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# NUTRITIVE VALUE OF FORAGE BIOMASS FROM MIXTURES OF ALFALFA WITH COCKSFOOT AND TALL FESCUE

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Forage quality characteristics of mixtures of alfalfa with cocksfoot (50:50%), alfalfa with tall fescue (50:50%), and the same with the addition of subterranean clover in their composition (33:33:33%) were measured. Alfalfa (cv. Pleven 6), cocksfoot (cv. Dabrava), tall fescue (cv. Albena), three subterranean clover subspecies, i.e. *Trifolium subterraneum ssp. brachycalicinum* (cv. Antas), *Trifolium subterraneum ssp. yaninicum* (cv. Trikkala) and *Trifolium subterraneum ssp. subterraneum* (cv. Denmark) were used. Forage biomass from mixtures of alfalfa with cocksfoot had generally higher forage quality than mixtures of alfalfa with tall fescue: higher crude protein (16.34% DM) and lower crude fiber contents (26.95% DM), higher, both, digestibility (62.41%) and protein feeding value (TDP-PDIN-PDIE, 134-114-93 g/kg DM). After the addition of subterranean clover in the composition of mixtures, forage biomass showed more balanced basic chemical composition: higher crude protein (with 0.32%) and lower crude fiber contents (with 0.63%) for cocksfoot mixtures; higher digestibility (with 1.67% for cocksfoot mixtures and with 3.24 for tall fescue mixtures); lower NDF, ADF and ADL (with 0.91, 2.23 and 1.16%) for tall fescue mixtures; lower degree of lignifications (with 0.84 for cocksfoot mixtures, and with 2.19 for tall fescue mixtures) and higher energy feeding value (UFL-UFV) (with 0.005-0.005) for tall fescue mixtures.
**Keywords:** Alfalfa, cocksfoot, tall fescue, subclover, *in vitro* digestibility, nutritive value.

## INTRODUCTION

The benefits of cultivation of legume and grasses in mixtures compared to monocultures from an agronomic, economic and environmental point of view are well known. One of them is the potential for obtaining a forage with balanced chemical composition, high energy and protein nutritive value for better absorption by animals (Luscher *et al.,* 2014). The forage quality depends on many factors, including the choice of suitable components (Pavlov, 1996; Arzani *et al*., 2001).
Alfalfa, commonly called "queen of feed", is a universal forage crops suitable for feeding animals as green forage or hay, silage and dehydrate preparation. It produces more protein per unit area compared to other legumes, has a high nutritive value and is an excellent forage for ruminants and horses, but have been also included in the rations of poultry, pig, rabbit (Kertikova, 2008; Baumont *et al.*, 2014). Alfalfa is grown in pure in mixtures with grasses (Berdahl *et al.*, 2001; Vuckovic, 2007). Alfalfa-cocksfoot mixtures are widely used in the practice, the forage biomass of which is of high quality (Posler *et al.*, 1993; Sleugh *et al.*, 2000; Vassilev, 2003/2004; 2004; Bijelić *et al.,* 2013). Cocksfoot is a competitive component in mixtures with alfalfa (Casler *et al.*, 1990). Tall fescue is a suitable grass component as well (Chamblee and Colins, 1988; Casler and Walgenbach, 1990; Nešić *et al.,* 2005). This crop has good growth capacity in mixtures with alfalfa (Hannaway *et al.,* 1999), but it is a less competitive compared to the cocksfoot. There are a plenty of data for the varying the quality of forage biomass from tall fescue – crude protein, crude fiber content, fiber components, *in vitro* digestibility of the dry matter and the correlation between them, and that the quality of the forage is not very high (Bughrara *et al*, 1991; de Santis *et al.,* 1997).
With a higher proportion of legume components in mixtures, mainly because of the crude protein content, the quality of the forage improves (Sleugh *et al.,* 2000; Samuil *et al.,* 2012). In view of this, it would be interesting to follow the indicators related to the basic chemical composition of forage biomass from mixtures of alfalfa with cocksfoot and with tall fescue after addition of a second legume component in their composition.
Subterranean clover (*Trifolium subterraneum* L.) is a legume crops relatively new to Bulgaria. It is strongly tolerant to grazing due to the prostrate habit (Evers and Newman, 2008; Ovalle *et al.,* 2008), the forage has high feeding value and good intake by animals when grazed, as well when fed as hay and silage (Ru and Fortune, 2001; Frame, 2005). Studies with subterranean clover during the last years showed that it has practical applicability under the climatic conditions of the country and is a suitable component for mixtures with widely used forage crops (Vasilev, 2006, 2009; Vasileva *et al.,* 2016). We received data for higher crude protein and lower crude fiber contents in the forage biomass from mixtures of subterranean clover with cocksfoot (Naydenova and Vasileva, 2016) and for more balanced basic chemical composition, higher digestibility, higher, both, net energy and protein feeding value of forage biomass from mixtures of subterranean clover with tall fescue (Naydenova and

Vasileva, 2016).
The aim of present work was to determine the quality characteristics, i.e. chemical composition, digestibility, energy and protein nutritive value of forage biomass from mixtures of alfalfa and cocksfoot (50:50%), alfalfa and tall fescue (50:50%), and the same with subterranean clover component in their composition (33:33:33%).

