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Nutritive value of forage biomass from sainfoin mixtures

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ABSTRACT

Forage quality characteristics of field-grown mixtures of sainfoin with cocksfoot (50:50%), sainfoin with tall fescue (50:50%), and the same with the addition of subterranean clover in their composition (33:33:33%) were measured. Forage biomass from the mixtures of sainfoin with cocksfoot had generally higher forage quality than mixtures with tall fescue. It had higher crude protein content (11.52% of dry matter (with 1.07% units), significantly higher digestibility (61.74%) (with 6.51% units), higher neutral detergent fiber content (53.42%) (with 3.22% units), higher nutritive value (Unité Fourragère Viande – Unité Fourragère Lait, 0.690–0.583) and higher protein feeding value (Total Digestible Protein – Protein digestible dans l'intestine in dependence of nitrogen – Protein digestible dans l'intestine in dependence of energy), 72–70–79 g/kg of dry matter. Forage biomass showed more balanced basic chemical composition after the addition of subterranean clover, i.e.: higher crude protein content (with 0.30% units) and lower crude fiber content (with 0.14% units) for mixtures with cocksfoot; higher digestibility (with 0.29% units) for mixtures with cocksfoot; lower neutral detergent fiber content (with 0.45% units) for mixtures with cocksfoot and with 3.15% units for mixtures with tall fescue, higher energy feeding value (Unité Fourragère Viande – Unité Fourragère Lait) (with 0.007–0.012 for mixtures with cocksfoot and with 0.009–0.014 for mixtures with tall fescue), higher protein feeding value for both mixtures with cocksfoot and tall fescue. Forage biomass from mixtures of sainfoin with cocksfoot and *Trifolium subterraneum* ssp. *brachycalycinum* had the highest crude protein (11.89% of dry matter), the lowest crude fiber content (27.07% of dry matter) and the highest digestibility (62.81% of dry matter).

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1. Introduction

Sainfoin is a perennial leguminous forage crops with a high content of crude protein, high palatability and nutritive value (Delgado et al., 2008). It is suitable for pastoral use due to the condensed tannins and does not cause the animals to swell while feeding on the green mass (Jacobs and Siddoway, 2007; Niderkorn et al., 2012). It can also be used for hay and silage (Vuckovic, 2004). In addition, sainfoin is also grown in mixtures with grasses, such as smooth brome grass, cocksfoot, perennial ryegrass, crested wheatgrass (Chakarov, 1998; Vasilev, 2008; Albayrak et al., 2011). The choice of a suitable component is very important (Pavlov, 1996; Peeters et al., 2006) with the aim of obtaining forage biomass with

a balanced protein and energy content (Chakarov and Vasilev, 1995; Demdoun et al., 2010). Tall fescue compared to the cocksfoot is a less competitive grass (Hannaway et al., 1999). 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 mainly the crude protein content is not very high (Bughrara et al., 1991; de Santis et al., 1997).

Legume components in mixtures, mainly because of the crude protein content improved the quality of the forage biomass (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 sainfoin with cocksfoot and with tall fescue after addition of a second legume component in their composition.

Subterranean clover (*Trifolium subterraneum* L.) is a relatively new legume crops 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 as, when fed as hay and silage

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(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 common used forage crops (Vasilev, 2006, 2009). It is acknowledged the information for higher crude protein and lower crude fiber content in the forage biomass from mixtures of subterranean clover with cocksfoot as well as 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).

Considering the above facts, the present work was, therefore, undertaken to determine the quality characteristics, (chemical composition, digestibility, energy and protein nutritive value) of forage biomass from mixtures of sainfoin and cocksfoot (50:50%), sainfoin and tall fescue (50:50%), and the same with subterranean clover component in their composition (33:33:33%).

2. Materials and methods

Experimental work was done in the Institute of Forage Crops, Pleven, Bulgaria (2011–2013). Sainfoin (*Onobrychis Adans*) (local population), 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 m². The following forage treatments were investigated 4 times replicated: (i) mixtures of sainfoin with cocksfoot: sainfoin + cocksfoot (50:50%); sainfoin + cocksfoot + *Trifolium subterraneum ssp. brachycalicinum* (33:33:33%); sainfoin + cocksfoot + *Trifolium subterraneum ssp. yaninicum* (33:33:33%); sainfoin + cocksfoot + *Trifolium subterraneum ssp. subterraneum* (33:33:33%) and (ii) mixtures of sainfoin with tall fescue: sainfoin + tall fescue (50:50%); sainfoin + tall fescue + *Trifolium subterraneum ssp. brachycalicinum* (33:33:33%); sainfoin + tall fescue + *Trifolium subterraneum ssp. yaninicum* (33:33:33%); sainfoin + tall fescue + *Trifolium subterraneum ssp. subterraneum* (33:33:33%). During the vegetative period neither fertilizers nor pesticides were applied. The swards were harvested at the budding stage for legumes and before earling for the grasses.

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 for 20 min at 105 °C 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 – crude protein (CP) by Kjeldahl (BDS-ISO 5983) and crude fibers (CF) (AOAC, 2007). Structural carbohydrates or cell wall components: Neutral-detergent fiber (NDF), Acid-detergent fiber (ADF), Acid-detergent lignin (ADL) as a percentage of the dry matter of the feed were determined by the method of Goering and Van Soest (1970) (EN ISO13906 2008). 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/NDF × 100 (Akin and Chesson, 1990). The *in vitro* enzyme digestibility of the dry (IVDMD) and organic (IVOMD) matter was determined as a

percentage by Aufrere two-step pepsin-cellulose enzyme method by Aufrere (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, and degradability of organic matter according to Aufrère (1982), Todorov et al. (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. Individual and mean values of the characteristics for feeding value of the forage were estimated. Data of one cut harvested on June 12, 2012 and two cuts, harvested on May 7 and July 5, 2013 was presented data were statistically processed using SPSS (2012).

