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Influence of cutting frequency and height on phytoestrogen levels in red clover and white clover

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Abstract

Phytoestrogens are a class of secondary compounds naturally produced by some forage legumes, including red clover (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.). Previous research showed that phytoestrogens can have beneficial and negative effects on animal health and reproductive function. Determining whether and how common agronomic practices influence the phytoestrogen profiles of legume forages would inform strategies for better managing ruminant exposure to phytoestrogens. A field experiment was conducted to evaluate how cutting frequency (cutting 3 vs. 5 times per growing season, hereafter 3x vs. 5x) and height (cutting to 5 cm vs. 10 cm residual height) influence phytoestrogen accumulation in red and white clover. The results revealed that total phytoestrogen concentrations were on average 5.01 mg g^{-1} dry matter in red clover and 0.07 mg g^{-1} dry matter in white clover. Formononetin and biochanin A were the major phytoestrogen compounds in both species, together accounting for 85% (red clover) and 65% (white clover) of the total measured phytoestrogens. Cutting frequency strongly influenced phytoestrogen concentrations in both species. In red clover, differences in total phytoestrogen concentration in the 5x relative to the 3x ranged from 33.6% to 83.5%, depending on the specific cutting event. In contrast to frequency, cutting height influenced phytoestrogen concentrations in white clover only. These findings indicate that harvest schedule may be an important management lever for moderating phytoestrogen concentrations in these common and important forage species.

Plain Language Summary

Red clover and white clover are important feeds for ruminants and both naturally produce chemicals called phytoestrogens, which can affect animal health and reproduction. Plant stress associated with harvest may influence the production of

Abbreviations: ANOVA, analysis of variance; DM, dry matter; HPLC, high-performance liquid chromatography; PLS, pure live seed.

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phytoestrogens in these species. The present study examined how harvesting 3 versus 5 times over the growing season, and at heights of 5 cm vs. 10 cm, influenced phytoestrogen concentrations. Red clover produced 72 times more phytoestrogens than white clover, and in both legumes, phytoestrogen concentrations varied across different harvest times in the 5-harvest but not in the 3-harvest system. Harvesting at a lower height reduced phytoestrogen concentration in white clover, but only in the 3-harvest system. This research sheds light on how management affects phytoestrogen levels in these important forages and suggests that harvesting red clover more frequently over the growing season can greatly increase its phytoestrogen content.

1 | INTRODUCTION

Legumes are an important component of the feed base that supports both conventional and organic ruminant production systems (Brito & Silva, 2020; Brito et al., 2022; Pereira et al., 2013). The organic and grass-fed dairy sectors, in particular, rely on perennial legume forages such as red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.), and alfalfa (*Medicago sativa* L.) because of their high crude protein content and palatability (Hatungimana et al., 2024; Kuhnen et al., 2015; Paulson et al., 2008). Compared to grass alone, legume forages increase feed intake, milk production (Brito et al., 2020; Steinshamn, 2010), milk components (i.e., fat, protein), and nutrient digestibility (Sleugh et al., 2000). Further, some forage legume species contain bioactive secondary metabolites such as condensed tannins, which prevent bloat, reduce the burden of intestinal parasites, and mitigate enteric methane emission in ruminants (Evans, 2018; Mueller-Harvey et al., 2019; Soder & Brito, 2023). Legumes also improve soil fertility due to their ability to "fix" atmospheric nitrogen (N) (Carlsson & Huss-Danell, 2003).

In addition to their numerous benefits to livestock agricultural systems, some species of forage legumes can also be a significant source of phytoestrogens. Phytoestrogens are plant-derived nonsteroidal secondary metabolites that are structurally and functionally similar to the mammalian primary female sex hormone estrogen (Hashem & Soltan, 2016). First isolated in Australia in the 1940s as the cause of dramatic and widespread reproductive abnormalities in sheep grazing on subterranean clover (*Trifolium subterraneum* L.), phytoestrogens have since been documented to be synthesized in a variety of economically important forage and grain legume species, including red clover, white clover, alfalfa, and soybean (*Glycine max* L.) (Franke et al., 1994; Marshall, 1973). A large and diverse body of subsequent research has demonstrated both positive and negative effects of phytoestrogen consumption on ruminant health and reproductive function, the nature of which appear to depend on a variety of factors, some of which are not well understood (e.g., Harlow et al., 2020; Sarelli et al., 2003; Tucak et al., 2020; Woclawek-Potocka et al., 2008). Further, milk and other dairy products from ruminants fed phytoestrogenic legumes can contain substantive concentrations of phytoestrogens, including the estrogenic metabolite equol, which may have positive and negative implications for human health (Canivenc-Lavier & Bennetau-Pelissero, 2023; Křížová et al., 2021).

The specific roles phytoestrogens play within legumes are not fully understood; however, substantial evidence suggests their synthesis aids in plant–microbe signaling and in mediating plant response to biotic and abiotic stress factors (De Rijke et al., 2005; Gierus et al., 2012; Subramanian et al., 2006; Tucker et al., 2010; Wasserman et al., 2013). Among perennial forage legumes that have been studied, red clover generally exhibits the highest concentrations of phytoestrogen compounds, and its phytoestrogen levels can be strongly influenced by both abiotic and biotic stressors, including insect herbivory (Mandal, Lima, et al., 2025; Mandal, Wallingford, et al., 2025; Saloniemi et al., 1995). Similarly, concentrations of phytoestrogens in white clover have also been shown to be influenced by biotic stress (Carlsen et al., 2008).

Given the apparent responsiveness of phytoestrogens to plant stress factors, it may be possible to modulate their levels in red clover and white clover via agronomic practices that mediate plant stress, such as cutting frequency (i.e., number of cutting events in a season) and cutting height (i.e., residual forage height after cutting). Although cutting frequency and height are important determinants of forage dry matter (DM) yield and nutritive value (Hunter et al., 2020), both factors can cause plant stress by driving the reallocation of energy and metabolic resources to plant regrowth, which can weaken and predispose the plant to other abiotic and biotic stress factors (Beuselinck et al., 1994; Friedrichsen, 2022). However, to the best of our knowledge, the potential impact of cutting frequency and height on the concentration and profile of phytoestrogens in red clover and white clover has not yet been examined. This question is especially relevant in the relatively cool and humid northeastern United States and eastern Canada, where farmers typically have flexibility in

the number of times they harvest over the growing season and warming climates and extended growing seasons may increase their ability to conduct additional harvests in the near future (Thivierge et al., 2023).

The present study aimed to quantify the effect of cutting frequency and height on phytoestrogen concentrations in two common perennial forage legumes, red clover and white clover. It was hypothesized that phytoestrogen concentrations would vary across cutting events, with more frequent and lower residual cutting height both leading to increased phytoestrogen concentrations in both species.

Core Ideas

- Red and white clover produce phytoestrogens, secondary compounds that mimic mammalian estrogen.
- Effects of cutting frequency (3x vs. 5x) and height (5 cm vs. 10 cm residual height) were examined.
- Total phytoestrogen concentration in red and white clover was 5.01 and 0.07 mg g^{-1} dry matter, respectively.
- Phytoestrogen concentrations varied extensively across cuttings in the 5x but not the 3x.
- Phytoestrogen concentrations were influenced by cutting height in white clover but not in red clover.

