Journal of Animal Science, 2021, Vol. 99, No. 5, 1–9

doi:10.1093/jas/skab058
Advance Access publication February 23, 2021
Received: 2 December 2020 and **Accepted:** 18 February 2021
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RUMINANT NUTRITION

Functional diversity vs. monotony: the effect of a multiforage diet as opposed to a single forage diet on animal intake, performance, welfare, and urinary nitrogen excretion

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Abstract

The objective of this study was to determine the effect of offering animals a multiforage choice (**MF**) of fresh herbages on dry matter intake (**DMI**), live weight gain, and animal welfare, in comparison with a monotonous diet of ryegrass (*Lolium perenne* L.). Twenty ram lambs (30.5 ± 0.9 kg initial live weight; mean ± SEM), were randomly allocated to either a diet consisting of diverse MF choice or a single forage ryegrass (**SF**) diet (n = 10 per treatment) for 35 d. Both diets were fed ad libitum; however, the MF diet was composed of set dry matter ratios of 24% chicory (*Cichorium intybus* L.), 30% lucerne (*Medicago sativa* L.), 25% plantain (*Plantago lanceolata* L.), and 21% ryegrass. The DMI of the MF lambs was 48% greater (P < 0.01) and the within animal day-to-day coefficient of variation (**CV**) of intake was 26% lower (P < 0.01) than the SF lambs. The average daily gain (**ADG**) of lambs offered the MF diet was 92% greater (P < 0.01) than the lambs offered the SF diet. The within-animal day-to-day CV of intake was negatively related to ADG (r = –0.59; P < 0.01). The MF lamb's urinary N concentration was 30% lower (P < 0.01) than that of the SF lambs. The SF lambs spent more time (P < 0.05) exhibiting stereotypic behaviors in the afternoon and spent more time observing other animals than the MF. Overall, allocating an MF choice of fresh herbages as opposed to a single forage diet of ryegrass increases DMI and thereby animal performance, while potentially reducing urinary N excretion.

Key words: diet, diversity, monotony, welfare, sheep

Introduction

The ancestors of today's ruminants evolved within environments containing a diverse array of plant species (Provenza et al., 2007). To compose their own diet within such diverse environments, individual animals selected from a multitude of unique forage species, of which availability, abundance, and chemical composition varied across space and through time (Provenza et al., 2007). This is in stark contrast with monotonous diets typically used by today's intensive pastoral livestock production systems, which generally offer binary mixes of a grass and a

Abbreviations

ADF	acid detergent fiber
ADG	average daily gain
ADMD	apparent dry matter digestibility
AM	0700 to 1200 h
ANOVA	analysis of variance
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
GLM	generalized linear model
GPx	glutathione peroxidase
LW	live weight
MF	multiforage
N	nitrogen
NDF	neutral detergent fiber
NH_3	ammonia,
NIRS	near-infrared spectrophotometry
OM	organic matter
OMD	organic matter digestibility
SF	single forage ryegrass
TAS	total antioxidant status
VFA	volatile fatty acid
WSC	water-soluble carbohydrate

legume (e.g., perennial ryegrass [*Lolium perenne* L.] and white clover [*Trifolium repens* L.]). Undoubtedly, such dietary strategies have aided to increase productivity and profitability of pastoral production systems by simplifying agronomic and grazing management of swards. Mixes of a perennial ryegrass and a legume ("simple pastures") are widely fed to livestock, as perennial ryegrass is not only a palatable and digestible forage (Delagarde et al., 2000), but also provides high herbage yields under a range of temperate environments and management conditions (Moser et al., 1996). Simple pastures can result in a 'monotonous' diet, as over time biotic (e.g., competition) and abiotic (e.g., soil moisture) factors affect plant persistence or as animals are unable to choose and sort at the bite level, or if they are able to graze selectively, will graze, so that only one species persists (Zydenbos et al., 2011; Gregorini et al., 2017). Moreover, the repeated allocation of such a single species pastures or simple pasture mixes induces dietary monotony, which is defined as tedious repetition or a lack of variety (Pearsall, 2001). Despite the advantages of 'simple pastures', in terms of grazing management, there has been little consideration of the consequences of its monotonous feature at the animal level.

Animal production, health, and, in turn, welfare can be compromised by dietary monotony, for example by inducing nutrient imbalances (Provenza et al., 2007; Hogan and Phillips, 2008). Nutrient imbalances can lead to phenomenon such as incidental restriction, where the upper threshold for a nutrient is reached and animals cease eating (i.e., nutrient-specific satiety), with an ensuing deficiency in nutrients present in lower concentrations within feeds (Raubenheimer, 1992; Provenza, 1995; Gregorini et al., 2017). Conversely, incidental augmentation is encountered when animals consume nutrients present in higher concentrations to excessive and potentially detrimental quantities to satisfy other nutrient or energetic needs (Raubenheimer, 1992; Provenza, 1995; Gregorini et al., 2017). Impaired nutrition can have a number of detrimental effects on animal health and wellbeing (Beck and Gregorini, 2020). Furthermore, nutritionally imbalanced diets are contributors to inefficient utilization and excretion of nutrient to the environment, therefore contributing to negative environmental footprint of pastoral production systems (Gregorini et al., 2017). Thereby, monotonous diets may have detrimental impacts on the environment as well as animal performance and welfare.

