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Sheep grazing *Trigonella balansae* had productivity, health and meat quality similar to sheep grazing subterranean clover or French serradella

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ABSTRACT

Context. An accession of the annual legume *Trigonella balansae* Boiss. & Reuter. has been selected for commercial release in Australia. **Aims.** As part of a duty-of-care assessment, we tested the hypothesis that sheep grazing the trigonella accession will have liveweight, condition scores and wool production similar to those of sheep grazing two widely adopted annual legumes, subterranean clover (subclover, *Trifolium subterraneum* L. cv. Dalkeith) and French serradella (*Ornithopus sativus* Brot. cv. Erica). We also hypothesised that sheep grazing the three legumes will have similar meat quality and health, as indicated by blood plasma panel analysis. **Methods.** Wethers ($n = 6$) grazed plots ($n = 4$) of three pasture species. Liveweight, condition scores and wool growth were measured, and a subset of animals were subject to blood sampling to assess health. Consumer sensory taste analysis was used to assess meat eating quality from two sheep per plot. Forage biomass, dry-matter digestibility, crude protein, fibre, mineral content and isoflavones were measured across the plant's lifecycle. **Key results.** There were no significant differences in liveweight, wool growth, wool yield or condition score that were associated with pasture species during the grazing period. There were differences in dry-matter digestibility, minerals and crude protein content of the pastures over time. As trigonella matured, it did not meet the minimum sulfur, phosphorus, manganese, molybdenum and copper requirements for mature sheep. We found some differences in indications of animal health; however, there were no significant systematic detrimental differences between sheep grazing trigonella and the other two species. In total, 2 of 24 individual sheep grazing trigonella had more than two plasma indicators of liver damage. There were no differences in meat tenderness, juiciness, flavour and overall consumer acceptability. **Conclusions.** The data supported the hypotheses. Under the conditions of this experiment, the pasture legumes had the same relative feeding value. Some care must be taken in interpreting the outcome as the trigonellacomprised only 18% of the dry matter on offer at the termination of grazing. **Implications.** Results of this study will be considered during the commercialisation process for *T. balansae*.

Keywords: forage quality, grassland management, ley farming system, meat flavour, pasture improvement, plant secondary compounds, predicted growth, ruminant modelling, sward composition.

Introduction

Grazing systems in the low- to medium-rainfall zone of southern Australia (300–450 mm rainfall annually) have traditionally relied on a narrow range of legumes that have been imported from the Mediterranean basin and domesticated for agriculture. These species have included subclover (*Trifolium subterraneum* L.) and annual medics (*Medicago* spp.). In the mixed crop and livestock systems in this zone, pasture legumes provide high-quality forage, fix atmospheric nitrogen in soils, and provide a break-crop option in cereal-dominant production systems. In the past 20 years, these species have been supplemented by annual legumes (with appropriate rhizobia) that have been selected to offer seed that is easily harvested and processed, with the ability to fill specific climatic and edaphic niches within systems. This new generation includes French and yellow serradella (*Ornithopus* spp.), biserrula (*Biserrula pelecinus* L.), bladder clover (*Trifolium spumosum* L.), eastern star clover (*Trifolium dasyurum* C.Presl.) and gland clover (*Trifolium glanduliferum* Boiss.; Howieson *et al.* 2000; Loi *et al.* 2005; Nichols *et al.* 2007).

An accession of *Trigonella balansae* (referred to as trigonella; acc. SA 5045 or CPI 19633, origin – University of Uppsala, Sweden) has shown agronomic potential on fine-textured soils in the low- to medium-rainfall zone of southern Australia. If released, it will be the first commercial cultivar of this species developed for agriculture in Australia. *T. balansae* is an annual legume of Eurasian origin. It is a self-compatible species, which requires vectors such as honeybees to bring about pollination and exhibits a mixed mating system (Nair *et al.* 2004). As it has an upright growth habit, and sets pods at the top of the canopy so seed can be harvested with cereal harvesters. This enables low-cost seed establishment (Loi *et al.* 2012), with a downside risk of seed removal via grazing. Average seed yields of trigonella across three locations in South Australia were 429 kg/ha (Howie *et al.* 2001). In a sheep grazing experiment after seed set, 39% (257 kg/ha) of the seed reserve remained after 14 days of grazing, indicating that the species should tolerate moderate grazing pressure when senesced (Howie *et al.* 2001). De Koning *et al.* (2001) reported that sheep grazing trigonella ate 61% of the seeds and only 10.3 kg/ha were still viable in faeces. Trigonella forms root nodules with the native populations of *Rhizobium meliloti* that are associated with southern Australian medic pastures (Howie *et al.* 2001) and can therefore contribute to increasing soil nitrogen. The pattern of hard seed breakdown within and between seasons favours long-term persistence and the ability to be sown in summer (Harrison *et al.* 2021; Nutt *et al.* 2021).

Subclover pastures with a high content of phytoestrogenic compounds can be associated with impaired reproduction in sheep, including infertility, dystocia, and perinatal lamb losses (Bennetts *et al.* 1946; Reid 1981). From 1940 to 1990, it was estimated that phytoestrogens in subclover rendered millions of ewes infertile in Western Australia (Adams 1998). In preliminary laboratory screening, trigonella appeared to have low concentrations of phytoestrogens, but it is possible that the plant contains other secondary compounds that can affect livestock health and product quality. The genus *Trigonella* contains several species with bioactive compounds that are used to flavour food and are used in traditional medications. Fenugreek (*Trigonella foenum-graceum* L.) is reported to contain yamogenin, gitogenin, tigogenin, and trigoneoside, fatty acids, alkaloids (trigonelline and gentianin), flavonoids (vitexin, isovitexin, orientin, vicenin, quercetin, and luteolin), saponin, nicotinamide and choline (Agustini *et al.* 2015; Yusharyahya *et al.* 2020). The bioactive compounds in some *Trigonella* species may improve sheep health and productivity. Hassan (2014) found that adding 10–20 g/day of fenugreek seeds to the diet of lactating Aissi sheep led to significantly higher red blood cells, white blood cells and haemoglobin, than in animals on a control diet. There were no significant differences among treatments in blood and plasma indicators of health, including packed cell volume (PCV), mean corpuscular volume (MCV), aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine. They concluded that fenugreek seeds could be used as supplement to increase milk yield (Hassan 2014).

Introduction of new plant species to Australia may present risks to livestock, production systems and the natural environment. For livestock, the risks include poor nutritive value, leading to suboptimal growth rates, secondary plant compounds that may have a negative impact on the health, performance and product quality of livestock and physical structures such as spines or burrs that injure animals or contaminate wool (Norman *et al.* 2005). Addressing the risk of new introductions is difficult due to the large number of potential compounds and their possible interactions (Revell and Revell 2007). It is common practice in Australia to conduct a grazing study to compare the feeding value of novel species with widely adopted and commercially successful cultivars prior to commercial release (Norman *et al.* 2005, 2013; Masters *et al.* 2006).

While incredibly useful as a screening tool, laboratory measurement of digestibility, crude protein, fibre, and field measurement of pasture growth rates, do not allow the full value of forage to be assessed. This is because laboratory analyses do not allow for voluntary feed intake and the impact of secondary plant compounds to be considered. Feeding value is a function of voluntary feed intake and the nutritive value of a forage species. Voluntary feed intake can account for up to 50% of the variation in animal performance when grazing forage and is related to factors such as digesta load in the rumen (Weston 1996), clearance rate of digesta from the rumen and palatability (Provenza and Pfister 1991; de Vega and Poppi 1997). By comparing liveweight changes and wool growth of sheep grazing a novel plant species, with those of sheep grazing a well known species (when herbage availability is not limiting intake), it is possible to determine the relative feeding value of a new species compared with a known and 'safe' option. The grazing studies also allow for assessment of animal health and product quality.

Subclover is the most common leguminous pasture species in southern Australia (Fortune *et al.* 1995). This paper reports the findings of an experiment designed to assess the feeding value of a cultivar accession of trigonella (acc. SA 5045) and French serradella (cv. Erica) compared with a commercial cultivar of subclover (cv. Dalkeith). The hypothesis tested is that the sheep grazing the different legume species will have similar liveweight, condition scores, wool production, meat eating quality and indicators of muscle, liver and kidney health.

Materials and methods

The experiment was conducted with approval from the CSIRO Wildlife, Livestock and Laboratory Animal Ethics Committee (AEC# 2017-04), in compliance with the Australian Code of Practice for care and use of animals for scientific purposes (8th edition 2013).

Experimental plots

The experiment was conducted in 12 0.5-ha plots at on a private farm, near Spencer's Brook, in Western Australia (31.75°S, 116.68°E). The plot area was previously managed as a single paddock and had been used to grow cereal and canola crops. Prior to sowing the legumes in 2020, the plots were sprayed twice with non-selective herbicides. The plots were adjacent to one another on a slight slope and the area was blocked into four blocks of three adjacent plots. Before seeding, the plots were subject to a double herbicide knockdown and residual insect spray. On 15 May 2020, the plots were sprayed with 2.0 L/ha of Glyphosate 450 (Nufarm®), 1% sulfate of ammonia and 0.75% Uptake® (paraffinic oil, non – ionic surfactant) at a spray rate of 100 L/ha. On the 8 June 2020, they were sprayed with 2.0 L/ha of Spray Seed 250® (Syngenta) and Chlorpyrifos 500 EC (Nufarm®) at a mixed spray rate of 100 L/ha. On the 9 June 2020, four of the plots (randomly allocated within each block) were sown (4-mm depth) with scarified seed being either subclover at 11 kg/ha, trigonella at 7 kg/ha or serradella at 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), which was deep-banded at the time of sowing. Seed of subclover, trigonella and serradella were inoculated with rhizobial strains WSM1325, RRI128 and WSM471 respectively, 2 h before sowing. The plots were sprayed with Talstar® (bifenthrin 100 g/L) to control insects and mites at a rate of 0.3 L/ha on the 12 August 2020.