2 | MATERIALS AND METHODS

2.1 | Experimental setup and design

Aboveground plant tissue samples were collected over the 2022 growing season from red clover and white clover plants subjected to harvest treatments (described below) that were part of a longer term field experiment established in late summer 2018 at the University of New Hampshire Kingman Research Farm in Madbury, New Hampshire, United States (43° 10′ 17.5′′ N, 70° 56′ 06.7′′ W). Soils at the study site are classified as Inceptisol-Charlton fine sandy loams (US Soil Taxonomy). Soil measurements conducted at the site prior to treatment establishment indicated that organic matter content (loss on ignition) was 3.2%; pH was 5.7; and concentrations of calcium, magnesium, potassium, and phosphorus were 622, 120, 248, and 133 mg kg^{-1}, respectively (Teixeira et al., 2023). Prior to establishing the field experiment, lime (2242 kg ha^{-1} of pulverized limestone [102.9% calcium carbonate equivalent]) and compost (32.7 Mg ha^{-1} dry weight; dairy bedding and food scraps; 1.5% organic N) were applied to the site based on soil test results. Other than a single application of 76 kg ha^{-1} P and K with 0-20-20 (N-P-K) in June of 2022 to address K needs, no additional fertilizers were applied during the experiment.

The field experiment included three factors applied as a randomized complete block with split-plot restrictions and five replications. Whole-plot factors were factorial combinations of cutting frequency (mechanical harvest 3 vs. 5 times per growing season [3x vs. 5x]) and height (setting the harvester blades to cut to a 5 cm vs. 10 cm residual forage height). The sub-plot factor was legume–grass mixture composition, randomized within each whole plot. The complete experiment included six legume–grass mixtures. However, the present study focused on red clover "Freedom!" and white clover "Alice," each planted in mixture with orchardgrass (*Dactylis glomerata* L.). The legume seeding rate was set at 754 pure live seed (PLS) m^{-2}, and the orchardgrass seeding rate at 323 PLS m^{-2}, aiming for a 70:30 legume:orchardgrass ratio. Experimental units (subplots) were 2.3 × 7.6 m. Legume–grass mixtures were seeded in late summer 2018, and the first cutting event (harvest) was initiated in early June 2019 and continued from June to late September each year through 2022. After each harvest event, all mechanically harvested plant material was removed from the field. Legume tissue samples for phytoestrogen analysis (described below) were collected over the growing season in 2022 just prior to each cutting event, starting from late May until late September. During this period in 2022, the abundance of red clover and white clover in their respective mixtures (as mean percentage of total dry matter across harvest treatments and sampling dates) was 12.4% and 2.0%, respectively. Monthly mean air temperatures and precipitation for 2022 are presented in Figure 1.

2.2 | Sample collection

Four of the five field replicates of the red clover and white clover experimental units were sampled prior to each cutting event (i.e., $n = 4$ for each). During each sample collection, a 0.25-m^2 quadrat was randomly placed in each subplot, and legume herbage, including leaves, stems, and flowers, was clipped from a height of 5 or 10 cm from ground level, depending on the treatment. Orchardgrass tissue was not sampled. Clipped plant materials were placed into 50-mL conical centrifuge tubes and placed in a cooler with ice packs. Next, samples were transported to the laboratory, flash frozen in liquid N_2, and stored at −80°C. Finally, samples were lyophilized and stored at −20°C until analysis.

2.3 | Analysis of phytoestrogens

Concentrations of phytoestrogens, including formononetin, biochanin A, genistein, daidzein, glycitein, prunetin, and coumestrol, were quantified using high-performance liquid chromatography (HPLC). Freeze-dried samples were ground

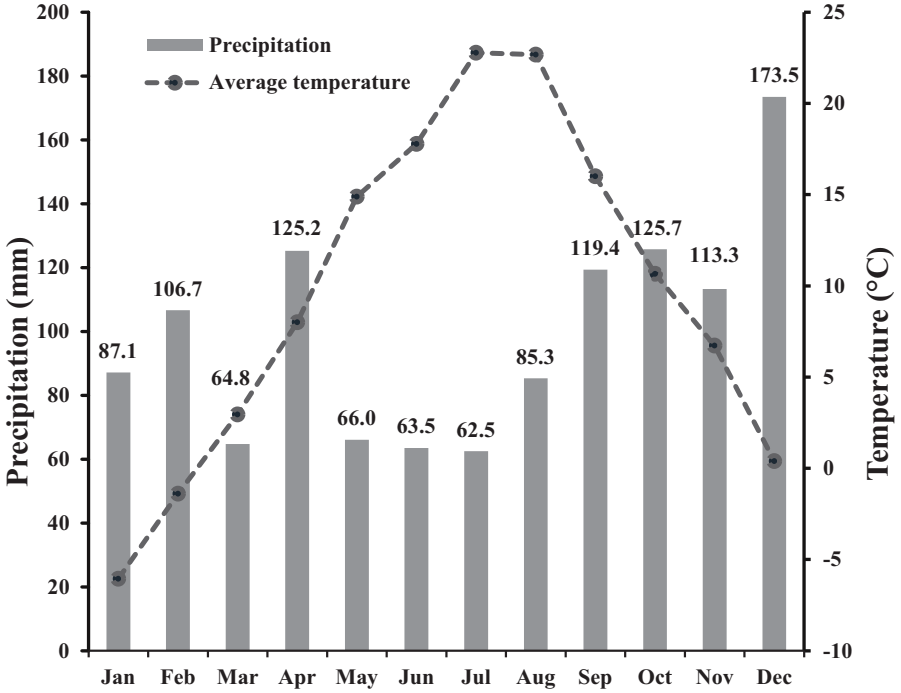


FIGURE 1 Monthly average temperature (˚C) and precipitation (mm) at the University of New Hampshire Kingman Research Farm, Madbury, New Hampshire, during 2022.

into powder before solvent extraction. Approximately 100 mg of plant powder was placed in 15-mL conical centrifuge tubes, and 4750 µL of 80% aqueous methanol plus 250 µL of flavone (400 ng μL^{-1} as internal standard) was added and vortexed for 5 s. Samples were sonicated for 10 min at room temperature, shaken at high speed in a multi-tube vortexer (VWR Scientific Products) for 2 h, and incubated overnight before centrifugation to sediment biomass. Finally, 1.5 mL supernatant was filtered through 0.45-µm PVDF syringe filter into a 2-mL HPLC vial and stored at 4˚C until analysis within next 24 h. An HPLC system with a diode array detector (Agilent Technology 1100 Series) was utilized to identify and quantify phytoestrogens in the samples, following the conditions outlined in Payette et al. (2021). The chromatographic separation was performed using an Atlantis T3 C18 column (150 × 2.1 mm, 3 µm; Waters Corp.) at 40˚C. The mobile phase consisted of solvent A (H_2O with 0.1% formic acid) and solvent B (acetonitrile), with separation achieved using a gradient ranging from 15% to 50% acetonitrile over the initial 35 min, followed by a rapid increase to 85% acetonitrile in the final 5 min of the run. The flow rate was maintained at 0.3 mL min^{-1} and detection set at 260 nm. Individual phytoestrogen compounds in the samples were identified by comparing their retention times and UV-visible spectra with analytical standards of formononetin, biochanin A, daidzein, genistein, glycitein, prunetin, and coumestrol. Calibration curves constructed with these standard compounds spiked with the internal standard (flavone) for concentrations ranging from 1.2 to 32 µg mL^{-1} were used to quantify the concentration of phytoestrogens. Finally, the concentration of phytoestrogens was calculated on a DM basis of the forage samples.

