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# Herbal leys increase forage macro- and micronutrient content, spring lamb nutrition, liveweight gain, and reduce gastrointestinal parasites compared to a grass-clover ley

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## ARTICLE INFO

## ABSTRACT

Commercial herbal leys (multispecies swards) are rapidly gaining popularity due to their potential to deliver an enhanced suite of ecosystem services. However, little is known about their impact on lamb production. A 2-ha split-field experiment using an herbal and grass-clover ley (0.33 ha paddock$^{-1}$, $n = 3$ per sward) aimed to evaluate the effect of sward-type on forage quality and lamb productivity. Lambs ($n = 40$ per sward) were rotationally grazed over two experimental seasons: autumn 2020 (males) and spring 2021 (females). Sward quality was measured at the start of each grazing season. Liveweight gain and faecal egg counts (FEC) were measured at week 0, 4 and 6 in autumn and week 0, 4, 9 and 11 in spring. Blood samples were analysed after 11-weeks in spring to assess mineral status. General sward nutritional quality (e.g., crude protein) did not improve under the herbal ley, however, higher sward macro- and micronutrient concentrations were observed in both seasons. Spring liveweight gain was significantly greater in lambs grazing the herbal (172 $\pm$ 7 g d$^{-1}$) *vs.* grass-clover ley (144 $\pm$ 7 g d$^{-1}$), while autumn liveweight gain showed no difference. Spring lambs grazing the herbal ley compared to the grass-clover ley had elevated plasma cobalt (2.0 $\pm$ 0.1 nmol l$^{-1}$ *vs.* 1.6 $\pm$ 0.1 nmol l$^{-1}$) and selenium (0.7 $\pm$ 0.04 $\mu$mol l$^{-1}$ *vs.* 0.5 $\pm$ 0.01 $\mu$mol l$^{-1}$), with lower blood urea (7.7 $\pm$ 0.3 nmol l$^{-1}$ *vs.* 10.4 $\pm$ 0.4 nmol l$^{-1}$). Spring FEC scores were reduced by 78 % in herbal grazed lambs; there were no significant differences in autumn FEC from either sward. In conclusion, the herbal ley resulted in seasonal improvements in sward micronutrient content and lamb parasite burden, mineral status, and productivity.

## 1. Introduction

Globally, grazing sheep for meat and milk production produces 67.3 and 29.9 million tonnes $CO_2$-equivalent greenhouse gas (GHG) emissions per annum respectively, with sheep production representing approximately 6.5 % of emissions from the livestock sector (Gerber et al., 2013). Improving the efficiency of grazing sheep may be achieved via feed additives and diet manipulation (e.g., altering the sward botanical composition) by improving their overall productivity and health, subsequently reducing GHG emissions and contributing towards achieving sustainable agricultural intensification (Gerber et al., 2013; Pulina et al., 2017).

In temperate regions, lowland sheep production systems are dominated by grass swards consisting of perennial ryegrass (*Lolium perenne*) and grass-clover swards utilising legumes such as white (*Trifolium repens*) and red clover (*Trifolium pratense*) due to their productivity, persistence under grazing, and high digestibility and sugar content (Waghorn and Clark, 2004; Fraser et al., 2022). However, *Lolium perenne* swards typically require additional N inputs to maintain yields, resulting in increased on-farm economic constraints from fertiliser costs and environmental implications, e.g., soil acidification, watercourse eutrophication, and increased nitrous oxide emissions (Bell et al., 2015; Kidd et al., 2017). Herbal leys (also known as multispecies swards) consisting of grasses, legumes and herbs are rapidly increasing in popularity as a

low N-input alternative to conventional pastures that can deliver greater ecosystem service benefits, such as improved sward productivity (Finn et al., 2013; Jordon et al., 2022; Baker et al., 2023; Hearn et al., 2024), sward quality (Jing et al., 2017; Grace et al., 2018), drought tolerance (Hofer et al., 2016; Grange et al., 2021), and reduced nitrous oxide emissions (Cummins et al., 2021).

Improved livestock productivity has been reported when grazing a herbal ley due to the enhanced sward nutritional quality and dry matter intake (Golding et al., 2011; Somasiri et al., 2015; Grace et al., 2019; Jerrentrup et al., 2020). Unlike *Lolium perenne* swards, deep-rooting herb species such as chicory (*Cichorium intybus*) and ribwort plantain (*Plantago lanceolata*) can access subsoil nutrient reserves and often accumulate greater macro- and micronutrient concentrations than most grasses, which may reduce the risk of sub-clinical micronutrient deficiencies and improve productivity in grazing livestock (Barry, 1998; Watson et al., 2012; Darch et al., 2020, 2022; Kao et al., 2020). Herb and legume species often used within a herbal ley also contain high concentrations of plant secondary metabolites which can reduce the gastrointestinal parasite burden of livestock and subsequently the need for anthelminthic interventions (Marley et al., 2003; Peña-Espinoza et al., 2018). Gastrointestinal parasites can cause significant productivity and economic losses in livestock production, with the repeated use of synthetic anthelminthics contributing to the rise of anthelminthic resistance (Hoste and Torres-Acosta, 2011). A previous study utilising a 6- or 9-speices herbal ley has shown a reduction in lamb faecal egg counts, increasing the duration between anthelminthic doses (Grace et al., 2019).

Operating a herbal ley system, however, is not without its challenges. For example, some herbs are vulnerable to selective grazing (Jing et al., 2017), and little is known about their impact on livestock productivity, health, persistence, and contribution to sward nutritional quality when included in a commercial herbal ley mixture (consisting of 10–18 species) under field conditions. Most grazing studies utilise low-diversity experimental herbal mixtures (e.g., 3–9 species) that do not fully encompass the complexity of commercial swards promoted via agri-environment schemes, resulting in a significant knowledge gap as farmers increase adoption of these swards.

This study aims to compare the impact of a commercial herbal ley on livestock productivity and health and sward quality compared to a conventional grass-clover ley. We hypothesise that i) sward nutritional quality and macro- and micronutrient content will be enhanced in the herbal ley due to greater accumulation of nutrients by herb species; and ii) weaned lambs will have greater productivity due to consuming feed with high nutritional quality and anthelminthic properties.

## 2. Materials and methods

### 2.1. Experimental design

In July 2020, a 2-ha lowland field located at the Henfaes Research Centre, Abergwyngregyn, North Wales, UK (10 m a.s.l., 53.240329 N, −4.014574 W) was ploughed and reseeded with either a 18-species herb- and legume-rich multispecies ley (herbal) or a conventional 5-species grass-clover ley (grass-clover) (Table 1) supplied by Cotswold Seeds (Cotswold Seeds Ltd., Moreton-in-Marsh, UK). The field was previously grassland prior to reseeding. The soil at the field site was classified as a sandy clay loam textured free-draining Eutric Cambisol, with an average annual rainfall and temperature of 1060 mm and 10.8 °C, respectively.

One hectare was dedicated to each sward type and subsequently split into three independent permanently fenced ~0.33 ha paddocks, allowing for the rotational grazing of weaned lambs. Lambs were moved between each paddock every 4–5 days throughout their experimental grazing season, with each paddock having approximately 8–10 days' rest before grazing occurred again. The field received no nitrogen fertiliser in autumn 2020, only receiving one application of 50 kg N ha$^{-1}$ ammonium nitrate, 20 kg P$_2$O$_5$ ha$^{-1}$, 60 kg K$_2$O ha$^{-1}$ fertiliser in March 2021 to encourage spring grass growth. Background soil characteristics and analytical methods of the field experiment have been evaluated in detail previously (see Cooledge et al., 2024). Briefly, the soil pH (0–10 cm) was 6.90 ± 0.08 and 6.99 ± 0.06 with an electrical conductivity of 51 ± 14 µS cm$^{-1}$ and 38 ± 5 µS cm$^{-1}$ for the herbal and grass-clover leys, respectively ($p > 0.05$).

