to flowering stage in the affected paddock). The most severly affected sheep showed variably extensive scabbing on the sun exposed unpigmented skin of the external ear and/or tissue loss (Fig. 4.7). Two days later 10 animals showing the worst clinical signs, and 5 showing no clinical signs (classified as 'subclinical') were identified and separated from the flock for further investigation.

3.5.3 Biological sampling

Venous blood samples were collected and processed, and necropsy performed as described above (section 3.3.7). Blood was collected from both clinical and subclinically affected lambs (n = 15) from the jugular vein, and EDTA preserved blood and serum were placed on ice and transported to the laboratory for a complete blood count (CBC) and serum biochemical analysis within 4 hours of collection (Table 4.1). In addition, 5 animals showing clinical signs were transported to the Veterinary Diagnostic Laboratory at Charles Sturt University, where they were housed overnight with free access to water and cut samples of their original *Biserrula* pasture. They were euthanased the following day by an intravenous barbiturate injection and subjected to a complete necropsy, where samples from a wide range of tissues were placed in 10% formalin for subsequent histopathological examination.

3.5.4 Statistical analysis

The values for each analyte were tested for normality using the Kolmogorov-Smirnov equation, using $p > 0.050$ to indicate a normal distribution. Levene's test was performed for homogeneity of variance between the clinical and subclinical groups, with values $p > 0.05$ indicating equal variance. Haematological and biochemical analytes were then compared by student's two-tailed t-test using IBM SPSS, Version 20.0.0.

3.6 Analysing temperature-dependent growth characteristics of *Biserrula pelecinus* under controlled growth conditions

Seed of *B. pelecinus* L. *cvs* 'Casbah' and 'Mauro' were planted in 1.5 L pots containing 6:4 potting mix:sand and inoculant, which were then well watered with Yates soluble fertiliser according to manufacturer's instructions. Pots were transferred to three growth chambers maintained at one of three temperature regimes: 20 day/15 night, 25 day/20 night, or 30/25 °C day/night temperatures for 14 h/10 h day/night regimes. Twelve specimens of each cultivar were placed in each chamber in six groups of four plants each, with two specimens of each cultivar in each group (positions regularly randomised within groups). Plants were watered as needed by sub-irrigation. Growth data were recorded for 12 weeks, after which the plants were harvested. Plant height (tallest plant to the nearest cm) was measured in each pot. Eight specimens of each cultivar were selected at random (excluding any plants in poor condition) and two additional measures were recorded: fresh weight (to the nearest 0.1g), and vigour, which was assessed visually and assigned by visual rating from zero (dead, brown senesced leaf tissue) to 5 (healthy, bright green leaf tissue). Data were analysed by factorial ANOVA (with temperature and cultivar as the factors) and polynomial regression to ascertain the relationship between temperature and growth parameters measured for each of two cultivars.

4 Results

4.1 Understanding incidence and distribution of outbreaks of photosensitisation in sheep and cattle associated with ingestion of biserrula. Producer survey.

A total of thirty two (32) producers responded to the survey; 23 in Western Australia and 9 in New South Wales. Their geographical location and self-reported prevalence of photosensitisation, are identified in Figs 4.1 and 4.2. Crosstab analysis showed a significant correlation between state and prevalence ($p = 0.023$), with a significantly higher prevalence observed in WA than NSW.

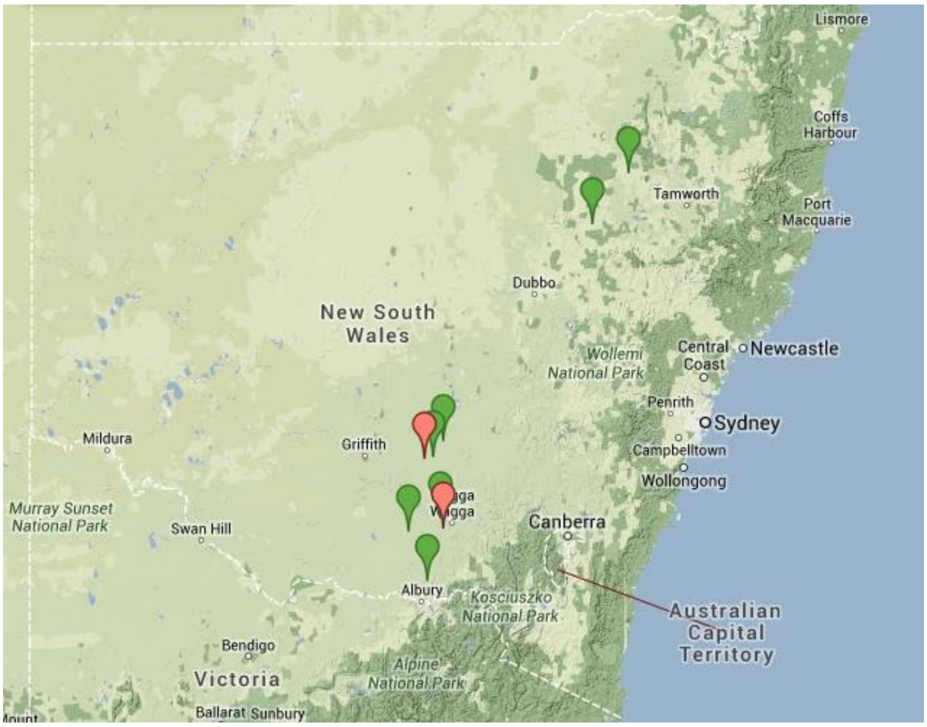

Figure 2. Geographical distribution of NSW biserrula producers (n = 9). Green, no photosensitisation reported; red, photosensitisation reported.

Figure 3. Geographical distribution of WA biserrula producers (n = 23). Green, no photosensitisation reported; orange, incidence unknown; red, photosensitisation reported.

Both wool (n=27) and meat (n=20) sheep producers were represented in the respondents, but only one beef cattle producer was present in the cohort. Most (31/32) survey respondents had sown cultivar 'Casbah' with the majority of pastures part of a crop rotation (93.3%), and most paddocks (70%) planted prior to 2008. Clay was the dominant parent soil material (33.3%) and the soil texture was typically either sandy loam (46.7%) or light clay (26.7%). Only 33.3% of producers had a current soil test certificate. The majority of paddocks had been treated with some type of applied fertiliser (92.6%). Crosstab analysis indicated no statistical correlation between soil type and prevalence of photosensitisation reported, and no correlation with soil type or production parameters (age, breed, sex of animals, size of property).

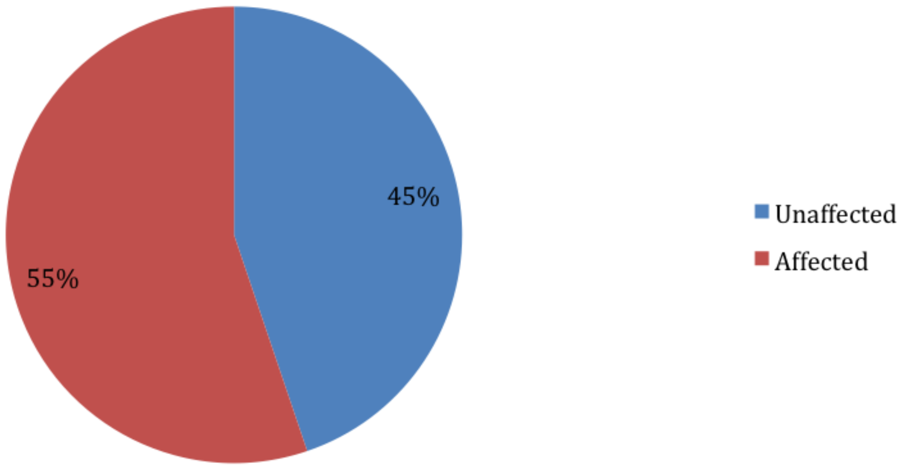

Figure 4. Percentage of livestock that had experienced photosensitivity whilst grazing biserrula, as indicated by survey responses. Three respondents did not answer the question (n=29).

Survey results identified that 55.2% of producers responding to the survey, and grazing livestock on biserrula, had experienced outbreaks of clinical photosensitisation (Fig. 4.3). Sheep of all ages and sexes were reported as affected, although ewes were the most common cohort reported to be affected (81% reported affected ewes, with 75% affected lambs of any age) (Fig. 4.4). Together, 80% of cases being identified by the producer as complying to descriptions of either mild or moderate photosensitisation (Fig. 4.5). No farmers reported photosensitisation likely to be caused by other plants grazed concurrently.

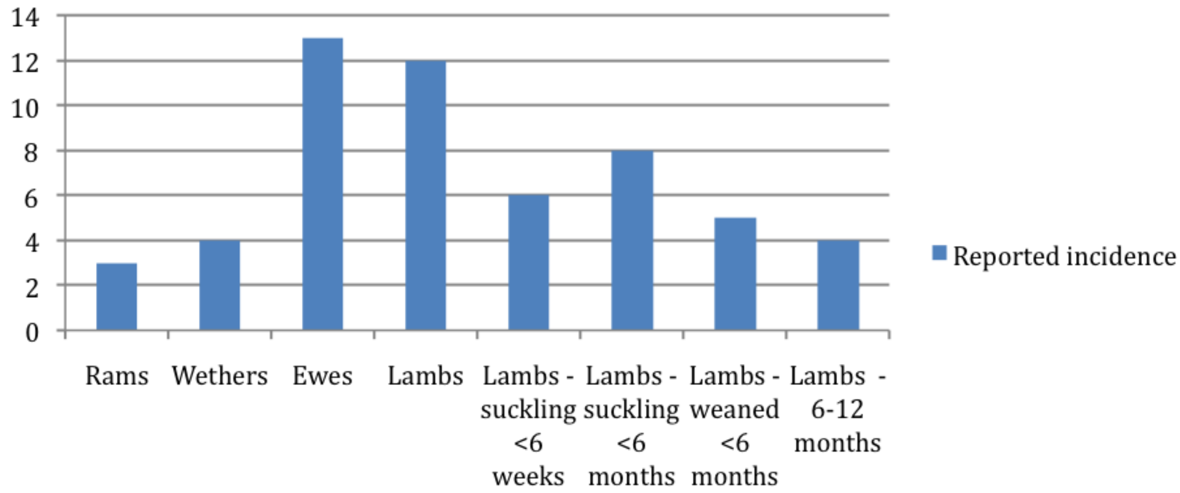

Figure 5. Prevalence in stock reported to be affected by photosensitisation, from 16 producers that reported photosensitisation in any stock class.

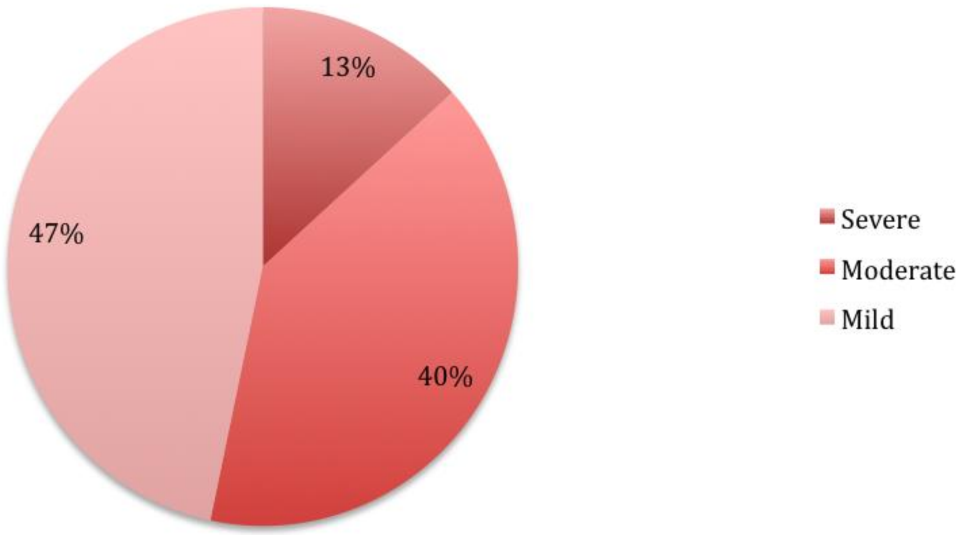

Figure 6. Self-reported severity of observed photosensitisation outbreaks. 46.7% of affected animals were described as 'mildly affected', 40% were 'moderately affected' and 13.3% were 'severely affected'.

Only 18.8% of producers that experienced photosensitisation contacted a veterinarian to confirm the diagnosis. On observation of photosensitisation in their livestock, 81% (13/16) of producers moved the animals to an alternate pasture, whilst 13% took no action. One producer did not answer this question. One producer (6%) also reported contacting a veterinarian at this point (once animals had been relocated to alternative grazing).

Thirty-two producers responded to the survey and while the sample size is not sufficiently large, it does give a representative sample of biserrula producers in Australia and represents 25% of the target population. More producers responded to this survey than had been achieved in the past where only 14 respondents from WA only were recruited (Hogg & Davis, 2009). The combination of survey distribution method (personal posting and / or email) with follow-up may have contributed to this relative success.

The vast majority of producers responding to the survey (31/32) reported use of the 'Casbah' cultivar of biserrula. This is indicative of the relative longevity of this variety in Australia compared to the more recent introduction of 'Mauro'. This profile remains similar to results obtained in a previous producer survey, where the majority (93%) of respondents grew 'Casbah' biserrula (Hogg & Davis, 2009). An unexpected outcome was that a majority of producers had experienced livestock photosensitisation when grazing biserrula (55%), and that all classes of animals were affected. This is a higher percentage than might be anticipated from previous anecdotal reports from the literature where incidence of photosensitisation has been described as 'sporadic' and that it does not present more commonly in any one animal population.

A small number of producers confirmed their diagnosis of photosensitisation with a veterinarian (18.8%). Low involvement of veterinarians in diagnosis and treatment of clinical cases suggests information on best practice for management of outbreaks needs to be delivered through agricultural extension officers, farmers groups and agronomists, as well as via veterinary channels.

4.2 Investigation of an on-farm outbreak of photosensitisation associated with ingestion of biserrula in sheep

Figure 7. *A* dominant pasture of *Biserrula pelenicus L. cv. '*Casbah' associated with an outbreak of primary photosensitisation in September 2013.

Figure 8. Lesions in the sun exposed skin of the pinnae in 4 of the affected lambs. Lambs A and B show severe crusting and desquamation of this skin, whilst lamb C is a more chronic lesion as crusts have lifted from the healing underlying skin. Note the healed pinnae in lamb D, although the apical tip has been lost.

Values for electrolytes, and the WBC differential were all within normal reference ranges, and were not significantly different between affected and unaffected animals. There was no significant difference between clinical and subclinical animals in any CBC values. No elevation in bilirubin or gamma glutamyl-transpeptidase (GGT) was recorded. Two lambs (12 and 15) showed very mild elevations in glutamate dehydrogenase (GLDH). GLDH enzyme is considered specific for hepatocellular necrosis, but this was not confirmed histologically in these animals. Six of the clinically affected animals also had mild elevations in aspartate aminotransferase (AST) which may be released from damaged hepatocytes and muscle cells; three of these lambs also had elevations in the muscle specific enzyme creatinine kinase (CK), two of which were marked (see Table 4.1).

