

Structural fibre components of the cell walls of forage legume grasses grown in mountain areas

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Abstract

In the period 2020–2022 in the experimental field of the Research Institute of Mountain Stockbreeding and Agriculture – Troyan a research experiment has been conducted with the following species of perennial leguminous meadow grasses: bird's-foot-trefoil (*Lotus corniculatus* L.), hybrid clover (*Trifolium hybridum* L.), alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.). The experiment aimed to monitor the composition of the structural fibre components of the forage cell walls average two-year period. The dry matter digestibility and energy nutritional value was determined. The legume crops studied were found to have different compositions of forage cell wall fractions. The forage mass of red clover showed the lowest content of neutral and acid detergent fibre (30.87 % and 21.49 %), and acid detergent lignin (8.11 %) and the highest dry matter digestibility (73.11 %). The alfalfa forage had the highest hemicellulose values (11.17 %) and that of hybrid clover had the maximum cellulose values (24.06 %). Good energy nutritive value was recorded by the forage of bird's-foot-trefoil and white clover.

Key words: cell wall fibre components, digestibility, legume grasses.

Introduction

Legume meadow grass species are suitable components for the establishment of grass mixtures (Bozhanska et al. 2016, Kirilov 2016, Arlauskienė et al. 2021) due to their high productivity, ability to fix nitrogen from air and their nutritional value,

which defines them as good forage for farm animals (Churkova et al. 2016). They are less often grown alone because in combination with perennial grasses they have higher productivity and higher forage quality, lower nitrogen requirements, higher resistance to diseases and pests, and better seasonal distribution of the for-

age obtained (Annicchiarico 2008). The inclusion of legumes in the composition of grasses increases the quality characteristics of forage and maintains a sustainable balance between the perennial and legume components (Naydenova et al. 2010). For the mountain conditions of Bulgaria, suitable legumes for obtaining forage with high nutritional value are red clover (Naydenova and Bozhanska 2014, Britaňák et al. 2018), white clover (Mihovski and Sabeva 2011) and bird's-foot-trefoil (Churkova and Churkova 2022). Alfalfa is a source of plant protein that determines the nutritional value of forage under changing climatic conditions (Marinova 2020).

The cell wall component contents of legume forage grasses are important indicators of dry matter uptake and utilization depending on the grass species. They determine forage quality due to being a source of energy for ruminants when they break down (Ivanova et al. 2019). Detergent analysis based on fibre composition and digestibility is the basis for determining forage digestion (Marković et al. 2023).

Acid detergent fibre (ADF) determines feed digestibility and neutral detergent fibre (NDF) determines feed utilization (Ertekin et al. 2022). The amount of neutrally detergent fibre in forage of self-seeded cropped bird's-foot-trefoil was $418.9 \text{ g}\cdot\text{kg}^{-1} \text{ DM}$ and was lower compared to growing it with perennial grasses (Churkova and Churkova 2020). In a study of self-seeded grassland from legume crops, Petkova et al. (2022) found that white clover had the highest dry matter digestibility ($722.63 \text{ g}\cdot\text{kg}^{-1} \text{ DM}$), red clover had the highest neutral-detergent fibre content ($418.03 \text{ g}\cdot\text{kg}^{-1} \text{ DM}$), and bird's-foot-trefoil biomass had the highest concentration of acid-detergent fibre ($291.98 \text{ g}\cdot\text{kg}^{-1} \text{ DM}$), acid-detergent lignin ($70.72 \text{ g}\cdot\text{kg}^{-1} \text{ DM}$), and cellulose

($221.27 \text{ g}\cdot\text{kg}^{-1} \text{ DM}$).

The study aimed to determine the fibre composition of cell wall components and the energy value of forage from perennial legume grasses grown under mountain conditions.

Material and Methods

The research experiment was carried out in 2020 in the experimental field of Research Institute of Mountain Stockbreeding and Agriculture, Troyan. The area is located at the northern foot of the Central Stara Planina Mountains (Bulgaria) at an altitude of 384 m a.s.l. The experiment includes the following species of meadow legume crops: bird's-foot-trefoil (*Lotus corniculatus* L.), hybrid clover (*Trifolium hybridum* L.), alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.) on light gray pseudopodzolic soil.

The experiment was laid out using the block method in four replications with a harvest plot size of 5 m^2 . A generally accepted technology for the cultivation of meadow legume crops for forage was applied (Churkova and Churkova 2024). The sowing rate for grass species was as follows: for bird's-foot-trefoil, white and hybrid clover – $12 \text{ kg}\cdot\text{ha}^{-1}$, for alfalfa and red clover – $25 \text{ kg}\cdot\text{ha}^{-1}$. The sowing of the grassland was carried out manually, scattered at different seeding rates according to the grass species.

