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METHODS

Fractionation of mixed grass and clover stands using a leaf stripper

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Abstract

Leys are an important part of northern European livestock production, particularly for ruminants since monogastric animals are limited in their ability to digest the fibres of the forage. Crop fractionation methods are a promising option to make forages more beneficial for monogastric animals and decrease the amount of imported protein feed. A leaf stripping harvesting technique was evaluated at Röbäcksdalen in northern Sweden in mixed grass-clover leys over 2 years. The PremAlfa Mini leaf stripper (Trust'ing-Alf'ing, Nantes, France) worked well in mixed stands, harvesting on average a third of the available forage biomass, primarily in the form of leaves and soft stems from the clover plants. It proved successful in producing a forage fraction that had a significantly higher crude protein (CP) concentration (+39.1%) and lower neutral detergent fibre (aNDFom) concentration (−21.4%) than the pre-harvest mixed sward (all significant at $p < .05$ level). Due to the remaining high level of aNDFom in the leaf stripper fraction, it is more suited for use as an energy source for monogastrics rather than as a protein supplement. Alternatively, the leaf stripper fraction could be used to increase digestibility and CP content in the feed rations of high producing dairy cows.

KEYWORDS
biorefinery, crude protein, forage, fractionation, monogastric, neutral detergent fibre, ruminant

1 | INTRODUCTION

As the need for protein feed for livestock has increased, so has the development of new techniques to create alternative, locally produced protein-rich feeds. The EU is heavily dependent on the import of soybean meal as the main source of protein for the livestock sector, but a new emphasis on biorefining of local forages paves the way for the production of a sustainable protein source in Europe (van Krimpen et al., 2013). Forages, particularly forage legumes, are an important protein source for ruminants. Monogastrics, however, require high quality protein with a specific amino acid profile and have limited ability to digest unprocessed forage fibres, thus limiting their usage of forage legume-based feed. Fractionation of forage legumes through biorefinery bypasses these limitations through the creation of a forage-based protein source with a high feed value and a balanced amino acid composition, ideal for monogastrics (Laudadio et al., 2014). Current methods of forage fractionation include sieving, pin milling, air classification, and twin-screw press juicing (Damborg et al., 2018; Laudadio et al., 2014; Wu & Nichols, 2005). Previous studies have shown that the protein-rich fraction created through forage biorefinery can act as a suitable replacement to soybean meal in the diet of chickens (Damborg et al., 2018; Laudadio et al., 2014; Stødkilde et al., 2020; Wu & Nichols, 2005). Additionally, the fibre-rich coproduct can serve as an alternative source of

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forage for dairy cows, thus increasing the sustainability of the system (Damborg et al., 2018). All fractionation methods mentioned above are post-harvest and require a multi-step process to achieve the end product. An alternative method that allows for fractionation during the harvest process could present a more streamlined approach.

A potential way to achieve harvest level fractionation is to consider how protein and fibre are partitioned throughout the plant. The leaves of forage legumes contain higher levels of soluble protein due to photosynthetic machinery, as well as lower levels of fibre due to their lower cell wall content when compared to stems (Fiorentini & Galoppini, 1983). The concentration of extractable true protein was also found to be higher in the leaf than the stem for both red clover (*Trifolium pratense* L.) and lucerne (*Medicago sativa* L.) (Hakl et al., 2016; Solati et al., 2018). This difference in nutritive value between the leaf and the stem presents an opportunity to fractionate forage legumes through the separation of leaf from stem, creating a protein-rich fraction consisting of leaves and a fibre-rich coproduct from the stems. The high crude protein and low fibre concentrations in leaves makes them a potential protein feed source for monogastrics. Additionally, the stems that remain are high in fibre and could be used as a forage source for ruminants.

The focus of this study is on leaf stripping, a harvest-level fractionation method which separates the leaves, containing easily digestible protein and a low fibre concentration, from the stems (Julier & Huyghe, 1997). Leaf stripping involves the use of harvest machinery that removes a high proportion of the leaves and the soft, upper portion of the stem, while leaving the fibrous portion of the stem behind. The remaining plant material can then be harvested using traditional methods and utilised as a high-fibre coproduct (Figure 1). Previous studies have explored the potential of leaf stripping using harvest machinery either modified or designed specifically for leaf stripping. They have shown promising results for the use of leaf stripping fractionation techniques to improve the nutritive value of the harvested material in comparison to conventional harvesting techniques (Andrzejewska et al., 2020; Liebhardt et al., 2022; Shinners et al., 2007).

Forage production in northern Europe is dominated by leys, in which forages are grown in rotation with annual crops to produce animal feed (Nilsdotter-Linde et al., 2019; Nykänen et al., 2000; Steinshamn et al., 2016). Leys are typically grown as either pure grass or mixtures of grasses, legumes and forbs. In addition to the production of forage for livestock, leys increase agrobiodiversity, sequester carbon, and provide other environmental benefits (Conant et al., 2001; Lemaire et al., 2015). The most prominent forage legume in northern Europe is red clover, which is generally grown in mixtures with various species of grasses, such as timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* Huds.), and perennial ryegrass (*Lolium perenne* L.), among others (Frankow-Lindberg, 2017). Mixed leys of grasses and legumes produce higher yields over time than pure legume stands and a superior nutritive value than pure grass (Finn et al., 2013; Lüscher et al., 2014). As no previous studies have investigated the potential of leaf stripping for mixed stands, it is essential to determine the plausibility of using the machinery and the nutritive value of the resulting fractions. Leaf stripping mixed leys could provide an opportunity to produce local protein feed using typical forage production systems in northern Europe.

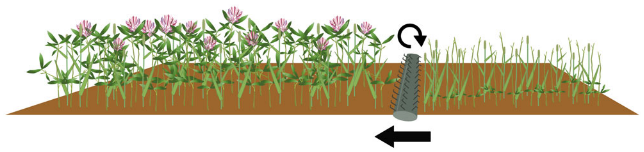

FIGURE 1 An illustration of the leaf stripping method used in this experiment. As the leaf stripper is driven through the plot, it removes leaves and the upper portion of the stem of the legume, which are collected in the machine. The more fibrous legume stem and the majority of the grass remain and are harvested using traditional harvesting methods.

