Forage Quality Analysis of Perennial Legumes-Subterranean Clover Mixtures

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ABSTRACT
Analysis of forage quality of alfalfa (*Medicago sativa* L.) and sainfoin (pure swards) and their mixtures with three subterranean clover subspecies at the next ratios was performed: legume + *Trifolium subterraneum* ssp. *brachycalicinum* (50: 50%), legume + *Trifolium subterraneum* ssp. *yaninicum* (50: 50%); legume + *Trifolium subterraneum* subsp. *subterraneum* (50: 50%). It was found that the inclusion of subterranean clover as a component of alfalfa and sainfoin affect the forage quality of mixtures. *Trifolium subterraneum* ssp. *brachycalicinum* and *Trifolium subterraneum* subsp. *yaninicum* contributed to reducing the degree of lignifications. The forage from alfalfa-*Trifolium subterraneum* subsp. *yaninicum* mixture was found to be with the highest protein content (18.97%) and the lowest crude fiber one (25.96%), the highest digestibility (65.94%) and protein feeding value (TDP 154; PDIN 125; PDIE 97 g kg\(^{-1}\) dry matter). Forage biomass from the mixtures of alfalfa had better quality as compared to sainfoin’s ones.

Key words: Alfalfa, sainfoin, crude protein, in vitro digestibility, feeding value

INTRODUCTION
To be ensured both, balanced feeding value and better intake of the forage obtained from perennial swards, the components in mixtures should be properly chosen\(^1\). Interest towards intercropped systems based on legumes is rising in recent years as sources of high quality forage for the intensive animal production. Legumes were preferable for mixtures with hay and grazing direction of use\(^2\). Legume component contributed to improvement of forage quality and animal productivity\(^3,4\).

The development of forage base is agreement with the needs of animals for the relaying of its productive potential but in the future and with the climate changes occurred\(^3,7\). Components adapted to the climate conditions will be included in mixtures\(^8\). At present the main interest in directed towards more drought resistant and drought tolerant plants. Sustainable management of forage production for ruminants will rely on the flexible use of different technological approaches including the introduction of new crops.

Subterranean clover (*Trifolium subterraneum* L.)-widespread component in the pastures and other grasslands of the temperate areas of Central and Northern Europe and America is a new crop for Bulgaria\(^9-12\). The subterranean clover is an annual drought resistant ephemeral legume with winter-spring type of development and ability for self-sowing\(^13,14\). It is strong tolerant to grazing, the forage has high feeding value and good intake from animals as grazing as well as hay and silage\(^15,16\). Subterranean clover’s forage is preferred for the feed rations of lambs and cows\(^17,18\).

Studies with subterranean clover during the last years showed that it has practical applicability under the climatic conditions of Bulgaria\(^19-23\). It is suitable component for mixtures with commonly used for forage production grasses (cocksfoot, tall fescue, wheatgrass) and legumes as well (alfalfa, birdsfoot trefoil, sainfoin)\(^24-26\).

In this study, alfalfa as one of the most important forage crops in Bulgaria and considered the main crop for hay and silage\(^27,28\). and sainfoin as a valuable component of mixtures with hay-pasture direction of use were included\(^29,30\).
The aim of present study was to determine the quality characteristics of the forage, obtained from alfalfa and sainfoin, pure grown and from the forage of their two-component mixtures with subterranean clover (50: 50%).

