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Analysis of Forage Quality of Grass Mixtures – Perennial Grasses with Subterranean Clover

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Abstract: The analysis of forage quality of cocksfoot (*Dactylis glomerata* L.) and tall fescue (*Festuca arundinacea* Schreb.) (pure swards) and their mixtures with three subterranean clover (*Trifolium subterraneum* L.) subspecies in the followed relations: grass + *Trifolium subterraneum ssp. brachycalicinum* (50:50%); grass + *Trifolium subterraneum ssp. yaninicum* (50:50%); grass + *Trifolium subterraneum ssp. subterraneum* (50:50%); grass + *Trifolium subterraneum ssp. brachycalicinum* +*Trifolium subterraneum ssp. yaninicum* + *Trifolium subterraneum ssp. subterraneum* (25:25:25:25%) was performed. The field experiment was carried out at the Institute of Forage Crops, Pleven, Bulgaria (2011-2013). It was found that *Trifolium subterraneum ssp. brachycalicinum* in mixture with cocksfoot improves principal chemical composition and *in vitro* digestibility of forage biomass dry matter. The energy feeding value (Feed units for milk and Feed units for growth) was the highest for the mixtures of cocksfoot with *Trifolium subterraneum ssp. brachycalicinum.* The protein feeding value (Total digestible protein, PDIN, PDIE) significantly increased for all kind of cocksfoot – subterranean clover mixtures. Their forage quality was improved in comparison with the same mixtures of tall fescue. The principal chemical composition of forage obtained from cocksfoot – subterranean clover mixtures was more balanced - *in vitro* digestibility, net energy and protein feeding value were found be higher.

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INTRODUCTION

In view of better relationship between forage production and animal husbandry the creation of high productive grasslands for hay and pasture direction of use is important. Mixtures with properly chosen components ensured both, balanced nutritive composition and better intake of forage by animals (Komarek et al., 2007).

Legumes are sources of high quality protein forages for intensive animal production (Anwar et al., 2010). Their participation on the composition of mixtures prolonged the period for ensuring the animals with forage, contributed to quality improvement and enchanced animal productivity (Porqueddu et al., 2003; Lemus, 2013).

The development of forage base is in agreement with the needs of animals for the relaying of its productive potential but in the future and with the climate changes occurred (Gornall et al., 2010; Mihovski and Kirilov, 2014; Luscher et al., 2014). Components adapted to the climate conditions will be included in mixtures (Lelièvre and Volaire, 2009). 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 (Frame et al., 1998; Pecetti and Piano, 1998, 2002; Kyriazopoulos et al., 2008) is a new crop for Bulgaria. The subterranean clover is an annual drought resistant ephemeral legume with winter-spring type of development and ability for self-sowing (Yakimova and Yancheva, 1986; Piano et al., 1996; Frame et al., 1998). It is strong tolerate to grazing, the forage has high feeding value and good intake from animals as grazing as well as hay and silage (Ru and Fortune, 2001; Frame, 2005). Subterranean clover's forage is preferred for the feed rations of lambs and cows (Stockdale et al., 1992; Mulholland et al., 1996).

Studies with subterranean clover during the last years showed that it has practical applicability under the climatic conditions of Bulgaria (Vasilev, 2006, 2009; Vasileva et al., 2011; Vasilev and Vasileva, 2012; Ilieva et al., 2015). It is suitable component for mixtures with commonly used for forage production grasses and legumes as well (Ilieva and Vasileva, 2011; Vasileva and Vasilev, 2012; Vasileva, 2015).

The aim of present work was to determine the quality characteristics of the forage, i.e. chemical composition, digestibility, energy and protein nutritive value from cocksfoot and tall fescue and their mixtures with subterranean clover.