This study is based on the idea that farmers could opportunistically fractionate their leys through leaf stripping in mixed stands with a high percentage of red clover. This may be particularly applicable in the second and third cuts in northern Europe, as the percentage of clover in the stand for these cuts is generally much higher than in the first cut. The following research questions are addressed in this study: (1) Can a leaf stripper machine be used in mixed leys to improve the nutritive value of the resulting fraction when compared to the mixed sward? (2) To what extent does leaf stripping mixed leys of red clover and grass improve the feed value compared to material harvested conventionally? (3) What measurable characteristics of mixed leys affect the nutritive value of the leaf stripper fraction?

2 | MATERIALS AND METHODS

2.1 | Data collection

This study was performed at Röbäcksdalen, a research station located in northern Sweden (63.81° N, 20.24° E). Plots used for sampling were typical mixed ley systems sown with timothy and red clover. Twenty sampling locations were selected each year in 2021 and 2022 ($n = 40$), based on having a visually homogeneous distribution of red clover. Samples were taken throughout the entire season and in swards of different compositions, heights, and phenological stages to obtain a diverse dataset representing a large range of potential nutritive value and yield. To define the sampling area, a four-metre long strip was measured and marked (Figure 2). The normalised difference vegetation index (NDVI) was measured over the length of the entire plot using the GreenSeeker hand-held crop sensor (Trimble, Sunnyvale, California, USA). NDVI is related to the chlorophyll content and leaf area, and thus is a widely used numerical index to evaluate the density and vigour of vegetation. NDVI can be defined as:

$$\mathrm{NDVI} = \frac{\mathrm{NIR} - \mathrm{R}}{(\mathrm{NIR} + \mathrm{R})} \qquad (1)$$

where NIR is the reflectance of near-infrared and R is the reflectance of the visible red. Values for NDVI range from -1 to $+1$, with

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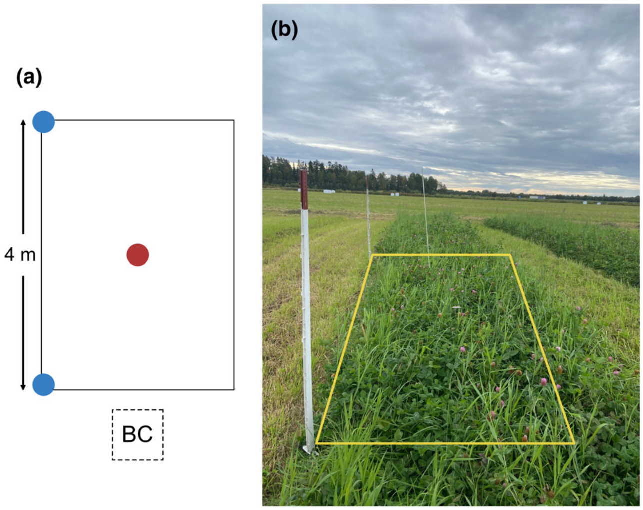

FIGURE 2 Representations of the sampling area: (a) Each blue dot represents a corner of the plot (4 m apart). The red dot represents the hoop used for measurements within the sampling area. The botanical composition (BC) square was placed outside the area used for leaf stripping; (b) The approximate harvested area is represented by the orange polygon. The stick in the middle represents the location of the hoop.

+1 representing an area with the highest possible density of green leaves.

A hoop (76 cm diameter) was placed in the middle of the sampling area and measurements were taken for the height of the tallest clover when stretched and clover phenological development stage (Nadeem et al., 2019). Clover phenological stage was described on a scale from 1.00, which signified first visible leaf, to 4.00, which signified seed formation. Four individual healthy clover plants in the hoop were measured with a Dualex 4 Scientific leaf-clip meter (Dx4, FORCE-A, Orsay, France) to determine the chlorophyll content index (CCI). The Dualex calculates CCI using the function:

$$f(I_o, I) = \left[\left(I_{(850)}/I_{o(850)}\right)/\left(I_{(710)}/I_{o(710)}\right)\right] - 1 \tag{2}$$

where I_o is the signal without the leaf, I represents the signal when the leaf is present in the leaf-clip, and the subscript values correspond to wavelengths (Cerovic et al., 2012). The CCI values presented in this article are the result of transformation using the formula:

$$M = k \times f(I_o, I) + c \tag{3}$$

where k is the proportionality constant used to convert the units to $\mu g\ cm^{-2}$, $f(I_o, I)$ is the result of the CCI function, and c is the constant to correct for model bias (Nauš et al., 2010). Measurements were performed with the adaxial leaf side facing the light source to mitigate for leaf heterogeneity. A location for collecting the botanical composition sample was selected at the end of the sampling strip (Figure 2), measured with the GreenSeeker, delineated with a sampling quadrat (50 cm × 50 cm), and cut by hand at a stubble height of approximately 8 cm. Prior to leaf stripping, the ends of the plot were trimmed with a lawnmower to form clean edges. The hoop was removed from the plot and an additional stick was placed at the edge of the plot, perpendicular to the midpoint of where the hoop had been placed.

The PremAlfa Mini electric leaf stripper prototype (Trust'ing-Alf'ing, Nantes, France) used in the experiment has tines that rotate opposite to the direction of the wheels when in forward motion to separate leaves from stems. The separated leaves are subsequently deposited into a container held within the machine. The harvesting width of the machine is 80 cm. Rotor height, rotor speed, and ground speed were adjusted according to the judgement and experience of the operator, aiming for the machine to operate at the height in the canopy where the majority of leaves was found. The height of the rotor ranged from 9 to 25 cm and on average was set to 14 cm. The rotation speed of the leaf stripper was set to approximately 260–280 rpm, however this dropped to an average of 240 rpm when the machine engaged with the plant canopy. Ground speed was set to approximately 1.7–2.5 km h^{-1} and was subsequently recorded as the leaf stripper passed through the plot. The actual ground speed ranged from 0.8 to 2.2 km h^{-1}, depending on the amount of biomass, slope of the selected plot, and other factors. The ratio of speed of the rotor tines to ground speed was on average 25.6, though was quite variable due to a wide range in ground speed. Harvesting loss was not measured, as visual assessment post leaf stripping indicated that nearly all material harvested by the leaf stripper was successfully collected in the machine.

