Primary photosensitivity in lambs grazing forage brassica

A thesis

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by

Gemma Box

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Photosensitisation: surveyed as the most prevalent animal disease of livestock grazing forage brassica in Australia.

Morton & Campbell, 1977

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Forage brassicas are a diverse range of important, high-quality annual feed crops grown on farms throughout New Zealand (300,000 ha) and Australia (200,000 ha). An animal health concern occasionally associated with lambs consuming forage brassica crops grown during summer is photosensitivity; a sporadic, non-contagious disease where the epidermis and dermis of ear pinna, eyelids, bridge of the nose and midline of the back is uncharacteristically responsive to sunlight due to the presence of a phototoxin. The most prevalent clinical sign of this disease was a change in ear thickness, due to oedematous accumulation, affecting animal welfare, and potentially production. While the phototoxin in forage brassica is unidentified, compounds including perylenequinone, aglycone anthraquinone, furanocoumarin or a chlorophyll metabolite may be responsible. In order to identify mitigating factors, document gross pathology and provide practical management tools for this disease, three studies were conducted. In the first study, evidence of photosensitivity was compared between forage rape and raphanobrassica under low and high nitrogen (N) applications. There was little difference between forage species with both forages eliciting photosensitivity, peaking three days after introduction to brassica then gradually resolving. On Day 3 raphanobrassica fed lambs had a slightly higher increase in mean ear thickness at +1.77 mm, 95% CI [1.47, 2.08], compared with forage rape at +1.20 mm, 95% CI [1.01, 1.40], being no difference thereinafter ($P > 0.1$). There was no effect of N on photosensitivity ($P = 0.531$). A lack of alteration of liver enzymes, as demonstrated by the presence of gamma-glutamyl transferase (GGT) and glutamate dehydrogenase (GLDH), indicated this disease is a form of primary photosensitivity. A second study compared severity, incidence and onset of photosensitivity in lambs assigned to four brassica grazing treatments or a non-brassica control, chicory. The four brassica treatment strategies were designed to influence the intake of plant parts (i.e. leaf or petiole/stem). The strategy encouraging the highest leaf intake resulted in the greatest severity, highest incidence and earliest onset of the disease

($P < 0.001$). There was little evidence of photosensitivity in lambs that consumed predominantly petiole and stem, signifying the phototoxin may be present in leaf material. In both studies, clinical signs resolved while lambs continued to consume affecting feed, suggesting a developed ability to detoxify the causative agent(s). In a final study, the duration of this detoxification or tolerance ability was determined by measuring photosensitivity in four groups of lambs who had initially exhibited photosensitivity on brassica. Lambs continuously fed brassica for over eight weeks or those transferred from brassica to chicory for eight weeks showed no indication of photosensitivity. Lambs grazing brassica continuously, with a four-day break on chicory, before being returned to brassica for two weeks showed no signs either. While lambs initially on brassica removed to chicory for 8 weeks and returned to brassica for 14 days developed clinical signs ($P < 0.001$). This suggests their ability to detoxify the causative agent lasted at least four days but not eight weeks. As this disease was traditionally associated with forage rape (‘rape scald’), we propose the term ‘brassica-associated primary photosensitivity’ (BAPP) to encompass evidence of it occurring in other forage brassica species. Grazing strategies that reduce brassica leaf intake and retain lambs on forage brassica throughout the summer risk period may be effective tools to mitigate BAPP in lambs. A tentative diagnosis of BAPP may be given to lambs exhibiting an ear pinna thickness > 3.6 mm taken at the midpoint of the ear pinna and/or presence of non-pitting bilateral oedema and/or presence of erythema with a history of recent introduction to a forage brassica, i.e. within the last week, during summer.

Keywords: *Brassica* spp., *Brassica napus* spp. *biennis, Raphanus x Brassica* L., forage brassica, forage rape, raphanobrassica, BAPP, primary photosensitivity, rape scald, ear thickness, live weight, erythrocyte integrity, gamma-glutamyl transferase, glutamate dehydrogenase, crop yield, ultraviolet radiation.

Disclaimer

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I am eagerly awaiting my next adventure.

Manuscripts

The first manuscript is currently under review in the Journal of New Zealand Grasslands

1) The development of Pallaton raphanobrassica for New Zealand farming systems (2021). A DUMBLETON, GM BOX, F FOLEY, CT WESTWOOD & EM WRIGHT

The following two manuscripts, covering the three experimental chapters, are temporarily embargoed until the release of this PhD, due to commercial sensitivity.

2) Primary photosensitisation (‘rape scald’) in lambs 1: Grazing forage rape or raphanobrassica. GM BOX, GK BARRELL, CT WESTWOOD
3) Primary photosensitisation in lambs 2: Mitigating strategies. GM BOX, RW HOFMANN, CT WESTWOOD, GK BARRELL

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List of Abbreviations

ADF	Acid detergent fibre
BAPP	Brassica associated primary photosensitivity
BALD	Brassica associated liver disease
CP	Crude protein
DAS	Days after sowing
DM	Dry matter
DMI	Dry matter intake
DM%	Dry matter percentage
DNA	Deoxyribonucleic acid
DOMD	Dry organic matter digestibility
ECF	Extracellular fluid
GLDH	Glutamate dehydrogenase
GGT	Gamma-glutamyl transferase syn. γ-glutamyl transferase
GSL	Glucosinolate
Hb	Haemoglobin
ICF	Intracellular fluid
ME	Metabolisable energy
MJ ME	Megajoules of metabolisable energy
N	Nitrogen
NDF	Neutral detergent fibre

RBC	Erythrocytes syn. red blood cells
ROS	Reactive oxygen species
SMCO	S-methyl-L-cysteine sulphoxide
SS	Soluble sugars
UV	Ultraviolet (radiation)
UVI	Ultraviolet index
WSC	Water soluble carbohydrate

Definitions in the context of this thesis

Clinical evidence of Brassica associated primary photosensitivity (BAPP) syn. ‘rape scald’ in lambs:

Main criterion is an increase in ear pinna thickness of ≥ 0.5 mm taken at the midpoint of the ear pinna, but may also include presence of non-pitting bilateral oedema and/or presence of erythema, with a history of recent introduction to a forage brassica, i.e. within the last week, during summer.

Clinical significance:

Oedema was easily able to be identified on palpation when the ear thickness had increased approximately ≥ 0.5 mm taken at the midpoint of the ear pinna from the initial measurement performed prior to brassica consumption. Depending on the individual lamb, increases in ear thickness ≥ 0.5 mm may also be associated with ventral displacement of the ear pinna from the typical position, i.e. drooping. At this point the weight of the ear, due to oedematous fluid accumulation, may begin to overcome the strength of the structural cartilaginous tissue. Therefore, we determined that an increase in ear pinna thickness of ≥ 0.5 mm was a cut off for clinical significance of BAPP.

Incidence of BAPP:

The proportion of lambs which developed BAPP during the study.

Chapter 1

General Introduction

1.1 Context

Brassica spp. (forage brassicas), are annual fodder crops grown on approximately 300,000 ha in New Zealand (de Ruiter, Dalley, Hughes, Fraser, & Dewhurst, 2007) and 200,000 ha in Australia (D. Thomas, personal communication, November 30, 2020) on sheep, beef and deer enterprises. Accumulating yields of 4 - 20 t DM/ha over 2 - 6 months (Fletcher & Chakwizira, 2012; Fletcher, Wilson, Maley, McCallum, & Shaw, 2010; Gowers & Armstrong, 1994; Jacobs, Ward, McDowell, & Kearney, 2001; Jung, Kocher, & Gilica, 1984), these crops provide additional high quality (de Ruiter, Dalley, Hughes, Fraser, & Dewhurst, 2007; Westwood & Mulcock, 2012) feed options to traditional pasture swards; particularly during periods of feed shortages due to low soil moisture (Fletcher, et al., 2010) and temperature (Andreucci, Black, & Moot, 2012; Andreucci, Moot, Black, & Sedcole, 2016; Dumbleton, et al., 2012).

Two examples of forage brassica crops commonly grown during summer are *Brassica napus* (forage rape) and a new-to-market product: *Raphanus x Brassica* L., cv 'Pallaton' (*xRaphanobrassica* syn. raphanobrassica); which had an initial limited commercial release in 2017. These two crops have a short maturity, whereby they are ready to graze 60 - 110 days after seed is sown (DAS) (de Ruiter, Dalley, Hughes, Fraser, & Dewhurst, 2007). Forage rape and raphanobrassica crops are well-suited for sheep enterprises, where their nutritional profile (Westwood & Mulcock, 2012) and timing of crop growth coincides with high lamb energy requirements, post-weaning.

While forage rape and raphanobrassica are agronomically excellent forage options, there are some animal health concerns occasionally associated with these crops. One of these concerns, which may sporadically appear in lambs grazing forage rape crops during the summer months, is photosensitisation (traditionally called 'rape scald'). Photosensitisation is a well-recognised clinical condition causing non-contagious dermatitis and sensitivity to sunlight (Filmer, 1947; Vermunt, West, & Cooke, Rape poisoning in sheep, 1993), comparable to spring eczema. Presenting animals can have swollen, droopy ears, lesions progressing to blistering and scabbing of the ears, facial swelling and may have burn-like skin damage on the dorsum of the lamb. The aetiology of this disease is unknown (Collett, 2013). There is some anecdotal evidence that this form of photosensitivity emanates not only in lambs grazing forage rape crops but raphanobrassica too. There is a gap in the literature on differences between summer brassica crops inducing photosensitivity and grazing recommendations to reduce the risk of this disease.

Photosensitisation is an animal welfare concern in the livestock industry. While the Animal Welfare Act 1999 does not specifically list photosensitivity, the act does require the “protection from, and rapid diagnosis of, any significant injury or disease” as a definition of physical, health and behavioural need (Animal Welfare Act 1999, s. 4E). The provision of shade when signs of photosensitivity are present is specifically listed in the Code of Welfare under the act (Code of Welfare Sheep and Beef Cattle 2018).

This thesis confirms photosensitivity may arise in both forage rape and raphanobrassica crops; measures disease incidence and severity between crops and various management strategies; documents gross pathology; and transforms this information into practical layman recommendations, which have subsequently been utilised on farm to reduce the risk of photosensitivity.

Recommendation

Traditionally, the condition forming the basis of this thesis has been called ‘rape scald’. However, we report it occurring in lambs grazing a non-rape plant (Chapter 3), causing us to propose a more encompassing term for the disease. We submit the term – brassica-associated primary photosensitivity (BAPP) – as an appropriate new name for this condition.

1.2 Justification for thesis

Raphanobrassica, a new forage brassica species, is an interspecific and intergeneric hybridisation of kale (*Brassica oleracea* L.) x radish (*Raphanus raphanistrum* subsp. Sativus L.). It is the first new forage brassica species or subspecies since the creation of leafy turnip in the 1980s. The initial limited commercial release of raphanobrassica, marketed by PGG Wrightson Seeds, to the commercial marketplace took place in 2017 with 3,500 ha grown that year (A. Dumbleton, personal communication, February 22, 2018). Sales volumes have subsequently grown exponentially (H. Mulcock, personal communication, November 30, 2020).

As a new product entering the marketplace, extensive testing and trialling has been undertaken on raphanobrassica (H. Mulcock, personal communication, November 30, 2020), including feed quality testing and assessment of potential anti-nutritional factors including the concentrations of glucosinolates (GSL) and S-methyl-L-cysteine sulphoxide (SMCO). The compound(s) responsible for primary photosensitisation remains unknown (Collet, 2019). *In vitro* screening of new brassica genetics for risk for photosensitisation in lambs remains problematic and currently cannot be incorporated into existing screening programs identifying the presence of antinutritional compounds.

On-farm evaluation for risk of photosensitisation currently remains the only effective way to assess the risk of primary photosensitisation in lambs grazing forage brassicas.

Anecdotal evidence of photosensitivity, similar to that seen in lambs grazing forage rape, occurred in lambs grazing raphanobrassica crops during on-farm studies in 2016/17 (C. Westwood, personal communication, February 6, 2017). Due to this unintended consequence, this project was immediately presented as a potential research thesis, ensuring rigorous due diligence was performed on one of PGG Wrightson Seeds leading brassica products. The first study (Chapter 3) pertaining to photosensitivity associated with raphanobrassica was conducted during summer 2017/18.

1.3 Nature and scope of the investigation

There are four objectives in this thesis on photosensitivity in lambs consuming forage brassica. The objective of the research contained within this thesis was twofold. Firstly, to quantify the risk of photosensitisation in lambs grazing raphanobrassica compared with lambs grazing forage rape. Secondly, a series of grazing management-based mitigation strategies were assessed ways to protect brassica-fed lambs against the effects of photosensitisation.

1. Review literature to identify potential causative agent(s) of photosensitivity of lambs grazing forage brassica. Note: *In vitro* phytochemical analysis, which could be utilised to identify and isolate the causative agent in Brassica spp. (Collett, 2019), is out of the scope of this research.

2. Empirically identify mitigating factors of photosensitisation of lambs grazing forage brassica which reduce severity and/or incidence including:

    A. Comparison between forage rape and raphanobrassica, two brassica species commonly grazed during summer;

    B. Additional crop nitrogen application;

    C. Comparison between three grazing management practices of 'set stocking' lambs onto crop, strip grazing and intermittent grazing;

    D. If prior exposure to forage brassica reduces severity and/or incidence.

3. Document progression of gross pathology of lambs clinically affected by photosensitivity whilst grazing forage brassica.

4. Transform information into practical management tools to reduce morbidity and disseminate associated production losses as a result of brassica associated primary photosensitivity on sheep farming enterprises:

    A. Identification of crop risk factors;

    B. Grazing management strategies during peak risk periods.

1.4 Thesis structure

The structure of the thesis is depicted below (

Figure 1 1). Following a literature review, three experimental chapters, Chapters 3-5, are presented to address objectives 2A, 2B, 2C and 2D. Gross pathology of clinical disease from these three studies are summarised in Chapter 6. Key findings are pulled together in the final chapter, Chapter 7, which discusses practical management strategies to reduce severity and/or incidence of brassica associated primary photosensitivity.

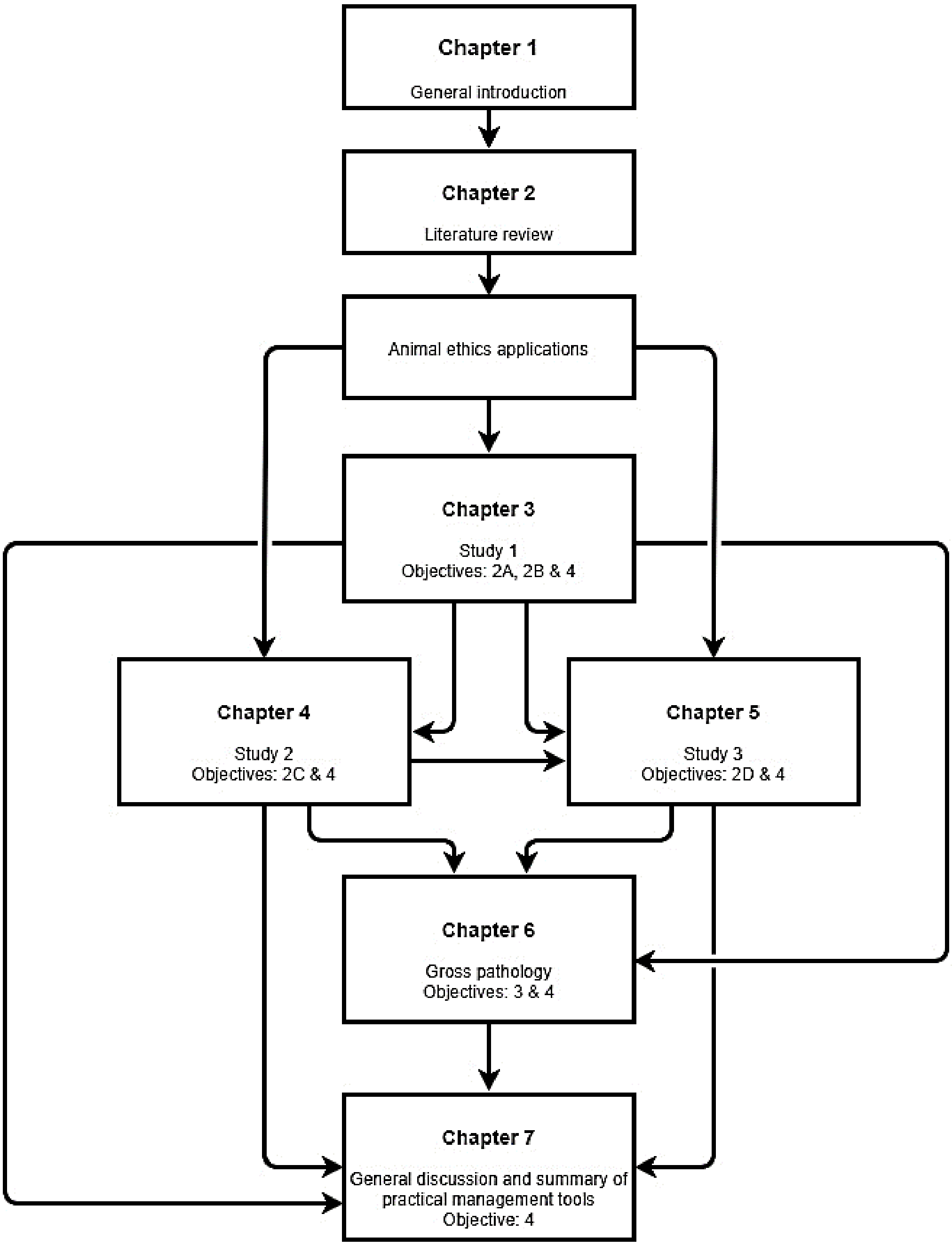


Figure 1: Schematic diagram of the thesis structure, depicting the relationship between thesis chapters

Chapter 2

Literature review

2.1 Forage brassica

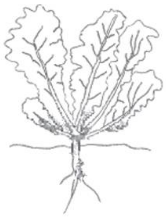
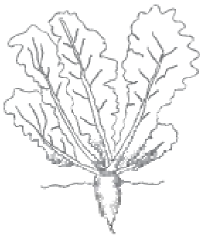
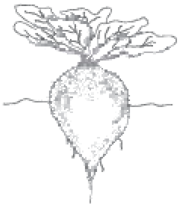
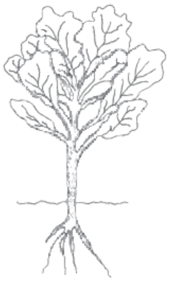
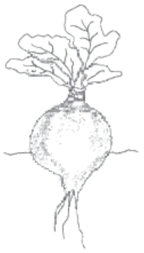
2.1.1 Agricultural use

Forage brassicas are annual feed crops commonly grown throughout Australasia on sheep, beef and deer enterprises. These crops are grazed by livestock *in situ*. There are several purposes for these crops. Firstly, they may be grown as an alternative to pastures, particularly during periods of feed shortages that occur due to low soil moisture and temperature (Dumbleton, et al., 2012). Forage brassica crops also offer a feed supplement to pastures and are especially useful to help mitigate pasture related animal health issues, such as internal parasite larval challenge or facial eczema spore count (de Ruiter, et al., 2009). Lastly, they may be grown as part of a crop rotation to reduce weed, pests and disease burdens (de Ruiter, et al., 2009).

2.1.2 Species and subspecies

There are six species or subspecies of forage brassica of importance for livestock feeding in Australasia (Table 1): forage rape (*Brassica napus* spp. *biennis*); raphanobrassica (*Raphanus x Brassica* L.); leafy turnip (*B. rapa* syn. *B. campestris*); bulb turnip (*B. rapa* syn. *B. campestris*); kale (*B. oleracea* spp. *acephala*); and swedes (*B. napus* spp. *napobrassica*). Each species or subspecies, and cultivars within these, have varying characteristics which fit into different farming systems (de Ruiter, et al., 2009). The focus of this thesis is on two species that are typically utilised as feed for lamb growth post-weaning during the summer period: forage rape and raphanobrassica. There is some evidence of BAPP occurring in lambs consuming leafy turnip crops too (Filmer, 1947), however, this is out of scope for this thesis. All six forage brassica feed options are briefly highlighted in Table 1.

Table 1: Forage brassicas most commonly used for animal feeding in Australasia adapted from Stewart & Charlton (2003) with approximate proportion of crop grown of all New Zealand brassica sales in 2019/20 (personal communication, A. Dumbleton, September 16, 2020).

Common name	Growth habit	Vegetative description	Proportion grown (%)
Forage rape		• Numerous leaves • Fibrous stem • No bulb or fleshy stem • Grows to various heights	35
Raphanobrassica		• Non-bulb producing • Tap root with single growing point • Able to regrow multiple times after grazing • Leafy	5
Leafy turnip		• Non-bulb producing • Swollen tap root with multiple growing points • Able to regrow after grazing • Leafy	5
Bulb turnip		• Fleshy bulb • No neck • White or yellow fleshed	10
Kale		• Large swollen stem with varying leaf percentage • Stem – woody outer layer, soft fleshy marrow • Grows to various heights	40
Swedes		• Fleshy bulb • Obvious neck • White or yellow fleshed	5

2.1.2.1 Forage rape

Forage rape (syn. rape) is traditionally sown in spring for consumption over the summer/autumn period as an alternative pasture species on lamb finishing enterprises. Newer cultivars, especially hybrids of rape x kale, provide increased options for different stock classes with varying crop maturities, from 70-110 days after sowing (de Ruiter, Dalley, Hughes, Fraser, & Dewhurst, 2007), and repeated grazing abilities. Compared with other brassica species, forage rape is more tolerant of low soil moisture and lower fertility soils (Keogh, McGrath, & Grant, 2011), although maximal yields are obtained under optimal growing conditions. Current forage rape cultivars available in the New Zealand marketplace are listed in Table 2.

Table 2: Forage rape (Brassica napus spp. *biennis*) cultivars available in New Zealand, adapted from de Ruiter *et al*. (2007) and Stewart, Kerr, Lissaman & Rowarth (2014). S = susceptible, T = tolerant.

Cultivar	Height	Maturity (days)	Aphid	Breeding	Marketer
'Cleancrop rape'	Tall	90-110	T	NZ	PGG Wrightson Seeds
'Goliath'	Tall	90-110	T	NZ	PGG Wrightson Seeds
'Greenland'	Medium	70-90	S	Europe	Seed Force
'Interval'	Tall	90-110	S	UK	Barenbrug
'Leafmore'	Medium	55-70	S	Europe	Barenbrug
'Mainstar'	Medium	70-90	T	NZ	Agricom
'Rangi'	Medium	70-90	S	NZ	Various
'Titan'	Medium	70-90	T	NZ	PGG Wrightson Seeds
'Spitfire'	Medium	90-100	T	NZ	Agricom
'Winfred'	Medium	70-90	S	Europe	Agricom

2.1.2.2 Raphanobrassica

A new forage brassica species, raphanobrassica, is a hybrid of kale x radish. Raphanobrassica provides farming enterprises with additional feed options, particularly throughout the summer-dry period. As this crop is new in the marketplace, there is currently no published data on raphanobrassica as a livestock forage. However, an extensive on-farm survey was compiled by PGG Wrightson Seeds during the product's early release which included yield and quality analysis. This information is publicly available in their marketing material. We have also produced a manuscript on the breeding and agronomy of raphanobrassica for the Journal of New Zealand Grasslands.

Raphanobrassica was developed under a joint venture between PGG Wrightson Seeds and Plant & Food Research, 'Forage Innovations', to provide greater genetic diversity in the plant breeding programme (Dumbleton *et al*., in publication). In turn, expanding forage brassica options for the grazing animal. Nationwide demand for prime land use for dairy production has resulted in sheep, beef and deer enterprises pushed to more marginal land areas. Thus, requiring more varied forage options, principally species and cultivars suitable for growth in agronomically unfavourable conditions.

Interspecific and intergeneric hybridisations between *Brassica* and *R. sativa* were of interest as these had yielded cultivars with increased biotic and abiotic stress resistance (Harberd & McArthur, 1980), particularly with their respective parent properties: superior dry matter yield, quality and palatability of kale; and increased drought, insect and disease tolerance of radish. However, crossing of kale and radish plants produce infertile offspring, requiring chromosome doubling (2n = 18), embryo rescue and multiple generations of backcrossing, before entry into a traditional breeding programme. Further *in vitro* selections for tolerance to clubroot (Pukekohe, Hawke's Bay and Southland strains) and increased water use efficiency were performed.

Early interspecific hybrids between *brassica* and *raphanus* species for human consumption produced largely sterile crosses, until works by Karpechenko in the early 1920s. Karpechenko (Karpechenko, 1927) found success with *R. sativus* and *B. oleracea* hybrids through the creation of allopolyploids, or gametes with doubled chromosome numbers, at the second generation. This allowed equal matching of chromosomes during cell division (4n = 36), producing fertile offspring, termed 'synthetic amphidiploid'.

Intergeneric hybrids are commonly cross-incompatible, resulting in the premature breakdown of endosperm and early abortion of embryos (Nishi, Kawada, & Toda, 1970). Weak and immature embryos were supported to a viable plant using various *in vitro* techniques, or embryo rescue. Following chromosome doubling and embryo rescue, viable interspecific and intergeneric progeny entered a traditional plant breeding programme undergoing extensive comparison with existing cultivars and breeding lines.

Raphanobrassica is sown at a rate of 8 kg ha^{-1}, can be grazed as early as 42 DAS (or as seed treatment recommendations allow), has no specific crop maturity requirement and an ideal first grazing height of 25 cm from the soil to the top of the canopy. Data from various studies throughout New Zealand comparing raphanobrassica with forage rape (*B. napus* spp. Biennis, cv 'Goliath'), found a 14% increased total cumulative dry matter yield from repeat harvests, a 38% increase in water use efficiency, and a 32% increase in aphid tolerance (Dumbleton, *et al.,* in publication). Raphanobrassica can persist for 4 – 5 grazing episodes over twelve months, particularly when initially grazed by livestock at 42 – 70 DAS using a rotational grazing system. There is one cultivar of raphanobrassica available in the marketplace as a livestock forage: ‘Pallaton’, marketed by PGG Wrightson Seeds.

2.1.3 Crop yield and nutritive value

Forage brassicas are considered high yielding crops with good nutritive values comparable to those of traditional spring pastures. They can accumulate large yields of 4 – 12 t DM/ha within a relatively short timeframe of 5 – 6 months (Fletcher & Chakwizira, Nitrate accumulation in forage brassicas, 2012; Fletcher, Wilson, Maley, McCallum, & Shaw, 2010; Gowers & Armstrong, 1994; Jacobs, Ward, McDowell, & Kearney, 2001; Jung, Kocher, & Gilica, 1984; Rao & Horn, 1986). Forage brassica tend to have a relatively low DM content at 10 – 14% (Table 3), with an ME content of 10 – 13 MJ ME/kg DM (Table 3). However, feed quantity and nutritive values vary between species (Figure 2) and can be inconsistent, due to environmental conditions, crop management and husbandry (Keogh, McGrath, & Grant, 2011).

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Figure 2: Nutritive characteristics of forage brassicas and pasture (ryegrass-clover) as measured in field trials in Australia (Thomas, et al., 2015; Ward & Jacobs, 2013) and New Zealand (Lindsay, Kemp, Kenyon, & Morris, 2007; Sun, et al., 2015; Westwood & Mulcock, 2012) retrieved from (Bell, Watt, & Stutz, 2020)

Forage rape is considered a highly nutritive crop that meets the metabolizable energy (ME) (Westwood & Mulcock, 2012), crude protein (CP) (de Ruiter, Dalley, Hughes, Fraser, & Dewhurst, 2007) and neutral detergent fibre (NDF) (de Ruiter, Dalley, Hughes, Fraser, & Dewhurst, 2007; Westwood & Mulcock, 2012) requirements of growing of lambs (Table 4). Thus, fast lamb growth rates circa 200 g/day are regularly reported (Judson, Ferguson, Cutts, & Moorehead, 2013; Lindsay, Kemp, Kenyon, & Morris, 2007). However, nutritive inconsistencies highlighted above were also demonstrated in some studies, particularly discrepancies with ME (de Ruiter, Dalley, Hughes, Fraser, & Dewhurst, 2007) and CP (Westwood & Mulcock, 2012).

Whilst there is currently no published data on raphanobrassica, marketing material from producer PGG Wrightson Seeds report crop yield of around 5,000 kg DM/ha at the first cut and 2,500 kg DM/ha for the second cut compared to forage rape, cultivar ‘Goliath’, at 3,500 and 1,500 kg DM/ha, respectively; equating to a 14% increase in yield (PGG Wrightson Seeds LTD., 2019). Nutritive feed values of DM, ME and CP are also reported as 11.3%, 13.3 MJ ME/kg DM and 19.2 % DM, respectively (n = 60) (PGG Wrightson Seeds LTD., 2019).

Table 3: Feed quantity and nutritional profile of forage rape (*Brassica napus* spp. *biennis*); leafy turnip (*B. rapa* syn. *B. campestris*); bulb turnip (*B. rapa* syn. *B. campestris*); kale (*B. oleracea* spp. *acephala*); and swedes (*B. napus* spp. *napobrassica*) in New Zealand. All values are expressed as percentage of DM. DM% = dry matter percentage; MJ ME = megajoules of metabolisable energy; DOMD = digestibility; CP = crude protein; ADF = acid detergent fibre; NDF = neutral detergent fibre; WSC = water soluble carbohydrate.

Species	DM %	MJ ME	DOMD	CP	ADF	NDF	WSC	Lipid	Ash	Leaf %	Petiole %
Forage rape (n = 28) (Westwood & Mulcock, 2012)	14.3	12.9	88.1	10.8	20.3	23.2	27.3	2.9	9.1	67.2	32.8
Forage rape (n = 10) (de Ruiter, Dalley, Hughes, Fraser, & Dewhurst, 2007)	12.4	10.8		16.3	23.7	25.7					
Leafy turnip (n = 12) (Westwood & Mulcock, 2012)	13.7	13.0	86.7	22.6	13.5	15.6	19.2	4.8	11.1	72.0	28.0
Bulb turnip (n =24) (Westwood & Mulcock, 2012)	10.1	11.7	89.0	14.2	18.9	22.5	27.0		10.4	55.4	44.6
Bulb turnip (n = 3) (de Ruiter, Dalley, Hughes, Fraser, & Dewhurst, 2007)	10.9	12.3		15.8	18.3	17.7	2.7				
Kale (n = 32) (Westwood & Mulcock, 2012)	17.3	11.2	77.0	9.7	23.5	28.0	33.4	2.1	7.0	28.7	71.3
Kale (n = 21) (de Ruiter, Dalley, Hughes, Fraser, & Dewhurst, 2007)	14.2	11.5		13.1	22.6	27.1	38.7				
Swedes (n = 24) (Westwood & Mulcock, 2012)	10.3	13.8	93.5	13.7	13.9	15.2	49.8	1.8	6.1	25.0	75.0
Swedes (n=6) (de Ruiter, Dalley, Hughes, Fraser, & Dewhurst, 2007)	10.4	11.6		11.9	17.5	21.3	48.1				

Lamb feed requirements

Knowledge of crop yield and feed nutritive value is vital for matching the energy demands of grazing livestock. There are numerous publications in animal nutrition, with four main models from the following organisations (Frater, Howarth, & McEwen, 2015):

1. National Research Council (NRC) (National Research Council 2007): American;
2. Commonwealth Scientific and Industrial Research Organisation (CSIRO) (CSIRO 2007): Australian. Formerly the Standing Committee on Agriculture (SCA) (SCA 1990);
3. Agriculture and Food Research Council (AFRC): United Kingdom. Formerly the Agriculture Research Council (AFRC 1993);
4. Institut National de la Recherche Agronomique (INRA) (Institut National de la Recherche Agronomique 1989): French.

Lamb live weight growth is often limited by the volume available for consumption, or dry matter intake (DMI) and nutritive value, namely high ME, medium-high CP and mid-range NDF (Table 4). Forage brassica crops traditionally grown during the summer period to coincide with lamb finishing, such as forage rape and leafy turnip, show a good fit between the crop profile (Table 3) and that of the requirements of the growing lamb as calculated by the National Research Council (NRC) (2007).

Table 4: Feed requirements of 30 and 40 kg lambs growing at 300 g/day. All values are expressed as percentage of DM. LW = live weight; DMI = dry matter intake; MJ ME = megajoules of metabolisable energy; CP = crude protein; NDF = neutral detergent fibre, adapted (National Research Council, 2007)

Lamb	DMI potential % LW	DMI	MJ ME	CP	NDF
Weaned lamb 30 kg	4.15%	1.25	11.95	12.4	≤ 29
Weaned lamb 40 kg	3.22%	1.29	11.97	12.4	≤ 37

2.1.4 Animal health concerns

As with any dietary source of feed, there are some animal health concerns. The most typical limitation of forage brassica is underperforming livestock (Keogh, McGrath, & Grant, 2011). Generally, this is due to a mismatch between feed requirements to maximise production and feed consumption (Barry, 1978; Geisler, Newton, Sheldrick, & Mohan, 1979). Examples are incorrect stock numbers, inaccurate energy calculations and/or incorrect estimations of area on offer, crop yield, crop dry matter (DM), or crop wastage.

Simplistically, the energy requirements of livestock can be calculated using the following equation (Nichol & Brookes, 2007; Pickering, 2011) deriving their information from AFRC (1993), NRC (1985) and SCA (1990):

$$ME_{\ total}(MJ\ ME\ ) = BASAL + 1.1\ x\ ME_p + \ ME_{graze}$$

Where: BASAL = ME requirements to maintain animal weight

ME_p = ME requirements used directly for milk production, conception/gestation and live weight gain

ME_{graze} = additional energy expenditure of a grazing animal compared with a similar housed animal

Energy requirements of livestock are also provided in nutritional tables (Nichol & Brookes, 2007), such as Table 4, or websites that may be a more practical approach for farmers than calculating these values. Examples of websites include https://www.feedsmart.co.nz/ based on AFRC (1993) and SCA (1990). Further information on energetics is out of the scope of this thesis.

Crop yield can be determined by harvesting quadrats representative of the crop at the desired height of post grazing residuals, weighing the fresh weight and calculating the mean weight of all quadrats. Subsamples are collected and weighed before and after material is dried in an appropriate oven between 60-90°C for 48 hours to calculate dry matter percentage (DM%). The total DM yield can then be calculated using this equation:

$$DM\ yield\ (kg\ DM/ha) = (Fresh\ weight\ (kg)\ x\ DM\ \%)\ x\ 10000$$

Subclinical or clinical disease in livestock grazing forage brassica may also occur. Although out of the scope of this thesis, the list of diseases related to forage brassica, but not limited to these crops, is varied and includes: bloat (Wang, Majak, & McAllister, 2012), acidosis (Brethour, 1955), nitrate toxicity (Guillard & Allinson, 1988), secondary photosensitivity (Collett, Stegelmeier, & Tapper, 2014), BAPP (subsequently discussed), mineral deficiencies i.e. selenium or copper (Barry, Reid, Millar, & Sadler, 1981), hypothyroidism and goitre (Russel, 1961), SMCO toxicity (Prache, 1994; Smith, 1980), clostridial disease (Barnett & Duncan, 1953), polioencephalomalacia or thiaminase deficiency (Allison, 1978), tryptophan toxicity (Allison, 1978), acute respiratory distress syndrome (Arnold & Lehmkuhler, 2014) and oxalate poisoning (Schmidt, MacDonald, & Brockman, 1971). Many cases of these diseases, such as bloat or acidosis, may be a function of poor stock adaptation to dietary change (Dalley, et al., 2015).

2.2 Photosensitisation

2.2.1 Definition

Photosensitisation is a non-contagious disease with numerous underlying aetiological causes (Rowe, 1989), where an animal has an exaggerated response to sunlight due to the presence of a phototoxin, or less commonly, a photoallergen within or on the dermis or epidermis (Clare, 1952).

2.2.2 Signs

Gross clinical signs of photosensitisation include inflammation of the skin (dermatitis) or eye membrane (keratoconjunctivitis), itching (pruritis), redness (erythema) and sensitivity (hyperaesthesia) (Clare, 1952; Epstein, 1999). Histopathology of dermatitis caused by photosensitisation has been described as thickening of the dermis (acanthosis), exaggeration of the normal pattern of keratinisation with no nuclei (orthokeratosis), multifocal infiltration by lymphocytes, eosinophils and plasma cells around blood vessels (de Araújo, et al., 2017; Drucker & Rosen, 2011). Generally, photosensitisation occurs on areas unprotected by wool or hair, such as the muzzle, eyes, ears, coronary bands, and dorsum, where there is greater interaction with sunlight (Clare, 1952; Quinn, et al., 2018). Melanin pigments are also protective (Collett, 2019). For example, black coated sheep (Tunisian Noire de Thibar) do not exhibit clinical photosensitisation when consuming *Hypericum perforatum* compared with their white coated (Queue fine de l'ouest) counterparts (Baazaoui, et al., 2020).

Early signs of photosensitisation in grazing livestock are animals seeking shade, pruritus, and may also display head shaking (Kaneko, Harvet, & Bruss, 1997). Clinical signs of photosensitivity are similar for sheep and cattle. Ears of affected lambs are typically oedematous and drooping, leading to deformity and discolouration. Ear thickness measurements can reveal oedematous changes in this region, thus objectively determining that clinical photosensitisation is present. These ear thickness measurements performed using vernier calipers were piloted on lambs in a previous study (Westwood & Nichol, unpublished).

Photosensitisation differs from sunburn: the characteristic formation of sunburn cells (syn. apoptotic keratinocytes) following UV exposure (Young, 1987), mainly UVB. Affected cells have pyknotic nuclei and an eosinophilic cytoplasm which keratinise abnormally and prematurely. Clinically, erythema develops 3 - 4 hours after sun exposure, peaking 12 - 24 hours later, which resolves over 4 - 7 days (McStay, 2018). Blistering may be present depending on the degree of burn. This mechanism is in contrast to sunburn, which transpires without the presence of a phototoxin or photoallergen and does not require oxygen (Collett, 2019). Similarly to photosensitisation, sunburn mainly occurs on animals in areas of reduced hair/wool which lack melanin pigment. It has been estimated at a latitude of 34° S that two consecutive clear days would induce sunburn in sheep in all spring, summer and autumn months (Chapman, Bennett, & Carter, 1984).

2.2.3 Oedema and interstitial fluid balance

The following is an overview of the regulation and pathology of interstitial fluid balance and the most significant clinical sign of photosensitisation: oedema. Oedema in lambs with BAPP is grossly seen as ventral drooping of ear pinna (Westwood & Nichol, unpublished). Oedema is not pathognomonic for photosensitivity.

2.2.3.1 Fluid

Total body fluid constitutes 50-60% of body weight (Levick, 2010). Variation between tissues also occurs with brain, skin, muscles and bones at 73%, 64%, 79% and 31% respectively (Mitchell, Hamilton, Steggerda, & Bean, 1945). Fluid is typically referred to as water, sodium and chlorine, although the different fluid compartments contain additional ions and solutes in small quantities (Dyce, Sack, & Wensing, 2009). Water is an essential medium of all cells as it acts to provide a medium for biological reactions; acts as a solvent and transport agent; diffuses through differentially permeable membranes; excellent thermal properties with high specific heat capacity; and lubricates tissues (Cunningham & Klein, 2007).

Animals consist mostly of fluid, so terrestrial animals must maintain balance whilst losing fluid to their dry environment (Sjaastad, Sand, & Hove, 2010). Maintenance of fluid within normal limits (homeostasis) is termed euvolemia. Practically, this is achieved through fluid loss and gain. Fluid is lost from excretion in urine and faeces, evaporation from the respiratory tract and body surfaces. Fluid can be obtained from the external environment by drinking, from food and catabolism by the oxidation of carbohydrates and fats (Sjaastad, Sand, & Hove, 2010).

Animals are comprised of three fluid compartments, separated by a differentially permeable membrane: intracellular fluid (ICF), interstitial fluid and plasma. These latter two comprise most of the extracellular fluid (ECF). There is a differentially permeable membrane between the three compartments: capillary wall between plasma and interstitial fluid; and lipid bilayer cellular wall between interstitial fluid and intracellular fluid (Chatterjea & Shinde, 2012). Approximately two-thirds of total body fluid is within cells.

Maintenance of fluid (water, sodium and chloride) within normal limits is termed ‘fluid homeostasis’, derived from the Greek words for ‘steady’ and ‘same’. Homeostasis refers to processes that actively maintain stable conditions sustainable for life (Cannon, 1932). Under normal conditions, fluids are maintained in more-or-less the same level, thus fluid homeostasis.

2.2.3.2 Interstitial fluid

Interstitial fluid is derived from filtration and diffusion from the capillaries (Sjaastad, Sand, & Hove, 2010). The interstitial space is comprised of two main solid elements: collagen fibres and proteoglycan filaments. Collagen fibre bundles extend long distances, are strong and provide most of the strength of the tissues. Proteoglycan filaments are very thin and comprise about 98% hyaluronic acid (Sjaastad, Sand, & Hove, 2010).