3. Results and discussion

Forage quality, expressed mainly with crude protein, crude fiber contents, digestibility and other associated characteristics are essential for animal productivity. Quality parameters varied for legumes and grasses. Thus, the components in grass mixtures influence the quality of the forage obtained (Whitehead, 1995). We suppose the inclusion of legume component which can improve the quality of forage biomass obtained from mixtures.

Table 1 presents the data on crude protein and crude fiber contents in forage biomass of the studied mixtures. Crude protein content in the first cut for the first year for cocksfoot mixtures was found higher than tall fescue mixtures (on average with 2.05% units).

In the second year, the crude protein content of three component mixtures (when added subclover to the composition) was found higher with 0.75% units for the mixtures with cocksfoot and with 0.77% units for the mixtures with tall fescue. In second cut, the influence of subterranean clover component was not recorded. It was due to the biological features of the clover. The crude protein content in the forage biomass from mixtures with cocksfoot was found by 1.24% higher than that of tall fescue mixtures.

On average, forage biomass from mixtures of sainfoin, cocksfoot and *Trifolium subterraneum ssp. brachycalicinum* (11.89% DM) and from sainfoin, cocksfoot and *Trifolium subterraneum ssp. yaninicum* (11.89% DM) showed the highest crude protein content exceeding that of sainfoin and cocksfoot mixtures with 0.37% units. Results for mean crude protein content of sainfoin and cocksfoot mixtures are in agreement with Stoycheva et al. (2017).

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 used by the animals. The 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).

In our study, the highest crude fiber content was recorded for the forage biomass from mixtures in the first year of 29.21% DM for cocksfoot mixtures and 30.46% DM for tall fescue mixtures.

Table 1
Main chemical composition of the forage of sainfoin mixtures (%DM).

Mixtures	I cut, 2012	I cut, 2013	II cut, 2013	Mean
<i>Crude protein</i>				
Sainfoin + cocksfoot	12.18	10.82	11.55	11.52
Sainfoin + cocksfoot + <i>Trs brach</i>	12.31	11.84	11.53	11.89
Sainfoin + cocksfoot + <i>Trs yanin</i>	11.59	11.82	11.61	11.67
Sainfoin + cocksfoot + <i>Trs subter</i>	13.90	11.05	10.72	11.89
Mean-three-components mixtures	12.60	11.57	11.29	11.82
Mean for all mixtures	12.50	11.38	11.35	11.74
STDEV	0.99	0.53	0.42	0.18
SE (P = 0.05)	0.31	0.16	0.13	0.05
<i>Sainfoin + tall fescue</i>				
Sainfoin + tall fescue	10.85	10.02	10.49	10.45
Sainfoin + tall fescue + <i>Trs brach</i>	9.51	9.76	11.06	10.11
Sainfoin + tall fescue + <i>Trs yanin</i>	11.50	11.03	10.23	10.92
Sainfoin + tall fescue + <i>Trs subter</i>	9.92	11.59	10.05	10.52
Mean-three-components mixtures	10.31	10.79	10.45	10.52
Mean for all mixtures	10.45	10.60	10.46	10.50
STDEV	0.90	0.86	0.44	0.33
SE (P = 0.05)	0.28	0.27	0.13	0.10
<i>Crude fiber</i>				
Sainfoin + cocksfoot	29.33	26.38	26.41	27.37
Sainfoin + cocksfoot + <i>Trs brach</i>	29.03	25.97	26.22	27.07
Sainfoin + cocksfoot + <i>Trs yanin</i>	29.61	25.97	25.78	27.12
Sainfoin + cocksfoot + <i>Trs subter</i>	28.86	26.37	27.24	27.49
Mean-three-components mixtures	29.17	26.10	26.41	27.23
Mean for all mixtures	29.21	26.17	26.41	27.26
STDEV	0.33	0.23	0.61	0.20
SE (P = 0.05)	0.10	0.07	0.19	0.06
<i>Sainfoin + tall fescue</i>				
Sainfoin + tall fescue	29.70	25.28	27.19	27.39
Sainfoin + tall fescue + <i>Trs brach</i>	31.20	26.63	24.95	27.59
Sainfoin + tall fescue + <i>Trs yanin</i>	31.44	25.22	26.19	27.62
Sainfoin + tall fescue + <i>Trs subter</i>	29.49	24.98	26.84	27.10
Mean-three-components mixtures	30.71	25.61	25.99	27.44
Mean for all mixtures	30.46	25.53	26.29	27.43
STDEV	1.00	0.75	0.99	0.24
SE (P = 0.05)	0.32	0.23	0.31	0.07

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

Bijelić et al. (2013) received higher crude fiber content in the forage biomass from mixtures of sainfoin with tall fescue. We found a negative correlation ($r = -0.077$) between CP and CF content for mixtures with cocksfoot.

When subterranean clover was added as a component, crude fiber content in the forage biomass declined for cocksfoot mixtures by 0.16% in the first year and by 0.28% for the first cut in the second year. On average from the cocksfoot mixtures, crude fiber content decreased by 0.14%. At most, 0.30% crude fiber content decreased in mixtures with *Trifolium subterraneum* ssp. *brachycalicinum*. Tall fescue mixtures had higher crude fiber content (with 0.16% units) comparing to cocksfoot mixtures.

The part of sainfoin in mixtures decreased and the part of cocksfoot increased during the years after sowing (Pavlov, 1996). That led to higher share of generative stems and effect the chemical composition of the forage biomass. When subterranean clover was added, the forage biomass from the mixtures with cocksfoot had more balanced basic chemical composition, crude protein content increased by 0.30% and crude fiber decreased by 0.14% units.

