The effects of organic grass and grass-birdsfoot trefoil pastures on Jersey heifer development: Herbage characteristics affecting intake

Journal:	*Journal of Dairy Science*
Manuscript ID	JDS.2020-19563.R3
Article Type:	Research
Date Submitted by the Author:	n/a

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Key Words:	dry matter intake, grass legume mixture, grazing, pasture

Running title: Herbage characteristics affect pasture intake

The effects of organic grass and grass-birdsfoot trefoil pastures on Jersey heifer development: Herbage characteristics affecting intake.

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ABSTRACT. Low dietary energy and decreased intake of herbage have been attributed to the reduced performance of grazing dairy cattle. We hypothesized that grasses with inherently greater energy would interact in a complementary way with condensed tannins (CT) in birdsfoot trefoil to increase herbage intake by grazing dairy heifers. Eight pasture treatments comprised of high-sugar perennial ryegrass (*Lolium perenne* L.; PR), orchardgrass (*Dactylis glomerata* L.; OG), meadow bromegrass (*Bromus riparius* Rehmann; MB), and tall fescue (*Schendonorus arundinaceus* [Schreb.] Dumort; TF) were established in Lewiston, Utah, USA as monocultures and binary mixtures with birdsfoot trefoil (*Lotus corniculatus* L; BFT). Pasture treatments were rotationally stocked by Jersey heifers for 105 days in 2017 and 2018, and herbage samples were collected pre- and post-grazing each 7-day grazing period and analyzed for herbage mass, nutritive value, and apparent herbage intake. We observed differences among pasture treatments in herbage quantity and nutritive value, as well as differences in herbage intake by grazing Jersey heifers. On average, grass-BFT mixtures had greater herbage intake than grass monocultures, and individually every grass-BFT treatment had greater herbage intake than their respective grass monocultures. Using multivariate analyses, we determined that approximately 50% of the variation in herbage intake was due to nutritive and physical herbage characteristics, with the most explanatory being characteristics related to fiber and energy, followed by those related to the percent of BFT in the herbage. Grass monocultures exhibited a range of inherent dietary energy, but there was indication that an energy to crude protein imbalance (e.g., protein deficient) reduced intake of grass monocultures. Moreover, there was some evidence of a complementary effect between increased dietary energy and CT, however, low CT levels made it impossible to determine the effect of CT on herbage intake per se. This study confirmed that chemical and physical characteristics inherent to different pasture species have a large effect on herbage intake by grazing cattle. Pastures planted to binary mixtures of nutritious grasses and birdsfoot trefoil increase herbage intake of temperate pastures by grazing Jersey heifers.

Key Words: dairy heifer, dry matter intake, grass legume mixture, grazing, herbage nutritive value, pasture.

INTRODUCTION

Pasture-based dairies and organic milk production are becoming more prevalent, with organic milk production being the fastest growing segment of organic agriculture (McBride and Greene, 2009, AgMRC, 2015). Over 60% of organic dairies use pastures as their primary (≥ 50%) source of forage and 90% use pastures for at least 25% of their forage (McBride and Greene, 2009, AgMRC, 2015). Organic milk companies often promote their product based upon the health and environmental benefits of milk from cows grazing pasture (Anon, 2020a) and usually require at least 120 grazing days per year for both lactating cows and replacement heifers (Anon, 2020b). However, milk production was 32% lower in organic dairies using the highest amount of pasture forage (75-100%) compared to those using 25% or less pasture forage (McBride and Greene, 2009). Research has shown that low forage dry matter intake (**DMI**) by grazing dairy cows is a major factor limiting milk production (Bargo et al., 2003). Producers have also observed that dairy cattle appear to be more selective grazers than beef cattle, with many dairy cattle showing strong preference for some pasture plants, resulting in even lower DMI of non-preferred traditional pasture species like tall fescue (G. Bingham, Dairy Exec. Committee Organic Valley Coop., Weston, Idaho, personal communication).

Pasture performance can be improved by introducing legumes into grass pastures (Stephenson and Posler, 1988, Hoveland et al., 1991). When grown in mixtures, perennial forage legumes can supply nitrogen to grasses (Mallarino et al., 1990, Nyfeler et al., 2011), potentially maintaining high grass forage yields with reduced nitrogen fertilizer (Cox et al., 2017). Grass-legume pastures can also improve livestock performance due to improved forage nutritive value. Birdsfoot trefoil (*Lotus corniculatus* L., **BFT**) is of particular interest because it is a non-bloating legume and contains condensed tannins (**CT**). Moderate CT concentrations reportedly enhance forage nutritive value by reducing rumen bacterial protein degradation and increasing protein degradation in the intestine, without reducing fiber digestion or voluntary intake by grazing ruminants (Min et al., 2003, Piluzza et al., 2014). Multiple researchers have reported greater steer average daily gains (**ADG**), as well as increased total grazing days, when grazing tall fescue-BFT trefoil mixtures compared to nitrogen-fertilized tall fescue monocultures (Hoveland et al., 1981, Wen et al., 2002, Waldron et al., 2020). Cows that graze BFT monoculture pastures have also shown higher DMI and milk production when compared to animals on grass pastures (Harris et al., 1998, Woodward et al., 2000, MacAdam et al., 2015).

Recently, a team of scientists conducted grass-legume pasture research at Utah State University. They reported that beef steers have better ADG when grazing tall fescue-legume mixtures than tall fescue monocultures, with the BFT mixture resulting in the highest gains and overall net profit (Waldron et al., 2020). Forage nutritive value was improved and forage mass was only slightly less for the grass-legume mixtures compared to fertilized tall fescue (Waldron et al., 2020), with small-plot studies indicating that certain grass-legume mixtures could be more productive than fertilized grass monocultures (Cox et al., 2017). Furthermore, digestion studies showed that grass-BFT mixtures produced less ammonia-nitrogen and methane (Noviandi et al., 2014b). Overall, it was concluded that increased herbage-based dietary energy was needed in grass and CT-containing legume mixture pastures in order to further improve utilization of crude protein (Noviandi et al., 2012, Noviandi et al., 2014a) and livestock growth performance (Waldron et al., 2020) .

'High sugar' grasses with elevated water soluble carbohydrates (**WSC**) have been touted as having concurrent increased digestibility and metabolizable energy (**ME**) (Miller et al., 2001, Edwards et al., 2007, Smith et al., 2007, Waghorn, 2007). These high sugar perennial ryegrass cultivars have also been shown to increase DMI in dairy and beef cattle (Lee et al., 2002, Moorby et al., 2006), which can partially be explained by increased rumen degradation rate, leading to reduced feed retention time and fewer limitations on DMI (Miller et al., 2001). Thus, a possible tool to simultaneously increase dietary energy levels and DMI of grass-legume pastures is using high sugar grasses. However, WSC levels in perennial ryegrass varieties have shown large fluctuations depending on the geographic location, time of year, soil moisture content, night temperatures, and/or day length and temperature (Parsons et al., 2004, Cosgrove et al., 2007, Cosgrove et al., 2014, Robins and Alan Lovatt, 2016). Furthermore, with few exceptions, high sugar grasses, especially high sugar orchardgrass cultivars, have not been widely evaluated in the irrigated pastures of the temperate United States (Robins and Alan Lovatt, 2016). Nor have these high energy grasses been extensively studied when planted in mixture with a CT-containing legume like BFT. Therefore, we undertook this study to investigate the potential to increase herbage intake by grazing binary mixtures of various grasses with BFT. We hypothesized that low levels of CT in BFT would interact in a complimentary way with grasses that had greater inherent energy to further improve herbage intake compared to other mixtures. Specific objectives were to: 1) determine if grass-BFT pastures resulted in greater herbage intake by dairy cattle compared to grass-monoculture pastures, and 2) elucidate which herbage characteristics largely contributed to differences in herbage intake, including if there was a complimentary effect between dietary energy and low levels of CT.

MATERIALS AND METHODS

Pasture Treatments and Pastures

Grazing terminology in this paper is based on Allen et al. (2011). This experiment was conducted at the Utah State University Intermountain Pasture Research Farm (41°57'01.85" North, 111°52'15.75" West, elev. 1,369 m, 46 cm annual precipitation and 56.1 precipitation days per year) located near Lewiston, UT, USA. The soils at the site are a Kidman fine sandy loam (Coarse-loamy, mixed, superactive, mesic Calcic Haploxerolls) and Lewiston Fine Sandy Loam (Coarse-loamy, mixed, superactive, mesic Calcic Haploxerolls). The site is within the semiarid Central Great Basin region of the western USA, characterized by hot, dry summers, and a majority of the annual precipitation as snowfall (Figure 1). In this particular area (Cache county, Utah, USA), the precipitation from winter-time snowfall is stored in reservoirs and used in the summer for irrigated crop production (Utah Climate Center, 2018). The experiment was arranged in a randomized complete block design with 8 pasture treatments in 3 blocks. Pasture treatments were endophyte-free tall fescue (‘Fawn’, **TF**), meadow bromegrass (‘Cache’, **MB**), high-sugar orchardgrass (‘Quickdraw’, **OG**), and high-sugar perennial ryegrass (‘Amazon’, **PR**) in monoculture and as binary mixtures with BFT (‘Pardee’). Seeding occurred in June 2015 with a Great Plains drill (Great Plains Ag, Salina, KS, USA) with double disk openers spaced 15.3 cm apart. Prior to planting, the pastures were prepared with conventional tillage equipment. For grass monocultures, TF, MB, and PR were seeded at 16.8 kg pure live seed/ha and OG at 15.1 kg pure live seed/ha. In binary mixtures, TF, MB, and PR were seeded at 10.1 kg pure live seed/ha, and OG was seeded at 9 kg pure live seed/ha, whereas, the BFT was seeded at 6.7 kg pure live seed/ha in all the grass-legume treatments. The BFT was seeded separately from the grasses to ensure proper depth (i.e., 1.0 and 0.5 cm for grasses and BFT, respectively) (Jensen et al., 2001). As per the recommendation of Waldron et al. (2020), our goal was to get 30 to 40% BFT in the herbage (by weight) and these seeding rates were based upon prior studies that achieved this proportion (Cox et al., 2017, Waldron et al., 2020).