MATERIALS AND METHODS

Experimental work was done in the Institute of Forage Crops, Pleven, Bulgaria (2011-2013). Cocksfoot (*Dactylis glomerata* L.) (cv. Dabrava) and tall fescue (*Festuca arundinacea* Schreb.) (cv. Albena), pure grown and in mixture with 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 studied. In field trial (long plot method, plot size of 70 m^2 and 4 replications), the next variants were investigated: Cocksfoot (100%); Cocksfoot + *Trifolium subterraneum ssp. brachycalicinum* (50:50%); Cocksfoot + *Trifolium subterraneum ssp. yaninicum* (50:50%); Cocksfoot + *Trifolium subterraneum ssp. subterraneum* (50:50%); Cocksfoot + *Trifolium subterraneum ssp. brachycalicinum* + *Trifolium subterraneum ssp. yaninicum* + *Trifolium subterraneum ssp. subterraneum* (25:25:25:25%); Tall fescue (100%); Tall fescue + *Trifolium subterraneum ssp. brachycalicinum* (50:50%); Tall fescue + *Trifolium subterraneum ssp. yaninicum* (50:50%); Tall fescue + *Trifolium subterraneum ssp. subterraneum* (50:50%); Tall fescue + *Trifolium subterraneum ssp. brachycalicinum* + *Trifolium subterraneum ssp. yaninicum* + *Trifolium subterraneum ssp. subterraneum* (25:25:25:25%). 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) (AOAC, 2007) (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 polisoides 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/NDFx100 (Akin and Chesson, 1990).

Enzymatic *in vitro* digestibility/degradability *in vitro* of dry (IVDMD, %) and organic (IVOMD, %) matter was performed by two stage pepsin-cellulase method of 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 (AOAC, 2001), and degradability of organic matter (Aufrere, 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.

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

RESULTS AND DISCUSSIONS

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 mixtures of cocksfoot with *Trifolium subterraneum ssp. brachycalicinum* increased by 1.92% in first cut after the year of sowing (Table 1). The effect of subterranean clover was found be higher during the next year when in mixture with *Trifolium subterraneum ssp. yaninicum*, crude protein content increased by 2.06%.

Data averaged for three cuts showed that in mixture of cocksfoot with *Trifolium subterraneum ssp. brachycalicinum* crude protein content was by 1.02% higher. There were any differences for the mixtures with *Trifolium subterraneum ssp. yaninicum* and *Trifolium subterraneum ssp. subterraneum*, as well for four-component mixtures.

In mixtures of tall fescue with *Trifolium subterraneum ssp. brachycalicinum* crude protein

content during the second year was found be increased by 0.17% for first and by 0.66% for the second cut, respectively.

Table 1. Principal chemical composition of forage from cocksfoot (*Dactylis glomerata* L.) and tall fescue (*Festuca arundinacea* Schreb.) in pure swards and in grass-legume mixtures with subrerranean clover (% of dry matter)

Plant species/Mixture *	Crude protein			
	I/2012	I/2013	II/2013	mean
Dactylis glomerata L.	11.58	9.40	10.35	10.44
D. glomerata + Trs brach	13.50	9.88	10.90	11.43
D. glomerata + Trs yanin	9.85	11.46	9.93	10.41
D. glomerata + Trs subter	11.35	10.09	10.11	10.52
D. glomerata + Tb+Ty+Ts	9.87	9.80	10.41	10.03
Mean-two-components mixtures	11.57	10.48	10.31	10.79
SE (P=0.05)	0.67	0.35	0.16	0.23
F. arundinacea Schreb.	10.59	9.64	9.71	9.98
F. arundinacea + Trs brach	10.37	9.81	10.37	10.18
F. arundinacea + Trs yanin	8.74	8.65	9.17	8.85
F. arundinacea + Trs subter	8.82	8.72	9.41	8.98
F. arundinacea +Tb+Ty+Ts	10.48	9.38	8.86	9.57
Mean-two-components mixtures	10.48	9.38	8.86	9.57
SE (P=0.05)	0.41	0.23	0.25	0.30
	Crude fiber			
Dactylis glomerata L.	30.41	27.61	26.75	28.26
D. glomerata + Trs brach	27.08	26.97	26.64	26.90
D. glomerata + Trs yanin	31.04	25.56	27.43	28.01
D. glomerata + Trs subter	29.43	27.95	28.27	28.55
D. glomerata + Tb+Ty+Ts	28.09	26.42	27.46	27.32
Mean-two-components mixtures	29.21	26.90	27.31	27.81
SE (P=0.05)	0.72	0.42	0.29	2.04
Festuca arundinacea Schreb.	29.99	27.25	24.42	27.22
F. arundinacea + Trs brach	31.00	29.38	25.69	28.69
F. arundinacea + Trs yanin	29.70	27.42	27.61	28.24
F. arundinacea + Trs subter	29.37	26.23	26.68	27.43
F. arundinacea +Tb+Ty+Ts	30.70	24.00	27.50	27.40
Mean-two-components mixtures	30.15	26.86	26.38	27.80
SE (P=0.05)	0.30	0.87	0.59	0.28