Meteorological data were recorded every 30 minutes from an automated on-site weather station located approximately 200 m from the

**Table 1**
Species composition and seeding rate of the herbal ley and grass-clover ley sown in July 2020.

| Plant type | Herbal ley | | | Grass-Clover ley | | |
|---|---|---|---|---|---|---|
| | Species and Cultivar | Proportion (%) | Seeding rate (kg ha$^{-1}$) | Species and Cultivar | Proportion (%) | Seeding rate (kg ha$^{-1}$) |
| Grass | Cocksfoot (*Dactylis glomerata*) cv. 'Amba' | 11.5 | 3.75 | Perennial ryegrass (*Lolium perenne*) cv. 'Glenstal' | 30.8 | 10.0 |
| | *Festulolium* cv. 'Lofa' | 11.5 | 3.75 | Perennial ryegrass (*Lolium perenne*) cv. 'AberMagic' | 16.9 | 5.50 |
| | Perennial ryegrass (*Lolium perenne*) cv. 'Oakpark' | 7.7 | 2.50 | Timothy (*Phleum pratense*) cv. 'Winnetou' | 15.4 | 5.00 |
| | Perennial ryegrass (*Lolium perenne*) cv. 'Glenstal' | 3.8 | 1.25 | Cocksfoot (*Dactylis glomerata*) cv. 'Amba' | 15.4 | 5.00 |
| | Timothy (*Phleum pratense*) cv. 'Winnetou' | 4.6 | 1.50 | Hybrid ryegrass (*Lolium perenne*) cv. 'Tetragraze' | 11.5 | 3.75 |
| | Tall fescue (*Festuca arundinacea*) cv. 'Kora' | 3.8 | 1.25 | | | |
| | Meadow fescue (*Festuca pratensis*) cv. 'Pardus' | 3.1 | 1.00 | | | |
| Legume | Sainfoin (*Onobrychis*) | 19.2 | 6.25 | Red clover (*Trifolium pratense*) cv. 'AberClaret' | 3.9 | 1.25 |
| | Sweet clover (*Melilotus*) | 6.2 | 2.00 | White clover (*Trifolium repens*) cv. 'AberDai' | 3.1 | 1.00 |
| | Red clover (*Trifolium pratense*) cv. 'Milvus' | 5.4 | 1.75 | White clover (*Trifolium repens*) cv. 'AberHerald' | 2.3 | 0.75 |
| | White clover (*Trifolium repens*) cv. 'AberHerald' | 3.8 | 1.25 | Wild white clover (*Trifolium repens*) cv. 'AberAce' | 0.8 | 0.25 |
| | Lucerne (*Medicago sativa*) cv. 'Luzelle' | 2.3 | 0.75 | | | |
| | Alsike clover (*Trifolium hybridum*) cv. 'Aurora' | 1.5 | 0.50 | | | |
| | Birdsfoot trefoil (*Lotus corniculatus*) cv. 'Bull' | 1.5 | 0.50 | | | |
| Herb | Burnet (*Sanguisorba minor*) | 5.4 | 1.75 | | | |
| | Chicory (*Cichorium intybus*) cv. 'Puna II' | 4.6 | 1.50 | | | |
| | Ribwort Plantain (*Plantago lanceolata*) cv. 'Endurance' | 1.5 | 0.50 | | | |
| | Sheep's Parsley (*Petroselenium crispum*) | 1.5 | 0.50 | | | |
| | Yarrow (*Achillea millefolium*) | 0.8 | 0.25 | | | |

field site (Campbell Scientific Ltd., Leicestershire, UK). Meteorological data can be found in Supplementary Figure 1.

### 2.2. Livestock management

Weaned Welsh mountain lambs (*Ovis aries*) ($n = 40$ per sward) at a stocking density of 3.2 LU ha$^{-1}$ were grazed over two experimental seasons: autumn 2020 (late September to late October) and spring 2021 (late April to early July). The breed and sex of lamb grazed each experimental season represented seasonal differences in the local grazing system, with male ram lambs approximately 6–7 months old (average starting liveweight 22.1 $\pm$ 0.4 kg) being conditioned for slaughter grazing in autumn 2020, and female ewe lambs approximately 10–11 months old (average starting liveweight 23.0 $\pm$ 0.3 kg) being conditioned for breeding grazing in spring 2021. Each lamb was fitted with an EID ear tag at the start of the experiment to allow for identification and use of the electronic 3-way manual weighing crate (Prattley, Temuka, New Zealand) with liveweight gain (LWG) recorded digitally (Tru-Test XR5000, Auckland, New Zealand). At the start of each grazing season, lambs were weighed and assigned to each sward type based on their starting weight to ensure an even split across the grazing groups. Lambs received no additional dietary supplementation throughout the experiment. Mains supplied fresh water was freely accessible via communal water troughs in each paddock.

Following the initial weighing at the start of grazing (week 0), lambs were weighed at week 4 and 6 in autumn 2020, and at week 4, 9 and 11 in spring 2021. Lambs used for excreta (urine and dung) collection (as described Cooledge et al., *in prep*; ca. $n = 6$ lambs per sward) and faecal egg count sampling were excluded from the LWG dataset due to the grazing disruption experienced during collection periods. Liveweight gain was calculated for the duration each lamb spent on the grazing trial. Due to grass limitations in the autumn 2020 grazing experiment, lambs that reached their target condition (data not recorded) after 4-weeks were removed from the experiment to prevent overgrazing and damage to the sward.

Body condition score (BCS) of the older ewe lambs was assessed at each weighing session in the spring 2021 grazing experiment by a single trained worker assessing back fat using a scale of 1 – 5 with 0.5 increments (Russel et al., 1969). Body condition score was not measured in the autumn 2020 grazing experiment.

### 2.3. Sward nutritional composition and standing biomass

At the start of each grazing season, sward samples were obtained by cutting four randomly assigned 1 m$^2$ quadrat sward samples with hand shears across each paddock at an approximate grazing height (ca. 4 – 5 cm). These samples were then bulked into one sample per paddock for nutritional and chemical composition analyses. A subsample from each paddock was obtained to determine dry matter (DM) content by drying in a forced draught oven at 80 °C for 24 hours. Fresh sward samples were analysed by wet chemistry by Sciantec Analytical (Sciantec, Yorkshire, UK) to determine sward quality properties (DM content, crude protein, sugar, metabolisable energy (ME), neutral-detergent fibre (NDF), D-value, ash, oil-A, nitrate-nitrogen, and buffering capacity) and macro- and micronutrient content (calcium, magnesium, sodium, potassium, cobalt, copper, iodine, manganese, selenium, and zinc). A final sward composition measurement after all grazing trials had been completed was taken in autumn 2021 (15/09/2021) using methods described previously (see Supplementary Materials for more information).

Sward standing biomass was measured regularly from each grazed paddock throughout each experimental season using a rising plate meter (Jenquip EC09 Plate Meter, Feilding, New Zealand) and taking 30 measurements in a 'W' shape transect across each paddock to determine average height and estimated yield. Sward standing biomass measurements were obtained by calibrating the height data from plate meter with the DM content of the sward at the start of each grazing season (see calibration equation in Farming Connect, 2017). Data is reported as tonnes DM ha$^{-1}$. Sward standing biomass at the start of each grazing season was 1.45 $\pm$ 0.06 t DM ha$^{-1}$ and 1.75 $\pm$ 0.12 t DM ha$^{-1}$ in autumn 2020 and 2.28 $\pm$ 0.10 t DM ha$^{-1}$ and 2.18 $\pm$ 0.04 t DM ha$^{-1}$ in spring 2021 for the herbal and grass-clover ley, respectively. However, as only limited DM measurements were conducted and no accurate calibration equation currently exists for utilising a rising plate meter on complex commercial herbal leys, the supporting sward standing biomass data are only provided as an indicator of growth (see Supplementary Materials).