Following harvest, the residual fraction left by the leaf stripper was sampled using the 50 × 50 cm quadrat placed where the hoop was located for pre-harvest measurements. Residual material within the quadrat was hand harvested at a cutting height of approximately 8 cm. The sampling included 40 independent sampling areas, 20 harvested between the 24th of June and 1st of September 2021 and 20 harvested between the 23rd of June and the 8th of September 2022, representing a typical forage harvest window for northern Sweden.

The material harvested by the leaf stripper was weighed (fresh) and a subsample of 1 kg fresh weight was taken for further analysis. The botanical composition sample was separated into three different fractions: grass, clover, and broad-leaf weeds. Grass weeds were included in the grass fraction. The leaf stripper subsample, botanical composition samples, and residual fraction sample were all weighed for fresh weight, dried at 60°C for at least 48 h until they reached a constant weight, and weighed again, to facilitate calculation of dry matter. The dried samples were milled to pass through a 1 mm screen and stored for chemical analysis.

2.2 | Description of the harvested fractions

The different pre-harvest biomass fractions represented the mixed sward and consisted of the grass fraction (GF), clover fraction

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(CF) and weed fraction (WF). The WF was small (the highest value was 5.8%), and thus there was not sufficient material for lab analysis of nutritive value. There were two post-harvest fractions: the fraction harvested by the leaf stripper (LSF) and the residual fraction (RF), which includes the grass, clover stems, and other material not harvested. Thus, the fractions that were assessed for nutritive value included GF, CF, LSF, and RF. Nutritive value of the mixed sward was calculated based on the weighted GF and CF results. Figure 3 shows the fractions in relation to each other.

2.3 | Nutritive value analysis

Dried samples were ground to 1 mm to prepare for chemical analysis. A subsample was re-dried at 103°C for 16 h and cooled in a desiccator before weighing, to determine dry matter (DM) concentration. Amylase-treated, ash-free neutral detergent fibre (aNDFom) was analysed using the method of Chai and Udén (Chai & Udén, 1998). Crude Protein (CP) was analysed using the Kjeldahl-N method, according to the Nordic Committee on Food Analysis (Nordic Committee on Food Analysis, 1976), using the 2520 Digestor, Kjeltec 8400 Analyser unit, and Kjeltec 8460 sampler unit (Foss, Hillerod, Denmark). Organic matter digestibility (OMD) was determined using the rumen degradable organic matter (VOS) method (Lindgren, 1979). Samples were transferred to a glass filter crucible. Rumen fluid from a cow fed a standardised ration at maintenance level with a forage to concentrate ratio of 70:30 was filtered and mixed with a buffer (pH 7, 38°C under anaerobic conditions). Samples were incubated with the rumen fluid-buffer mixture for 96 h. After incubation, the fluid was filtered through a sintered glass disc, washed with deionised water and acetone, and dried overnight. The crucible was then weighed, ashed, and weighed again.

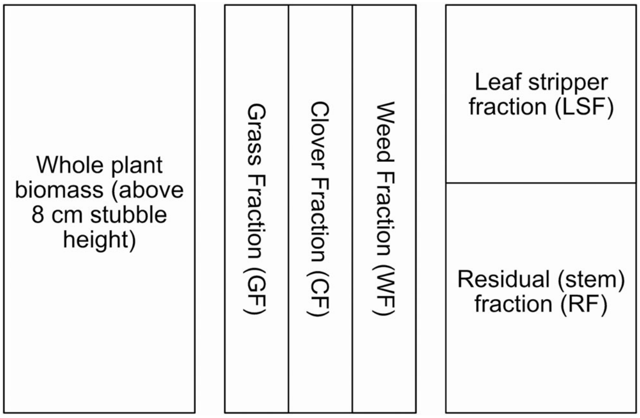

FIGURE 3 Representations of the biomass fractions. The botanical composition sample was separated into the grass fraction (GF), clover fraction (CF), and weed fraction (WF). Following leaf stripping, the relevant fractions were the leaf stripper fraction (LSF) and the residual fraction (RF).

2.4 | Statistical analysis

To assess the differences in nutritive value characteristics, (CP, aNDFom, VOS digestibility, and ash) between different plant fractions, each output variable was analysed using general linear mixed model procedures in PROC GLIMMIX (SAS software version 9.4, SAS Institute Inc., 2008). Plant fraction and year were treated as fixed effects. Denominator degrees of freedom were approximated using the Kenward-Roger method. The RANDOM statement was used for fraction, with sample ID as the subject, using an unstructured covariance structure. Quantile-quantile plots and distributions of studentised residuals were assessed for normal distributions and homoscedasticity. Tukey's statistic was used to test differences ($p < .05$) among means when only the main effect of fraction was significant. When the interaction between fraction and year was significant, the Holm-Bonferroni method was used to test comparisons ($p < .05$) between the same fraction over both years and all fractions within each year.

The Shapiro–Wilk test was used to determine if the data followed a normal distribution (R Studio software version 2022.12.0 + 353, R Core Team, 2022). As the data did not follow a normal distribution, the correlation between each explanatory variable and each response variable was evaluated using the Kendall correlation method (R Studio software version 2022.12.0 + 353, R Core Team, 2022). To build multiple regression models for predicting nutritive value characteristics, PROC GLMSELECT (SAS software version 9.4, SAS Institute Inc., 2008) was used. To estimate the post-harvest nutritive value of the LSF, two multiple regression models were constructed for each variable (CP, aNDFom, VOS digestibility, and CP Yield). One model was constructed using only pre-harvest field measurements (referenced further as field model). The other was constructed using pre-harvest field measurements and pre-harvest nutritive value measurements (referenced further as full model). The explanatory variables are summarised in Table 1. Two datasets were used to construct the eight models outlined above, one with all explanatory variables and another limited to explanatory variables with moderate or strong correlation to the response variable. Variable selection was performed using the STEPWISE option and the PRESS statistic as the criterion, with 0.05 and 0.10 specified as the significance levels for variable entry and removal, respectively. The resultant models were assessed using PROC REG with the PARTIAL option to assess the linearity of partial regression plots. For reporting data, the adjusted r-squared criterion (R^2) was used.