## MATERIALS AND METHODS

Experimental work was done in the Institute of Forage Crops, Pleven, Bulgaria (2011-2013). Alfalfa (*Medicago sativa* L.) (cv. Pleven 6), cocksfoot (*Dactylis glomerata* L.) (cv. Dabrava), tall fescue (*Festuca arundinacea* Schreb.) (cv. Albena), three subterranean clover subspecies, i.e. *Trifolium subterraneum ssp. brachycalicinum* (cv. "Antas"), *Trifolium subterraneum ssp. yaninicum* (cv. "Trikkala") and *Trifolium subterraneum ssp. subterraneum* (cv. "Denmark") were used. Field trial was performed with plot size of 70 $m^2$. The following forage treatments were investigated 4 times replicated: 1) mixtures of alfalfa with cocksfoot: alfalfa + cocksfoot (50:50%); alfalfa + cocksfoot + *Trifolium subterraneum ssp. brachycalicinum* (33:33:33%); alfalfa + cocksfoot + *Trifolium subterraneum ssp. yaninicum* (33:33:33%); alfalfa + cocksfoot +*Trifolium subterraneum ssp. subterraneum* (33:33:33%) and 2) mixtures of alfalfa with tall fescue: alfalfa + tall fescue (50:50%); alfalfa + tall fescue + *Trifolium subterraneum ssp. brachycalicinum* (33:33:33%); alfalfa + tall fescue + *Trifolium subterraneum ssp. yaninicum* (33:33:33%); alfalfa + tall fescue +*Trifolium subterraneum ssp. subterraneum* (33:33:33%). During the vegetative period neither fertilizers nor pesticides were applied. The swards were harvested when the plants reached 20 cm height.
The comparative analysis of the composition and *in vitro* digestibility of dry matter in dry forage biomass was performed. Both, crude protein and crude fiber contents 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 consisted of the aboveground part of the whole plants. Sample preparation was done through oven drying at 65ºC for 20 min at 105ºC and milling to particle size of 1.0 mm, successively lab mills QC 136 and QB 114, Labor Mim, Hungary, an optional sieving was performed.
Crude protein and crude fiber contents of the forage biomass were determined by Weende system (AOAC, 2007). The plant cell wall components were determined using the systematic detergent analysis proposed by Goering and Van Soest (1970) (AOAC, 2007) (EN ISO13906 2008) as a percent of dry matter. The following fiber fractions were determined: Neutral-detergent fiber (NDF), Acid-detergent fiber (ADF) and Acid-detergent lignin (ADL). Both, hemicellulose and cellulose as components of plant cell wall were calculated as follows: Hemicellulose = NDF - ADF; Cellulose = ADF - ADL. The degree of lignifications was presented as a coefficient calculated as ADL/NDFx100 (Akin and Chesson, 1990).
Enzymatic *in vitro* digestibility/degradability *in vitro* of dry (IVDMD, %) and organic (IVOMD, %) matter were determined by two stage pepsin-cellulase method of Aufrere (1982) (Todorov et al., 2010).
Potential energy feeding value was estimated by the French system UFL-UFV (INRA, 1988), on the basis of equations for legumes, according to the experimental values of crude protein and crude fibers (AOAC, 2001), and degradability of organic matter according to Aufrere (1982) (Todorov, 2010). The coefficient of digestibility of organic matter dMO *in vivo* was determined by Andrieu and Demarquilly (1989), 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 (INRA, 1988) 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. Data of one cut harvested on June 12, 2012 and two cuts, harvested on May 7 and July 5, 2013 was presented and discussed.

## RESULTS AND DISCUSSION

Forage quality, expressed mainly with crude protein, crude fiber contents, digestibility and other associated characteristics is essential for animal productivity. Quality parameters vary for legumes and grasses. Thus, the components in grass mixtures influence the quality of the forage obtained (Whitehead, 1995; Hakl *et al.*, 2016). Table 1 presents the data on crude protein and crude fiber contents in forage biomass of studied mixtures.
The lowest crude protein content was found in the first experimental year due to the faster development of grasses compared to legume components. Crude protein content in forgae biomass of alfalfa-cocksfoot mixtures was 12.99% DM. Sleugh *et al*. (2000) received a higher crude protein content (14.1% DM) in the first and second year (21.3% DM) (vs. 20.18% DM in our study). In the forage biomass of all mixtures of alfalfa with cocksfoot and subterranean clover, crude protein content during the first year was higher (with more than 4%), varying in narrow limits (from 16.34 to 16.37% DM) and there was no proven difference for the three sub-clover subspecies. On average, from the mixtures with cocksfoot crude protein content was 15.52% DM, and from the mixtures with tall fescue - 12.29% DM.
In the second experimental year crude protein content from the mixtures with cocksfoot was found 1.45% higher than that of tall fescue mixtures. In terms of the influence of sub-clover as a legume component in mixtures with cocksfoot, crude

protein content was found to increase (on average by 0.32%). There was no positive impact of sub-clover on the values of this characteristics for mixtures with tall fescue, except for *Trifolium subterraneum ssp. yaninicum*.

