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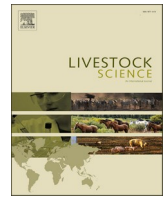
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Grazing lambs on a low-input, multispecies pasture for an extended period has no detrimental effect on meat nutritional or sensory quality

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HIGHLIGHTS

- Lambs grazing BD had lower daily live weight gain than those grazing PRG.
- Meat from BD lambs had higher 18:2 n-6 content than that of PRG lambs.
- Pasture type (PRG or BD) did not affect other fatty acids or TBARS content of lamb meat.
- Pasture type (PRG or BD) did not affect sensory properties of lamb meat.

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ABSTRACT

There is an increasing interest in low-input, multispecies swards as a sustainable forage for ruminant production, but the impact of grazing these over extended periods of time on meat nutritional quality (fatty acid (FA) profile, trace minerals and oxidisability risk) and sensory properties is unclear. Thirty lambs were grazed for 133 days on either a perennial ryegrass (PRG) or botanically diverse (containing twelve plant species, BD), pasture. The effects on the FA composition of *musculus longissimus thoracis* (lean and subcutaneous fat), zinc and iron content (lean), glutathione peroxidase (GSH-Px) activity (lean and plasma) and thiobarbituric acid reactive substances (TBARS) content (lean) were determined, and cooked meat was assessed for sensory properties using a trained sensory panel. BD lambs were lighter ($P = 0.014$) and had a lower weekly live weight gain ($P = 0.017$) than PRG, which was probably due to the nutritional quality of the pasture. BD pasture increased ($P < 0.05$) 18:2 n-6 content in lean and subcutaneous fat, and there was no effect ($P > 0.05$) on other polyunsaturated FA (PUFA), GSH-Px activity or TBARS content. BD pasture increased ($P = 0.038$) lean tissue zinc content compared with PRG but did not affect iron ($P > 0.05$). Pasture type had no impact ($P > 0.05$) on meat aroma and flavour sensory properties. It was concluded that grazing BD for an extended period did not negatively affect the nutritional and eating quality of lamb meat compared with PRG, and as such is suitable for finishing lambs. In order to benefit economically from including a BD pasture in a lamb finishing system, producers may need to consider an earlier slaughter target weight, depending on live weight gain.

1. Introduction

Within temperate regions the use of monoculture perennial ryegrass (PRG; *Lolium perenne*) for lowland sheep production systems is widespread. However, to maintain high yields PRG requires a high input of inorganic nitrogen, which can result in nitrous oxide losses (Harty et al., 2016), contributing to greenhouse gas emissions. In addition, PRG (which is shallow-rooted) grown as a monoculture is less drought tolerant when compared with other pasture species and multi-species

mixes (Hofer et al., 2016), which may have implications for pasture resilience as global temperatures increase. Therefore, there is increasing interest in low-input multi-species pastures as a sustainable forage for grass-based ruminant production systems (Lüscher et al., 2014).

The impact of such pastures on lamb productivity is varied depending on the plant species present and proportion of different species within the sward. Kenyon et al. (2017) reported a greater per hectare production of lamb over three years with a more botanically diverse pasture compared with a PRG-clover mixture, possibly due to

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higher nutritional quality of the former. Kliem et al. (2018) observed no difference in live weight between a conventional (mainly PRG) and botanically diverse (PRG plus six legume/forb species) pasture over a 10-week period. More recently, Grace et al. (2019) reported heavier live weights and body condition scores (BCS) in lambs consuming 6- and 9-plant species mix swards, from spring turnout to target slaughter weight. Yet Cooledge et al. (2024) reported no difference in daily live-weight gain between lambs grazing either a grass clover or herbal ley pasture, during the Autumn months. These inconsistent results reflect unpredictable growing conditions on pastures from year to year, or within year, as well as the number of species present and their contribution to the grazing biomass DM. The nutritional quality of the comparative treatment will also affect any pasture comparison; the lack of difference observed for liveweight gain by Cooledge et al. (2024) can be attributed to very little difference between the two treatment pastures in terms of sward nutritional quality. Multispecies pastures are dynamic systems by design, with fluctuations in plant species contribution to biomass occurring throughout the year (Barker, 2022). However they remain productive into winter months, and little is known about their impact on livestock production in extended grazing situations, which may exceed typical periods of vegetative growth for some of the diverse species.

The presence of different plant species in botanically diverse pastures can also impact upon the nutritional quality of lamb meat, in terms of fatty acid (FA) profile. In ruminant muscle and subcutaneous fat, botanically diverse pasture systems have been associated with enhanced polyunsaturated FA (PUFA) concentrations (Campidonico et al., 2016; Kliem et al., 2018), and Lourenço et al. (2007) reported increased concentrations of docosahexaenoic acid (a very long-chain (VLC) n-3 PUFA) in the intramuscular fat of lambs consuming a botanically diverse forage compared with a predominantly ryegrass forage. This is due to increased availability of the main plant PUFA (18:2 n-6 and 18:3 n-3) for tissue incorporation, following (i) inhibition of initial lipolysis of plant PUFA prior to rumen biohydrogenation, as sometimes occurs with certain plant species containing condensed tannins (chicory, plantain) or polyphenol oxidase (red clover), (ii) inhibition of rumen biohydrogenation resulting in PUFA escaping this process, and (iii) increased rate of passage through the rumen, as has been observed with pastures containing white clover (Dewhurst et al., 2006). An increase in lamb meat PUFA (particularly n-3 PUFA, and a decrease in n-6:n-3 ratio) could contribute to a more healthy human diet (Prache et al., 2022). However this could also compromise oxidative stability (Leticia et al., 2017) and affect meat aroma and flavour sensory properties. Less desirable flavours detected by trained sensory panels have been associated with oxidation products of meat PUFA when cooked (Watkins et al., 2013; Prache et al., 2022). One of the primary mechanisms preventing oxidative damage in tissues is the action of the glutathione peroxidase (GSH-Px) enzyme family, which contain selenium as an essential component. It has been suggested that expression and activity of these enzymes is upregulated in situations of higher oxidative stress, when selenium status is adequate (Touat-Hamici et al., 2014). Therefore, it was hypothesised that an increase in meat PUFA would increase oxidative stress and, providing animals are replete in selenium, the activity of GSH-Px would increase in response to counteract this.

