Final report

Novel dual purpose perennial cereals for grazing

Project code: P.PSH.1036

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Abstract

This project arose due to a growing interest in perennial grains to improve environmental outcomes of grain production systems. Previous research identified that deployment of perennial wheat in Australia would likely be as a dual-purpose grain and graze crop. However, no previous research had examined the suitability of perennial wheat forage for grazing livestock. As with perennial grass-based pastures, perennial crops are envisaged to be grown in mixtures with a legume, rather than as monocultures like conventional crops, to maximise productivity over the longer term and reduce inputs, such as nitrogenous fertiliser.

A multi-disciplinary team spanning agroecology, agronomy, mineral nutrition, sheep health and meat science, came together to deliver a program of research that involved many discreet experiments conducted over a 5-year period, 2018-2022. Two intensive feeding studies, plus a final grazing experiment in 2021, were used to monitor sheep responses to various wheat or perennial wheat diets fed with and without legumes and/or mineral supplements. Field experiments were established to evaluate different perennial wheat breeding material, their compatibility with legumes, the effects of common agronomic practices such as nitrogen fertiliser and plant density, and screen the mineral profile of a range of candidate legumes.

This project established the suitability of perennial wheat forage for sheep, which was observed to be quite similar in quality and mineral profile to conventional grazing wheat. The inclusion of a legume increased dietary intake, enhanced the concentration of health-claimable compounds in the meat of grazing lambs and reduced the need for mineral supplementation compared to a pure cereal diet, but grain yields were generally reduced. Lucerne was suggested to be a poor choice of companion due to its low forage sodium (Na) concentrations and perennial growth habit. Self-regenerating annual legumes such as subterranean clover or serradella, both of which have higher forage Na concentrations than lucerne, may be better companions with perennial wheat, although this needs to be validated over a longer timeframe.

Executive Summary

The *Novel dual-purpose perennial cereals for grazing* project, P.PSH.1036, set out to establish the feasibility of perennial wheat as a viable dual-purpose, grain and graze crop for Australian environments. It drew together a multi-disciplinary team to examine aspects of perennial wheat agronomy as well as consequences to sheep health and meat quality after grazing perennial wheat. It established a series of three feeding experiments in 2019-2021 beginning with pen studies where either lambs or late-gestation, twin-bearing ewes were provided different forages in a cut and carry system, culminating in a field experiment where lamb grazing behaviour was more representative of a paddock environment. In addition, 12 'agronomy' experiments were established to compare attributes of diverse perennial wheat breeding lines and examine the compatibility of a range of legume species to be grown as companions with perennial wheat. Agronomy practices, such as planting density and nitrogen nutrition, were also examined on a subset of perennial lines.

The project confirmed the suitability of perennial wheat as a dual-purpose crop when grazed by sheep. Lambs were similarly productive on perennial wheat compared to conventional grazing wheat and both forages produced lamb meat categorised as 'good everyday eating quality'. Compared to annual wheat, perennial wheat forage offered more calcium (Ca), magnesium (Mg), potassium (K) and less sodium (Na) and phosphorus (P). Similar to annual wheat, the mineral profile of perennial wheat forage was imbalanced with excessive concentrations of K and insufficient Na, presenting a risk of metabolic disorders such as hypocalcaemia and hypomagnesaemia to vulnerable livestock. This mineral imbalance is managed in conventional grazing cereals by the provision of mineral supplements including lime ($CaCO_3$), causmag (MgO) and salt (NaCl). Perennial wheat is envisaged to be grown in mixtures, such as with legumes, and it was hypothesised that grazing a cereal/legume biculture diet would reduce the risk of metabolic disorder and the need for mineral supplements. The series of experiments conducted throughout the project generally supported the hypothesis, noting that legumes could not entirely eliminate all mineral supplements required by high-producing livestock and that, at times, the inclusion of legumes reduced overall biomass production.

The provision of lucerne in diets of the feeding studies that included either lambs (2019) or late-gestation twin-bearing ewes (2020) increased daily intake, as well as the forage Ca:P ratio and lowered the tetany index compared to a pure cereal diet. The addition of lucerne alleviated the need for Ca supplementation but despite some improvement in Na and Mg supply, was insufficient to meet total requirements and so supplementation of those minerals was still required. Although the concentration of Na in lucerne forage was an order of magnitude higher than that found in cereal forage, it was still deficient for animal requirements. Providing Na lowered the K:Na + Mg ratio and offering Na with lucerne lowered that ratio further, but neither Na supplementation nor adding lucerne lowered the K:Na ratio or the dietary cation-anion difference (DCAD) sufficiently. Other legumes, such as subterranean clover or serradella, were found to have higher concentrations of Na than lucerne, offering scope for further exploration into the mineral balance brought about by biculture forage mixes

Analysis of carcass traits from the lamb feeding study showed that the concentration of saturated and omega-6 polyunsaturated fatty acids, as well as of thiobarbituric reactive substances and vitamin E were higher when lucerne was included, compared to a pure cereal diet. This is an important finding as it demonstrates an opportunity for farmers to select production systems to enhance the concentration of health-claimable compounds in the meat of grazing lambs. Whether this result is specific to lucerne or if the inclusion of other legume species would lead to a similar outcome remains to be determined.

An objective was to inform choice of companion species to grow with perennial wheat. Although far from an exhaustive evaluation of all possible legume options, some useful insights are offered. Lucerne represents a less desirable alternative, especially in semi-arid environments. In addition to its low forage Na concentration outlined above, lucerne is a strong competitor that dries the soil over summer and reduces resources available to the perennial wheat. Well-adapted self-regenerating species, such as subterranean clover or serradella, may reduce the level of competition over summer and improve survival of perennial wheat under dry summer conditions. However, competition still exists with any companion species, and the lower year 1 grain yields in the perennial wheat plus subterranean clover treatment in the grazing experiment is a reminder that any well-adapted species can inhibit performance of other species in mixtures. Competition is dynamic among plants and this question warrants continued investigation over a longer timeframe. Vetch and field pea are both species that warrant a mention as they represent a different class of legume that may offer appeal in some systems. Both species can be harvested for seed/grain and offer potential for a second grain harvest from the same field if grown in mixtures with perennial wheat. Strategies to manage such a system on a commercial scale require significant further research.

The optimal target for legume abundance in mixtures with perennial wheat was also examined, as trade-offs in production are inevitable. We now know that sowing legumes in alternate drill rows with a cereal is ill-advised, due to the negative impacts on production. This is highlighted in the 2021 grazing experiment where perennial wheat grain yield was, on average, 33% lower in mixtures with a legume compared to pure perennial wheat. In mixtures, perennial wheat was the dominant species contributing more biomass than the legume component. Care is therefore required when substituting perennial wheat with a less productive legume species, as total biomass may ultimately be reduced. To avoid undue yield reductions, the legume should comprise <50 % of the mixture. This conclusion is supported by the feeding studies in which lucerne never comprised more than about 50% of the *ad libitum* diet, under a cut and carry regime. A realistic target range for legumes is likely to be around 25 - 40%, being mindful that the higher the legume content within this range, the more nitrogen fixation that will occur and the greater impact the legume will have in balancing the mineral profile of the cereal-based diet.

Several unanticipated scientific outputs flowed from the network of experiments, which promise to add further value to industry. For example, initial evidence suggests that monitoring pH decline in the lower value and more accessible *semitendinosus* muscle of lamb carcases may be just as effective as sampling of *longissimus lumborum* muscle, as is present industry practice. If validated across a broader range of lambs, this finding serves to reduce costs to meat processors. Similarly, the 2020 feeding experiment presented an opportunity to evaluate the effectiveness of measuring the health status of late gestation ewes using the Optium Neo™ handheld device to monitor blood glucose and *β*-hydroxybutyrate (BHB) concentrations. Results showed that readings from this device could be sufficiently accurate, once suitable correction factors are applied and more data is recorded across a wider range of concentrations, paving the way for better on-farm monitoring of some metabolic disorders in vulnerable livestock.

Synergies with multiple other initiatives enabled the team to leverage additional investment over the life of the project to increase the collective output and add value to the broader research program. Notable among these was the Agrifutures project PRJ-012277 *Assessing artisan perennial wheat material as a new food crop*, led by Matthew Newell. Surplus grain, derived in part from seed multiplication activities in the existing project, was used to examine the grain functionality and potential end-use in food applications. In a similar vein, an Honours project at the University of Tasmania utilised grain from four agronomy experiments to explore the baking properties of perennial wheat. Collectively, both initiatives provide increased confidence in the marketability of perennial wheat, aiding future adoption by farmers.

The feeding studies provided opportunities to add value to other projects by additional sampling of the lambs under investigation. A project variation sought by the MLA-sponsored B.GBP.0024 'Better doers' project enabled sampling of the rumen wall and analysis of the rumen epithelium of lambs in the 2019 feeding study and demonstrated that nutrient transporters in the rumen wall varied with diet. Carcases of the same lambs were also sampled for two other projects led by Stephanie Fowler to relate carcase characteristics to spectrometry data. The 2019 feeding study fostered another Honours project, this time with Charles Sturt University.

Substantial obstacles were overcome to deliver beyond contracted obligations. These included the fundamental requirement for seed multiplication, as no external perennial wheat seed source was available to underpin research activities. A paucity of seed early in the project prompted some creative thinking by the project team to utilise the scarce resource efficiently, such as planting the seed multiplication blocks as replicated field experiments that were concurrently used as evaluation experiments to compare different perennial wheat lines. The severe drought year of 2019 posed risks to all field activities planned that year, most significantly to the first pen feeding study. The installation of temporary irrigation infrastructure helped mitigate risks to the feeding study. New methodology in the pen-feeding experiments demanded a lot of effort in refining practices, as well as investment in new infrastructure at the Cowra Agricultural Research and Advisory Station, requiring a pilot experiment to be run in 2018. The feeding studies proved highly successful and the facility at Cowra remains available for use in future projects. Finally, COVID 19-21 presented everyone with unforeseen challenges that could not be anticipated. This placed particular pressure on the intensive feeding/grazing experiments that each had high demands on staff in and around periods of general lockdown. The dedication of the staff that worked hard to achieve project outcomes despite these challenges is gratefully acknowledged (Figure 1).

Fig. 1. The project team at NSW DPI Cowra, conducting the 2020 ewe study under COVID lock-down restrictions. L-R: Richard Hayes, Ben Holman, George Carney, David Cupitt, Gordon Refshauge, Phil Goodacre, David Hopkins, Meaghan Vials, Matthew Newell, Neil Munday, Lance Troy. Absent: Susan Langfield, Kylie Cooley, Matthew Kerr.

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1. Background

1.1 Rationale

Frequent disturbance of land by tillage and/or herbicides is necessary to cultivate annual grain and forage crops but depletes soil organic carbon and degrades soil (Crews et al. 2016). Perennial species not only protect the soil resource but increase the utilisation of water and nutrients in the soil volume through a more developed root system and longer growing season (Dunin and Passioura 2006). Scientists globally have long thought that the development of perennial grain crops would help to improve the efficiency and environmental sustainability of food production systems (Wagoner 1990) but few viable perennial grains presently exist worldwide.

Research to understand the feasibility of perennial grains in Australian farming systems has been ongoing since 2008 at the NSW Department of Primary Industries agricultural research facility at Cowra. An initial evaluation of over 150 perennial wheat derivatives showed promising results with over a third of the entries tested persisting to yield grain over two successive years in the Cowra environment, demonstrating a proof of concept that a novel perennial whet could be developed (Hayes et al. 2012). However, from the outset it was clear that for perennial grains to be commercially viable in Australia they would most likely be used as a grain and graze crop, to utilise the large amount of biomass that a perennial crop produces (Newell and Hayes 2017). Prior to P.PSH.1036 *Novel dual-purpose perennial cereals for grazing* (the project), no study had examined the suitability of perennial wheat forage for grazing livestock.

The development of perennial grains represents not only the development of a new crop but also a new cropping system (Hayes et al. 2013). Inherent in the 'perennial vision' is a shift away from production systems reliant upon monoculture crops, towards multifunctional swards grown as mixtures thought to be better placed to utilise available resources (Ryan et al. 2018). Legumes are obvious candidate companion species due to their capacity for nitrogen (N) fixation, potentially reducing inputs of fertiliser N and improving the utilisation of N in the system (Crews and Peoples 2005). Early research in Australia suggested that subterranean clover grown in mixtures with perennial wheat may be able to fix adequate N to support grain yields of 2 t/ha (Hayes et al. 2017).

1.1.1 Identifying the knowledge gap

The question in this emerging field of research was not 'what is the knowledge gap?' but rather 'which knowledge gap should we target?'. After all, this project represented a proof-of-concept that perennial grains could be a novel grain and graze option for the Australian red meat industry. To assess the likely consequences on livestock, the project relied heavily on a pilot study that examined the forage quality and mineral profile of selected perennial wheat lines and identified a potential animal health risk associated with mineral imbalances in the forage (Newell and Hayes 2017).

1.1.2 The research questions

Being the first study in the world to graze sheep on perennial wheat forage, the most pressing question was whether perennial wheat was a safe forage for sheep. If the answer was no, there was little point continuing to develop perennial wheat for a grain and graze system. The experience from grazing wheat was that risks of metabolic disorder exist due to a high potassium:sodium + magnesium (K:Na + Mg) ratio in the forage, reducing the availability of calcium (Ca) and Mg (Dove et al. 2016) increasing the risk of significant production losses in high-producing livestock such as growing lambs or late gestation/early lactation ewes. It was thought that the inclusion of a legume would improve the grazing value of perennial crops by increasing the diversity of the diet. The second research question was whether a perennial wheat/legume biculture diet would reduce the risk of metabolic disorder in livestock compared to a pure cereal, due to a changed mineral profile of the diet.

1.1.3 The target audience

The research community was the primary target audience for this pioneering research as it was acknowledged from the outset that perennial crops would not be widely available to industry in the near future. Nevertheless, some findings of this study were likely to be transferable to the mixed farming zone where dual-purpose wheat is commonly used in rotations and where there is increased interest in mixed forage crops.

2. Objectives

2.1 Contracted outputs

The project will deliver the following outputs to the red meat industry:

1. Recommendations for farmers and their advisors on how to more efficiently grow and graze dual purpose cereals with companion legumes
2. A network of stakeholder engagement sites which demonstrate the novel concept, and explore practical considerations (species selection, herbicide options, seasonal growth patterns) which farmers and advisors will need to consider
3. At least two field days annually to extend research findings to stakeholders
4. Defined benefits to meat production and trade-off's in growing cereal crops in mixtures with legumes
5. Defined thresholds of the legume abundance required in a cereal-based diet in order for financial benefits in lamb production to be achieved
6. Defined meat quality parameters of pH, intra-muscular fat content, colour and tenderness for animals produced from grazing cereals
7. At least 3 scientific conference papers within the life of the project describing the results of growing dual-purpose cereal crops in mixtures
8. At least 2 papers within the life of the project submitted to an appropriate international scientific journal
9. A plain-English fact sheet delivered at the end of the project which describes the trade-off's and key learnings of growing dual purpose crops with companion legumes

2.1 Success in meeting objectives

2.1.1 Recommendations for farmers and their advisors on how to more efficiently grow and graze dual purpose cereals with companion legumes

This objective was met. The grazing experiment conducted in 2021 demonstrated crop yields and lamb production potential of perennial wheat grown in mixtures with either lucerne, subterranean clover or French serradella compared to perennial wheat grown alone. It showed that where legumes were grown with perennial wheat, grain yield was reduced. In part this was due to the sowing configuration where legumes were sown in alternate drill rows with the crop. This had a negative effect on crop yields because perennial wheat was the dominant species in the sward but sown at only half the sowing rate in the mixtures treatments compared to where it was sown alone. However, legume choice also played a part. Grain yields were reduced further where subterranean clover was sown, due to a high level of competition with the crop in spring.

In addition, the range of agronomy experiments assists in identifying potential companion legumes to be grown with perennial wheat. For example, vetch was shown to be more productive than most, highlighting a potential to increase legume biomass and nitrogen inputs. Along with field pea, vetch offers some prospect of harvesting seed, which, when combined with cereal grain yield, was shown to outyield other legume mixtures. The option to augment the cereal grain yield with legume seed yield is not readily available with most forage legume options. However, greater biomass in vetch and field pea treatments did lead to greater competition with perennial wheat, which sometimes reduced total biomass production.

Experiments demonstrated that all cereals tested, including barley, Kernza and mountain rye, presented a similar risk of metabolic disorder due to consistently low concentrations of forage sodium. Results suggest that there is little prospect for selecting perennial wheat genotypes that might offer a lower risk of metabolic disorder in grazing livestock. By contrast, large site effects were evident in the mineral profile data indicating that the risk of metabolic disorder on grazing cereals is not uniform across soil types and environments. Farmers are advised that livestock management on novel perennial cereals should be similar to existing practices used for conventional grazing wheat.

The agronomy experiments highlighted the different mineral profiles of the range of legume species available that may be used to mitigate risks of metabolic disorder in livestock. Subterranean clover and French serradella exhibited substantially higher concentrations of forage Na and Mg compared to lucerne, justifying their inclusion in the 2021 grazing experiment.

Taken together, the recommendation for farmers looking to grow dual purpose cereals with companion legumes is to **proceed cautiously**. The inclusion of legumes increased forage intake and improved the quality of the diet through changed mineral profile of the diet and, in the case of subterranean clover and serradella in the grazing experiment, increased metabolizable energy. However, the inclusion of the companion species must not come at the expense of cereal production, otherwise the yield penalty observed may off-set any benefit of a mixed planting. We now know that planting the cereal and legume in alternate drill rows is an unwise approach. Rather, farmers need to maximise plant cover by reducing drill row spacings and all species should be planted in all drill rows to maximise production. Some legumes, such as subterranean clover, may benefit from having additional seed scattered between the drill rows to further increase plant cover. Evidence supporting this recommendation was presented in detail in the Milestone 5 report drawing on a broader range of experiments (Hayes et al. 2017; 2022). Results from the 2021 grazing study (see section 4.5) certainly support a cautious approach.

2.1.2 A network of stakeholder engagement sites which demonstrate the novel concept, and explore practical considerations…

This objective was met. At its conclusion, the project maintained six experimental sites spanning NSW DPI research stations at Cowra, Orange, Glen Innes and Wagga Wagga, as well as two remote sites located on private farms at Dry Plain (Cooma) and Mandurama.

The strategic location of experiments at Research Stations fostered many visits from a range of stakeholders over the life of the project, including (at different times) the NSW Minister for Agriculture, the Governor-General, high level public servants working for Federal Government agencies, industry representatives as well as the general public at open days and special events. In addition, the project team collaborated with the Central West LLS and the Grassland Society of NSW to conduct three field days at the Mandurama site, the latest occurring on 27 May 2022.

2.1.3 At least two field days annually to extend research findings to stakeholders

The Project exceeded this objective (see Table 1). The list of extension events includes more than just field days, as was necessary during COVID lockdown periods, and covers diverse aspects of perennial crop management including mineral balance of forage, grain production and mixtures with legumes. Several of the communication initiatives remain available on various websites (Table 1).

Table 1. Major communication events showcasing the perennial cereals research over the life of the project.

Date	Event	Location	Details
21/9/19	DPI open day	Cowra Research and Advisory Station	R. Hayes & G. Refshauge; approx. 80 community members, overview of perennial wheat research
18/10/19	Southern Advisory Committee meeting	Mandurama	R. Hayes and M. Newell; SAC members, overview of research and site inspection
22/10/19	LLS/Delta Ag field walk	Mandurama field site	M. Newell & R. Hayes; approx. 20 advisors and producers, overview and inspection of perennial wheat field trials
30/10/19	LLS/DPI paddock walk	Glen Innes Research Station	R. Hayes; approx. 20 advisors and producers, overview and inspection of perennial wheat field trials
10/9/20	NSW DPI Central Cluster Research Roundup	Webinar	Matt Newell presented 'Novel dual-purpose perennial cereals for grazing'
10/9/20	NSW DPI Central Cluster Research Roundup	Webinar	Gordon Refshauge presented 'Plasma and urine mineral balance in lambs fed perennial wheat with and without lucerne'
10/9/20	NSW DPI Central Cluster Research Roundup	Webinar	Ben Holman presented 'Quality and mineral composition of loin and topside cuts from lambs fed perennial wheat'
9/11/20	Annual meeting of the Tri Societies	Virtual Conference, (USA)	Richard Hayes presented 'Managing competition in perennial polycultures through changed spatial configurations at sowing'.
9/11/20	Annual meeting of the Tri Societies	Virtual Conference, (USA)	Matt Newell presented 'Novel dual purpose perennial wheat for grazing'.
9/11/20	Annual meeting of the Tri Societies	Virtual Conference, (USA)	Ke Hong (Alexis) Tang presented 'Investigating the grain functionality of perennial wheat.'
16/11/20	Site visits by the Senior Management of the Federal Dept.	Cowra Agricultural Research and Advisory Station	Hayes/Newell/Refshauge/Holman/Fowler 'Overview of perennial wheat research'

	Agriculture, Water and Environment		
20/11/20	LPP Southern Advisory Committee	Canberra	Presentation to leading producers and consultants and discussion about the project.
2/2/21	33rd Conference of the Australian Association of Animal Sciences	Fremantle/Virtual	Steph Fowler presented the paper to over 200 conference delegates.
9/3/21	Riverina LLS Pasture research update	Griffith	Gordon Refshauge presented 'Mineral balance in sheep grazing cereals', (approximately 60 growers and advisors)
9/3/21	Riverina LLS Pasture research update	Griffith	Richard Hayes presented 'Getting the most out of lucerne pastures'
10/3/21	Riverina LLS Pasture research update	Wagga Wagga	Gordon Refshauge presented 'Mineral balance in sheep grazing cereals', (approximately 140 growers and advisors)[1]
10/3/21	Riverina LLS Pasture research update	Wagga Wagga	Richard Hayes presented 'Getting the most out of lucerne pastures'[2]
24/3/21	Australian Grasslands Association	Virtual symposium	Richard Hayes presented 'Sowing configuration changes competition and persistence of lucerne in mixed pasture swards'[3] (87 views as at 14 July 21)
25/3/21	SALRC SNSW Regional Committee meeting	Orange	Presentation by Matt Newell providing an overview of the LPP Legume, perennial grains and tropical pastures projects (19 committee members and guests present)
15/6/21	Perennial Artisan Grains Conference	Cowra/virtual	Matt Newell presented 'Multifunctional perennial grain systems for Australia' (40 attendees in total)
15/7/21	NSW DPI Science Matters forum	Cowra/virtual	Matt Newell presented an overview of the perennial grains research to 40+ staff across NSW DPI.
10/10/21	Evoke Ag Podcast	Virtual	Matt Newell described the prospects for perennial grains in Australia[4]
27/5/22	CW LLS Field walk	Mandurama	Matt Newell & Richard Hayes present final results and discuss prospects and challenges of perennial grains in Australia

[1] https://www.lls.nsw.gov.au/regions/riverina/articles,-plans-and-publications/2021-riverina-pastures-research-update/mineral-balance-in-sheep-grazing-cereals-gordon-refshauge-dpi-riverina-pastures-research-update

[2] https://www.lls.nsw.gov.au/regions/riverina/articles,-plans-and-publications/2021-riverina-pastures-research-update/Getting-the-most-out-of-lucerne-pastures-Richard-Hayes-DPI

[3] https://youtu.be/l8mgYDHSjng

[4] https://podcasts.apple.com/us/podcast/perennial-wheats-purpose-bred-for-sustainability/id1458966229?i=1000540674970

1/6/22	Riverina LLS Pasture Research Update	Wagga Wagga	Gordon Refshauge described the use of legumes to mitigate the risk of mineral disorder in livestock, 180 attendees[5]
2/6/22	Riverina LLS Pasture Research Update	Whitton	Gordon Refshauge described the use of legumes to mitigate the risk of mineral disorder in livestock, 55 attendees

2.1.4 Defined benefits to meat production and trade-offs in growing cereal crops in mixtures with legumes

The gross margin analysis helps to illustrate the trade-off in meat production in growing cereal crops in mixtures with legumes, which is associated with reduced biomass production that leads to reduced stocking rates and to lower grain yields. Although not a comprehensive economic analysis, the gross margin presents a message of caution about growing grazing cereals in mixtures with legumes. A more sophisticated analysis is required to take account of financial implications of a perennial cereal system over a longer time period so that sowing costs etc are appropriately depreciated and that other system benefits, such as nitrogen cycling, can be properly included. These items have undoubtedly skewed the present analysis.

Nevertheless, there is evidence of improvements in health-claimable compounds such as polyunsaturated fatty acids where lucerne is added compared to a pure cereal diet (Holman et al. 2022). Despite the fact that these benefits were not rewarded with higher carcass prices in these experiments, positive health attributes are seen as an advantage for future marketability of Australian lamb and need to be quantified. Whether similar benefits can be observed through feeding legume species other than lucerne remains the topic of ongoing investigation and is one aspect being explored in the analysis of the 2021 grazing experiment. Indeed, preliminary analysis of the fatty acid data suggests that lambs grazing perennial wheat, with or without a companion legume, will produce meat that is a classifiable as a ‘good source of health claimable omega-3 fatty acids’.

2.1.5 Defined thresholds of the legume abundance required in a cereal-based diet in order for financial benefits in lamb production to be achieved

The question of optimum legume abundance is somewhat context specific. For example, maximising N inputs to soil requires a higher proportion of legume in the sward, but maximising livestock production may require a lower legume content in some situations, to maximise biomass production. From our range of experiments, we can say that legumes should comprise no more than 50% of a cereal-based sward. In our stage 2 (2019) feeding study, lucerne comprised <50 % of total intake in the diets in which it was offered (Newell et al. 2020). In the Stage 3 (2020) study, legume comprised slightly more than 50 % of intake when lucerne was provided. Under paddock conditions in the Stage 4 (2021) grazing study where lambs could graze and select their diet under more normal conditions, preliminary results suggest that legume intake was substantially less than 50 %. There is little point growing vastly more legume than livestock will eat, especially when this leads to reduced total production as was observed in the lucerne abundance experiments. On the other hand, too little legume over the long term constrains production through inadequate inputs of nitrogen (Hayes

[5] 2022 Riverina Pastures Research Update - Presentation - Website - Local Land Services (nsw.gov.au)

et al. 2017). As we continue to refine our understanding with further analysis of all the experiments, we expect that optimal legume abundance of a cereal-based sward to be around 25-40 % of total biomass.

2.1.6 Defined meat quality parameters of pH, intra-muscular fat content, colour and tenderness for animals produced from grazing cereals

This objective has been met with results documented in detail in several earlier scientific publications from the lamb feeding study conducted in 2019 (Holman et al. 2021a; 2021b; 2022; Newell et al. 2020b). In summary, carcasses of saleable weight and quality were produced when lambs were fed perennial wheat, with or without lucerne. These parameters were assessed as hot carcass weight, dressing percentage, cold carcass weight, tissue depth at the GR site, subcutaneous fat coverage, eye muscle area, pH decline rates, glycogen concentrations, and fresh colour measures. An untrained consumer panel confirmed perennial wheat to have no detrimental effects on the eating quality of meat from grazing lambs. These findings were affirmed by comparisons to consumer thresholds for tenderness, overall liking, and acceptance of retail colour – based on intramuscular fat content, shear force, particle size, a* (redness) values, etc. The mineral associated nutritional value of the topside and loin cuts were comparable for all the experimental lambs and within expected ranges for Australian lambs. The effect of wheat type on the concentration of fatty acids in the meat of grazing lambs were observed to be minimal, especially when compared to the observed effects of lucerne on the SFA and n-6 PUFA concentrations in the meat. TBARS and vitamin E concentrations in the meat of grazing lambs were increased with the inclusion of lucerne, demonstrating a dietary effect on the oxidative status of meat.

Preliminary analysis of the data from the 2021 grazing experiment has shown that lambs grazing perennial wheat can deliver carcasses with weights, fat coverage, and compositions that align with industry expectations and past research of Australian prime lambs. Evaluation of pH decline parameters demonstrated that all the lambs, irrespective of legume companion species, could provide meat of acceptable tenderness, colour, and eating quality. Laboratory analyses for these same parameters (i.e., cooking loss, drip loss, intramuscular fat, purge, sarcomere length, shear force, total moisture, and total volatile basic nitrogen) provide some evidence for consumer satisfaction with meat from lambs grazing perennial wheat. Comparisons further demonstrated a robust shelf-life for the meat from lambs grazing perennial wheat, with eating quality and spoilage markers within acceptable ranges even after 56 days of refrigerated storage (wet ageing). The mineral composition of lamb meat was consistent between the perennial wheat combinations, each of which supported lamb meat that was a rich dietary source for iron and zinc. The fatty acid profile of meat from all the experimental lambs confirmed their classification as ‘a good source of healthy omega-3 fatty acids’. In addition, there was a trend whereby total omega-6 fatty acid concentrations were lower in the meat from lambs grazing a cereal only diet, compared to those grazing combinations of perennial wheat and legume. More sophisticated analysis will help to extrapolate the significance of this outcome.

2.1.7 At least 3 scientific conference papers within the life of the project describing the results of growing dual-purpose cereal crops in mixtures

The project has exceeded this objective, delivering a total of seven Conference papers over the project life. For convenience, citations to all conference papers reporting results from this project are listed below (in alphabetical order):

1. Fowler SM, Holman BWB, Newell MT, Refshauge G, Hayes RC, Hopkins DL (2021). Prediction of eating quality of lamb loin using Raman Spectroscopic technologies. Proceedings of the '33rd Conference of the Australian Association of Animal Sciences', 1-3rd February 2021, Fremantle p. 89.
2. Fowler SM, Holman BWB, Newell MT, Refshauge G, Hayes RC, Morris S, Hopkins DL (2021). Preliminary investigation into the prediction of and factors influencing the eating quality of lamb loin using Raman spectroscopy. Proceedings of the '67th International Congress of Meat Science and Technology', 23-27th August 2021, Warsaw: Poland.
3. Holman BWB, Hayes RC, Newell MT, Hopkins DL, Refshauge G (2022). The carcass and meat quality of Australian lambs diagnosed with acute, subclinical hypocalcaemia. Proceedings of the '34th Conference of the Australian Association of Animal Sciences', 5-7th July 2022, Cairns: Aus. p. lxxxi.
4. Holman BWB, Refshauge G, Newell MT, Hopkins DL, Hayes RC (2022). The meat quality of lambs grazing perennial wheat with different companion legumes. Proceedings and Abstracts of the 68th International Congress of Meat Science and Technology, August 22-25, Kobe, Japan.
5. Newell M, Holman B, Refshauge G, Hopkins D, Hayes R (2020) Novel dual purpose perennial wheat for grazing. Annual meeting of the tri societies (American Society of Agronomy, Crop Science Society of America, Soil Science Society of America) Arizona, USA (Virtual meeting), 9-13 November.
6. Newell MT, Munday N, Hayes RC (2023). The effect of nitrogen rates and plant density on grain yield components and persistence in intermediate wheatgrass (*Thinopyrum intermedium*) and mountain rye (*Secale strictum*). Proceedings of the International Grasslands Congress, Covington, KY, USA. (in press).
7. Refshauge G, Holman BWB, Newell MT, Hopkins DL, McGrath SM, Vials M, Bond J, Hayes RC (2022). The accuracy of the Optium Neo™ handheld glucose-ketone meter for testing ewe blood samples. Proceedings of the '34th Conference of the Australian Association of Animal Sciences', 5-7th July 2022, Cairns: AUS. p. xlv.
8. Tang KH, Penrose B, Hayes RC, Wilson M, Blanchard CL, Newell MT (2020). Investigating the grain functionality of perennial wheat. Annual meeting of the tri societies (American Society of Agronomy, Crop Science Society of America, Soil Science Society of America) Arizona, USA (Virtual meeting), 9-13 November.

2.1.8 At least 2 papers within the life of the project submitted to an appropriate international scientific journal

The project has exceeded this objective with five scientific journal articles already published, all from the 2019 lamb feeding study. For convenience, those publications are listed here. In addition to those listed are the draft scientific publications appended to this report (Fowler et al. 2023; Pleming et al. 2023; Appendices 1-3).

1. Holman BWB, Fowler SM, Refshauge G, Hayes RC, Newell MT, Clayton EH, Bailes KL, Hopkins DL. (2022). The effect of perennial and annual wheat forages, fed with or without lucerne, on the fatty acid profile and oxidative status of lamb meat. *Veterinary and Animal Science,* **15**, 100230. doi:https://doi.org/10.1016/j.vas.2022.100230
2. Holman BWB, Hayes RC, Newell MT, Refshauge G, McGrath SR, Fowler SM, Shanley AR, Hopkins DL. (2021). The quality and mineral composition of the longissimus lumborum and semimembranosus muscles from lambs fed perennial or annual wheat forage with or without lucerne. *Meat Science,* **180**, 108564. doi:https://doi.org/10.1016/j.meatsci.2021.108564
3. Holman BWB, Kerr MJ, Refshauge G, Diffey SM, Hayes RC, Newell MT, Hopkins DL. (2021). Post-mortem pH decline in lamb semitendinosus muscle and its relationship to the pH decline parameters of the longissimus lumborum muscle: A pilot study. *Meat Science,* **176**, 108473. doi:https://doi.org/10.1016/j.meatsci.2021.108473
4. Newell MT, Holman BWB, Refshauge G, Shanley AR, Hopkins DL, Hayes RC. (2020). The effect of a perennial wheat and lucerne biculture diet on feed intake, growth rate and carcass characteristics of Australian lambs. *Small Ruminant Research,* **192**, 106235. doi:https://doi.org/10.1016/j.smallrumres.2020.106235
5. Refshauge G, Newell MT, Hopkins DL, Holman BWB, Morris S, Hayes RC. (2022). The plasma and urine mineral status of lambs offered diets of perennial wheat or annual wheat, with or without lucerne. *Small Ruminant Research,* **209**, 106639. doi:https://doi.org/10.1016/j.smallrumres.2022.106639

2.1.9 A plain-English fact sheet delivered at the end of the project which describes the trade-off's and key learnings of growing dual purpose crops with companion legumes

Three Plain English Fact sheets have been developed as PrimeFacts, following the NSW DPI template (Appendices 3-5). Pending internal approval, these FactSheets will be made available alongside other resources on the NSW DPI website.

3. Methodology

This multi-disciplinary study comprises several distinct components spanning the fields of agronomy, livestock production and meat quality that focus on particular aspects of the perennial cropping system. Progress on each activity is reported below.

One change that occurred since the milestones were initially written was a re-ordering of the sequence of feeding experiments. The milestones are written as if the sheep nutrition (ewe) study was to precede the meat quality study conducted with lambs. However, early in the project a decision was taken, in consultation with the Southern Advisory Committee, to reverse this order. The justification was that there was uncertainty in the methodology to be used in the feeding experiments and embarking on such an experiment with twin-bearing ewes which are more vulnerable to metabolic disorder than lambs was seen as a high-risk approach, both to animal welfare and to achieving the research outcomes. Therefore, when interpreting the milestones, the ‘sheep nutrition experiment’ should substitute the ‘meat quality experiment’, and *vice-versa*. Those terms have been substituted in the remainder of this report.

3.1 Seed multiplication

The perennial grains study is unique because there is no commercial seed production of perennial cereals, therefore seed supplies need to be generated in-house. Experimentation is therefore constrained by seed production, and activities in future years are impacted by yields in the previous year.

Two approaches have been taken for seed multiplication in this project. The first is a series of dedicated seed multiplication plantings which are managed as a commercial grain crop. The second is through grain harvested as a by-product from experimental plots conducted under other activities in the project.

3.2 Agronomic evaluation

Several types of agronomic field evaluation experiments were established to improve our understanding of the relative performance of best bet perennial wheat germplasm, and their interaction with companion legumes. All experiment types assist in the seed multiplication of particular perennial wheat lines, as well as with increasing stakeholder engagement as they are a focal point of field activity across a relatively diverse geographic footprint. The companion legumes provide a relative assessment of the legume species that might be considered as companions in perennial wheat crops, and the legume abundance experiments examine the relative competition between the legume and the crop. Considering these experiments together it is envisaged that the project can answer practical questions about perennial wheat agronomy, specifically: which crop? Which legume? And how much of each might be grown?

3.2.1 Perennial wheat evaluations

Two experiments were established in 2018 with four additional experiments sown in 2019. Plots were 1.8 x 7.5 m and sown as pure stands with a cone seeder, replicated three times. Evaluation experiments established in both years (Table 2) compared seven perennial wheat derivatives with two perennial grasses (Mountain Rye and Kernza (CPI-148055) and three annual cereals (wheat cv. Wedgetail, barley cv. Hindmarsh and triticale cv Endeavour) The dual-purpose oat variety Eurabbie was include at Mandurama in 2018. The 2018 evaluations were sown at the Mandurama site and Cowra Agricultural Research and Advisory Station on 3 and 17 May, respectively. Both sites received an application of 2L/ha Tri-allate (500g/L) and 2L/ha glyphosate prior to sowing with a cone seeder fitted with narrow points and press wheels on 18cm row spacings. Fertiliser was applied at 120kg/ha Starter 15 (15% N, 13% P) and seeding rate of each entry was adjusted based on seed size and germination to target 150 plants/m^2. A subset of these evaluation entries was sown at Cowra, Orange, Glen Innes and Dry Plains (near Cooma) in 2019 (Table 1) and followed a similar process to the 2018-sown experiments.

Dry matter assessments were taken at growth stage 30 (Zadocks *et.al* 1974) and again at anthesis for each entry at each site. Samples from the early dry matter assessment were analysed for forage mineral content to determine difference between species and site on macronutrient content, particularly calcium, magnesium, potassium and sodium which is of relevance to the feeding studies in this project. All entries were harvested by hand due to the large difference in maturity times between entries. Yield components were determined (seed size, tiller number harvest index) and final grain yield recorded. Entries were monitored for persistence over summer with further dry matter assessments taken in March at each site. Sites continued to be monitored for persistence in subsequent years following establishment. Grain samples from the Cowra and Mandurama sites in 2018, and Cowra and Orange sites in 2019, were used as the basis of an Honours project (K H Tang, University of Tasmania) examining grain quality, and are reported in a manuscript presently in preparation for *Frontiers in Plant Science* (Appendix 2).

Table 2. Entries included in the perennial wheat evaluation experiments sown in 2018 and 2019 at Cowra (Ca), Mandurama (Ma), Orange (Oe), Glen Innes (GI) and Dry Plain (DP).

Line	Pedigree	Ca18	Ma18	Ca19	Oe19	GI19	DP19
11955	*Triticum*/Agropyron hybrid (ex. USA)	●	●	●	●	●	●
20238	*T. durum*/*Th. elongatum* (ex. Mexico)	●	●	●	●		●
CPI-148055	*Thinopyrum intermedium* (ex. USA)	●	●	●	●	●	●
CPI-147235a	*T. aestivum*/*Th. ponticum* (ex. USA)	●	●	●	●	●	●
CPI-147251b	*T. aestivum*/*Th. intermedium* (ex. USA)	●	●	●	●		
OK7211542	*T. aestivum*/*Th. ponticum* (ex. USA)	●	●	●	●		●
Summer 1	*T. aestivum*/*Th. intermedium* (ex. China)	●	●	●	●		
Ot-38	*T. aestivum*/*Th. intermedium* (ex. Russia)	●	●	●	●		
Ostan (915)	*T. aestivum*/*Th. intermedium* (ex. Russia)	●					
P15	*T. aestivum*/*Th. intermedium* (ex. USA)	●					
M706-M3	*T. aestivum*/*Th. intermedium* (ex. USA)	●					
Wedgetail	*T. aestivum*	●	●	●	●		●
Hindmarsh	*Hordeum vulgare*		●	●	●		
Endeavour	*TriticoSecale* X	●	●	●	●	●	●
Eurabbie	*Avena sativa*		●				
Mtn rye	*Secale montanum* (ex. Australia)			●	●	●	●

For determination of mineral profile, samples of each species were collected at growth stage 25 (Zadoks et al. 1974) at Cowra and growth stage 30 at Orange and Dry Plain. Samples were dried at 60°C for 48 hrs. Ground samples were analysed by Department Primary Industries AgEnviro laboratory, Wollongbar, NSW to determine N, P, S, Cu, Zn, Mn, K, Na, Mg, Ca, B, Fe and nitrate contents, through closed vessel microwave nitric acid and hydrogen peroxide digestion (SPAC 1998) and processed using ICP-OES (Agilent 5110, ICP_OES). Chloride was extracted in 1:125 water solution using the ferricyanide method (SPAC 1998) on a flow injection analyser (QuikChem 8000, Lachat).

Mineral indices were determined as follows: The K:(Na+Mg) ratio was calculated from the percentage of mineral in the dry matter using the formula (K/0.039)/[(Na/0.023)+(Mg/0.012)] (Dove *et al.* 2016). The tetany index was calculated as (K/0.039)/[(Mg/0.012)+(Ca/0.02)] (Kemp and 't Hart 1957) and the dietary cation anion difference (DCAD; meq/100 g) was calculated using the formula (Na/0.023 + K/0.039)-(Cl/0.0355 +S/0.016) (Takagi and Block 1991). The Ca:P ratio was calculated from the percentage of each mineral in the diet.

3.2.2 Companion legume experiments

Two legume compatibility experiments were sown at the Cowra Agricultural Research and Advisory Station, one in 2019 and one in 2020. The experiments were sown in replicated, randomised designs in 1.8 m x 7.5 m plots. Cereal crops, which included grazing wheat (*Triticum aestivum* cv EGA Wedgetail), a perennial wheat hybrid (*T. aestivum* x *Agropyron* spp. breeding line 11955) and a Kernza breeding line (*Thinopyrum intermedium* CPI 148055) were highly contrasting in their early vigour and capacity to persist. Legumes included subterranean clover (*Trifolium subterraneum*), white clover (*T. repens*), red clover (*T. pratense*), lucerne (*Medicago sativa*), birdsfoot trefoil (*Lotus corniculatus*), vetch (*Vicia sativa*) and field pea (*Pisum sativum*).

The 2019 experiment was sown 30th April 2019 in an area which had been chemically fallowed for 12 months. Immediately prior to sowing 2L/ha of diquat (115g/L) + paraquat (135g/L) was applied and 100kg/ha of triple superphosphate (17%P, 11%S) was broadcast to the area. The trial was sown with a seeder which had two seed delivery cones, each connected to five sowing tynes. Tyne arrangements were set on 17cm spacings fitted with narrow points and press wheels. Each species was confined to alternate rows so that the cereals and legumes were spatially separated in different drill rows. Sowing rates of each species were half the nominal seeding rate per hectare so the plant density per meter of row was maintained as if the same species was sown in every row. Following sowing, 40ml/ha bifenthrin (250 g/L) and 400ml/ha chlorpyrifos (500g/L) was applied. The experiment had to be resown in 2020 due to severe drought conditions in 2019. In 2020, 2L/ha Tri-allate (500g/L) and 100kg/ha Starter 15 (14%N, 12%P) was applied prior to sowing. The field pea treatment was substituted with a wide row (34cm) cereal-only treatment with no companion legume, to provide a comparison of crop response at that lower plant density. The Wedgetail treatments were resown into existing plots in 2021 which contained the regrowing legumes from the previous year. All companion vetch treatments were also resown in 2021 as they did not regenerate and 80kg/ha of DAP (18%N, 20%P) was broadcast in all plots prior to sowing.