2.4 | Statistical analysis

Total phytoestrogen concentrations measured over the 2022 growing season were 72-fold greater in red clover compared to white clover; therefore, to detect treatment effects, statistical analyses were conducted separately for each species. For each species, we used a mixed model analysis of variance (ANOVA) with repeated measures. Response variables were the concentrations of individual phytoestrogen compounds as well as total phytoestrogen concentrations (sum of all phytoestrogen compounds). Fixed factors in the model were cutting frequency, cutting height, and cutting time (month of sampling) nested in cutting frequency. The model included all possible two-way interactions. The estimation of the three-way interaction was not feasible due to unequal numbers of cutting time points across frequencies (3 for 3x and 5 for 5x). Block was considered a random factor, and repeated observations were handled at the sampling-unit (plot) level using an AR(1) covariance on numeric time within the subject. If the effect of cutting frequency was significant, cutting time was analyzed separately within each frequency (3x and 5x). Additionally, a reduced model was used to examine treatment effects on total phytoestrogen concentrations at each of the three timepoints that were common to both the 3x and 5x cutting frequency treatments (May, July, and September).

TABLE 1 Results of analysis of variance (ANOVA; degrees of freedom, DF; and *p*-value) indicating the effects of cutting frequency, height, and time (nested in cutting frequency) of cutting on phytoestrogen concentrations in red clover and white clover.

Species	Treatment	DF	Total phytoestrogen	Formononetin	Biochanin A	Daidzein	Genistein	Glycitein	Prunetin
Red clover	Frequency (F)	1, 45	**0.0057**	**0.0003**	0.0508	**0.0074**	**0.0146**	**0.0006**	0.2807
	Height (H)	1, 45	0.5865	0.1453	0.5375	0.4030	0.2072	0.3316	0.2017
	Time (TIF)	6, 45	**0.0003**	**<0.0001**	**0.0019**	0.2532	**<0.0001**	**<0.0001**	0.0576
	F × H	1, 45	0.7220	0.5287	0.6336	0.3754	0.5046	0.4852	0.9659
	H × time (TIF)	6, 45	0.7259	0.6907	0.9197	0.8724	0.4869	0.5389	0.6475
White clover	Frequency (F)	1, 45	**0.0373**	0.9224	0.7321	**0.0001**	0.0982	0.4112	—
	Height (H)	1, 45	**0.0105**	**0.0261**	**0.0495**	0.9852	**0.0138**	0.6383	
	Time (TIF)	6, 45	**<0.0001**	**0.0008**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	
	F × H	1, 45	**0.0122**	**0.0272**	0.0550	0.5994	**0.0155**	0.6394	
	H × time (TIF)	6, 45	0.6093	0.8947	0.8803	0.2928	0.8366	0.9944	

Note: Bold values indicate significant main effects or interactions. "—" indicates data were not analyzed because the concentration was below the detection level.

This reduced model included the two cutting treatments and their interaction as fixed factors, as well as block. Prior to all analyses, the homogeneity of variance and the distribution of residuals were analyzed using the Levene and Shapiro–Wilk tests, respectively. The nonparametric Kruskal–Wallis test was used for response variables that did not satisfy the variance and distributional assumptions of ANOVA after transformation. When the ANOVA or Kruskal–Wallis test indicated significant treatment effects, Tukey HSD ($\alpha = 0.05$) was used to determine treatment differences. All analyses were performed using JMP (Student Edition 18.0.2).

3 | RESULTS

3.1 | Total phytoestrogen concentrations

In red clover, both cutting frequency ($p < 0.01$) and cutting time ($p < 0.01$) affected total phytoestrogen concentration (Table 1). Total phytoestrogen concentrations in red clover were on average 34% greater in the 5x (5.5 mg g^{-1} of DM) compared to 3x (4.1 mg g^{-1} of DM) treatment (Figure 2A). Further assessment of cutting time within each frequency treatment indicated that the effect was significant only in the 5x treatment ($p < 0.01$). In the 5x treatment, total phytoestrogen concentration varied by as much as 129% depending on cutting time, ranging from a high of 7.8 mg g^{-1} of DM at the June cutting to a low of 3.4 mg g^{-1} of DM at the August cutting (Figure 2B). There was no effect of cutting time on total phytoestrogen concentration in the 3x treatment ($p > 0.05$; Figure 2B). There was no significant effect of cutting height, and interactions between height and frequency or time were nonsignificant (all $p > 0.05$; Table 1).

In white clover, the effect of cutting frequency on total phytoestrogen concentration depended on the cutting height treatment ($p < 0.05$; Table 1). Total phytoestrogen concentrations were on average over 100% higher in the 3x treatment cut at 5 cm compared to the other 3x or the 5x treatments, none of which differed from one another (Figure 3A). Cutting time also affected total phytoestrogen concentration ($p < 0.01$; Table 1). Consistent with red clover, the effect of cutting time within frequency treatments was significant only in the 5x treatment ($p < 0.01$). Phytoestrogen concentrations in white clover managed under 5x ranged from 0.024 mg g^{-1} of DM at the first cutting (May) to as high as 0.152 mg g^{-1} of DM at the July cutting (Figure 3B).

3.2 | Phytoestrogen concentrations at three time points

Three cutting events coincided in the 3x and 5x treatments (May, July, and September cuttings were performed on the

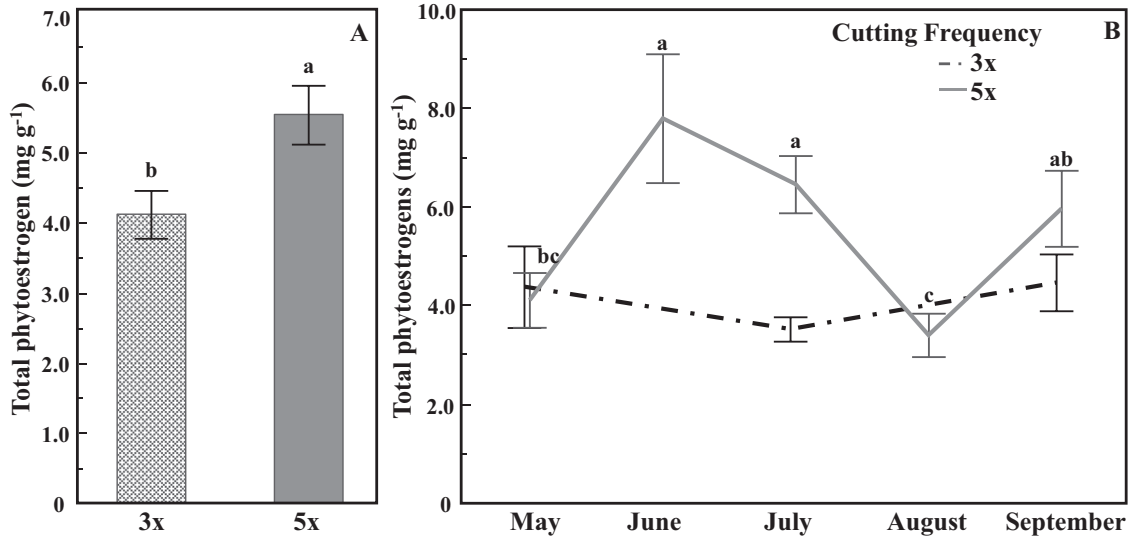


FIGURE 2 Effects of cutting frequency on total phytoestrogen concentrations (mg g^{-1} of DM) in red clover, averaged across the two cutting heights: (A) overall mean and (B) seasonal variation. Whiskers indicate ±1 standard error (SE) of the mean (in panel A, $n = 24$ for 3x and 40 for 5x; in panel B, $n = 8$ for 3x and 5x). Treatments (panel A) and cutting events within the 5x treatment (panel B) sharing the same letter are not significantly different ($\alpha = 0.05$) as determined by Tukey's honestly significant difference (HSD) test.

