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Ruminant Nutrition

RUMINANT NUTRITION

Varied diets: implications for lamb performance, rumen characteristics, total antioxidant status, and welfare

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Abstract

Intensive pastoral systems have moved away from diverse and varied diets towards overly simple monotonous diets. Feed choice through time is an obsolete way of providing forage to animals, as intensive management schemes generally allocate a single herbage or a dyad mixed sward. Monotonous feeding regimes impose nutritional repetition, which may impair animal performance and welfare. The objective of this experiment was to determine the impact of a diverse diet [DIV; free choice from perennial ryegrass (*Lolium perenne* L.), plantain (*Plantago lanceolata* L.), alfalfa (*Medicago sativa* L.), and chicory (*Cichorium intybus* L.) at all times], a varied diet [VAR; choice from ryegrass and plantain in the AM (0700–1600 h), and chicory and alfalfa in PM (1600–0700 h)], and a single forage diet of alfalfa [SFA; alfalfa at all times], on DMI, performance, and welfare of lambs. Six-month-old Coopworth ram lambs (n = 21) were offered their respective fresh-forage treatment (n = 7) diet indoors for 20 d. The DIV lambs consumed 1.64 ± 0.03 kg DM/d (mean ± SEm), which was 6% more ($P < 0.05$; 1.54 ± 0.03 kg DM/d) than the SFA and were not different ($P > 0.05$; 1.59 ± 0.03 kg DM/d) to the VAR lambs. Average daily gain (ADG) of DIV (296 g/d) and VAR (378 g/d) was 30% and 67% greater ($P < 0.05$) than that in the SFA lambs (227 g/d), respectively. The VAR lambs had 28% greater ($P < 0.05$) ADG than the DIV lambs. Differences among treatments were detected ($P < 0.05$) for the proportion of the day spent conducting the following behaviors: eating, ruminating, idling, lying, and standing. In addition, the number of bouts of stereotypic behaviors recorded from the SFA lambs (13.2 ± 2.2) was 150% greater ($P < 0.05$) than the DIV (5.1 ± 1.0) and VAR (5.5 ± 1.0) lambs. Our results suggest that the varied diet offered can improve animal performance and welfare compared to a monotonous SFA diet. Feeding management to provide a varied diet can improve performance relative to giving lambs free choice from taxonomically diverse forage options. Moreover, performance is affected by more than the primary chemical composition of the diet consumed, but how the diet is presented through time and the herbage species and quantities of each that are consumed to reach that chemical composition.

Key words: alfalfa, diverse, monotony, sheep, varied

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Abbreviations

ADF	acid detergent fiber
ADG	average daily gain
ADMD	apparent dry matter digestibility
CP	crude protein
DM	dry matter
DMD	dry matter digestibility
DMI	dry matter intake
DOMD	digestible organic matter in dry matter
FCE	feed conversion efficiency
GPx	glutathione peroxidase
LW	live weight
NDF	neutral detergent fiber
NIRs	near infrared spectrophotometry
OM	organic matter
OMD	organic matter digestibility
PSC	plant secondary compounds
SFA	single forage diet of alfalfa
TAS	total antioxidant status
VFA	volatile fatty acid
WSC	water soluble carbohydrate

Introduction

Ancestors of today's ruminants selected from a range of biochemically diverse plant species within their given foodscape, of which species availability, abundance, and chemical composition changed over time and space (Provenza et al., 2007). As such diversity is multifaceted and encompasses a) species or component richness, b) the abundance of each of the given species or component, c) how the species or component are distributed through space, d) the individuality within species (e.g., genotypic variation and resource utilization, and how a–d vary through the temporal scale (Tilman et al., 1997, Purvis and Hector, 2000; Mason et al., 2005; Mouillot et al., 2005). Due to the term diversity encompassing the availability of feeds at a given site and how they vary through time, we propose for the purpose of this paper that a diverse diet will describe the availability of feeds at a given site and that a varied diet will describe changing feed availability through time (within the day scale). Varying diet availability can be used as a grazing management tool and is being implemented successfully in extensive pastoral systems to increase animal performance and enhance ecosystems health (Meuret and Provenza, 2015). Intensive pastoral systems though have turned away from diverse and varied diets towards repeated allocation (monotony) of simple diets where animals do not get to make a feed choice, as single or dyad mix sward favor easier, less complex practical implementation and management.

Monotonous feeding environments impose nutritional repetition, which may impair animal performance and welfare. Monotonous diet presentation reduces intake or productivity relative to animals provided choice from a diverse diet (Provenza et al., 2007; Rodríguez et al., 2007; Dixon and Pasinetti, 2010; Garrett et al., 2021). This can be the result of nutrient specific satiation, as the upper threshold for a specific nutrient is reached despite deficiencies in other nutrients existing (Raubenheimer, 1992; Early and Provenza, 1998; Gregorini et al., 2017), or sensory-specific satiety, where the repeated oro-sensorial experience (i.e., taste) saturates the intake-related sensorial neurons and reduces the response for a particular feed (Early and Provenza, 1998; Epstein et al., 2009; Gregorini et al., 2017). On the other hand, diverse diets that allow selectivity, enable individual animals to choose from feeds of differing nutrient and oro-sensorial profiles and can result in improved intake or performance (Villalba and Provenza, 1997; Papachristou et al., 2007; Rodríguez et al., 2007; Mote et al., 2008; Catanese et al., 2013; Garrett et al., 2021). In addition, varied diets can also improve DMI relative to repeated allocation of a single feed and specific sequences of diet allocation can improve intake relative to other sequences (Mote et al., 2008; Jensen et al., 2013). For example, Mote et al. (2008) reported that offering sheep feed rich in tannins before a feed rich in terpenes doubled intake compared with a meal offered in the reverse order. Although there is information regarding increased DMI when feeding a range or specific sequences of plant secondary compounds (PSC; e.g., tannins and terpenes), less information is known on the effect of varied diets of fresh forages can have on the DMI, performance, and welfare of animals compared with those consuming a diverse diet of the same components or a single monotonous diet.

We hypothesize that a varied diet of fresh forages over the day will improve intake, performance, and welfare of lambs compared to dietary monotony and dietary diversity of the same herbage species on offer at the same time, all day. We also hypothesize that a diverse diet of fresh forages over the day will improve intake, performance, and welfare of lambs compared to single forage monotony. As a result, our objective was to compare the DMI, ADG, and welfare of lambs fed a monotonous single forage diet of alfalfa (SFA), choice from diverse (DIV) diet components ryegrass (*Lolium perenne* L.), plantain (*Plantago lanceolata* L.), alfalfa (*Medicago sativa* L.), and chicory (*Cichorium intybus* L.), and a varied (VAR) diet comprised of the diverse diet components offered in a sequence through time. Alfalfa was chosen to be fed in monotony and to be compared against the DIV and VAR diets as it is often used within New Zealand farming systems as a specialty finishing diet due to its ability to provide large amounts of high-quality forage (Brown et al., 2000; Avery et al., 2008; Anderson et al., 2014; Moot et al., 2019). The chosen diverse multi-forage diet increases DMI and ADG relative to a monotonous ryegrass diet (Garrett et al., 2021). Comparing this diverse diet to another species, known as a high-performing and quality diet for finishing lambs in dryland settings, will allow us to determine if the effects reported in previous work from our laboratory were diet specific. Further, comparing the VAR and DIV diet will allow us to determine if a temporal approach to grazing management can improve performance relative to animals with free choice from the same species.