Proteoglycan filaments make a very fine reticular meshwork (Cunningham & Klein, 2007). Minute spaces among the proteoglycan filaments entrap interstitial fluid, forming the characteristics of a gel-like substance. Due to the structure, fluid does not flow easily through the gel. Instead of moving by bulk flow, it diffuses at a rate that is almost the same speed as it would through free fluid (Chatterjea & Shinde, 2012). This provides adequate transport for nutrients, water, and other metabolites.

Although most of the fluid is within the tissue gel, the chemical environment of proteoglycans can change as small streams of free form fluid are released (Sjaastad, Sand, & Hove, 2010). Most of this movement by bulk flow occurs near the surfaces of collagen fibres. When there is an increase in interstitial fluid, i.e. oedema, the amount of free fluid increases and >50% of the fluid becomes free-flowing rather than embedded proteoglycan gel-like substance (Sjaastad, Sand, & Hove, 2010).

The lymphatic system recovers fluid and colloids which may have been transported from circulation or escaped from the interstitial space, returning them to blood. Approximately 10% of fluid leaving capillaries ends up in the lymphatic system (Sjaastad, Sand, & Hove, 2010). Without the lymphatic system, large substances would be unable to return to the blood. Fluid can return through the capillary walls into plasma via diffusion. As Starling’s force suggests, maintaining tissues free from the excess fluid by removal of colloids is vital in preserving fluid homeostasis (Sjaastad, Sand, & Hove, 2010).

Endothelial cells of lymphatic capillaries overlap, forming one-way valve flaps which open into the interior of the lymphatic capillary. Interstitial fluid and suspended particles push against these valves, opening them, and flow directly into the lymphatic system. Particles are not only limited to colloids and proteins but also bacteria and other foreign particles where they are conveyed to lymph nodes. There are also much larger valves throughout the lymphatic system, resulting in the flow of fluid on a pathway towards the central venous system where they empty (Sjaastad, Sand, & Hove, 2010).

Lymphatic flow is dependent on interstitial pressure (Sjaastad, Sand, & Hove, 2010). When interstitial fluid pressures are ≤6 mm Hg, lymph flow is almost zero (Chatterjea & Shinde, 2012). As the pressure of interstitial fluid approaches 0 mm Hg, flow increases almost 20-fold (Chatterjea & Shinde, 2012). When interstitial pressures are >2 mm Hg, lymph flow is unable to increase due to external compression of lymphatic capillaries (Chatterjea & Shinde, 2012).

Diffusion of water across the differentially permeable membrane is termed osmosis. Plasma proteins within the capillary lumen are normally [7 g/dL] compared with interstitial proteins at [0.2 g/dL] (Chatterjea & Shinde, 2012). This difference creates an osmotic imbalance or colloid osmotic pressure. Combined with hydrostatic pressure differences, Starling's hypothesis (Levick, 2010) states that there is a net movement of water. Starling's resulting equation is:

$$Net\ driving\ force = (P_c|P_i)|(\pi_c|\pi_i)$$

Where P = Hydrostatic pressure in either capillary (c) or interstitial space (i)

π = Oncotic pressure

The balance of these forces allows the calculation of the net driving pressure for filtration.

On the arterial side, there is a larger hydrostatic pressure in the capillaries compared to that of the interstitial fluid. Water is driven out of the capillaries: filtration. However, as this fluid leaves the oncotic pressure in the capillaries, near the venous end, becomes greater than the oncotic pressure in the interstitial fluid and water is driven back into the capillaries: absorption. The net result is +1 mm Hg, a small driving pressure for fluid to move from the capillaries to the interstitium. When the net pressure is combined with the coefficient, or leakiness, it is possible to determine the transcapillary water flux (Levick, 2010).

As per Starling's Law, any changes in pressure alters the movement of fluid out of the capillaries into the interstitial space. One way of altering the normal fluid balance is increased capillary hydrostatic pressure (>35 mm Hg), due to a rise in arterial pressure or decrease in alveolar resistance. Elevated pressure within the capillaries results in an increase in net movement of plasma fluid into the interstitial fluid. Likewise, increased venous hydrostatic pressure (>15 mm Hg) from blood backing up in the venous system results in excessive capillary filtration (Sjaastad, Sand, & Hove, 2010).

Net oncotic pressure is dependent on protein and colloid concentrations in plasma (25 mm Hg) and interstitial fluid (Cunningham & Klein, 2007). Any alterations in the concentration of plasma proteins alters the plasma oncotic pressure. Normally, protein and colloids are too large to pass through the capillary pores and clefts (Sjaastad, Sand, & Hove, 2010). A small amount of protein passes from plasma to interstitial fluid via transcytosis. Tissue inflammation may allow increased protein and colloid movement into the interstitial fluid, thus affecting fluid balance.

2.2.3.3 Oedema

Oedema is caused by changes in fluid balance, affecting Starling's Law. Interstitial hydrostatic pressure is usually slightly sub-atmospheric at -7mm Hg (Cunningham & Klein, 2007). Removal of interstitial fluid into the lymphatic system decreases the interstitial hydrostatic pressure. Nevertheless, when interstitial fluid hydrostatic pressure rises above atmospheric pressure, the accumulation of interstitial fluid becomes clinically noticeable as swelling and oedema occurs. Oedema may occur when: lymphatic function is depressed; or excessive filtration out of capillaries due to reduced plasma hydrostatic colloid pressure or increased venous hydrostatic pressure (Cunningham & Klein, 2007).

There are three safety mechanisms to reduce elevated interstitial fluid levels (and increased interstitial hydrostatic pressure). Firstly, increased interstitial hydrostatic pressure itself reduces excessive filtration out of the capillaries. Even a small change in interstitial hydrostatic pressure, for example from -7 mm Hg to +1 mm Hg assists in changing the net balance in the direction of reducing excessive filtration (Cunningham & Klein, 2007). Secondly, increased interstitial fluid promotes lymphatic flow and limits the degree of oedema. Thirdly, lymphatic flow indirectly removes proteins and colloids from the interstitium. Proteins exert a small but significant oncotic pressure. By reducing interstitial osmotic pressure, filtration is reduced.

2.2.4 Mechanism of photosensitisation

Most examples of photosensitisation in livestock are from the ingestion of a phototoxin, or a precursor to a phototoxin; or topical contact with a phototoxin, or a precursor. Photoallergens are not discussed. In a review by DeRosa & Crutchley (2002), the general process of photosensitisation is:

1. Absorbing
2. Transforming
3. Oxidising

Absorbing: Animal consumes feed/plant containing phototoxin(s) which is then either absorbed through the gastrointestinal tract and moves through the systemic circulation of livestock; or is absorbed locally in sufficient concentration for effect. Areas of the animal which are more exposed to sunlight, such as those with little to no pigment are most affected. In these areas, more sunlight, namely UVA, is absorbed into the dermis.

Transforming: In the spectral region of the excitation light, likely that of UVA at 320 – 400 nm, the phototoxin must have a high absorption coefficient to promote electrons within the molecule from a stable state to an excited state. There are two excited states: singlet ($^1\Sigma^+_g$) or triplet ($^3\Sigma^+_g$) that differ by the π-antibonding orbitals where the last two electrons, those with the highest energy, have antiparallel spins (Table 5). The first state, termed singlet, is where the last two electrons have opposite spins, whereas the last two electrons in a triplet have the same directional spin (Collett, 2019). The efficacy of a phototoxin to be a successful clinical photosensitiser is dependent on the triplet state, thus the phototoxin must have a high yield of the triplet state, at $\Phi T > 0.4$, and a long triplet state lifetime, at $\tau T > 1$ µs and have enough stability for the reactions to take place (DeRosa & Crutchley, 2002).

Table 5: Primitive representations of molecular oxygen lowest singlet and triplet states, adapted from DeRosa & Crutchley (2002)

State	Orbital assignment	
$^1\Sigma^+_g$	↑ π	↓ π
$^1\Delta_g$	↑↓ π	π
$^3\Sigma^+_g$	↑ π	↑ π

Oxidising: A phototoxin itself may act as a radical in an excited triplet state ($ET \geq 95$ kJ mol^{-1}), undergoing oxidation and releasing hydrogen or electrons (DeRosa & Crutchley, 2002). This mechanism is termed 'Type I phototoxicity' (Foote, 1991; Núñez Montoya, et al., 2005). When sequentially reducing molecular oxygen, it can create reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, hydroxyl radicals. Alternatively, energy may be transferred directly to oxygen (O_2) to form another radical: singlet oxygen ($^1O_2$), termed 'Type II phototoxicity' (Foote, 1991; Núñez Montoya, et al., 2005). These molecules are highly oxidising, and when produced in sufficient quantities cause oxidative stress, along with structural damage to macromolecules such as nucleic acids, proteins and lipids. Vascular permeability is increased due to the release of hydrolytic enzymes from ruptured lysosomes (Phaniendra, Jestadi, & Periyasamy, 2015; de Jager, Cockrell, & Du Plessis, 2017). Necrotic damage follows, causing erythema, oedema, scaling and exudation from the dermis.

Oxidative stress also cause changes to erythrocytes (syn. red blood cells, RBC). Such oxidative damage to RBC appears as Heinz body formation: round projections of the RBC membrane (Valenciano, Cowell, Rizzi, & Tyler, 2014; Wilson, 2012) which are evident on histology (Figure 3). Consumption of brassicas by sheep has been associated with increased Heinz body formation that can lead to haemolytic anaemia after 1-3 weeks (Haskell, 2008). Heinz body inclusions increase the rigidity of the RBC membrane, making them susceptible to fragmentation in the systemic circulation (haemolytic anaemia) (Kumar, Abbas, Fausto, & Aster, 2009). The presence of Heinz bodies causes premature phagocytosis of RBC in the spleen, reducing the number in circulation. Haematology to measure RBC, Hb concentration and Heinz bodies can demonstrate oxidative damage associated with clinical and subclinical photosensitisation.

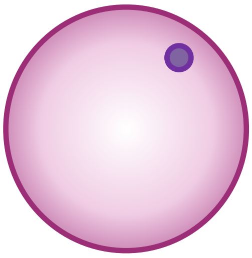

Figure 3: Presence of Heinz bodies (purple) on erythrocytes, adapted from S Bhimji (National Institutes of Health, 2020)

Toxicity from SMCO in livestock consuming brassicas also produces Heinz bodies and causes haemolytic anaemia (Smith, 1980), which may present some difficulty discerning between oxidative damage due to photosensitivity or that of SMCO toxicity. All brassica species contain some secondary S-containing compounds such as SMCO (Barry, 2013). SMCO is metabolised to dimethyl disulphide in the rumen, where it is absorbed across the rumen wall into the systemic circulation (Fales, Gustine, Bosworth, & Hoover, 1987). Oxidation from dimethyl disulphide also induces Heinz body inclusions on RBC.

2.2.5 Light

Solar light is composed of 6 – 7% UV light, 42% visible light and 51% near infra-red; each of which has a variable intensity of energy (Figure 4) at 290 – 400 nm, 400 – 700 nm, 700- 2400 nm, respectively (Figure 4). Of these, UVB, 290 – 320 nm, and UVA, 320 – 400 nm, are typically responsible for phototoxicity due to a large amount of energy reaching either the epidermal layer, in the case of UVB, or subcutaneous layer, UVA (Kim, Park, & Lim, 2015).

UVB is a carcinogen (Young, 1995), by which it tends to be absorbed directly by DNA causing photochemical damage and gene mutations. UVB is 1000-fold stronger than UVA at inflicting DNA damage or burns (Kim, Park, & Lim, 2015), however is far less abundant than UVA. In contrast, little UVA is absorbed by DNA. Rather, UVA is absorbed by other molecules, which can result in the generation of free radicals, such as ROS, that damage membranes, proteins, DNA and other susceptible cellular constituents by way of oxidation (Gruijl, 2000). Therefore, it is suggested that UVA is likely to be responsible for photosensitivity.

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Figure 4: Solar spectra as a function of wavelength (Cleaveland & Morris, 2013)

Variation in UV intensity varies seasonally and geographically (Figure 5), with the largest recordings occurring in latitudes in the Northernmost locations in New Zealand (36° S), compared with the Southernmost (46° S). Prior to commencing research, anecdotal evidence suggested sheep farming enterprises in the North Island of New Zealand exhibited a greater number of BAPP cases during summer, which may be due to increased UVA levels.

Furthermore, UV radiation reaching the earth’s surface has compounded in New Zealand and Australia due to the depletion of the ozone layer. In 1992, the difference between UV irradiances was almost a factor of two-fold greater in New Zealand (45° S) compared to those in the same season in Germany (48° N) (Seckmeyer & McKenzie, 1992). However, this is largely due to an increase in UVB which may not affect the severity and/or incidence of photosensitivity diseases. For example, in 1999, there was a 12% increase in recorded UVB levels compared to the previous decade, but little change in UVA (McKenzie, Connor, & Bodeker, 1999). Further work into the relationship between UV and BAPP is out of scope for this research, although a likely research topic may be the application of a long-lasting topical compound during the risk period of BAPP to reflect UVA photons such as titanium dioxide or zinc oxide; or absorb UVA photons such as bemotrizinol or bisoctizole.

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Figure 5: Ultraviolet index forecasts with seasonal and geographical differences between Auckland and Invercargill (McKenzie, et al., 2014)

2.2.6 Classification of photosensitivity

Originally Clare (1952) first described a four-category classification of photosensitisation in accordance with the pathogenesis. However, more recently Type I (triplet) and Type II (excited singlet molecular oxygen) mechanisms of photosensitivity have been identified (Foote, 1991; Núñez Montoya, et al., 2005) which often adds confusion (Collett, 2019).

1. Type I (Primary photosensitisation)
2. Type II (Aberrant endogenous porphyria)
3. Type III (Hepatogenous photosensitisation)
4. Type IV (Unknown origin/idiopathic)

Type I photosensitisation (Primary photosensitisation): Occurs when the phototoxic compounds are ingested/administered, absorbed then travel to the skin systemically. These compounds, or their metabolites, then interact with UV light resulting in clinical photosensitivity before being excreted. Examples of primary photosensitisers in New Zealand are *Hypericum spp.* (St John's wort) and *Erodium spp.* (Storksbill). Following consumption of St John's-wort, hypericin circulates in the blood. When in the presence of UV light, hypericin generates reactive oxygen species (ROS) leading to apoptosis and/or necrosis of cells (Constable, Hinchcliff, Done, & Grünberg, 2017). The photodynamic agent of Storksbill has not yet been identified but has been associated with spring eczema in 10-14-week-old dairy calves (Collett, 2005).

Type II photosensitisation (Aberrant endogenous porphyria): An inherited metabolic defect in cattle. Instead of forming series III porphyrin isomers for haeme production, series I isomers are produced instead, with the series I isomers manifesting as photosensitisation in affected cattle (Clare, 1952).

Type III photosensitisation (Hepatogenous photosensitisation): A hepatotoxin that has been ingested causes hepatic necrosis. Hepatic necrosis leads to reduced detoxification and/or the excretory ability of the liver, resulting in an accumulation of photosynthetic toxins (Parton, Bruere, & Chambers, 2001). Consequently, levels of the photosynthetic agent phytoporphyrin are increased in blood plasma, along with hepatic enzymes: mainly gamma-glutamyl transferase (GGT) and glutamate dehydrogenase (GLDH) (Osweiler, 2018). Clinically, livestock display signs of photosensitisation.

Gamma-glutamyl transferase (GGT) is an enzyme present in various tissues throughout the body, however, the main source of GGT in the blood is from the liver (hepatocytes). GGT is primarily found on biliary epithelial cells, and sinusoidal and canalicular surfaces of hepatocytes in the liver (Whitfield, 2001). As increased levels of hepatic enzymes in plasma indicate secondary photosensitisation, measuring blood GGT is a specific and sensitive method for detecting liver damage (Pauli, 1983; Collett, Bryan, & Tapper, 2014). Increased GGT levels in plasma are indicative of cholestasis or biliary hyperplasia (Centre, 2007).

Glutamate dehydrogenase (GLDH) is a mitochondrial enzyme (Stewart, Mann, & Fentem, 1980). Similarly to GGT, GLDH originates in many tissues throughout the body, although most blood GLDH emanates from the liver (hepatocytes). An increase of GLDH levels in blood indicates leakage from necrotic hepatocytes, thus the measurement of blood GLDH is a specific and sensitive indicator of hepatic disease (Stewart, Mann, & Fentem, 1980).

An example of secondary photosensitisation is facial eczema (pithomycotoxicosis), which is well documented in New Zealand (Brook & Mutch, 1964; Di Menna, Smith, & Miles, 2009). Spores from the fungus *Pithomyces chartarum* grow in the litter at the base of pasture swards. This fungus produces a toxin, sporidesmin. When these are ingested in large quantities by livestock, sporidesmin causes hepatic damage leading to blockage of bile ducts and the accumulation of the photosynthetic agent, phytoporphyrin resulting in clinical photosensitisation. The highest risk period in New Zealand is January to May during high moisture and temperature conditions.

*Type IV photosensitisation (Unknown origin/idiopathi*c*)*: In examples where the disease aetiology has not been described, the hypothesised pathological photodynamic agent is classified as idiopathic. Lambs consuming forage rape is considered an example of idiopathic photosensitisation (Parton, Bruere, & Chambers, 2001). Measuring hepatic pathology by way of liver enzyme concentration in systemic circulation will add to the body of evidence to determine if it does or does not involve hepatic dysfunction. Other potentially photosensitising plants, such as birdsfoot trefoil (*Lotus* corniculatus) is described in review articles as idiopathic photosensitisation (Collett, 2019) and in others as a source of primary photosensitisation (Stafford, West, Alley, & Waghorn, 1995). In agreeance with the work by Collett (2019), without knowledge of the causative compounds, these plants should be considered idiopathic, particularly due to the sheer volume of compounds that have the potential to induce photosensitisation in livestock.

Collett (2019) proposed remodelling of Clare's classification to logically clarify diseases of photosensitisation into (Table 6): primary (traditionally Type I), secondary (traditionally Type III) with pytoporphryin as the phototoxic agent, endogenous (traditionally Type II), and idiopathic (traditionally Type IV). Thus, this remodelling of classification allows differentiation between the classification system and the photosensitivity mechanism (Type I, triplet, and Type II, excited singlet molecular oxygen).

Table 6: Unambiguous classification of the photosensitisation diseases of animals (Collett, 2019)

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2.2.7 Photosensitivity and forage brassica

There are at least two examples of differing photosensitivity pathology associated with livestock consuming forage brassica. The first class of photosensitivity (Brassica-associated liver disease, or BALD) generally occurs sporadically in cattle and is associated with bulb turnips and swedes (Collett, Westwood, & GIll, 2015; Connor, 1977). An unprecedented outbreak occurred in dairy cattle grazing swede (*Brassica napus* ssp. *napobrassica*) in Southland and Otago, New Zealand in 2014. Approximately 200 cattle and 300 ewes died from BALD due to severe hepatic damage, weight loss and photosensitivity in Southland during winter 2014. Much of the focus was on dairy cattle due to the relatively higher financial value of each animal. Affected animals developed acute hepatotoxicity, which may also be followed by recumbency and death within days (Dalley, et al., 2015). Non-pathognomonic clinical signs of animals affected by BALD include (Collett, 2018):

- Downer cattle with no apparent signs of injury, that are unresponsive or very poorly responsive to metabolic treatments or treatments for other suspected conditions, e.g. methylene blue for nitrate toxicity;
- Dull, depressed cattle, reluctance to move, anorexia;
- Colic/abdominal discomfort and kicking at the belly;
- Clinical photosensitisation on non-pigmented areas of the skin or udder;
- Clinical jaundice (icterus) can occur in cattle;
- Excessive, rapid loss of body condition and/or live weight where other underlying predisposing causes such as other debilitating conditions, severe footrot, etc., have been excluded.

Causes of clinical photosensitivity are indistinguishable, thus serum biochemistry is essential for diagnosis. A no-cost-to-the-farmer outreach programme was undertaken whereby serum samples of affected animals (identifying elected GGT, GLDH and/or creatinine) and necropsy samples of euthanised animals or those recently dead (liver, kidney, pancreas, affected skin and/or udder, ventral rumen wall and abomasum) were collated, analysed and reported by Dr Mark Collett.

Collett & Matthews (2014) then proposed a catabolised product of GSL as the pathological agent causing BALD. These GSL are sulphur compounds found in varying concentrations in brassicas (Kushad, et al., 1999), accumulating higher concentrations in leaf and flowers compared to the crown and bulb (Verkerk, et al., 2008). There are over 100 different compounds of GSL (Collett & Matthews, 2014) which have a range of properties, from the distinguishing brassica flavour to potential cancer-protective properties (Cartea & Velasco, 2008). One group of GSL, progoitrin: the most prominent GSL, is incriminated as the potential causative agent in BALD and accompanying clinical photosensitivity in New Zealand livestock (Collett & Matthews, 2014).

The process of mastication releases progoitrin (Figure 6), which is broken down into biochemically unstable intermediary products in the rumen (Collett, Stegelmeier, & Tapper, 2014). The intermediate product formed theoretically depends on the pH of the rumen (Collett & Matthews, 2014). Cattle that have rumen acidosis, where pH < 5.5 (Malmo, Vermunt, & Parkinson, 2010), are at higher risk of developing BALD Where rumen pH < 4.0 $progoitrin \rightarrow nitrile \rightarrow crambene$. Crambene has been implicated in pancreatic damage in numerous studies on rats (Cao, et al., 2006) although the effects on livestock have not been described. Where rumen pH 4.0 - 6.0 $progoitrin \rightarrow epithionitrile \rightarrow thiirane$. Thiirane was suggested as the likely cause of hepatic and bile duct necrosis causing BALD (Collett & Matthews, 2014), however, crambene is also implicated (Collett, Matthews, & Parton, 2020). Both nitrile and epithionitrile formation require the presence of cofactors nitrile specifier protein (NSP) or epithiospecifier protein (ESP) respectively, as well as ferrous ions (Collett & Matthews, 2014).

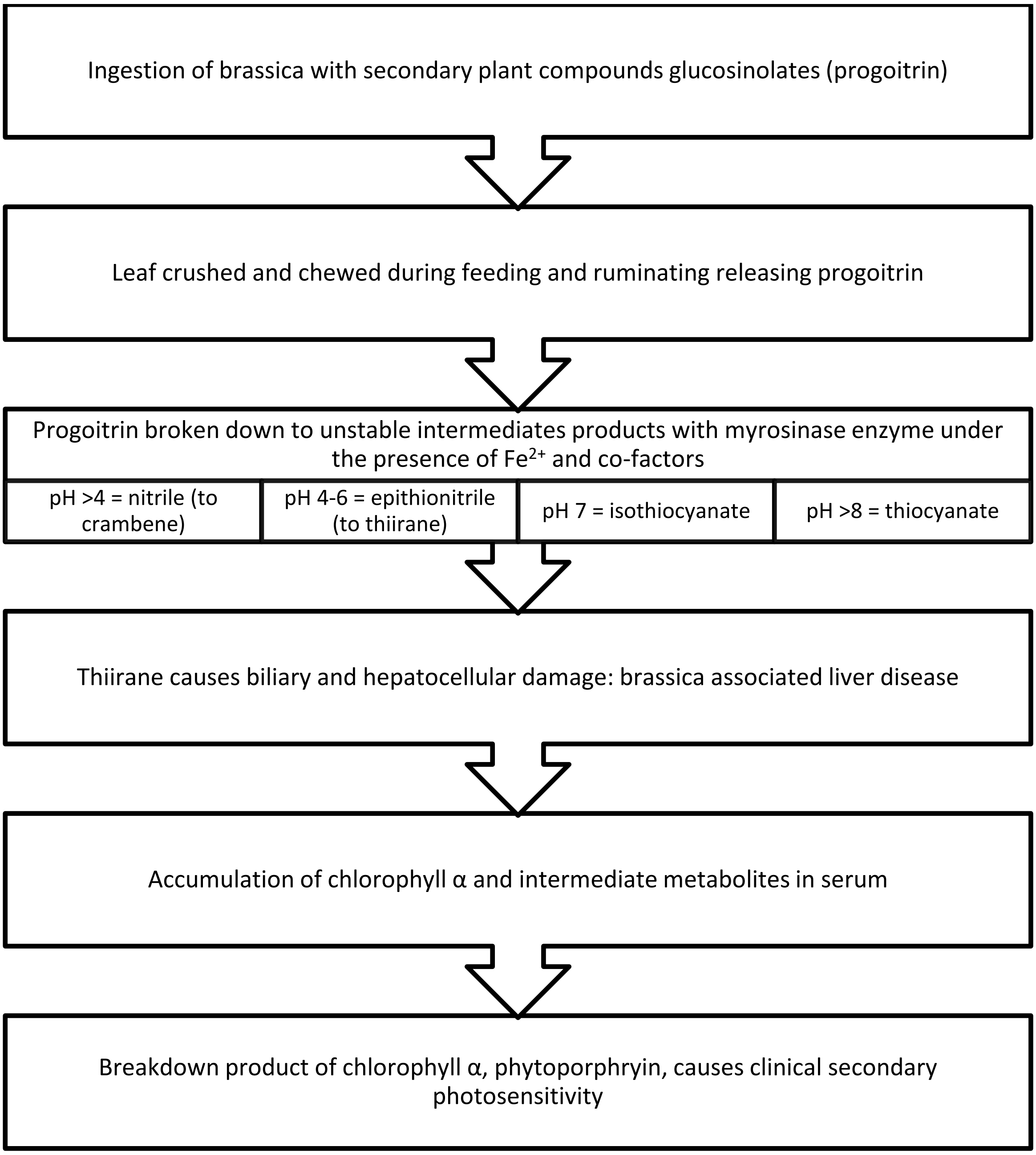

Figure 6: Proposed aetiology of clinical photosensitivity from consumption of brassica, adapted (Collett, Stegelmeier, & Tapper, 2014)

During the outbreak, cattle grazing affected swedes which presented with clinical photosensitivity revealed elevated liver enzymes, a proxy for hepatic necrosis. Mean (GGT) was 893 U/l (normal range

0 - 36), GLDH was 464 U/l (normal range 8 - 41), and bilirubin 12 µM (normal range 0 - 13) (Collett & Matthews, 2014). Due to hepatic and biliary damage, chlorophyll a and intermediate products are unable to be excreted in bile. Affected cattle had serum phytoporphyrin, a catabolised product of chlorophyll a, of 0.6 µM (Collett & Matthews, 2014), although no normal range has been established.

Histological examination revealed distinctive bile duct lesions which Collett (2014) describes as "microscopic cholangiectasis, concentric periductal fibrosis and bile duct necrosis that progresses to obliterative sclerosis, that differ from hepatic lesions associated with facial eczema".

Mortality of BALD in pre-calving dairy cattle mobs was 1.1% compared to 3.4% in calving and early lactation mobs (Dalley, et al., 2015). Subclinical BALD was widespread throughout all swede cultivars, with liver damage revealed in clinically normal cattle. A stronger statistical relationship was found between cattle grazing HT swede and BALD compared to other cultivars, likely due to increased GSL levels. Other risk factors from this report were also found to have a compounding effect on BALD incidence. These are particularly notable as BALD had not been reported in the previous three years that HT swede had been on the market for. The multifactorial situation causing widespread BALD included the following risk factors (Dalley, et al., 2015):

- Warmer climatic effects resulting in swedes retaining more leaf:
    - Leaf has higher levels of GSL compared from bulb at 42.43 and 20.21 µmol/g dry matter respectively;
- Grazing past DAS recommendations:
    - Reproductive plant parts have levels of GSL up to 30-100 times greater (Collett, Stegelmeier, & Tapper, 2014);
- Physiological status of the animal:
    - Cows in late pregnancy and early lactation are at greater risk;
- Feed management practices:
    - Allowing animals to only graze leafy material;
    - Not supplementing feed with hay or balegae at 20-30% of the diet;
    - Relationship between hunger, low rumen fill and increased incidence of rumen acidosis (Malmo, Vermunt, & Parkinson, 2010).

The second example of photosensitivity, which is the focus of this thesis, appears to occur when lambs consume forage rape. This form of photosensitivity appears to be primary (Cunningham, Hopkirk, & Filmer, 1942; Clare, 1952; Connor, 1977; Vermunt, West, & Cooke, Rape poisoning in sheep, 1993) although further work is required to confirm this classification. The aetiology of this disease is not understood (Collett, 2013).

Anecdotal evidence from companies producing brassica seed for sale, veterinarians and farming groups in New Zealand and Australia report BAPP occurring in immature forage rape crops before they have matured with a purple tinge to leaves (Cambridge Vets, 2018; Vermunt, West, & Cooke, 1993). This may be the result of the plant producing a phototoxin in immature leaves as a response to predation in any of the following classes, as it is in the plant's best interest to protect immature leaf material for future growth and survival.

Other proposed risk factors also include climate events, such as a period of dull overcast days, immediately followed by bright sunny days; poor management of transition onto crop; and applications of nitrogenous (Morton & Campbell, 1977) or sulphur fertilisers (Westwood & Nichol, unpublished).

2.2.8 Primary photosensitisation

Morbidity of proposed primary photosensitisation in *Ovis aries* (sheep) varies greatly in peer-reviewed literature. Morbidity of suspected photosensitisation in three case studies of lambs consuming *Lotus corniculatus* (Birdsfoot trefoil) in New Zealand was 33.3% (N = 30), 7.5% (N = 40) and 26.8% (N = 56) over consecutive years (Stafford, West, Alley, & Waghorn, 1995). Two examples of suspected primary photosensitivity outbreaks in lambs consuming *Biserrula pelecinus* in Australia revealed morbidities of 100% (N = 167) (Quinn, et al., 2018) within 72 hours and 25% (N = 120) (Kessell, Ladmore, & Quinn, 2015), respectively. A further study of primary photosensitisation in sheep grazing *Medicago sativa* (lucerne) revealed a 24.3% (N = 1850) morbidity (Ferrer, et al., 2007), however, the authors of the latter propose the agent may be from the infestation of *Coccinella septempunctata* 'ladybirds' (containing perylenequinone). There was no mortality from primary photosensitivity reported in any of these studies. The phototoxin(s) responsible for photosensitisation have not been determined in any of these plant species (Collett, 2019).

Cases of primary photosensitivity in sheep and cattle consuming *Froelichia humboldtiana* (Ervanço) in semi-arid environments in Brazil have been shown to have morbidities of 100% (N = 5) (Pimentel, et al., 2007) and 38.6% (N = 70) (Souza, et al., 2012), respectively. Another Brazilian weed, *Malachra fasciata* (Malachra), which grows during the rainy period, had a morbidity of 100% (N = 3) in sheep (de Araújo, et al., 2017). Both these plant species are not common in New Zealand. The authors hypothesised the responsible agents as naphthodianthrone derivate due to the lack of keratoconjunctivitis (Pimentel, et al., 2007), and monoterpenes (de Araújo, et al., 2017). Although the latter did not rule out the presence of coumarins and furocoumarin as a potential causative agent. There was a lack of hepatic pathology in sheep consuming both species as hepatic enzymes, alanine-aminotransferase (AST) and gamma-glutamyl transferase (GGT) were within normal ranges of clinical animals (Pimentel, et al., 2007) and gross liver pathology was normal (de Araújo, et al., 2017).

There is further evidence of primary photosensitisation occurring in *Equus caballus* (horses) consuming predominantly lucerne hay with several large outbreaks occurring in California (Puschner, Chen, Read, & Affolter, 2016). Two investigations of 70 and 116 horses revealed morbidity of 100% and 6.9% respectively, with one case of mortality.

Overall, cases identified in the literature as being primary photosensitising have a range of morbidity from 7.5% to 100%. There is little evidence of mortality in these proposed primary photosensitivity examples, with the associated dermatitis resolving upon removal from direct sunlight and affecting feed. Current literature is largely limited to case study examples. Furthermore, proposed species causing suspected primary photosensitisation may contradict the classification proposed by Collett (2019). Crops may be labelled as primary photosynthesising; however, these may contain weeds/fungi/insects which may be the causative agent (Ferrer, et al., 2007).

2.2.9 Treatment

Recovery from primary photosensitisation is symptomatic (Underwood & Schoell, 2015). Affected livestock should be removed from offending plant material and preferably into shade is suggested (Parton, Bruere, & Chambers, 2001). Secondary bacterial infection may occur.

2.3 Hypothesised phototoxins causing BAPP

There are four main phototoxic classes associated with photosensitisation in livestock (Figure 7): perylenequinones, aglycone anthraquinones, furanocoumarins, and derivatives of chlorophyll such as pheophorbide *a* and phytoporphyrin (Collett, 2019). To date, no primary photosensitizing compound has been identified in any of the brassicas (Collett, 2013). However, there is a large volume of literature available on causative agents of primary photosensitisation in humans, given 8% of cutaneous adverse events are from drug-induced photosensitivity (Monteiro, Rato, & Martins, 2016). These causative agents tend to have low molecular weights of 200 – 500 Daltons (Moore, 2002) have heteroatoms in their structures and have planar, tricyclic or polycyclic configurations.

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Figure 7: The structures depicting potential phototoxic agent(s): Top row, perylenequinones (basic structure in red), comprising hypericin (1) from Hypericum spp., fagopyrin (2) from Fagopyrum esculentum, erythroaphin (3) from the cowpea aphid Aphis craccivora, and the mycotoxin cercosporin (4). Middle row shows an aglycone anthraquinone (5) (for rubiadin, R1 = OH, R2 = H; for soranjidiol R1 = H, R2 = OH), the linear furanocoumarin psoralen (6) (two other important linear furanocoumarins are xanthotoxin [methoxy group at position 8] and bergapten [methoxy group at position 5]), and the angular furanocoumarin angelicin (7). Bottom row shows the two chlorophyll derivatives, pheophorbide a (8, note the single bond at 17–18 making it a chlorin), and phytoporphyrin (9, porphyrins have a double bond at 17–18), as well as the alkaloid perloline (10). Phytoporphyrin (phylloerythrin) is the phototoxin in secondary photosensitisation. From Collett (2019).

The following are hypothesised agents of brassica from the known main phototoxic classes (the causative agent in BAPP may be a compound or metabolite of unknown origin).

2.3.1 Perylenequinones

There are several sources of perylenequinones that can affect forage brassica crops in New Zealand. Some perylenequinones, such as cercosporin, and aphin have chemical structures like those of hypericin, a known photosensitiser found in St John's Wort (Thompson, 1971; Halder, et al., 2005).

A fungal disease of brassica, *Pseudocercosporella capsellae* ('white leaf spot disease' or 'white spot'), is of the family Cercospora. These are known as producers of light-activated perylenequinone toxins such as cercosporin (Gunasinghe, You, & Barbetti, 2016). White spot can be identified by grey-brown circular spots on leaves surrounded by a yellow halo, which can affect crops in the North Island and sometimes in the South Island (Harvey, 2007). White spot can be controlled with a range of fungicides.

Often referred to as 'leaf spot', *Alternaria brassicicola* (producing black leaf spots) and *A. brassicae* (producing brown/grey leaf spots) are common fungal diseases of forage brassicas, particularly prevalent where brassicas are grown intensively (Harvey, 2007). Again, these mycotoxins (Alternaria toxins) are perylenequinones (Zhang, et al., 2012) which may induce photosensitisation. There are no fungicides with specific label claims in New Zealand, however, cyproconazole, chlorothalonil, difenoconazole and copper-based foliar-applied fungicides may be useful against *Alternaria* spp. (Harvey, 2007).

Aphids (family *Aphididae*), such as the cabbage aphid (*Brevicoryne brassicae*) attacks a wide range of forage and vegetable crops, including brassica, directly feeding on the plants leading to wilting, distorted crinkling of leaves, yellowing and stunted growth (Harvey, 2007). Aphids have been implicated in photosensitisation (Ferrer, et al., 2007) likely due to the aphin molecules (Halder, et al., 2005). Although, other major pigments in aphids may also be responsible including carotenoids, a, b and γ-carotene, melanin, lycopene and torulene (Tsuchida, 2016).

2.3.2 Aglycone anthraquinones

Anthraquinones have been found in *Isatis microcarpa* of the *Brassicaceae* family at a rate of 0.61 g/100gDM (Emam & El-Moaty, 2009). Further work is required to identify the presence of these compounds in forage brassica species, and whether these are capable of inducing photosensitivity.

2.3.3 Furanocoumarins

There is some evidence of linear furanocoumarin biosynthesis occurring in *B. oleracea* to the bergapten step (National Library of Medicine, 2020). This is stimulated by stressors such as wounding, or fungal elicitors (Matern, Luer, & Kreusch, 1999). Further research is required to determine if this pathway occurs in forage rape or raphanobrassica.

2.3.4 Derivatives of chlorophyll

Another hypothesised photodynamic agent causing photosensitivity from ingestion of brassicas is phytoporphyrin (syn. phylloerythrin), a catabolised product of chlorophyll a (Scheie, Smith, Cox, & Flaoyen, 2003; Tapper, Lohrey, Howe, & Allison, 1975; Towers, 1980). Plasma concentrations of phytoporphyrin detected in clinically affected cattle and sheep are 0.4 – 1.8 and 0.9 – 2.8 μM, respectively (Campbell, Dombroski, Sharma, Partridge, & Collett, 2010). However, no reference ranges for phytoporphyrin in healthy animals have been established for comparison with these values. The intermediate metabolites, pyropheophorbide a (Huang, Ou, Tao, Yin, & Tu, 2016; Tu, et al., 2017) and pheophorbide a (Pruzinska, Tanner, Anders, Roca, & Hortensteiner, 2003) are also photosensitising agents causing cellular apoptosis and necrosis. (See Figure 8 for reaction sequence).

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Figure 8: Breakdown of chlorophyll a from phytoporphyrin via intermediate metabolites. From Campbell *et al*. (2010)

Animals consuming large quantities of chlorophyll-rich feed may overwhelm the hepatic chlorin (i.e. pheophorbide) or porphyrin (i.e. phytoporphrin) excretion capacity. The resulting photodynamic chlorophyll a metabolites may accumulate in dermal capillaries resulting in clinical photosensitisation without hepatic pathology.

During an outbreak of photosensitivity in lambs consuming rape in Manutuke, affecting rape crop was collected and fed to guinea pigs, which subsequently became photosensitive (Filmer, 1947). However, when repeated a week later no photosensitivity could be induced, suggesting a phototoxin concentration may have altered during the course of the week. A non-descriptive chromatography analysis revealed no novel pigments not usually found in green leafy plants, although the ability to identify compounds now is likely to be far superior.

In a study where lucerne hay was inducing photosensitisation in horses, the content of chlorophyll a, b and pheophorbide a of the affecting feed was compared to control samples of lucerne and orchard grass (Puschner, Chen, Read, & Affolter, 2016). Chlorophyll ranged from 0.90 – 2.30 mgg^{-1} and 1.37 – 2.94 mgg^{-1}, respectively. Similarly, pheophorbide a ranged from 3.36 – 89.87 μgg^{-1} and 42.33 – 81.39 μgg^{-1}, respectively. There was little difference between the affected and control samples, indicating there was no causal link between chlorophyll or pheophorbide and photosensitivity in this case.

Chlorophyll a is a large porphyrin ring molecule (Louda, Li, Liu, Winfree, & Baker, 1998). It is the principal photosynthetic pigment of plants and interacts directly with UV light (Jansson, 1994). Chlorophyll b is an accessory pigment and transfers light energy to chlorophyll a. The ratio of chlorophyll a to chlorophyll b is normally 3:1, however, this ratio can change depending on the intensity of light exposure (Chappelle, Kim, & McMurtrey, 1992).

2.3.5 Identification of a photosensitive disease

While outside the scope of this thesis, Blum (1941) proposed the following postulates to positively identify a phototoxin:

1) To distinguish the condition from sunburn, the signs of photosensitisation are elicited by exposure to sunlight through window glass;

2) The phototoxin must be isolated in pure form and upon dosing/injection in experimental animals produce the same clinical signs when exposed to sunlight;

3) Wavelengths producing sensitivity in the above postulates are identical;

4) Similarities between the absorption spectrum of the purified phototoxin and action spectrum of the tissue(s) of the photosensitised animal.

2.4 Study designs

Many outbreaks of photosensitisation are sporadic or transient (Mauldin & Peters-Kennedy, 2016) with a large portion of the works cited being case studies. Anecdotal evidence of lamb growth studies by PGG Wrightson Seeds also suggests photosensitisation on brassica crops does not occur every year. However, cases tend to occur following prolonged dull and overcast weather conditions, on immature crops (Vermunt, West, & Cooke, Rape poisoning in sheep, 1993), which suggests some level of difficulty in reproducing a series of reliable study with enough cases of disease to quantify BAPP. Therefore, literature outside of the topic of photosensitisation was relied upon to guide the study design.

A review of 20 experimental designs in the field of plant components on animal production revealed approximately 60% used individual stalls, most of which had a Latin square design. Field experiments made up around 30% and used either a complete randomised block design (75%) or matched pair design (25%). Feed treatments were usually limited to less than three treatments plus controls and replicates were sometimes limited to the number of animals in the experiment. Publications from Europe and America tended to use individual stalls, compared to Australasian studies which used field experiments. The duration ranged from 1 - 120 days. *In vitro* studies were the shortest followed by individual stalls, with field studies having the longest duration. ANOVA and mixed model of procedure were common initial statistical methods of analysis.

Following a pilot study by Westwood & Nichol (unpublished), it was determined that three large scale field experiments would be the most appropriate method of comparing severity and/or incidence of photosensitivity. Dr David Baird calculated the total number of animals required for each experiment using data from the pilot study by way of a two-sided test with a 5% significance and 80% power.