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 the 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 forage, a prerequisite for their energy and protein nutrition, and an indicator where the forages are compared to each other. According to Bal et al. (2006) with the advancing the phase of development of

sainfoin decreased the digestibility of forage. Reducing the forage quality (expressed as crude protein and crude fiber contents) as the age of sainfoin and cut increased was found by Kaplan (2011). Similar results for the quality of forage from tall fescue were reported by de Santis et al. (1997), and from cocksfoot by Ammar et al. (1999).

Subterranean subspecies differ in digestibility of dry matter (McLaren and Doyle, 1994; Ru and Fortune, 2000) and this had an impact on the digestibility of forage biomass from mixtures in which they participate 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/subterranean-clover.html>. Lilley et al. (2001) found a decreasing of *in vitro* digestibility with the advancing the age of subterranean clover.

The studied 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 cocksfoot mixtures was 61.45%. The digestibility of forage biomass from tall fescue mixtures was significantly lower (55.52%) (with 5.93% units).

The digestibility of forage biomass in the second cut in the second year decreased and was found lower as compared to the first cut by 4.22% for the cocksfoot mixtures and by 4.69% for the tall fescue mixtures. This is related to the formation of generative stems from sainfoin in the second cut. The leaf/stems ratio is an important characteristics and indicator of quality and intake of forage (Ammar et al., 1999). The leaves of sainfoin are richer with mineral substances compared to the stems (Vuckovic, 2004) and their part decreases as the age advancing, which decreased the forage quality (Albrecht and Marvin, 1995).

Table 2
Digestibility of dry matter of the forage of sainfoin mixtures (%).

Mixtures	I cut, 2012	I cut, 2013	II cut, 2013	Mean
Sainfoin + cocksfoot	62.23	62.22	60.78	61.74
Sainfoin + cocksfoot + <i>Trs brach</i>	62.78	66.43	59.22	62.81
Sainfoin + cocksfoot + <i>Trs yanin</i>	61.33	65.84	60.50	62.56
Sainfoin + cocksfoot + <i>Trs subter</i>	59.46	62.80	59.91	60.72
Mean-three-components mixtures	61.19	65.02	59.88	62.03
Mean for all mixtures	61.45	64.32	60.10	61.96
STDEV	1.46	2.12	0.69	0.94
SE (P = 0.05)	0.46	0.68	0.22	0.29
Sainfoin + tall fescue	57.03	57.06	51.61	55.23
Sainfoin + tall fescue + <i>Trs brach</i>	52.86	56.03	52.74	53.88
Sainfoin + tall fescue + <i>Trs yanin</i>	56.70	59.42	54.09	56.74
Sainfoin + tall fescue + <i>Trs subter</i>	55.47	56.84	52.15	54.82
Mean-three-components mixtures	55.01	57.43	52.99	55.14
Mean for all mixtures	55.52	57.34	52.65	55.17
STDEV	1.89	1.46	1.07	1.19
SE (P = 0.05)	0.60	0.46	0.34	0.37

The legend as in Table 1.

On average from the three cuts the digestibility of forage biomass from mixtures of sainfoin and cocksfoot with added sub-clover component (62.03%) was found significantly higher (with 6.89%) as compared to that from mixtures of sainfoin with cocksfoot (61.74%).

Forage biomass from mixtures of sainfoin with cocksfoot and *Trifolium subterraneum ssp. brachycalicinum* was found with the highest crude protein (11.89% DM), the lowest crude fiber contents (27.07% DM) and the highest digestibility (62.81% DM).

Mixtures of sainfoin with cocksfoot had significant higher digestibility of the forage (61.74%) as compared to the mixtures with tall fescue (55.23%). On average from the all mixtures studied the digestibility of forage biomass from cocksfoot mixtures was found by 6.79% units higher as compared to that from tall fescue mixtures, and by 6.89% when the second legume component was added in their composition.

The data obtained are related to the competition of tall fescue (Hannaway et al., 1999), as well as to the presence of more leaflets

of cocksfoot that are more digestible (Ammar et al., 1999). Increasing digestibility leads to an increase in forage intakes as digestibility and uptake are in positive correlation (Van Soest, 1982).

Structural polysaccharides 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 like neutral detergent fibers (NDF), acid detergent fibers (ADF) and acid detergent lignin (ADL) (Scotti and Julier, 2014). In summer, the content of NDF, ADF and lignin increased faster in legumes than in grasses (Elgersma and Soegaard, 2017). The NDF content in forage biomass from mixtures of sainfoin with cocksfoot was found 53.08% (Fig. 1). In the case of the clover component, the NDF content of forage biomass of mixtures with cocksfoot decreased on average by 0.45% and significantly decreased in these with tall fescue by 3.15%, which is