All treatments were harvested using a Kingaroy plot harvester. In 2019 harvesting occurred on the 12 November 10 December, and 6 February 2020 for Wedgetail, 11955 and Kernza treatments, respectively. Vetch and field pea grain was harvested at the same time as the cereals and separated to calculate yields of each species. Harvesting followed a similar pattern in 2020 and 2021 based on the maturity of each cereal genotype.

A further experiment established at Mandurama under another LPP project, P.PSH.1030 *Extending the boundaries of legume adaptation through better soil management*, was sampled in spring 2019 to compare herbage mineral concentrations among a broader range of species. The experiment was sown at Mandurama on 2 April 2019 and included 20 legume species (Table 3) grown in 1.8 x 7.5 m plots, replicated 3 times. The paddock in which the trial was located, had lime applied at 2.5t/ha and incorporated by chisel plough approximately three months before sowing. One week before sowing, 2L/ha glyphosate (450g/L) plus 2L/ha trifluralin (480g/L) were applied to the area and incorporated by harrows. Immediately prior to sowing 2L/ha of diquat (115g/L) + paraquat (135g/L) was applied followed by 40ml/ha bifenthrin (250 g/L) and 400ml/ha chlorpyrifos (500g/L) after sowing.

Table 3. List of legume treatments planted at Mandurama (in project P.PSH.1030) to be sampled for forage quality analysis in spring 2019.

Species	Cultivar/line	Comment
Caucasian clover	Kuratas	A strong perennial in alpine environments, limited testing of forage quality
Caucasian x white clover hybrid	Aberlasting	From the UK, a genuinely novel perennial legume option
Lucerne	SARDI Grazer	A 6-7 winter activity type selected specifically for its grazing tolerance
Lucerne	Titan 9	A more winter active type compared to SARDI Grazer
French serradella	Margurita	A self-regenerating annual legume. Sampling this adds value to the RnD4Profit P-efficient pastures project also.
Yellow serradella	Avila	A self-regenerating annual legume with promising persistence in higher rainfall environments of NSW
Yellow serradella	Yellotas	A self-regenerating annual legume with promising persistence in higher rainfall environments of NSW. Limited testing of forage quality.
Red clover	Relish	Selected for its tolerance to grazing
Red clover	Rubitas	Selected for adaptation to higher rainfall environments
Sainfoin	Melrose	A species identified as of interest by the Advisory Committee, this species reportedly exhibits non-bloating compounds
Strawberry clover	Palestine	A relatively common legume species in Australia, but relatively few reports on forage quality
Subterranean clover	Leura	A common legume used in grazing systems
Sulfur clover	Tas0433	A new perennial legume species that was promising in previous evaluations (Li *et al*. 2008)
Talish clover	Permatas	A new perennial legume species
Birdsfoot trefoil	LC07AUYF	Bred for lower rainfall regions and known to exhibit non-bloating compounds (tannins)
White clover	Haifa	The most common cultivar used in Australia
White clover	Trophy	Commercially unavailable, but arguably the most advanced cultivar for persistence under Australian conditions
White clover	Tribute	Arguably, the most advanced cultivar for New Zealand conditions
White clover	Nomad	A cultivar commonly sold in Australia
White clover	Storm	A cultivar commonly sold in Australia

3.2.3 Lucerne abundance experiments

Two experiments were established on 11 and 15 April 2019 at Cowra and Mandurama respectively, in 3.6 x 7.5 m plots on 17cm row spacings, both in a replicated randomised block design. Plots of annual wheat (cv, Wedgetail), perennial wheat (11955) and lucerne (cv. Titan 9) were established as monocultures sown at 71, 62 and 10 kg/ha respectively. The lucerne seed was inoculated with the Group AL rhizobia 4 days prior to sowing with slurry of 4g peat / 100g seed. A further set of treatments used combinations of wheat or perennial wheat sown with lucerne in different row configurations. The sowing row combinations were varied to produce alternate rows of cereal with lucerne (1:1), two rows of cereal next to one row of lucerne (2:1) or one row of cereal next to two rows of lucerne (1:2). Sowing rates of each species were reduced on an area bases in the row spacing treatments, so as to maintain the same density of seedlings that were established per meter of row in the monoculture plots. The trials were established with 100kg/ha triple superphosphate (18%P, 11%S) applied immediately prior to sowing. For each wheat and perennial wheat monoculture plots, separate treatments were imposed where nitrogen was applied (+N) or withheld (–N). The added nitrogen treatments received 100kg/ha urea on 26 June at Mandurama and 28 June at Cowra in 2019. Because of drought in 2019, all cereal treatments were resown in the first week of April 2020, following the same process as above into the established lucerne plots at the appropriate row configuration. 100kg/ha Starter 15 (14% N,12% P) was applied to all plots at planting. A split application of urea was applied to the +N cereal treatments at the end of June and beginning of August at each site with 50kg/ha N applied on each occasion. In 2021, the cv. Wedgetail plots were re-seeded into the existing Wedgetail treatments in the third week of April at both sites. 80kg/ha of DAP (18%N, 20%P) was broadcast to all treatments prior to sowing. Again, a split application of 100kg/ha urea was applied to the +N treatments with similar timing as described for 2020 at both sites.

Estimates of biomass were taken at anthesis for each cereal species by cutting two 0.5 m x 0.5 m quadrats per plot to a height of ~ 10 mm. Samples were separated for cereal and lucerne and dried to determine dry matter production, expressed as kg/ha for each species. Due to the difference in maturity between the cereals, anthesis sampling occurred for Wedgetail on 9 and 10 September, with 11955 sampled on 14 and 29 October 2019 at Cowra and Mandurama, respectively. At maturity all plots were harvested with a Kingaroy plot harvester to determine final grain yield (kg/ha). Wedgetail plots were harvested on 5 and 11 December 2019 at Cowra and Mandurama, respectively, with the 11955 plots harvested 8 February 2020. The later-maturing perennial wheat plots were not harvested at Cowra as no seed set occurred due to the extremely dry conditions in 2019. Harvesting in 2020 and 2021 followed a similar process as detailed above, with Wedgetail wheat harvested on 4 and 14 December 2020 at Cowra and Mandurama, respectively. The later maturing perennial wheat line 11955 was harvested on 20 December at Cowra and on 19 January 2021 at Mandurama. At the Cowra trial sown in 2021, the Wedgetail treatments and perennial wheat treatments were harvested on 7 and 24 December, respectively. At Mandurama, harvest times were extended due to mild summer conditions with Wedgetail harvested on 11 January 2022 and 11955, one month later, on the 11 February.

3.2.4 Crop density

Kernza (CPI-148055) and Mountain Rye (Mtn Rye) have the greatest near-term prospect for release as perennial grains in Australia due to their superior longevity compared to hybrid perennial wheats. Both species are perennial forage grasses and little is known how to manage either species for grain production, at least in an Australian context. An experiment was established at the Orange Agricultural Institute in 2020 to examine the effect of plant population and nitrogen rates on grain yield components. The experiment was sown on 21 April in a randomised design with three replicates. The area had been chemically fallowed since September 2019 and then cultivated after 3t/ha lime ($CaCO_3$) was applied on 17 February 2020. The experiment consisted of two species (Kernza, Mtn Rye) sown at three plant populations (50, 100 and 200 plants/m^2) with three nitrogen rates (0, 100, 200 kg/ha N). The experiment was repeated in 2021 in a different field at the Orange Agricultural institute and sown using the same method and treatments as above.

3.3 Pilot feeding study (Stage 1)

A series of feeding experiments was conducted over consecutive years during this project. The 'Stage 1' experiment was a preliminary experiment conducted in 2018 designed to test the methodology used in subsequent experiments, approved by the NSW DPI Animal Ethics Committee under Authority ORA 18/21/001. At the outset of the project there was uncertainty whether we could successfully house sheep in individual pens and that they would eat the prescribed diets. The Stage 1 experiment provided confidence that the research objectives and animal welfare standards could be met using this approach (see Fig. 2). This preliminary experiment was reported in detail in the Milestone 3 report, but no further mention will be made of it here as it does not contribute directly to project outputs and outcomes.

Fig. 2. Illustrating the approach used in the pen feeding experiments, including (left) the pens that housed individual sheep for the duration of the experiment, and (right) the daily routine of weighing feed rations to be provided to each individual in large plastic tubs.

3.4 Finishing lambs on grazing cereals (Stage 2)

A pen feeding experiment, approved by the NSW DPI Animal Ethics Committee under Authority ORA 15/21/022, commenced at Cowra on 15 May 2019. Poll Dorset x Merino ewe lambs (n=48), approximately 12 weeks of age, were housed individually in adjacent pens for a period of 28 days, which included a 7-day induction period. Lambs were each fed one of four diets, which included perennial wheat (PW), annual wheat (W), perennial wheat plus lucerne (PW+L) or wheat plus lucerne (W+L) forage. Each forage type was grown on site, sown in three blocks sown at different times to vary plant physiological development at the time of cutting. Forage was cut daily using a sickle bar mower, weighed and placed in plastic tubs delivered to each lamb twice daily. Uneaten forage was collected each morning and weighed and fresh forage was provided to each lamb shortly thereafter.

Bulk samples of each forage type were taken each day and dried a 60 ^{o}C in a fan forced dehydrator to determine moisture content and later ground to pass through a 1 mm sieve. At the conclusion of the study, samples were sent to the Feed Chemistry Laboratory at Wagga Wagga and analysed for forage quality including acid and neutral detergent fibre, dry matter digestibility, crude protein, metabolizable energy and water-soluble carbohydrate using near infra-red (NIR; and also included in the LPP NIR project dataset, P.PSH.1202). A subsample of each forage sample was also sent to the Nutrient Advantage laboratory, Werribee Victoria and analysed for mineral content including P, S, Cu, Zn, Mn, K, Na, Mg, Ca, B, Fe, and nitrates using nitric acid digestion.

Samples of plasma and urine were taken from each lamb at the commencement of the study (D1) and at weekly intervals until the final sampling on D28. Lambs were walked to a nearby yard facility in replicate groups. Duplicate 9 ml jugular vein blood samples (lithium heparin) were taken from each individual lamb and stored on ice prior to centrifugation which occurred within 1 hour of sampling. After blood collection, samples of urine (up to 50 ml). Liveweight and condition score of lambs were recorded before they were loaded onto a trailer and transported back to their designated feeding pen.

At the conclusion of the feeding experiment, lambs were transported (approx. 200 km) to a commercial abattoir. Following an overnight lairage period, lambs were slaughtered as a single flock in accordance with standard industry practice. Carcasses were exposed to medium voltage electrical stimulation, underwent trimming and dressing and were held under refrigeration for 24 hr post-mortem prior to boning out and further processing. Samples retained by the research team for further analysis included paired *longissimus lumborum* (LL) and the left-side *semimembranosus* (SM) muscles. An unaged portion of the left-side LL was removed and stored at -25 ^{o}C to be later tested for mineral composition and fatty acid profile. The pH decline of the *semitendinosus* (ST) and LL muscles was recorded at 30 minutes *post-mortem* and repeated at 4 hr intervals until the final sampling at 24 hr *post-mortem*.

3.5 Balancing the nutritional requirements of ewes (Stage 3)

A second pen-feeding experiment was established at Cowra in 2020 under Animal Research Authority ORA 19/22/026. The experiment was similar in design to the Stage 2 experiment described previously but included twin-bearing ewes, a class of livestock more vulnerable to metabolic disorder associated with mineral imbalance, compared with lambs, and did not follow sheep through to slaughter as in the Stage 2 experiment. The experiment commenced on 12 May and ewes were released from the feeding pens at D21 on 2 June. The following hypotheses were tested:

1. The addition of salt to a diet of PW will improve the sodium status of late-pregnancy twin-bearing ewes.
2. The addition of lucerne to a diet of PW will improve the magnesium status of twin-bearing ewes in late-pregnancy.
3. The addition of lucerne and salt to a diet of PW will improve the sodium and magnesium status of twin-bearing ewes in late-pregnancy.

A cohort of merino ewes (n=76) were selected from the main adult ewe flock at the Cowra station, scanned pregnant with twins and of similar gestation (according to ram harness raddle marks made during mating and confirmed by two pregnancy scans estimating litter size and foetal age). Among the 76 ewes, the experimental ewes (n=48) were selected for having the smallest possible range in liveweight (14 kg). We included another 4 ewes for spares, which were the next heavier (n=2) and lighter (n=2) outside the selected 48. One ewe from the PW + S treatment was removed after three days due to low intake and not replaced, leaving 47 ewes in the study.

Ewes were housed in individual pens and allocated to one of four diets, two of which were common to the Stage 2 experiment: PW, PW + L, perennial wheat + salt (PW + S) and PW + L + S. Forage was cut with a sickle bar mower, weighed and delivered twice daily. Perennial wheat and lucerne were each grown on site in three blocks that were sown at different times to vary physiological development. Areas with a high weed burden were avoided. Each morning (9:00 – 9:30am) the feed tubs were replaced immediately after the refusals were collected (Fig. 3). The ewes were fed a second time in the afternoon, at about 3:00pm, which involved placing the fresh new forage into the existing tub, on top of any forage still in the tub. In this way, the refusals collected in the morning were from the previous 24-hour period.

Table salt (NaCl) was added to the + Salt diets. This was done by sprinkling approximately 11.3 grams onto their feed (half in the morning, half in the afternoon). The addition of lucerne was offered in a separate tub to the perennial wheat. This facilitated faster and more accurate weighing of the refusals. The PW + L + S diet had salt added to each feed type (salt sprinkled onto the forage in each tub). Similarly, in the PW + S diet, salt was sprinkled onto the forage in each tub.

Fig. 3. Illustration of (left) feeding time in the Stage 3 study where all ewes would quickly come to the front of the pen to explore the fresh forage provided. (Right) Refusals were collected each morning to calculate intake. It was common to see almost all cereal forage eaten but a high quantity of lucerne forage refused.

On the first day in the pens, the ewes were offered 5 kg fresh forage. Those on lucerne received 2.5 kg of lucerne and 2.5 kg of perennial wheat. The next day the amount increased to 10 kg for each diet, including 5 kg PW and 5 kg L in both mixed diets. Each week the amount offered increased, to 11 kg by the end of Week 2 and to 13 kg in week 3. The mixed diets were always 50:50 PW and L by weight.

Blood and urine were collected a total of FOUR times during this experiment. These include the baseline sample, and then weekly thereafter for THREE weeks (Day 0, D7, D14, D21). The baseline samples were collected and then the ewes (in groups of 6) were moved to the pens. In addition to plasma minerals, blood glucose and blood beta-hydroxybutyrate (BHB) were also measured. Blood glucose and BHB were recorded in the yards with a hand-held FreeStyle Optium Neo device, but we also tested the plasma (after centrifugation) for glucose and BHB using laboratory-controlled methods. More details are provided in Appendix 3.

From urine we collected mineral concentration and also measured specific gravity, pH and temperature. Ewes were weighed and body condition scored weekly, immediately before blood and urine collection.

At the end of the three-week pen study, the ewes were moved to a pasture paddock as one mob for lambing. Ewes were monitored daily until the end of lambing and the number of lambs born, lamb birth weight, lamb gender, date of birth and lamb weight at marking were recorded. Lambing data are not strictly related to treatment as lambing commenced 8 days after ewes were removed from pens and lasted for 12 days.

3.6 Lamb grazing study (Stage 4)

A need was identified to examine grazing behaviours under more normal paddock conditions. The pen studies were excellent in enabling a robust estimate of intake which enabled a robust analysis that related forage intake with animal health and production outcomes. However, it was acknowledged that the pen experiment was a contrived environment that may foster modified behaviour and grazing habits. To have confidence that the results were applicable to industry it was deemed necessary to move to a grazing study which enabled sheep to i) graze under more normal conditions, ii) select forage as they normally would rather than the cut forage provided in the pens, and iii) to be exposed to the diets for a longer period than was possible in the pens. This experiment was not conceived in the initial project plan but was a product of design-led thinking.

A field experiment was conducted at the Cowra Agricultural Research and Advisory Station in 2021. Plots, 10 x 100 m, were sown to one of four forages including perennial wheat only, or a mixture of PW and lucerne (cv. Titan 9; PW+Luc), PW and subterranean clover (cv. Leura; PW+Sub) or PW and French serradella (cv. Margurita; PW+Ser). In the mixtures of PW and legumes, species were spatially separated in alternate drill rows at 17 cm row spacings. Loose lick feeders were installed in plots sown only to PW to provide mineral supplement, a mixture of lime, causmag and salt, as is considered standard industry practice due to the known risks of mineral deficiency on grazing cereals. This treatment name is abbreviated to PW+min to avoid confusion with previous feeding experiments where PW was offered without supplement.

This study followed a split-split-plot design, wherein 3 ewe lambs (sub-plots) grazed each of 4 forage types (plots) that were rotationally grazed in alternating blocks (sub-plots), replicated 6 times. There were 72 first cross (Merino x White Suffolk) ewe lambs in total, that were sourced from the NSW DPI flock at Trangie. Lambs were selected from a total drop of approximately 350 head to ensure the cohort of 72 selected for the experiment had the tightest possible liveweight range (around 32 -38 kg) at the commencement of the study. An additional 72 wether lambs were also selected from the same drop and were included in the study primarily to manage pasture growth and ensure the feed did not go rank during the study. The wether lambs were excluded from the study when pasture growth rates declined, the timing of which depended on treatment. Whilst in the study, liveweight of wether lambs was recorded weekly but no other sampling was taken.

Sampling of ewe lambs occurred weekly and included recording of liveweight, urine and intravenous blood samples similar to as described for the Stages 2 and 3 studies, above. Fresh faecal samples were collected on three occasions by staff from the LPP NIR project (J. Piltz et al.), immediately after lambs were taken out of plots. At the end of each fortnight, all lambs were rotated onto the alternate plot ascribed to their respective mob. Weekly assessments of pasture included quadrat cuts both inside and outside of stock exclusion cages (see Fig. 4) to estimate pasture growth rates and relative intake, using a 0.25 x 0.25 m quadrat. Grab samples of each forage type were also taken from plots to estimate the forage quality in each plot at the start of each grazing week. Lambs entered the experiment on 8 June 2021 (D0) and were removed on 13 September (D97) for their final sampling and transported to a commercial abattoir for slaughter following an overnight lairage. Samples were retained from each carcass, similar to as described for the Stage 2 study (see Newell et al. 2020), although no samples were retained for a consumer sensory panel.

Fig. 4. Photographs of the Stage 4 grazing experiment including (left) small mobs of lambs grazing a PW+ser plot, with a stock exclusion cage in the foreground, and (right) weekly sampling of all lambs in the experiment at the temporary yard facility established adjacent to the plots.

Permanent stock exclusion areas were constructed in half of all plots (n=24; treatments replicated 3 times) to assess the grain yield potential of perennial wheat in the absence of grazing. Sheets of mesh held in place with star pickets were used to create exclusion areas that were 6 m x 4 m. Following the final grazing, additional focus areas of 6 x 4 m were selected in every plot (n=48) to monitor grain yields. Urea was applied at 150 kg/ha on 28 September 2021, just prior to rain, to a 3 x 4 m area within each of the 6 m x 4 m areas (n=72) to create a +/- nitrogen (N) contrast. Total DM and the composition of legume and cereal biomass at that time was estimated when the PW reached anthesis by taking 2 x 0.5 m^2 quadrats in each focus area, sorting legume and cereal biomass and weighing separately after drying to constant weight in a fan-forced dehydrator. At crop maturity, harvest index cuts were taken using the same approach taking care to avoid areas that were previously sampled. The remainder of grain in all plots was then harvested with a commercial Case axial flow harvester and plot yields calculated in kg/ha.

4 Results and discussion

4.1 Seed multiplication

The dedicated seed multiplication plantings that were sown are listed in Table 4. In each instance, seed multiplication was undertaken at NSW DPI research stations. The total grain produced was sufficient to satisfy all requirements for this and the sister Agrifutures Artisan grain project, PRJ-012277.

Table 4. Yields of dedicated perennial wheat seed multiplication plantings, 2018-2021.

Year	Line	Area sown (ha)	Location sown	Total grain (kg)
2018	11955	1.0	Cowra	848
2018	CPI-147235a	0.5	Cowra	180
2018	Mtn rye	0.5	Cowra	35
2018	OK7254112	0.02	Cowra	3
2018	M706-M3	0.002	Cowra	0.6
2019	11955	2.3	Cowra	550
2020	11955	3.0	Cowra	4500
2020	Kernza (CPI 148055)	0.02	Cowra	50
2020	Mtn rye	0.9	Cowra	300
2020	11955	0.7	Wagga	1000
2020	CPI-147235a	1.25	Wagga	1500
2020	OT-38	0.16	Wagga	180
2020	OK7254112	0.16	Wagga	150
2020	Mtn rye	0.59	Wagga	200
2020	20238	0.16	Wagga	90
2020	Summer 1	0.16	Wagga	800
2020	Kernza (CPI 148055)	0.33	Wagga	45
2021	11955	0.7	Wagga	600
2021	CPI-147235a	1.25	Wagga	20
2021	OT-38	0.16	Wagga	22
2021	OK7254112	0.16	Wagga	26
2021	Mtn rye	0.59	Wagga	122
2021	20238	0.16	Wagga	57

2021	Summer 1	0.16	Wagga	90
2021	Kernza (CPI 148055)	0.33	Wagga	85
2021	Kernza (CPI 148055)	0.02	Cowra	42
2021	Mtn rye	0.9	Cowra	140
2021	11955	0.0135	Cowra	14.5
2021	OT-38	0.0067	Cowra	1.1
2021	OK7254112	0.16	Cowra	13.4
2021	CPI-147235a	0.0135	Cowra	6.9
2021	20238	0.0135	Cowra	14
2021	Summer 1	0.0135	Cowra	45
2021	Kernza (C51549/C56573)	0.0135	Cowra	12.5
2021	16F 3570	0.0135	Cowra	40
2021	16F 3852	0.0135	Cowra	24
2021	CPI-147280b	0.0135	Cowra	21
2021	11955	5	Cowra	6500

#WSU = Washington State University Bread Lab

*TLI = The Land Institute, Kansas.

4.2 Agronomic evaluation

4.2.1 Perennial wheat evaluations

Yield results for four sites are presented in Figure 5. The experimental year of 2018 was characterised by lower-than-average rainfall which had a negative impact on grain yields at both Cowra (Fig. 5a) and Mandurama (Fig. 5b), especially for later-maturing lines. In general, grain yields were higher for the annual cereals (Wedgetail wheat, Endeavour triticale, Eurabbie oat and Hindmarsh barley). The better yielding perennial wheat lines were Summer 1 (43% and 56% of the yield of Wedgetail at Cowra and Mandurama, respectively) and 11955 (36% and 33% of the yield of Wedgetail at Cowra and Mandurama, respectively), which had consistently higher grain yields than the other perennial genotypes at both sites. A grain yield target of 40% of an annual wheat is thought to be profitable for perennial cereals as long as there is greater dry matter production compared to conventional wheat that can be utilised by grazing animals (Bell et al. 2008). In 2019 there was a worsening of drought conditions which had a greater effect on perennial wheat yields compared to the 2018 experiments (Figs 5c & d). In both years low rainfall and high temperatures restricted regrowth in the year subsequent to establishment, however there was some regrowth in genotypes at the Orange site. Similar results were observed at the Dry Plain site near Cooma, however, a late frost severely impacted grain yields with only Mtn Rye producing grain which could be harvested (data not shown). There was better yields and persistence into 2020 from the subset of perennial wheat genotypes included at Glen Innes in 2019, presumably due to cooler and wetter conditions over summer (data not shown).

The herbage mineral profile was determined at three sites to identify genotypes that may be of lower risk of metabolic disorder in grazing livestock. Broadly speaking, there was little material difference among the lines tested. All lines exhibited very low Na concentrations at all sites, which generally pushed the important metabolic indices beyond established thresholds (Table 5). Notably, there was little advantage in mineral profile of other cereal species such as Kernza, barley or mountain rye compared to the wheat-derived lines. More commonly, there was a significant site effect on mineral profile most likely suggesting differences in mineral availability attributable to soil attributes. For example, K concentrations were generally higher at Cowra compared to either Dry Plain or Orange; concentrations of Mg were generally lower at the Orange site. Whilst there is little in these data to suggest opportunity to target specific lines for lower risk of metabolic disorder in grazing livestock, it serves as a reminder that risk of metabolic disorder will vary markedly with soil type and environment.

The postgraduate project examined grain attributes in more detail, extending beyond the scope of the present project but adding significant value to the broader research program. A brief summary of that component of work is copied below, with results described in more detail in Appendix 2.

Perennial cereals are new to modern agriculture but are being developed to improve the environmental sustainability of grain production systems. For viable perennial crops to be developed, knowledge of the end-use potential of the grain is warranted. This study examined the suitability of several perennial wheat breeding lines for baking bread. Several breeding lines exhibited better baking performance than conventional bread wheat, with good loaf volume and appealing loaf colour and crumb structure. Other breeding lines demonstrated inferior dough and baking properties. The perennial wheats differed from the expected behaviours attributed to conventional bread wheat for a range of attributes. For example, perennial wheats with softer grain and only moderate gluten strength unexpectedly exhibited better baking performance than the conventional wheat control. Furthermore, milling yield and flour water absorption were found to decrease with increasing grain hardness, the opposite of that normally observed for annual wheat. These results cast some doubt over the suitability of applying relationships developed for bread wheat to perennial wheat. Overall, this study has demonstrated encouraging results from some breeding lines, suggesting that with appropriate breeding along with refinement of baking practices, perennial wheat has potential to deliver highly functional grain for food applications.

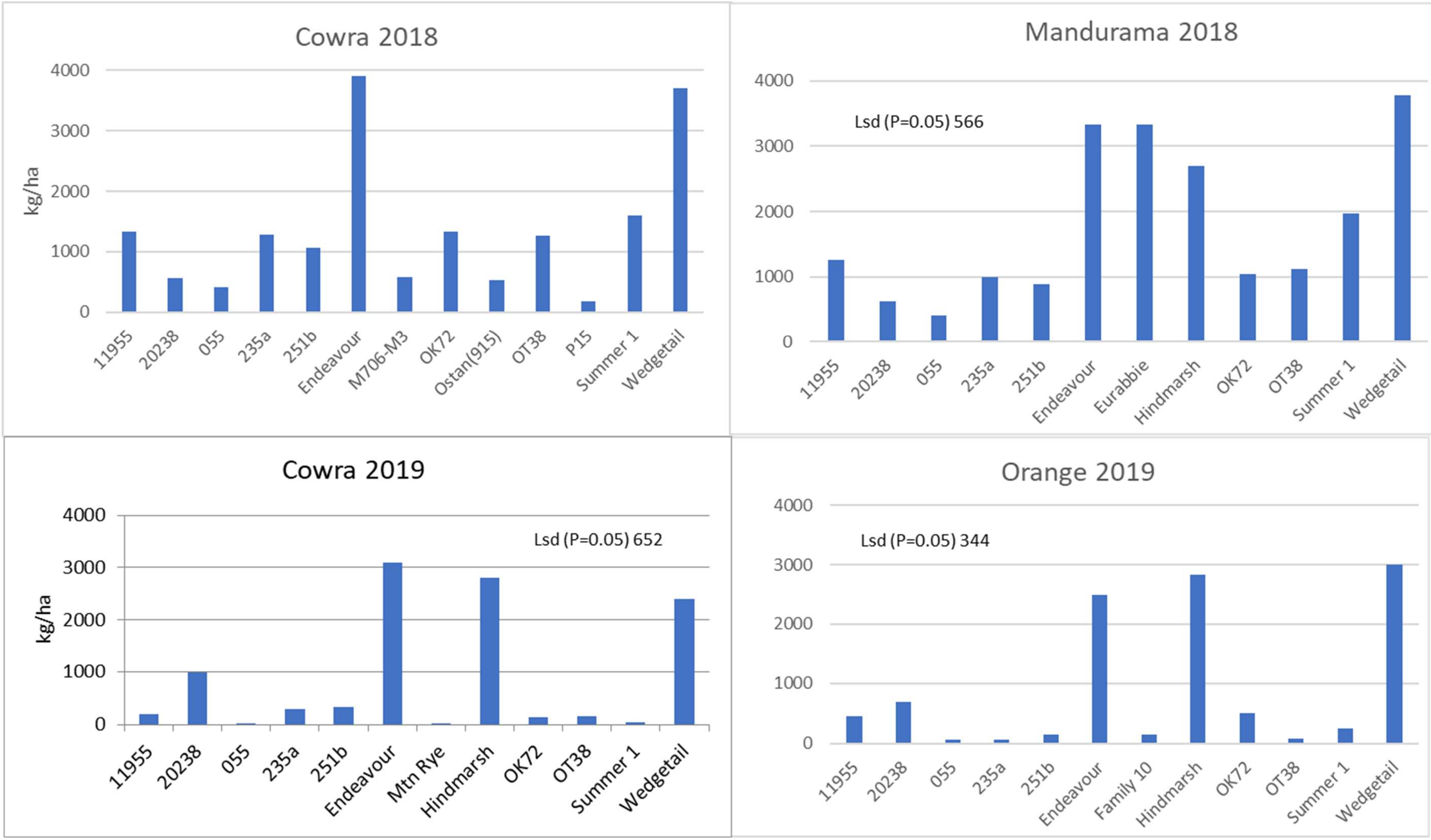

Fig. 5. grain yields for perennial grain genotypes compared to annual cereals (Endeavour triticale, Wedgetail wheat, Hindmarsh barley and Eurabbie oat at a) Cowra 2018, b) Mandurama 2018, c) Cowra 2019 and d) Orange 2019.

Table 5. Forage concentration of calcium (Ca), magnesium (Mg), phosphorous (P), potassium (K), sodium (Na) and sulfur (S) from Cowra (CARS) , Orange (OAI) and Dry Plain (DP) field evaluations, and mineral indices which reflect risk of mineral imbalance (DCAD, dietary cation-anion difference). Figures in bold are beyond established thresholds.

Entry	Ca			Mg			K			Na			Ca:P			DCAD			Tetany			K: (Na+Mg)		
Requirement	>0.28[A]			>0.09[A]			<3[A]			>0.06[A]			1.2[A]			<12[B]			<2.2[C]			<6[D]		
Site	CARS	OAI	DP	CARS	OAI	DP	CARS	OAI	DP	CARS	OAI	DP	CARS	OAI	DP	CARS	OAI	DP	CARS	OAI	DP	CARS	OAI	DP
Kernza	0.42		0.38	0.17		0.17	2.90		1.27	**0.004**		**0.012**	1.33			**32.88**		4.50	**2.30**		0.75	**6.27**		1.33
11955	0.41	0.31	0.31	0.17	0.10	0.17	**3.80**	2.70	1.70	**0.006**	**0.010**	**0.014**	1.75	1.66		**51.9**	**39.7**	**15.13**	**3.15**	**3.07**	1.32	**9.00**	**9.16**	2.45
20238	0.41	0.36	0.34	0.17	0.10	0.17	**3.90**	**3.10**	1.57	**0.022**	**0.027**	0.214	1.55	1.73		**48.68**	**12.59**	**12.59**	**3.42**	**3.31**	1.10	**10.17**	**11.06**	1.47
235a	0.44		0.26	0.17		0.17	2.63		1.50	**0.004**		**0.016**	1.95			**28.24**		8.92	2.06		1.18	**6.15**		1.90
251b	0.37			0.17			**3.13**			**0.005**			1.55			**41.38**			**2.97**			**8.53**		
Triticale	0.36	0.28	**0.12**	0.17	0.10	0.17	**4.13**	1.47	**3.13**	**0.007**	**0.010**	**0.007**	1.18	1.09		**56.3**	**50.45**	**12.77**	**3.71**	**3.60**	**2.21**	**9.64**	**9.44**	3.38
Mtn Rye	0.45		**0.25**	0.17		0.17	**3.23**		1.43	**0.006**		**0.006**	1.30			**41.14**		9.52	**2.36**		1.17	**6.37**		1.94
Barley	0.64	0.50		0.18	0.11		**4.00**	2.33		**0.021**	**0.052**		2.40			**43.1**	**34.4**		**2.21**	1.73		**6.67**	**5.16**	
OK 72	0.42	0.37	**0.24**	0.15	**0.08**	0.17	**3.57**	2.27	1.40	**0.005**	**0.016**	**0.010**	1.89	1.87		**45.05**	**27.95**	8.77	**2.84**	2.12	1.35	**8.08**	**6.13**	2.34
OT 38	0.39	0.28		0.14	**0.07**		2.73	2.12		**0.004**	**0.009**		1.39	1.29		**28.61**	**30.7**		**2.27**	**2.47**		**6.20**	**7.08**	
Summer 1	0.41	0.36		0.14	**0.07**		**3.17**	2.79		**0.005**	**0.009**		1.33	1.50		**35.69**		**39.99**	**2.59**	**2.82**		**7.35**	**8.75**	
Wedgetail	0.39	**0.25**	**0.19**	0.15	0.09	0.15	**3.30**	2.70	1.30	**0.009**	**0.015**	**0.018**	1.54	1.23		**41.27**	**41.83**	6.52	**2.84**	**3.56**	1.35	**7.95**	**9.40**	2.09
Lsd (5%)	0.07			ns			0.52			0.040			0.30			12.03			0.57			1.75		

[A] Requirement for a 50kg lamb, derived from National Research Council (2007)

[B] Estimated from Takagi and Block (1991)

[C] Kemp and t' Hart (1957)

[D] (Dove *et al.* 2016)

4.2.2 Companion legume experiments

The longer-term goal for perennial cereals is to combine them with other functional species to increase resource use efficiency and reduce inputs (Hayes et al. 2017; Ryan et al. 2018). In this instance a mix of perennial wheat and legumes could supply nitrogen to support crop growth and improve forage quality for grazing. Here we investigated the productivity of seven legume species in combination with a hybrid perennial wheat derivative (11955), a perennial grass selected for grain yield (Kernza) and an annual wheat (cv. Wedgetail). Cereal and companion legumes were sown in separate drill rows to reduce interspecific competition. Legume biomass measured at cereal anthesis was observed to be higher when combined with Kernza compared to the other cereal genotypes (Fig. 6a & b) in the establishment year of 2019 and again when the trial was resown in 2020. Kernza has a longer maturity than 11955 or Wedgetail and had lower early dry matter production compared to the other two cereals (Fig. 6a & b). This reduced competition between Kernza and the legume species allowing an increase in legume growth. In 2021 there was significant regrowth in the Kernza, with only minor regrowth from the perennial wheat derivative (11955). Predation from mice decreased the wheat density, which reduced dry matter production at anthesis compared to 11955. This highlights an advantage of perennial crops in that, once established, they can avoid some of the risks involved in re-seeding annual crops. In contrast to previous years, Kernza in 2021 had the highest anthesis dry matter (Fig. 6c), which tended to reduce the legume growth compared to where there was less cereal growth in the other two cereal genotypes.

There were significant differences between cereal genotypes in terms of grain yield, with Wedgetail yielding 80% and 64% more than 11955 in 2019 and 2020, respectively, across companion legume treatments (Fig. 7a & b). Over the same period Kernza grain yields averaged only 109kg/ha and 317kg/ha. The higher grain yields of the perennial species in year two of the experiment were reflective of the increased rainfall in 2020. The addition of the companion legumes reduced the grain yield of the three cereal species compared to the monoculture cereal treatments in 2020 (Fig. 7b). We observed that this reduction in yield was statistically significant in the Wedgetail treatments across most companion legumes. However, there were no significant difference in grain yield between companion legume treatments compared to monoculture treatments for Kernza or 11955 in the same period. In 2021 there where reductions in grain yield from regrowing perennial grains compared to 2021 as well as in Wedgetail (Fig. 7c). In the case of 11955 and Wedgetail this was due to reduced plant populations; however, the cause is unclear in Kernza, given the higher dry matter production at anthesis. Similar observations of declining grain yields in the year after establishment have been reported in overseas studies with Kernza and possibly linked to increased intraspecific competition (Jungers et al. 2017). Of note was the opportunity to harvest grain from vetch and field pea treatments across all harvests. This augmented the cereal grain yield and, in some cases the combined grain out yielded other legume treatments, particularly when combined with Kernza.

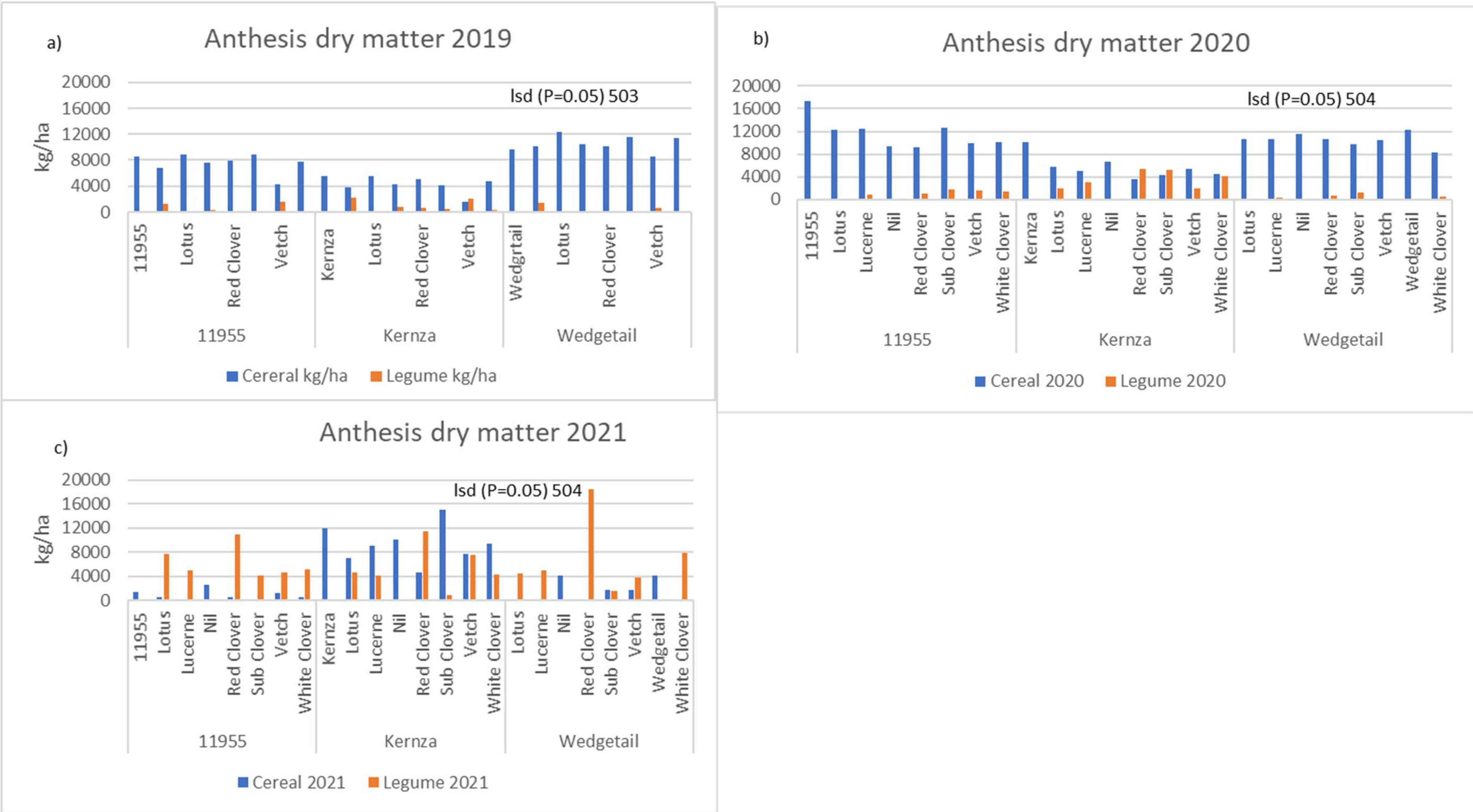

Fig. 6. Dry matter production (kg/ha) for legume species (lotus, lucerne, red clover, sub clover, vetch, white clover and field pea) grown in combination with cereals measured at anthesis for perennial wheat (11955), Kernza and annual wheat (Wedgetail) in a) 2019 (year of sowing), b) 2020 (year of sowing) and c) 2021 (year after sowing). Treatments labelled 11955, Kernza and wedgetail are monocultures of cereal and nil treatments have no companion legume with a wide cereal row spacing.

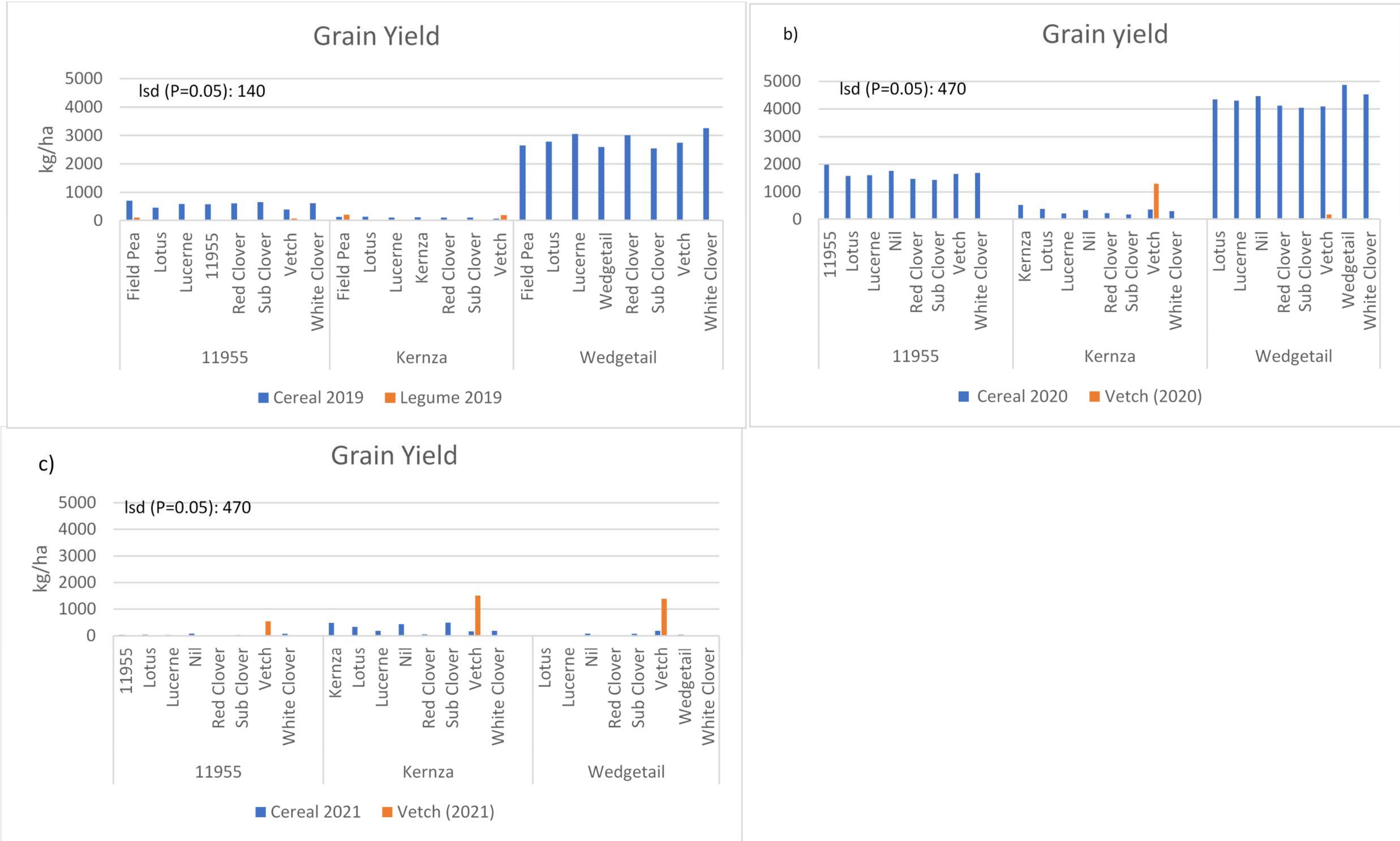

Fig. 7: Grain yield (kg/ha) for perennial wheat (11955), Kernza and annual wheat (Wedgetail) measured in a) 2019, b) 2020 and c) 2021 when grown in combination with lotus, lucerne, red clover, sub clover, vetch, white clover and field pea. Treatments labelled 11955, Kernza and Wedgetail are monocultures of cereal and nil treatments have no companion legume with a wide cereal row spacing. Grain yield of vetch and field pea are indicated.