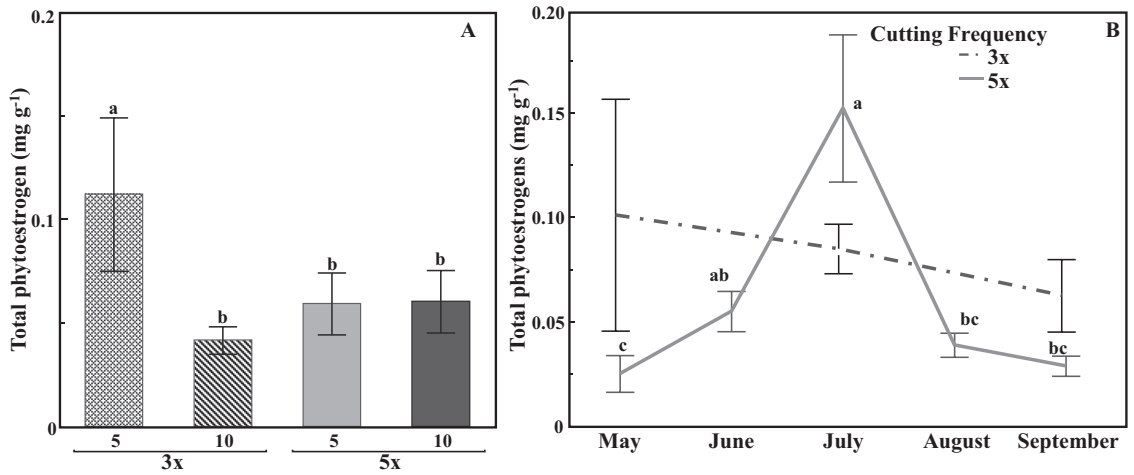


FIGURE 3 Effects of cutting frequency on total phytoestrogen concentrations (mg g^{-1} of DM) in white clover: (A) treatment effect and (B) seasonal variation averaged across the two cutting heights. In panel A, cutting height treatments are 5 and 10 cm residual forage height. Whiskers indicate ±1 standard error (SE) of the mean (in panel A, $n = 12$ for 3x and 20 for 5x; in panel B, $n = 8$ for 3x and 5x). Treatments (panel A) and cutting events within the 5x treatment (panel B) sharing the same letter are not significantly different ($\alpha = 0.05$) as determined by Tukey's honestly significant difference (HSD) test.

same day in both treatments), allowing direct comparison of phytoestrogen concentrations between the cutting treatments at those time points. Total phytoestrogen concentrations in red clover were 83% higher in the 5x compared to the 3x treatment at the July time point but did not differ at the other two time points (Figure 4A). In contrast, total phytoestrogen concentrations in white clover differed between the 3x and 5x treatments at all three times, though the nature of those differences varied depending on the time point (Figure 4B).

3.3 | Individual phytoestrogen compounds in red clover and white clover

Formononetin and biochanin A were the most abundant phytoestrogens in both species, making up 85% of the total phytoestrogen concentration in red clover and 67% in white clover. In red clover, formononetin alone accounted for nearly half of the total phytoestrogen concentration. In both clover species, patterns of variation in formononetin and biochanin

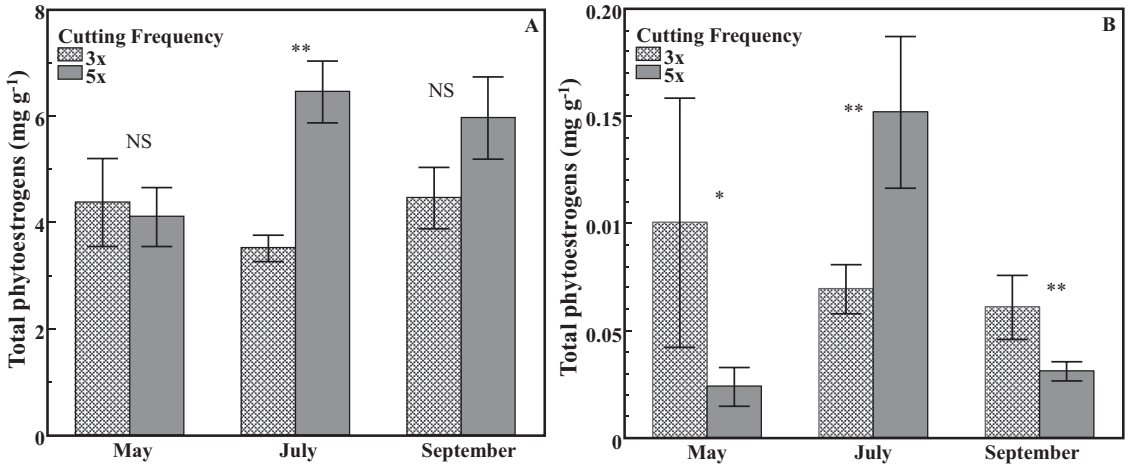


FIGURE 4 Effects of cutting frequency on total phytoestrogen concentrations averaged across the two cutting heights (mg g^{-1} of dry matter [DM]) at three harvest times in red clover (A) and white clover (B). Whiskers indicate ±1 standard error (SE) of the mean ($n = 8$). Asterisk (*) represents statistical differences (**$p = 0.01$; *$p = 0.05$) between the two cutting frequency treatments at that time point ($\alpha = 0.05$). NS, nonsignificant.

A concentrations in response to the cutting treatments roughly mirrored those observed for total phytoestrogen concentration. In the case of red clover, formononetin and biochanin A concentrations differed among different cutting events in 5x ($p < 0.01$). In the 3x treatment, biochanin A remained consistent across cutting events ($p > 0.05$; Table 2), and although formononetin concentrations varied across events, these differences were not statistically significant ($p \geq 0.05$; Table 2). In the 5x treatment, formononetin concentrations showed seasonal fluctuations, with peaks at the second (June) and fifth (September) cuttings. Biochanin A followed a similar pattern, reaching its greatest concentration during the second cutting (Table 2). Concentrations of other phytoestrogens, such as genistein and glycitein, also varied with cutting events, while daidzein and prunetin concentrations remained relatively stable across the cutting events in both 3x and 5x treatments (Table 2).

In white clover, formononetin concentration differed among cutting events in both 3x ($p < 0.01$) and 5x ($p < 0.01$) treatments (Table 2). In contrast, biochanin A concentration varied across cuttings in the 5x treatment ($p < 0.01$) but not in the 3x treatment ($p > 0.05$). Genistein concentration varied across cuttings in both 3x ($p < 0.01$) and 5x ($p < 0.01$), whereas daidzein and glycitein concentrations only differed in the 5x (each $p < 0.01$; Table 1). Coumestrol concentrations were below the detection limit in all cutting events, whereas prunetin concentrations were below the quantification limit in most white clover samples (Table 2).

4 | DISCUSSION

This study was conducted to examine the effects of two important components of perennial forage harvest management, the number of times over the growing season that forages are cut (cutting frequency) and the height at which those forages are cut (residual forage height), on the concentrations of phytoestrogen compounds of two agriculturally important perennial legumes, red clover and white clover. The results showed that phytoestrogen concentrations were several orders of magnitude higher in red clover compared to white clover, which is in line with earlier studies conducted with the same species (Andersen et al., 2009; Mandal, Wallingford, et al., 2025; Saloniemi et al., 1993). Total phytoestrogen concentrations (5.01 mg g^{-1} of DM) and the relative proportions of individual phytoestrogen compounds in red clover were comparable to those observed in previous greenhouse studies using the same cultivar (Mandal et al., 2024; Mandal, Lima, et al., 2025). Formononetin and biochanin A were the two most abundant phytoestrogens in both clover species, a finding consistent with previous studies (Andersen et al., 2009; Mustonen et al., 2018; Popović et al., 2019).

