Domestication of the annual legume *Trigonella balansae* for mixed farming systems in southern Australia

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Abstract
An accession of the annual legume *Trigonella balansae* Boiss. and Reuter. has been selected for commercial release in Australia. The annual legume has significant potential as a self-regenerating pasture within mixed farming systems. As part of a duty-of-care assessment, we tested the hypothesis that sheep grazing the trigonella cultivar will have similar liveweight, condition scores, health and wool production to sheep grazing two widely adopted annual legumes, subterranean clover (*Trifolium subterraneum* L. cv Dalkeith) and French serradella (*Ornithopus sativus* Brot. cv. Erica). Forage dry matter digestibility (DMD), crude protein (CP), fibre, mineral content and isoflavones were measured across the plant's lifecycle. The data supported the hypotheses and there were no significant differences in liveweight, wool growth, wool yield or condition score that were associated with pasture species. The mineral content of trigonella requires further investigation.

Introduction
Australian agronomists have been actively seeking to diversify the range of annual legumes that are available for mixed farming systems in Mediterranean-climate areas of southern Australia. In these systems annual pasture legumes provide high quality forage for sheepmeat and wool production, fix atmospheric nitrogen in soils and provide a break-crop for management of weeds and diseases in cereal-dominant production systems. To reduce establishment costs, the legume is expected to regenerate from seedbanks after a series of crop monocultures. Traditional options such as subclover (*Trifolium subterraneum* L.) and annual medics (*Medicago* spp.) have more recently been supplemented by a new generation of annual legumes. These species that have been selected to fit specific climatic and edaphic niches, present seed that is easily harvested and processed, and have a seed dormancy pattern that optimises regeneration after the cropping phase. Species include French and yellow serradella (*Ornithopus* spp.), biserrula (*Biserrula pelecinus* L.), bladder clover (*Trifolium spumosum* L.) and gland clover (*Trifolium glanduliferum* Boiss.) (Loi et al.2005).

An accession of *Trigonella balansae* (SA 5045, CPI 19633) has shown potential for fine textured soils in the low to medium rainfall zones (300-450 mm annual rainfall). If commercialised, it will be the first cultivar of this species developed for agriculture in Australia. The plant has an upright growth habit, and the seed is relatively easy to harvest with conventional cereal harvesters (facilitating low establishment costs). The pattern of hard seed breakdown within and between seasons favours long-term persistence and the ability to sow seed in either summer or autumn (Nutt et al.2021). The genus *Trigonella* contains species with bioactive compounds that are used to flavour food and or in traditional medications, including fenugreek (*Trigonella foenum-graecum* L).

This paper reports key findings from an experiment that was designed to compare the feeding value of a cultivar accession of trigonella with French serradella (cv Erica) and subclover (cv Dalkeith). The hypothesis tested was that the sheep grazing the different legume species will have similar liveweight, condition scores, wool production, meat eating quality and plasma indicators of health.

Methods
The experiment was conducted in twelve 0.5 ha plots on a farm in Northam, Western Australia (31.75^{o}S, 116.68^{o}E). The plot area was previously managed as a single paddock. Long-term average annual rainfall at the experimental site was 445 mm, with 350 mm falling during the annual plant growing season in winter and spring (May to October). In 2020, the experimental site received 212

mm during the growing season, only 60 % of the long-term average. On the 9 June 2020, three treatments (randomly allocated) replicated in four blocks were sown with scarified seed of subclover (11 kg/ha), trigonella (7 kg/ha) or serradella (7 kg/ha). The plots were fertilised with 85 kg/ha of 3:2 superphosphate/potash (0.055 P, 0.066 S, 0.20 K) and seeds were inoculated with appropriate rhizobial strains two hours before sowing. The experiment was conducted with approval from the CSIRO Wildlife, Livestock and Laboratory Animal Ethics Committee (AEC# 2017-04).

On 15 September 2020, 72 clean shorn, healthy 14-month-old Merino wethers with a mean liveweight of 51.9 ± 3.31 kg and mean condition scores of 2.65 ± 0.23 units (using method of Suiter 1994) were randomly allocated to each of the plots, leading to a stocking density of 12 sheep/ha. All animals were utilised to compare liveweight and condition score changes every 2 weeks during grazing. Three sheep per plot were subject to wool growth measurement, using 100 cm^2 midside patches (Langlands and Wheeler 1968). The same sheep were subject to blood sampling (10 mL per jugular vein) to assess health status on 29 October 2020 (day 44) (https://vetpath.com.au). All animals remained on the plots until 4 November 2020, a total of 50 days of grazing. After 50 days of grazing two sheep per plot (n=24) were transported to a commercial abattoir and placed in lairage with access to water for 12 hours. The following day they were slaughtered (in random order), according to normal commercial protocols. Hot carcass weight was recorded five minutes post slaughter. Fatness at the GR site (between 12th and 13th ribs) and ultimate muscle pH were recorded 24 hours post slaughter. Meat sensory analysis methods are described in Wilmot et al. (2023).