MATERIALS AND METHODS

Experimental work was done in the Institute of Forage Crops, Pleven, Bulgaria (2011–2013). Alfalfa (cv. “Pleven 6”) and sainfoin (population of the same Institute), pure grown and in mixture with three subterranean clover subspecies, i.e. *Trifolium subterraneum* subsp. *brachycalicinum* (cv. “Antas”), *Trifolium subterraneum* subsp. *yaninicum* (cv. “Trikkala”) and *Trifolium subterraneum* subsp. *subterraneum* (cv. “Denmark”) were studied. In field trial (long plot method, plot size of 70 m² and 4 replications), the next variants were investigated: Alfalfa (100%); Alfalfa+*Trifolium subterraneum* subsp. *brachycalicinum* (50: 50%); Alfalfa+*Trifolium subterraneum* subsp. *yaninicum* (50: 50%); Alfalfa+*Trifolium subterraneum* subsp. *subterraneum* (50: 50%); Sainfoin (100%); Sainfoin+*Trifolium subterraneum* subsp. *brachycalicinum* (50: 50%); Sainfoin+*Trifolium subterraneum* subsp. *yaninicum* (50: 50%); Sainfoin+*Trifolium subterraneum* subsp. *subterraneum* (50: 50%). During the vegetation no fertilizers and pesticides were applied. The swards were harvested in pasture maturity. The data from one cut harvested on June 12, 2012 and two cuts, harvested on May 7 and July 5, 2013 are shown in the present work.

The comparative analysis of the composition and enzyme in vitro digestibility of dry matter in dry forage biomass was performed. Both, the principal chemical composition and digestibility of dry matter were determined in the forage obtained from all cuts and other characteristics-in the forage obtained from two cuts in 2013.

Forage biomass consist the aboveground part of the whole plants. Sample preparation was done through fen drying at 65°C to embitterment at a prefixation for 20 min at 105°C and milling to the size of parts 1.0 mm successively lab mills QC 136 and QB 114, Labor Mim, Hungary, an optional sieving was performed.

The principal chemical composition of the forage was determined by Weende system for crude protein and crude fiber content. The plant cell walls components content was found by systematic detergent analysis of Goering and Van Soest (1970) (EN ISO13906 2008) as a percent of dry matter. The next fiber fractions were determined: Neutral-Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL). The polisoids both hemicellulose and cellulose as components of plant cell walls, containing in the fiber fraction: Hemicellulose = NDF-ADF; Cellulose = ADF-ADL. The degree of lignifications was presented as a coefficient calculated as ADL/NDF.

Enzymatic in vitro digestibility/degradability in vitro of dry (IVDMD%) and organic (IVOMD%) matter was performed by two stage pepsin-cellulase method of Aufrere. Potential energy feeding value was estimated by the French system UFL-UFV on the basis of equations for legumes, according to the experimental values of crude protein and crude fibers and degradability of organic matter. The coefficient of digestibility of organic matter dMO in vivo was determined by Andrieu and Demarquilly, after dependence used in vitro degradability of organic matter, experimentally determined. The potential protein feeding value (PDIN=PDIA+PDIMN and PDIE=PDIA+PDIME) was estimated by the French system by the parameters: TDP/PBD-Total Digestible Protein/Protein Brute Digestible, PDIN-Protein digestible dans l’intestine in dependence of nitrogen and PDIE-Protein digestible dans l’intestine in dependence of energy. Individual and mean values of the characteristics for feeding value of the forage were estimated.

Experimental data was statistically processed by the SPSS (2012) software.

RESULTS AND DISCUSSION

Forage quality is mainly expressed by crude protein, crude fiber content, digestibility and other related characteristics having importance for the animal performance.

In this study, the inclusion of subterranean clover as a component effected the composition of mixtures. Crude protein content in first cut after the year of sowing increased in mixtures of alfalfa (Table 1). On average for the cuts crude protein content increased in mixtures of alfalfa with *Trifolium subterraneum* subsp. *yaninicum* and *Trifolium subterraneum* subsp. *subterraneum*.