Chapter 3

Effect of brassica species and nitrogen on severity and incidence of idiopathic photosensitisation (‘rape scald’) in lambs

3.1 Abstract

Forage brassicas are commonly grown throughout New Zealand in summer for feeding weaned lambs. However, their consumption has been associated with idiopathic photosensitisation (‘rape scald’). This study aimed to determine if idiopathic photosensitisation differed for lambs grazing the new-to-market brassica, raphanobrassica (*Raphanus x Brassica* L.) compared with forage rape (*Brassica napus* spp. Biennis, cv ‘Titan’) under the presence of either non-nitrogen (N) boosted or N-boosted fertiliser, using a 2 x 2 factorial design. During the summer of 2017 - 2018, 240 cryptorchid NZ Romney lambs were introduced to 16 plots, each of 0.25 ha, of raphanobrassica or forage rape, 70 days after sowing. Soon after, there was an initial increase in ear thickness for all lambs (mean of 1.49 mm, measured on Day 3, $P<0.001$), with ears 0.32 mm thicker for lambs grazing raphanobrassica compared with those grazing forage rape. Subsequently, ear thickness declined about 0.5 mm during the next three weeks and was not significantly different ($P>0.05$) between treatments. There was a temporary elevation in incidence of Heinz bodies, along with reductions in erythrocyte count and haemoglobin levels suggestive of minor forage-associated pathology. There was little effect of nitrogen fertiliser applications on severity and/or incidence of photosensitisation. Primary photosensitisation occurred in lambs grazing both crops, with raphanobrassica having a similar risk of primary photosensitisation in sheep. Standard management practices with attention to this risk will still be required.

Keywords: primary photosensitisation, rape scald, forage rape, raphanobrassica

3.2 Introduction

Forage brassicas are annual fodder crops grown as part of the feed rotation systems in New Zealand. These crops are grazed as a monoculture *in situ* and in summer are generally grown to meet periods of feed shortages due to soil moisture limitations and reduced pasture growth (Dumbleton, et al., 2012). Compared with traditional pasture swards (perennial ryegrass [*Lolium perenne* L.]) and white clover [*Trifolium repens* L.]), forage brassicas are associated with superior dry matter yields (Judson & Edwards, 2008) and higher nutritional quality (Westwood & Mulcock, 2012).

Two brassica species commonly grown on lamb finishing enterprises (post-weaning to slaughter period) are forage rape (*Brassica napus* spp. Biennis) and a new species, raphanobrassica (*Raphanus x Brassica* L.), a hybrid of kale (*Brassica oleracea* L.) x radish (*Raphanus raphanistrum* subsp. Sativus L.). Raphanobrassica was first released into the New Zealand marketplace in 2017, with 3,500 ha grown that year (A. Dumbleton, personal communication). Forage rape cultivar 'Titan' is a hybrid of rape x kale (*Brassica oleracea* spp. acephala) with a maturity of 70 – 90 DAS (Westwood & Mulcock, 2012). Raphanobrassica can be grazed as early as 42 DAS through to 100 DAS, although the latter limits plant utilisation and regrowth potential (A. Dumbleton, personal communication, February 22, 2018).

While forage brassicas are agronomically excellent forages, they can cause negative animal performance and health issues. For example, lambs grazing forage brassicas occasionally develop idiopathic photosensitisation, commonly termed 'rape scald'. Forage rape has traditionally been associated with clinical photosensitivity. However, there is anecdotal evidence of similar effects arising from grazing raphanobrassica.

This photosensitivity clinically presents as marked oedema of the ears, face and occasionally along the midline of the back (Cunningham, Hopkirk, & Filmer, 1942; Vermunt, West, & Cooke, Rape poisoning in sheep, 1993). Haemoglobinuria without liver damage may also be observed (Bruere, Cooper, & Dillon, 1990). Although the pathophysiology of rape scald is not well understood (Collett, 2013), it has been documented as a potential primary photosensitisation disease unaccompanied by hepatic damage (Bruere, Cooper, & Dillon, 1990; Collett, 2013)

Primary photosensitisation occurs following the ingestion of a photodynamic agent which reaches the systemic circulation (Parkinson, Vermunt, & Malmo, 2010). The agent undergoes photoxidation in the presence of ultraviolet (UV) light, which leads to cell necrosis through enzyme inactivation, membrane destruction, plus cytoplasmic and/or nuclear changes (Towers, 1980). The prognosis is generally favourable when livestock are removed from the feed (Parkinson, Vermunt, & Malmo, 2010). Common diseases in New Zealand causing primary photosensitisation include rape scald (Collett, 2013; Collett, 2010) and spring eczema (Collett, Sharma, Bullock, Officer, & Partridge, 2008). The cause(s) of these diseases remain elusive (Collett, 2006).

This differs from secondary photosensitisation, where a hepatotoxin that has been ingested causes hepatic necrosis. Hepatic necrosis leads to reduced detoxification and/or excretory ability of the liver, resulting in an accumulation of photosynthetic toxins (Parton, Bruere, & Chambers, 2001). Consequently, levels of hepatic enzymes: mainly gamma-glutamyl transferase (GGT) and glutamate dehydrogenase (GLDH) are elevated (Osweiler, 2018). Clinically, livestock display signs of photosensitisation.

To date, no primary photosensitizing compound has been identified in any of the brassicas (Collett, 2013). One hypothesised aetiology of rape scald and spring eczema is the consumption of plants containing high concentrations of chlorophyll (Collett, Bryan, & Tapper, 2014; Collett, Sharma, Bullock, Officer, & Partridge, 2008). Where high levels of chlorophyll are not detoxified, chlorophyll (or its metabolites) remains in blood plasma causing clinical photosensitisation. Phytoporphyrin, a catabolised product of chlorophyll a, has been proposed as the photodynamic agent from the ingestion of brassica, which causes the clinical signs of primary photosensitivity (Scheie, Smith, Cox, & Flaoyen, 2003; Tapper, Lohrey, Howe, & Allison, 1975; Towers, 1980). Large quantities of chlorophyll-rich feed may overwhelm the hepatic chlorin (i.e. pheophorbide) or porphyrin (i.e. phytoporphrin) excretion capacity. The resulting photodynamic chlorophyll a metabolites may accumulate in dermal capillaries resulting in clinical photosensitisation without hepatic pathology (Collett, et al., unpublished).

Circulating concentrations of chlorophyll and phytoporphyrin in livestock may be influenced by the application of nitrogenous fertiliser to fodder crops. A pilot study (Westwood & Nichol, unpublished) found an interaction with N application and photosensitivity. Nitrogen is a macronutrient incorporated in proteins and nucleic acids (Bojovic & Markovic, 2009) and is absorbed by plant roots as nitrate. It has a two-fold effect on plant growth: it is utilised for cell synthesis, increasing plant leaf area and surface area for photosynthesis to occur (Prioul, Brangeon, & Reyss, 1980); and it increases the formation of photosynthetic proteins (Bojovic & Markovic, 2009; Mooney, 1986). Chlorophyll a is a large porphyrin ring molecule (Louda, Li, Liu, Winfree, & Baker, 1998), and the principal photosynthetic pigment of plants and interacts directly with UV light (Jansson, 1994).

Since nitrogen is transposed throughout the plant, leaf nitrogen is dependent on available soil nitrogen (Pillai & De, 1979). Chlorophyll content in plants is approximately proportional to leaf nitrogen levels (Evans, 1983). A quantified example of this relationship is in *Oryza sativa* plants in which high nitrogen fertiliser application (14.7 kg/ha) produced higher plant chlorophyll concentrations than occurred following the application of low nitrogen fertiliser nitrogen (10.3 kg/ha) at 1.01 and 0.47 mg/g chlorophyll respectively (Sun, Ye, Peng, & Li, 2016). Optimal nitrogen fertiliser application on brassicas depends on timing, soil cultivation, chemical composition (variety), site conditions and weather events (Rathke, Behrens, & Diepenbrock, 2006).

The initial aim of this study was to determine the incidence and/or severity of rape scald in lambs grazing either forage rape or raphanobrassica. We hypothesised that whilst raphanobrassica is a new species of forage brassica, that the incidence of rape scald in lambs grazing raphanobrassica would not differ significantly from that in lambs grazing forage rape. This hypothesis was addressed by comparing lambs grazing these forage species for evidence of rape scald which included measurement of ear thickness (clinical), haematology (sub-clinical and clinical), and live weight changes (sub-clinical and clinical). A second hypothesis is that chlorophyll a, or its metabolites (e.g. phytoporphyrin), are specific inducers of clinical photosensitisation. This was tested by supplying a high nitrogen fertiliser (+N) treatment to increase the plant levels of chlorophyll a and comparing the incidence of clinical measures of rape scald in lambs grazing these forages with that of lambs grazing the non-boosted (-N) forages.

3.3 Methods and materials

3.3.1 Experimental design

The study was conducted at PGG Wrightson Seeds Research Centre, Kimihia, Lincoln, Canterbury, New Zealand (-43.62, 172.47) during summer (2017 – 2018). Treatments were: variety of brassica (forage rape cv. 'Titan' and raphanobrassica); and applications of N fertiliser (+N: 2 x 100 kg urea ha^{-1}) and no additional fertiliser (-N). The study design was a 2 x 2 complete randomised block study with four replicates. Treatments were allocated to four different paddocks using GENSTAT Version 19.1. All animal procedures were approved by the Lincoln University Animal Ethics Committee (Appendix 4). Lambs were blocked by their live weight three weeks before the beginning of the study. Fifteen lambs were assigned to each 0.25 ha plot, totalling 240 lambs.

3.3.2 Study Husbandry

To ensure adequate establishment of the crops, the study site was treated with diammonium phosphate (DAP) fertiliser at a rate of 200 kg/ha, providing 36 kg N/ha and 40 kg P/ha. Seeds were sown on 1 November 2017. Best management practices provided by the seed company (PGG Wrightson Seeds, Lincoln, New Zealand) in terms of herbicide and insecticide applications and use of irrigation were followed, thus minimising alternative causes of primary photosensitivity in terms of aphids and weed species. An electric fence system provided a physical barrier between the treatment plots. In addition to the electric fence system, a 1 m boundary area between plots was sprayed with herbicide (glyphosate) to ensure the potential effect of fertiliser applications overlap was eliminated and lambs were unable to graze neighbouring plots. Treatment replicates were separated with permanent fencing materials. Fresh water was provided from troughs. Treatments requiring additional nitrogenous fertiliser (+N) received two fertiliser applications: 27 November (3 weeks post sowing) and 20 December (7 weeks post sowing), of 100 kg urea/ha, providing 2 x 46 kg N/ha.

All lambs were cryptorchid Romney males from a single source flock. Three weeks prior to the study, they were weighed after 24 hours of fasting and their mean live weight was 30.4 kg (range 22.5 – 40.0 kg). These live weights were used to allocate the lambs into groups that were balanced for this parameter (blocked). Appropriate animal health management in terms of clostridial vaccinations, anthelmintic treatments, selenium and cobalt supplements, inguinal wool clipping (crutching) and a topical insecticide application were conducted before commencement of the study. The lambs were grazed in paddocks containing chicory and white clover between 21 December and the beginning of the study on 15 January 2018. Lambs were introduced into their treatment plots 74 days after seed sowing at a lax stocking rate with no other supplemented feed, which we hypothesised would assist in inducing clinical rape scald. Throughout the study, lambs grazed on their assigned plots and were monitored by field staff who surveyed them by walking through the plots daily for the first two weeks of the study.

3.3.3 Measurements

Lambs were mustered from the study plots and moved to a yard facility for measurements on Day 0, and twice weekly for the first 14 days of the study, then once weekly thereafter. Measurements included: live weight, ear thickness, subjective ear damage score, haematology and blood biochemistry. Live weight (kg) was measured with electronic scales (Tru-Test™, Auckland, New Zealand). Electronic vernier callipers (Jobmate, Palmerston North, New Zealand) were used to measure ear thickness (in mm) 10 mm proximal to an existing ear mark on the caudal border of the right ear pinna. Three measurements were taken on each sheep and the median value was recorded. One person measured all ear thicknesses for the first three-time points. On day 10, two people measured ear thicknesses of the first 100 lambs providing a cross-checking system where the values were found to be within 0.2 mm between the two measurers. Following this, the second person performed all ear thickness measurements for the remainder of the study. Ear damage was recorded using a 5-point scale (1 = no damage, 5 = severe blistering and necrosis of external ear tissue).

Blood sampling (10 mL) was performed once weekly on 128 lambs (8 lambs/plot, same individuals each time) using venepuncture of the left external jugular vein. All lambs assigned to blood testing had wool clipped from the ventral neck region. 20-gauge needles were used to evacuate 10 mL of blood into 16 x 100 mm vacutainers containing the anticoagulant Na-ethylenediaminetetracetate. Vacutainers were immediately placed on ice. Thereafter (i.e. within 3 h) the chilled blood samples were transported 18 km to a commercial laboratory (Gribbles Veterinary Pathology Laboratories, Christchurch, New Zealand) for analysis of RBC, Hb, Heinz bodies, gamma-glutamyl transferase (GGT) and glutamate dehydrogenase (GLDH) using an automated haematology analyser.

Plant measurements were largely performed prior to the commencement of the study. Dry matter yield (DM) was measured on Day -7. Three 1 m^2 quadrat samples per plot were cut 3 cm above soil level and manually separated into leaf and stem. Subsamples of approximately 400 g of leaf and stem were weighed, dried in an oven for 48 h at 90 °C and re-weighed to calculate dry matter percentage. Dry matter yield was calculated using the equation:

$$DM\ Yield\ (kg\ DM/ha\ ) = (Leaf\ fresh\ weight\ x\ DM\ \%) + (Stem\ fresh\ weight\ x\ DM\ \%) x\ 10000$$

Another plant subsample of approximately 300 g was chilled and transported to a commercial laboratory (Hill Laboratories, Christchurch, New Zealand) for wet chemistry analysis of metabolisable energy, crude protein, digestibility of organic matter in dry matter and nitrogen content. The median of each result is termed in the text as ‘sample mean’. Three whole plants per plot were freeze-dried and transported to the same laboratory for analysis of glucosinolate concentrations.

Twenty-five leaf samples were taken from each plot in replicate 4. The newest, fully unfolded leaf with a sun orientation of 70 - 80°, in the top third of the canopy was selected. Leaves were immediately wrapped in aluminium foil and transferred to an ice-cold insulated container. Disks (1 cm diameter) were removed from the outer edge of the leaf. Cell contents were extracted for 48 h using the solvent N, N-dimethylformamide (99%), as described by Inskeep & Bloom (1985). Fluid containing cell contents was placed in a spectrophotometer (ThermoFisher Scientific, Shanghai, China) to determine light absorption at wavelengths of 664.5 and 647 nm. Quantities of chlorophyll a, b and total were calculated using the following equations:

$$Chlorophyll\ a\ (mg/m^2) = \frac{\big((12.7\ x\ 664.5\ reading - 2.79\ x\ 647\ reading)\ x\ 5\big)\ x\ 11978.2}{1000}$$

$$Chlorophyll\ b\ (mg/m^2) = \frac{\big((20.7\ x\ 647\ reading - 4.62\ x\ 664.5\ reading)\ x\ 5\big)\ x\ 11978.2}{1000}$$

$$Total\ Chlorophyll\ (mg/m^2) = \frac{\big((17.9\ x\ 647\ reading - 8.08\ x\ 664.5\ reading)\ x\ 5\big)\ x\ 11978.2}{1000}$$

3.3.4 Data analysis

The study ran for a 35-day period, from 15 January to 19 February 2018. Raw data were recorded into Microsoft Excel and analysed using the statistical software GENSTAT Version 19.1. Data were tested for homogeneity of variances with no transformation required. Statistical tests included two-way blocked analysis of variance (ANOVA) or two way blocked repeated measures ANOVA, Fisher's protected least significant difference (LSD), coefficient of variation (CV) and estimated standard error of the mean (SEM). Graphical representations were performed on Microsoft Excel using combined means, standard deviations and error values based on LSD.

3.4 Results

3.4.1 Ear thickness

Transfer of the lambs from grazing chicory/white clover crops to the brassica forages was accompanied by a major increase in mean ear thickness of about 1.5 mm (P < 0.001, mean 55%, range 1 – 312%, Figure 9) in all lambs. This effect was more pronounced in the lambs grazing raphanobrassica (P = 0.023) compared to forage rape, particularly on Day 3 (P < 0.001). Ear thickness increased by ≥ 0.5 mm in 223 lambs, equating to an overall clinically significant incidence of rape scald of 93%. Thereafter, there was a slight reduction in mean ear thickness throughout the study, with a temporary elevation (P < 0.001) in all groups (see Day 10). There was no difference between the N-fertiliser treatments (P = 0.531). All values remained about 1 mm above pre-treatment means.

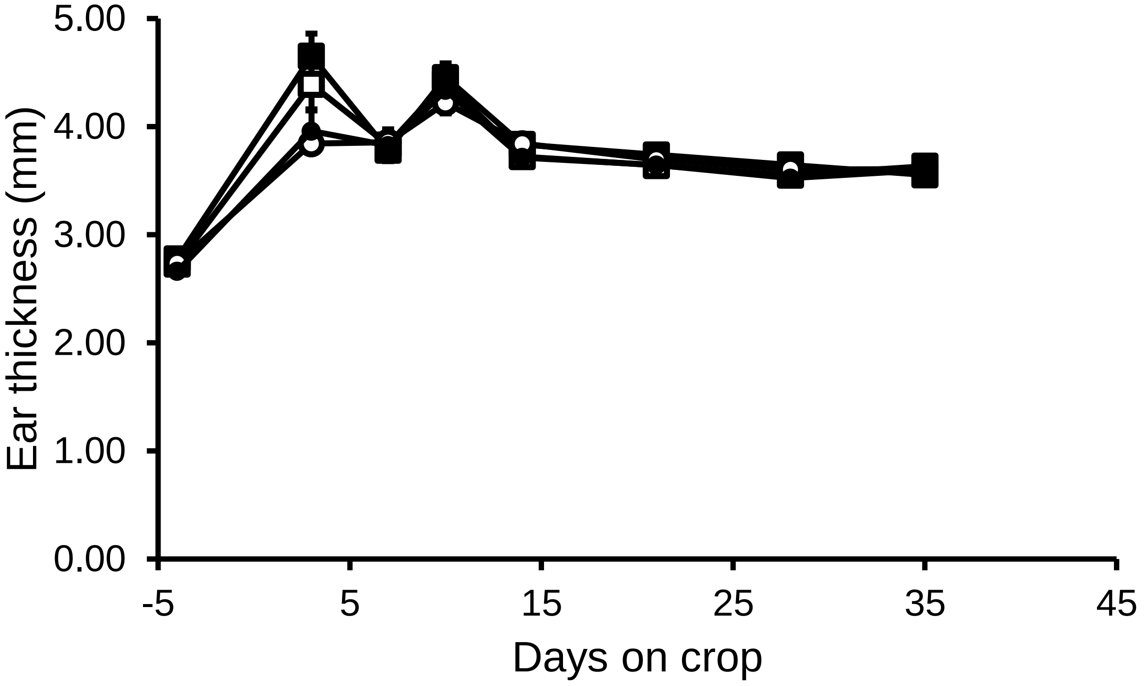

Figure 9: Mean (N = 240) ear thickness of lambs consuming forage rape either with (●) or without (○) additional N fertiliser or raphanobrassica either with (■) or without (□) additional N fertiliser. Error bars ± SEM.

3.4.2 Live weight

There was a marked effect (P < 0.001) of stage of the study on lamb live weight (Figure 10). During the first week and a half (10 days) of the study mean live weight generally decreased (P < 0.001) by about 4 kg, then steadily recovered (P < 0.001) so that by Day 35 (i.e. 5 weeks) live weights had recovered to, or slightly exceeded, the start values. Lambs consuming raphanobrassica tended to have a greater reduction in live weight (P = 0.093) compared with those grazing forage rape, which was more pronounced when the raphanobrassica crop had additional nitrogenous fertiliser applied (P = 0.005). Other than this interaction, there was no effect of N-fertiliser applications (P = 0.677) on the mean live weight of the lambs.

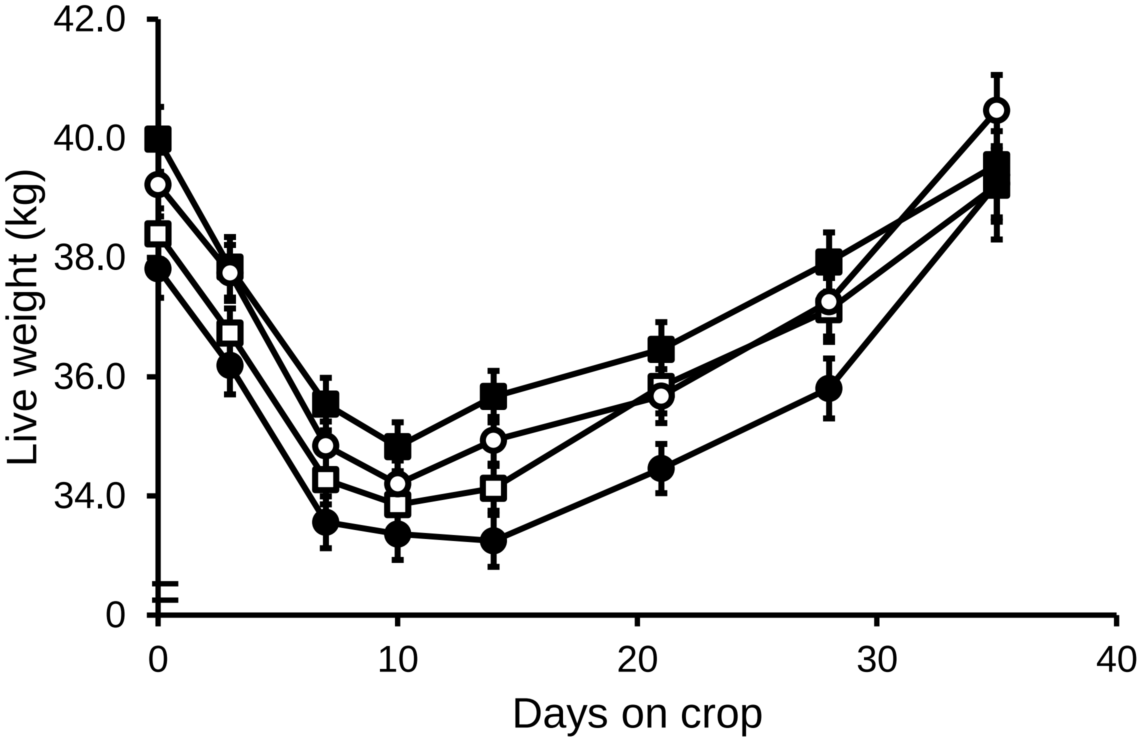

Figure 10: Mean (N = 240) live weight of lambs consuming forage rape either with (●) or without (○) additional N fertiliser or raphanobrassica either with (■) or without (□) additional N fertiliser. Error bars ± SEM.

3.4.3 Haematology

Lambs grazing these forage brassicas had gradual reductions in mean RBC, and Hb; reducing by approximately 2.5 x 10^6 μL^{-1} and 20 gL^{-}, respectively ($P < 0.001$) until about Day 21 (Figure 11A & B). The reductions were more severe in lambs grazing forage rape ($P < 0.001$), of which some individuals had mild anaemia. Nitrogenous fertiliser applications tended to be associated with greater decreases in RBC ($P = 0.101$) and Hb ($P = 0.062$), particularly when N had been applied to forage rape (RBC $P = 0.121$; Hb $P = 0.014$). RBC ($P = 0.080$) and Hb ($P = 0.094$) of lambs grazing forage brassica with N-fertiliser applications tended to fall more rapidly over time compared with the non N-boosted crops.

The initial incidence of Heinz bodies in lambs was low, although there was a gradual increase to Day 14 for lambs grazing forage rape and Day 21 for lambs grazing raphanobrassica ($P < 0.001$, Figure 11C). Lambs grazing forage rape had greater increases in Heinz bodies ($P < 0.001$) compared with those grazing raphanobrassica.

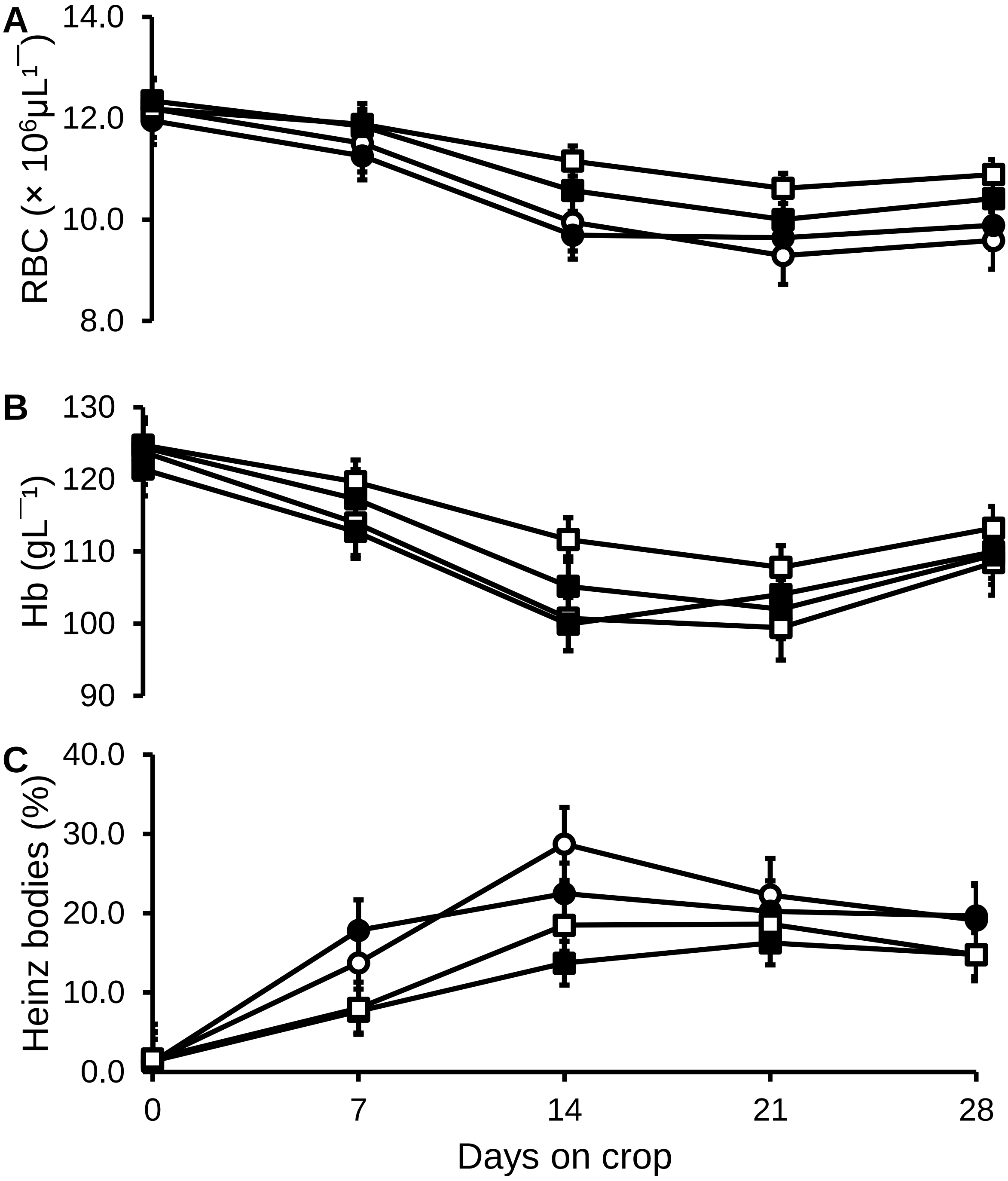

Figure 11: Mean (N = 128) A) erythrocyte count (RBC), B) haemoglobin concentration (Hb) and C) Heinz body percentage in blood of lambs consuming forage rape either with (●) or without (○) additional N fertiliser or raphanobrassica either with (■) or without (□) additional N fertiliser. Reference range of RBC and Hb in clinically normal sheep are 9.0 – 15.0 × 106 µL^{-1} and 80 – 140 gL^{-1} respectively. There is no established reference range for Heinz bodies. Error bars ± SEM.

3.4.4 Enzyme changes

Throughout the grazing study period, there were steady changes in levels of the enzyme markers of liver function (Figure 12). Mean plasma GGT concentrations increased ($P < 0.001$) and those of GLDH decreased ($P < 0.001$). Lambs grazing forage rape had higher levels of GGT throughout the study ($P < 0.001$) compared with those grazing raphanobrassica. There was no effect ($P = 0.489$) of nitrogen fertiliser applications on GGT of lambs grazing these forage brassicas. However, GLDH levels of lambs grazing these brassicas with additional nitrogenous fertiliser applications fell more rapidly over time compared with non-nitrogen fertilised crops ($P = 0.029$).

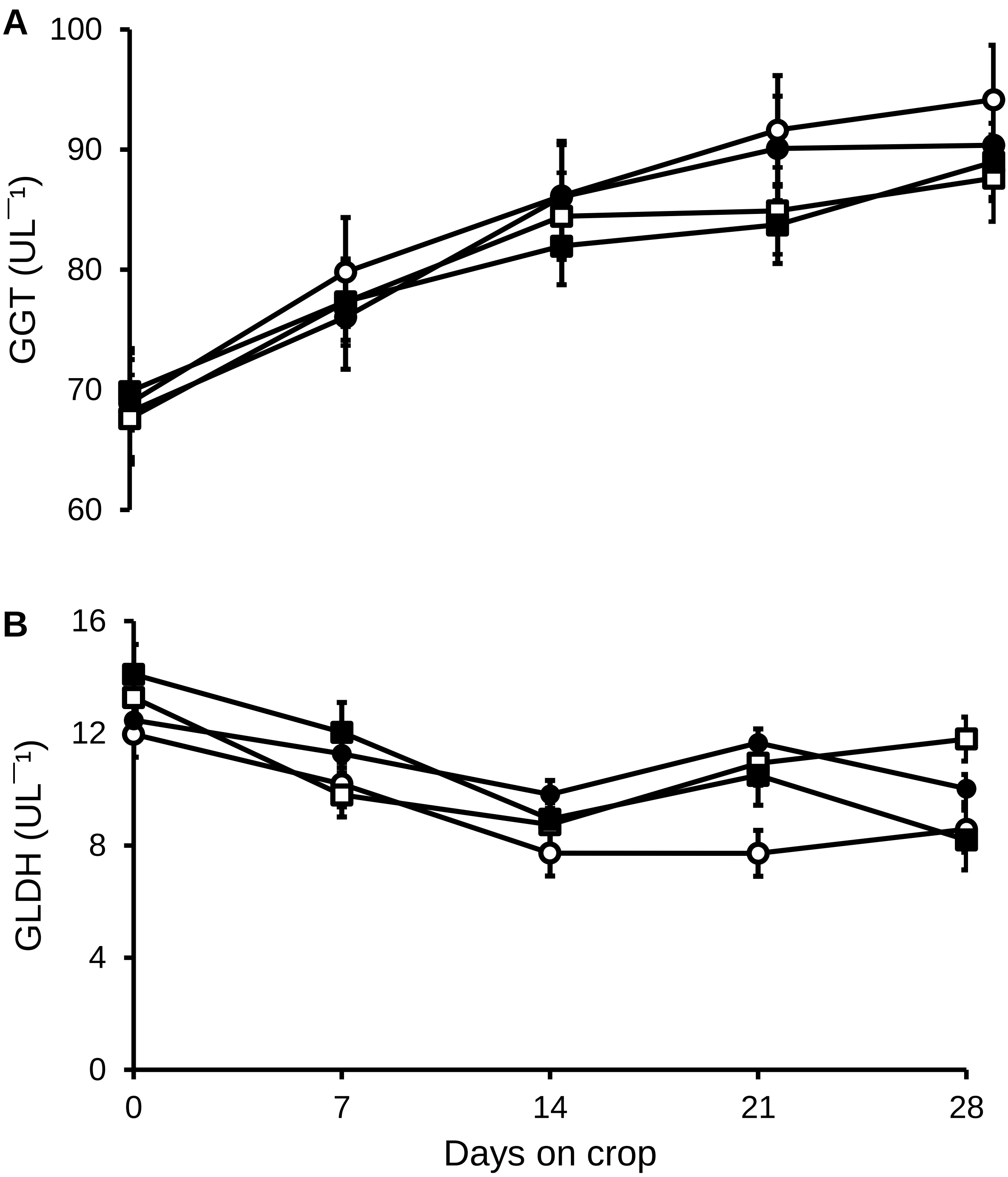

Figure 12: Mean (N = 128) A) gamma-glutamyl transferase concentration (GGT), B) glutamate dehydrogenase concentration (GLDH) in blood of lambs consuming forage rape either with (●) or without (○) additional N fertiliser or raphanobrassica either with (■) or without (□) additional N fertiliser. Reference range of GGT and GLDH in clinically normal sheep are 0 - 60 µL^{-1} and 0 - 15 µL^{-1} respectively. Error bars ± SEM.

3.4.5 Feed profile

Applications of nitrogen fertiliser (2 x 100 kg urea/ha) to the brassica crops increased (P < 0.001) yield, CP, and N content (Table 7). This effect did not differ between the feed types for yield (P = 0.447), CP (P = 0.078) or N content (P = 0.085). Energy content, digestibility and NDF also did not differ between the feed types (P > 0.1) nor were they affected by applications of nitrogen fertiliser (P > 0.1).

Table 7: Effect of forage type (forage rape and raphanobrassica) and nitrogenous fertiliser (± N) applications on crop yield and nutrient content. Data are means ($\bar{x}$) and standard deviations (s), n = 12. All values are expressed as a percentage of DM. DM% = dry matter percentage, MJ ME = megajoules of metabolisable energy, SS = soluble sugars, CP = crude protein, DOMD = digestibility of organic matter, NDF = neutral detergent fibre, N = nitrogen

			Yield	**Feed Profile**					
Treatment			Total DM (kg DM/ha)	MJ ME	SS	CP	DOMD	NDF	N
Forage rape	-N	$\bar{x}$	4275^b	12.33	19.11^a	19.30^b	77.10	22.25	3.09^b
		s	1325	0.37	3.69	3.56	2.25	5.16	0.57
	+N	$\bar{x}$	5187^a	12.35	17.47^b	23.09^a	77.22	22.34	3.69^a
		s	2680	0.50	3.87	4.37	3.12	5.57	0.70
Raphanobrassica	-N	$\bar{x}$	3954^c	12.32	16.84^b	20.20^b	77.03	21.93	3.23^b
		s	1734	0.49	3.91	4.65	3.09	4.98	0.74
	+N	$\bar{x}$	5073^a	12.10	14.84^c	24.17^a	75.58	21.73	3.86^a
		s	1470	0.50	2.67	2.85	3.10	3.37	0.46
Sample mean		$\bar{x}$	4623	12.28	17.09	21.68	76.73	22.07	3.47
		s	1892	0.47	3.84	4.34	2.93	4.77	0.69

Superscript = Fisher's Least Significant Difference groupings ($LSD_{0.05}$ and $p < 0.05$).

3.4.6 Chlorophyll

Nitrogenous fertiliser applications tended to raise the levels of chlorophyll a (P = 0.153), b (P = 0.071) and total chlorophyll (P = 0.124, Table 8) compared to non-N boosted forages. There was no effect of brassica species on altering chlorophyll a, b and total chlorophyll levels (P > 0.2).

Table 8: Effect of forage type (forage rape and raphanobrassica) and nitrogenous fertiliser (± N) applications on chlorophyll a, b and total concentration. Data are means (x̅) and standard deviations (s), n = 25

Treatment			**Chlorophyll concentration mg/m²**		
			a	b	Total
Forage rape	-N	x̅	394	111	505
		s	70	19	88
	+N	x̅	409	117	526
		s	76	20	95
Raphanobrassica	-N	x̅	380	114	494
		s	46	15	59
	+N	x̅	399	121	519
		s	54	16	68
Sample mean		x̅	396	116	512
		s	63	18	79

No significant differences between groupings ($LSD_{0.05}$ and $p < 0.05$).

3.5 Discussion

In this study, lambs grazing raphanobrassica have shown a similar incidence of BAPP to those grazing forage rape. Ear thickness data indicated a larger effect induced by raphanobrassica on Day 3 only, but subsequent biochemical measures of erythrocyte integrity and liver function could ascribe a greater subclinical effect to the grazing of forage rape.

Ear thickness

All lambs in the study experienced an increase in ear thickness within 72 hours after transfer to these forage brassicas. To date, there have not been any published studies that quantify rape scald in lambs, nor detail of incidence in affected flocks. However, a previous pilot study revealed all ear thickness changes in lambs occurred within the first 7 days (Westwood & Nichol, unpublished). Another study of primary photosensitivity in 167 lambs grazing the pasture legume *Biserrula pelecinus* L. produced similar results, whereby mild ear oedema was observed between 12 - 72 hours after exposure with 89% of animals affected (Quinn, et al., 2018).

The magnitude of ear thickness increase was briefly (on Day 3 only) higher in the lambs grazing raphanobrassica; there being no subsequent forage species-related differences, although ear thickness did remain elevated above the pre-brassica grazing values. It is difficult to place much weight on the greater ear thickness recorded from lambs grazing raphanobrassica because this effect appears to have been transient. Practically, although there was a statistical difference in ear thickness measurements associated with both species on Day 3, it is unlikely that this represents a difference in clinical severity. However, additional measurements of ear thickness conducted during the first 72 hours may provide a clearer picture of the rapidity of onset of the condition, which could have differed between the two forages. Overall we conclude that upon first grazing these two brassica species, clinical primary photosensitivity was slightly more severe for lambs grazing raphanobrassica (Day 3), but thereafter both brassica species have caused similar increases in ear thickness of these lambs.

After the initial increase in lamb ear thickness, there was a downward trend except for Day 10, which may be due to climatic conditions. The normal mean ambient temperature in January 2018 was 16.9 °C, whereas Day 10 of the study had an overnight low of 23°C and a daily high of 28°C. These elevated ambient temperatures may have exacerbated the effects on ear thickness.

The gradual reduction in ear thickness throughout the study may suggest the lambs may have developed tolerance to the presence of a causative agent, indicating a homeorhetic adjustment, activation of a detoxification pathway, or developed tolerance.

This possibility is akin to the observation that lambs grazing forage rape in another study (Westwood & Nichol, unpublished) did not have increases in ear thickness during a second period of exposure to the crop. However, this re-grazing occurred in late autumn which is outside the summer risk period for photosensitisation. There may be physiological mechanisms that enable lambs to develop tolerance to forage toxicity deserves further investigation. A comparative study of lambs that have previously been consuming forage brassica and naïve animals would assist in determining if lambs develop a degree of adaptation in terms of clinical measures and blood changes when recurrently supplied with a photodynamic agent.

Despite the reduction in ear thickness throughout the latter period of the study, recorded values remained well above the pre-treatment levels. This may be due to the continued supply of the causative agents in these crops or that these oedematous changes had become permanent. Persistent, untreated oedema is associated with longer wound healing time due to ambient fluid pressure reducing local blood supply (Hess, 2011), thus decreasing phagocytotic cell movement, nutrient flow and limiting angiogenesis (Leaper & Harding, 1998). In some individual lambs, oedema resolved with no other clinical signs. In others, leathering of the epidermal layers, external necrosis and excessive cartilaginous growth caused twisting of the distal pinna. The amount of distortion appeared to be a function of the degree of ear thickness developed from the initial fluid accumulation.

Haematology

Supporting evidence of pathology in lambs resulting from grazing these forage brassicas is provided by the subclinical data showing changes in erythrocyte number and integrity. A peak of Heinz bodies was evident on Day 14, two weeks after the introduction of lambs onto the brassica crops, with the lambs grazing forage rape having the larger increase. This effect was larger in forage rape-fed lambs. Heinz body presence after Day 21 was reduced in all treatments. The presence of Heinz bodies can reflect oxidative damage caused by a photodynamic agent, however, the oxidative damage may have been caused, in part, by a non-photodynamic agent. This is because the peak of oxidative damage in the systemic circulation (Day 14) lagged behind ear thickness changes, which peaked on Day 3. Rapid increases in Heinz bodies have also been seen in lambs grazing other species of forage brassicas, such as turnips (*Brassica rapa* L.) (Cox-Ganser, Jung, Pushkin, & Reid, 1994) and kale (*Brassica oleracea* L.) (Barry, Manley, & Millar, Nutritional evaluation of kale (Brassica oleracea) diets:4. Responses to supplementation with synthetic S-methyl-L-cysteine sulphoxide (SMCO), 1982) where there has been no link to clinical photosensitivity.

Further blood changes were found in RBC count and Hb concentrations of lambs grazing these forage brassicas. RBC and Hb gradually decreased, although largely remained within the normal reference range of healthy sheep, as the study progressed to Day 21, then stabilising. In contrast to ear thickness measurements, which suggested slightly greater ear thickness pathology in raphanobrassica-fed lambs on Day 3, lambs grazing forage rape had the largest reductions in RBC and Hb, with two lambs exhibiting mild haemolytic anaemia. This implies that anti-nutritional compounds which are often implicated in reducing erythrocytes (haemolytic anaemia), such as SMCO (Barry, Manley, & Millar, 1982), may be present in higher concentrations in forage rape than raphanobrassica. Significant decreases in RBC and Hb are a common finding in sheep grazing forage brassicas due to the presence of SMCO, although the values often remain within the normal reference range for healthy sheep (Valderrabano, Uriarte, & Munoz, 1986). It would be useful to determine the comparative SMCO concentrations between these two brassica species.