The main objective of this study was to assess whether an established multispecies pasture containing twelve sown plant species could produce good quality lamb meat, maintaining levels of nutrients such as monounsaturated FA and PUFA, iron and zinc, when grazed over an extended period of time, without detrimentally affecting oxidation risk or sensory characteristics, compared with a less sustainable (environmentally and economically) PRG monoculture pasture.

2. Material and methods

2.1. Plant species and establishment of pastures

Plots (0.8 ha) were established in 2016 as part of the Diverse Forages project (BBSRC project BB/N004353/1; Humphries et al., 2021). Plots contained either PRG or had been sown with a seed mix containing 12 plant species (botanically diverse, BD; species included in the seed mix were perennial ryegrass *Lolium perenne*, timothy *Phleum pratense*, cocksfoot *Dactylis glomerata*, festulolium, meadow fescue *Festuca pratensis*, red clover *Trifolium pratense*, white clover *Trifolium repens*, alsike clover *Trifolium hybridum*, black medick *Medicago lupulina*, lucerne *Medicago sativa*, plantain *Plantago lanceolata*, chicory *Cichorium intybus*). The plots were rotationally grazed by cattle from March to September 2019, before being left to over winter (2019/2020). Plots received no additional fertiliser during this time.

2.2. Experimental animals and the grazing study

Thirty Mule male castrated lambs, born in March 2020, were obtained for the grazing study on weaning in June 2020. On arrival the lambs were weighed (mean weight 29.3 kg \pm 0.63 SEM), and 15 lambs were randomly allocated to each plot to ensure a similar mean live-weight within each plot/treatment (29.5 kg and 29.1 kg for PRG and BD pasture, respectively). Individual lambs were the experimental unit. Lambs were strip grazed (allocated 10 \times 40 m every week) to ensure pastures were grazed completely. Lambs had access to water *ad libitum*, and were weighed weekly throughout (from week 6 body condition score, BCS, was also assessed using a 5-point scale by palpation of the loin area). The grazing period continued until December 2020 (week 19), by which stage most lambs had exceeded the target weight of 45 kg, due to Covid-19 disruption affecting the availability of slaughterhouses. In the 24 h prior to slaughter, blood samples were taken from each lamb via jugular venupuncture, into heparinised tubes. Whole blood was centrifuged at 3000 rpm at 4 °C for 15 min to obtain plasma samples, which were frozen immediately, and then stored at -80 °C until analysed for GSH-Px activity. Animals were transported to the University of Bristol for slaughter, according to European Union Welfare guidelines. On arrival animals were stunned by captive bolt followed by abrupt exsanguination. Carcasses were prepared, graded, deboned and aged for 7 d. Tissue samples were taken for study from *musculus longissimus thoracis* (four loin chops) from the left-hand side of each animal, vacuum packed and transported to the laboratory chilled. In addition, one leg and four loin chops (*musculus longissimus thoracis*) from the right-hand side of randomly selected animals (n = 6 per treatment) were also taken, vacuum packed and stored at -20 °C until analysed for aroma and flavour sensory properties.

On arrival at the laboratory, 100 mg lean tissue from the left-hand side loin chops were removed in duplicate and the tissue washed in phosphate buffered saline at 4 °C. Tissue samples were then homogenised using a Dounce homogeniser with 200 μ l cold buffer from a commercially available GSH-Px kit (ab102530, Abcam, Cambridge, UK). Homogenised tissue was centrifuged for 15 min at 10 000 g at 4 °C, and the supernatant was stored at -80 °C prior to analysis for GSH-Px. The remaining left hand side chops were weighed, separated into lean tissue and visible subcutaneous fat, and these were homogenised to a paste in a food blender. A proportion of this blended lean tissue and subcutaneous fat was then frozen (-20 °C) and freeze dried, before grinding in a mortar and pestle. The remaining blended tissue was stored at -20 °C until analysed.

2.3. Pasture sampling

Samples of above ground biomass were sampled prior to the start of the study (June), during the summer (August) and towards the end of the study (October). A 50 cm² quadrat was randomly thrown and all

above-ground biomass was cut and separated according to species. This was repeated so that five quadrats were sampled from each of the two pasture plots, on the three occasions, and samples were stored at -20 °C. Samples were then freeze dried and dry weights of plant species for the BD pasture plots were used to calculate percentage of each species present. After this, dried plants from the five quadrats per plot per month were combined to create a representative sample of pasture available, and this was milled to pass a 1 mm screen. Dried, milled pasture samples were stored at room temperature prior to analysis.

2.4. Sample analysis

Dried, milled pasture samples were analysed for chemical composition (organic matter, OM; neutral detergent fibre, NDF; acid detergent fibre, ADF; crude protein CP, water-soluble carbohydrates) according to reference procedures outlined previously (Kliem et al., 2008). Freeze dried lean and subcutaneous tissue and dried pasture samples were analysed for FA profile using a modified one-step transesterification method from Sukhija & Palmquist (1988). Briefly, to 400 mg duplicate samples 1 ml of internal standard (1 mg/ml methyl heneicosanoate, H3265, Sigma Aldrich Company Ltd., Dorset, UK in toluene), 1 ml of toluene, and 3 ml 2 % H₂SO₄ in methanol was added. Tubes were mixed thoroughly, sealed under nitrogen and incubated at 60 °C for 3 h. Tubes were left to cool to room temperature, after which 5 ml of 6 % K₂CO₃ and 2 ml toluene was added and tubes mixed. Tubes were centrifuged at 2 500 rpm for 10 min, and the resulting upper phase was transferred to a tube containing 1.0 g Na₂SO₄, and left at room temperature for 1 h. Following centrifuging (5 000 rpm for 5 min), supernatant was transferred to vials. Resulting FA methyl esters were separated using a 100 m CP-Sil 88 column (Agilent, UK) in a Bruker 450 gas chromatograph equipped with a flame ionisation detector, using a temperature programme (Kliem et al., 2013). Identification of FA methyl esters was completed using an external standard (GLC463, Nu-Chek Prep, MN, USA). All results were expressed as g/100 g FA. Due to issues with the internal standard, total lipid in lean and subcutaneous tissue was quantified using the method of Folch et al. (1957), and results were used to calculate mg/100 g fresh tissue of each FA.