The finding that cutting frequency strongly influenced the total concentration of phytoestrogens in both species is noteworthy; however, this result may be more practically relevant to red clover given its exceedingly high phytoestrogen concentration relative to white clover. Further, the fact that concentrations of both formononetin and biochanin A in red clover herbage varied from cut-to-cut under the higher frequency cutting regime (5x), but not the lower frequency regime (3x), is especially intriguing. While the five-harvest system evaluated here represents an especially intensive management scenario that may be somewhat atypical of what is practiced on most farms in New Hampshire or other cool-season regions with similarly short growing periods, it is practiced on some farms in some years when growing seasons are favorable and may become a more common practice in the future as the climate continues to warm in these regions. It has

TABLE 2 Results of analysis of variance (ANOVA) and Kruskal–Wallis test (*p) conducted on concentrations (mg g^{-1} of dry matter [DM]) of individual phytoestrogen compounds in red clover "Freedom!" and white clover "Alice" during each cutting event in 3x and 5x (treatment means, $n = 8$) in 2022.

			Concentration (mg g^{-1})						
Species	**Frequency**	**Harvest**	**Formononetin**	**Biochanin A**	**Daidzein**	**Genistein**	**Glycitein**	**Prunetin**	**Coumestrol**
Red clover	3x	May	2.21	1.65	0.37	0.08b	0.03b	0.03	
		July	1.18	1.52	0.42	0.33a	0.05a	0.02	n.d.
		Sept.	2.35	1.62	0.36	0.09b	0.04b	0.02	
		Time	$p = 0.05$	$p = 0.97$	$p = 0.47$	$p < 0.01$	$p < 0.01$	$p = 0.07$	
	5x	May	2.09bc	1.55bc	0.34	0.04b	0.02bc	0.05	
		June	4.35a	3.11a	0.24	0.05b	0.02c	0.02	
		July	3.35ab	2.58ab	0.31	0.15a	0.03ab	0.02	n.d.
		Aug.	1.54c	1.33c	0.35	0.13a	0.05a	0.04	
		Sept.	3.59a	1.88abc	0.32	0.10a	0.04a	0.03	
		Time	$p < 0.01$	$p < 0.01$	$p = 0.35$	$p < 0.01$	$*p < 0.01$	$p = 0.12$	
White clover	3x	May	0.019b	0.042	0.01	0.021a	0.003	0.003	
		July	0.027ab	0.015	0.014	0.006a	0.007	n.d.	n.d.
		Sept.	0.04a	0.011	0.006	0.002b	0.001	n.d.	
		Time	$*p < 0.01$	$*p = 0.30$	$p = 0.10$	$*p < 0.01$	$p = 0.07$	$*p = 0.15$	
	5x	May	0.013b	n.d. c	0.001b	0.01ab	n.d. b		
		June	0.014ab	0.017ab	0.017a	0.003b	0.002b		
		July	0.056a	0.04a	0.028a	0.016a	0.011a	n.d.	n.d.
		Aug.	0.015ab	0.015ab	0.004b	0.002b	0.003b		
		Sept.	0.019ab	0.012b	n.d. b	n.d. b	n.d. b		
		Time	$p < 0.01$	$*p < 0.01$	$*p < 0.01$	$*p < 0.01$	$*p < 0.01$	—	

Note: For each phytoestrogen compound within a species and a cutting treatment, means sharing the same letter are not significantly different ($\alpha = 0.05$). "—" indicates data were not analyzed because the concentration was below the detection level.

Abbreviation: n.d., not detected (concentration below the detection limit).

been reported that formononetin concentrations ≥3 mg g^{-1} of DM may negatively impact the reproductive health of ruminant species such as sheep and cows (McDonald et al., 1994; Wyse et al., 2024). Formononetin is a precursor of the potent estrogenic compound equol, which is formed after action of ruminal microorganisms, while biochanin A is mostly demethylated into genistein and non-estrogenic metabolites, also by bacteria in the rumen (Batterham et al., 1965; Mustonen et al., 2009). In sheep, concentrations of formononetin as low as 3 mg g^{-1} of DM resulted in an increased risk of infertility, but the level of phytoestrogens associated with poor reproductive performance in other ruminants remains unknown (Little, 1996; Marshall et al., 1976). Hence, given that we observed formononetin concentrations in red clover herbage that exceeded the 3 mg g^{-1} of DM threshold considered "safe" at three of the five harvest time points under the higher frequency cutting regime, but never under the lower frequency regime (Table 2), is cause for concern. In contrast, formononetin concentrations in white clover averaged 0.025 mg g^{-1} of DM, well below the 3 mg g^{-1} of DM threshold considered problematic in sheep.

There may be several potential explanations for these findings. After each cutting, plants regrow their leaves to supply energy for continued growth. In the 5x treatment, plants had less time to recover, which likely altered their physiological activity by interrupting energy supply and triggering the redistribution of assimilates and mobilization of carbohydrate reserves (Briske & Richards, 1993). Thus, the frequent cutting likely resulted in a stress response in both clover species, potentially triggering the upregulation of phytoestrogens. Stress-induced upregulation of secondary metabolites, including phytoestrogens, is a well-documented defense mechanism in plants (Bennett & Wallsgrove, 1994; Dixon & Paiva, 1995).

Variation in isoflavone phytoestrogen concentrations at different cutting times over the growing season may also have been associated with weather patterns over the study period (Sivesind & Seguin, 2005). Previous research showed that drought stress can more than double the concentrations of phytoestrogens in red clover (Mandal et al., 2024). During the second (June) and third (July) cutting events in 5x, the monthly mean air temperature was relatively high, around 20°C, and monthly precipitation was relatively low, only 6 cm, which may have led to short-term drought conditions that increased the phytoestrogen levels in the plants. In both red clover and white clover, total phytoestrogen concentrations increased during the second and third cutting events compared to the first cutting event in May. Future research can clarify the relative roles of other possible factors, such as temperature, plant developmental stage, and nutrient availability, in driving these patterns.

It is also possible that concentrations of some phytoestrogen compounds are naturally dynamic. In previous research, both genistein and daidzein contents in red clover were observed to increase from June to July, whereas biochanin A and formononetin concentrations peaked in early September (Booth et al., 2006). Such temporal shifts are often linked to metabolic activity, phenological stage, or environmental cues such as photoperiod, soil moisture regimes, or temperature. A similar temporal trend was not observed for genistein and daidzein concentrations in the present study; however, formononetin and biochanin A concentrations did increase during the June and September cuttings, a trend consistent with Booth et al. (2006). Determining whether these dynamics are related more to ontogeny or environmental cues will require additional research.

The phytoestrogen coumestrol was not detected in red clover, which is consistent with the findings from previous studies (Andersen et al., 2009; Mustonen et al., 2018). Coumestrol is typically associated with plant responses to biotic stress, particularly fungal infection, which triggers its accumulation as part of the plant's defense mechanism (Saba et al., 1972; Saloniemi et al., 1993). Although previous studies have detected coumestrol in white clover, it was below the detection level in the present experiment, possibly due to the absence of fungal pathogens in the study system (Saba et al., 1972; Saloniemi et al., 1995).