Pastures of each treatment were considered the experimental unit and consisted of 0.45 ha (i.e., 8 treatments × 3 blocks = 24 experimental units, totaling 10.7 ha for the entire experimental area) divided evenly into five 0.09-ha paddocks with a single strand of poly-wire charged with a battery-powered fence energizer (Gallagher USA, Riverside, MO). The study was conducted using organic dairy grazing protocols, so no treatment received commercial fertilizer. However, in 2017 and 2018, approved organic sources of nitrogen were applied to the treatments at yearly rates of 91 and 28 kg nitrogen/ha for grass monocultures and mixtures, respectively, as described herein. Chilean nitrate (sodium nitrate, 15-0-2, N-P-K) (SQM, Santiago, Chile) was applied at 28 kg nitrogen/ha in April to all treatments (both monoculture and mixtures). In addition, grass monocultures also received a second application of 28 kg nitrogen/ha of Chilean nitrate in July, and further received 35 kg nitrogen/ha in the form of hydrolyzed poultry feathers in June 2017 and March 2018 (12.8% nitrogen) as a slow-release source of nitrogen. Pastures were sprinkler irrigated regularly from mid-May to mid-September each year with 7.6 cm water applied in 12-h applications every 14 to 20 days (e.g., approximately 100% evapotranspiration replacement). In 2016, pastures were mechanically harvested in June, and then a preliminary grazing study was conducted throughout the rest of the growing season. Due to differences in how the forage sampling and grazing was conducted, including timing of such events, data from 2016 were not included in the analyses.

Livestock Grazing

Livestock used in the study were 81 (per year) Jersey dairy heifers, with mean initial body weights (**BW**) of 209±47 and 183±72 kg in 2017 and 2018, respectively. Animals were cared for with the approval, and in accordance with the guidelines of the Institutional Animal Care and Use Committee at Utah State University (IACUC protocol #2777 and #10063). Three heifers (testers) were randomly allocated to each of the 8 pasture treatments (TF, MB, OG, PR, TF+BFT, MB+BFT, OG+BFT, and PR+BFT) within each block. Grazing was initiated on the same calendar date for all treatments, when most grasses had reached the E0 stem elongation stage (Moore et al., 1991) and were approximately 25 cm in height (e.g., mid-May). In addition, three replicates of three control feedlot heifers were fed a total mixed ration (**TMR**) formulated to meet the nutritional needs of an ADG target of 0.8 kg/day. Feed offered and refused each day was dried and weighed to calculate DMI.

A fixed stocking rate of 6.7 heifers/ha (i.e., 3 heifers/0.45 ha experimental unit) was used throughout the study. This stocking rate was determined based upon presumed herbage intake of 2.5% BW, previous estimates of these grasses and grass+BFT mixtures herbage mass (Cox et al., 2017), and the objective to ensure excess herbage (e.g., high herbage allowance) in order to emphasize the nutritive value effects on DMI and heifer performance (Baudracco et al., 2010, Sollenberger and Vanzant, 2011). There were no herbage target end-points for grazing a particular paddock. Rather, rotational stocking was used with a set stocking period of 7 days, followed by a rest period of 28 days for each of the five paddocks, such that the entire rotation cycle was 35 days. There were three rotation cycles each year, thus, heifers were on pasture for a total of 105 days (17 May to 30 August, 2017 and 16 May to 29 August, 2018). In a few instances, a tester was removed due to illness and no longer used in herbage intake measures, and a spare heifer was placed in the treatment in order to keep stocking rate the same for each treatment and rotation. The total BW of heifers in each experimental unit (e.g., pasture) were recorded, and later converted to standard animal units (**AU**) to equalize all treatments over the grazing season. The standard AU was defined as a 250 kg Jersey heifer (i.e., mean final heifer BW), thus AU was calculated as the total observed metabolic live BW (i.e., BW $kg^{0.75}$) divided by the metabolic live BW for a 250 kg heifer (i.e., 62.9 kg) (Allen et al., 2011). Paddocks were mowed to a uniform stubble height of 15 cm with a rotary mower at the end of each 7-day stocking period to reduce confounding effects of remaining residue on herbage mass and nutritive value in subsequent grazing rotations. All heifers had access to water and trace mineral supplement. Heifers were weighed at the beginning of the study, and after each 35-day rotation cycle to determine BW as reported by Hadfield et al. (In Review).

Herbage Evaluation and Herbage Intake

Pre-grazing and post-grazing herbage samples were collected throughout the experiment 24 hours prior to (pre-) and immediately after (post-) heifer rotation to the next paddock, by hand-clipping four random quadrats (0.25 m^2) per paddock to a stubble height of 7.6 or 3.8 cm, in 2017 and 2018, respectively. Stubble height was lowered in 2018 to reduce sampling inconsistencies. Post-grazing samples were taken immediately adjacent to the pre-grazing samples, unless it was in an area where heifers had defecated or lain. Herbage samples were placed into a paper bag and dried to a constant weight at 60°C and weighed to determine herbage mass (as dry matter). Pre- and post-grazing compressed sward heights (cm) were measured each time herbage was clipped with a rising plate meter (Jenquip, Fielding NZ) directly over each preand post-grazing clipped quadrat and as the mean of 30 measurements taken in a 'w' pattern throughout each paddock. Individual quadrat herbage mass measurements were regressed against respective rising plate meter measurements, forcing a zero intercept as described by Dillard et al. (2016), to develop herbage mass prediction equations within each year and treatment (R^2 ranging from 0.78 to 0.97). Paddock-based pre- and post-grazing herbage mass were then predicted using these equations and the 30-measurement rising plate meter mean herbage height. Because of the tall height of the herbage in the first rotation cycle, rising plate meter measurements were not reliable for paddocks 3, 4 and 5 in 2017 and paddocks 4 and 5 in 2018 and not used in the calibration equations. An estimate of daily herbage accumulation rate (kg/ha per day) during the grazing period was determined as:

$$\text{Daily herbage accumulation} = \frac{\frac{Rot_n\ pregrazing\ herbage\ mass}{Rot_n\ pregrazing\ HT\ -\ Rot_n\ sample\ stubble\ HT} \times (Rot_n\ pregrazing\ HT\ -\ Rot_{n-1}\ postgrazing\ mowed\ stubble\ HT)}{28\ day\ rest\ period}$$

, where Rot_n represents each successive grazing rotation cycle. The same daily herbage accumulation was assumed for both rotation cycles 1 and 2.

Herbage mass was converted to herbage allowance as described by Sollenberger et al. (2005) for rotational stocking. Briefly, herbage allowance (kg herbage/kg BW) was calculated as: $Herbage\ allowance = \frac{\frac{pregrazing\ herbage\ mass}{BW} + \frac{postgrazing\ herbage\ mass}{BW}}{2}$,

where heifer BW was that from the beginning of each rotation cycle. This method of 'mid-point' calculation addresses questions concerning point-in-time requirements for herbage allowance, and accounts for changes in herbage mass during the 7-day stocking period (Sollenberger et al., 2005).

Dried herbage samples were ground to pass through a 1-mm screen using a Thomas Wiley Laboratory Model 4 mill (Arthur H Thomas Co, Swedesboro, NJ, USA), and were scanned with a Foss XDS near-infrared reflectance spectroscopy (**NIRS**) instrument (Foss, Eden Prairie, MN, USA) to determine herbage nutritive value. The appropriate 2018 NIRS Forage and Feed Testing Consortium (Hillsboro WI, USA) equations were used (i.e., grass hay-18gh50 for monocultures, and mixed hay-18mh50 for the grass-BFT mixtures) resulting in estimates of crude protein (**CP**), neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**), acid detergent lignin (**ADL**), in vitro true digestibility (**IVTD**), 48-hour NDF digestibility (**NDFD**), fatty acids, and ash. Total digestible nutrients (**TDN**) were calculated using the appropriate formulas for grass monocultures or grass-legume mixtures as per Saha et al. (2010) (e.g., not ADF-based). Metabolizable energy was calculated as TDN × 0.04409 × 0.82 (National Research Council, 2000).

An existing grass-legume NIRS equation developed by Waldron et al. (2020) was calibrated using NIRSystem software to predict the proportion of BFT (e.g., % BFT) in the herbage. One-half of all clipped grass-BFT samples were hand separated of which 50% were used for additional equation development and 50% were used for equation validation. Following hand separation, grass and BFT components were dried and weighed to determine actual % BFT in the herbage mass. Components were then ground separately, and a sub-sample recombined at the original ratio was scanned for NIRS analysis. The validation for percent legume was R^2 = 0.94, and standard error of prediction was 6.20. Condensed tannin concentrations in the pre-grazing BFT were predicted using the separated BFT samples and a previously developed NIRS equation (Grabber et al., 2014, Grabber et al., 2015). The equation resulted in prediction statistics of R^2 = 0.88, and standard error of prediction = 3.79 (not validated with an independent sampling). Concentrations of CT in the herbage (%) were calculated as: BFT CT × % BFT, assuming that BFT CT concentration did not change significantly between pre-grazing and post-grazing samples. Consistent with herbage allowance, all herbage nutritive value data, herbage height, % BFT, and % CT in herbage data are presented on a dry matter basis and as the mid-point value between pre- and post-grazing for each 7-day grazing period.

Estimates of apparent herbage intake were based upon herbage disappearance between the pre-grazing and post-grazing herbage mass estimates for each paddock (Macoon et al., 2003), with adjustments made for daily herbage accumulation and grazing efficiency. Grazing efficiency (the proportion of herbage consumed by livestock compared to the total that disappears due to all other activities) increases as grazing pressure increases (Allison et al., 1982, Smart et al., 2010, Baudracco et al., 2013). Estimates of grazing efficiency (on a paddock basis) were calculated by regressing modified herbage allowance data from this study (modified as kg herbage/kg BW/day) using an equation developed from the Allison et al. (1982) comparisons of herbage allowance and grazing efficiency (i.e., grazing efficiency = 105.11 – 463.30 × modified herbage allowance; R^2=0.93). Overall, herbage intake was estimated as:

$$Herbage\ Intake\ = ((pregrazing\ herbage\ mass\ + (daily\ herb.accumul.\ \times 7)) - postgrazing\ herbage\ mass) \times Grazing\ efficiency,$$

and reported as kg ha^{-1}, kg heifer^{-1} day^{-1}, and additionally as kg AU^{-1} day^{-1} to account for differences in heifer growth among pasture treatments (an AU was defined as a 250 kg Jersey heifer, see livestock grazing). For comparison, predicted herbage intake based upon nutritive value was calculated using, 1) a weighted average of the grass and legume DMI equations in Saha et al. (2010), and 2) the all-forage diet DMI equation for growing cattle in National Research Council (2000).

Statistical Analysis

Pastures were defined as the experimental units, and the five paddocks within each pasture experimental unit were observational/sampling units. Therefore, the mean of all herbage samples and apparent herbage intake calculations within a rotation (n=20) were used for statistical analysis. Herbage data and herbage intake were analyzed across years as a randomized complete block design using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA). Pasture-type (monoculture vs mixture), pasture treatment within type, and rotation cycle were considered fixed effects, whereas year and block were considered random. Rotation cycle was considered a repeated measure and the best covariance models for each trait (most often heterogeneous compound symmetry) were determined and used in the analysis (Littell et al., 2006). Mean comparisons were made using Fisher's protected least significant difference (**LSD**) test at the $P = 0.05$ level of probability. Significant pasture treatment × rotation interactions were plotted, using the interaction means and SEM, and examined for patterns. For herbage intake analyses, the TMR treatment was included as an additional experimental unit.