For the mixtures of sainfoin crude protein content varied in first cut after the year of sowing and it was found to be lower than pure sainfoin. During the next experimental year crude protein content was lower in mixture with *Trifolium subterraneum* subsp. *yaninicum* and *Trifolium subterraneum* subsp. *subterraneum*, as well on average for the cuts.
Table 1: Chemical composition of the forage from alfalfa and sainfoin, pure grown and in mixture with subterranean clover (dry matter %)

|                | I/2012 | I/2013 | II/2013 | Mean±SD |
|----------------|--------|--------|---------|---------|
| **Crude protein** |        |        |         |         |
| Alfalfa         | 17.01  | 17.92  | 19.91   | 18.28±1.48 |
| Alfalfa + T. subterraneum subsp. brachycalicinum | 17.41  | 16.86  | 20.73   | 18.97±1.80 |
| Alfalfa + T. subterraneum subsp. yaninicum | 17.88  | 19.46  | 19.31   | 18.88±0.87 |
| Average for two components mixtures | 17.48  | 18.45  | 19.99   | 18.64±1.27 |
| SE (p=0.05)     | 0.19   | 0.58   | 0.29    | 0.22    |
| Sainfoin        | 13.57  | 14.28  | 15.45   | 14.43±0.95 |
| Sainfoin + T. subterraneum subsp. brachycalicinum | 12.83  | 14.38  | 15.48   | 14.23±1.33 |
| Sainfoin + T. subterraneum subsp. yaninicum | 12.83  | 12.68  | 15.83   | 13.78±1.25 |
| Sainfoin + T. subterraneum subsp. subterraneum | 12.58  | 14.28  | 15.01   | 13.96±1.25 |
| Average for two components mixtures | 12.75  | 13.78  | 15.44   | 13.99±1.36 |
| SE (p=0.05)     | 0.21   | 0.40   | 0.16    | 0.14    |
| **Crude fiber** |        |        |         |         |
| Alfalfa         | 29.22  | 28.06  | 27.20   | 28.16±1.01 |
| Alfalfa + T. subterraneum subsp. brachycalicinum | 24.18  | 27.02  | 26.00   | 25.73±1.44 |
| Alfalfa + T. subterraneum subsp. yaninicum | 25.46  | 26.02  | 26.41   | 25.96±0.48 |
| Alfalfa + T. subterraneum subsp. subterraneum | 26.87  | 23.48  | 27.60   | 25.98±2.20 |
| Average for two components mixtures | 26.43  | 26.15  | 26.80   | 26.46±0.33 |
| SE (p=0.05)     | 1.07   | 0.98   | 0.36    | 0.57    |
| Sainfoin        | 28.14  | 25.36  | 25.51   | 26.34±1.56 |
| Sainfoin + T. subterraneum subsp. brachycalicinum | 25.50  | 24.18  | 25.52   | 25.07±0.77 |
| Sainfoin + T. subterraneum subsp. yaninicum | 30.21  | 26.15  | 26.93   | 27.76±2.15 |
| Sainfoin + T. subterraneum subsp. subterraneum | 26.99  | 22.87  | 27.55   | 25.80±2.56 |
| Av. for two components mixtures | 27.71  | 24.64  | 26.38   | 26.24±1.54 |
| SE (p=0.05)     | 0.99   | 0.71   | 0.51    | 0.56    |
| SE: Standard error |      |        |         |         |

As a whole in the two-component mixtures of alfalfa higher crude protein content (by 4.65%) was found compared to the same in mixtures of sainfoin.

Regarding the crude fiber content-in all alfalfa mixtures crude fiber content decreased when subterranean clover was included (in a higher degree in the first cut of two experimental years). As a rule crude fiber content during the summer is higher due to temperatures, which stimulated structural carbohydrates accumulation in the plants. On average for all cuts received crude fiber content in the two-component mixtures of alfalfa was lower as compared to pure alfalfa by 1.7%.

For the mixtures of sainfoin crude fiber content showed different values and the tendency for the decrease in the presence of subterranean clover was no permanent. So, in mixture of sainfoin with Trifolium subterraneum subsp. brachycalicinum and Trifolium subterraneum subsp. subterraneum crude fiber content showed lower values as compared to pure alfalfa. There were no differences in crude fiber content for the forage of alfalfa and sainfoin.

Digestibility of dry matter significantly increased in the forage of the first cut for all mixtures (Table 2). Variation is results were observed in the second cut. On average for the three cuts the digestibility increased in mixtures with subterranean clover by 1.92%. Subterranean subspecies differ in digestibility of dry matter in the forage. In our study the highest digestibility was found in the mixtures of alfalfa with Trifolium subterraneum subsp. yaninicum (65.94%).