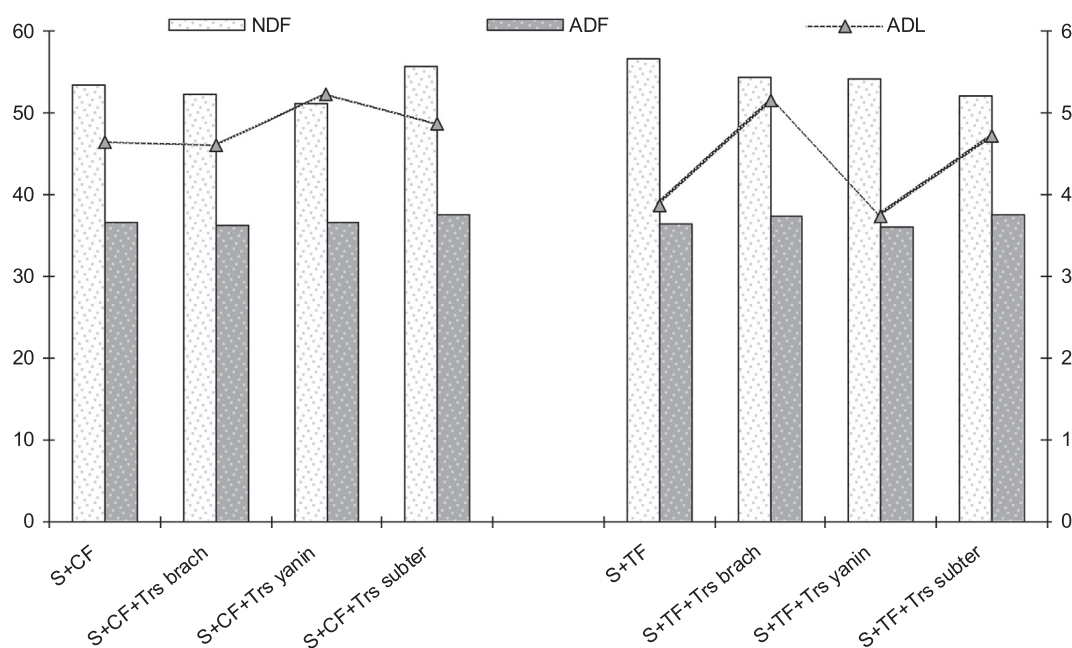


Fig. 1. Composition and digestibility of the forage of sainfoin + cocksfoot + subterranean clover [(SE (P = 0.05) NDF, 0.62; ADF, 0.18; ADL, 0.09) and sainfoin + tall fescue + subterranean clover mixtures [(SE (P = 0.05) NDF, 0.60; ADF, 0.23; ADL, 0.21), %].

important given the lower quality of forage biomass from tall fescue.

Forage biomass from cocksfoot mixtures had lower content both of NDF and ADF. By comparing the two types of mixtures, the ADL content was lower in tall fescue mixtures (by 0.48%) as compared to that of cocksfoot mixtures. According to Niderkorn et al. (2012) cocksfoot is richer in the NDF.

In this study, we found a negative correlation between the digestibility of forage biomass and the content of fiber fractions ADF ($r = -0.9162$ for cocksfoot mixtures and $r = -0.8523$ for tall fescue mixtures), which is in agreement with other authors (Scotti and Julier, 2014; Adamovic et al., 2017).

Lower values of NDF in forage biomass from mixtures with included subclover component, NDF 52.97% (by 0.45%) for cocks-

foot mixtures and 53.50% (by 3.15%) for tall fescue mixtures are related to the NDF content in the forage from pure grown subclover (NDF 41.26%), which is lower than that of other leguminous forage crops (Naydenova and Vasileva, 2015).

A lower degree of lignifications was found in the forage biomass from the mixtures of sainfoin with tall fescue (coefficient 6.80) compared to those with cocksfoot (coefficient 8.71) (Fig. 2). The same tendency was observed when subclover component was included. On average from the mixtures of sainfoin with tall fescue and subclover, the degree of lignifications was 8.57 (coefficient), and that of the mixtures of sainfoin with cocksfoot and subclover –9.29 (coefficient).

Pollysoides chemicellulose and cellulose were determined and presented in Fig. 3. The content of hemicellulose was found lower

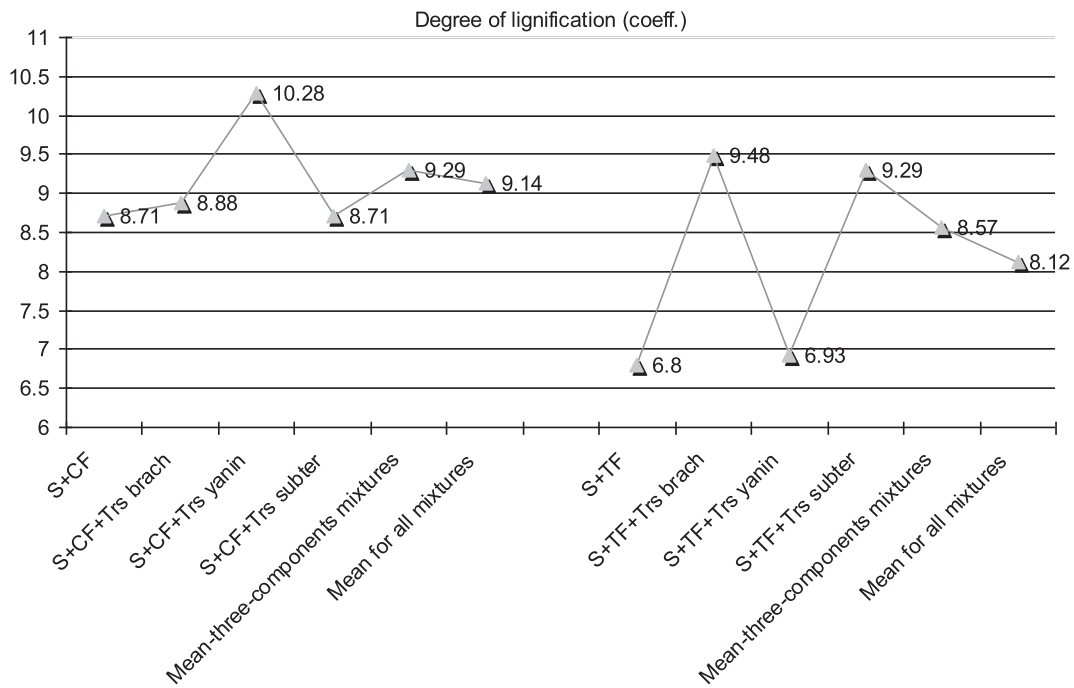


Fig. 2. Degree of lignifications of forage of sainfoin + cocksfoot + subterranean clover [(SE (P = 0.05) 0.24) and sainfoin + tall fescue + subterranean clover mixtures (coeff.), [(SE (P = 0.05) 0.46].