Providing ruminant livestock with diets diverse in flavor or biochemical composition has been shown to increase dry matter intake (**DMI**) or feed conversion efficiency (**FCE**; Champion et al., 1998; Rogosic et al., 2006; Distel et al., 2007; Villalba et al., 2011), improve animal health (Provenza et al., 2007; Dixon and Pasinetti, 2010), and productivity (Rodríguez et al., 2007; Al-Marashdeh et al., 2020). Offering animal's choice from taxonomically diverse diets affords animals the opportunity to select plant combinations that meet their nutrition and therapeutic needs, while negating nutrients that are in excess or that are causing malaise (Villalba et al., 2010). Furthermore, providing choice from taxonomically diverse plants may provide greater benefits than when individual plant species are consumed alone (Tilman, 1982; Gregorini et al., 2017). Although the promising effects of dietary diversity on improved animal performance and productivity have been identified, much of this research has been conducted using concentrates or conserved forage, with little information regarding the effect of fresh forages. Therefore, we hypothesized that as opposed to a monotonous diet of perennial ryegrass, a taxonomically diverse MF diet would increase DMI, improve animal performance, reduce urinary N excretion, and enhance welfare. The objective of this study was to determine whether feeding animals a diet of equal proportions of cut fresh herbages: ryegrass, lucerne (*Medicago sativa* L.), chicory (*Cichorium intybus* L.), and plantain (*Plantago lanceolata* L.) alter DMI, animal performance, N excretion, and animal welfare relative to a conventional monotonous diet of ryegrass.

Materials and Methods

The study was conducted at the Johnstone Memorial Laboratory at Lincoln University (43°38′57″S, 172°27′01″E), according to the methods approved by the Lincoln University Animal Ethics Committee (AEC 2018–49) prior to experiment initiation.

Animal management and dietary treatments

Twenty 6-month-old Coopworth rams (30.5 ± 0.9 kg initial live weight [**LW**]; mean ± SEM) were housed in individual pens (3.6 × 1.0 m) indoors for 35 d starting on 4 March 2019. Animals were randomly allocated to one of two treatments (n = 10): single forage ryegrass (**SF**) or a multi-forage diet (**MF**) consisting of a selection of equal parts dry matter (**DM**) of ryegrass, lucerne (*M. sativa* L.), chicory (*C. intybus* L.), and plantain (*P. lanceolata* L.). Ryegrass, chicory, and lucerne were all in a vegetative state, whereas plantain contained 35% reproductive stem and seed head. The chicory was first year with a standing height of 35 cm prior to harvesting, ryegrass was at the three-leaf stage of growth, and lucerne ranged from mid to late vegetative state over the course of the trial (Hall, 1996). Animals offered the MF diet were presented all four feeds simultaneously, with each feed occupying one half of a split bin placed at each ends of the pen. The half-bin that each forage species was offered in was randomly assigned for each pen. Both the SF and MF treatments received ad libitum access to fresh cut herbage of their respective diets at

0730 h and, if required, additional herbage was supplied at 1600 h. Herbage was cut using a Haldrup forage harvester (Haldrup GmbH, Ilshofen, Germany) that cut 3 cm above ground level. All herbages were stored at 4 °C until they were fed, any feed excess to daily requirement was stored for up 2 d after harvest as a reserve. Herbage refusals from the previous day were weighed before each morning feeding. Each pen was cleaned daily, and water was freely available to animals at all times.

Animal sampling and measurements

Samples of allocated herbage and orts were taken at each morning feeding time. All animals were weighed (Prattley 3-Way Manual Weigh Crate, Temuka, New Zealand with a Tru-test XR300 weigh head, Auckland, New Zealand) once weekly before morning feeding. Average daily gain (**ADG**) for each animal was determined as the slope of a regression line fitted for live weight across time for each individual animal. Samples of feces, blood, and rumen fluid were collected on days 1, 20, and 35. Fecal samples were collected by rectal grab. Blood samples were obtained by jugular venepuncture and collected with a 20G by 1" multisample collection needle (Greiner Bio-One, Kremsmünster, Austria) into a 10-mL heparinized blood tube (Greiner Bio-One, Kremsmünster, Austria). A 2-mL heparinized whole blood subsample was removed from the blood tube and stored at −20 °C until analysis. The remaining blood was centrifuged (Megafuge 1.0R, Heraeus Holding GmbH, Hanau, Germany) at 2,300 × g and 4 °C for 15 min; plasma was then aspirated and stored at −20 °C until analysis. Ruminal fluid was obtained via esophageal tubing. Ruminal fluid was subsampled into three containers: one acidified with sulphuric acid (10 μL of 98% sulphuric acid; Fisher Scientific, Loughborough, UK) and two without.

On days 23 and 24, five animals from each treatment were housed in metabolism crates for 48 h, with the remaining animals housed within the crates on days 25 to 26, to determine total daily fecal and urine output. Representative samples of feces and urine were collected from the metabolism crates for each 24-h period that animals were in the crates. Collection trays for urine contained ~250 mL of 5% sulphuric acid, so that the urine was immediately acidified to prevent ammonia (**NH_3**) volatilization.