Herbage dry matter (DM) and *in vitro* nutritive value were determined on six occasions during the growing season. Herbage mass was estimated using calibrated quadrat estimation. Sward composition was estimated using the BOTANAL technique (Mannetje and Haydock 1963). The plant quality analyses; *in vitro* (pepsin-cellulase) DMD, neutral detergent fibre (NDF), acid detergent fibre (ADF), CP and organic matter (OM) were estimated using near infrared spectroscopy (NIRS) according to the methods described in Norman *et al.* (2020). Mineral analyses were measured at a commercial laboratory according to their standard methods (CSBP Soil and Plant Analysis Laboratory, Bibra Lake, WA, Australia).

Results and Discussion

Introductions of novel annual legumes to Australia presents risks to livestock, farming systems and the natural environment. For livestock systems, the risks include poor nutritive value, secondary plant compounds that could impact the health, performance and product quality from animals and physical structures such as spines or burrs that injure animals or contaminate wool (Norman *et al.*2005). Laboratory quantification of nutritive traits and known secondary compounds does not necessarily account for factors that influence voluntary feed intake. Addressing the risk of new introductions is difficult to achieve through small-plot studies due to the large number of potential compounds and their possible interactions (Revell and Revell 2007). Comparative grazing studies, with commercially successful cultivars, offer an opportunity to explore these risks prior to commercial release (Norman et al. 2005; 2013).

As hypothesised, there were no significant differences in mean liveweight or condition score between plots of sheep before or at any time during grazing the three legume species ($P > 0.05$). The species therefore had similar feeding value under the conditions of the experiment. Daily liveweight change averaged 219 g/head/day when the pastures were in the vegetative phase and -15 g/head/day when the pastures were mature. There were no significant differences associated with pasture species for clean wool growth, hot or cold carcass weights, carcass fat score and ultimate pH of the meat ($P > 0.05$). The results from plasma panel analysis showed no significant differences in mean indicators of health, as extrapolated by muscle, liver and kidney enzymes. Although some individuals fell outside the standard reference ranges, there were no consistent or significant negative consequences associated with grazing trigonella.

There were no significant differences in mean feed on offer between the pasture treatments at any stage during grazing ($P > 0.05$). Biomass ranged from a mean of 2.07 t DM/ha of biomass at the start

of grazing to 1.44 t DM/ha at the termination of grazing. Herbage availability was unlikely to limit voluntary intake during the experiment. The trigonella appeared highly palatable, based on defoliation. Pasture DMD and CP are presented in Figure 1. Trigonella had significantly higher *in vitro* DMD than subclover and serradella during the vegetative phase at the start of grazing until podding when the DMD of trigonella declined much more rapidly than the serradella. It is likely that the deep root system of serradella and indeterminate growth habit allowed it to remain green later in the season (as described by Hackney et al. 2013). Serradella had significantly higher crude protein content than the other species from the second month of spring. By the end of October, the crude protein content of trigonella was only at the maintenance requirement for mature animals and could have limited sheep growth.