Recovery from the pathological sequelae of oxidative damage to RBC (Kaneko, Harvet, & Bruss, 1997) resulting in premature degradation, i.e. a reduction in RBC and Hb, is evident from reduced Heinz bodies (after Day 14) and stabilisation of RBC and Hb (Day 21) (Wilson, 2012). This could mean that either, a) the forage brassicas changed over four weeks resulting in less production of oxidative compound(s) or, b) lambs adapted to the presence of oxidative compound(s) and were able to detoxify the agent as the study progressed. The ability to activate detoxification pathways deserves further investigation.

Enzyme changes

Plasma concentration of the liver enzyme GGT was elevated above the normal reference range for healthy sheep at Day 0 and continued to increase throughout the study. This was more pronounced in lambs grazing forage rape compared with those grazing raphanobrassica, indicating the possible presence of an antinutritional factor that is higher in forage rape.

In the literature, rape scald is proposed as a primary photosensitivity (Vermunt, West, & Cooke, Rape poisoning in sheep, 1993). Primary photosensitivity diseases do not involve hepatic dysfunction, which in turn causes photodynamic agents to accumulate in the systemic circulation. Thus, rape scald is not typically associated with increased circulating liver enzyme concentrations (Vermunt, West, & Cooke, Rape poisoning in sheep, 1993). The changes in GGT documented here are much lower than those typically recorded in cases of secondary photosensitivity (Collett & Matthews, 2014). Thus, the elevated plasma GGT levels recorded in this study may be unrelated to the forages, or instead could be an artefact of brassica consumption but unrelated to photosensitivity. Lambs can also acquire GGT through passive transfer, with initial blood levels up to 140 times the normal adult levels, before stabilising at 60% higher than in ewes at 24 - 45 Days (Pauli, 1983). Another study revealed blood GGT levels in neonatal lambs remained elevated for 6 weeks (Britti, Massimini, Peli, Luciani, & Boari, 2005).

Also, the downward trend in plasma GLDH levels rules out any hepato-pathology in these lambs. Collectively, these unremarkable values for liver enzymes support the contention that the rape scald occurring in sheep grazing forage brassicas is a primary photosensitivity disease.

Live weight

Further pathological effects may be inferred from the live weight changes of lambs grazing forage brassicas, particularly as this study was set up to induce photosensitivity with a lax stocking rate and no supplementary feed. These lambs were gaining weight during the 25 day period before the study commenced. Once introduced to these forage brassicas, all lambs lost weight irrespective of plant species. Lambs consuming raphanobrassica tended to have a greater reduction in live weight, corroborating the ear thickness data and indicating that rape scald may be more severe in lambs grazing raphanobrassica compared to those on forage rape.

The large loss in live weight over the first ten days is likely to be a combination of three factors: pathology, neophobia and/or changes in rumen microbial populations. Discussions of the latter two are in Chapter 7. Live weight loss may be evidence of anorexia as a result of subclinical and clinical pathology. Anorexia has long been associated with illness in livestock (Sykes & Coop, 1976; Stromberg, et al., 2012). For example, a study of sheep with high intestinal parasite burdens grazing pasture showed reduced grazing duration in each grazing bout, decreased bite size, and a lower daily herbage intake compared with non-infected sheep (Hutchings, Gordon, Robertson, Kyriazakis, & Jackson, 2000). Disease processes causing pain, particularly around the head area, are also likely to decrease voluntary intake (Sneddon, Elwood, Adamo, & Leach, 2014). Observation of lamb behaviour identified head shaking, pruritic ear pinna and shade seeking within the first 24 hours of consuming these forage brassicas, which may have reduced grazing time. The gain in live weight of these lambs after Day 10 largely aligned with the recovery in ear thickness and the stabilisation of RBC and Hb. By Day 10, the lambs would have become familiar with the forage brassica, thus have a reduced feed neophobia and, simultaneously, have experienced suitable adjustment of rumen microbial populations.

Chlorophyll and nitrogen

The second hypothesis, that chlorophyll a or its metabolites, e.g. phytoporphyrin, are specific inducers of clinical photosensitisation, was to be tested by supplying a high nitrogen fertiliser (+N) treatment to increase the plant levels of chlorophyll a and comparing the incidence of clinical measures of rape scald in lambs grazing these forages with that of lambs grazing the non-boosted (-N) forages. However, the nitrogen fertiliser application only caused a tendency for plant levels of chlorophyll a, b and total chlorophyll to rise. As these results were not statistically significant, it is not possible to examine any possible link between chlorophyll a or its metabolites and rape scald. These limitations may be due to N not being limited in the fields, or to chlorophyll samples being taken only from plants in replicate 4, i.e. 1 plot of each treatment, at a single time point. This was due to the time constraint for processing samples in the laboratory within an 8-hour period and the requirement to select plants of a similar size with leaves at a similar growth stage, sun orientation, and geographical distribution within the leaf canopy. Furthermore, some works (Collett, et al., unpublished) question the measurement of chlorophyll or pheophorbide concentrations in feedstuffs, proposing that the plasma concentrations are of greater diagnostic value and are recommended for future studies.

Although the effects of N fertiliser on plant chlorophyll levels were minimal, there were some changes in both the crops and blood in these lambs. Application of N increased yield crude protein and plant N, irrespective of forage type. These findings are consistent with the effects of N fertiliser reported in the literature (McKenzie, Hampton, White, & Harrington, 2000). Nitrate is absorbed by plant roots (Valentine & Matthew, 2000) and incorporated in plants as proteins (Bojovic & Markovic, 2009), thus the +N treatment (92 kg N/ha) used here raised crude protein and plant N levels in these crops as expected.

Lambs in the present study grazing N-boosted forage brassica crops developed blood changes more rapidly than those on the non-boosted crops. For example, Heinz bodies levels fell more rapidly, although there was no related effect on RBC and Hb content, and GLDH levels increased faster. The blood changes may be partially explained by an accumulation of SMCO within the plants, which may be accentuated by applications of N fertiliser (Fletcher, Wilson, Maley, McCallum, & Shaw, 2010).

3.5.2 Conclusions

This study is the first to examine clinical and subclinical signs indicative of rape scald in lambs grazing both raphanobrassica and forage rape. Raphanobrassica appears to cause a similar incidence of clinical rape scald in grazing lambs to that which occurs with rape itself. The surge in lamb ear thickness recorded here within the initial 72 hours of grazing demonstrates that both crops can induce clinical rape scald; although the more sudden increase in ear thickness of lambs grazing raphanobrassica on Day 3 may indicate a heightened propensity of rape scald compared with lambs grazing forage rape. In contrast, biochemical measures of erythrocyte integrity provide some evidence that forage rape may cause more severe subclinical pathology in lambs, which may be due, in part, to the higher concentrations of antinutritional factors present in forage rape. Further research on quantifying the concentrations of antinutritional factors in these forages would assist in determining whether the brassica-related pathology resulted from the presence of these antinutritional factors or from subclinical rape scald. Unremarkable changes in the presence of hepatic enzymes, suggest rape scald is a primary photosensitising disease. Application of additional nitrogenous fertiliser to these forages may be a risk factor for this pathology. However, in the present study additional N fertiliser had a very limited effect on chlorophyll levels of the forages, making it difficult to reach any conclusion about a possible association between chlorophyll levels and rape scald. This may be resolved in future by investigating blood levels of pyropheophorbide a or phytoporphryrin (chlorophyll a metabolites) in lambs grazing these forages.

Addendum

Traditionally, the condition forming the basis of the present study has been called ‘rape scald’. However, this report of it occurring in lambs grazing a non-rape plant causes us to propose a more encompassing term for the disease. We submit the term – brassica associated primary photosensitivity (BAPP) – as an appropriate new name for this condition.

Chapter 4

Effect of grazing management on the incidence and severity of brassica associated primary photosensitivity (BAPP) in lambs grazing raphanobrassica

4.1 Abstract

Raphanobrassica (*Raphanus x Brassica* L.), forage rape (*Brassica napus* spp. biennis) and leafy turnips (*Brassica rapa*; syn. *B. campestris*) are forage brassica grown throughout Australasia, often as a high-quality feed for weaned lambs in summer and autumn. One animal health concern occasionally associated with the grazing of these summer brassicas is 'brassica associated primary photosensitivity' (BAPP), representing an animal welfare issue and loss of production. The purpose of this study was to determine if the severity and/or incidence of BAPP could be altered for lambs grazing raphanobrassica utilising different grazing treatments, which, more or less, changed the intake of plant parts. Signs of BAPP was measured in 276 lambs assigned to one of four grazing treatments of raphanobrassica: predominantly leaf (LF), alternating between grazing raphanobrassica and pasture (IN), grazing small areas daily (SG), consuming predominantly petiole or stem (PS) or to a control feed of chicory (CH), equating to 20 plots, each 0.2 – 0.3 ha, 60 days after sowing. Ear thickness, from oedematous accumulation, was more pronounced in the LF treatment [+2.31 mm (95% CI 1.51 to 3.1)], had a higher incidence (31%) and the disease curve peaked earlier (Day 3); and was intermediate in grazing treatments which had a midway consumption of leaf material IN [+1.47 mm (95% CI 0.853 to 2.09), 31%, Day 6, respectively]; and SG [+1.02 mm (95% CI 0.649 to 1.39), 22%, Day 6, respectively]. Lambs grazing the PS treatment had similar ear thickness changes [+0.40 mm (95% CI 0.253 to 0.547)] to those in the control, CH [+0.39 mm (95% CI 0.242 to 0.538)]. There was little effect of BAPP on lamb live weight ($P > 0.05$) and a poor correlation with blood changes. Grazing strategies that reduce leaf intake may be an effective mitigation tool to reduce BAPP in lambs grazing summer forage brassicas.

Keywords: Brassica associated primary photosensitivity, raphanobrassica, mitigation strategies

4.2 Introduction

Annual fodder crops, such as forage brassicas, are often grown on sheep enterprises during summer and autumn throughout New Zealand (Westwood & Mulcock, 2012). These crops are typically grazed *in situ* by lambs to maximise growth potential, particularly when feed shortages occur due to soil moisture and temperature (Dumbleton, et al., 2012).

One of the forage brassicas available is a new-to-market product: raphanobrassica, a hybrid of kale (*Brassica oleracea* L.) x radish (*Raphanus raphanistrum* subsp. *Sativus* L.). Raphanobrassica has been selectively bred over eight years for high forage yields, plant persistence under multiple grazing events, drought tolerance through water use efficiency, and flexibility of initial grazing from 42 to 120 days after sowing (A. Dumbleton, personal communication, March 13, 2019).

As in the case of forage rape and leafy turnips, lambs consuming raphanobrassica has been shown to induce idiopathic primary photosensitivity, commonly termed ‘rape scald’ or ‘brassica-associated photosensitivity’ (BAPP) (Box, *et al*., unpublished). Although the aetiology of BAPP is not understood (Collett, 2013), the proposed pathophysiological hypothesis is that the ingestion of a photodynamic agent reaches systemic circulation (Parkinson, Vermunt, & Malmo, 2010) and undergoes photoxidation in exposed dermal layers under the presence of ultraviolet light (Towers, 1980) and/or visible light (Moore, 2002). Dermal areas with reduced melanin and hair, such as ear pinna are the most affected (Campbell, Dombroski, Sharma, Partridge, & Collett, 2010).

Photoxidation induces oxidative stress causing damage to circulating erythrocytes, necrosis of surrounding tissues and notable localised oedema (Parkinson, Vermunt, & Malmo, 2010). This produces the typical initial presentation of BAPP in lambs as marked oedema of the ears, face and occasionally along the midline of the back (Cunningham, Hopkirk, & Filmer, 1942; Vermunt, West, & Cooke, Rape poisoning in sheep, 1993), which may be present as early as 72 hours after first consuming the feed (Box, *et al*., unpublished).

In cases of severe oedematous fluid accumulation, the structural integrity of the outer dermal layers may become compromised, leading to skin sloughing 1 to 2 weeks after the initial clinical presentation (Westwood & Nichol, unpublished). Following this, a typical dermal healing process (Levenson, et al., 1965) is evident. The prognosis is generally favourable when livestock are transferred onto another feed (Parkinson, Vermunt, & Malmo, 2010), although oedematous changes were shown to resolve whilst lambs remained grazing raphanobrassica (Box, *et al*., unpublished). While photosensitivity diseases, in general, are well documented, there is limited literature available on the disease progression of BAPP in lambs grazing forage brassica, with an on-farm focus of ear pinna loss weeks after introduction to brassica crops.

One method of controlling feed intake, and perhaps the ingestion of a photodynamic compound, is through grazing management. Livestock prefer feed that is easier to consume and is highly palatable. Leaf material in forage brassica has a lower portion of structural carbohydrates (Westwood & Mulcock, 2012) and is often consumed in preference to stem material (Judson & Edwards, 2008). If offered *ad libitum* forage brassica, Box, Barrel and Westwood (unpublished) found lambs preferentially consume greater volumes of leaf material.

The initial aim of this study was to determine if the incidence of BAPP can be altered through changes in grazing management. This hypothesis was addressed by comparing lambs grazing raphanobrassica utilising four different grazing management strategies and a control of chicory for evidence of BAPP, which included measurement of ear thickness (clinical), haematology (sub-clinical and clinical), and live weight changes (sub-clinical and clinical).

4.3 Materials and methods

4.3.1 Experimental design

The study was conducted at PGG Wrightson Seeds Research Centre, Kimihia, Lincoln, Canterbury, New Zealand (-43.62, 172.47) during the summer (2018-19). The study design was a 4 x 5 complete randomised block study, with treatments allocated using GENSTAT Version 19.1. All animal procedures received approved by the Lincoln University Animal Ethics Committee (Appendix 4). Prior to the commencement of the study, crop dry matter (DM) yields were measured (Day -1) to determine that a total of 276 lambs was required to consume all available feed in the 20 plots of 0.2 to 0.3 ha. The grazing treatments were:

- LF (leaf): lambs were laxly stocked (crop offered provided significantly higher energy supply than lamb energy demands) on raphanobrassica plots. This treatment served as a positive control as Box, Barrell and Westwood (unpublished) found lambs laxly stocked on forage brassica preferentially consumed high levels of leaf blade as opposed to leaf petiole and stem.

- IN (intermittent): for the first seven days, lambs grazed raphanobrassica plots during the day (8 hours) and adjacent Italian ryegrass pasture plots (16 hours) overnight. Following Day 7, lambs remained on raphanobrassica plots.

- SG (strip grazing): lambs were confined to a set area of raphanobrassica by use of electrified net fencing to encourage concurrent consumption of leaf blade, leaf petiole and stem. The electrified fencing was shifted on alternate days to provide a fresh area of raphanobrassica.

- PS (petiole and stem): at Day -4, a mulcher removed the top (vertical) 1/3rd of the raphanobrassica crop in these plots, leaving very large quantities of leaf petiole and stem compared with leaf blade. Mulching was performed to simulate ewes grazing ahead of lambs. Lambs grazed the remaining (lower 2/3rd of the canopy) raphanobrassica.

- CH (chicory): lambs consumed a sward comprising only chicory and red and white clover.

4.3.2 Study husbandry

To ensure the adequate establishment of the crops, the study site was treated with fertiliser (YaraMila ACTYVA S, Balance Agrinutrients, 1099 Bluff Highway, Awarua 9877) at a rate of 200 kg/ha, providing 30 kg N/ha, 14 kg P/ha and 26 kg K/ha. Seeds were sown on 15 November 2018; followed by one application of urea fertiliser 21 days post sowing, at a rate of 65 kg/ha, to provide an additional 30 kg N/ha. Best management practices provided by the seed company (PGG Wrightson Seeds, Lincoln, New Zealand) in terms of herbicide and insecticide applications and use of irrigation were followed, thus minimising alternative causes of primary photosensitivity by infestation by insects, e.g. aphids, and weed species (Collett, et al., unpublished). An electric fence system provided a physical barrier between the treatment plots. In addition to the electrified fence system, a 1 m boundary area between plots was sprayed with herbicide (glyphosate) to ensure the potential effect of fertiliser applications overlap was eliminated and lambs were unable to graze neighbouring plots. Treatment replicates were separated with permanent fencing materials. Fresh water was provided from reticulated troughs.

All lambs were cryptorchid Romney males from a single source flock. Before arrival at the Kimihia Research Centre, lambs had previously grazed forage brassicas followed by 4 weeks of grazing brown top grass pasture. Upon arrival (Day -7) lambs were weighed after 24 hours of fasting and their mean live weight was 30.4 kg (range 19.5 – 46.5 kg). These live weights were used to allocate the lambs into groups that were balanced for this parameter (blocked). Appropriate animal health management in terms of clostridial vaccinations, anthelmintic treatments, selenium supplementation, inguinal wool clipping (crutching) and a topical insecticide application was conducted before commencement of the study. The lambs were grazed in paddocks containing chicory, red clover and white clover for 7 days prior to the commencement of the study. Lambs were introduced into the treatment plots 61 days after seed sowing. Throughout the study, lambs were monitored daily by field staff who surveyed them by walking through the plots.

4.3.3 Measurements

Lambs were mustered from the study plots and moved to yard facilities for measurements on Days 0, 3, 7, 14, 21 and 28. Due to the large number of blood samples and limited laboratory resources, the study was staggered over two days for each measurement period. Lambs grazing plots in replicates 1 and 2 entered the study one day prior to those grazing replicates 3 and 4. There was little effect of this timing on any result ($P > 0.05$). Measurements included: live weight, ear thickness, subjective ear damage score, haematology and blood biochemistry. Live weight (kg) was measured with electronic scales (Tru-Test™, Auckland, New Zealand). Electronic vernier callipers (Jobmate, Palmerston North, New Zealand) were used to measure ear thickness (in mm) 10 mm proximal to an existing ear mark on the caudal border of the right ear pinna. Three measurements were taken on each lamb and the median value was recorded. For consistency, the same person performed all ear thickness measurements throughout the study.

Subjective measurements were also performed during daily monitoring, whereby field staff would walk the length of each plot (approximately 100 m). The number of lambs that were clinically affected in each plot was recorded. Visually, the ears of clinically affected lambs were oedematous and drooping below the level of the eyes (Chapter 6).

Blood sampling (10 mL) was performed on all lambs on measurement days, except for Day 3, using venepuncture of the left external jugular vein. All lambs had wool clipped from the ventral neck region and 20-gauge needles were used with manual restraint to draw 10 mL of blood into 16 x 100 mm vacutainers containing the anticoagulant Na-ethylenediaminetetracetate. Vacutainers were immediately placed on ice. Thereafter (i.e. within 3 h) the chilled blood samples were transported 18 km to a commercial laboratory (Gribbles Veterinary Pathology Laboratories, Christchurch, New Zealand) for analysis of red blood cell number, haemoglobin content and presence of Heinz bodies using an automated analyser.

Seven lambs succumbed to moderately severe oedematous accumulation within the ear pinnae and face. Subjectively, these lambs had reached the threshold for removal from the study as per the Animal Ethics application. On Day 7, these lambs were relocated to a shaded paddock to consume a non-brassica feed to aid recovery. They were from the following treatments: two LF lambs in two different replicates; two SG lambs in two different replicates; two IN lambs in the same replicate; and one CH lamb. Ongoing measurements were treated as missing values in this dataset but continued to be recorded. Details of their recovery are discussed in Chapter 6.

Crop yields were estimated prior to the commencement of the study on Day -1. Three 1 m^2 quadrat samples per plot were cut 3 cm above soil level and weighed (fresh weight). Subsamples of approximately 400 g were weighed, dried in an oven for 48 h at 90 °C and re-weighed to calculate dry matter percentage (DM%). Dry matter yield was calculated using the equation:

$$DM\ Yield\ \left(kg\ DM/ha\quad\right) = (Fresh\ weight\ x\ DM\ \%)\ x\ 10000$$

Stocking rate (number of lambs per plot) was calculated from the basis of mean crop yield per plot along with the following assumptions:

There was little variation in yield throughout the plots (Appendix 3: Crop yield). Lambs were assumed to gain on average 250 g/day and feed requirements were assumed to average 2.4 kg DM offered/lamb/day, which is equivalent to 7% of the mean lamb live weight $\frac{(30.4\ kg\ starting\ weight + 37.4\ kg\ finishing\ weight)}{2}$ throughout the study:

$$\begin{aligned}&Number\ of\ lambs\ per\ plot\\ &\qquad = DM\ yield\ (kg\ DM/ha)\ x\ Plot\ area\ (ha)\ x\ 2.4\ (kg\ DM\ offered\\ &\qquad /lamb)\ x\ 28\ (days)\end{aligned}$$

While this provided stocking rates for the raphanobrassica crop which were in line with current grazing recommendations by the seed company (PGG Wrightson Seeds, Lincoln, New Zealand), it resulted in a statistically unbalanced study design.

Climate data, such as solar radiation and ambient temperature was measured throughout the study using a Halo weather station at five-minute intervals, across six locations within Kimihia Research Centre.

4.3.4 Data analysis

The study ran for a 28-day period, from 15 January to 12 February 2019. Raw data were uploaded into Microsoft Excel and analysed using the statistical software GENSTAT Version 19.1. Statistical tests included Repeated Measures Correlation Model by Restricted Maximum Likelihood (REML), Approximate Least Significant Differences 5% of REML, Linear Mixed Models and one-way blocked Analysis of Variance (ANOVA). A significance value of a = 0.05 was used for rejecting the null hypothesis. The area under the disease progress curve was calculated using:

$$A_k = \sum_{i=1}^{N_i-1} \frac{(y_i + y_{i+1})}{2}(t_{i+1} - t_i)$$

4.4 Results

4.4.1 Climate

Mean daily solar radiation and ambient temperature ranged throughout the study period (Figure 13) between 126 to 356 W/m² and 12.56 to 22.85°C, respectively. There was little interaction in this study ($R^2 < 0.05$) between lamb ear thickness and mean solar radiation or ambient temperature.

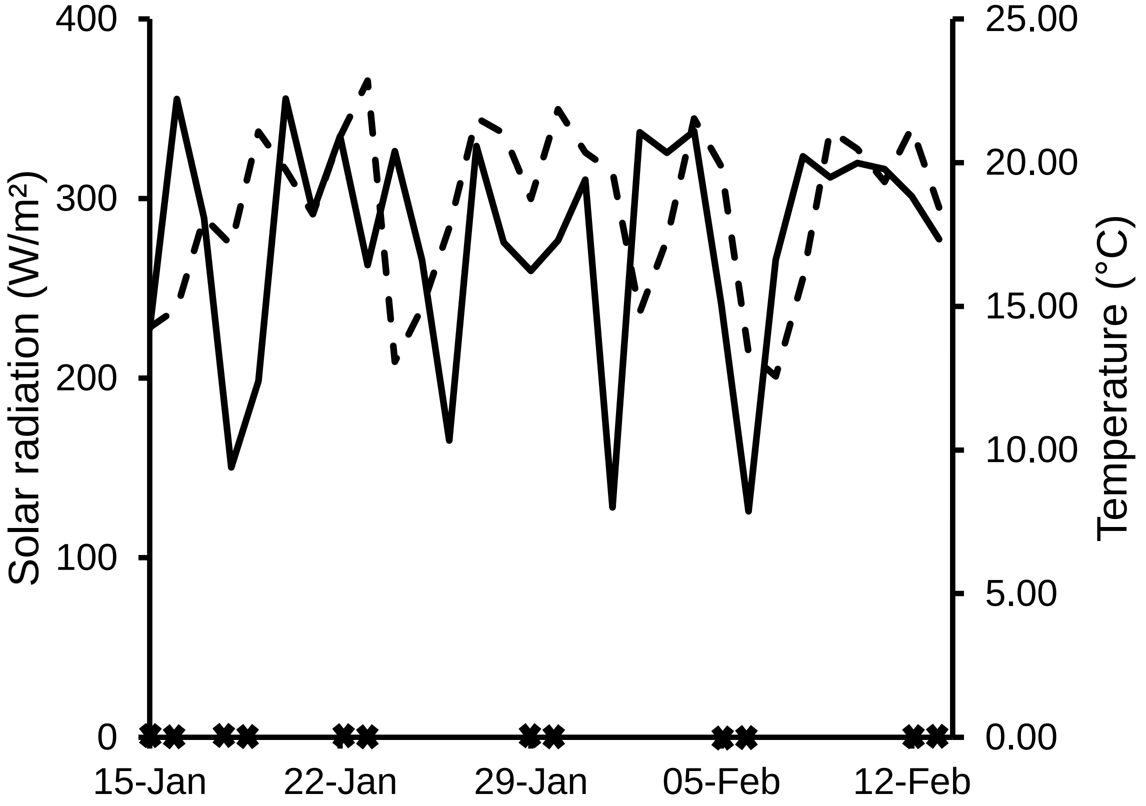

Figure 13: Mean daily solar radiation (-) and temperature (- -) whilst lambs grazed chicory and raphanobrassica crops. X symbols indicate days where lambs had clinical and subclinical measurements performed.

4.4.2 Ear thickness

The mean ear thickness of lambs increased by more than 0.5 mm when they were transferred to plots containing raphanobrassica, except in the case of the PS treatment (Figure 14). There was a significant ($P < 0.001$) difference between lambs grazing the LF (Day 3, 7 and 14), IN (Day 3, 7 and 14) and SG (Day 7 and 14) treatments compared to PS and CH treatments. The largest mean ear thickness values were recorded during this period (Days 3 to 14) but were declining by Day 14 and thereafter (Day 21) and were reduced to control group levels in all treatments at Day 28.

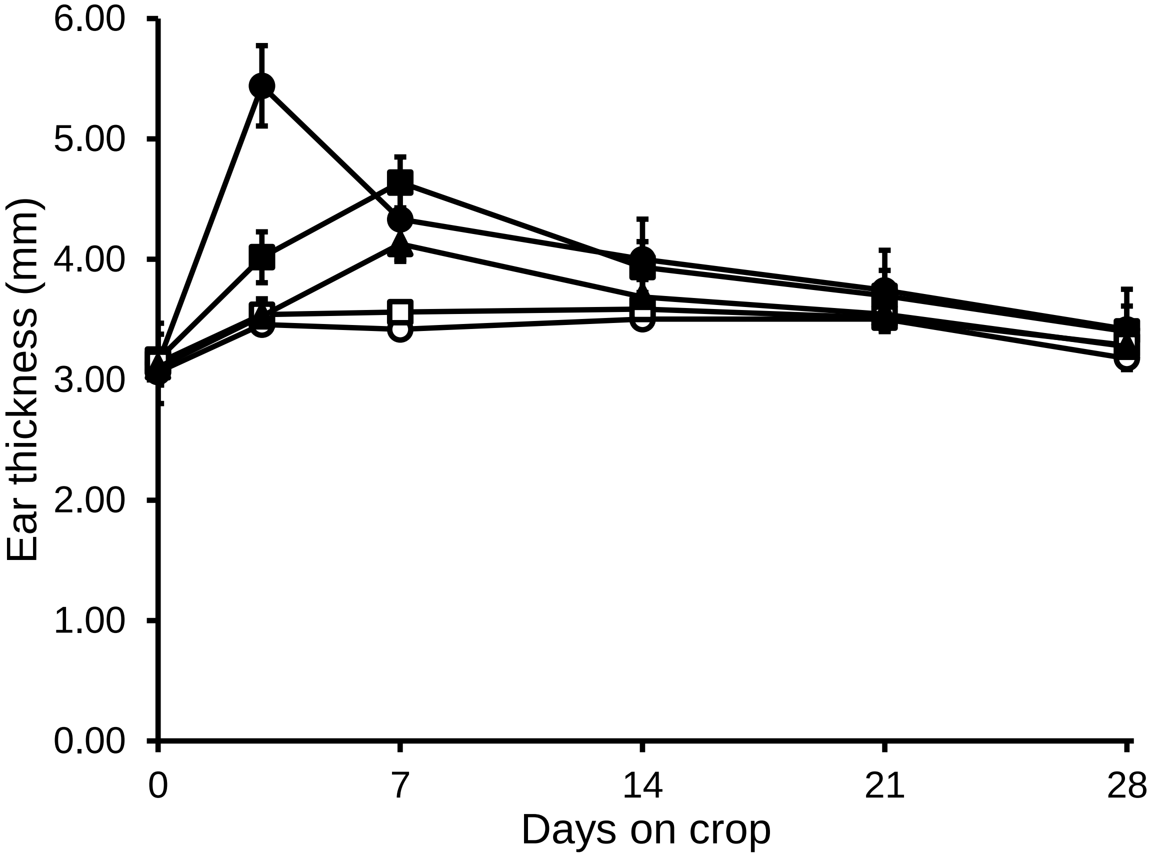

Figure 14: Mean (N = 276) ear thickness of lambs consuming either chicory, red clover and white clover (○) or raphanobrassica. Lambs consuming raphanobrassica were subjected to strip grazing (▲) or intermittent grazing (■); or consuming predominantly leaf (●) or petiole/stem (□), Error bars ± SEM.

4.4.3 Clinical cases

Subjective recording of clinically affected lambs (oedematous ears, drooping below the level of the eye, Figure 15) identified cases as early as 24 hours (one day) after being introduced to these crops. There was a strong positive correlation ($R^2 = 0.986$) between subjectively scoring morbidity in the field and lambs with ear thicknesses > 4.5 mm measured using electronic Vernier callipers (Day 3 and 7). The largest increases in clinical BAPP incidence occurred within 24 hours for lambs grazing LF treatment (20%), followed by lambs grazing raphanobrassica treatments IN (13%) and SG (12%) between Day 4 and 5. The number of clinically affected lambs grazing LF treatment peaked at Day 3 (n = 22), resulting in an area under the disease progress curve (AUDPC) at 175% (Figure 15). Lambs grazing IN treatment had a similar number clinically affected (n=25) as the LF treatment, however, the peak occurred later at Day 6, with an AUDPC of 102%. Clinically affected lambs in the SG treatment again peaked at Day 6 (n = 15), although the AUDPC was 56.7%. Lambs assigned to the CH and PS treatments were largely unaffected, with changes in ear thickness only occurring in a few individuals resulting in a low AUDPC 12.3% and 7.89%, respectively.

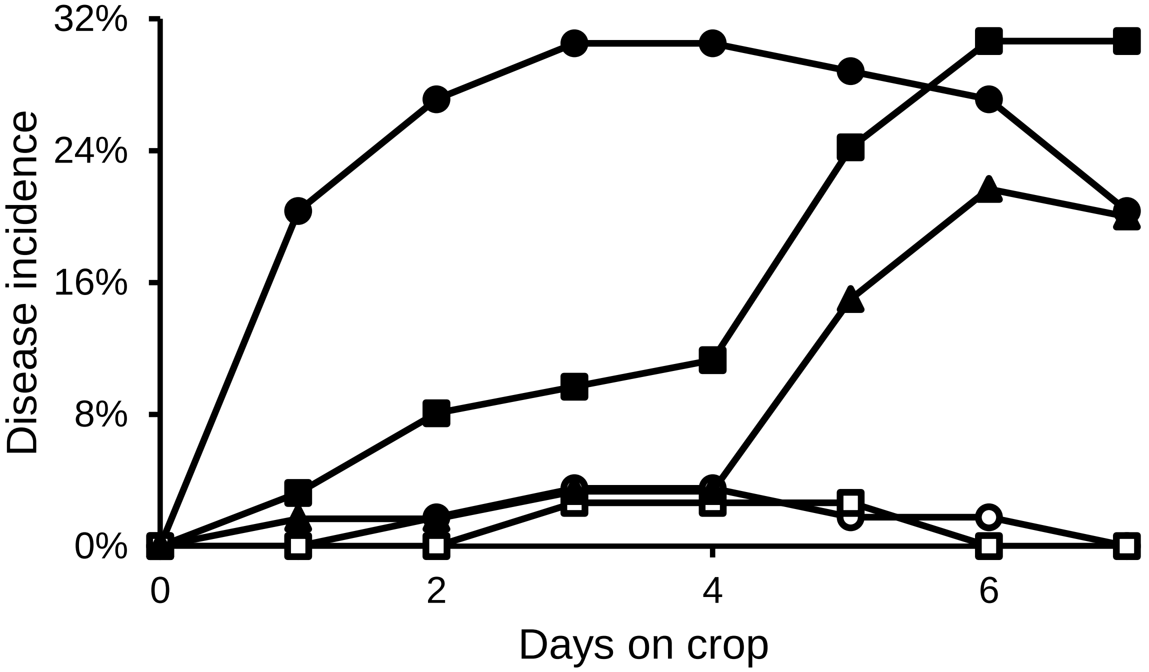

Figure 15: Incidence of lambs with clinical ear oedema, subjectively causing drooping of the ear pinna below the level of the eye (see Chapter 6) whilst consuming either chicory, red clover and white clover (○) or raphanobrassica. Lambs consuming raphanobrassica were subjected to strip grazing (▲) or intermittent grazing (■); or consuming predominantly leaf (●) or petiole/stem (□).

4.4.4 Live weight

There was a steady increase in mean lamb live weight throughout the study ($P < 0.001$). Lamb live weights on Day 28 were approximately 10 kg heavier than their initial starting value (Figure 16). Overall, live weight gain was greater in lambs grazing the CH and LF treatments so as by Day 28, lambs in these two treatments were heavier ($P = 0.019$) than lambs assigned to the SG treatment.

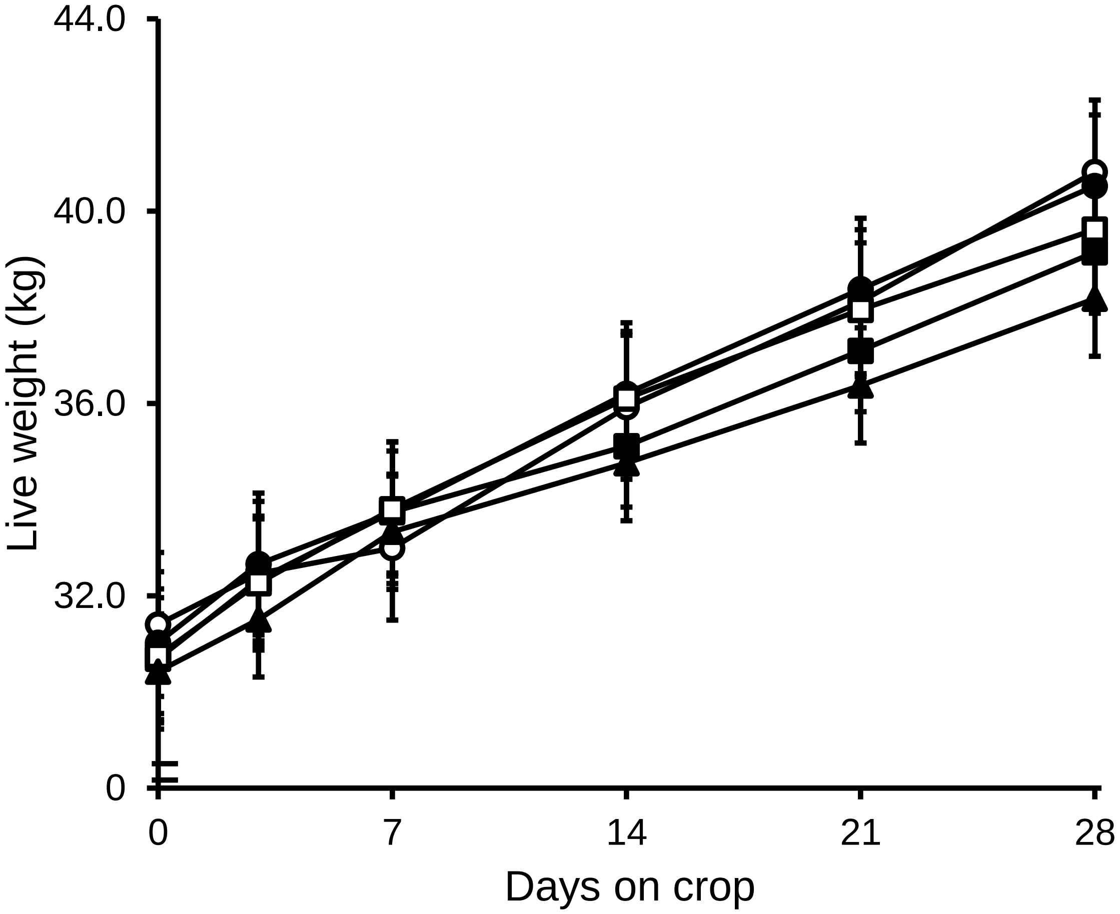

Figure 16: Mean (N = 276) live weight of lambs consuming either chicory, red clover and white clover (○) or raphanobrassica. Lambs consuming raphanobrassica were subjected to strip grazing (▲) or intermittent grazing (■); or consuming predominantly leaf (●) or petiole/stem (□), Error bars ± SEM.

4.4.5 Haematology

Throughout the study, lambs grazing all raphanobrassica treatments had a reduction in erythrocyte count ($P < 0.001$) and Hb concentration ($P < 0.001$) combined with an increased Heinz body incidence ($P < 0.001$) compared with lambs consuming the control feed (Figure 17). Following the initial changes, these blood changes tended to stabilise from Day 14 in raphanobrassica-fed lambs. The pattern of change over time was relatively consistent across all groups grazing raphanobrassica ($P > 0.05$).

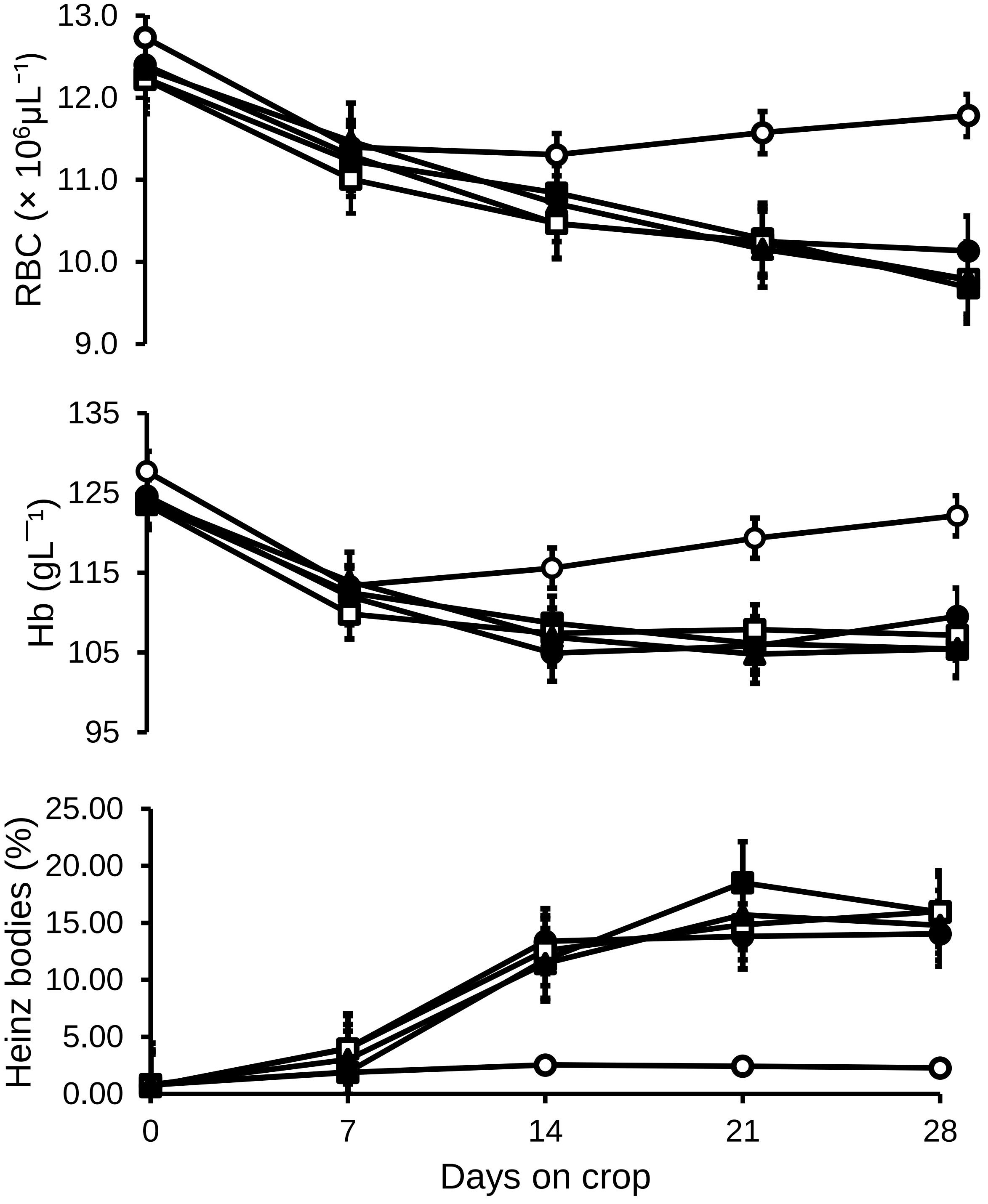

Figure 17: Mean (N = 276) A) erythrocyte count (RBC), B) haemoglobin concentration (Hb) and C) Heinz body percentage in blood of lambs consuming either chicory, red clover and white clover (○) or raphanobrassica. Lambs consuming raphanobrassica were subjected to strip grazing (▲) or intermittent grazing (■); or consuming predominantly leaf (●) or petiole/stem (□), Reference range of RBC and Hb in clinically normal sheep are 9.0 – 15.0 × 106 µL⁻¹ and 80 – 140 gL⁻¹ respectively. There is no established reference range for Heinz bodies. Error bars ± SEM.