The sward was cut once during spring 2021 grazing season (21/04/21) to maintain grass quality for grazing (Supplementary Figure 4). Briefly, when the sward height exceeded ca. 10 cm and was not managed by grazing, this was then mown to ca. 5 cm with a Malone Procut 2.4 m disc mower (Malone Farm Machinery, Claremorris County Mayo, Ireland). The mown biomass was not removed from the field.

### 2.4. Sward botanical composition

The botanical composition of each sward was assessed in July 2021 at the end of the spring 2021 grazing experiment. Five 4 m$^2$ quadrats were evaluated in each paddock, accounting for spatial variability, with the resulting Domin scores then converted to percentage cover using the Domin 2.6 transformation described in Currall (1987). Sward botanical composition and overhead photographs of the sward are provided in Supplementary Figures 2 and 3, respectively.

### 2.5. Gastrointestinal parasite burden

Faecal samples were collected from selected lambs from each grazing group ($n = 6$ per sward, per season) at each LWG measurement occasion using the excreta collection pens described previously (see Cooledge et al., *in prep*) (Bangor University ethics committee approval code COESE2019EC01A). Briefly, lambs were held in individual excreta collection pens fitted with slatted livestock flooring (Rimco Ltd., Yorkshire, UK) raised ca. 10 cm above the floor to allow dung to collect below the animal on muslin cloth mesh screens (as described in Marsden et al., 2020). The excreta collection period lasted for approximately 6 hours a day (occurring between 10:00–16:00), with samples immediately collected upon natural defaecation and stored at 4 °C prior to analysis within 24-hours. Lambs were provided with freshly cut forage and water during the collection period, then returned to their respective pastures once a dung sample was obtained.

Individual faecal samples were homogenised prior to analysis using the modified McMaster technique (Zajac and Conboy, 2012) to determine faecal egg count (FEC). Briefly, 2 g of faecal matter was floated in 28 ml of saturated NaCl solution (ca. 6 M) before straining and adding to a glass two-chamber McMaster slide. Gastrointestinal parasitic eggs identified included *Moniezia*, *Nematodirus*, and *Strongylids*. *Coccidia* oocysts were not included in the total FEC score.

Male lambs in autumn 2020 were dosed with Supaverm (10 mg kg$^{-1}$) and Levacur SC (0.25 ml kg$^{-1}$) two months and one month, respectively, before the start of grazing to treat any pre-existing gastrointestinal parasite burden. Female lambs in spring 2021 received Zolvix™ (2.5 mg kg$^{-1}$) 6 weeks prior to the start of grazing. If FEC scores exceeded 750 epg (eggs per gram) at any point in the grazing experiment, then lambs were dosed with Zolvix™ (2.5 mg kg$^{-1}$) to reduce the impact on LWG.

### 2.6. Spring 2021 lamb blood analysis

Blood samples were obtained from spring ewe lambs ($n = 10$ per sward) at the end of the 11-week grazing period to determine differences in general haematology parameters and macro- and micronutrient content between the sward types. Blood was collected via jugular venepuncture under ASPA licence using a 20 g $\times$ 2.5 cm Vacuette® needle (Griener Bio One Ltd, Stonehouse, UK) and stored in a lithium heparin

(LH) vacuum tube (BD vacutainer, Plymouth, UK) and a CAT serum clot activator vacuum tube (BD vacutainer, Plymouth, UK) at 4 °C until extraction.

Blood analysis was conducted by the Nottingham University Veterinary Nutritional Analysis (NUVetNA) lab (University of Nottingham, UK). General haematology parameters haemoglobin and haematocrit were determined by colorimetric assay (Randox Laboratories Ltd., UK) according to manufacturer's instructions on a clinical chemistry analyser (RX IOMA, Randox Laboratories Ltd., UK) and determined by capillary tube, respectively. Superoxide dismutase, caeruloplasmin, β-hydroxybutyrate, NEFA, urea, total protein, albumin, and globulin were determined either via colorimetric assay or using the RX series diagnostic kits (Randox Laboratories Ltd, UK). Glutathione peroxidase was determined following the addition of 25 μl of heparinised blood to 1 ml of RANsel diluent, then analysed using the RANsel kit (Randox Laboratories Ltd, UK). A subsample of plasma was analysed for Vitamin B12 at Axiom Laboratories (Axiom Veterinary Laboratories Ltd., Devon, UK).

Macro- and micronutrients in the serum were analysed by ICP-MS following a 1:20 dilution. Briefly, 0.5 ml serum in 10 ml of dilutant containing 0.5 % $HNO_3$ + 4 % MetOH + Internal standard: Sc (25 μg $l^{-1}$), Ge (10 μg $l^{-1}$), Rh (5 μg $l^{-1}$), and Ir (2.5 μg $l^{-1}$) was placed into 14 ml polypropylene tubes for multi-element analysis. The ICP-MS (Thermo-Fisher iCAP-Q, Thermo-Fisher Scientific, Loughborough, UK) was set to 'flatpole collision cell' mode charged upstream of the analytical quadrupole with helium gas for all elements except Se which was charged with hydrogen gas to reduce polyatomic interferences. Samples were introduced via a covered autosampler (Cetac ASX-520) through a 1317090 pfa-st nebulizer (ESI) (Thermo-Fisher Scientific, Loughborough, UK) with sample processing completed using the 'Qtegra' software (Thermo-Fisher Scientific, Loughborough, UK). Plasma inorganic iodine was determined via ICP-MS using an adapted method by Aumont and Tressol (1987).

### 2.7. Statistical analysis

Data were analysed in R Studio (version 4.2.1) with graphical images produced using the 'ggplot2' package (version 3.3.6, Wickham 2016). Prior to analysis, all data was tested for normality using the Shapiro Wilks test (R core stats package) and homogeneity of variance using the Levene's test ('car' package, version 3.1.1). Sward (autumn 2021: cobalt, selenium and oil-A) and livestock (blood: caeruloplasmin, NEFA, β-hydroxybutyrate, vitamin B12, plasma selenium and plasma inorganic iodine) data that did not meet these assumptions were log transformed. If the log transformation did not work then an alternative non-parametric test (e.g., Kruskal-Wallis test) was conducted where appropriate.

Livestock measurements (liveweight gain and blood characteristics) were analysed within each season only using a t-test; no comparisons were made between grazing seasons due to seasonality differences in lamb sex used in the experiment. Spring lamb body condition score was assessed using a Mann-Whitney-Wilcoxon test. Gastrointestinal parasite burden (i.e., FEC scores) were assessed at the end of each grazing experiment using either a t-test (autumn 2020) or a Kruskal-Wallis test (spring 2021).

As the autumn 2021 sward composition data was not part of the grazing experiments, this was analysed separately using a t-test (see Supplementary Materials for more). Sward standing biomass was analysed using a two-way mixed measures ANOVA, followed by a post-hoc pairwise comparison. The two-way mixed measures ANOVA model included sward type and date as the fixed effects, paddock as the random effect, and explored sward type × date as the interaction. A two-way ANOVA was used to analyse general sward properties and macro- and micronutrient content. The model of the two-way ANOVA assessed sward chemical composition (e.g., crude protein content) and season as the fixed effects, with sward chemical composition × season as the

interaction.