Materials and Methods

This study was conducted at the Lincoln University Johnstone Memorial Laboratory (43°38'57"S, 172°27'01"E), as per the methods approved by the Lincoln University Animal Ethics Committee (#2019-33A).

Animal management and dietary treatments

Six-month-old Coopworth rams (n = 21) with an average live weight (LW) of 33.55 ± 0.51 kg (mean ± SEm) were housed indoors in individual pens for 20 d starting on March 3, 2020. Animals were randomly allocated to one of three treatments: SFA (monotony of alfalfa), DIV (free choice of ryegrass, alfalfa, chicory, and plantain), or VAR (selection from ryegrass and plantain in the AM [0700 h to 1600 h] and selection from alfalfa and chicory in the PM [1600 h to 0700 h]). The sequence was selected as it was one where lambs performed better in terms of

DMI within a trial by Garrett et al. (Unpublished) where animals received all possible feeding combination. Further, this sequence provided animals with access to a legume for the greatest proportion of their time. When offered the choice ruminants have a greater partial preference towards legumes, allocating on average 70% of their time to grazing legumes in a grass or legume choice scenario (Rutter, 2010). Prior to experiment initiation all animals had been grazing alfalfa and had been reared together, thereby had the same early life dietary experience. Animals in the DIV diets were presented all four feeds in individual bins simultaneously, with two feeds placed at each end of the pen. The diet options available to the VAR animals at a given time were presented simultaneously, with one forage option available from bins at each end of the pen. The end of the pen that each forage occupied was randomly assigned for each pen and maintained for the duration of the trial.

All treatments were offered fresh forage daily at 0700 h and pens were cleaned prior to forage allocation. The VAR treatment was presented their PM options and the AM options were removed at 1600 h. Lambs on the SFA and DIV diets were also presented a PM diet allocation of their respective diets at 1600 h to eliminate any frequency of feed presentation effects. Each sheep had *ad libitum* access to their allocated treatment diet and fresh water. Orts from the previous feeding were weighed at 0700 and 1600 h prior to the allocation of fresh feed.

Herbage composition, establishment, and harvesting

Planting preparation included defoliation of existing herbages and application of glyphosphate (Weedmaster Ts540; 4 L/ha), fluroxypyr (Starane Xtra Herbicide; 1 L/ha), Carfentrazone-E (Hammer Force; 0.1 L/ha), and Polyalkyleneoxide (Slikka; 0.15 L/ha). Seven days after spraying the area was ploughed and power harrowed. The areas planted with alfalfa, and chicory had Trifluralin (2 lts/ha) applied and incorporated appropriately. On October 26, 2019, a direct drill calibrated to each forage species with 7.6 cm row spacing was used to plant each species as a monoculture in spatially separated strips. Seeding rate was 25, 12, 16, and 14 kg/ha for ryegrass (cv. Legion), chicory (cv. Choice), alfalfa (cv. Titan), and plantain (cv. Agritonic), respectively. The established plantain and ryegrass were treated with Dicamba (Kamba 500; 0.4 L/ha) and application of Flumetsulum (Preside; 60g/ha) and mineral oil (Uptake; 1 L/ha) occurred for chicory, clover, and alfalfa pastures. The area was fertilized with 250-kg diammonium phosphate.

Fresh herbage was cut daily ~3 cm above ground level with a Haldrup forage harvester (Haldrup GmbH, Ilshofen, Germany). Feed was otherwise fed whole and un-cut. Once cut, feed was stored in a walk-in refrigerator (4 °C) until it was allocated, and unutilized feed was disposed of within 2 d post-harvest. Feed stored for more than a day was kept for topping up herbage if the fresh forage from the relevant day ran short. Samples of allocated and refused herbages were taken at each feeding to determine the feed quality and DM consumed.

Herbage chemical composition of the individual species included in the diets is presented in Table 1, and the average chemical composition of the diets consumed is presented in Table 2. Chicory and alfalfa were all in a vegetative state, whereas plantain and ryegrass contained 19.0% and 6.9% stem, respectively. The extended shoot leaf height of the chicory, alfalfa, plantain, and ryegrass was 26.6 ± 2.6, 50.2 ± 2.4, 32.0 ± 2.9, and 21.9 ± 2.9 cm, respectively.

Animal sampling and measurements

On days 13 and 18, blood samples were collected at (09:30 h [0 h], 15:30 h [6 h], and 21:30 h [12 h]) to determine total antioxidant status (TAS) and glutathione peroxidase (GPx) concentration. Blood samples were obtained via jugular venipuncture and collected in 10-mL lithium heparinized blood tubes (Greiner Bio-One International GmbH, Kremsmünster, Austria). Whole blood subsamples were collected, plasma samples were collected by centrifuging (Megafuge 1.0R, Heraeus Holding GmbH, Hanau, Germany) the remaining sample at 2,300 × *g* and 4 °C for 15 min, and samples were then stored at –20 °C until analysis. Rumen fluid was obtained via esophageal tubing on days 1 and 17, an hour after the allocation of feed during the AM and PM, to allow comparison of rumen characteristics (e.g., ammonia concentration). Rumen samples were sub-sampled into three 2-mL Eppendorf tubes, one acidified with sulphuric acid (10-µL of 98% sulfuric acid; Fisher Scientific, Loughborough, UK) and two without. Animals live weights were measured every 5 d, before the morning feed allocation. Average daily gain (ADG) was estimated for each individual animal by regression and feed conversion efficiency (FCE; g ADG/kg DMI) was calculated.

Trained observers conducted behavioral observations during daylight hours on days 9 (0700–2010 h) and 20 (0734–1942 h). During daylight hours throughout the trial, artificial lighting was used. Observers scan sampled (Altmann, 1974; Villalba et al., 2015), recording the behavior of each animal every 2 min. An ethogram of the behaviors is presented in

Table 1. Chemical composition of the herbages eaten composing the total diets

	Herbage				
Item[1]	Chicory	Alfalfa	Plantain	Ryegrass	SEm[2]
DM, % as-is	13.34[b]	21.67[a]	12.56[b]	22.86[a]	0.63
OM, %DM	86.80[c]	91.00[b]	91.72[ab]	92.01[a]	0.36
CP, % DM	12.27[c]	21.11[a]	13.39[c]	16.17[b]	0.60
NDF, % DM	16.85[c]	25.46[b]	24.54[b]	49.91[a]	1.17
ADF, % DM	18.59[c]	23.27[b]	21.90[b]	30.94[a]	0.79
WSC, % DM	18.17[b]	11.81[c]	29.40[a]	16.71[b]	0.98
DMD, %DM	83.86[a]	72.98[bc]	75.00[b]	71.46[c]	1.16
OMD, % OM	88.61[a]	76.62[c]	81.39[b]	76.47[c]	1.27
ME, MJ/kg DM	12.80[a]	11.13[b]	12.53[a]	11.70[b]	0.26

[1]DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; WSC, water soluble carbohydrates; DMD, dry matter digestibility; OMD, OM digestibility; ME, metabolizable energy.
[2]SEm, standard error of the mean.
[a–d]Means in a row with different superscripts are statistically different ($P \leq 0.05$).