4.5 Discussion

Primary photosensitivity in lambs consuming brassica, typically forage rape, is a well-recognised disease on Australasian sheep farming enterprises (Vermunt, West, & Cooke, Rape poisoning in sheep, 1993). However, due to the sporadic nature of the disease, research has been to be limited to case studies. This was the first study to identify management strategies that may reduce the severity and/or incidence of BAPP in lambs grazing forage brassica. A clear relationship between leaf intake and BAPP existed in these lambs, whereby strategies increasing leaf intake had greater BAPP severity and elevated incidence, compared to a diet consisting of predominantly petiole and stem. While biochemical measures of erythrocyte integrity indicated some mild pathological trends compared to those consuming a control diet of chicory, these changes remained within the range of normal limits in sheep and were similar between brassica-fed treatments. Specific phototoxin(s) eliciting BAPP in brassica-fed lambs remains unclear, however, results from this study indicate it is likely found in the leaf portion of the brassica plant.

Ear thickness

Changes in ear pinna thickness due to localised oedema is a classical sign of BAPP. Mean ear pinna thickness in raphanobrassica-fed lambs differed between grazing treatments but was greatest in lambs assigned to the LF treatment. The two treatments which somewhat reduced leaf intake by either consuming a different feed, IN, or limiting the volume of leaf available each day, SG, had moderate increases in mean ear thickness. Raphanobrassica-fed lambs assigned to these three treatments had the largest ranges in ear thickness, whereby data were skewed by a small number of individuals, with relatively large Q3 - Q2 interquartile ranges. For example, on Day 3 lambs grazing the LF treatment had a mean ear thickness of 5.88 mm but a median of 3.97 mm. Elevation in of ear thickness of this magnitude and with this pattern are supported by findings in the previous study, whereby lambs introduced to raphanobrassica and grazing the crop in a similar manner to the LF treatment, had a mean increase in ear thickness of 2.4 mm which peaked at Day 3. These changes are both statistically and clinically significant (≥ 0.5 mm increase over Day 0 measurement) compared to mean ear thicknesses of lambs assigned to the PS treatment and CH control. Following Day 7, there was a steady reduction in lamb ear thickness throughout the remainder of the study so by Day 28, mean ear thicknesses were comparable to starting values.

Not only was BAPP more severe in raphanobrassica-fed lambs assigned to the LF treatment, there was also an earlier onset of BAPP. These clinical cases of BAPP in lambs were evident within 24 hours after introduction to raphanobrassica, with the largest number of affected animals peaking on Day 3. There was a similar number of affected lambs grazing the IN as the LF treatment, however, the peak number of lambs affected occurred later at Day 6. Again, lambs assigned to the SG treatment had an intermediate response to BAPP, peaking at Day 6.

Additionally, more lambs succumbed to the clinical signs of BAPP (≥ 0.5 mm) in the LF, IN and SG raphanobrassica-fed treatments compared to those in the PS and control treatments. These results are comparable to the incidence of primary photosensitivity in lambs grazing *Lotus corniculatus* (Stafford, West, Alley, & Waghorn, 1995) measured over three consecutive years at 33.3%, 7.5% and 26.8%. However, the previous study (Chapter 3) found a larger clinical incidence in lamb ear thickness increases (≥ 0.5 mm) than that of this study at 93%, where both measurements were performed 72 hours after introduction to raphanobrassica.

Lambs assigned to the PS treatment had lower elevations in mean ear thickness and a reduced incidence of BAPP. This suggests that the compound(s) or metabolites responsible for BAPP is likely to be in the green, leafy plant parts rather than the pale petiole and stems. This is demonstrated from lambs grazing the LF treatment which had the largest increase in mean ear thickness, the earliest peak in clinical cases and the greatest AUDPC. Since lambs behaviourally appear to prefer leaf material, low stocking rates of lambs onto brassica crops may heighten the risk of BAPP during the summer period. Lambs grazing an intermediate volume of leafy material which was diluted through either an additional feed source (IN) or by reducing the area to graze resulting in similar volumes of plant parts, i.e. leaf, petiole and stem, (SG) had an intermediate response by which there was an increase in ear thickness, however, this occurred slightly later, was not as severe, and had a lower AUDPC than those on the LF treatment. This result may indicate the compound(s) or metabolites responsible for BAPP had accumulated in a significant quantity to cause clinical BAPP.

It was noted that lambs assigned to the IN raphanobrassica treatment for the first seven days, by which they alternated between adjacent crops of raphanobrassica for 8 hours or Italian ryegrass for 16 hours, were not consuming large quantities of ryegrass. Proposed causes of this include the presence of rust on the ryegrass crop affecting palatability (Cruickshank, 1957; Cunningham, Blumenthal, Anderson, Prakash, & Leonforte, 1994); and a sward height >15 cm. Grazing management similar to the IN treatment has previously been a tool utilised to reduce photosensitisation in lambs grazing *Brachiaria* spp. (Castro, et al., 2018). Repeating this study may elucidate whether intermittent grazing using a palatable crop is an appropriate tool to reduce the severity and/or incidence of BAPP in lambs.

Live weight

Lamb live weight steadily increased throughout the study and did not appear to be affected by increased in ear thickness nor was it statistically different from the control treatment, CH. This contrasts with the previous finding where live weight was negatively affected for the first ten days, i.e. lambs lost approximately 4 kg within the first ten days of consuming the crop. The discussion in the previous study (Chapter 3) proposed weight loss may be associated with neophobia and reduced rumen fill, behavioural and social changes of the lambs being exposed to a novel feed source, trends regularly identified in farming enterprises. Lambs in this study had previously consumed forage brassica prior to their arrival to the study site (approximately 6 weeks) and appeared to immediately consume the crops in the respective raphanobrassica-fed treatments.

A slight difference in live weight gain occurred throughout the study between lambs assigned to the LF and CH treatments compared with the SG treatment, equating to approximately 1.5 kg/lamb difference in live weight after 28 days. This is likely due to controlling the intake of SG lambs compared to the *ad libitum* intake of those on all other treatments.

Haemotology

Heinz bodies, which reflect oxidative damage on erythrocytes, was increased in all forage brassica treatments, peaking at around 15% on Day 21. While there is no reference range for clinically normal lambs, values were significantly larger than those in lambs consuming chicory. This aligns with the findings in the previous study by which Heinz bodies increased from 0% at Day 0 to 10 – 30% at Day 14. While RBC count and Hb concentration remained within the normal range for clinical normal sheep, there was a reduction in both measures, a likely consequence of Heinz bodies, in raphanobrassica-fed lambs throughout the study compared to the control treatment, CH, which tended to stabilise around Day 14. The pattern of change and the degree of reduction was comparable to the results from the previous study.

A comparison of these blood changes between the raphanobrassica-fed PS treatment and control treatment, CH, where little BAPP pathology occurred highlights these measures do not appear to be related to the pathology of BAPP. There is also a poor correlation between these haematological measurements and lamb ear thickness, suggesting this pathology may be due to an anti-nutritional compound rather than a true indicator of subclinical photosensitivity disease. Literature suggests Heinz bodies increases in animals exposed to forage brassica (Cox-Ganser, Jung, Pushkin, & Reid, 1994; Barry, Manley, & Millar, 1982) without the presence of clinical photosensitivity.

Climate

Anecdotally, the incidence of BAPP differs throughout the year and between regions in Australasia. Box *et al.* (unpublished) found a potential relationship between the temperature on one measurement day and increased ear thickness. However, there was little interaction in this study between lamb ear thickness and mean solar radiation, which includes UV light, or ambient temperature. Further research into a potential interaction between UV radiation, particularly in the range of UVA at 320 – 400 nm, is required.

4.5.2 Conclusions

This study examined clinical and subclinical signs of BAPP in lambs subjected to four different grazing management strategies of raphanobrassica and a control feed of chicory. The grazing treatment which promoted the largest intake of leaf material was associated with the highest disease incidence, the most severe increases in lamb ear thickness, and the earliest peak of disease incidence. The grazing treatment which mechanically removed most of the leaf material, resulting in high intakes of petiole and stem, had lamb ear thicknesses comparable to the control treatment. Practically farming enterprises could mimic the PS treatment by grazing lambs with ewes, or grazing ewes ahead of the lambs to initially remove large volumes of leaf. Further research determining if there is a degree of adaptation to the toxin(s) responsible for BAPP will aid in providing practical management tools around when lambs could begin consuming leafy material. Both ear thickness changes and haematological changes mirrored those in the previous study (Chapter 3). A poor correlation between increased ear thickness and erythrocyte pathology suggests erythrocyte changes may be associated with an anti-nutritional compound rather than subclinical BAPP. In contrast to the previous study, live weight changes steadily increased, suggesting lamb live weight changes may not indicate disease, rather neophobia of a novel feed source. Early observation and treatment of BAPP in lambs is essential for predicting animal welfare outcomes. There should be some degree of caution as this study provides mitigation strategies to a disease where the underlying cause is unknown.

Chapter 5

Effect of prior exposure on the incidence and severity of brassica associated primary photosensitivity (BAPP) in lambs grazing raphanobrassica

5.1 Abstract

Forage brassica crops are a common source of feed on sheep, beef and deer enterprises throughout New Zealand and Australia. However, during the summer months, forage brassica crops such as forage rape and raphanobrassica have been associated with 'brassica associated primary photosensitivity' (BAPP). The aim of this study was to determine if lambs can detoxify the causative agent(s) of BAPP, thus influencing the severity and incidence of BAPP. Lambs were randomly assigned to a negative control feed of chicory (CHI), continuously grazed raphanobrassica (CON), did not graze any forage brassica for 56 days prior to the study, only chicory (NAÏ), or were grazing raphanobrassica then were removed for four days to consume chicory immediately prior to the study, then reintroduced to raphanobrassica (PEX), using a complete random block design with four replicates. During autumn 2019, 240 lambs from a single source were introduced to 16 plots of 0.25 ha of either raphanobrassica or chicory. On Day 3, NAÏ lambs had elevated ear pinna thickness, a result of accumulation of excessive interstitial fluid between the skin and cartilage of the auricle, of +0.56 mm (95% CI [0.43, 0.70]) compared with CHI, CON and PEX at +0.13 (95% CI 0.07, 0.19); +0.02 (95% CI -0.05, 0.09); and +0.02 mm (95% CI -0.09, 0.13), respectively. Ear thickness changes in these NAÏ lambs gradually resolved after Day 3, so as they were similar to starting values by Day 14. Live weight and apparent feed intake did not appear to be affected regardless of disease status. Heinz bodies gradually reduced in CHI lambs (-1.03%/day), slowly increased in CON (+2.50%/day) and PEX lambs (+2.91%/day) and increased significantly in NAÏ lambs (+4.96%/day, $P<0.001$). RBC and Hb remained within the normal ranges for healthy animals but did trend downwards in raphanobrassica-fed lambs. These haematological changes suggest that they are unrelated to BAPP and may instead be due to the presence of an antinutritional compound. BAPP occurred in NAÏ lambs but was reduced in CON and PEX raphanobrassica-fed lambs. Lambs appear to have developed an ability to detoxify the causative agent(s) of BAPP which lasts as long as four days, but not as long as 56 days. We recommend lambs are maintained on brassica crops to reduce the future risk of developing BAPP. Whilst this study was conducted using raphanobrassica, results are of relevance for lambs grazing all summer forage brassica crops.

Keywords: primary photosensitisation, BAPP, detoxifying, forage brassica, raphanobrassica

5.2 Introduction

Forage brassicas are annual fodder crops that occupy the largest area of cultivated land in New Zealand at 300,000 ha grown annually (de Ruiter, et al., 2009). Approximately 60 - 70% are utilised as summer/autumn or winter crops on sheep, beef and deer enterprises (A. Dumbleton, personal communication, March 13, 2019). One issue occasionally associated with forage rape (*Brassica napus* spp. *biennis*), raphanobrassica (*Raphanus sativa x Brassica oleracea)* and leafy turnip (*Brassica rapa*; syn. *B. campestris*) crops during summer months is brassica associated primary photosensitivity (BAPP). Whereby lambs develop a non-contagious inflated sensitivity to sunlight in areas with reduced melanin pigment, hair and wool such as ear pinna (Clare, 1952). Presenting animals typically have oedematous ear pinna, which may extend to eyelids, bridge of the nose or midline of the back. The causative agent of BAPP remains elusive (Collett, 2013).

As demonstrated in the two previous studies (Chapters 3 and 4), mild to moderate clinical cases of BAPP have resolved whilst lambs continue to consume the affecting feed, suggesting a developed ability to detoxify the causative agent(s). This ability appears to establish approximately one week after initial exposure to forage brassica, whereby the initial increase in ear pinna thickness, due to oedematous accumulation, begins to resolve (Westwood & Nichol, unpublished).

There are numerous cases of toxin adaptation in sheep. Specifically, lambs grazing *Brachiaria* have shown adaptation to steroidal saponins, causing photosensitivity, using three different mechanisms: changes in grazing management, whereby lambs were restricted to grazing the affecting crop for only two hours per day; rumen microbial changes using ruminal transfaunation; and in the long term, inherited resistance (Castro, et al., 2018). In another example, sheep administered with *Galega officinalis* L., causing toxicosis when given at 0.8 g DM/kg body weight/day, over consecutive days were then able to tolerate doses 5 – 10 times the lethal dose pre-treatment with no clinical effects (Keeler, Johnson, Stuart, & Evans, 1986).

A reduction in expected live weight gain, or live weight loss, may be associated with disease, due to alterations in energy (Hindson & Winter, 1990), or protein (van Houtert & Sykes, 1996) requirements for disease recovery, or anorexia (Sykes & Coop, 1976). In the first study (Chapter 3), lambs lost weight over the initial ten days following introduction to forage brassica, which coincided with BAPP pathology. However, in the second study (Chapter 4), lambs gained weight even in the presence of mild to moderate presentation of clinical BAPP, suggesting a need to revisit a potential relationship between live weight and clinical BAPP.

The aim of this study was to determine if lambs develop an ability to detoxify the causative agent(s) of BAPP, thus altering disease severity, incidence and onset. This aim was addressed by measuring evidence of BAPP between treatments of lambs which 1) had not consumed any forage brassica for 8 weeks, or 56 days prior to the study; 2) had not consumed any forage brassica for four days prior; 3) continuously grazed raphanobrassica before and during the study; and 4) consumed a negative control treatment of chicory. Lambs assigned to all non-control treatments were reintroduced to brassica at the beginning of the study. An additional hypothesis is that clinically affected lambs have a reduction in live weight. This was tested by measuring lamb live weight and apparent intake.

5.3 Materials and methods

5.3.1 Experimental design

The study was conducted at PGG Wrightson Seeds Research Centre, Kimihia, Lincoln, Canterbury, New Zealand (-43.62, 172.47) during the summer (2018-19). The study design was a 4 x 4 complete randomised block study, with treatments allocated using GENSTAT Version 19.1. All animal procedures received approved by the Lincoln University Animal Ethics Committee (Appendix 4). Prior to the commencement of the study (Day -58), 240 lambs were assigned to their treatment groups to be maintained on either raphanobrassica or chicory (*Chichorium intybus* cv. 'Puna II') forage crops. Crop dry matter (DM) yields were measured (Day -1) and lambs received a full wool clip. On Day 0, the 240 lambs were separated into their assigned plots (15 lambs per plot).

The treatments were:

- Chicory (CHI): prior to the study, on Day -56 to Day -1, lambs consumed forage brassica, then were introduced to a sward comprised predominantly of chicory, with minor amounts of red and white clover at the beginning of the study.

- Naïve (NAÏ): lambs that had previously grazed raphanobrassica were removed on Day -56 and did not graze any forage brassica crop, but instead grazed chicory, for the 8-week period prior to the study commencing, then were reintroduced to raphanobrassica from Day 0 onwards. This treatment served as a positive control as we noted on-farm lambs which had suffered clinical BAPP and removed from grazing forage brassica suffered BAPP again 8 weeks later once reintroduced to raphanobrassica.

- Continuous (CON): lambs were introduced to raphanobrassica on Day -56 and continued to feed on this crop for the duration of the study.

- Prior exposure (PEX): lambs that had been consistently grazing raphanobrassica were removed (Day -4) for 4 days prior to the commencement of the study to graze chicory, then reintroduced to raphanobrassica from Day 0 onwards.

5.3.2 Study husbandry

To ensure the adequate establishment of crops, the study site was treated with fertiliser (YaraMila ACTYVA S, Balance Agrinutrients, 1099 Bluff Highway, Awarua 9877) at a rate of 200 kg/ha, providing 30 kg N/ha, 14 kg P/ha and 26 kg K/ha. Seeds were sown on 15 November 2018; followed by one application of urea fertiliser 21 days post sowing, at a rate of 65 kg/ha, to provide an additional 30 kg N/ha. These crops were then grazed between 15 January and 29 February 2019 and allowed to regrow. Best management practices provided by the seed company (PGG Wrightson Seeds, Lincoln, New Zealand) in terms of herbicide and insecticide applications and use of irrigation were followed, thus minimising alternative causes of primary photosensitivity in terms of infestation by insects, e.g. aphids, and weed species (M. G. Collett, Z. M. Matthews, K. H. Parton, B. A. Tapper & K. C. Harrington, unpublished). An electric fence system provided a physical barrier between the treatment plots. In addition to the electrified fence system, a 1 m boundary area between plots was sprayed with herbicide (glyphosate) to ensure the potential effect of fertiliser applications overlap was eliminated and lambs were unable to graze neighbouring plots. Treatment replicates were separated with permanent fencing materials. Fresh water was provided from reticulated troughs.

All lambs were cryptorchid Romney males from a single source flock. As lambs were assigned to their feed treatments on 22 January 2019 (Day -56), there was some variation in starting live weight between the treatments (mean: 40.8 kg, range: 27.5 – 51.0 kg). Appropriate animal health management in terms of clostridial vaccinations, anthelmintic treatments, selenium supplementation, full body wool clipping (shorn) and a topical insecticide application was conducted prior to commencement of the study. Lambs were monitored by field staff daily, who surveyed them by walking through the plots, throughout the study.

5.3.3 Measurements

Lambs were mustered from the study plots and moved to yard facilities for measurements on Days 0, 3, 7 and 14. Measurements included: live weight, ear thickness, subjective ear damage score, haematology and blood biochemistry. Live weight (kg) was measured with electronic scales (Tru-Test™, Auckland, New Zealand). Electronic vernier callipers (Jobmate, Palmerston North, New Zealand) were used to measure ear thickness (in mm) 10 mm proximal to an existing ear mark on the caudal border of the right ear pinna. Three measurements were taken on each lamb and the median value was recorded. For consistency, the same person performed all ear thickness measurements throughout the study. Lambs that had an increase in ear thickness ≥ 0.5 mm above Day 0 values were recorded as clinical cases of BAPP.

Blood sampling (10 mL) was performed on 12 lambs per plot (same lambs each time) on measurement days, except for Day 3, using venepuncture of the left external jugular vein. 20-gauge needles were used with manual restraint to draw 10 mL of blood into 16 x 100 mm vacutainers containing the anticoagulant Na-ethylenediaminetetracetate. Vacutainers were immediately placed on ice. Thereafter (i.e. within 3 h) the chilled blood samples were transported 18 km to a commercial laboratory (Gribbles Veterinary Pathology Laboratories, Christchurch, New Zealand) for analysis of red blood cell number, haemoglobin content and presence of Heinz bodies using an automated analyser.

Crop yields were estimated prior to the commencement of the study on Day -1 and every 3 days thereinafter. Three 1 m^2 quadrat samples per plot were cut 3 cm above soil level and weighed (fresh weight). Subsamples of approximately 400 g were weighed, dried in an oven for 48 h at 90 °C and re-weighed to calculate dry matter percentage (DM%). Dry matter yield was calculated using the equation:

$$DM\ yield\ (kg\ DM/ha) = (Fresh\ weight\ (kg)\ x\ DM\ \%)\ x\ 10000$$

Apparent intake was estimated from DM yield data. Feed utilisation of 75% was also incorporated in calculating apparent intake as there was approximately 25% wastage in the field. Apparent intake was calculated using the equation:

$$Intake\ (kg\ DM/head/day) = \frac{\left(\frac{(DM\ yield\ pre - DM\ yield\ post)}{3\ days}\right)}{15\ lambs\ per\ plot}\ x\ 75\%\ feed\ utilisation$$

Climate data, such as solar radiation and ambient temperature was measured throughout the study using a Halo weather station at five-minute intervals, across six locations within Kimihia Research Centre.

5.3.4 Data analysis

The study ran for a 14-day period, from 19 March to 2 April 2019. Raw data were uploaded into Microsoft Excel and analysed using the statistical software GENSTAT Version 19.1. Statistical tests included Repeated Measures Correlation Model by Restricted Maximum Likelihood (REML), Approximate Least Significant Differences 5% of REML, Linear Mixed Models, one-way blocked Analysis of Variance (ANVOA) and one-way blocked Repeated Measures ANOVA. A significance value of a = 0.05 was used for rejecting the null hypothesis.

5.4 Results

5.4.1 Climate

The ambient climate was relatively consistent throughout the two-week study with daily mean temperatures remaining at 18°C ± 1.5 for the initial 9 days (Figure 18). Mean daily solar radiation plummeted during the middle days of the study.

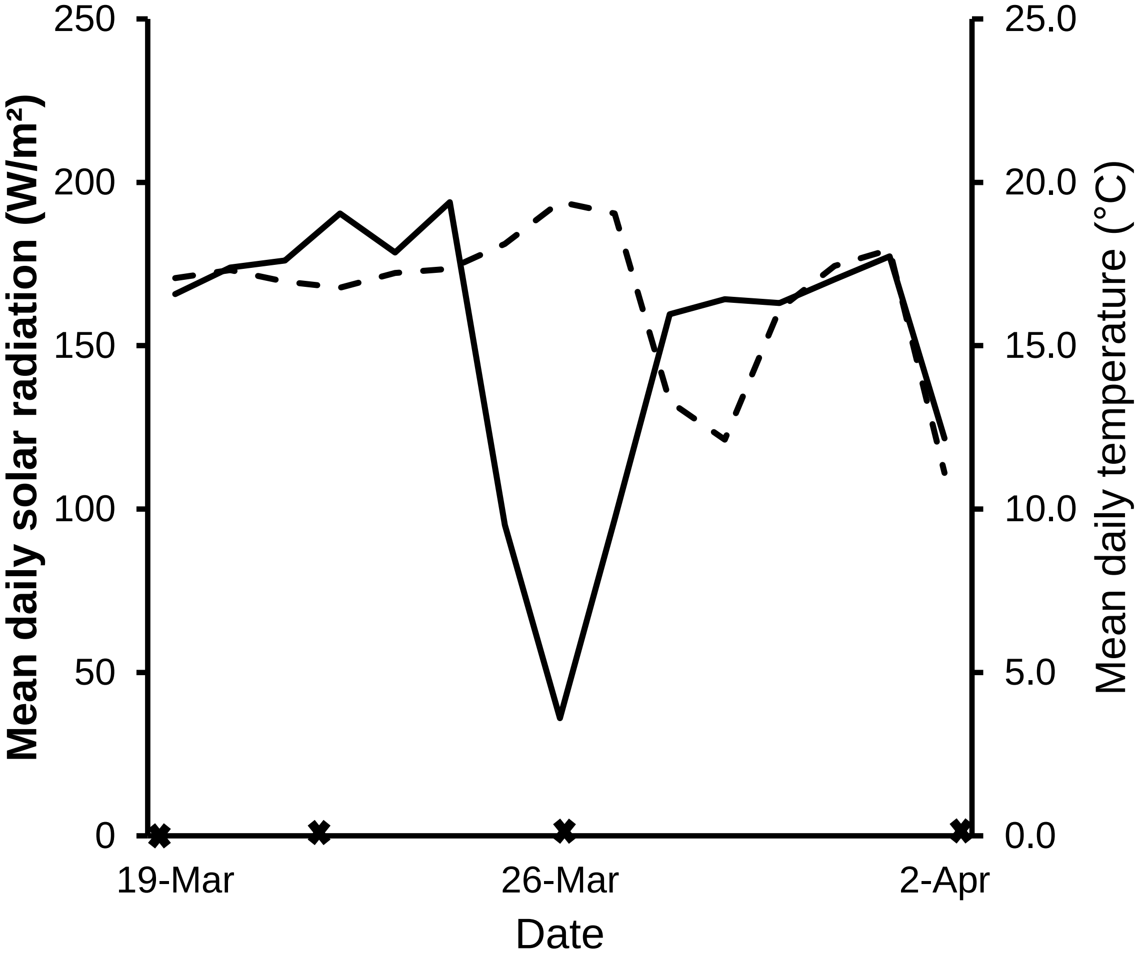

Figure 18: Mean daily solar radiation (-) and temperature (- -) whilst lambs grazed chicory and raphanobrassica crops. X symbols indicate days where lambs had clinical and subclinical measurements performed.

5.4.2 Ear thickness

Lambs assigned to the NAÏ treatment had the largest increase in ear thickness of approximately 0.5 mm (Figure 19, see Day 3, P<0.001) once reintroduced to raphanobrassica. The largest ear thickness changes occurred in these lambs on Day 3, which returned to their initial ear thicknesses by the end of the study (Day 14). Previous exposure of lambs to raphanobrassica, either by continuously grazing (CON) or a short removal of 4 days (PEX) revealed little change in mean ear thicknesses throughout the study and was comparable to lambs grazing the negative control of chicory (CHI).

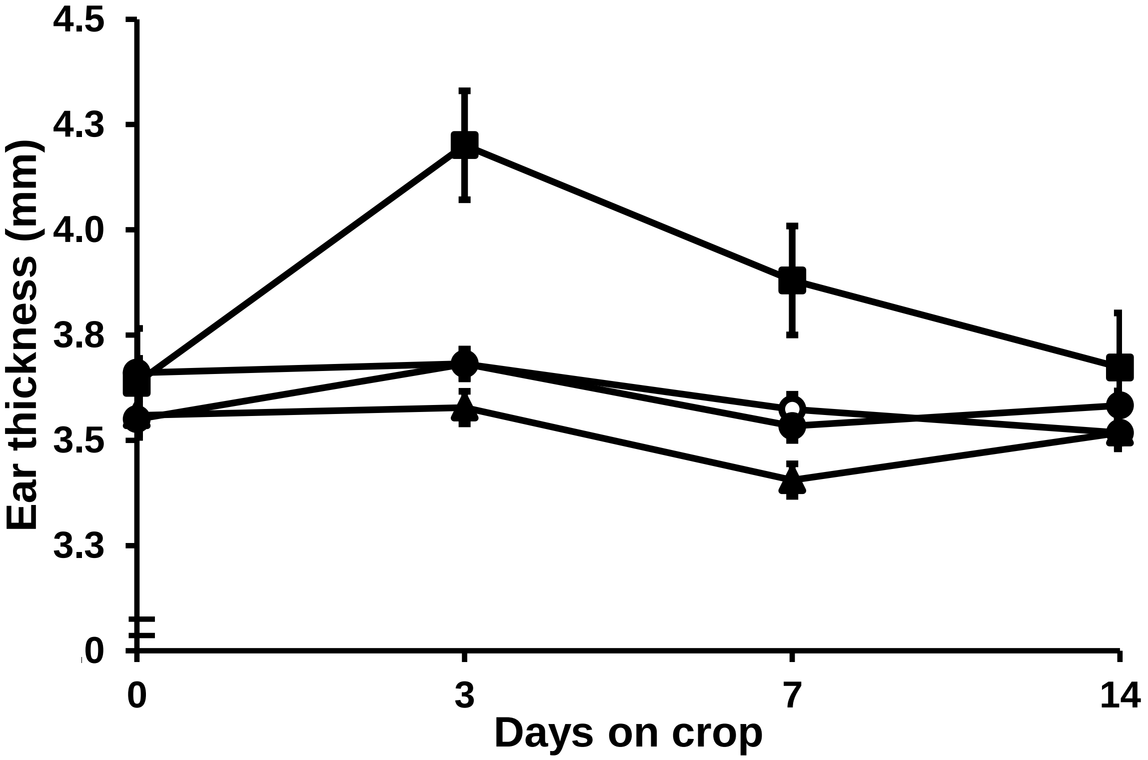

Figure 19: Mean (N = 240) ear thickness of lambs consuming either raphanobrassica or chicory (○) forage crops. Prior to the study, lambs assigned to consume raphanobrassica were either consistently grazing raphanobrassica (CON) (●), initially grazed raphanobrassica followed by non-brassica crops for -4 days (PEX) (▲) or initially grazed raphanobrassica followed by non-brassica crops for -56 days (NAÏ) (■). Error bars ± SEM.

5.4.3 Clinical cases

Incidence of BAPP, where ear thickness was elevated ≥0.5 mm over the initial Day 0 measurements was largest on Day 3 (Figure 20) and affected nearly half of the individuals assigned to the NAÏ treatment. The AUDPC of lambs assigned to the NAÏ treatment was 239.84%, but in those assigned to the CHI, CON and PEX treatments was 24.60%, 13.78% and 36.08%, respectively.

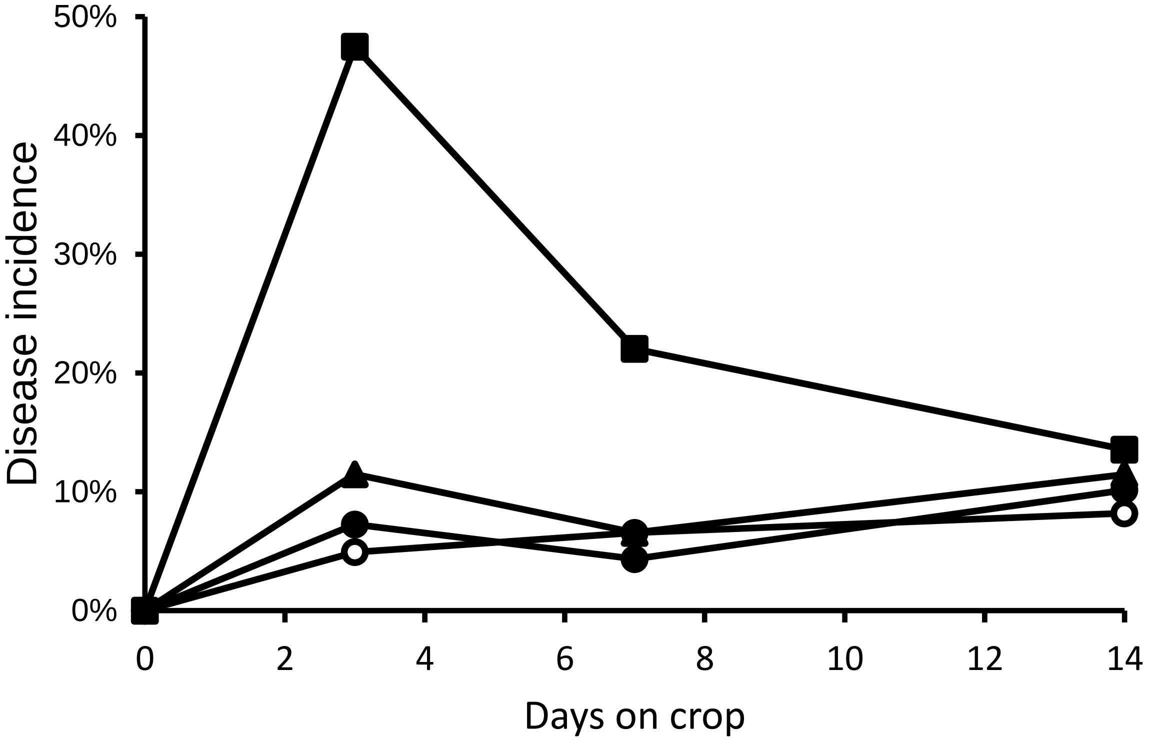

Figure 20: Incidence of lambs with clinical ear oedema (≥0.5 mm increase) whilst consuming either raphanobrassica or chicory (○) forage crops. Prior to the study, lambs assigned to consume raphanobrassica were either consistently grazing raphanobrassica (CON) (●), initially grazed raphanobrassica followed by non-brassica crops for -4 days (PEX) (▲) or initially grazed raphanobrassica followed by non-brassica crops for -56 days (NAÏ) (■).

5.4.4 Live weight

Overall, there was a positive live weight gain (P < 0.001, Figure 21) throughout the two-week study of approximately 5 kg. Lambs that were assigned to the CON grazing treatment, and consumed raphanobrassica before and throughout the study tended to have a larger increase in live weight (P = 0.110). Although, variations in grazing management before the commencement of the study resulted in a disparity of starting mean lamb live weights between the highest (CON) and lowest (CHI) treatments of 5.76 kg (P < 0.001).

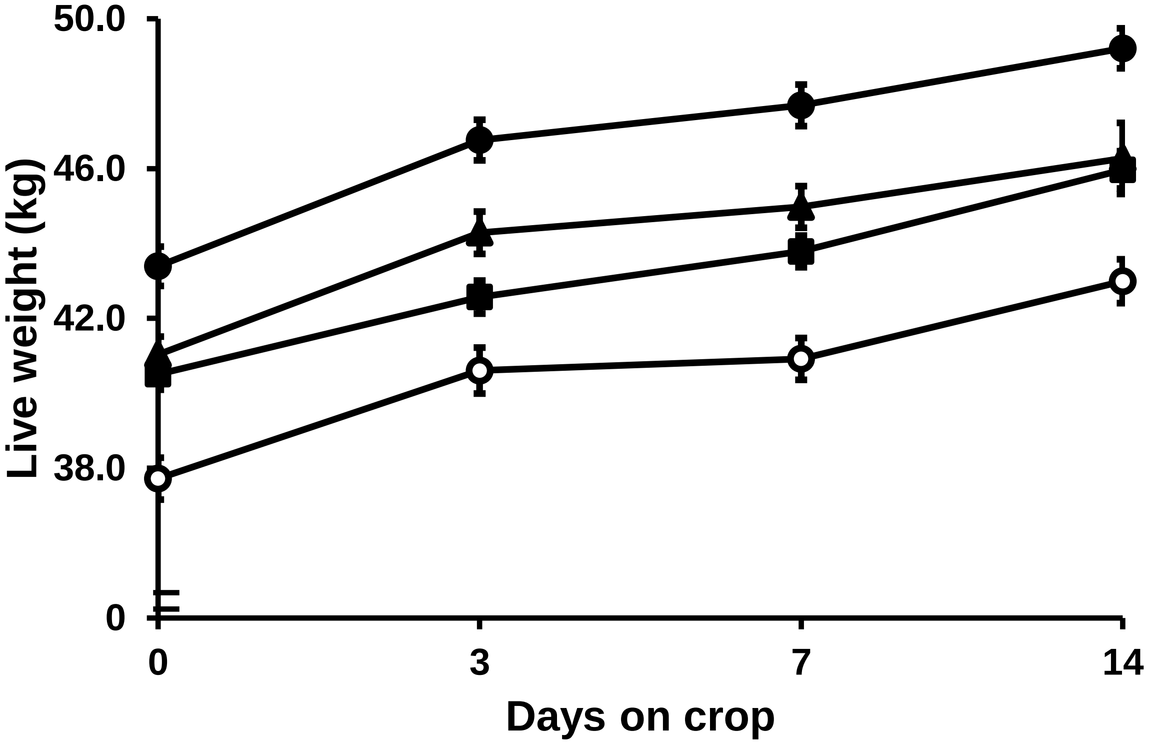

Figure 21: Mean (N = 240) live weight of lambs consistently consuming raphanobrassica (●), after 4 days of not consuming brassica (▲), after 56 days of not consuming brassica then allowed to consume raphanobrassica (■) or grazing chicory (○). Error bars ± SEM.

5.4.5 Apparent intake

Both raphanobrassica and chicory forage crops were consumed at a consistent rate throughout the study (P = 0.287, Table 9). Overall, chicory plots had a lower amount of available feed (P < 0.001), but this was not enough to limit intake.

Table 9: Crop DM yield (kg DM ha^{-1}) and apparent intake of lambs grazing raphanobrassica and chicory forage crops. Letters indicate Fisher's Least Significant Difference groupings (LSD$_{0.05}$). N = 240.

DM Yield (kg DM/ha)		Feed treatment				Significance
		CHI	CON	NAÏ	PEX	
Day -1	$\bar{x}$	2363^{c}	2932^{a}	2805^{b}	2805^{b}	P<0.001
	s	223	314	272	261	
Day 2	$\bar{x}$	2264^{b}	2775^{a}	2681^{a}	2666^{a}	P<0.001
	s	306	250	235	277	
Intake (kgDM/h/day)		1.7^{a}	2.6^{a}	2.1^{a}	2.3^{a}	P=0.880
Day 5	$\bar{x}$	2112^{b}	2611^{a}	2596^{a}	2571^{a}	P<0.001
	s	215	252	294	337	
Intake (kgDM/h/day)		2.5^{a}	2.7^{a}	1.4^{a}	1.6^{a}	P=0.764
Day 8	$\bar{x}$	1995^{b}	2495^{a}	2496^{a}	2430^{b}	P<0.001
	s	298	211	198	306	
Intake (kgDM/h/day)		1.9^{a}	1.9^{a}	1.7^{a}	2.4^{a}	P=0.961
Day 11	$\bar{x}$	1982^{b}	2352^{a}	2329^{a}	2268^{a}	P<0.001
	s	314	284	308	267	
Intake (kgDM/h/day)		1.7^{a}	2.4^{a}	2.8^{a}	2.7^{a}	P=0.774
Day 14	$\bar{x}$	1726^{b}	2184^{a}	2217^{a}	2137^{a}	P<0.001
	s	269	332	251	235	
Intake (kgDM/h/day)		2.8^{a}	2.8^{a}	1.9^{a}	2.2^{a}	P=0.867

5.4.6 Haematology

Throughout the study, lambs that had not consumed brassica in the 56 days prior had the greatest reduction in RBC count (-0.50 x 10^6 µ/L/day, P=0.027, Figure 22), Hb (-6.56 g/L/day, P<0.001) and the largest increase in the presence of Heinz bodies (4.95%/day, P<0.001), throughout the study compared to CHI (-0.17 x 10^6 µ/L/day, -3.75 g/L/day and -1.03%/day, respectively), CON (-0.17 x 10^6 µ/L/day, -0.99 g/L/day and 2.50%/day, respectively) and PEX treatments (-0.32 x 10^6 µ/L/day, -5.21 g/L/day and 2.91%/day, respectively). Lambs consuming the control feed, CHI, still showed some minor changes in RBC and Hb but had a steady reduction in Heinz bodies. Lambs that continuously consumed raphanobrassica (CON) maintained high levels of Heinz bodies, with the most consistent levels of RBC and Hb throughout the study.