(*Trs brach - Trifolium subterraneum ssp. brachycalicinum; Trs yanin - Trifolium subterraneum ssp. yaninicum; Trs subter - Trifolium subterraneum ssp. subterraneum; Tb+Ty+Ts - Trifolium subterraneum ssps. brachycalicinum+yaninicum+subterraneum*)

Subterranean clover in mixture with cocksfoot had bigger effect on crude protein content as compared to mixture with tall fescue.

On averaged from two-component mixtures of cocksfoot crude protein content was found be higher by 1.22%.

Crude fiber content in forage biomass of all mixtures studied was found be higher during the first experimenta year, when prolonged drougth was occured. 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).

In mixtures of cocksfoot with subterranean clover crude fiber content decreased in the first year – by 3.33% for *Trifolium subterraneum ssp. brachycalicinum* and by 2.32% for four-component mixtures.

Crude fiber content in mixtures of cocksfoot with *Trifolium subterraneum ssp. brachycalicinum* decreased by 1.36% on average for three cuts.

Crude fiber content in mixtures of tall fescue decreased in the first cut for two experimental years.

Subterranean subspecies differ in digestibility of dry matter in the forage (McLaren and Doyle, 1994; Ru and Fortune, 1999, 2000).

In first cut in the year after sowing for mixtures of cocksfoot with *Trifolium subterraneum ssp. brachycalicinum* and with *Trifolium subterraneum ssp. subterraneum* the digestibility, especially with *Trifolium subterraneum ssp. brachycalicinum* was found be increased significantly (Table 2). During the next experimental year for the two cuts obtained, mixtures of cocksfoot with *Trifolium subterraneum ssp. brachycalicinum* and *Trifolium subterraneum ssp. yaninicum* showed higher digestibility.

Table 2. Digestibility of forage from cocksfoot (*Dactylis glomerata* L.) and tall fescue (*Festuca arundinacea* Schreb.) in pure swards and in grass-legume mixtures with subrerranean clover (% *in vitro* dry matter digestibility)

Plant species/Grass-Legume mixture	Digestibility of DM			
	I/2012	I/2013	II/2013	mean
Dactylis glomerata L.	59.75	61.68	60.01	60.48
D. glomerata + Trs brach	68.39	63.30	64.24	65.31
D. glomerata + Trs yanin	53.80	65.01	62.74	60.52
D. glomerata + Trs subter	61.84	60.49	59.84	60.72
D. glomerata + Tb+Ty+Ts	57.64	61.97	59.74	59.78
Mean-two-components mixtures	61.34	62.93	62.27	62.18
SE (P=0.05)	2.42	0.77	0.92	0.99
Festuca arundinacea Schreb.	43.66	53.74	53.97	50.46
F. arundinacea + Trs brach	58.02	52.93	51.41	54.12
F. arundinacea + Trs yanin	51.36	56.85	54.77	54.33
F. arundinacea + Trs subter	54.52	56.92	52.43	54.62
F. arundinacea + Tb+Ty+Ts	61.86	60.29	53.74	58.63
Mean-two-components mixtures	54.63	55.57	52.87	54.36
SE (P=0.05)	3.09	1.31	0.59	1.29

Forage quality of subterranean clover was 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/.

Forage biomass from mixture of cocksfoot with *Trifolium subterraneum ssp. brachycalicinum* showed higher digestibility (65.31).