Table 2. Chemical composition of the single forage alfalfa (SFA) diet and the calculated chemical composition of the diverse (DIV) and varied (VAR) diet consumed by the ram lambs

	Treatment diet[2]				
Item[1]	SFA	DIV	VAR	SEM	P[3]
DM, % as-fed	21.67[a]	16.82[b]	16.75[b]	0.76	<0.01
OM, % DM	91.33[a]	90.10[b]	90.29[b]	0.39	0.03
CP, % DM	20.49[a]	17.17[b]	16.41[b]	0.68	<0.01
NDF, % DM	28.27	25.18	26.63	1.49	0.22
ADF, % DM	25.08	22.89	22.88	0.58	0.07
WSC, % DM	11.29[b]	17.33[a]	18.36 [a]	1.11	<0.01
DMD, % DM	70.87[b]	75.94[a]	76.40[a]	1.17	<0.01
OMD, % DM	74.08[b]	81.08[a]	81.03[a]	0.90	<0.01
ME, MJ/kg DM	10.78[b]	11.94[a]	12.08[a]	0.16	<0.01

[1]ME, metabolizable energy; DM, dry matter; OM, organic matter; OMD, OM digestibility; WSC, water-soluble carbohydrates; NDF, neutral detergent fiber; ADF, acid detergent fiber; CP, crude protein.
[2]Values for diverse diet chemical composition were calculated by using the percentage of the Item value that each dietary component accounted for. SFA, monotonous alfalfa diet; DIV, free choice of diverse diet components: chicory, ryegrass, plantain, and alfalfa; VAR, free choice of plantain and ryegrass in the morning and chicory and alfalfa in the afternoon.
[3]P, t-test *P*-value.
[a–c]Means in a row with different superscripts are statistically different ($P \leq 0.05$).

Table 3. Ethogram of recorded behavioral activities and the category they were considered under

Behavior	Description
Eating	Eating specific was recorded
Idle	Sheep not engaged in any of the other listed behaviors
Ruminating	Sheep is ruminating
Position	
Standing	Sheep is in an upright position
Lying	Sheep is lying down
Stereotypic behaviors	
Pacing	Walking in a distinct pattern, such as frequent walking back and forth, weaving, or moving in circles
Chewing pen fixtures	Chewing pen fixtures (e.g., feed bin and bars)
Head butting pen fixtures	Butting pen fixtures
Head hanging	Standing quietly with head drooped down
Crouching	Crouching in fear (usually to human activity)
Pawing or stamping	Striking ground with forelegs
Rearing	Head raised with forelegs on pen or off ground, back legs on ground
Grooming behaviors	
Scratching	Scratching self
Rubbing	Rubbing on pen fixtures

Table 3. The behaviors recorded were based on previous studies (Done-Currie et al., 1984; Lauber et al., 2012; Catanese et al., 2013): eating (consumption of which feed was specified for VAR and DIV animals), ruminating, idle, and position of the animal (standing or lying down) were recorded. In addition to the scan samples, observers also documented the occurrences of stereotypic behaviors, which are repeated behaviors with no apparent function and are indicative of poor welfare (Broom, 1991; Catanese et al., 2013), and grooming behaviors. Stereotypic behaviors were considered to be the sum of incidences of pacing, chewing, head butting, head hanging, pawing or stamping, rearing, or crouching (cowering). Grooming was considered as the sum of time spent scratching one's self and rubbing on pen fixtures.

Sample analysis

Herbage samples were thoroughly mixed and subsampled into three parts, to determine botanical composition, DM, and herbage chemical composition. The subsample of herbage taken to determine DM was weighed, dried at 60 °C for 7 d, and re-weighed dry. Botanical and chemical composition samples were analyzed every 4 d. The botanical sub-samples of the herbage were also dried at 60 °C for 7 d after sorting into leaf, stem, weeds, and dead material. The chemical composition of freeze-dried and ground (ZM200; Retsch, Haan, Germany; 1mm screen) herbage samples was determined using near-infrared spectrophotometry (NIRS; Model: FOSS NIRS Systems 5000, Maryland, USA). Chemical composition values used for NIRS calibration were derived before sample analysis for DM (AOAC International, 1990; method 930.15), organic matter (OM; 100% minus ash%; AOAC International, 1990; method 942.05), neutral detergent fiber (NDF; Van Soest et al., 1991), acid detergent fiber (ADF; AOAC International, 1990; method 973.18), water-soluble carbohydrates (WSC; MAFF, 1986), digestible OM in DM (DOMD), DM digestibility (DMD), OM digestibility (OMD; Iowerth et al., 1975), and crude protein (CP) by combustion (Variomax CN Analyser; Elementar Analysensysteme, Hanau, Germany). The NIRS calibration equations all had R^2 values greater than 0.90 and were within the calibration range. Herbage metabolizable energy (ME) was estimated using the Primary Industries Standing Committee (2007) equation:

$$[ME\ (MJ/kgDM) = digestible\ OM\ in\ DM,\ \%\ (DOMD) \times 0.16] \quad [1]$$

The GPx content of the whole blood samples was determined using an enzymatic-based protocol (RANSEL; Cat. No. RS504) and a clinical analyzer (Randox Rx Daytona, Crumlin, Co. Antrim, UK). The TAS content of the plasma was determined using a colormetric method on the clinical analyzer using a commercial kit (Cat. No. NX2332; Randox Rx Daytona, Crumlin, Co. Antrim, UK).

Ammonia concentration of the acidified rumen samples was measured using a clinical analyzer (Randox Rx Daytona, Crumlin, Co. Antrim, UK) and a commercial test kit (Cat. No. AM3979; Randox; Crumlin, Co. Antrim, UK) based on the enzymatic UV method described by Neeley and Phillipson (1988). Rumen volatile fatty acid (VFA) concentration of the non-acidified samples was determined using a Gas Chromatograph (GC: Shimadzu GC-2010, Kyoto, Japan with AOC-20i auto-sampler) fitted with a SGE BP21 30 m × 530 µm × 1 µm wide-bore capillary column as described by Chen and Lifschitz (1989). The lactate concentration of the non-acidified rumen fluid was determined using a separate commercial kit (Cat. No. LC2389; Randox; Crumlin, Co. Antrim, UK) and the Randox Rx Daytona clinical analyzer.