Significance level was set at $p < 0.05$ for all statistical tests. Values presented in the text represent mean ± SEM unless otherwise stated.

## 3. Results

### 3.1. Sward nutritional quality

General sward properties were not influenced by sward type within either grazing season ($p > 0.05$, Table 2 and S1). However, a two-way ANOVA indicated that season had a significant impact on sward properties such as DM ($F_{(1,8)} = 174.5$, $p < 0.001$), crude protein ($F_{(1,8)} = 13.661$, $p = 0.006$), ash ($F_{(1,8)} = 33.937$, $p < 0.001$), and sugar content ($F_{(1,8)} = 23.123$, $p = 0.001$). Dry matter and sugar concentration of both swards were higher in spring 2021 than autumn 2020, where concentrations of crude protein and ash were highest.

Macronutrient concentrations were strongly affected by both season and sward type, with a two-way ANOVA finding a significant interaction between sward and season for sodium, calcium, magnesium, and potassium (Table 2 and S1). Concentrations of sodium ($F_{(1,8)} = 27.780$, $p < 0.001$), calcium ($F_{(1,8)} = 48.270$, $p < 0.001$), and magnesium ($F_{(1,8)} = 77.540$, $p < 0.001$) were greater in the herbal ley than the grass-clover ley in both seasons (Table 2 and S1). Highest levels were measured in

**Table 2**

General sward composition measured from a herbal or a grass-clover ley in autumn 2020 (06/10/20) or spring 2021 (18/05/21). Data represents mean ± SEM, $n = 3$ per sward type, expressed on a dry-weight basis. Lowercase and uppercase letters indicate statistical differences within and between seasons, respectively, following a two-way ANOVA, $p < 0.05$ = significance level, indicated by *.

| Sward Properties | Autumn 2020 | | Spring 2021 | | Interaction between Sward and Season | |
|---|---|---|---|---|---|---|
| | Grass-Clover | Herbal | Grass-Clover | Herbal | $F_{(1,8)}$ | *p*-value |
| Dry matter (g $kg^{-1}$) | 131.0 ± 3.5 [aA] | 133.0 ± 9.7 [aC] | 259.3 ± 13.3 [aB] | 293.3 ± 5.7 [aD] | 1.534 | 0.251 |
| Crude protein (g $kg^{-1}$) | 252.3 ± 3.28 [aA] | 206.7 ± 33.1 [aC] | 126.3 ± 14.7 [aB] | 172.3 ± 23.7 [aC] | 4.465 | 0.068 |
| Sugar (g $kg^{-1}$) | 110.3 ± 8.0 [aA] | 138.3 ± 37.8 [aC] | 213.7 ± 11.1 [aB] | 235.7 ± 11.3 [aC] | 1.436 | 0.265 |
| Metabolisable energy (MJ $kg^{-1}$) | 12.0 ± 0.1 [aA] | 11.5 ± 0.2 [aA] | 12.0 ± 0.1 [aA] | 12.0 ± 0.1 [aA] | 3.658 | 0.092 |
| Neutral-detergent fibre (g $kg^{-1}$) | 401.7 ± 18.6 [aA] | 419.3 ± 52.2 [aA] | 361.3 ± 14.1 [aA] | 351.7 ± 16.6 [aA] | 0.018 | 0.896 |
| D-value (%) | 76.5 ± 0.5 [aA] | 73.0 ± 1.6 [aA] | 76.5 ± 0.8 [aA] | 76.8 ± 0.6 [aA] | 3.780 | 0.088 |
| Ash (g $kg^{-1}$) | 109.3 ± 3.5 [aA] | 108.0 ± 8.5 [aC] | 78.7 ± 5.2 [aB] | 74.0 ± 3.2 [aC] | 0.292 | 0.604 |
| Oil-A (g $kg^{-1}$) | 35.3 ± 0.01 [aA] | 31.3 ± 0.2 [aA] | 30.3 ± 1.9 [aA] | 24.7 ± 2.2 [aA] | 2.612 | 0.145 |
| Nitrate-nitrogen (%) | 0.1 ± 0.1 [aA] | 0.5 ± 0.1 [aA] | 0.1 ± 0.01 [aA] | 0.02 ± 0.1 [aA] | 1.921 | 0.203 |
| Buffering capacity (meq $kg^{-1}$) | 417.0 ± 1.7 [aA] | 411.0 ± 4.2 [aA] | 381.3 ± 11.3 [aA] | 402.7 ± 16.3 [aA] | 1.796 | 0.217 |
| Calcium (g $kg^{-1}$) | 7.2 ± 1.0 [aA] | 14.4 ± 0.3 [bC] | 4.2 ± 0.3 [aB] | 7.8 ± 1.0 [bD] | 39.37 | < 0.001* |
| Magnesium (g $kg^{-1}$) | 1.9 ± 0.1 [aA] | 3.0 ± 0.1 [bC] | 1.1 ± 0.1 [aB] | 1.5 ± 0.1 [bD] | 66.540 | < 0.001* |
| Potassium (g $kg^{-1}$) | 40.9 ± 1.3 [aA] | 37.0 ± 1.0 [aC] | 25.7 ± 0.7 [aB] | 27.1 ± 0.6 [aD] | 6.004 | 0.040* |
| Sodium (g $kg^{-1}$) | 2.1 ± 0.4 [aA] | 6.9 ± 0.8 [bC] | 0.4 ± 0.1 [aB] | 0.9 ± 0.1 [bD] | 26.730 | < 0.001* |

autumn 2020, where concentrations of sodium, calcium, and magnesium were 229 %, 100 %, 58 % greater in the herbal ley, respectively. Potassium concentrations were only affected by season ($F_{(1,8)} = 1933.335$, $p < 0.001$), sward type did not have an effect.

A significant interaction between sward and season was found for iodine ($F_{(1,8)} = 8.245$, $p = 0.021$) and manganese ($F_{(1,8)} = 8.856$, $p = 0.018$) concentrations following a two-way ANOVA (Table 3 and S1). Sward type influenced copper ($F_{(1,8)} = 16.472$, $p = 0.004$), iodine ($F_{(1,8)} = 34.127$, $p < 0.001$), and zinc ($F_{(1,8)} = 6.644$, $p = 0.033$), where concentrations were greater in the herbal ley than the grass-clover in both seasons. However, copper ($F_{(1,8)} = 155.46$, $p < 0.001$), cobalt ($F_{(1,8)} = 25.063$, $p = 0.001$), iodine ($F_{(1,8)} = 84.794$, $p < 0.001$), selenium ($F_{(1,8)} = 6.670$, $p = 0.033$), and zinc ($F_{(1,8)} = 37.705$, $p < 0.001$) were also influenced by seasonal differences, with higher concentrations of each micronutrient found in autumn 2020. Sward micronutrient content was above the recommended content by Kao et al. (2020) for growing lambs in autumn 2020 for cobalt, manganese, and selenium in both swards, but below this recommendation in spring 2021. Copper and zinc only exceeded the recommended level in autumn 2020 in the herbal ley, the grass-clover ley did not reach the recommendation. Iodine did not meet the recommended nutritional content in either season or sward type.