Forage biomass from mixtures of alfalfa, cocksfoot and *Trifolium subterraneum ssp. subterraneum* showed the highest crude protein content (17.02% DM) - higher than that of mixtures of alfalfa with cocksfoot by 0.68%.

**Table 1: Main chemical composition of the forage biomass of alfalfa mixtures (%DM).**

| Mixtures | I cut, 2012 | I cut, 2013 | II cut, 2013 | Mean |
|---|---|---|---|---|
| | **Crude protein** | | | |
| Alfalfa+cocksfoot | 12.99 | 20.18 | 15.84 | 16.34 |
| Alfalfa+cocksfoot+*Trs brach* | 16.34 | 14.26 | 19.06 | 16.55 |
| Alfalfa+cocksfoot+*Trs yanin* | 16.39 | 15.75 | 17.10 | 16.41 |
| Alfalfa+cocksfoot+*Trs subter* | 16.37 | 17.11 | 17.59 | 17.02 |
| Mean-3-components mixtures | 16.37 | 15.71 | 17.92 | 16.66 |
| Mean for all mixtures | 15.52 | 16.83 | 17.40 | 16.58 |
| SE (P=0.05) | 0.84 | 1.26 | 0.66 | 0.15 |
| Alfalfa+tall fescue | 11.96 | 16.09 | 18.24 | 15.43 |
| Alfalfa+tall fescue+*Trs brach* | 11.25 | 14.77 | 15.17 | 13.73 |
| Alfalfa+tall fescue+*Trs yanin* | 15.07 | 18.54 | 16.46 | 16.69 |
| Alfalfa+tall fescue+*Trs subter* | 10.86 | 17.94 | 15.15 | 14.65 |
| Mean-3-components mixtures | 12.39 | 17.08 | 15.59 | 15.02 |
| Mean for all mixtures | 12.29 | 16.84 | 16.26 | 15.13 |
| SE (P=0.05) | 0.95 | 0.86 | 0.72 | 0.62 |
| | Crude fiber | | | |
| Alfalfa+cocksfoot | 30.66 | 21.53 | 28.66 | 26.95 |
| Alfalfa+cocksfoot+*Trs brach* | 25.23 | 25.14 | 26.83 | 25.73 |
| Alfalfa+cocksfoot+*Trs yanin* | 26.27 | 26.40 | 28.21 | 26.96 |
| Alfalfa+cocksfoot+*Trs subter* | 26.60 | 25.01 | 27.15 | 26.25 |
| Mean-3-components mixtures | 26.03 | 25.52 | 27.40 | 26.32 |
| Mean for all mixtures | 27.19 | 24.52 | 27.71 | 26.47 |
| SE (P=0.05) | 1.19 | 1.04 | 0.43 | 0.29 |
| Alfalfa+tall fescue | 30.14 | 26.23 | 25.66 | 27.34 |
| Alfalfa+tall fescue+*Trs brach* | 27.75 | 23.79 | 27.35 | 26.30 |
| Alfalfa+tall fescue+*Trs yanin* | 28.23 | 22.90 | 28.35 | 26.49 |
| Alfalfa+tall fescue+*Trs subter* | 26.96 | 21.39 | 28.42 | 25.59 |
| Mean-3-components mixtures | 27.65 | 22.69 | 28.04 | 26.13 |
| Mean for all mixtures | 28.27 | 23.58 | 27.45 | 26.43 |
| SE (P=0.05) | 0.67 | 1.01 | 0.64 | 0.35 |

(*Trs brach - Trifolium subterraneum ssp. brachycalicinum; Trs yanin - Trifolium subterraneum ssp. yaninicum*; *Trs subter - Trifolium subterraneum ssp. subterraneum*)

Crude fiber content is considered as a major indicator from the chemical composition when determining the energy feeding value of the forage (Krachunov, 2007). It is related to the quality and digestibility of the forage and the use by animals. A high crude fiber content is an indicator of low digestibility and energy feeding value of the forage. As a rule crude fiber content during the summer is higher due to temperatures, which stimulated structural carbohydrates accumulation in the plants (Wilson *et al.,* 1991; Stockdale, 1992; Mulholland *et al.*, 1996). Higher protein and lower fiber contents are prerequisite for higher digestibility of the whole plant (Frame, 2005). As the crude protein content increases, the crude fiber content decreases (Pavlov, 1996).

The highest crude fiber content was found in forage biomass from mixtures of alfalfa with cocksfoot during the first year (30.66% DM). Bijelić *et al*. (2013) reported similar results (31.03% DM).

Crude fiber content during the first year in our study was found to decrease in two types of mixtures when subclover was included as a component. On average from the mixtures higher crude fiber content was recorded for these with tall fescue (28.27 vs. 27.19% DM). The data showed lower crude fiber content when a second legume component was included in the composition of mixtures - for those with cocksfoot by 0.63%, and with tall fescue, less, by 0.19%. Crude fiber content on average for both types of mixtures was close in value (26.47 and 26.43% DM).