Gross post mortem findings in the clinically affected lambs were confined to the conjunctiva and skin of the face and ears, although one lamb had an area of wool loss in the skin of the dorsal lumbar midline, and one lamb had a focal 1 cm area of ulceration and bleeding in the skin of the upper eyelid (this lesion suggestive of self trauma). All clinically affected animals showed moderate bilateral reddening of the conjunctiva and variable epiphora, some animals also showed mild swelling and crusting over the midline, hairless anterior portion of the muzzle. All animals showed variable, and sometimes marked changes within the skin of the sun exposed dorsal surface of the ear that consisted of extensive crusting and ulceration. In some animals complete loss of a variable portion of the distal pinna was observed (Fig. 4.7). All other organ systems were closely inspected and judged to be grossly of normal morphology, including the liver.

Significant histopathological lesions were confined to the sun exposed skin of the face and ears in the grossly affected lambs, where there was a variably severe necrotising, crusting and pustular dermatitis accompanied at times by re-epithelisation (Fig. 4.8). In all animals the non-pigmented skin of the face contained scattered small intracornual accumulations of degenerate neutrophils, accompanied by a mild perivascular lymphoid infiltrate in the superficial dermis. In the non-pigmented sun exposed skin of the external pinna there were variably severe changes. Mildly affected skin contained scattered intracornual pustules, that alternated with areas in which the skin surface was variably covered by a crust that consisted of serum admixed with neutrophils, which was either sitting on top of, or within, the cornified epidermal layer. In more severely affected areas there was a variable coagulative necrosis of the epidermis and apical dermis, characterised by hyper-eosinophilia with retention of architectural details but loss of nuclear staining. Necrosis often extended into the apical 1/3rd of the follicle, and was accompanied a similar necrosis within adnexal dermal elements, and sometimes partial extrusion of hyper-eosinophilic necrotic sebaceous glands into the follicular lumen. In worst affected areas shed necrotic epidermis and superficial dermis combined with the sero-neutrophilic crust to form a variably thick “shed” layer sitting on top of a variably ulcerated dermis (Fig. 4.8). In most animals there was a mild superficial dermal fibroplasia, and in some early attempts at surface re-epithelisation were evident.

Table 4.1. CBC and selected biochemical values from subclinical (animals 1-5) and clinically affected sheep (animals 6-15). Animals 11-15 were submitted for necropsy as being the most severely affected cases. Values in **bold** are outside the normal reference range.

	Animal															
Analyte	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**Reference range**
PCV	0.36	0.37	0.39	0.39	0.37	0.34	0.34	0.36	0.37	0.38	0.34	0.34	0.35	0.30	0.32	0.29-0.40 L/L
WBCC	6.3	6.8	8.7	5.5	7.7	6.4	7.3	8.2	5.1	6.4	6.1	7.8	6.4	6.6	8.3	4.1-13.0 x 10^9/L
Fibrinogen	4.0	7.0	8	6	9	3	8	9	5	6	8	5	3	8	3	3-8 g/L
Tot Protein	71	68	74	70	72	68	70	74	73	68	70	71	73	**58**	67	60/82 g/L
Albumin	40	**42**	41	39	37	39	**42**	40	39	39	38	34	38	**18***	38	29-41 gL
Globulin	31	26	33	31	35	29	28	34	34	29	32	37	35	40	29	30-42 gL
Bilirubin	3	3	4	3	3	2	3	3	2	3	2	3	2	3	2	<3□mol/L
GLDH	31	10	6	**37**	10	10	5	14	14	31	28	**68**	8	3	**38**	0-33 U/L
GGT	44	55	62	46	58	54	91	63	70	54	58	59	51	68	77	47-95 U/L
AST	**149**	**146**	**149**	**141**	117	119	103	**149**	**163**	**139**	**201**	112	129	**341**	**141**	59-130 U/L
CK	714	561	933	548	**1428**	546	385	**1397**	**10007**	992	**10489**	269	394	574	589	0-1194 U/L
Urea	5.7	4.9	7.5	6.2	4.9	5.9	6.1	6.6	5.2	5.3	3.7	6.0	5.2	5.0	7.3	5.0-11.4 mmol/L
Creatinine	57	68	65	65	63	52	58	55	52	51	41	43	46	50	48	44-72 □mol/L
USG	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	1.022	1.044	1.040	1.014	1.026	

* Animal found to have multiple ileal ulcerations at necropsy, which might explain this hypoalbuminaemia.

NT, not tested; PCV, Packed Cell Volume; WBCC, white blood cell count; GLDH, glutamate dehydrogenase; GGT, gamma-glutamyltransferase; AST, aspartate transaminase; CK, creatinine kinase; USG, urine specific gravity.

Figure 9. High power view of epidermal crust, which consists of serum admixed with large numbers of neutrophils. Note some adnexal elements within the crust: sebaceous glands (smaller arrows) and follicular structures (larger arrows)- indicative of coagulative necrosis of the dermis, with subsequent shedding and re-epithelialisation.

Incidental histological changes found in other tissues of affected animals consisted of small numbers of nematode worms consistent with *Teladorsagia sp*. within the abomasum, small numbers of coccidial life cycle stages within the small intestine, and a mild, multifocal lymphocytic periportal infiltration in the liver, which could be considered background lesions in lambs of this age. Sections of diaphragm and tongue in all necropsied animals showed a mild multifocal lymphocytic myositis with intralesional sarcocysts. Additional skeletal muscle samples from the hind leg, foreleg, and psoas muscle were examined in lamb 11 because of the marked elevation in CK noted in the biochemical results, and the only lesions found were the mild myositis associated with the sarcocysts noted in all necropsied lambs, which was considered a background lesion in this age of lamb.

No hepatic involvement was identified in association with this on-farm outbreak. Histologically there were no significant hepatic lesions within any of the clinical animals sampled. These findings strongly suggests that ingestion of *Biserrula pelecinus* L. *cv.* ‘Casbah’ causes primary photosensitivity in domestic livestock.

4.3 Controlled grazing trial 1 – spring 2013 grazing trial using cross-bred and merino lambs

4.3.1 Defining clinopathogenesis of photosensitisation

Biserrula monoculture plots (plot numbers 3, 8 and 12) were not uniform in their consitution; feed analysis showed biserrula to contribute 88%, 83% and 80% respectively to each of these areas. After 4 days on pasture, 11 of the 12 animals grazing in plot 3, the area which contained the highest proportion of biserrula , showed clinical sign of mild/moderate photosensitisation (Figs 4.9A and B) as graded by the scoring system shown in Appendix 3.

The mean PS score of clinical cases was 5.41, with individual animals ranging from 0 to 13. The most severely affected animals were removed from the trial at this point and subjected to necropsy (Fig. 4.9). The animals remaining on biserrula plot 3 were noted to have corneal ulceration,lachrymation, swollen eyelids and muzzles, drooping/oedematous ears and mild skin lesions(Fig. 4.10).

The remaining animals were allowed to continue grazing *Biserrula* as they were not deemed sufficiently badly affected to warrant withdrawal from the trial. Their clinical signs began to resolve with reducing ambient temperatures.

Fifteen days after entry to the trial, all animals were removed from their plots, scored for signs of photosensitisation, routine blood samples taken for haematology and biochemistry and tissue biopsies removed from one (subterranean subclover only or biserrula:subterranean subclover split plots) or both ears for histopathological analysis (biserrula only plots). A total of 12 animals were selected for routine necropsy, two of each genotype from each treatment. Other than lesions to the ears, no remarkable findings were noted at necropsy in any animal from any pasture.

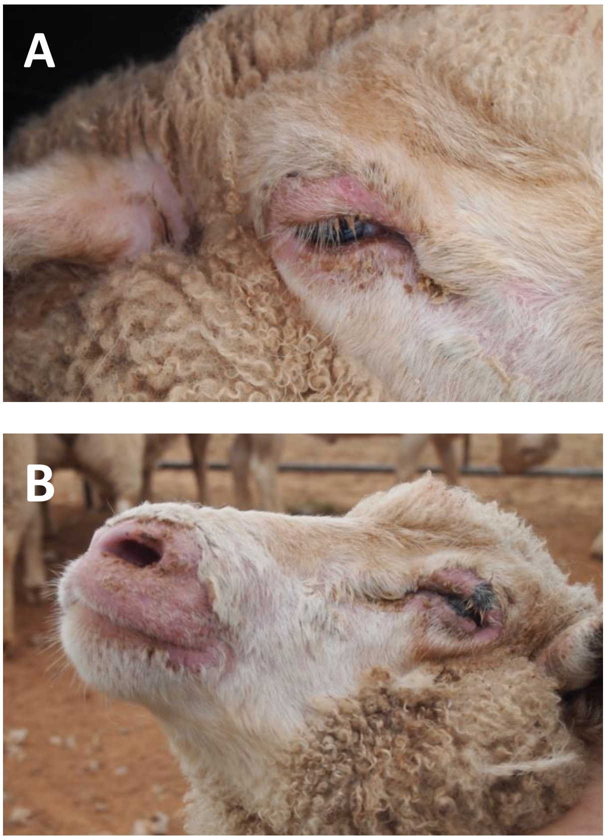

Figure 10. Early A) mild and B) moderate signs of photosensitisation in sheep grazing Biserrula elecinus. A) Swelling and reddening of the skin around the eyes, crusting around the eyes. The pinnae of the ears are mildly swollen. B) A more severely affected animal showing swelling around the eyes and muzzle, pinnae were oedematous and blackened around the distal margins and mild corneal ulceration was apparent.

Ambient temperatures were high during the first 96 hours of the trial with temperatures peaking on day 3 at 35 ºC. After this temperature spike, ambient temperatures began to fall, stabilising around 25 ºC for the remainder of the trial. This had a significant impact on the progression of clinical signs as it was that clinical signs of photosensitisation began to resolve as ambient temperatures dropped.

Figure 11. (A-I) Early, mild clinical signs of photosensitisation in lambs grazing biserrula dominant pastures. Key observations are blephitis, redding arounf the margins of the eyelids, and moderate excudate from the eyes which was be observed to stain the fleece of the face.

4.3.2 Haemaology and biochemistry

A reference range of parameters was established from the initial sampling to ensure that the age/sex/genotype bias of the experimental cohort was taken into account for subsequent interpretation. All haematological and biochemical parameters were compared using a multivariate analysis to take into account effects of both genotype and plot, using SPSS™.

No effect of genotype was observed on any parameter measured in the pre-trial or post-trial bloods. Haematology showed no significant changes in any parameter analysed between the treatment groups pre- or post-trial. Significant differences were observed between treatments in a number of biochemical parameters analysed including fibrinogen, creatinine, AST and bilirubin. However, none gave rise to biochemical changes indicative of significant (or any) pathology (Fig.12).

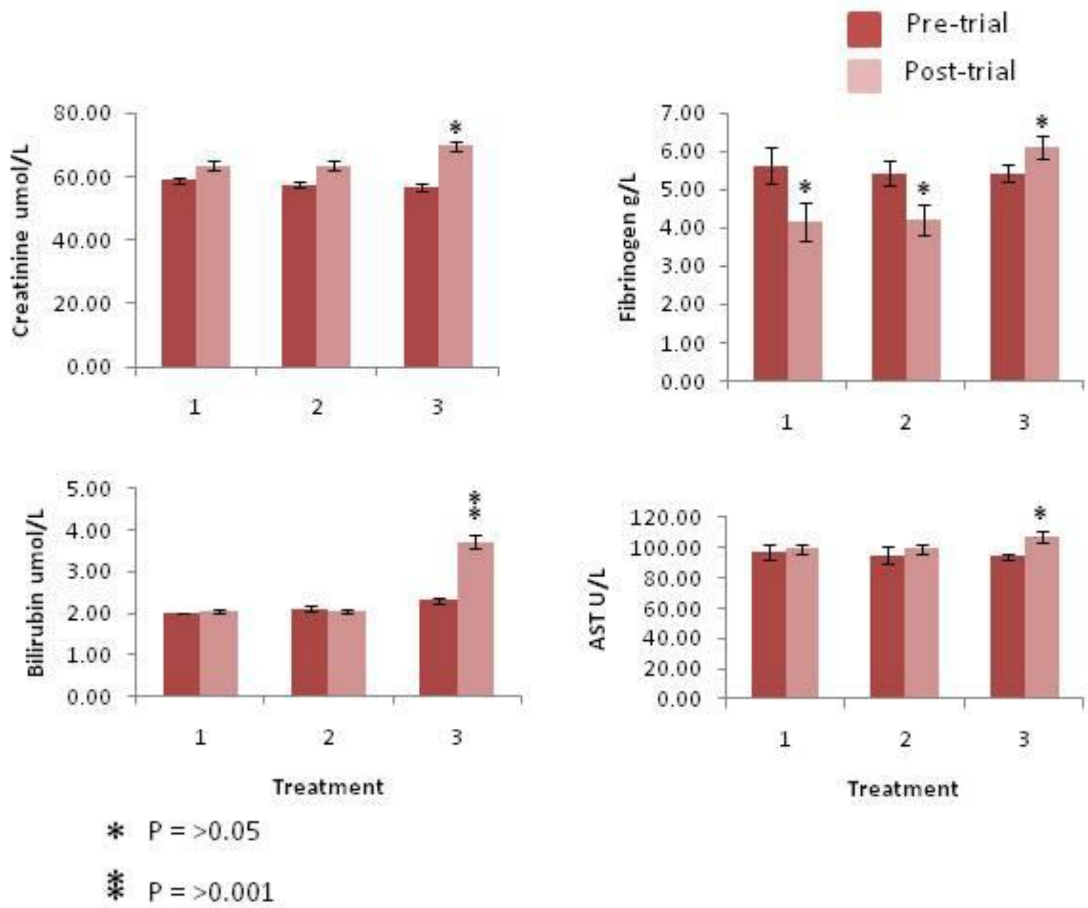

Figure 12. Biochemical analytes from venous blood samples collected prior to and at the end of the trial period. Animals grazing on biserrula pelecinus 'Mauro' (treatment 3) showed significant increases in creatinine, fibrinogen, bilirubin and AST although only bilirubin values fall outside new reference ranges for the experimental cohort (treatment 3: mean bilirubin 3.72, s.e.m. 1.76 µmolL/, n = 72; reference range 1.8-2.6 µmol/L).Although differences between groups were significant, the parameter values were still within what is considered the normal range (see Appendix 2). Treatments: 1) subterranean clover dominant; 2) 50:50 biserrula : subterranean clover dominant; 3) biserrula dominant.

In particular, no significant differences were observed in liver enzyme activity between animals grazing biserrula pastures and control pastures suggesting that liver pathology is not induced by ingestion of *B. pelecinus* L. 'Mauro".