## 3.2. Lamb productivity and health

### 3.2.1. Daily liveweight gain

Sward type only had a significant effect on spring ewe lamb liveweight gain (Fig. 1). In spring 2021, ewe lambs consuming the herbal ley had a 19 % increase in LWG compared to lambs consuming the grass-clover ley (172 ± 7 g d$^{-1}$ herbal *vs.* 144 ± 7 g d$^{-1}$ grass-clover; $T_{(65)} = -2.8677$, $p = 0.006$). However, in autumn 2020, despite a 23 % increase in LWG in ram lambs consuming the herbal ley compared to their grass-clover counterparts this was not statistically significant (146 ± 15 g d$^{-1}$ herbal *vs.* 119 ± 10 g d$^{-1}$ grass-clover; $K_{(1)} = 0.981$, $p = 0.322$).

### 3.2.2. Spring 2021 lamb body condition score

Spring ewe lamb BCS was not affected by sward type ($p > 0.05$, Supplementary Table 3). Lambs consuming either the herbal ($n = 32$) or the grass-clover ley ($n = 36$) only experienced a 0.6 ± 0.1 unit increase in BCS over the 11-week grazing period (W = 556.5, $p = 0.810$). Final lamb BCS was 3.6 ± 0.1 and 3.5 ± 0.1 units in the grass-clover and herbal ley, respectively. Body condition score was not measured in autumn ram lambs.
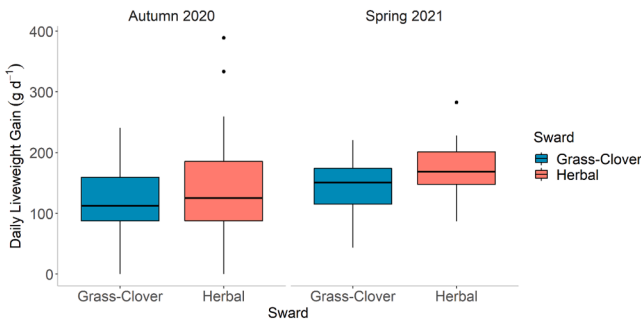


**Fig. 1.** Daily liveweight gain of Welsh mountain lambs consuming either a grass-clover or herbal ley, measured from ram lambs over 6 weeks in autumn 2020 ($n = 34$ grass-clover, $n = 32$ herbal) and ewe lambs over 11 weeks in spring 2021 ($n = 36$ grass-clover, $n = 32$ herbal). Boxplot displays median and interquartile range, with whiskers showing minimum and maximum values in the data, and dots indicating potential outliers.

### 3.2.3. Spring 2021 lamb blood analysis

General haematology parameters (haematocrit and haemoglobin) did not differ significantly and remained within the normal range for ewe lambs consuming either sward ($p > 0.05$, Table 4 and S4). Diet did not affect energy indicators (e.g., Non-Esterified Fatty Acids (NEFAs) or β-hydroxybutyrate) measured at the end of the 11-week grazing period ($p > 0.05$, Table 4). However, concentrations of blood urea were significantly higher in lambs grazing the grass-clover ley ($T_{(18)} = 5.340$, $p < 0.001$; Table 4), with levels 46.4 % and 8.4 % above the normal reference range for lambs grazing a grass-clover and herbal sward, respectively, which may indicate a potential energy-protein imbalance in both diets. Albumin was also elevated above normal ranges in both swards. This was significantly higher in grass-clover ley grazed lambs than the herbal ley lambs ($T_{(18)} = 2.132$, $p = 0.047$), indicative of either potential dehydration or a high protein diet.

Glutathione peroxidase (GSHPx) concentrations were 76 % higher in lambs consuming the herbal ley, placing them within the normal reference range compared to grass-clover grazed lambs ($T_{(18)} = -3.765$, $p = 0.001$). Levels of GSHPx in grass-clover lambs indicated a potential decline in functional selenium status, which was reflected in borderline deficient plasma selenium levels (Table 4). However, despite no statistical difference in concentrations of sward selenium or cobalt, levels of plasma selenium and cobalt in herbal ley grazed lambs were 40 % ($T_{(18)} = -5.183$, $p < 0.001$) and 25 % greater ($T_{(18)} = -2.642$, $p = 0.017$), respectively, than their grass-clover counterparts. Despite higher levels

**Table 3**

Sward trace element content measured from a herbal or a grass-clover ley in autumn 2020 (06/10/20) or spring 2021 (18/05/21). Data represents mean ± SEM expressed on a dry-weight basis. Lowercase letters indicate statistical differences within seasons, uppercase letters denote between season statistical differences following a two-way ANOVA, significance level is set at $p < 0.05$, $n = 3$ per sward type. [1] = Recommended micronutrient content values and typical levels in UK pastures are obtained from Kao et al. (2020). [†] Degrees of freedom = 1,2.

| Micronutrient content (mg kg$^{-1}$ DW) | Recommended micronutrient content for growing lambs[1] (mg kg$^{-1}$ DW) | Typical micronutrient content of UK pastures[1] (mg kg$^{-1}$ DW) | Autumn 2020 | | Spring 2021 | | Interaction between Sward and Season | |
|---|---|---|---|---|---|---|---|---|
| | | | Grass-Clover | Herbal | Grass-Clover | Herbal | $F_{(1,8)}$ | $p$-value |
| Cobalt | 0.2 | 0.05 – 0.25 | 0.42 ± 0.12 $^{aA}$ | 0.31 ± 0.02$^{aC}$ | 0.07 ± 0.006$^{aB}$ | 0.07 ± 0.003$^{aD}$ | 0.871 | 0.377 |
| Copper | 11.0 | 2.0 – 15.0 | 10.37 ± 0.59 $^{aA}$ | 12.27 ± 0.15$^{bC}$ | 4.73 ± 0.03$^{aB}$ | 6.53 ± 0.68$^{bD}$ | 0.012 | 0.915 |
| Iodine | 0.8 | 0.1 – 0.5 | 0.31 ± 0.02 $^{aA}$ | 0.46 ± 0.01$^{aC}$ | 0.20 ± 0.01$^{aB}$ | 0.25 ± 0.02$^{aD}$ | 8.245 | 0.021* |
| Manganese | 40 | 25.0 – 250 | 90.77 ± 8.68 $^{aA}$ | 73.13 ± 4.19 $^{aA}$ | 63.67 ± 2.8 $^{aA}$ | 80.57 ± 5.8 $^{aA}$ | 8.856 | 0.018* |
| Selenium | 0.2 | 0.02 – 0.15 | 0.30 ± 0.06 $^{aA}$ | 0.36 ± 0.16$^{aA}$ | 0.08 ± 0.01$^{aB}$ | 0.12 ± 0.02$^{aD}$ | $^†$0.022 | 0.885 |
| Zinc | 33 | 20.0 – 60.0 | 29.87 ± 1.95 $^{aA}$ | 35.77 ± 3.12 $^{aA}$ | 18.06 ± 0.32$^{aB}$ | 22.63 ± 1.68 $^{aA}$ | 0.108 | 0.751 |

**Table 4**
General haematology, serum biochemistry, and mineral parameters for Welsh mountain ewe lambs measured after 11 weeks of grazing either a herbal ($n = 10$) or grass-clover ($n = 10$) ley in Spring 2021. Results reported as mean $\pm$ SEM. * = indicates a significant result, significance level is $p < 0.05$. [1] = normal reference range values provided by NUVetNA lab, [2] = normal reference range values obtained from MSD Veterinary Manual (Merck & Co., 2022).