The mineral profile among the range of legumes grown at the Mandurama site indicated a 30-fold difference in Na concentration between the lowest value in sainfoin (*Onobrychis vicifolia* cv. Othello; 0.015%) and the highest value in subterranean clover (*Trifolium subterraneum* cv. Leura; 0.50%). Herbage of the two lucerne cultivars at this site, at this one sampling time, were at around the minimum level theoretically required by actively growing lambs (National Research Council 2007; Fig. 8). Some evidence of intraspecific variation in relative Na concentration was observed in white clover (*T. repens*) which was represented by six cultivars in this experiment, and yellow serradella (*Ornithopus compressus*; n=2) although in both cases values of Na were at or above the nominal threshold. The K:Na ratio was, broadly speaking, inversely proportional to Na concentrations depicted in Fig. 8 with all cultivars shown to the right of cv. Haifa found to have a K:Na ratio ranging from 9.5 (Haifa white clover) – 1.2 (Leura subterranean clover). Given that indicative forage values grown elsewhere of wheat (0.009%) and perennial wheat (0.005%) are even lower than the lowest value legume at the Mandurama, the thinking is that a suitable companion legume would need to be at the high end of the Na spectrum in order to compensate the cereal and provide a balanced diet.

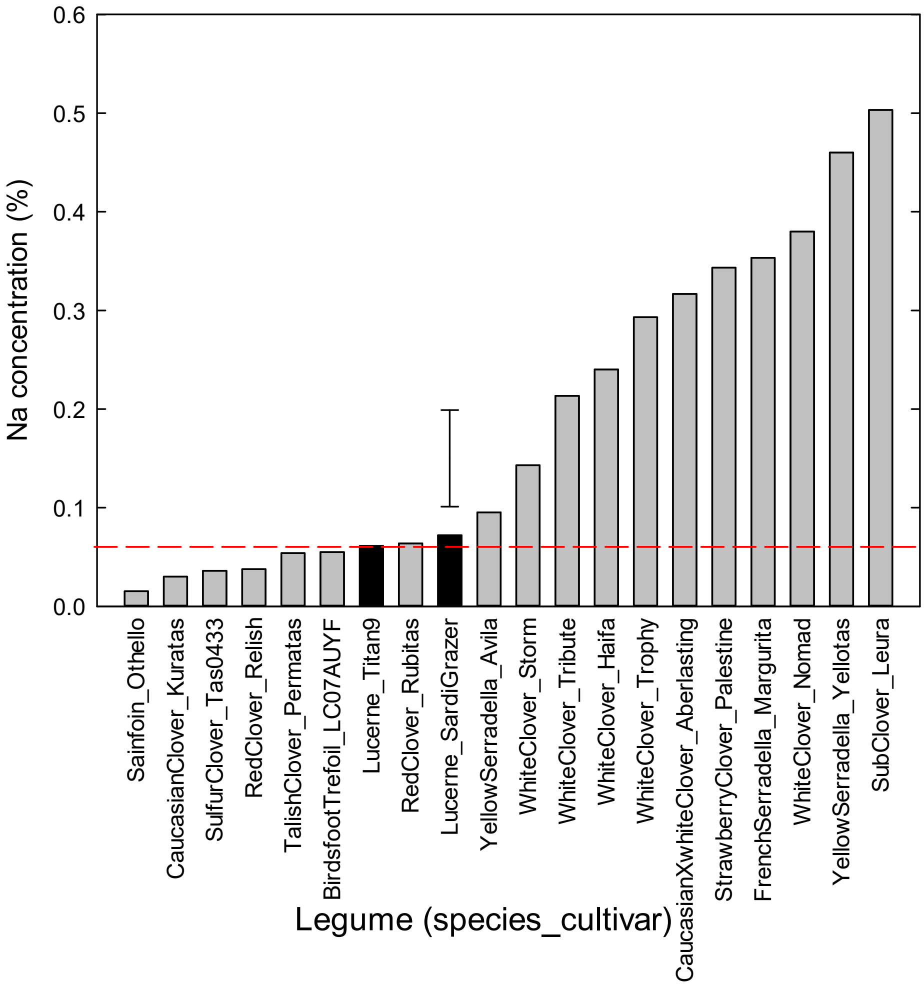

Fig. 8. **Herbage sodium (Na) concentration (%) in a range of legume species sampled in spring year 1 (2019) at the Mandurama site. Error bars show l.s.d. at *P* = 0.05.** Red dashed line indicates nominal threshold above which is required by growing lambs (National Research Council 2007).

Further examination of those data highlights the range of mineral profiles on offer in this narrow range of legume species. In terms of animal health, it is the relative balance of minerals that is of central importance in understanding the risk profile of a particular feed. Figure 9 illustrates the variation in two important mineral ratios among the legumes at Mandurama, the K:(Na+Mg) and Ca:P. A range of adapted legume species were identified as having a K:(Na+Mg) ratio below the nominal threshold for growing lambs. This is thought to be necessary in any companion legume to reduce the risk of metabolic disorder in grazing cereals. Yellow serradella (cvv. Avila and Yellotas), French serradella (cv. Margurita) and subterranean clover (cv. Leura) had among the lowest values at this sampling while both lucerne cultivars were marginally above the established threshold. By contrast, all legume species were shown to have Ca:P ratios in excess of the optimal suggested for growing lambs (Fig. 9b) with values for lucerne observed to be up to 3 times greater than for other legumes. A high Ca:P ratio generally indicates a lower P supply and is implicated in disorders such as osteoporosis and rickets in vulnerable livestock.

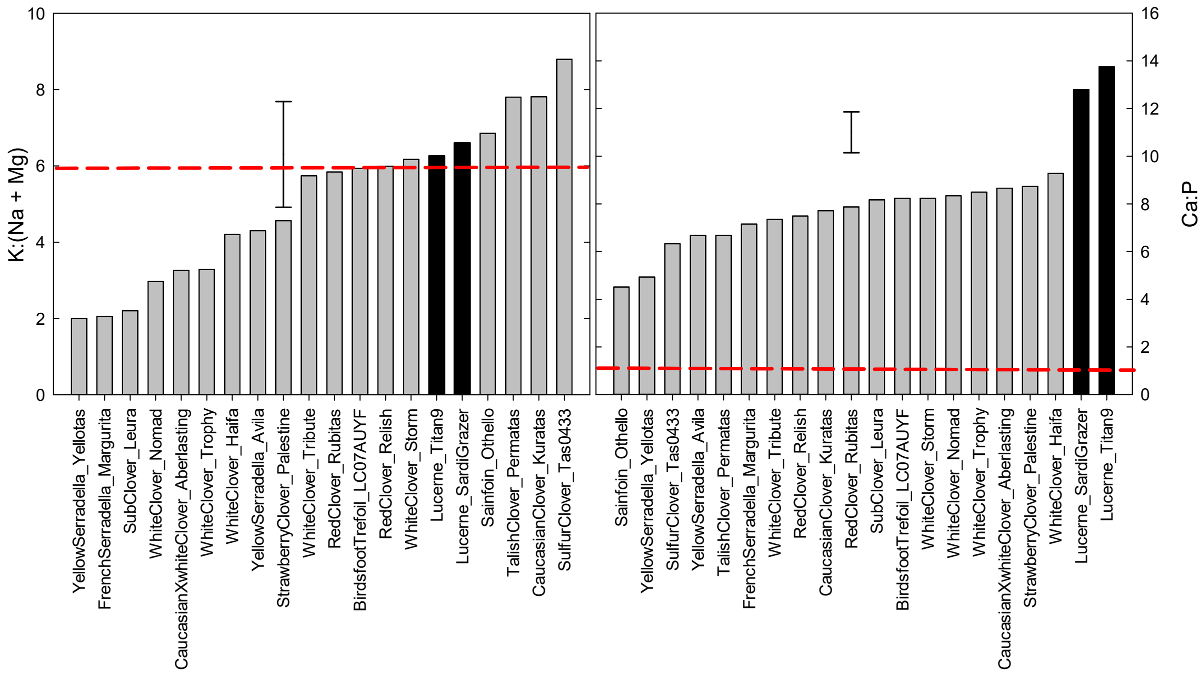

Fig. 9. Ratios of a) K:(Na+Mg) and b) Ca:P in herbage of a range of legume species sampled in spring year 1 (2019) at the Mandurama site. Error bars show l.s.d. at *P* = 0.05. Red dashed line indicates nominal threshold below which is optimal in growing lambs (National Research Council 2007).

The mineral profile of different legume species was examined over two years at Cowra. Concentrations of Na were lower than at the Mandurama experiment, perhaps reflecting differences in soil properties, and generally lower in 2020 compared to 2019, perhaps reflecting differences in growing conditions. Concentrations of Na were generally higher in subterranean clover (average 0.05% in both years) with white clover and vetch the next highest (Tables 6 and 7). The K:Na+Mg was higher in lucerne than any other species (average 7.4 and 5.0 in 2019 and 2020, respectively).

Table 6: Mean dietary concentration (%) of calcium (Ca), magnesium (Mg), phosphorous (P), potassium (K), sodium (Na) and sulfur (S), and mineral indices which reflect risk of mineral imbalance (DCAD, dietary cation-anion difference) from the cereal/legume intercropping field evaluation 2019.

	Cereal Component							
	Ca	Mg	K	Na	Ca:P	DCAD	Tetany	K:(Na+Mg)
Requirement	>0.28[A]	>0.09[A]	<3[A]	>0.06[A]	1.2[A]	<12[B]	<2.2[C]	<6[D]
11955	0.28	0.07	2.63	0.009	0.95	43.97	3.44	10.95
11955 + Field Pea	0.31	0.08	2.77	0.011	0.93	44.48	3.22	10.30
11955 + Lotus	0.28	0.06	2.30	0.009	0.96	36.39	3.11	10.97
11955 + Lucerne	0.63	0.09	2.93	0.012	2.07	49.38	2.38	9.49
11955 + Red clover	0.38	0.09	3.23	0.012	0.96	53.70	3.14	10.45
11955 + Sub Clover	0.43	0.11	3.93	0.014	0.96	65.42	3.33	10.73
11955 + Vetch	0.41	0.10	3.97	0.013	0.87	68.30	3.58	11.67
11955 + White Clover	0.42	0.11	3.87	0.013	0.85	64.81	3.36	10.57
Kernza	0.41	0.11	3.27	0.012	0.93	54.01	2.80	8.57
Kernza + Field Pea	0.39	0.12	3.70	0.015	0.86	62.88	3.22	8.92
Kernza + Lotus	0.41	0.12	3.33	0.013	0.88	51.83	2.82	8.11
Kernza + Lucerne	0.33	0.10	2.90	0.012	0.86	48.35	3.02	8.63
Kernza + Red clover	0.36	0.11	2.93	0.013	0.92	50.40	2.81	7.96
Kernza + Sub Clover	0.39	0.11	3.27	0.014	0.92	54.75	2.91	8.41
Kernza + Vetch	0.41	0.12	3.50	0.018	0.87	56.68	2.90	8.13
Kernza + White Clover	0.36	0.11	3.13	0.012	0.85	50.48	2.93	8.08
Wedgetail	0.35	0.09	3.43	0.016	0.75	62.75	3.59	11.49
Wedgetail + Field Pea	0.34	0.09	3.43	0.018	0.84	63.83	3.60	10.55
Wedgetail + Lotus	0.33	0.09	3.33	0.016	0.84	60.61	3.59	10.95
Wedgetail + Lucerne	0.37	0.09	3.57	0.016	0.82	66.56	3.51	11.21
Wedgetail + Red clover	0.35	0.09	3.37	0.015	0.89	59.68	3.53	10.90
Wedgetail + Sub clover	0.32	0.08	3.37	0.011	0.77	62.08	3.85	12.45
Wedgetail + Vetch	0.32	0.08	3.43	0.013	0.81	64.16	3.86	11.73
Wedgetail + White Clover	0.33	0.08	3.37	0.014	0.80	59.35	3.75	12.02

Lsd (5%)	0.17	0.02	0.55	0.006	0.80	11.00	0.58	1.48

	Legume Component							
	Ca	Mg	K	Na	Ca:P	DCAD	Tetany	K:(Na+Mg)
Requirement	>0.28[A]	>0.09[A]	<3[A]	>0.06[A]	1.2[A]	<12[B]	<2.2[C]	<6[D]
11955 + Field Pea	0.95	0.14	2.70	0.026	2.61	53.15	1.17	5.45
11955 + Lotus	1.20	0.25	3.30	0.023	3.33	63.57	1.05	3.88
11955 + Lucerne	1.02	0.12	3.53	0.021	2.68	64.54	2.10	9.13
11955 + Red clover	1.57	0.22	3.17	0.018	4.20	62.41	0.84	4.25
11955 + Sub Clover	1.53	0.16	3.30	0.066	4.39	63.14	0.94	5.34
11955 + Vetch	1.00	0.15	3.50	0.018	2.33	67.96	1.44	6.76
11955 + White Clover	1.43	0.17	3.40	0.032	3.34	66.74	1.01	5.52
Kernza + Field Pea	1.21	0.14	2.13	0.022	4.33	42.30	0.78	4.34
Kernza + Lotus	1.57	0.26	3.23	0.021	4.42	58.57	0.84	3.73
Kernza + Lucerne	1.70	0.13	2.73	0.019	4.52	51.06	0.73	5.91
Kernza + Red clover	1.67	0.22	2.53	0.018	5.11	50.65	0.64	3.47
Kernza + Sub Clover	1.67	0.16	2.50	0.051	6.00	48.40	0.67	4.20
Kernza + Vetch	2.17	0.20	3.03	0.039	6.15	58.07	0.62	4.31
Kernza + White Clover	1.60	0.18	2.70	0.058	5.73	53.84	0.73	3.96
Wedgetail + Field Pea	-	-	-	-	-	-	-	-
Wedgetail + Lotus	1.29	0.23	3.23	0.042	1.10	54.51	1.05	3.98
Wedgetail + Lucerne	1.27	0.14	3.40	0.022	2.29	64.48	1.18	7.07
Wedgetail + Red clover	1.33	0.18	2.73	0.017	3.41	53.61	0.85	4.37
Wedgetail + Sub clover	1.63	0.14	2.83	0.037	3.79	51.75	0.80	5.57
Wedgetail + Vetch	0.41	0.09	3.80	0.044	0.84	80.60	3.44	10.00
Wedgetail + White Clover	1.45	0.16	3.25	0.030	3.01	64.52	1.00	5.93
Lsd (5%)	0.34	0.04	0.55	0.018	0.94	13.08	0.76	1.74

[A] Requirement for a 50kg lamb, derived from National Research Council (2007)

[B] Estimated from Takagi and Block (1991)

[C] Kemp and t' Hart (1957)

[D] Dove et al 2016

Table 7: Mean dietary concentration (%) of calcium (Ca), magnesium (Mg), phosphorous (P), potassium (K), sodium (Na) and sulfur (S), and mineral indices which reflect risk of mineral imbalance (DCAD, dietary cation-anion difference) from the cereal/legume intercropping field evaluation 2020.

	Cereal Component							
Requirement	Ca >0.28[A]	Mg >0.09[A]	K <3[A]	Na >0.06[A]	Ca:P 1.2[A]	DCAD <12[B]	Tetany <2.2[C]	K:(Na+Mg) <6[D]
11955	0.39	0.11	3.03	0.007	1.09	45.30	2.71	8.36
11955 + Lucerne	0.36	0.10	2.73	0.006	1.06	42.69	2.66	8.15
11955 + Red clover	0.37	0.10	2.80	0.009	1.08	43.10	2.71	8.55
11955 + Sub Clover	0.36	0.10	3.07	0.008	0.94	49.02	2.96	9.14
11955 + Vetch	0.37	0.10	2.77	0.013	1.04	44.25	2.70	8.79
11955 + White Clover	0.32	0.09	2.60	0.006	1.00	40.75	2.88	9.31
11955 + WR	0.33	0.08	2.50	0.006	0.99	42.00	2.80	9.58
Kernza	0.23	0.07	1.90	0.005	0.81	34.68	2.74	7.79
Kernza + Lotus	0.29	0.09	2.20	0.006	1.05	40.82	2.63	7.68
Kernza + Lucerne	0.34	0.10	2.97	0.008	0.82	57.37	3.02	9.04
Kernza + Red clover	0.29	0.10	2.60	0.006	0.89	46.48	2.95	8.04
Kernza + Sub Clover	0.28	0.09	2.87	0.007	0.77	55.39	3.39	9.32
Kernza + Vetch	0.33	0.09	2.50	0.005	0.95	46.18	2.68	8.02
Kernza + White Clover	0.31	0.09	2.70	0.007	0.80	50.43	2.99	8.59
Kernza + WR	0.27	0.08	2.20	0.005	0.89	37.48	2.77	7.86
Wedgetail	0.28	0.08	2.93	0.008	0.79	52.20	3.58	10.55
Wedgetail + Lotus	0.34	0.11	3.00	0.010	0.96	49.60	2.96	8.06
Wedgetail + Lucerne	0.37	0.11	2.80	0.011	1.12	43.41	2.56	7.22
Wedgetail + Red clover	0.35	0.11	3.23	0.012	0.87	54.42	3.12	9.00
Wedgetail + Sub clover	0.40	0.12	3.13	0.017	0.98	49.99	2.68	7.34
Wedgetail + Vetch	0.33	0.10	2.83	0.012	0.90	46.01	2.84	8.08
Wedgetail + White Clover	0.34	0.11	2.87	0.013	1.00	47.18	2.81	7.95
Wedgetail + WR	0.33	0.12	3.17	0.017	0.96	50.95	3.07	7.35

Lsd (5%)	0.065	ns	ns	0.005	0.178	7.68	0.438	ns
				Legume Component				
	Ca	Mg	K	Na	Ca:P	DCAD	Tetany	K:(Na+Mg)
Requirement	>0.28[A]	>0.09 [A]	<3 [A]	>0.06 [A]	1.2 [A]	<12[B]	<2.2[C]	<6[D]
11955 + Lotus	0.87	0.21	1.70	0.013	2.91	29.66	0.71	2.42
11955 + Lucerne	1.05	0.14	2.00	0.011	2.70	35.03	0.78	4.11
11955 + Red clover	0.72	0.16	1.67	0.010	2.72	33.22	0.86	3.09
11955 + Sub Clover	0.85	0.14	1.50	0.023	3.23	25.60	0.71	2.97
11955 + Vetch	0.92	0.13	1.65	0.010	2.57	26.96	0.72	3.76
11955 + White Clover	0.79	0.15	1.87	0.009	2.78	37.66	0.92	3.66
Kernza + Lotus	0.90	0.22	1.97	0.005	3.18	39.78	0.82	2.79
Kernza + Lucerne	1.23	0.13	2.43	0.016	4.05	46.09	0.86	5.31
Kernza + Red clover	1.13	0.20	2.43	0.012	4.10	52.38	0.85	3.57
Kernza + Sub Clover	1.20	0.17	2.27	0.103	3.91	49.89	0.78	3.12
Kernza + Vetch	1.11	0.16	2.37	0.014	3.73	44.20	0.89	4.37
Kernza + White Clover	1.23	0.21	2.33	0.023	4.26	49.53	0.76	3.24
Wedgetail + Lotus	1.20	0.30	2.90	0.011	2.82	51.94	0.86	2.92
Wedgetail + Lucerne	1.53	0.14	2.63	0.009	3.67	49.09	0.77	5.63
Wedgetail + Red clover	1.23	0.23	2.37	0.008	3.43	46.86	0.76	3.11
Wedgetail + Sub clover	1.63	0.19	2.27	0.035	4.54	39.87	0.60	3.36
Wedgetail + Vetch	0.69	0.14	1.37	0.008	2.15	20.90	0.76	3.06
Wedgetail + White Clover	1.10	0.18	2.57	0.011	2.76	47.37	0.94	4.27
Lsd (5%)	0.175	0.036	ns	0.01	0.931	15.36	ns	1.15

[A] Requirement for a 50kg lamb, derived from National Research Council (2007)

[B] Estimated from Takagi and Block (1991)

[C] Kemp and t' Hart (1957)

[D] Dove et al 2016

WR = wide row spacing, ie. No intercrop legume

The mineral profile of both the perennial cereal and legume was altered by the presence of a companion species but trends were difficult to define. For example, in the 2020 experiment the Ca concentration was highest in 11955 where the perennial wheat was grown in the absence of a legume, contrasting that of Kernza and Wedgetail wheat where Ca concentrations were lowest in the absence of a legume. Those trends existed in the 2019 Ca data (Table 6) but were less obvious than in 2020 (Table 7). There was no difference (P>0.05) in either the Mg or K concentrations among any cereal/legume combination in 2020. In 2019, differences existed but consistent trends were again hard to distinguish. For example, the highest cereal K concentration was observed in 11955 grown with vetch but the lowest K values was also observed in 11955 where it was grown alone. It is not clear if there is any biological basis for this result or it is merely a type II statistical error.

4.2.3 Lucerne abundance experiments

To sustain production in perennial cereal crops over a long term, a source of nitrogen will be required. Other studies have shown available soil N tends to decline in grassland soils and could similarly become depleted in perennial cereal systems. Intercropping with legumes could reduce the need for fertiliser inputs into a perennial grain crop and increase the efficiency of N use, as less legume derived N is lost from landscapes than with fertilizer N (Crews and Peoples 2004). The inclusion of a legume component could also provide some of the mineral requirements for grazing ruminants which are lacking in the forage of most cereals, particularly calcium and magnesium (Newell and Hayes 2017). In this experiment we compared intercropping lucerne with a perennial wheat derivative (11955) with an annual grazing wheat (Wedgetail). The composition of lucerne was varied between treatments by adjusting the number of sowing rows containing lucerne and all treatments compared to nitrogen fertilised (+N) and unfertilised (-N) monoculture cereal treatments.

Total cereal dry matter production averaged across the three years of experimentation was 24% higher in the perennial wheat treatments compared to the annual wheat treatments at Mandurama (Fig. 10a). Data in this figure was the sum of dry matter production at cereal anthesis along with the dry matter production following harvest into autumn for the perennial species. In contrast, at the Cowra site the N fertilised perennial wheat treatment had the highest dry matter production of all treatments, however the legume intercropped annual wheat treatments produced on average 12% more cereal dry matter than the corresponding perennial wheat treatments (Fig. 10b). Drought conditions in 2019 severely impacted lucerne persistence into 2020 at Cowra, with negligible lucerne survival. Most of the legume growing in the intercrop plots at this site consisted of background annual clovers. Legume dry matter production at both sites increased as the number of drill rows sown to cereal decreased. At the Mandurama site there was 14% more lucerne dry matter produced in the annual wheat intercrop treatments, on average, compared to the perennial wheat intercrop treatments. All intercrop treatments at this site averaged over 3000kg/ha lucerne dry matter which might equate to approximately 75kg/ha legume N being returned to the soil (Peoples et al. 2012). Increasing the number of intercropped lucerne rows significantly reduced total dry matter production in the perennial wheat treatments compared to the unfertilised monoculture perennial wheat treatment at both sites. However, in the annual wheat treatments there was less reduction in dry matter by intercropping, with similar total dry matter between intercrop treatments and the unfertilised control. This suggests that the percentage of perennial wheat in the total forage was driving production more so than in the annual wheat treatments.

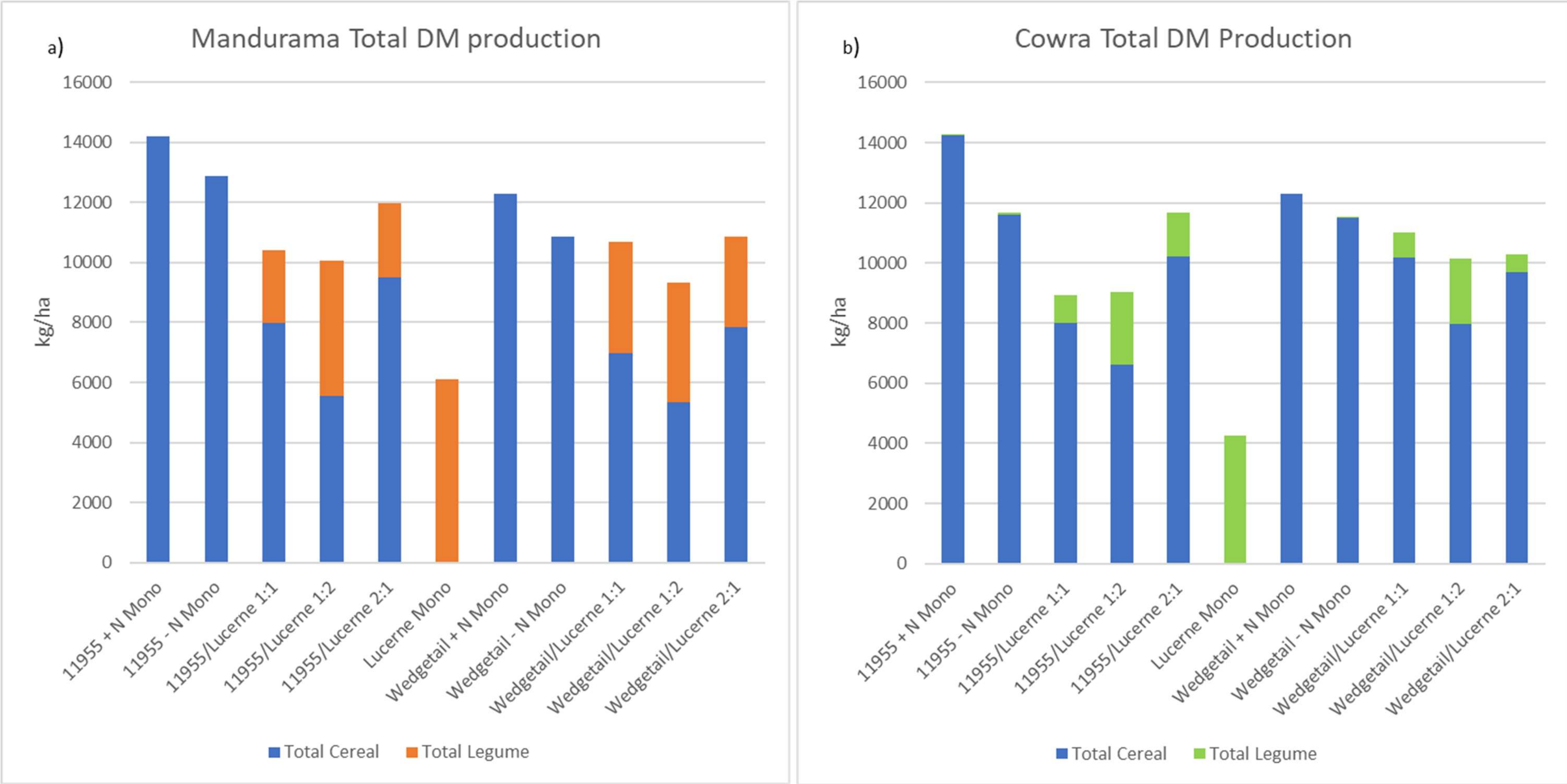

Fig. 10. Cumulative dry matter production for perennial wheat (11955) and annual wheat (Wedgetail) under contrasting row configurations with lucerne; alternate rows (1:1), two rows of cereal next to one row of lucerne (2:1) or one row of cereal next to two rows of lucerne (1:2) at a) Mandurama and b) Cowra measured between 2019-2021. Data include production from anthesis measurements and post-harvest autumn DM.

Grain yields were severely reduced by the dry conditions in 2019 at both sites and subsequently there was little response to fertiliser N (Fig. 11a & b). The hot dry conditions at the Cowra (Fig. 11b) interrupted seed set in the perennial wheat treatments (no grain produced) and very little annual wheat was harvested (av. 300kg/ha). At the Mandurama site in 2019, annual wheat yields were 87% higher (av. 2218 kg/ha) than perennial wheat treatments with no significant differences between treatments. The later maturing perennial wheat lines were impacted more by dry conditions. At the Mandurama and Cowra sites in 2020, perennial wheat average yield was approximately 35% of the annual wheat yield. At Mandurama all intercropping treatments reduced grain yield in both cereal crops, but at the Cowra site grain yields were similar between intercropping treatments and the monoculture cereal treatments, except for the Wedgetail/lucerne 1:2 treatment which had a 25% reduction in yield compared to the fertilised control plots. This suggest there was some compensation in grain yield when the number of cereal rows were reduced and requires further investigation. In 2021 there was good regrowth in perennial wheat treatments at both sites, however grain yields were similar between treatments and sites for perennial wheat. At the Mandurama site annual wheat yields averaged 1350 kg/ha in the monoculture treatments and there was no significant difference in annual and perennial wheat yields across the intercropping treatments. Perennial wheat yields were reduced by low plant populations in the monoculture plots, but more root disease symptoms were observed in the annual wheat treatments which may have reduced grain yields in 2021. At the Cowra site the fertilised annual wheat treatment averaged 5067kg/ha which was 34% higher than the unfertilised treatment. There were similar yields between the unfertilised annual wheat monoculture treatment and the Wedgetail/lucerne1:1 treatment, but yields were reduced in the Wedgetail/lucerne 1:2 and 2:1 treatments compared to the unfertilised monoculture, suggesting the annual wheat could not compensate for the reduced number of cereal rows.

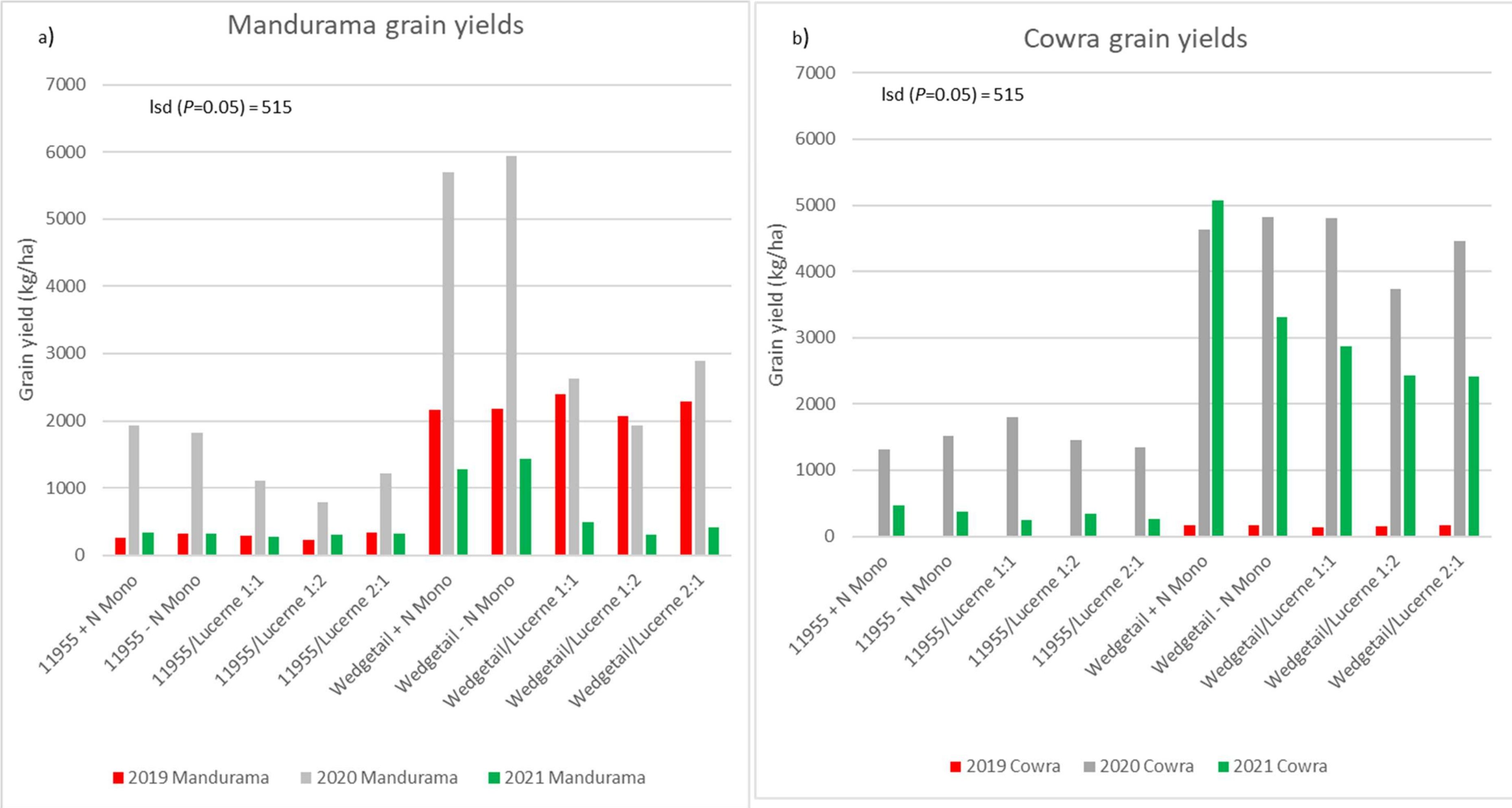

Fig. 11. Grain yields for perennial wheat (11955) and annual wheat (Wedgetail) under contrasting row configurations with lucerne; alternate rows (1:1), two rows of cereal next to one row of lucerne (2:1) or one row of cereal next to two rows of lucerne (1:2), at a) Mandurama and b) Cowra measured between 2019-2021.

4.2.4 Crop density

Intermediate wheatgrass, a perennial forage grass, is undergoing selection in the USA for domestication traits to develop the species into a perennial grain crop called Kernza (Dehaan et al. 2020). Although there is an increasing area sown to Kernza in North America, there is little experience with this species in Australian agriculture. By contrast, Mountain Rye (Mtn Rye) was developed as a perennial forage grass for higher altitude environments of Australia and may offer potential as a candidate perennial grain via a similar 'domestication' pathway as used for Kernza. However, for both species, basic agronomic practices to maximise grain yields, persistence and profitability remain unclear in Australian environments.

In year 1 of the experiment at Orange, a positive response in mature tiller numbers to increasing rates of nitrogen fertiliser (0, 100 & 200kg/ha N) was observed across the three population densities for each species. We observed that Mtn Rye produced significantly more tillers than Kernza (Fig. 12a). This translated into higher yields for Mtn Rye overall, however we found a reduction in yield with higher rates of N across populations (Fig. 12b). This was due to increased crop lodging with increasing N in the Mtn Rye plots however there was less evidence of this in the Kernza treatments and no clear response between plant population and nitrogen treatment. There was no significant difference in seed occupancy of florets between plant populations with increasing N rates, however the inflorescence of Kernza contained more seeds per spikelet than Mtn Rye (Fig. 12c). Small decreases in seed size were observed with increasing N rate at all densities of both species and on average, Mtn Rye had 20% larger seeds than Kernza (Fig. 12d).

Nitrogen responses in crops generally follow a sigmoidal response curve in which production increases with increasing rates of N, before plateauing and sometimes declining. The current study was located on a site with high soil N levels at sowing. Therefore, we maybe observing responses at the top end of the response curve with increasing rates of N. Overall, Mtn Rye had higher grain yields than Kernza. With further selection for floret fertility and seed size, Mtn Rye could prove a successful candidate for a perennial grain crop via domestication in Australia.

Initial results of this study are reported in a paper recently accepted by the International Grasslands Congress, in Covington, Kentucky USA, 14-18 May 2023 (Appendix 7).

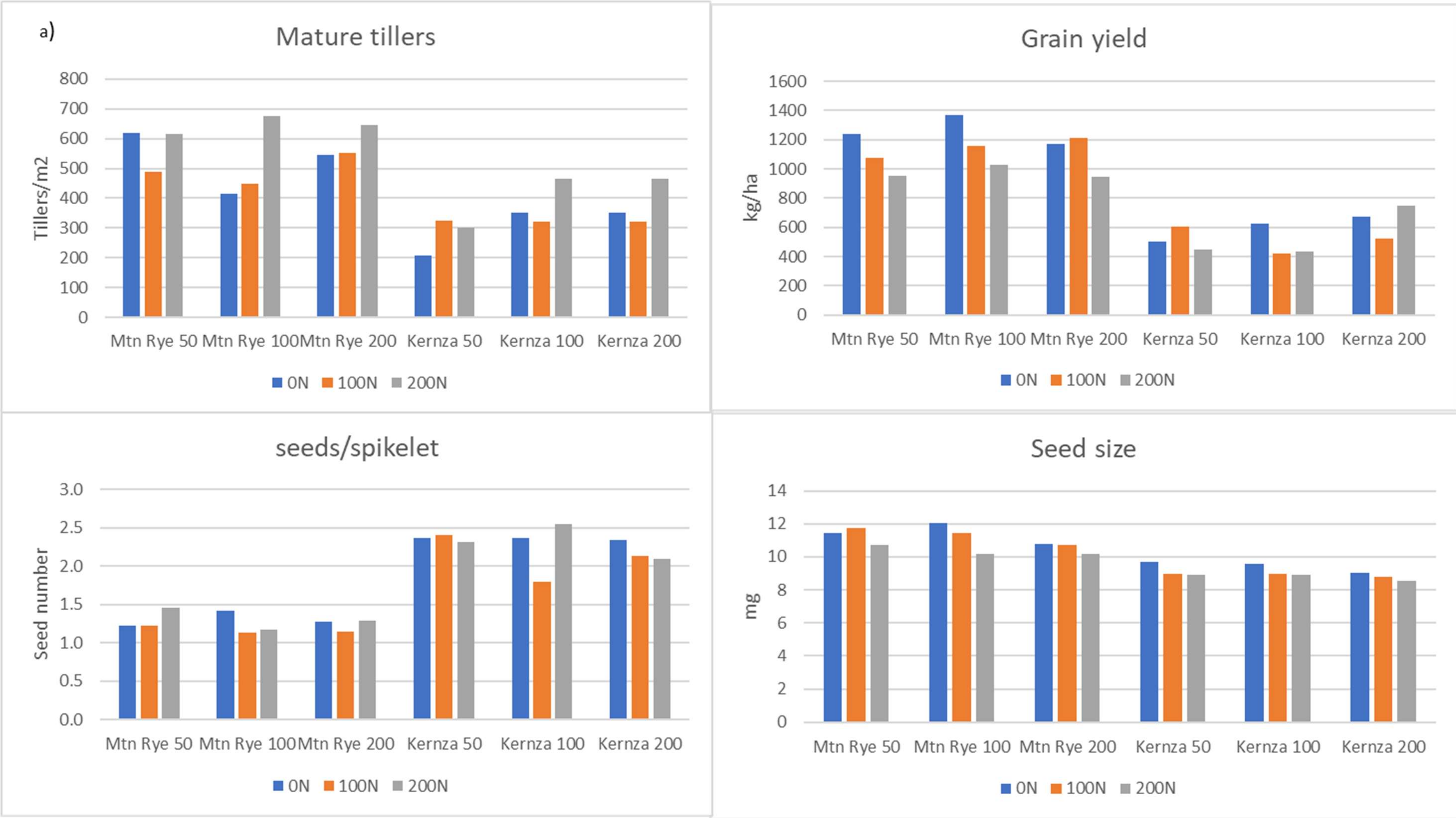

Fig. 12. Grain yield components of a) mature tillers at harvest, b) grain yield, c) seeds per spikelet and d) seed size for Mtn Rye and Kernza sown at three plant populations (50, 100 & 200 plants/m^2) and three nitrogen treatments (0,100 & 200 kg/ha N).

4.3 Finishing lambs on grazing cereal (Stage 2)

The results of this experiment have been substantially reported in scientific journal articles appended to this report. Briefly, the addition of lucerne increased intake compared to lambs on pure cereal diets but there was no treatment effect detected on lamb liveweight, with growth rates observed to be modest (55-110 g per head per day) for all lambs in the study regardless of diet (Newell et al. 2020b). No diet was able to satisfy the sodium (Na) requirement of fast-growing lambs, when compared to established industry thresholds, due to the low Na concentration in all forages provided. The perennial wheat forage was shown to have negligible Na concentrations in the herbage, often below the limit of detection of laboratory instruments (Newell et al. 2020b). This is thought to be attributable to the tall wheatgrass parentage of the 11955 perennial wheat line used. Tall wheatgrass is renowned for relative tolerance to saline/waterlogged conditions, due in part to its ability to exclude Na^+ (Jenkins et al. 2010), although few studies have directly compared Na^+ uptake of wheat and tall wheatgrass under contrasting conditions.

Analysis of plasma and urine from lambs taken during the study (Refshauge et al. 2022) revealed that the addition of lucerne increased the dietary supply of Ca, Mg and Na and decreased K and P. When compared to wheat, perennial wheat offered more Ca, Mg, K, and less Na and P. Compared to established industry thresholds, all diets offered high dietary cation anion difference (> 45), and high K: (Na + Mg) and K: Na ratios, while the addition of lucerne increased the Ca: P ratio and improved the Tetany index (K: (Mg + Ca)). Taken together the results indicate that the greater dietary intake of Ca, Mg and Na due to the addition of lucerne was insufficient to overcome the primary mineral imbalance of high dietary K and low Na in both cereal forages. Furthermore, the addition of lucerne to the diet further decreased P intake and greatly increased the Ca: P ratio. Our calculations suggest lambs grazing any of the diets still required supplementation with Na and Mg, but the addition of lucerne alleviated the need for Ca supplementation.