Multivariate analyses were conducted to determine which physical and chemical herbage characteristics were primarily associated with differences in herbage intake following the procedures outlined by Yeater and Villamil (2017). All multivariate analyses were performed using R v3.6.1 (R Core Team, 2019) and the R packages MASS (Venables and Ripley, 2002) and FACTOEXTRA (Kassambara and Mundt, 2020). First, as collinearity was expected among the 18 measured herbage characteristics, a principal component analysis (**PCA**) was performed using the correlation matrix (e.g., to account for different units of measure) with the *princomp*() function. Second, multiple regression was conducted on measures of herbage intake versus the first four principal components (**PC**) (e.g., those with a eigenvalue greater than '1.0') using the *lm*() function. Third, canonical discriminant analysis using the linear discriminants from the first three PC was conducted using the *lda*() and *predict*() functions to determine ability of our herbage characteristics model to discriminate among the predefined pasture treatments. Fourth, PCA biplots were created using the *fviz_pca_var*() function to examine relationships among herbage characteristics. Finally, the herbage characteristics that contributed most to each PC were identified by examining absolute loading scores (e.g., mostly > 0.3) (Yeater and Villamil, 2017) and as a function of loading scores and PC standard deviations (Kassambara and Mundt, 2020). These contributing herbage characteristics were considered to be largely explanatory of the variation in herbage intake and used for further discussion.

RESULTS

Herbage Intake and Trait Differences

Pasture-type (e.g., average of mixture vs monoculture), pasture treatment, and rotation all had a significant ($P < 0.001$) effect on the amount of herbage intake by grazing Jersey heifers. Pasture treatment interactions with rotation were also significant (P = 0.001 to 0.015, Table 1), primarily due to herbage intake decreasing from rotation 1 to 2 and then rebounding in rotation 3. However, PR and PR+BFT were exceptions as herbage intake did not recover in rotation 3 (Supplemental Figure S1; https://doi.org/10.3168/jds.20XX-XXXXX). On average, herbage intake of mixture pastures was greater ($P < 0.05$) than monocultures (Table 1). Herbage intake was also greater ($P < 0.05$) in rotation 3 than rotations 1 and 2 (Table 1), but herbage mass did not limit herbage intake, with only 22 to 47% of herbage utilized (treatment × rotation basis; data not shown). Individual pasture treatments also differed, with the PR+BFT, MB+BFT, and OG+BFT pastures exhibiting the greatest ($P < 0.05$) herbage intake, whereas, PR had the least herbage intake (Table 1). Grass-binary mixtures with BFT consistently increased ($P < 0.05$) herbage intake for all grasses, compared to their respective monocultures (Table 1; Supplemental Figure S1; https://doi.org/10.3168/jds.20XX-XXXXX). Apparent herbage intake of heifers consuming TMR was greater ($P < 0.05$) than pasture treatments and in close agreement with predicted herbage intake (based upon TMR nutritive value) (Table 1). In contrast, herbage intake of pasture treatments was somewhat less than predicted, especially for the PR treatment (Table 1).

Details on the effects of pastures on the measured herbage characteristics are given in Tables 2 through 5. In brief, most main effects were significant ($P < 0.05$), with the exception of pasture-type on IVTD. As expected, pasture treatments exhibited a wide range ($P < 0.05$) of pre- and post-grazing herbage height, herbage mass, and daily herbage accumulation (Table 2). These herbage characteristics were the basis of calculating estimates of herbage intake and herbage allowance, and thus not included in multivariate analyses. Pasture treatment × rotation interactions were also significant ($P = 0.001$ to 0.013) for physical characteristics of herbage mass, herbage allowance and herbage height, and all nutritive value measures. Most of these interactions were changes in magnitude from rotation to rotation, and rarely involved major rank change among pasture treatments. Therefore, herein, the results are primarily presented as the means of the main effects. However, treatment × rotation interactions of highly explanatory herbage characteristics were also explored by plotting interaction means and presented as supplementary material (Supplemental Figures S1-S4; https://doi.org/10.3168/jds.20XX-XXXXX). On average, mixtures had greater ($P < 0.001$) herbage allowance, herbage height, CP, NFC, and ME, and more favorable (i.e. lesser) ($P < 0.001$) NDF and ADF, but less ($P < 0.001$) favorable ADL, NDFD, WSC, and fatty acids than grass monocultures (Tables 3-5). The effect of individual pasture treatments was also significant ($P < 0.001$) for all herbage characteristics, with individual grass+BFT mixtures differing ($P < 0.05$) from their respective grass monocultures for all physical characteristics, and CP, NDF, ADF, ADL, fructans, and ME (Tables 3-5). Metabolizable energy results confirmed that chosen grass entries exhibited a range of inherent dietary energy with PR being greatest, OG and MB intermediate, and TF the least ($P < 0.05$) (Table 3).

Explanatory Herbage Characteristics

The first four PC from PCA of all measured herbage variables explained 38, 27, 19, and 6% of the variation found in the data, respectively (i.e., 90% cumulatively). Multiple regression on these four PC resulted in significant models ($P < 0.001$) with all contributing ($P < 0.001$), and R^2 values of 0.53, 0.51, and 0.53 for measures of apparent herbage intake of kg ha^{-1}, kg AU^{-1} d^{-1}, and kg $heifer^{-1}$ d^{-1}, respectively. Canonical discriminant analysis resulted in the first three linear discriminants explaining 75, 19, and 6% of the variation between the pre-defined pasture treatments, respectively (100% cumulatively). Furthermore, the first linear discriminant was primarily influenced by PC 1 and 2 as determined by coefficients. However, cross-validation determined that the accuracy of discriminating among pasture treatments was on average only 58%, with the PR and PR+BFT treatment most likely to be characterized (89%) and the MB treatment least characterized (22%) (Supplemental Table S5 https://doi.org/10.3168/jds.20XX-XXXXX).

The first PC included all but three of the 16 herbage characteristics, but NDF, ADF, NFC, ME, IVTD, and WSC contributed the most to this PC (Figure 2). Principle component 1 showed a contrast between NDF and ADF on one hand, and NFC, ME, IVTD, and WSC on the other, indicative that the pasture treatments mainly differed in their digestibility and resulting energy (Figure 2). On average, PR+BFT had the least (most favorable) ($P < 0.05$) ADF and NDF, followed closely by PR, whereas, NDF was greatest ($P < 0.05$) in MB and ADF greatest in MB and OG (Table 3). The PR and PR+BFT treatments exhibited the greatest ($P < 0.05$) concentrations of NFC and WSC validating the claim of it being a "high-sugar" perennial ryegrass cultivar, whereas, these carbohydrate fractions in the putative "high-sugar" OG and OG+BFT were the least (i.e., NFC; $P < 0.05$) or not different (i.e., WSC) compared to the remaining pasture treatments (Table 3). Metabolizable energy followed a similar pattern as carbohydrate concentrations, however, ME in MB+BFT and OG+BFT were equivalent to PR and PR+BFT in rotations 2 and 3 (Supplemental Figure S2; https://doi.org/10.3168/jds.20XX-XXXXX).

The second PC showed a contrast between % BFT, % CT in herbage, CP, and ADL versus NDFD, suggesting that the variation explained by this PC was primarily related to the amount of BFT in the herbage (Figure 2). On average, PR had the greatest ($P < 0.05$) NDFD and least ($P < 0.05$) ADL (e.g., most favorable values), whereas, PR+BFT had the least favorable levels of both these characteristics corresponding to the greatest ($P < 0.05$) BFT proportion (Table 4; Supplemental Figures S1, S3, and S4; https://doi.org/10.3168/jds.20XX-XXXXX). Similarly, CP was greater ($P < 0.05$) in all grass-BFT mixtures than their respective monocultures. A notable pasture treatment × rotation interaction resulted from PR+BFT in which NDFD decreased, and ADL, % BFT, and % CT in herbage increased between rotations 2 and 3, in contrast to the other treatments (Supplemental Figure S4; https://doi.org/10.3168/jds.20XX-XXXXX).

The third PC included explanatory variables of herbage allowance, herbage height, and fructan, and grouped herbage allowance and herbage height versus fructan, somewhat suggesting an herbage mass effect (Figure 3). Herbage allowance and herbage height both decreased from rotation 1 to 2 but very little from rotation 2 to 3 (e.g., treatment × rotation interaction significance of $P = 0.002$). This herbage allowance decrease was more so for MB, TF, and OG and their mixtures than the shorter statured, PR, and PR+BFT (data not shown). Overall, herbage allowance was greatest ($P < 0.05$) for MB+BFT and TF+BFT, and nearly double the least found in PR. The fourth PC only explained 6% of the variation among treatments, with the primary explanatory characteristic of fatty acids (Figure 3). With the exception of TF and TF+BFT, monocultures had greater ($P < 0.05$) fatty acids than respective mixtures (Table 5).

DISCUSSION

Herbage Intake Differences

Pasture-based milk production is the fastest growing segment of U.S. organic agriculture; but such dairies experience up to 32% decrease in milk production (McBride and Greene, 2009), due to reduced DMI by grazing dairy cows (Bargo et al., 2003). Thus, characterizing pasture herbage characteristics that are associated with herbage intake is useful in identifying the optimum pasture mixtures. In this study we observed variation among pasture treatments in both herbage intake, as well as in herbage quantity and quality. On average, grass-BFT mixtures had greater ($P < 0.05$) herbage intake than grass monocultures (6.1 and 4.5 kg/AU per day, respectively), but both were less than heifers consuming TMR (7.9 kg/AU per day) (Table 1). These levels of apparent herbage intake equate to 2.4 and 1.8% of heifer BW for grass-BFT mixtures and grass monocultures, respectively, with the monoculture herbage intake considerably less than norms (e.g., 2.6% BW) for growing cattle within this weight class consuming a diet with similar ME (National Research Council, 2000). The two measures of predicted herbage intake are for all forage diets, and for the most part in agreement with each other (Pearson's correlation [r_P] of 0.92 and Spearman's rank correlation [r_S] of 0.83). In contrast, there was a discrepancy between measured and predicted herbage intake (kg/AU per d) (r_P=0.32 and 0.54, for Saha and NRC estimates, respectively) (Table 1), suggesting our herbage intake estimates may have been low. While pasture intake is expected to be less than TMR intake (Bargo et al., 2003), our lesser values could also be reflective of the difficulties of measuring pasture intake using the disappearance method. As such, one possible explanation could be unaccounted herbage accumulation (e.g., growth/regrowth) during the 7-day grazing period. However, our estimated herbage accumulation rates of 55 and 51 kg/ha per day align with previous reports of fescue and orchardgrass regrowth rates of 20 to 60 kg/ha per day (Belesky and Fedders, 1994, Bonesmo and Skjelvåg, 1999), suggesting that our model sufficiently accounts for regrowth. The most notable difference between observed and predicted herbage intake was in perennial ryegrass. This might suggest that the rising plate meter was less effective at measuring disappearance of perennial ryegrass, which is lower in stature and more closely grazed by livestock than the other grass species (e.g., as previously noted we reduced stubble height for clipped samples between years based upon these observations). However, the methodology of separate rising plate meter prediction equations for each treatment/year combination and resulting regression R^2 of 0.81 and 0.85 for PR in 2017 and 2018, respectively, indicate this was not the case. Overall, we conclude that our apparent intake measures of pasture are mostly reasonable estimates of actual herbage intake, and provide reliable relative comparisons among pasture treatments, with interpreting results of the PR treatment requiring some caution.