For mixtures of tall fescue in first cut in the year after sowing digestibility was found be significantly higher, especially in *Trifolium subterraneum ssp. brachycalicinum*, followed by *Trifolium subterraneum ssp. subterraneum* and *Trifolium subterraneum ssp. yaninicum*. During the next experimental year for the two cuts obtained higher digestibility was observed in mixtures with *Trifolium subterraneum ssp. yaninicum*.

Digestibility of forage biomass was significantly higher in all tall fescue-subterranean clover mixtures studied and varied for two- component mixtures in narrow limits. The highest digestibility was found in four-component mixtures (58.63) due to the higher part of subterranean clover.

As a whole mixtures of cocksfoot with subterranean clover were significantly more digestible as compared to these of tall fescue (on average by 7.83%). Forage from mixture of cocksfoot with *Trifolium subterraneum ssp. yaninicum* showed the lowest content of Acid-detergent fiber (ADF) (35.33) (Table 3). Forage

Table 3. Plant cell walls fiber components content of forage from cocksfoot (*Dactylis glomerata* L.) and tall fescue (*Festuca arundinacea* Schreb.) in pure swards and in grass-legume mixtures with subrerranean clover (% of dry matter)

Plant species/Mixture	NDF			ADF			NDF/ADL		
	I/2013	II/2013	mean	I/2013	II/2013	Mean	I/2013	II/2013	mean
Dactylis glomerata L.	57.14	60.52	58.83	37.63	38.02	37.82	3.59	4.87	4.23
D. glomerata + Trs brach	53.68	57.95	55.82	35.99	37.29	36.64	4.06	3.43	3.74
D. glomerata + Trs yanin	51.56	57.48	54.52	32.27	38.39	35.33	3.95	4.26	4.10
D. glomerata+ Trs subter	54.37	57.32	55.84	38.37	38.93	38.65	4.40	4.01	4.20
D. glomerata+Tb+Ty+Ts	51.79	58.99	55.39	37.30	37.87	37.58	3.88	3.53	3.70
Mean-two-component mixtures	53.20	57.58	55.39	35.54	38.20	36.87	4.14	3.90	4.01
SE (P=0.05)	1.01	0.59	0.72	1.08	0.27	0.56	0.13	0.26	0.11
Festuca arundinacea Schreb.	56.14	59.92	58.03	37.98	36.02	37.00	3.72	3.81	3.76
F.arundinacea + Trs brach	58.39	54.55	56.47	39.22	36.56	37.89	4.26	4.31	4.28
F.arundinacea + Trs yanin	56.09	59.51	57.80	40.19	38.29	39.24	4.69	3.33	4.01
F.arundinacea + Trs subter	55.47	60.52	58.00	36.21	38.31	37.26	3.56	3.94	3.75
F.arundinacea + Tb+Ty+Ts	51.74	59.51	55.62	34.95	38.60	36.78	3.61	3.91	3.76
Mean-two-component mixtures	56.65	58.19	57.42	38.54	37.72	38.13	4.17	3.86	4.01
SE (P=0.05)	1.07	1.07	0.48	0.95	0.52	0.44	0.21	0.15	0.10

biomass from mixture of cocksfoot with three subterranean clover subspecies and these of cocksfoot with *Trifolium subterraneum ssp. yaninicum* had the lowest lignin content (3.70 and 3.74). For mixtures of cocksfoot the lowest degree of lignification (6.74) showed these with *Trifolium subterraneum ssp. brachycalicinum* and four-component mixtures (Figure 1).

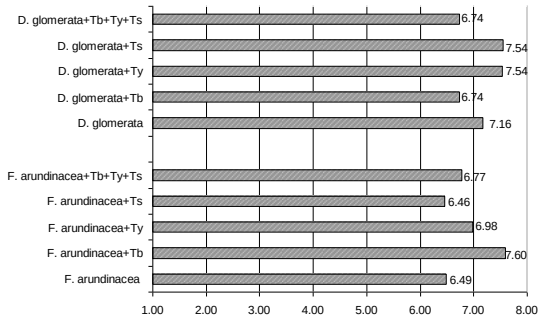

Figure 1. Degree of lignification of forage from cocksfoot (*Dactylis glomerata* L.) and tall fescue (*Festuca arundinacea* Schreb.) in pure swards and in grass-legume mixtures with subrerranean clover (coeff.)