Samples for chemical analysis were determined for two consecutive years (2021 and 2022) and were averaged over the period.

The harvesting of the leguminous meadow grasses (*Lotus corniculatus*, *Medicago sativa*, *Trifolium hybridum*, *T. pratense*, *T. repens*) was carried out in

June at the beginning of flowering.

Samples were taken immediately before harvesting in the budding-early flowering stage. Dried samples were ground with a grinder through a 1 mm mesh sieve before analysis. The structural carbohydrates of the cell walls were determined in each sample, the nutritive value and *in vitro* enzymatic digestibility of the dry matter were calculated.

The fibre composition of the cell walls was determined in % and included:

- NDF;
- ADF;
- Acid-detergent lignin (ADL) – determined by the detergent analysis of Van Soest et al. (1991);
- Degree of lignification (expressed as percentage of ADL/NDF);
- Hemicellulose (calculated by NDF–ADF);
- Cellulose = ADF–ADL.

Nutritional value of the forage was estimated using the Bulgarian system as feed units for milk (FUM) and feed units for growth (FUG) and calculated based on equations (1) and (2) according to the experimental values of CP, CF, CF and NFE, recalculated using the digestibility factors proposed by Todorov et al. (2010).

$$GE = 0.0242 \cdot CP + 0.0366 \cdot CF + 0.0209 \cdot CF + 0.017 \cdot NFE - 0.0007 \cdot Zx, \quad (1)$$

where: GE is gross energy, MJ·kg⁻¹ DM; CP is crude protein, %; CF is crude fibre, %; NFE is nitrogen free extractable substances, %; Zx is sugars, %.

$$EE = 0.0152 \cdot DP + 0.0342 \cdot D + 0.0128 \cdot DF + 0.0159 \cdot DNFE - 0.0007 \cdot Zx, \quad (2)$$

where: EE is exchangeable energy, MJ·kg⁻¹ DM; DP is digestible protein, %; D is digestibility, %; DF is digestible fibre, %; DNFE is digestible nitrogen free extractable substances, %; Zx is sugars, %.

FUM and FUG were calculated using

equations (3) and (4), respectively.

$$FUM = EE \cdot (0.075 + 0.039 \cdot q), \quad (3)$$

$$FUG = EE \cdot (0.04 + 0.1 \cdot q), \quad (4)$$

where: *q* is exchange rate.

In vitro enzyme digestibility of dry matter (%) was determined according to Aufrere (1982) by the two-phase pepsin-cellulose method. In the first phase, a pre-treatment with pepsin-hydrochloric acid was carried out to digest the protein, and in the second phase, the residue of the feed was treated in an acid condition with the enzyme cellulose to digest the cellulose.

Statistical treatment of data is represented by mean (X), standard deviation (SD); minimum (min) and maximum (max) limits (Lidanski 1988). The degree of variation of the parameters of individual indicators was determined by the coefficient of variation (CV) according to Mamaev's scheme: up to 7 % – very low; above 7.0 to 12.0 % – low; above 12.0 to 20.0 % – moderate; above 20.0 to 40.0 % – high; above 40.0 % – very high. The CV measures the variability relative to the mean and is found by dividing the SD by the mean. For this purpose, the software products Analysis Toolpak for Microsoft Excel 2010 and Statgraphics Plus v.2.1 were used.

Results and Discussion

To estimate the forage quality of different meadow legume crops in Table 1 are presented data for neutral and acid detergent fibre, ADL, hemicellulose and cellulose.

Cell wall components of legume crops are closely related to variety, stem anatomy (Engels and Jung 1998), and stage of plant development (Doblin et al. 2010). NDF is a key indicator for predicting the

Table 1. Structural fibre components of cell walls of forage from meadow legume crops averaged over the period 2021–2022.

Variants	NDF, %	ADF, %	ADL, %	Hemicellulose, %	Cellulose, %	Degree of lignification (coefficient)
<i>Lotus corniculatus</i> L.	45.18	34.90	13.17	10.28	21.74	28.79
<i>Trifolium hibridum</i> L.	43.34	33.07	9.02	10.26	24.06	20.70
<i>Medicago sativa</i> L.	42.21	31.04	11.25	11.17	19.80	26.06
<i>Trifolium pratense</i> L.	30.87	21.49	8.11	9.38	13.38	27.20
<i>Trifolium repens</i> L.	37.08	27.80	8.20	9.28	19.60	22.09
X	39.73	29.66	9.95	10.07	19.71	24.97
SD	5.80	5.27	2.20	0.77	3.97	3.44
CV	14.59	17.78	22.10	7.67	20.16	13.78
Min	30.87	21.49	8.11	9.28	13.38	20.70
Max	45.18	34.90	13.17	11.17	24.06	28.79

free uptake of forage by animals, while ADF influences forage degradability and digestibility. Red clover had the lowest content of NDF (30.87 %), ADF (21.49 %) and ADL (8.11 %).