Rainfall during the growing season

Long-term average annual rainfall at the experimental site is 445 mm, with 350 mm falling during the growing season from May until the end of October (Fig. 1). In 2020, the site received 318 mm of rainfall through the year and 212 mm during the annual pasture-growing season. This represented only 60% of the long-term growing-season average and was one of the driest seasons on record.

Liveweight changes and wool growth

On the 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 the method of Suiter 1994) were selected from a large commercial flock. Six wethers were randomly allocated to each of the 12 plots, leading to a stocking density of 12 dry sheep equivalents (DSE)/ha. All animals were vaccinated with 1 mL of GlanVac 6® (Zoetis) and given an oral drench of Moximax Moxidectin® Dalgety) at a dose rate of 10 mL per animal the day before introduction (14 September 2020). At the commencement of grazing, the animals were randomly allocated as 'core' (three wethers per plot), 'spare' (one wether per plot) or 'meat quality' (two wethers per plot). All six animals per plot were utilised throughout the experiment to compare liveweight and condition score changes. Each weighing occurred at the same time of the day and within 30 min of removal from the plots. For the three core animals per plot, wool samples were collected from 100-cm^2 mid-side patches at the start (to clean the area to bare skin) and end of grazing (to compare wool growth during grazing). The wool was scoured prior to

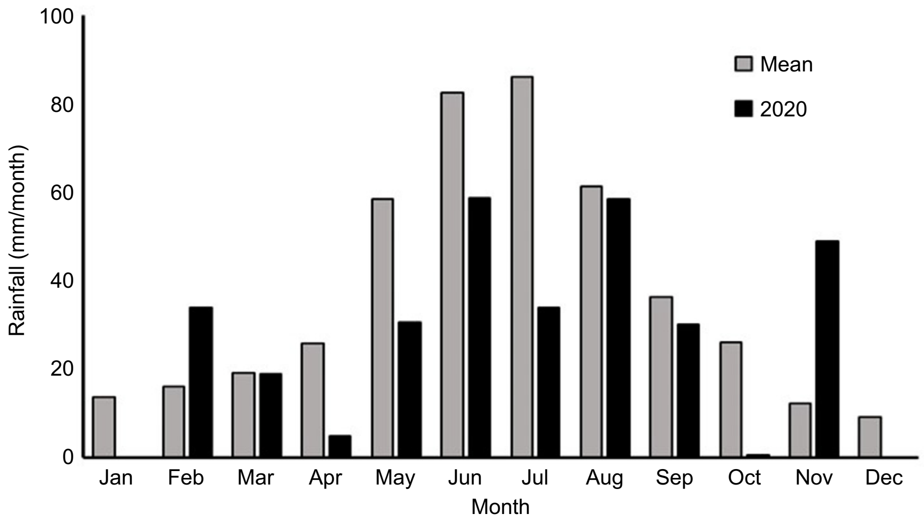

Fig. 1. Long-term average monthly rainfall at the experimental site and rainfall received in 2020.

weighing and clean wool growth was calculated as g per 100 cm^2 per day (Langlands and Wheeler 1968). The three core animals per plot were subject to blood sampling to assess health status on 29 October 2020 (Day 44). All animals remained on the plots until 4 November 2020, so that there was a total of 50 days of grazing.

Plot composition, herbage mass and quality

Herbage mass and *in vitro* nutritive value were determined on six occasions during the season (24 August, 14 September, 6 October, 19 October, 29 October and 4 November 2020), with pasture sampling corresponding to animal measurements. Herbage mass was estimated using visual estimation within quadrats, assessed every 5 m along three transects within each plot. Visual estimation was calibrated with 25 herbage mass cuts (cut to ground level with a knife). Herbage mass in plots was compared using ANOVA. At four measurement times, sward composition was estimated in each quadrat drop using the BOTANAL technique (Mannetje and Haydock 1963). Plant herbage samples from each legume species within each plot were collected through grab samples along three transects within plots at the same time as herbage mass was measured. Volunteer plant species were sampled and bulked to provide one representative sample of aboveground biomass per plant species per sampling time. The samples were dried at 65°C for 48 h and ground to pass through a 1-mm screen by using a Tecator Cyclone© mill.

The plant-quality analyses; *in vitro* (pepsin–cellulase) dry-matter digestibility (DMD), neutral detergent fibre (NDF), acid detergent fibre (ADF), crude protein (CP) and organic matter (OM) were estimated by near-infrared spectroscopy (NIRS) according to the methods described in Norman *et al.* (2020). Briefly, the methods used to build and test the NIRS calibrations included pepsin–cellulase *in vitro* DMD (in duplicate and calibrated with *in vivo* standards in each run). NDF and ADF were measured sequentially on the same samples by using an Ankom 200/220 Fibre Analyser in accordance with the operating instructions for this equipment (Ankom® Tech. Co. Fairport, NY, USA). Total nitrogen (N) was determined by combustion by using a Leco FP-428 N Analyser (Sweeney and Rexroad 1987) and CP was estimated by multiplying N by 6.25. Mineral analyses were conducted at a commercial analytical laboratory according to their standard methods (CSBP Soil and Plant Analysis Laboratory, Bibra Lake, WA, Australia). Seed samples were analysed for oil content by using a Soxtec 2050 extraction unit (Foss Tecator; Foss Pacific, Sydney, NSW, Australia). Samples for fatty acid composition were freeze-dried and ground using a Foss Knifetec grinder. Fatty acid composition was determined by direct synthesis of fatty acid methyl esters (O'Fallon *et al.* 2007) and measured on an Agilent 7890 gas chromatograph.

Animal health

Blood samples (10 mL) were taken from the jugular veins of three core animals per plot immediately prior to weighing on Day 44 of grazing. Half the blood was decanted into a heparinised tube, placed on ice and within an hour spun in a centrifuge to separate the plasma (2000*g* for 10 min). The remainder was placed in ice as whole blood. The samples were sent to Vetpath Laboratory Services (https://vetpath.com.au) for a panel analysis to identify any damage to muscle, liver or kidneys, measurement of minerals in the plasma and analysis of whole blood. The plasma panel analyses included creatinine kinase (CK), AST, gammaglutamyl transferase (GGT), glutamate dehydrogenase (GLDH), total bilirubin, urea, creatinine, B-hydroxybutyrate (BOHB), glucose and albumin. Mineral analyses included sodium, potassium, chloride, calcium, phosphorus and magnesium. Whole-blood assessment included haemoglobin, PCV, red blood cell count, mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), MCV, white blood cell count, neutrophils, lymphocytes, monocytes, eosinophils, basophils and fibrinogen.

For all animal-health analyses, plot means (of the three core animals per plot) were used to compare the impact of forage species by using ANOVA. Data were compared with reference data to identify abnormalities. Data from individual animals were also compared to reference values.

Meat sensory analysis

After 50 days of grazing, the two 'meat-quality' sheep per plot (n = 24) were transported to a commercial abattoir and placed in lairage with access to water for 12 h. The following day they were slaughtered (in a random order) according to normal commercial protocols (Food Security International, TA Corrigin Meatworks, Corrigin, Western Australia). Hot carcass weight was recorded 5 min post-slaughter. Fatness at the GR site (between 12th and 13th ribs) and ultimate muscle pH were recorded 24 h post-slaughter (using an Orion 250A pH meter, Cat. No. 0250A2, Orion Research Inc. Boston, MA, USA). The backstrap muscle (*m. longissimus thoracis et lumborum*) was carefully removed the following day, vacuum-sealed and aged at 4°C for 4 days before freezing at −20°C. Freezer temperature was monitored and did not deviate more than 2°C during storage. After thawing, samples of the backstrap muscle (*m. longissimus thoracis et lumborum*) were subsampled and analysed for total intramuscular fat content by using a Soxtec 2050 extraction unit (Foss Tecator; Foss Pacific, Sydney, NSW, Australia) and following Foss application note '3127: Extraction of Fat in Meat and Meat Products'. Fatty acid composition was determined by direct synthesis of fatty acid methyl esters (O'Fallon *et al.* 2007).