Behavioral observations were determined by scan sampling each animal at 2-min intervals and recording the displayed behavior, during daylight hours (0712 to 2010 h) on days 11 and 32 (Altmann, 1974; Villalba et al., 2015). During this daylight period, artificial lighting was used. Behaviors recorded were as follows: idle, eating, ruminating, pacing, chewing pen fixtures, head butting pen fixtures, head hanging, crouching, pawing or stamping, rearing, scratching, rubbing, observing other sheep, and observing humans (Table 1). These activities were selected as behaviors of interest based on previous studies (Done-Currie et al., 1984; Lauber et al., 2012; Catanese et al., 2013). Pacing, chewing, head butting, head hanging, pawing or stamping, rearing, and crouching were grouped as stereotypic behaviors. Stereotypic behaviors are those that are repeated with no apparent function and are indicative of poor animal welfare (Broom, 1991; Catanese et al., 2013). Grooming was considered as the incorporation of scratching one's self and rubbing on pen fixtures as defined by Mattiello et al. (2019).

Table 1. Ethogram of recorded behavioral activities

Behavior	Description
Eating	Eating (specific feed was recorded)
Idle	Sheep not engaged in any of the following behaviors
Ruminating	Sheep is ruminating
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, 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
Scratching	Scratching self
Rubbing	Rubbing on pen fixtures
Observing other sheep	In an alert state, ears pricked, or actively looking with attention directed to other sheep (the treatment of the animal being observed was recorded)
Observing humans	In an alert state, ears pricked, or actively looking with attention directed to other sheep

Sample analysis

Herbage samples were frozen (−20 °C), freeze dried, ground by a centrifugal mill (ZM200; Retsch, Haan, Germany; 1mm screen), and analyzed using near infrared spectrophotometry (**NIRS**; Model: FOSS NIRS Systems 5000, MD) to determine chemical composition. Chemical composition values used for NIRS calibration were derived prior to sample analysis for DM (AOAC, 1990; method 930.15), organic matter (**OM**; 100%-ash%; AOAC, 1990; method 942.05), neutral detergent fiber (**NDF**; Van Soest et al., 1991), acid detergent fiber (**ADF**; AOAC, 1990; method 973.18), water-soluble carbohydrates (**WSC**; MAFF, 1986), digestible OM in DM (**DOMD**), DM digestibility (**DMD**), and OM digestibility (**OMD**; Iowerth et al., 1975), and crude protein (**CP**) by combustion (Variomax CN Analyser; Elementar Analysensysteme, Hanau, Germany). The NIRS calibration equations used all had R^2 values greater than 0.97 and were within the calibration range. The metabolizable energy (**ME**) of herbages was estimated based on the Primary Industries Standing Committee (2007) equation:

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

Fecal samples were frozen (−20 °C), freeze dried, ground to pass through a 1-mm screen using a centrifugal mill (ZM200; Retsch, Haan, Germany), and analyzed for total N by combustion (Variomax CN, Elementar Analysensysteme, Hanau, Germany). Due to a sampling error, fecal DM for individual animals was not available, and other research (Garrett et al., unpublished data) has shown no difference in fecal DM% between lambs provided similar dietary treatments (i.e., a ryegrass compared with a diverse, herb containing diet). Thereby for the purpose of this work, an average fecal DM (20.23%) was assumed for calculating digestibility. Apparent dry matter digestibility (**ADMD**) was determined using the following equation:

$$\text{ADMD} = \frac{(\text{DMI, kg/d} - \text{DM Faeces, kg/d})}{\text{DMI, kg/d}} \times 100 \quad (2)$$

Whole blood glutathione peroxidase (**GPx**), plasma total antioxidant status (**TAS**), and urine urea concentrations were measured with a Randox Rx Daytona clinical analyzer (Crumlin, Co. Antrim, UK) using kits RANSEL (Cat. No. RS504, Cat. No. NX2332, and Cat. No. UR3825, respectively). Urine total N was determined by combustion (Vario MAX CN, Elementar Analysensysteme, Hanau, Germany).

The NH_3 concentration of the acidified rumen samples was measured using the clinical analyzer (Randox Rx Daytona, Crumlin, Co. Antrim, UK) and a commercial test kit (Cat. No. AM3979; Randox; Crumlin, Co.) based on the enzymatic UV method described by Neeley and Phillipson (1988). The volatile fatty acid (**VFA**) concentration within nonacidified rumen samples was determined using a gas chromatograph (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). Rumen lactate concentration was analyzed by the Randox Rx Daytona clinical analyzer with a commercial kit (Cat. No. LC2389; Randox; Crumlin, Co.) using enzymatic determination of L-lactate.