After each cutting event, both legume species rely on their remaining leaf area and root reserves to recover (Meuriot et al., 2004; Teixeira et al., 2007). It has been shown that the contribution of reserves for plant regrowth is dependent on the remnant leaf biomass after cutting (Meuriot et al., 2004). Cutting forages at a lower residual height reduces the remaining leaf area and increases the plant's dependence on stem reserves for sustaining regrowth (Sinclair et al., 2007). An interactive effect between cutting frequency and cutting height on total phytoestrogen concentrations was observed in white clover, whereas no cutting height effect was detected in red clover. It is possible that the difference in cutting height treatments (5 cm vs. 10 cm residual height) was not large enough to result in a substantive difference in red clover residual leaf area or root reserves and, therefore, a consequent stress response in the production of phytoestrogens. Unfortunately, residual red clover leaf area or root reserves after the cutting events were not measured and therefore this possibility cannot be ruled out.

Although this study used mechanical harvest, grazing ruminants exert different types of stress on forage plants. Grazing typically results in uneven and selective removal of plant materials, trampling, and deposition of excreta, which can further influence plant regrowth and secondary metabolite production (Pereira et al., 2018). Studies have shown that grazing by cattle increases the crude protein content of legumes like red clover due to the wounding and regrowth processes associated with herbivory (Randall

et al., 2023). Additionally, animal saliva has been reported to induce plant chemical defenses, potentially affecting the phytoestrogen profile differently than mechanical cutting (Felton et al., 2014; Parnell et al., 2024). Therefore, while these results indicate that frequent cutting increases phytoestrogens concentration in red clover, actual grazing could induce even stronger or more variable phytoestrogen responses, especially under high stocking rates.

The present study has several limitations. Tissue sampling was conducted in the fourth production year of stand development, beyond the typical window of red clover persistence in many production systems. Whether the phytoestrogen response observed in red clover to harvest management would be similar in younger, more productive stands remains to be investigated. Additionally, this study evaluated only a single cultivar of red clover and white clover at one site during a single growing season. Future research examining cultivar-level variation across diverse genetic backgrounds, together with multi-site and multi-year experimentation, will be necessary to determine the generality of harvest-management effects on phytoestrogen dynamics in these two important perennial forage legumes.

5 | CONCLUSION

The results of this study indicate that the frequency of harvest can strongly influence the concentrations of phytoestrogens present in two globally important perennial forage legumes, red clover, and white clover. Concentrations of two phytoestrogen compounds, formononetin and biochanin, were especially high in red clover and varied between harvest events under the higher frequency cutting regime but not under the lower frequency regime. Under the higher frequency cutting regime, concentrations of formononetin in red clover herbage exceeded levels thought to be impactful to ruminant health and reproductive function (i.e., ≥3 mg g^{-1} of DM) in three out of the five harvest events, while the concentrations never exceeded this threshold under the lower frequency cutting regime. To the extent that farmers growing these forage legume species have flexibility in their harvest schedule, the data suggest that they may be able to manipulate phytoestrogen exposure to their ruminant herds by adjusting harvest interval (and possibly timing relative to plant stress status). Of course, such decisions will have tradeoffs in terms of the quantity and nutritive value of the resulting dry matter that must be weighed against the potential positive or negative impacts on ruminant health and reproduction associated with phytoestrogen consumption.

AUTHOR CONTRIBUTIONS

Palash Mandal: Data curation; formal analysis; funding acquisition; investigation; visualization; writing—original draft. **Marta R. M. Lima**: Funding acquisition; methodology; project administration; writing—review and editing. **Nicholas D. Warren**: Conceptualization; investigation; methodology; writing—review and editing. **André F. Brito**: Funding acquisition; project administration; writing—review and editing. **Richard G. Smith**: Conceptualization; funding acquisition; project administration; resources; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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REFERENCES

Andersen, C., Nielsen, T. S., Purup, S., Kristensen, T., Eriksen, J., Søegaard, K., Sørensen, J., & Fretté, X. C. (2009). Phyto-oestrogens in herbage and milk from cows grazing white clover, red clover, lucerne or chicory-rich pastures. *Animal*, *3*(8), 1189–1195. https://doi.org/10.1017/S1751731109004613

Batterham, T. J., Hart, N. K., Lamberton, J. A., & Braden, A. W. H. (1965). Metabolism of oestrogenic isoflavones in sheep. *Nature*, *206*(4983), 509. https://doi.org/10.1038/206509a0

Bennett, R. N., & Wallsgrove, R. M. (1994). Secondary metabolites in plant defence mechanisms. *New Phytologist*, *127*(4), 617–633. https://doi.org/10.1111/j.1469-8137.1994.tb02968.x

Beuselinck, P. R., Bouton, J. H., Lamp, W. O., Matches, A. G., McCaslin, M. H., Nelson, C. J., Rhodes, L. H., Sheaffer, C. C., & Volenec, J. J. (1994). Improving legume persistence in forage crop systems. *Journal of Production Agriculture*, *7*(3), 311–322. https://doi.org/10.2134/jpa1994.0311

Booth, N. L., Overk, C. R., Yao, P., Totura, S., Deng, Y., Hedayat, A. S., Bolton, J. L., Pauli, G. F., & Farnsworth, N. R. (2006). Seasonal variation of red clover (*Trifolium pratense* L., Fabaceae) isoflavones

and estrogenic activity. *Journal of Agricultural and Food Chemistry*, *54*(4), 1277–1282. https://doi.org/10.1021/jf052927u

Briske, D. D., & Richards, J. H. (1993). Physiology of plants recovering from defoliation. *Proceedings of the XVII International Grassland Congress* (pp. 85–94). New Zealand Grassland Association.

Brito, A. F., Almeida, K. V., & Oliveira, A. S. (2022). Production performance, nutrient use efficiency, and predicted enteric methane emissions in dairy cows under confinement or grazing management system. *Translational Animal Science*, *6*(2), etxac028. https://doi.org/10.1093/tas/txac028

Brito, A. F., Lange, M. J., & Silva, L. P. H. (2020). *The key role of forage legumes in organic dairy diets: Effects on your bottom line*. NODPA. https://nodpa.com/n/945/The-Key-Role-of-Forage-Legumes-in-Organic-Dairy-434

Brito, A. F., & Silva, L. H. P. (2020). Symposium review: Comparisons of feed and milk nitrogen efficiency and carbon emissions in organic versus conventional dairy production systems. *Journal of Dairy Science*, *103*(6), 5726–5739. https://doi.org/10.3168/jds.2019-17232

Canivenc-Lavier, M.-C., & Bennetau-Pelissero, C. (2023). Phytoestrogens and health effects. *Nutrients*, *15*(2), 317. https://doi.org/10.3390/nu15020317

Carlsen, S. C. K., Understrup, A., Fomsgaard, I. S., Mortensen, A. G., & Ravnskov, S. (2008). Flavonoids in roots of white clover: Interaction of arbuscular mycorrhizal fungi and a pathogenic fungus. *Plant and Soil*, *302*(1–2), 33–43. https://doi.org/10.1007/s11104-007-9452-9

Carlsson, G., & Huss-Danell, K. (2003). Nitrogen fixation in perennial forage legumes in the field. *Plant and Soil*, *253*(2), 353–372. https://doi.org/10.1023/A:1024847017371