Statistical analysis

Statistical analysis was conducted using R (R Core Team, 2020, v.3.6.0). All normally distributed data ($P > 0.10$; Shapiro–Wilk test) that had homogenous variance ($P > 0.10$; Bartlett's test) were analyzed using an analysis of variance (ANOVA) using the 'aov' function. Data analyzed using 'aov' function included DMI, ADG, and FCE. Non-normally distributed data were analyzed by a generalized linear model (GLM) using the 'glm' function of R (R Core Team, 2020) with the distribution used for the model selected based on qq-plots of the residuals. Non-normally distributed data included rumen ammonia, rumen glucogenic VFA, rumen non-glucogenic VFA, and total VFA. Differences in chemical composition among herbages and diets were tested using the 'lme' mixed model function, with day as a random effect. The d1 samples (pre-treatment) for the rumen variables were explored as covariates and included in the model as they explained a significant ($P < 0.05$) amount of the variation in rumen parameters. The ANOVA and GLM models that contained repeated measures (i.e., blood and rumen variables) included diet, time, and the diet × time interaction as fixed effects. The models for variables of averaged data or that were not repeatedly measured (i.e. DMI, ADG, and FCE) contained diet as fixed effects. The behavior data model included the treatment, observation time (morning = dawn to noon; afternoon = noon to dusk), observation days, and their interactions as fixed effects. Upon significance of the ANOVA, means separation among treatments was conducted by a pairwise t-test using the 'emmeans' package (Lenth, 2018). The DMI data were used to calculate the within animal day-to-day coefficient of variation (CV) of DMI. Pearson's correlation co-efficient between the day-to-day variability in DMI (CV) and DMI, ADG, and FCE was determined using the 'cor.test' function of R (R Core Team, 2020). Statistical significance was declared at $P \leq 0.05$ with tendencies declared at $0.05 < P \leq 0.10$.

Results

Diet composition

The DM of the alfalfa and ryegrass herbages was not different from one another ($P > 0.05$); however, their DM was 72% greater ($P < 0.05$) than chicory and plantain, which were not different from one another ($P > 0.05$; Table 1). Although the ME of alfalfa and ryegrass was not different ($P > 0.05$), they were 10% lower than chicory and plantain ($P < 0.05$), which were not different ($P > 0.05$). Chicory and plantain had the least ($P > 0.05$) CP, ryegrass was intermediate ($P < 0.05$), and alfalfa had the greatest CP content ($P < 0.05$). The chemical compositions of each diet consumed are reported in Table 2. There were no differences ($P > 0.05$) in the chemical composition (e.g., ME, DM, DMD, OM, OMD, WSC, and CP) between the DIV and VAR diets. There were no treatment differences for NDF contents of the diets consumed ($P = 0.22$); however, there was a tendency ($P = 0.07$) for the SFA diet to have a greater ADF content than the DIV and VAR diets. The ME and WSC content of the DIV and VAR diets was greater ($P < 0.05$) than the SFA diet. Conversely, the CP content of the SFA diet was greater ($P<0.05$) than both the DIV and VAR diets. Leaf comprised 87.4%, 77.6%, 96.1%, and 81.4 ± 2.9% of the DM, respectively, for chicory, plantain, alfalfa, and ryegrass, respectively. The DM comprised of weed for chicory, plantain, alfalfa, and ryegrass was 11.3%, 0.9%, 3.7%, and 0.8 ± 2.9%, respectively, whereas dead matter made up 1.3%, 2.5%, 0.2%, and 10.9 ± 2.9% for chicory, plantain, alfalfa, and ryegrass, respectively. Only plantain and ryegrass had any stem material, with 19.0% and 6.9 ± 3.1% each.

Forage DMI and ADG

The DIV lambs consumed 6% more ($P = 0.01$) total DM compared with SFA lambs, whereas the VAR lambs were intermediate and not different ($P = 0.15$) compared to the other treatments (Table 4). Although the DMI of the DIV and VAR treatments was not different, the proportions of species they consumed to reach that level of intake differed. The SFA treatment consumed 163% more ($P < 0.01$) alfalfa on a DM-basis compared to the DIV or VAR lambs, which did not differ ($P > 0.05$) in alfalfa intake. The VAR lambs had a 14% greater chicory intake ($P < 0.05$), but 26 % less plantain intake ($P < 0.05$) compared with the DIV lambs. There was a tendency for the VAR lambs to consume more DM as ryegrass than the DIV lambs ($P < 0.10$). The within animal between days DMI CV was 30% greater for the SFA treatment compared with the VAR treatment ($P < 0.05$). There was a tendency for the DIV lambs to have a lower within animal between day DMI CV compared to the SFA lambs ($P = 0.08$); however, there was no difference ($P > 0.05$) among the DIV and VAR lambs ($P > 0.05$). The ADG of DIV lambs (296 g/d) was 30% greater ($P < 0.05$) compared with SFA (227 g/d). The ADG for VAR lambs (378 g/d) was 28% and 67% greater ($P < 0.05$) than the DIV and SFA lambs, respectively. The FCE of VAR was 63% greater ($P < 0.01$) than the SFA lambs and 30% greater ($P < 0.05$) than the DIV lambs. The DIV lambs tended ($P < 0.10$) to have a 25% greater FCE compared with the SFA lambs.

Rumen and blood

Rumen ammonia (NH_3) concentration at the morning sampling (0800 h) was 287% greater for the SFA lambs compared with the VAR lambs, whereas DIV was intermediate and different ($P < 0.05$) from SFA and VAR (Table 5). At the afternoon sampling (1700 h), rumen NH_3 concentrations of VAR and DIV were not different ($P > 0.05$), but they were both lower ($P < 0.05$) than the SFA lambs. Although the rumen NH_3 concentrations did not differ ($P > 0.05$) between the morning and afternoon for the SFA and VAR, the rumen NH_3 of the DIV treatment was lower ($P < 0.05$) in the afternoon compared to the morning.

There was no interaction between time of day and treatment ($P = 0.50$) and there was no overall treatment effect ($P = 0.13$) on total VFA concentration, but there was a time of day effect ($P = 0.02$), with total VFA concentration being greater in the afternoon. A time of day × treatment interaction ($P = 0.03$) was detected for the acetate to propionate ratio, with VAR having lower ($P < 0.05$) acetate to propionate ratio than the DIV and SFA in the morning, but no effect was detected ($P > 0.05$) in the afternoon. There was a tendency ($P = 0.07$) for a time of day × treatment interaction for the percentage of VFA that were

Table 4. Mean dry matter intake, growth, and feed conversion efficiency of ram lambs fed a varied (VAR), diverse (DIV), and single forage alfalfa (SFA) diet over 20 d