Forage biomass from mixtures of alfalfa with cocksfoot has higher crude protein and lower crude fiber contents compared to mixtures with tall fescue, by 0.91% and by 0.39%, respectively. Forage biomass showed more balanced basic chemical composition after the addition of second legume component -crude protein in mixtures with cocksfoot was found to increase (with 0.32%) and crude fiber contents to decrease (with 0.63%). On average, crude protein content from mixtures with cocksfoot was 1.45% higher as compared to that with tall fescue.

According to the NRC (2001) the daily needs of small and large ruminants (sheep and cattle) for the specific content of crude protein in forage varied from 9.1 to 15.0% DM for sheep and from 7.4 to 16.6% DM for cattle. Comparing the results of analysis of crude protein in forage biomass with data from the NRC (2001), we recognize a very good quality of forage of studied mixtures that can fully meet the daily requirements of sheep and cattle.

The digestibility of dry matter is another important characteristic of the forage quality. It is a key indicator of the nutritive value of forages, a prerequisite for their energy and protein nutrition, and an indicator where the forages are compared to each other. According to Baumont *et al*. (2014) with the advancing the phase of development of alfalfa the digestibility of forage decreased. Reducing the forage quality (expressed as crude protein and crude fiber content) as the age of alfalfa and cut increased was found by Popovic *et al.* (2001). Santis *et al.* (1997) reported similar results for the quality of forage from tall fescue.

Subterranean subspecies differ in digestibility of dry matter in the forage (McLaren and Doyle, 1994; Ru and Fortune, 1999, 2000) and this had an impact on the digestibility of forage biomass from mixtures in which they prticipate as a component. Forage quality of subterranean clover is the highest from the period of initial growth to early summer and decreased with advancing the vegetation http://msucares.com/crops/forages/legumes/cool/subterraneanclover.html). Lilley *et al*. (2001) found a decreasing of *in vitro* digestibility with the advancing the age of subterranean clover.

The data on the digestibility of dry matter in the forage biomass in mixtures studied are presented in Table 2. In the first cut, the digestibility of the forage biomass from mixtures of alfalfa with cocksfoot was 59.78% and is close to the results obtained by Sleugh *et al*. (2000). When sub-clover was added in the composition of mixtures as a component, the digestibility increased by 3.37% for *Trifolium subterraneum ssp. brachycalicinum*, by 7.88% for *Trifolium subterraneum ssp. yaninicum* and by 11.09% for *Trifolium subterraneum ssp. subterraneum*.

In both experimental years the digestibility values of the forage biomass in the first cut were close (65.37, 67.23%).

**Table 2: Digestibility of dry matter of the forage biomass of alfalfa mixtures (%).**

| Mixtures | I cut, 2012 | I cut, 2013 | II cut, 2013 | Mean |
|---|---|---|---|---|
| Alfalfa+cocksfoot | 59.78 | 70.96 | 56.49 | 62.41 |
| Alfalfa+cocksfoot+*Trs brach* | 63.15 | 65.37 | 58.42 | 62.31 |
| Alfalfa+cocksfoot+*Trs yanin* | 70.87 | 66.97 | 58.21 | 65.35 |
| Alfalfa+cocksfoot+*Trs subter* | 67.66 | 65.60 | 60.51 | 64.59 |
| Mean-3-components mixtures | 67.23 | 65.98 | 59.05 | 64.08 |
| Mean for all mixtures | 65.37 | 67.23 | 58.41 | 63.67 |
| SE (P=0.05) | 2.44 | 1.29 | 0.82 | 0.76 |
| | | | | |
| Alfalfa+tall fescue | 52.46 | 63.21 | 63.47 | 59.71 |
| Alfalfa+tall fescue+*Trs brach* | 60.64 | 65.89 | 58.34 | 61.62 |
| Alfalfa+tall fescue+*Trs yanin* | 65.51 | 68.47 | 60.49 | 64.82 |
| Alfalfa+tall fescue+*Trs subter* | 57.47 | 69.49 | 60.22 | 62.39 |
| Mean-3-components mixtures | 61.21 | 67.95 | 59.68 | 62.95 |
| Mean for all mixtures | 59.02 | 66.77 | 60.63 | 62.14 |
| SE (P=0.05) | 2.74 | 1.40 | 1.06 | 1.05 |

The legend as in Table 1

The digestibility of forage biomass in the second cut in the second year decreased as Baumont *et al*. (2014) as well found, and was lower comparing to the first cut by 14.47% for the mixtures of alfalfa with cocksfoot and less, from 5.09 to 8.76% (on average by 6.93%) for the mixtures with added sub-clover component (i.e. two times less).

The data for digestibility of forage biomass from mixtures of alfalfa with cocksfoot coincide with the results obtained by Sleugh *et al.* (2000). On average, the digestibility of forage biomass from mixtures of alfalfa and cocksfoot with subclover component (64.08%) was found higher by 1.67% compared to mixtures of alfalfa with cocksfoot (62.41%). Forage biomass from mixtures of alfalfa with cocksfoot and *Trifolium subterraneum ssp. subterraneum* had the highest crude protein (17.02% DM), the lowest crude fiber contents (25.73% DM) and was the most digestible (64.59%).