The greater herbage intake of grass-BFT mixtures compared to grass monocultures, coincides with previous studies that have concluded that legumes increase forage intake. For instance, Woodward et al. (2000) found that cows fed freshly harvested BFT in a feed bunk had increased forage intake compared to cows fed freshly cut perennial ryegrass, and MacAdam et al. (2015) reported that dairy cows grazing BFT monocultures had greater herbage intake than those grazing grass monocultures. Ribeiro Filho et al. (2003, 2005) found that grass-clover swards with clover contents of 42% increased herbage intake over the grass monocultures, but swards with 27% clover did not ($P > 0.05$). In contrast, our BFT proportion ranged from 14 to 41% of herbage (Table 1) and significantly increased ($P < 0.05$) herbage intake by 0.85 to 3.60 kg/AU per day over respective grass monocultures, regardless of BFT percentage.

It is often difficult to obtain significant differences in grazing studies given the limited replication and high spatial and biological variability of pastures and cattle (Bransby, 1989, Giesbrecht, 1989). However, multivariate analysis utilizes correlated variables, such as herbage characteristics, and given the response data, can point to which variables drive even subtle differences among the treatments (Yeater and Villamil, 2017). As such, multiple regression using the first four principal components from PCA explained up to 53% of the variation in herbage intake by the Jersey heifers with the most explanatory herbage characteristics corresponding to fiber and energy (PC 1) and BFT-related characteristics (PC 2). The inability of our models to explain 100% of the variation indicates that there are unidentified variables associated with herbage intake, possibly including environmental conditions, heifer breeding and background, and measuring errors. Nevertheless, we found differences ($P < 0.001$) among pasture treatments for herbage intake, and the moderately high R^2 of 53% and 58% from PCA-regression and canonical discriminant analysis, respectively, indicate that herbage intake differences were associated with the variation in herbage.

Herbage Characteristics Associated with Differences in Pastures and Herbage intake

Fiber and energy (PC 1). Fiber in forage diets has been reported to be the single best nutritive predictor of forage intake (Waldo, 1986) and is the main source of energy for ruminants (Wilson, 1994). As such, it is not surprising that fiber concentrations, digestibility, and energy contributed the most to PC1, indicative of their importance in explaining the differences among pasture treatments. Although it has been proposed that NDF intake of lactating dairy cattle on mixed rations will not exceed 1.3% of BW, research indicates that animals on pastures with high herbage allowance often consume greater than 1.3% NDF (Vazquez and Smith, 2000). In this study, herbage allowance greatly exceeded metabolic need (e.g., ~2.0 to 2.5 % of BW) and average apparent NDF intake was 1.0 and 1.3% of BW, for grass monocultures and BFT mixtures, respectively (based upon herbage intake and NDF estimates). However, the MB+BFT and OG+BFT treatments exhibited the greatest apparent NDF intake at 1.4% BW, but along with PR+BFT were the most consumed pasture treatments. Fiber constituents such as NDF are highly variable and influenced by multiple factors including plant maturity. Yet, the relative differences observed among pastures were consistent with previous geographically close studies for orchardgrass (51-61% NDF) (Robins et al., 2015, Robins et al., 2016) and tall fescue (50 to 55% NDF) (Waldron et al., 2020). Thus, this among-study relative consistency for species-inherent NDF may help explain why the NDF component was of such explanatory importance in multivariate and regression analyses.

Total dietary energy (i.e., ME), non-structural carbohydrates (**NSC**) (i.e., NFC and WSC), and digestibility (i.e., IVTD) were the other predominate explanatory variables in PC1.

On average, grass-BFT mixture ME was greater ($P < 0.05$) than that of grass-monoculture, and every individual grass-BFT pasture ME was greater ($P < 0.05$) than its corresponding grass-monoculture. Given these differences, and the fact that energy is often the most limiting nutrient on pasture (Kolver and Muller, 1998, Bargo et al., 2003), it is not surprising that ME was associated with pasture treatment and herbage intake differences. Non-fiber carbohydrates were closely correlated with ME (Figure 2), with the PR+BFT and OG+BFT treatments having greater ($P < 0.05$) NFC than their respective monocultures. Interestingly, multiple authors have concluded that feeding high NFC supplements to grazing animals reduced intake of pasture, which they attributed to reduced ruminal pH and a lower rate of fiber digestion (Vazquez and Smith, 2000, Baudracco et al., 2010). However, Stakelum and Dillon (2003) found that supplementing with fibrous concentrates, had a less depressing effect on grass intake than cereal (starchy) based concentrates, possibly helping to explain our results.

Multiple authors have indicated that grasses with increased WSC have more efficient digestibility and increased metabolizable energy (ME) levels (Miller et al., 2001, Edwards et al., 2007, Smith et al., 2007, Waghorn, 2007). We also found that digestibility and WSC were highly correlated with each other and also with ME (Figure 2). Mayland et al. (2000) examined the effect that different types of NSC have on animal preference in tall fescue and found that animals preferred grasses with greater NSC, but no specific sugar fraction influenced animal preference. While the relationship between preference and herbage intake is nebulous, it is clear that livestock prefer grasses with greater total carbohydrates and WSC (Mayland et al., 2000, Cougnon et al., 2018). Overall, our study indicates that inherently greater carbohydrate and dietary energy concentrations in the herbage enhance grazing intake.

Birdsfoot trefoil-related characteristics (PC2). Previous researchers reported that dairy cattle grazing BFT monoculture pastures have greater herbage intake compared to those grazing grass pastures (Harris et al., 1998, Woodward et al., 2000, MacAdam et al., 2015). Our study adds to these reports by finding that BFT at a range of proportions in mixtures with grass was also correlated with increased herbage intake compared to grass monoculture (Tables 1 and 4). Waldron et al. (2020) recommended 30+% BFT in mixtures for optimal livestock performance, and in 2017 all +BFT treatments approached or exceeded this BFT proportion during rotations 2 and 3. However, %BFT declined substantially from 2017 to 2018, with only PR+BFT consistently at or above the 30% level in both years (Supplemental Figure S4; https://doi.org/10.3168/jds.20XX-XXXXX). Nevertheless, our study indicates that BFT comprising just 14 to 41% of grass-BFT mixed herbage results in increased herbage intake compared to grass monoculture. Furthermore, PCA indicated that the CT, protein, and lignin concentrations were highly correlated with BFT proportion, and as such were positively correlated with differences among pasture treatments (Figure 2).

Piluzza et al. (2014) suggested that low levels of CT improve herbage intake, and we hypothesized that CT in the BFT would interact in a complementary way with inherently greater grass energy (e.g., WSC) to further increase herbage intake. This was partially validated, as WSC and ME were included as a secondary traits in this tannin-containing PC (i.e., PC2), whereas the % CT in the herbage was also included as a secondary trait in the energy-related PC (i.e., PC1). Furthermore, within the +BFT treatments, there was a trend of increasing ME, WSC and CT levels to be associated with increased herbage intake. However, the full effect of CT in this study was probably confounded by extremely low CT concentrations. Low levels of CT from forage legumes have been shown to improve protein use efficiency and livestock performance (Min et al., 2003), but those benefits are usually realized at CT concentrations of 1 to 2.5% (MacAdam, 2019). This threshold CT concentration is double our 0.5% CT in the PR+BFT herbage, and 4 to 6-times greater than that in other +BFT treatments. It is important to note that the BFT CT levels were similar amongst all +BFT treatments (Table 4), indicating that differences in % CT in herbage were entirely due to differences in % BFT (as opposed to differential CT synthesis). Thus, inasmuch as three +BFT treatments had greater ($P < 0.05$) herbage intake than all other treatments, but also exhibited widely ranging % CT in herbage (0.15 to 0.48%), it is impossible to draw a conclusion as to the effect of CT per se on grazing intake. Overall, the PC2 data supports that BFT proportion and its association with CT and CP influenced herbage intake by Jersey heifers.

Moore et al. (1999) found that CP increased ruminant intake when TDN:CP ratio was greater than 7 (e.g., deficient in nitrogen). In our study, the TDN:CP ratio was 5.2 for +BFT and 7.2 for monoculture pasture types, with ratios of 7.1, 6.8, 7.3, and 7.5 for PR, MB, OG, and TF treatments, respectively. Fisher (2002) also reported that CP less than 6-9% was closely associated with forage intake, but that digestibility and NDF had greater influence on intake when protein was over 9%. Average CP of our monoculture pastures was 9.7 and 8.6 in rotations 1 and 2, respectively, compared to 13.8 and 14.4 for mixtures. Therefore, it is likely that monoculture treatments experienced a ME:CP imbalance such that they were deficient in CP, providing support for consistently lesser herbage intake of monocultures compared with their respective mixtures.

It has been hypothesized that superior cell wall digestibility (e.g., increased NDFD and reduced lignin) increases forage intake due to improved digestive passage rate, however, Brink and Soder (2011) were unable to validate this in several cool-season grasses varying in NDFD.

In our study, NDFD was negatively associated with PC2 corresponding to greater NDFD on average in grass monocultures compared to grass+BFT mixtures (Table 4). Furthermore, PR+BFT and TF+BFT pastures had less ($P < 0.05$) NDFD and all individual grass-BFT mixtures exhibited up to 64% greater ($P < 0.05$) highly indigestible lignin (ADL) than their respective grass-monocultures, making these results counter-intuitive to the greater herbage intake observed for all grass+BFT pastures. Overall, these results indicate that the effect of % BFT masked any putative positive effects of improved cell wall digestibility on grazing intake.