For mixtures with tall fescue the lowest degree of lignification (coef. 6.46) showed these with *Trifolium subterraneum ssp. subterraneum.* Mixtures of tall fescue with subterranean clover showed lower degree of lignification.

Pollysoides chemicellulose and cellulose were determined. Their content decreased in all mixtures studied (Table 4).

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. (McLaren and Doyle, 1994). Individual plant parts have different digestibility. The leaves are more digestible as compared to stems and have higher nitrogen content (Stockdale, 1992; Mulholland et al., 1996).

The energy feeding value (Feed units for milk and Feed units for growth) was highest for mixtures of cocksfoot with *Trifolium subterraneum ssp. brachycalicinum* (UFL 0.717, UFV 0.616) and with *Trifolium subterraneum ssp. yaninicum* (UFL 0.718, UFV 0.615) (Table 5).

Table 4. Hemicellulose and Cellulose content in forage from cocksfoot (*Dactylis glomerata* L.) and tall fescue (*Festuca arundinacea* Schreb.) in pure swards and in grass-legume mixtures with subrerranean clover (% of dry matter)

Plant species/Mixture	Hemicellulose		
	I/2013	II/2013	mean
Dactylis glomerata L.	19.51	22.50	21.01
D. glomerata + Trs brach	17.69	20.66	19.18
D. glomerata + Trs yanin	19.29	19.09	19.19
D. glomerata + Trs subter	16.00	18.39	17.19
D. glomerata + Tb+Ty+Ts	14.49	21.12	17.81
Mean-two-component mixtures	17.66	19.38	18.52
SE (P=0.05)	0.96	0.73	0.66
Festuca arundinacea Schreb.	18.16	23.90	21.03
F. arundinacea + Trs brach	19.17	17.99	18.58
F. arundinacea + Trs yanin	15.90	21.22	18.56
F. arundinacea + Trs subter	19.26	22.21	20.74
F. arundinacea + Tb+Ty+Ts	16.79	20.91	18.84
Mean-two-component mixtures	18.11	20.47	19.29
SE (P=0.05)	0.66	0.96	0.54
		Cellulose	
Dactylis glomerata L.	34.04	33.15	33.59
D. glomerata + Trs brach	31.93	33.86	32.90
D. glomerata + Trs yanin	28.32	34.13	31.23
D. glomerata + Trs subter	33.97	34.92	34.45
D. glomerata + Tb+Ty+Ts	33.42	34.34	33.88
Mean-two-component mixtures	31.41	34.30	32.86
SE (P=0.05)	1.07	0.29	0.55
Festuca arundinacea Schreb.	34.26	32.21	33.24
F. arundinacea + Trs brach	34.96	32.25	33.61
F. arundinacea + Trs yanin	35.50	34.96	35.23
F. arundinacea + Trs subter	32.65	34.37	33.51
F. arundinacea + Tb+Ty+Ts	31.34	34.69	33.02
Mean-two-component mixtures	34.37	33.86	34.12
SE (P=0.05)	0.76	0.60	0.39

In mixtures of tall fescue the highest energy feeding value had forage from mixtures with *Trifolium subterraneum ssp. subterraneum* (UFL 0.642, UFV 0.530) and with *Trifolium subterraneum ssp. yaninicum* (UFL 0.646, UFV 0.536).

The energy feeding value of two-components mixtures of cocksfoot with subterranean clover was higher as compared to the same from two-component mixtures of tall fescue with subterranean clover (for UFL by 0.076 and for UFV by 0.086).