3 | RESULTS

3.1 | Summary of data

The field and nutritive value data collected are summarised in Table 1. The total yield (clover, grass, and weeds) before harvesting is not a focus of this study, as the data are approximations based on a single sample quadrat.

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TABLE 1 Summary statistics and description of field-measured variables and of the nutritive value of the pre-harvest sward (mixed, clover, and grass).

Variables	Name	Unit	Mean	Minimum	Maximum	Description
Field-measured	Clover stage		3.3	2.3	4.0	Clover stage of the furthest advanced plant
	Clover fraction	%	62.2	33.3	98.1	Fraction of clover in the sward, on a DM basis
	Grass fraction	%	37.1	1.5	66.6	Fraction of grass in the sward, on a DM basis
	Weed fraction	%	0.75	0.00	5.80	Fraction of weed in the sward, on a DM basis
	CCI	$\mu g\ cm^{-2}$	30.5	25.8	44.0	Chlorophyll content index from Dualex measurement on red clover leaves
	Day of the year		203	174	251	Day of the year starting from January 1st
	NDVI		0.87	0.82	0.91	NDVI measurement taken with the GreenSeeker across the whole length of the plot
	Tallest clover	cm	67.4	48.0	92.0	Clover height measurement from the ground to the top of the longest stretched plant
	Total yield	kg DM/ha	3554	2126	5368	Total yield calculated from the sample taken for analysis of botanical composition
	LSF yield	kg DM/ha	1164	533	2386	Yield of the leaf stripper fraction (LSF), calculated from the area harvested using the leaf stripper.
	RF Yield	kg DM/ha	2390	1376	4532	Yield of the residual fraction (RF), calculated from the area harvested using the leaf stripper.
	LSF in total yield	%	32.2	16.5	57.0	The LSF, as a fraction of total yield, calculated from the sample taken for analysis of botanical composition and the area harvested using the leaf stripper.
Sward nutritive value	CP	g/kg DM	168	90.9	214	Crude protein using the Kjeldahl-N method
	aNDFom	g/kg DM	422	321	517	aNDFom concentration
	Digestibility	g/kg DM	823	757	877	Organic matter digestibility using the VOS method
Clover nutritive value	CP	g/kg DM	186	129	236	Crude protein using the Kjeldahl-N method
	aNDFom	g/kg DM	344	268	422	aNDFom concentration
	Digestibility	g/kg DM	815	670	870	Organic matter digestibility using the VOS method
Grass nutritive value	CP	g/kg DM	136	59.6	231	Crude protein using the Kjeldahl-N method
	aNDFom	g/kg DM	551	485	642	aNDFom concentration
	Digestibility	g/kg DM	830	694	902	Organic matter digestibility using the VOS method

Abbreviations: aNDFom, neutral detergent fibre; CP, crude protein; DM, dry matter; LSF, leaf stripper fraction.

3.2 | Nutritive value of the resultant fractions

The different dry matter fractions, both pre-harvest (clover, grass, and mixed sward), and post-harvest (leaf stripper and residual) had different compositions in terms of nutritive value. All statements of significance are at the $p < .05$ level. For CP concentration, there was a significant interaction between year and fraction (Figure 4a). The CF, GF, and sward were not significantly different to each other in 2021. In 2022, the CF had a significantly higher CP concentration than both the GF and the sward. Following leaf stripping, the LSF had a higher CP concentration than all other fractions in both years. In both years, the RF had a lower CP concentration than the LSF, CF, and sward, but a higher CP concentration than the GF. There was no significant difference between the CP concentrations of the same fraction between years for any of the fractions.

There was also a significant interaction between year and fraction for aNDFom concentration (Figure 4c). All fractions had significantly different aNDFom concentrations in 2021, with the GF having the highest and the LSF having the lowest. In 2022, the aNDFom concentrations of the GF, sward, and RF were significantly higher than the LSF and the CF. The CF and the LSF had the lowest aNDFom concentration in both years. In 2021, the LSF has a significantly lower aNDFom concentration than the CF, whereas in 2022 there was no significant difference between the two fractions. For both years, GF had the highest aNDFom concentration, even higher than the RF. The GF, sward, LSF, and RF had significantly higher aNDFom concentrations in 2022 than in 2021, while there was no significant difference between the CF across years.

As there was no significant interaction between fraction and year for VOS digestibility or ash concentration, results are presented for the pooled 2021 and 2022 datasets. The pre-harvest fractions were not significantly different from each other in regards to the VOS digestibility (Figure 4b). The VOS digestibility of the LSF was significantly higher than all other fractions, while the RF was significantly lower than all fractions apart from the CF. The only fractions with significantly different ash concentrations were the GF and the RF, with the GF being significantly higher than the RF (Figure 4d). All other fractions were not significantly different from the GF or RF.