4.3.3 Pathology

The most severely clinically affected animal (shown in Fig. 10B) was found to have severe, multifocal coalescing abcessation of the liver with neutrophilic cholangitis. It is possible that this pathology contributed to the early and severe onset of photosensitisation in this animal. Skin lesions on this animal showed moderate to severe dermal and epidermal superficial necrosis with early multifocal re-epithelialisation consistent with an ongoing photosensitiation

(Ladmore, 2014). Because of the underlying liver pathology, this animal should be excluded from the trial results. No liver lesions were seen any of the other animals examined by necropsy.

Histological examination of tissue biopsies taken from the lateral margins of the ear of animals grazing on control or monocultural stands of biserrula showed histological changes consistent with early, acute changes resulting from dermal photosensitization. These included mild oedema at the dermal-epidermal junction with variable amounts of haemorrhage and epidermal thickening (Ladmore, 2014, Kessell et al., 2015).

4.4 Controlled grazing trial 2

4.4.1 Spring 2014 – two cultivar comparison and effect of pigmentation on expression of photosensitisation

Animals entered the trial on 24 September 2014 (crossbred lambs) or 2 October 2014 (Merino ewes and lambs), orientation of plots are shown in Fig. 13. In all cases FOO had reached or exceeded 1800 kg/DM/ha (Fig. 14). Plants of both cultivars were at late vegetative/early flowering stage by analysis of flowering composition (Fig. 15).

Figure 13. Orientation and Biserrula cultivar (M, 'Mauro'; C, 'Casbah') associated with grazing trial plots 2014. Four replicates of each cultivar were further divided into subplots for 8 total sub-plots monitored for plant growth and biochemical analyses.

First clinical signs of photosensitivity were noted in unpigmented animals only, grazing ‘Mauro’ and ‘Casbah’, on day 3 and 7, respectively. Onset of clinical signs was not strongly correlated with dramatic increases in ambient temperature (Fig. 16) suggesting that relative total (high) intake of novel pasture plays an important role in onset of clinical signs rather than high ambient temperatures. In particular, onset of clinical signs in animals grazing ‘Mauro’ was associated with maximum daily temperatures under 25 ^{o}C. Interestingly, there was a marked palatability difference between ‘Mauro’ and ‘Casbah’ with ‘Mauro’ being significantly more palatable that ‘Casbah’. This is reflected in pasture composition data collected during the trial where presence of ‘other’ species in pasture remains constant in proportion in ‘Mauro’ plots suggesting equal grazing pressure on both biserrula and other species, whilst biserrula becomes dominant in ‘Casbah’ where preferential grazing of ‘other’ species reduced their contribution to the pasture over time (Fig. 4.13). i.e. Mauro appears to be more palatable than Casbah.

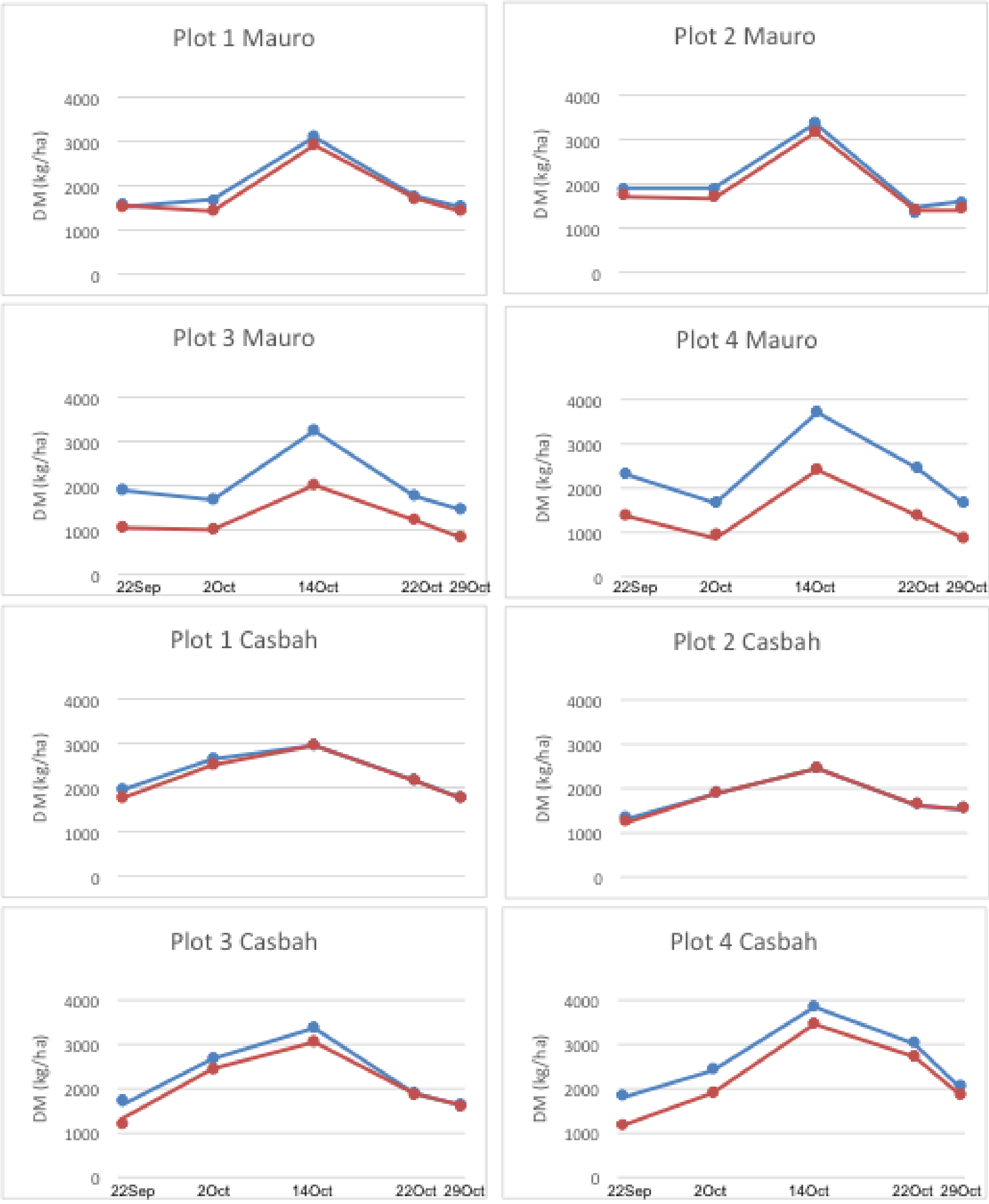

Figure 14. Plot composition of biserrula plots for 2014 grazing trial. Blue line represents total biomass for each plot with the red line representing biomass associated with biserrula alone. 'Casbah' plots 1-4 show convergence of data points indicating reduction (to zero in plots 1 and 2) of biomass associated with volunteer species, with lines reaching convergence by the middle of the trial in 'Casbah' plots 3 and 4. Data lines fail to reach convergence in Mauro plots 1 and 2 until the end of the trial, and never reach convergence in plots 3 and 4, suggesting potentially greater palatability of 'Mauro' and reduced grazing of volunteer species in these plots. Agronomic parameters were monitored by Dr Belinda Hackney, Central West Local Landcare Services.

The difference in photosensitization observed among cultivars suggests that in ‘Casbah’ plots, grazing sheep exhibited a preference for weedy volunteer species rather than biserrula itself. This hypothesis is supported by the observed estimated reductions in proportion of biomass contributed by volunteer species across the duration of the trial in plots sown to ‘Casbah’ (Fig. 14).

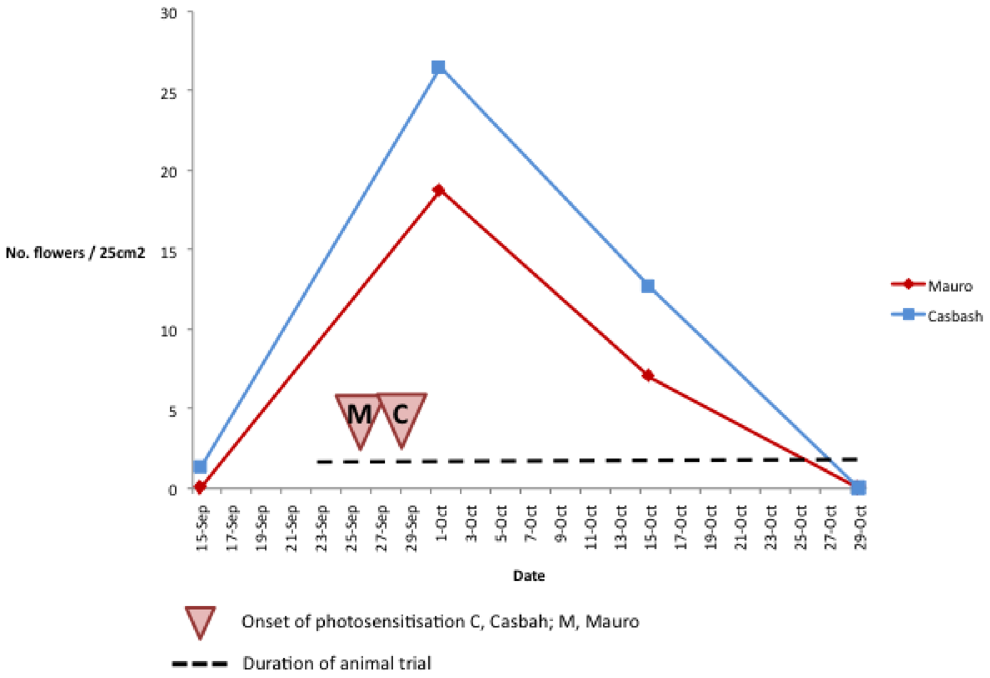

Figure 15. Contribution of flowering plants per 25 cm^2 for Biserrula ‘Mauro’ (red) and ‘Casbah’ (blue) during grazing. Relative percentage of flowering plants was greater in ‘Casbah’ plots compared to ‘Mauro’ on entry of animals to the trial (24th September, dotted line), indicative of relative earlier maturity of this cultivar compared to ‘Mauro’ at the same date. Both cultivars reach the peak of reproductive activity at the beginning of October, with flower number diminishing to zero by trial completion. First observation of clinical cases of photosensitisation for each cultivar is indicated by a red triangle (M, ‘Mauro’; C, ‘Casbah’).

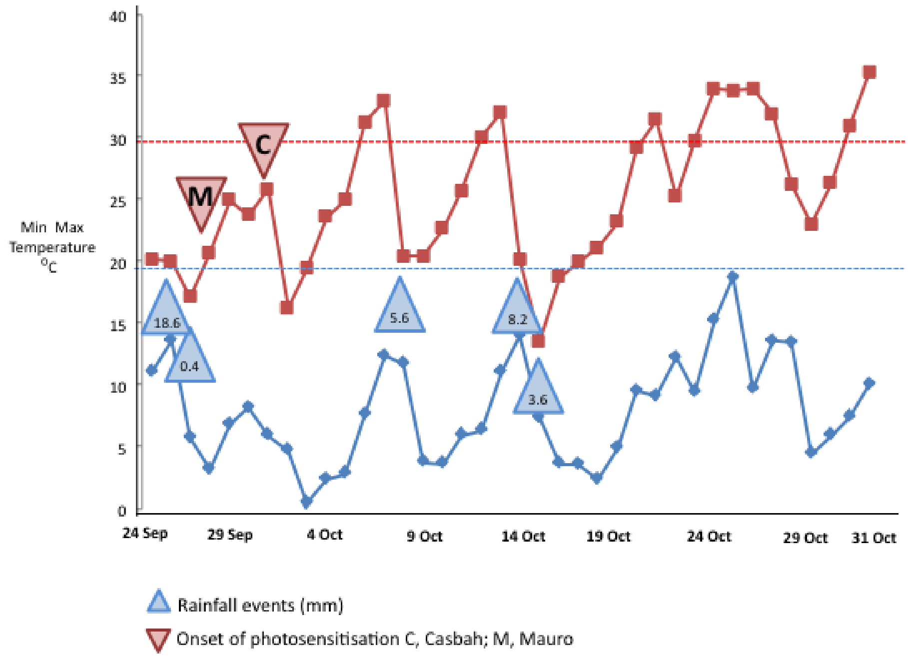

Figure. 16. Minimum, (blue), maximum (red) and average high/low temperatures recorded during the duration of the grazing trial. Dotted lines represent average temperatures (blue, minimum; red, maximum) according to more than 50 years of data recorded from the Wagga Wagga Bureau of Meteorology weather station. Rainfall events (mm) are indicated with blue triangles, first incidence of clinical signs of photosensitisation are indicated by a red triangles (M= Mauro; C= Casbah).

Unpigmented animals (white crossbred lambs) showed the earliest clinical signs of photosensitisation (Day 3 in animals grazing 'Mauro', and Day 7 in animals grazing 'Casbah'; Figs 15 and 16), while pigmented animals in the same plots remained unaffected. Earliest clinical signs of photosensitization noted were pitting oedema of the ears, facial oedema and scratching of affected ears resulting in open lesions (Fig. 4.16) and shade-seeking behaviour.

Figure 17. White crossbred lambs affected with early clinical signs of photosensitisation after grazing biserrula cultivar 'Casbah' for 7 days. Note facial swelling and oedematous drooping ears (animal facing with green ear tag) and evidence of ear-scratching (animal, side on with red ear tag).

Photosensitisation scoring identified the white crossbred lambs showed the highest cumulative percentage of clinically affected on both cultivars of biserrula (Fig. 4.17). The total % of white crossbred animals identified to be suffering from clinical signs of photosensitisation across the duration of the trial was 71% and 100% for animals grazing 'Mauro' and 'Casbah' respectively, although more animals presented with clinical signs of photosensitisation grazing 'Mauro' on any given sampling day bar Day 30 (Fig. 17). The highest accumulated photosensitisation scores were also recorded for white crossbred lambs animals grazing 'Casbah' (highest score = 6.5, mean of 1.77; see Appendix 3 for scoring criteria). By comparison, incidence of photosensitisation in pigmented animals grazing biserrula varieties was 42% and 57% for 'Mauro' and 'Casbah' respectively with most animals only recording a positive score on 1-2 sampling dates during the trial, compared to 3-5 occasions for their white counterparts. The highest photosensitisation score recorded for a pigmented crossbred animal grazing any cultivar was 2 (in a lightly pigmented animal on Day 37 of the trial) with a mean of 0.45 across the cohort. In all animals sampled, the majority of lesions were recorded on the face and ears in animals grazing either variety and cumulatively constituted signs indicative of a mild to moderate outbreak in the flock. Together, these data suggest that pigment affords significant protection against both onset and severity of photosensitisation in animals grazing biserrula but that ongoing signs of photosensitisation can be observed in unpigmented (or lightly pigmented) crossbred sheep grazing either cultivar. These data also suggest that although very mild signs of photosensitivity can be noted in pigmented stock on close clinical examination, this is transient and resolving and would be unlikely to be noted by the producer during normal management procedures.