Statistical analysis

Statistical analyses were conducted using R (R Core Team, 2020, v.3.4.4). All data that were normally distributed ($P > 0.10$; Shapiro–Wilk test) and had homogenous variance ($P > 0.10$; Bartlett's test) was analyzed by analysis of variance (**ANOVA**) using the *aov* function of R. Data analyzed using *aov* function included: herbage chemical composition, DMI, ADG, FCE (ADG/kg DMI), GPx, urinary urea, rumen, water consumption through feed, water intake, fecal water, urine water, and water out. If data was not normally distributed it was analyzed by a generalized linear model (**GLM**), using the *glm* function of R. Data analyzed using *glm* included: TAS, total nitrogen in urine, water drunk, water balance, and the proportion of water intake excreted as urine, feces, and accounted for by the water balance. Values for samples collected on day 1 were used as covariates for rumen, blood, fecal, and urine variables as these samples explained a significant ($P < 0.05$) amount of variation. The ANOVA and GLM models that contained repeated measures (i.e., blood, urine, and fecal variables) included diet, day, and the diet × day interaction as fixed effects. The models for variables of averaged data or that were not repeatedly measured (i.e., rumen, DMI, ADG, and FCE) contained diet as fixed effects. Herbage chemical composition was assessed using a mixed model, using the *lme* function, with day as a random effect. Behavior data were averaged across observation days and analyzed by GLM using the *glm* function of R, with the distribution used for the model selected based on qq-plots of the residuals. The model for the animal behavior that was averaged across observation days included the treatment, observation time (AM or PM), and their interaction as fixed effects. Upon significance of the ANOVA, means separation between diets were done using a pairwise *t*-test using the *emmeans* package (Lenth, 2018), when a multiple comparison was needed, such as when comparing more than two means which occurred in the event of a significant interaction term for the repeated measures. Pearson's correlation coefficient between the day-to-day variability in DMI (CV) and DMI, ADG, and FCE was determined using the *cor.test* function of R. Statistical significance was declared at $P \leq 0.05$ with tendencies declared at $0.05 < P \leq 0.10$.

Results

Diet composition

The chemical composition of each herbage comprising the SF and MF diets is presented in Table 2. The CP content (24.9 %DM) of the ryegrass component of each the SF and MF diet was 15%, 23%, and 39% greater than that of the chicory, lucerne, and plantain, respectively ($P < 0.05$). The WSC content of herbages decreased from chicory (22.0 %DM) to plantain and then ryegrass and lucerne, which were not different to one another. These values were used to calculate the chemical composition of the total diet of each animal and the average chemical composition of each diet was compared between the dietary treatments (Table 3). There was no difference in ADF between the SF and MF diets ($P > 0.10$). Ryegrass, which made up 100% the SF diet, had a greater ME ($P = 0.04$) and greater DM, OM, OMD, NDF, and CP content compared with the average MF diet ($P < 0.01$). However, the WSC content was greater for the MF diet compared with the SF diet ($P < 0.01$).

Intake and performance

The DMI of lambs fed MF was 48% greater ($P < 0.01$) than lambs fed SF (Table 4). The DMI CV within animal between day was 26% greater ($P < 0.01$) for the SF lambs than the MF lambs. In addition, the FCE (ADG/kg DMI) of lambs offered MF was 36% greater than the SF lambs ($P < 0.01$). There was a negative correlation between the CV of day-to-day DMI and average DMI ($r = -0.74$; $P < 0.01$). The ADG of the MF lambs was 92% greater than that of the SF lambs (Table 4). Overall, there was a negative correlation between CV of day-to-day DMI and ADG ($r = -0.60$; $P < 0.01$) and no correlation ($r = -0.37$; $P = 0.11$) between CV of day-to-day DMI and FCE.

During the metabolism crate portion of this study, the DMI remained different ($P < 0.01$; Table 5). However, the SF lambs showed a 0.06-kg decrease in DMI and the MF lambs consumed 0.14 kg more DM, compared with their average DMI over the trial. This resulted in a greater magnitude of difference between MF and SF for DMI, while in the metabolism crates compared with when they were not (73% and 49% difference, respectively). The MF lambs tended to excrete 21% more feces than the SF lambs ($P = 0.08$). Lambs offered MF had greater apparent DMD than SF lambs ($P = 0.02$; Table 5).

Table 2. Chemical compostion of the herbages composing the single forage (perennial ryegrass only) and a taxonomically diverse MF diet of equal proportions of fresh cut herbage of ryegrass, lucerne (*Medicago sativa* L.), chicory (*Cichorium intybus* L.), and plantain (*Plantago lanceolata* L.)

	Herbage				
Item	Ryegrass	Lucerne	Plantain	Chicory	SEM
ME, MJ/kg of DM	11.0^{b}	8.8^{c}	10.8^{b}	13.2^{a}	0.3
DM, % as-is	19.7^{a}	21.7^{a}	14.3^{b}	8.7^{c}	0.10
OM, % DM	90.6^{b}	93.8^{a}	88.5^{c}	86.4^{d}	0.8
OMD, % DM	76.9^{b}	58.9^{c}	74.5^{b}	91.9^{a}	2.1
WSC, % DM	7.9^{c}	5.7^{c}	13.1^{b}	22.0^{a}	1.9
NDF, % DM	49.5^{a}	46.0^{a}	30.3^{b}	16.1^{c}	2.2
ADF, % DM	26.1^{b}	35.9^{a}	24.5^{b}	17.5^{c}	1.2
CP, % DM	24.9^{a}	20.3^{bc}	17.9^{c}	21.7^{b}	1.3

$^{a-c}$Means in a row with different superscripts are statistically different ($P < 0.05$).