Results for the digestibility in sainfoin varied from 52.27-65.23% and there were no significant differences for the variants studied.

Subterranean clover affected the digestibility of the forage of alfalfa mixtures and they were more digestible (by 4.02%) as compared to those of sainfoin.

Forage quality of subterranean clover is the highest from the period of initial growth to early summer and decreased with advancing the vegetation. For the two-component mixtures of alfalfa and sainfoin with subterranean clover the highest forage quality characteristics were found in mixtures with alfalfa-protein content (18.64% mean value), crude fiber content (26.46% mean value) and digestibility 63.33%. These quality characteristics of the two-component mixtures of alfalfa with subterranean clover were better as compared to the same in the forage from pure grown alfalfa. The best quality forage from two-component mixtures of alfalfa with subterranean clover was the mixture with T. subterraneum subsp. yaninicum (crude protein 18.97% and digestibility 65.94%).
Content of NDF, ADF and ADL decreased in the mixtures of alfalfa with subterranean clover (Table 3).

Acid-detergent lignin decreased significantly in the mixtures of the first cut. On average for the two cuts ADF content in mixtures with subterranean clover was lower as compared to pure sainfoin by 0.85%. As a whole NDF content in mixtures of alfalfa was lower by 0.63, ADF by 1.23 and ADL by 0.44%, respectively.

Subterranean clover decreased the degree of lignifications of the forage for the mixtures of alfalfa and sainfoin (Fig. 1). In mixtures of alfalfa the degree of lignifications was the lowest (17.72-17.92) when components were *Trifolium subterraneum* subsp. *brachycalicinum* and *Trifolium subterraneum* subsp. *yaninicum* (17.46). Significantly lower degree of lignifications showed two-component mixtures of alfalfa with subterranean clover.

The polisoides both hemicellulose and cellulose were determined. Their content decreased in all mixtures tested (Fig. 2). Hemicellulose content in mixture of alfalfa was higher (on average by 0.61) and cellulose content lower (on average by 0.80%).

Energy as well protein feeding value of the forage from pure grown and mixed swards was estimated. It is known with advancing the vegetation nutritive value of the forage is going to change. Individual plant parts of subterranean clover as a component have different digestibility. The leaves are more digestible as compared to stems and have higher nitrogen content.
Fig. 2: Hemicelluloses and cellulose content in forage of alfalfa and sainfoin, pure grown and in mixture with subterranean clover (% dry matter)

Table 3: Composition and digestibility of alfalfa and sainfoin, pure and in mixture with subterranean clover (% dry matter)