Clinical photosensitisation was also observed in Merino ewes and lambs grazing both cultivars of biserrula (Fig. 18). In comparisons to crossbred animals grazing the same plots, Merino ewes showed a lower incidence of photosensitisation on both cultivars (Mauro, ewes: 45%; lambs, 55%; Casbah ewes: 26%; lambs: 50%).

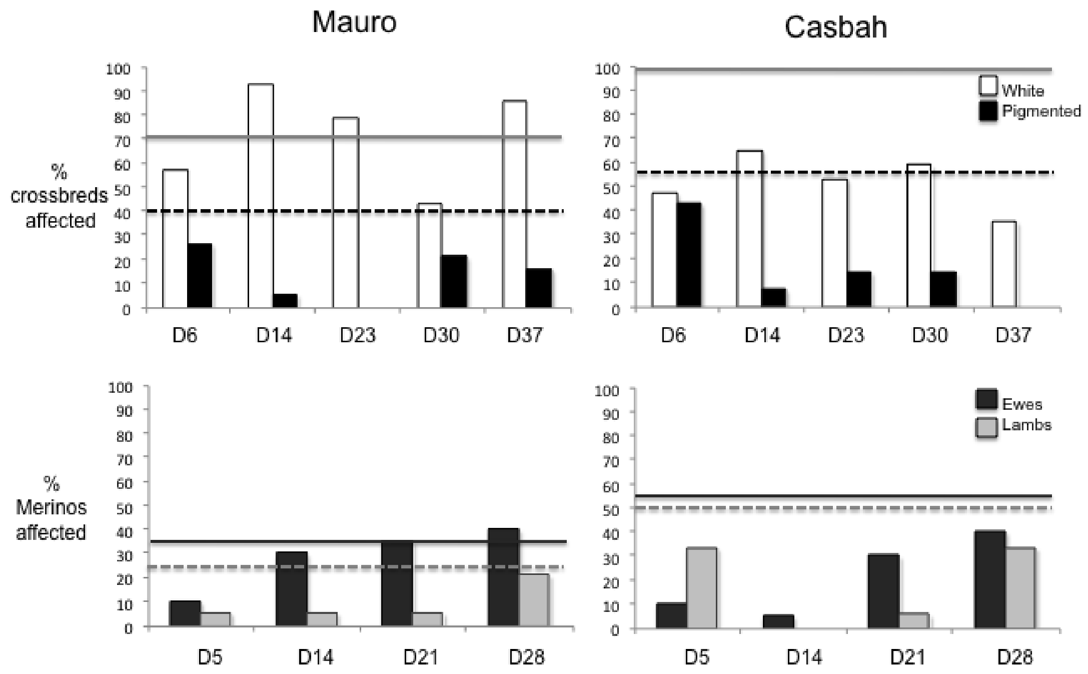

Figure 18. Percentage of animals presenting with clinical signs of photosensitisation grazing the biserrula cultivars 'Mauro' and 'Casbah' over time (days on pasture, D). Upper graphs represent, white and pigmented crossbred lambs (white and black bars respectively) and lower graphs, Merino ewes (dark grey bars) and lambs (light grey bars). In the upper graphs, the solid grey bar indicates the total % of white crossbred animals affected across the duration of the trial (cv 'Mauro', white: 71%; cv 'Casbah' white: 100%), the dotted bar represents the total % pigmented animals affected (cv 'Mauro', pigmented: 42%; cv 'Casbah', pigmented: 57%). In the lower graphs, the dark grey bar indicates the total % Merino ewes (cv 'Mauro', ewes: 45%; cv 'Casbah' ewes: 55%), and total % Merino lambs (cv 'Mauro', Merino lambs: 26%; cv 'Casbah' Merino lambs: 50%), showing clinical signs of photosensitisation. Crossbred lambs: cv 'Casbah' white, n = 15, pigmented = 17; cv 'Mauro' white, n = 17, pigmented = 15; Merino ewes: n = 20 per cultivar; Merino lambs: 'Mauro' n = 19; 'Casbah' n = 21).

Similarly to that observed in white crossbred lambs, Merino ewes also showed highest photosensitisation scores on the cultivar 'Casbah' (highest score 7, mean = 0.54). Merino lambs showed the least incidence of photosensitisation on either cultivar, receiving minimal scores for photosensitivity (<=1) on only 1 or 2 individual sampling dates. Together these data suggest that Merinos as a breed may be afforded more protections against clinical signs of photosensitisation, possibly due to greater fleece coverage, lower feed intake or more selective grazing behaviour, or a combination of these factors.

Statistical analysis using multivariate regression showed pigment to be a significant factor in defining observation of photosensitisation ($p = 0.002$), with sex and cultivar exerting no statistically significant effect. These data indicate that both biserrula cultivars are equally likely to cause clinical photosensitisation in unpigmented (white) sheep with onset occurring rapidly (within 3 to 7 days) post-introduction to biserrula pastures, depending on the amount of ingested material.

A marked difference in severity of photosensitisation and associated lesions was noted at the end of the trial between the two pigment cohorts (Fig. 18). Little or no damage was apparent in pigment crossbred lambs with mild to moderate resolving skin lesions apparent on the ears of white crossbred lambs in all plots. Merino ewes showed only mild resolving lesions of the muzzle with little or no apparent damage to the ears, very few Merino lambs showed any lesions at the end of the trial, the most significant being mild crusting over skin lesions on the distal tips of the pinnae and the tip of the muzzle in two cases.

4.4.2 Clinical and histopathology

Laboratory analysis of venous blood samples collected from affected white animals after 7 days on pasture, or at onset of clinical signs later in the trial, showed no evidence of hepatopathy (Table 4.2). As statistical analysis had not indicated a significant effect of cultivar on incidence of photosensitisation, both varieties were considered together. Values for white animals after 7 days grazing were not significantly different from entry values, nor from their pigmented counterparts. Specifically, no elevation in bilirubin, glutamate dehydrogenase (GLDH) or gamma glutamyl-transpeptidase (GGT) levels were recorded in any affected lambs (Table 4.2). Together these data indicate no underlying hepatopathy associated with photosensitisation post ingestion of *Biserrula pelecinus,* as previously reported (Kessell *et al.*, 2015). One pigmented animal showed a significant elevation in creatinine kinase (CK), without obvious pathology (Table 4.2; CK 1522). The significance of this finding is unknown. Values for electrolytes were all within normal reference ranges, as were seen in the first controlled grazing trial reported in this study (see section 4.3.) and on-farm case analyses (Kessell *et al.*, 2015). Similar findings were recorded for Merino ewes, (data not shown) with no indication of hepatopathy. Elevation in AST was also noted in two Merino ewes with unknown aetiology. Merino lambs were not sampled for biochemical analysis.

Table 4.2. Selected biochemical values from white crossbred lambs at onset of clinical signs of photosensitisation in white animals grazing biserrula varieties and their pigmented counterparts, n = 32 in both cases. Values in **bold** are outside the normal reference range.

Analyte	White	Pigmented	Reference range
Fibrinogen	4.0	7.0	3-8 g/L
Tot Protein	70-72	69-72	60/82 g/L
Bilirubin	2-3	2-3	<3μmol/L
GLDH	3-26	10-29	0-33 U/L
GGT	55-69	60-80	47-95 U/L
AST	80-115	104-116	59-130 U/L
CK	129-210	181-**1522**	0-1194 U/L

Abbreviations: GLDH, glutamate dehydrogenase; GGT, gamma-glutamyltransferase; AST, aspartate transaminase; CK, creatinine kinase.

Figure 19. Moderate resolving photosensitisation observed in an unpigmented crossbred lamb, compared to its pigmented counterpart who shows no obvious pathology.

Biopsy samples taken from the ears of affected animals at the end of the trial showed similar histopathological changes to those reported previously, in this case for resolving lesions associated with photosensitisation secondary to ingestion of *Biserrula pelecinus*.

4.4.3 Live weight gain in pigmented and unpigmented sheep grazing two cultivars of *Biserrula pelecinus* L.

Increases in live weight were noted across all classes of animals grazing biserrula, of either variety, and of both pigment types for the crossbred lambs (Fig. 4.19). Multivariate analysis showed no effect of sex or colour on weight gain in crossbred lambs suggesting that photosensitisation did not appear to exert a significant negative impact on weight gain, despite clear differences in clinical signs between pigmented and unpigmented sheep.

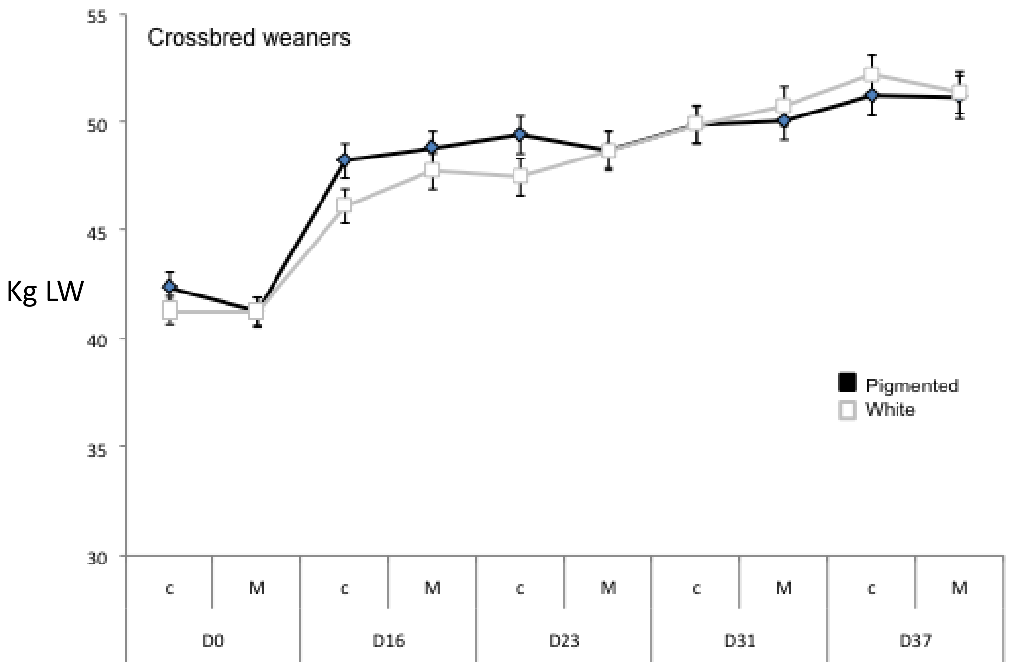

Figure 20. Mean live weight gain (kg) +/- s.e. by cultivar for pigmented (closed) and white (open boxes) crossbred lambs grazing Mauro (M) and Casbah (C).

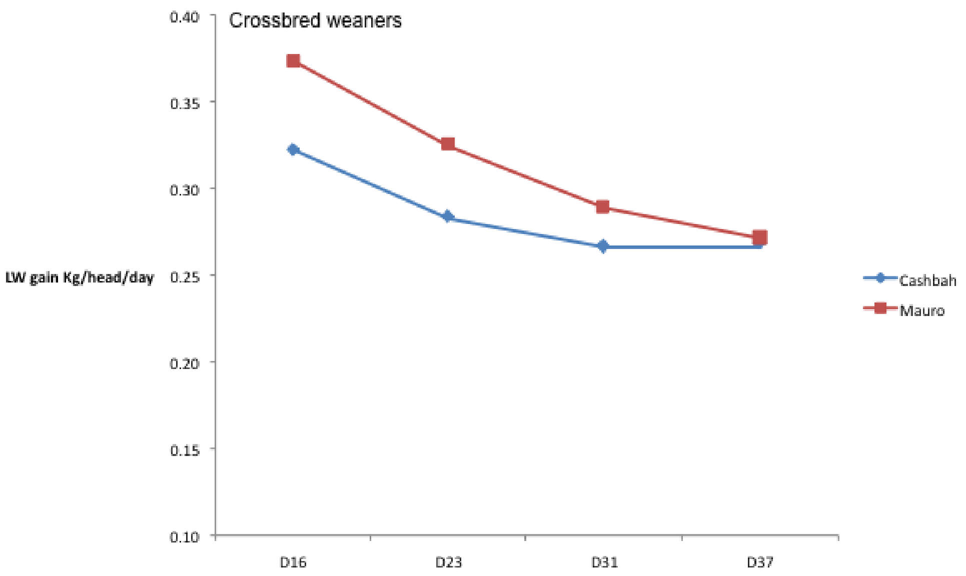

Figure 21. Comparison of live weight gain per head per day for crossbred lambs grazing two cultivars of *Biserrula pelecinus*, 'Casbah' (blue, n = 32) and 'Mauro' (red, n = 32).

When both cultivars were considered separately, live weight gain per head per day was found to be higher at all time points monitored for Mauro pastures, with the exception of the final collection at day 37 (Fig. 4.20). It is possible that this relative difference in live weight gain was due to the difference observed in stage of plant maturity of 'Mauro' compared to 'Casbah' at the beginning of the trial. In this case, 'Mauro' was still primarily in a late vegetative phase of growth at trial onset while 'Casbah' was nearing initiation of flowering. On both varieties, live weight gain decreased for both varieties over the duration of the trial, suggesting that rate of ingestion of pasture was decreasing (Figs 4.19 and section 4.20). Whether this was a direct effect of appetite suppressing compounds in the pasture, or learned avoidance and therefore reduction in intake is not known. Conversely, lactating merino ewes did not show a reduction in weight gain over the period of the trial, suggesting that physiological status and breed grazing selectivity may also be playing a role (Figs 4.21 and 4.22).

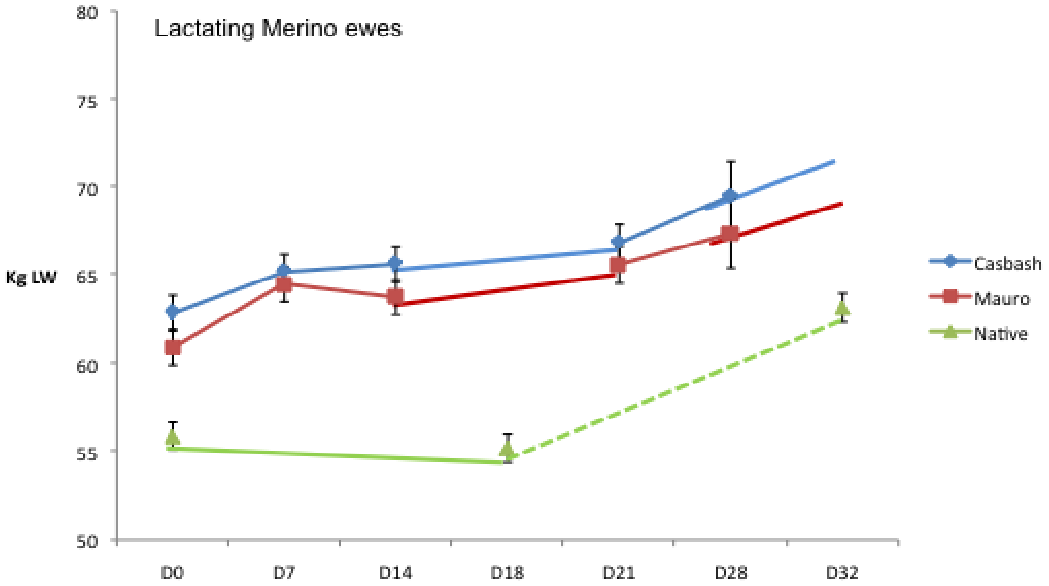

Figure 22. Comparison of mean live weight gain (kg) for lactating Merino ewes grazing two cultivars of *Biserrula pelecinus*, 'Casbah' (blue, n = 20) and 'Mauro' (red, n = 20) and naturalised pasture (solid green line). Merino ewes grazing naturalised pasture (green, n = 186). Time at which point the mob was moved to improved pasture (dotted green line).