De Rijke, E., Aardenburg, L., Van Dijk, J., Ariese, F., Ernst, W. H. O., Gooijer, C., & Brinkman, U. A. T. (2005). Changed isoflavone levels in red clover (*Trifolium pratense* L.) leaves with disturbed root nodulation in response to waterlogging. *Journal of Chemical Ecology*, *31*(6), 1285–1298. https://doi.org/10.1007/s10886-005-5286-1

Dixon, R. A., & Paiva, N. L. (1995). Stress-induced phenylpropanoid metabolism. *The Plant Cell*, *7*(7), 1085–1097. https://doi.org/10.1105/tpc.7.7.1085

Evans, B. (2018). The role ensiled forage has on methane production in the rumen. *Animal Husbandry, Dairy and Veterinary Science*, *2*(4), 1–4. https://doi.org/10.15761/AHDVS.1000143

Felton, G. W., Chung, S. H., Hernandez, M. G. E., Louis, J., Peiffer, M., & Tian, D. (2014). Herbivore oral secretions are the first line of protection against plant-induced defences. *Annual plant reviews* (pp. 37–76). Wiley. https://doi.org/10.1002/9781118829783.ch2

Franke, A. A., Custer, L. J., Cerna, C. M., & Narala, K. K. (1994). Quantitation of phytoestrogens in legumes by HPLC. *Journal of Agricultural and Food Chemistry*, *42*(9), 1905–1913. https://doi.org/10.1021/jf00045a015

Friedrichsen, A. (2022). There are consequences for cutting too low. *Hay and Forage Magazine*. https://www.hayandforage.com/article-2829-don%E2%80%99t-cut-it-too-close.html

Gierus, M., Koch, M., & Schulz, H. (2012). Phytoestrogen carryover into cow's milk from legumes-an overview along the food chain. *Berichte über Landwirtschaft*, *90*(3), 354–379.

Harlow, B. E., Flythe, M. D., Kagan, I. A., Goodman, J. P., Klotz, J. L., & Aiken, G. E. (2020). Isoflavone supplementation, via red clover hay, alters the rumen microbial community and promotes weight gain of steers grazing mixed grass pastures. *PLoS ONE*, *15*(3), e0229200. https://doi.org/10.1371/journal.pone.0229200

Hashem, N. M., & Soltan, Y. A. (2016). Impacts of phytoestrogens on livestock production: A review. *Egyptian Journal of Nutrition and Feeds*, *19*(1), 81–89. https://doi.org/10.21608/ejnf.2016.74871

Hatungimana, E., Darby, H. M., Soder, K. J., Ziegler, S. E., Brito, A. F., Kissing Kucek, L., Riday, H., & Brummer, E. C. (2024). Assessing forage research and education needs of organic dairy farms in the United States. *Renewable Agriculture and Food Systems*, *39*, e9. https://doi.org/10.1017/S1742170523000455

Hunter, M. C., Sheaffer, C. C., Culman, S. W., Lazarus, W. F., & Jungers, J. M. (2020). Effects of defoliation and row spacing on intermediate wheatgrass II: Forage yield and economics. *Agronomy Journal*, *112*(3), 1862–1880. https://doi.org/10.1002/agj2.20124

Křížová, L., Křešťáková, V., Dadáková, K., & Kašparovský, T. (2021). Production of bovine equol-enriched milk: A review. *Animals*, *11*(3), e735. https://doi.org/10.3390/ani11030735

Kuhnen, S., Stibuski, R., Honorato, L., & Filho, L. (2015). Farm management in organic and conventional dairy production systems based on pasture in Southern Brazil and its consequences on production and milk quality. *Animals*, *5*(3), 479–494. https://doi.org/10.3390/ani5030367

Little, D. L. (1996). Reducing the effects of clover disease by strategic grazing of pastures. *Australian Veterinary Journal*, *73*(5), 192–193. https://doi.org/10.1111/j.1751-0813.1996.tb10026.x

Mandal, P., Lima, M. R. M., Wallingford, A. K., Warren, N. D., Brito, A. F., & Smith, R. G. (2025). Short-term exposure to elevated temperature and CO_2 alters phytoestrogen production in red clover. *Scientific Reports*, *15*, e9105. https://doi.org/10.1038/s41598-025-92250-y

Mandal, P., Mortensen, D. A., Brito, A. F., Wallingford, A. K., Lima, M. R. M., Warren, N. D., & Smith, R. G. (2024). Water stress influences phytoestrogen levels in red clover (*Trifolium pratense*) but not kura clover (*T. ambiguum*). *Journal of Agricultural and Food Chemistry*, *72*(18), 10247–10256. https://doi.org/10.1021/acs.jafc.4c00300

Mandal, P., Wallingford, A. K., Lima, M. R. M., Warren, N. D., Brito, A. F., & Smith, R. G. (2025). Effects of insect herbivory on phytoestrogen concentrations in three *Trifolium* species. *Arthropod-Plant Interactions*, *19*(6), e99. https://doi.org/10.1007/s11829-025-10206-5

Marshall, T. (1973). Clover disease: What do we know and what can we do. *Journal of the Department of Agriculture, Western Australia, Series 4*, *14*(3), 198–206.

Marshall, T., Beetson, B. R., & Lightfoot, R. J. (1976). A study of reproductive wastage among commercial sheep flocks grazing non-oestrogenic pastures in south Western Australia. *Australian Society of Animal Production, Proceedings, Adelaide (Australia)*, *11*, 229–232.

McDonald, M. F., Anwar, M., & Keogh, R. G. (1994). Reproductive performance of ewes after grazing on G27 red clover, a low formononetin selection in cultivar Pawera. *Proceedings of the New Zealand Society of Animal Production*, *54*, 231–234.

Meuriot, F. (2004). Influence of initial organic N reserves and residual leaf area on growth, N uptake, N partitioning and N storage in alfalfa (*Medicago sativa*) during post-cutting regrowth. *Annals of Botany*, *94*(2), 311–321. https://doi.org/10.1093/aob/mch144

Mueller-Harvey, I., Bee, G., Dohme-Meier, F., Hoste, H., Karonen, M., Kölliker, R., Lüscher, A., Niderkorn, V., Pellikaan, W. F., Salminen, J.-P., Skøt, L., Smith, L. M. J., Thamsborg, S. M., Totterdell, P., Wilkinson, I., Williams, A. R., Azuhnwi, B. N., Baert, N., … Brinkhaus, A. G. (2019). Benefits of condensed tannins in forage legumes fed to ruminants: Importance of structure, concentration, and

diet composition. *Crop Science*, *59*(3), 861–885. https://doi.org/10.2135/cropsci2017.06.0369

Mustonen, E. A., Tuori, M., Kurki, P., Isolahti, M., Taponen, J., & Vanhatalo, A. (2018). Variety, time of harvest and conditions during growing season have impact on red clover isoflavone content. *Agricultural and Food Science*, *27*(2), 102–109. https://doi.org/10.23986/afsci.69781

Mustonen, E. A., Tuori, M., Saastamoinen, I., Taponen, J., Wähälä, K., Saloniemi, H., & Vanhatalo, A. (2009). Equol in milk of dairy cows is derived from forage legumes such as red clover. *British Journal of Nutrition*, *102*(11), 1552–1556. https://doi.org/10.1017/s0007114509990857

Parnell, D., Merchant, A., & Ingram, L. (2024). Is animal saliva a prominent factor in pasture regrowth? *Crop and Pasture Science*, *75*(1), eCP23201. https://doi.org/10.1071/CP23201

Paulson, J., Jung, H., Raeth-Knight, M., & Linn, J. (2008). Grass vs. legume forages for dairy cattle. *Proceedings of the 69th Annual Minnesota Nutrition Conference* (pp. 119–133). University of Minnesota. Minnesota Extension Service.