The meat sensory analysis occurred over two sessions on 14 October and 15 November 2021, after delays associated with COVID restrictions. The procedure was based on the methods described by Pearce *et al*. (2008) and Norman *et al*. (2005). Briefly, both backstrap samples from each of the slaughtered sheep were used for the sensory analysis (allocated as left and right samples). Meat was defrosted at 4°C 48 h prior to the experiment. From each of these samples six pieces of meat (2.0 cm × 4 cm) were cut from the 12th rib down the loin and labelled 1 to 6. Each animal was therefore represented by 12 pieces of meat. On each date, each of 24 panellists who regularly eat sheep meat was given six pieces of meat to assess the flavour, aroma, tenderness, juiciness, residual mouth feel and overall acceptability, one piece at a time, in six consecutive cooking runs. Thirteen panellists participated in both sessions. The allocation of meat to panellist was random with three exceptions. First, each panellist received two pieces of meat from sheep grazing different plots of each of the three legume species. Second, each panellist received one piece of meat from each treatment in a random order. The three treatments were then repeated in the same order using samples from different plots. It was attempted to ensure that all the possible treatment orders were equally represented. And finally, panellists who participated in both sessions did not receive more than one piece of meat that originated from the same animal or plot (so they did not sample the same animal twice or its paddock mate). All 12 cuts from 10 sheep were tasted on first date and all 12 cuts from another 10 sheep were tasted on the second date. For the remaining four sheep, all cuts from one side were tasted on one date and cuts from the remaining side were tasted on the other date. In each session, panellists all received a training sample from a commercial sample of the same cut of lamb, so they assessed seven samples in total. Meat was cooked in a commercial electric grill for 2 min until cooked to a medium level (Silex Grills Australia Pty Ltd, Sydney, NSW, Australia). Panellists were seated and presented with a series of pieces of meat on a bamboo plate. After sampling each piece, panellists were asked to 'cleanse their palate' with a small piece of brown bread and a sip of apple juice. Meat attributes were evaluated using a 10-point continuous scale (from 1, low values for a trait, to 10, high values for a trait). The tasting was conducted with CSIRO Human Ethics approval (2021-024-LR).

Statistical analyses

Power analysis of this design was conducted using measured variation from a similar small-plot experiment where two groups of four plots were compared, with four sheep per plot. (White *et al*. 1992). The results from the power analysis indicated that we could detect a 50 g/day difference in liveweight gain at the $P < 0.05$ level with a power of 80%.

An ANOVA (GenStat Ver. 21, Lawes Agricultural Trust, Rothamsted Experimental Station, UK) was performed to test the effect of pasture treatment on each productivity parameter. Variance among treatments was compared with the residual variance among pasture plots. Weight on the first day of the experiment was used as a covariate for all subsequent analyses of weights and condition score on the first day of the experiment as a covariate for all subsequent measures of fatness. Plasma and blood parameters and ultimate pH were analysed in the same way but with no covariates.

Each of the meat-tasting measurements, namely aroma, tenderness, juiciness, flavour and overall satisfaction, were analysed using a linear mixed mode in GenStat (Ver. 21). The fixed model included pasture species, meat cut within loin, pasture species × meat cut, pasture species of the preceding piece of meat and covariates, initial weight and initial condition score. The random model included terms for tasting date, panellist within tasting date, pasture plot, and sheep. The remaining variance among scores for each piece of meat is the residual variance.

- Fixed model: initial liveweight + initial condition score + ultimate pH + previous meat treatment + meat cut within loin + pasture species + meat cut within loin. pasture species
- Random model: Date of tasting. Panelist + pasture plot + Sheep identity + sheep identity. meat cut within loin. side of sheep

The order of terms in the fixed model ensured that effects of pasture species were adjusted for any effects of initial liveweight and condition score, ultimate pH, meat cut or tasting order.

An ANOVA (GenStat Ver. 21) was performed to test the effect of pasture treatment on all other parameters. Residual variance among pasture plots was compared with the variance among treatments. Plots were divided into four blocks (plots within blocks were adjacent and pasture species was allocated randomly within a block) for the plant productivity analyses. Residual plots were used to check the underlying assumptions for linear models.

GrazFeed modelling

The observed sheep growth rates were compared with growth rates that were predicted using the GrazFeed ruminant nutrition model (Freer *et al*. 1997). Sheep liveweight and age, pasture biomass (all green, no dead material), percentage legume in the sward and measured DMD of the legume species were used to generate the predictions.

Results

Liveweight and wool growth

Sheep in all plots gained weight from the start of grazing in mid-September through to early November (50 days in total) when plots were de-stocked due to biomass becoming restrictive. There were no significant differences in mean liveweight or condition score among plots of sheep before or during grazing the three legume species (Fig. 2). During the first 21 days of grazing, the sheep gained a mean of 210, 220 and 226 g/head.day (for subclover, serradella and trigonella respectively). During the next phase (Days 21–34), the sheep gained a mean of 178, 215 and 191 g/head.day (for subclover, serradella and trigonella respectively). During the final phase from Day 34 to Day 50, the sheep grazing serradella gained a mean of 14 g/head.day, while sheep grazing trigonella and subclover lost a mean of 25–33 g/head.day.

There were no significant differences in wool growth over the 50 days or clean wool yield, as measured by mid-side patches (Table 1) There were no significant differences in hot and cold carcass weights, carcass fat score, crude fat in the backstrap muscle and ultimate pH of meat from plots of sheep grazing three different pasture species over 50 days (Table 1). There were no significant differences in fatty acid composition of the backstrap muscle (data not presented).

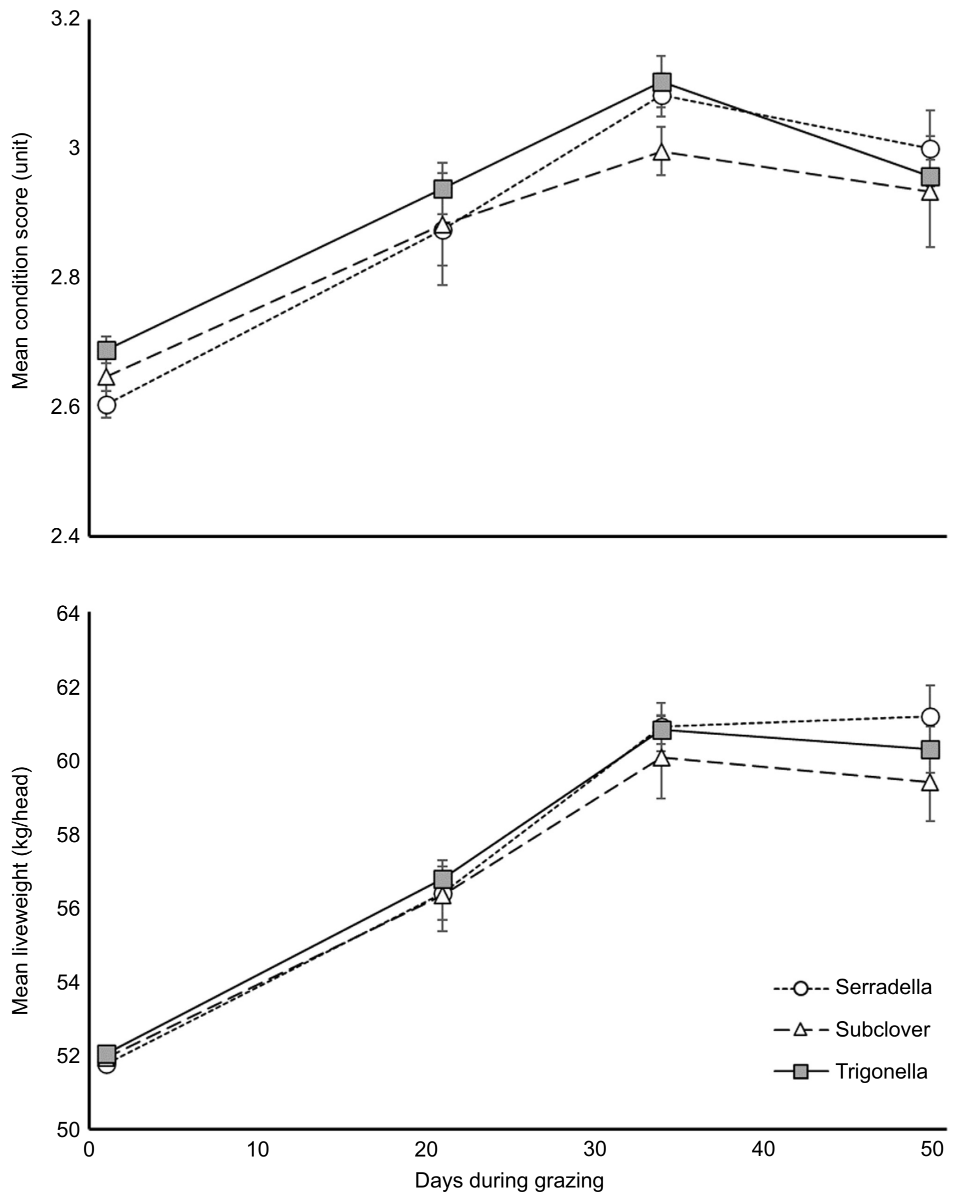

Fig. 2. Mean liveweight and condition scores of plots of sheep grazing subclover (Δ), trigonella (□) or serradella (○) during 50 days of grazing from mid-September to early November 2020. There were no significant differences associated with pasture species at any time point.

Table 1. Daily wool growth, wool yield, hot and cold carcass weights, carcass fat score, crude fat in the backstrap muscle and ultimate pH (of muscle) of wethers grazing three different pasture species.

Trait	Unit	Treatment mean			P-value
		Trigonella	Serradella	Subclover	
Hot carcass weight	kg	28.0	28.6	26.6	n.s.
Cold carcass weight	kg	27.3	27.5	26.1	n.s.
Ultimate pH	units	6.28	6.30	6.27	n.s.
Carcass fat score	units	2.49	2.38	2.13	n.s.
Crude fat in muscle	% DM	15.9	18.3	16.7	n.s.
Clean wool growth	g/100 cm^2.day	0.136	0.148	0.138	n.s.
Clean wool yield	%	66.5	70.6	69.8	n.s.

n.s, not significant ($P > 0.005$).