Table 3. Chemical composition of the monotonous diet (perennial ryegrass only, SF) and the calculated chemical composition of a taxonomically diverse MF diet of equal proportions of fresh cut herbage of perennial ryegrass (*Lolium perenne* L.), lucerne (*Medicago sativa* L.), chicory (*Cichorium intybus* L.), and plantain (*Plantago lanceolata* L.) offered to the ram lambs

	Treatment diet			
Item[1]	SF	MF	SEM	P-value[2]
ME, MJ/kg DM	11.0	10.8	0.1	0.04
DM, % DM	19.7	18.5	0.2	<0.01
DMD_p, % DM	73.3	71.5	0.6	<0.01
OM, % DM	90.6	90.1	0.2	<0.01
OMD, % DM	76.9	74.4	0.7	<0.01
WSC, % DM	7.9	11.8	0.3	<0.01
NDF, % DM	49.5	35.9	0.6	<0.01
ADF, % DM	26.1	26.7	0.4	0.17
CP, % DM	24.7	21.1	0.1	<0.01

[1]Values for diverse diet chemical composition were calculated by using the percentage of the Item value that each dietary component accounted for.
[2]t-test *P*-value.

Table 4. Dry matter intake and growth performance of Coopworth lambs fed for a 35-d period either a monotonous diet (perennial ryegrass only, SF) or a taxonomically diverse MF diet of equal proportions of fresh cut herbage of perennial ryegrass (*Lolium perenne* L.), lucerne (*Medicago sativa* L.), chicory (*Cichorium intybus* L.), and plantain (*Plantago lanceolata* L.)

	Treatments			
Item[1]	SF	MF	SEM	P-value[2]
Initial LW, kg	31	30	0.9	0.46
DMI, kg/d	0.99	1.47	0.01	<0.01
DMI CV, %	21.3	15.8	0.8	<0.01
Daily gain, g LW/d	97	187	10	<0.01
FCE, gLWgain/kg DMI	89	121	7	<0.01

[1]DMI CV, day-to-day DMI co-efficient of variation.
[2]t-test *P*-value.

Water and N dynamics

Within the metabolism crates, SF lambs drunk 0.80 L/d of water, four times more than the MF (0.19 L/d; Table 5; $P < 0.01$). However, the MF lambs consumed 80% more water from feed than the SF lambs ($P < 0.01$). Total water consumption (water drunk + water from feed) of the MF lambs was 58% greater compared with the SF lambs ($P < 0.01$). The MF lambs excreted 74% more urine than SF lambs ($P < 0.01$). There was no difference in the amount of water excreted within the feces between the treatments ($P > 0.10$). The total amount of water excreted (64%) and the amount accounted for by the water balance (51%) was greater for the MF lambs compared with the SF lambs ($P < 0.01$). Although the percentage of water intake excreted in the feces was greater for the SF (10.29 ± 1.40%) compared with the MF (8.39 ± 1.40%; $P = 0.04$), there was no difference in how the percentage of total water intake was partitioned into urine (44.58 ± 5.73%; mean ± SEM) or the water balance (46.08 ± 6.2%) between treatments ($P > 0.10$).

The MF lambs consumed 51% more N than the SF lambs ($P < 0.01$; Table 6). Apparent N digestibility tended to be greater for the MF lambs compared to the SF ($P = 0.09$). Although there was no difference in the amount (g/d) of urinary N excreted between treatments ($P = 0.26$), the urine N concentration was 30% less for the MF compared with the SF lambs ($P < 0.01$). The MF lambs retained 15.38 g more N than the SF lambs ($P < 0.01$). The percentage of the N consumed excreted in the feces was less for the MF (11.01 ± 1.73%; mean ± SEM) compared with the SF (13.79 ± 1.73%; $P = 0.05$). Furthermore, the MF (33.80 ± 5.50%) tended to excrete a lower percentage of the consumed N in the urine compared with the SF (42.60 ± 5.50%; $P = 0.08$). Consequently, the N retention was 12% greater for the MF (55.19 ± 6.97%) compared with the SF (43.61 ± 6.97%; $P = 0.05$).

Table 5. DMI, water consumption, and fecal and urine output information obtained from a 48 h period within metabolism crates of Coopworth lambs offered a monotonous diet (perennial ryegrass only, SF) or a taxonomically diverse MF diet of equal proportions of cut fresh herbage of perennial ryegrass (*Lolium perenne* L.), lucerne (*Medicago sativa* L.), chicory (*Cichorium intybus* L.), and plantain (*Plantago lanceolata* L.)

	Treatments			
Item	SF	MF	SEM	P-value[1]
DMI, kg/d	0.93	1.61	0.06	<0.01
Feces, kg DM/d	0.14	0.17	0.01	0.07
ADMD, % DM	85.18	88.79	2.34	<0.01
Water intake, L/d				
Trough	0.80	0.19	0.23	<0.01
Feed	4.90	8.82	0.24	<0.01
Total	5.70	9.01	0.27	<0.01
Water excretion, L/d				
Feces	0.59	0.75	0.09	0.07
Urine	2.54	4.40	0.37	<0.01
Total	3.13	5.15	0.43	<0.01
Water balance, L/d	2.57	3.86	0.65	<0.01

[1]t-test *P*-value.

Table 6. Nitrogen dynamics from information obtained from a 48-h period within metabolism crates of Coopworth lambs fed either a monotonous diet (perennial ryegrass only, SF) or a taxonomically diverse MF diet of equal proportions of fresh cut herbage of perennial ryegrass (*Lolium perenne* L.), lucerne (*Medicago sativa* L.), chicory (*Cichorium intybus* L.), and plantain (*Plantago lanceolata* L.)