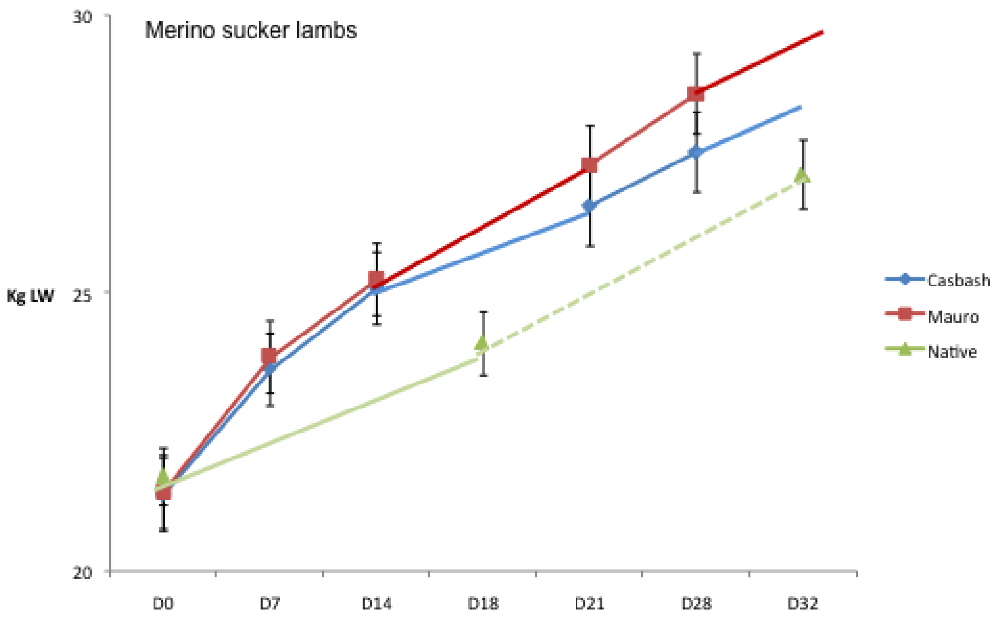

Figure 23. Comparison of mean live weight gain (kg) for Merino suckling lambs grazing two cultivars of *Biserrula pelecinus*, 'Casbah' (blue) and 'Mauro' (red, n = 20 for each cultivar) and naturalised pasture (solid green line, n = 96). The dotted line represented the time point at which the mob was moved to improved pasture.

An additional factor contributing to the relatively lower weight gain observed in animals grazing cultivar 'Casbah' was the relative availability of digestible high quality feed. Biserrula components contributing to feed on offer (FOO) changed considerably over the duration of the grazing experiment (Fig. 4.13). An increase in the proportion of pod as a component of total FOO was more rapid in 'Casbah' plots compared to 'Mauro plots' due to the earlier flowering of 'Casbah' as a variety (Fig. 4.14). This relative difference in growth characteristics between 'Casbah' and 'Mauro' may have contributed to the higher weight gain observed in animals grazing 'Mauro' as digestibility of this variety was comparatively higher than 'Casbah' at all time points analysed.

4.5 Identification of temperature-dependent growth characteristics in *Biserrula pelecinus* L.

Temperature-dependent growth responses of 'Casbah' and 'Mauro' cultivars in controlled environment pot experiments were similar (Figs 4.24 - 4.26). Plant height was found to be similar for both cultivars at 20 and 25 °C, but declined as temperature increased to 30 °C (Fig. 4.24). The effect of temperature on growth characteristics was highly significant and was best fit by a second-degree polynomial response (linear and quadratic terms both significant at $P < 0.0001$).

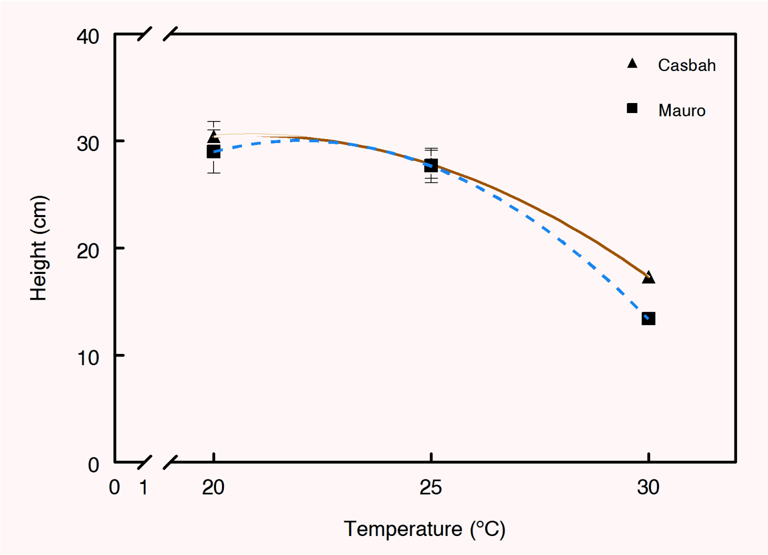

Figure 24. Height of *Biserrula pelecinus* L. *cvs* 'Casbah' and 'Mauro' recorded in a controlled environment experimentation at three daily temperature regimes. Error bars represent s.e.m.

Fresh weight of both cultivars declined significantly with increasing temperature (Fig. 4.25). The relationship between fresh weight and temperature was adequately described by a linear relationship, which was also highly significant (*P* < 0.0001). Interestingly, the rate of decline in growth with increasing temperature was higher for the cultivar ‘Casbah’ than for ‘Mauro’ (interaction term *P* < 0.0001). Vigour ratings for ‘Casbah’ did not vary significantly with temperature, but significantly declined with increasing temperature for ‘Mauro’ (P < 0.001, Fig. 4.25).

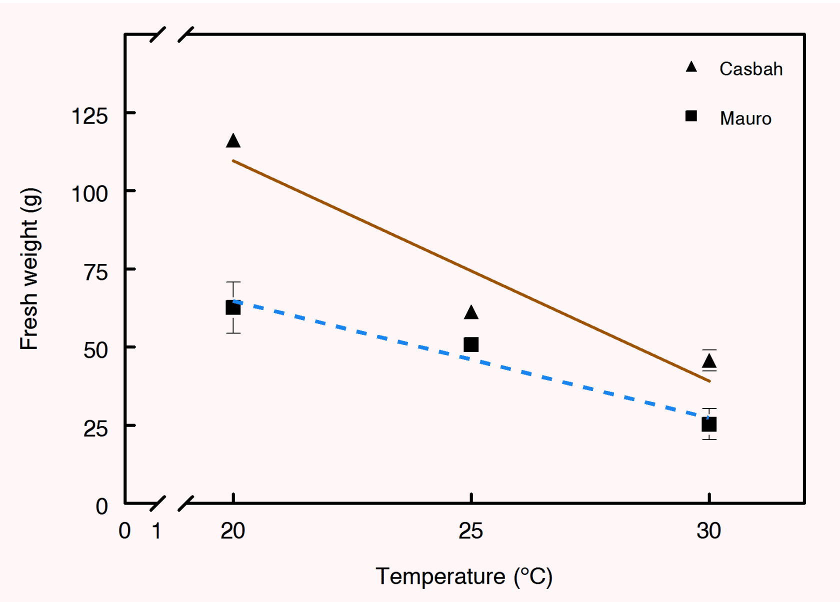

Figure 25. Fresh weight of *Biserrula pelecinus* 'Casbah' and 'Mauro' recorded in controlled environment experimentation at three temperature regimes. Error bars represent s.e.m.

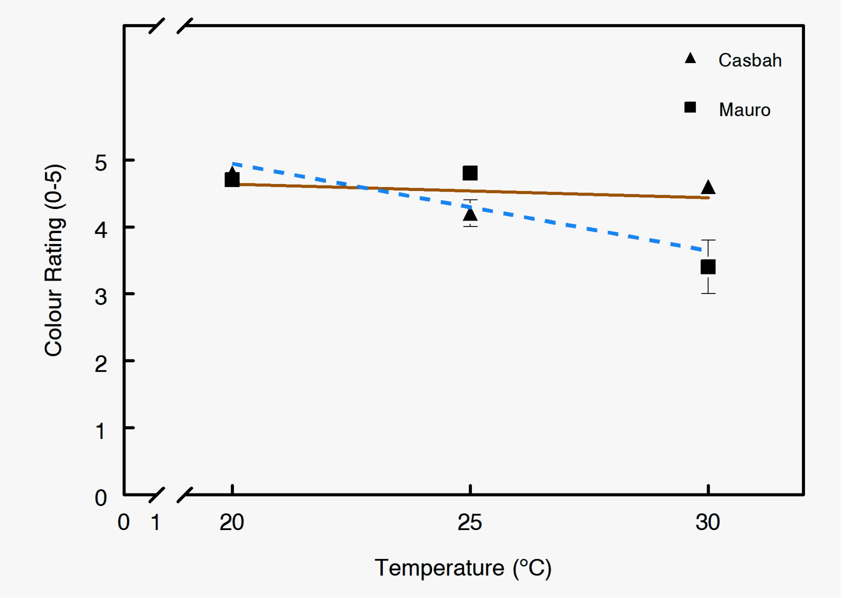

Figure 26. Vigour of *Biserrula pelecinus* 'Casbah' and 'Mauro' recorded in controlled environment experiments at three temperature regimes. Error bars represent s.e.m.

For biomass accumulation and growth rate, both ‘Casbah’ and ‘Mauro’ performed best at cooler temperatures over the range of temperatures evaluated, suggesting that growth under Australian field conditions will likely be most vigorous during the cooler part of the year in late winter and spring.

5 Discussion

5.1 Summary of major findings

- *Biserrula pelecinus L. cvs.* ‘Mauro’ and *‘Casbah’* were confirmed by the results of this trial to cause photosensitisation in sheep.
- Hepatic dysfunction was not a primary clinical factor in *Biserrula* photosensitization suggesting that the causal agent is a primary photosensitising agent.
- Compromised liver function may predispose to photosensitisation..
- The earliest clinical signs observed in animals grazing *Biserrula pelecinus* L. ‘Mauro’ were weeping and swelling of the eyes and eyelid margins. Corneal ulceration can occur within 96 hours of exposure in the presence of high ambient temperatures (high UV).
- Histopathological analysis of exposed tissue (skin from the ears) showed early clinical changes associated with dermal photosensitisation.

- Together these findings suggested that the primary causal agent is a primary photosensitising agent and is present in the plant during the vegetative / flowering phase of plant growth.

5.2 Success in meeting the project objectives

5.2.1 Define the critical growth stage window of phototoxicity associated with Biserrula pelecinus L.

Studies in this report identify that Biserrula pelecinus L. is photocytotoxic at all stages of the plant's life-cycle, bar when the plant is senescent. (see section 4.6.4)

5.2.2 Determine the aetiology and pathogenesis of biserrula photosensitisation, defining this disorder as either a primary or secondary hepatotoxic photosensitivity.

On-farm case study analysis and controlled grazing trials confirm no apparent hepatopathy associated with photosensitisation in livestock concurrent with ingestion of biserrula pastures, confirming the aetiology to be one of a primary photosensitisation in nature (see section 4.2.). Controlled grazing studies confirm that both commercially available biserrula cultivars 'Casbah' and 'Mauro' cause photosensitisation in livestock (see sections 4.3. - 4.4.) and analysis by in-vitro bioassay (see section 4.6).

5.2.3 Survey producers who have implemented this pasture species on their experience with *Biserrula* photosensitivity.

Surveys of producers who have implemented biserrula as a pasture species in their farming system showed an incidence of 55%, with mild, moderate and severe outbreaks experienced. Both sheep and cattle were reported to have experienced photosensitisation association with consumption of biserrula pastures, and all ages of animals were reported to be affected. (see section 4.1).

5.3 Photosensitisation outbreaks in animals grazing biserrula pelecinus are common

Fewer than 200 producers were estimated to be utilising Biserrula species as a pasture in Australia in 2013 therefore our potential sample size of over 100 producers represented approximately 50% of all those suspected to be utilising this pasture species. Thirty two producers responded to the survey and whilst the sample size is not sufficiently large for statistical analysis, it does give a representative sample of biserrula producers in Australia. More producers responded to this survey that had been encouraged to do so in the past where only 14 respondents from WA only were recruited (Hogg and Davis, 2009a) to 15 (Hogg and Davis, 2009b) . The combination of survey distribution method (personal posting and / or email) with follow-up may have contributed to this relative success. Further surveying of producers is recommended to try to elucidate links between factors that may contribute or protect against photosensitivity. Incentives may help to increase response rates for future surveys.

In 2013, most (31/32) producers reported use of the 'Casbah' cultivar of biserrula. This may be indicative of the relative long history of this cultivar in Australia compared to the more recent introduction of ‘Mauro’. This profile remains similar to results obtained in a previous producer survey, where the majority (93%) of respondents grew the cultivar ‘Casbah’ (Hogg and Davis, 2009b). An unexpected outcome was that a majority of producers had experienced livestock photosensitisation when grazing biserrula (55%). This is a higher percentage than might be anticipated from previous anecdotal reports from the literature where incidence of photosensitisation has been described as ‘sporadic’.

The demographic of animals reported to be affected by photosensitisation showed a predominance of ewes, followed by lambs. It is possible that this reflects the animals placed on biserrula pastures rather than any predilection for photosensitivity. Future surveys should aim to represent both the animals grazing biserrula, as well as the animals affected by photosensitivity for a better description of the factors involved.

It is interesting that a small number of producers confirmed their diagnosis of photosensitisation with a veterinarian (18.8%). The low levels veterinary involvement in the diagnosis and treatment recommendations necessitates a tailored approach for the dissemination of this information to producers who are unlikely to recruit specialist veterinary advice for this pathology. The findings of this survey need to be delivered through agricultural extension officers, farmers groups and agronomists, rather than via veterinary channels.