Table 5. Energy feeding value in forage from cocksfoot (*Dactylis glomerata* L.) and tall fescue (*Festuca arundinacea* Schreb.) in pure swards and in grass-legume mixtures with subrerranean

Plant species/Mixture	UFL			UFV		
	I/2013	II/2013	mean	I/2013	II/2013	mean
Dactylis glomerata L.	0.712	0.666	0.689	0.610	0.557	0.584
D. glomerata + Trs brach	0.703	0.731	0.717	0.600	0.631	0.616
D. glomerata + Trs yanin	0.723	0.712	0.718	0.621	0.609	0.615
D. glomerata + Trs subter	0.693	0.694	0.694	0.588	0.589	0.589
D. glomerata + Tb+Ty+Ts	0.698	0.683	0.690	0.594	0.576	0.585
Mean-two-component mixtures	0.706	0.712	0.710	0.603	0.610	0.607
SE (P=0.05)	0.005	0.001	0.006	0.005	0.001	0.007
Festuca arundinacea Schreb.	0.623	0.622	0.623	0.510	0.509	0.510
F. arundinacea + Trs brach	0.615	0.610	0.613	0.501	0.495	0.498
F. arundinacea + Trs yanin	0.651	0.642	0.646	0.542	0.531	0.536
F. arundinacea + Trs subter	0.658	0.625	0.642	0.549	0.512	0.530
F. arundinacea + Tb+Ty+Ts	0.688	0.634	0.661	0.583	0.522	0.552
Mean-two-component mixtures	0.641	0.626	0.634	0.531	0.513	0.521
SE (P=0.05)	0.001	0.005	0.008	0.001	0.006	0.009

Table 6. Protein feeding value in forage from cocksfoot (*Dactylis glomerata* L.) and tall fescue (*Festuca arundinacea* Schreb.) in pure swards and in grass-legume mixtures with subrerranean clover

Plant species/Mixture	PBD/TDP			PDIN			PDIE		
	I/2013	II/2013	mean	I/2013	II/2013	mean	I/2013	II/2013	mean
	g kg^{-1} dry matter								
Dactylis glomerata L.	54	64	59	60	65	63	75	76	76
D. glomerata + Trs brach	59	69	64	62	68	65	77	81	79
D. glomerata + Trs yanin	74	60	67	72	62	67	81	78	80
D. glomerata + Trs subter	60	61	61	63	64	64	75	77	76
D. glomerata + Tb+Ty+Ts	58	64	60	62	65	64	76	77	77
Mean-two-component mixtures	64	63	64	66	65	65	78	79	78
SE (P=0.05)	3.4	1.5	1.4	2.1	0.9	0.6	1.1	0.8	0.8
Festuca arundinacea Schreb.	56	57	57	61	61	61	70	71	71
F. arundinacea + Trs brach	58	64	61	62	65	64	70	71	71
F. arundinacea + Trs yanin	47	53	50	54	58	56	70	72	71
F. arundinacea + Trs subter	48	55	52	55	59	57	71	71	71
F. arundinacea +Tb+Ty+Ts	54	50	52	59	56	58	74	71	73
Mean-two-component mixtures	51	57	54	57	61	59	70	71	71
SE (P=0.05)	2.1	2.3	2.0	1.5	1.5	1.4	0.7	0.2	0.4

Protein feeding value significant increased in all mixtures of cocksfoot with subterranean clover (Table 6). The highest protein feeding value was found in mixtures with *Trifolium subterraneum ssp. yaninicum* regarding the charactersitics PDIN-Protein digestible dans l'intestine in dependence of nitrogen (67 g kg^{-1} dry matter) and PDIE–Protein digestible dans l'intestine in dependence of energy (80 g kg^{-1} dry matter) as well total digestible protein (67 g kg^{-1} dry matter).

In mixtures of tall fescue the protein nutritive value was the highest in mixtures with *Trifolium subterraneum ssp. brachycalicinum* regarding the charactersitics PDIN-Protein digestible dans l'intestine in dependence of nitrogen (64 g kg^{-1} dry matter) and total digestible protein (61 g kg^{-1} dry matter).

As a whole the protein nutritive value of mixture of cocksfoot with subterranean clover was higher (for total digestible protein by 9.7 g kg^{-1} dry matter, for PDIN by 6.0 g kg^{-1} dry matter and for PDIE by 7.0 g kg^{-1} dry matter) as compared to this of mixtures with tall fescue.

Forage obtained from mixtures of cocksfoot and tall fescue with subterranean clover showed better quality characteristics as compared to these from pure grown grasses.