Herbage allowance, height, and fructan (PC3). The importance of herbage allowance on herbage intake has been well documented in the literature (Vazquez and Smith, 2000, Sollenberger et al., 2005, Baudracco et al., 2010, Sollenberger and Vanzant, 2011, Baudracco et al., 2013). In general, at low herbage allowance, non-nutritional factors like herbage mass largely drive herbage intake and as such intake by cattle increases as herbage allowance increases (Baudracco et al., 2010, Sollenberger and Vanzant, 2011). In contrast, at high herbage allowance, increased herbage mass has little effect, whereas, herbage nutritional factors largely control herbage intake (Baudracco et al., 2010, Sollenberger and Vanzant, 2011). For instance, Bargo et al. (2002) showed that as herbage allowance increased from 20 to 40 kg herbage mass/cow per day (i.e., based upon 631 kg cow and a 20-d grazing period equivalent to 0.79 and 1.27 kg herbage mass/kg BW, respectively), herbage intake also increased from 2.9% to 3.4% of BW of dairy cattle. In contrast, Brink and Soder (2011) evaluated Holstein heifers grazing in Wisconsin and found no relationship between herbage intake and herbage allowance. They noted that they purposely set herbage allowance high (i.e., based upon the Spring and Summer data equivalent to 0.99 to 1.97 kg herbage mass/kg BW) for ad libitum intake, in order to reduce the confounding effect of herbage allowance on the intake-sward structure relationships. Likewise, we also purposely used low stocking rates to reduce the confounding of grasses varying widely in herbage mass on the intake-herbage nutritive value relationship. As such, our herbage allowance (i.e., 1.51 and 1.93 kg herbage mass/kg BW, for monocultures and BFT-mixtures, respectively) are similar to theirs and also suggest ad libitum intake with only 35 and 34% utilization of the grass-BFT and grass monoculture pastures, respectively. Thus, it is not surprising that PC3, which is largely driven by herbage allowance, only explained 19% of the variation among pasture treatments. Nevertheless, PC3 was a significant factor ($P < 0.001$) associated with herbage intake in multiple regression, and every grass-BFT mixture had greater ($P < 0.05$) herbage allowance and corresponding greater herbage intake than their respective grass monocultures. Even so, it is possible that differences in herbage allowance among pasture treatments still partially confounded the actual relationship between nutritional value and +BFT on herbage intake. Future grazing studies with these pasture species and mixtures at a similar herbage allowance for all treatments (preferably approaching ad libitum herbage intake) would exclude this confounding and help elucidate how the inherent differences among these treatments in nutritional value and +BFT are associated with herbage intake.

The inclusion of herbage height with herbage allowance in this PC is consistent with other studies. For instance, Tharmaraj et al. (2003) reported that herbage intake was not only greater in perennial ryegrass swards with herbage allowance of 70 kg herbage mass/cow per day compared to 35 kg herbage mass/cow per day, but that intake increased in both herbage allowance regimes when pre-grazing herbage height went from 14 to 28 cm. In comparison, we observed that pre-grazing herbage height for all +BFT treatments were taller on average by 3.8 cm and had greater herbage intake than their respective monocultures. Fructans are a subcomponent of total NSC, which are generally positively associated with livestock preference

(Mayland et al., 2000). Thus, the biplot contrast of fructan with the herbage allowance-height complex makes fructan's inclusion in PC3 counterintuitive. Especially given that all +BFT treatments, except for PR+BFT, had greater herbage allowance, herbage height, and fructan than their respective monocultures. These results suggest that the PR treatment, which had the greatest fructan levels but also the least herbage allowance and herbage height, was the primary driver of fructan's inclusion in PC3, making fructan's association with our herbage intake inconclusive.

Fats (PC4). PC4 only explained 6 of the variation among treatments, but was still a significant contributor to our regression model and identified fatty acids as contributing herbage characteristic not previously discussed. Bargo et al. (2003) conducted an extensive review and concluded that fat-supplemented dairy cows on pasture generally do not significantly differ in DMI compared to non-supplemented animals. Likewise, on average grass-monoculture pastures had greater ($P < 0.05$) fat (as estimated by fatty acids), but lesser ($P < 0.05$) herbage intake than grass-BFT mixtures. Schroeder et al. (2004) also hypothesized that since typical pasture diets are relatively low in fat content, a growth response from minimal additional fat may be expected. Our heifers received all dietary fat from grazed herbage (ranging from 2-3%), and perhaps as hypothesized (Schroeder et al., 2004), even minimal differences had an effect on herbage intake, especially in the OG and OG+BFT treatments which exhibited greater ($P < 0.05$) fat in both monoculture and BFT mixture than the other monoculture and mixture pastures, respectively.

CONCLUSION

We observed differences among pasture treatments in herbage quantity and nutritive value, as well as differences in herbage intake by grazing Jersey heifers. The study showed that grass+BFT binary mixtures increased herbage intake over grass monocultures, regardless of the nutritive value of the grass. Approximately 50% of the variation in herbage intake was explained by nutritive and physical herbage characteristics, including primarily fiber and energy (NDF, ADF, NFC, ME, IVTD, and WSC) and those characteristics related to the proportion of BFT in the herbage (% BFT, % CT in herbage, ADL, CP, and NDFD). Grasses exhibited a range of inherent dietary energy, and there was evidence that resulting ME:CP imbalances (e.g., CP deficient) reduced intake of grass monocultures. We had hypothesized that CT in the birdsfoot trefoil would interact in a complementary way with greater energy in the grasses to increase herbage intake, which was partially validated by the high CT and ME treatment (PR+BFT) ranking first for herbage intake. However, three +BFT treatments had equivalent herbage intake, but widely ranging CT levels (0.15 to 0.48%) making it impossible to determine the effect of CT on herbage intake per se. Overall, pastures consisting of binary mixtures of high-energy grasses and as little as 14% birdsfoot trefoil increased herbage intake by grazing Jersey heifers.

ACKNOWLEDGEMENTS

This research was supported in part by the U.S. Department of Agriculture, Agricultural Research Service, and the USDA National Institute of Food and Agriculture (NIFA) through, 1) Western Sustainable Agriculture Research and Education program (WSARE) grant no. 2016-38640-25383/subaward SW17-046, and, 2) Organic Agriculture Research and Extension Initiative (OREI) grant no. 2017-51300-26866/project accession no UTA01375. USDA is an equal opportunity employer and service provider. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture or Utah State

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Table 1. Measures of estimated grazing efficiency (GE), and apparent and predicted herbage intake by Jersey heifers (HF) from a grazing study in Lewiston, Utah, USA in 2017 and 2018. Pasture treatments included monocultures of meadow brome (MB), orchardgrass (OG), perennial ryegrass (PR) and tall fescue (TF), and each grass in a binary mixture with birdsfoot trefoil (BFT).

		Apparent Herbage Intake[2,3]				Predicted intake[4]	
Rotation	GE[1], %	kg/ha	kg/AU per day	kg/HF per day	%BW	Saha kg/AU per day	NRC kg/AU per day
1	75[b]	1020[b]	5.51[b]	4.59[b]	2.39[a]	7.23[a]	6.45[b]
2	86[a]	1010[b]	5.21[b]	4.62[b]	1.99[b]	6.11[c]	6.11[c]
3	87[a]	1280[a]	6.08[a]	5.72[a]	2.30[a]	6.62[b]	6.67[a]
SEM	4.3	37	0.14	0.20	0.15	0.10	0.07
Pasture type							
Mixture	80[b]	1290[a]	6.09[a]	5.45[a]	2.44[a]	6.94[a]	6.88[a]
Mono	85[a]	920[b]	4.52[b]	3.92[b]	1.81[b]	6.36[b]	5.95[b]
SEM	4.2	37	0.23	0.33	0.09	0.09	0.06
TYPE*ROT *P*-val.	0.032	0.519	0.701	0.484	0.701	0.217	0.041
Pasture trmt							
TMR	---	---	7.95[a]	7.31[a]	3.18[a]	7.31[a]	7.00[b]
PR	91[a]	630[d]	3.05[f]	2.65[e]	1.22[f]	7.00[b]	6.37[c]
PR+BFT	80[d]	1410[a]	6.65[b]	5.97[b]	2.66[b]	7.52[a]	7.61[a]
MB	83[bc]	1140[b]	5.55[cd]	4.82[c]	2.22[cd]	6.17[d]	5.83[d]
MB+BFT	78[e]	1360[a]	6.43[b]	5.77[b]	2.57[b]	6.76[c]	6.88[b]
OG	85[b]	1070[b]	5.30[d]	4.55[c]	2.12[d]	6.29[d]	5.85[d]
OG+BFT	82[c]	1310[a]	6.25[bc]	5.56[b]	2.50[bc]	6.90[b]	6.51[c]
TF	83[c]	860[c]	4.19[e]	3.65[d]	1.68[e]	5.97[e]	5.73[d]
TF+BFT	79[de]	1060[b]	5.04[d]	4.50[c]	2.02[d]	6.60[c]	6.51[c]
SEM	4.3	52	0.30	0.31	0.19	0.08	0.12
TRMT*ROT *P*-val.	0.002	0.001	0.015	0.002	0.015	<0.001	<0.001

[a-f]Mean values within columns of rotation, treatment type, or pasture treatment with different superscripts are significantly different ($P = 0.05$).

[1]Grazing efficiency, defined as the proportion of herbage consumed by livestock compared to the total that disappears due to all activities, was estimated as a function of herbage allowance.

[2]Apparent herbage intake measured as the disappearance of herbage mass as determined via a calibrated rising plate meter at pre- and post-grazing of 7-day grazing periods. Estimated herbage accumulation during the 7-day grazing period and grazing efficiency included in estimates.

[3]The number and body weight of heifers (HF) in each paddock were recorded and converted to animal units (AU) where for this study one AU = a 250 kg Jersey heifer (Allen et al., 2011).

[4]Predicted herbage intake calculated using a weighted average of the grass and legume DMI equations in Saha et al. (2010) and the all-forage diet DMI equation for growing cattle in National Research Council (2000).

Table 2. Herbage height, mass, and accumulation of pasture treatments in a Jersey heifer grazing study conducted in Lewiston, Utah, USA in 2017 and 2018. The main effects and interactions among rotation, pasture type, and pasture treatment for these herbage characteristics are shown. Pasture treatments included monocultures of meadow brome (MB), orchardgrass (OG), perennial ryegrass (PR) and tall fescue (TF), and each grass in a binary mixture with birdsfoot trefoil (BFT).