Consumer sensory panel feedback showed 'good eating quality' for all treatments tested, supporting the use of perennial wheat as a viable alternative forage option (Holman et al. 2021a). There were few differences between lambs attributable to diet in standard meat quality parameters, although a small but significant difference was observed in ultimate pH, which was higher in lambs fed annual wheat compared to perennial wheat. Concentrations of Na, sulphur (S) and zinc (Zn) were also higher in meat from lambs fed annual wheat compared to perennial wheat. Further analysis showed that the concentration of long-chain saturated and omega-6 polyunsaturated fatty acids, as well as of thiobarbituric reactive substances and vitamin E were higher when lucerne was included compared to pure cereal diets (Holman et al. 2022). The latter is an important finding as it demonstrates an opportunity for farmers to select production systems to enhance the concentration of health-claimable compounds in meat of grazing lambs. Whether this result is specific to the inclusion of lucerne in the diet or may also occur with the inclusion of other legume species remains a topic of ongoing investigation.

In addition to the key findings described above, this experiment delivered a number of 'add on' benefits to industry through opportunistic sampling and linkages with existing projects. The first is detailed in Holman et al. (2021b), which reports a comparison of pH decline in carcasses by sampling the higher value *longissimus lumborum* muscle (standard practice) compared to the lower value *semitendinosus* muscle. Results showed that there were few practical differences in pH decline parameters when the sampling of the different muscles was compared. Although further validation is required, the implication is that cost savings may be available to the meat processing supply chain if destructive sampling to monitor pH decline in carcasses occurs in a lower value cut than is presently sampled.

The study also provided an opportunity to investigate the potential of Raman spectroscopy to be used as a non-destructive measure to predict eating quality through an analysis of the "chemical fingerprint" of proteins, collagen and fats in the carcass. For this technology to be adopted by industry, a significant amount of work is required to validate instrumentation on a range of carcasses from a range of livestock classes across multiple environments. The 48 carcasses in the Stage 2 experiment were sampled for this purpose and preliminary results were reported in conference papers (see Section 2.1.7) A comprehensive analysis has since been undertaken and results summarised in a draft manuscript (Fowler *et al*. 2023; Appendix 1). This activity represented collaboration with two projects, P.PSH.1034 *Verification of grass fed beef using spectroscopic technologies* and *The assessment of Near Infra-red spectroscopy to determine the intramuscular fat content of lamb* (funded by Australian Livestock Measurement Technologies, ALMTech project 2) to explore potential application of NIR and Raman scanning technologies.

Linkage to the MLA 'Better doers' project, B.GBP.0024 was also established to enable rumen samples from the lambs in the Stage 2 experiment to be analysed. A project variation was granted by MLA to that project to provide $37 000 additional funding for analysis. Results of that sampling have been reported in the 'Better doers' final report (Bond and Hudson 2019).

4.4 Balancing the nutritional requirements of ewes (Stage 3)

4.4.1 Intake

Ewes fed only PW had the lowest average daily DM intake of all treatments. The addition of salt increased average daily intake compared to PW, the addition of lucerne increased intake again compared to PW+S but the highest average daily intake was observed in the PW+L+S treatment (Fig. 13). There was a high level of variability between individual sheep in the relative intake of cereal and lucerne forage for those fed a mixed ration, with some examples of low preference for lucerne forage. However, averaged across all pens there was slightly higher daily intake of lucerne compared to cereal in the mixed forage diets, especially earlier during the experiment (Figs 14 & 15). Further analysis is required to determine whether these differences were statistically significant.

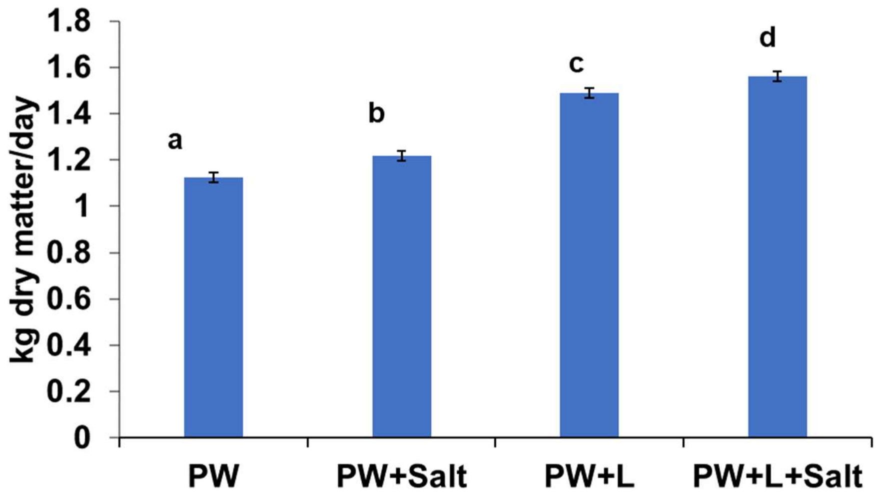

Fig. 13. Average daily dry matter intake (kg/day) of twin bearing ewes in late pregnancy in the Stage 3 study. Bars with the same superscript are not significantly different at P=0.05.

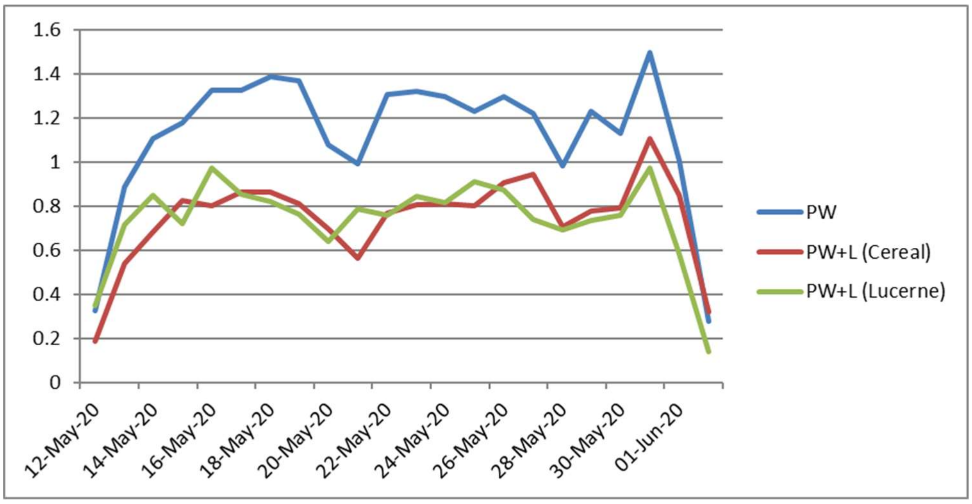

Fig. 14. Average daily dry matter intake (kg/day) of cereal and lucerne forage in twin bearing ewes fed the PW+L diet. Average daily dry matter intake of the PW treatment is provided for comparison.

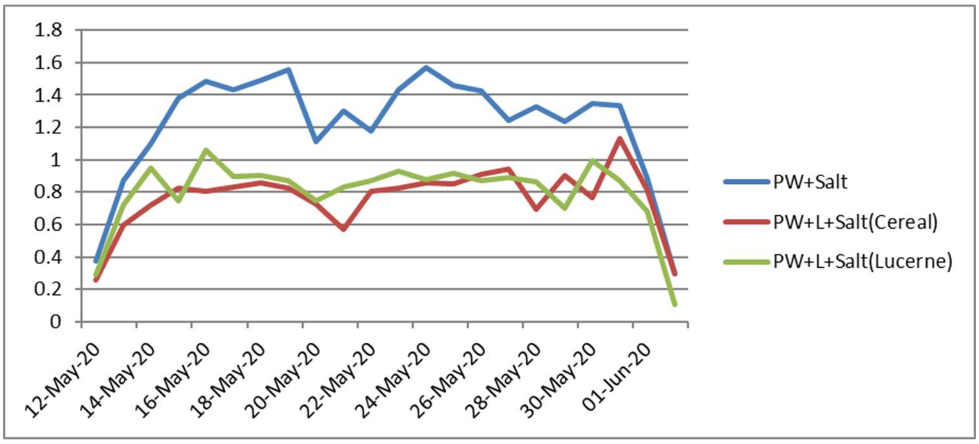

Fig. 15. Average daily dry matter intake (kg/day) of cereal and lucerne forage in twin bearing ewes fed the PW+L+S diet. Average daily dry matter intake of the PW treatment is provided for comparison.

4.4.2 Plasma

The concentration of Ca in plasma was lower on Day 7, when compared to all other days of the study ($P<0.01$, Fig. 16.). When compared to the plasma Ca concentration of PW ewes, there were increases observed in PW+L ($P<0.01$) and PW+S+L ($P<0.001$) on Day 7 of the study. There was a tendency for plasma Ca to be lower in PW+S on Day 14 ($P=0.051$) and by Day 21 there were reductions in calcium in the PW+S ewes ($P<0.01$), with increases observed in PW+L ($P<0.05$) while PW+L+S was tending to increase ($P=0.059$).

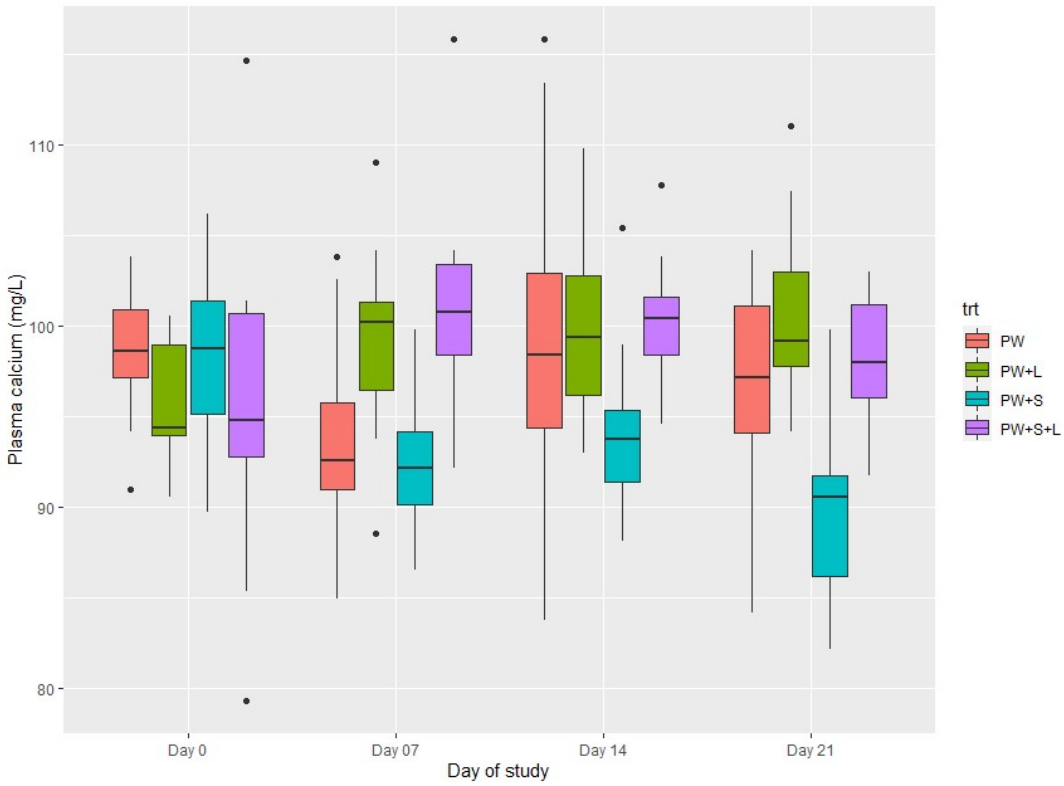

Fig.16. Boxplot distributions for plasma calcium concentration at each week.

Plasma Mg did not differ statistically between treatments throughout the study, although there were differences between sampling dates. When compared to the mean concentration at Day 0 (Fig. 17), the lowest concentrations were observed on Day 7 (P<0.001), and still lower than Day 0 on Days 14 (P<0.05) and Day 21 (P<0.01).

Plasma Na concentrations (Fig. 18) were elevated in PW+L (P<0.05), PW+S (P<0.01) and PW+L+S (P<0.001) on Day 21.

Plasma potassium (Fig. 19) increased on Day 14 and 21 (P<0.001). Among the dietary interactions, potassium concentration was high in PW+L+S on Day 7 (P<0.01).

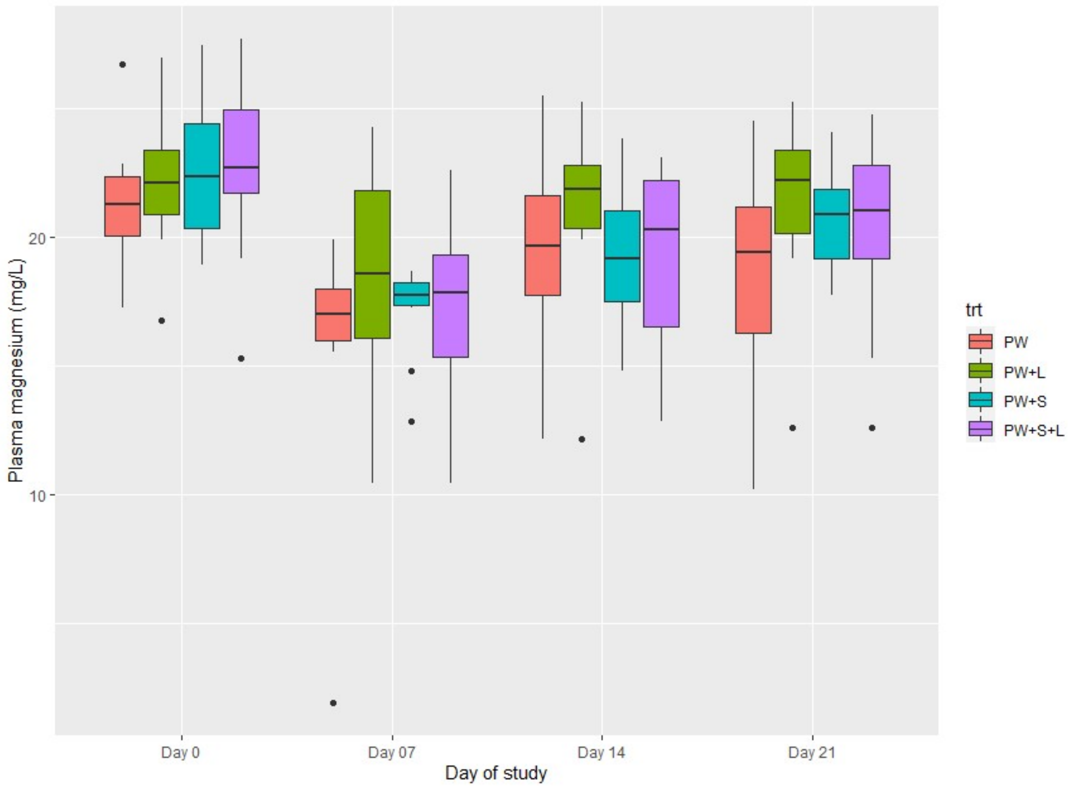

Fig 17. Boxplot for plasma magnesium concentration at each week.

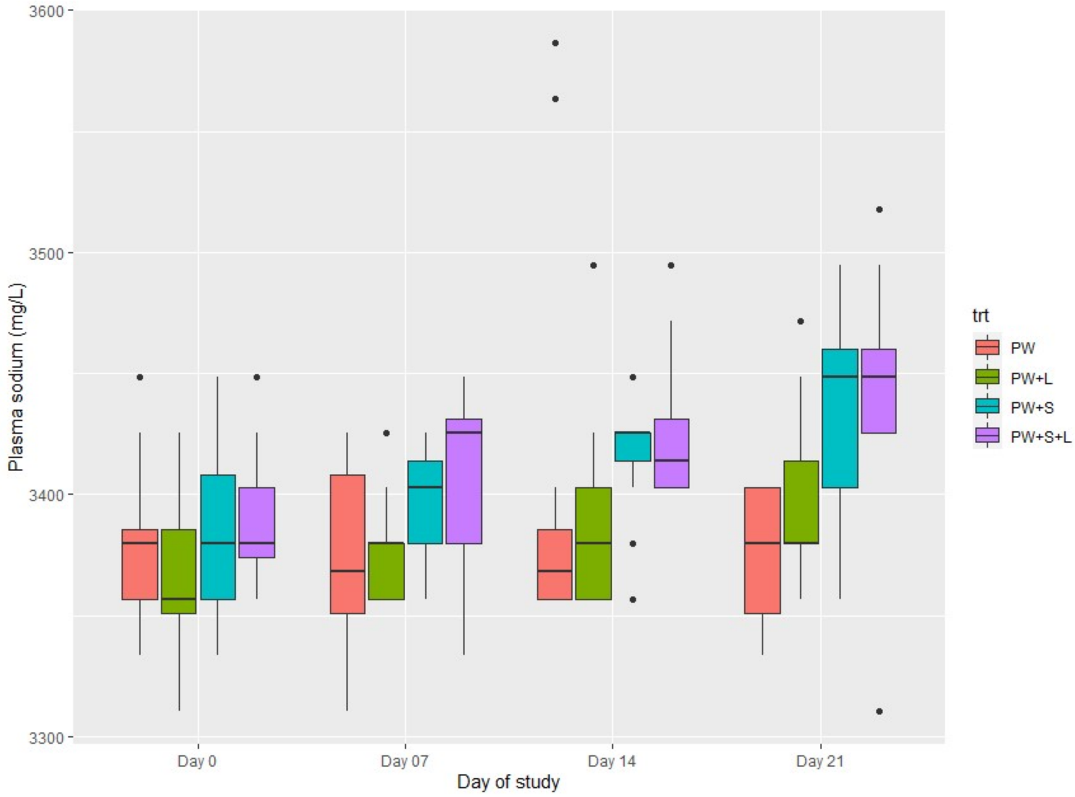

Fig. 18. Boxplot for plasma sodium concentration for each week.

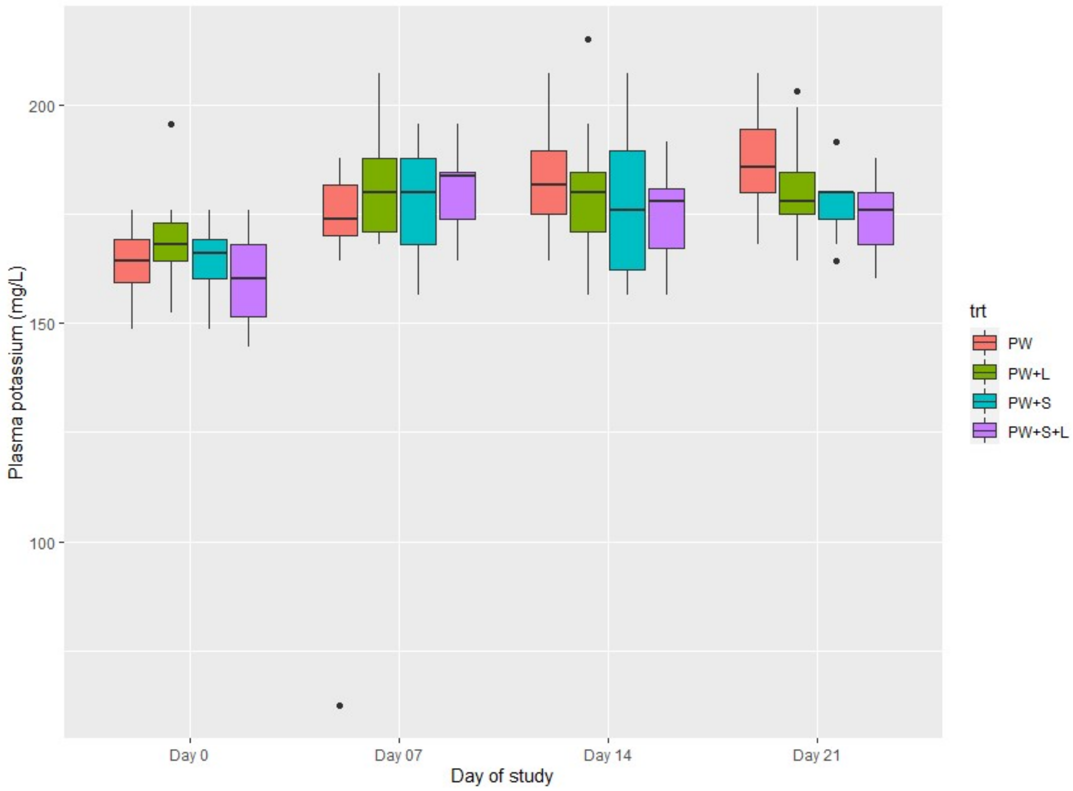

Fig. 19. Boxplot for plasma potassium concentration for each week.

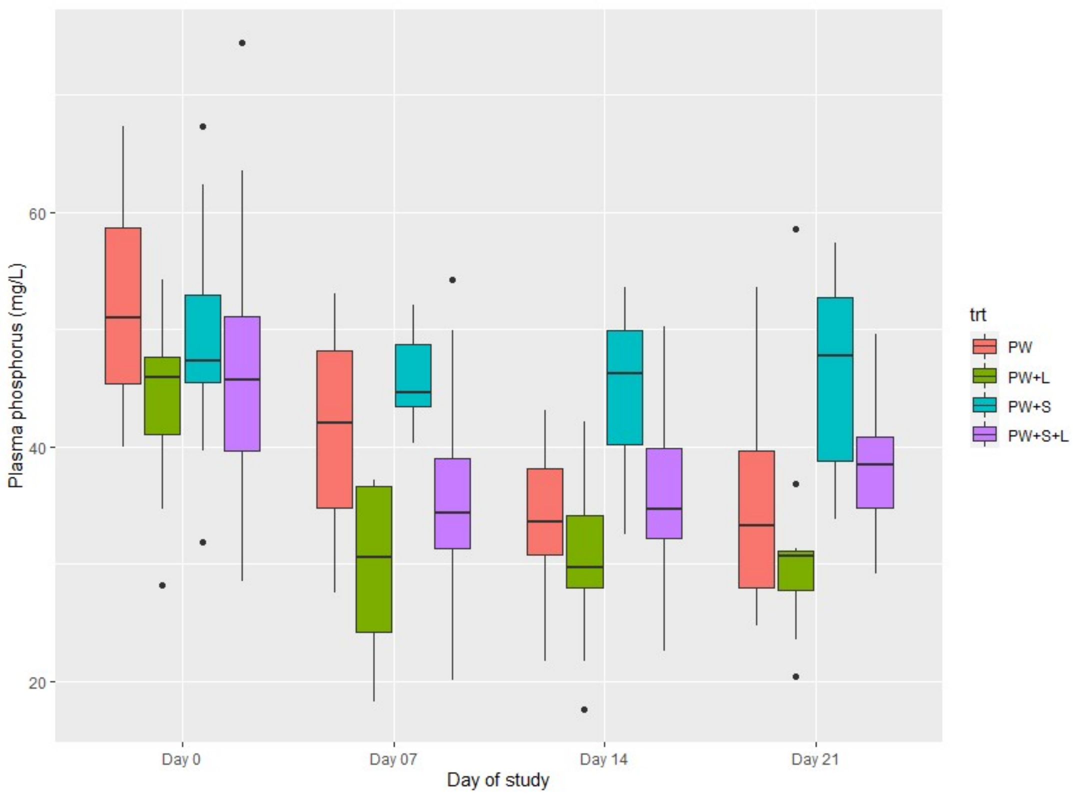

Fig. 20. Boxplot for plasma phosphorus concentration for each week.

When compared to PW, the mean plasma P concentration was significantly lower in all diets although differences were not significant in the PW+S (P=0.29), or PW+L+S (P=0.06) diets. When compared to the base level (Day 0), there were lower plasma P concentrations at Day 7 and Day 14, with Day 21 remaining lower but no longer different to Day 14 (Fig. 20). Throughout, ewes fed PW+S maintained levels equivalent to their baseline samples at Day 0, remaining higher than the other diets on Day 7 (P<0.05), on Day 14 and Day 21 (P<0.001). The PW+S ewes had higher concentrations than PW at Day 14 (P<0.05) and on Day 21 (P<0.01).

4.4.3 Urine mineral to creatinine ratios

The excretion of calcium in urine, as a ratio of the excretion of creatinine, was significantly elevated in PW+L on Day 7, Day 14 and Day 21 (P<0.001, Fig. 21) compared to PW. On Day 7 and Day 14, the ratio was also elevated in PW+S+L (P<0.001) and remained high on Day 21 (P<0.01).

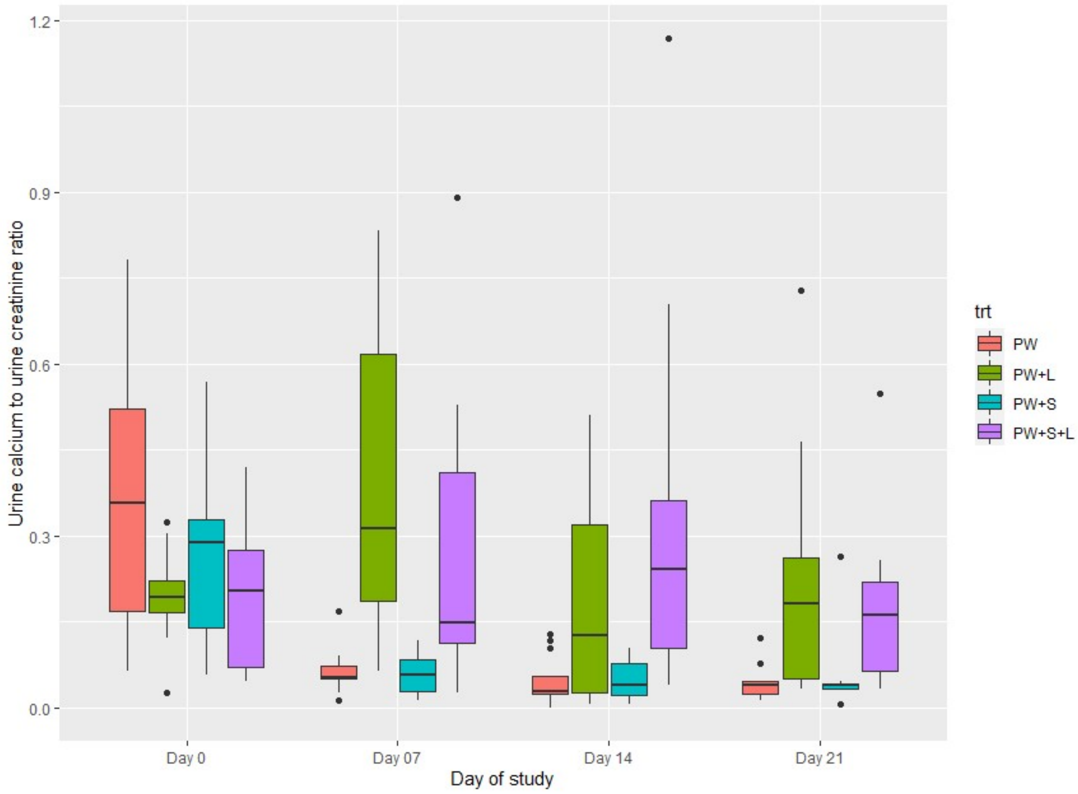

Fig. 21. Boxplot for ratio of calcium excretion to creatinine excretion in urine for each week.

More magnesium was excreted in PW+L on Day 7 (P<0.001) and in PW+S+L (P<0.05) compared to PW. On Day 14 the PW+L and PW+S+L ewes continued to have higher excretion rates of magnesium (Fig. 22) than PW and PW+S fed ewes (P<0.05). At Day 21, the PW+L was tending to differ statistically (P=0.08), while PW+S+L remained significantly elevated compared to PW ewes at Day 21 (P<0.05).

The excretion of sodium (Fig. 23) was significantly elevated in PW+S and PW+S+L ewes at Day 7 (P<0.001), Day 14 (P<0.001), and Day 21 (P<0.01).

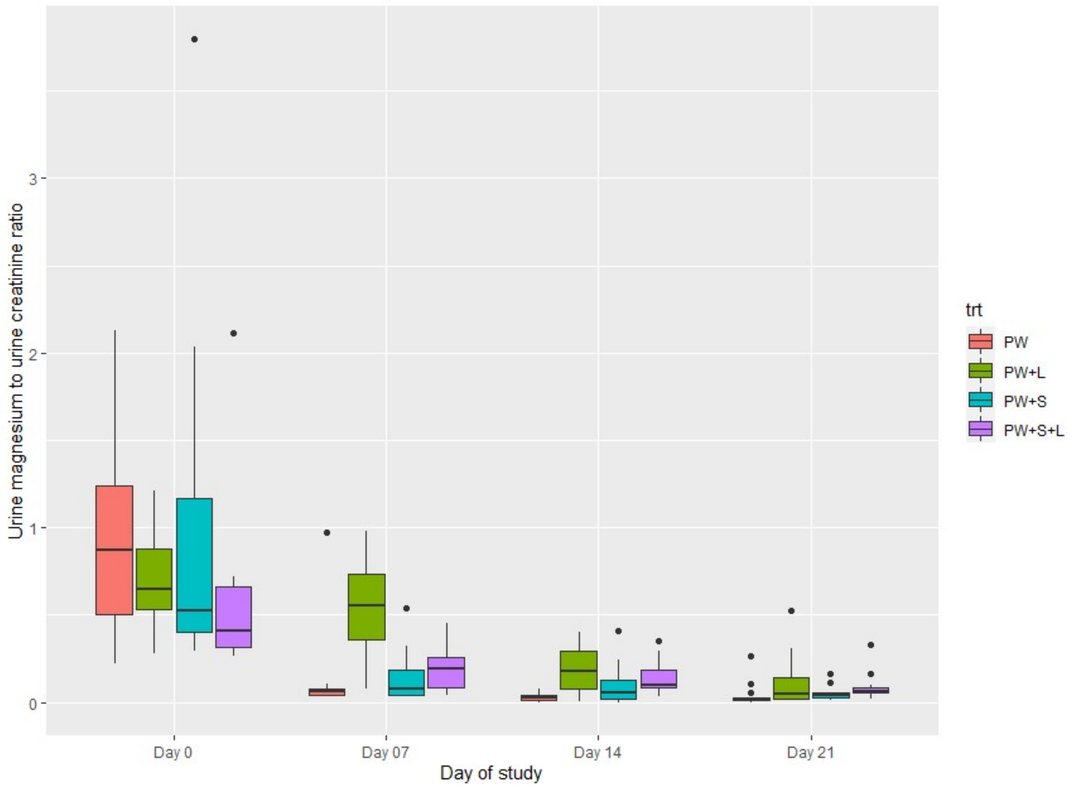

Fig. 22. Boxplot for ratio of magnesium excretion to creatinine excretion in urine for each week.

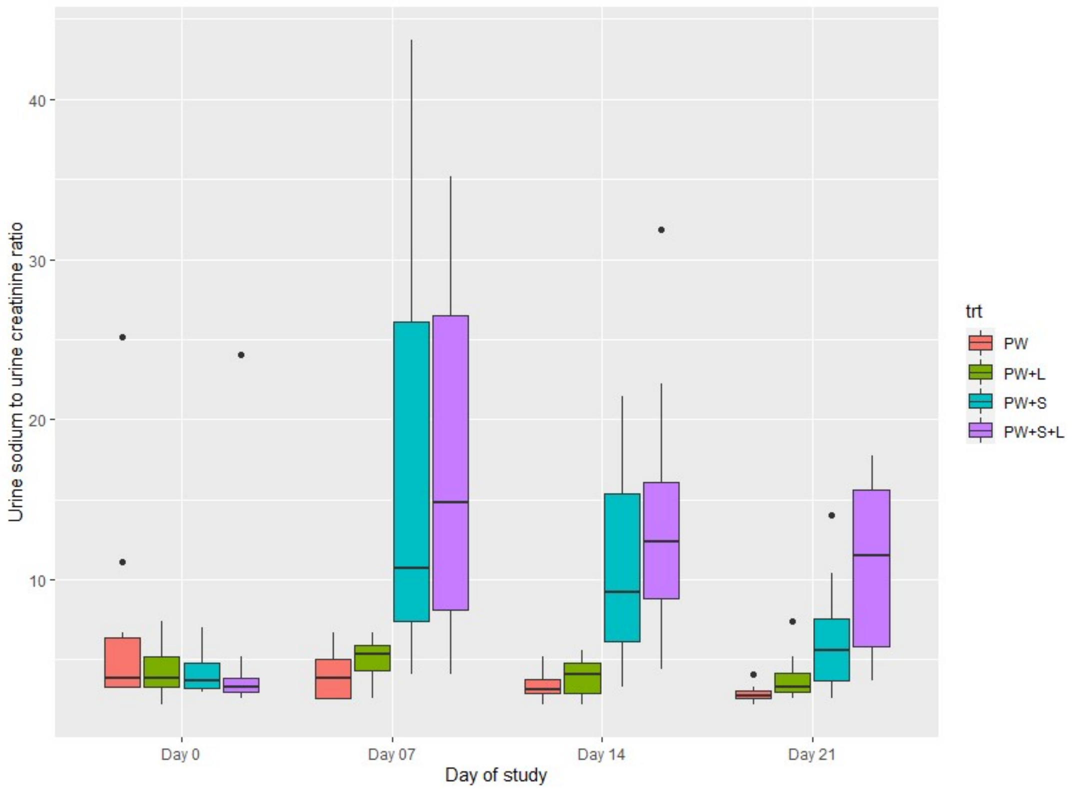

Fig. 23. Boxplot for ratio of sodium excretion to creatinine excretion in urine for each week.

Potassium excretion was higher at Day 7 (P<0.05) and in the PW+L ewes at that time (P<0.05, Fig. 24).

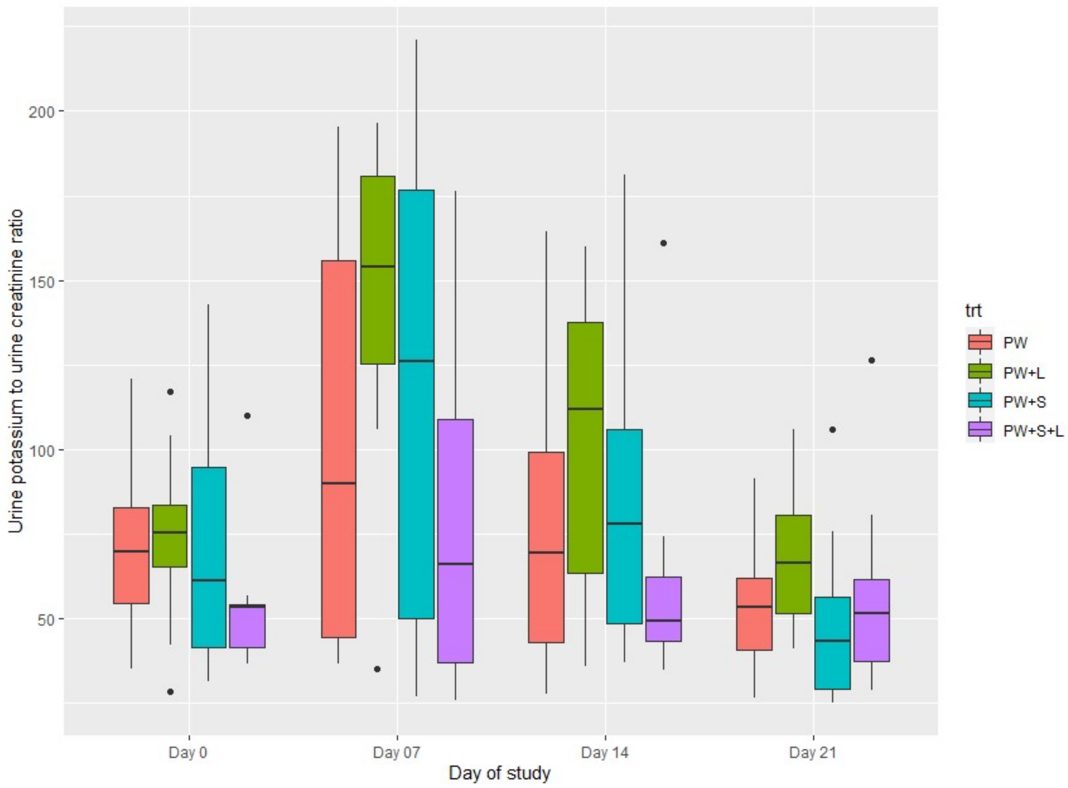

Fig. 24. Boxplot for ratio of potassium excretion to creatinine excretion in urine for each week.

4.4.4 Ewe liveweight and body condition score

By Day 21 ewe liveweight was significantly higher than at Day 0 (P<0.001, Fig. 25). At Day 7 ewes grazing PW+L (P<0.05), PW+S and PW+S+L (P<0.01) were heavier than PW. At Days 14 and 21 all ewes were heavier than the PW ewes at the respective time point (P<0.001). At Day 21, PW+S+L ewes were 4.0 ± 0.67 kg heavier than PW ewes.

Over the duration of the study the ewes continued to lose body condition (Fig. 26) and by Day 21 were 0.3 ± 0.06 score leaner than at Day 0 (P<0.001). At Day 7, PW+S were leaner than the PW ewes (P<0.05).

Betahydroxybutyrate (BHB) was highest throughout the study in the ewes offered the PW+S+L diet (P<0.001), being 0.44 ± 0.13 mmol/L higher than PW (Fig. 27). Ewe body condition score (BCS) was included in the model and had a significant effect (P<0.05), in particular the PW+S+L fed ewes had a significant interaction with BCS and their BHB concentrations (P<0.001, Fig 28). There was a tendency for BHB to decrease in PW+S+L ewes at Day 21 (P=0.055).

In the glucose model BCS was removed due to no significant effects. Plasma glucose was lowest on Day 14 of the study, when compared to Day 0 (P<0.05) and there were no other significant effects (Fig. 29).

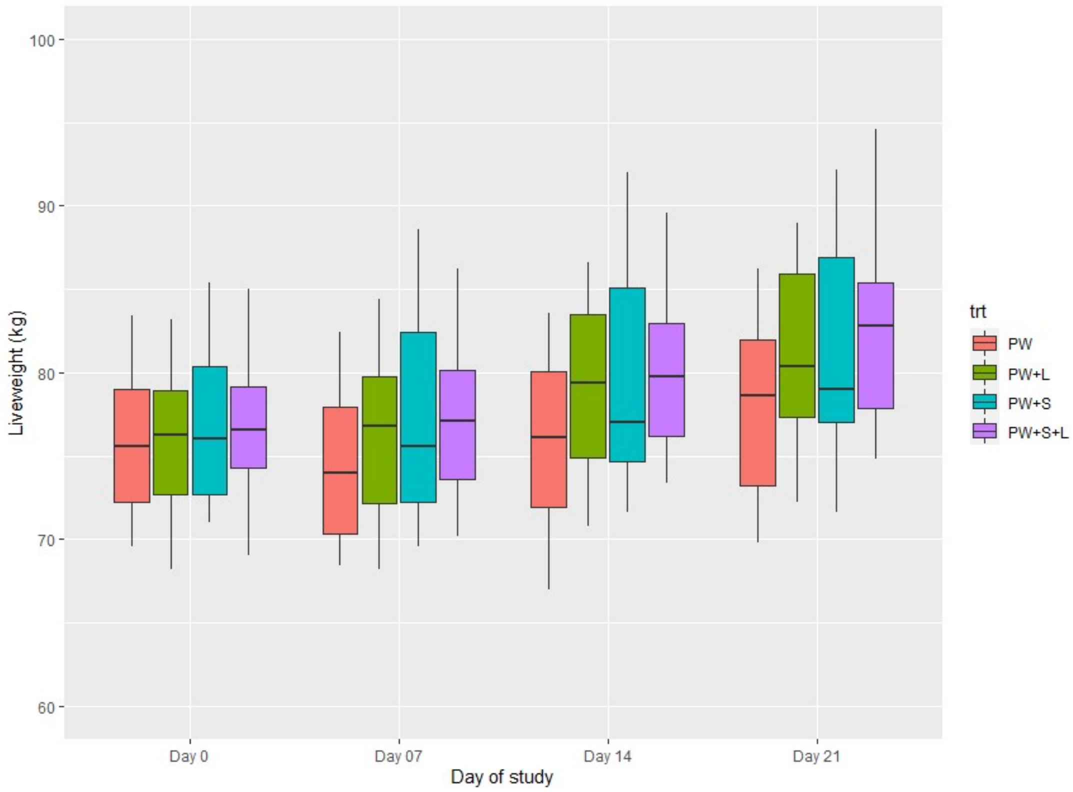

Fig. 25. Boxplot for weekly ewe liveweight (kg).

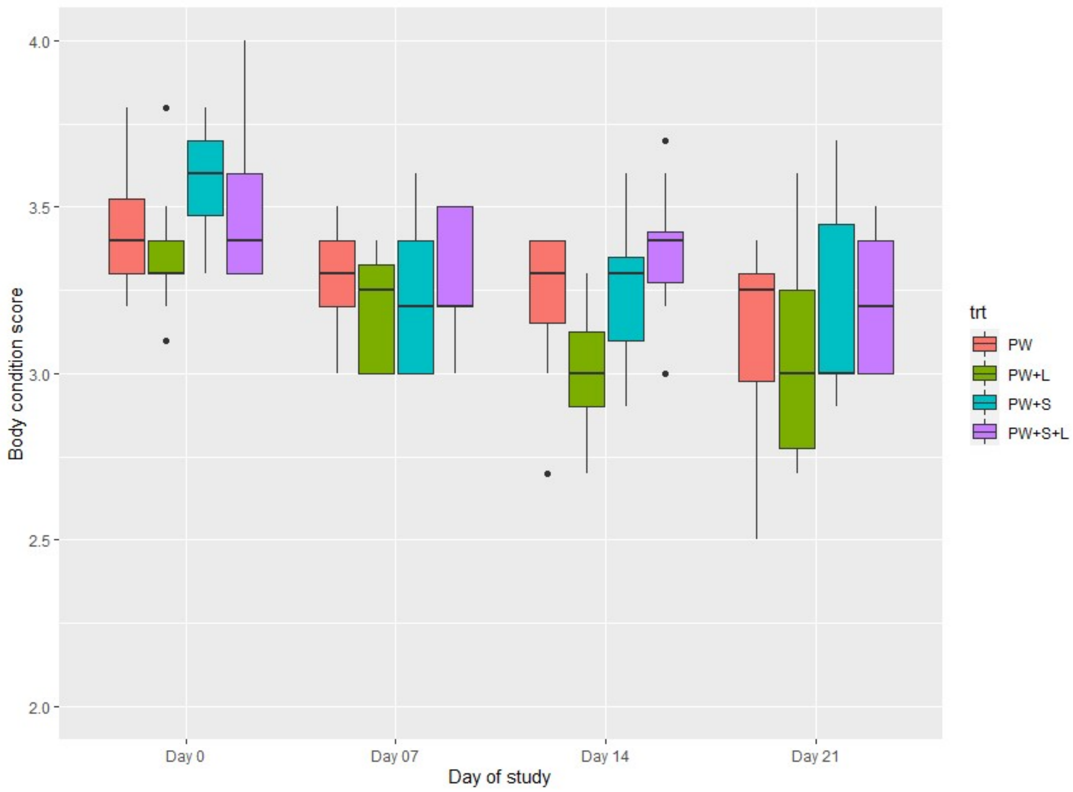

Fig. 26. Boxplot for weekly ewe body condition score.