Mixtures of alfalfa with cocksfoot had higher digestibility of forage (62.41%) compared to mixtures of alfalfa with tall fescue (59.71%). With a second legume component included, the digestibility of forage biomass increased by 1.67% for mixtures with cocksfoot and by 3.24% for mixtures with tall fescue. On average from all mixtures studied the digestibility of forage from cocksfoot mixtures was found 1.53% higher as compared to tall fescue mixtures and by 1.14% with a second legume component in their composition. The data obtained are related to the competition of tall fescue in mixtures with alfalfa (Hannaway *et al.*, 1999).

Structural pollysoides of forage plants make up 30-80% of the dry matter of the forage and are the main source of energy for ruminants, with less than 50% of them being digested and utilized (Fahey and Hussein, 1999). The nutritive value of forage is mainly the result of the chemical composition and, in particular, of the crude protein content and the fiber fractions (neutral detergent fibers, acid detergent fibers, acid detergent lignin) (Scotti and Julier, 2014). In summer, the content of NDF, ADF and lignin increases faster in legumes than in grasses (Elgersma and Soegaard, 2017). The content of NDF in our study (Table 3) in the forage biomass from mixtures of alfalfa with cocksfoot (46.33%) was lower than that obtained from Sleugh *et al*. (2000) (42.40%). NDF content in forage biomass from mixtures of cocksfoot with subclover component increased by 2.20% on average, and tended to decrease in mixtures of tall fescue. Comparing two types of mixtures, higher NDF content was found in cocksfoot mixtures (by 1.07%).

According to Sleugh *et al*. (2000) the content of NDF in forage biomass from mixtures of alfalfa with cocksfoot was lower than that of mixtures of birdsfoot trefoil with cocksfoot. The present study confirms the conclusion of the authors - NDF content in the forage biomass of alfalfa mixtures with cocksfoot was 46.33% and we received a higher value for mixtures of birdsfoot trefoil and cocksfoot (53.88%) in another study (Vasileva and Naidenova, 2017).

The content of ADF decreased on average by 2.23% for mixtures of alfalfa with tall fescue and subclover as a component. In all mixtures tested ADF content decreased when subclover was added, lower, with 0.29% for cocksfoot mixtures, and more, with 1.16% for tall fescue ones. By comparing the two types of mixtures ADF content was lower with 1.01% in tall fescue mixtures compared to cocksfoot mixtures.

We found a negative correlation between the digestibility of forage biomass and the content of fiber fractions ADF ($r = -0.7766$) for tall fescue mixtures, which is in agreement with other authors (Scotti and Julier, 2014; Adamovic *et al.*, 2017). The lower values of NDF, 46.69% (by 1.84%), ADF, 33.40% (by 3.16%) and ADL 4.75% (by 1.23%) in the forage biomass from mixtures of alfalfa with tall fescue and subclover are related to low NDF, ADF and ADL contents in forage biomass from pure grown subclover (Naydenova and Vasileva, 2015).

**Table 3: Composition and digestibility of the forage biomass of alfalfa mixtures (%).**

| Mixtures | I cut, 2013 | II cut, 2013 | Mean |
|---|---|---|---|
| | | NDF | |
| Alfalfa+cocksfoot | 39.14 | 53.52 | 46.33 |
| Alfalfa+cocksfoot+*Trs brach* | 46.69 | 49.67 | 48.18 |
| Alfalfa+cocksfoot+*Trs yanin* | 44.04 | 53.02 | 48.53 |
| Alfalfa+cocksfoot+*Trs subter* | 45.15 | 52.63 | 48.89 |
| Mean-3-components mixtures | 45.29 | 51.77 | 48.53 |
| Mean for all mixtures | 43.76 | 52.21 | 47.98 |
| SE (P=0.05) | 1.63 | 0.86 | 0.56 |
| Alfalfa+tall fescue | 48.65 | 46.54 | 47.60 |
| Alfalfa+tall fescue+*Trs brach* | 45.88 | 52.39 | 49.14 |
| Alfalfa+tall fescue+*Trs yanin* | 39.45 | 51.32 | 45.39 |
| Alfalfa+tall fescue+*Trs subter* | 40.66 | 50.41 | 45.54 |
| Mean-3-components mixtures | 51.37 | 46.69 | 46.69 |
| Mean for all mixtures | 43.66 | 50.17 | 46.91 |
| SE (P=0.05) | 2.17 | 1.27 | 0.89 |
| | | ADF | |
| Alfalfa+cocksfoot | 29.93 | 39.65 | 34.79 |
| Alfalfa+cocksfoot+*Trs brach* | 35.24 | 37.39 | 36.32 |
| Alfalfa+cocksfoot+*Trs yanin* | 36.15 | 37.48 | 36.82 |
| Alfalfa+cocksfoot+*Trs subter* | 35.39 | 37.73 | 36.56 |
| Mean-3-components mixtures | 37.53 | 36.56 | 36.56 |
| Mean for all mixtures | 34.18 | 38.06 | 36.12 |
| SE (P=0.05) | 1.42 | 0.53 | 0.45 |
| Alfalfa+tall fescue | 37.70 | 33.56 | 35.63 |
| Alfalfa+tall fescue+*Trs brach* | 34.03 | 37.22 | 35.63 |
| Alfalfa+tall fescue+*Trs yanin* | 30.32 | 34.52 | 32.42 |
| Alfalfa+tall fescue+*Trs subter* | 29.72 | 34.61 | 32.17 |
| Mean-3-components mixtures | 35.45 | 33.40 | 33.40 |
| Mean for all mixtures | 32.94 | 34.98 | 33.96 |
| SE (P=0.05) | 1.85 | 0.78 | 0.96 |
| | | ADL | |
| Alfalfa+cocksfoot | 4.07 | 8.47 | 6.27 |
| Alfalfa+cocksfoot+*Trs brach* | 4.17 | 8.19 | 6.18 |
| Alfalfa+cocksfoot+*Trs yanin* | 5.67 | 6.58 | 6.13 |
| Alfalfa+cocksfoot+*Trs subter* | 4.89 | 6.37 | 5.63 |
| Mean-3-components mixtures | 7.05 | 5.98 | 5.98 |
| Mean for all mixtures | 4.70 | 7.40 | 6.05 |
| SE (P=0.05) | 0.37 | 0.54 | 0.14 |
| Alfalfa+tall fescue | 6.25 | 5.57 | 5.91 |
| Alfalfa+tall fescue+*Trs brach* | 4.34 | 5.72 | 5.03 |
| Alfalfa+tall fescue+*Trs yanin* | 4.86 | 4.76 | 4.81 |
| Alfalfa+tall fescue+*Trs subter* | 3.97 | 4.84 | 4.41 |
| Mean-3-components mixtures | 5.11 | 4.75 | 4.75 |
| Mean for all mixtures | 4.86 | 5.22 | 5.04 |
| SE (P=0.05) | 0.49 | 0.24 | 0.31 |