Rotation	Pre-HT,[1] cm	Post-HT, cm	Pre-MASS, kg/ha	Post-MASS, kg/ha	DHA, kg/ha per day
1	42.3 ^{a}	29.9 ^{a}	3400 ^{a}	2400 ^{a}	55.8 ^{b}
2	31.3 ^{c}	21.3 ^{b}	2480 ^{c}	1690 ^{b}	55.8 ^{b}
3	33.2 ^{b}	19.9 ^{c}	2640 ^{b}	1580 ^{c}	61.6 ^{a}
SEM	0.56	0.81	313	227	3.6
Pasture type[2]					
Mixture	38.0 ^{a}	24.7 ^{a}	3270 ^{a}	2130 ^{a}	68.5 ^{a}
Mono	33.2 ^{b}	22.6 ^{b}	2420 ^{b}	1650 ^{b}	46.9 ^{b}
SEM	0.64	0.70	312	226	3.6
TYPE*ROT *P*-val.	0.527	0.620	0.144	0.166	0.896
Pasture trmt					
PR	24.5 ^{d}	17.4 ^{g}	1760 ^{e}	1250 ^{f}	27.4 ^{d}
PR+BFT	32.0 ^{c}	19.6 ^{f}	3370 ^{a}	2070 ^{b}	69.9 ^{a}
MB	35.3 ^{b}	22.7 ^{e}	2750 ^{c}	1770 ^{d}	55.1 ^{c}
MB+BFT	40.2 ^{a}	25.8 ^{bc}	3510 ^{a}	2260 ^{a}	70.8 ^{a}
OG	36.4 ^{b}	23.3 ^{de}	2530 ^{d}	1610 ^{e}	50.7 ^{c}
OG+BFT	39.9 ^{a}	24.6 ^{cd}	3030 ^{b}	1860 ^{cd}	64.4 ^{b}
TF	36.5 ^{b}	27.2 ^{b}	2610 ^{cd}	1960 ^{bc}	54.6 ^{c}
TF+BFT	39.8 ^{a}	28.9 ^{a}	3160 ^{b}	2310 ^{a}	69.0 ^{ab}
SEM	0.85	0.81	315	229	3.9
TRMT*ROT *P*-val.	0.002	<0.001	0.013	<0.001	<0.001

$^{a\text{-}g}$Mean values within columns of rotation, treatment type, or pasture treatment with different superscripts are significantly different ($P = 0.05$).

[1]Abbreviations: Compressed height pre-grazing and following a 7-day grazing period (Pre-HT and Post-HT, respectively), herbage mass pre-grazing and following a 7-day grazing period (Pre-MASS and Post-MASS, respectively), and estimated daily herbage accumulation as calculated from herbage growth during 28 day rest period between grazing rotation cycles (DHA).

[2]For pasture-type and pasture-treatment, values represent the mean of three 35-d rotations.

Table 3. Measures of herbage characteristics[1] of pasture treatments in a Jersey heifer grazing study conducted in Lewiston, Utah, USA in 2017 and 2018. These characteristics were identified as contributing the most to principal component (PC) 1 following PC analysis of the variation among pasture treatments (PC1 explained 38% of variation). Pasture treatments included monocultures of meadow brome (MB), orchardgrass (OG), perennial ryegrass (PR) and tall fescue (TF), and each grass in a binary mixture with birdsfoot trefoil (BFT).

Rotation	NDF, %	ADF, %	NFC, %	ME, Mcal/kg	IVTD, %	WSC, %
1	52.1 [c]	32.8 [c]	26.7 [a]	2.84 [a]	80.2 [a]	9.7 [a]
2	58.2 [a]	38.2 [a]	19.2 [b]	2.55 [c]	73.6 [c]	5.8 [c]
3	54.7 [b]	35.6 [b]	19.0 [b]	2.64 [b]	76.2 [b]	6.3 [b]
SEM	2.05	0.88	2.04	0.030	0.63	0.73
Pasture type[2]						
Mixture	52.5 [b]	34.4 [b]	22.0 [a]	2.74 [a]	76.8 [a]	6.9 [b]
Mono	57.5 [a]	36.6 [a]	21.2 [b]	2.61 [b]	76.5 [a]	7.6 [a]
SEM	2.16	0.88	2.03	0.029	0.63	0.72
TYPE*ROT *P*-val.	0.060	0.126	0.075	0.832	0.121	0.065
Pasture trmt						
PR	49.5 [e]	31.1 [d]	27.7 [b]	2.78 [b]	81.5 [a]	11.2 [a]
PR+BFT	42.1 [f]	30.2 [e]	29.5 [a]	2.85 [a]	79.0 [b]	8.5 [b]
MB	60.5 [a]	40.3 [a]	19.4 [cd]	2.60 [c]	76.2 [cd]	6.4 [c]
MB+BFT	55.9 [d]	36.2 [c]	19.4 [cd]	2.76 [b]	76.4 [c]	6.4 [c]
OG	61.2 [a]	37.3 [b]	18.1 [e]	2.58 [c]	73.0 [e]	6.4 [c]
OG+BFT	57.3 [c]	35.9 [c]	19.1 [d]	2.75 [b]	75.7 [cd]	6.5 [c]
TF	58.9 [b]	37.7 [b]	19.4 [cd]	2.49 [d]	75.3 [d]	6.4 [c]
TF+BFT	54.7 [d]	35.2 [c]	20.0 [c]	2.60 [c]	76.2 [cd]	6.1 [c]
SEM	2.09	0.92	2.04	0.031	0.69	0.74
TRMT*ROT *P*-val.	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a-f]Mean values within columns of rotation, treatment type, or pasture treatment with different superscripts are significantly different ($P = 0.05$).

[1]Herbage characteristic abbreviations: acid detergent fiber (ADF), in-vitro true digestibility (IVTD), metabolizable energy (ME), neutral detergent fiber (NDF), non-fiber carbohydrates (NFC), and water-soluble carbohydrates (WSC).

[2]For pasture-type and pasture-treatment, values represent the mean of three 35-d rotations.

Table 4. Measures of herbage characteristics[1] of pasture treatments in a Jersey heifer grazing study conducted in Lewiston, Utah, USA in 2017 and 2018. These characteristics were identified as contributing the most to principal component (PC) 2 following PC analysis of the variation among pasture treatments (PC2 explained 27% of variation). Pasture treatments included monocultures of meadow brome (MB), orchardgrass (OG), perennial ryegrass (PR) and tall fescue (TF), and each grass in a binary mixture with birdsfoot trefoil (BFT).

Rotation	BFTPERC, %	BFTCT, %	HERBCT, %	CP, %	ADL, %	NDFD, %NDF
1	14.6 [b]	0.81 [b]	0.14 [c]	11.8 [b]	3.9 [c]	64.3 [a]
2	26.4 [a]	1.15 [a]	0.27 [b]	11.5 [b]	4.3 [a]	57.2 [c]
3	27.7 [a]	1.35 [a]	0.35 [a]	13.9 [a]	4.0 [b]	59.6 [b]
SEM	7.1	0.08	0.06	0.20	0.07	0.40
Pasture type[2]						
Mixture	22.9	1.11	0.25	14.7 [a]	4.6 [a]	58.5 [b]
Mono	---	---	---	10.1 [b]	3.6 [b]	62.1 [a]
SEM				0.23	0.09	0.56
TYPE*ROT *P*-val.				0.043	0.001	0.203
Pasture trmt						
PR	---	---	---	10.8 [d]	3.3 [f]	66.2 [a]
PR+BFT	41.0 [a]	1.25 [a]	0.48 [a]	17.6 [a]	5.4 [a]	55.4 [d]
MB	---	---	---	10.5 [de]	3.8 [e]	60.6 [b]
MB+BFT	20.7 [b]	1.10 [ab]	0.22 [b]	15.3 [b]	4.4 [b]	59.9 [b]
OG	---	---	---	9.8 [ef]	3.2 [f]	60.6 [b]
OG+BFT	16.1 [c]	0.92 [b]	0.15 [c]	13.1 [c]	4.1 [cd]	61.0 [b]
TF	---	---	---	9.2 [f]	4.0 [de]	61.1 [b]
TF+BFT	13.8 [c]	1.15 [a]	0.16 [bc]	13.0 [c]	4.3 [bc]	57.9 [c]
SEM	7.2	0.07	0.06	0.32	0.10	0.73
TRMT*ROT *P*-val.	0.145	0.336	0.001	<0.001	<0.001	<0.001

[a-f]Mean values within columns of rotation, treatment type, or pasture treatment with different superscripts are significantly different ($P = 0.05$).

[1]Herbage characteristic abbreviations: acid detergent lignin (ADL), proportion of birdsfoot trefoil in herbage (BFTPERC), percent condensed tannin in the birdsfoot trefoil (BFTCT), crude protein (CP), percent condensed tannin in the total (i.e., grass+BFT) herbage (HERBCT), and neutral detergent fiber digestibility (NDFD).

[2]For pasture-type and pasture-treatment, values represent the mean of three 35-d rotations.

Table 5. Measures of herbage characteristics[1] of pasture treatments in a Jersey heifer grazing study conducted in Lewiston, Utah, USA in 2017 and 2018. These characteristics were identified as contributing the most to principal components (PC) 3 and 4 following PC analysis of the variation among pasture treatments (PC3 and PC4 explained 19 and 6% of variation, respectively). Pasture treatments included monocultures of meadow brome (MB), orchardgrass (OG), perennial ryegrass (PR) and tall fescue (TF), and each grass in a binary mixture with birdsfoot trefoil (BFT).

	PC3			PC4
Rotation	HA, kg/kg BW	HT, cm	Fructan, %	FA, %
1	2.27 [a]	36.1 [a]	1.21 [c]	2.33 [c]
2	1.50 [b]	26.3 [b]	1.50 [b]	2.39 [b]
3	1.40 [b]	26.5 [b]	1.64 [a]	2.81 [a]
SEM	0.325	0.71	0.047	0.030
Pasture type[2]				
Mixture	1.93 [a]	31.4 [a]	1.46 [a]	2.35 [b]
Mono	1.51 [b]	27.9 [b]	1.44 [b]	2.67 [a]
SEM	0.324	0.55	0.045	0.035
TYPE*ROT *P*-val.	0.032	0.571	0.598	0.030
Pasture trmt				
PR	1.10 [e]	21.0 [e]	1.77 [a]	2.86 [b]
PR+BFT	1.93 [b]	25.8 [d]	1.70 [b]	2.12 [g]
MB	1.68 [cd]	29.0 [c]	1.31 [f]	2.54 [d]
MB+BFT	2.07 [a]	33.1 [ab]	1.36 [d]	2.35 [e]
OG	1.56 [d]	29.9 [c]	1.35 [de]	3.03 [a]
OG+BFT	1.74 [c]	32.3 [b]	1.43 [c]	2.73 [c]
TF	1.70 [c]	31.9 [b]	1.32 [ef]	2.27 [ef]
TF+BFT	1.99 [ab]	34.4 [a]	1.36 [d]	2.19 [fg]
SEM	0.327	0.72	0.046	0.045
TRMT*ROT *P*-val.	0.002	0.001	<0.001	<0.001

[a-g]Mean values within columns of rotation, treatment type, or pasture treatment with different superscripts are significantly different ($P = 0.05$).

[1]Herbage characteristic abbreviations: fatty acids (FA), herbage allowance (HA), and pre-grazing compressed height (HT).

[2]For pasture-type and pasture-treatment, values represent the mean of three 35-d rotations.

Figures

Figure 1. Total monthly precipitation, and average minimum and maximum monthly temperatures in 2016, 2017, and 2018 for dairy heifer grazing study in Lewiston, Utah, USA. (Utah Climate Center, Station Name: Richmond, Station ID: USC00427271).