Animal health

Residual plots indicated that the underlying assumptions for linear mixed models are valid for all measurements. The results from liver, kidney and muscle panel analysis showed no significant differences in mean plasma CK, GGT, GLDH, total bilirubin, urea, BOHB, glucose and albumin of sheep grazing the three pasture species ($P > 0.05$, Table 2). Sheep grazing subclover plots had significantly lower mean plasma AST, than did sheep grazing serradella or trigonella ($P = 0.063$, Table 2). All AST means and all individual animals were within the reference range. Sheep grazing serradella had significantly lower plasma creatinine than did sheep grazing subclover ($P < 0.011$). Sheep grazing trigonella had creatinine similar to that of sheep grazing subclover. Means for all pasture treatments were within the reference values and no individual animal had plasma creatinine that was outside of the reference values (Table 2). For CK, 19% of individuals were outside the reference range but this was not associated with a particular pasture species (trigonella $n = 3$, serradella $n = 2$, subclover $n = 2$, Table 2). While there were no significant differences in treatment means in GLDH, more individuals grazing trigonella ($n = 5$) were outside the reference range than for the other two species ($n = 1$, Table 2). For plasma glucose, 66% of animals had higher concentrations than the reference range (2.78–4.44 mmol/L); however, this was not associated with a particular pasture species (trigonella $n = 8$, serradella $n = 6$, subclover $n = 8$, Table 2). For gammaglutamyl transferase (GGT), eight individuals had plasma concentrations outside the reference range, but this was not associated with a particular pasture species (trigonella $n = 3$, serradella $n = 3$, subclover $n = 2$, Table 2). For the minerals in plasma (Na, K, Cl, Ca, P and Mg), there were no significant differences associated with pasture species ($P > 0.05$, Table 2).

There were no significant differences between plot means of sheep grazing the pastures in the whole-blood parameters, including haemoglobin, PCV, red blood cell count, MCHC, MCH, MCV, white blood cell count, neutrophils, lymphocytes, monocytes, eosinophils, basophils and fibrinogen. Most animals had individual measurements within the reference range, and when there were individuals outside the values, they were not associated with a particular pasture species (Table 2).

Meat analysis

The results of sensory evaluation demonstrated no effects of pasture species on any of the meat-tasting measurements (Table 3). Initial liveweight was associated with meat aroma ($P = 0.054$). Previous treatment was significantly associated with scores for juiciness ($P = 0.012$) and overall satisfaction ($P = 0.010$) and there was a trend towards previous meat treatment being associated with tenderness ($P = 0.07$). Cut within the loin was associated with tenderness ($P = 0.060$). According to the ratings, meat samples from the sheep grazing all species of clover were acceptable to consumers.

Botanical composition, herbage mass and quality

There were no significant differences in mean feed on offer (kg DM/ha) among the pasture treatments at any stage during grazing ($P > 0.05$). There was a mean of 2.07 t DM/ha of biomass at the start of grazing in mid-September and 1.44 t DM/ha at the termination of grazing in early November (Fig. 3). Herbage mass availability was not likely to limit animal production during the experiment. The pasture species supported 12 sheep/ha, despite very low growing-season rainfall.

The plots were not monocultures of the species being compared. At the commencement of grazing, 38% of herbage in the trigonella treatments and 29% in the French serradella and subclover treatments were derived from species other than the legumes sown (Fig. 3). All of the pasture legume species were actively grazed (visual observations, data not presented). By November, 86% of the biomass in the trigonella plots was from volunteer species. In contrast, 58% of biomass in the serradella plots and 60% of biomass in the subclover plots

Table 2. Indicators of muscle, liver and kidney health through panel analysis of plasma samples and whole-blood measurements taken from sheep after 45 days of grazing the plots.

Trait	Unit	Reference range	Treatment mean			*P*-value	Individuals outside reference range		
			Trigonella	Serradella	Subclover		Trigonella	Serradella	Subclover
Creatinine kinase (CK)	U/L	<500	346	356	290	n.s.	3	2	2
Aspartate aminotransferase (AST)	U/L	60–280	147.0	132.8	106.4	0.063	0	0	0
Gammaglutamyl transferase (GGT)	U/L	23–67	60.3	56.2	59.4	n.s.	3	3	2
Glutamate dehydrogenase (GLDH)	U/L	<20	21.6	10.9	9.3	n.s.	5	1	0
Total bilirubin	µmol/L	<15	3.25	2.50	3.17	n.s.	0	0	0
Urea	mmol/L	3.3–12.0	6.27	7.34	6.14	n.s.	1	0	0
Creatinine	µmol/L	50–150	85.6	77.60	92.4	0.011	0	0	0
B-hydroxybutyrate (BOHB)			0.37	0.34	0.32	n.s.			
Glucose	mmol/L	2.78–4.44	5.00	4.70	4.60	n.s.	8	6	8
Albumin	g/L	29–40	35.58	35.17	34.58	n.s.	0	0	0
Sodium	mmol/L	139–152	148.7	148.30	147.1	n.s.	2	0	0
Potassium	mmol/L	3.9–5.4	4.66	4.68	4.48	n.s.	0	0	0
Chloride	mmol/L	101–105	110.0	10.60	108.2	n.s.	11	12	10
Calcium	mmol/L	2.88–3.20	2.65	2.64	2.60	n.s.	12	12	12
Phosphorus	mmol/L	1.62–2.36	1.61	1.53	1.85	n.s.	12	9	12
Magnesium	mmol/L	0.90–1.31	0.92	0.94	0.86	n.s.	6	6	9
Haemoglobin	g/L	90–150	115.2	115.70	113.7	n.s.	1	0	1
Packed cell volume	L/L	0.27–0.45	0.36	0.36	0.36	n.s.	1	0	0
Red blood cell count	x10^6 cells/µL	9–15	11.64	11.66	11.46	n.s.	1	0	1
Mean corpuscular haemoglobin (MCHC)	g/L	310–340	321.2	325.80	315.5	n.s.	4	2	6
Mean corpuscular haemoglobin (MCH)	pg	8–12	10.0	10.00	10.2	n.s.	0	0	0
Mean corpuscular volume (MCV)	fL	28–40	30.95	30.46	31.71	n.s.	0	0	1
White blood count	x10^3/µL	7.2–17.7	8.11	8.58	7.97	n.s.			
Neutrophils	%	10–50	26.2	35.20	27.7	n.s.	0	1	1
Lymphocytes	%	40–75	66.6	59.00	65.5	n.s.	3	2	2
Monocytes	%	0–6	2.00	1.58	1.83	n.s.	0	0	0
Eosinophils	%	0–10	1.47	1.31	1.51	n.s.	1	1	1
Basophils	%	0–3	0.50	0.50	0.25	n.s.	0	0	0

Reference range refers to the expected range for healthy animals (data supplied by Department of Agriculture and Food WA Animal Health Laboratory and Jones and Allison (2007).

n.s, not significant ($P > 0.005$), the l.s.d. (5%) value for creatinine was 8.5 µmol/L.

was from volunteer species. The largest contaminants of the plots were volunteer clovers (*Trifolium michelianeum* Savi., *T. tomemntosum* L. and *T. glomeratum* L.), oats (*Avena sativa* L.), annual ryegrass (*Lolium rigidum* Gaudin.), silver grass (*Vulpia myuros* L.), toadrush (*Juncus bufonius* L.), capeweed (*Arctotheca calendula* L.) and crassula (*Crassula decumbens* Thunb.; Fig. 3).

At the beginning of grazing, when the pasture legumes were in the vegetative phase, trigonella had a significantly higher DMD and a lower fibre content (NDF and ADF) than did the other legume species (Table 4). These differences remained significant throughout flowering until mid-October. Subclover had a higher DMD than serradella until the late reproductive phase of development. While serradella had a lower DMD during most of the experiment, the rate of decline during senescence was slower than for the other two species. By the termination of grazing in November, there were no significant differences in DMD, NDF or ADF among the species. The legumes did not differ significantly in CP content during the vegetative phase. Serradella had significantly higher CP than did the other species during flowering on the 6 and 19 October.

Table 3. Sensory evaluation of meat samples from sheep grazing paddocks sown to three pasture legume species. The terms in the fixed model are added sequentially to ensure that effects of pasture species were adjusted for any effects of initial liveweight and condition score, ultimate pH, meat cut or tasting order.

Sequentially adding terms	d.f.	Aroma	Tendernes	Juiciness	Flavour	Overall satisfaction
		F_{prob}	F_{prob}	F_{prob}	F_{prob}	F_{prob}
Initial weight	1	0.054	n.s.	n.s.	n.s.	n.s.
Initial condition	1	n.s.	n.s.	n.s.	n.s.	n.s.
Previous sample treatment	3	n.s.	0.07	0.012	n.s.	0.01
Cut within loin	5	n.s.	0.06	n.s.	n.s.	n.s.
Pasture species	2	n.s.	n.s.	n.s.	n.s.	n.s.
Cut within loin × pasture species	10	n.s.	n.s.	n.s.	n.s.	n.s.
Means of treatments						
Serradella		64.2	66.8	60.8	70.6	67.1
Subclover		64.7	57.1	57.0	67.9	63.1
Trigonella		64.9	55.9	58.1	68.5	61.7
Average SED		1.5	5.4	4.4	3.5	3.7

n.s, not significant ($P > 0.005$).