5.4 On farm outbreak and controlled grazing trials identify ingestion of biserrula to cause primary photosensitisation in sheep

Photosensitivity is either primary, when photodynamic agents are absorbed into the bloodstream directly, or secondary, when impaired hepatobiliary excretion results in photodynamic phylloerythrin circulating in the bloodstream. Regardless of the aetiology and/or the photodynamic agent involved, the clinical signs of photosensitisation are similar. The presence of the skin pigment melanin blocks UV penetration through the epidermis to the sensitive underlying dermal layers, giving significant protection from the excitation ability of UV light. Thus dermal lesions typically develop in unpigmented skin exposed to sunlight. Lightly haired non pigmented facial areas are thus the most sensitive to photosensitisation in livestock. Initial clinical signs of photosensitisation include excessive lacrimation, poorly demarcated erythema and oedema of these skin areas (Radostits, 2006). Corneal oedema has also been reported. Local oedema can be severe, leading to drooped pinnae, swollen eyelids with secondary entropian and epiphora that may progress to blepharospasm, and oedema of the lips and muzzle. Initial signs may include behavioural changes such as restlessness, head-shaking and self-mutilation secondary to intense pruritus and photophobia (Pfister, 1999).

The use of an objective photosensitivity monitoring score has not been utilised previously to the author’s knowledge. An objective score highlighted the differences in manifestations between individuals. Using objective criterion minimised potential biases and observer error. The compartmentalising of clinical signs into eye, ear, skin and fleece categories also helps to group confounding factors.

Whilst the eye lesions seen in sheep grazing biserrula did not show a statistically significant difference in incidences to that of the mixed or subterranean clover pastures, although there may have been greater severity in biserrula animals. Other causes of blepharospasm, conjunctivitis and epiphora need to be considered, including Chlamydia, environmental factors and secondary bacterial infection. Chlamydia PCR analysis of the most affected animals did not confirm this as a diagnosis for the conjunctivitis observed in these animals suggesting that corneal sensitivity was related to biserrula ingestion in this instance.

Other abnormalities observed on necropsy, in both experimental and on-farm cases, were largely incidental findings to be expected in sheep of this age and/or production status. The abomasitis observed in one sheep is likely to be caused by parasitism, specifically *Telodorsagia* spp. (formerly *Ostertagia* spp.). The animal with liver abscessation uidentified at necropsy in our 2013 controlled grazing trial had no clinical signs of photosensitisation on entry to the biserrula pasture, despite an inflammatory haematology profile and biochemical changes suggestive of hepatic lesions at entry (elevated GGT, GLDH, and bilirubin), consistent with histological evidence that the lesions were chronic. In this case alone, the severity of the clinical signs observed in this animal are suggested be correlatyed, in part, to it's poor liver function which would in turn impede normal metabolism of toxic constituents ingested in it's food.

Together, the on-farm and experimental data presented in this report strongly suggest that the photosensitisation observed following ingestion of biserrula was unlikely to caused by hepatic damage. Although the single worst affected animal also had moderate liver abscessation, plasma bile acids were within normal limits, which may suggest adequate liver function(Thrall, 2006). This, combined with histology suggesting a bacterial pathogenesis for the liver lesions, suggests that this is not hepatogenous photosensitivity. However it is possible, perhaps likely, that compromised liver function may accelerate the rapidity and severity of clinical signs, yet this needs further investigation to be confirmed.

On necropsy and histopathological inspection of animals from both on-farm outbreaks and controlled grazing trials, the only consistent, clinically relevant lesions were acute skin lesions (see sections 4.2. – 4.5.). Observed clinical signs and lesions on the on the unpigmented skin of the face, ears and tail of affected animals, including loss of all or parts of the pinnae of the ear, after consumption of biserrula are consistent with photosensitivity (Hogg, 2010). Together these data strongly support a hypothesis that the disease mechanism undelying photosentisisation in livestock ingesting biserrula is primary (non-hepatogenous) in nature. The lack of enzymatic, histologic or gross pathological lesions add further evidence that the toxin(s) involved in biserrula photosensitisation is/are a primary photosensitisation agent.

5.4.1 Both commercially-available cultivars of biserrula cause photosentisition in livestock

Prior to this report, isolated cases of photosensitisation have occurred in sheep grazing the cultivar 'Casbah' throughout the Australian wheatbelt (Loi *et al.*, 2006, Loi, 2005, Loi, 2010, Hackney, 2012), and cases of biserrula photosensitivity have been noted in spring in Western Australia in both ewes and lambs (Pfister, 1999). Previous to this study, anecdotal reports suggest that the plant is toxic during the flowering stage (Salam, 2010), and has reduced or absent toxicity when dried or ensiled (Revell, 2008, Swinny et al., 2015). Data generated in this report confirms this finding at a biochemical level (see section 4.9.1. and Fig. 4.30). Our experimental data also supports anecdotal evidence of a swift onset of clinical signs, with the first clinical changes including epiphora, oedema of the face, muzzle or pinnae, evident within 96 hours of sheep grazing the biserrula pasture (see Fig. 4.15).

Previously, anecdotal evidence has suggested that clinical signs of photosensitisation were observed in naïve lambs when introduced to late vegetative or flowering *Biserrula pelecinus cv*. 'Mauro' monoculture pastures (personal communiations to J. Quinn and data from section 4.1.). Although the cultivar 'Mauro' is less commonly used in Australia at present, we have now provided strong evidence that this cultivar also causes a primary photosensitisation in livestock (Fig. 18). As spring / summer are the seasons most commonly associated with outbreaks of photosensitisation on biserrula pastures, our growth chamber data suggests that increasing biomass due to cooler growing conditions is related to increased availability of the plant for animal consumption, leading to a relative increase in production of phototoxic constituents during this phase of the plant's growth cycle.

In conclusion, anecdotal reports of photosensitisation of sheep whilst grazing *Biserrula pelecinus cv.* 'Mauro' can be supported by this clinical example and findings in this report confirm that both commercial available cultivars of biserrula cause photosensitisation in livestock.

5.4.2 Casbah and Mauro cultivars of biserrula show different palatabilities for grazing livestock

To avoid any risk of the controversial aversion responses observed when sheep graze biserrula, all sheep used in the controlled grazing trials reported were naïve to biserrula pastures. Despite early conjecture about whether fasting sheep does (Arnold *et al.*, 1964) or does not (Sidahmed *et al.*, 1977) affect dietary selectivity, it was resolved that hunger has no effect on diet selectivity, but satiation increases selectivity (Jung and Koong, 1985). The hunger after fasting perhaps negated dietary selectivity upon first admission to biserrula pastures in the trials reported, and may also therefore have contributed to the rapidity of the onset of clinical signs.

Evidence of selective grazing was observed in the controlled grazing trials (see section 4.5.1.2.; Fig. 4.14.) where pasture composition of grazing pplots containing *Biserrula pelecinus cv*. 'Mauro' were observed to remain constance between relative contribution of biserrula and 'other' species, suggesting that animals were ingesting 'Mauro' in the same relataive proportion as other pasture species on offer. In comparison, relative composition of *Biserrula pelecinus cv*. 'Casbah' in matched plots showed a reduction in the contribution of 'other' species in these plots over time, suggesting that animals were preferentially grazing the other pasture species available in preference of grazing 'Casbah'.

This hypothesis is also supported by evidence that onset of photosensitisation in animals grazing the cultivar 'Mauro' was earlier (Day 3 on pasture) than their counterparts grazing 'Casbah' (Day 6 on pasture, Fig. 4.15). This is also strongly suggestive that the relative intake of 'Mauro' was higher in animals grazing this pasture species initially, as they expressed no grazing preference which would exclude it from their diet, with those animals grazing 'Cashbah' only reaching critical threshold of ingestion for onset of clinical signs some days later.

To date, this is the first time that evidence has been presented for a difference in palatability or grazing preference for *Biserrula pelecinus cv*. 'Mauro' compared to *Biserrula pelecinus cv*.

'Casbah'. Simple taste analysis of the two cultivars confirms that *Biserrula pelecinus cv*. 'Casbah' has a sharp, bitter taste whilst *Biserrula pelecinus cv*. 'Mauro' does not (J. Quinn and L. Weston – personal communication). Further biochemical analysis could identify the compounds present in these two varieties which give rise to a difference in palatability and / or preference in livestock.

5.4.3 Breed, age and pigment can affect incidence and severity of photosensitiation in sheep grazing biserrula

Controlled grazing studies were undertaked to examine the effect of pigmentation on expression of clinical signs of photosensisation in meat lambs grazing biserrula pastures (see section 4.5.). To achieve this aim, animals of similar genotype but mixed colour (white sussex x dorper first cross lambs) were compared for rate of onset and appearance and severity of clinical signs of photosensitisation when indroduced to the two commercially available biserrula cultivars, *Biserrula pelecinus cv*. 'Mauro' and *cv*. 'Casbah'.

Clear differences both in onset of clinical signs and severity were observed (Fig. 4.17) with the highest proportions of affected animals being those of white skin pigmentation grazing *Biserrula pelecinus cv*. 'Mauro'. Although some pigmented animals were observed to present with clinical signs of photosensitisation, in general their severity was mild (mean PS score across the duration of the trial in this group was 2) and they resolved more quickly than their non-pigmented counterparts. Interestingly, in this particular trial, no difference was observed in live weight gain between the two groups, (Fig. 4.19). This is suggested to be due to the relatively mild nature of the 'outbreak' initiated in this grazing trial, rather than an inherent lack of effect of clinical photosensitisation on production. . The relatively low ambient temperatures experienced during the trial and presentation of only mild to moderate clinical signs of photosensitization throughout the experiment may be associated with the lack of significant response. In a further grazing trial carried out at CSU in winter 2015 (data not shown as beyond scope of this project), severe photosensitisation was observed which precluded any animals remaining on biserrula pastures. In this event production was affected and this was reflected in live weights recorded during the duration of this trial.

Overall, data in this report shows that clinical signs of photosensitisation relative to ingestion of biserrula pastures can be mitigated by presence of pigmentation in the skin of grazing animals, and in mild outbreak seasons this can preclude production loss in these cohorts. Together these data suggest that the presence of pigment in sheep grazing biserrula affords some significant mitigation to photosensitisation and that Merino ewes and lambs may show a breed-related mitigation of clinical presentation, possibly due to lower feed intake and/or greater fleece coverage of the face and ears. However, caution needs to be implemented when grazing any animals of biserrula pastures as severity of outbreak cannot necessarily be predicted by season, only by rate of senescence of biserrula pastures.

5.4.4 Merino sheep may show reduced clinical signs of photosensitisation due to breed specific characteristics

Annecdotal verbal reports had suggested that Merino sheep may show a lower susceptibility to photosensitisaton on biserrula pastures than other breeds. To test this hypothesis, naieve Merino ewes with suckling lambs at foot were inctroduced to two cultivars of *Biserrula* *pelecinus* during our 2014 grazing trial, and their incidence and severity of photosensitisation, along with production (live weight gain) was compared.

Similar to their crossbred counterparts, Merino ewes showed increased weight gain across time grazing both cultivars of biserrula (Fig. 4.21), particularly in comparision to Merino ewes grazing ‘native’ unimproved pastures. Their lambs showed a similar trend (Fig. 4.22). Interestingly, Merino ewes did present lower photosensitisation scores than their crossbred counterparts (Fig. 4.17), mainly as clinical signs were restricted to those areas of the face which were not covered with fleece (muzzle only). Their lambs showed reduced incidence and severity compared to their mothers.

Together this evidence suggests that Merino sheep may be afforded a relative level of protection from photosensitisation when grazing biserrula due to their more extensive fleece covering, and that suckling labs are afforded some relative protection due to their diet containing little fresh grazed material.

This evidence also must be taken with the caveat that the overall ‘outbreak’ was mild, and that grazing in other seasons, or older lambs, may not be afforded this relative protection. Indeed, verbal reports of severe outbreaks of photosensitisation in newly weaned lambs grazing *Biserrula pelecinus cv*. ‘Casbah’, and our own experiences with clinical cases presented to our Diagnostic Clinic at CSU of shedding lambs and rams grazing biserrula pastures, suggests that variation in breed-specific presentiation is complex and that protection cannot be assumed by the producer in any animal grazing biserrula pastures unless they are senescent.

5.5 Key extension messages

Key extension messages have been identified by the work described in this report. They have been condensed into the ‘Tips and Tools’ format brochure presented in Appendix 4.

These include:

- Identifying for producers the difference between primary and secondary photosensitisation;
- Identifying that photosensitisation can occur in livestock grazinf biserrula throughout it’s life cycle, excepting when the plant is senescent;
- That the onset of clinical signs of photosentisiation in animals grazing biserrula pastures is rapid;
- That animals can recover providing that intervention is provided quickly;
- That any other underlying health issue can increase the severity of clinical signs of photosensitisation and make recovery times longer;
- That presence of other pasture species can mitigate, to some extend, the severity of clinical signs of photosensitisation, but can not mitigate against them completely in all animals due to inter-animal differences in grazing preference;

- That sheep breed and genotype can influence both incidence and severity of clinical signs of photosentisiation in sheep grazing biserrula pastures;
- That the only truly 'safe' biserrula pasture is one that is fully senescent.

6 Conclusions/recommendations

Biserrula pelecinus is a useful addition to the repertoire of self-regenerating legumes available to farmers in the temperate regions of southern Australia, but consumption of the plant may be associated with primary photosensitivity that can result in serious disease and production losses.

Close observation of animals grazing biserrula can detect the initial behavioural and skin changes as they occur and allow management strategies to be implemented to prevent the more severe consequences of prolonged toxic exposure. However, this requires constant physical monitoring of stock, including close physical inspection as severity of lesions cannot necessarily be accurately assessed at a distance from the animal, and is therefore expensive in terms of time and labour.

7 Key messages

Biserrula is a useful addition to the pasture toolbox but incidence of photosensitisation in animals grazing this pasture cannot be ignored.

Currently, both commercially vailable cultivars 'Casbah' and 'Mauro' can cause photosensitisation in livestock with prevalence and severity being linked to ingestion of fresh, green, growing plant material. Sheep breed can mitigate clinical signs to some extent, as can presence of pigmentation in this skin, but neither can prevent manifestation of clinical signs in 100% of animals.

As such, caution should be applied when grazing biserrula pastures, with animals rotated in and out every 3 days or less to ensure minimal effect on animals on pasture. Utilising this pasture in this manner may pre-empt severe outbreaks of photosensitisation occurring.

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9 Appendices

Appendix 1 - Producer survey

Information and declaration of consent

Dear Participant,

This survey is part of a research project being undertaken by Veterinary Science Honours student Georgia Ladmore who is investigating the issues of Biserrula photosensitisation. Her supervisors on this project are Jane Quinn (BSc (Hons),PhD) a senior research scientist in the School of Animal and Veterinary Sciences and Leslie Weston (BSc, PhD), Professor of Plant Biology in the School of Agriculture and Wine Science. This project is part of a wider research project involving Belinda Hackney, Research agronomist and John Piltz, Livestock Research Officer at NSW DPI as well as researchers at the Department of Agriculture and Food in Western Australia.

We hope you consider participating in this study. Data obtained from this exercise will help inform best use of this important legume species as a grazing crop for sheep and other animals.