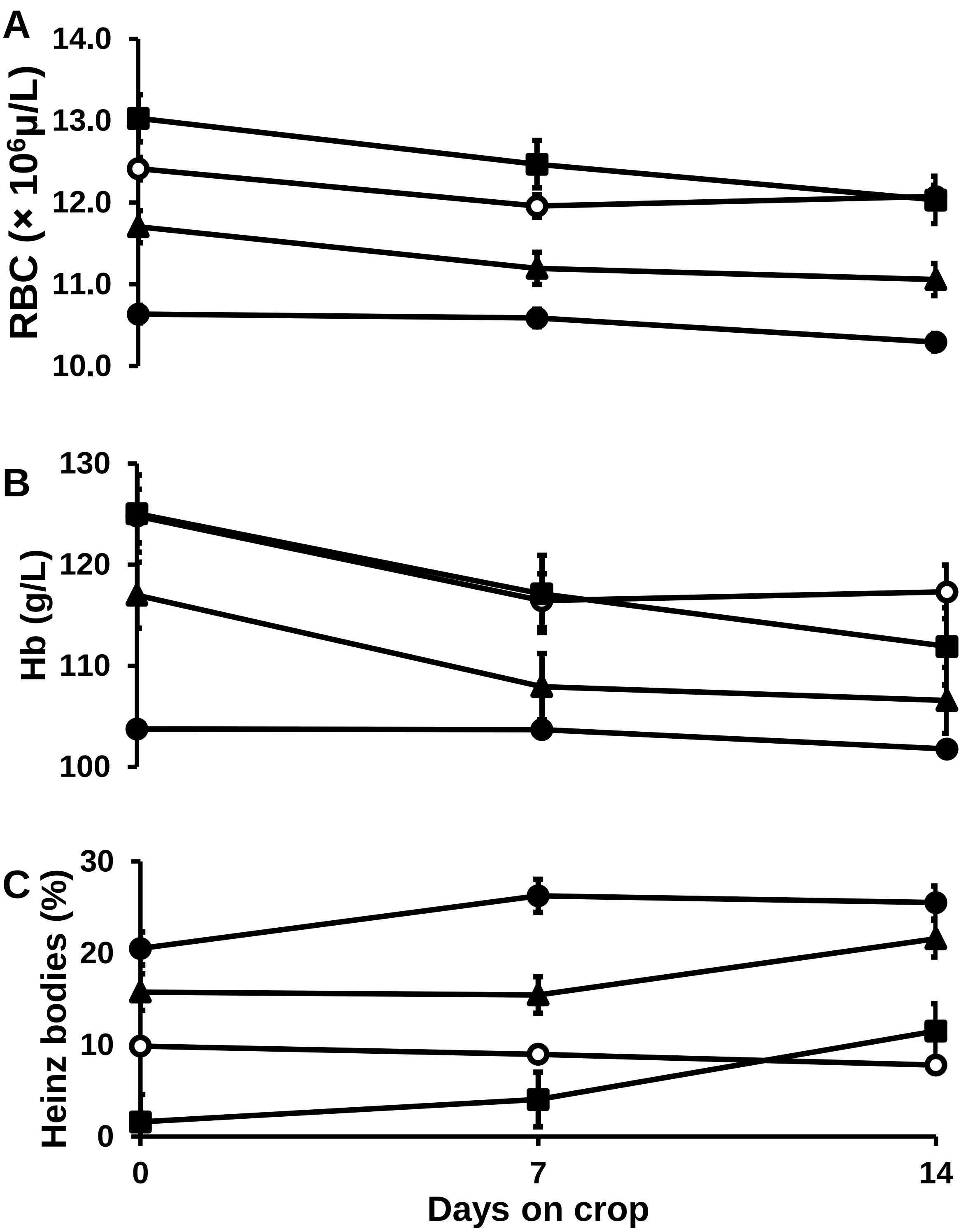

Figure 22: Mean (N = 192) A) erythrocyte count (RBC), B) haemoglobin concentration (Hb) and C) Heinz body percentage in blood of lambs continuously consuming raphanobrassica (●), after 4 days of not consuming brassica (▲), after 56 days of not consuming brassica then allowed to consume raphanobrassica (■) or grazing CHI (○). Reference range of RBC and Hb in clinically normal sheep are 9.0 – 15.0 × 10^6 µL^{-1} and 80 – 140 gL^{-1} respectively. There is no established reference range for Heinz bodies. Error bars ± SEM.

5.4.7 Pearson's correlation matrix

There was a relatively poor correlation between ear thickness and lamb live weight in all studies (Table 10), suggesting live weight loss (first study; Chapter 3) or gain (second and third studies; Chapters 4 and 5) may not be directly related to BAPP. Ear thickness had a low to moderate correlation (0.3 - 0.5) with haematological changes in the first study, although this was not repeatable in the second and third studies. Hepatic enzymes, GGT and GLDH (first study; Chapter 3), and feed intake (third study; Chapter 5) were also poorly correlated (0.0 - 0.3) with ear thickness changes. Discussion related to this table is in Chapter 7: General discussion.

Table 10: Pearson's Correlation Matrix between measured clinical signs of BAPP in lambs grazing forage brassica. ET = ear thickness (mm), LW = live weight (kg), RBC = erythrocytes (syn. Red blood cells) (x $10^6 \mu L^{-1}$), Hb = haemoglobin count (g/L), HBZ = Heinz bodies (%), GGT = gamma-glutamyl transferase (u/L), GLDH = glutamate dehydrogenase (u/L), SR = solar radiation (W/m^2), TE = ambient temperature (°C), IN = intake (kg DM/head/day).

Study 1	**ET**	**LW**	**RBC**	**Hb**	**HBZ**	**GGT**	**SR**	**TE**
ET								
LW	-0.13							
RBC	-0.26	0.06						
Hb	-0.31	0.09	0.88					
HBZ	0.32	-0.15	-0.59	-0.54				
GGT	0.28	0.12	-0.32	-0.29	0.30			
GLDH	-0.11	0.13	0.26	0.26	-0.24	-0.10		
Study 2								
ET								
LW	-0.02							
RBC	-0.13	-0.27						
Hb	-0.17	-0.19	0.87	-				
HBZ	0.09	0.24	-0.60	-0.53				
Study 3								
ET								
LW	0.02							
RBC	0.03	-0.20						
Hb	-0.03	-0.27	0.76					
HBZ	-0.04	0.31	-0.50	-0.48				
SR	-0.01	0.12	-0.04	-0.04	0.10			
TE	-0.02	0.42	-0.15	-0.29	0.13		-0.15	
IN	-0.04	0.08	-0.05	0.01	0.03		0.15	-0.15

5.5 Discussion

In this study, lambs that had not consumed any brassica for 56 days (NAÏ) had the greatest severity and incidence of BAPP upon reintroduction of raphanobrassica. Lambs assigned to both other raphanobrassica-fed treatments had similar ear thickness results to those grazing the control feed of chicory. Live weight and apparent feed intake were not affected, regardless of disease status, which may be an indication of the mildness of clinical BAPP, compared to cases in the previous two studies. Haematological changes were indicative of mild pathological changes, particularly the presence of Heinz bodies, however, there was a poor correlation to BAPP.

Ear thickness

Ear pinna thickness was elevated in lambs with clinical BAPP due to oedematous fluid accumulation. As indicated by this increase in ear thickness, NAÏ lambs had the greatest severity of BAPP upon reintroduction of raphanobrassica. However, while clinically significant, this increase in ear thickness is not as severe as the changes shown in the previous two studies of this thesis (Chapters 3 and 4). A result likely due to reduced intensity of light and exposure time (Parton, Bruere, & Chambers, 2001) with this study being conducted in autumn rather than summer. Of note, ear thickness was reduced on Day 7 in CHI, PEX and NAÏ treatment groups, which coincided with a lowered mean solar radiation compared to the mean solar radiation throughout the rest of the study period. This was similar to the result found by Westwood & Nichol (unpublished), where no BAPP was exhibited in lambs regrazing a forage rape crop in autumn.

Incidence of BAPP was also greater in those assigned to the NAÏ treatment, with almost half of the lambs clinically affected (≥0.5 mm increase in ear thickness) on Day 3. These ear thickness changes then trended downwards with a shorter duration of clinical disease compared to those in the first two studies (Chapters 3 and 4). The AUDPC in NAÏ lambs was approximately ten times that of the CHI, CON and PEX treatments, although as this is a calculation of total numbers affected, no statistical inference has been made. Interestingly, even individuals assigned to the CHI treatment had slight increases in ear pinna thickness with some individuals exhibiting ventral drooping of ear pinna, suggesting that there may be one or more plant-associated compounds not limited to forage brassica causing clinical photosensitivity. The number of animals affected in the CHI, CON and PEX treatments tended to decrease on Day 7 then increased slightly on Day 14, which coincides with changes in solar radiation and temperature measured on-site.

Ear thickness in CON lambs was unchanged compared to those in the control group, CHI. This finding suggests that once lambs have adapted to any forage brassica, including but not limited to raphanobrassica, they are unlikely to succumb to BAPP. This is supported in the two previous studies

(Chapters 3 and 4), by which after an initial increase in ear thickness coinciding with an introduction to forage brassica crops, there is a reduction in ear thickness over time, towards starting values, whilst the animals remain grazing the affecting feed. Additionally, PEX lambs had ear thicknesses that were unchanged compared to the control group. Again, this provides evidence that lambs have adapted to this feed, which may last at least four days. Adaptation to a toxin is not a novel concept. In a series of three studies Castro *et al*. (2018) found lambs could adapt to *Brachiaria* spp., a photosensitising plant, and reduce clinical photosensitivity either by grazing management; transferring rumen fluid from adapted sheep to non-adapted lambs; or inherited resistance to the toxin (steroidal saponins). Ruminal transfaunation of lambs from brassica-fed sheep may be an area of further research to reduce BAPP.

Haematology

Heinz bodies presence, reflective of oxidative injury to the erythrocyte (Heinz, 1890), were elevated on Day 0 in lambs assigned to CHI, PEX and CON treatments. All lambs in these treatments had consumed forage brassica immediately prior to, or within four days of the study commencing. The level of Heinz bodies slowly reduced in non-brassica-fed lambs, but slightly increased in these two brassica-fed treatments, reinforcing forage brassica may induce subclinical oxidative damage (Herman & Javaid, 1950). Removal of lambs from grazing a brassica crop resolved the presence of Heinz bodies by about 20% in two weeks.

NAÏ lambs initially had negligible Heinz bodies. However, these levels elevated with consumption of the brassica crops and continued to increase throughout the study. Heinz bodies levels were poorly correlated with ear thickness measurements (Table 10) and were highest on Day 14 when ear pathology had resolved. The presence of Heinz bodies is likely to affect RBC count and Hb concentration, whereby premature degradation of RBC may occur (Herman & Javaid, 1950). While blood changes remained within the reference ranges of clinically normal animals, the pattern of change of RBC count and Hb concentration potentially indicated some mild pathology with the greatest change occurring in lambs assigned to the NAÏ treatment. Lambs that were either assigned to CON or the control treatment, CHI, had relatively stable RBC count and Hb concentrations throughout the study. As these two treatments yielded similar severity and incidence of BAPP, we can conclude that these blood changes are unlikely to be due to BAPP.

Heinz body may be due to an antinutritional compound in brassicas, such as SMCO presence or thiosulphate (Collett, Bryan, & Tapper, 2014), which is often compounded in selenium deficient livestock (Herman & Javaid, 1950). While selenium status was not investigated nor reported in the current study, all lambs received an oral drench of selenium and an assumption has been made that lamb selenium status did not differ between the four treatments.

Another antinutritional compound of note in brassica are GSLs, which are involved as a plant defence mechanism (Nour-Eldin, et al., 2017). These compounds have been implicated in inducing secondary photosensitivity in sheep and cattle (Collett, Bryan, & Tapper, 2014; Collett & Matthews, 2014), however, there is no current evidence of them acting as a primary photosensitiser. While the concentration of GSLs were not quantified in the series of studies in this thesis, levels are often greater in leaf material (Dalley, et al., 2015; Mithen, 1992), which appeared to be preferentially consumed in all three studies. Livestock fed rations containing high levels of GSL have displayed adverse effects such as anaemia, an exacerbated mechanism of blood changes identified in this study; reduced feed intake; gastrointestinal irritation; hepatic and renal lesions; and goitre (Bichoff, 2016).

Live weight and intake

The second hypothesis was that lambs which clinical BAPP have a reduction in live weight. Live weight is typically associated with disease status; whereby if energy is required for recovery, less is available for live weight gain in lambs (Alley, 1986). However, lamb live weight was unaffected in this study regardless of treatment, which may be attributed to the low severity of BAPP. Greater disease severity may negatively affect lamb production, although further work is required in this area.

Lambs had similar growth curves regardless of treatment, except CHI lambs, which were slightly lower by Day 14. This may be due to slightly lower feed availability in the chicory plots. In the first study (Chapter 3), brassica consumption coincided with a loss in live weight during the initial ten days. Therefore, it might be expected that NAÏ lambs may have a live weight loss, or a poor live weight gain. However, the trend over time was similar to other raphanobrassica-fed lambs. Live weight and the relationship to BAPP is further discussed in Chapter 7: General discussion.

Live weight is generally proportionate to feed intake. Apparent intake of feed was relatively consistent throughout the study, even in the NAÏ treatment which exhibited clinical BAPP. Again, this could be associated with low disease severity, or that BAPP does not generally affect livestock productivity. All these lambs had previously consumed forage brassica, therefore neophobia was not evident in these lambs. We do, however, note the difficulty in relating quadrat cuts over an area of 0.25 ha to lamb intake. This may be mitigated in future studies by performing a smaller study in metabolic crates to measure individual feed intake.

5.5.2 Conclusions

This study was the first to observe a relationship between clinical BAPP and a potential ability to detoxify the affecting agent(s) in lambs grazing raphanobrassica plots. Lambs were able to maintain this ability for at least four days, but not as long as 56 days. Further work as to the maximum duration of this ability is required, although we recommend maintaining lambs on forage brassica crops to reduce the future risk of developing BAPP. NAÏ lambs had the greatest incidence and severity of BAPP, with all other brassica-fed treatments comparable to the control feed, CHI. Ear thickness changes were only mild, even in the NAÏ treatment, likely due to conducting the study in autumn, rather than summer. Haematological changes, particularly when comparing those between brassica-fed lambs without BAPP and non-brassica fed lambs suggests these blood changes are likely unrelated to BAPP and may be due to another compound in brassicas. Live weight and apparent feed intake were not affected, regardless of disease status albeit mild cases of BAPP. Repeating this study during the summer period would be useful for comparing disease severity to live weight.

Chapter 6

Disease progression of clinical brassica associated primary photosensitisation (BAPP) in lambs grazing forage brassica

6.1 Abstract

Forage brassicas are annual feed crops grown on dairy, sheep, beef and deer farming enterprises. These crops occupy large areas of cultivated land in New Zealand and Australia at 300,000 ha and 200,000 ha, respectively. Brassica associated primary photosensitivity (BAPP) is a condition in lambs occasionally associated with the grazing of forage brassica species such as forage rape (*Brassica napus* spp. *biennis*), raphanobrassica (*Raphanus x Brassica* L.) and leafy turnip (*B. rapa* syn. *B. campestris*). Three studies, as detailed in chapters 3, 4 and 5 of this thesis, were conducted in Canterbury, New Zealand to identify mitigating factors of BAPP in lambs. This chapter summarises the general disease progression of BAPP in these lambs. There was a range of clinical disease amongst lambs, with some not exhibiting any clinical signs. The dermatopathy pattern in these affected lambs showed similar progressions over a four-week period, whereby some initially presented with erythema, pruritis, photophobia and head-shaking within hours to days of introduction to affecting crops. This was followed by oedematous changes in some individuals of the ear pinnae, eyelids, bridge of the nose, and/or midline of the back. The sequelae of the disease appeared to depend on the degree of oedematous accumulation: in mild to moderate cases that remained grazing these crops, or in individuals removed from affecting crops, oedema resolved quickly and an uncomplicated, complete recovery was observed; in moderate to severe cases sloughing of the epidermis from the dermis from the dorsal pinnae and face occurred; in severe cases where pinna cartilage failed to remain intact necrosis of the pinna tip was observed. Treatment of lambs depends on the severity of BAPP, in line with the Animal Welfare Act 1999; mild to moderate cases appear to be self-limiting. Veterinary advice is recommended when animal welfare is compromised due to severe cases of BAPP.

Keywords: primary photosensitisation, BAPP, forage brassica, summer, erythema, oedema, pruritis

6.2 Introduction

Photosensitivity is a non-contagious disease of the epidermis of the dermis by which an animal has a heightened response to sunlight due to a phototoxin or photoallergen (Clare, 1952). There are numerous examples of photosensitivity disease, one example which is associated with lambs grazing forage brassicas during the summer months is 'brassica associated primary photosensitivity' (BAPP). While photosensitivity diseases are generally well defined in the literature (Clare, 1952; Collett, 2019), there is little detail describing the gross pathology of BAPP.

Brassica associated primary photosensitivity is assumed to have a similar mechanism to other primary photosensitivity diseases in livestock. In the first instance, a phototoxin is either ingested and moves through systemic circulation or is absorbed topically. In both situations, photosensitivity arises on dermal areas unprotected by wool or hair (Clare, 1952) where considerable interaction with UV light occurs (DeRosa & Crutchley, 2002). The phototoxin must have a high absorption coefficient to promote electrons to an excited triplet state, have a high yield of triplet state molecules, a long triplet state lifetime and stability for oxidation reactions to proceed (Collett, 2019; DeRosa & Crutchley, 2002). Oxidisation and oxidative stress are either caused directly by the phototoxin, which acts like an excited state triplet radical itself (Type I phototoxicity), or via the transfer of energy to oxygen (Type II phototoxicity) (Foote, 1991), manifesting as localised structural injury to macromolecules and gross necrotic damage.

The purpose of this chapter is to collate the generic disease progression of BAPP using information from the three experimental studies (Chapters 3, 4 and 5) and document the typical development of clinical signs over four weeks from lambs with moderately severe cases of BAPP. This was done through a collection of photographs of clinical cases; subjective recording of gross changes; comparing ear thickness and live weight data from severely affected lambs with those lambs with either no BAPP or very mild cases; and predicting disease outcomes given initial oedematous changes. Veterinary advice does not replace the findings of this chapter.

6.3 Case history

This case study relates to two mobs of lambs enrolled in three studies, conducted at PGG Wrightson Seeds Research Centre, Kimihia, Lincoln, Canterbury, New Zealand (-43.62, 172.47) over consecutive years; summer 2017/18 (mob one) and two studies in summer/autumn 2018/2019 (mob two). Lambs were assigned to consume various forage brassica treatments, as defined in their respective chapters, to induce clinical BAPP. Lambs from the chicory control in the second study (Chapter 4) were re-enrolled in the third study (Chapter 5), along with other lambs which had also been maintained on chicory but were not included in the prior study. All animal procedures were approved by the Lincoln University Animal Ethics Committee (Appendix 4).

Both mobs were cryptorchid Romney male lambs, each from a single source flock. The first mob was enrolled in the first study (Chapter 3), while a second mob was enrolled in either the second and third studies (Chapters 4 and 5). Prior to their enrolment into their respective studies, they were weighed, received appropriate animal health management in terms of clostridial vaccinations, anthelmintic treatments, selenium and cobalt supplements. Inguinal wool clipping (crutching) and a topical insecticide application were conducted prior to the commencement of the study. Lambs were maintained on a pastoral sward of perennial ryegrass and white clover until the beginning of the respective studies.

Throughout the studies, lambs grazed on their assigned brassica plots and were monitored by field staff who surveyed these lambs by walking through the plots daily. Similar generic patterns of disease progression occurred throughout the three trials, which were subsequently documented using objective measures such as ear thickness, as recognised in the previous three chapters of this thesis; or subjectively, with visual trends in gross pathology and recorded using photographs. Visual observations were recorded by a single person in the second and third studies during the daily walks, whereby the number of lambs per plot which displayed displacement of ears from the typical upright position to a downward position due to the weight of the ear. This subjective information was collated on a score sheet. On objective measurement days, lambs were mustered from the study plots and moved to a yard facility where photographs were taken of gross pathological changes. Photographs did not document the change in the same lambs, rather the generic changes over time. Details of objective measurements can be obtained in the material and methods sections in the previous three chapters. The studies ran for 35, 28 and 14 days, respectively.

6.4 Results

6.4.1 Typical progression of BAPP

The most objective sign of clinical BAPP was oedematous fluid accumulation, which appeared within hours (Figure 23). Excessive oedema in the ear pinnae of some lambs equated to an increased ear thickness of approximately 300% ($P < 0.001$). Oedema also occurred on the bridge of the nose, upper and lower eyelids (Figure 24A), and the midline of the back. The latter was evident when determining body condition score but may otherwise be missed. Lachrymation (Figure 24B) and excessive eye and nasal drainage (Figure 24A) were noted in some individuals.

Figure 23: Severe oedema observable from afar in three of the seven lambs (Study 2: Intermitted grazing treatment) 48 hours post forage brassica exposure. Clinically affected lambs are numbered 1-3.

Figure 24: Initial presentation of lambs (72 hours post-exposure to forage brassica) clinically affected by BAPP with A) severe oedema of ear pinna with erythema and lachrymation, B) oedema and erythema of the upper and lower eyelids and C) oedema on the bridge of the nose

After Days 3 – 7 ear pinnae returned to their original conformation. However, further clinical signs developed approximately 7 days after initial brassica exposure in lambs with moderately severe BAPP. These signs included vesicles, ulcers, serum exudation and scabbing, loss of epidermal and dermal tissue (Figure 25 and, in severe cases, necrosis of the dermis and underlying cartilage. Mild haemostasis was also present in some areas (Figure 25B).

Figure 25: Initial recovery phase of lambs grazing forage brassica (14 days post-exposure to forage brassica). A) facial oedema is no longer present. B, C & D) Superficially cutaneous layers have sloughed off.

Wound maturation was evident approximately three weeks after initial brassica exposure (Figure 26). However, in some instances, there was dorsal curling of pinnae tips (Figure 26A), palpable changes of the epidermis and dermis and/or partial loss of distal tips.

Figure 26: Recovery phase of lambs grazing forage brassica >21 days post initial exposure. A) dorsal curling of distal ear pinna. B) Proliferation and C) maturation of superficial ear pinna damage. Ears have a thick, leathery texture on palpation.

6.4.2 Quantitative disease recovery

In the second study (Chapter 4), seven lambs displayed moderately severe oedematous accumulation within the ear pinnae and face. Ear pinnae were four-fold greater in thickness than their starting values (Figure 27A). The epidermis of the pinnae tips were cool and grey. These lambs were removed from the study and moved to a shaded paddock to consume a non-brassica feed. The ear thickness and live weight of these lambs continued to be measured to document disease recovery.

Oedema of the ear pinna resolved in all seven lambs so as by Day 28 their ear thickness was like that of their starting values and not statistically different to those remaining on the study (Figure 27A). Although there was some sloughing of the epidermal layers from the dermis as outlined as an example of pathological consequences above. Live weight in these lambs was not negatively affected and followed a similar positive linear trend as the population of lambs grazing brassica crops (Figure 27B).

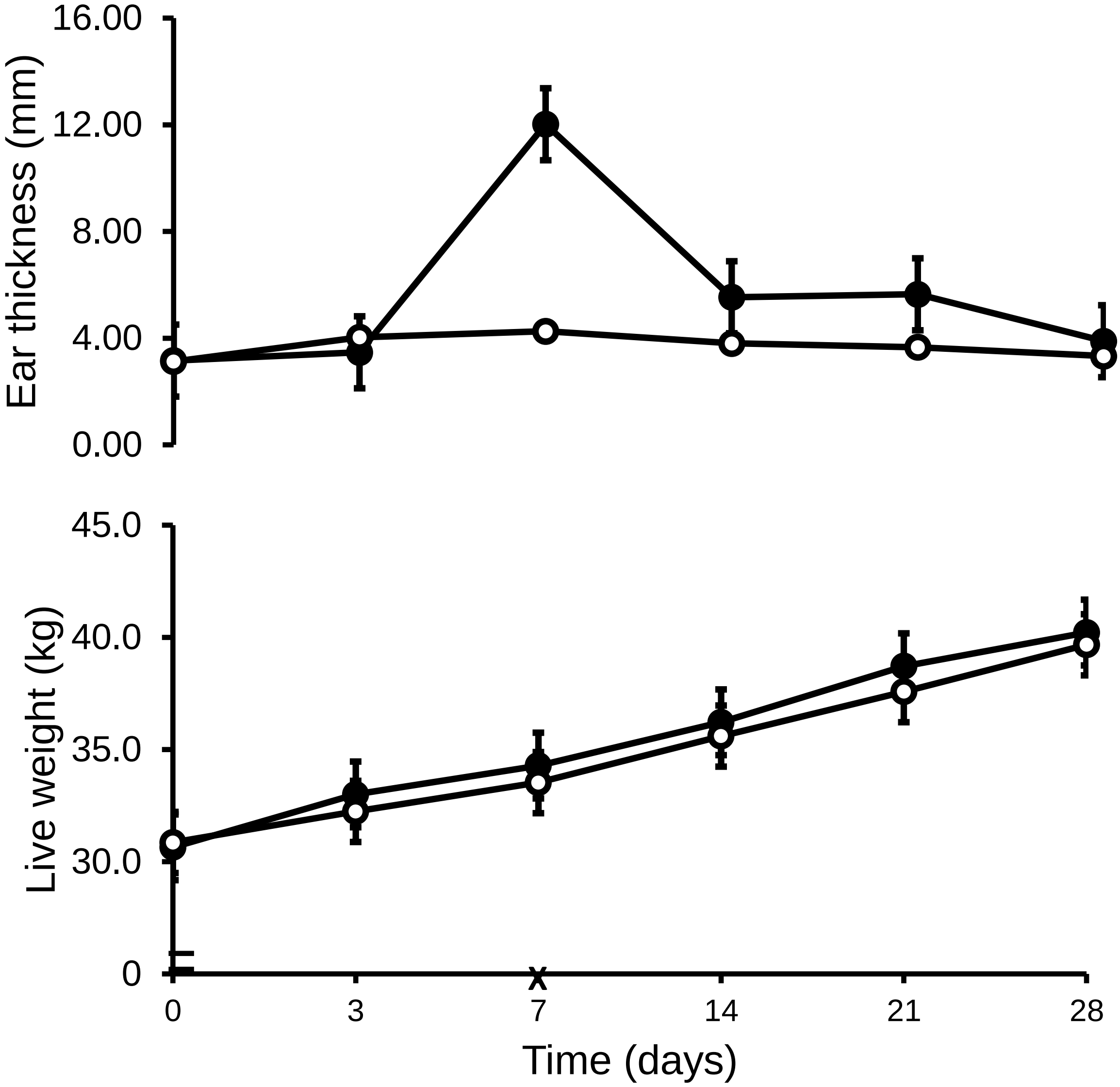

Figure 27: Comparison of A) ear thickness and B) live weight of seven lambs severely affected with BAPP (●) from the remaining population (○) of raphanobrassica grazed lambs. Error bars ± SEM, cross on x-axis indicates when severely affected lambs were removed from grazing raphanobrassica.

6.4.3 Typical progression and outcomes of BAPP

The following table outlines the typical disease progression in these lambs over time, with a list of clinical signs which may be present in clinically affected individuals, Table 11. Not all lambs in the flock may exhibit gross clinical signs and attention to those most affected is recommended.

Table 11: Disease progression over four weeks of lambs exhibiting clinical signs of BAPP

Days on crop	Clinical signs
0 – 1	Erythema; pruritis; and/or photophobia; may be followed by oedema
0 – 6	Erythema; pruritis; lachrymation; oedematous changes in ear pinnae, eyelids, bridge of the nose, and/or midline of the back; areas with excessive oedema cool on palpation; and/or photophobia and reduced feed intake
7 - 14	Uncomplicated and complete reabsorption of oedematous changes in mild to moderate cases; sloughing of epidermal layers affected by excessive oedema in moderate to severe cases
15 - 28	Proliferation and maturation of wound(s) in moderate to severe cases

We have adapted the following scoring of the extent of the pathology of BAPP from Westwood & Nichol (unpublished) and propose an updated classification of severity of BAPP for on-farm use along with a suggested treatment (Table 12).

Table 12: Classification of severity of BAPP in lambs seven days after initial introduction to forage brassica. Recommended treatment should not proceed advice given by attending veterinarian.

Score	Evidence of BAPP within 7 days of brassica exposure	Recommended treatment	Expected outcome four weeks after brassica exposure
1	No damage	Maintain lambs on brassica crop	No clinical signs
2	Minor erythema and oedema of ear pinna	Maintain lambs on brassica crop with increased monitoring	Clinical signs resolve
3	Mild erythema and major oedema of ear pinna causing ventral drooping	Consider removing affected lambs from brassica crop, offering shade. Increase monitoring	Clinical signs resolve
4	Major oedema of ear pinna with ventral drooping, mild facial and/or midline oedema. Erythema may or may not be present	Remove affected lambs from brassica crop and provide shade from sunlight. Consult veterinarian. Symptomatic treatments may include, but not be limited to, application of topical zinc oxide ointment, antihistamines, topical or systemic steroidal or non-steroidal treatments as deemed necessary. Analgesia used as necessary.	Severe blistering and necrosis of ear, face with most affected epidermal tissues sloughing off
5	Major oedema of ear, facial and/or midline tissue, with some areas experiencing blood occlusion. Erythema may or may not be present	Remove affected lambs from brassica crop into an area of full shade from sunlight. Consult veterinarian. Symptomatic treatments may include, but not be limited to, application of topical zinc oxide ointment, antihistamines, topical or systemic steroidal or non-steroidal treatments as deemed necessary. Analgesia used as necessary.	Major loss of tissue such as ear pinna loss

6.5 Discussion

Clinical photosensitisation in lambs is not characterised by a single pathognomonic sign, rather the condition is progressive and time-dependent, such that dermatopathy symptoms of photosensitisation change over time, following the onset of brassica ingestion by lambs. This information is outlined in Table 11. Oedematous changes in ear pinna thickness was the most objective clinical sign of BAPP, as detailed in the three experimental chapters, however erythema, pruritis and photophobia was also evident.

There was a range of severity of clinical BAPP in these lambs from mild to moderate. Since undertaking this project, we have seen a variety of individual cases throughout New Zealand with some severe examples of BAPP. Most of these cases are identified by farmers and veterinarians approximately two weeks after introduction to forage brassica, whereby oedema has been so immense that systemic circulation has been occluded and ear pinnae have consequently been lost; and the cutaneous layers of the epidermis on the face and along the midline of the back has sloughed off from the dermis.

Traditionally animal welfare concerns of BAPP in lambs have been focused on the skin sloughing process, characteristically occurring 1 - 2 weeks following initial presentation, by which stage the damage has already been caused. However, clinical photosensitisation in these lambs appeared within hours or days after being introduced to brassica, then gradually resolved over four weeks. While these studies did not measure when the dermatopathological changes first occurred, it has been noted in human medicine that cellular damage from phototoxins may appear within minutes to hours following exposure (Monteiro, Rato, & Martins, 2016). There is a large variation in the literature on the onset of clinical signs of photosensitisation in livestock following a dietary change, likely due to low reporting of these diseases, sporadic nature, and the exacerbation of signs over the initial week. Findings varied from within 24 hours (Nieman, Schaefer, Maroney, Nelson, & Albrecht, 2020), to days (Witte & Curry, 1993), to several weeks (Casteel, et al., 1991).

Live weight in lambs eliciting moderate to severe BAPP appeared to not be negatively affected; whereby live weight change over time followed a similar positive linear trend as the remainder of the flock. This was a novel finding as when an animal typically succumbs to disease, particularly one affecting feeding behaviour, live weight tends to be reduced, or lower gains than expected due to the energetic demands of disease recovery (Hindson & Winter, 1990; van Houtert & Sykes, 1996). Anecdotally, farmers describe a weight loss associated with BAPP. Further work around live weight changes in lambs clinically affected by BAPP may be required to quantify this effect, particularly in those animals which have greater clinical signs than these seven lambs.

Acute phase

Initial erythema, from capillary congestion, occurred on areas of reduced wool/hair (Clare, 1952) such as ear pinnae, eyelids and midline of the back (Figure 24A); along with pruritis and apparent localised discomfort. This was followed by excessive migration of fluid from capillaries into the interstitial space between the skin and cartilage of the auricle (Sjaastad, Sand, & Hove, 2010), presenting clinically as non-pitting bilateral oedema, with overt drooping due to the excessive weight of the oedematous pinnae. The excessive eye and nasal drainage via the nasolacrimal duct noted in some individuals is a likely consequence of keratoconjunctivitis (Collett, 2019). The distal pinna tip of some individuals exhibiting excessive oedema felt cool on palpation because of malperfusion of the capillaries, likely secondary to inflammatory changes. In these individuals, the skin was grey in colour indicating reduced arterial perfusion.

The onset of erythema and/or oedema was accompanied by lamb behavioural changes including photophobia, with lambs avoiding direct sunlight by seeking shade beneath the canopy of the brassica crop or in the shade of other lambs. Some lambs appeared unsettled and shook their heads implying a degree of pruritis and/or discomfort. These behavioural changes are evident in other photosensitising diseases in sheep such as facial eczema (Ozmen, Sahinduran, Haligur, & Albay, 2008) and *Microcystis aeruginosa* toxicity (Carbis, Waldron, Mitchell, Anderson, & McCauley, 1995).

Intermediate phase

If lambs were immediately removed from brassica crops when erythema and/or oedema was seen, swelling resolved quickly and lambs typically underwent a relatively complete and uncomplicated recovery over two to four days. Recovery may be aided by the provision of access to shade and supportive treatment for the management of symptoms including but not limited to topical treatments such as zinc creams and/or systemic use of anti-inflammatory treatments to relieve pain and inflammatory changes, as in line with the Animal Welfare Act 1999. If lambs remained on crop after initial erythema and oedema were observed, and lambs continue to ingest brassica, ongoing exposure to phototoxins may have contributed to more severe lesions of the pinnae, a potential dose-dependent response (Monteiro, Rato, & Martins, 2016).

After Days 3 - 7 of pinnae oedema, swelling and drooped ear position; resorption of interstitial fluid took place, signifying the cause of oedema was not permanent. This resulted in the return of pinnae to the original non-droopy conformation. In mild to moderate cases, a complete recovery was observed whilst animals continued to consume affecting crops, appearing as a self-limiting disease (de Araújo, et al., 2017; Parkinson, Vermunt, & Malmo, 2010). In moderate to severe clinical cases, lambs developed a range of signs 1 – 2 weeks after initial exposure, secondary to oedematous changes. Typical scenarios included: the formation of vesicles; ulcers, secondary to vesicular ulceration; serum exudation and scabbing; loss of epidermal and dermal tissue and accompanying hair or wool (Figure 25) and, in severe cases, necrosis of the dermis and underlying cartilage. In these lambs, skin sloughing was predominantly limited to the ears, although three lambs in the first study (Chapter 3) also had facial skin sloughing. Mild haemostasis was also present in some areas (Figure 25B). These findings were also presented in lambs with BAPP by Westwood & Nichol (unpublished) and in cattle with secondary photosensitivity by Nieman, Schaefer, Maroney, Nelson, & Albrecht (2020).

While not exhibited in these three studies, theoretically, areas where the epidermis has been compromised has an elevated risk for secondary infection (Baird, 2000; Devriese, 1990; Mitchell G. B., 1988) and flystrike (Fels, 1971).

Recovery phase of moderately severe cases

Necrotic changes and loss of pinnae were characteristically seen in moderately severe cases of BAPP between 1 – 2 weeks after lambs first start to graze brassica. Following this, wound maturation, proliferation and remodelling were occurring approximately three weeks after initial brassica exposure (Figure 26). At this stage affected pinnae had a thick, leathery texture on palpation, that was likely fibrotic (Keast, Despatis, Allen, & Brassard, 2014); with either partial loss of or dorsal curling of the distal ear pinnae. The remaining tissue appeared to follow a typical dermal healing process with proliferation and maturation of superficial damage. This process was seen in the first two studies (Chapters 3 and 4). The prognosis has previously been noted as being generally favourable when livestock are transferred onto another feed (Parkinson, Vermunt, & Malmo, 2010; de Araújo, et al., 2017), although oedematous changes were shown in this thesis to resolve whilst lambs remained grazing brassica, suggesting some adaption to the toxin(s) responsible for BAPP.

Observing lambs regularly, i.e. daily, within the first week of exposure to brassica is vital. Where required, treating lambs early is likely to have a better result in terms of animal welfare, lamb performance and speed of recovery. Therefore, the classification of severity of BAPP with suggested treatments offers a practical solution for on farm use. Due to the transient nature of BAPP, there were not any lambs that would have scored ‘5’ in any of the studies performed in this thesis. Lambs with overt signs of BAPP must be removed from affecting crop to a shaded area with fresh stock water, veterinary advice should be sort, and symptomatic treatment performed as necessary. The expected outcome of lambs scoring ‘5’ has been approximated based on individual cases seen throughout New Zealand. We also recommend considering paddock selection which offers shade in the initial introduction phase.

6.6 Conclusions

Clinical signs of BAPP manifested in these lambs within twenty-four hours of transferring them to forage brassica crops and was not characterised by a single sign, but a combination of pathological changes evident within the epidermis and dermis. The most objective clinical sign was ear pinna oedema, resulting in ventral drooping. In more severe cases signs of BAPP also developed on the upper and lower eyelids, the bridge of the nose and the midline of the back. The severity of oedematous accumulation within the initial week may predict the likelihood of epidermal sloughing 1 - 2 weeks later. In these lambs, BAPP was self-limiting and gradually recovered upon removal from forage brassica. We recommend removal of lambs from affecting crops in cases where lambs score >4 on Table 12 and treatment in line with the Animal Welfare Act 1999. However, recovery whilst lambs continued to graze forage crops was evident in mild to moderate cases of BAPP. Further work documenting the outcome of lambs exhibiting larger clinical signs and histopathology is recommended.

Chapter 7

General Discussion

7.1 Overview

Forage brassicas are fast-growing crops that provide an easily digestible, high-quality feed during periods of feed shortage (de Ruiter, Dalley, Hughes, Fraser, & Dewhurst, 2007; Dumbleton, et al., 2012; Westwood & Mulcock, 2012). However, brassica species such as forage rape, raphanobrassica and leafy turnip, under some conditions, can cause photosensitivity, through radical and reactive oxygen species damage resulting in clinical oedema, erythema and non-contagious dermatitis. This disease ranges in clinical severity, causing potential animal welfare concerns and possible production losses in lambs grazing forage brassica during the summer months throughout Australasia.

Initially, this type of photosensitivity occurring in lambs grazing forage brassica only arose in forage rape crops, hence the traditional term ‘rape scald’. Following the identification of this disease-causing clinical signs not only in forage rape crops but in raphanobrassica, we proposed the term ‘brassica associated primary photosensitivity’ (BAPP) as a more accurate description of this photosensitising disease. In the first study (Chapter 3), there were only minor increases in GGT and GLDH, reinforcing the suggestion in literature that BAPP is a primary photosensitivity disease. Increases in ear pinna thickness due to oedema was the most apparent measurable sign of BAPP and can be used to identify acutely affected individuals within the first week of introduction to a brassica crop. A tentative diagnosis of BAPP may be given to lambs exhibiting an ear pinna thickness > 3.6 mm taken at the midpoint of the ear pinna and/or presence of non-pitting bilateral oedema and/or presence of erythema with a history of recent introduction to a forage brassica, i.e. within the last week, during summer.

7.1.1 Thesis progression

This thesis was an orchestration of science, management strategies, and demand for practical solutions from sheep farmers. In the first 2 x 2 factorial study, we compared severity, incidence and onset of BAPP using two forage brassica species and additional N fertiliser application. Ear thickness, live weight and haematological data all showed evidence of clinical pathology. Yet, without a non-brassica control feed nor a brassica feed treatment that did not induce clinical BAPP, we were unable to determine if these haematological changes were associated with BAPP. The minor changes in GGT and GLDH suggested BAPP is an example of a primary photosensitising disease.

The first study (Chapter 3) provided two main observations, which would later assist farmers in crop management to reduce BAPP. Initially, we noted there were many clinical cases of BAPP in lambs that consumed predominantly leaf material, as induced by laxly stocking lambs on a high DM yielding crop, which encouraged the preferential selection of leaf but not petiole or stem by lambs. This formed the basis of the second study (Chapter 4), which acted to manipulate the dietary intake of plant parts. Leaf intake appeared to be proportionate to BAPP severity, incidence and onset. As there were both a non-brassica control and a brassica treatment which did not induce BAPP but tended to display haematological changes, we surmised RBC count, Hb concentration and presence of Heinz bodies are unlikely to relate to BAPP and may be due to an antinutritional compound. Future studies of BAPP may not require haematological measurements. Studying different management strategies to manipulate leaf intake in various sites over multiple years will further add to feed management knowledge to mitigate BAPP. Live weight gain in lambs in this study (Chapter 4) contradicted that of the previous study (Chapter 3), suggesting live weight may not be affected in mild to moderate cases of BAPP where lambs had previous exposure to forage brassica.

From the first two studies (Chapters 3 and 4), we also noted that the clinical signs of BAPP resolved whilst lambs continued to graze the affecting crops, suggesting lambs develop an ability to detoxify the agents causing BAPP. Again, we compared severity, incidence and onset of BAPP across treatments with varying levels of prior exposure to forage brassica crops. When this study occurred, we had not yet determined the lack of relationship between haematological changes and BAPP, thus these blood measures were performed. We found those maintained on crops, or temporarily removed for four days, did not develop clinical BAPP, thus surmised detoxification of and/or adaptative mechanism of the causative agent(s) of BAPP. The exact length of time of this detoxification or tolerance ability moved out of scope. Practically, we were able to recommend to farmers that if lambs are maintained on forage brassica crops, they are unlikely to develop clinical BAPP in the future. Once more, live weight increased in these lambs, even in the treatment which exhibited BAPP.

We are mindful of providing a list of mitigating factors and practical management tools for a disease where we are unsure of the mechanism. While we would have preferred to also determine the causative agent(s) of BAPP, the course of action, which recently identified the phototoxin in *Heterophyllaea pustulata* at Universidad Nacional de Córdoba (Micheloud, et al., 2017) was the result of four Doctor of Philosophy degrees.

7.1.2 Key findings

The course of study provided four objectives (Figure 1): 1) review potential causative agents in forage rape and raphanobrassica; 2) identify mitigating factors; 3) document gross pathology; and 4) transform this information into practical management tools for on-farm use. Findings for these objectives are depicted in Figure 28.