| Crop/mixtures                     | NDF          | ADF          | ADL          |
|-----------------------------------|--------------|--------------|--------------|
|                                   | I/2013       | II/2013 Mean±SD | I/2013       | II/2013 Mean±SD | I/2013       | II/2013 Mean±SD |
| Alfalfa                           | 42.49        | 44.08        | 43.28±1.12   | 36.61         | 35.73         | 36.17±0.62     | 8.06          | 7.87          | 7.96±0.13     |
| Alfalfa + *T. subterraneum* subsp. brachycalicinum | 40.75        | 45.01        | 42.88±3.01   | 34.88         | 33.70         | 34.29±1.99     | 6.93          | 8.27          | 7.60±0.95     |
| Alfalfa + *T. subterraneum* subsp. yaninicum | 37.83        | 43.65        | 40.74±4.12   | 33.66         | 33.99         | 33.82±0.23     | 7.15          | 7.44          | 7.30±0.21     |
| Average for two components mixtures | 39.61        | 44.74        | 42.17±3.63   | 34.28         | 36.39         | 35.34±1.49     | 7.17          | 8.03          | 7.60±0.61     |
| SE (p = 0.05)                     | 1.22         | 0.56         | 0.37         | 0.97          | 0.95          | 0.57            | 0.32          | 0.24          | 0.13          |
| Sainfoin                          | 37.80        | 46.64        | 42.22±6.25   | 34.19         | 38.62         | 36.40±3.13     | 8.58          | 9.20          | 8.89±0.44     |
| Sainfoin + *T. subterraneum* subsp. brachycalicinum | 37.56        | 45.64        | 41.60±5.71   | 33.39         | 36.83         | 35.11±2.43     | 5.89          | 8.52          | 7.20±1.86     |
| Sainfoin + *T. subterraneum* subsp. yaninicum | 42.00        | 46.68        | 44.34±3.31   | 35.43         | 39.02         | 37.22±2.54     | 5.81          | 9.68          | 7.74±2.74     |
| Sainfoin + *T. subterraneum* subsp. subterraneum | 35.69        | 50.17        | 43.03±10.24  | 32.06         | 43.02         | 37.54±7.75     | 5.21          | 11.42         | 8.32±4.39     |
| Average for two components mixtures | 38.26        | 47.28        | 42.80±6.38   | 35.77         | 39.37         | 36.57±3.96     | 6.37          | 9.71          | 8.04±2.36     |
| SE (p = 0.05)                     | 1.33         | 0.99         | 0.59         | 0.70          | 1.30          | 0.54            | 0.75          | 0.61          | 0.36          |
| SE: Standard error, NDF: Natural detergent fiber, ADF: Acid detergent fiber, ADL: Aid detergent lignin |

Table 4: Energy feeding value of forage of alfalfa and sainfoin, pure grown and in mixture with subterranean clover

| Crop/mixtures | UFL          | UFV          |
|---------------|--------------|--------------|
|               | I/2013       | II/2013 Mean±SD | I/2013       | II/2013 Mean±SD |
| Alfalfa       | 0.684        | 0.703        | 0.694±0.01   | 0.572         | 0.592         | 0.582±0.01     |
| Alfalfa + *T. subterraneum* subsp. brachycalicinum | 0.776        | 0.668        | 0.722±0.08   | 0.677         | 0.552         | 0.600±0.09     |
| Alfalfa + *T. subterraneum* subsp. yaninicum | 0.725        | 0.713        | 0.719±0.01   | 0.618         | 0.602         | 0.610±0.01     |
| Alfalfa + *T. subterraneum* subsp. subterraneum | 0.750        | 0.662        | 0.706±0.06   | 0.645         | 0.546         | 0.596±0.07     |
| Av. for two components mixtures | 0.734        | 0.687        | 0.710±0.03   | 0.628         | 0.573         | 0.597±0.04     |
| SE (p = 0.05) | 0.002        | 0.001        | 0.006        | 0.002         | 0.001         | 0.005          |
| Sainfoin      | 0.715        | 0.653        | 0.684±0.04   | 0.610         | 0.539         | 0.574±0.05     |
| Sainfoin + *T. subterraneum* subsp. brachycalicinum | 0.750        | 0.641        | 0.696±0.08   | 0.649         | 0.526         | 0.588±0.09     |
| Sainfoin + *T. subterraneum* subsp. yaninicum | 0.733        | 0.641        | 0.687±0.07   | 0.632         | 0.526         | 0.579±0.07     |
| Sainfoin + *T. subterraneum* subsp. subterraneum | 0.796        | 0.626        | 0.711±0.12   | 0.703         | 0.509         | 0.606±0.14     |
| Av. for two components mixtures | 0.749        | 0.640        | 0.695±0.08   | 0.649         | 0.525         | 0.587±0.09     |
| SE (p = 0.05) | 0.001        | 0.005        | 0.006        | 0.001         | 0.006         | 0.007          |
| SE: Standard error |