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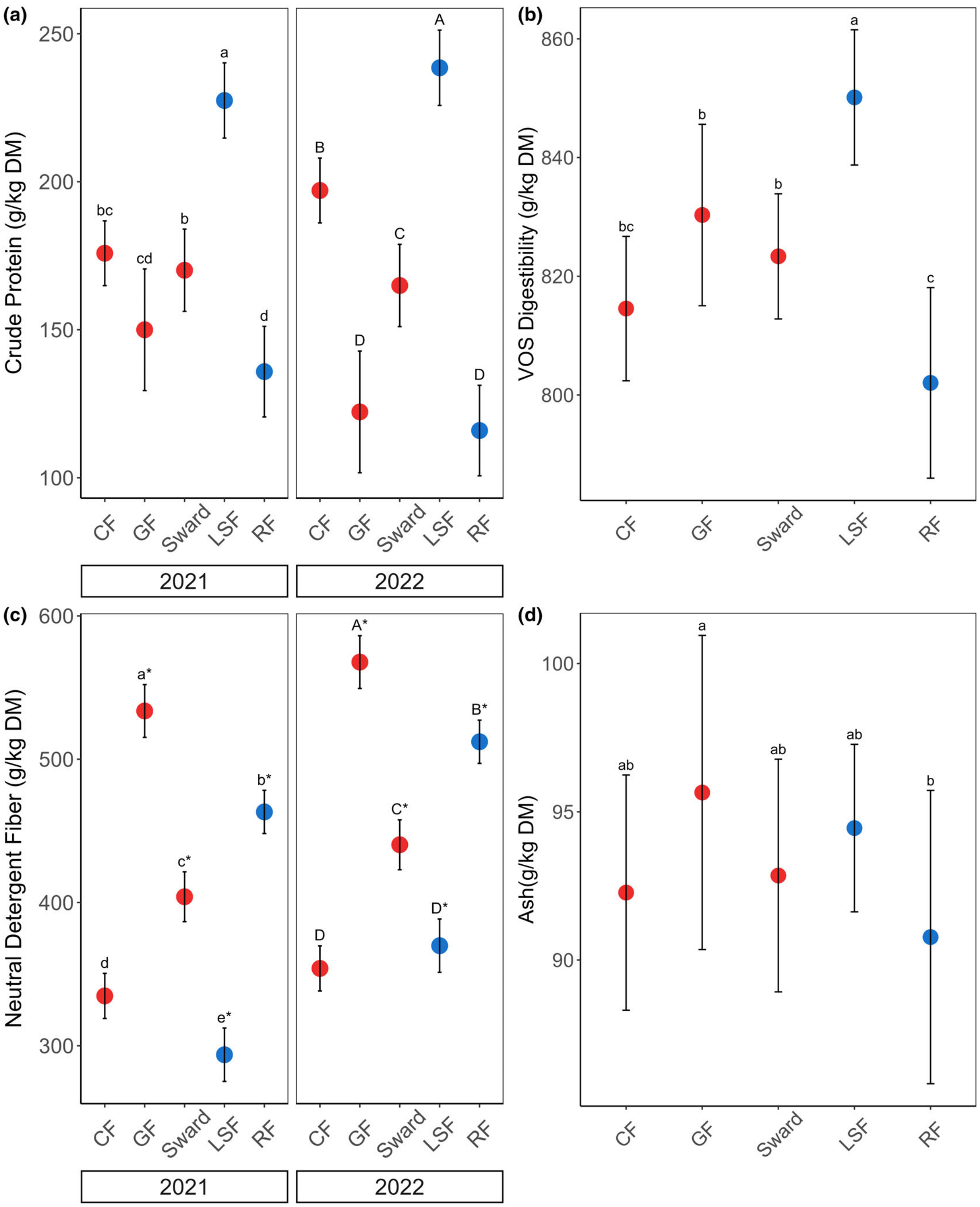

FIGURE 4 Crude protein (a), VOS digestibility (b), neutral detergent fibre (c), and ash (d) of different plant fractions, pre-harvest (red) and post-harvest (blue) using a leaf stripper (LS) in mixed grass-clover leys. Points are least squares means ($n = 20$ for (a) and (c) and $n = 40$ for (b, d). Error bars are 95% confidence intervals. Means with common letters are not significantly different ($p < .05$) according to the Holm-Bonferroni method for subfigures (a) and (c) or Tukey's test for subfigures (b, d). Sub-figures (a, c) present nutritive value data for the interaction of year and fraction. Same fractions across years 2021 and 2022 that are statistically different are denoted with an asterisk. The clover fraction (CF) and grass fraction (GF) constitute the sward pre-harvest. The post-harvest fractions include the leaf stripper fraction (LSF) and residual fraction (RF).

3.3 | Correlations between the leaf stripper fraction nutritive value and the pre-harvest measurements and nutritive value

The level of correlation between explanatory variables (field measurements and nutritive value of the pre-harvest fractions) and the response variables (nutritive value of the LSF) was assessed to identify potential predictors for a regression analysis (Table 2). LSF CP was strongly correlated to the CP of all three pre-harvest fractions. Explanatory variables year, aNDFom of the clover, grass, and sward fractions, and VOS digestibility of the clover and sward fractions were strongly correlated to the aNDFom of the LSF. The LSF VOS digestibility was strongly correlated to the LSF yield, the aNDFom of the CF, and the VOS digestibility of all three pre-harvest fractions. The

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TABLE 2 Correlation between nutritive value characteristics (CP, crude protein; aNDFom, neutral detergent fibre; VOS, organic matter digestibility using the VOS method; CP Yield, crude protein yield) of the leaf stripper fraction (LSF) and field measurements and nutritive value characteristics (CP, aNDFom, VOS) of the clover fraction (CF), grass fraction (GF), and sward.