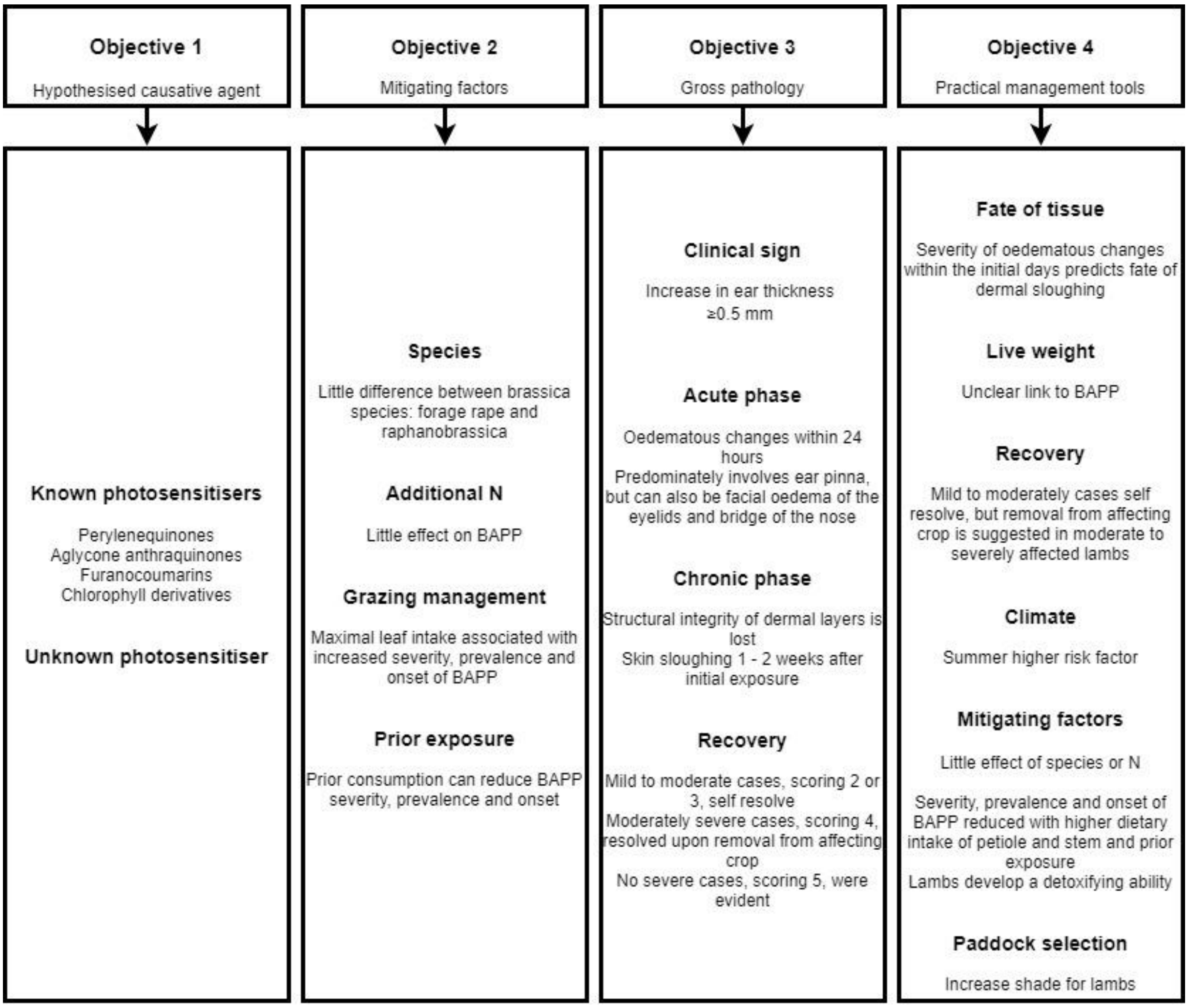

Figure 28: Schematic diagram depicting the four objectives of this thesis and overall findings.

7.2 Potential causative agents

The first objective of reviewing the literature for a potential causative agent of lambs grazing forage rape and raphanobrassica revealed an array of molecules that may induce photosensitisation in grazing livestock to a varying degree. There are four main classes of photosensitising agents known in livestock, including perylenequinones, aglycone anthraquinones, furanocoumarins, and derivatives of chlorophyll (pheophorbide *a* and phytoporphyrin), all of which may be present in these forage brassica species. Following the works of Tapper *et al.* (1975), we originally assumed the causative agent was chlorophyll metabolite phytoporphyrin. However, we were unable to successfully alter these levels within the plant material. The causative agent of BAPP could also be another compound, or metabolite, which is not yet identified. Substantial further work is required to rule out chlorophyll a, or phytoporphyrin, or identify another potential causative agent causing BAPP in lambs grazing forage brassica.

7.3 Mitigating factors

7.3.1 Species comparison

Photosensitisation occurred in both forage rape and raphanobrassica crops, with maximal clinical disease measured on Day 3 and all lambs exhibited some degree of ear thickness increase. The severity was briefly higher in lambs grazing raphanobrassica on Day 3, accounting for a statistical difference, but not a clinical difference (< 0.5mm). Thereinafter, no differences between the two forages arose. In both species, the highest risk factor was in the first week of grazing these crops. While further work of increasing measurements within the initial 72 hours of forage brassica exposure may ascertain the species differences, based on these data we can conclude that incidence of BAPP was similar for lambs grazing forage rape and raphanobrassica. There is a risk of BAPP in both crops.

7.3.2 Nitrogen application

Although N application significantly increased forage N content, there was little association between increased N and BAPP, as measured by ear thickness, in the first study (Chapter 3), perhaps due to crops being N replete. The low N treatment may not have been sufficiently low, thus masking an absence of N effect. This was noticeable when examining the feed profile (Table 7), whereby even the non N-boosted crops had relatively high amounts of CP compared to published ranges (Westwood & Mulcock, 2012). Although soil tests performed prior to conducting these experiments (Appendix 5) suggested potentially available N (15 cm depth) and anaerobically mineralizable N was low. There was an application of fertiliser, containing N, to all treatments at sowing.

This contrasts to the finding from an Australian survey which correlated additional N inputs and photosensitivity (Morton & Campbell, 1977) as well as Westwood & Nichol (unpublished) discovering a relationship between N and BAPP. Further works with higher N application rates or an increased number of N applications may be required to rule out N as a risk for BAPP. The relevance of N as a risk factor for influencing BAPP severity and/or incidence for BAPP may be reduced in the future with the development of Freshwater Regulations. Currently, as of December 2020, national and regional environmental legislation in New Zealand limit N input in pastoral areas to 190 kg N/ha/year with the most sensitive loss of N from the soil profile between 1 May and 30 September. Legislation may become more relevant for summer forage crops in time.

There were, however, some interactions between N and blood changes in these lambs, where lambs grazing N-boosted forage brassica crops developed pathological changes more rapidly than those on the non-boosted crops. Again, this may be explained by N fertiliser accentuating the accumulation of antinutritional components, such as but not limited to SMCO, within plants (Fletcher, Wilson, Maley, McCallum, & Shaw, 2010).

7.3.3 Grazing management

Grazing strategies that increased leaf intake, compared with petiole and stem, had higher incidence and severity with an earlier onset of BAPP. These included low stocking rates, reducing individual competition for feed and encouraging lambs to consume leaf only, in preference to petiole and/or stem; accessing crop without a full rumen, that is, hungry; and the leaf treatment in the second study (Chapter 4). As a result, we hypothesise that the causative agent is potentially found in leaf material but not petiole and/or stem material and may represent a dose-dependent response with leaf intake. Establishing a correlation between leaf intake and the incidence of BAPP should be the focus of future research.

Strategies that were intermediate for leaf intake had a moderate response of BAPP, with reduced incidence, severity and/or disease onset compared to those encouraging maximal leaf intake. These treatments included strip grazing and intermittently consuming forage brassica with pasture for the initial seven days (Chapter 4). Further work teasing out if intermittently grazing forage brassica with a highly palatable crop in the same paddock for individual lamb selection, rather than tall, rusty perennial ryegrass, is required to determine if this is a practical measure to mitigate BAPP.

The strategy of mechanically removing the top third of the crop canopy, promoting high intakes of petiole and stem intake had a similar outcome to the non-brassica control, whereby there was no evidence of BAPP. While this treatment may not be always practical on-farm, grazing ewes ahead of lambs to remove the bulk of the leaf material is already being used as a strategy on commercial farms as one approach to BAPP mitigation and may prove to be as effective without crop wastage.

7.3.4 Tolerance ability

In the first two studies (Chapters 3 and 4), lambs that exhibited mild to moderate clinical signs of BAPP recovered whilst they continued to consume the affecting forage. This led to the supposition of the third study (Chapter 5): lambs develop an ability to detoxify and/or become more tolerant of the causative agents of BAPP over time. Lambs could maintain this ability, without gross clinical changes, for at least four days, but not for eight weeks. This could further be tested as a tool to mitigate BAPP entirely by grazing lambs on forage brassica crops before the summer risk period and maintaining them on these crops for the duration of the summer.

7.4 Gross pathology

The most common objective sign of BAPP in these lambs was bilateral oedematous accumulation in the ear pinna, which developed within twenty-four hours of transferring lambs to forage brassica crops. The oedema was often so profound that it overcame the structural integrity of the cartilaginous tissue resulting in ventral dropping of the pinnae. In some lambs, oedema also occurred on the upper and lower eyelids and bridge of the nose, particularly around the mucocutaneous junctions. Wool hides potential erythema and oedema along the midline and rump, which often is not noticed until wool and epidermal loss 1 – 2 weeks following introduction to brassica crops. By the time of epidermal, dermal and/or wool loss occurring, there is no longer pathological changes associated with BAPP, except for an elevated risk of secondary sunburn (Townend, 1987), infection (Baird, 2000; Devriese, 1990; Mitchell G. B., 1988) and flystrike (Fels, 1971). In mild to moderate clinical cases, BAPP was self-limiting and gradually recovered upon removal from forage brassica. We recommend removal of lambs from affecting crops, and advice from veterinarians who may recommend symptomatic treatment, in cases where lambs score >4 on

Table 12.

The degree of ear pathology of these lambs was also much greater in the studies conducted during summer (Chapters 3 and 4) than in the third study (Chapter 5) in autumn. This suggests some relatedness to a climatic feature, likely UV exposure, as also found by Westwood & Nichol (unpublished). We therefore suggest that summer, with increased light intensity and duration; and lack of shade for lambs grazing forage brassica crops may be risk factors for BAPP. While there was a poor correlation between ear thickness and either solar radiation or temperature measured in the third study (Table 10; Chapter 5) recreating this study during the summer months may reveal some relationship between these variables. Anecdotally, farmer groups in the North Island, particularly along the Eastern Coast, are more concerned about BAPP occurring in their lambs than those in the South Island, which may also be a function of sunlight differences between these two geographically distinct locations (Cleaveland & Morris, 2013). Repeating studies in the North Island during summer, or in the South Island during winter are likely to yield data to further relate climate variables to BAPP. If there is a relationship, then climate change may extend the risk of BAPP in Australasia.

There were no examples in any of these studies of photosensitivity manifesting on coronary bands (Clare, 1952) and skin sloughing was limited to ear and face. However, since undertaking this research, many farming groups have discussed and provided photographic evidence of severe examples of BAPP occurring. Therefore, we do not rule out more severe examples of BAPP occurring throughout Australasia.

In the first study (Chapter 3), we nominated an increase in ear thickness of ≥ 0.5 mm upon introduction of lambs to forage brassica crops as being a threshold for clinical significance. While slightly ambiguous, a measurement increase of 0.5 mm above pre experimental measurement had a strong relationship with oedematous accumulation being a palpable change and increases over this may be so large as to overcome the stability of the ear pinna, causing ventral drooping. Based on initial ear thicknesses in the first two studies (Chapters 3 and 4), with no pre-existing damage, of 2.7 mm (95% CI 2.67 to 2.73) and 3.1 mm (95% CI 3.07 to 3.13), respectively, we suggest lambs with an ear thickness greater than 3.6 mm taken at the midpoint of the ear pinna and/or presence of non-pitting bilateral oedema and/or presence of erythema would be tentatively diagnostic for BAPP together with the history of recent introduction, i.e. within the last week, to a summer forage brassica, provided there are no other causes of bilateral ear thickness changes such as injury, misadventure, dermatophilosis, bacterial infection, viral papillomatosis, lice (*Bovicola ovis*) or neoplasia.

7.5 Results and their application

7.5.1 Ear thickness

Ear thickness, or pathological oedematous accumulation, was used to measure the incidence and severity of BAPP in these lambs. Treatments that encouraged maximal leaf intake and by *ad libitum* access to crop, facilitating ingestion of just leaf, with low competition from other animals (low stocking rate) had the largest number of animals affected and the most severe cases of clinical BAPP. Lambs that had objective ear thickness elevations ≥ 0.5 mm subjectively appeared to have swollen oedematous ear pinna whereby the oedematous accumulation within the ear pinna was palpable. This measurement was then used as a proxy for BAPP incidence and clinical disease significance. There was a 93%, 30.5% and 47.5% incidence of BAPP in treatments purposefully created to initiate BAPP (low stocking rate, large leaf intake, sudden non-brassica dietary change) in the three respective studies. Similar to the variation in incidence findings of Stafford *et al.* (1995), between years of lambs grazing birdsfoot trefoil, the differences between the three studies of this thesis reinforce the transient nature of photosensitivity.

The change in ear thickness was comparable between the first two studies (Chapters 3 and 4), whereby ear thickness increased by approximately 300% in some treatment groups. In the third study (Chapter 5), the severity of BAPP was reduced, with most affected lambs having an increase in ear thickness of approximately 100%. Oedematous severity in all studies in this thesis only reached a maximum of 4/5 on the subjective scoring scale (Westwood & Nichol, unpublished) with no associated deaths. In the second study (Chapter 4), ear thickness also increased in two individuals grazing the control, non-brassica treatment, presenting a possible correlation with photosensitivity in other plant species or insects. Oedematous changes resolved, however, some individuals exhibited secondary epidermal, dermal and/or wool sloughing, and some developed fibrosis of pinnae, a likely result of inflammatory changes (Keast, Despatis, Allen, & Brassard, 2014). Equivalently to Westwood & Nichol (unpublished), most cases resolved whilst lambs remained grazing affecting crops, which is useful for brassica grazing management recommendations in the face of mild to moderate BAPP in lambs.

There was a poor correlation between ear thickness and lamb live weight in all studies (Table 10), suggesting live weight loss (first study; Chapter 3) or gain (second and third studies; Chapters 4 and 5) may not be directly related to BAPP. As apparent feed intake was measured in the third study, BAPP appears also to be unrelated to total intake. Ear thickness was moderately correlated (0.3 - 0.5) with blood changes in the first study, although this was not repeatable in the second and third studies.

7.5.2 Live weight

Live weight loss, or a reduction in expected live weight gains, are often associated with subclinical and clinical disease due to anorexia (Hutchings, Gordon, Robertson, Kyriazakis, & Jackson, 2000; Sykes & Coop, 1976) and/or increased energy (Hindson & Winter, 1990) or protein (van Houtert & Sykes, 1996) requirements for disease recovery. In the first study (Chapter 3), where lambs had not consumed forage brassica before, there was a significant loss in lamb live weight of approximately 4 kg over the first 10 days, followed by a steady increase in live weight. This appeared to coincide with disease progression and potentially the theory of anorexia associated with subclinical and clinical disease.

However, the subsequent two studies (Chapters 4 and 5), where lambs had previously consumed forage brassica, revealed no initial live weight losses even in the presence of clinical disease. This was an unexpected finding, leading to the proposal that the lack of live weight loss may be attributed to lambs having prior knowledge of grazing brassica crops (therefore, absence of neophobia).

Reduction in live weight may be seen when introducing animals to a novel feed (neophobia), which initially decreases intake (Le Du, Combellas, Hodgson, & Baker, 1979). Dietary changes of odour and flavour, particularly related to the odour and flavour of forage brassicas imparted by the presence of GSL (Horbowicz, 2003), can induce feed neophobia (van Tien, Lynch, Hinch, & Nolan, 1999). This effect is more pronounced when lambs are moved into new social groupings (Leme, Titto, Titto, Pereira, & Neto, 2013). A grazing history of pasture, chicory and clover in the first study (Chapter 3), and the dramatic change in odour, flavour associated with forage brassicas, combined with the stress of weaning, then transport by truck from their farm of origin, and alteration of social groupings (15 lambs/plot), were all novel experiences for these lambs and are likely to cause a degree of stress and influence feed neophobia. This effect was observed at the commencement of the first study (Chapter 3), where although lambs were allowed unrestricted access to feed within their assigned 0.25 ha plots, lambs were observed to move cautiously around the edge of the plots, just nibbling on small amounts of leaf material during the first week.

Live weight loss may be due to changes in the rumen microbial and bacterial population, altering the volatile fatty acid profile and reducing nutrient availability. There is a time period required for the rumen microbial population to adjust to novel diets (Barry, 2013; Rattray, Brookes, & Nicol, 2009; West, Bruere, & Ridler, 2009), particularly those with low nutrient detergent fibre (NDF) (< 30%) and high water soluble carbohydrates (WSC)(> 20%) (Russell, Minton, Sexten, Kerley, & Hansen, 1997). Feeds with low NDF and high WSC, such as forage brassicas, are also associated with increased rumen dysfunction as feed flows through the gastrointestinal tract (particularly the foregut) at a rapid rate, exacerbating nutrient loss due to a high rumen outflow rate (Offer & Dixon, 2000). Hence transitioning periods of 7 - 10 days is recommended for crops such as forage brassicas (Dalley D. E., 2014). Lambs in the first two studies (Chapters 3 and 4) were not transitioned onto these brassicas: they were allowed full, unrestricted access to the novel forage brassicas right from commencement and had no opportunity to undergo a gradual transition. Further work, particularly in locations of more severe cases of BAPP is required to determine a correlation between BAPP and live weight loss.

7.5.3 Blood changes

Liver enzymes, GGT and GLDH were largely unremarkable. GGT levels, were slightly above the reference range of normal animals, and did not rise to the same degree as in examples of hepatopathology in secondary photosensitivity diseases (Collett & Matthews, 2014). The increase in GGT in the first study (Chapter 3) may be attributed to either an incidental finding relative to crop acclimation, or an artefact of young lambs (Britti, Massimini, Peli, Luciani, & Boari, 2005; Pauli, 1983), particularly as the levels were above the reported reference range upon entry into this study. We support the previously unstudied hypothesis in the literature that BAPP is an example of a primary photosensitivity disease (Cunningham, Hopkirk, & Filmer, 1942; Clare, 1952; Connor, 1977; Vermunt, West, & Cooke, Rape poisoning in sheep, 1993). To confirm this, we could repeat the first study (Chapter 3) with the inclusion of a negative control, comparing GGT and GLDH levels with lambs grazing a non-brassica feed.

Haematological changes appear unrelated to BAPP. This was most evident in the second two studies (Chapters 4 and 5), as the first (Chapter 3) lacked a non-brassica control, where data from brassica-fed lambs without clinical signs of BAPP could be compared to that on non-brassica fed lambs. Although some brassica treatments did not induce clinical BAPP ear thickness changes, Heinz bodies were elevated and resulting trends of mild reductions in erythrocyte counts and haemoglobin concentrations, but largely within reference ranges, may be due to premature phagocytosis or fragmentation of erythrocytes (Kumar, Abbas, Fausto, & Aster, 2009). We suggest these blood changes may instead be due to an antinutritional compound in brassicas.

Soil sulphur reserves prior to the commencement of these studies were low (Appendix 5) with sulphate sulphur, extractable organic sulphur and total sulphur levels at 4 - 9 mg/kg (reference range 10 – 12), 5 - 6 mg/kg (reference range 15 – 20) and 207 - 279 mg/kg (reference range 600 – 1000), respectively. This suggests that there is a likely low presence of sulphur-containing antinutritional compounds (Fletcher, Wilson, Maley, McCallum, & Shaw, 2010), such as SMCO (Smith, 1980) or thiosulphate (Collett, Bryan, & Tapper, 2014; Smith, 1980), and blood changes found may be due to the presence of alternative compounds.

7.6 Management strategies

The following are a list of management strategies developed empirically or theoretically from this thesis which have been offered as advice to farmers concerned with BAPP:

- Oedematous changes within the first few days of exposure to a forage brassica crop appear to predict the subsequent fate of superficially affected tissue.
- Live weight loss may not be associated with BAPP in mild to moderate cases, instead may be due to neophobia and/or social and environmental changes.
- In mild to moderate cases of clinical BAPP, ear thickness changes resolve whilst lambs remain grazing the crop. Individuals exhibiting moderate to severe cases should be removed from the crop and offered shade and symptomatic treatment, veterinary advice may also be warranted.
- By the time epidermal, dermal and/or wool loss occurs 1 – 2 weeks after initial exposure, we found no more pathological changes associated with BAPP, however, lambs may benefit from access to shade, symptomatic treatment and observation for secondary sunburn, infection and flystrike.
- Summer appears to have a higher risk factor for BAPP than autumn. We predict the presence of shade may reduce the severity of BAPP and aid in animal welfare.
- Both forage rape and raphanobrassica as summer feed crops for lambs induced BAPP to a similar degree.
- While nitrogenous fertiliser did not influence BAPP in this study, it may impact the presence of other antinutritional compounds which negatively affects blood changes.
- Grazing strategies that reduced leaf intake and increase petiole/stem intake had a lower incidence and severity of clinical BAPP.
- Mechanical removal of the top third of the canopy was the most successful treatment at reducing BAPP and may also be achieved by grazing ewes ahead of lambs.
- Lambs appear to develop an ability to detoxify and/or develop a tolerance to the phototoxin causing BAPP whilst grazing forage brassica, which may last at least four days when removed from crops.

- Future risk of BAPP is minimised when lambs remain grazing affecting crop.
- Selection of paddocks that provide shade for lambs is likely to increase animal welfare outcomes if BAPP occurs.

7.7 Future work

The findings point out several areas for future work:

- Identification of the plant phototoxin in *Brassica* spp. causing BAPP:
    1. Make a bibliographic search of the compounds present in the genus,
    2. Source plant material that has induced photosensitivity,
    3. Identify compounds by HPLC-MS in this plant material,
    4. Isolate the compound,
    5. Prove the isolated compound induces photosensitivity (Blum, 1941).
- Communicate identified phototoxin to brassica plant breeders to negatively select against plants containing high levels of the identified compound.
- Validation of results over a range of sites and years focusing on selecting geographical locations which commonly exhibit severe clinical signs of BAPP in lambs.
- Investigate the effect of climate, in particular UVA intensity, on BAPP severity and/or incidence.
- Further work into the effect of nitrogenous fertiliser application and BAPP presence.
- Investigation into the cause of oxidative damage resulting in elevated Heinz bodies when lambs graze forage brassica, and identification of any potential loss of production associated with these blood changes.
- Further work into the reference range of Heinz bodies present in clinically normal lambs.
- Investigation into the level of serum phytoporphrin in lambs grazing forage brassica with clinical BAPP signs.
- Quantification of the maximum duration lambs can be removed from a brassica crop and successfully detoxify the phototoxin causing BAPP.

- Determine if BAPP can be entirely mitigated by grazing lambs on forage brassica before the summer risk period, then throughout the summer.

- Investigation into a genetic basis of protection from BAPP in lambs.

- Investigation into ruminal transfaunation given by oral bolus as a tool to mitigate BAPP in lambs.

- Quantification of live weight changes associated neophobia in forage brassica.

- Further work with individual cases of lambs exhibiting severe clinical signs of BAPP, principally looking at histopathological changes of ear pinna, hepatic enzyme changes and determining the ratio of mild, moderate to severe cases.

7.8 Conclusions

This thesis developed the current understanding of primary photosensitivity occurring in lambs grazing forage brassica. The three main empirical findings identified that BAPP occurred in lambs grazing both forage rape and raphanobrassica crops; the causative agent is likely found in leaf material, whereby grazing strategies which alter leaf intake in favour of petiole and stem intake, reduce severity, incidence and delay onset of BAPP; and lambs develop an ability to detoxify or tolerate the causative agent(s) whilst grazing affecting brassica crops. Live weight and blood changes appear to not be a causative function of BAPP, but rather a normal process of consuming novel forage brassica crops, although more elucidation may be required. Generically, a tentative diagnosis of BAPP may be given to lambs exhibiting an ear pinna thickness > 3.6 mm taken at the midpoint of the ear pinna and/or presence of non-pitting bilateral oedema and/or presence of erythema with a history of recent introduction to a forage brassica, i.e. within the last week, during summer.

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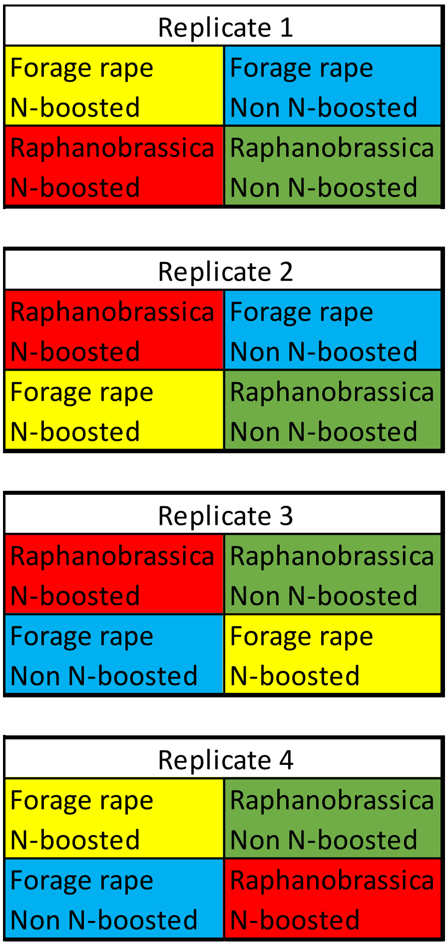
Appendices

Appendix 1: Study designs

Appendix 1A: Physical design of Study One (Chapter 3), consisting of a 2x2 factorial design in a complete randomised block with four replicates. Treatments are: forage species (forage rape or raphanobrassica) and nitrogen (N; N-boosted with two additional applications of 100 kg urea/ha or non N-boosted).



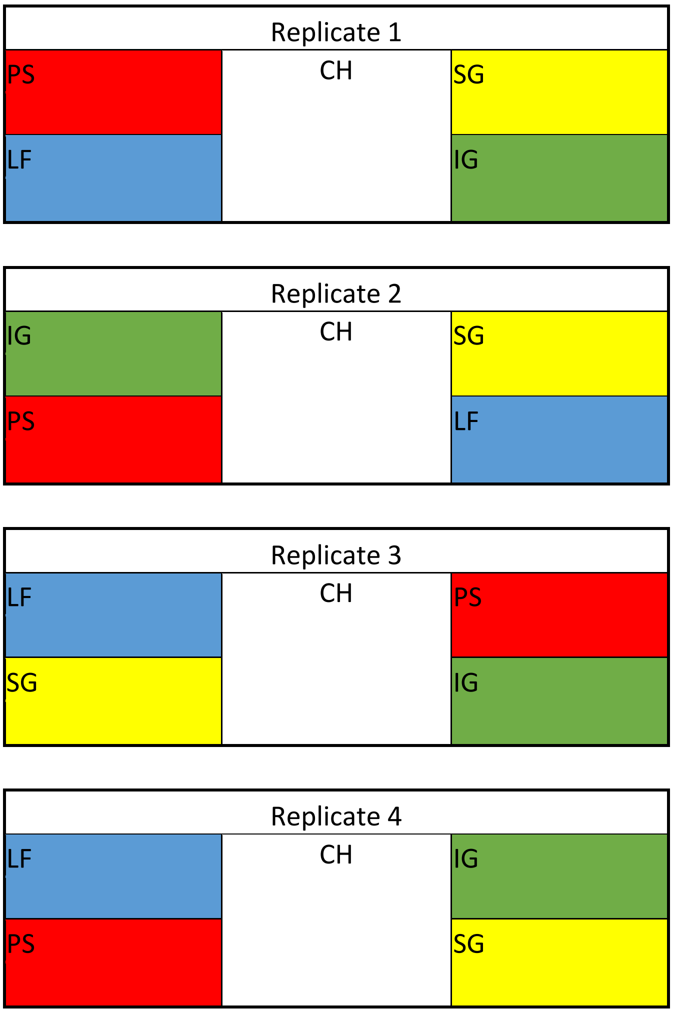
Replicate 1	
Forage rape N-boosted	Forage rape Non N-boosted
Raphanobrassica N-boosted	Raphanobrassica Non N-boosted

Replicate 2	
Raphanobrassica N-boosted	Forage rape Non N-boosted
Forage rape N-boosted	Raphanobrassica Non N-boosted

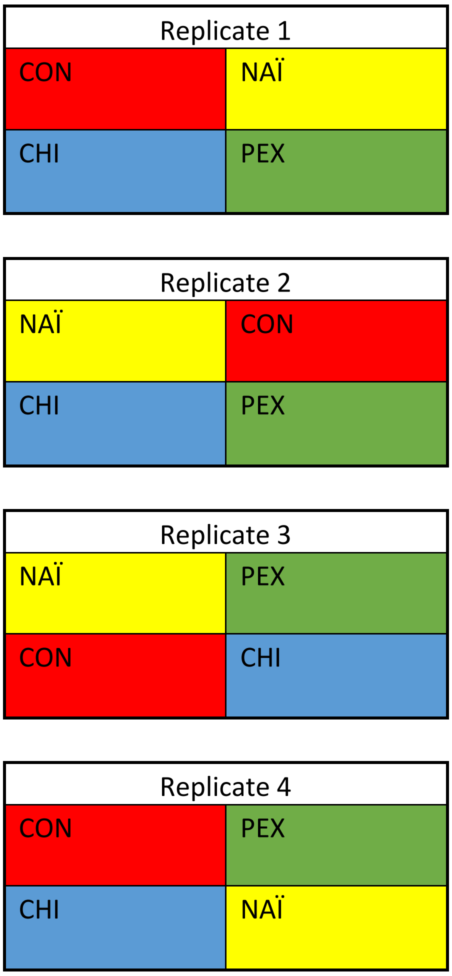
Replicate 3	
Raphanobrassica N-boosted	Raphanobrassica Non N-boosted
Forage rape Non N-boosted	Forage rape N-boosted

Replicate 4	
Forage rape N-boosted	Raphanobrassica Non N-boosted
Forage rape Non N-boosted	Raphanobrassica N-boosted

Appendix 1B: Physical design of Study Two (Chapter 4), consisting of five treatments in a complete randomised block design with four replicates. Lambs consumed either chicory (CH) or raphanobrassica. Lambs consuming raphanobrassica were subjected to strip grazing (SG) or intermittent grazing (IG); or consuming predominantly leaf (LF) or petiole/stem (PS)



Appendix 1C: Physical design of Study Three (Chapter 5), consisting of four treatments in a complete randomised block design with four replicates. Lambs consumed either chicory (CHI) or raphanobrassica. Raphanobrassica fed lambs were determined prior to the study, lambs assigned to consume raphanobrassica were either consistently grazing raphanobrassica (CON), initially grazed raphanobrassica followed by non-brassica crops for -4 days (PEX) or initially grazed raphanobrassica followed by non-brassica crops for -56 days (NAÏ).



Replicate 1	
CON	NAÏ
CHI	PEX

Replicate 2	
NAÏ	CON
CHI	PEX

Replicate 3	
NAÏ	PEX
CON	CHI

Replicate 4	
CON	PEX
CHI	NAÏ

Appendix 2: Primary photosensitisation ('rape scald') in lambs grazing forage rape or raphanobrassica

GM BOX[a*], GK BARRELL[b] and CT WESTWOOD[a]

[a]*PGG Wrightson Seeds Limited, 742 Tancreds Road, Lincoln 7676, New Zealand,* [b]*Faculty of Agriculture and Life Sciences, Lincoln University, PO Box 85084, Lincoln 7647, New Zealand*

**Corresponding author*. Email: gbox@pggwrightsonseeds.co.nz

Abstract

Forage brassicas are commonly grown throughout New Zealand in summer for feeding weaned lambs. However, forage brassica consumption has been associated with primary photosensitisation ('rape scald'). This study aimed to determine whether incidence and/or severity of primary photosensistation was greater for lambs grazing the new-to-market brassica, raphanobrassica (*Raphanus x Brassica* L.) compared with forage rape (*Brassica napus* spp. Biennis, cv 'Titan'). During summer of 2017-2018, 240 cryptorchid NZ Romney lambs were introduced to 16 plots, each of 0.25 ha, of raphanobrassica or forage rape, 70 days after sowing. Soon after, there was an initial increase in ear thickness for all lambs (mean of 1.49 mm, measured on Day 3), with ears 0.32 mm thicker for lambs grazing raphanobrassica ($P < 0.001$) compared with those grazing forage rape. Thereinafter, ear thickness declined about 0.5 mm during the next three weeks, and was not significantly different ($P > 0.05$) between treatments. There was a temporary elevation in incidence of Heinz bodies, along with reductions in erythrocyte count and haemoglobin levels suggestive of minor forage-associated pathology. Primary photosentisation occurred in lambs grazing both crops, with raphanobrassica having similar risk of primary photosensitisation in sheep. Standard management practices with attention to this risk will be required.

Keywords: primary photosensitivity; rape scald; forage rape; raphanobrassica

Introduction

Forage brassicas are grown as annual fodder crops for part of the feed rotation systems in New Zealand. These crops are grazed as a monoculture *in situ* and are generally grown to meet periods of feed shortages in summer due to low soil moisture and reduced pasture growth (Dumbleton, et al., 2012). Compared with traditional pasture swards (perennial ryegrass [*Lolium perenne* L.]) and white clover [*Trifolium repens* L.]), forage brassicas have superior dry matter yields (Judson & Edwards, Survey of management practice of dairy cows grazing kale in Canterbury, 2008) and higher nutritional quality (Westwood & Mulcock, 2012).

Two brassica species commonly grown on lamb finishing enterprises (post weaning to slaughter period) are forage rape (*Brassica napus* spp. Biennis) and a new species, raphanobrassica (*Raphanus x Brassica* L.), a hybrid of kale (*Brassica oleracea* L.) x radish (*Raphanus raphanistrum* subsp. Sativus L.). Raphanobrassica was first released into the New Zealand marketplace in 2017, with 3,500 ha grown that year (A. Dumbleton, personal communication). Whilst forage brassicas are agronomically excellent forages, they can cause negative animal performance and health issues. For example, lambs grazing forage brassicas occasionally develop idiopathic primary photosensitisation, commonly termed ‘rape scald’. Forage rape, specifically, has been traditionally associated with the occurrence of clinical idiopathic primary photosensitisation in sheep. However, there is anecdotal evidence for similar effects arising from grazing raphanobrassica.

Idiopathic primary photosensitisation clinically presents as marked oedema of the ears, face and occasionally along the midline of the back (Cunningham, Hopkirk, & Filmer, 1942; Vermunt, West, & Cooke, Rape poisoning in sheep, 1993). Although the pathophysiology is not well understood (Collett, 2013), it has been documented as a primary photosensitisation unaccompanied by hepatic damage (Bruere, Cooper, & Dillon, 1990; Collett, 2013). To date no primary photosensitizing compound has been identified in any of the brassicas (Collett, 2013). Primary photosensitisation occurs following the ingestion of a photodynamic agent which reaches the systemic circulation (Parkinson, Vermunt, & Malmo, 2010). As the agent moves through the systemic circulation of livestock, exposed skin areas which receive the most interaction with UV light can develop photodynamic dermatitis. With increased interaction between the photodynamic agent and UV light, cell necrosis occurs through a combination of enzyme inactivation, membrane destruction, plus cytoplasmic and/or nuclear changes (Towers, 1980) and generation of reactive oxygen species (ROS) (Parton, Bruere, & Chambers, 2001). These ROS induce oxidative stress, causing structural changes in lipid components of cell membranes which increases vascular permeability (Phaniendra, Jestadi, & Periyasamy, 2015; de Jager, Cockrell, & Du Plessis, 2017). Necrotic damage follows, causing erythema, oedema, scaling and exudation from the dermis. ROS also cause changes to erythrocytes, which can appear as Heinz body formation: round projections of the erythrocyte membrane (Wilson, 2012; Valenciano, Cowell, Rizzi, & Tyler, 2014) that are evident histologically. Consumption of brassicas by sheep has been associated with increased Heinz body formation that can lead to haemolytic anaemia after 1-3 weeks (Haskell, 2008). Heinz body inclusions increase the rigidity of the erythrocyte membrane, making erythrocytes susceptible to fragmentation in the systemic circulation (haemolytic anaemia) (Kumar, Abbas, Fausto, & Aster, 2009). Also, the presence of Heinz bodies causes premature phagocytosis of erythrocytes in the spleen, which also reduces red blood cell count (RBC). Haematology to measure RBC, haemoglobin (Hb) and Heinz bodies can provide evidence of oxidative damage associated with clinical and subclinical photosensitisation. Early signs of photosensitisation in grazing livestock are animals seeking shade, pruritus, and in the case of sheep, head shaking (Kaneko, Harvet, & Bruss, 1997). Clinical signs of photosensitivity are similar for sheep and cattle. Dermal areas with reduced melanin and hair are the most affected (Campbell, Dombroski, Sharma, Partridge, & Collett, Photodynamic chlorophyll a metabolites, including phytoporphyrin (phylloerythrin), in the blood of photosensitive livestock: Overview and measurement, 2010) including: the udder, ears and coronary bands. Ears of affected lambs are typically oedematous and drooping, leading to deformity and discolouration. Ear thickness measurements can reveal oedematous changes in this region, thus objectively determining that clinical photosensitisation is present. The prognosis for recovery is generally favourable when livestock are removed from the feed (Parkinson, Vermunt, & Malmo, 2010).

The aim of this study was to determine whether raphanobrassica causes a lower incidence or severity of idiopathic primary photosensitisation in grazing lambs than forage rape. This hypothesis was addressed by comparing lambs grazing these forage species for evidence of photosensitisation from measurement of ear thickness and examination of haematological indices.

Materials and methods

Experimental design

The study was conducted at PGG Wrightson Seeds Research Centre, Kimihia, Lincoln, Canterbury, New Zealand (-43.62, 172.47) during summer (2017 – 2018). The study design was a complete randomised block study with four replicates and two feed treatments: forage rape (cv. 'Titan') and raphanobrassica. Treatments were allocated using GENSTAT Version 19.1. All animal procedures were approved by the Lincoln University Animal Ethics Committee. Thirty lambs were assigned to each 0.5 ha plot, totalling 240 lambs.

Study husbandry

To ensure adequate establishment of the crops, the study site was treated with diammonium phosphate (DAP) fertiliser at a rate of 200 kg ha^{-1}, providing 36 kg N ha^{-1} and 40 kg P ha^{-1}. Seeds were sown on 1 November 2017. Best management practices provided by the seed company (PGG Wrightson Seeds, Lincoln, New Zealand) in terms of herbicide and insecticide applications and use of irrigation were followed, thus minimising alternative causes of primary photosensitivity such as aphids and weed species (Collett, *et al*., unpublished). An electric fence system provided a physical barrier between the treatment plots. In addition to the electric fence system, a 1 m boundary area between plots was sprayed with herbicide (glyphosate) to ensure the potential effect of fertiliser overlap was eliminated and lambs were unable to graze neighbouring plots. Treatment replicates were separated with permanent fencing materials. Fresh water was provided from reticulated troughs.

All lambs were cryptorchid Romney males from a single source flock. Three weeks prior to the study, they were weighed after 24 h of fasting and their mean live weight was 30.4 kg (range 22.5 – 40.0 kg). These live weights were used to allocate the lambs into groups that were balanced for this parameter. Appropriate animal health management in terms of clostridial vaccinations, anthelmintic treatments, selenium and cobalt supplementation, inguinal wool clipping (crutching) and a topical insecticide application were conducted prior to commencement of the study. The lambs were grazed in paddocks containing chicory and white clover between 21 December 2017 and the beginning of the study (15 January 2018). Lambs were introduced into their treatment plots 74 days after seed sowing. Throughout the study lambs grazed on their assigned plots and were monitored by field staff who surveyed them by walking through the plots daily.

Measurements

Lambs were moved to a yard facility for measurements on Day 0, then twice weekly for the first 14 days of the study and once weekly thereafter. Measurements included: ear thickness, haematology and blood biochemistry. Electronic vernier callipers (Jobmate, Palmerston North, New Zealand) were used to measure ear thickness (in mm) 10 mm proximal to an existing ear mark on the caudal border of the right ear pinna. Three measurements were taken on each sheep and the median value was recorded.

Blood sampling (10 mL) was performed once weekly on 128 lambs (16 lambs/plot, same individuals each time) by jugular venepuncture using vacutainers containing Na-ethylenediaminetetracetate as anticoagulant. The vacutainers were immediately placed on ice and thereafter (i.e. within 3 h) the chilled blood samples were transported to a commercial laboratory (Gribbles Veterinary Pathology

Laboratories, Christchurch, New Zealand) for analysis of RBC, Hb, Heinz bodies, gamma glutamyl transferase (GGT) and glutamate dehydrogenase (GLDH).

Data analysis

The study ran for a 35-day period, from 15 January to 19 February 2018. Raw data were recorded into Microsoft Excel and analysed using the statistical software GENSTAT Version 19.1. Statistical tests included one-way blocked analysis of variance (ANOVA) or one way blocked repeated measures ANOVA. Variation among data was measured as the coefficient of variation (CV) and standard error of means (SEM). Graphical representations were performed on Microsoft Excel using combined means.