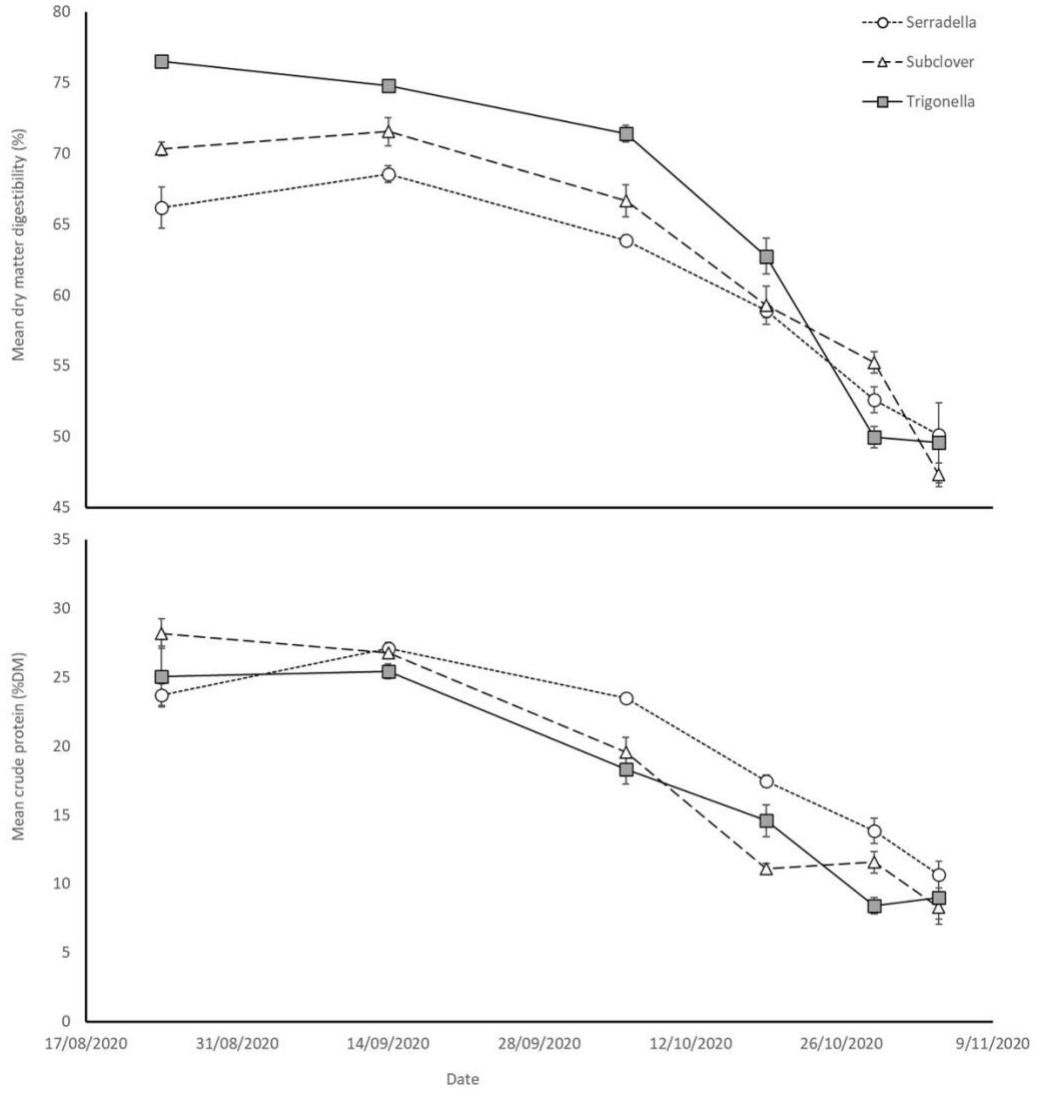

Figure 1. Mean dry matter digestibility and crude protein content of the legume species.

As trigonella matured, the DM did not meet the minimum requirements for mature sheep for sulphur, phosphorus, manganese, molybdenum and copper (Freer et al.2007). This had not translated to apparent deficiency in the sheep as evidenced by blood plasma analyses (Wilmot et al. 2023). Phosphorus deficiency can impact feed intake and lower growth rates. Copper is important for wool growth and manganese deficiency leads to skeletal abnormalities and reduced reproductive performance (Freer et al.2007). It is likely the sheep were able to obtain the minerals from volunteer plants in the sward, they had sufficient reserves or apparent deficiency was masked by a concomitant lack of protein and energy. A more thorough comparison of mineral content is required across target soils before drawing further conclusions and consideration of supplementation strategies. There was

no evidence of phytoestrogens in trigonella or serradella. More data, including the meat sensory analysis will be available soon (Wilmot et al. 2023).

Conclusion and implications

Domesticating new forage species for commercial agriculture carries greater inherent risks than selection of new cultivars within species, where negative traits are likely to be well defined. Duty of care experiments, such as the experiment reported in this paper, cannot ensure that all possible negative consequences to animals and their products are identified because concentrations of plant secondary compounds will vary with growing environment while animals may differ in their physiological responses to particular compounds (Revell and Revell 2007). Regardless of the limitations, comparative feeding value experiments are valuable in that they incorporate both voluntary feed intake and nutritional value of the feed.

The results from this 50-day grazing comparison indicate that sheep grazing trigonella (SA 5045) and French serradella (cv Erica), were as productive, healthy and had similar meat-eating quality to sheep grazing subclover (cv Dalkeith). While we found little evidence of trigonella impacting sheep productivity or health, it must be noted that the experiment was conducted in an unusually dry season and the plots were not monocultures of the species being tested. Given biomass availability, it was possible for sheep to have selected a varied diet.

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