	Treatments			
Item	SF	MF	SEM	P-value[1]
Nitrogen intake, g/d	36.05	54.56	1.68	<0.01
Apparent nitrogen digestibility, %	86.26	88.63	1.70	0.09
Urinary nitrogen concentration, g/L	6.27	4.39	0.41	<0.01
Nitrogen excretion, g/d				
Feces	5.09	6.06	0.92	0.13
Urine	15.66	17.83	2.50	0.26
Total	20.75	21.06	2.82	0.86
Apparent nitrogen retention, g/d	15.29	30.67	2.92	<0.01

[1]t-test *P*-value.

Rumen, blood, and plasma parameters

There were no differences between treatments for rumen NH_3 ($P = 0.70$) or total VFA concentrations ($P = 0.81$; Table 7). There was a tendency for SF lambs to have a greater acetate-to-propionate ratio compared with the MF lambs ($P = 0.10$). The percentage of total VFA accounted for by valerate was greater for the MF lambs compared with the SF lambs ($P = 0.02$). There were no differences between treatments in the percentage of VFA composed by propionate, iso-butyrate, butyrate, and iso-valerate ($P > 0.10$). No differences were detected in the measured plasma and blood parameters. There was no difference in the levels of TAS for the SF (1.11 ± 0.03 mmol/L; mean ± SEM) and MF (1.16 ± 0.03 mmol/L; $P = 0.17$) or in the GPx levels between the SF (36.7 ± 589 U/mL) and the MF (35.6 ± 590 U/mL; $P = 0.21$).

Behavioral observations

The proportion of time observed for each behavior is given in Table 8. For eating, there was a treatment × time interaction ($P = 0.01$), whereby no difference was observed between treatments in the morning (0712 to 1200 h; $P > 0.05$), but a 12% and 27% increase was observed in the afternoon compared with the morning for the SF and MF, respectively ($P < 0.01$). The MF lambs spent 17.1% more time eating in the afternoon than the SF lambs ($P < 0.05$). For ruminating, there was an interaction between time and treatment whereby SF and MF lambs spent +2.7% and −6.5% time ruminating in the afternoon than the morning, respectively ($P < 0.05$). Furthermore, in the afternoon, MF lambs spent 7.0% less time ruminating than SF lambs ($P < 0.05$). For the percent of time spent idle, there was an effect of time and treatment ($P = 0.01$), and a tendency for an interaction between the two terms ($P = 0.09$). The SF lambs spent more time idle than MF lambs, regardless to the time of the day, and both treatments spent less time idle in the afternoon than the morning.

There was a treatment × time interaction on the percent of time spent displaying stereotypic behaviors ($P = 0.05$). The SF lambs exhibited less stereotypic behaviors in the afternoon than in the morning ($P < 0.05$), both of which were not different to the percentage of stereotypic behavior exhibited by the MF lambs in the morning ($P > 0.10$). However, the MF lambs exhibited less stereotypic behaviors in the afternoon, compared with the morning and the SF at any time of day ($P < 0.05$). There were no effects observed for the percentage of time spent grooming ($P > 0.10$). For the percent of time spent observing SF and MF, there was only a treatment effect ($P = 0.05$ and $P < 0.01$, respectively), with the MF lambs spending less time observing SF or MF lambs. For observing humans, there was only an effect of time ($P < 0.01$), reflecting an increased proportion of time observing humans in the morning compared with the afternoon.

Table 7. Rumen NH_3 and rumen VFA profile of ram lambs on days 20 and 35 fed either a monotonous diet (perennial ryegrass only, SF) or a taxonomically diverse MF diet of equal proportions of fresh cut herbage of perennial ryegrass (*Lolium perenne* L.), lucerne (*Medicago sativa* L.), chicory (*Cichorium intybus* L.), and plantain (*Plantago lanceolata* L.)

	Treatments			
Item[1]	SF	MF	SEM	P-value[2]
NH_3, mmol/L	8.74	9.15	0.67	0.70
Total VFA, mmol/L	35.84	36.92	3.00	0.81
Ace:Prop	4.45	4.23	0.09	0.10
VFA profile, % of total VFA				
Acetate	69.39	68.21	0.48	0.10
Propionate	15.62	16.30	0.33	0.17
Iso-butyrate	2.40	2.48	0.21	0.80
Butyrate	8.61	9.10	0.24	0.16
Iso-valerate	1.10	0.97	0.09	0.31
Valerate	0.09	1.06	<0.01	0.02

[1]Hexanoic and lactic acid were not included as the amounts present were below the detection limit gas chromatogram. Ace:Prop acid, proportion of acetate to propionate.
[2]t-test P-value.