	Treatment[2]				
Item[1]	SFA	DIV	VAR	SEM	P[3]
Initial LW, kg	33.9	33.9	32.8	1.0	0.63
Total DMI, kg DM/ d	1.54[b]	1.64[a]	1.59[ab]	0.03	0.04
AM	0.70[a]	0.70[a]	0.41[b]	0.02	<0.01
PM	0.84[c]	0.94[b]	1.18[a]	0.03	<0.01
Alfalfa DMI, kg DM/ d	1.54[a]	0.62[b]	0.55[b]	0.03	<0.01
AM	0.70[a]	0.30[b]	–	0.01	<0.01
PM	0.84[a]	0.32[c]	0.55[b]	0.02	<0.01
Chicory DMI, kg DM/ d	–	0.58[b]	0.66[a]	0.02	<0.01
AM	–	0.23	–	0.01	<0.01
PM	–	0.35[b]	0.66[a]	0.01	<0.01
Plantain DMI, kg DM/ d	–	0.31[a]	0.23[b]	0.01	<0.01
AM	–	0.12[b]	0.23[a]	0.01	<0.01
PM	–	0.19	–	0.01	<0.01
Ryegrass DMI, kg DM/ d	–	0.13	0.15	0.01	0.08
AM	–	0.05[b]	0.15[a]	0.01	<0.01
PM	–	0.08	–	0.01	<0.01
DMI CV, %	22.34	17.44	17.13	2.35	0.10
ADG, g BW/d	227[c]	296[b]	378[a]	22	<0.01
FCE, gBWgain/ kg DMI	146[b]	183[b]	238[a]	14	<0.01

[1]Initial LW, initial live weight; DMI, dry matter intake; DMI CV, day-to-day DMI co-efficient of variation; ADG, average daily gain; FCE, feed conversion efficiency; AM, 0700 to 1600 h; PM, 1600 to 0700 h.
[2]SFA, single forage diet of alfalfa; DIV, free choice of diverse diet components: chicory, ryegrass, plantain, and alfalfa; VAR, free choice of plantain and ryegrass from 0700 to 1600 h and chicory and alfalfa between 1600 and 0700 h.
[3]P, t-test P-value.
[a–c]Means in a row with different superscripts are statistically different ($P \leq 0.05$).

Table 5. Rumen ammonia (NH_3) and rumen volatile fatty acid (VFA) profile of ram lambs on day 17 in the morning and afternoon

	Treatments[2]								
	SFA		DIV		VAR		P-value[3]		
Item[1]	AM	PM	AM	PM	AM	PM	Time	TRT	Time×TRT
NH_3, mmol/L	16.13[a] ±1.06	14.04[a]±1.06	8.72[b] ±1.11	5.57[c] ±1.11	4.16[c] ±1.06	5.28[c] ±1.03	0.14	<0.01	0.11
Total VFA, mmol/L	139[ab]± 9	145[a] ±9	113[bc] ±10	141[a] ±10	112[c] ±10	134[abc]±10	0.02	0.13	0.50
Ace:Prop, ratio	3.06[a]±0.16	2.90[a]±0.16	2.88[a]±0.17	2.67[ab]±0.17	2.31[b]±0.16	2.86[a]±0.16	0.34	0.04	0.03
VFA profile, %									
Glucogenic	23.98±1.06	24.75±1.06	24.87±1.16	26.38±1.16	27.57±1.07	24.40±1.07	0.66	0.28	0.07
Nonglucogenic	76.02±1.06	75.25±1.06	75.13±1.16	73.62±1.16	72.43±1.07	75.60±1.07			

[1]NH_3, ammonia, mmol/L; Total VFA, total volatile fatty acid (mmol/L); Ace:Prop ratio, ratio of acetate to propionate; Gluc., glucogenic VFAs; Non., non-glucogenic VFAs. Hexanoic and lactic acid were not included as the amounts present were below the detection limit gas chromatogram.
[2]SFA, single forage diet of alfalfa; DIV, free choice of diverse diet components: chicory, ryegrass, plantain, and alfalfa; VAR, free choice of plantain and ryegrass from 0700 to 1600 h and chicory and alfalfa between 1600 and 0700 h; Mean ± Standard error of the mean.
[3]t-test P-value.
[a–c]Means in a row with different superscripts are statistically different ($P \leq 0.05$).

glucogenic and non-glucogenic, with the VAR treatment having a greater percentage of glucogenic VFA in the morning.

Time of day × treatment interaction for plasma TAS ($P < 0.01$) was detected (Figure 1). No difference was detected among treatments at 0 ($P > 0.05$); however, at 6 h the VAR treatment TAS (1.36 ± 0.02 mmol/L) was lower than the DIV (1.44 ± 0.03 mmol/L; $P < 0.01$) and SFA (1.43 ± 0.02 mmol/L; $P = 0.02$), which were not different from one another ($P = 0.69$), and at 12 h the VAR treatment (1.60 ± 0.03 mmol/L) had a TAS concentration that was 11% greater than the SFA (1.44 ± 0.03 mmol/L; $P < 0.01$). At 12 h, the DIV lambs' TAS concentration (1.56 ± 0.03 mmol/L) was 8% greater than the SFA ($P = 0.03$) but not different from the VAR treatment ($P = 0.56$). The TAS concentration of all treatments was greater at the 12 h measurement compared to the 0 h measurement ($P < 0.05$). There was only a time of day effect on GPx concentration ($P < 0.05$), with GPx concentrations being greater earlier in the day.

Behavioral observations

There was a treatment ($P < 0.01$; Table 6) and time effect ($P < 0.01$) on the proportion of time spent eating in the morning and afternoon, and a treatment effect ($P < 0.01$) over the whole day.

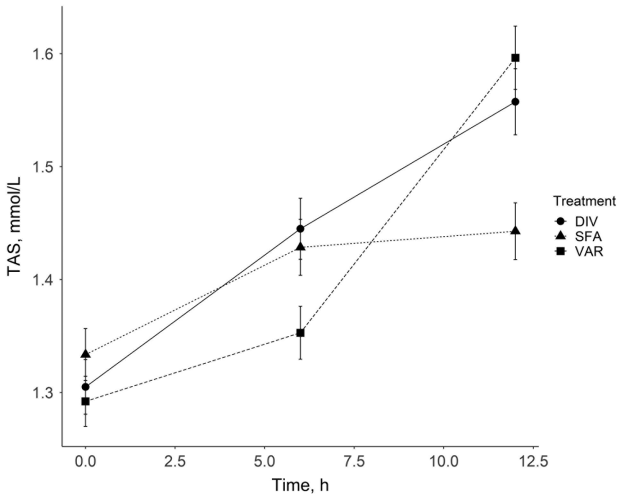

Figure 1. Total anti-oxidant status (TAS) of ram lambs fed a DIV (free choice of diverse diet components: chicory, ryegrass, plantain, and alfalfa), SFA (single forage diet of alfalfa), or VAR (free choice of plantain and ryegrass from 0700 to 1600 h and chicory and alfalfa between 1600 and 0700 h diets at three time points over the day (09:30 [0 h], 15:30 [6 h], and 21:30 [12 h]).

For each time of day and over the whole day, the proportion of time eating was greatest for the SFA lambs, intermediate for DIV lambs, and lowest for the VAR lambs, all of which were different ($P < 0.05$) from one another. Similarly, there was a treatment effect ($P = 0.03$) on the proportion of time spent ruminating in the morning, afternoon, and over the whole day. In addition, there was a time effect ($P < 0.01$), but no treatment × time interaction ($P = 0.53$) on the proportion of time spent ruminating in the morning and afternoon. During the morning and afternoon, and over the whole day the VAR lambs spent more time ($P < 0.05$) ruminating than both the SFA and DIV treatments, which were not different ($P > 0.05$). There was a treatment ($P < 0.01$) effect on the proportion of time lambs spent idle in the morning and afternoon, and over the whole day, with the SFA treatment spending less time idle than the DIV and VAR treatments, which were not different ($P > 0.05$).