Results

Ear thickness

Transfer of the lambs from chicory/white clover grazing to the brassica forages was accompanied by a major increase in ear thickness of about 1.5 mm ($P < 0.001$, Fig. 1). This effect was more pronounced in the lambs grazing raphanobrassica ($P = 0.023$) compared with those on forage rape, particularly at Day 3 (Fig. 1). Thereafter there was a slight reduction in mean ear thickness throughout the study, with a temporary elevation ($P < 0.001$) in both groups (see Day 10, Fig. 1). All values remained about 1 mm above the pre-treatment means (Fig. 1). All lambs in the study ($N = 240$) experienced an increase in ear thickness (mean 55%, range 1 – 312%) within 72 hours after transfer to these forage brassicas.

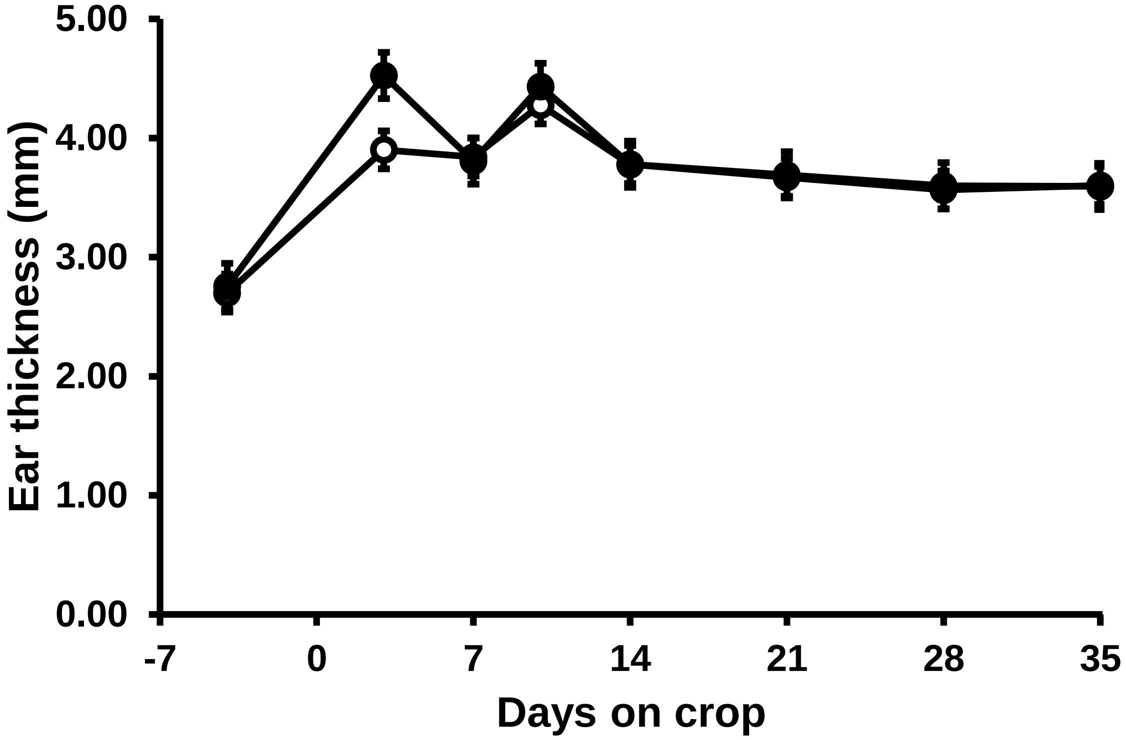


Figure 1: Mean (n=120) ear thickness of lambs grazing forage rape (o) or raphanobrassica (●). Error bars ± SEM.

Haematology

Lambs grazing these forage brassicas had gradual reductions in mean RBC and Hb concentration; reducing by approximately 2.5×10^6 μL^{-1} and 20 gL^{-1}, respectively ($P < 0.001$) until about Day 21 (Figs. 2a and 2b). The reductions were more severe in the lambs grazing forage rape ($P < 0.001$), of which some individuals had mild anaemia. There was a considerable increase in the incidence of Heinz bodies in lambs (Fig. 2c), which peaked at Day 14 and was greater in those grazing forage rape compared with those grazing raphanobrassica ($P < 0.001$). Heinz bodies remained elevated in these lambs by around 15% compared with their initial incidence of around 1.5%.

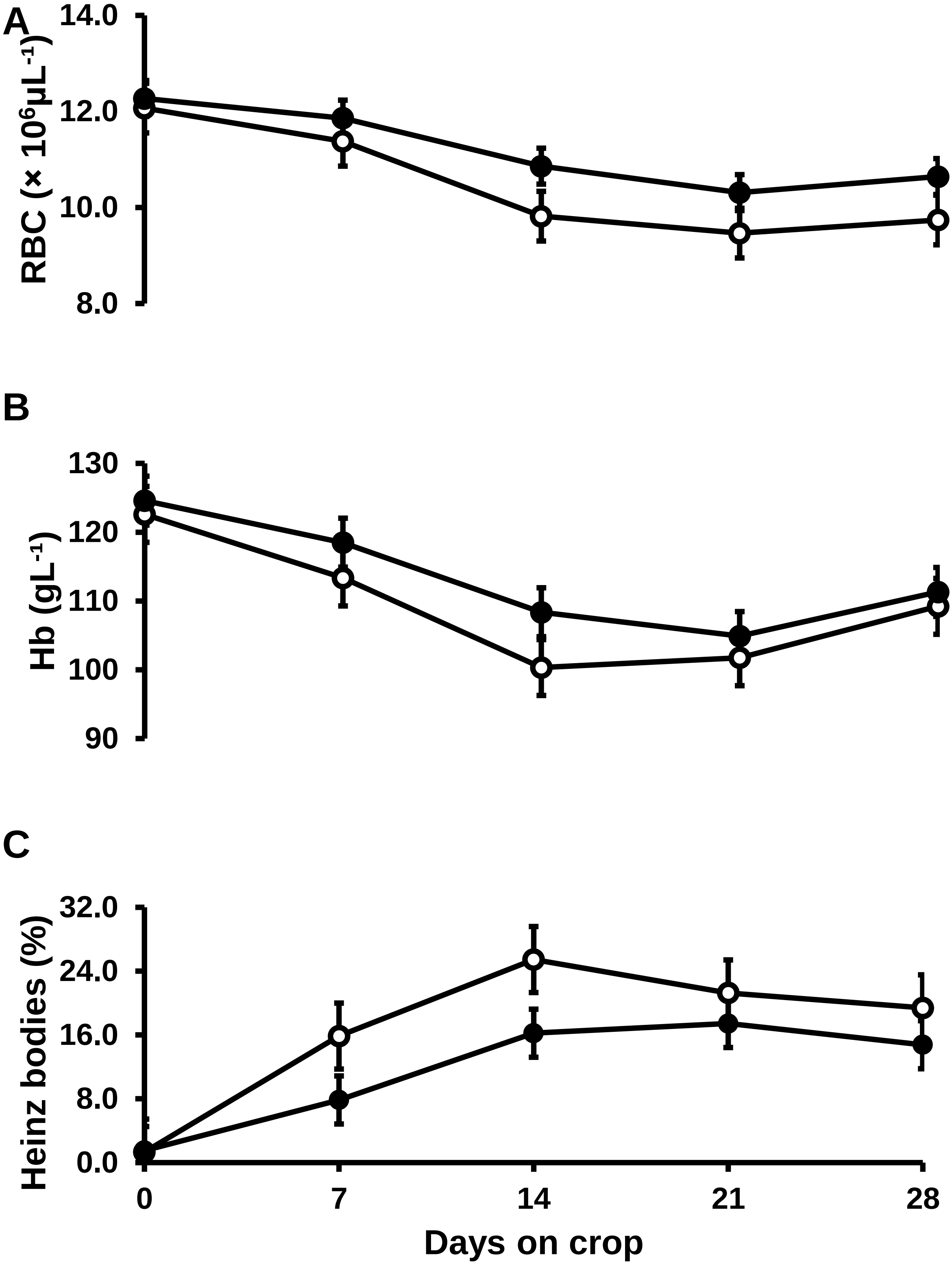


Figure 2: Mean (n = 120) A) erythrocyte count (RBC), B) haemoglobin concentration (Hb) and C) Heinz body percentage in blood of lambs grazing forage rape (o) or raphanobrassica (●). Reference range of RBC and Hb in clinically normal sheep are 9.0 – 15.0 × 10^6 μL^{-1} and 80 – 140 gL^{-1} respectively. There is no established reference range for Heinz bodies. Error bars ± SEM.

Blood biochemistry

Throughout the grazing study there were steady changes in levels of the enzyme markers of liver function (Fig. 3). Mean plasma GGT concentrations increased (P < 0.001) and those of GLDH decreased (P < 0.001). Lambs grazing forage rape had higher levels of GGT throughout the study (P < 0.001) compared with those grazing raphanobrassica.

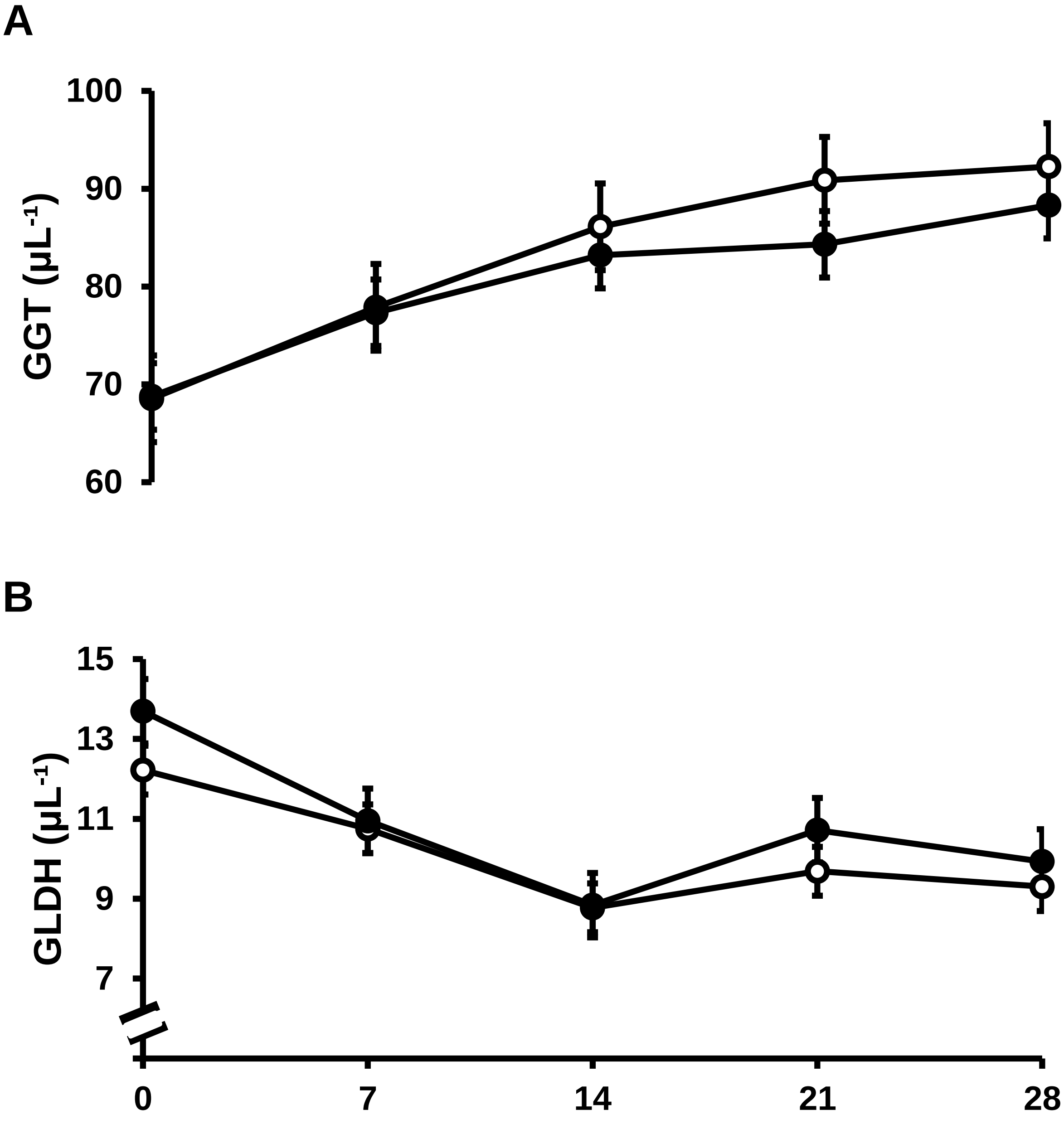


Figure 3: Mean (n = 120) A) plasma concentration of gamma-glutamyl transferase (GGT) and B) glutamate dehydrogenase (GLDH) of lambs grazing forage rape (o) or raphanobrassica (•). Reference range of GGT and GLDH in clinically normal sheep are 0 – 60 µL^{-1} and 0 – 15 µL^{-1} respectively. Error bars ± SEM.

Discussion

In this study, lambs grazing raphanobrassica have shown a similar severity of clinical and subclinical idiopathic primary photosensitivity to those grazing forage rape. The ear thickness data indicated a larger clinical effect induced by raphanobrassica, but subequent biochemical measures of erythrocyte integrity and liver function could ascribe a greater subclinical effect to the grazing of forage rape.

Within 72 hours of transfering these lambs onto forage brassica, all lambs exhibited an increase in ear thickness (100% incidence rate). To date there have not been any published studies that quantify idiopathic primary photosensitivity in lambs or detail its incidence in affected flocks. However, a previous pilot study revealed that all ear thickness changes in lambs occurred within the first 7 days (Westwood & Nichol, unpublished). Another study of primary photosensitivity in lambs grazing the pasture legume *Biserrula pelecinus* L. produced similar results, whereby mild ear oedema was observed between 12-72 h after exposure, with 89% of animals being affected (Quinn, et al., Acute-onset high-morbidity primary photosensitisation in sheep associated with consumption of the Casbah and Mauro cultivars of the pasture legume Biserrula, 2018).

The increased ear thickness was briefly (on Day 3 only) higher in the lambs grazing raphanobrassica; there being no forage species-related differences thereafter, although ear thickness did remain elevated above the pre-brassica grazing values. It is difficult to place much weight on the greater ear thickness initially recorded from lambs grazing raphanobrassica because this effect appears to have been transient. Practically, although there was a statistical difference in ear thickness measurements associated with both species on Day 3, it is unlikely that this represents a difference in clinical severity. However, additional measurements of ear thickness conducted during the first 72 hours may provide a clearer picture of the rapidity of onset of the condition, which could have differred between the two forages. Overall we conclude that upon first grazing these two brassica species, clinical primary photosensitivity was slightly more severe for lambs grazing raphanobrassica (Day 3), but thereafter both brassica species have caused similar increases in ear thickness of these lambs. After the initial increase in ear thickness, there was a downward trend with the exception of Day 10 (Fig. 1), which may be due to climatic conditions. Normal mean ambient temperature in January 2018 was 16.9 °C, whereas Day 10 of the study had an overnight low of 23 °C and daytime high of 28 °C. These elevated ambient temperatures may have exacerbated the effects on ear thickness.

The gradual reduction in ear thickness throughout the latter course of the study suggests the lambs may have developed tolerance to the presence of a causative agent, indicating a homeorhetic adjustment or activation of a detoxification pathway to developed tolerance. This possibility is akin to the observation that lambs grazing forage rape in another study (Westwood & Nichol, unpublished) did not have increases in ear thickness during a second period of exposure to the crop. However, this re-grazing occurred in late autumn which is outside the summer risk period for photosensitisation. There may be physiological mechanisms which enable lambs to develop tolerance to forage toxicity deserves further investigation.

Despite the reduction in ear thickness throughout the latter period of the study, the values remained well above the pre-treatment levels. This may be due to continued supply of the causative agents in these crops or that these oedematous changes had become permanent. Persistent, untreated oedema is associated with longer wound healing time due to ambient fluid pressure reducing local blood supply (Hess, 2011), thus decreasing phagocytotic cell movement, nutrient flow and limiting angiogenesis (Leaper & Harding, 1998). In some individual lambs the oedema resolved with no other clinical signs. In others, leathering of the epidermal layers, external necrosis and excessive cartilaginous growth caused twisting of the distal pinna. Although no subjective measurements of wound appearance were taken, the amount of distortion appeared to be a function of the degree of ear thickness developed from the initial fluid accumulation.

Supporting evidence of pathology in lambs resulting from grazing these forage brassicas is provided by the subclinical data showing changes in erythrocyte number and integrity. A peak of Heinz bodies was evident on Day 14 (Fig. 2c), two weeks after introduction of lambs onto the brassica crops, with the lambs grazing forage rape having the larger increase. The presence of Heinz bodies can reflect oxidative damage caused by a photodynamic agent, however the oxidative damage may have been caused, in part, by a non-photodynamic agent. This is because the peak of oxidative damage in the systemic circulation (Day 14) lagged behind ear thickness changes, which peaked on Day 3. Rapid increases in Heinz bodies have also been seen in lambs grazing other species of forage brassicas, such as turnips (*Brassica rapa* L.) (Cox-Ganser, Jung, Pushkin, & Reid, 1994) and kale (*Brassica oleracea* L.) (Barry, Manley, & Millar, Nutritional evaluation of kale (Brassica oleracea) diets:4. Responses to supplementation with synthetic S-methyl-L-cysteine sulphoxide (SMCO), 1982) where there has been no link to clinical photosensitivity.

The gradual decreases in RBC and Hb concentration (Figs. 2a and 2b) could represent further subclinical effects resulting from grazing these forages, although the values were largely within the normal reference ranges for healthy sheep. These effects were slightly greater in lambs grazing forage rape, with two of these exhibiting mild haemolytic anaemia and having the highest incidence of Heinz bodies. This introduces the possibility that high levels of other potential agents, such as S-methyl cysteine sulphoxide (SMCO) (Barry, Manley, & Millar, Nutritional evaluation of kale (Brassica oleracea) diets:4. Responses to supplementation with synthetic S-methyl-L-cysteine sulphoxide

(SMCO), 1982), may have been present in the forage rape. Although SMCO levels in have reported as being lower than those in swedes, turnips and kale (Stewart & Judson, 2004; Sun, et al., 2011). Although SMCO levels in have reported as being lower than those in swedes, turnips and kale (Stewart & Judson, 2004; Sun, et al., 2011). Reductions in RBC and Hb concentration are commonly found in sheep grazing forage brassicas due to the presence of SMCO, although the values often remain within the normal reference range (Valderrabano, Uriarte, & Munoz, 1986).

Primary photosensitivity diseases typically do not cause hepatic dysfunction, as determined by elevated circulating liver enzyme concentrations (Vermunt, West, & Cooke, Rape poisoning in sheep, 1993). In the present study, plasma concentration of GGT was elevated, especially for lambs grazing forage rape, above the normal reference range for healthy sheep (0 - 60 μL^{-1}) at Day 0 and continued to increase throughout the study, so that by Day 35 (i.e. at 5 weeks) the mean increase in GGT throughout the study was 32% (Fig. 5a). However, the changes recorded here may be normal for immature animals. Lambs can acquire GGT through passive transfer, with initial blood levels up to 140 times the normal adult levels, before stabilising at 60% higher than in ewes at 24-45 Days (Pauli, 1983). Another study revealed blood GGT levels in neonatal lambs remained elevated for 6 weeks (Britti, Massimini, Peli, Luciani, & Boari, 2005). Thus, the elevated plasma GGT levels recorded in this study may be totally unrelated to the forages. Also, the downward trend in plasma GLDH levels rules out any hepato-pathology in these lambs. Collectively, these unremarkable values for liver enzymes support the contention that the rape scald occurring in sheep grazing forage brassicas is a primary photosensitivity disease (Collett, 2006; Collett, 2013; Cunningham, Hopkirk, & Filmer, 1942; Parton, Bruere, & Chambers, 2001).

Conclusions

This study is the first to examine clinical and subclinical signs indicative of idiopathic primary photosensitivity in lambs grazing both raphanobrassica and forage rape. Raphanobrassica appears to cause a similar incidence and severity of clinical idiopathic primary photosensitivity in grazing lambs to that which occurs with rape itself. The surge in lamb ear thickness recorded here within the initial 72 hours of grazing demonstrates that both crops can induce clinical idiopathic primary photosensitivity; although the more sudden increase in ear thickness of lambs grazing raphanobrassica may indicate a heightened propensity of idiopathic primary photosensitivity for this crop compared with lambs grazing forage rape. In contrast, the biochemical measures of erythrocyte integrity provide some evidence that forage rape may cause more severe subclinical pathology in lambs, indicating the possibility of there being higher concentrations of antinutritional factors in forage rape.

Addendum

Traditionally, the condition forming the basis of the present study has been called 'rape scald'. However, this report with sheep grazing a non-rape plant causes us to propose a more encompassing term for the disease. We submit the term – brassica associated primary photosensitivity (BAPP) – as an appropriate new name for this condition.

Acknowledgements

This research was supported by Primary Growth Partnership between PGG Wrightson Seeds and the New Zealand Ministry of Primary Industries. We thank Dr Mark Collett who provided insight and expertise that greatly assisted the research.

Appendix 3: Crop yield

Table 13: Crop yield of raphanobrassica and chicory, red clover and white clover on Day -1 measured for Study Two (Chapter 4). Data are means (x̅) and standard deviations (s), n = 60.

		Yield (kg DM/ha)				
Treatment		Replicate				Mean
		1	2	3	4	
CH	x̅	1511	2032	2121	1614	1819
	s	511	553	13	223	432
LF	x̅	4345	5434	5313	4135	4807
	s	649	904	436	686	839
IN	x̅	4579	4331	4262	4329	4375
	s	261	776	1165	728	693
PS	x̅	2419	2450	1605	2379	2213
	s	714	181	159	903	622
SG	x̅	4216	5071	4761	3953	4500
	s	694	1130	151	1095	868

Appendix 4: Animal ethics application numbers

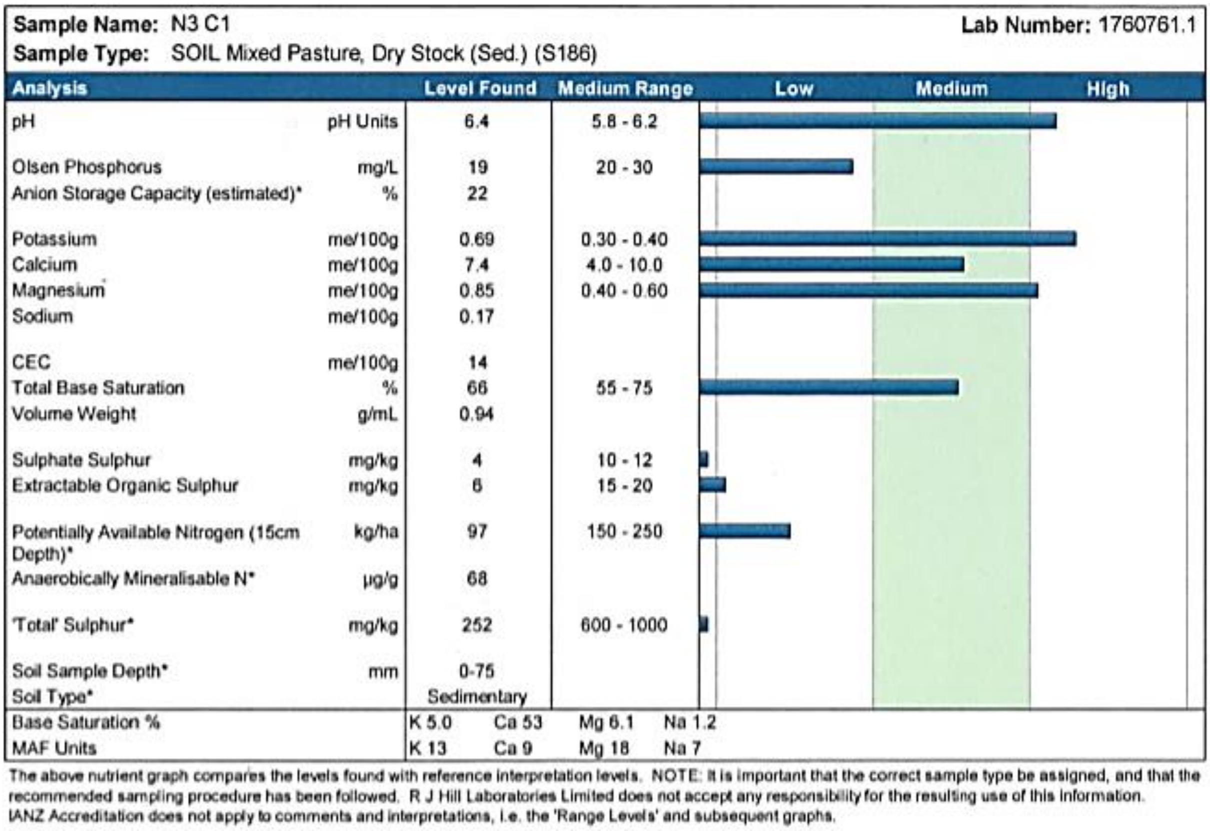
All animal ethics applications were accepted by Lincoln University's Animal Ethics Committee in line with approving to use animals for research, testing or teaching in the Animal Welfare Act 1999, section 100. Final reports to the committee were provided within six months of the completion of the studies.

Study one application: AEC 2017-33

Study two and three animal ethics application: AEC 2018-43A

Appendix 5: Soil analysis reports

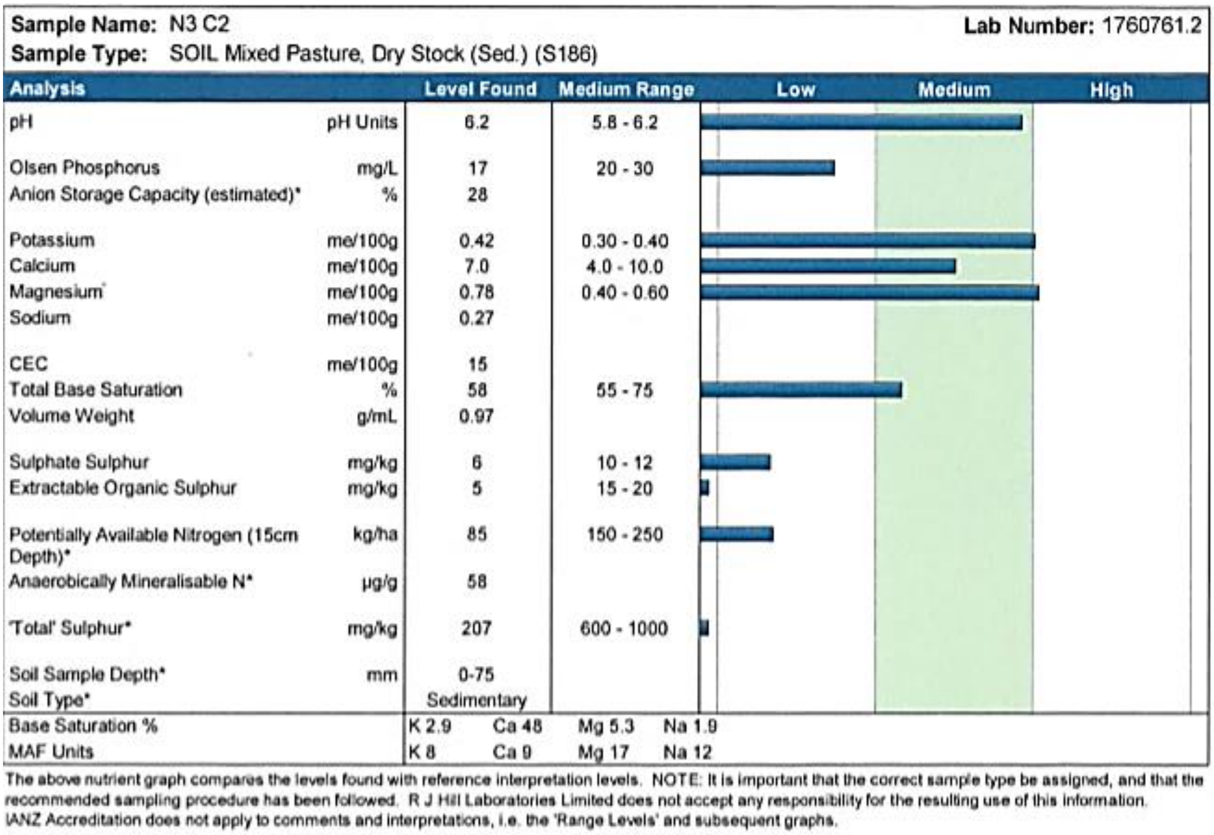
Measurements of the nutrient status of the soil as quantified by Hill Laboratories. Soil samples were taken six months prior to the studies (Chapters 3, 4 and 5) being performed.



Sample Name: N3 C1 **Lab Number:** 1760761.1
Sample Type: SOIL Mixed Pasture, Dry Stock (Sed.) (S186)

Analysis		Level Found	Medium Range	Low	Medium	High
pH	pH Units	6.4	5.8 - 6.2			
Olsen Phosphorus	mg/L	19	20 - 30			
Anion Storage Capacity (estimated)*	%	22				
Potassium	me/100g	0.69	0.30 - 0.40			
Calcium	me/100g	7.4	4.0 - 10.0			
Magnesium	me/100g	0.85	0.40 - 0.60			
Sodium	me/100g	0.17				
CEC	me/100g	14				
Total Base Saturation	%	66	55 - 75			
Volume Weight	g/mL	0.94				
Sulphate Sulphur	mg/kg	4	10 - 12			
Extractable Organic Sulphur	mg/kg	6	15 - 20			
Potentially Available Nitrogen (15cm Depth)*	kg/ha	97	150 - 250			
Anaerobically Mineralisable N*	µg/g	68				
'Total' Sulphur*	mg/kg	252	600 - 1000			
Soil Sample Depth*	mm	0-75				
Soil Type*		Sedimentary				
Base Saturation %		K 5.0 Ca 53	Mg 6.1 Na 1.2			
MAF Units		K 13 Ca 9	Mg 18 Na 7			

The above nutrient graph compares the levels found with reference interpretation levels. NOTE: It is important that the correct sample type be assigned, and that the recommended sampling procedure has been followed. R J Hill Laboratories Limited does not accept any responsibility for the resulting use of this information.
IANZ Accreditation does not apply to comments and interpretations, i.e. the 'Range Levels' and subsequent graphs.



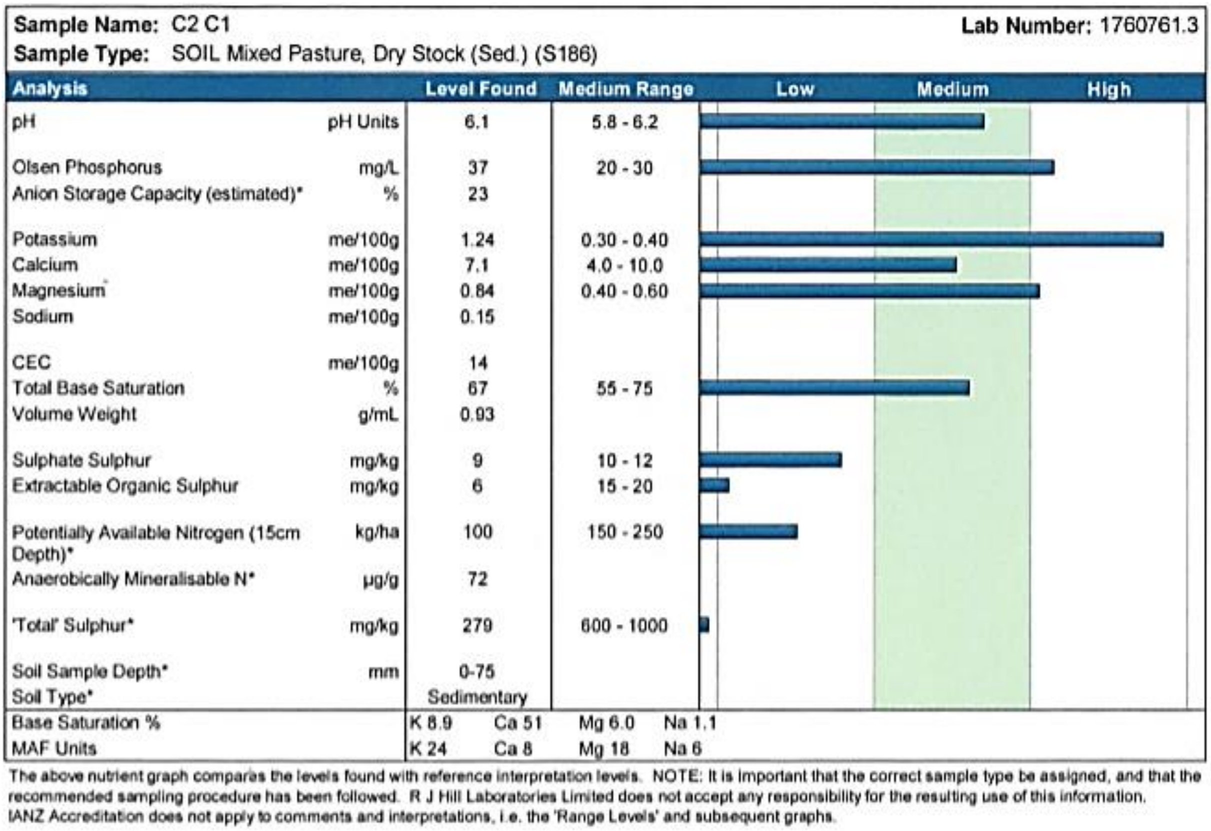
Sample Name: N3 C2 **Lab Number:** 1760761.2

Sample Type: SOIL Mixed Pasture, Dry Stock (Sed.) (S186)

Analysis		Level Found	Medium Range
pH	pH Units	6.2	5.8 - 6.2
Olsen Phosphorus	mg/L	17	20 - 30
Anion Storage Capacity (estimated)*	%	28	
Potassium	me/100g	0.42	0.30 - 0.40
Calcium	me/100g	7.0	4.0 - 10.0
Magnesium	me/100g	0.78	0.40 - 0.60
Sodium	me/100g	0.27	
CEC	me/100g	15	
Total Base Saturation	%	58	55 - 75
Volume Weight	g/mL	0.97	
Sulphate Sulphur	mg/kg	6	10 - 12
Extractable Organic Sulphur	mg/kg	5	15 - 20
Potentially Available Nitrogen (15cm Depth)*	kg/ha	85	150 - 250
Anaerobically Mineralisable N*	µg/g	58	
'Total' Sulphur*	mg/kg	207	600 - 1000
Soil Sample Depth*	mm	0-75	
Soil Type*		Sedimentary	

Base Saturation %	K 2.9	Ca 48	Mg 5.3	Na 1.9
MAF Units	K 8	Ca 9	Mg 17	Na 12

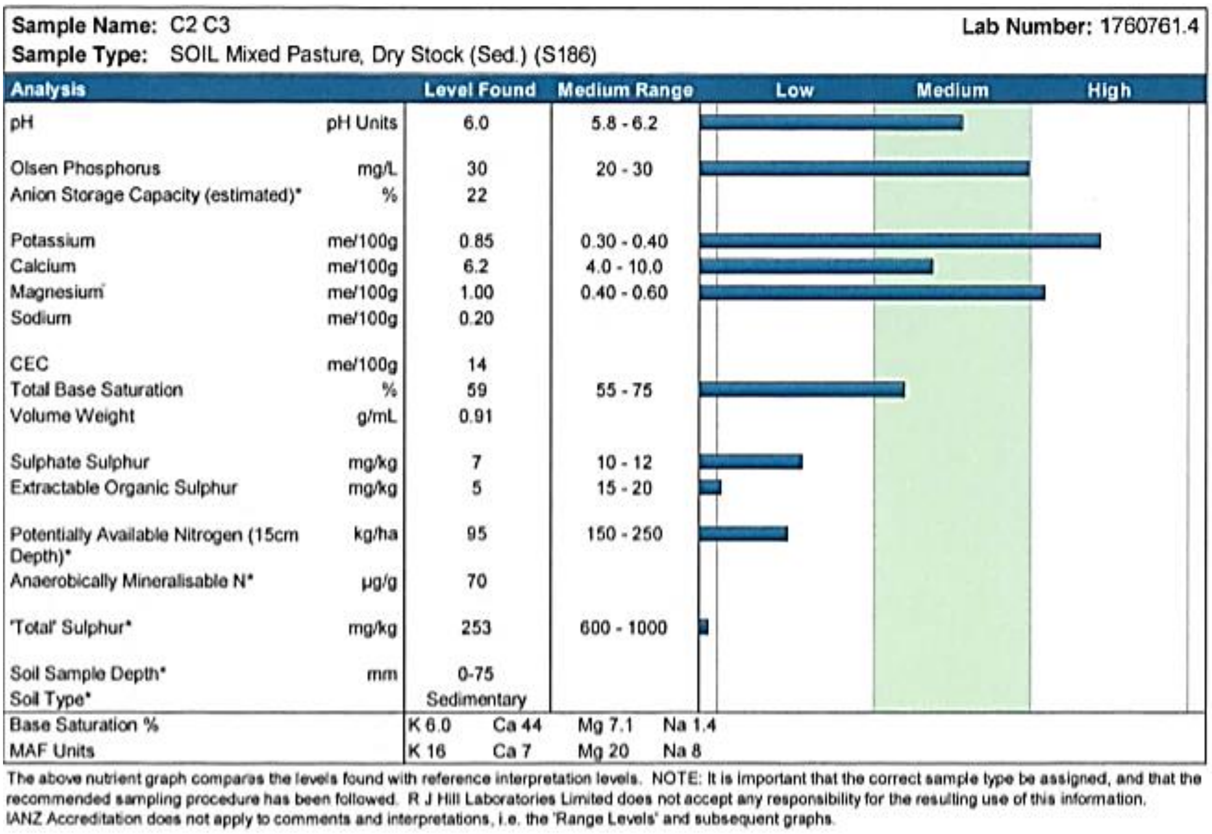
The above nutrient graph compares the levels found with reference interpretation levels. NOTE: It is important that the correct sample type be assigned, and that the recommended sampling procedure has been followed. R J Hill Laboratories Limited does not accept any responsibility for the resulting use of this information. IANZ Accreditation does not apply to comments and interpretations, i.e. the 'Range Levels' and subsequent graphs.



Sample Name: C2 C1 **Lab Number:** 1760761.3
Sample Type: SOIL Mixed Pasture, Dry Stock (Sed.) (S186)

Analysis		Level Found	Medium Range
pH	pH Units	6.1	5.8 - 6.2
Olsen Phosphorus	mg/L	37	20 - 30
Anion Storage Capacity (estimated)*	%	23	
Potassium	me/100g	1.24	0.30 - 0.40
Calcium	me/100g	7.1	4.0 - 10.0
Magnesium	me/100g	0.84	0.40 - 0.60
Sodium	me/100g	0.15	
CEC	me/100g	14	
Total Base Saturation	%	67	55 - 75
Volume Weight	g/mL	0.93	
Sulphate Sulphur	mg/kg	9	10 - 12
Extractable Organic Sulphur	mg/kg	6	15 - 20
Potentially Available Nitrogen (15cm Depth)*	kg/ha	100	150 - 250
Anaerobically Mineralisable N*	µg/g	72	
'Total' Sulphur*	mg/kg	279	600 - 1000
Soil Sample Depth*	mm	0-75	
Soil Type*		Sedimentary	
Base Saturation %		K 8.9 Ca 51	Mg 6.0 Na 1.1
MAF Units		K 24 Ca 8	Mg 18 Na 6

The above nutrient graph compares the levels found with reference interpretation levels. NOTE: It is important that the correct sample type be assigned, and that the recommended sampling procedure has been followed. R J Hill Laboratories Limited does not accept any responsibility for the resulting use of this information.
IANZ Accreditation does not apply to comments and interpretations, i.e. the 'Range Levels' and subsequent graphs.



Sample Name: C2 C3 **Lab Number:** 1760761.4

Sample Type: SOIL Mixed Pasture, Dry Stock (Sed.) (S186)

Analysis		Level Found	Medium Range
pH	pH Units	6.0	5.8 - 6.2
Olsen Phosphorus	mg/L	30	20 - 30
Anion Storage Capacity (estimated)*	%	22	
Potassium	me/100g	0.85	0.30 - 0.40
Calcium	me/100g	6.2	4.0 - 10.0
Magnesium	me/100g	1.00	0.40 - 0.60
Sodium	me/100g	0.20	
CEC	me/100g	14	
Total Base Saturation	%	59	55 - 75
Volume Weight	g/mL	0.91	
Sulphate Sulphur	mg/kg	7	10 - 12
Extractable Organic Sulphur	mg/kg	5	15 - 20
Potentially Available Nitrogen (15cm Depth)*	kg/ha	95	150 - 250
Anaerobically Mineralisable N*	µg/g	70	
'Total' Sulphur*	mg/kg	253	600 - 1000
Soil Sample Depth*	mm	0-75	
Soil Type*		Sedimentary	

Base Saturation %	K 6.0	Ca 44	Mg 7.1	Na 1.4
MAF Units	K 16	Ca 7	Mg 20	Na 8

The above nutrient graph compares the levels found with reference interpretation levels. NOTE: It is important that the correct sample type be assigned, and that the recommended sampling procedure has been followed. R J Hill Laboratories Limited does not accept any responsibility for the resulting use of this information. IANZ Accreditation does not apply to comments and interpretations, i.e. the 'Range Levels' and subsequent graphs.