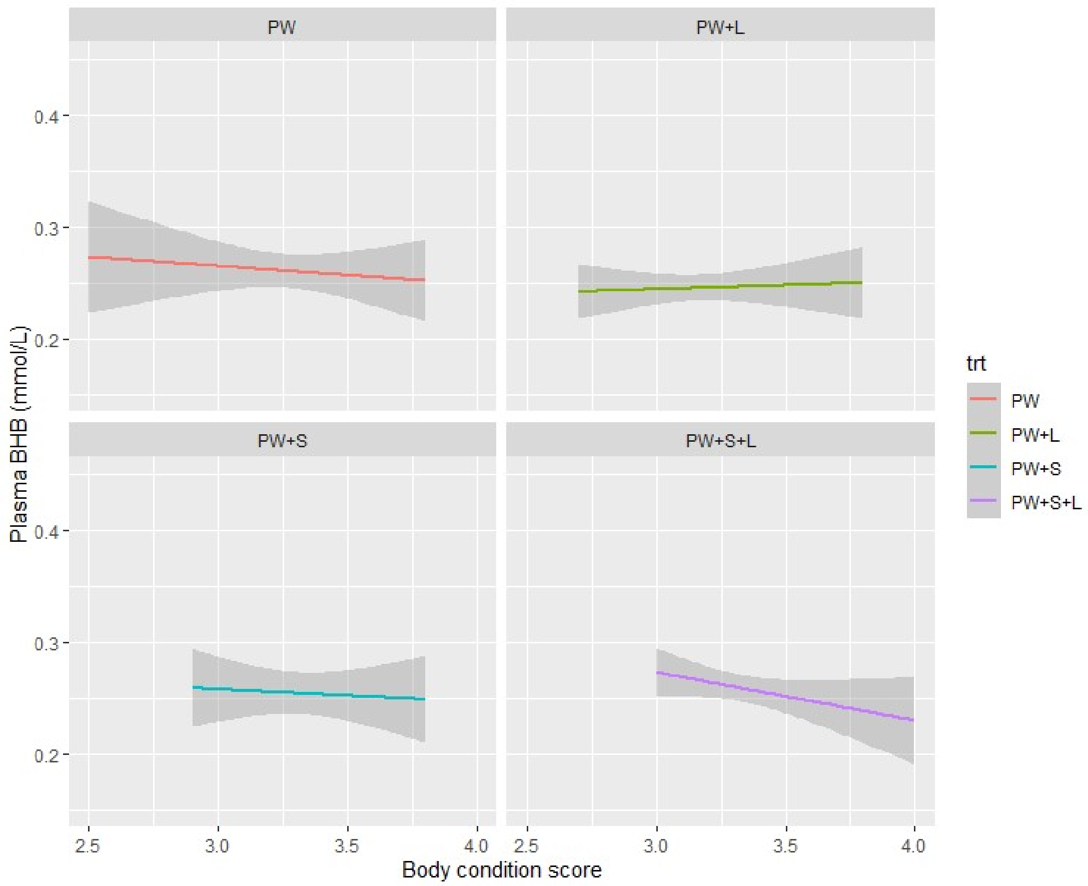

Fig. 27. Plasma betahydroxybutyrate (BHB, mmol/L) and body condition score for each diet.

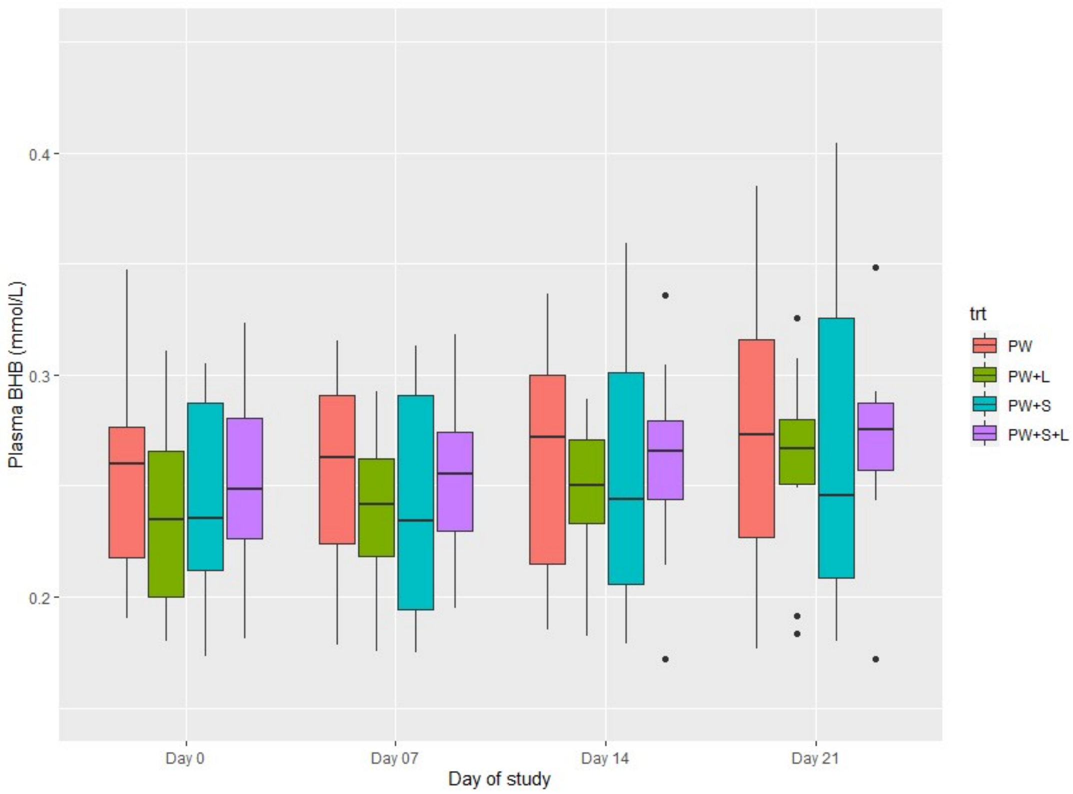

Fig. 28. Boxplot for weekly ewe plasma betahydroxybutyrate (BHB, mmol/L).

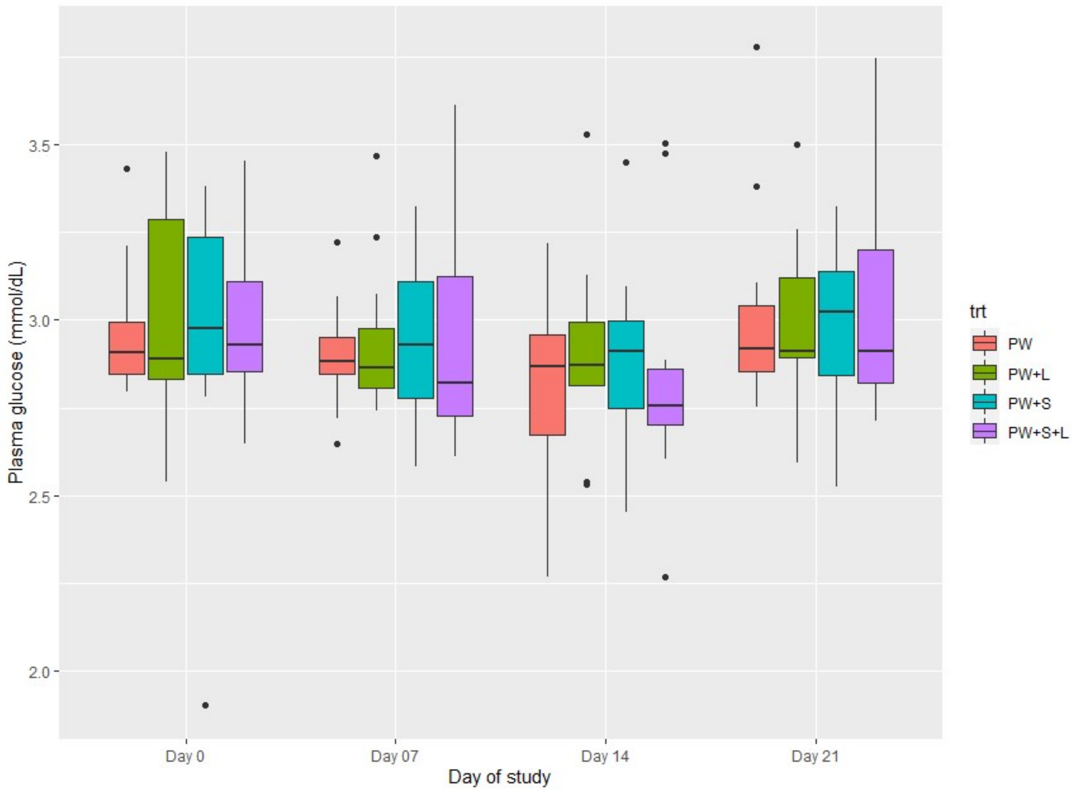

Fig. 29. Boxplot for weekly ewe plasma glucose (mmol/dL).

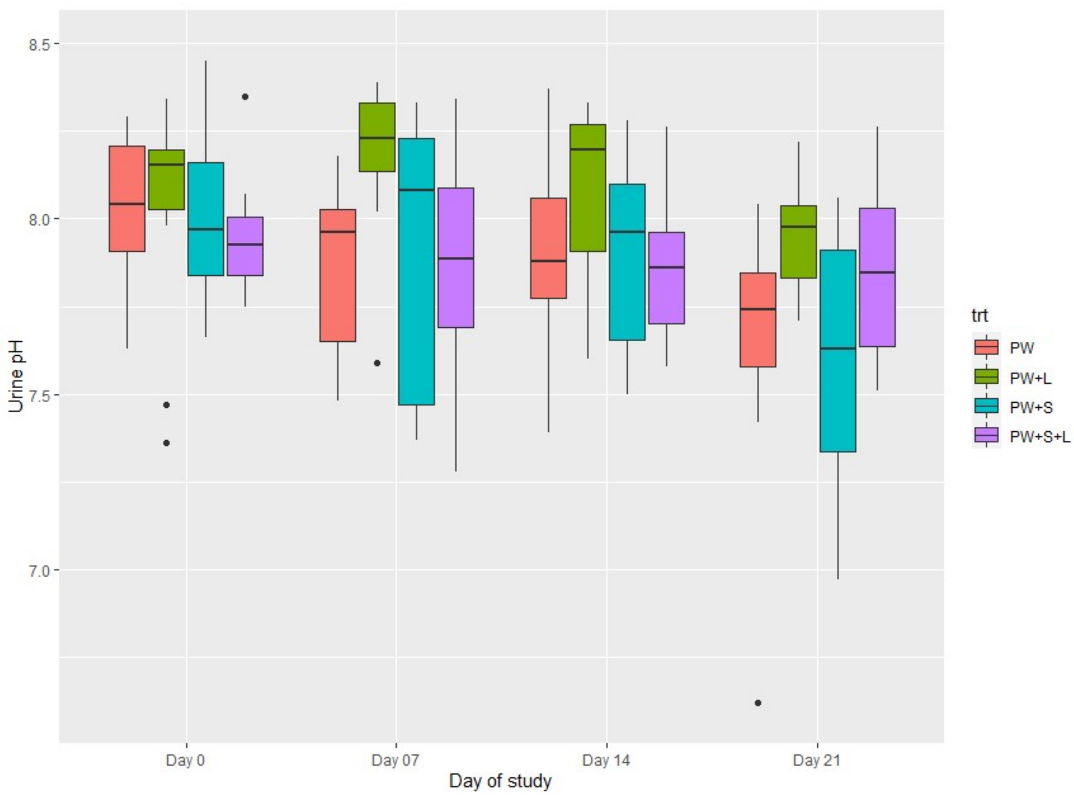

Fig. 30. Boxplot of weekly ewe urine pH.

Urine pH decreased over the course of the study, being 0.38 ± 0.1 units lower at Day 21 when compared to Day 0 (P<0.001, Fig. 30). When compared to PW ewes at Day 7, the PW+L ewes had higher urine pH (P<0.05). Similarly, at Day 21 the PW+L ewes had higher urine pH than the PW ewes (P<0.05). The PW+S+L ewes had higher urine pH (P<0.05) than PW ewes only at Day 21.

4.4.5 Ewe effects at lambing

The following analysis is of the ewe and does not permit ewe as a random term but does include the blocking as a random term. Ewe weight and BCS at lambing were included as covariates and tested for interaction with the main effects. Average lamb birthweight was higher (0.7 ± 0.3 kg) in the lambs born to the ewes fed the PW+S diet (P<0.05), when compared to the PW lambs (Fig. 31). Lamb birthweight was heavier in heavier ewes (P<0.01, Fig. 32) and tended to be lower in ewes with higher BCS (P=0.052).

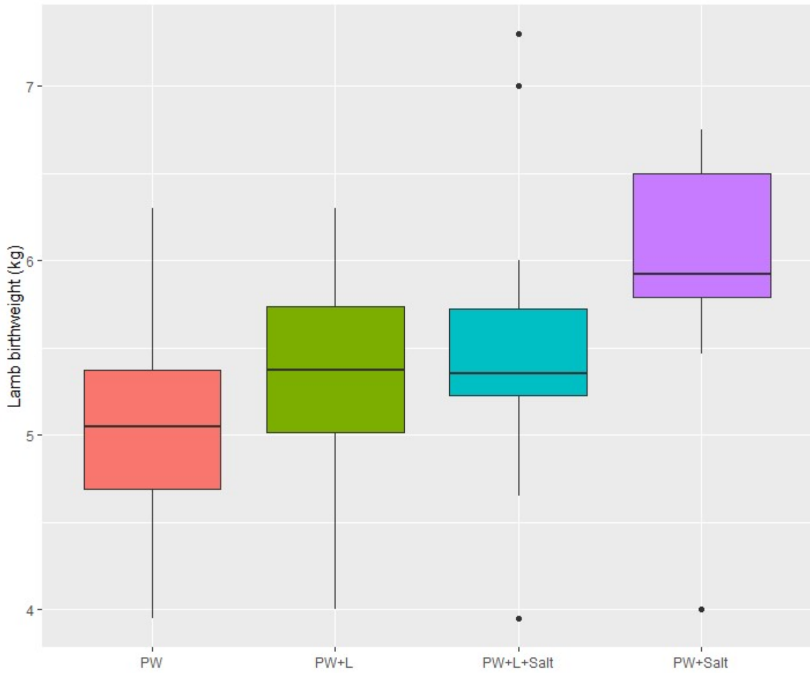

Fig. 31. Boxplot of lamb birthweight (kg) for each treatment group.

Analysis of total weight of lamb marked included the age of the lamb at the time of marking, calculated from the day of their birth. Total weight of lamb marked did not differ between diets and tended (P=0.053) to be higher in ewes that were heavier at the start of lambing. Ewe BCS at lambing and age of the lamb at marking were not significant effects.

At the time of marking, ewe liveweight did not differ significantly between the diets and was not affected by the age of the lamb, nor by the total weight of lamb marked. There were no diet effects on ewe BCS at marking, although the total weight of lamb marked was lower when ewes were more forward in condition (P<0.05, Fig. 33).

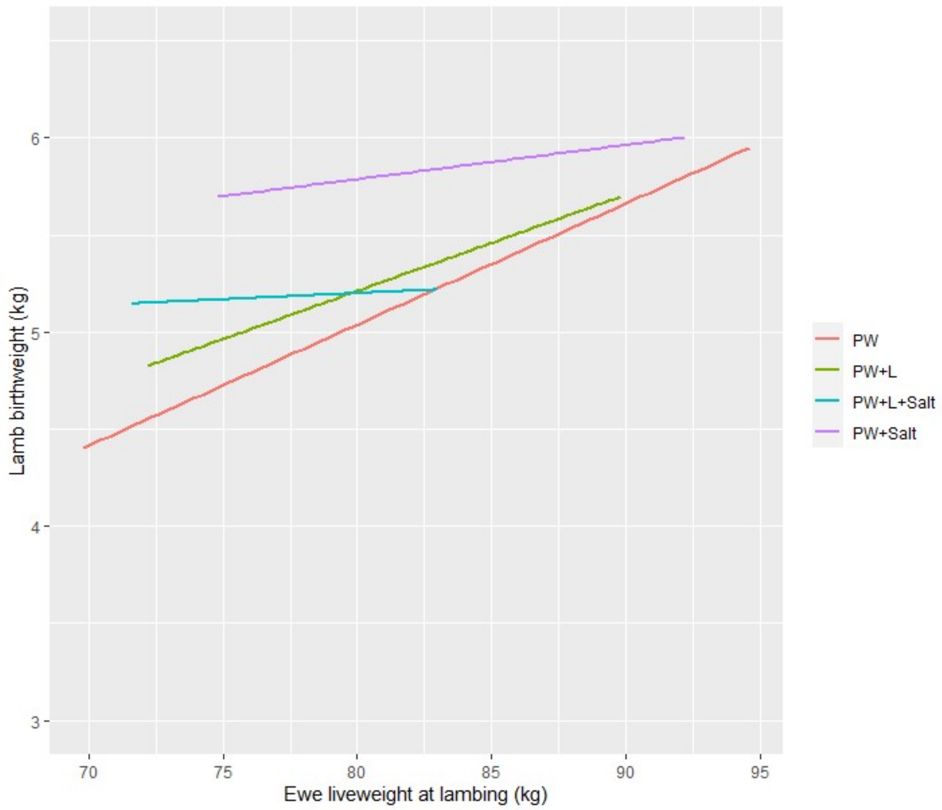

Fig. 32. Lamb birthweight (kg) increases with ewe liveweight (kg).

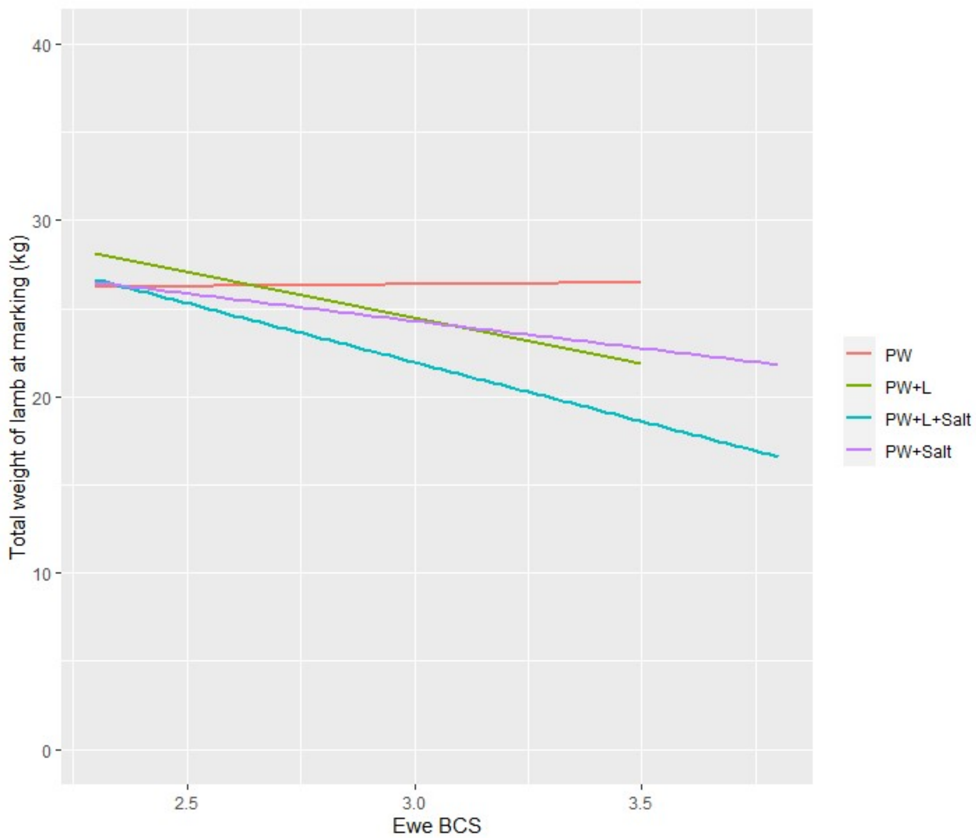

Fig. 33. Total weight of lamb at marking (kg) and ewe body condition score (BCS).

4.4.6 Lamb birthweight, marking weight and mortality

These analyses are of the lamb and permit the animal to be a random term, as well as block. At the time of lambing, there was typical confusion around the maternal parentage for some lambs. Some ewes were only found with one lamb, with no other lamb to be found, and in another instance one ewe had triplets at foot, while a nearby ewe had a single and it was impossible to determine which lamb belonged to which dam. Therefore, number of lambs born is used in the analysis. The number of lambs born is treated as a fixed effect in the model, as is lamb gender.

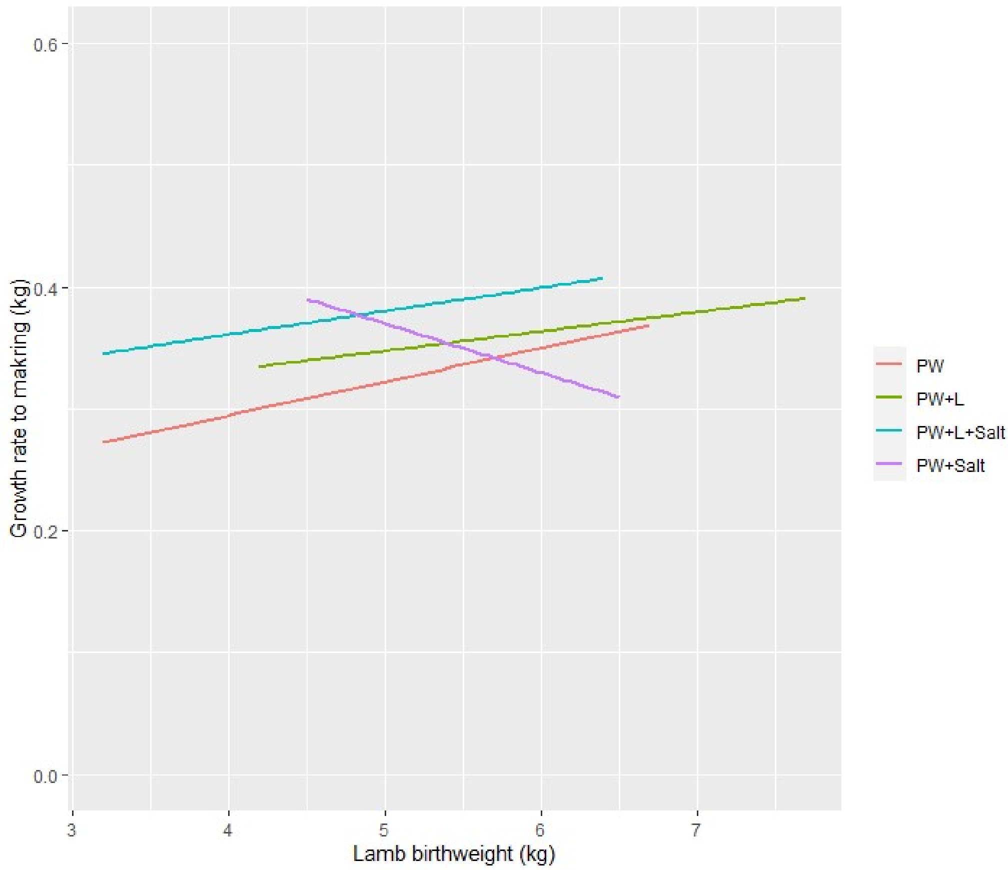

Fig. 34. Growth rate to marking (kg) and lamb birthweight (kg).

There were no diet effects on lamb birthweight, while ram lambs tended to be heavier (P=0.1). Ram lambs were 0.94 ± 0.45 kg lighter at birth when their dams were fed PW+S+L (P<0.05).

Analysis of lamb marking weight include lamb birthweight as a covariate and tested for two-way interactions with lamb gender and treatment. Lambs heavier at birth were heavier at marking 1.21 ± 0.55 kg (P<0.05). Ram lambs born to the ewes fed PW+S were 3.62 ± 1.25 kg heavier at marking (P<0.01), when compared to ram lambs born to PW fed ewes.

Analysis of growth rate of the lambs to marking revealed lambs born to ewes fed PW+S grew 0.52 ± 0.18 kg faster than PW lambs (P<0.01, largely due to the ram lambs in this group (P<0.01). However, lambs that were born to PW+S ewes had lower growth rates to marking if their birthweight was heavier (P<0.01, Fig. 34).

Mortality of lambs at birth was not affected by diet, lamb gender or lamb birthweight. There were no factors affecting mortality of lambs to lamb marking.

4.4.7 Stage 3 discussion

4.4.7.1 Minerals

The addition of salt (NaCl) to the diet was expected to improve both plasma and urine excretion of Mg, Na and to a lesser extent, Ca. While this was observed for plasma and urine Na, it was not observed in Ca or Mg.

The analysis shows that the addition of lucerne to the diet of PW increased plasma Ca and this was supported by the fractional excretion of Ca from ewes whose diets contained lucerne. An unexpected finding was the decrease in plasma Ca concentration in the ewes offered PW+S. This observation is also supported by the limited excretion of Ca in urine in PW+S. No ewes had plasma Ca concentrations below the threshold considered to represent clinical hypocalcaemia in adult sheep (70 mg/L). Sub-clinical hypocalcaemia has a plasma Ca concentration threshold of 90 mg/L. Throughout the study, 1 PW+S ewe had plasma Ca concentrations lower than the sub-clinical indicator on 3 occasions, while another 3 PW+S ewes on two occasions each. In total, 19 samples returned subclinical concentrations of plasma Ca, and PW+S ewes accounted for 11 of these, with PW+S+L accounting for 2, PW accounting for 5 and PW+L just once. These instances occurred mostly on Day 7 (7 samples) and Day 21 (6 samples) with Day 0 and Day 14 having 3 each.

The primary response in plasma Mg was a decrease over time, when compared to the baseline concentration on Day 0. This observation is mirrored by the declining urinary excretion of Mg. The addition of lucerne to the diets has supported higher levels of Mg excretion. While the PW+S+L diet excreted more Mg – when compared to PW - this is likely to be an artefact due to the inclusion of lucerne in the diet. This suggests that providing a source of Mg is more important to Mg status than is the provision of salt (NaCl) as a targeted method to correct the imbalanced ratios of K:Na and K:Na+Mg. Given the continued decline in Mg excretion, neither lucerne, nor salt – when provided at the rates in this experiment – provide adequate protection from the risk of hypomagnesaemia (grass tetany). Plasma Mg concentrations <18mg/L are considered deficient and 28% of all samples collected were lower than this concentration. On Day 7 of the study, 59.6% of all ewes had plasma Mg lower than this indicator level. There was no sign of grass tetany, although 2 ewes were replaced on Day 3 due to low feed intake. One further ewe was removed and not replaced and its plasma sample from Day 0 is included but not thereafter.

The addition of salt to the diet, at 11.3 g/hd/day, is evident in the plasma concentrations and urine fractional excretion. The addition of lucerne had a minor, statistically significant effect on plasma Na, while the addition of salt was much clearer in its effect, but these effects were not statistically significant until Day 21. However, in urine, there were differences due to the addition of salt (PW+S and PW+S+L) from Day 7 and these persisted through to Day 21. Overall, the objective of the inclusion of salt was to observe improvements in plasma Mg and Ca, and in urine excretion of Mg in particular, and these responses do not appear to have occurred. It is certain that the inclusion of salt has not restored mineral status of the ewes consuming those diets. The data does provide a great deal of confidence in these findings because the fractional excretion of Na points to the consumption of that mineral; evidence that the mineral was offered and was eaten.

It is well established that the concentration of K in the diet of PW is excessive, and that effect is evident with the plasma K increasing from Day 0 to Day 7 and maintaining at that elevated level. Plasma K appears to decline in the PW+L, PW+S and PW+S+L diets at Day 21, but this effect is not significant. Urinary excretion of K elevates after Day 0, which is to be expected, and decreased over time to be not different to the baseline levels at Day 21. This suggests that forage K content declines by Day 21, because the ewes eating the PW diet also had decrease in plasma K at Day 21.

Phosphorus is only analysed in plasma. It shows that the addition of lucerne to the diet decreased plasma P, while the ewes eating the PW+S diet maintained Na concentrations equivalent to their baseline. There may be some effect on plasma P due to the addition of salt to the diet, as there appear to be consistent increases in the PW+S+L diet. The plasma P concentration in the PW+S ewes is very interesting. In this dietary group, plasma Ca continued to decline and was lower than the other diets, whereas the reverse was true in plasma P. The two minerals are similarly weighted and structured and should respond in similar ways. In the 2019 (Stage 2) lamb study, there were also clear decreases in plasma P over time, although there were no differences between the diets (PW, PW+L W, W+L). The finding of the present study suggests the addition of L to the diets decreases plasma P and the consumption of salt offsets that effect. Some reviewers will be sceptical of the P results and some caution is advised in their interpretation. However, these effects are the same as those observed in the 2019 study and perhaps more should be made of them.

4.4.7.2 Animal performance

As expected, the ewes gained weight over the duration of the study. Differences emerged in weight immediately, when on Day 7 all diets were heavier than PW, and this relationship persisted. Interpretation of this finding requires consideration of the feed intake results (not presented). Concurrently, the ewes were gradually losing body condition, indicating their dietary requirements were not being met by any of the diets.

Despite the loss of condition, the ewes were not under metabolic duress, as glucose and BHB were normal. The maximum BHB concentration observed in the study was 0.4 mmol/L. The risk indicator for ketosis is plasma BHB exceeding levels of 0.8 mmol/L. Curiously, the PW+S+L ewes, which were the heaviest, had the highest BHB concentration. BHB is a marker for metabolic energy status. It will increase in ewes failing to consume sufficient energy and thus, their body fat reserves are utilised for energy. This catabolism occurs in the liver via a process called gluconeogenesis. A by-product of fat being catabolised for energy is the production of ketone. The dominant ketone is BHB and as its concentration increases in the blood, there are negative feedback signals for satiety. Ewes with higher BHB have metabolic signals suggesting they do not need to eat. This leads to increase fat catabolism and a downward spiral towards death. There is a general relationship between feed intake and body fatness in late pregnancy twin-bearing ewes, which suggests late term ewes in high body condition are also less likely to eat and more likely to have elevated BHB. This isn't observed in the present study, whereas for most diets, BHB was lower in ewes that were fatter in body condition.

Blood glucose is maintained by homeostasis, in interaction with insulin (data not collected), and there were no dietary effects on glucose concentrations. It should not be surprising that there are no diet interactions with blood glucose, despite some diet effects in BHB. The BHB levels are normal, and so too are the blood glucose concentrations.

Urine pH appeared to have been increased by the addition of lucerne to the diet. An indicator for increased urine alkalinity can be the dietary cation anion difference (DCAD). That data is not available elsewhere but is required for further interpretation of urine pH.

4.4.7.3 Lambing

The effect of higher ewe liveweight in the PW+S+L group translated to heavier lamb birthweights in the lambs born to those ewes, likely due to the ram lambs. Birthweight is highly significantly associated with lamb survival, but the scale of the present study is quite small to examine such effects. As a result, there was no statistical differences in ewe or lamb mortality, or due to lamb gender or birthweight. Lamb mortality on the day of birth was 6.6%, which is quite low for twin bearing ewes. By the time of lamb marking, mortality had increased to 14%, with no dietary effects. Normal rates of twin lamb mortality are between 15% and 30%, as such the performance of the Merino ewes in the present study is high and suggestive that no lasting or carry-over negative effects, if any, were attributable to the diets.

Lambs that were heavier at birth, were generally heavier at lamb marking, but ram lambs born to PW+S ewes were heaviest at marking, while ram lambs born to PW+S+L ewes were the heaviest at birth. In terms of growth rate, PW+S lambs grew faster than lambs born to PW-fed ewes, likely due to the ram lambs in the cohort.

Overall, there were no statistically significant effects that were detrimental to lamb survival due to the diets. The effects of diet on ewe weight did not translate into higher mortality.

4.5 Stage 4 grazing experiment

4.5.1 Forage intake

There were significant ($P<0.05$) differences in perennial wheat intake between treatments over the course of the study (Table 8). The average forage intake across rotation plots showed an increase in perennial wheat intake over the first five weeks of grazing with lambs consuming on average ≈30% more perennial forage in the PW and PW+C treatments than the PW+L and PW+S treatments (Fig. 35). From week five through to week seven, perennial wheat consumption declined across all treatments, however, intake was on average 27% higher in the perennial wheat treatment. Apart an early increase in perennial wheat intake, there was a plateau in cereal intake between weeks 8-10. Over the last two weeks of grazing, perennial wheat intake increased, however compared to the previous weeks, intake of perennial wheat in the PW+L treatment was 37% higher on average than the other two legume intercrops but similar to intake of the PW monocrop treatment, perhaps reflecting lower lucerne intake compared to serradella and clover.

The lower perennial wheat forage intake in the legume intercrop treatments was somewhat reflective of legume intake (Fig. 35). In the initial weeks of grazing, intake of serradella was 86% higher than either clover or lucerne but declined over the course of the experiment. Lucerne intake was also initially higher than clover, however declined to negligible levels by week eight of the experiment. The decline in lucerne and serradella intake coincided with an increase in perennial wheat consumption in their respective treatments. Conversely, intake of clover increased over the grazing period and by the last week of the study was 44% and 98% higher than serradella and lucerne intake, respectively. This was also associated with a subsequent decrease in the proportion of perennial wheat consumed in the clover treatment. Overall, the combined forage intake of each treatment showed higher total consumption in the PW+C and PW+S treatments (33%) on average compared to the PW or PW+L in the first seven weeks of the experiment. In the last two weeks of the experiment there was little difference in total intake between treatments, however, on average total intake in the PW+L diet tended to be lower across all assessment dates.

Table 8: Wald statistics for total weekly intake (Measurement date) of lambs grazing treatments (PW, PW+C, PW+L and PW+S)

Strata/Description	Effect [A]	NDF [B]	DDF [B]	F-value	P-value
		Perennial wheat intake			
Treatment	F	3	283.9	3.0	<0.05
Measurement date	F	1	284.0	2.4	>0.05
Treatment x Measurement date	F	3	284.0	3.8	<0.05
Residual	R				
		Legume intake			
Treatment	F	2	222	8.1	<0.001
Measurement date	F	1	222	0.03	>0.05
Treatment x Measurement date	F	2	222	6.7	<0.01
Residual	R				

[A] F, fixed effect; R, random effect. [B] Numerator Degrees of Freedom, Denominator Degrees of Freedom

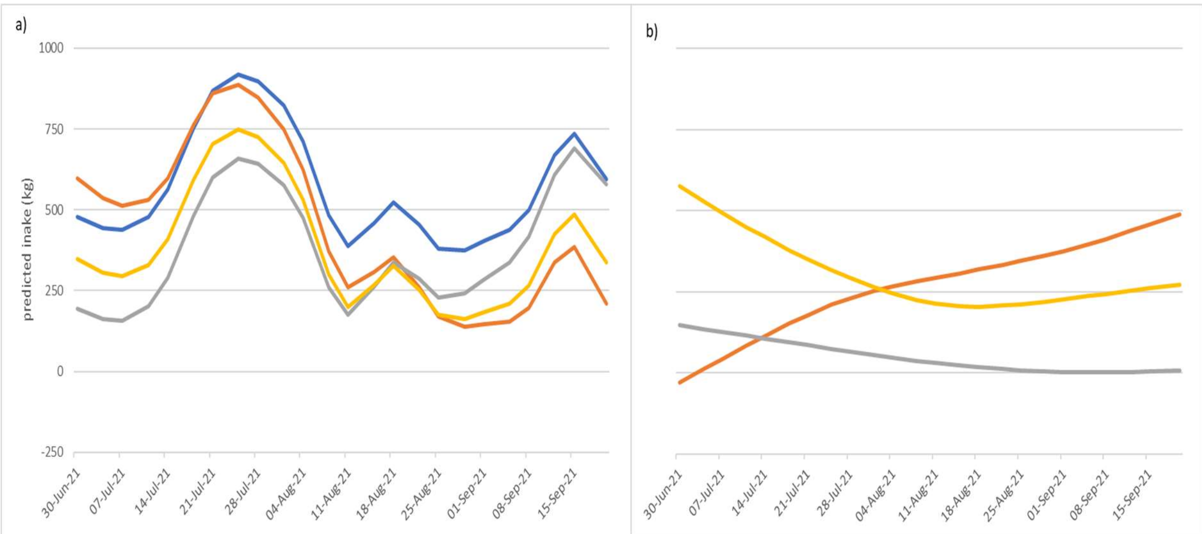

Fig. 35: Average forage intake of a) perennial wheat and b) legume by lambs grazing perennial wheat (PW —), perennial wheat + subterranean clover (PW+C —), perennial wheat + lucerne (PW+L—) and perennial wheat + serradella (PW+S—).

Table 9. a) Mean dietary concentration of calcium (Ca), magnesium (Mg), phosphorous (P), potassium (K), sodium (Na), sulfur (S), chloride (Cl) and b) mineral indices which reflect risk of mineral imbalance (DCAD, dietary cation-anion difference) as well as dry matter digestibility (DMD), neutral detergent fibre (NDF) crude protein (CP) and metabolisable energy (ME) across forage components of each treatment. Figures in bold are outside required limits.

a)	Ca% [A]		Mg% [A]		P% [A]		K% [A]		Na% [A]		S% [A]		Cl% [A]	
Lamb required level	>0.28		>0.09		>0.24		<3		>0.06		>0.18		>0.04	
	Cereal	Legume	Cereal	Legume	Cereal	Legume	Cereal	Legume	Cereal	Legume	Cereal	Legume	Cereal	Legume
PW	0.39		0.13		0.42		**3.50**		**0.01**		0.40		0.40	
PW+C	0.42	1.15	0.15	0.24	0.44	0.39	**3.49**	2.79	**0.01**	0.09	0.43	0.29	0.43	0.29
PW+L	0.38	1.41	0.13	0.20	0.41	0.45	**3.40**	2.80	**0.01**	**0.02**	0.39	0.33	0.39	0.33
PW+S	0.40	0.99	0.15	0.25	0.43	0.46	**3.65**	2.73	**0.01**	0.08	0.45	0.26	0.45	0.26
se	0.022		0.003		0.008		0.044		0.003		0.007		0.007	

b)	Ca:P [A]		DCAD [B]		Tetany Index [c]		K:Na+Mg [D]		DMD (%)		NDF (%)		CP (%) [A]		ME (MJ/kg DM) [A]	
Lamb required level	1.2		<12		<2.2		<6				40		9.4		12	
	Cereal	Legume	Cereal	Legume	Cereal	Legume	Cereal	Legume	Cereal	Legume	Cereal	Legume	Cereal	Legume	Cereal	Legume
PW	**0.95**		**51.94**		**4.44**		**8.09**		76.64		42.01		24.57		**11.73**	
PW+C	**0.98**	2.98	**50.96**	**51.71**	**4.10**	1.22	**6.93**	3.03	78.19	80.6	39.8	26.84	29.02	29.66	12.04	12.33
PW+L	**0.95**	3.20	**51.61**	**45.21**	**4.34**	1.04	**7.62**	4.06	77.34	77.3	40.95	27.36	25.89	30.89	**11.81**	**11.94**
PW+S	**0.94**	2.16	**53.05**	**49.41**	**4.45**	1.37	**7.38**	2.95	78.13	74.56	39.84	28.28	29.14	23.43	12.04	11.64
se	0.061		1.025		0.068		0.106		0.507		0.583		0.504		0.094	

[A] Requirement derived from National Research Council (2007)
[B] Estimated from Takagi and Block (1991)
[C] Kemp and t' Hart (1957)
[D] (Dove *et al.* 2016)

4.5.2 Forage mineral concentration

Daily mineral concentrations were averaged across measurement dates to provide a representative concentration over the grazing period. This value was used for comparisons between the supply of minerals from forages of perennial wheat (Cereal) and the legume component of each treatment, compared with the published requirements for a 50 kg lamb growing 250 g/day (Table 9a). All forages had more than adequate concentrations of Ca, Mg, P, S and Cl to supply the requirements of a fast growing 50 kg lamb, however the Ca and Mg concentrations in the legumes were 63% and 37% higher on average, respectively. Perennial wheat had forage Na concentrations which were 16% below the published requirement for this class of sheep, with concurrently 83% higher than required K concentrations. Concentrations of Na were more than adequate for lamb requirements in both clover and serradella, however lucerne Na concentrations was 66% below requirements. Legume K concentrations were also 21% lower than the concentration of K in the corresponding perennial wheat forage.

Key mineral ratios (Table 9b) indicate the availability of Ca and Mg in perennial wheat forage were well beyond the thresholds for the tetany index (two-fold higher) and K:(Na + Mg) ratio (24% higher). These same indices were approximately half the threshold levels in the legumes in each treatment group. On average the DCAD (<12) for all treatments was four-fold higher than the published requirement across all forage components. The Ca:P ratio was slightly below requirements for the perennial wheat forage, while all forage treatments had more than adequate ME, except for the PW and PW+L treatments.

4.5.3 Liveweight Gain

Liveweight of all lambs were observed to increase as weekly measurements progressed over the course of grazing in each treatment (Table 10, ΔLWT). The highest live weight increase occurred in lambs grazing the PW+C forage which had 18% and 8% heavier lambs than those grazing in the PW+L and PW+S treatments. Overall increases in liveweight was similar in lambs grazing the PW and PW+C treatments.

4.5.4 Anthesis dry matter and grain yield components

The effect of grazing on anthesis dry matter and grain yield is presented in Table 11. Anthesis was delayed in the grazed treatments by 12 days and anthesis dry matter was two-fold higher in the ungrazed area compared the grazed plots. Legume dry matter, measured at perennial wheat anthesis, was on average of 64% lower in the grazed plots compared to the ungrazed areas. Higher amounts of lodging was observed in the ungrazed areas compared to the grazed plots, but there was no difference (P>0.05) in grain yields or tiller number between grazed and ungrazed plots. Nevertheless, HI and TKW were reduced (P<0.01) by 30% and 10% respectively in the ungrazed area.

Grain yield through mechanical harvest, was significantly reduced (P<0.001) in the PW+C treatment, producing approximately half the amount of grain than in the other three treatments. However, the HI in this treatment was on average 16% higher (Table 10). The legume intercrop treatments also had lower (cereal) tiller density, as consequence of the reduced number of cereal rows, although the TKW was similar between all four treatments.