| Blood Characteristic | Normal Reference Range for Sheep | Grass-Clover | Herbal | *p*-value |
|---|---|---|---|---|
| Haematocrit (%) | - | 38.7 ± 1.1 | 37.7 ± 1.0 | 0.507 |
| Haemoglobin (g dl$^{-1}$) | - | 13.2 ± 0.3 | 13.3 ± 0.4 | 0.837 |
| Glutathione peroxidase (U ml$^{-1}$ PCV) | 80 – 150[1] | 47.8 ± 6.2 | 84.4 ± 7.5 | 0.001* |
| Plasma Selenium (µmol l$^{-1}$) | 0.5 – 1.0[1] | 0.5 ± 0.01 | 0.7 ± 0.04 | < 0.001* |
| Caeruloplasmin (mg dl$^{-1}$) | 15.0 – 35.0[1] | 27.3 ± 3.2 | 26.7 ± 1.5 | 0.919 |
| Plasma Copper (µmol l$^{-1}$) | 12.0 – 19.0[1] | 15.4 ± 1.2 | 15.4 ± 1.0 | 1.000 |
| Caeruloplasmin/plasma copper (ratio) | 1.7 – 2.0[1] | 1.7 ± 0.1 | 1.7 ± 0.1 | 0.995 |
| Superoxide dismutase (U g$^{-1}$ Hb) | > 2000[1] | 1672 ± 170 | 1528 ± 101 | 0.070 |
| β-hydroxybutyrate (mmol l$^{-1}$) | 0.47 – 0.63[1] | 0.38 ± 0.03 | 0.33 ± 0.02 | 0.214 |
| Non-Esterified Fatty Acids (mmol l$^{-1}$) | < 0.4[1] | 1.1 ± 0.2 | 0.8 ± 0.1 | 0.052 |
| Urea (mmol l$^{-1}$) | 2.8 – 7.1[2] | 10.4 ± 0.4 | 7.7 ± 0.3 | < 0.001* |
| Total Protein (g l$^{-1}$) | 67 – 88[2] | 84 ± 1.2 | 83 ± 1.9 | 0.539 |
| Albumin (g l$^{-1}$) | 24 – 30[2] | 35 ± 0.4 | 34 ± 0.6 | 0.047* |
| Globulin (g l$^{-1}$) | 35 – 57[2] | 49 ± 1.4 | 49 ± 2.0 | 0.968 |
| Plasma Calcium (mmol l$^{-1}$) | 2.88 – 3.2[2] | 2.4 ± 0.02 | 2.4 ± 0.04 | 0.787 |
| Plasma Cobalt (µmol l$^{-1}$) | > 5.0[1] | 1.6 ± 0.1 | 2.0 ± 0.1 | 0.017* |
| Vitamin B12 (pmol l$^{-1}$) | > 400[1] | 353 ± 38.4 | 453 ± 61.4 | 0.261 |
| Plasma Inorganic Iodine (µg l$^{-1}$) | > 100[1] | 48 ± 5.8 | 43 ± 3.5 | 0.562 |
| Plasma Magnesium (mmol l$^{-1}$) | 0.9 – 1.31[2] | 1.1 ± 0.03 | 1.0 ± 0.04 | 0.227 |
| Plasma Manganese (µmol l$^{-1}$) | > 0.9[1] | 0.03 ± 0.001 | 0.05 ± 0.01 | 0.706 |
| Plasma Potassium (mmol l$^{-1}$) | 3.9 – 5.4[2] | 7.9 ± 0.8 | 7.1 ± 0.7 | 0.545 |
| Plasma Sodium (mmol l$^{-1}$) | 139–152[2] | 136.3 ± 1.9 | 138.9 ± 1.7 | 0.315 |
| Plasma Zinc (µmol l$^{-1}$) | 12.3 – 18.5[1] | 12.2 ± 0.3 | 11.6 ± 0.8 | 0.821 |

of sward copper in spring 2021, there was no effect on plasma copper ($p > 0.05$, Table 4).

### 3.2.4. Gastrointestinal parasite burden

Faecal egg count measurements throughout each grazing season are presented in Fig. 2. In autumn 2020, herbal ley sheep #5 was identified as an extreme outlier in week 0 with a starting FEC score of 4550 epg, therefore was removed from the dataset prior to data analysis. By week 4, FEC scores in both grazing groups exceeded the 750 epg dosing threshold, requiring intervention with Zolvix™. Statistical analysis was only conducted on week 4 prior to dosing where there was no difference between grazing groups ($T_{(10)} = -0.348$, $p = 0.735$) and the herbal ley did not suppress gastrointestinal parasite burden. No further FEC measurements were included in the analysis for autumn 2020 as samples measured after week 4 were under both the influence of artificial and natural anthelminthic effects.

Anthelminthic intervention was not required during the grazing experiment in spring 2021. However, sward type did affect the final FEC score in week 11, where the herbal ley reduced gastrointestinal parasite burden by 78 % relative to the grass-clover control ($K_{(1)} = 4.246$, $p = 0.039$).

## 4. Discussion

### 4.1. Sward nutritional quality

The nutritional quality of grasslands is intrinsically linked to its botanical composition, with the inclusion of grasses primarily increasing sward digestibility and fiber content (Belesky et al., 2001; Wilson et al., 2020) and legumes and herbs increasing crude protein and macro- and micronutrient concentrations (Scales et al., 1995; Sanderson, 2010; Darch et al., 2020). Previous research has shown that increasing sward complexity through herbal leys can improve key elements of sward quality, such as metabolisable energy, crude protein content and digestibility (Deak et al., 2009; Komainda et al., 2022). Our study has shown that despite no difference in general nutritional quality (e.g., crude protein content, sugar, metabolisable energy, digestibility) between the herbal and grass-clover ley, the herbal ley had greater concentrations of certain macro- and micronutrients (Tables 2 and 3), allowing us to accept our first hypothesis.

The lack of differences in general nutritional quality between sward types may be explained by the low proportion of herbs (13.8 %) sown in the herbal ley relative to the proportion of grasses (46 %) and legumes (39.9 %), whereas in the grass-clover ley grasses and legumes comprised 90 % and 10 % of the mixture respectively. Although species presence and absence will vary seasonally, a sward botanical survey conducted in July 2021 revealed that of the 18 species sown in the herbal ley, only 11 species persisted after two grazing seasons (Supplementary Figure 2), with key deep-rooting herbs such as *Plantago lanceolata*, *Achillea millefolium* and *Cichorium intybus* comprising 4.9 ± 1.2 %, 6.2 ± 0.7 %, and 21.1 ± 1.3 % of the sward composition, respectively. While the proportion of herbs present in the mixture in this study reflects the typical botanical composition of a commercial herbal ley, the higher proportions of grasses and legumes combined with selective grazing pressure and poor establishment of some species, e.g., *Onobrychis*, can dilute the potential nutritional benefits, resulting in a similar composition to the grass-clover ley (Jing et al., 2017; Grace et al., 2018). Herb-clover swards predominately containing *Cichorium intybus*, *Plantago lanceolata*, *Trifolium pratense* and *Trifolium repens* have shown higher organic matter digestibility and greater metabolisable energy for grazing lambs than herb-grass (containing only *Plantago lanceolata* as the herb), grass-clover, or grass-only mixtures (Golding et al., 2011). Seasonal differences where sward DM, sugar, ME content and digestibility (D-value) were higher in spring than in autumn was likely driven by changes in plant maturity and botanical composition, where maturing plants cause a decline in nutritive quality (Sanderson, 2010).