There was a treatment ($P < 0.01$) effect on the proportion of time spent lying down in the morning, afternoon, and over the whole day. In addition, there was a time ($P < 0.01$) effect on the proportion of time spent lying down in the morning and afternoon. At each of the time periods and over the whole day, the time spent lying was not different ($P > 0.05$) for the VAR and DIV treatment; however, they spent more time ($P < 0.05$) lying than the SFA treatment. Conversely, the SFA lambs spent a greater ($P < 0.05$) proportion of time standing at each time point analyzed over the day compared with the DIV and VAR lambs, which did not differ ($P > 0.05$) from one another.

There was a treatment × time interaction ($P < 0.01$) on the number of stereotypic behavior bouts recorded in the morning and afternoon (Table 7). Over the whole day, the SFA lambs had a greater ($P < 0.05$) number of stereotypic behavior bouts than the DIV and VAR lambs, which did not differ ($P > 0.05$). In the morning all treatments had a different number of stereotypic behavior bouts ($P < 0.05$), the SFA treatment had the greatest number bouts, followed by the DIV, and then VAR. In the afternoon, the SFA lambs had 81% more bouts ($P < 0.05$) of stereotypic behaviors than the DIV lambs. During this afternoon period, the incidence of stereotypic behavior bouts by VAR treatment was intermediate and not different ($P > 0.05$) from the SFA and DIV treatments. There was a treatment ($P < 0.01$) and time ($P < 0.01$) effect on the number of bouts of grooming recorded. In addition, over the whole day, there was a treatment effect ($P < 0.05$). At each of the periods examined, the VAR treatment had a greater ($P < 0.05$) number of grooming bouts compared to the SFA and DIV treatments, which were not different ($P > 0.05$) from one another.

Discussion

We hypothesized that a diverse and varied diet of fresh forages over the day will improve intake, performance, and welfare of lambs compared to dietary monotony and that the varied diet of fresh forages over the day would improve intake, performance, and welfare of lambs compared to dietary diversity of the same herbage species on offer at the same time. Based on the results, we accept that diverse and varied diets can improve performance and welfare relative to a single forage monotony, however reject that a varied diet will improve intake within this context. We also accept that a varied diet can improve performance compared with a diverse diet. The following sections will discuss the intake and performance of the SFA treatment compared to the DIV and VAR and then compare the DIV and VAR treatments. The rumen, blood, and behavior data of the treatments are then discussed collectively to give inferences on potential welfare differences. Finally, we outline areas for future research as identified by this work.

Intake and performance: SFA vs. DIV and VAR

A diverse diet that is varied through time can improve performance relative to alfalfa or a free choice diverse diet that is presented in a monotonous manner. The DIV and VAR lambs both had greater ADG than the SFA, largely explained by differences in the nutritional composition of the diet, namely, the greater DMD and ME content of the DIV and VAR diets. Other studies have reported similar results with lower DMI from treatments offered a flavorally or biochemically uniform diet compared to those offered choice from a diverse range of feeds (Keskin et al., 2004; Atwood et al., 2006; Distel et al., 2007; Villalba et al., 2011; Garrett et al., 2021). The magnitude of the effect from choice of diverse feeds on increased DMI is lower within the present study than that reported in other studies. For example, Garrett et al. (2021) reported a 48% increase in DMI from lambs of the same age and similar weights offered choice from a set ratio of chicory, alfalfa, plantain, and ryegrass compared with those repetitively fed ryegrass. Discrepancies between Garrett et al. (2021) and the current study may be a result of the different forage species (i.e., ryegrass vs. alfalfa) and their chemical composition. Greater DMI by lambs and other ruminants grazing alfalfa have been reported compared to those grazing ryegrass, even when the in vivo digestibility was similar (Niezen et al., 1993; Fraser et al., 2004). Greater DMI of alfalfa diets compared to ryegrass diets is a result of legume forages being more susceptible to ingestive and digestive particle breakdown, increasing rumen clearance rate, and thereby reducing the physical constraint of intake (Waghorn et al., 1989; Jamot and Grenet, 1991; Mertens, 1994). Greater ingestive and digestive particle breakdown of alfalfa may also explain why, despite having a greater predicted digestibility (+7.5%), the DIV and VAR did not have greater intakes, respectively, than the SFA diet. Varying the availability of diverse diet components throughout the day can reduce the DMI CV compared to repeatedly allocating alfalfa. The greater DMI CV of the SFA treatment

Table 6. Observed behavioral differences within daylight hours of ram lambs fed a SFA, DIV, or VAR diet on days 9 and 20

	Treatments (TRT)[2]				P-value[4]		
Behavior, % of time[1]	SFA	DIV	VAR	SEM[3]	TRT	Time	TRT x Time
Eating							
Morning	45.05[a]	38.27[b]	31.94[c]	2.03	<0.01	<0.01	0.29
Afternoon	54.53[a]	47.75[b]	41.42[c]				
Total	50.49[a]	44.39[b]	38.20[c]	2.00	<0.01	–	–
Ruminating							
Morning	28.96[b]	28.71[b]	33.53[a]	1.74	0.03	0.01	0.53
Afternoon	24.50[b]	24.50[b]	29.07[a]				
Total	26.72[b]	26.91[b]	31.24[a]	1.67	0.03	–	–
Idle							
Morning	23.86[b]	31.37[a]	32.48[a]	2.40	<0.01	0.42	0.20
Afternoon	21.73[b]	29.24[a]	30.35[a]				
Total	22.75[b]	28.67[a]	30.36[a]	1.99	<0.01	–	–
Position, % of time							
Lying							
Morning	44.78[b]	52.62[a]	57.88[a]	2.97	<0.01	<0.01	0.72
Afternoon	35.26[b]	42.80[a]	44.95[a]				
Total	38.77[b]	46.56[a]	49.75[a]	2.16	<0.01	–	–
Standing							
Morning	53.11[a]	45.37[b]	41.33[b]	3.62	<0.01	<0.01	0.38
Afternoon	65.05[a]	57.30[b]	53.27[b]				
Total	61.54[a]	53.52[b]	50.13[b]	2.09	<0.01	–	–

[1]Morning (0700 to 1200 h on day 9 and 0734 to 1200 h on day 20); Afternoon (1200 to 2010 h on day 9 and 1200 to 1942 h on day 20).
[2]SFA, single forage diet of alfalfa; DIV, free choice of diverse diet components: chicory, ryegrass, plantain, and alfalfa;VAR, free choice of plantain and ryegrass from 0700 to 1600 h and chicory and alfalfa between 1600 and 0700 h.
[3]The values reported in this table are least-squares means ± the standard error of the mean for the proportion of time spent doing a specific behavior.
[4]t-test *P*-value.
[a–c]Means in a row without similar superscripts differ between treatments at each time ($P \leq 0.05$).