The degree of lignification for both, cocksfoot and tall fescue mixtures was lower in all variants with subclover component, for cocksfoot mixtures from 0.40 to 1.64 and for tall fescue mixtures, from 1.61 to 2.73. For both types of mixtures, the lowest degree of lignification showed the forage biomass with *Trifolium subterraneum ssp. subterraneum*, respectively for mixtures with cocksfoot – coefficient 11.47 and with tall fescue – coefficient 9.68 (Figure 1).

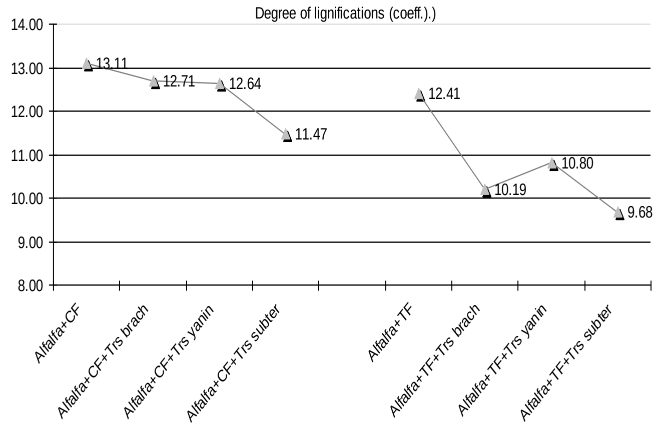


**Figure 1: Degree of lignifications of forage biomass of alfalfa + cocksfoot + subterranean clover [(SE (P = 0.05) 0.35] and alfalfa + tall fescue + subterranean clover mixtures (coeff.), [(SE (P = 0.05) 0.59].**

Pollysoides chemicellulose and cellulose were determined (Fig. 2).

In all mixtures with subclover as a component, the hemicellulose content was found higher - for mixtures with cocksfoot, on average by 0.43%, and for mixtures with tall fescue, on average by 1.31%.

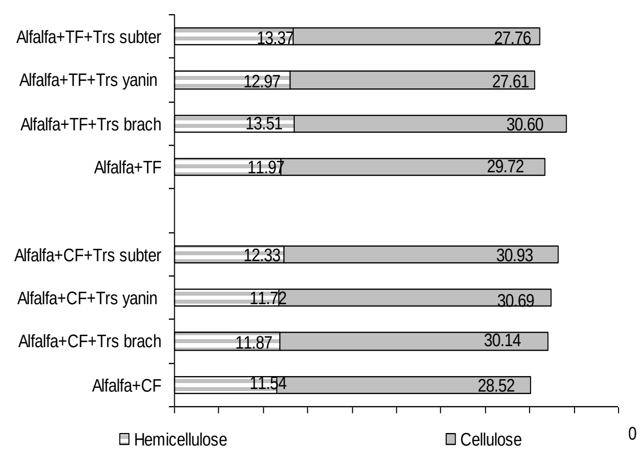


**Figure 2: Hemicelluloses and cellulose content in forage biomass of alfalfa mixtures (% dry matter).**

Regarding the cellulose content, for cocksfoot mixtures also there was an increase in the three-component mixtures, on average by 2.07% compared to the mixtures of alfalfa with cocksfoot. Cellulose content was found higher in tall fescue mixtures with sub-clover *Trifolium subterraneum ssp.*

*brachycalicinum* and lower for the remaining sub-clover subspecies as compared to the cellulose content in forage biomass from mixtures of alfalfa and tall fescue.