Dried lean tissue and pasture samples were also analysed for selected trace minerals (lean tissue for iron and zinc; pasture for selenium). Samples (0.4 g feed, 0.2 g tissue) were prepared for selenium quantification using an alkaline (1.0 ml 25 % v/v tetramethylammonium hydroxide) extraction for 3 h at 90 °C as outlined by BSI (2017), and selenium was quantified by comparing to a range of selenium standards (E3Se6s, Romil Ltd., Cambridge, UK) using an Agilent 7000 inductively coupled plasma mass spectrometer (ICP-MS; Agilent Technologies, Singapore). Tellurium was used as an internal standard (E3Te6s, Romil Ltd., Cambridge, UK), and 125 and 75 were used as masses for tellurium and selenium, respectively. For iron and zinc, ashed tissue samples (1.5 g) underwent heated acid extraction (1:4 v/v HNO₃:HCl) for 5 min, before filtering. Iron and zinc were quantified by comparing to a range of standards (E3F24s and E3Zn4s, Romil Ltd., Cambridge, UK), analysed by ICP-MS (Enamorado-Báez et al., 2013).

Previously blended and frozen lean tissue was analysed for thiobarbituric acid reactive substances (TBARS) using the method of Subbarao et al. (1990). Briefly, 0.5 g tissue was thawed and homogenised using a Dounce homogeniser in 2 x volume of 0.9 % (w/v) NaCl. The homogenate was diluted in 0.9 % NaCl and distilled water before being incubated at 37 °C for 20 min. After this, 600 µl cold 0.8 M HCl containing 12.5 % trichloroacetic acid and 780 µl 1 % thiobarbituric acid was added, tubes sealed and then boiled for 20 min. After cooling to 4 °C, tubes were centrifuged at 1 500 g for 10 min, and the absorbance of the supernatant was measured at 532 nm (Cecil CE2040 spectrophotometer). Results were compared to a standard curve using 1,1,3,3-tetraethoxypropane. Results were expressed as mg malondialdehyde per kg fresh tissue.

Plasma and tissue supernatant were analysed for GSH-Px activity

using the GSH-Px kit (ab102530, Abcam, Cambridge, UK), so that the activity of GSH-Px isoenzymes 3 and 4 (predominant isoenzymes in plasma and tissue, respectively) could be determined. Results were expressed as nmol/min/ml.

2.5. Sensory analysis

Frozen chops and legs (n=6 from each treatment) were thawed at 4 °C 18 h before the day of sensory testing. Samples (with adhered fat attached) were roasted to an internal temperature of 71 °C (AMSA, 2016). The temperature of the oven was set at 160 °C, and a hand-held digital thermometer (Thermopen, Worthing, UK) was used to monitor the temperature in the middle of the samples. Once ready, the samples were allowed to rest for 5 min in an aluminium foil. After that, the subcutaneous fat was removed and the samples were cut into pieces of 1.5 cm (length x width x height) each. For the leg, samples were retrieved from *musculus semimembranosus*. Samples were wrapped again in the same foil and held on a hot plate before served to the panellists.

The trained sensory panel (n = 10) at the Sensory Science Centre, University of Reading, with previous experience in meat assessment, was used to develop a sensory profile to describe the aroma and flavour sensory characteristics of the samples and the characteristics were estimated quantitatively. Samples were presented to each assessor on a glass saucer. During the development of the sensory profile of the samples, assessors smelled and tasted the samples (from three animals per treatment) to produce as many descriptive terms as seemed appropriate, focusing more on aroma and flavour modalities. After discussion as a group and with the help of the panel leader, a consensus vocabulary was developed comprising seven aroma terms, six taste/flavour terms, one mouthfeel term and five after effects terms. The quantitative assessment of the samples (the other three animals per treatment) took place in isolated booths equipped with iPads, under artificial daylight and with controlled room temperature (22 °C). The sensory data were acquired using Compusense Cloud software (Compusense, Guelph, ON, Canada). The assessors were asked to sniff the samples first to score the aroma attributes, then taste the samples and score the taste/flavour and mouthfeel attributes. After 45 s pause, assessors scored the after effects attributes. All scoring was conducted on triplicate samples (three animals per treatment) in separate sessions and samples were presented monodically in a balanced presentation order, coded with three-digit random codes. The intensity of each attribute was recorded on a 100-point unstructured line scale (0 for low intensity, 100 for high). Panelists cleansed their palate with water between samples.

2.6. Data analysis

Dry weight proportions (g/kg) of plant species present in BD at different sampling points were analysed using an ANOVA general linear model (Statistical Analysis System software package version 9.4, SAS Institute, Cary, NC, USA), including the random effect of quadrat and sampling time. BD plant species data was also combined to calculate grasses, legumes and forbs species, and data was analysed using the same model. Nutritional composition of pooled pasture samples was analysed using a two sample t-test to assess effect of treatment. FA composition (g/100 g FA and mg/100 g fresh tissue), tissue mineral content, TBARS and GSH-PX activity data were analysed using an ANOVA Mixed model, which included the fixed effect of treatment and random effect of lamb. Sensory data were analysed using an ANOVA Mixed model, including fixed effect of treatment and random effect of assessor, within each meat cut. Estimated pasture DM intake (DMI) was calculated using pasture NDF contents, lamb weights and Eq. (2) of Starks and Brown (2018). Lamb live weight, weekly live weight gain, estimated DMI and BCS data were analysed using an ANOVA Mixed model including the fixed effects of treatment, week of study and treatment by week interaction, and a random effect of lamb (with week as a repeated effect within lamb). Compound symmetry, heterogeneous

compound symmetry, first-order autoregressive or heterogeneous first-order regressive were used for repeated measures analysis, based on goodness of fit criteria for each variable. Data are presented as least squares means with SEM, and differences were deemed significant when $P < 0.05$.