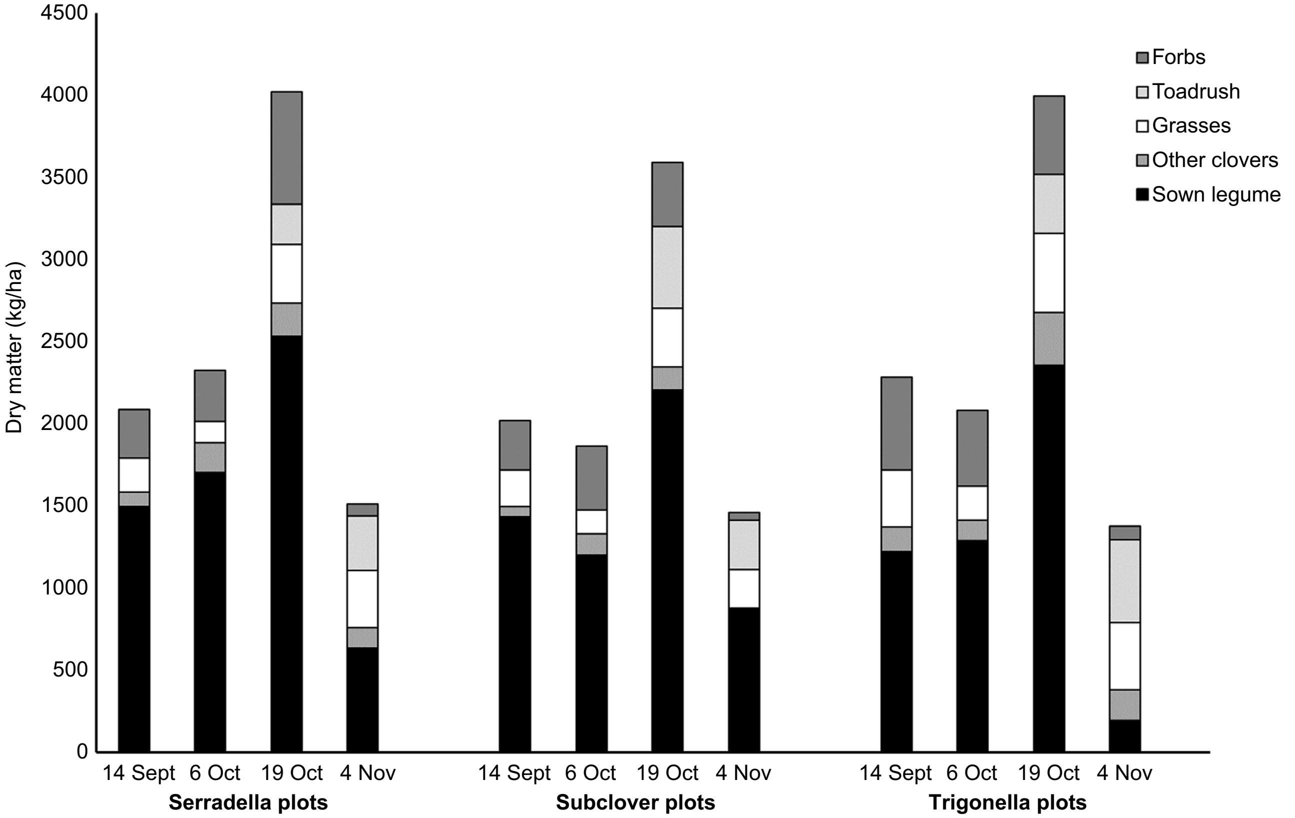

Fig. 3. Mean dry matter (kg/ha) and botanical composition of the pasture plots during grazing. Grasses include volunteer oats, ryegrass and silvergrass, forbs include capeweed and crassula, and other clovers include balansa and woolly clovers.

Capeweed maintained a high DMD throughout the experiment (Table 5). The grasses were characterised by rapid loss of DMD and CP and an increasing ADF during maturity. Towards the end of grazing, few volunteer species (apart from capeweed and volunteer clovers) had higher digestibility or CP than did the treatment legumes.

Sheep liveweight and age, pasture biomass (all green, no dead), percentage legumes in the sward and measured

Table 4. Mean nutritional value traits of three pasture legume species.

Trait	Day	Trigonella		Serradella		Subclover		*P*-value	l.s.d. (5%)
		Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.		
DMD (%)	24/08/2022	76.5	0.16	66.2	1.43	70.3	0.49	<0.001	2.97
	14/09/2020	74.8	0.23	68.6	0.60	71.6	1.02	0.002	2.26
	6/10/2020	71.4	0.58	63.9	0.35	66.7	1.14	0.003	3.16
	19/10/2020	62.8	1.26	58.9	0.48	59.3	1.36	0.044	3.13
	29/10/2020	50.0	0.77	52.6	0.93	55.2	0.74	0.014	2.99
	4/11/2020	49.6	2.83	50.1	0.26	47.3	0.82	n.s.	
CP (%DM)	24/08/2020	25.1	2.22	23.7	0.78	28.2	1.10	n.s.	
	14/09/2020	25.4	0.55	27.1	0.43	26.8	0.44	n.s.	
	6/10/2020	18.3	1.09	23.5	0.35	19.6	1.09	0.031	3.62
	19/10/2020	14.6	1.16	17.5	0.41	11.1	0.35	0.001	2.14
	29/10/2020	8.4	0.61	13.9	0.92	11.6	0.79	0.011	2.95
	4/11/2020	9.0	1.93	10.7	0.97	8.3	0.89	0.044	3.32
NDF (%DM)	24/08/2020	19.2	0.68	33.2	2.41	29.2	0.86	0.002	5.59
	14/09/2020	21.6	0.58	31.1	0.80	28.9	0.89	<0.001	2.45
	6/10/2020	25.4	0.57	32.5	0.39	31.7	1.07	0.002	2.89
	19/10/2020	40.4	2.17	40.5	0.49	46.2	1.86	0.054	
	29/10/2020	61.2	0.95	51.7	2.16	53.6	0.89	0.015	5.71
	4/11/2020	61.6	5.00	60.5	5.16	61.0	1.95	n.s.	
ADF (%DM)	24/08/2020	14.9	0.61	24.5	1.07	21.5	0.48	<0.001	2.50
	14/09/2020	16.2	0.34	22.4	0.43	20.2	0.32	<0.001	0.85
	6/10/2020	18.8	0.52	24.5	0.44	22.9	0.84	0.005	2.58
	19/10/2020	27.8	1.54	31.0	0.29	39.6	3.06	0.006	3.28
	29/10/2020	45.8	1.27	39.9	1.80	40.9	0.93	0.071	5.28
	4/11/2020	42.8	1.12	40.4	2.62	45.4	4.62	n.s.	

n.s, not significant ($P > 0.005$).

DMD of the legumes were used to generate predictions of sheep growth rates by using GrazFeed (Freer *et al.* 1997). These predictions were regressed against measured growth rates (Fig. 4). During the first 21 days of grazing the vegetative and flowering pastures, sheep grazing the serradella grew at a faster rate than predicted, on the basis of the nutritive value of the biomass (14 g/day better growth than predicted), while the sheep grazing subclover and trigonella grew more slowly than predicted (43 and 48 g/day less than the prediction respectively). During the second period of growth (from 21 to 34 days), the GrazFeed model predicted that sheep grazing the species should be growing from 175 to 273 g/day. The sheep grazing serradella again grew at a higher rate than predicted, with an additional 40 g/day. Sheep grazing subclover and trigonella grew less than predicted (−34 and −82 g/day respectively). In the final grazing period (Days 34–50), the model accurately predicted the moderate growth of sheep grazing serradella and liveweight loss of sheep grazing subclover. Performance of sheep grazing trigonella was not accurately predicted by the model (+10 g/day predicted and −25 g/day measured).

Table 6 presents the mineral composition of the pasture species at the vegetative and the reproductive stages of growth. All species had sufficient calcium, magnesium, potassium, sodium, cobalt, iron and zinc. Reproducing trigonella and subclover had significantly lower sulfur concentrations than did serradella ($P = 0.02$) and did not meet the minimum requirement for sheep (Freer *et al.* 2007). Trigonella in the reproductive stage of growth had significantly lower and inadequate phosphorus concentrations, when compared with subclover and serradella (Freer *et al.* 2007). While differences among species in selenium concentration were not significant, at both sampling times, the mean for trigonella was lower than the minimum requirement for sheep (Freer *et al.* 2007). Reproductive trigonella had a significantly lower manganese concentration than did the other species and it was lower than sheep requirements (Freer *et al.* 2007). Serradella and trigonella had significantly lower molybdenum than did subclover at the reproductive phase ($P = 0.024$), and the

Table 5. Dry-matter digestibility (DMD), crude protein (CP) and fibre content of the volunteer species growing in the pasture plots.

Day	Group	Species	DMD (%)	CP (%DM)	NDF (%DM)	ADF (%DM)
14/09/2020	Forb	Cape Weed	74.8	11.7	33.3	17.5
		Crassula	67.5	6.6	24.5	13.6
	Grass	Oats	64.7	9.0	45.5	25.5
		Ryegrass	69.8	9.3	40.8	22.9
	Legume	Other clovers	69.1	18.5	28.4	20.2
6/10/2020	Forb	Cape Weed	74.7	9.7	33.0	21.9
		Crassula	61.4	5.3	34.4	21.1
	Grass	Oats	55.6	7.7	54.4	34.3
		Ryegrass	61.2	6.9	51.1	29.0
		Silver grass	57.7	7.4	59.3	30.9
	Legume	Other clovers	70.3	18.7	26.8	19.4
19/10/2020	Forb	Cape Weed	73.5	16.1	37.1	23.7
		Crassula	59.2	4.6	45.0	27.0
	Grass	Oats	45.0	4.2	67.1	43.1
		Ryegrass	56.2	3.6	53.7	31.2
		Silver grass	57.0	8.8	60.4	28.9
	Legume	Other clovers	65.8	19.2	30.9	22.1
29/10/2020	Forb	Cape Weed	68.9	11.3	43.8	32.1
		Crassula	51.0	6.7	52.0	34.1
	Grass	Toad Rush	34.9	5.1	75.3	48.3
		Oats	46.1	4.2	66.0	38.9
		Ryegrass	47.9	3.8	68.7	40.3
		Silver grass	45.6	5.2	77.2	41.8
	Legume	Other clovers	56.1	10.2	53.5	39.5

concentrations were insufficient to meet sheep requirements (Freer *et al.* 2007). While all pasture met the zinc requirements for sheep, serradella maintained significantly higher concentrations than did trigonella or subclover. Species varied in copper concentrations at both sampling times; reproductive trigonella had copper concentrations below sheep requirements. All species had a K: (Na + Mg) ratio of <5, suggesting that major mineral imbalance was not a major issue.