Energy nutritive value is an indicator that is important for the balance between forage quantity and quality (Radovic *et al.*, 2003). The mean data in our study showed that for cocksfoot mixtures UFL-UFV decreased by 0.009 - 0.022 g/kg DM when sub-clover was a component and for tall fescue mixtures UFL-UFV increased, even low, by 0.005-0.005 g/kg DM, respectively (Table 4).

Forage biomass from tall fescue mixtures had a higher energy feeding value (UFL-UFV) compared to cocksfoot mixtures, with 0.036-0.041 g/kg DM. The highest energy nutritive value was found for forage biomass from mixtures of alfalfa, tall fescue and *Trifolium subterraneum ssp. subterraneum* (UFL-UFV 0.728-0.622 g/kg DM).

**Table 4: Energy feeding value of forage biomass of alfalfa mixtures (g/kg DM).**

| **Mixtures** | **UFL** | | |
|---|---|---|---|
| | **I cut 2013** | **II cut 2013** | **mean** |
| Alfalfa + cocksfoot | 0.765 | 0.640 | 0.703 |
| Alfalfa + cocksfoot + *Trs brach* | 0.698 | 0.659 | 0.679 |
| Alfalfa + cocksfoot + *Trs yanin* | 0.730 | 0.640 | 0.685 |
| Alfalfa + cocksfoot + *Trs subter* | 0.705 | 0.669 | 0.687 |
| Mean-3-components mixtures | 0.711 | 0.656 | 0.684 |
| Mean for all mixtures | 0.725 | 0.652 | 0.688 |
| SE (P=0.05) | 0.001 | 0.007 | 0.005 |
| | | | |
| Alfalfa + tall fescue | 0.707 | 0.722 | 0.715 |
| Alfalfa + tall fescue + *Trs brach* | 0.738 | 0.680 | 0.709 |
| Alfalfa + tall fescue + *Trs yanin* | 0.759 | 0.686 | 0.723 |
| Alfalfa + tall fescue + *Trs subter* | 0.766 | 0.689 | 0.728 |
| Mean-3-components mixtures | 0.754 | 0.685 | 0.720 |
| Mean for all mixtures | 0.743 | 0.694 | 0.718 |
| SE (P=0.05) | 0.001 | 0.009 | 0.004 |
| | UFV | | |
| Alfalfa + cocksfoot | 0.662 | 0.525 | 0.594 |
| Alfalfa + cocksfoot + *Trs brach* | 0.591 | 0.543 | 0.567 |
| Alfalfa + cocksfoot + *Trs yanin* | 0.625 | 0.524 | 0.575 |
| Alfalfa + cocksfoot + *Trs subter* | 0.596 | 0.555 | 0.576 |
| Mean-three-components mixtures | 0.604 | 0.541 | 0.572 |
| Mean for all mixtures | 0.619 | 0.537 | 0.578 |
| SE (P=0.05) | 0.001 | 0.007 | 0.005 |
| | | | |
| Alfalfa + tall fescue | 0.600 | 0.615 | 0.608 |
| Alfalfa + tall fescue + *Trs brach* | 0.635 | 0.569 | 0.602 |
| Alfalfa + tall fescue + *Trs yanin* | 0.656 | 0.575 | 0.616 |
| Alfalfa + tall fescue + *Trs subter* | 0.665 | 0.579 | 0.622 |
| Mean-3-components mixtures | 0.652 | 0.574 | 0.613 |
| Mean for all mixtures | 0.639 | 0.585 | 0.612 |
| SE (P=0.05) | 0.001 | 0.001 | 0.004 |