Second place in terms of indicators defining a balanced quality composition of the forage was occupied by white clover, with NDF 37.08 %, ADF 27.80 % and ADL 8.20 %, respectively. More significant changes were observed in NDF, ADF and ADL contents of bird's-foot-trefoil forage mass compared to those of other legumes. The mean excess of NDF was 14.31 %, of ADL – 13.41 %, and of ADF – 5.06 % compared with the values of red clover forage. Hybrid clover had elevated values for these three parameters. NDF exceeded those of red clover by 12.47 % and white clover by 6.26 %. The same trend was observed for ADF and ADL content. Lignin plays an important role in structural integrity of the cell wall, stem strength, water transport, mechanical support and protection against plant pathogens (Wang et al. 2013, You et al. 2013), but has an abrasive effect on digestion by degrading plant cell walls (Lamb et al. 2007). This requires a search for crop species with reduced lig-

nin content with appropriate composition leading to increased lignin degradation and increased energy efficiency of the forage (Jung and Engels 2002).

Therefore, the forage mass of red clover has the most suitable quality composition in terms of lignin due to its reduced content of 8.11 %. Bird's-foot-trefoil had the highest levels of NDF, ADF and ADL accordingly: 45.18 %, 34.90 %, 13.17 %. Pavlov and Naydenova (2000) found that the content of the three components increased with increasing age of the bird's-foot-trefoil. Alfalfa had values of these parameters higher than those of white and red clover, and lower than those of bird's-foot-trefoil. According to the data obtained on CV, neutral (CV = 14.59 %) and ADF (CV = 17.78 %), have a medium degree of variability, and ADL has a high degree of variability (CV = 22.10 %). These results indicate a significant difference among the species of legume crops in relation to ADL and less in relation to neutral and acid detergent fibres.

Forage lignification correlates negatively with its digestibility. Feed uptake depends not only on the components of the cell walls, but also on their volume

and degradability. The degree of lignification had the highest values for the bird's-foot-trefoil forage (28.79) and the lowest for the hybrid clover forage (20.70). Vasileva and Naydenova (2017) also obtained high values for this indicator of fodder from self-seeded bird's-foot-trefoil. The degree of lignification of red clover slightly exceeded that of alfalfa, by 1.14. White clover forage (22.09) had higher values for degree of lignification than hybrid clover. Hemicellulose had similar values in the forage mass of red (9.38 %) and white (9.28 %) clovers, as well as bird's-foot-trefoil (10.28 %) and hybrid clovers (10.26 %). The highest value was recorded for the fodder mass of alfalfa (11.17 %) with an average value of $X = 10.07$ %.

The variation of hemicellulose in the forage of the studied meadow legume grasses according to the CV had a very low degree of variability ($CV = 7.67$ %). The cellulose polysaccharide content varied from 13.38 % to 24.06 %, with a maximum value in hybrid clover and a minimum in red clover. The average value

of cellulose content was 19.71 %, and according to the CV, the degree of variability of this indicator was high ($CV = 20.16$ %). Of the meadow legume grasses studied, red clover forage had the most favourable cellulose content regarding forage quality.

Feed intake is considered to be physiologically regulated if the digestibility of organic substance is lower than 67 % (van Vuuren 1994), and higher digestibility is an indicator of high feed quality. In our study, high values for the digestibility (Fig. 1) of the dry matter of the feed of red clover (73.11 %) and white clover (68.13 %) were obtained. The lowest digestibility of dry matter is the feed mass of bird's-foot-trefoil (62.54 %), which is in line with the high levels of neutral and acid-detergent fibres and ADL, which corresponded to an established relationship between the digestibility of dry matter and the fibrous components of cell walls (Naydenova and Pavlov 2005, Glindemann et al. 2009).

This was confirmed by the highest digestibility of red clover feed, which had the lowest values of NDF, ADF and ADL,