	Response variable			
Explanatory variable	LSF CP (g/kg DM)	LSF aNDFom (g/kg DM)	LSF VOS (g/kg DM)	LSF CP yield (kg DM/ha)
Year	0.147	__0.580***__	_−0.245_	0.129
Clover stage	−0.020	−0.101	−0.171	−0.118
Clover fraction (%)	0.159	−0.144	−0.073	__0.308**__
Grass fraction (%)	−0.154	0.154	0.073	__−0.328**__
CCI (μg cm^{-2})	0.067	_0.221*_	−0.153	0.026
Day of the year	0.093	−0.194	0.081	__−0.383***__
NDVI	0.192	−0.003	−0.035	__0.322**__
Tallest clover (cm)	_−0.289**_	0.054	_−0.247*_	_0.227*_
Total yield (kg DM/ha)	−0.122	0.081	−0.110	_0.299**_
LSF Yield (kg DM/ha)	−0.077	_0.292**_	__−0.301**__	__0.841***__
RF Yield (kg DM/ha)	−0.074	_0.228*_	−0.176	_0.259*_
LSF in total yield (%)	−0.036	0.169	_−0.201_	__0.544***__
CP Clover (g/kg DM)	__0.515***__	0.162	−0.055	−0.003
CP Grass (g/kg DM)	__0.344**__	−0.113	0.040	0.036
CP Sward (g/kg DM)	__0.426***__	−0.108	0.065	0.092
aNDFom Clover (g/kg DM)	0.005	__0.369***__	__−0.407***__	__0.374***__
aNDFom Grass (g/kg DM)	−0.115	__0.387***__	_−0.219*_	_0.223*_
aNDFom Sward (g/kg DM)	−0.192	__0.454***__	−0.194	−0.049
VOS Clover (g/kg DM)	0.023	__−0.336**__	__0.527***__	__−0.362**__
VOS Grass (g/kg DM)	0.087	_−0.287**_	__0.468***__	__−0.385***__
VOS Sward (g/kg DM)	0.072	__−0.344**__	__0.561***__	__−0.326**__

Note: The correlation coefficients (Kendall's tau) were calculated using the Kendall rank correlation test. Correlation coefficients denoted with asterisks are significant at the levels 0.05*, 0.01**, or 0.001***. Correlation coefficients in bold have strong correlation. Coefficients in italics have moderate correlation. All other coefficients have weak correlation.

response variable LSF CP yield was strongly correlated to many field measurements and pre-harvest nutritive value parameters, totaling 10 of the 21 explanatory variables. All response variables had either strong or moderate correlation to at least one of the response variables.

3.4 | Explanatory variables for nutritive value of the leaf stripper fraction

For all eight models, the dataset using all explanatory variables produced stronger or equal models than the dataset limited to the explanatory variables with strong or moderate correlation to the response variable. All models presented in this article were produced using the dataset with all explanatory variables to maximise fit of the final models (Table 3).

For the full model for CP of the LSF, as expected, the CP of the CF was the most important explanatory variable, explaining 0.56 of the variability. To explain up to 0.67 of the variability, the CP of the sward and the CF percent were also included. In the field model, the variable tallest clover was the first added and explained only 0.15 of the variability. The only other variable included in the model was CF percent, which resulted in an adjusted R^2 of 0.25 for the model.

To estimate aNDFom of the LSF, the field model included only one field measurement, alongside two nutritive value parameters. The aNDFom of the sward fraction was the first variable selected, explaining only 0.47 of the variability. LSF yield and clover CP were added, respectively, to increase the variability explained by the model to 0.69. Similar to the full model, LSF yield was included in the field model and accounted for 0.11 of the variability. The percent CF was then added to increase the adjusted R^2 of the model to 0.31.

The full model estimated the VOS digestibility of the LSF by first including VOS of the sward to explain 0.54 of the variability. Subsequently, total yield was the only other variable included in the model to achieve an R^2 of 0.66. The only field measurement variable included in the full model, total yield, was not included in the field model. The most important explanatory variable for the field model was LSF yield, explaining 0.13 of the variability. The other variable added was clover stage, which only increased the adjusted R^2 of the model to 0.27.

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TABLE 3 Multiple regression models ($n = 40$) for predicting nutritive value characteristics (CP, crude protein; aNDFom, neutral detergent fibre; VOS, organic matter digestibility using the VOS method, CP Yield, crude protein yield) of the leaf stripper fraction (LSF). No further variables were added when doing so would not result in a significant ($p < .05$) decrease in the PRESS statistic†.

Explanatory variable set	Response variable	R^2	RMSE	PRESS	Model
Full	LSF CP (g/kg DM)	0.67	16.2	12,358	50.1 + 0.61(CFCP) + 0.28(SCP) + 36.7(CF)
	LSF aNDFom (g/kg DM)	0.69	30.9	43,360	−272 + 0.96(SNDF) + 0.04(LSFY) + 0.77(CFCP)
	LSF VOS (g/kg DM)	0.66	21.6	21,343	−122 + 1.10(SVOS) + 0.02(TY)
	LSF CP Yield (kg DM/ha)	0.64	72.2	253,543	734–0.87(GFVOS) + 0.06(RFY) + 1.60(CFCP) + 238(CF) − 1.63(DOY)
Field	LSF CP (g/kg DM)	0.25	24.4	26,071	270–1.14(TC) + 63.8(CF)
	LSF aNDFom (g/kg DM)	0.31	46.4	91,959	364 + 0.07(LSFY) − 184(CF)
	LSF VOS (g/kg DM)	0.27	31.5	43,080	989–0.03(LSFY) − 30.4(CS)
	LSF CP Yield (kg DM/ha)	0.49	86.2	337,110	195 + 369(CF) − 1.4(DOY) + 0.05(RFY)

Abbreviations: $^{\dagger}R^2$, coefficient of determination; CF, clover fraction %; SNDF, sward aNDFom (g/kg DM); CFCP, clover fraction crude protein (g/kg DM); CFVOS, clover fraction VOS digestibility (g/kg DM); CS, clover stage; DOY, day of the year; GFVOS, grass fraction VOS digestibility (g/kg DM); LSFY, leaf stripper fraction yield (kg DM/ha); PRESS, predicted residual sum of squares; RFY, residual fraction yield (kg DM/ha); RMSE, root mean square error; SCP, sward crude protein (g/kg DM); SVOS, sward VOS digestibility (g/kg DM); TC, tallest clover (cm); TY, total yield (kg DM/ha).

The datasets used to predict the CP yield of the LSF were modified to exclude LSF yield as an explanatory variable, as it was used to calculate the LSF CP yield. The full model for predicting the LSF CP yield first included GF VOS digestibility to explain 0.36 of the variability. An additional four variables, RF yield, CF CP, CF percent, and day of the year, were also included to explain 0.64 of the variability. The model initially included NDVI of the entire plot, but this variable was dropped by the stepwise process once additional variables were added to increase the adjusted R^2 from 0.63 to 0.64. CF percent was the most important explanatory variable for the field model and explained 0.31 of the variability. After including variables day of the year and RF yield, the model had an adjusted R^2 of 0.49, the highest of all field models.