Figure 2. PCA biplot of principal components (PC) 1 versus PC 2 (Dim1 and Dim2, respectively) from analysis of the physical and chemical herbage characteristics inherent to the pasture species of a Jersey heifer grazing study in Lewiston, Utah, USA in 2017 and 2018. Contribution of each trait to the PC (i.e., labeled "contrib") as a proportion of 100% is shown. Herbage trait abbreviations: proportion of birdsfoot trefoil in herbage (BFTPERC), herbage allowance (HA), percent condensed tannin in the total (i.e., grass+BFT) herbage (HERBCT), compressed height (HT), in-vitro true digestibility (IVTD), NDF digestibility (NDFD), and water-soluble carbohydrates (WSC).

Figure 3. PCA biplot of principal components (PC) 3 versus PC 4 (Dim3 and Dim4, respectively) from analysis of the physical and chemical herbage characteristics inherent to the pasture species of a Jersey heifer grazing study in Lewiston, Utah, USA in 2017 and 2018. Contribution of each trait to the PC (i.e., labeled "contrib") as a proportion of 100% is shown. Herbage trait abbreviations: proportion of birdsfoot trefoil in herbage (BFTPERC), herbage allowance (HA), percent condensed tannin in the total (i.e., grass+BFT) herbage (HERBCT), compressed height (HT), in-vitro true digestibility (IVTD), NDF digestibility (NDFD), and water-soluble carbohydrates (WSC).

Supplemental Figures and Tables

Supplemental Figure S1. Herbage intake by Jersey dairy heifers (A), herbage allowance (B), and percent condensed tannins in the BFT (C) over the grazing season for pasture treatments of a grazing study conducted in Lewiston, Utah, USA in 2017 and 2018. Pasture treatments included monocultures of meadow brome (MB), orchardgrass (OG), perennial ryegrass (PR) and tall fescue (TF), and each grass in a binary mixture with birdsfoot trefoil (BFT). Intake was compared to heifers feed a total mixed ration (TMR) in feedlot. Rotational stocking was used with three 35-day cycles. Bars represent plus or minus the standard error of the means (0.73, 0.33, and 0.16 for A, B, and C, respectively, n=6).

Supplemental Figure S2. NDF (A), ME (B), and WSC (C) over the grazing season for pasture treatments of a grazing study conducted in Lewiston, Utah, USA in 2017 and 2018. Pasture treatments included monocultures of meadow brome (MB), orchardgrass (OG), perennial ryegrass (PR) and tall fescue (TF), and each grass in a binary mixture with birdsfoot trefoil (BFT). Intake was compared to heifers feed a total mixed ration (TMR) in feedlot. Rotational stocking was used with three 35-day cycles. Bars represent plus or minus the standard error of the means (2.17, 0.03, and 0.82 for A, B, and C, respectively; n=6).

Supplemental Figure S3. Proportion of BFT in the herbage, averaged across years (A), in 2017 (B), and in 2018 (C) over the grazing season for pasture treatments of a grazing study conducted in Lewiston, Utah, USA in 2017 and 2018. Pasture treatments included monocultures of meadow brome (MB), orchardgrass (OG), perennial ryegrass (PR) and tall fescue (TF), and each grass in a binary mixture with birdsfoot trefoil (BFT). Intake was compared to heifers feed a total mixed ration (TMR) in feedlot. Rotational stocking was used with three 35-day cycles. Bars represent plus or minus the standard error of the means (7.38, 3.28, and 3.29 for A, B, and C, respectively; n=6 for A and n=3 for B and C).

Supplemental Figure S4. Condensed tannins in the herbage (A), CP (B), and NDFD (C) over the grazing season for pasture treatments of a grazing study conducted in Lewiston, Utah, USA in 2017 and 2018. Pasture treatments included monocultures of meadow brome (MB), orchardgrass (OG), perennial ryegrass (PR) and tall fescue (TF), and each grass in a binary mixture with birdsfoot trefoil (BFT). Intake was compared to heifers feed a total mixed ration (TMR) in feedlot. Rotational stocking was used with three 35-day cycles. Bars represent plus or minus the standard error of the means (0.06, 0.87, and 0.47 for A, B, and C, respectively; n=6).

Supplemental Table S5. Accuracy of canonical discriminate analysis (CDA) to discriminate amongst pasture treatments using PCA of the herbage traits from a grazing study in Lewiston, Utah, USA in 2017 and 2018. Pasture treatments included monocultures of meadow brome (MB), orchardgrass (OG), perennial ryegrass (PR) and tall fescue (TF), and each grass in a binary mixture with birdsfoot trefoil (BFT).

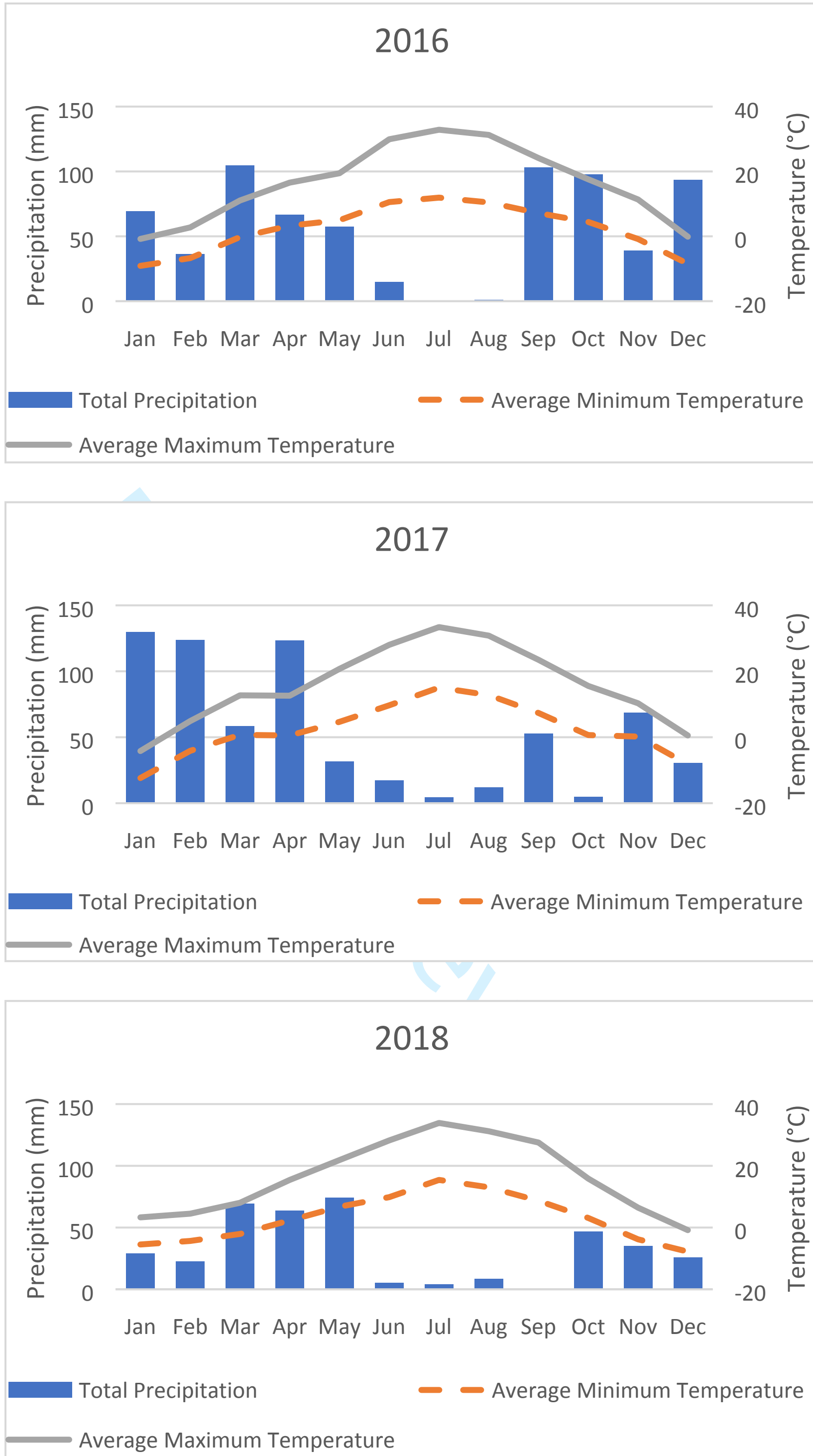
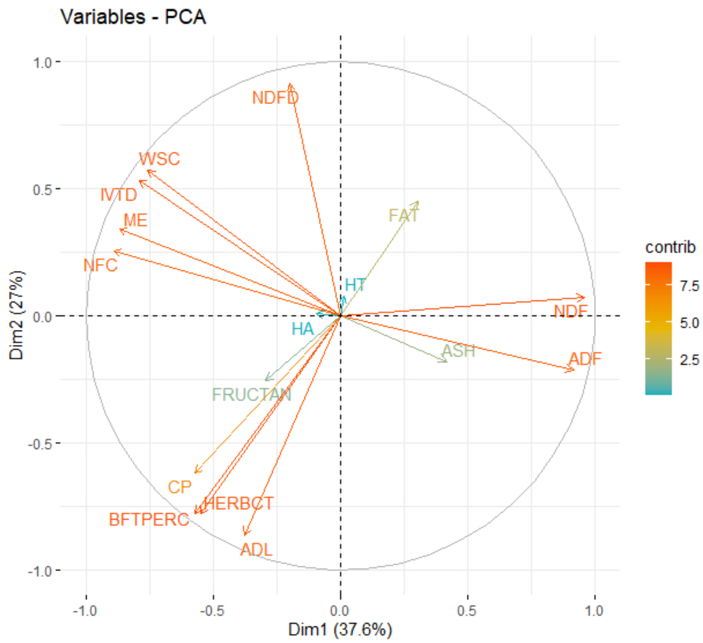


Figure 1. Total monthly precipitation, and average minimum and maximum monthly temperatures in 2016, 2017, and 2018.Data is from a dairy heifer grazing study in Lewiston, Utah, USA. (Utah Climate Center, Station Name: Richmond, Station ID: USC00427271).