Table 10: Grain harvest parameters determined through hand harvest for perennial wheat (PW), perennial wheat + subterranean clover (PW+C), perennial wheat + lucerne (PW+L) and perennial wheat + serradella (PW+S). Grain yield data determined through mechanical harvest

	Grain Yield kg/ha	HI*	Tillers/m^2	TKW(g)	ΔLWT (kg)	Anthesis Legume DM (kg/ha)	TLO
PW	1695	0.20	377.5	31.48	16.6		1794.6
PW+C	719	0.24	186.1	31.03	17.1	1182	2003.6
PW+L	1382	0.22	250.2	32.63	14.1	305	1771.6
PW+S	1272	0.22	223.0	32.13	15.8	1316	2682.8
P value	<0.001	<0.001	<0.001	>0.05	<0.001	<0.01	
lsd (5%)	192.6	0.01	39.27	ns	1.2	747.2	

*Harvest Index

Thousand Kernel Weight

Table 11: herbage production assessed at perennial wheat anthesis and grain production parameters determined from hand harvest for the ungrazed treatments compared to the grazed rotation A and rotation B treatments

	Anthesis Dry Matter		Harvest Grain Parameters			
	Cereal (kg/ha)	Legume kg/ha	Grain Yield kg/ha	HI*	Tillers/m^2	TKW#
Rotation A	6474	752	2108	0.23	218.2	33.7
Rotation B	6051	227	1787	0.22	240.7	32.4
Ungrazed	12702	1374	1962	0.15	249.1	30.9
P value	<0.001	<0.01	>0.05	<0.001	>0.05	<0.001
lsd (5%)	1279.8	647.1	ns	0.02	ns	1.37

*Harvest Index

Thousand Kernel Weight

4.5.6 Carcass characteristics

Table 12 shows there to be no effects from forage type on lamb carcass composition (P > 0.05). There were no effects from forage type and their interactions with ageing period on the meat quality of grazing lambs (P > 0.05; Table 13). Purge and TVB-N both increased with ageing period (P < 0.001), whereas shear force values demonstrated the opposite trend and decreased with ageing period (P < 0.001) (Table 14). There were no effects of forage type on the meat colour parameters, of grazing lambs, and their variation over retail display (P > 0.05; Table 15). L*, b*, and Chroma values increased, initially, and then declined as display period continued (P < 0.001). R630/580 and a* values declined with each increase to display period (P < 0.001), and Hue values increased with each increase to display period (P < 0.001). There were no effects from forage type on the mineral composition of the meat from grazing lambs (P > 0.05; Table 16).

Table 12: The effect of forage type on experimental lamb carcass composition and quality parameters. Means, standard error (SEM), and the level of significance (P-values) are included.[1]

Carcass composition	Forage type				SEM	P-value
	PW+C	PW+S	PW+L	PW		
CCW, kg	24.3	23.2	23.7	24.0	0.3	0.136
DEXA Bone, %	13.0	13.4	13.0	13.3	0.1	0.168
DEXA Fat, %	34.7	34.4	35.4	34.3	0.5	0.350
DEXA Lean, %	52.3	52.2	51.6	52.4	0.4	0.438
DRESS, %	51.1	50.2	50.8	49.6	0.4	0.072
EMA, cm2	14.5	15.5	15.8	15.1	0.4	0.127
FatC, mm	4.7	4.7	5.1	4.9	0.4	0.909
GR tissue depth, mm	17.8	17.7	19.4	17.8	0.9	0.466
HCW, kg	24.9	23.8	24.4	24.5	0.3	0.163
PH18, U	6.36	6.35	6.37	6.41	0.04	0.746
PH24, U	5.55	5.59	5.56	5.56	0.03	0.801
TEMP6, °C	11.3	11.5	10.4	9.1	0.7	0.087

[1]Other abbreviations included perennial wheat and subterranean clover (PW+C), perennial wheat and French serradella (PW+S), perennial wheat and lucerne (PW+L), perennial wheat and a mineral salt supplement (PW), cold carcass weight (CCW), dual energy x-ray absorptiometer (DEXA), dressing (DRESS), eye muscle area (EMA), Fat C depth (FATC), girth rib site (GR), hot carcass weight (HCW), pH at 18 °C (PH18), final pH (PH24), and temperature at pH 6 (TEMP6).

Table 13: The effect of forage type, ageing period (days), and their interaction on the meat quality of the experimental lambs. Means, standard error (SEM), and the level of significance (P-values) are included.[1]

Meat quality	Forage type				SEM	Ageing Period		SEM	P-value		
	PW+C	PW+S	PW+L	PW		5	56		Forage	Age	Interaction

CL, %	35.6	35.8	35.9	36.4	0.8	36.3	35.6	0.6	0.805	0.240	0.818
DL, %	0.47	0.49	0.53	0.56	0.10	0.48	0.54	0.07	0.793	0.402	0.717
PURGE, %	4.97	5.11	5.23	4.85	0.31	3.73a	6.35b	0.19	0.649	< 0.001	0.956
SF, N	24.2	25.4	24.6	25.2	1.0	28.8a	20.8b	0.6	0.590	< 0.001	0.278
TVB-N, mg/100 g	6.43	6.25	6.11	6.31	0.23	6.00a	3.55b	0.2	0.569	< 0.001	0.500

[1]Means within fixed effects with different superscripts are significantly different (P < 0.05). Other abbreviations include perennial wheat and subterranean clover (PW+C), perennial wheat and French serradella (PW+S), perennial wheat and lucerne (PW+L), perennial wheat and a mineral salt supplement (PW), cooking loss (CL), drip loss (DL), purge loss (PURGE), shear force (SF), and total volatile basic nitrogen (TVB-N).

Table 14: The effect of forage type on the meat quality of the experimental lambs. Means, standard error (SEM), and the level of significance (P-values) are included.[1]

Meat quality	Forage type				SEM	P-value
	PW+C	PW+S	PW+L	PW		
Sarcomere length,	1.71	1.66	1.74	1.72	0.04	0.343
pHu, U	5.50	5.54	5.48	5.53	0.04	0.619
Total moisture, %	73.2	73.4	73.1	73.3	0.3	0.798

[1]Other abbreviations included perennial wheat and subterranean clover (PW+C), perennial wheat and French serradella (PW+S), perennial wheat and lucerne (PW+L), perennial wheat and a mineral salt supplement (PW), and ultimate pH (pHu).

Table 15: The effect of forage type, display period (days), and their interaction on the colour stability of meat from the experimental lambs. Means, standard error (SEM), and the level of significance (P-values) are included.[1]

Colour parameter	Forage type				SEM	Display Period				SEM	P-value		
	PW+C	PW+S	PW+L	PW		0	1	2	3		Forage	Display	Interaction
L*	37.1	36.9	37.2	36.2	0.5	36.3b	37.8a	36.8b	36.5b	0.3	0.293	< 0.001	0.857
a*	16.5	16.4	17.1	17.0	0.4	17.5a	17.5a	16.7b	15.3c	0.2	0.237	< 0.001	0.964
b*	15.5	15.4	15.4	15.5	0.3	14.9c	16.2a	15.5b	15.1c	0.2	0.947	< 0.001	0.986
Hue	43.3	43.3	42.0	42.4	0.7	40.5d	42.8c	43.0b	44.6a	0.3	0.236	< 0.001	0.995
Chroma	22.6	22.5	23.1	23.0	0.4	23.0b	23.9a	22.8b	21.6c	0.3	0.374	< 0.001	0.968
R630/580	3.82	3.80	4.02	4.09	0.14	5.18a	3.91b	3.53c	3.10d	0.09	0.125	< 0.001	0.971

[1]Means within fixed effects with different superscripts are significantly different (P < 0.05). Other abbreviations include perennial wheat and subterranean clover (PW+C), perennial wheat and French serradella (PW+S), perennial wheat and lucerne (PW+L), perennial wheat and a mineral salt supplement (PW), and the ratio of reflectance at 630 and 580 nm.

Table 16: The effect of forage type on experimental lamb carcass composition. Means, standard error (SEM), and the level of significance (P-values) are included.[1]

Mineral composition	RDI, mg[2]	Forage type, mg/100 g fresh wt.				SEM	P-value
		PW+C	PW+S	PW+L	PW		
Calcium	1000	34.89	34.57	34.98	34.71	0.45	ns
Copper	1.2-1.7	0.12	0.12	0.12	0.12	< 0.01	ns
Iron	8-18	2.15	2.13	2.15	2.14	0.03	ns
Magnesium	310-420	29.53	29.25	29.60	29.37	0.38	ns
Phosphorous	1000	249.63	247.31	250.22	248.33	3.18	ns
Potassium	2800-3800	322.11	319.11	322.86	320.43	4.10	ns
Sodium	460-920	45.63	45.21	45.74	45.39	0.58	ns
Zinc	8-14	2.63	2.61	2.64	2.62	0.03	ns

[1]Other abbreviations included recommended daily intake (RDI), perennial wheat and subterranean clover (PW+C), perennial wheat and French serradella (PW+S), perennial wheat and lucerne (PW+L), perennial wheat and a mineral salt supplement (PW), and not significant (P>0.05) (ns).
[2]Recommended daily intake derived from National Health and Medical Research Council (2006). These nutrient reference values are for adults (19 to 50 years of age).

4.6 Gross Margin analysis

Using results of the 2021 grazing study a gross margin analysis was undertaken to compare the 4 dietary treatments, perennial wheat + mineral supplement (PW+MS), or perennial wheat grown in combination with one of three companion legumes, lucerne (PW+L), subterranean clover (PW+C) or French serradella (PW+S). A grain price of $600/t and a lamb price of $8.60/kg carcass weight were assumed for this analysis, which included a price sensitivity analysis examining the effects of price movements of 25% increments in either direction (Table 17).

The analysis includes some real costs incurred in the course of the experiment, including seed and fertiliser costs to establish the pasture, one drench and two vaccination treatments as well as the loose-lick mineral supplements offered in the PW+MS treatment.

Importantly, we only include in this analysis the carcass weight gain attributable to treatments therefore the purchase price and sale price of lambs are both excluded. We calculate weight gain as liveweight at the end of the experiment (lwt_u) minus initial liveweight (lwt_i), multiplied by lamb meat yield which is assumed to be 52%.

$$Cwt\ (kg) = (lwt_u - lwt_i) \times 0.52$$

Table 17. Gross margin and sensitivity analysis generated from the grazing experiment comparing four dietary treatments. Value in bold for each treatment represents the baseline assumptions of $8.60/kg lamb carcass weight and $600/t for grain.

Grain price	Lamb price (Carcass weight; $/kg) $4.30	$6.45	$8.60	$10.75	$12.90
			Perennial wheat + mineral supplement		
$ 300	$ 1,008	$ 1,587	$ 2,167	$ 2,747	$ 3,326
$ 450	$ 1,262	$ 1,842	$ 2,421	$ 3,001	$ 3,581
$ 600	$ 1,516	$ 2,096	**$ 2,676**	$ 3,255	$ 3,835
$ 750	$ 1,770	$ 2,350	$ 2,930	$ 3,509	$ 4,089
$ 900	$ 2,025	$ 2,604	$ 3,184	$ 3,764	$ 4,343
			Perennial wheat & lucerne		
$ 300	$ 393	$ 836	$ 1,279	$ 1,723	$ 2,166
$ 450	$ 600	$ 1,043	$ 1,487	$ 1,930	$ 2,373
$ 600	$ 807	$ 1,251	**$ 1,694**	$ 2,137	$ 2,580
$ 750	$ 1,015	$ 1,458	$ 1,901	$ 2,344	$ 2,788
$ 900	$ 1,222	$ 1,665	$ 2,108	$ 2,552	$ 2,995
			Perennial wheat & subterranean clover		
$ 300	$ 532	$ 1,156	$ 1,779	$ 2,402	$ 3,026
$ 450	$ 640	$ 1,264	$ 1,887	$ 2,510	$ 3,134
$ 600	$ 748	$ 1,371	**$ 1,995**	$ 2,618	$ 3,241
$ 750	$ 856	$ 1,479	$ 2,103	$ 2,726	$ 3,349
$ 900	$ 964	$ 1,587	$ 2,210	$ 2,834	$ 3,457
			Perennial wheat & French serradella		
$ 300	$ 467	$ 1,026	$ 1,586	$ 2,146	$ 2,705
$ 450	$ 657	$ 1,217	$ 1,777	$ 2,337	$ 2,896
$ 600	$ 848	$ 1,408	**$ 1,968**	$ 2,527	$ 3,087
$ 750	$ 1,039	$ 1,599	$ 2,158	$ 2,718	$ 3,278
$ 900	$ 1,230	$ 1,790	$ 2,349	$ 2,909	$ 3,469

Results revealed that the highest gross margin was achieved with the perennial wheat grown alone, with mineral supplements offered. Perennial wheat plus lucerne had the lowest gross margin.

The gross margins for subterranean clover and serradella treatments were quite similar to each other at the baseline prices, but 26% lower than the PW+MS treatment. At lower grain prices and as lamb price increased, the margin between the PW+MS and PW+C treatments narrowed to <10% yet for the PW+S, the margin between the PW+MS treatment narrowed to only 19%. This reflected the higher carrying capacity of the PW+C treatment compared to other legumes, and the fact that at lower grain prices the yield penalty observed in the PW+C treatment had less impact on gross margins of that treatment.

This gross margin analysis helps to highlight the trade-offs in growing cereals with legumes and that further research is required to integrate cereal/legume mixtures without incurring financial penalty.

5 Success in meeting the milestone

5.1 Report on success against each objective

All project objectives were met, as detailed earlier in this report (see Section 2.1).

5.2 Recommendations on design-led thinking approach

Design-led thinking enabled the project to tweak activities in response to new information and feedback from stakeholders. An early example of design-led thinking in action was changing the order of feeding experiments; running a lamb experiment first before the ewe feeding experiment was conducted in 2020. This sensible change came about due to the realisation that there were many elements in the proposed feeding experiment methodology that were untested and that may place vulnerable livestock at risk. Design-led thinking enabled this change to occur without the administrative burden of a contract variation.

Another example in this project was the establishment of the Stage 4 grazing experiment in 2021. This experiment was not part of the original research plan but was deemed necessary to get a more complete understanding of grazing preference under more realistic grazing conditions. This feedback was received from a number of sources including the Industry Advisory Committee, who were always keen to explore the relevance of findings to existing farm practices. Other sources of feedback included social media, such as this exchange on Twitter with reputable sheep nutritionist Dr David Masters, where he asked a simple and obvious question 'Why cut and carry?' (Fig. 36). The project team also recognised a clear need to explore a greater range of legume options.

Of course, it was not possible in the early years to run anything other than a cut and carry feeding experiment due to the paucity of perennial wheat seed available. The pen feeding experiments also provided the advantage that daily intake could be estimated with a high degree of accuracy. However, after having run two successful pen feeding experiments in 2019 and 2020, and with early successes in the seed multiplication activity despite drought conditions in 2018 and 2019, the project team had the seed, the time, the budget and the confidence to conduct an additional grazing experiment in response to constructive feedback from stakeholders and the gaps that were obvious after analysis of early results.

Fig. 36. An example of feedback on social media. From Twitter during the Stage 3 experiment we are asked why we are using a cut and carry system. The inference is, quite rightly, that a grazing experiment would be more industry relevant.

Based on experience from this project, the recommendation to MLA is to maintain design-led thinking as an element of all research projects, to facilitate timely and appropriate changes to research plans without increased administrative burdens. Research is, by definition, about finding out what is not already known. Therefore, it is anticipated that plans will deviate as the project progresses. The high level of output from this project is evidence that deviations from the initial plan can be a positive outcome for research and should be encouraged, where appropriate.

5.3 Plain-English Fact sheet

The project has exceeded its obligations on this milestone, delivering three industry-relevant factsheets on aspects of this project including Agronomy, Animal health and Grain Properties and End Uses (Appendices 4-6). These factsheets use the standard NSW DPI PrimeFact template and are in the process of being loaded alongside other resources on the NSW DPI website.

5.4 Update on distribution actions of the information packaged from M6

A primary audience for this project was the research community, due to the early stage of development of perennial wheat globally. Accordingly, the project team placed high priority on publication of results in broadly accessible journals and conferences. The project far exceeded its contracted outputs and with several draft manuscripts in preparation (see Appendices 1-3), continues to deliver basic research to industry.

Some elements of the work are immediately applicable to industry, especially around the question of using legumes to manage the mineral balance of livestock on grazing cereals. There is a growing array of online resources available from this project. These include two presentations from Dr Gordon Refshauge delivered at the 2021 and 2022 Pasture Research Updates hosted by the Riverina LLS, and a podcast interview with Matthew Newell talking about perennial grains more generally (links to those resources are in the foot notes of Table 1 in Section 2.1.3 of this report). In addition, the three plain-English factsheets described previously will soon be accessible on the NSW DPI website.

6 Conclusions

This proof-of-concept study established the suitability of perennial wheat forage for grazing sheep. The forage quality of perennial wheat was broadly comparable to conventional grazing wheat, with no additional risks to animal health or well-being identified. Similar to wheat, the mineral profile of perennial wheat forage was imbalanced with excessive concentrations of K and insufficient Na, presenting a risk of metabolic disorders such as hypocalcaemia and hypomagnesaemia that would need to be managed in vulnerable livestock, such as by provision of mineral supplements. Consideration was given to the role of companion legumes to be grown with perennial wheat to achieve multiple functions, including N additions through biological N-fixation and improved forage quality associated with a changed mineral profile. The addition of lucerne increased total forage intake compared to a cereal-only diet and alleviated the need for Ca supplementation. However, supplementation of Mg and Na was still required to meet requirements, due to the low Na concentrations in lucerne forage. Alternative legume species were identified with higher concentrations of Na than lucerne, including subterranean clover and serradella. The inclusion of a legume always led to a reduction in perennial wheat grain yield, prompting recommendations that optimal legume composition in a cereal-based sward be in the range of 25-40% of total biomass.

Perennial wheat is only at an early stage of the delivery pipeline, explaining why the research community was a primary target audience and why many of the project objectives related to delivery of scientific outputs. During the life of the project, 8 Conference papers and 5 scientific journal papers have been published with another 5 at various stages of development, far exceeding contracted obligations. The project also delivered a good range of extension and outreach outputs to farmers and broader industry, informing decisions around legume mixtures, mineral balance in livestock and the novel concept of perennial grains in general. Material for a general audience is still available on various websites in the form of PrimeFacts, presentations (YouTube), NSW Country Hour (ABC Radio Listen app), and one podcast.

The project successfully met all its contracted obligations. In addition, linkages were formed with a number of other projects which collectively added significant value to the broader research program. The value-add opportunities included additional sampling in the first feeding study of the rumen epithelium and of lamb carcases using spectroscopy, screening the mineral profile of a broader range of legume species, and detailed evaluation of the end use and grain functionality of a range of perennial breeding lines. The project sponsored three Honours projects (two completed) at Charles Sturt University and the University of Tasmania, and one PhD candidate at the University of Southern Queensland that remains in progress.

6.1 Key findings

6.1.1 Plasma and urine mineral status of lambs and ewes

The objective of the series of studies was to explore the value of a biculture diet, using lucerne as the compliment to perennial wheat, to offer a higher dietary concentration of Ca, Mg and Na. Each study differed from the previous with new knowledge informing the following set of treatments.

The first study (2019, lambs) evaluated the effect of adding lucerne to perennial wheat, or annual wheat, examining the value lucerne has for Ca, Mg and Na status. This study showed that adding lucerne to the diet increased feed intake and mineral intake for Ca, Mg and Na. However, the increased mineral intake was insufficient to overcome the primary mineral imbalance in the perennial wheat forage, led by the high concentration of K and low Na. A key observation was seen in both plasma and urine Mg and Na, which declined over the study. The primary finding of this study was that mineral supplementation including Mg and Na was still required to improve mineral status of growing lambs. The conventional mineral supplementation recommended for ruminants grazing wheat forages includes Ca as well as Mg and Na and the findings of the 2019 study suggested Ca was not required when lucerne was included in the diet with wheats.

The second study (2020) used late-pregnancy twin bearing Merino ewes and nuanced dietary differences. A departure from the 2019 lamb study was the use of the ewes, which was to increase the demand for mineral intake for Mg and Na. Further changes were made to the design, which involved dropping annual wheat from the diets to allow the research to focus on perennial wheat, with or without lucerne. To overcome the health risks associated with using a livestock class with a higher demand for dietary Mg and Na, salt (NaCl) was provided with the forages. The diets were able to meet the recommended dietary thresholds for K:Na and K:Na + Mg. Despite this, however, the inclusion of salt to improve plasma Mg and Ca, and urinary Mg excretion in particular, do not appear to have occurred.

Our findings suggest sheep grazing any of the diets examined continue to require supplementation with Na and Mg, but the addition of lucerne alleviated the need for Ca supplementation.

6.1.2 Meat quality

The 2019 experiment showed that perennial wheat forage fed as either a monoculture or with lucerne can deliver lamb meat that meets consumer expectations. This observation is based on consumer sensory scores and instrumental measures for tenderness, flavour, juiciness and overall liking. The colour and oxidative status of meat from lambs fed perennial wheat were within ranges defined for consumer satisfaction with product freshness. There were lower concentrations of sodium, sulphur and zinc in the meat from lambs fed perennial wheat diets, although in practical terms, these differences may be considered to be negligible. The nutritional value of lamb meat that is associated with omega-3 fatty acid concentrations was comparable between the combinations of cereal and lucerne, although lucerne did increase the concentrations of omega-6 and saturated fatty acids.

The meat quality of grazing lambs do, somewhat, reflect the results obtained in the pen study. There were no apparent differences between the colour and laboratory measures of eating quality for the meat from lambs grazing perennial wheat in combination with salt, lucerne, clover, and/or serradella. The mineral composition of the meat from all the experimental lambs confirmed its high nutritional value to consumers. Likewise, preliminary analysis of the fatty acid data shows comparable antioxidative properties and concentrations of health claimable omega-3 fatty acids. The trend observed for the omega-6 concentrations requires further investigation, because of its associated health implications.

6.1.4 Productivity and legume compatibility

A primary focus of the project was examining the effect of intercropping with cereals and legumes with a particular focus on lucerne. In northern hemisphere studies, the combination of perennial cereals and lucerne have shown a degree of facilitation between the intercropped species over a longer time frame, which indicate less competition between components. In the current project our findings suggest that lucerne is unable to compensate for the nutrient deficiencies in wheat when offered together as a forage for grazing ruminants. Furthermore, intercropping perennial wheat with lucerne reduced overall dry matter production compared to similar annual cereal combinations, as perennial wheat was the dominant species in terms of dry matter accumulation. This, combined with higher competition for resources over summer, would suggest that in an Australian context, lucerne may not be the best companion legume to use with perennial wheat.

Intercropping cereals with forage legumes generally had a trade-off with grain yield, with some resources devoted to growing the legume. If the extra dry matter produced by the legume can be used to balance the mineral intake of grazing animals, as well as provide nitrogen to the system, then some of this trade-off can be compensated. In this regard annual legume species such as subterranean clover or serradella may offer a viable alternative to lucerne, as both these species had substantially higher Na concentration compared to other species tested. Their annual growth habit also reduced the competition for resources over summer compared to a perennial wheat/lucerne intercrop.

6.1.5 Grain functionality and end use

Grain functionality and end use was beyond the scope of the present project that was focused on the grazing value of perennial wheat. Nevertheless, the substantial effort in seed multiplication fostered two daughter activities which both relied on access to reasonable quantities of perennial grain. These included the Agrifutures Artisan Grains project, which supplied grain of several perennial lines to artisan bakers and brewers to evaluate the range and quality of products that could be manufactured, and an Honours project undertaken by Alexis Tang at UTAS characterising the properties of grain harvested from four of the perennial wheat evaluation experiments in 2018 and 2019.

The key learning from both activities is that novel perennial cereals demonstrate good utility across a range of products from brewing to processing for breakfast cereals, biscuits and breads, with desirable attributes for flavour and function. This is an important finding because the end-use (and value) of the grain will be important for profitability and a primary driver impacting adoption by growers. Importantly, the normal putative tests for grain function in wheat are less accurate in predicting performance of perennial wheat grain. There is still work required to further develop both the markets and techniques to create various products, but knowledge that perennial grain is more than just a feed grain raises prospects of viable perennial crops in future Australian farming systems. For more detail on the products and end use attributes of perennial grains, the reader is directed to the Perennial Artisan Grains Conference Proceedings (Newell 2021) and to the draft manuscript appended to this report (Pleming *et al*. 2023; Appendix 2).

7 Future research and recommendations

7.1 Research and development

Perennial grains remain an emerging technology globally and as such, there are many aspects that require further research. For the Australian context, the highest priority is, arguably, to improve the longevity of perennial crop genotypes through breeding and selection. The existing hybrid perennial wheat breeding material does not persist reliably beyond the second year, which is an inadequate timeframe to monitor yield and competition dynamics to sufficiently value the new cropping system and to realise the environmental benefits that a perennial crop offers. The domesticated breeding lines of Kernza and mountain rye are longer-lived than the hybrid material, and probably represent the best near-term prospect for a viable perennial crop in Australia. The superior grain characteristics and yields, along with the prior breeding that has occurred in Australia to develop it as an alternative forage (Oram 1996), probably makes mountain rye the primary candidate perennial crop to be developed in Australia. The favourable grain functionality and end use identified in mountain rye in this project (Newell 2021) adds confidence in the potential marketability of the grain of this species for food applications. Further breeding and selection of this species would likely target increased yields and longevity by addressing floret infertility and selecting for earlier-maturing genotypes.

Several lines of investigation initiated in this project warrant further attention. For example, the increased concentrations of health-claimable compounds in lamb meat from a mixed lucerne/perennial wheat diet compared to a pure cereal diet demonstrates an opportunity for farmers to develop farming systems to deliver a higher quality end-product to a discerning market. This would seem to be one avenue worth investigating to further increase the value of premium Australian lamb. This point is of particular relevance for perennial cereals as plant maturity can affect its fatty acid composition and therefore nutritional value to grazing livestock. A better understanding of the role of mixtures in developing these traits is required, as well as an understanding of whether similar principles apply in beef as was identified in lamb in the current project.

The methodologies developed in this project have application in other red meat industry research. For example, the pen feeding studies employed were demonstrated to be an effective means of monitoring the effects of diet on the nutritional profile of sheep and on carcass traits. Pens that housed individual sheep afforded more precision when estimating daily intake compared to standard mob or paddock studies. As demonstrated by the contrasts in Na concentrations of the various diets, the approach gave researchers a detailed insight of the role of that nutrient within the sheep by following changes in the blood, urine, meat, and even in the rumen wall. This approach could be applied to other nutrients of interest, or to any research where there is a need to understand the effects of diet on the chemical compounds formed in the rumen (compounds such as methane, for example) and their deposition within the animal/carcass.

Immediate adoption of this research is inhibited by the fact that perennial crops are not available to farmers in Australia. Nevertheless, momentum in perennial crop development continues to increase globally as pressure mounts to produce food and fibre with the lowest environmental footprint possible. By examining experiences elsewhere around the world, such as with Kernza in the USA (DeHaan et al. 2020) or perennial rice in China (Zhang et al. 2022), it seems that perennial crops in Australia are inevitable. It is therefore important to continue to investigate the role they may play in future production systems and to quantify the environmental benefit that the Australian agricultural industries might expect from perennial cropping systems.

7.2 Extension and adoption

Despite the lack of perennial crops in Australian agriculture, results from this research may be applied to conventional production systems. For example, the similarity in forage quality and mineral profile of perennial wheat and conventional grazing wheat suggests that the increased understanding of mineral balance attributable to this project applies directly to conventional grazing wheat systems. There is also increased interest in mixed annual fodder crops due to perceived benefits of a mixed sward. The findings of this project relating not only to changed mineral balance in grazing livestock, but also to changes in biomass production associated with the addition of new species, are immediately relevant. The approach taken in the present project provides a mechanistic understanding that may be applied to a range of contexts.

Experience shows that the story of perennial crops appeals to a much broader audience than does most typical research. This was highlighted in this project by the range of visitors that engaged with the research sites including (at different times) the Governor General of Australia, the NSW Minister for Primary Industries, Federal Department of Agriculture senior managers, farmers, advisors and the general public. As was demonstrated in the Agrifutures Artisan Grains project, there is a large, untapped (often urban) audience that is inspired by the perennial grains story. This broad audience represents not only red meat producers but also, red meat consumers. Perennial grains therefore present an opportunity to engage with its market and the broader community and tell a positive story of the red meat industry striving to meet environmental imperatives in its future production systems.

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Appendix 1 – Fowler *et al*. 2023

Target Journal: *Meat Science*

The potential for Raman Spectroscopy to measure lamb meat quality and sensory traits

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Abstract

This study explored the potential for Raman spectra to predict a wide range of meat and eating quality traits. To this end, 48 lamb loins were measured using a 785nm Raman device before being analysed for traits including shear force, retail colour stability, purge, drip loss, particle size, ultimate pH, moisture, mineral and fatty acid (FA) composition, intramuscular fat (IMF) content as well as eating quality determined by an untrained sensory panel. Modelling the relationship between principal component (PC) scores and these traits revealed shear force, pHu, iron and lightness (L*) values at all days of display had a significant relationship with PC1 of the raw spectra. Furthermore, copper, total moisture, a* values at 2 days and fatty acids including C14:1n-5, C16:1n-7, C14:0, C12:0, C15:0, C12:1n-7 had a significant relationship with PC1 calculated from the pre-processed spectra. Consequently, Raman spectroscopy has the potential to predict industry relevant traits including FAs and mineral composition, however peaks postulated to characterise myoglobin dominated the spectra and further research is required to ascertain the validity of these associations.

Keywords: Raman spectroscopy; Grazing cereals; Sheep meat; Eating quality; Fatty acids; Mineral composition; Colour stability

1. Introduction

Australian domestic lamb consumption patterns have changed as consumer's have become more conscious of their food choices, seeking higher quality products. To meet the changing consumer demands, the Australian lamb supply chain has undertaken extensive work to increase the consumer appeal of lamb cuts (Fowler et al., 2018). However, as identified by Hopkins, Holman, Fowler & Hoban (2015) there is an absence of meat and eating quality data available for a wide range of lamb cuts. Due to the resource intensity and subjectivity of sensory panels, other indicators of meat quality are used as a proxy for eating quality, such as shear force, particle size, intramuscular fat, ultimate pH (pHu), and cooking loss (Hopkins, Hegarty, Walker & Pethick, 2006). Yet industry application of these methods are limited as they are destructive and expensive (Toohey, van de Ven & Hopkins, 2018). A non-destructive, non-invasive method for routinely measuring eating quality of lamb could improve the efficacy and timeliness of determining eating quality indicators.

Raman spectroscopy has been identified as a potential tool due to the development of handheld and miniaturised lasers and spectrometer devices suitable for use in commercial processing plants (Damez & Clerjon, 2008). Recent research has focused on the application of Raman spectroscopy to predict lamb quality traits given the lack of carcase assessment methods as carcases are not split or quartered prior to boning out (Fowler, Schmidt, Scheier & Hopkins, 2017). While a small number of studies have considered the ability of Raman spectra to predict shear force, pHu, and group fatty acid composition of lamb, these studies were limited in the traits measured, consequently it was difficult to associate spectral variation with traits related to consumer acceptance and health (Fowler, Schmidt, van de Ven, Wynn & Hopkins, 2014b; Fowler, Ponnampalam, Schmidt, Wynn & Hopkins, 2015a; Fowler, Schmidt, van de Ven, Wynn & Hopkins, 2015b). Consequently, a study was conducted to explore Raman spectra of lamb loins and a diverse range of meat and eating quality traits to explore relationships between the biophysical and biochemical components of the meat and spectra.

2. Materials and Methods

2.1. Samples

In order to measure samples with variation in the population measured, the right *M. longissimus lumborum* was collected at 24 h post slaughter from the carcases of 48 Poll Dorset × Merino lambs were fed diets consisting of annual wheat (*Triticum aestivum* L. cv. Wedgetail) or Perennial Wheat (*T. aestivum* × *Thinopyrum* spp. breeding line 11955), with or without Lucerne (*Medicago sativa* L. cv. Titan 9) for 21 d and processed as per commercial abattoir processes, as described by Newell et al. (2020) and Holman et al. (2022).

2.2. Raman spectroscopy

Raman spectroscopy measurements were conducted at 24 h post slaughter using a Mira® 785 nm handheld Raman spectroscopic device calibrated using the manufacture's standard. Samples were measured using an integration time of 5 s, 3 averages (total scan time 15 s), and a laser power of 5. Three positions across the muscle were measured, perpendicular to the muscle fibre.

2.3. Meat Quality Traits

Meat quality traits including shear force, retail colour stability, purge, drip loss, particle size, ultimate pH, moisture, mineral composition, and intramuscular fat (IMF) content were analysed as previously described by Holman et al. (2022).

In short, sections of muscle were cooked in a water bath set to 71 °C for 35 min before being submerged in cold water for 30 min. The percentage change in the weight of the meat before and after cooking was recorded as the cook loss. Cuboidal strips were sectioned (cross-sectional area: 1 cm^2) and tested using a texture analyser (model LRX, Lloyd Instruments, Hampshire, UK) fitted with a vee-shaped Warner-Bratzler blade in order to determine shear force (Hopkins, Toohey, Kerr & van de Ven, 2011).

Retail colour display of 5 d aged samples was measured using a calibrated spectrophotometer (model 45/0-L, MiniScan 7237, HunterLab Associates Laboratory Inc., Hong Kong, PRC) on slices of 3-4 cm thickness which were prepared and arranged onto individual black Styrofoam trays overwrapped with PVC Food Film (thickness: 15 µm). Samples were displayed for a total of 3 d at 2.5 °C (± s.d. 0.2), and under continuous fluorescent lighting (58 W NEC Tubes that delivered ~ 1000 lux to the sample surfaces, verified using a handheld lux meter). Subsequent colorimetric assessments were made at 24 h intervals (1, 2, and 3 days). The colorimetric data recorded included lightness (L^*), redness (a^*), yellowness (b^*) and the calculated reflectance ratio of 630 nm and 580 nm (R630/580).

Purge was calculated as a percentage of weight loss during ageing over 5 d with sample weights collected prior to ageing and after samples were aged and patted down with paper towel. Drip losses were determined on sample cores of 2.5 cm using the EZ-DripLoss method (Holman, Alvarenga & Hopkins, 2020).

Particle size at 1 and 5 days post slaughter was determined using duplicate samples (~ 1.0 g) homogenised with a total of 20.0 mL phosphate buffer consisting of 0.1 KCl, 1 mM EDTA (di-sodium), 7 mM KH2PO4, 18 mM K2HPO4, pH 7.0 at 4 °C (Karumendu, van de Ven, Kerr, Lanza & Hopkins, 2009). The homogenised muscle in buffer was then added drop-wise to a laser diffraction particle size analyser with water connection (model LS13-320, Beckman Coulter Ltd., Miami, USA).

Mineral composition was determined on 24 h post-mortem freeze dried samples using a microwave digestion and coupled plasma-optical emission spectroscopy or inductively coupled plasma mass spectroscopy method (Carrilho, Gonzalez, Nogueira, Cruz & Nóbrega, 2002).

The moisture content was measured as the mean percentage weight change of duplicate samples (~ 25 g) before and after being freeze-dried at -50 °C (ScanVac CoolSafe, LaboGene Ltd., Lynge, DEN). These were then ground and held at -80 °C to be analysed further. Intramuscular fat (IMF) was determined on a 2.5g subsample extracted with 85 mL of hexane for 60 min using the Soxhlet extraction method as adapted by Holman, Bailes, Meyer & Hopkins (2019).

Approximately 100 mg of sample was homogenised with 500 µL of a radio-immunoprecipitation assay (RIPA) buffer (Item No. 10010263, Cayman Chemical Company Ltd., Missouri, USA) using micro-tube pestles (Zhang et al., 2019). The supernatant was isolated and the content of thiobarbituric acid reactive substances (TBARS) was determined as per Holman, Bailes, Kerr & Hopkins (2019).

Ultimate pH (pHu) was measured by removing ~ 1 g of frozen sample and homogenising at 19 000 rpm for 2 bursts of 15 s (Ystral homogeniser: Series X10/25, Ystral, Germany) in 50 mL falcon tubes containing 6 mL of buffer solution as described by Dransfield, Etherington & Taylor (1992).

Fatty acid composition was analysed using a one-step extraction methylation after samples were frozen, freeze-dried and homogenised using a Foss KnifeTech® grinder. The extraction method was based on the method of Lepage & Roy (1986) where fatty acids were extracted using 10 mL of chloroform/methanol mixture (2:1 v/v) and evaporated with nitrogen gas. The mixture was methylated using 2 mL of methanol/toluene mixture (4:1 v/v) containing 200 µL of acetyl chloride, C13:0 (4 µg/mL) and C19:0 (4 µg/mL) as internal standards and 5 mL of a 6 % potassium carbonate solution. Fatty acids were then identified using an Agilent 6890N gas chromatograph (GC) equipped with an SGE BPX70 analytical column, a fused carbon-silica column, coated with cyanopropyl phenyl (BPX70, 30 m × 0.25 mm i.d. and 0.25 µm film thickness, SGE Analytical Science, Victoria, AUS). Helium was the carrier gas with a split ratio of 10:1, total flow rate of 12.4 mL per min and a column flow of 0.9 mL per min. The inlet pressure was 107.8 kPa, its temperature was 250 °C, and injection volume was 3.0 µL into a focused inlet liner (4 mm i.d., no. 092002, SGE Analytical Science, Victoria, AUS). Retention times with those of the internal standard C19:0 were used to identify sample peaks which were quantified using Agilent Chemistation (Version B.01.03). The peaks for some branched-chain fatty acids (FA) were identified by comparison to published data (Or-Rashid, Fisher, Karrow, Al Zahal & McBride, 2010). Total saturated FAs, monounsaturated, polyunsaturated and omega FA were calculated as the sum of the relevant individual FA. All FA are expressed in mg/100 g fresh weight.

2.3. Eating Quality

Once spectra were collected, the loins were vacuum packed and aged for 5 d at 2.5 °C (± s.d. 0.2) prior to being sectioned into 5 slices and frozen. The samples were tested using untrained sensory panels, as described by Holman et al. (2021) which is based on the method described by Thompson et al. (2005). Briefly, samples were allocated to consumers based on having each loin sampled by 10 consumers, with each consumer eating 8 samples representing all feed treatments. Prior to sensory testing, loin slices were thawed at 1 – 2 °C for 21 h and were subsequently grilled to an internal temperature of 71 °C, rested for 2 min, cut in half and presented to two consumers simultaneously. Sensory attributes were recorded on a 100 mm line scale anchored with tenderness (not tender to very tender), juiciness (not juicy to very juicy), liking of flavour (dislike extremely to like extremely), and overall liking (dislike extremely to like extremely) and panellists were asked to rate the quality of the sample (unsatisfactory, good everyday quality, better than everyday quality or premium quality). The position of the panellists mark on the scale was then converted into a score for that trait, i.e. a mark at 76 mm was taken as a score of 76 (De Brito et al., 2016).

2.5. Statistical Analysis

The 10 consumer sensory scores for tenderness, juiciness, flavour and overall liking were averaged for each loin. Spectra were examined for unusual patterns that may associate with misapplication or operator error, but none were highly different and no spectra were removed prior to being averaged for each loin.

The average spectra for each loin was pre-processed to remove non-Raman background, fluorescence and noise which can obscure the Raman signal using the baseline package (lambda = 6, p = 0.05, maxit = 20) (Liland & Mevik, 2010) and smoothed using a standard normal variate transformation. Principal component analysis (PCA) was then undertaken on the raw and pre-processed spectra as previous research has suggested that the net intensity which is removed with pre-processing may be related to characteristics such as shear force (Fowler, Schmidt, van de Ven, Wynn & Hopkins, 2014a).

The first 2 Principal Component (PC) scores were saved as a reflection of the variation within spectra. Measured meat quality and sensory traits were then regressed against the score(s). Furthermore, meat quality traits were regressed against sensory traits and fatty acids were regressed against IMF. Traits were considered significantly associated with the scores when the F-ratio test for the regression sums of squares tested at $P < 0.05$ for either score. All data analysis and graphics was undertaken in the R environment (R Core Team, 2021) with particular use of the prospectr package (Stevens & Ramirez-Lopez, 2014).

3. Results

3.1. Meat and Eating Quality Traits

The summary statistics for meat and eating quality traits measured are given in Tables 1, 2 and 3.

Insert Tables 1-3.

3.2. Spectral Analysis

Raw Raman spectra are shown in Fig 1, which illustrates peaks typically found in meat at 900cm^{-1}, 1450cm^{-1} and 1650cm^{-1} as well as peaks and troughs at 638cm^{-1}, 1348cm^{-1} and 1538cm^{-1}.

Insert Fig 1.

Spectra pre-processed spectra using baseline correction are shown in Fig 2, which illustrates that the main peaks were at 900, 1080, 1450 and 1650, and the large peaks and troughs are evident at 687, 1348 and 1538 cm^{-1}.

Insert Fig 2.

Linear modelling of traits against PC scores derived from the raw spectra indicated that shear force, pHu, iron and *L** values at all sampling times (d 0 - 3) had a relationship ($P < 0.05$) with PC1 which explained 95% of the variation. Furthermore, the concentrations of calcium, magnesium, sulphur as well as fatty acids C12:0, C22:0, C23:0, C24:0 and C24:1n-9 had a relationship ($P < 0.05$) with PC2 which represented a further 2% of spectral variation.

Further modelling indicated copper, total moisture, *a** values at 2 days, and fatty acids C14:1n-5, C16:1n-7, C14:0, C12:0, C15:0, C12:1n-7 had a significant relationship with the variation of pre-processed spectra as expressed by PC1 which represented 53% of the variation. Additionally, calcium and *b** values at 2 days were significantly associated with the spectral variation in PC2 which accounted for a further 7.3% of spectral variation.

4. Discussion

Although, there is a paucity of data collected using Raman spectroscopy on lamb using an 781nm Raman device, a comparison with previous research conducted by Fowler et al. (2014b) shows clear peaks with significantly greater intensities at wavelengths 687cm^{-1}, 1348 cm^{-1} and 1538 cm^{-1} which has not been previously described. As such PCA computes variables based on the spectral features which manifest the highest degree of variability and therefore the peaks found at 687cm^{-1}, 1348 cm^{-1} and 1538 cm^{-1} would contribute greatly to PCA scores derived from the raw spectra (Bonnier & Byrne, 2012). Further analysis of scores suggests PC1 which contains 95% of spectral variation was strongly associated with shear force, pHu, iron and *L** values at all days (0, 1, 2 and 3). Although these peaks have never been characterised for lamb, work completed by Almohammedi et al. (2015) to monitor the intracellular redox state of myoglobin isolated from rat cardiomyocytes has characterised the peak at 1358 cm^{-1} as deoxygenated myoglobin (Mb^{II}) and the peak at 1538 cm^{-1} as oxygenated myoglobin (Mb^{II}-O_2). Given the significance of these peaks and the association between PC1 lightness and iron, it is hypothesised this is similar for lamb and these bands are characteristic of the amounts and state of myoglobin which is present.