Table 7. Observed behavioral differences within daylight hours of ram lambs fed a SFA, DIV, or VAR diet on days 9 and 20

	Treatments (TRT)[2]			*P*-value[3]		
Behavior, count[1]	SFA	DIV	VAR	TRT	Time	TRT×Time
Stereotypic						
Morning	6.72[a] ± 1.20	1.55[b] ± 0.42	0.35[c] ± 0.16	<0.01	<0.01	<0.01
Afternoon	6.50[a] ± 1.17	3.59[b] ± 0.76	5.10[ab] ± 0.96			
Total	13.22[a] ± 2.17	5.14[b] ± 0.99	5.45[b] ±1.01	<0.01	—	—
Grooming					—	—
Morning	3.79[b] ± 0.38	4.37[b] ± 0.43	5.32[a] ± 0.48	<0.01	<0.01	0.70
Afternoon	8.14[b] ± 0.67	9.38[b] ± 0.78	11.40[a] ± 0.81			
Total	11.93[b] ± 0.92	13.75[b] ±1.07	16.72[a] ± 1.09	<0.01	—	—

[1]Morning (0700 to 1200 h on day 9 and 0734 to 1200 h on day 20); Afternoon (1200 to 2010 h on day 9 and 1200 to 1942 h on day 20).
[2]SFA, single forage diet of alfalfa; DIV, free choice of diverse diet components: chicory, ryegrass, plantain, and alfalfa; VAR, free choice of plantain and ryegrass from 0700 to 1600 h and chicory and alfalfa between 1600 and 0700 h.
[3]t-test *P*-value. The values reported in this table are least-squares means ± the standard error of the mean for the proportion of time spent doing a specific behavior.
[a–c]Means in a row without similar superscripts differ between treatments at each time ($P \leq 0.05$).

suggests that the lambs within the treatment were forming short-term aversions to alfalfa, thus creating cyclic patterns of intake (Provenza, 1996). Improved performance (i.e., ADG) with reduced CV has been reported by several studies (Allison, 1985; Galyean et al., 1992; Horn et al., 2005; Williams et al., 2018), which allow us to suggest that the greater ADG of DIV and VAR could have been due to more consistent feed consumption as well as differences in the primary composition of diets, compared with the SFA. Further, choice of diet components as available to the DIV and VAR treatments has been shown to allow animals to better fulfil their nutrient requirements, thereby increasing the feed conversion efficiency of the consumed herbage (Atwood et al., 2001). For example, Atwood et al. (2001) offered calves free-choice from a diet components comprising a total mixed ration (TMR) or TMR and found that on average both treatments consumed a diet of similar energy to protein ratios; however,

the results suggested that individual intake of diet components varied greatly within the free-choice group, suggesting that the no-choice TMR group was potentially over-ingesting energy to meet their protein needs.

Utilizing first principles based on energy requirements for maintenance and growth and the measured diet nutritive value, an estimated ADG of 274, 354, and 349 g/d for the SFA, DIV, and VAR treatments was calculated, respectively (Nicol and Brookes, 2007). These calculations predict similar ADG for the DIV and VAR treatments (354 vs. 349 predicted g/d, respectively), which were not similar to the recorded ADG (296 vs. 378 g/d) for the DIV and VAR treatments. We argue that such a model only accounts for the intake of primary nutrients and we now know that PSC can impact animals at a range of levels including their intake and performance. For example, alfalfa is known to be a rich source of plant secondary compounds, particularly saponins, but also flavonoids and phenolics (Rafińska et al., 2017). Saponins from alfalfa are known to reduce microbial fermentation, protozoa numbers, and the digestion of nutrients and have been suggested to adversely affect rumen microbial protein production (Lu and Jorgensen, 1987). Thereby as animal production (growth, development, and reproduction) is greatly influenced by nutrient utilization, it is reasonable to assume that these anti-nutritional properties of alfalfa may contribute to the lower feed conversion efficiency and production of the SFA diet.

The single forage diet had a greater rumen NH_3 concentration compared with the VAR and DIV treatments, whom could reduce their rumen NH_3 concentration through dilution of protein intake from alfalfa, thereby balancing the soluble protein to energy ratio (Hill et al., 2009). As a result of the excessive dietary nitrogen (evidenced by the elevated rumen NH_3 concentration), it is likely that the SFA animals experienced the resulting negative post-ingestive effects to a greater degree than the other treatments. Elevated rumen NH_3 is associated with increased blood NH_3, both of which represent a toxin burden to the animal which requires negation through assimilation into amino acids or excretion via urine (Hill et al., 2009). Plant primary and secondary components act in a nutrient to toxin concentration gradient, where the actions of ingested compounds have dose-dependent effects (Raubenheimer et al., 2009; Beck and Gregroini, 2020). The finding of elevated NH_3 is similar to the findings of Dziba and Provenza (2007) and Dziba et al. (2006) who found that intake of high levels of monoterpenes (a PSC) resulted in negative post-ingestive feedback and induced satiety. The DIV and VAR diets would have ingested a greater range of different kinds and quantities PSC compared to the SFA, which would be detoxified through different mechanisms at different rates (Freeland and Janzen, 1974), thereby reducing the chance of a detoxifying pathway being saturated and thus reducing any associated negative effects. For example, the DIV and VAR treatments had access to chicory which is rich in tannins, flavonoids, coumarins, sesquiterpene lactones, and alkaloids (Nwafor et al., 2017) plantain rich in iridoid glycosides, aucubin and catapol, and tannins (Rumball et al., 1997), and perennial ryegrass is rich in endophyte alkaloids, flavonoids, and phenolics (Cao et al., 2017; Kagan, 2021). Therefore, perhaps diverse and varied diets providing a range of primary and secondary compounds can negate some of the nutritional inefficiencies or toxic effects encountered when a monotonous diet is supplied. For example, each the DIV and VAR diets consumed chicory which contains tannins that form complexes with proteins, altering protein digestion and aiding in alleviated the adverse effects of too much protein in the diet (Naumann et al. (2017).