The legend as in Table 1

**Table 5: Protein feeding value of forage forage biomass of alfalfa mixtures (g/kg DM).**

| Mixtures | **TDP** | | |
|---|---|---|---|
| | **I cut 2013** | **II cut 2013** | **mean** |
| Alfalfa+cocksfoot | 158 | 116 | 137 |
| Alfalfa+cocksfoot+*Trs brach* | 101 | 147 | 124 |
| Alfalfa+cocksfoot+*Trs yanin* | 115 | 128 | 122 |
| Alfalfa+cocksfoot+*Trs subter* | 129 | 133 | 131 |
| Mean-3-components mixtures | 115 | 136 | 126 |
| Mean for all mixtures | 126 | 131 | 128 |
| SE (P=0.05) | 12 | 6 | 3 |
| | | | |
| Alfalfa+tall fescue | 118 | 134 | 126 |
| Alfalfa+tall fescue+*Trs brach* | 106 | 109 | 108 |
| Alfalfa+tall fescue+*Trs yanin* | 142 | 122 | 132 |
| Alfalfa+tall fescue+*Trs subter* | 136 | 110 | 123 |
| Mean-3-components mixtures | 128 | 114 | 121 |
| Mean for all mixtures | 126 | 119 | 122 |
| SE (P=0.05) | 8 | 6 | 5 |
| | PDIN | | |
| Alfalfa+cocksfoot | 127 | 100 | 114 |
| Alfalfa+cocksfoot+*Trs brach* | 90 | 120 | 105 |
| Alfalfa+cocksfoot+*Trs yanin* | 99 | 107 | 103 |
| Alfalfa+cocksfoot+*Trs subter* | 107 | 111 | 109 |
| Mean-3-components mixtures | 99 | 113 | 106 |
| Mean for all mixtures | 106 | 110 | 108 |
| SE (P=0.05) | 8 | 4 | 2 |
| | | | |
| Alfalfa+tall fescue | 101 | 115 | 108 |
| Alfalfa+tall fescue+*Trs brach* | 93 | 95 | 94 |
| Alfalfa+tall fescue+*Trs yanin* | 116 | 103 | 110 |
| Alfalfa+tall fescue+*Trs subter* | 113 | 95 | 104 |
| Mean-3-components mixtures | 107 | 98 | 103 |
| Mean for all mixtures | 106 | 102 | 104 |
| SE (P=0.05) | 5 | 5 | 3 |
| | PDIE | | |
| Alfalfa+cocksfoot | 102 | 83 | 93 |
| Alfalfa+cocksfoot+*Trs brach* | 86 | 91 | 89 |
| Alfalfa+cocksfoot+*Trs yanin* | 90 | 87 | 89 |
| Alfalfa+cocksfoot+*Trs subter* | 92 | 90 | 91 |
| Mean-3-components mixtures | 89 | 89 | 89 |
| Mean for all mixtures | 93 | 88 | 90 |
| SE (P=0.05) | 3 | 2 | 1 |
| | | | |
| Alfalfa+tall fescue | 89 | 94 | 92 |
| Alfalfa+tall fescue+*Trs brach* | 88 | 85 | 87 |
| Alfalfa+tall fescue+*Trs yanin* | 97 | 89 | 93 |
| Alfalfa+tall fescue+*Trs subter* | 96 | 86 | 91 |
| Mean-3-components mixtures | 94 | 87 | 90 |
| Mean for all mixtures | 93 | 89 | 91 |
| SE (P=0.05) | 2 | 2 | 1 |

The legend as in Table 1

The highest protein nutritive value per indicators of protein, digestible in the small intestine, depending on energy; protein, digestible in the small intestine, depending on nitrogen, and total digestible protein, have mixtures of alfalfa with tall fescue and *Trifolium subterraneum ssp. yaninicum*, PDIN (110 g/kg DM), PDIE (93 g/kg DM) and total digestible protein (132 g/kg DM) (Table 5). Naydenova and Vasileva (2015) found that subclover as a component increased the protein nutritive value of forage biomass from mixtures of alfalfa and had no effect in mixtures of sainfoin. In the present study, in the presence of cocksfoot as a grass component in mixture with alfalfa such dependence has not been established.

However, when tall fescue was a grass component, *Trifolium subterraneum ssp. yaninicum* contributes to a higher total digestible protein content with 6 g/kg DM, and less, with 2 and 1 g/kg DM for PDIN and PDIE. Mixtures with cocksfoot in terms of total digestible protein and PDIN have higher values of 6 and 4 g/kg DM, respectively.

In general, mixtures with a grass component cocksfoot had better quality of forage biomass compared to those with tall fescue. They have a higher crude protein content (16.58% DM) (by 1.45%), higher digestibility (63.67%) (with 1.53%) and higher protein nutritive value relative to total digestible protein (128 g/kg DM) and PDIN (108 g/kg DM) (with 6 and 4 g/kg DM, respectively). Better quality characteristics of forage biomass from mixtures of alfalfa are also related to the fact that cocksfoot grows faster than tall fescue after cutting (Jacobs and Siddoway, 2007).

***Conclusions*:** Forage biomass from mixtures of alfalfa with cocksfoot had generally higher forage quality than that of mixtures of alfalfa with tall fescue. It had a higher crude protein (16.34% DM) (with 0.91) and lower crude fiber contents (26.95% DM) (with 0.39%), higher digestibility (62.41%) (with 2.70) and higher protein feeding value (TDP-PDIN-PDIE, 134-114-93 g/kg DM) (with 11, 6 and 1 g/kg DM). After the addition of subterranean clover in the composition of mixtures, forage biomass showed more balanced basic chemical composition: higher crude protein (with 0.32%) and lower crude fiber contents (with 0.63% for mixtures with cocksfoot and with 3.24 for mixtures with tall fescue); lower NDF, ADF and ADL contents (with 0.91, 2.23 and 1.16%) for mixtures with tall fescue; lower degree of lignifications (with 0.84 for mixtures with cocksfoot, and with 2.19 for mixtures with tall fescue); higher energy feeding value (UFL-UFV) (with 0.005-0.005 for mixtures with tall fescue). Forage biomass from mixtures of alfalfa with cocksfoot and *Trifolium subterraneum ssp. subterraneum* had the highest crude protein (17.02% DM), the lowest crude fiber contents (25.73% DM) and the highest digestibility (64.59% DM).

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