The herbal ley consistently produced higher concentrations of key macro- and micronutrients than the grass-clover ley across both grazing seasons in this study, with the effect observed to persist in autumn 2021 (see Supplementary Materials for more information). Individual plant species within the herbal ley, e.g., *Cichorium intybus*, are known to accumulate greater concentrations of beneficial and antagonistic nutrients than other common grassland species, e.g., *Lolium perenne* (Scales et al., 1995; Belesky et al., 2001). Higher concentrations of sward sodium, calcium and magnesium within the herbal ley despite no differences in topsoil macronutrient content agrees with previous research, and is likely driven by the dominance of *Cichorium intybus* in the sward in this study and its greater nutrient accumulation ability (Barry, 1998; Høgh-Jensen et al., 2006). *Cichorium intybus* also has deep-rooting capabilities, allowing it to access subsoil nutrients which may explain the higher concentration of sward copper in the herbal ley across both grazing seasons (Belesky et al., 2001). However, the increase in sward copper content in the herbal ley was subject to seasonal variability, only
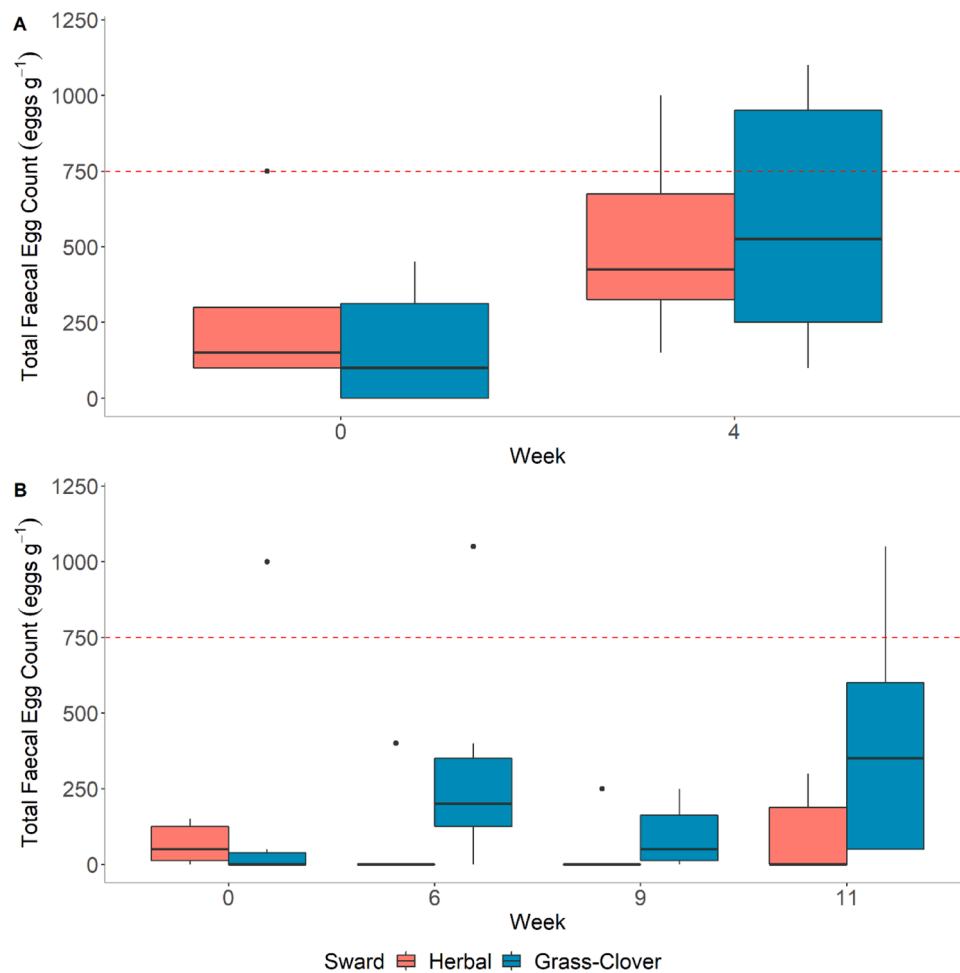
**Fig. 2.** Total faecal egg count (FEC) measured in autumn 2020 (male lambs, $n = 6$ per sward; panel A) and spring 2021 (female lambs, $n = 6$ per sward; panel B) respectively. Total FEC (eggs per gram) includes identified *Moniezia*, *Nematodirus* and *Strongylids* eggs. Dashed line indicates anthelminthic dosing threshold, set at 750 epg. Boxplot displays median and interquartile range, with whiskers showing minimum and maximum values in the data, and dots indicating potential outliers.

meeting the grazing requirements for lambs in autumn 2020, not spring 2021. While promising, the low frequency of sward chemical composition measurements in this study only provides a snapshot of the nutritional benefits of using herbal leys, as these results are influenced by seasonal factors and require further investigation (Kao et al., 2020).

It is difficult to compare our results to previous research as in addition to the botanical composition of the sward varying between studies, the macro- and micronutrient content can be heavily influenced by geographical location (e.g., distance from the sea), field management (e. g., grazing *vs.* mowing), seasonal nutrient availability (e.g., soil moisture and temperature), soil factors (e.g., pH, redox potential, organic matter, microbial activity), and geochemical abundance in the underlying parent material (Watson et al., 2012; Kao et al., 2020; Darch et al., 2022). In this study, the field experiment had a neutral soil pH of ca. 6.9–7.0 which did not limit nutrient availability but was located approximately 450 m from the sea, so may have been subjected to aerosol deposition of salt spray providing an additional external selenium source (Watson et al., 2012). Due to the underlying soil condition, it was not possible to measure soil micronutrient content or subsoil (10–100 cm) macronutrient content in this study. To our knowledge, this is the first study reporting the macro- and micronutrient content of a commercial herbal ley under field conditions, as previous studies have often only examined the chemical composition of species in isolation (e. g., Hamacher et al. 2021) or following pot-scale trials (e.g., Lindström et al. 2013; Darch et al. 2020; Darch et al. 2022). A key limitation of this study that future research should explore is the soil macro- and

micronutrient content in greater detail throughout the soil profile to better understand the relationship between root depth, nutrient availability and uptake in herbal leys using a conventional grass-clover ley as a comparison.

### 4.2. Lamb productivity and health

It was anticipated that the herbal ley would increase lamb liveweight gain and reduce gastrointestinal parasite burden due to the improved sward nutritional quality, greater DM intake, and higher plant secondary metabolite content providing anthelminthic properties (Kenyon et al., 2010; Grace et al., 2019; Jordon et al., 2022). In this study, LWG was only significantly greater in the herbal ley than grass-clover grazed lambs in spring 2021, with ewe lambs gaining an additional 28 g d$^{-1}$ (Fig. 1). This agrees with previous research, where ewes consuming a 6- or 9-species herbal ley had greater LWG than those consuming a *Lolium perenne* sward (Grace et al., 2019). Similarly, lambs consuming a herb-clover or pure herb stands frequently had a greater liveweight gain (Golding et al., 2011; Somasiri et al., 2015; Jerrentrup et al., 2020). This is likely due to the improved sward nutritional quality combined with higher concentrations of plant secondary metabolites present in key plant species (e.g., *Cichorium intybus*, *Plantago lanceolata*) within a herbal ley reducing rumen protein degradation, therefore increasing absorption in the intestines and overall protein utilisation in grazing ruminants (Jordon et al., 2022). This mechanism of improving protein utilisation, combined with a potential increase in DM intake and improved macro-

and micronutrient content, may explain the greater liveweight gain in herbal ley grazed lambs in spring 2021.