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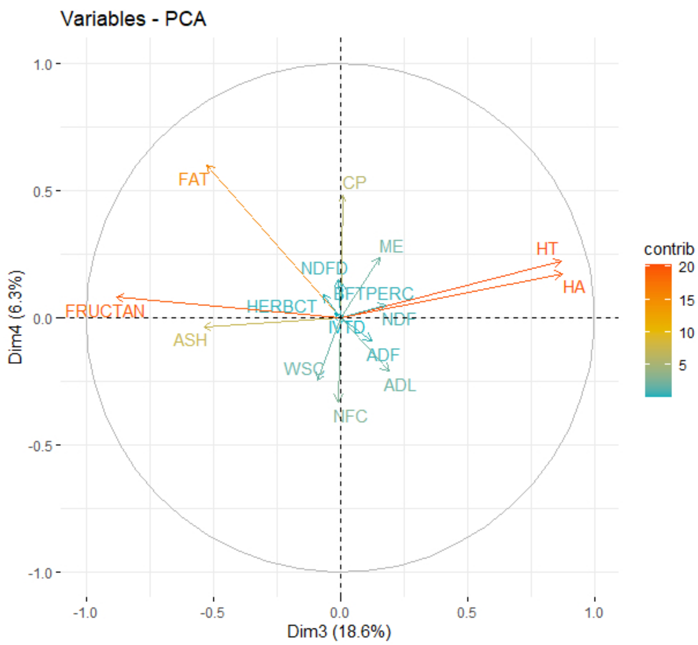

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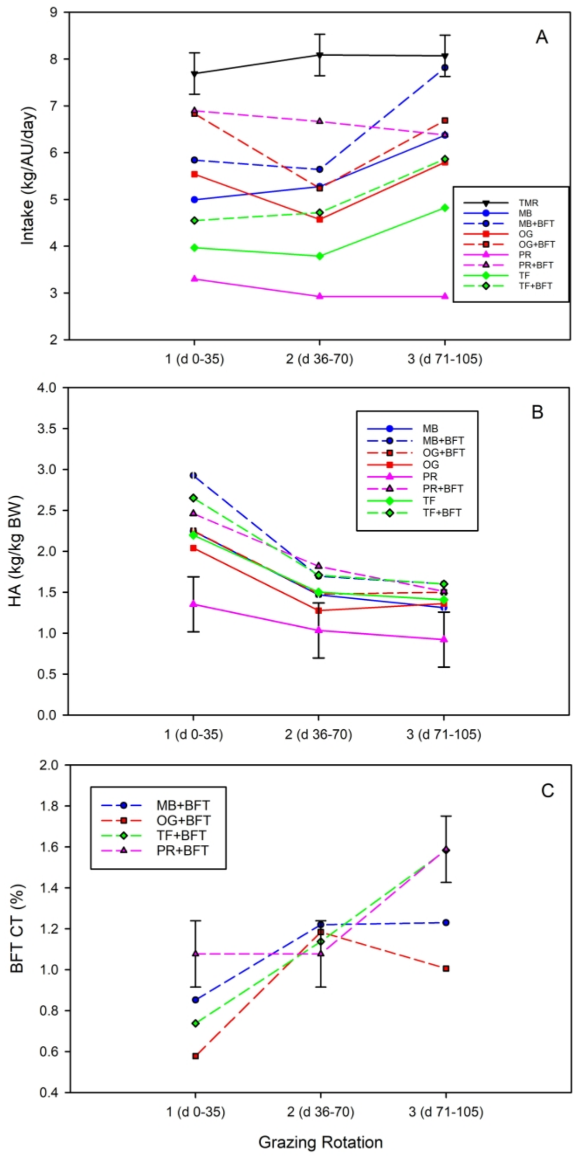

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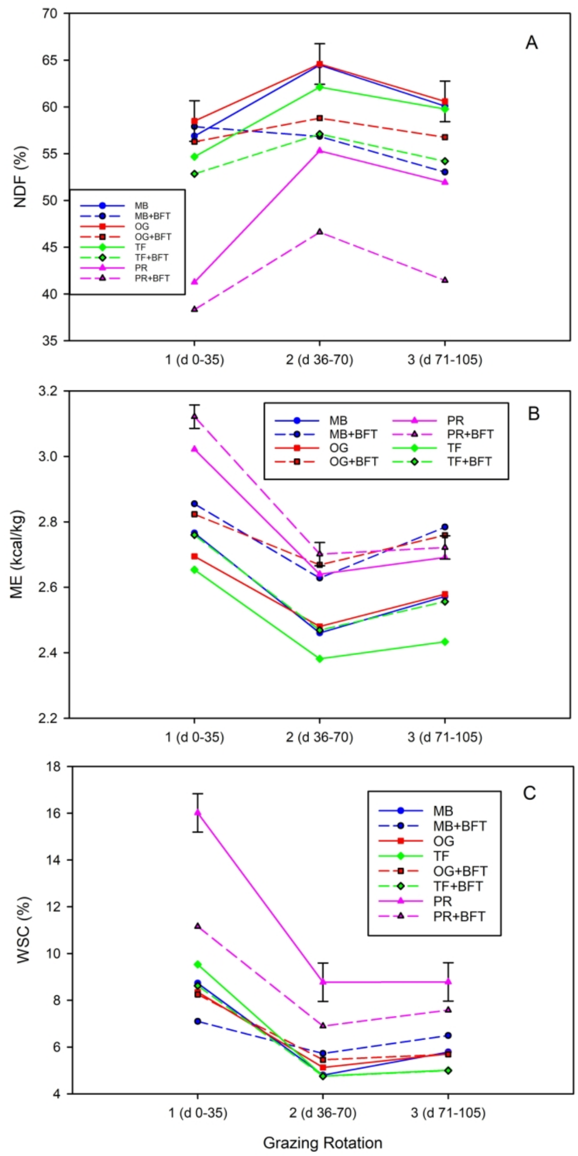

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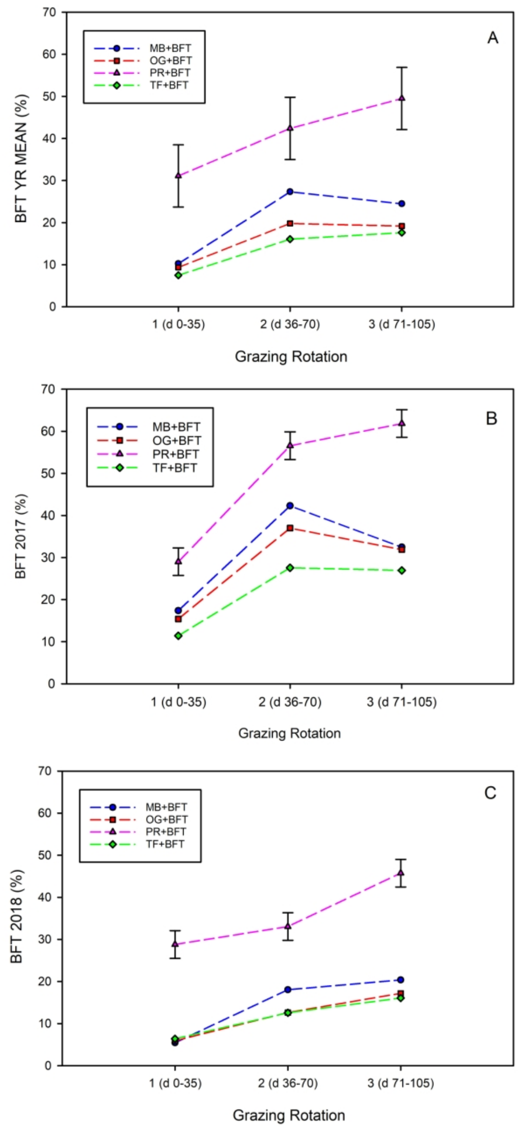

Supplemental Figure S3. Proportion of BFT in the herbage, averaged across years (A), in 2017 (B), and in 2018 (C) over the grazing season for pasture treatments of a grazing study conducted in Lewiston, Utah, USA in 2017 and 2018. Pasture treatments included monocultures of meadow brome (MB), orchardgrass (OG), perennial ryegrass (PR) and tall fescue (TF), and each grass in a binary mixture with birdsfoot trefoil (BFT). Intake was compared to heifers feed a total mixed ration (TMR) in feedlot. Rotational stocking was used with three 35-day cycles. Bars represent plus or minus the standard error of the means (7.38, 3.28, and 3.29 for A, B, and C, respectively; n=6 for A and n=3 for B and C).

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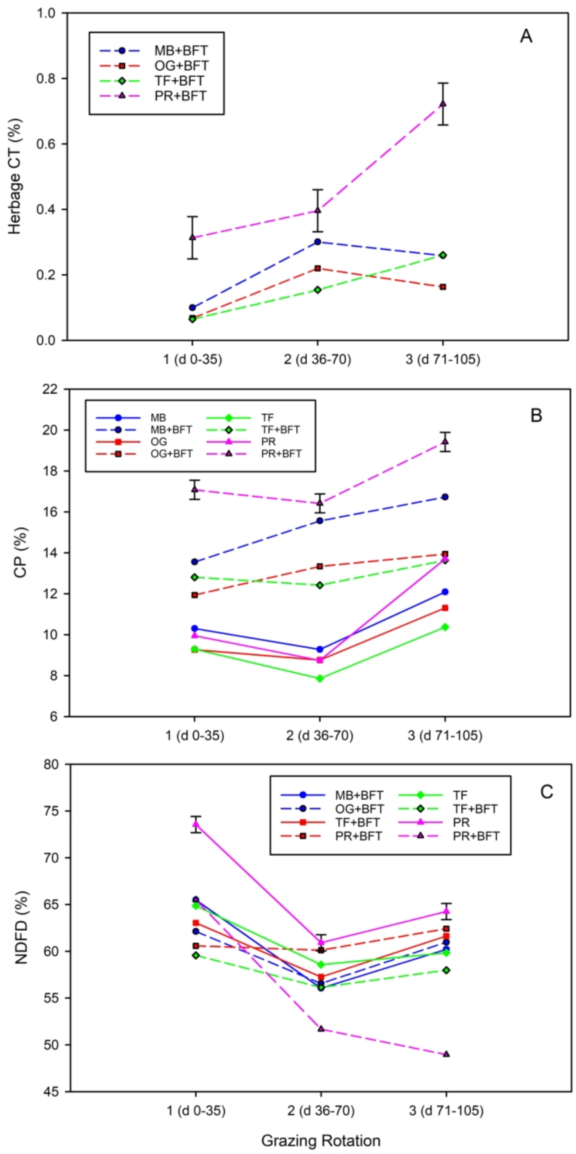

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	Prediction (%)								
True	MB	MB+BFT	OG	OG+BFT	PR	PR+BFT	TF	TF+BFT	Error rate[1] (%)
MB	22	0	33	0	6	0	33	6	78
MB+BFT	0	44	0	17	0	22	0	17	56
OG	33	0	56	0	0	0	11	0	44
OG+BFT	6	17	6	50	0	0	0	22	50
PR	0	0	0	0	89	0	11	0	11
PR+BFT	0	11	0	0	0	89	0	0	11
TF	22	0	11	0	11	0	56	0	44
TF+BFT	0	22	0	11	0	0	6	61	39
Ave. True	58								
Ave. Error	42								

[1]Error rate represents the percent of treatments incorrectly identified by CDA.