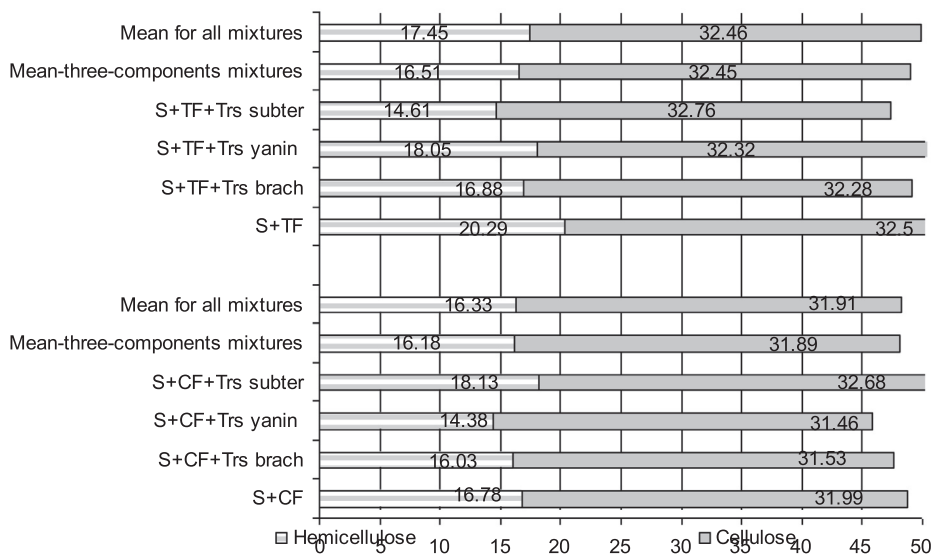


Fig. 3. Hemicelluloses and cellulose content in forage of sainfoin + cocksfoot + subterranean clover [(SE (P = 0.05), 0.49, 0.17) and sainfoin + tall fescue + subterranean clover mixtures [(SE (P = 0.05), 0.76, 0.06] (% dry matter).

Table 3
Energy feeding value of forage of sainfoin mixtures (g/kg DM).

Mixtures	UFL		
	I cut, 2013	II cut, 2013	Mean
Sainfoin + cocksfoot	0.699	0.680	0.690
Sainfoin + cocksfoot + <i>Trs brach</i>	0.736	0.673	0.705
Sainfoin + cocksfoot + <i>Trs yanin</i>	0.735	0.670	0.703
Sainfoin + cocksfoot + <i>Trs subter</i>	0.703	0.664	0.684
Mean-three-components mixtures	0.725	0.669	0.697
Mean for all mixtures	0.718	0.672	0.695
STDEV	0.020	0.007	0.010
SE (P = 0.05)	0.006	0.002	0.003
Sainfoin + tall fescue	0.654	0.613	0.634
Sainfoin + tall fescue + <i>Trs brach</i>	0.651	0.611	0.631
Sainfoin + tall fescue + <i>Trs yanin</i>	0.680	0.643	0.662
Sainfoin + tall fescue + <i>Trs subter</i>	0.668	0.623	0.646
Mean-three-components mixtures	0.666	0.626	0.646
Mean for all mixtures	0.663	0.623	0.643
STDEV	0.013	0.015	0.014
SE (P = 0.05)	0.004	0.004	0.004
	UFV		
Sainfoin + cocksfoot	0.594	0.572	0.583
Sainfoin + cocksfoot + <i>Trs brach</i>	0.636	0.565	0.601
Sainfoin + cocksfoot + <i>Trs yanin</i>	0.634	0.561	0.598
Sainfoin + cocksfoot + <i>Trs subter</i>	0.598	0.555	0.577
Mean-three-components mixtures	0.623	0.560	0.592
Mean for all mixtures	0.616	0.563	0.589
STDEV	0.023	0.007	0.012
SE (P = 0.05)	0.007	0.002	0.003
Sainfoin + tall fescue	0.544	0.498	0.521
Sainfoin + tall fescue + <i>Trs brach</i>	0.541	0.495	0.518
Sainfoin + tall fescue + <i>Trs yanin</i>	0.573	0.532	0.553
Sainfoin + tall fescue + <i>Trs subter</i>	0.559	0.510	0.535
Mean-three-components mixtures	0.558	0.512	0.535
Mean for all mixtures	0.554	0.509	0.532
STDEV	0.015	0.017	0.016
SE (P = 0.05)	0.004	0.005	0.005

The legend as in Table 1.