Intake and performance: DIV vs. VAR

In addition to greater capability of avoiding or negating toxicosis, animals offered a diverse diet are also thought to have greater capability of ingesting an appropriate dose of compounds that allows for increased utilization of beneficial therapeutic properties (Provenza et al., 2007; Dixon and Pasinetti, 2010), and have even been suggested to increase the efficiency of rumen fermentation (Frutos et al., 2008). Although the plant primary chemical composition of the VAR and DIV diets was not different, the proportions of species consumed and thereby the quantities of ingested PSC to reach this composition likely differed and contributed to the differences in performance seen. The ingestion of PSC from different plants can have synergistic effects, offering greater benefits than what an individual plant species can offer (Tilman, 1982; Gregorini et al., 2017). Thereby ingestion of a different quantity of a particular PSC or a different ratio of PSC may have resulted in greater exploitation of a property or synergistic effect that increased efficiency of the VAR treatment in comparison with the DIV treatment. For example, the differences in ruminal parameters between the DIV and VAR provide evidence for differences in nutrient use efficiency between the VAR and DIV lambs. For example, the VAR treatment had a lower acetate:propionate ratio compared with the DIV in the AM, which is indicative of increased energy retention by the animal (Wolin, 1960; Russel,1998). Further, in the AM the DIV had a greater rumen NH_3 compared with the VAR treatment. Elevated NH_3 can indicate inefficiencies as the nitrogen availability exceeds the capacity for microbial utilization (Chanu et al., 2020). The VAR treatment consumed its DM through small quantities of ryegrass and plantain in the morning. Plantain, which has a lower CP concentration, reduces production of rumen NH_3 through the presence of aucubins and acetocides (Navarrete et al., 2016; Nkomboni, 2017), when it is increased at a level of 30% or greater as it is in the AM by the VAR. In the PM when the VAR treatment consumed most of its protein through alfalfa, there was no rise in NH_3 levels, indicating that protein was effectively utilized. It is possible that the greater intake of chicory by the VAR animals aided in this effect, with the chicory tannins binding some of the protein, reducing the amount of rumen degradable protein and increasing the portion of non-ammonia N reaching the small intestine and thereby the ratio of essential amino acids to energy (Waghorn et al., 1987; Villalbla et al., 2015). However, the DIV treatment paired a greater number of feeds during any feeding period; perhaps, this resulted in the complexation of PSC to negate any increases in nutrient use inefficiencies. Further, the DIV treatment had a lower intake of chicory, thereby would likely have had a corresponding decrease in the quantity of tannins ingested and the beneficial properties associated with this. At the same level of intake with no differences in dietary CP, the VAR treatment had lower rumen NH_3 levels in the AM and no difference in the PM than the DIV treatment, indicating reduced release of ammonia from soluble protein.

The results indicate that diverse and varied diets can improve production further than a currently common and high performing feed (e.g., SFA). Although, future testing of the PSC profiles of herbages offered is required, and a control diet containing a homogenized un-sortable mixture of the diverse plants as a dietary control or offering a monotonous diet of all single forages comprising the diverse diet could allow for treatment comparisons of more similar PSC profiles. Another contributing cause of comparatively lower performance from the DIV treatment relative to the VAR treatment, despite similar DMI and primary chemical composition of diets, could be that the DIV treatment experienced a greater level of stress,

potentially indicated by the differences in stereotypic behavior in the AM. For example, elevated levels of glucocorticoids in the blood of stressed animals elicit physiological responses that result in reduced feed conversion efficiency (Llonch et al., 2016). This premise may explain why the FCE (g ADG/kg DMI) was not different between the DIV and SFA treatments but was greater for the VAR treatment.

Rumen, blood, and behavior indicating differences in welfare: SFA vs. DIV vs. VAR

Greater animal performance (Roche et al., 2009; Barrell, 2019) and more consistent DMI (McGuffey et al., 1997) have been associated with reduced health incidents and improved welfare. Thereby the order of increasing ADG; SFA < DIV < VAR may also be indicative of the hierarchy of welfare among the treatments. In addition, excessive levels of dietary N can have detrimental effects on animal health and thereby welfare (Pacheco and Waghorn, 2008). Thus, the elevated concentration of rumen NH_3 of the SFA relative to the DIV and VAR may be suggestive of reduced welfare. The VAR treatment had a greater number of grooming bouts compared with the DIV and SFA treatments, which can be considered an indicator of positive welfare (Napolitano et al., 2009). Further, the VAR and DIV treatments exhibited fewer bouts of stereotypic behaviors than the SFA treatment. Although stereotypic behaviors are only partial indications of impaired welfare (Mason, 1991), we argue, as per Garrett et al., (2021), that such behaviors should be minimized where possible to enhance animal wellbeing.

Varying allocation of diverse diet components can alter diurnal patterns of DMI, which is perhaps the cause of differences in TAS levels during subsequent measurements. Dietary antioxidants (e.g., vitamin E and PSC) are a major exogenous defense against oxidative damage. As such, greater antioxidant status can be a sign of improved internal state and well-being of ruminants (Beck and Gregorini, 2020). At 15:30 h, the VAR treatment had a lower TAS than either DIV or SFA, likely a result of consuming very little feed containing antioxidants prior to this measurement. We do not believe that the lowered TAS level at this time is indicative of elevated stress due to the VAR treatment also exhibiting fewer bouts of stereotypic behaviors than both other treatments over this period. At 21:30 h, the VAR and DIV treatments had a greater TAS than the SFA treatment, perhaps because their diet likely containing greater levels of antioxidants, in the period leading up to this sampling. Alternatively, the SFA may have experienced a greater level of oxidative stress over the day, supported by their greater levels of stereotypic behavior throughout the day, depleting their TAS levels, relative to the DIV and VAR treatments.

Overnight grazing activity is typically reduced as it diminishes alertness and thereby increases the risk of predation (Gregorini, 2012). Thereby, based on the elevated TAS levels at nightfall for the VAR and DIV animals, we speculate that these treatments are better prepared to cope with stressors encountered overnight, which is considered a stressful time as animals are more susceptible to predation at night (Tyler et al., 2016). We therefore argue that, by offering a diverse or varied diet, farmers can enhance animal welfare by increasing the antioxidant levels available to combat oxidative stress or perhaps aid in preventing stress that would otherwise deplete antioxidants overnight.

Collectively, our results—reduced DMI CV, increased ADG and 12 h TAS, reduced bouts of stereotypic behavior, and increased grooming (VAR only)—present compelling evidence in support of the DIV and VAR lambs having increased welfare relative to the SFA lambs. Further, the increased ADG and grooming bout number of the VAR treatment compared to the DIV may be indicative of improved welfare but further research is with more definitive measures of welfare are required, e.g., cortisol in blood, saliva, feces, or wool.

Future research

Our research is some of the first to depict that varying forage availability through time can improve performance of lambs relative to those offered continuous access to the same diverse forages. Although our research was some of the first to depict such a phenomenon, future research repeating such experimentation with greater animal numbers would further strengthen the results seen. Due to limitations of resources, the current experiment was only able to examine the effect of one feeding sequence, future research exploring different forage combinations and the possible sequences could elucidate patterns of forage offerings that could enhance performance. Future examination of sequence effects should be done in grazing situations to evaluate and examine how diurnal fluctuations in forage composition impact the results reported here. Further research is also required on the duration at which varied diet sequences still elicit an effect, for example, would 1 wk on ryegrass and plantain and then another week on chicory an alfalfa still elicit the benefits seen or is the time scale used within the present study important.

Conclusions

The diverse and varied diets explored improve animal performance relative to high performance diet of alfalfa fed monotonously. Further, temporal management of diverse diets (to create varied diets, e.g., VAR) can improve performance relative to animals given free choice diversity at all times. Moreover, the diverse and varied diets may enhance animal welfare in comparison with a monotonous alfalfa diet, and a varied diet may provide welfare advantages to the repetitive presentation of free choice diversity. Although the exact mechanism for this increased performance of the varied diet compared with the diverse is unclear within the present study, therefore requiring further evaluation, it highlights that it is more than merely the primary chemical composition of the diet consumed but rather how the diet is presented through time, and the herbage species and quantities of each species consumed to reach that primary composition that influences performance and animal welfare.

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Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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