Discussion

Animal ADG, DMI, and digestibility

Animal performance was enhanced by offering the MF diet compared with the SF diet. The ADG was 92% greater and FCE 36% greater for the MF lambs compared with the SF lambs, allowing the suggestion that offering alternatives to a monotony of ryegrass can have positive benefits on animal production. These observations are consistent with previous reports in which lambs offered forages consisting of herbs and clover had a 200 g/d greater ADG than those on a sward composed of a single forage (Golding et al., 2011). In addition, Al-Marashdeh et al. (2020) reported that lambs grazing a three species sward had a 50% greater ADG than lambs grazing a ryegrass and white clover association. However, results have been inconsistent. Raeside et al. (2017) fed spatially separated strips and different combinations of the same forage species used within the present study and reported no difference in ADG compared with animals grazing a monoculture of lucerne. Thereby, further investigation into other monotonous diets is required, to determine whether the effects reported here are diet specific (e.g., limited to monotony of ryegrass). Within the present study, the greater performance and FCE appeared to have a nutrition

Table 8. Behavior within daylight hours of ram lambs on days 11 and 32 being fed either a monotonous diet (perennial ryegrass only, SF) or a taxonomically diverse MF diet of equal proportions of fresh cut herbage of perennial ryegrass (*Lolium perenne* L.), lucerne (*Medicago sativa* L.), chicory (*Cichorium intybus* L.), and plantain (*Plantago lanceolata* L.)

	Treatments[1]						
	SF		MF		P-value		
Behavior, % of time	AM	PM	AM	PM	TRT	Time	TRT×Time
Eating	35.29 ± 1.27[c]	47.28 ± 1.61[b]	37.34±1.27[c]	64.44 ± 2.19[a]	<0.01	<0.01	0.01
Ruminating	21.64 ± 2.09[ab]	24.30 ± 2.35[a]	23.82 ± 2.30[a]	17.34 ± 1.68[b]	0.26	0.36	0.02
Idle	33.88 ± 2.49	26.38 ± 1.82	26.53 ± 1.84	21.70 ±1.38	0.01	0.01	0.09
Stereotypic behavior	1.14 ± 0.31[a]	0.47 ± 0.13[b]	0.93 ± 0.25[ab]	0.19 ± 0.05[c]	0.59	<0.01	0.05
Groom	0.48 ± 0.12	0.77 ± 0.22	0.49 ± 0.12	0.80 ± 0.23	0.93	0.12	0.81
Observing SF	0.89 ± 0.41	0.33 ± 0.14	0.28 ± 0.11	0.18 ± 0.05	0.05	0.10	0.98
Observing MF	1.43 ± 0.31	2.06 ± 0.31	0.14 ± 0.31	0.77 ± 0.31	<0.01	0.15	0.54
Observing humans	3.59 ± 0.93	0.41 ± 0.07	4.95 ± 1.28	0.42 ± 0.08	0.38	<0.01	0.28

[1]The values reported in this table are least-squares means ± SEM for the proportion of time spent doing a specific behavior.
[a–c]Means in a row without similar superscripts differ ($P < 0.05$).

basis and can be almost fully explained by the different levels of feed intake. Utilizing first principles based on energy requirements for maintenance and growth (Nicol and Brookes, 2007) and the measured diet quality, an estimated ADG of 102 and 224 g/d for the SF and MF treatments were predicted using the mean intakes of each treatment and was close to the actual gain measured. Furthermore, the greater FCE reported may reflect a dilution of maintenance requirement for the MF lambs due to their great intake. This is further supported by a modest (3%) difference in apparent DM digestibility between the diets and a lack of any effect of diet treatment on N digestibility or rumen NH_3 or VFA profiles, with the exception of valerate which only consisted of 1% of total VFA present. As such, the benefit to animal performance appears to be as a result of allowing greater levels of nutrient intake.

The reasons for the greater intake achieved by MF lambs are not fully apparent. The daily DM consumption was 3% and 4.5% of LW for the SF and MF, respectively, values for the latter being close to what may be expected as a physiological maximum. In part, this difference in intake may be explained by diet composition, apparent DMD, and NDF, which is associated with reductions in DMI due to rumen digesta outflow rates and thereby increasing the likelihood of physical limitation as an intake constraint (Mertens, 1994). Much of these differences in DMD are likely due to chemical and physical characteristics of the forages fed. For instance, the SF diet had 27.5% more NDF than the MF diet and dietary NDF have been reported to have a strong negative correlation with DMD (Du et al., 2016). The MF diet was composed of ~50% herbs (i.e., plantain and chicory), which contain less structural cell arrangements, termed girder structures, than grasses. Thereby, differences in dietary DMD helped to facilitate the increment in DMI by lambs offered MF. However, the difference in apparent DMD detected between the two diets (89.6% and 85.4% for the MF and SF diets, respectively), was not of a magnitude that could fully explain the difference in DMI that was observed completely. In addition to expressing a greater DMI, the MF lambs exhibited a lower within animal day-to-day CV of DMI compared with the lambs provided the SF diet, indicating the animals offered the MF diet had a more consistent daily feed intake. Similar results were reported by Villalba et al. (2011), who found DMI was more consistent over time when given the choice of the same feed, but with different flavors, compared with a diet monotonous in flavor. Furthermore, our results present a strong negative correlation between CV and total intake, which is similar to the value of −0.82 derived from Ingvartsen et al. (1992). Our results showed that as CV was reduced, and DMI and ADG increased. Improved performance (i.e., ADG) with reduced CV has been reported by a number of studies (Allison, 1985; Galyean et al., 1992; Horn et al., 2005; Williams et al., 2018), which allows the suggestion that the greater intake, and subsequent performance of MF was due to more consistent feed consumption. Although it remains unclear from the current study what the reasons for this may be, it may be speculated that a multitude of other factors including sensory and postingestive signals that also contribute to satiety and intake regulation, and likely contributed to the increased DMI of the MF lambs. It is possible that SF treatment resulted in nutrient-specific satiety or incidental restriction where intake ceased as one nutrient reached a physiological threshold despite other nutrient deficiencies existing (Raubenheimer, 1992; Early and Provenza, 1998; Gregorini et al., 2017). The SF may have resulted in satiety due to the repetitive oro-sensorial experience (i.e., taste and smell) as the intake-related sensorial neurons response saturates and ceases for that particular feed or its nutrient profile, resulting in sensory-specific satiety (Early and Provenza, 1998; Gregorini et al., 2017). Conversely, the joint intake of herbages comprising the MF lambs may have enabled greater intake by reducing the habituation of neurons (Epstein et al., 2009), through altering the consumption sequence. Therefore, due to the incomplete explanation of an increased DMI by MF lambs by dietary NDF and DMD, we argue that the treatment difference in DMI could be explained by a more integrated appreciation of the morphological and thereby phytochemically diversity of the MF diet, that is, combination of basic nutritional and oro-sensorial factors.