If you have any questions or concerns regarding this survey you can contact any of us on the numbers below:
Georgia Ladmore 0423 359527 gladmo1@postoffice.csu.edu.au
Dr Jane Quinn 02 6933 4208 jquinn@csu.edu.au
Professor Leslie Weston 02 6933 2429 leweston@csu.edu.au
John Piltz 02 6938 1869 John.piltz@dpi.nsw.gov.au
Belinda Hackney 02 6330 1200 Belinda.hackney@dpi.nsw.gov.au

We would like you to fill in a short survey for each paddock of Biserrula sown on your property. Please fill in a separate survey for each paddock, or, if all paddocks are identical please state this in your survey. This should take no more than 20 minutes to complete for each paddock.

Please be aware that by completing this survey, you are providing your consent to participate in this study and you are agreeing to the terms of this study detailed below.

Participation is open to adults (over 18 years of age, according to NSW guidelines) and is anonymous and voluntary. There is no obligation to consent. You are free to decline or withdraw from the survey at any time without affecting your relationship with Charles Sturt University or NSW DPI. If you do withdraw, any data entered will not be included. All questionnaire data will be held in the strictest of confidence by the researchers.

Any personal details included in this survey will be kept in strictest confidence and will not be divulged to any third parties.

The results, once analysed, will be part of a research report that will be submitted to Charles Sturt University as part of this honours dissertation as well as forming part of an ongoing research project. It is also hoped that the results of Georgia's studies will be published in a peer-reviewed journal in the future.

The School of Animal and Veterinary Sciences Ethics Committee has approved this project. If you have any reservations about the ethical conduct of this project, you may contact the Committee through the executive officer:

Dr Raf Freire
School of Animal and Veterinary Sciences
Tel: (02) 6933 4451
Email: rfreire@csu.edu.au

Any issues you raise will be treated in confidence and investigated fully and you will be informed of the outcome.

DECLARATION OF CONSENT:
The following consent is implied on commencement and completion of the online or printed questionnaire:

1. I understand that I am free to withdraw my participation in the research at any time, and that if I do I will not be subjected to any penalty or discriminatory treatment.
2. The purpose of the research has been explained to me and I have read and understood the information sheet.
3. I understand that any information or personal details gathered in the course of this research about me are confidential and that no identifying information will be published or shared without my written consent

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Producer and Property general information

You will now be asked for a few details about yourself and your property.

1. Please enter your name:

2. Would you prefer to be contacted by phone to complete this survey?

- ○ No, I am happy to complete this survey online
- ○ Yes. Please give a contact phone number, or email address to arrange a mutually agreeable time

Producer and Property general information

3. Please state the location of your property:

4. What state is your property in?

- ○ WA
- ○ SA
- ○ VIC
- ○ NSW
- ○ ACT
- ○ QLD
- ○ NT
- ○ TAS

5. What is the total size of your property in hectares?

6. Are you happy to be contacted with the results of this study in the future?

- ○ Yes
- ○ No

7. Are you happy to be contacted to seek further assistance with this study?

- ○ Yes
- ○ No

8. Please insert an email address, or current contact number, if you are happy to be contacted for further assistance or with the results of the study, as you MAY have indicated above.

Information on cropping and pasture species

You will now answer a series of questions identifying the types of crops and pastures sown on your property.

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9. What proportion of your property is sown to the following crops on an annual basis?

	<10%	<20%	<30%	<40%	<50%	<60%	<70%	<80%	<90%	100%
Pasture - grass dominant	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pasture - grass / legume mix	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pasture - legume dominant	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Oil Seed	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Summer crop	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Winter cereal	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lucerne	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other pulses	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Other (please specify)

10. How many paddocks do you have sown with Biserrula? Please indicate the size in hectares in the box provided, and indicate here if all paddocks are IDENTICAL

Paddock 1

Paddock 2

Paddock 3

Paddock 4

Paddock 5

More than 5 paddocks: please state the number & sizes of other paddocks

Your livestock

You will now be asked a few questions about the livestock you hold.

11. What type of stock do you hold?

☐ Wool sheep ☐ Beef cattle ☐ Alpacas

☐ Meat sheep ☐ Dairy cattle ☐ Horses

☐ Other (please specify)

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12. Indicate the approximate number of each livestock species on your property.

Wool sheep

Meat sheep

Beef cattle

Dairy cattle

Alpacas

Horses

Other

Information on each Paddock sown to Biserrula.

You will now be asked to fill in a separate set of questions for each of your paddocks sown to Biserrula.

Please answer all the questions as completely as you can.

On completion of your first paddock you will be asked to if you have further paddocks sown to Biserrula, please select 'yes' to fill in information for subsequent paddocks or 'no' to exit the survey.

You will be able to enter information for 3 paddocks in this survey. If you have more than 3 biserrula paddocks please complete the first three, and either provide contact details somewhere so that we can contact you for further information on the other paddocks, or re-enter the survey to add the other paddock information.

13. State the name or number of your first biserrula paddock.

14. What is the area of this paddock? Please insert the area in heactares.

15. In what year was the current crop of Biserrula sown in this paddock?

- prior to 2008
- 2008
- 2009
- 2010
- 2011
- 2012

16. What variety of Biserrula is sown in this paddock?

- Casbah
- Mauro
- Don't know

17. Is this paddock part of a crop rotation?

- Yes
- No
- Not at present

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18. Is this paddock part of a long term pasture?

- ◯ Yes
- ◯ No
- ◯ Not at present

Paddock 1: Cropping paddock

You will now enter information for your Biserrula CROPPING paddock.

19. What herbicides / pesticides do you use in cropping years (including pre-emergent herbicides)?

Product 1
Product 2
Product 3
Product 4
Product 5

20. At what rate did you use these products?

Product 1
Product 2
Product 3
Product 4
Product 5

Paddock 1: Pasture paddock.

You will now enter information on your Biserrula PASTURE paddock.

21. What herbicides / pesticides have you used on this pasture paddock?

Product 1
Product 2
Product 3
Product 4
Product 5

22. At what rate did you use these products? Please indicate L/ha or g/ha.

Product 1
Product 2
Product 3
Product 4
Product 5

Paddock 1: Soil type

23. What is the parent material of the soil in this paddock?

- [ ] Basalt
- [ ] Slate / shale
- [ ] Clay
- [ ] Granite
- [ ] Limestone
- [ ] Don't know
- [ ] Other (please specify)

24. Describe the texture of the soil in your paddock.

- [ ] Sand
- [ ] Light clay
- [ ] Other
- [ ] Sandy loam
- [ ] Medium clay
- [ ] Loamy sand
- [ ] Heavy clay
- [ ] Other (please specify)

25. Do you have a current soil test?

- Yes
- No

26. If yes, would you be willing to send this to the research team? If you say 'yes' to this question you will be contacted by mail or email to request a copy of your soil test.

- No
- Yes. Please insert a phone, email or mail contact in the box below

27. Had fertilizer been applied to the paddock?

- Yes
- No

28. If yes, please state which fertlizer used, time of application (e.g. 2006, 2007) and rate of application.

Fertilizer 1 (name, year of application and rate)

Fertilizer 2 (name, year of application and rate)

Fertilizer 3 (name, year of application and rate)

Fertilizer 4 (name, year of application and rate)

Fertilizer 5 (name, year of application and rate)

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29. Does your paddock suffer from any trace element deficiences?

- ◯ No
- ◯ Don't know
- ◯ Yes. Please specify

Paddock 1: Grazing livestock.

You will now be asked a series of questions about the livestock you graze on this Biserrula paddock.

30. What is the main species you graze on this paddock?

- ☐ Cattle
- ☐ Sheep
- ☐ Alpacas
- ☐ Horses
- ☐ Other

31. What is the main BREED you graze on this pasture?

32. In the case of sheep, what was the months of wool growth on the animals when they entered the paddock?

33. In the case of SHEEP, did they have lambs at foot or did they lamb in the paddock?

- ☐ They were not in lamb
- ☐ They were in lamb
- ☐ They lambed in the paddock
- ☐ They had lambs at foot

34. What is their main production purpose? E.g. Fat lambs, wool, beef stock, dairy, no production purpose.

35. What is your stocking density in this paddock?

Paddock 1: Photosensitisation

36. Have you had any animals exhibit signs of photosensitisation whilst grazing in this paddock?

- ◯ Yes
- ◯ No

Paddock 1: Stock photosensitisation

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37. Please select the ages / types of animals affected by photosensitisation grazing in this paddock. You can select multiple types.

- [ ] Adult sheep - ram
- [ ] Adult sheep - wether
- [ ] Adult sheep - ewe
- [ ] Lambs - suckling <6 weeks
- [ ] Lambs - suckling <6 months
- [ ] Lambs - weaned <6 months
- [ ] Lambs - 6-12 months
- [ ] Cattle - bull
- [ ] Cattle - steer
- [ ] Cattle - cow
- [ ] Cattle - heifer
- [ ] Unweaned Calves
- [ ] Weaner Calves
- [ ] Horses
- [ ] Foals
- [ ] Alpacas
- [ ] Other (please specify)

38. How long had these animals been grazing biserrula when photosensitisation was first noticed?
(number of days/weeks/months etc)

39. What action did you take? Please write a short answer.

40. What proportion of the paddock is biserrula?

Paddock 1: Stock photosensitisation

41. Was a diagnosis of photosensitivity given or confirmed by a veterinarian?

- ○ Yes
- ○ No
- ○ Wasn't required

42. If the animals were seen by a veterinarian, what treatment did they advise? You can select more than one option.

- [ ] No treatment
- [ ] Ointments / creams for skin
- [ ] Pain relief
- [ ] Surgical removal of dead / affected tissues
- [ ] Move animals to new pasture
- [ ] Move animals to shed
- [ ] Supplement feed with hay on Biserrula pasture
- [ ] Other (please specify)

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43. Did your veterinarian carry out any tests on your affected animals?

- ◯ Yes
- ◯ No

44. Do you have any test results that you are willing to share with the research team?

- ◯ No
- ◯ Yes. Please give a phone, email or mail contact so we can contact you for these results

Paddock 1: Stock photosensitisation.

You have indicated that your stock showed signs of photosensitisation whilst grazing on Biserrula. You will now be asked a series of questions relating to the onset, signs and severity of this photosensitisation.

45. What proportion of the animals in the paddock were:

	up to 10%	up to 25%	up to 50%	up to 75%	up to 100%
Lambs / calves	☐	☐	☐	☐	☐
Immature males (stock under 1 year)	☐	☐	☐	☐	☐
Immature females (stock under 1 year)	☐	☐	☐	☐	☐
Mature males	☐	☐	☐	☐	☐
Mature females	☐	☐	☐	☐	☐

46. Did any animals in the paddock show signs of photosensitivity NOT related to Biserrula?

- ☐ Yes
- ☐ No
- ☐ Maybe
- ☐ I'm not sure

47. If 'YES, do you know the causal agent?

48. Were any of the following species present in the pasture?

- ☐ Lantana
- ☐ Heliotrope
- ☐ Fireweed
- ☐ Patterson's Curse
- ☐ Bishop's weed
- ☐ Caltrop
- ☐ Other weeds (please specify)

Paddock 1: Severity of clinical signs.

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Please read the following descriptions carefully and then answer the questions regarding the severity of clinical signs of photosensitisation you observed in your stock.

MILD = Skin is red, weepy and swollen but not blackened. Eyes, muzzle and ears are affected. Swelling of the ears in young stock. Mild dermatitis, affecting exposed skin areas only. Some eye and nasal discharge may be present. Irritation and pain will be evident by scratching of head and face. Udders will be affected in dairy cattle. Animals may show reduction in appetite.

MODERATE = Skin is very red, blackened and flaking. There will be severe swelling of ears and soft tissues of the face. Focal or generalised hair or wool loss. Dermatitis affecting whole body. Animals may show significant nasal and eye discharge. Eyes may be swollen shut. Animals will show difficult in eating and / or reduced appetite. Irritation and pain will be evident by continuous scratching of affected regions. Animals will preferentially seek shade or stand in dams to reduce irritation.

SEVERE = The skin will be sloughing and necrotic in patches with weeping lesions. Severe dermatitis including secondary lesions. Significant loss of wool or fur. Animals will be depressed and have poor appetite. They will seek shade, show skin scratching and pain related behaviours or may, in extreme cases, show a complete lack of movement or response to normal stimuli.

49. Of those animals affected, most of them were.........

○ Mild
○ Moderate
○ Severe

50. What total proportion of the animals in the paddock were affected?

Total % affected ______

51. What total proportion of the animals in the paddock were SEVERLY affected?

% severely affected ______

52. The animals which were SEVERLY affected were........
(select from the following, you can choose more than one)

☐ Adult sheep - ram
☐ Adult sheep - wether
☐ Adult sheep - ewe
☐ Lambs - suckling <6 weeks
☐ Lambs - suckling <6 months
☐ Lambs - weaned <6 months
☐ Lambs - 6-12 months
☐ Cattle - bull
☐ Cattle - steer
☐ Cattle - cow
☐ Cattle - heifer
☐ Unweaned Calves
☐ Weaner Calves
☐ Horse
☐ Foal
☐ Alpaca

53. What total proportion of the animals in the paddock were MODERATELY affected?

% moderately affected ______

54. The animals which were MODERATELY were.....
(select from the following, you can choose more than one)

- [ ] Adult sheep - ram
- [ ] Adult sheep - wether
- [ ] Adult sheep - ewe
- [ ] Lambs - suckling <6 weeks
- [ ] Lambs - suckling <6 months
- [ ] Lambs - weaned <6 months
- [ ] Lambs - 6-12 months
- [ ] Cattle - bull
- [ ] Cattle - steer
- [ ] Cattle - cow
- [ ] Cattle - heifer
- [ ] Unweaned Calves
- [ ] Weaner Calves
- [ ] Horse
- [ ] Foal
- [ ] Alpaca

55. What total proportion of the animals in the paddock were MILDLY affected?

% mildly affected ____________________

56. The animals which were MILDLY affected were..........
(select from the following, you can choose more than one)

- [ ] Adult sheep - ram
- [ ] Adult sheep - wether
- [ ] Adult sheep - ewe
- [ ] Lambs - suckling <6 weeks
- [ ] Lambs - suckling <6 months
- [ ] Lambs - weaned <6 months
- [ ] Lambs - 6-12 months
- [ ] Cattle - bull
- [ ] Cattle - steer
- [ ] Cattle - cow
- [ ] Cattle - heifer
- [ ] Unweaned Calves
- [ ] Weaner Calves
- [ ] Horse
- [ ] Foal
- [ ] Alpaca

57. Did any animals die or were euthanised as a result of thier clinical signs? If 'yes' please state the % in the comment box below.

- [ ] Yes
- [ ] No
- [ ] %

____

58. Please describe, in general, the age / breed / status of the animals affected. E.g. weaner merino, first cross lambs etc.

____

Paddock 1: Feeding and husbandry information on animals grazing in this Bis...

You will now be asked a short series of questions on what these animals were fed prior to and on entering the Biserrula paddock and also any husbandry procedures that were undertaken on this flock or herd whilst grazing Biserrula in this paddock.

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59. What were your animals grazing on PRIOR to enterring the paddock of Biserrula?