Depending on morphological traits, subterranean subspecies formed different leaf biomass. Energy feeding value was found higher in mixtures of alfalfa with subterranean clover. In mixtures with *Trifolium subterraneum* subsp. brachycalicinum (UFL-UFV 0.722-0.600) and with *Trifolium subterraneum* subsp. yaninicum (UFL-UFV 0.719-0.610 (Table 4).
Table 5: Protein feeding value of forage of alfalfa and sainfoin, pure grown and in mixture with subterranean clover

| Crop/mixtures | TDP/PBD | PDIN | PDIE | TDP/PBD | PDIN | PDIE | TDP/PBD | PDIN | PDIE |
|---------------|---------|------|------|---------|------|------|---------|------|------|
| Dry matter (g kg$^{-1}$) | I/2013 | II/2013 | Mean±SD | I/2013 | II/2013 | Mean±SD | I/2013 | II/2013 | Mean±SD |
| Alfalfa | 136 | 154.0 | 145±12.7 | 112 | 125.0 | 118±9.2 | 91 | 95.0 | 93±2.8 |
| Alfalfa + T. subterraneum subsp. brachycalicinum | 124 | 155.0 | 140±21.9 | 106 | 125.0 | 116±13.4 | 93 | 94.0 | 94±0.7 |
| Alfalfa + T. subterraneum subsp. yanicum | 146 | 162.0 | 154±11.3 | 120 | 130.0 | 125±7.1 | 96 | 98.0 | 97±1.4 |
| Alfalfa + T. subterraneum subsp. subterraneum | 151 | 149.0 | 150±1.4 | 122 | 121.0 | 122±0.7 | 98 | 92.0 | 95±4.2 |
| Av. for two components mixtures | 139 | 155.0 | 147±11.3 | 115 | 125.0 | 120±7.1 | 95 | 95.0 | 95 |
| SE (p = 0.05) | 5.9 | 2.6 | 3.0 | 3.6 | 1.8 | 2.0 | 1.5 | 1.2 | 0.8 |
| Sainfoin | 100 | 111.0 | 106±7.8 | 90 | 97.0 | 94±4.9 | 83 | 82.0 | 83±0.7 |
| Sainfoin + T. subterraneum subsp. brachycalicinum | 101 | 112.0 | 107±7.8 | 90 | 97.0 | 94±4.9 | 87 | 82.0 | 85±3.5 |
| Sainfoin + T. subterraneum subsp. yanicum | 85 | 115.0 | 100±21.2 | 80 | 99.0 | 90±13.4 | 83 | 82.0 | 83±0.7 |
| Sainfoin + T. subterraneum subsp. subterraneum | 101 | 107.0 | 104±4.2 | 90 | 94.0 | 92±2.8 | 91 | 80.0 | 85±7.7 |
| Av. for two components mixtures | 97 | 111.0 | 104±9.9 | 88 | 97.0 | 93±6.4 | 86 | 82.0 | 84±2.8 |
| SE (p = 0.05) | 3.9 | 1.6 | 1.5 | 2.5 | 1.0 | 1.0 | 1.9 | 0.5 | 0.6 |

Mixtures of alfalfa with *Trifolium subterraneum* subsp. *yanicum* and with *Trifolium subterraneum* subsp. *subterraneum* showed the highest protein feeding value by parameters as total digestible protein, PDIN and PDIE. For the mixtures of alfalfa with *Trifolium subterraneum* subsp. *yanicum* total digestible protein content was 154, PDIN 125 and PDIE 97 g kg$^{-1}$ dry matter (Table 5).

For the mixtures of alfalfa with *Trifolium subterraneum* subsp. *subterraneum* total digestible protein content were 150, PDIN 122 g kg$^{-1}$ and PDIE 95 g kg$^{-1}$ dry matter.

Mixtures of sainfoin with subterranean clover were not affected by subterranean clover in terms of protein feeding value characteristics.

**CONCLUSION**

The inclusion of subterranean clover as a component of alfalfa and sainfoin affect the forage quality. Mixtures of alfalfa with subterranean clover showed higher crude protein content, they were more digestible, had lower fiber content as NDF, ADF and ADL, lower degree of lignifications, as well and higher energy and protein feeding value.

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