Nitrogen dynamics

Although the N intake was 51% greater for the MF compared with the SF lambs, there was no difference in the quantity of N excreted in the feces or urine; in fact, there was a tendency for a reduction in the proportion of consumed N excreted in the urine and a reduction of that excreted in the feces. A greater proportion of N consumed was retained (N use efficiency) by the MF. Furthermore, urinary N concentration (g/L) was 30% lower for the MF compared with the SF, which could suggest a likewise 30% reduction in N loading (kg N per ha) onto pasture at the urine patch level. Evidence for a difference in N loading at the patch level is strengthened by at the same quantity of nitrogen excreted in a greater volume of urine over the day. There is a known curvilinear relationship between N loading at the urine patch level and N leaching (Di and Cameron, 2007; Li et al., 2012). This relationship suggests that the magnitude of difference between SF and MF could be 30%, or even greater, for nitrate leaching. Reductions in nitrogen deposition onto pastures is desirable as excess N has negative environmental implications through its volatilization into the potent greenhouse gas nitrous oxide or by directly leaching into ground water, reducing water quality, and causing eutrophication (Cameron et al., 2013). Although much research is concentrating on reducing the environmental impact of cattle, it is likely that other pasture-based livestock production systems will be encouraged to also reduce their environmental impacts, thereby and based on our results, a dietary solution of multiple forages can be used to reach this goal while improving animal performance.

Welfare and behavioral observations

There was limited evidence in the current study that lambs offered the MF diet had an improved welfare status. Animals within the MF treatment had greater ADG, which is a proxy for welfare status (Barrell, 2019) and there was a reduction in day-to-day CV of DMI, which has been linked to improvements in animal health and welfare (McGuffey et al., 1997, Forbes, 2007). For example, McGuffey et al. (1997) reported a 4% increase in the prevalence of adverse health incidents (e.g., metabolic or digestive disorder) in cows for every 1% increase in day-to-day intake CV within the first 21 d of lactation. However, there was no effect of treatment on the spot samples of TAS or GPx, and only modest effects on observed behaviors. The MF animals spent less time displaying stereotypic behaviors in the afternoon than the SF animals. Although some speculate that stereotypies help animals cope with their environment, the general consensus is that stereotypies are indicative of poor welfare (Broom, 1991). Although the proportion of the day spent conducting stereotypies is small, there would be more concern for animals spending a greater percentage of time exhibiting stereotypic behavior (Philbin, 1998). Thereby, we believe that such behaviors should be minimized where possible. We speculate that a potential cause for this behavioral difference may have been that the environment of the MF lambs was more stimulating, as they had choice from different flavors, textures, and "make your own" feed combinations, allowing more freedom to express individual personality and normal behaviors. Furthermore, time spent observing other sheep, which was greater for the SF, has been suggested as a mechanism to alleviate boredom in an environment with fewer stimulations (Done-Currie et al., 1984). Conversely, less time observing sheep has also been suggested as withdrawn and nonalert state to alleviate the stress of a situation (Done-Currie et al., 1984). However, when we consider that the MF spent more time conducting other "busy" activities (i.e., eating) and less time idle, and exhibited other signs of improved welfare (e.g., greater ADG), the former explanatory speculation is more likely. Again, although the proportion of the day accounted for by such observatory behaviors is small, statistically our results suggest a treatment difference between the MF and SF lambs for such indicators of welfare, indicating further research and refined measurement is needed. Further stressing the importance for consideration of both physiological and behavioral measurements when assessing animal welfare, to build up the most complete picture possible.

Conclusions

Providing animals with a multiforage diet that is taxonomically diverse can provide a win-win-win by increasing DMI and reducing day-to-day variability of intake, improving performance (ADG), and reducing the environmental impact by lowering urinary N excretion, while potentially improving animal welfare. The improvements to animal performance from a multiforage diet appear to have some nutritional basis; however, further research is required on the potential mechanism for improved intake and mild behavioral and welfare differences detected within the current work. Our results provide the basis and outline of potential benefits to designing and establishing functionally diverse pastures; however, more research is required in grazing settings.

Acknowledgments

We thank Agricom (Christchurch, New Zealand), Glenn Judson, and the other team members for providing the forage used during this experiment. We also thank the Vernon Willey Trust Fellowship and the William Machin Doctoral Scholarship for Excellence for funding K.G.'s Ph.D. studies.

Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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