CONCLUSIONS

The inclusion of *Trifolium subterraneum ssp. brachycalicinum* as legume component in mixtures with cocksfoot improved the principal chemical composition of forage obtained from mixtures (crude protein content increased by 1.02% and crude fiber content decreased by 1.36%).

The highest digestibility of dry matter showed forage biomass of mixtures of cocksfoot with *Trifolium subterraneum ssp. brachycalicinum* (65.31%).

The lowest degree of lignification (coef. 6.46) showed mixtures of tall fescue with *Trifolium subterraneum ssp. subterraneum.*

The energy feeding value (Feed units for milk and Feed units for growth) was the highest for mixtures of cocksfoot with *Trifolium subterraneum ssp. brachycalicinum* (UFL 0.717, UFV 0.616) and with *Trifolium subterraneum ssp. yaninicum* (UFL 0.718, UFV 0.615).

The protein feeding value (total digestible protein, PDIN, PDIE) significantly increased for all kind of cocksfoot – subterranean clover mixtures. The highest protein feeding value was found in mixtures of cocksfoot with *Trifolium subterraneum ssp. yaninicum* regarding protein, digestible dans l'intestine in dependence of nitrogen (PDIN 67 g kg^{-1} dry matter) and protein digestible dans

l'intestine in dependence of energy (80 g kg^{-1} dry matter) as well total digestible protein (67 g kg^{-1} dry matter).

As a whole, mixtures of cocksfoot with subterranean clover showed better quality of forage biomass as compared to mixtures with tall fescue. They have more balanced principal chemical composition (higher crude protein content average by 1.22%), higher digestibility (average by 7.83%), higher both net energy and protein feeding value (for total digestible protein by 9.7 g kg^{-1} dry matter, for PDIN by 6.0 g kg^{-1} dry matter and for PDIE by 7.0 g kg^{-1} dry matter, respectively).

REFERENCES

Akin, D.E., & Chesson, A. (1990). Lignification as the major factor limiting forage feeding value especially in warm conditions. In: Proceedings XVI Int. Grassland Cong., Vol. III. Association Francaise pour la Production Fourragere. Versailles, France. pp. 1753-1760.

Andrieu, J. & Demarquilly, C. (1989). Prediction of the digestible and metabolisable energy content of forages from their chemical composition and organic matter digestibility. In: Proceedings (p. 875-876). Presented at 16. International Grassland Congress, Nice, FRA (1989-10-04-1989-10-11). Versailles, FRA: Association Française pour la Production Fourragère.

Anwar, A., Ansar, M., Nadeem, M., Ahmad, G., Khan, S., & Hussain, A. (2010). Performance of non-traditional winter legumes with oats for forage yield underrainfed conditions. *J. Agric. Res*., 48(2), 171–179.

AOAC, (2007).Official methods of analysis. 17-th ed. Association of Analytical Chemists, Gaitensburg , MD, USA.

Aufrère J., (1982). Etude de la prévision de la digestibilité des fourrages par une méthode enzymatique. *Ann. Zootech*., (31), 11-30.

Availabale from: /http://msucares.com/crops/forages/legumes/cool/subterraneanclover.html/

Frame, J., (2005). Forage legumes for temperate grasslands. Rome: Food and Agriculture Organization of the United Nations. Plymouth UK: Science Publishers Inc. 320 p.

Frame, J., Charlton, J.F.L., & Laidlaw, A.S. (1998). Temperate Forage Legumes. CAB International, Wallingford, p. 327.

Goering, H. K. and Van Soest, P.J. (1970). Forage Fiber Analysis (apparatus, reagents, prosedures and some applications). *USDA Agricultural Handbook* No. 379.

Gornall, J., Betts, R., Burke, E., Clark, R., Camp, L., Willett, K., & Wiltshire, A. (2010). Implications of climate change for agricultural productivity in the early twenty-first century. *Philosophical Transactions of the Royal Society* B 365, 2973–2989.

Ilieva, A., Vasileva, V. & Katova, A. (2015). The effect of mixed planting of birdsfoot trefoil, sainfoin, subterranean clover, and tall fescue on nodulation, and nitrate reductase activity in shoots. *Journal of Global Agriculture and Ecology*, 3 (4), 222-228.