While pHu and shear force are not directly associated with myoglobin, PC1 and these peaks may be related as variations in eye muscle area as selection for muscularity affects shear force, pHu and colour through increased muscle fibre diameter, changes to the muscle oxidative and glycolytic fibre types as well as variation in intramuscular fat (Calnan, Jacob, Pethick & Gardner, 2014; Kelman, Pannier, Pethick & Gardner, 2014; Wu et al., 2020; Thomas et al., 2021). Indeed, studies conducted on beef suggest higher pH values enhance mitochondrial respiration, depleting oxygen that binds with surface myoglobin leading to the formation of more Mb^{II} and therefore altering the redness of samples (Wu et al., 2020). However, as this is the first time these peaks have been reported in lamb, further research is needed to determine if the relationship between these peaks, myoglobin, and meat quality traits are consistent, and consequently whether these peaks which dominate PC1 are useful in the prediction of meat quality traits using a commercially available Raman spectroscopic device.

Overall, this study does agree with earlier research conducted by Fowler et al. (2015b) who demonstrated the potential for Raman spectra to predict pHu in lamb. While it was previously hypothesised Raman spectra were able to detect the signals which characterised creatine phosphate (PCr), creatine, glucose 6-phosphate (G6P), lactate, adenosine triphosphate (ATP), inosine monophosphate (IMP) and phosphoric acid concentrations (Fowler et al., 2014a.), it is unlikely that the same mechanisms are evident in the current study given the raw spectra are largely dominated by background signal and the peaks are associated with myoglobin and fatty acids. Yet it is plausible that the association between spectral variation, L^*, shear force and pHu is mediated through the structural changes occurring during the early post-mortem period because pH and temperature induce transverse shrinkage of the muscle fibres (Hughes, Clarke, Purslow & Warner, 2020). Consequently, it is thought the formation of extracellular space increases the light scattering, the extent to which is determined by the pH decline. This also alters the integrity of the sarcomeres and myofibrillar proteins and the sarcoplasmic proteins to myofibrils and myofilaments thereby linking shear force, pHu, and lightness to the microstructure of the meat (Hughes et al., 2020). Although, meat colour, pH, and shear force are vital attributes affecting consumer acceptance of meat products, there is a paucity of research that has examined the spectral variation and their relationship with the biophysical properties of meat. Larger studies are required to determine the validity of this hypothesis and explore relationships between spectral peaks and background, meat microstructures, myoglobin and quality traits and whether such traits can be accurately predicted using Raman spectroscopy.

While PC2 represented only 2% of the variation in the raw spectra, it was associated with calcium, magnesium, sulphur, as well as the fatty acids C12:0, C22:0, C23:0, C24:0 and C24:1n-9. Given the non-polar acyl chains in their structures, the peaks which characterise fatty acids are pronounced within the spectra (Czamara et al., 2015). Thus, despite the significant contribution of the large peaks deemed to be myoglobin, the peaks which characterise the CH_2 methylene scissor formations and the C=C olefinic stretch of fatty acids at 1450 cm^{-1} and 1650 cm^{-1} are still evident (Beattie, Bell, Borggaard, Fearon & Moss, 2007). Interestingly, the fatty acids associated with PC2 are not the fatty acids which are most abundant in lamb meat, palmitic (C16:0) and stearic acids (C18:0) (Díaz et al., 2005). However this may be the result of the feeding treatments as the different feed types did increase the variation of the data, the concentration of long-chain saturated fatty acids were higher in the muscle when lucerne was included in the diet (Holman et al., 2022). Although the sample size in the present study are too low to provide a robust predictions, the association between these fatty acids and PC scores suggest the potential to predict the fatty acid composition of lamb using a 785nm Raman device without separating the protein and fat signals, as done in previous research (Fowler et al., 2015a). Therefore, a larger study with greater numbers from commercially produced carcases from lambs of differing feed types which reflect lambs typically produced in Australia is warranted.

Pre-processing of spectra is a commonly used technique to remove unwanted variation associated with equipment to enhance predictive power (Mishra, Biancolillo, Roger, Marini & Rutledge, 2020). This explains why the peaks which charaterise CH_2 methylation at 1450 cm^{-1} and C=C olefinic stretch at 1650 cm^{-1} are more evident in the pre-processed spectra than in the raw spectra. Subsequently the association between the first PC score and longer chain fatty acids and mono-unsaturated fatty acids was identified, but which varied, in particular C14:1n-5, C16:1n-7, C14:0, C12:0, C15:0, and C12:1n-7. Yet, the variation in fatty acid composition of these samples were not detected as previous analysis has shown lucerne in the diet altered long chain polyunsaturated fatty acids including C18:2n-6, C20:2n-3, C20:4n-6, C22:4n-6 and C22:5n-6, resulting in significant difference in the n-6:n-3 ratio (Holman et al., 2022). It is hypothesised that overlapping spectral features may affect the detection of fatty acids in muscles as while differences due to diet are evident in the subcutaneous fat of beef carcases, this finding agrees with previous research on lamb (Fowler et al., 2015a; Logan, Hopkins, Schmidtke, Morris & Fowler, 2021). Nevertheless, the ability of Raman spectroscopy to predict the fatty acid content of intact lamb remains promising given the association between both raw and pre-processed spectra and individual fatty acids. However, further work is needed to further understand the impact of spectral overlap on the prediction of fatty acids in intact meat.

This is the first study to investigate the potential of Raman spectra to determine the mineral composition of meat. Raman spectra are capable of classifying minerals and mineral mixes, however it is challenging due to overlapping spectral features and, therefore, analyses are required to accurately label individual components of the spectra (Cochrane & Blacksberg, 2015). In the present study, calcium, magnesium, and sulphur were associated with PC1 and copper was associated with PC2, thus it is plausible that mineral composition could be determined using Raman spectroscopy. Further research is required to investigate whether the spectral peaks of interest for determining minerals can be elucidated given the peaks at 1358 cm^{-1} and 1538 cm^{-1} dominate PC1. The ability to characterise minerals could very important if consumers demand more detailed nutritional labelling of lamb in the future.

Overall, the range of the meat quality traits are consistent with what would be expected for lamb in Australia. However, the addition of lucerne to the cereal forage diet resulted in greater variation in pHu, fatty acids, and minerals then would typically be expected (Newell et al., 2020; Holman et al., 2022). Yet, other traits such as the sensory scores for samples did not vary as much as could be expected for commercially produced lamb (Hopkins et al., 2006), which may contribute to the lack of association between sensory and PC scores. PCA was chosen as a data analysis method for the current study as it a powerful and adaptive method for exploratory data analysis in spectral data sets which require compression of the data dimensionality while minimising the loss of information and the aim of the study was to explore the potential for Raman spectroscopy to detect a large number of possible traits (Biancolillo & Marini, 2018). While non-linear regression analysis methods are becoming increasingly used in the field of food analysis to overcome limitations such as a lack of variation, the number of samples in the current study limit their use and thus they have not been applied (Rocha, Prado & Blonder, 2020). Therefore, a study which measures a larger number of samples is required to fully explore the potential to predict or classify samples into eating quality classes.

It is interesting to note, when pre-processing techniques were applied to the current spectra, associations between the spectral variation as captured by the PC scores and meat quality traits changed. Indeed, copper, total moisture, a^* values at 2 days and fatty acids had a significant relationship with the variation of pre-processed spectra as expressed by PC1, whereas shear force, pHu, iron, and L^* were associated with PC1 as calculated from the raw spectra. As fluorescence and the background can be 2 to 3 times greater than the Raman signal, baseline correction is required to determine the composition of traits which have distinct peaks such as fatty acid composition, while traits such as shear force, sensory traits and pH have both biophysical and biochemical aspects which are likely to be reflected in both the raw and pre-processed spectra (Mishra et al., 2020; Ryabchykov, Schie, Popp & Bocklitz, 2022). Given this, the most optimal prediction models for some traits such as pHu may be achieved by combining both the pre-processed and raw spectra. However, these methods of analysis have not yet been established for meat quality assessment.

5. Conclusion

Overall, this research demonstrates the potential for a commercially available 785 nm Raman spectroscopic device to predict lamb quality traits including shear force, pHu, fatty acid, and mineral composition. Despite an increase in studies using Raman spectroscopy for meat quality assessment, there remains a paucity of data for lamb and consequently further research is required to determine if the peaks found at 1348 cm^{-1} and 1538 cm^{-1} are indicative of myoglobin as postulated.

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Appendix 2 – Pleming *et al*. 2023

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Perennial wheat breeding lines exhibit favourable baking performance.

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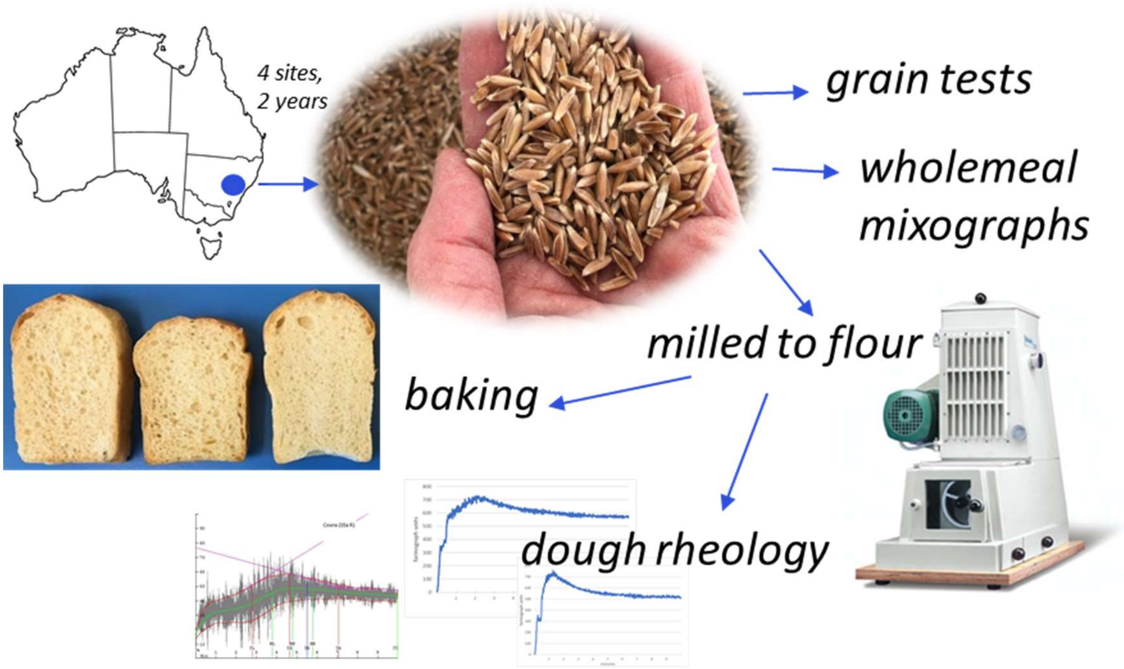
Keywords: perennial wheat, baking, rheology, milling, predictive tests

Graphical abstract



Abstract

Perennial grains are being developed to improve the environmental sustainability of grain production systems. However, to maximise their commercial viability, a clearer understanding of food processing properties is required. In this study, the functional properties of selected perennial wheat breeding lines grown at sites in central New South Wales, Australia, were compared to an annual bread wheat cultivar. Lines were assessed for grain yield parameters, rheological properties (wholemeal and refined flour), starch properties, milling yield and refined flour baking quality. Perennial wheats were found to differ from expected behaviours attributed to annual wheat, offering novel combinations of grain characteristics. Despite softer grain and rheological tests indicating only moderate gluten strength, several lines exhibited better baking performance than the conventional bread wheat control. Furthermore, milling yield and flour water absorption were found to decrease with increasing grain hardness, the opposite of that normally observed for annual wheat. The results demonstrated that with appropriate breeding and selection, perennial wheat offers good potential for baking.

1 Introduction

Perennial wheats (PW) are derived from crosses between wheat (either common, *Triticum aestivum*, or durum, *T. durum*) and wheatgrass (*Thinopyrum spp.*), with original breeding efforts occurring in Russia in the 1920's (Wagoner, 1990). Perennial crops are of interest due to their sustainability and environmental attributes – primarily the improved resource-use efficiency attributable to longer growing seasons (Zhang et al., 2011; Crews et al. 2016) and multifunctionality (Ryan et al., 2018) compared to annual crops. Perennial crops also mitigate soil erosion through permanent vegetative cover and reduced soil disturbance, and their extensive root biomass development can increase carbon accumulation in soils (Shi et al., 2011). A diversity of PW breeding lines have been evaluated across a range of locations in recent years (Hayes et al., 2012; 2018; Larkin et al., 2014). Trials have largely focused on PW potential as a dual-purpose crop, providing grazing of forage and opportunistic grain production for lower input cost (Newell and Hayes 2017; Hayes et al., 2017). In addition to associated environmental benefits, PW grain has also been shown to possess several health advantages compared to annual cereal grains including greater concentrations of bioactive compounds (yellow pigments, polyphenols and 5-alkylresorcinal), dietary fibre and resistant starch (Pogna et al., 2014). This provides an opportunity for development of perennial grain food products with health and nutritional benefits for consumers. However, little is known about its baking performance and other functional traits. An initial study of F_5 PW breeding lines derived from a *Th. ponticum*/Chinese Spring//Madsen[6] population indicated the perennial lines had inferior baking and loaf characteristics compared to bread wheat (Murphy et al., 2009), but no information exists for a broader range of PW pedigrees.The present paper investigates the year one grain characteristics and functional properties of a diverse range of perennial wheat lines grown in central New South Wales (NSW), Australia, during 2018 and 2019.

2 Materials and methods

2.1 Experimental sites & germplasm

Four field experiments were established at Cowra (site A, C18) and Mandurama (M18) in 2018; and in 2019 at Cowra (site B, C19) and Orange (O19) in NSW, Australia. Each

[6] Murphy et al. (2009) initially described this pedigree as *Th. elongatum*/Chinese Spring//Madsen, but Curwen-McAdams and Jones (2017) later clarified the perennial parent as being *Th. ponticum.*

experiment comprised small plots (2 × 7.5m) in randomised block designs with three replicates. Seven PW genotypes and the bread wheat cultivar, Wedgetail, were trialled at each site (Table 1). The PW lines represent a diversity of parentage and were selected based on performance in previous field assessments (Hayes et al. 2012; 2018; Larkin et al. 2014). The 2019 harvest year experienced drier conditions than 2018, receiving less rainfall during the flowering period but minimum and maximum temperatures recorded in all experimental sites were relatively similar to the long-term average (Figure 1).
Grain was harvested from each plot in each experiment at the end of year one. Due to lower yields of some genotypes, and the severe drought year of 2019, sufficient quantities of grain were not always available for all tests. As a consequence, three sets of samples were used at various stages in this study, dictated by grain availability or suitability: Set A (all entries from both 2018 and 2019 sites); Set B (all entries at 2018 sites only, composite samples of grain from each genotype at each site); and Set C (2018 sites only, composite samples of four perennial lines, plus Wedgetail).

2.2 Set A analyses (all entries at all sites): Grain yield; harvest index; grain weight, colour, protein; and using composite samples from all plots per site for each genotype, wholemeal mixograph.

Harvest and grain yield sampling were targeted to the maturity of genotypes at each site. Once each genotype had ripened, a representative sample of biomass was removed from each plot by cutting two adjacent rows at ground level, along a 50 cm ruler (total area = 0.36 m^2) at either end of the plot. This sample was weighed, and grain removed to determine the proportion of grain to total biomass produced (harvest index, HI). All remaining grain heads were removed from each plot by hand due to the large difference in maturity times between genotypes. This material was then threshed and cleaned with a stationary thresher (Kimseed, Perth) from which final grain yield (kg/ha) was determined for each plot.

Grain length and width (mm) were measured with digital callipers on three randomly selected grains from each plot. Length was measured from the germ to the brush end of each kernel, and width measured from dorsal to ventral (crease) side (DV) and also from left to right of each kernel. Grain weight (g) was determined in triplicate by counting 100 grains (Contador seed counter, Pfeuffer GmBH, Denmark), weighing to 3 decimal places and multiplying by 10; reported as thousand kernel weight (Kwt). Grain colour was measured in triplicate using a CR-400 chromameter (Minolta, Japan) and reported in CIE colour space L* a* b* where L* represents brightness, a* redness, and b* yellowness. For protein determination, 5 g grain from each plot was ball milled (MM200, Retsch, Germany) for 1 min at 25hz, then sifted (1 mm sieve) and stored at room temperature until analysed. Nitrogen content was determined in triplicate using a CHN analyser (2400 series II, Perkin Elmer, USA) and converted to protein content (as is) by applying a multiplication factor of 5.7 (Mariotti et al., 2008).

For mixograph analysis approximately 10g from each field plot was combined to provide a single sample per site of each genotype. Grain was ground in a hammer mill (3100 laboratory mill, Perten Instruments, Australia) fitted with a 0.8 mm sieve, and moisture content determined in duplicate by heating approximately 1.5 g meal to 130 $^{\circ}$C in a thermo-gravimetric analyser (TGA701, Leco Corporation, USA), and holding until constant weight achieved. Adjusting sample weight to 11% moisture basis (mb), samples were then analysed in duplicate using a 10g mixograph (National Manufacturing, USA) and Mixsmart for Windows software V1.0.404 according to Approved Method 54-40.02 (AACC, 2010). The standard equation to determine water addition was modified through trial and error to account for the use of wholemeal (rather than refined flour) and to achieve midline peaks in the desired mid-chart range (50 to 65%) for mixograph analysis. Thus, PW received (% water) 1.5*protein content of sample + 41, while Wedgetail wheat received 1.5*protein content + 46. Reported parameters were peak time, peak height, peak width, peak integral (peak time x mins), width and height at 5.5 minutes, breakdown (defined as peak height minus height at 5.5 mins), and descending slope (defined as breakdown/5.5 minus peak time).

2.3 Set B analyses (2018 sites only; combined field plots): Grain hardness, moisture and ash content.

Grain hardness was determined (in duplicate) according to Symes (1961) utilizing a disc grinder at the finest setting (Buhler-Miag, Germany) and an EFL2000/1 sieve shaker (Endecotts Ltd, UK) fitted with 75 µm sieves. Higher numbers indicate softer grains. Ash and moisture content (duplicate) was determined using approximately 1.5 g meal in a thermo-gravimetric analyser (TGA701, Leco Corporation, USA). Samples were dried to constant weight at 130 °C to determine moisture content, then ramped to 600 °C and again held until constant weight to determine ash content (reported on 11% mb).

2.4 Set C analyses (2018 sites, composite sample of selected lines only): Grain test weight, kernel weight, colour, and flour extraction; wholemeal colour; flour (combined mill duplicates) protein, ash, moisture, colour, total starch, starch pasting, dough rheology and baking.

Test weight was determined in duplicate using a mini chondrometer (Wagga Wagga Agricultural Institute, Australia) calibrated to a Franklin chondrometer (Franklin Instruments, Sydney, Australia) and reported as kg per hectolitre. Kernel weight (duplicate) was determined as described for Set A except 250 grains were counted and the weight multiplied by four. Whole grain and wholemeal colour were measured in duplicate using a CR-410 chromameter (Minolta, Japan) fitted with a 50mm head and granular materials attachment (CR-A50), reported in CIE colour space L* a* b* as described above.

Duplicate 150 g grain samples were conditioned to approximately 12.5% moisture and milled in a Quadrumat junior mill (Brabender, Germany) in randomised order. Flour extraction was the flour weight as a percentage of total products (bran plus flour). Flour samples from mill duplicates were then combined for all remaining tests.

Flour moisture and ash were determined as described for Set B, with ash reported on 14% mb. Flour nitrogen content was determined in triplicate using a Trumac (Leco Corp, USA) standardised with EDTA, and protein (N% x 5.7) was reported on 14% mb. Flour colour was determined as described for wholemeal. Flour total starch content was determined in duplicate using the rapid total starch method K-TST (Megazyme, Ireland) and reported on dry matter basis. Starch pasting parameters of initial gelatinisation temperature, peak viscosity, breakdown, final viscosity and setback were obtained using standard 1 profile on S4 rapid visco-amylograph (RVA) (Perten Instruments, Australia). Rheological tests were performed in duplicate using a 4 g DoughLAB (Perten Instruments, Australia) mixing at 150 rpm to a target peak of 630 farinograph units. Water absorption, time to peak (dough development time = DDT) and stability were reported according to AACC Approved Method 54-21.02 (AACC, 2010), as well as width at peak, energy (work input) to peak and softening (decrease in midline peak height) at 3 mins after peak. Flour mixographs were performed in duplicate as described for Set A, using 14% flour moisture basis and % water addition equation of 1.5*protein content + 45.3 for all samples.

Straight dough baking formulation comprised 50 g flour (100%), 1.5% salt, 1% sugar, 0.1% ammonium chloride, 1.1% Lesaffre red label instant yeast and 0.5% Saunders malted barley extract. Bakery water addition was taken from DoughLAB water absorption without adjustment. Baking was performed in duplicate, yielding 4 loaves per genotype. Doughs were mixed to development at 150rpm (DoughLAB 2500, Perten Instruments, Australia), then scaled to 2 x 40 g dough pieces and fermented for a total of 75 minutes at 30 °C, with a first knock and mould at 60 min (80% total ferment time). Each dough was then lightly knocked down and moulded again in a bun moulder (Domex, UK) before placing in open square bake tins and proofing for 45 min at 34 °C and 85% relative humidity. Loaves were baked at 214 °C in a bakery oven (Rotel 2, Moffatt, Australia) for 15 min. After removal from the oven, loaves were turned out of tins and cooled for 45 min before determining weight and (duplicate) volume (seed displacement). Specific volume was reported as weight divided by volume. Loaves were stored overnight before subjective assessment of external appearance and internal crumb structure, instrumental crumb colour (quadruplicate per loaf) and crust colour (duplicate) using a 210 chromameter fitted with 8 mm head (Minolta, Japan), and instrumental crumb texture (single 20 mm thick slice per loaf) using a texture analyser (TATX2, Stable Microsystems, UK) fitted with a 25mm cylindrical head according to TA.XTPlus application study BRD2/P36R (Stable Micro Systems, 2006).

2.5 Data analysis

Data were analysed using linear models and ANOVA using the R language (R Core Team, 2020). For harvest index, the data were natural logged to reduce heteroskedacity. Grain yield, 1000 kernel weight, harvest index and protein concentration were analysed using a linear model with the agronomic variable as the explanatory variable and genotype and site/year and the interaction between genotype and site/year as the fixed variables. For Sets A and B data where composite samples of each genotype were used for laboratory testing (see Tables 3 and 4), ANOVA with genotype as the fixed variable and site/year as the random variable was conducted in Genstat v. 20 (VSN International), with least significant differences calculated at P=0.05. The baking and flour parameters (Set C) were not analysed statistically but were described qualitatively with presented data for each genotype representing the average of duplicate samples (see Tables 5-8).

3 Results

3.1 Grain yield, harvest index, kernel weight, grain protein, colour and morphology.

Averaged across all site/years, the grain yield of Wedgetail wheat was 4-fold greater than the PW cultivars (Table 2). Among PW, Summer 1 had the highest grain yield, although this was not significantly higher than 11955 at P=0.05. A similar trend was observed in grain size, with 11955 and Summer 1 both having a higher kernel weight than the remaining perennial lines, but both smaller than Wedgetail wheat. The perennial lines 251b, 235a and Ot38 generally had the lowest grain yields and harvest index of all lines tested. The harvest index of Summer 1 approached that of Wedgetail, with only 9% difference between the two genotypes across sites and was 50% higher than all other PW lines tested. Yields, harvest index and grain size were all lower in the 2019 experiments compared to 2018, reflecting much drier seasonal conditions (Figure 1). This in turn influenced protein content, with higher GP values in 2019 and a significant negative correlation between GP and Kwt (R^2 = 0.56, P<0.001).

3.2 Grain hardness; wholemeal moisture, ash, protein and mixograph parameters

Grain hardness (PSI, determined for 2018 sites only) varied greatly among genotypes, with 20238 recording equivalent PSI to Wedgetail and characterised as medium hard. The highest PSI (softest grain) was recorded for 11955, characterised as extra soft (Table 3). Grain moisture was similar between all lines, ranging from 9.5 to 9.8% (Table 3). Grain protein (GP, 11% mb) was lower in Wedgetail (16.1%) than all PW lines, although not significantly different to Summer 1, whilst highest protein concentration was observed in 251b and OK72 (Table 3). Mixograph water addition for individual sites ranged from 66.1% (Summer 1 at C18) to 72.8% (Wedgetail at O19) and was on average (across sites) 0.66% lower per protein unit for PW compared to Wedgetail to achieve equivalent targeted peak height of 50 to 65%. Lowest peak height was 52.9% for 20238, and highest 65.5% for OK72. Time to peak was significantly lower for PW in comparison to Wedgetail (P<0.001), except 235a which had an extended peak time, on average 47% higher than all other PW lines (P<0.001). Peak width was also highest in 235a, which along with 11955, was greater than for Wedgetail (P<0.001). Greatest peak torque integral value was recorded for 235a, which was 50% higher than all other PW lines, and similar to Wedgetail (P<0.001). Lowest breakdown and descending slope were observed in Wedgetail and 235a, and greatest in 20238 and Ot38 (Table 3). Greater grain length was seen in 20238 than all other genotypes, being 27% and 30% longer than Wedgetail and 235a respectively. Wedgetail grain width on average was greater than all PW, particularly in the drier growing conditions of 2019 (Table 4). While grain colour of the PW lines was comparable with that expected of annual bread wheats, 20238 was significantly less bright and less yellow than all other genotypes (Table 4). Grain ash (11% moisture basis, 2018 sites only) was significantly lower for Wedgetail (1.3%) than all PW genotypes. Among PW, Summer 1 (1.5%) was significantly lower than all others, and highest ash content (2.07%) was recorded for OK72 (Table 4).

3.3 Milling extraction, grain, flour & wholemeal colour, starch quality, rheology and baking performance

Milling was performed on Set C, comprising lines chosen on the basis of wholemeal mixograph results to cover a range of dough strength types. Lines 235a, 251b, and Wedgetail were included from both sites in 2018 but 11955 and OK72 were only from one 2018 site each, Cowra and Mandurama respectively. Flour extraction was on average ~20% higher for Wedgetail than the PW genotypes (Table 5). DoughLAB rheology parameters indicative of dough strength (development time, stability, and work to peak) were also higher for Wedgetail compared to PW genotypes, of which 235a exhibited the highest values for these parameters and 251b the lowest (Table 5). Within PW genotypes, DoughLAB water absorption was associated with higher (softer) PSI. Starch pasting curves of PW, while typical of wheat flour, were generally of lower peak viscosity, final viscosity, and setback than Wedgetail, with 251b displaying greatest peak and final viscosity amongst PW lines (Table 5). In flour mixographs, 235a was again identified as the PW genotype consistently exhibiting stronger dough parameters, while 251b recorded the lowest peak time, peak integral and width at 5.5 minutes indicating weaker dough strength (Table 6; Figure 2).

At each site, Wedgetail flour colour was less bright (L*) and more red (a*) than the PW, and 251b was more yellow (b*) than all other lines. Grain colour was not a good indicator of wholemeal or flour colour, particularly redness, however flour colour was better predicted by wholemeal colour, especially brightness and yellowness (Table 7).

Averaged duplicate results (4 loaves) of baking tests are shown in Table 8. Bakery mix time ranged from 1.5 min for 251b to 4.3 min for Wedgetail, and loaf volume from 114 cc (251b at M18) to 132 cc (OK72 at M18). Wedgetail, 11955 and OK72 exhibited very good internal crumb structure, while external loaf appearance of 251b at M18 was judged to be unsatisfactory (Figure 3). From grain produced at Cowra in 2018, PW lines 11955 and 251b produced softer (average 382 gF) crumb than Wedgetail (523 gF), while at Mandurama in the same year Wedgetail (337 gF) was softer than all perennial lines (average 469 gF). Crumb brightness (L*) was consistently greatest for Wedgetail, and 251b from Cowra was noted as being much brighter compared to Mandurama. Crumb yellowness (b*) and redness (a*) were lower in Wedgetail than all PW, while crust L* was greater. Loaf internal and external character is shown in Figure 3

4 Discussion

4.1 Baking performance

Baking performance of the PW lines in this study was not uniformly inferior to traditional annual wheat, with 11955 and OK72 producing loaves of very good quality compared to Wedgetail. A major determinant of baking performance is gluten protein quality, with balance between strength (or elasticity) and extensibility required for best results (Carson & Edwards, 2009). Such balance allows for the retention of CO_2 produced during the fermentation stage without impeding the expansion of gas cells during proofing and baking. Weak (inelastic) doughs are unable to retain gas without rupturing, producing low volume breads featuring coarse open crumb with thick-walled cells. Inextensible doughs impede cell expansion, producing low volume breads with dense, firm crumb. Thus, good baking quality is reflected in good loaf volume but also in appealing crumb structure of small, even cells with fine walls and this was seen in 11955 and particularly OK72, which both outperformed Wedgetail for these traits. The greatest volume and specific volume of all lines was recorded for OK72, while 11955 produced softer crumb than Wedgetail at the one site it was examined. With rheological parameters indicating greater gluten strength, 235a was expected to have superior baking quality however, like 251b, internal structure scores were low due to open, thick-walled crumb cell structure, particularly from grain produced at Mandurama in 2018.

Amongst PW lines, volume and specific volume were found to increase with increasing mixograph peak height, while loaf external appearance improved with greater DoughLAB energy to peak and lower softening. Crumb firmness can be a function of loaf volume because limited gas retention and expansion during baking results in a firmer, less airy crumb structure. However, within PW genotypes, no relationship was observed between volume and firmness indicating crumb structural characteristics rather than volume were responsible for texture differences. Line 251b, which was identified as having weak gluten in rheological tests, was the poorest PW baking performer with short bakery mix times and poor external appearance and internal crumb structure scores, particularly from grain produced at Mandurama in 2018. Of the four PW genotypes assessed for baking quality in the present study, only 251b was found by Pogna et al. (2014) to possess high molecular weight glutenin subunits (HMW-GS) likely inherited from the wheatgrass parent. Processing of 251b was problematic in the present study with very sticky dough persisting throughout all handling stages, particularly from grain produced at Mandurama in 2018. Bakery water absorption for this line (and 11955) was reduced by 2% which improved handling but still resulted in sticky dough for 251b immediately after mixing.

A negative association was observed between PSI and crumb firmness, with softer grain PW producing softer crumb. While wholemeal colour was a good indicator of flour colour parameters, PW grain colour was found to be a poor predictor of wholemeal colour, especially a* values, in agreement with Adams et al. (2013) who also found a negative correlation between grain and wholemeal redness in annual wheat. PW flour colour was strongly aligned with crumb colour, especially b*. Crumb colour of 235a and 251b were visually described as yellow-brown while 11955 and OK72 were a more appealing creamy-yellow colour. Crust colouration in bread is due to Maillard reactions between sugars and proteins (Murata, 2021), and higher protein content would lead to greater browning as reflected in higher a* for PW compared to Wedgetail.

The strong baking performance of PW lines in this study contrasts with that of Murphy et al. (2009), who concluded that PW baking qualities were inferior to that of annual wheat. In their study, all PW breeding lines tested shared a pedigree similar to 235a and 251b. Recent testing of a greater diversity of PW material among grain industry end users generally observed high functional qualities among most PW lines tested, with utility to form a range of products. For example, PW lines such as Summer1, 11955, OK72 and even 235a demonstrated that they could produce a high-quality product using artisan baking methods, rating well for external and internal loaf characteristics (Newell 2021). The favourable baking properties observed in the present study suggests that there is substantial opportunity for selection of PW lines with desirable flour attributes, for both artisan and general baking applications alike.

4.2 Rheology

High values for mixograph peak time and integral are interpreted as indicators of high gluten strength (Isaak et al., 2019). The wholemeal mixograph results are suggestive of 235a having good dough quality, and 251b, 20238 and Ot38 all being weaker. The ability to maintain curve width and height over sustained mixing (5.5 mins) is also indicative of good dough strength, and this was again seen in 235a. Overall 235a was highly comparable to Wedgetail. This supports the work of Pogna et al. (2014) where this line was found to have significantly higher sedimentation volume, another indicator of gluten quality, compared to other PW lines evaluated. Weaker dough strength is indicated by low peak time and integral, and by high breakdown and descending slope values, as seen in 20238 and Ot38. Within PW lines, protein was not closely related with any mixograph parameters, in line with data presented by Murphy et al. (2009) showing no relationship between protein and PW bakery mixing time.

Flour mixograph data were very closely aligned with those obtained from wholemeal samples, particularly peak time and integral; and bakery mix time trended closely with both mixograph peak time and DoughLAB dough development time. Pogna et al. (2014) found PW lines commonly inherit HMW-GS from the wheat parent; and Hayes et al. (2012) reported encouragingly high unextracted polymeric protein (UPP) contents in a broad range of PW pedigrees. These observations support the expectation that PW lines can possess good dough mixing and breadmaking potential. The PW mixing curves obtained from both the mixograph (Figure 2) and DoughLAB were characteristic of doughs with significant gluten content, notwithstanding variability in quality. Good agreement was found between both instruments for characterisation of dough strength (e.g., mixograph peak time and integral, and DoughLAB development time and stability) and of weakness (e.g., mixograph breakdown and width at 5.5 mins, and DoughLAB softening at 3 mins). A negative association was found between DoughLAB water absorption and PSI in PW, which is the opposite of what is observed in common wheat. High water absorption is due to high arabinoxlyan content, a component of endosperm cell walls, and has been selectively bred for in hard bread wheats (Delcour & Hoseney, 2010). On the other hand, high water absorption is undesirable in soft wheat flours. PW have not been subjected to the same selection pressure, and it is probable that the soft-grained genotypes in this study have high arabinoxylan contents (thick endosperm cell walls), conferring high water absorption.

4.3 Milling extraction and hardness

As expected, Wedgetail had much greater flour extraction than all other entries, being 11 to 17.5% higher than best and poorest PW, respectively (Table 5). This differential is consistent with Murphy et al. (2009), where extraction reported for PW genotypes were between 12.3 and 16.2% lower than annual wheat. Test weight and kernel weight were also substantially greater for Wedgetail in the present study. Test weight is generally regarded as an indicator of milling quality and has also been found to have a positive relationship with kernel weight, however the evidence for either claim is not conclusive (Wang & Fu, 2020). Lower flour extraction in PW compared to common wheat may be attributed to selective breeding in the latter to achieve larger spherical features i.e., shorter length and greater width (Gegas et al., 2010), effectively increasing the endosperm to bran ratio. In wheat, higher extraction is associated with harder grain character (Martin et al., 2007) and is attributed to the rougher surface and smaller size of soft wheat flour particles leading to aggregation and lower sifting efficiency (Williams, 2011; Delcour & Hoseney, 2010). It was therefore anticipated that the softest PW would result in the lowest extraction. However, the opposite was observed with a strong positive trend observed between hardness (PSI) and extraction. With a PSI value of 41 (extra soft), 11955 was the best milling PW, while 235a, the "hardest" PW milled (PSI of 24, medium soft) had the lowest extraction. This finding casts some doubt over the suitability of applying relationships developed for bread wheat to other grains such as perennial wheat.

Kernel hardness in wheat is controlled by puroindoline genes located on the D chromosome, commonly referred to as PIN genes, which govern the degree of adhesion between starch granules and the protein matrix, thus influencing milling behaviour (Chen et al., 2006; Pasha et al., 2010). Soft PW lines such as 251b, OK72 and 11955 possess wild type alleles PINa-D1a and PINb-D1a inherited from common wheat and which code for softness (Pogna et al., 2014; Gazza et al., 2016). However, 235a was found to possess novel previously undescribed PIN-A and PIN-B alleles (presumably inherited from the perennial parent) and this may have influenced the milling behaviour observed in this study. Murphy et al. (2009) found PW on average produced softer grains than annual wheat, and with the exception of the durum-derived 20238, that was the case in this study. With three lines in common there was agreement in hardness ranking with results reported by Gazza et al. (2016), but poorer agreement with Hayes et al. (2012) (5 lines in common), particularly for 251b which those authors characterised as medium hard but was found to be soft in the current study. It is noted that the PW lines with highest flour extraction at both sites in 2018 were also higher in kernel weight, while no trend was observed for test weight. Total starch contents, while lower for PW than Wedgetail, were all above 72% apart from OK72 (68.5%) and showed a negative trend against flour protein content across all entries.

4.4 Yield, harvest index, grain morphology and protein

PW lines were generally longer and narrower than Wedgetail, reflecting the selection preference in modern wheat breeding programs for larger rounded grains which positively effects milling yield (Gegas *et al*., 2010). Yield results varied between the four sites and were largely affected by environmental conditions during the growing season. The experimental year of 2018 was characterised by lower-than-average rainfall which had a negative impact on grain yields at both Cowra and Mandurama, especially for later-maturing lines that struggled to fill grain under dry conditions. A worsening of drought conditions in 2019 had a greater effect on perennial wheat yields than observed in 2018. Grain weight, a direct function of dry matter accumulation during grain filling (Xie *et al*., 2015), was on average 27% lower than in 2018 reflecting the truncated grain filling period. Phenology plays an important part in determining wheat grain yield as quicker maturing genotypes can have up to a 16% yield advantage under conditions which constrain growing season rainfall (Qaseem et al., 2019; Cann et al., 2023). In general, grain yields were higher for the annual cereal (Wedgetail) which was the earliest to maturity at each site (data not shown). The better yielding perennial wheat lines were Summer 1 and 11955 (30% and 25% of the yield of Wedgetail across sites respectively), which had consistently higher grain yields than the other perennial genotypes. These higher yielding PW lines approach the yield target of 40% of an annual wheat suggested by Bell (2013) to be as profitable as conventional wheat in a mixed farming system, assuming additional biomass in the PW genotypes was available for grazing. The promising yields reported under highly challenging seasonal conditions, along with favourable baking and rheological properties of some of PW genotypes bodes well for the development of viable perennial wheat lines in the future. Further research to investigate opportunities to increase grain yield of PW genotypes by selecting earlier maturing types is warranted.

5 Conclusion

The variation in grain parameters, flour milling yield, dough rheology and breadmaking quality was anticipated. However, there were unexpected combinations of traits which would suggest caution in applying traditional assumptions based on wheat. Lines 11955 and OK72, despite classification as extra soft and soft respectively, displayed higher flour extraction and water absorption than harder grained lines. Furthermore, although rheological tests indicated moderate gluten strength only, these lines exhibited better baking performance than the line 235a which displayed good gluten strength more comparable to Wedgetail wheat. Although these findings are based on a small sample set, they nevertheless indicate that general expectations and predictive tests used for common wheat do not necessarily translate to PW. This paper has investigated baking performance with very encouraging results and further studies utilising PW in other products is warranted. With refinement of formulations and conditions to maximise performance, PW can clearly offer good functionality for food applications given the gluten and starch properties observed in this study. Further assessments of PW lines would provide an opportunity to apply selective breeding to this crop to produce lines combining both longevity, higher grain yield and food functionality.

6 Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

7 Author contributions

All authors contributed to conception and design of the study. DP & BP organized the database. BP and RH performed the statistical analysis. DP wrote the first draft of the manuscript. KHT, BP, MN and RH wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the final version.

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10 Data Availability Statement

All data contained in this study can be made available upon reasonable request to the authors.

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Table 1. Identification (ID) and pedigree details of genotypes.

ID	Pedigree
11955	*Triticum spp. / Thinopyrum ponticum*
20238	*T.durum (S) / Th.elongatum*
CPI-235a (235a)	*T.aestivum (CS) / Th.ponticum / T.aestivum (M)*
CPI-147251b (251b)	*T.aestivum (CS) / Th.ponticum / T.aestivum (M)*
OK7211542 (OK72)	*Partial amphiploid derived from T.aestivum / Th.ponticum*
Otrastajuscaja 38 (Ot38)	*Partial amphiploid derived from T.aestivum / Th.intermedium*
Summer 1	*Partial amphiploid derived from T. aestivum / Th.intermedium*
Wedgetail	*T.aestivum*

Chinese Spring (CS) and Madsen (M) are cvv. of *T.aestivum*; Stewart (S) is a cv. of *T. durum*

Table 2. Significant main effects of genotype and site/year on grain yield, harvest index (HI), thousand kernel weight (Kwt) and grain protein (GP).

Treatment	Grain yield (kg/ha)	HI (%)	Kwt (g)	GP* (%)
		Genotype main effect (P<0.001)		
11955	806	17.8	28.5	19.0
20238	712	16.5	25.4	19.8
235a	654	11.7	21.7	19.7
251b	603	12.2	22.4	20.7
OK72	753	12.6	25.8	20.1
Ot38	651	10.9	20.6	20.6
Summer 1	966	26.5	27.8	18.6
Wedgetail	3219	28.9	33.2	17.4
$l.s.d._{0.05}$	*168.3*	*1.94*	*1.16*	*0.56*
		Site/year main effect (P<0.001)		
Cowra 2018	1515	26.3	27.9	18.7
Cowra 2019	567	0.9	23.0	20.8
Mandurama 2018	1455	30.4	29.7	18.4
Orange 2019	645	11.0	22.1	20.0
$l.s.d._{0.05}$	*119.0*	*1.37*	*0.82*	*0.40*

* not corrected for moisture content

Table 3. Grain hardness (PSI), wholemeal moisture, protein and mixograph parameters of perennial lines compared to Wedgetail wheat, determined from grain produced at the four experimental sites in 2018 and 2019.

Genotype	PSI	PSI descriptor	GM %	GP %	Mw %	PkT min	PkH %	PkW %	PkI Tq*min	5.5H %	5.5W %	BDn %	DSI %/min
11955	41	Extra soft	9.8	18.4	68.5	1.5	62.4	30.5	73	48.0	7.5	14.5	3.5
20238	17	Med hard	9.5	18.4	68.6	1.4	52.9	19.9	59	35.6	14.3	17.3	4.2
235a	24	Med soft	9.8	18.3	68.4	2.9	63.4	31.0	143	54.5	17.3	8.8	3.3
251b	30	Soft	9.7	19.4	70.1	1.4	57.1	24.9	65	42.6	4.8	14.3	3.5
OK72*	34	Very soft	9.6	19.4	70.0	1.6	65.5	29.8	84	50.4	3.9	14.6	3.9
OT38*	23	Med soft	9.6	19.3	69.9	1.3	57.4	24.2	57	39.4	1.7	17.7	4.2
Summer 1	27	Soft	9.6	17.3	67.0	1.9	58.5	27.8	88	43.4	12.9	15.1	4.1
Wedgetail	17	Med hard	9.8	16.1	70.2	2.6	60.3	26.9	125	51.5	12.5	8.9	3.0
$l.s.d_{0.05}$	*4.5*	-	*ns*	*1.2*	*1.72*	*0.38*	*4.39*	*3.07*	*17.2*	*7.24*	*8.17*	*6.26*	*ns*

PSI = particle size index (determined on 2018 sites only); GM = Grain moisture; GP = grain protein 11% mb; Mw = mixograph water addition; PkT = mixograph time to peak; PkH = peak height; PkW = peak width; PkI = peak integral; 5.5H =

height at 5.5 mins; 5.5W = width at 5.5 mins; BDn = breakdown; DSl = descending slope; ns, differences not significant at *P*=0.05.*OK72 lacks mixograph data from C19 site; Ot38 lacks data from C19 and O19 sites.