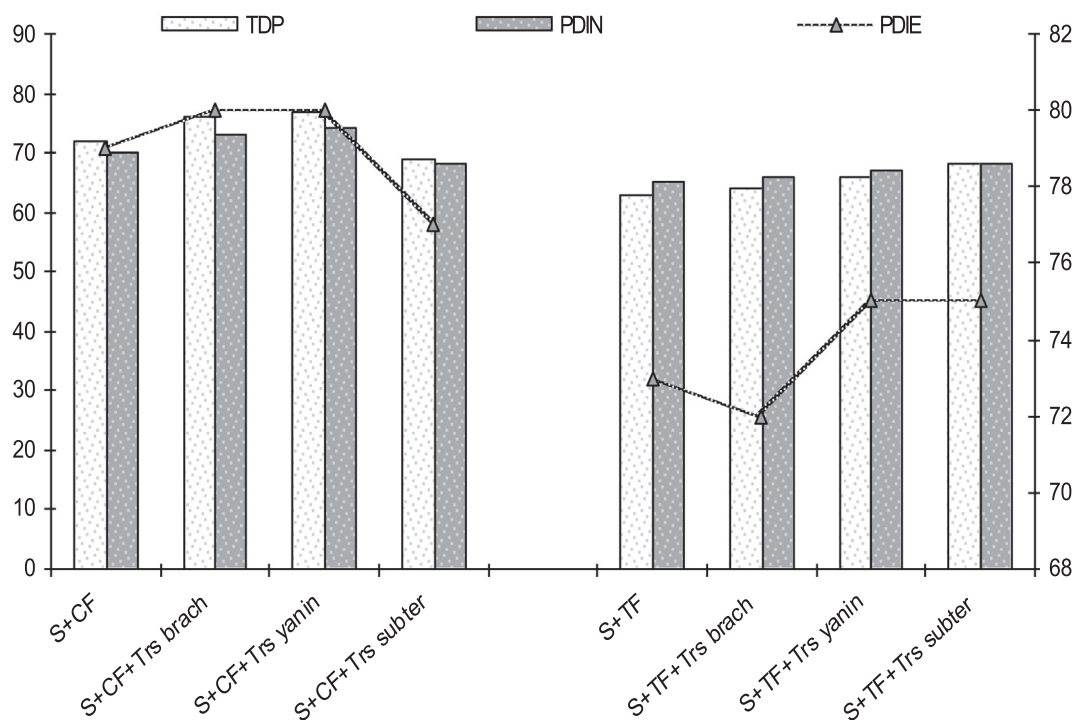


Fig. 4. Protein feeding value of forage of sainfoin + cocksfoot + subterranean clover [(SE (P = 0.05) TDP, 1.1; PDIN, 0.8; PDIE, 0.4)] and sainfoin + tall fescue + subterranean clover mixtures [(SE (P = 0.05) TDP, 0.7; PDIN, 0.4; PDIE, 0.4), (g/kg DM).

in mixtures with subclover, an average of 0.60% for mixtures with cocksfoot, and an average of 3.78% for mixtures with tall fescue.

Concerning the content of cellulose, there was also a decrease in the content for the mixtures with subclover component, and an average of 0.10% for mixtures with cocksfoot but 0.05% for mixtures with tall fescue.

Higher content of both, hemicellulose of 1.13% and cellulose of 0.55% was found in forage biomass of mixtures when tall fescue was a grass component.

Energy nutritive value is an important indicator for the balance between forage quantity and quality (Radovic et al., 2003). The average data in our study showed that the UFL-UFV increased when subterranean clover was added as a component – for mixtures with cocksfoot with 0.007–0.009 g/kg DM, and for mixtures with tall fescue with 0.012–0.044 g/kg DM (Table 3).

Forage biomass from mixtures with cocksfoot had higher energy feeding value (UFV-UFL 0.695–0.589) compared to the mixtures with tall fescue (UFV-UFL 0.643–0.532), or by 0.052–0.057 g/kg DM, respectively.

Mixtures of sainfoin with tall fescue and *Trifolium subterraneum* ssp. *brachycalicinum* was found with the highest energy nutritive value (UFV-UFL 0.705–0.601 g/kg DM). There was a strong positive correlation between UFV-UFL and the digestibility of the dry matter of the forage (for mixtures with cocksfoot $r = +0.9715$ and for mixtures with tall fescue $r = +0.8601$). Krachunov (2007) found that the content of digestible organic matter closely correlated with metabolizable and net energy (FUM and FUG) – (r from 0.93 to 0.99).

Higher protein feeding value (TDP-PDIN-PDIE, 73–71–79 g/kg DM) (with 8–5–5 g/kg DM) had forage biomass from mixtures with cocksfoot (Fig. 4).

When subclover was added in the composition of mixtures, the protein feeding value was found to increase for mixtures with

cocksfoot (TDP-PDIN, with 1–1 g/kg DM), and for the mixtures with tall fescue (TDP-PDIN-PDIE, with 3–2–1 g/kg DM).

In general, mixtures with grass component cocksfoot had higher crude protein (11.74% DM) (with 1.24% units) and lower crude fiber contents (27.26% DM) (with 0.16% units), and they are significantly more digestible (61.96%) (with 6.79% units).

Better qualitative characteristics of forage biomass from mixtures of sainfoin are also related to the fact that the cocksfoot grows faster than tall fescue after cutting (Jacobs and Siddoway, 2007). The inclusion of legume component as subterranean clover improved the quality of forage biomass obtained from mixtures.

4. Conclusions

Forage biomass from the mixtures of sainfoin with cocksfoot showed generally higher forage quality than mixtures with tall fescue, i.e. higher crude protein content (with 1.07% units), significantly higher digestibility (with 6.51% units), higher NDF content (with 3.22% units), higher nutritive value (with 0.056–0.062% units), and higher protein feeding value (with 9–5–6 g/kg DM).

Subterranean clover included in the composition of mixtures improved the basic chemical composition of forage biomass such as higher crude protein and lower crude fiber contents as well higher digestibility for mixtures with cocksfoot, lower NDF content and higher energy and protein feeding value for both mixtures.