4 | DISCUSSION

4.1 | Performance of the leaf stripper

The leaf stripper worked well in mixed stands, removing on average a third of the available forage biomass. This is roughly equivalent to 50% of the available clover biomass; however, small amounts of grass were also included in the LSF. The success of the machine in removing clover leaves in mixed stands likely depends on the height and maturity of the plants, as well as machine settings such as the height of the leaf stripper rotor, rotational speed, and ground speed. Proper adjustment of the machine requires the user to observe the composition of the LSF and RF, and make adjustments accordingly. In order to fully understand the performance of the leaf stripper, additional work is needed to determine the proportion of clover leaves collected in the LSF. This could be achieved by hand sorting the LSF and RF post-harvest to calculate the percent of clover leaves collected through leaf stripping. The leaf stripper setting and the biomass of stand, however, will heavily influence these results.

4.2 | Effects on nutritive value

The results clearly showed that the LSF had a significantly higher CP concentration than all other fractions. Following leaf stripping, the CP concentration of the LSF was 39.1% higher than the sward and 25.0% higher than the CF. A previous study comparing conventional harvesting to leaf stripping for pure stands of red clover reported a 32.3% higher CP concentration in the LSF than the clover harvested conventionally, considerably higher than the results presented here from the mixed stands (Liebhardt et al., 2022). The smaller increase in CP concentration of the LSF compared to the CF seen in our results can be attributed to the inclusion of grass in the LSF fraction. As the CP concentration of the GF was 27.0% lower than the CF, the inclusion of grass in the LSF decreases its CP concentration. Although higher CP concentrations could be achieved when leaf stripping pure clover stands, mixtures of grass and clover are preferable in northern Europe due to their higher yields, longer persistence, and increased sustainability.

The impact of leaf stripping on aNDFom was less consistent however. The LSF had a significantly lower aNDFom concentration than both the sward (−110 g/kg DM) and the CF (−41 g/kg DM) in 2021. In 2022, however, the aNDFom of LSF was only significantly lower than the sward (−70.5 g/kg DM), as the aNDFom of the LSF and CF were not significantly different. The aNDFom concentration of the LSF was significantly higher in 2022 than in 2021 (Figure 4). Considering the LSF is made up of clover leaves and grass and that the aNDFom concentration of the CF was not significantly different between 2021 and 2022, the change in aNDFom concentration of the LSF must be explained by the changes in the GF. For the GF, the aNDFom concentration (+34.1 g/kg DM) and the sward percentage (+7.48%) increased in 2022 compared to 2021 (Figure 4). This higher aNDFom concentration and increased amount of grass in the sward likely contributed to the higher aNDFom of the LSF in 2022. Though not included in the analysis due to missing data, the grass stage at

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harvest could also have contributed the difference in aNDFom concentration between years. Previous studies on leaf stripping have only been done in legume monocultures and thus the results are not directly comparable when considering pre-harvest fractions and their influence on the LSF. An experiment performed in pure lucerne reported that the RF contained the highest concentration of aNDFom compared to the LSF and whole lucerne plant (Sikora et al., 2019). This trend is also seen in the data presented here, as the RF contained a higher aNDFom concentration than both the LSF and CF (Figure 4).

Leaf stripping had a significant effect on the VOS digestibility when compared to the sward and the CF (Figure 4). The LSF had 3.25% higher VOS digestibility than the sward and 4.37% higher than the CF. Leaf stripping had a small effect on ash concentration, with the only significant difference being between the GF and the RF (Figure 4). Based on the improved nutritive value of the LSF compared to the CF and sward, the largest effect of the leaf stripper was increasing the CP concentration of the resultant product, while decreasing the aNDFom concentration and slightly increasing the VOS digestibility were secondary effects.

4.3 | Potential use of the forage fractions

The higher CP and lower aNDFom concentrations in the LSF fractions compared to the sward increases the feed value for monogastrics such as pigs. Pigs can utilise some amount of forage, which can be beneficial for gut health. The CP concentration in the LSF in this study averaged 23% DM, significantly lower than the CP contained in soybean meal which ranges from 45% to 50% DM (Sauvant et al., 2004). The successful integration of the leaf stripper machine into current production systems will require reliable methods of preservation. Though the nutritive value of the LSF is significantly better than that of the mixed sward, the ensiling process has been shown to result in protein degradation, leading to silage with protein concentrations too low to serve as a protein feed (Renaudeau et al., 2022). Due to this, it is not reasonable for the preservation process to be overly expensive. The average moisture content in the LSF (202 g/kg DM) was similar to that of the GF (217 g/kg DM). On-field wilting of leaf material is not possible, thus requiring the inclusion of grain or additives during the ensiling process to achieve an adequate DM content. Previous studies have shown potential for inclusion of crushed barley grain or ground corn to achieve a suitable moisture content for ensiling (Renaudeau et al., 2022; Shinners et al., 2007). Alternatively, formic acid has been successful in inhibiting clostridial fermentation when ensiling forage leaves with a high moisture content (Muck et al., 2010; Shinners et al., 2007). Due to the post-ensiling nutritive value of leaf stripped material, Renaudeau et al. (2022) concluded that legume leaf silages should be considered as an energy source rather than a protein source for pig feeding. If the goal is to develop a protein feed for pigs, further processing (such as juicing of the LSF) to increase protein content and remove fibre may be necessary.