	Sheep	Cattle	Other
Native pasture	☐	☐	☐
Perennial or annual ryegrass	☐	☐	☐
Lucerne	☐	☐	☐
Stubble	☐	☐	☐
Mixed pasture (clover, cocksfoot, ryegrass)	☐	☐	☐

Other (please specify)

60. Were these animals fed any feed supplements prior to entering the paddock?

- ◯ Yes
- ◯ No

61. If YES, what supplements were they fed?

Supplement 1

Supplement 2

Supplement 3

62. Did they continue to be supplemented when grazing Biserrula?

- ◯ Yes
- ◯ No
- ◯ Just for a short while

63. Were the animals subjected to any of the folllowing husbandry procedures immediately prior to entering the Biserrula paddock?

- ☐ Drenching
- ☐ Vaccinations
- ☐ Shearing
- ☐ Docking
- ☐ Ringing
- ☐ Weaning
- ☐ Other (please specify)

64. Were the animals subjected to any husbandry practices AFTER entering the Biserrula paddock?

- ☐ Drenching
- ☐ Vaccinations
- ☐ Shearing
- ☐ Docking
- ☐ Ringing
- ☐ Weaning
- ☐ Other (please specify)

Paddock 1: Stage of plant growth at the onset of photosensitivity.

You will now be asked a short series of questions relating to the stage of plant growth of the Biserrula in the paddock in which you noticed photosensitivity.

65. What proportion of the ground do you estimate to be covered by Biserrula when the animals first entered the paddock?

proportion of ground covered [ ]

66. What was the stage of growth of the Biserrula when the animals first entered the paddock?

- [ ] Vegetative
- [ ] Flowering
- [ ] Early pod formation
- [ ] Senescing
- [ ] Dried
- [ ] Mixed, all of the above

Other (please specify)

[ ]

67. What was the stage of growth of the biserrula when the animals FIRST showed signs of photosenstisation?

- [ ] Vegetative
- [ ] Flowering
- [ ] Early pod formation
- [ ] Senescing
- [ ] Dried
- [ ] Mixed, all of the above
- [ ] Other (please specify)

[ ]

68. How much TOTAL pasture (kg DM / ha) was available to the animals when they entered the paddock?

- [ ] <500
- [ ] <1000
- [ ] <1500
- [ ] <2000
- [ ] <3000
- [ ] more than 3000
- [ ] Don't know

69. What other species were present in the pasture? Please state the proportion of ground cover of each in your response.

Species 1

Species 2

Species 3

Species 4

Species 5

70. Additional comments about Paddock 1

Next paddock........

Please write your contact details if you have more Paddocks to add.

71. Do you have another Biserrula paddock to add to this survey?

Yes

No

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End of survey

Thank you for taking the time to fill in this survey. Your responses are important and will help to guide future use of this important pasture species in Australia.

If you have asked to be called for this survey, will be in contact soon to complete this survey over the phone, or via email to arrange a mutually agreeable time to complete this survey.

If you have consented to be contacted about other ways to assist with this study, we may contact you. Similarly, if you have requested to be informed of the outcomes of the study we will be contacting you in the future to update you with this information.

If you have any further comments or questions about this survey please feel free to contact the research team:
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Thank you for your time and assistance in this study.

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Appendix 2. Defined clinical pathology references ranges for 6-9 month old cross-bred lambs.

Full Blood Count	Standard range	Defined Range	Unit
Red blood cell count	9.0 - 15.0	**9.5 - 13.5**	x 10^{12}/L
Haemoglobin	90 - 150	**95 - 135**	g/L
Haematocrit	0.27 - 0.45	**0.25 - 0.45**	L/L
MCV	28 - 40	**25 - 35**	fL
MCH	8 - 12	**8.5 - 11.5**	pg
MCHC	310 - 340	**315 - 375**	g/L
Platelets	250 - 750	**750 - 2250**	x 10^9/L
White Blood Cell Count	4.0 - 12.0	**4 - 12**	x 10^9/L
Neutrophils	0.7 - 6.0	**1 - 7**	x 10^9/L
Band Neutrophils	0 - 0.2	**0.002 - 0.12**	x 10^9/L
Lymphocytes	2.0 - 9.0	**3 - 6**	x 10^9/L
Monocytes	0.0 - 0.9	**0.0 - 0.3**	x 10^9/L
Eosinophils	0.0 - 0.1	**0.0 - 0.15**	x 10^9/L
Basophils	0.0 - 0.3	**0.0 - 0.45**	x 10^9/L
Fibrinogen	1.0-5.0	**4.0 – 7.0**	g/L
Total protein	60 - 83	**63 - 75**	g/L
Albumin	25 - 40	**31 - 38**	g/L
Globulins	30 - 42	**26-42**	g/L
A:G ratio	0.4 - 0.7	**0.4 - 0.7**	
Creatinine	70 - 120	**47 - 67**	□M/L
Urea	2.8 - 7.2	**7 - 10**	mM/L
CK*	69 - 182	**40 - 70**	U/L
AST*	53 - 153	**77 - 112**	U/L
GLDH*	0 - 20	**2 - 15**	U/L
Bilirubin	0 - 9	**1.8 - 2.6**	□M/L
GGT*	30 - 66	**52 - 94**	U/L
Potassium	3.9-5.4	**4.6 - 5.2**	mM/L
Sodium	139 - 152	**139 - 144**	mM/L
Na:K ratio	>29.0	**<31**	
Anion gap	10 - 20	**14 - 19**	mM/L
Bicarbonate		**21.5 - 27.5**	mM/L
Calcium	2.40 - 3.20	**2.8 - 3.2**	mM/L
Phosphate	1.61 - 2.35	**1.4 - 2.2**	mM/L
Ca:PO_4 ratio	1.0 - 2.25	**1.25 - 2.25**	
Chloride	95 - 103	**103 - 108**	mM/L

*CK, creatinine kinase; AST, aspartate aminotransferase; GLDH, glutamate dehydrogenase, GGT, gamma glutamyl transferase.(Kessell et al., 2015)

Appendix 3. Photosensitisation scoring system.

Photosensitisation scoring system.
MILD = 1 to 2
MODERATE = 3 to 4
SEVERE = 5

Facial skin and muzzle:
0: Normal
1: Skin is mildly red, and swollen but not blackened or weepy
2: Skin is red, and swollen but not blackened or weepy. Noticeable but not severe swelling of ears and soft tissues of the face.
3: Skin is red and flaking. There will be severe swelling of ears and soft tissues of the face. Some focal or generalised hair or wool loss.
4: Skin is very red, blackened and flaking. There will be severe swelling of ears and soft tissues of the face. Focal or generalised hair or wool loss.
5: The skin will be sloughing and necrotic in patches with weeping lesions. Severe dermatitis including secondary lesions. Significant loss of wool or fur. Severe swelling of soft tissues of the face.

.
Eyes:
0: Normal
1: Some weeping from the eyes.
2. Weeping eyes, conjunctival swelling noticeable. Some swelling of soft tissues around the eyes with noticeable redness.
3. Eyes weeping and significant palpebral and conjunctival redness and swelling. Soft tissues around the eyes swollen.
4. Severe swelling around the eyes, palpebral and conjunctival swelling. Eyes beginning to close. Ulceration of the cornea may be visible.
5. 4. Severe swelling around the eyes, palpebral and conjunctival swelling. Eyes beginning to close. Corneal ulceration.

Ears:
0: Normal
1: Drooping of ears with mild oedema.
2. Pitting oedema and mild swelling of pinnae.
3. Significant pitting oedema of pinnae, curling of ear ends, some dermal lesions may be visible. Skin flaking.
4. Significant pitting oedema of pinnae, curling of ear ends, dermal lesions visible. Skin flaking and areas of necrotic changes. Some tissue loss from rubbing may be evident. Moderate dermatitis with weeping lesions.
5. Significant pitting oedema of pinnae, curling of ear ends and loss of ear tissue, dermal lesions visible including blackened dead skin. Skin flaking and areas of necrotic changes. Severe dermatitis with weeping lesions.

Fleece / body:
0: Normal
1: Mid reddening of exposed areas.
2: Significant reddening of exposed areas.
3: Patchy fleece loss, reddening of underlying skin.
4. Significant fleece loss and dermatitis of exposed skin.
5. Widespread fleece loss, severe dermatitis of exposed skin.

Appendix 4. Producer ‘Tips and Tools’ brochure.

Information collated from the producer survey (section 4.1), on-farm outbreaks (section 4.2) and controlled grazing trials (sections 4.4 and 4.5) was condensed into a two page document suitable for dissemination to producers or presentation on producer-focussed websites. This brochure was presented to producers at the Sheep Field Day in October 2016 for producer feedback, which was positive regarding usefulness of the information presented.

The Graham Centre is an alliance between Charles Sturt University and NSW Department of Primary Industries
www.grahamcentre.net

Understanding Photosensitisation in Sheep Grazing Biserrula Pastures

Background

Biserrula (*Biserrula pelecinus*) is an annual pasture legume native to Europe and North Africa (Figure 1). In its native environment, biserrula grows in association with other annual legumes such as subterranean clover (*Trifolium subterraneum*) and serradella (*Ornithopus* spp.). The first cultivar of biserrula, Casbah, was released in 1997; followed by Mauro in 2002. Biserrula is used mainly in low to medium rainfall (325-650 mm) zones of southern Australia as break pasture in a cropping; usually as a monoculture and it can be very weed suppressive.
Sheep growth rates on biserrula can exceed 350 g/head/day, **however**, livestock of all ages grazing biserrula can be affected by mild to severe photosensitisation

A number of grazing experiments were undertaken at Charles Sturt University (CSU) in Wagga Wagga NSW to better understand factors contributing to photosensitisation in sheep grazing biserrula and investigate options to reduce the incidence and severity of the disorder.

Figure 1. Biserrula (cv. Casbah) leaf, flower and pod.

Tips and Tools for managing sheep grazing biserrula pastures

Tip 1: Understand the different types of photosensitisation

Tool 1: There are different types of photosensitisation. Primary photosensitisation occurs when animals ingest plants that cause direct damage to skin in the presence of sunlight, Removal of animals from pastures containing these plants results in resolution of the problem – this is the type of photosensitisation caused by biserrula.
Secondary photosensitisation occurs when ingested compounds contained in plants such as, witch grass (*Panicum capillare*) and caltrop (*Tribulus terrestris*), affect liver function which then causes photoreactive pigments to circulate under the skin where they react with sunlight to cause tissue damage. Photosensitisation can also occur through direct contact with plants such as pathenium weed (*Parthenium hysterophorus*) that produce exudates which react with sunlight. ***Biserrula causes primary photosensitisation.***

Tip 2: Photosensitisation can occur in sheep grazing biserrula-dominant pastures (both Casbah and Mauro) throughout the active growth phase of the plant with variation in the number of animals affected and severity of outbreak

Tool 2: It is important to observe livestock grazing biserrula-dominant pastures at regular intervals during the active growth (green) phase of the plant in order to detect early signs of photosensitisation. This can occur as soon as Day 3 on pasture.
One winter and three spring grazing studies have now been completed at CSU. The outbreak experienced in winter was more severe than in spring. The reason for this is as yet unclear but colder-than-average winter temperatures at the time of this experiment may have influenced increased biserrula intake through environmental effects on plant nutritive characteristics.

Tip 3: The initial signs of photosensitisation can arise quickly (48-72 hours)

Tool 3: Become familiar with the range and severity of symptoms exhibited by affected animals (Figure 2) so you can act quickly to minimise the number of animals affected and the severity of symptoms.

Figure 2. Lambs showing mild (top left), moderate (top right) and severe acute (bottom left) photosensitisation after grazing biserrula-dominant pasture. Fleece loss is also commonly observed in severe cases (bottom right).

Tip 4: The sooner you recognise the symptoms and take management action, the more rapidly affected animals will recover.

Tool 4: Promptly remove animals from biserrula-dominant pasture as soon as mild signs appear (Figure 2). Provision of an alternative feed source (e.g. hay or grass pasture) reduces the intensity of injury and improves recovery time. Remove animals to shaded areas where possible.

Tip 5: If animals have other underlying health issues (e.g. worms, liver fluke), they may be more rapidly and severely affected by photosensitisation

Tool 5: Ensure health disorders such as parasites are controlled, regardless of pasture to be grazed, and be particularly vigilant in ensuring animals grazing biserrula are healthy, underlying health issues will increase the likelihood and severity of photosensitisation.

Tip 6: Presence of other species in the pasture sward reduces the incidence and severity of photosensitisaton

Tool 6: In CSU studies, 10-30% annual ryegrass in the pasture at the commencement of grazing reduced the severity of photosensitisation. Higher levels of ryegrass were required with the cultivar Mauro compared to Casbah. Mauro is less bitter in taste than Casbah and animals tend to consume it more readily.

Figure 3. Pigmented (foreground) and non-pigmented (background) lambs grazing biserrula dominant (>90% biserrula) pasture. Note lack of tissue damage in the pigmented animal.

Tip 7: Sheep breed and genotype affects both the incidence and severity of photosensitisation

Tool 7: Use of less susceptible breeds/genotypes can reduce the incidence and severity of photosensitisation. Pigmented sheep generally do not exhibit visible signs of photosensitisation (Figure 3). Merino sheep appear to be less affected by photosensitisation than cross-bred sheep. This is most likely due to merinos having better wool coverage around the ears and face. Keep in mind shearing time may affect susceptibility. Non-pigmented (white), shedding sheep are most susceptible to photosensitisation.

Tip 8: Dried (senescent) biserrula does not cause photosensitisation

Tool 8: Photosensitisation has not been recorded in any animals in CSU trials when grazing fully senesced biserrula pasture, regardless of the percentage of biserrula in the sward (Figure 4). As a dried forage biserrula has a high protein content (14%) and a maintenance energy level for mature dry sheep (7 MJ ME/kg DM). Rainfall received over summer can affect the quality of senesced pasture. It is important to observe pasture over the summer period and particularly after rain for summer weeds, such as witch grass and caltrop, which can cause secondary photosensitisation.

Figure 4. Dried or senescent biserrula pasture.

Other strategies used in industry

Some producers have reported success in using low quality hay as a supplement in biserrula paddocks to reduce photosensitisation. Some producers also use rapid rotational grazing (7 days or less on pasture) incorporating biserrula paddocks with non-biserrula containing paddocks, whilst this may mitigate severe ourbreaks it cannot prevent photosensitisation in all animals in the mob, particularly in older or younger stock.

If you are interested to read more:

Kessell, A., Ladmore, G., Quinn, J. (2015). An outbreak of primary photosensitisation in lambs secondary to consumption of *Biserrula pelecinus* (biserrula). *Aust. J. Vet. Sci.* 93:174-178

Ladmore, G. (2014). Investigating Livestock Production with the Legume *Biserrula pelecinus*: Aetiology and Pathogenesis of Photosensitivity. Honours Thesis. Charles Sturt University.

Quinn, J.C., Kessell, A., Weston, L.A. (2014). Secondary plant products causing photosensitisation in grazing herbivores: their structure, activity and regulation. *Intl. J. Mol. Sci*. 15: 1441-1465.

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