3. Results

For PRG pasture, perennial ryegrass contributed to a mean of 995 g/kg dry weight across the three collection dates, with the remainder being non-established species ingress. There was no effect ($P > 0.05$) of cutting month on species contribution to the BD pasture DM (means \pm SEM: cocksfoot 282 \pm 71.6 g/kg, perennial ryegrass 187 \pm 45.3 g/kg, timothy 166 \pm 30.4 g/kg, white clover 156 \pm 48.6 g/kg, plantain 97 \pm 54.9 g/kg, chicory 71 \pm 64.8 g/kg, lucerne 27.4 \pm 30.1 g/kg, red clover 14 \pm 19.9 g/kg, meadow fescue 0.4 \pm 0.82 g/kg). There was also no effect ($P > 0.05$) of cutting month on grasses, legumes or forbs dry weight proportions in BD (means \pm SEM: grasses 635 \pm 44.3 g/kg, legumes 197 \pm 34.6 g/kg, forbs 168 \pm 46.3 g/kg).

Nutritional composition of the two pasture types is reported in Table 1. There was very little difference ($P > 0.05$) in nutritional composition of both pastures across the three sampling points. However there was a trend ($P = 0.061$) for BD to have a higher ADF content. Selenium content of BD was higher ($P = 0.001$) than that of PRG. FA profiles of both pastures were also different, with BD containing more ($P < 0.05$) 18:2 *cis*-9, *cis*-12, and less ($P < 0.05$) 16:0 and 18:0, than PRG.

Lambs in both treatment groups increased ($P < 0.001$) in weight over time (Fig. 1a). There was also an overall effect of treatment, with PRG lambs being heavier ($P = 0.014$) than BD lambs. This effect was particularly pronounced at weeks 5-11, 14, 16 and 19 (Fig. 1a). Weekly live weight gain was greater ($P = 0.017$) for PRG than BD (1.34 vs 1.11 kg/week). Estimated pasture DMI was overall greater ($P < 0.001$) for PRG than BD (Fig. 1b). BCS also increased ($P < 0.001$) with both treatments over time (Fig. 1c), but there was no overall effect ($P = 0.174$) of treatment on BCS. For live weight, weekly live weight gain, estimated pasture DMI and BCS there was a treatment by time interaction ($P < 0.01$).

In terms of meat FA content (mg/100 g fresh tissue), there was very little difference between treatments apart from 18:2 *cis*-9, *cis*-12, which was higher in lean tissue ($P = 0.037$) and subcutaneous fat ($P = 0.014$) from lambs which had grazed the BD pasture (Table 2). Higher 20:3 n-6 content following grazing on BD pasture was also observed in lean tissue (6.0 vs 4.8 mg/100 g tissue; $P = 0.029$), and there was a tendency for

Table 1

Nutritional composition of two pasture types (perennial ryegrass, PRG and botanically diverse, BD; least squares means \pm s.e.m. from three sampling times during the grazing period (June, August and October). g/kg DM unless specified).

Composition	Pasture type		SEM	P^1 Treatment
	PRG	BD		
DM (g/kg fresh)				
Organic matter	931	929	2.0	0.450
CP	138	164	18.3	0.282
NDF	516	529	25.8	0.729
ADF	297	346	18.0	0.061
Water soluble carbohydrates	147.9	98.8	25.5	0.199
Selenium (μ g/kg DM)	37.7	147.8	21.7	0.001
Fatty acids				
16:0	2.7	2.2	0.11	0.001
18:0	0.31	0.27	0.011	0.008
<i>cis</i> -9 18:1	0.57	0.56	0.067	0.891
18:2 n-6	2.1	2.8	0.11	<0.001
18:3 n-3	7.0	5.3	0.92	0.14
Total lipid	14.0	19.4	1.91	0.056

CP – crude protein; NDF – neutral detergent fibre; ADF – acid detergent fibre.

20:3 n-6 to be higher in subcutaneous fat from BD lambs (9.2 vs 6.2 mg/100 g fat; $P = 0.065$; Supplementary tables 1 and 2). These treatment differences did not affect the sum totals of n-6 PUFA, or affect n-6:n-3 ratio in either tissue analysed ($P > 0.05$; Table 2). When assessed as FA profile (g/100 g FA), 18:2 n-6 had a higher ($P < 0.01$) proportion in both tissues (Table 3). In subcutaneous fat from BD lambs there was a tendency for a lower ($P = 0.052$) amount of *cis*-9 18:1, which was then reflected in a tendency for lower ($P = 0.071$) total *cis*-MUFA (Table 3).

There was no effect of pasture type on lean tissue iron content when expressed on either a dry or fresh tissue basis ($P > 0.05$; Table 4). Lean tissue zinc content was greater ($P = 0.038$) in BD than PRG meat when expressed on a dry tissue basis, and this difference was not observed when expressed on a fresh tissue basis ($P = 0.418$). There was no effect of pasture type on tissue TBARS ($P = 0.507$) or GSH-Px activity in plasma ($P = 0.937$) or tissue ($P = 0.747$).

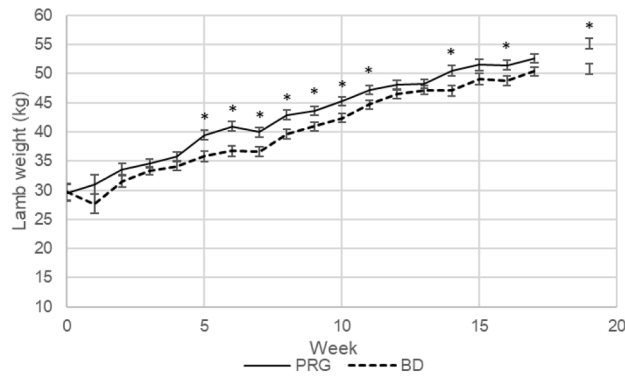
Meat from lambs grazed on BD did not differ ($P > 0.05$) from that of lambs grazed on PRG in terms of aroma and flavour sensory characteristics after assessment by the trained taste panel (Table 5).

4. Discussion

The BD pasture was established four years prior to the current study, with the original seed mix containing twelve different plant species. However during the current study only eight species were identified in the quadrats sampled at each sampling time, with festulolium, meadow fescue, alsike clover and black medick not observed. It is likely these species were out competed by others present and did not establish. Previous research using the same BD seed mix concluded that after three years the mean number of species present had declined to just over 9 (Barker, 2022). This was thought to be due to soil and environmental conditions in the area not favouring all sown species.