Table 7 presents the isoflavone analysis of the legume species. Trigonella did not have any evidence of high concentrations of daidzein, genistein, formononetin or biochanin A. Serradella had some evidence of very low concentrations of formononetin. The Dalkeith subclover had 0.3–0.11 mg/g of daidzein, 1.25–5.21 mg/g of genistein, 0.08–2.34 mg/g of formononetin and 0.12–9.32 mg/g of biochanin A.

Discussion

Sheep grazing the trigonella (acc. SA 5045) and French serradella (cv. Erica) had mean liveweight, condition, wool growth and wool yield similar to sheep grazing subclover (cv. Dalkeith) over 50 days of grazing. The three legumes therefore had a similar relative feeding value during this comparison period, supporting our hypothesis. Pasture species were not significantly associated with differences in hot carcass weight, cold carcass weight, carcass fat score, crude fat in muscle, ultimate pH of muscle and fatty acid composition of the muscle.

Trigonella had significantly higher *in vitro* energy value (metabolisable energy (ME) 11.3 MJ/kg DM) than did subclover (ME 10.4 MJ/kg DM) and serradella (ME 9.7 MJ/kg DM) during the vegetative phase at the start of grazing, and for the majority of the plant's lifecycle. Digestibility of the trigonella declined much more rapidly than did that of the serradella at the end of October as the plants started podding. There was virtually no rainfall in October; it is likely that the deep root system of serradella and indeterminate growth habit allowed it to better access moisture and enabled it to remain green later in the season (as described by Hackney *et al.* (2013)). Serradella had a significantly higher CP content than did the other species from the second month of spring. By the end of October, the CP content of trigonella was at the maintenance requirement for mature animals and could have limited growth. The

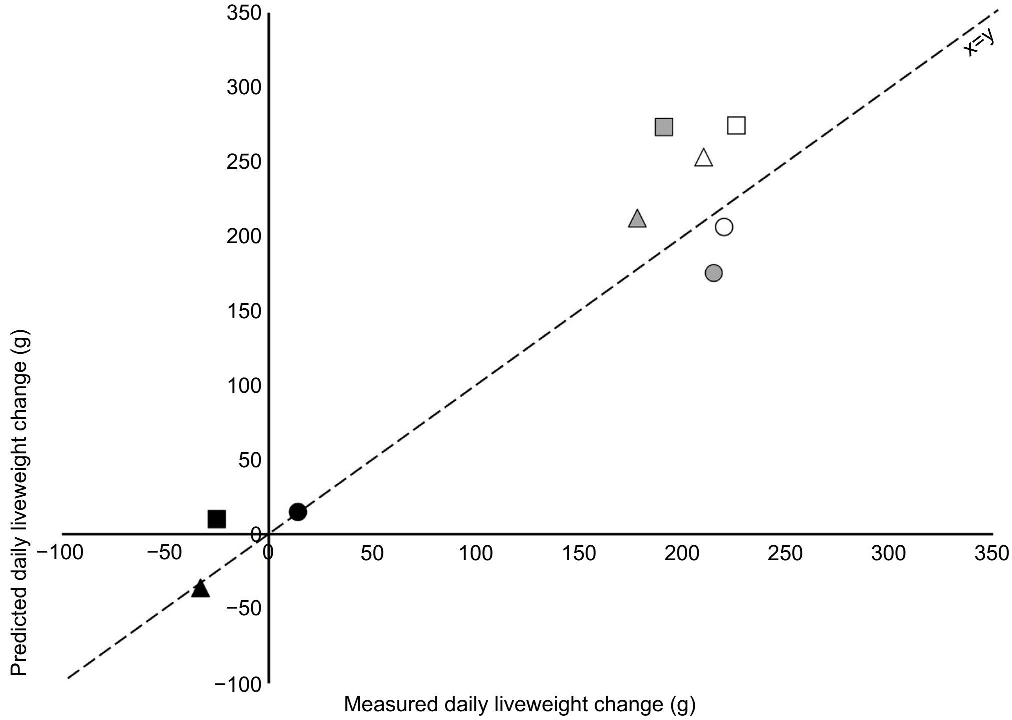

Fig. 4. Actual vs predicted liveweight for wethers grazing subclover (triangles), trigonella (squares) or serradella (circles) across three measurement periods. Growth rates are shown for Days 1–21 (open symbols), Days 21–34 (grey symbols) and Days 34–50 (black symbols). Predicted data were generated using the GrazFeed model and measured biomass, legume composition and dry-matter digestibility values.

measurement of higher nutritional value of subclover, when compared with serradella, contrasted with *in vitro* data from New South Wales suggesting that they have similar digestibility (Hackney *et al.* 2013). Both studies found no differences in sheep growth rates.

As trigonella matured, it did not meet the minimum requirements for mature sheep for sulfur, phosphorus, manganese, molybdenum and copper (Freer *et al.* 1997). The impact of these deficiencies is likely to be greater for rapidly growing animals. A reduction in feed intake is the primary impact of phosphorus deficiency and this leads to lower growth rates (Freer *et al.* 2007). Copper needs are hard to quantify accurately due to interactions with other minerals; however, wool contains 4 mg/kg of copper, so merino sheep are thought to have higher daily requirements than are other sheep and cattle (Underwood and Suttle 1999; Freer *et al.* 2007). Manganese is a component responsible for activation of numerous enzymes, and its deficiency leads to skeletal abnormalities and reduced reproductive performance (Underwood and Suttle 1999; Freer *et al.* 2007). Despite the suboptimal concentrations of critical minerals in mature trigonella, we found very little evidence that this compromised health or reduced productivity. This may be because sheep were eating volunteer plants to meet needs, or deficiency was masked by a concomitant lack of protein and energy (Masters *et al.* 2019). The mineral content of the plants is influenced by tolerance to the soil conditions that affect root growth, nutrient acquisition and rhizobial survival and productivity. A more thorough comparison of mineral content is required across target soils before drawing further conclusions and consideration of supplementation strategies.

The GrazFeed modelling tool, informed by biomass availability and DMD, consistently over-predicted growth of sheep grazing trigonella (ranging from 32 g/day to 82 g/day). The model under-predicted growth of the sheep grazing serradella, and over-predicted growth of sheep grazing subclover, during the vegetative and reproductive phases. It was accurate for serradella and subclover during the final grazing phase. It is possible that sheep offered trigonella during the early grazing were unfamiliar with the plant and took time to reach optimal intake levels. Given the rapid decline in trigonella content in the swards over time, and the visual evidence of heavily grazed plants, they were actively eating it during the latter stages of grazing. The measured underperformance of sheep grazing trigonella could be associated with difficulty in pre-hending biomass due to the reduced density in the sward, consumption of less nutritious volunteer species, a nutritional constraint or possibly lower than assumed intake levels. Sheep grazing serradella could have achieved higher growth rates by selecting more nutritious parts of the plants, more nutritious volunteer plants or eating more than predicted to achieve the higher growth

Table 6. Mineral composition of dry matter from the pasture legume species at the vegetative and reproductive stages of growth.

Mineral	Unit	Reference range[A]	Date	Trigonella	Serradella	Subclover	Mean	*P*-value	l.s.d. (5%)
Ca	%	0.14–0.7	24/08/2020	1.12	1.32	1.39	1.28	0.063	
			29/10/2020	0.79	0.88	1.15	0.94	0.001	0.128
Mg	%	0.09–0.12	24/08/2020	0.18	0.20	0.21	0.20	n.s.	
			29/10/2020	0.23	0.31	0.19	0.24	0.064	
P	%	0.09–0.3	24/08/2020	0.27	0.30	0.29	0.29	n.s.	
			29/10/2020	0.06	0.17	0.13	0.12	0.002	0.039
K	%	0.5–3.0	24/08/2020	2.16	1.99	2.34	2.16	n.s.	
			29/10/2020	0.66	1.52	1.01	1.06	0.011	0.461
Na	%	0.07–0.1	24/08/2020	0.33	0.35	0.37	0.35	n.s.	
			29/10/2020	0.55	0.73	0.58	0.62	n.s.	
S	%	0.20	24/08/2020	0.29	0.23	0.26	0.26	n.s.	
			29/10/2020	0.10	0.32	0.15	0.19	0.020	0.085
Cu	mg/kg	4–14	24/08/2020	9	23	14	16	0.093	
			29/10/2020	3	5	13	7	0.016	5.9
Fe	mg/kg	40	24/08/2020	324	458	393	392	n.s.	
			29/10/2020	122	527	777	476	n.s.	
Mn	mg/kg	20–25	24/08/2020	31	59	39	43	n.s.	
			29/10/2020	16	53	79	49	0.004	27.3
Zn	mg/kg	9–20	24/08/2020	18	30	19	22	0.019	7.8
			29/10/2020	10	19	14	15	0.056	
Co	ug/kg	80–150	24/08/2020	223	466	268	319	n.s.	
			29/10/2020	241	215	408	288	n.s.	
Mo	ug/kg	5000–10 000	24/08/2020	3216	17 717	4243	8392	n.s.	
			29/10/2020	581	2503	8681	3922	0.024	5362.0
Se	ug/kg	50	24/08/2020	41	142	59	81	n.s.	
			29/10/2020	27	187	197	137	n.s.	