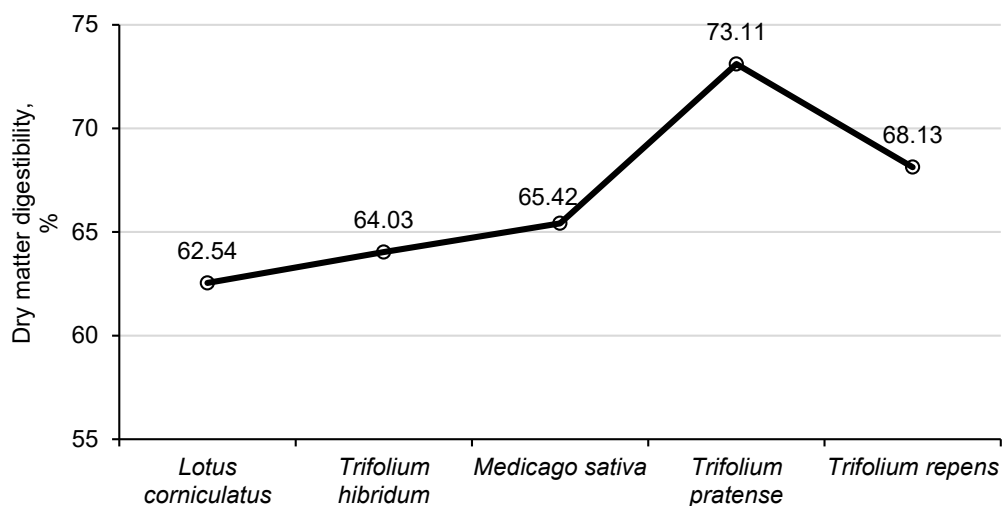


Fig. 1. Dry matter digestibility of meadow legume grass forage.

Note: Dry matter digestibility determined according to the method of Aufrere (1982).

and counter the feed mass of bird's-foot-trefoil, which had the highest content of these indicators, had the lowest digestibility. A trend was observed that proves that with an increase in CF, NDF and ADF, the enzymatic digestibility of the dry matter decreases, and with an increase in CP, the digestibility increases (Stoycheva et al. 2016). Hybrid clover (64.03 %) and alfalfa (65.42 %) have almost similar values of digestibility of the dry matter of the feed. The relative and absolute proportion of digestible cell content decreases rapidly during the growth of grass species, while the proportion of ADF, CF and hemicellulose increases slowly and then remains at the same level (Carlier et al. 2011). The absolute proportion of digestible cell walls in the total dry matter content reaches a maximum in the middle of the development phase, then decreases.

Energy nutritional value determines the balance between the quantity and quality of the forage (Vasileva and Naydenova 2017). The difference between the gross and exchangeable energy of forage (Table 2) from legume grasses ranged from 17.39 to 17.90 MJ·kg⁻¹ DM, with the highest value for forage from bird's-foot-trefoil. The same trend was observed for exchange energy.

FUM in bird's-foot-trefoil and white clover forages were similar (0.70 pieces) and alfalfa forage recorded the lowest values

(0.62 pieces). FUG of forage from white clover were highest (0.64 pieces), followed by the forage mass of bird's-foot-trefoil (0.63 pieces) and lowest for alfalfa (0.54 pieces), with the trend being identical to that of feed units for milk. Vateva and Stoeva (2007) obtained values of FUM from a self-seeded bird's-foot-trefoil – 0.698 pieces, and of FUG – 0.590 pieces, which are close to those obtained by us. According to literature sources (Carlier et al. 2011), the FUM of alfalfa varies from 0.6 to 0.9 pieces in 1 kg of dry matter, and in the scientific experiment the reported value is 0.62 pieces, the difference being due to the vegetation phase. The most favourable balance between quality and yield is obtained in the early flowering phase when the stands of the studied legume grasses are harvested.

Conclusions

The meadow legume grasses from bird's-foot-trefoil, hybrid clover, alfalfa, red and white clover showed different composition of forage cell wall fractions. The forage mass of red clover registered the lowest content of neutral and acid detergent fibres, and acid detergent lignin. The high values of the same parameters of the forage of bird's-foot-trefoil make it less suitable for cultivation compared to the rest

Table 2. Energy nutritional value of meadow legume grass feed.

Legume grasses	GE, MJ·kg ⁻¹ DM	EE, MJ·kg ⁻¹ DM	FUM, numbers	FUG, numbers
<i>Lotus corniculatus</i> L.	17.90	7.66	0.70	0.63
<i>Trifolium hybridum</i> L.	17.39	7.48	0.69	0.62
<i>Medicago sativa</i> L.	17.49	6.83	0.62	0.54
<i>Trifolium pratense</i> L.	17.84	7.24	0.66	0.58
<i>Trifolium repens</i> L.	17.51	7.64	0.70	0.64

Note: GE is gross energy, EE is exchangeable energy, FUM is feed units for milk, FUG is feed units for growth.

tested legume grasses in terms of forage quality. Alfalfa forage had the highest hemicellulose values and that of hybrid clover had the maximum cellulose values.

Low values of indicators of neutral and acid detergent fibre and acid detergent lignin determine the high dry matter digestibility of red clover forage.

The high crude protein content of bird's-foot-trefoil and white clover fodder predetermines the high nutritional value of their fodder.

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