Payette, M., Lima, M. R. M., Coleman, W. M., & Ashraf-Khorassani, M. (2021). Separation optimization and quantitative analysis of phytoestrogens employing reverse-phase high-performance liquid chromatography with UV-VIS detection. *Journal of Liquid Chromatography & Related Technologies*, *44*(17–20), 888–896. https://doi.org/10.1080/10826076.2022.2110118

Pereira, A. B. D., Brito, A. F., Townson, L. L., & Townson, D. H. (2013). Assessing the research and education needs of the organic dairy industry in the northeastern United States. *Journal of Dairy Science*, *96*(11), 7340–7348. https://doi.org/10.3168/jds.2013-6690

Pereira, L. E. T., Silva, S. C. d., Matthew, C., López, I. F., & Sbrissia, A. F. (2018). Grazing management for sustainable grazing systems. *Improving grassland and pasture management in temperate agriculture* (pp. 79–122). Burleigh Dodds Science Publishing. https://doi.org/10.1201/9781351114561

Popović, S., Ravlić, M., Viljevac Vuletić, M., Krizmanić, G., Čupić, T., Horvat, D., & Tucak, M. (2019). The characterization of isoflavone content in the Croatian red clover collection. *Poljoprivreda*, *25*(1), 1–11. https://doi.org/10.18047/poljo.25.1.1

Randall, K. J., Ellison, M. J., Yelich, J. V., Price, W. J., & Johnson, T. N. (2023). Changes in forage quality and cattle performance with short-duration grazing of mesic meadows in the intermountain west. *Rangeland Ecology & Management*, *87*, 13–21. https://doi.org/10.1016/j.rama.2022.10.005

Saba, N., Drane, H. M., Hebert, C. N., Newton, J. E., & Betts, J. E. (1972). Effect of disease on the oestrogenic activity and coumestrol content of white clover and lucerne. *The Journal of Agricultural Science*, *78*(3), 471–475. https://doi.org/10.1017/S002185960002640X

Saloniemi, H., Kallela, K., & Saastamoinen, I. (1993). Study of the phytoestrogen content of goat's rue (*Galega orientalis*), alfalfa (*Medicago sativa*) and white clover (*Trifolium repens*). *Agricultural and Food Science*, *2*(6), 517–524. https://doi.org/10.23986/afsci.72677

Saloniemi, H., Wahala, K., Nykanen-Kurki, P., Kallela, K., & Saastamoinen, I. (1995). Phytoestrogen content and estrogenic effect of legume fodder. *Proceedings of the Society for Experimental Biology and Medicine*, *208*(1), 13–17. https://doi.org/10.3181/00379727-208-43825

Sarelli, L., Tuori, M., Saastamoinen, I., Syrjälä-qvist, L., & Saloniemi, H. (2003). Phytoestrogen content of birdsfoot trefoil and red clover: Effects of growth stage and ensiling method. *Acta Agriculturae Scandinavica, Section A — Animal Science*, *53*(1), 58–63. https://doi.org/10.1080/09064700310002053

Sinclair, K., Lowe, K. F., & Pembleton, K. G. (2007). Effect of defoliation interval and height on the growth and quality of *Arachis pintoi* cv. Amarillo. *Tropical Grasslands*, *41*(4), 260–268.

Sivesind, E., & Seguin, P. (2005). Effects of the environment, cultivar, maturity, and preservation method on red clover isoflavone concentration. *Journal of Agricultural and Food Chemistry*, *53*(16), 6397–6402. https://doi.org/10.1021/jf0507487

Sleugh, B., Moore, K. J., George, J. R., & Brummer, E. C. (2000). Binary legume–grass mixtures improve forage yield, quality, and seasonal distribution. *Agronomy Journal*, *92*(1), 24–29. https://doi.org/10.2134/agronj2000.92124x

Soder, K. J., & Brito, A. F. (2023). Enteric methane emissions in grazing dairy systems. *JDS Communications*, *4*(4), 324–328. https://doi.org/10.3168/jdsc.2022-0297

Steinshamn, H. (2010). Effect of forage legumes on feed intake, milk production and milk quality—A review. *Animal Science Papers and Reports*, *28*(3), 195–206.

Subramanian, S., Stacey, G., & Yu, O. (2006). Endogenous isoflavones are essential for the establishment of symbiosis between soybean and *Bradyrhizobium japonicum*. *The Plant Journal*, *48*(2), 261–273. https://doi.org/10.1111/j.1365-313x.2006.02874.x

Teixeira, C. d. S., Castillo, B. T., Bernhardt, L., Warren, N. D., Petry, C., Ernakovich, J. G., Smith, R. G., & Frey, S. D. (2023). Frequent defoliation of perennial legume-grass bicultures alters soil carbon dynamics. *Plant and Soil*, *490*(1–2), 423–434. https://doi.org/10.1007/s11104-023-06091-7

Teixeira, E. I., Moot, D. J., Brown, H. E., & Pollock, K. M. (2007). How does defoliation management impact on yield, canopy forming processes and light interception of lucerne (*Medicago sativa* L.) crops? *European Journal of Agronomy*, *27*(1), 154–164. https://doi.org/10.1016/j.eja.2007.03.001

Thivierge, M., Bélanger, G., Jégo, G., Delmotte, S., Rotz, C. A., & Charbonneau, É. (2023). Perennial forages in cold-humid areas: Adaptation and resilience-building strategies toward climate change. *Agronomy Journal*, *115*(4), 1519–1542. https://doi.org/10.1002/agj2.21354

Tucak, M., Čupić, T., Horvat, D., Popović, S., Krizmanić, G., & Ravlić, M. (2020). Variation of phytoestrogen content and major agronomic traits in alfalfa (*Medicago sativa* L.) populations. *Agronomy*, *10*(1), e87. https://doi.org/10.3390/agronomy10010087

Tucker, H. A., Knowlton, K. F., Meyer, M. T., Khunjar, W. O., & Love, N. G. (2010). Effect of diet on fecal and urinary estrogenic activity. *Journal of Dairy Science*, *93*(5), 2088–2094. https://doi.org/10.3168/jds.2009-2657

Wasserman, M. D., Milton, K., & Chapman, C. A. (2013). The roles of phytoestrogens in primate ecology and evolution. *International Journal of Primatology*, *34*(5), 861–878. https://doi.org/10.1007/s10764-013-9699-3

Watson, C. A., Atkinson, D., Gosling, P., Jackson, L. R., & Rayns, F. W. (2002). Managing soil fertility in organic farming systems. *Soil Use and Management*, *18*(s1), 239–247. https://doi.org/10.1111/j.1475-2743.2002.tb00265.x

Woclawek-Potocka, I., Piskula, M. K., Bah, M. M., Siemieniuch, M. J., Korzekwa, A., Brzezicka, E., & Skarzynski, D. J. (2008). Concentrations of isoflavones and their metabolites in the blood of pregnant and non-pregnant heifers fed soybean. *Journal of Reproduction and Development*, *54*(5), 358–363. https://doi.org/10.1262/jrd.20013

Wyse, J. M., Nevard, R. P., Loy, J., Weston, P. A., Gurusinghe, S., McCormick, J., Weston, L. A., & Stephen, C. P. (2024). Effects of mixed pasture legume phytoestrogens on superovulatory response and embryo quality in angus cows. *Animals*, *14*(7), e1125. https://doi.org/10.3390/ani14071125

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