Plant secondary metabolites, e.g., sesquiterpene lactones, condensed tannins and saponins, can also provide anthelminthic properties when consumed. Further, livestock have been observed to self-medicate by selectively grazing certain plant species to reduce gastrointestinal parasite burden while carefully balancing any unintended toxicity effects (Peña-Espinoza et al., 2018; Costes-Thiré et al., 2019). Suppression of the naturally acquired gastrointestinal parasite burden was observed in spring 2021, where the herbal ley reduced FEC scores by 78 % relative to the grass-clover control in week 11 (Fig. 2). Although research into the gastrointestinal parasite burden of livestock consuming herbal leys is limited, this finding supports previous research by Grace et al. (2019), which showed lambs consuming a 6- or 9-species herbal ley required less anthelminthic interventions than their grass or grass-clover counterparts. In addition to the plant secondary metabolite content reducing parasite motility, egg production and survival *in vivo*, utilising grassland management options such as increasing the sward height and including plants such as *Cichorium intybus* can inhibit parasitic larvae migration up the plant towards the leaves, thus preventing consumption by the next host (Marley et al., 2003; Hoste et al., 2006; French, 2018; Peña-Espinoza et al., 2018; Jordon et al., 2022). This may partially explain why the parasite burden was suppressed in the herbal ley in the spring 2021 grazing period, as sward height ranged between 10–13 cm and 8–11 cm for the herbal and grass-clover ley respectively.

The lack of differences in both lamb liveweight gain and the reduction in parasite burden in the autumn 2020 grazing season may be due to the high initial FEC scores at the start of the experiment that did not respond to natural anthelminthic intervention when consuming the herbal ley. This required intervention with a broad spectrum synthetic anthelminthic as lambs on both diets hosted a mixed infection of *Moniezia*, *Nematodirus*, and *Strongylids*. This would have subjected lambs to additional physiological stress, with the resulting nutrient depletion and potential reduced voluntary DM intake arising from the high burden diminishing any additional nutritional benefit of grazing the herbal ley (Houdijk et al., 2017). Combined with the high pre-existing burden, potential reduced DM intake, seasonal differences in plant secondary metabolite availability and abundance, along with a shorter sward height during the autumn 2020 grazing period of 4–7 cm and 5–9 cm for the herbal and grass-clover ley respectively, may have contributed to the insignificant increase in LWG (Gilleard et al., 2021). However, the small grazing groups (ca. $n = 40$ per sward) used each season due to field experiment limitations may have hidden any natural variation in LWG. Similarly, the plant secondary metabolite content of both swards and DM intake of grazing lambs was not measured in this study. To our knowledge, there is no available data of the plant secondary metabolite content of commercial herbal ley mixtures that account for the potential dilution effect not explored in single species pot-scale experiments, e.g., Hamacher et al. (2021).

Although no blood measurement was obtained in autumn 2020, the spring 2021 samples obtained after 11-weeks of grazing from the ewe lambs showed an overall positive effect of grazing the herbal ley, with higher concentrations of GSHPx, plasma cobalt and plasma selenium bringing lambs closer to the normal range for their growth stage (Table 4). These improvements were surprising as there was no significant increase in the sward cobalt or selenium content in the herbal ley in spring 2021 (Table 3), however, it is important to note that the bioavailability of these micronutrients was not assessed and may vary with seasonality. It is therefore likely that the increase in plasma selenium and cobalt may be driven by a potential higher DM intake in herbal grazed lambs combined with reduced parasitism (Jordon et al., 2022), or lower cobalt requirements due to slower growth rates in older ewe lambs (ca. 12–13 months old) after 11-weeks of grazing. The reduced blood urea content in herbal grazed lambs despite a similar sward crude protein (126–172 g kg$^{-1}$), sugar (214 – 236 g kg$^{-1}$), and ME (12 MJ kg$^{-1}$) content between diets may be indicative of increased urine urea

excretion caused by plant secondary metabolites such as aucubin (Deaker et al., 1994), or condensed tannins increasing urea 'recycling' within the animal (Azuhnwi et al., 2013).

Alternatively, the higher blood urea content in the grass-clover lambs may be due to a dietary protein and energy imbalance, driven by higher gastrointestinal parasite burden and potential differences in voluntary DM intake. However, the mechanisms driving this are complex, and as it was only possible to measure one timepoint from a limited number of lambs ($n = 10$ per sward), any temporal changes in sward and plasma macro- and micronutrient content may have been missed. While there is currently no literature available on the haematological or plasma biochemical composition of livestock grazing herbal leys to compare our study to, research into lambs grazing single herb species has shown a significant decline in serum urea when lambs consume *Plantago lanceolata* (7.42 mmol l$^{-1}$) compared to *Lolium perenne* (9.41 mmol l$^{-1}$) (Deaker et al., 1994). Similar findings were also reported in dairy heifers consuming a pasture mixture or pure *Cichorium intybus*, where plasma urea concentrations declined from 0.32 g l$^{-1}$ to 0.24 g l$^{-1}$, reducing overall nitrogen excretion (Cheng et al., 2017).

Optimising the plasma cobalt and selenium status in lambs and reducing the need for synthetic anthelminthic interventions through grazing herbal leys has wider economic and environmental implications. Altering the grazing pasture with herbal leys can reduce on-farm greenhouse gas emissions associated with grazing and consequently the carbon footprint of the finished livestock product by removing barriers to improving livestock efficiency, such as micronutrient deficiencies and parasitic challenges (Houdijk et al., 2017). Diet manipulation can reduce the risk of clinical (e.g., white muscle disease, anaemia) and sub-clinical (e.g., limited growth, reduced fertility) diseases arising from micronutrient deficiencies and thus can improve overall livestock health and tolerance to stressors (e.g., gastrointestinal parasite burden) (Kao et al., 2020). This may also reduce additional production costs through reducing the need for additional supplementation (e.g., mineral licks, boluses) and veterinary interventions (Kao et al., 2020).

However, while the lambs in our study consuming the herbal ley did have a reduced deficiency risk for vitamin B12 and selenium compared to grass-clover grazed lambs, lambs on both diets were below the normal range for multiple macro- and micronutrients such as manganese, iodine, cobalt, and calcium which is likely caused by the sward quality not meeting nutrient requirements. Additional supplementation was not provided in our study to allow us to evaluate sward-only effects in a typical lowland grazing system. These nutrient deficiencies are known to be highly soil and site specific. Therefore, future research or farm adoption of herbal leys may face similar challenges and further research is needed across multiple sites to account for botanical and geographical variations and their impact on livestock health. Following these results, we can accept our second hypothesis.

## 5. Conclusion

Improving lamb productivity and health through diet manipulation is vital to reducing the environmental impacts of lowland meat production. This study has shown the seasonal potential for herbal leys to improve ewe lamb LWG, reduce gastrointestinal parasite burden, reduce the risk of micronutrient deficiency disease through increasing plasma cobalt and selenium, and reduce nutrient losses by reducing blood urea content. These differences were potentially driven by higher DM intake and plant secondary metabolite content but require further investigation. Despite an increase in sward macro- and micronutrient content in the herbal ley, no differences were found in general sward nutritional quality or productivity between diets.

To our knowledge, this is the first study to show a commercial herbal ley under field conditions, their effect on sward macro- and micronutrient content and the subsequent impact on lamb health and productivity. While these results show the promise of adopting herbal leys,

further research is needed using longer-term studies across multiple geographical locations to account for variations in sward productivity, composition, climate, and soil type to fully capture the benefits and challenges of wide-scale adoption of herbal leys for livestock production.

## Ethical statement

Liveweight gain and faecal egg count measurements were approved by the Bangor University Ethics Committee (code COESE2019EC01A). Blood samples were obtained under ASPA licence.

## CRediT authorship contribution statement

**Nigel R. Kendall:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Data curation. **Emily C. Cooledge:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **David R. Chadwick:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Conceptualization. **Jonathan R. Leake:** Writing – review & editing, Resources, Funding acquisition. **Davey L. Jones:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Methodology, Funding acquisition, 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.

## Data Availability

Data will be made available on request.

## *Declaration of interest*

The authors declare they have no competing interests.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2024.108991.

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