References

- Adamovic, A., Gutmane, I., Katamadze, M., 2017. The quality of multicomponent grass swards for grazing on three soil types. *Grassland Sci. Europe* 22, 286–288.
- Akin, D.E., Chesson, A., 1990. Lignification as the major factor limiting forage feeding value especially in warm conditions. In: Proc. XVI Int. Grassland Cong. Association Francaise pour la Production Fourragere, Versailles, France, pp. 1753–1760.
- Albayrak, S., Turk, M., Yuksel, O., Yilmaz, M., 2011. Forage yield and the quality of perennial legume-grass mixtures under rainfed conditions. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 9 (1), 114–118.
- Albrecht, K.A., Marvin, H.H., 1995. Hay and silage management. In: Barnes, R.F., Miller, D.A., Nelson, C.J. (Eds.), *Forages vol. I: An Introduction to Grassland Agriculture*. Iowa State University Press, Ames, Iowa, USA, pp. 155–174.
- Ammar, H., Lopez, S., Bochi-Brum, O., Garcia, R., Ranilla, M.J., 1999. Composition and *in vitro* digestibility of leaves and stems of grasses and legumes harvested from permanent mountain meadows at different stages of maturity. *J. Anim. Feed Sci.* 8, 599–610.
- Andrieu, J., Demarquilly, C., 1989. Prediction of the digestible and metabolizable energy content of forages from their chemical composition and organic matter digestibility. In: Proceedings Presented at 16. International Grassland Congress, Nice, FRA (1989-10-04-1989-10-11). Association Française pour la Production Fourragère, Versailles, FRA, pp. 875–876.
- AOAC, 2007. Official methods of analysis. Association of Analytical Chemists, Gaithersburg, MD, USA.
- Aufrère, J., 1982. Etude de la prévision de la digestibilité des fourrages par une méthode enzymatique Available from: *Annales de Zootechnie* 31, 11–30.
- Bal, M.A., Ozturk, D., Aydin, R., Erol, A., Ozkan, C.O., Ata, M., Karakas, E., Karabay, P., 2006. Nutritive value of sainfoin (*Onobrychis viciifolia*) harvested at different maturity stages. *Pak. J. Biol. Sci.* 9 (2), 205–209.
- Bijelić, Z., Tomić, Z., Ružić-Muslić, D., Mandić, V., Simić, A., Vučković, S., 2013. Yield potential and quality of forage mixtures of alfalfa with cocksfoot and tall fescue depending on the nitrogen fertilization. *Biotechnol. Anim. Husbandry* 29 (4), 695–704.
- Bughrara, S.S., Sleper, D.A., Krause, G.F., 1991. Genetic variation in tall fescue digestibility estimated using a prepared cellulase solution. *Crop Sci.* 31, 883–889.
- Chakarov, R., 1998. Nutrient content and productivity of pure grasses and mixtures with legumes. *Plant Sci.*, S. 35 (9), 749–754 (In Bg).
- Chakarov, R., Vasilev, E., 1995. Influence of the intercutting period on the productivity of some perennial grass swards. *Plant Sci.* 32 (6), 149–151.
- De Santis, G., Chiaravalle, E., Martiniello P., 1997. Variability for chemical composition in tall fescue progenies, yield and traits associated with *in vitro* digestibility. In: Proc. XVIII International Grassland Congress, Winnipeg and Saskatoon, (Canada), vol. 2, 17, pp. 97–98.
- Delgado, I., Salvia, J., Andrés, C., 2008. The agronomic variability of a collection of sainfoin accessions. *Span J. Agric. Res.* 6 (3), 401–407.
- Demdoum, S., Munoz, F., Delgado, I., 2010. Forage production of a collection of sainfoin over a three year period. In: *The Contributions of Grasslands to the Conservation of Mediterranean Biodiversity*. Options Méditerranéennes, pp. 101–104.
- Elgersma, A., Soegaard, K., 2017. Changes in nutritive value and herbage yield during extended growth intervals in grass–legume mixtures: effects of species, maturity at harvest, and relationships between productivity and components of feed quality. *Grassland Forage Sci.* <https://doi.org/10.1111/gfs.12287>, 00: 1–16.
- Evers, G.W., Newman, Y., 2008. Arrowleaf, crimson, rose, and subterranean clover growth with and without defoliation in the southeastern United States. *Agron. J.* 100, 221–230.
- Fahey, G.C., Hussein, H., 1999. Forty years of quality research: accomplish and impact from animal nutrition perspective. *Crop Sci.* 39, 4–12.
- Frame, J., 2005. *Forage legumes for temperate grasslands*. Rome: Food and Agriculture Organization of the United Nations. Science Publishers Inc, Plymouth UK. 3 20.
- Goering, H.K., Van Soest, P.J., 1970. Forage fiber analysis (apparatus, reagents, procedures and some applications). USDA Agricultural Handbook No. 379.
- Hannaway, D., Fransen, S., Cropper, J., Teel, M., Chaney, M., Griggs, T., Halse, R., Hart, J., Cheeke, P., Hansen, D., Klinger, R., Lane, W., 1999. Tall Fescue. PNW504 Oregon State University Extension Service, Corvallis, OR, USA.
- INRA, 1988. *Alimentation des bovins, ovins et caprins*. INRA Publ., Versailles, France, p. 471.
- Jacobs, J., Siddoway, J., 2007. Tame Pasture Grass and Legume Species and Grazing Guidelines, Plant Materials Technical Note Number MT-63 December 2007, pp. 1–3.
- Kaplan, M., 2011. Determination of Potential Nutritive Value of Sainfoin (*Onobrychis sativa*) Hays Harvested at Flowering Stage. *J. Anim. Veter. Adv.* 10 (11), 2028–2031.
- Krachunov, I., 2007. Estimation of energy feeding value of forages for ruminants II. Energy prediction through crude fiber content. *J. Mt. Agric. Balkans* 10 (1), 122–134.
- Lilley, J.M., Bolger, T.P., Peoples, M.B., Gifford, R.M., 2001. Nutritive value and the nitrogen dynamics of *Trifolium subterraneum* and *Phalaris aquatica* under warmer, high CO₂ conditions. *New Phytologist* 150, 385–395.
- McLaren, S.E., Doyle, P.T., 1994. Dry matter digestibility of subterranean clover during senescence and after death. *Proc. Austral. Soc. Anim. Prod.* 20, 221–224.
- Mulholland, J.G., Nandra, K.S., Scott, G.H., Jones, A.W., Coombes, N.E., 1996. Nutritive value of subterranean clover in a temperate environment. *Aust. J. Exp. Agric.* 36, 803–814.
- Naydenova, Y., Vasileva, V., 2015. Forage quality analysis of perennial legumes – subterranean clover mixtures. *Sci. Int.* 3 (4), 113–120.
- Naydenova, Y., Vasileva, V., 2016. Analysis of forage quality of grass mixtures – perennial grasses with subterranean clover. *J. Basic Appl. Res. (Jbaar)* 2 (4), 534–540.
- Niderkorn, V., Mueller-Harvey, I., Le Morvan, A., Aufrère, J., 2012. Synergistic effects of mixing cocksfoot and sainfoin on *in vitro* rumen fermentation. Role of condensed tannins. *Anim. Feed Sci. Technol.* 178, 48–56.
- NRC, 2001. National Research Council, (2001): *Nutrient Requirements of Dairy Cattle*. Natl. Acad. Sci., Washington.
- Ovalle, C., Fernández, F., del Pozo, Y.A., 2008. ¿Cómo manejar especies de leguminosas anuales de crecimiento erecto? Efecto de la época de utilización sobre la producción de fitomasa y semilla. 53–54. In: XXXIII Reunión Anual de la Sociedad Chilena de Producción Animal (SOCHIPA), Valdivia. 29–31 de octubre. Libro de resúmenes. SOCHIPA, Santiago, Chile.
- Pavlov, D., 1996. Productivity, nutritive value, quality characteristics of different groups forage plants and possibilities of their prediction, (Doctoral thesis MSc), Sofia, Bulgaria, pp. 569.
- Peeters, A., Parente, G., Gall, A., 2006. Temperate legumes: key –species for sustainable temperate mixtures. *Grassland Sci. Europe* 11, 205–220.
- Radovic, J., Dinic, B., Pudio, V., 2003. Productivity and quality of some birdsfoot trefoil (*Lotus corniculatus* L.) varieties. *Grassland Sci. Europe* 8, 118–121.
- Ru, Y.I., Fortune, J.A., 2000. Variation in nutritive value of plant parts of subterranean clover (*Trifolium subterraneum* L.). *Aust. J. Exp. Agric.* 40, 397–403.
- Ru, Y.I., Fortune, J.A., 2001. Seed yield and nutritive value of dry, mature subterranean clover (*Trifolium subterraneum* L.). *Aust. J. Exp. Agric.* 41 (2), 169–175.
- Samuil, C., Vintu, V., Sirbu, C., Surmei, G.M., 2012. Behavior of fodder mixtures with alfalfa in north-eastern Romania. *Romanian Agric. Res.* 29, 227–235.
- Scotti, C., Julier, B., 2014. Improving alfalfa forage quality. *Legume Perspect.* 4, 31.
- Sleugh, B., Moore, K.J., George, J.R., Brummer, E.C., 2000. Binary legume-grass mixtures improve forage yield, quality, and seasonal distribution. *Agron. J.* 92, 24–29.
- SPSS, 2012. SPSS Version 20.0. SPSS Inc., 233 S. Wacker Drive, Chicago, Illinois.
- Stockdale, C.R., 1992. The nutritive value of subterranean clover herbage grown under irrigation in Northern Victoria. *Aust. J. Agric. Res.* 43, 1265–1280.
- Stoycheva, I., Naydenova, Y., Vasileva, V., 2017. Changes in composition, plant cell walls fiber components and enzyme digestibility of temporary and natural pasture. In: 14th International Symposium of Animal Biology and Nutrition, Romania, Book of Abstracts, 56–57. Sept. 28–29, 2017, Baloteshi, Romania.