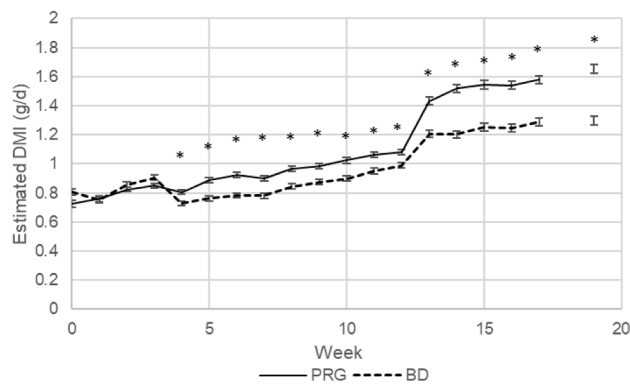
The grazing period was longer than that used in previous studies due to the pandemic, which meant that it incorporated months of year not usually associated with vegetative growth. However species contribution to the available DM in the BD pasture was consistent over the sampling timepoints, with no one species contributing to more than 28 % of the total DM. Unlike other lamb grazing studies involving botanically diverse pastures (Kliem et al., 2018; Grace et al., 2019; Coolidge et al., 2024), lambs grazing BD in the current study were on average lighter throughout than those grazing PRG, and the weekly live weight gain was less than that of PRG. The weekly gain for PRG was similar to the mean target weekly gain for pasture-grazed post-weaning lambs (1.38 g/week; Keady, 2012) but BD lambs fell short of this target. Estimated DMI was calculated to be lower for BD than PRG, particularly during the later weeks. There was a tendency for the BD pasture to have a higher ADF content than PRG, indicating a lower digestibility of BD. Plant maturity over the growing season causes a decline in nutritive quality, which can be measured via an increase in NDF or ADF content and a decrease in organic matter digestibility (Hurley et al., 2021). If the original target slaughter weight of 45 kg was adhered to, PRG lambs reached this on average 10 weeks into the study, whereas BD lambs required 11 weeks (equating to a mean of 179 and 186 days to slaughter for PRG and BD lambs, respectively). Taking into account average total production costs (including purchase cost) of £75.58 per head lamb for a 95 day finishing period (AHDB, 2016) and the stocking density used in this study, the cost of producing lamb on these pastures can be calculated as £83.54 per week per ha. Current (2024) costs to establish 1 ha PRG and BD amount to £253 and £195, respectively, taking into account similar seed mix costs (“Intensive Dairy Grass” and “Simple Herbal Ley Mix”; Cotswold Seeds, UK) and fertiliser required for PRG. Therefore the slightly longer time to slaughter weight for lambs finished on BD would result in a £25 higher cost to the producer, on a per hectare basis. Mean days to 45 kg slaughter weight for both groups was less than that of Earle et al. (2017) and comparable to that of Grace et al. (2019). Higher weaning weights can lead to improved production via less days to slaughter (Galvani et al., 2014). Data on weaning weights and diets prior

(a)



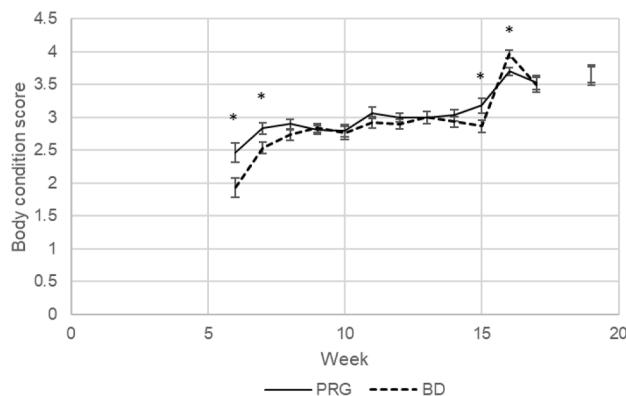
Mixed procedure concluded an effect of time ($P < 0.001$), treatment ($P = 0.014$) and an interaction between time and treatment ($P < 0.001$). Where significant treatment slice differences at each timepoint are indicated by *.

(b)



Estimated DMI calculated using equation (2) of Starks and Brown (2018), lamb weights and NDF values of pasture. Mixed procedure concluded an effect of time ($P < 0.001$), treatment ($P < 0.001$) and an interaction between time and treatment ($P < 0.001$). Where significant treatment slice differences at each timepoint are indicated by *.

(c)



Mixed procedure concluded an effect of time ($P < 0.001$), and an interaction between time and treatment ($P = 0.001$). Where significant treatment slice differences at each timepoint are indicated by *.

Fig. 1. The mean live weight (a), estimated pasture DM intake (DMI) (b) and body condition score (c) of lambs grazing either perennial ryegrass (PRG, solid line) or botanically diverse (BD, dashed line) pasture (least squares means of $n = 15$ per treatment).

Table 2

Fatty acid composition (selected fatty acids and groups, mg/100 g tissue) of tissues from lambs grazing either perennial ryegrass (PRG) or botanically diverse (BD) pastures (least squares means of n = 15 per treatment).

	Pasture type		SEM	P ^a Treatment
	PRG	BD		
<i>M. longissimus thoracis</i>				
16:0	2458	2591	184.6	0.608
18:0	2715	2762	219.4	0.880
18:1 <i>cis</i> -9	2935	2987	240.5	0.876
18:1 <i>trans</i> -11	448	489	33.4	0.380
18:2 <i>cis</i> -9, <i>cis</i> -12	187	233	15.1	0.037
18:3 n-3	127	138	11.2	0.492
Total SFA ^b	5605	5775	420.0	0.773
Total <i>cis</i> -MUFA ^c	3357	3455	273.8	0.798
Total <i>trans</i> -MUFA ^d	613	670	40.9	0.326
n-3 PUFA ^e	206	208	15.9	0.959
n-6 PUFA ^f	323	357	21.7	0.260
Total PUFA ^g	561	596	38.2	0.517
Total CLA	108	114	9.1	0.662
n-6:n-3	1.63	1.76	0.081	0.253
VLC n-3 ^h	59.0	57.0	4.64	0.765
Total lipid	10268	10639	749.2	0.724
Subcutaneous fat				
16:0	18993	18715	659.0	0.756
18:0	21135	21002	886.1	0.912
18:1 <i>cis</i> -9	22393	20663	775.7	0.109
18:1 <i>trans</i> -11	4298	4403	311.0	0.802
18:2 <i>cis</i> -9, <i>cis</i> -12	817	945	36.4	0.014
18:3 n-3	760	813	43.5	0.371
Total SFA	43062	42667	1229.2	0.813
Total <i>cis</i> -MUFA	25727	23914	854.4	0.126
Total <i>trans</i> -MUFA	5973	6027	360.0	0.911
n-3 PUFA	979	953	57.8	0.741
n-6 PUFA	1640	1568	72.3	0.463
Total PUFA	2909	2742	130.6	0.349
Total CLA	912	939	64.3	0.760
n-6:n-3	1.7	1.7	0.08	0.806
VLC n-3	136	122	12.2	0.392
Total lipid	78902	76555	2000.1	0.390

SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, CLA – conjugated linoleic acid, VLC – very long chain (>C20)

^a Significance of the effect of pasture treatment for n = 15 lambs

^b Sum of 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, 19:0, 20:0, 22:0, 24:0

^c Sum of *cis*-9 12:1, *cis*-9 14:1, *cis*-10 15:1, *cis*-9 16:1, *cis*-10 17:1, *cis*-9 18:1, *cis*-11 18:1, *cis*-5 20:1, *cis*-8 20:1, *cis*-13 22:1, *cis*-15 24:1

^d Sum of *trans*-9 16:1, *trans*-9 18:1, *trans*-11 18:1, *trans*-12 18:1, *trans*-16 18:1

^e Sum of 18:3 n-3, 20:3 n-3, 20:5 n-3, 22:3 n-3, 22:5 n-3, 22:6 n-3

^f Sum of *trans*-9, *trans*-12 18:2, *trans*-9, *cis*-12 18:2, *cis*-9, *cis*-12 18:2, 18:3 n-6, 20:2 n-6, 20:3 n-6, 20:4 n-6, 22:2 n-6, 22:4 n-6

^g Sum of n-3 and n-6 PUFA

^h Sum of 20:3 n-3, 20:5 n-3, 22:5 n-3, 22:6 n-3

to the start of the trial were not available, but lambs were weaned two weeks prior to the start of grazing, when mean live weights were 29.5 and 29.7 for PRG and BD, respectively. To incorporate this type of pasture into lamb finishing systems, producers would need to aim for a high weaning weight, and may consider slaughtering at a slightly lower weight, depending on the price of lamb, to ensure efficiency.

Total FA content of lean *m. longissimus thoracis* was greater than that reported by earlier studies comparing more biodiverse forages with a PRG control (Lourenço et al., 2007; Kliem et al., 2018). However the grazing period in the current study was longer (133 days) than those in the earlier studies (77 and 70 days, respectively) due to the pandemic. Older lambs would be expected to have a higher intramuscular fat content, as carcass fat percentage increases with age (Prache et al., 2022). Pasture type had a small impact on both lean tissue and subcutaneous fat FA content and profile. Kliem et al. (2018) reported no difference in individual FA content of *m. longissimus thoracis*, but the biodiverse treatment did result in a greater concentration of 18:2 n-6 and 18:3 n-3 in lean tissue from *m. semimembranosus* and subcutaneous

Table 3

Fatty acid profile (selected fatty acids and groups, g/100 g total fatty acids) of tissues from lambs grazing either perennial ryegrass (PRG) or botanically diverse (BD) pasture (least squares means of n = 15 per treatment).

	Pasture type		SEM	P ^a Treatment
	PRG	BD		
<i>M. longissimus thoracis</i>				
16:0	23.7	24.4	0.39	0.184
18:0	26.6	25.8	0.85	0.509
18:1 <i>cis</i> -9	28.3	27.9	0.54	0.613
18:1 <i>trans</i> -11	4.7	4.7	0.30	0.986
18:2 <i>cis</i> -9, <i>cis</i> -12	1.8	2.2	0.10	0.008
18:3 n-3	1.2	1.3	0.06	0.344
Total SFA ^b	54.5	54.3	0.61	0.792
Total <i>cis</i> -MUFA ^c	32.3	32.2	0.62	0.890
Total <i>trans</i> -MUFA ^d	6.4	6.4	0.37	0.884
n-3 PUFA ^e	2.0	2.0	0.10	0.990
n-6 PUFA ^f	3.2	3.4	0.13	0.156
Total PUFA ^g	5.5	5.7	0.20	0.427
Total CLA	1.1	1.1	0.06	0.958
n-6:n-3	1.62	1.76	0.077	0.201
VLC n-3 ^h	0.57	0.56	0.042	0.863
Subcutaneous fat				
16:0	24.1	24.3	0.49	0.754
18:0	26.8	27.7	0.86	0.461
18:1 <i>cis</i> -9	28.4	26.8	0.55	0.052
18:1 <i>trans</i> -11	5.4	5.7	0.32	0.522
18:2 <i>cis</i> -9, <i>cis</i> -12	1.0	1.2	0.04	<0.001
18:3 n-3	0.97	1.07	0.052	0.187
Total SFA	54.5	55.9	0.72	0.190
Total <i>cis</i> -MUFA	32.7	31.1	0.60	0.071
Total <i>trans</i> -MUFA	7.5	7.8	0.33	0.545
n-3 PUFA	1.2	1.3	0.07	0.964
n-6 PUFA	2.1	2.1	0.08	0.856
Total PUFA	3.7	3.7	0.15	0.842
Total CLA	1.1	1.2	0.07	0.682
n-6:n-3	1.7	1.7	0.07	0.991
VLC n-3	0.17	0.16	0.013	0.508

SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, CLA – conjugated linoleic acid, VLC – very long chain (>C20)

^a Significance of the effect of pasture treatment for n = 15 lambs

^b Sum of 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, 19:0, 20:0, 22:0, 24:0

^c Sum of *cis*-9 12:1, *cis*-9 14:1, *cis*-10 15:1, *cis*-9 16:1, *cis*-10 17:1, *cis*-9 18:1, *cis*-11 18:1, *cis*-5 20:1, *cis*-8 20:1, *cis*-13 22:1, *cis*-15 24:1