Table 4. Grain length, dorsal-ventral width (DV wd), left-right width (LR wd), colour (brightness L*, redness a*, yellowness b*) and ash of perennial lines compared to Wedgetail wheat, determined from grain produced at the four experimental sites in 2018 and 2019.

Genotype	length	DV wd	LR wd	Grain colour			Ash*
	mm	mm	mm	L*	a*	b*	%
11955	7.01	2.04	2.37	48.3	5.1	14.0	1.89
20238	7.71	1.93	2.27	45.6	4.4	10.5	1.75
235a	5.94	1.99	2.32	49.3	4.5	13.6	1.96
251b	6.62	1.79	2.28	48.0	4.9	14.2	2.02
OK72	6.85	2.06	2.36	48.9	5.1	14.2	2.07
Ot38	6.48	1.92	2.09	49.2	4.8	13.3	1.91
Summer 1	6.60	2.14	2.51	47.6	4.9	13.5	1.53
Wedgetail	6.06	2.40	2.73	49.2	4.4	14.0	1.32
l.s.d	*0.34*	*0.15*	*0.15*	*1.2*	*0.4*	*0.8*	*0.16*
	<0.001	*<0.001*	*<0.001*	*<0.001*	*=0.001*	*<0.001*	*<0.001*

DV wd = dorsal ventral width; LR wd = left-right width; Ash = ash at 11% moisture basis, *determined on 2018 entries only.

Table 5. Flour extraction, protein, ash, and total starch; RVA parameters; and DoughLAB parameters of three PW genotypes compared to Wedgetail wheat using grain from two sites sown in 2018.

Genotype	FE	FP	Ash	TS	Pv	Fv	Sb	WA	DDT	Stab	Soft3	PkE
	%	%	%	%	cP	cP	cP	%	min	min	FU	Wh/kg
Cowra18												
235a	48.6	15.1	0.58	73.6	1980	2448	1140	59.5	2.23	1.9	108	24.1
251b	53.0	17.0	0.48	72.6	2652	2748	996	61.3	0.91	0.7	223	19.7
11955	55.1	16.3	0.45	75.4	1632	1824	876	62.9	1.25	1.1	170	21.8
Wedgetail	66.1	13.5	0.45	78.1	2340	3132	1464	59.7	2.68	2.4	118	24.0
Mandurama18												
235a	50.1	16.3	0.63	72.8	1500	1908	948	59.2	2.11	2.1	103	24.1
251b	52.5	17.9	0.58	73.0	2316	2604	1068	61.5	0.83	0.5	285	18.8
OK72	54.6	17.8	0.50	68.5	2052	2376	1080	60.5	1.25	1.2	150	23.0
Wedgetail	64.6	13.9	0.45	77.6	2796	3180	1476	59.0	3.05	3.1	85	24.4

FE = flour extraction; FP = flour protein 14% mb; TS = total starch; Pv = RVA peak viscosity, in centipoise (cP); Fv = final viscosity; Sb = setback; WA = DoughLAB water absorption; DDT = dough development time; Stab = stability; Soft3 = softening at 3 minutes past peak, in Farinograph Units (FU); PkE = total energy to peak (watt hours per kg).

Table 6. Flour mixograph parameters of three PW genotypes compared to Wedgetail wheat using grain from the two sites sown in 2018.

Genotype	Mw	PkT	PkH	PkW	PkI	5.5H	5.5W	BDn	DSl
	%	min	%	%	Tq*min	%	%	%	%/min
Cowra18									
235a	67.9	4.0	53.4	24.8	171	51.5	15.8	2.0	1.4
251b	70.1	1.4	54.3	22.7	63	42.7	3.7	11.6	2.8
11955	69.7	1.8	58.8	28.6	87	47.3	4.6	11.6	3.2
Wedgetail	65.5	4.6	56.2	24.4	206	54.3	20.6	1.9	2.0
Mandurama18									
235a	69.7	3.9	57.8	25.9	169	53.3	9.3	4.5	2.7
251b	72.1	1.3	49.9	21.5	55	38.3	2.7	11.6	2.8
OK72	72.1	2.2	56.3	21.1	98	45.7	5.2	10.5	3.1

Wedgetail	66.2	5.0	59.7	27.1	227	58.7	26.8	1.0	1.6

Mw = mixograph water addition; PkT = mixograph time to peak; PkH = peak height; PkW = peak width; PkI = peak integral; 5.5H = height at 5.5 mins; 5.5W = width at 5.5 mins; BDn = breakdown; DSl = descending slope

Table 7. Flour colour of whole grain, wholemeal and flour, of three PW genotypes compared to Wedgetail wheat using grain from the two sites sown in 2018 (brightness L*, redness a*, yellowness b*).

Genotype	Whole grain colour			Wholemeal colour			Flour colour		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
Cowra18									
235a	56.9	5.7	15.3	82.8	0.9	11.8	91.5	-2.1	10.3
251b	56.8	7.1	17.6	84.0	0.5	11.8	92.0	-2.3	10.7
11955	58.8	6.4	16.5	85.0	0.6	10.6	92.5	-2.1	9.1
Wedgetail	61.2	6.0	18.9	83.2	1.1	12.6	90.3	-1.4	10.0
Mandurama18									
235a	57.8	5.9	15.9	83.4	0.6	11.5	91.3	-1.9	9.6
251b	55.9	6.8	16.4	83.6	0.7	11.4	91.2	-2.0	10.0
OK72	55.9	6.5	16.3	83.0	0.9	10.5	91.7	-1.9	8.9
Wedgetail	57.3	6.2	16.4	82.9	1.3	12.4	90.1	-1.2	9.6

Table 8. Bake test parameters of three PW lines compared to Wedgetail wheat using grain from the two sites sown in 2018.

Genotype	BWA	Mix	LV	SpV	ExtA	IntS	Firm	Crumb colour			Crust colour		
	%	min	cc	cc/g	of 5	of 5	gF	L*	a*	b*	L*	a*	b*
Cowra18													
235a	63.6	3.15	125	3.75	4.3	3.0	511	69.1	-5.6	22.9	58.3	8.2	35.7
251b	63.7	1.75	128	3.94	3.0	3.0	383	69.2	-6.3	23.0	58.4	7.6	36.7
11955	66.9	2.25	129	4.00	3.5	4.0	381	71.1	-6.3	20.5	60.2	7.5	38.1
Wedgetail	63.5	3.65	130	3.92	4.0	4.5	523	72.0	-5.6	17.0	63.2	6.2	36.8
Mandurama18													
235a	63.4	2.95	129	3.88	4.5	2.5	480	68.7	-5.2	21.8	55.2	9.3	34.1
251b	64.1	1.50	114	3.49	2.0	1.0	487	66.0	-5.2	21.7	54.7	9.4	35.1
OK72	64.6	2.35	132	4.06	5.0	5.0	441	70.8	-5.5	19.6	56.4	9.1	36.6
Wedgetail	62.8	4.30	127	3.86	4.0	5.0	337	71.4	-5.3	16.8	62.6	6.0	36.0

BWA = bakery water absorption; Mix = bakery mix time; LV = loaf volume; SpV = specific volume; ExtA = external appearance score; IntS = internal structure score; Firm = crumb firmness (gF = grams force); L*,a*,b* = crumb and crust colour.

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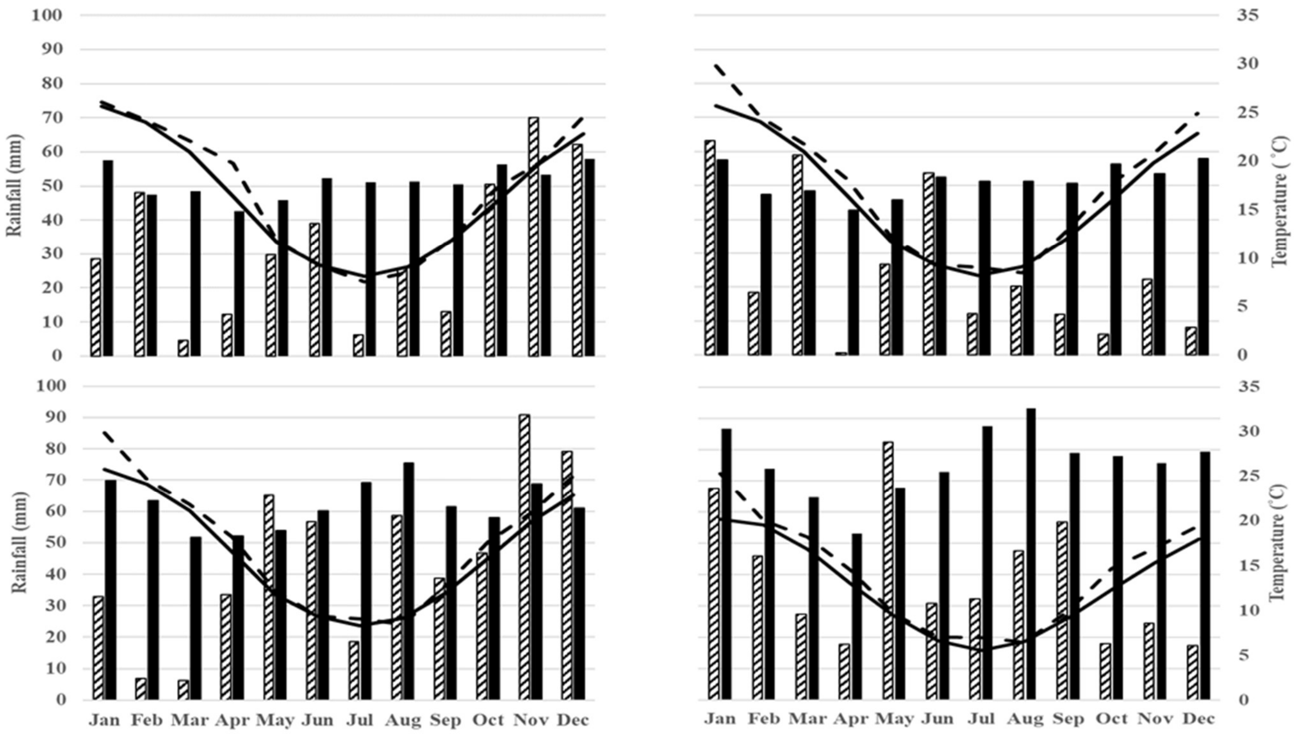
Figure1. Mean monthly temperature (——) along with monthly long-term average (- –) as well as total monthly rainfall (▨) and the monthly long-term average (■) recorded at each site or nearby weather station. A = Cowra 2018; B = Mandurama 2018; C = Cowra 2019; D = Orange 2019.

Figure 2. Flour mixographs: A, B, C, D = grain from Cowra18, genotypes 235a, 251b, 11955, Wedgetail. E, F, G, H = grain from Mandurama18, genotypes 235a, 251b, OK72, Wedgetail.

Figure 3. Internal and external appearance of loaves: A, B, C, D = grain from Cowra 2018, genotypes 235a, 251b, 11955, Wedgetail. E, F, G, H = grain from Mandurama 2018, genotypes 235a, 251b, OK72, Wedgetail.



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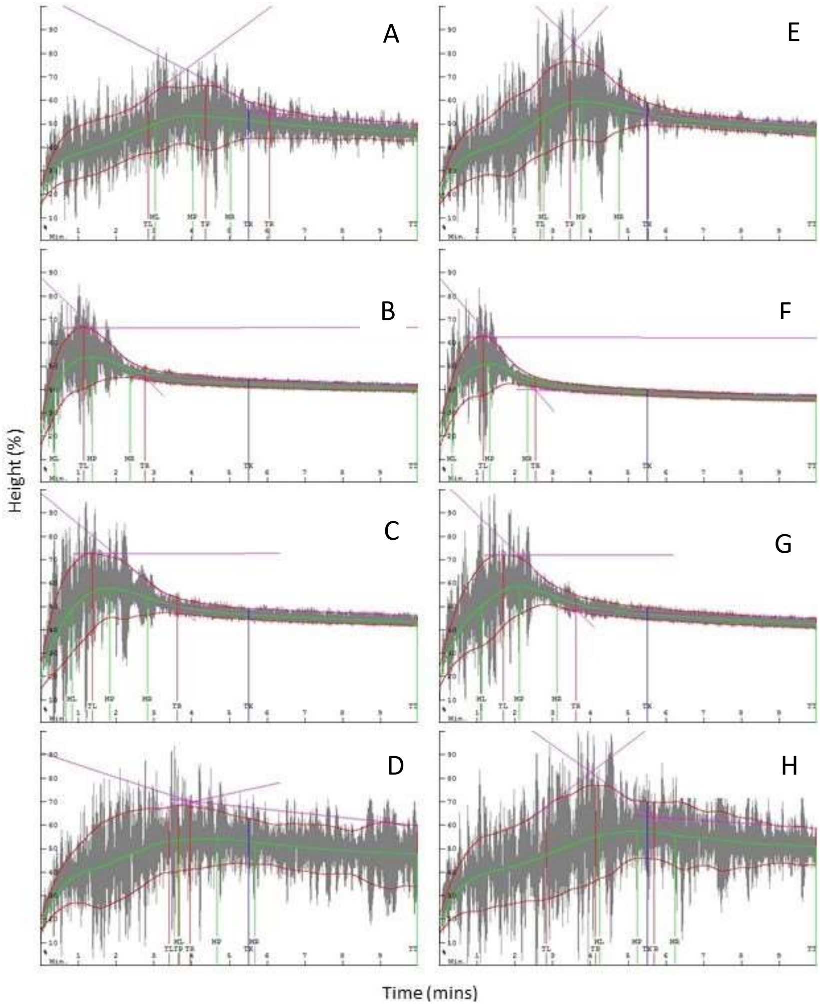

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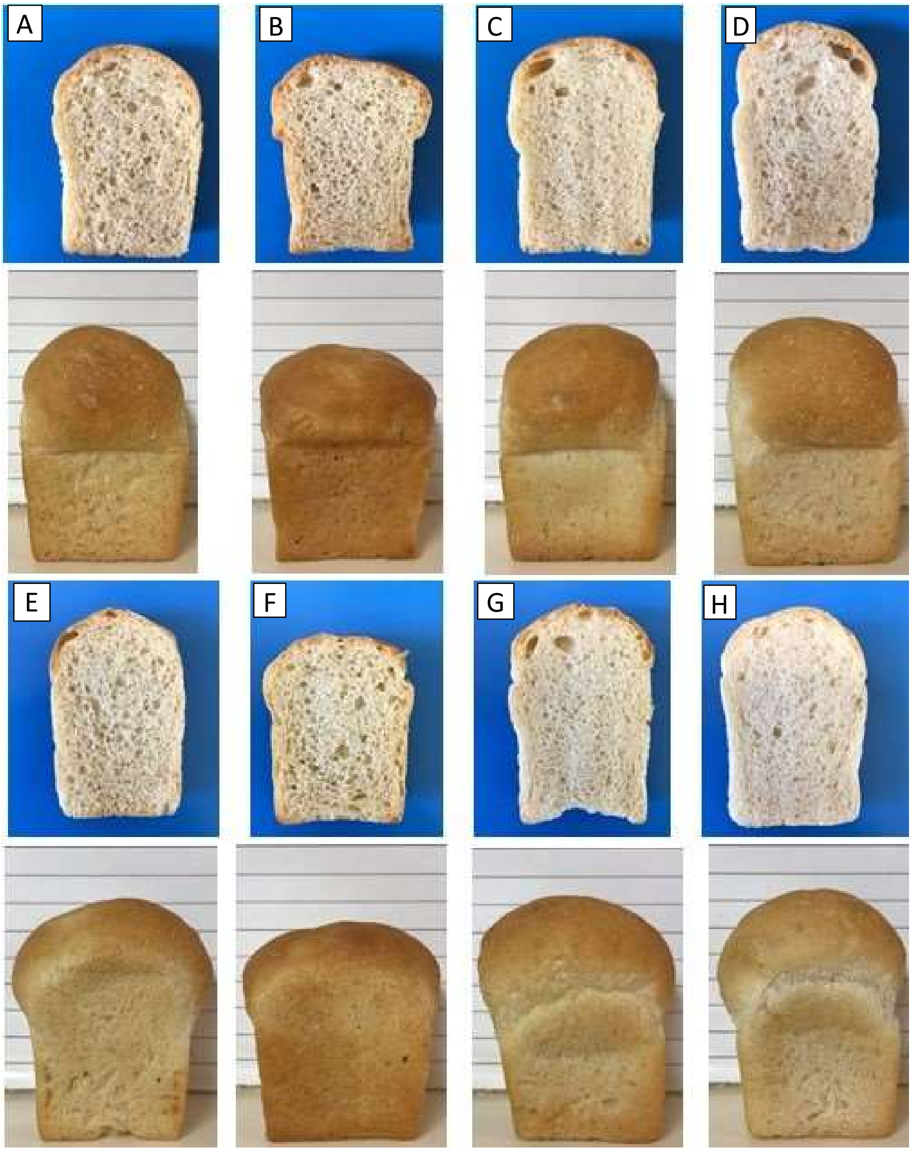

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BRIEF REPORT

The accuracy of the Optium Neo™ handheld glucose-ketone meter when field testing blood sampled from pregnant ewes

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ABSTRACT

Aims: To compare the β-hydroxybutyrate (BHB) and glucose concentrations of twin-bearing pregnant ewes when measured using laboratory methods or an Optium Neo™ handheld meter.
Methods: Blood samples were collected via jugular venepuncture from 48 twin-bearing adult Merino ewes, identified by ultrasound diagnosis, on four occasions, at weekly intervals, over 28 consecutive days in the final trimester. Each blood sample was tested within 10 min of collection utilising glucose and ketone test strips for their corresponding Optium Neo™

handheld meter. Plasma samples were also analysed for glucose and BHB concentrations using laboratory based diagnostics, i.e. colorimetric analytics and a benchtop spectrophotometer. Data were analysed to determine correlation, concordance, and collinearity.

Results: There were positive correlations (r = 0.47) and moderate concordance found for the glucose blood test results (ρ_c = 0.35; n = 187). The BHB results of the two methods were discorded (ρ_c = 0.04) with weak correlations (r = 0.24; n = 187). Bland-Altman analyses demonstrated agreement between the methods, although there were linear trends apparent in the plots and the Optium Neo™ tended to overestimate both glucose and BHB concentrations in the blood. In addition, the Optium Neo™ data had a greater coefficient of variation for both parameters when compared to laboratory methods. Linear regression models confirmed the positive relationship between the blood test results of either method and proposed correction factors (β_1) to account for the Optium Neo™ overestimations of BHB (0.03; β_0 = 0.24) and glucose (0.15; β_0 = 2.49) concentrations. These correction factors apply within the range observed in the present study.

Conclusions: The Optium Neo™ handheld device can be used when field-testing the blood of pregnant ewes. To achieve accuracy that is comparable to Laboratory methods, Optium Neo™ glucose and BHB results must, however, be adjusted.

Clinical relevance: This information will have value to the development of a ovine specific handheld blood testing device as well as to those using the Optium Neo™ to diagnose pregnant ewes with hypoglycaemic stress or pregnancy toxaemia.

KEYWORDS: Ovine; Diagnostic test; Beta-hydroxybutyrate; Hyperketonaemia; Glucose; Twin-bearing ewes

Introduction

Handheld ketone and glucose meters that were developed for clinical use in humans have also been used by veterinarians and researchers in animal science, to monitor the health status of domestic and commercial livestock. The Optium Neo™ is a handheld device that was designed to be a mobile, quick, less invasive, and an inexpensive alternative to laboratory blood tests. This device was developed to test human blood, but if validated, it could be useful as a diagnostic tool for the field testing of blood from pregnant ewes. Early detection using these devices *in situ* allows the manager of the flock to treat ewes affected by metabolic disturbances immediately. Specifically, elevated β-hydroxybutyrate (BHB) is indicative of sub-clinical ketosis or pregnancy toxaemia, whereas low glucose concentration is indicative of hypoglycaemic stress (Pethick and Lindsay 1982; Schlumbohm and Harmeyer 2004;2008). These disorders are, usually, associated with low energy in the diet and compounded by reduced rumen volume, as a result of pregnancy. These conditions are exacerbated when ewes are bearing twins, due to increased energy demands of the fetuses and lower feed intake. Although glucose levels in the blood are tightly controlled by hormonal regulation (normal levels of glucose in a dry ewe are 1.7-2.9 mmol/dL are similar to healthy pregnant ewes) foetal demands in late gestation deplete glucose reserves (Lindsay and Leat 1975). In response to the negative energy balance fat stores are mobilised resulting in increased ketone bodies in the blood, such as BHB.

Previous research has recommended other handheld diagnostic devices for testing livestock blood for BHB and glucose concentrations. The Nova Vet™ was found to provide highly accurate and precise measures of BHB in the blood of dairy cattle, noting that this device was developed to be bovine specific (Rodriguez *et al.* 2021). The Optium Xceed™ was reported to detect mild or severe pregnancy toxaemia in the blood of dry ewes with induced hyperketonaemia (Araújo *et al.* 2020). In addition, the strong correlations between the results of the Precision Xceed® meter and laboratory analysis, prompted the formers recommendation as a diagnostic tool for use with dairy sheep (Panousis *et al.* 2012). Irrespective of the conclusions made, the results in each of these studies indicate that the handheld devices overestimate blood BHB or glucose concentrations, when compared to established laboratory assays. If correct, and unaccounted for, the use of these devices may result in the misdiagnosis of pregnant ewe health status. These observations highlight an imperative, to determine the accuracy of the Optium Neo™ and support its use as an alternative device for field testing the blood of pregnant ewes.

This study aimed to compare the blood test results from the Optium Neo™ and laboratory methods for glucose and BHB determination. Subsequently, it aimed to define Optium Neo™ correction factors for use when testing the blood of pregnant twin-bearing ewes.

Materials and methods

Animals and sample collection

Approval for this study was granted by the NSW DPI Animal Ethics Committee (ORA19/22/026). Blood samples were collected via jugular venepuncture (K^+ EDTA, 9 mL Vacutainers) from 48 twin-bearing adult Merino ewes, identified by ultrasound diagnosis, on four occasions, at weekly intervals, over 28 consecutive days in the final trimester. Samples were collected in the morning. One ewe was removed from the study after its initial sampling. Lambing commenced 8 days after the last blood collection and finished 15 days thereafter (Refshauge *et al.* 2022).

Blood testing

Each blood sample was tested within 10 min of collection utilising glucose and ketone test strips for their corresponding Optium Neo™ handheld meter (FreeStyle, Abbott Laboratories, UK).

Blood samples were placed on ice and, within 2 h of collection, were centrifuged (CM-7S, ELMI, USA) for 15 min at 2,300 × g. The plasma was decanted and frozen at -20 °C until laboratory testing. Plasma samples were thawed at room temperature and analysed for glucose concentration (mmol/dL) as per the Glucose Assay Kit Technical Bulletin (MAK263, Sigma-Aldrich, USA). Technical duplicates were prepared and analysed using a benchtop spectrophotometer (FLUOstar OPTIMA™, BMG Labtechnologies, AUS) set to measure absorbance at 570 nm. The same thawed plasma samples were analysed for BHB concentrations (mmol/L) as per the β-hydroxybutyrate Assay Kit Technical Bulletin (MAK041, Sigma-Aldrich, USA). Samples were prepared and analysed using the same benchtop spectrophotometer, to measure absorbance at 450 nm.

Statistical analysis

Data were analysed in Stata/IC version 14.2 (StataCorp., www.stata.com). Precursory tests for normality, outliers, errors, and cook's distance were applied. No data were omitted. Pearson's correlation and Lin's concordance correlation coefficients were both used to quantify the relationship between the methods (Optium Neo™ and Laboratory) for glucose or BHB determination. The limits of agreement between the methods were visualised using Bland-Altman plots. Scatterplots fitted with the two methods were evaluated and linear regression models were observed to be appropriate for the data. Glucose and BHB data were analysed using individual linear mixed models that were fitted with method as the fixed effect, and both animal and collection time point as random effects. The level of significance was set at 5%.

Results and discussion

Table 1 shows the summary statistics for the glucose and BHB results of both methods. These are within the ranges expected for at-risk ewes and align with those reported in the literature (Harmeyer and Schlumbohm 2006; Panousis *et al.* 2012; Araújo *et al.* 2020). This distribution represents a constraint to the research findings, whereby their extrapolation beyond the reported ranges for glucose and BHB is ill-advised. For measurements of both glucose and

BHB concentrations, a higher coefficient of variation was observed in the data collected from the Optium Neo device compared to the laboratory methods.

There were moderate positive correlations between the Laboratory and Optium Neo™ results for glucose (r = 0.47) and BHB (r = 0.24). These results demonstrate some linear covariance between the blood test methods, however, additional analysis was needed to determine the reliability or agreement between the two methods (Schober *et al.* 2018). The Lin's concordance correlation coefficient for glucose ($\rho_c = 0.35 \pm 0.05$; $P < 0.001$) shows moderate agreement between the methods. The Lin's concordance correlation coefficient for BHB ($\rho_c = 0.04 \pm 0.01$; $P = 0.002$) shows low agreement between the methods. It is noted that the interpretation of Lin's concordance coefficients is somewhat subjective when $\rho_c \neq 0$ or 1 – these values being indicative of perfect discordance and accordance, respectively.(Lin 1989) The data were therefore analysed using Bland-Altman's limits of agreement.

Evaluation of the Bland-Altman's analyses found the majority of glucose (0.07 ± 0.54) and BHB (0.29 ± 0.34) results were within the limits of agreement (mean ± one standard deviation) (Bland and Altman 1986). On face value, this suggests comparability between the methods, although, the Optium Neo™ was shown to overestimate the Laboratory results for both glucose and BHB. There were strong correlations between the difference (Laboratory minus Device) and the mean glucose (r = 0.70) and BHB (r = 0.96) blood test results. These linear trends are obvious from the Bland-Altman plots (Figure 1.). Similar linear trends are evident in other Bland-Altman plots, in the literature, that have compared the BHB results from a handheld device to the laboratory method (Pineda and Cardoso 2015; Araújo *et al.* 2020). Alternatively, the results from the handheld device evaluated by Rodriguez *et al.* (2021) does not share this trend, although their observation may be the result of a correction factor developed for Nova Vet™, being a bovine specific device. There were large coefficients of variation in the data, also apparent from Figure 1., that would affect the precision of any exchange or direct comparison of the blood test results determined using either the Optium Neo™ or Laboratory method.

Linear regression models were used to define correction factors for Optium Neo™ blood test results, adjusted against those from the Laboratory method (Table 2). For glucose, a positive relationship was found between the methods ($P < 0.001$) that suggested a correction factor (β_1) of ~ 0.15 ($\beta_0 = 2.49$) could be used to adjust glucose results of the Optium Neo™. Likewise, for BHB, a positive relationship was found between the methods ($P = 0.001$) and a correction factor of ~ 0.03 ($\beta_0 = 0.24$) could be used to adjust Optium Neo™ blood test results. The significant linear relationships support the previous correlation analyses. In addition, the regression analyses both demonstrated that the Optium Neo™ overestimated the BHB and glucose concentrations in the blood of pregnant ewes, when compared to the Laboratory method. This finding supports the observations made from the Bland-Altman analyses as well as the results presented in the literature. The correction factor proposed for glucose was greater (~ 3-fold) than for BHB, which suggests the Optium Neo™ would be better applied to diagnose BHB associated disorders in pregnant ewes. This observation is supported by research of a point-of-care glucose and BHB meter, that found their device could adequately monitor BHB concentrations in the blood of pregnant ewes, but not glucose (Hornig *et al.* 2013). That said, the proposed correction factors may offer a means to modify the Optium Neo™ to become an ovine-specific device for monitoring both blood glucose and BHB concentrations.

In conclusion, this study demonstrates that the Optium Neo™ handheld device can be used when field-testing the blood of pregnant ewes. To achieve accuracy that is comparable to Laboratory methods, Optium Neo™ glucose and BHB results must, however, be adjusted. The recommended correction factors, presented herein, would be useful to avoid the misdiagnosis of pregnant ewes with metabolic disorder. Research with additional animals and greater ranges in blood glucose and BHB concentration would further aid this outcome. Nonetheless, the authors acknowledge that it is the individual users responsibility to formulate acceptable limits for accuracy and precision when using this device as a diagnostic tool.

Acknowledgements

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Table 1. The mean, standard deviation (SD), coefficient of variation (CV%), median and range of glucose and β-hydroxybutyrate (BHB) concentrations in the blood of pregnant ewes.

Summary statistics	**Glucose, mmol/dL**		**BHB, mmol/L**	
	Laboratory	Device	Laboratory	Device
No.	187	187	187	187
Mean	3.0	3.0	0.3	0.5
SD	0.3	0.6	0.1	0.4
CV%	9.4	20.1	19.1	64.6
Median	2.9	3.0	0.3	0.4
Minimum	1.9	1.4	0.2	0.2
Maximum	3.8	4.8	0.4	2.1

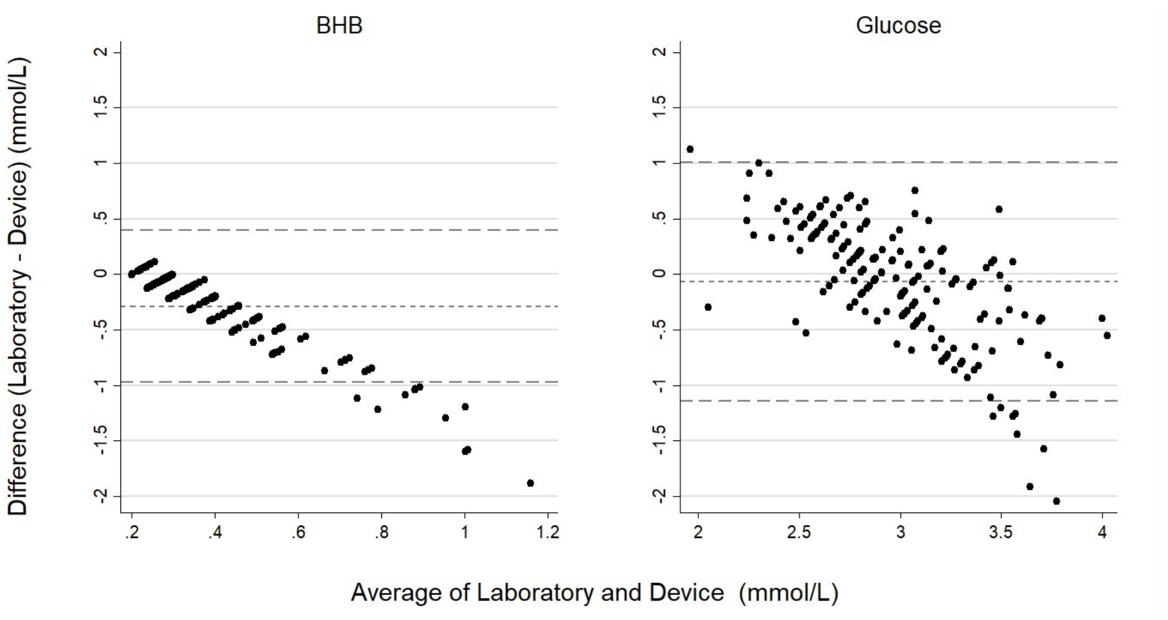

Figure 1. Bland-Altman plots representing the differences between the Optium Neo™ (Device) and laboratory-based method (Laboratory) measurements of β-hydroxybutyrate (BHB) and glucose in the blood of pregnant ewes. The middle short dashed lined represents the mean difference and the upper and lower long dashed line represents the limit of agreement (± one standard deviation).

Table 2

Linear regression models (± standard error) for the 'correction' of Optium Neo™ against the laboratory method results for glucose and β-hydroxybutyrate (BHB) concentrations in the blood of pregnant ewes. Data were adjusted for animal and collection interval effects.

Parameter	**Model**	*P* value
BHB	0.0265 (± 0.0076) + 0.2401 (± 0.0078) mmol/L	0.001
Glucose	0.1519 (± 0.0240) + 2.4940 (± 0.0816) mmol/dL	< 0.001

Appendix 4 – Animal Health PrimeFact

The Animal Health Primefact is attached to this report in a separate file to preserve formatting.

Appendix 5 – Grain Properties PrimeFact

The Grain Properties Primefact is attached to this report in a separate file to preserve formatting.

Appendix 6 – Agronomy PrimeFact

The Agronomy Primefact is attached to this report in a separate file to preserve formatting.

Appendix 7 – Newell *et al*. 2023

Newell MT, Munday N, Hayes RC (2023). The effect of nitrogen rates and plant density on grain yield components and persistence in intermediate wheatgrass (*Thinopyrum intermedium*) and mountain rye (*Secale strictum*). Proceedings of the International Grasslands Congress, Covington, KY, USA. (in press).

The effect of nitrogen rates and plant density on grain yield components and persistence in intermediate wheatgrass (*Thinopyrum intermedium*) and mountain rye (*Secale strictum*)

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Key Words: Perennial grains; Kernza; perennial grass; harvest index: grain size.

Abstract: Intermediate wheatgrass (IWG; *Thinopyrum intermedium*) and Mountain Rye (Mtn Rye; *Secale strictum*) have potential for release as dual-purpose (grazing and grain production) perennial grains in Australia due to their superior longevity compared to hybrid perennial wheats. Initially developed as perennial forage grasses, few management guidelines exist to inform agronomic practices to maximise grain yields and profitability in Australian environments. An experiment was established in 2020 to examine the effect of plant density and nitrogen rates on grain yield components. The experiment compared the two species (IWG, Mtn Rye) sown at three plant densities (50, 100 and 200 plants/m^2) with three nitrogen rates (0, 100, 200 kg/ha N). Overall, in the first year of production, Mtn Rye had higher grain yields than Kernza although yield decreased with increasing N rates. With further selection for floret fertility and seed size, Mtn Rye could prove a successful candidate for a perennial grain crop in Australia.

Introduction

Globally, annual grain crops provide 70% of human calorific requirements and occupy 70% of agricultural land (Glover and Reganold 2010). However, these grain production systems are associated with higher levels of soil disturbance, either through cultivation or the maintenance of chemical fallows between cropping sequences. Consequently, annual grain production has become associated with higher rates of soil loss, changed landscape hydrology, declining soil organic carbon and nutrient runoff. Changing the life cycle of grain crops from annual to perennial could offer a new production system which is more sustainable, resistant to climate mediated impacts, and with higher rates of carbon sequestration.

One method being pursued to develop perennial crops is domestication of perennial grasses to derive perennial cereals. Intermediate wheatgrass (IWG; *Thinopyrum intermedium*) , a perennial forage grass, has undergone selection for domestication traits and is one of the first perennial grain crops to be commercialised in the USA, under the name Kernza (DeHaan *et al.* 2020). Although there has been a significant increase in area sown to Kernza in North America, there is little experience with this species in Australian agriculture. Mountain Rye (Mtn Rye; *Secale strictum*) has a similar Eurasian origin to IWG and has been developed as a forage grass suited to acid duplex soils of south eastern Australia (Oram 1996). Improvements in seed harvestability led to a commercial cultivar of Mtn Rye being released in Australia, although was never widely adopted. Both IWG and Mtn Rye have the greatest near-term prospect for release as perennial grains in Australia, due to their superior persistence compared to hybrid wheats (Hayes *et al.* 2012). However, for both species, basic agronomic practices to maximise grain yields and profitability remain unclear in Australian environments. This study set out to understand the nitrogen (N) response and optimal planting density for these novel crops.

Methods

An experiment was established at the Orange Agricultural Institute (S33°19.565', E149°4.818') in 2020 to examine the effect of plant population and nitrogen rates on grain yield components of IWG and Mtn Rye. Soil type was a brown ferrosol (Isbell 1996) with $pH_{(CaCl2)}$ 5.5, 124 mg/kg and 36 mg/kg of mineral nitrogen and phosphorus (Colwell) in the top 10cm respectively. The experiment was sown on 21 April in a randomised design with three replicates. The area had been chemically fallowed since September 2019 and then cultivated after 3t/ha lime ($CaCO_3$) was applied on 17 February 2020. All treatments were sown using a cone seeder with 18cm row spacing, to produce plots which were 1.5m wide x 10m long. Seeding rates were adjusted according to seed size and germination percentage, to produce populations of each species that were 50, 100 and 200 plants/m^2. Nitrogen was applied as urea (46%N) in a split applications prior to rainfall, in June, July and September 2020. In total each plant population received one of three N rates, 0, 100, or 200 kg/ha.

Once each species had ripened, a representative sample of biomass was removed from each plot by cutting two rows at ground level, along a 50cm ruler (total area = 0.36 m^2) at either end of the plot in February 2021. This sample was used to determine the number of mature tillers/m^2. The grain was removed from this sample through a stationary thresher (Kimseed, Perth), from which the number of kernels were counted and weighed to determine relative seed size. A further sampling of five random inflorescences were selected from each plot. From these the number of spikelets where counted on each inflorescence and the seed removed and counted. This data was used to calculate the average number of seeds contained in each spikelet (floret occupancy).

Prior to harvesting plant height from ground level to the top of the tallest plant was measured in three random locations within each plot. At the same time, the level of lodging in each treatment was assessed using a visual (1-10) score, where 1= all plants standing and 10 = 100% plants laying horizontal to the soil surface. At harvest all grain was removed using a plot harvester (Kingaroy Engineering, Kingaroy) from which final grain yield was determined in kg/ha.

To determine plant survival over summer, assessments of basal frequency was taken in autumn following harvest for each treatment (Brown 1954). Fixed quadrats (1.0 x 1.0 m) divided into 100 cells (each 0.1 x 0.1 m) were located centrally in each plot. The number of cells which contained a live plant base was counted.

Data was analysed using a linear mixed model (Genstat, 21st edition) fitted with crop type, population and nitrogen rate and interactions as fixed effects, with replicate as the random term. All data was analysed at the 95% significance level (*P* =0.05).

Results and Discussion

There was no interaction between crop type, plant population and increasing rates of N fertilisation. Mountain Rye produced 71% more tillers than IWG (Table 1) while there was a 21% increase in the number of mature tillers from increasing the N rate from 0 to 200kg/ha for both species ($P = 0.007$). Overall Mtn Rye produced twice as much grain as IWG, however yields declined with increasing rates of N (P=0.064). In a similar experiment with IWG, Fernandez *et al.* (2020) found that increasing N application had no effect on grain yield in the first year of experimentation with positive responses in mature tillers to increasing plant population and nitrogen rate. Nitrogen responses in crops generally follow a sigmoid response curve in which production increases with increasing rates of N, before plateauing and sometimes declining (Russell 1963). The current study was located on a site with high soil N levels at sowing. Therefore, we maybe observing responses at the top end of the response curve showing little response to increasing rates of N. Unlike annual grain crops, response to N can be complicated by the perennial nature of these crops and variability in N demand as the perennial crop ages (Jungers *et al.* 2017). Further monitoring over the longer term will be important to gain insight into optimising N rates in both species.

There was no significant difference in seed occupancy of florets between plant populations and increasing N rates. However, the inflorescence of IWG contained more seeds per spikelet than Mtn Rye (Table 1) while Mtn Rye had a higher spikelet number/inflorescence. Small decreases in seed weight were observed with increasing N application ($P = 0.009$) at all densities ($P < 0.001$) of both species. On average, Mtn Rye had 22% larger seeds than IWG. Floret site utilisation and higher fecundity along with grain weight are major drivers of grain yield. The higher seed weight of Mtn Rye compared to IWG offers potential to improve grain yield in this species more quickly with an emphasis of selection

Table 1: Grain yield components of mature tillers at harvest, grain yield, seeds per spikelet seed size and lodging score, along with plant frequency (%) for IWG and Mtn Rye sown at three plant populations (50, 100 & 200 plants/m2) and three nitrogen treatments (0,100 & 200 kg/ha N).

	Crop		Plant Population (plants/m^2)			Nitrogen Rate (Kg/ha)			*P* value			lsd (5%)		
	IWG	Mtn Rye	50	100	200	0	100	200	Crop	Pop*	N#	Crop	Pop*	N#
Mature Tillers/m^2	455.2	783.7	600.1	582.1	676.3	588.9	576.0	713.5	<0.001	0.131	0.007	79.9	ns¥	97.8
Grain Yield (kg/ha)	553.1	1126.8	803.9	838.9	877.3	929.5	832.2	758.0	<0.001	0.585	0.064	116.8	ns	ns
Seed/ spiklet	2.3	1.3	1.9	1.7	1.7	1.8	1.6	1.8	<0.001	0.605	0.267	0.2	ns	ns
Spikelets/ inflorescence	21.4	37.5	28.3	30.3	89.7	29.4	29.7	29.2	<0.001	0.936	0.105	2.1	ns	ns
Seed weight (mg)	9.0	11.0	10.3	10.2	9.7	10.4	10.1	9.7	<0.001	0.009	<0.001	0.3	0.4	0.4
Plant Height (mm)	1603.0	1358.0	1511.0	1524.0	1407.0	1494.0	1484.0	1464.0	<0.001	0.007	0.722	62.7	76.8	ns
Lodging Score	0.3	5.3	2.8	2.3	3.8	2.6	2.6	3.2	<0.001	0.157	0.315	0.793	ns	ns
Plant Frequency (%)	50.2	29.8	34.1	41.8	43.7	39.7	40.9	39.1	<0.001	0.001	0.748	4.2	5.1	ns

*Plant population
#Nitrogen rate
¥ not significant

for improved floret fertility (Altendorf *et al.* 2021). Although, neither trait responded to increasing plant population or N rates in the first year of the current study.

The IWG grew taller than Mtn Rye, however there was a higher incidence of crop lodging in the Mtn Rye treatments, with no clear response between plant population and nitrogen treatment. In the study by Fernandez et al. (2020) there was limited lodging in IWG, however, high rates of N at high planting density increased the incidence of lodging. The higher rates of lodging in Mtn Rye suggest a weaker straw strength, compared to IWG and would indicate selection for improvement in this trait could lead to better harvestability and grain yield. Breeding programs have identified genetic markers for plant height and increased stem base dry weight in IWG, which lead to better seed yield (Fernandez *et al.* 2020).

Measurement of plant frequency demonstrated that both species had adequate survival through the first summer. IWG had 40% higher frequency scores by the start of the second growing season, which is indicative of its rhizomatous nature (Jungers *et al.* 2017) compared to Mtn Rye which is more caespitose. As expected, increasing plant population resulted in in an increase in plant frequency for both species, particularly when the higher plant densities (100 & 200 plants/m^2) where compared to the low plant density (50 plants/m^2, Table 1). Plant frequency will need to be monitored over the longer term to ascertain the optimal plant density to maintain persistence of each species and whether increased N rates are required to maintain plant numbers and grain yield.

Conclusions

In the first year of assessment crop type was the main driver of responses in grain yield components of both IWG and Mtn Rye, with no interaction between crop type, plant population and increasing rates of N fertilisation. The impact of plant density and N rate will need to be monitored over a longer period so that these factors can be optimised for production, while limiting any adverse effects from excess N. Overall, Mtn Rye had higher grain yields than IWG. As a forage grass Mtn Rye has had no selection for grain yield characteristics. With further emphasis for floret fertility and seed size, Mtn Rye could prove a successful candidate for a perennial grain crop via domestication in Australia.

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