The LSF has similar characteristics to typical forages harvested for ruminants, but with higher protein and digestibility, and lower fibre concentrations. It could be used to increase digestibility and CP content in the feed rations of lactating dairy cows, potentially reducing the need for grain-based concentrates. However, increased CP from clover in dairy diets will not necessarily increase production. The digestion of red clover protein in dairy cows is limited in the utilisation of the protein flowing from the rumen to the small intestine (Vanhatalo et al., 2009), and thus cows fed silages high in red clover may not fully utilise the protein for productive purposes, at least partially due to the presence of polyphenol oxidase (Lee, 2014). The RF, with lower CP and digestibility could potentially be fed to dry dairy cows, heifers, or other ruminants requiring feed with less energy and CP.

4.4 | Modelling nutritive value of the leaf stripper fraction

The purpose of the modelling component was to assess whether there are measurable characteristics of mixed leys that affect the nutritive value of the LSF. In general, the models for CP, aNDFom, VOS digestibility, and CP Yield of the LSF were quite poor and, based on these results, are not useful methods for assessing the potential nutritive value of the LSF pre-harvest. Although the full models were able to explain on average 70% of the variability, they relied heavily on the nutritive value of the pre-harvest fractions, data that is not typically available prior to harvest. The results of the field models give a better picture of the prediction potential one might have pre-harvest. For LSF CP, aNDFom, and VOS digestibility, the field models only explained on average 28% of the variability. The model for LSF CP yield was able to explain 49% of the variability, though this parameter is directly correlated to the amount of biomass in the field and thus easier to estimate pre-harvest.

Surprisingly, the variables NDVI and CCI were not included in any of the models. Considering these variables represent vegetation greenness and chlorophyll concentration (Cerovic et al., 2012; Tang et al., 2022), one might have expected them to be better indicators of CP concentration. The NDVI reading from the GreenSeeker contains information about the leaf area of the canopy and the chlorophyll content of the measured area. These variables can be highly correlated, especially in the case of non-stressed conditions. Moreover, NDVI is known to be prone to saturation for high levels of biomass (Mutanga & Skidmore, 2004), that is, the vegetation index cannot account for changes in biomass or chlorophyll content. Saturation results in a limited NDVI range (Sharma et al., 2015), which is consistent with the small amount of variability in the NDVI readings between plots in this study, regardless of their differences in yield and botanical composition. The CCI data obtained with the Dualex did not accurately represent the chlorophyll content of the LSF, as the Dualex leaf clip was only used on clover leaflets. The LSF is made up of clover leaflets, petioles, and stems, as well as grass, and thus the CCI data would need to take into account the chlorophyll content of all components of the LSF to provide an accurate indication of its CP concentration. Improving field models could potentially be achieved by the inclusion of additional equipment, capable of predicting nutritive value. Field spectrometers

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have been shown to have success in estimating nutritive value (Morel et al., 2022; Zhou et al., 2019), however currently the price is an obstacle for practical application. Alternatively, NIR sensors or spectrometers mounted to the harvest machinery, such as John Deere's HarvestLab or Zeiss' Corona extreme, could allow for continual adjustment of leaf stripper settings based on nutritive value measurements in real time.

The botanical composition of the sward, represented by the CF percent in this analysis, can be an important factor in determining the nutritive value of the LSF fraction and was included in three of the four field models (Table 3). Though not done in this study, a botanical separation of the LSF could provide additional information about how much grass the leaf stripper harvests. Previous leaf stripping studies have focused on hand or air separation of the LSF to gain insight into leaf proportion of the LSF. The only published study on leaf stripping of red clover showed that in pure red clover stands, 82% of the LSF was comprised of red clover leaves (Liebhardt et al., 2022). Understanding this mechanism will be essential in understanding the makeup of the LSF, as well as its nutritive value.

4.5 | Further development

This study was an initial investigation of using a leaf stripping machine designed primarily for lucerne in mixed stands of red clover and grass. It is clear that the PremAlfa Mini was suitable for fractionation of red clover in mixed stands. This was evident from visually assessing the resultant LSF, and from the clearly significant differences between the nutritive value parameters of the fractions.

Nevertheless, further investigation is needed to build up a database of samples and accompanying agronomic data. Increased understanding of how the machine functions with changing levels of clover content and increasing levels of biomass is necessary to develop machine setting recommendations based on stand characteristics to ensure consistent efficiency in fractionation. The machine performance likely impacts the resulting nutritive value and yield of the LSF, thus maintaining consistent machine settings across diverse stands will be essential in ensuring a homogenous end product. Variables such as the ratio of rotor speed to forward speed and location on the plant in which the tines fractionate should be further investigated to determine appropriate settings for the intended LSF composition. Additionally, further machine modification may be necessary to optimise fractionation in mixed stands. With further development, it could be possible to suggest the optimal rotor height based on the height of the sward and the botanical composition. The rotor speed when using a full-scale leaf stripping harvester would likely be less influenced by increased biomass due to increased available power, so these issues may not persist once shifting to large scale leaf stripping.

Further processing of the LSF could help achieve a more suitable CP and aNDFom concentration for utilization as a monogastric protein feed. Fractionation of forages through twin screw-press juicing has shown great promise in northern Europe to produce protein feed with suitable nutrient composition for monogastrics. The combination of these two fractionation methods could potentially produce a concentrated protein-feed product high in protein and low in fibre for monogastrics. Based on results of previous studies, juicing of the LSF could achieve a product with a significantly lower fibre content than leaf stripping alone (Colas et al., 2013; Digman et al., 2013; Hansen et al., 2022; Jørgensen et al., 2022).

5 | CONCLUSIONS

This study showed that the PremAlfa Mini leaf stripper machine could successfully separate clover leaf from clover stem and grass in mixed stands. The leaf stripping process increased CP concentration and digestibility, and reduced aNDFom concentration, in comparison to the original sward. The resultant nutritive value of the LSF signifies that it is more suitable as an energy source rather than a protein source for pig feeding. The LSF could however be used to upgrade the nutritional content of forages used for selected ruminants and offer feeds of different nutritive value to classes of animals with different nutritional requirements. The regression models developed to identify measureable characteristics that impact the nutritive value of the LSF are likely not useful for prediction at their current stage. Further development is needed to determine if additional spectrometer measurements can improve the ability of models based on pre-harvest data to predict the nutritive value of the LSF.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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