^d Sum of *trans*-9 16:1, *trans*-9 18:1, *trans*-11 18:1, *trans*-12 18:1, *trans*-16 18:1

^e Sum of 18:3 n-3, 20:3 n-3, 20:5 n-3, 22:3 n-3, 22:5 n-3, 22:6 n-3

^f Sum of *trans*-9, *trans*-12 18:2, *trans*-9, *cis*-12 18:2, *cis*-9, *cis*-12 18:2, 18:3 n-6, 20:2 n-6, 20:3 n-6, 20:4 n-6, 22:2 n-6, 22:4 n-6

^g Sum of n-3 and n-6 PUFA

^h Sum of 20:3 n-3, 20:5 n-3, 22:5 n-3, 22:6 n-3

Table 4

Lamb lean tissue selenium, zinc and iron content, thiobarbituric acid reactive substances (TBARS), and glutathione peroxidase (GSH-Px) activity in lamb lean tissue and plasma from lambs grazing either perennial ryegrass (PRG) or botanically diverse (BD) pasture (least squares means of n = 15 per treatment).

		Pasture type		SEM	P ^a Treatment
		PRG	BD		
Selenium	ug/kg dry tissue	207.1	213.6	14.40	0.753
	ug/kg fresh tissue	75.9	76.2	5.96	0.973
Iron	ug/kg dry tissue	64.8	66.3	2.03	0.604
	ug/kg fresh tissue	24.3	23.9	1.60	0.852
Zinc	ug/kg dry tissue	61.8	68.6	2.20	0.038
	ug/kg fresh tissue	23.0	24.6	1.40	0.418
TBARS	MDA ^b (mg/kg tissue)	1.1	1.0	0.05	0.507
GSH-PX	mU/min/ml plasma	211.8	211.2	5.50	0.937
	mU/min/g tissue	52.5	54.3	4.05	0.747

^a Significance of the effect of pasture treatment for n = 15 lambs

^b Malonaldehyde

Table 5

Aroma and flavour sensory assessment by a trained taste panel, of chop and rump cuts from lambs grazed on either perennial ryegrass (PRG) or botanically diverse (BD) pasture (least squares means of n = 6 per treatment; 100-point unstructured line scale).

		Chop				Rump			
		Pasture type		SEM	P Treatment	Pasture type		SEM	P Treatment
		PRG	BD			PRG	BD		
Aroma	Overall intensity	54.2	53.1	3.25	0.737	57.3	58.9	2.21	0.577
	Toasty	40.0	33.9	3.35	0.206	42.8	46.2	5.23	0.478
	Metallic/bloody	38.6	38.8	4.70	0.970	31.0	32.8	3.28	0.701
	Fatty	25.9	24.1	2.37	0.607	22.8	23.7	2.97	0.788
	Savoury	37.4	36.7	2.57	0.837	36.3	37.4	3.42	0.819
	Sweet	19.6	21.5	3.08	0.493	26.3	24.4	3.08	0.458
	Sulphury	1.98	3.32	1.054	0.372	1.31	2.65	1.11	0.397
Flavour	Overall intensity	47.5	47.0	3.49	0.850	45.8	46.7	3.35	0.752
	Fatty	21.4	19.1	3.20	0.483	18.5	19.3	2.01	0.773
	Metallic/bloody	34.5	34.6	3.59	0.980	32.5	30.3	3.47	0.665
Taste	Sweet	17.7	18.7	2.74	0.708	23.2	22.1	2.81	0.643
	Sour	7.84	7.73	2.883	0.975	4.24	4.42	2.784	0.949
	Umami	27.7	27.7	4.59	0.996	28.7	30.3	4.60	0.704
Texture	Chewy	41.4	42.7	4.73	0.759	34.0	37.0	4.99	0.492
	After effects								
After effects	Metallic/bloody	36.5	35.1	3.41	0.779	33.4	35.8	2.69	0.534
	Fatty	17.1	16.0	3.28	0.744	14.6	17.0	2.02	0.412
	Salivating	34.3	33.0	2.42	0.656	32.7	36.1	2.33	0.312
	Drying	25.7	23.1	3.43	0.501	24.9	27.1	3.07	0.591
	Tooth packing	13.2	12.8	5.13	0.927	7.11	9.73	5.312	0.571

fat, contributing to a higher n-6 and n-3 PUFA content overall. It was hypothesised that the increased plant diversity of BD and longer grazing time in the current study compared with that of Kliem et al. (2018) may have resulted in a greater difference between BD and PRG in terms of both PUFA families in lean tissue and subcutaneous fat. The FA concentration of the two pastures was variable, but BD had a higher 18:2 n-6 content than PRG, which, when coupled with the potential presence of secondary plant compounds that minimise rumen biohydrogenation, may have led to the observed increase in meat 18:2 n-6 content. As well as the lipolysis-minimising effects of polyphenol oxidase in red clover (Dewhurst et al., 2006), chicory and plantain contain condensed tannins (Waghorn et al., 1995), which can inhibit ruminal lipolysis and biohydrogenation of plant PUFA (Nikerdorn and Jayanegara, 2021), increasing their availability for absorption. In addition, the inclusion of white clover in forage can increase the rate of passage through the rumen, leading to more plant PUFA bypassing rumen biohydrogenation. Grazing lambs for 35 days on a plantain-chicory-white clover pasture resulted in *longissimus* meat higher in 18:2 n-6 and 18:3 n-3, with a concomitant increase in longer chain n-3 and n-6 PUFA due to elongation and desaturation of the former, compared with a grass-based pasture (Rodriguez et al., 2020). Furthermore, the meat 18:3 n-3 content from lambs grazed a chicory-arrowleaf clover pasture was greater than that of meat from other treatment pastures in a study conducted over 49 days, despite the 18:3 n-3 content of the chicory-arrowleaf clover pasture being lower (De Brito et al., 2017). This suggests that chicory has the potential to improve the bioavailability of its PUFA to the grazing ruminant. The lack of effect of plant species diversity on lean and fat 18:3 n-3 and n-3 PUFA content in the current study is unclear, but the contribution of herbs and forbs to the available biomass in winter months would be expected to be lower (Kemp et al., 2010) than that observed in earlier studies, which occurred during spring and summer. Future research needs to incorporate more detailed assessments of plant intake, to gain further understanding as to which plants might be responsible for beneficial impacts observed.