[A]Reference ranges from Freer *et al.* (2007).
n.s, not significant ($P > 0.005$).

rates. The differences between measured and modelled outcomes highlights the importance of paddock-scale comparative feeding experiments when comparing pasture species. Voluntary feed intake can account for up to 50% of the variation in animal performance when grazing forage and is related to factors such as digesta load in the rumen (Weston 1996), clearance rate of digesta from the rumen and palatability (Provenza and Pfister 1991).

Grazing pasture can lead to differences in flavour and odour of sheepmeat (see review by Schreurs *et al.* (2008)). Meat from sheep grazing trigonella or serradella did not differ in ultimate pH, tenderness, juiciness, flavour or overall acceptability to consumers, compared with meat from sheep grazing subclover. While meat from sheep fed legume-based forage has been reported to have a more intense flavour and odour than sheep fed grass (Cramer *et al.* 1967; Czochanska *et al.* 1970), there is little evidence, and no evidence from this study, that legume species differ in their effect on meat flavour.

There were very few differences in animal health, as extrapolated by muscle, kidney and liver panel analysis of plasma samples, among sheep grazing the pasture legume species. Although some individuals fell outside the standard reference ranges, there were no consistent or significant negative consequences associated with grazing trigonella. Sheep grazing subclover had significantly higher plasma creatinine, an indicator of kidney function, than did sheep grazing serradella. Creatinine treatment means and values for individuals were all within the 'healthy' reference values. Sheep grazing subclover plots had significantly lower mean plasma AST, an indicator of liver and/or muscle injury, than did sheep grazing serradella or trigonella. AST observations of individual animals were within the 'healthy' reference range. While there were no significant differences in treatment means for GLDH, an indicator of liver health, 42% (or 5 of 12) individuals grazing trigonella were outside the reference range we used (<20 U/L, Department of Agriculture and

Table 7. Isoflavone concentration of the pasture legumes at Mokine during 2020.

Species	Date	Daidzein (mg/g)		Genistein (mg/g)		Formononetin (mg/g)		Biochanin A (mg/g)	
		Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.
Trigonella	14/09/2020	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000
	6/10/2020	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000
	19/10/2020	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000
	29/10/2020	0.02	0.015	0.01	0.010	0.05	0.049	0.00	0.000
	4/11/2020	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000
Serradella	14/09/2020	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000
	6/10/2020	0.05	0.028	0.05	0.027	0.17	0.098	0.03	0.016
	19/10/2020	0.00	0.000	0.01	0.010	0.02	0.019	0.00	0.003
	29/10/2020	0.00	0.000	0.01	0.008	0.01	0.013	0.00	0.002
	4/11/2020	0.00	0.003	0.03	0.013	0.03	0.012	0.01	0.003
Subclover	14/09/2020	0.03	0.023	4.68	0.163	2.34	0.060	9.32	0.864
	6/10/2020	0.11	0.032	5.21	0.609	1.42	0.204	6.37	0.405
	19/10/2020	0.04	0.021	1.87	1.061	0.76	0.482	0.97	0.593
	29/10/2020	0.11	0.029	1.63	0.306	0.41	0.120	0.57	0.109
	4/11/2020	0.08	0.014	1.25	0.293	0.08	0.009	0.12	0.016

Comparison samples of known oestrogenic clovers (grown at another location) were tested for reference.

Food, WA Animal Health Laboratory), compared with the other two species (with 8%). Frye *et al.* (2022) were unable to establish reference intervals for GLDH in sheep in the USA, but found that 95% of GLDH results fell below 60 U/L and the mean of 119 sheep was 20.7 U/L. If other indicators of liver damage, AST, GGT and total bilirubin, are considered, two individuals (both grazing trigonella) had more than one plasma indicator that was outside the 'healthy' reference range. For plasma glucose, 66% of animals had higher concentrations than the 'healthy' reference range (2.78–4.44 mmol/L); however, this was not associated with a particular pasture species. There were no significant differences in minerals in plasma or haematology associated with the pasture treatments.

The main active oestrogenic compounds in subclover are genistein and biochanin A, and a gut microflora-metabolised compound that is derived from formononetin (Adams 1998). There was no evidence that trigonella or serradella accumulated significant amounts of phytoestrogens. Interestingly, the cv. Dalkeith subclover had isoflavone concentrations that differed from those typically reported for the cultivar. Oestrogen concentrations in subclover can be affected by growing conditions, including moisture stress (Reed 2016); an advantage of the serradella and trigonella is that this is not a concern during dry seasons. We measured 0.7% biochanin A in dry matter, whereas typical values have been reported to be 0.1% (Nichols *et al.* 2013). We also measured 0.18% formononetin, but it is generally reported as 'trace' (Nichols *et al.* 2013). There did not appear to be background subclover contamination prior to sowing and this was reinforced by lack of subclover volunteers in the seradella and trigonella paddocks. The isoflavone data suggested that the subclover that was sown was not purely the Dalkeith cultivar.

While we found little evidence of trigonella affecting 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 diet from a range of species. By the end of grazing, sheep had eaten most of the trigonella in the plots and it comprised only 18% of the total biomass. This suggests high relative palatability when compared with the volunteer species. Approximately 67% of the biomass in the trigonella plots was from grasses with submaintenance levels of energy and CP; thus, the feeding value of trigonella could have been underestimated as sheep were unlikely to have consumed just trigonella. The subclover and serradella plots had higher residual quantities of legume biomass at the end of the experiment (60% and 42% respectively).

Domesticating new forage species for commercial agriculture carries greater inherent risks than does 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 the growing environment, and 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 indicated that sheep grazing trigonella (acc. SA 5045) and French serradella (cv. Erica), were as productive, healthy as, and had meat quality similar to sheep grazing subclover (cv. Dalkeith). Sheep grazing French serradella grew at a faster rate than was predicted using measured energy and CP values of the sward. It is unclear whether the deviation was due to problems with the laboratory pepsin cellulase method of predicting energy, selectivity for high nutritional-value components or nutritional traits within serradella that allowed a greater rate of digestion and, therefore, higher intake levels. Both trigonella and French serradella are aerial seeders, so heavy grazing in late spring can affect the seedbank and future regeneration. Both species have been selected to be easily harvestable by using conventional cereal harvesting equipment, therefore, allowing producers to graze stands heavily and resow at a relatively low cost when compared to subclover.

References

Adams NR (1998) Clover phyto-oestrogens in sheep in Western Australia. *Pure and Applied Chemistry* **70**(9), 1855–1862. doi:10.1351/pac199870091855

Agustini K, Sriningsih S, Effe J (2015) Acute toxicity study of ethanolic extract of fenugreek seeds (*Trigonella foenum-graecum* L.) on white rats. *Journal of Indonesian Medicinal Plants* **8**, 9–13.

Bennetts HW, Underwood EJ, Shier FL (1946) A specific breeding problem of sheep on subterranean clover pastures in Western Australia. *Australian Veterinary Journal* **22**, 2–12. doi:10.1111/j.1751-0813.1946.tb15473.x

Cramer DA, Barton RA, Shorland FB, Czochanska Z (1967) A comparison of the effects of white clover (*Trifolium repens*) and of perennial ryegrass (*Lolium perenne*) on fat composition and flavour of lamb. *The Journal of Agricultural Science* **69**, 367–373. doi:10.1017/S0021859600019031

Czochanska Z, Shorland FB, Barton RA, Rae AL (1970) A note on the effect of the length of the resting period before slaughter on the intensity of flavour and odour of lamb. *New Zealand Journal of Agricultural Research* **13**, 662–663. doi:10.1080/00288233.1970.10421612

de Vega A, Poppi DP (1997) Extent of digestion and rumen condition as factors affecting passage of liquid and digesta particles in sheep. *The Journal of Agricultural Science* **128**, 207–215. doi:10.1017/S0021859696004078

de Koning CT, Beale PE, Duncan AJ, Hughes S, Haskard K (2001) A technique for assessing seed survival of new pasture legumes following grazing by sheep. In 'Proceedings of the XIX international grasslands congress', San Paulo Brazil, 11–21 February 2021. Published Online. (Fundacao de Estudos Agrarios Luiz de Queiroz)

Fortune JA, Cocks PS, Macfarlane CK, Smith FP (1995) Distribution and abundance of annual legume seeds in the wheatbelt of Western Australia. *Australian Journal of Experimental Agriculture* **35**, 189–197. doi:10.1071/EA9950189

Freer M, Moore AD, Donnelly JR (1997) GRAZPLAN: Decision support systems for Australian grazing enterprises: II. The animal biology model for feed intake, production and reproduction and the Grazfeed DSS. *Agricultural Systems* **54**, 77–126. doi:10.1016/S0308-521X(96)00045-5

Freer M, Dove H, Nolan JV (2007) 'Nutrient requirements of domesticated ruminants.' (CSIRO Publishing: Melbourne)