- Todorov, N., Atanassov, A., Ilchev, A., Gantchev, G., Mihaylova, G., Girginov, D., Penkov, D., Shindarska, Z., Naydenova, Y., Nedelkov, K., Chobanova, S., 2010. In: *Practicum in Animal Nutrition*, p. 463. East-West, Sofia, Bulgaria Europe.
- Van Soest, P.J., 1982. *Nutritional Ecology of ruminants* O and B books Inc. Corvallis.
- Vasilev, E., 2006. Productivity of subterranean clover (*Tr. subterraneum* L.) in pasture mixtures with some perennial grasses for the conditions of Central North Bulgaria. *Rastenievadni nauki* 43(2), 149–152.
- Vasilev, E., 2008. Dry mass yield from sainfoin in binary mixtures with ryegrass and cocksfoot. *Options Mediterraneennes. Series A* 79, 241–244.
- Vasilev, E., 2009. Chemical composition of subclovers forage (*Trifolium subterraneum* L.) and crude protein yield in pasture mixtures with grasses. *J. Mt. Agric. Balkans* 12, 329–341.
- Vuckovic, S., 2004. *Grasses*. Belgrade University, Serbia, p. 506.
- Whitehead, D.C., 1995. *Grassland Nitrogen*. CAB International, Wallingford, UK.
- Wilson, J.R., Deinum, B., Engels, F.M., 1991. Temperature effects on anatomy and digestibility of leaf and stem of tropical and temperate forage species. *Neth. J. Agric. Sci.* 39, 31–48.