The difference in tissue 18:2 n-6 content between treatments led to a difference in proportion of 18:2 n-6 when expressed on a g/100 g total FA basis. The lipid of subcutaneous fat from BD lambs also contained a lower proportion of *cis*-9 18:1 and total *cis*-MUFA than that of PRG lambs. *Cis*-9 18:1 in adipose tissue is derived from both dietary *cis*-9 18:1 that has escaped rumen metabolism, and also desaturation of 18:0 from the complete rumen biohydrogenation of dietary PUFA by tissue Δ^9

desaturase. The lower proportion of *cis*-9 18:1 in BD meat could indicate that dietary PUFA did not undergo complete biohydrogenation (especially as there were no treatment effects on proportion of 18:0).

Lamb meat is classed as being a “rich source” of zinc (providing > 30 % nutrient reference value; AHDB, 2019), and is a good source of nutritionally available haem iron (Prache et al., 2022). Iron and zinc contents of muscle tissue are related to type of muscle fibre within the muscle, with more oxidative muscle types containing more iron and zinc, and these muscle types increasing with lamb age (Pannier et al., 2013). Nutrition can also impact upon meat trace mineral content, not only in terms of dietary supply, but also (for iron and zinc particularly) in terms of restricted nutrition causing decreased muscle development and therefore lower iron and zinc concentration (Pannier et al., 2013). In the current study it was found that the lower live weight observed with BD lambs did not appear to affect iron concentration in muscle, indicating that muscle development was similar between PRG and BD lambs. Meat from BD lambs did have a higher zinc content when expressed on a DM basis but this was not apparent on a fresh weight basis. Grazing lambs on BD pasture did not have a negative impact on nutritionally important microminerals in meat, when compared with PRG pasture, despite the lower live weight gain.

With a significant but small treatment effect on meat PUFA content, it is perhaps not surprising that no treatment differences were observed for both GSH-Px isoenzymes measured (GSH-Px3 in plasma, GSH-Px4 in tissue). Research has demonstrated that the activity of blood GSH-Px (isoenzymes 1 and 3) can be increased after supplementing lamb diets with 0.1 mg/kg DM organic selenium (Qin et al., 2007), and a recent study in broiler chickens reported that tissue GSH-Px activity is enhanced in the presence of more tissue PUFA (therefore increased susceptibility to oxidation), but only when birds were Se-replete (Juniper et al., 2022). Growing lambs require a daily intake of around 0.2 mg/kg DM Se (National Research Council, 2007) which the BD pasture almost achieved, but PRG had a low Se concentration in comparison. Therefore it could be assumed that lambs grazing BD were Se-replete. The TBARS results were consistent with the lack of treatment difference in meat PUFA content. Ponnampalam et al. (2017) observed a higher *longissimus* TBARS concentration in lambs grazed on a lucerne/clover pasture compared with a ryegrass pasture. However dietary treatments in that study resulted in a significant difference in the more unsaturated PUFA, such as 18:3 n-3, 20:5 n-3 and 22:6 n-3. TBARS content reflects the susceptibility of tissue to oxidation, and this

increases with increasing unsaturation of FA. Therefore, it is possible that TBARS was not different in the current study as the numerical difference in meat PUFA was due to 18:2 n-6 and not 18:3 n-3, which is more unsaturated. If peroxidisability index (a measure of risk of tissue peroxidation, weighting PUFA on their degree of unsaturation; Erickson, 1992) is calculated for PRG and BD meat there is no significant difference between PRG and BM (1230 and 1288, respectively; $P = 0.626$).

It is known that meat from lambs consuming fresh forage-based diets is associated with the presence of pastoral flavours (Schreurs et al., 2008). These are derived from chemical components of pasture-based diets, and are formed both during rumen fermentation and also during cooking. Several compounds responsible for pastoral flavours can be linked to diets with higher digestible protein levels (such as those containing legumes such as white clover), when liberated peptides and amino acids are deaminated by rumen microflora (Schreurs et al., 2007). This, together with the anticipated higher meat PUFA content in BD (which can result in increased off-flavours as a result of greater oxidation) was why meat from BD lambs was expected to have different taste panel scores than meat from PRG lambs. There was no difference between treatments in terms of meat flavour in this study, even though the BD pasture had a numerically higher crude protein content than PRG pasture. This could be due to a lower overall digestibility of BD, as indicated by the higher ADF content. The only flavour attribute to be consistently numerically higher for BD than PRG meat across both cuts was “sulphury”. The sulphury attribute could be due to rumen fermentation of sulphur-containing amino acids from the diet (Schreurs et al., 2008). Although BD increased 18:2 n-6 in meat, there appeared to be no negative impact in terms of aroma and flavour. An early study reported detection of “sweet” or unacceptable aromas from meat from lambs fed a supplementary sunflower oil (Park et al., 1974) and a later study involving a rumen protected 18:2 n-6-rich supplement highlighted that lamb meat had a “grassy” flavour. The concentration of 18:2 n-6 in the diets of these studies was much higher than that of the present study, which may explain why no aroma/flavour differences were observed in meat from lambs grazing the PRG or BD pastures.

In conclusion, results of the current study suggest that grazing lambs on a botanically diverse ($n=8$ species) pasture for 133 days increased 18:2 n-6 content of meat without increasing oxidation risk, and maintained the content of selected trace minerals and eating quality, when compared with lambs grazing on a perennial ryegrass-based pasture. This BD pasture resulted in longer time to reach slaughter target weight, highlighting the need to consider adjustments to practice (such as ensuring good weaning weights, or slaughtering at a lower target weight) to benefit from incorporation into lamb finishing systems.

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All experimental procedures and sampling were undertaken in accordance with the Animals (Scientific Procedures) Act, 1986.

CRedit authorship contribution statement

K.E. Kliem: Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **D.J. Humphries:** Writing – review & editing, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **S. Lignou:** Writing – review & editing, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **D.T. Juniper:** Writing – review & editing, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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