Frye EA, Behling-Kelly EL, Lejuene M, Webb JL (2022) Complete blood count and biochemistry reference intervals for healthy adult sheep in the northeastern United States. *Veterinary Clinical Pathology* **51**, 119–125. doi:10.1111/vcp.13059

Hackney B, Rodham C, Piltz J (2013) 'Using French serradella to increase crop and livestock production.' (Meat & Livestock Australia: Sydney, NSW, Australia) Available at http://www.mla.com.au/News-and-resources/Publication-details?pubid=6124 [Verified 2 September 2020]

Harrison RJ, Howieson JG, Yates RJ, Nutt BJ (2021) Long-term storage of forage legumes greatly alters the hard seed breakdown pattern *in situ*. *Grass and Forage Science* **76**, 72–81. doi:10.1111/gfs.12490

Hassan WM (2014) Influence of fenugreek seeds (*Trigonella foenum graecum*) on blood parameters, kidney, liver and mammary gland function for parturited Aissi ewes. *Al-Qadisiya Journal for Agricultural Sciences* **4**(1), 1–12. doi:10.33794/qjas.2014.93953

Howie JH, Ballard RA, de Koning C, Sandral G, Charman N (2001). *Trigonella balansae* – a new pasture legume for the alkaline soils of southern Australia? In 'Proceedings of the 10th Australian agronomy conference', Hobart. (Australian Society of Agronomy). Published online. Available at https://scholar.google.com/scholar?q=Trigonella%20balansaea%20new%20pasture%20legume%20for%20the%20alkaline%20soils%20of%20southern%20Australia

Howieson JG, O'Hara GW, Carr SJ (2000) Changing roles for legumes in Mediterranean agriculture: developments from an Australian perspective. *Field Crops Research* **65**, 107–122. doi:10.1016/S0378-4290(99)00081-7

Jones ML, Allison RW (2007) Evaluation of the ruminant complete blood cell count. *Veterinary Clinics of North America: Food Animal Practice* **23**, 377–402. doi:10.1016/j.cvfa.2007.07.002

Langlands JP, Wheeler JL (1968) The dyebanding and tattooed patch procedures for estimating wool production and obtaining samples for the measurement of fibre diameter. *Australian Journal of Experimental Agriculture and Animal Husbandry* **8**, 265–269. doi:10.1071/EA9680265

Loi A, Howieson JG, Nutt BJ, Carr SJ (2005) A second generation of annual pasture legumes and their potential for inclusion in Mediterranean-type farming systems. *Australian Journal of Experimental Agriculture* **45**, 289–299. doi:10.1071/EA03134

Loi A, Nutt BJ, Howieson JG, Yates RJ, Norman HC (2012) Preliminary assessment of bladder clover (*Trifolium spumosum* L.) as an annual legume for ley farming systems in southern Australia. *Crop & Pasture Science* **63**, 582–591. doi:10.1071/CP11337

Mannetje L, Haydock KP (1963) The dry-weight-rank method for the botanical analysis of pasture. *Grass and Forage Science* **18**, 268–275. doi:10.1111/j.1365-2494.1963.tb00362.x

Masters DG, Mata G, Revell CK, Davidson RH, Norman HC, Nutt BJ, Solah V (2006) Effects of Prima gland clover (*Trifolium glanduliferum* Boiss cv. Prima) consumption on sheep production and meat quality. *Australian Journal of Experimental Agriculture* **46**, 291–297. doi:10.1071/EA05036

Masters DG, Norman HC, Thomas DT (2019) Minerals in pastures – are we meeting the needs of livestock? *Crop & Pasture Science* **70**, 1184–1195. doi:10.1071/CP18546

Nair RM, Dundas IS, Wallwork M, Verlin DC, Waterhouse L, Dowling K (2004) Breeding System in a population of *Trigonella balansae* (Leguminosae). *Annals of Botany* **94**, 883–888. doi:10.1093/aob/mch216

Nichols PGH, Loi A, Nutt BJ, Evans PM, Craig AD, Pengelly BC, Dear BS, Lloyd DL, Revell CK, Nair RM, Ewing MA, Howieson JG, Auricht GA, Howie JH, Sandral GA, Carr SJ, de Koning CT, Hackney BF, Crocker GJ, Snowball R, Hughes SJ, Hall EJ, Foster KJ, Skinner PW, Barbetti MJ, You MP (2007) New annual and short-lived perennial pasture legumes for Australian agriculture – 15 years of revolution. *Field Crops Research* **104**, 10–23. doi:10.1016/j.fcr.2007.03.016

Nichols PGH, Foster KJ, Piano E, Pecetti L, Kaur P, Ghamkhar K, Collins WJ (2013) Genetic improvement of subterranean clover (*Trifolium subterraneum* L.). 1. Germplasm, traits and future prospects. *Crop & Pasture Science* **64**, 312–346. doi:10.1071/CP13118

Norman HC, Masters DG, Rintoul AJ, Wilmot MG, Jayasena V, Loi A, Revell CK (2005) The relative feeding value of a new pasture legume, eastern star clover (*Trifolium dasyurum*), compared with subterranean clover (*Trifolium subterraneum*). *Australian Journal of Agricultural Research* **56**, 637–644. doi:10.1071/AR04271

Norman HC, Loi A, Wilmot MG, Rintoul AJ, Nutt BJ, Revell CK (2013) Sheep grazing bladder clover (*Trifolium spumosum* L.) had similar productivity and meat quality to sheep grazing subterranean clover (*Trifolium subterraneum* L.). *Animal Production Science* **53**, 209–216. doi:10.1071/AN12185

Norman HC, Hulm E, Humphries AW, Hughes SJ, Vercoe PE (2020) Broad near-infrared spectroscopy calibrations can predict the nutritional value of >100 forage species within the Australian feedbase. *Animal Production Science* **60**, 1111–1122. doi:10.1071/AN19310

Nutt BJ, Loi A, Hackney B, Yates RJ, D'Antuono M, Harrison RJ, Howieson JG (2021) 'Summer sowing': a successful innovation to increase the adoption of key species of annual forage legumes for agriculture in Mediterranean and Temperate environments. *Grass and Forage Science* **76**, 93–104. doi:10.1111/gfs.12516

O'Fallon JV, Busboom JR, Nelson ML, Gaskins CT (2007) A direct method for fatty acid methyl ester synthesis: application to wet meat tissues, oils, and feedstuffs. *Journal of Animal Science* **85**, 1511–1521. doi:10.2527/jas.2006-491

Pearce KL, Norman HC, Wilmot M, Rintoul A, Pethick DW, Masters DG (2008) The effect of grazing saltbush with a barley supplement on the carcass and eating quality of sheepmeat. *Meat Science* **79**, 344–354. doi:10.1016/j.meatsci.2007.10.014

Provenza FD, Pfister JA (1991) Influence of plant toxins on food ingestion by herbivores. In 'Recent advances on the nutrition of herbivores'. (Eds YW Ho, HK Wong, N Abdullah, ZA Tajuddin) pp. 199–206. (Malaysian Society of Animal Production: Kuala Lumpur, Malaysia)

Reed KFM (2016) Fertility of herbivores consuming phytoestrogen-containing *Medicago* and *Trifolium* species. *Agriculture* **6**, 35. doi:10.3390/agriculture6030035

Reid RL (1981) Livestock disease. In 'A manual of Australian agriculture'. 4th edn. (Ed. RL Reid) pp. 489–525. (The Australian Institute of Agricultural Science, William Heinemann: Australia)

Revell C, Revell D (2007) Meeting 'duty of care' obligations when developing new pasture species. *Field Crops Research* **104**, 95–102. doi:10.1016/j.fcr.2007.03.017

Schreurs NM, Lane GA, Tavendale MH, Barry TN, McNabb WC (2008) Pastoral flavour in meat products from ruminants fed fresh forages and its amelioration by forage condensed tannins. *Animal Feed Science and Technology* **146**, 193–221. doi:10.1016/j.anifeedsci.2008.03.002

Suiter J (1994) 'Body condition scoring of sheep and goats.' (Farmnote, Department of Agriculture: Perth, WA, Australia)

Sweeney RA, Rexroad PR (1987) Comparison of LECO FP-228 'Nitrogen Determinator' with AOAC copper catalyst Kjeldahl method for crude protein. *Journal of Association of Official Analytical Chemists* **70**, 1028–1030. doi:10.1093/jaoac/70.6.1028

Underwood EJ, Suttle NF (1999) 'The mineral nutrition of livestock.' (3rd edn). (CAB International Publishing: Wallingford, UK)

Weston RH (1996) Some aspects of constraint to forage consumption by ruminants. *Australian Journal of Agricultural Research* **47**, 175–197. doi:10.1071/AR9960175

White CL, Masters DG, Peter DW, Purser DB, Roe SP, Barnes MJ (1992) A multi element supplement for grazing sheep. I. Intake, mineral status and production responses. *Australian Journal of Agricultural Research* **43**, 795–808. doi:10.1071/AR9920795

Yusharyahya SN, Bramono K, Hestiantoro A, Edwar SQ, Kusuma I (2020) Fenugreek (*Trigonella foenum-graceum*) increases postmenopausal fibroblast-associated COL1A1 and COL3A1 production dominantly through its binding to estrogen receptor beta. *Journal of Applied Pharmaceutical Science* **10**, 22–27. doi:10.7324/JAPS.2020.104004

Data availability. The data that support this study cannot be publicly shared due to ethical or privacy reasons and may be shared upon reasonable request to the corresponding author if appropriate.

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