Ilieva, A. and Vasileva V. (2011). Study on nodule formation and nitrogenase activity in some grass mixtures. *Journal of Mountain Agriculture on the Balkans Agricultural Academy*, (14), 513-530.

INRA, (1988). Alimentation des bovins, ovins et caprins. R. Jarrige (ed.) INRA Publ., Versailles, France, 471 pp.

Komarek, P., Nerušil, P., Kohoutek, A., & Odstrčilova, V. (2007). The effect of repeted direct sowing of grass-legume seed mixtures into grasslands on forage production and quality. *Grassland Science in Europe* (12), 39-42.

Kyriazopoulos, A. P., Abraham, E. M., Parissi, Z. M., & Nastis, A.S. (2008). Herbage production and nutritive value of *Dactylis glomerata* L. and *Trifolium subterraneum* L. alone and in mixtures. *Options Méditerranéennes* (79), 211 - 214.

Lelièvre, F., & Volaire, F. (2009). Current and Potential Development of Perennial Grasses in Rainfed Mediterranean Farming Systems. *Crop Science*, 49, 6, 2371-2378. DOI: 10.2135/cropsci2009.06.0324

Lemus, R., (2013). Self-reseeding Potential of Annual Clovers. *Forage News*, Mississippi State University, (6), 1-2.

Luscher, A., Mueller-Harvey, I., Soussana, J.F., Rees, R.M., & Peyraud, J.L. (2014). Potential of legume-based grassland–livestock systems in Europe: a review. *Grass and Forage Scienc*e, (69), 206-228.

McLaren, S.E., & Doyle, P.T. (1994). Dry matter digestibility of subterranean clover during senescense and after death, Proc. Aust. Soc. Anim. Prod., 20, 221-224.

Mihovski, Ts., & Kirilov, A. (2014). State of ruminant animals' stockbreeding and the respective forage base in Bulgaria. Aktualni poznatky v pestovani, slechteni, ochrane rostlin a zpracovani produktu, "Uroda 12/2014, vedecka priloha casopisu", eds. Badalikova B. and Bartlova J., ISSN 0139-6013, 105-110.

Mulholland, J.G., K.S. Nandra, G.H. Scott, A.W. Jones, & Coombes, N.E. (1996). Nutritive value of subterranean clover in a temperate environment. *Australian Journal of Experimental Agriculture* (36), 803-814.

Pecetti, L., & Piano, E. (1998). Leaf size variation in subterranean clover *(Trifolium subterraneum* L. sensu lato). *Genetic Resources and Crop Evolution*, (45), 2, 161-165.

Pecetti, L., & Piano, E. (2002). Variation of morphological and adaptive traits in subterranean clover populations from Sardinia (Italy). *Genetic Resources and Crop Evolution*, (49), 2, 189-197.

Piano, E., Pecetti, L., & Carroni, A.M. (1996). Climatic adaptation in subterranean clover populations. *Euphytica*, (92), 1-2, 39-44.

Porqueddu, C., Parente, G., & Elsaesser, M. (2003). Potential of grasslands. In: A. Kirilov, N. Todorov & I. Katerov (eds.). *Grassland Science in Europe*, (8), 11-20.

Ru, Y. I., & Fortune, M., (2001). Seed yield and nutritive value of dry, mature subterranean clover (*Trifolium subterraneum* L.). Australian Journal of Experimental Agriculture, (41), 2, 169-175.

Ru, Y. J., & Fortune, J. A. (1999). Sward characteristics and Nutritive Value of two Ciltivars of Subterranean Clover. *Asian-Aus, J. Animal Science*, (12), 8, 1192-1199.

Ru, Y. J., & Fortune, J. A. (2000). Variation in nutritive value of plant parts of subterranean clover (*Trifolium subterraneum* L.). *Australian Journal of Experimental Agriculture*, (40), 397-403.

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. Australian Journal of Agricultural Research (43), 1265-1280.

Todorov, N., Atanassov, A., Ilchev, A., Gantchev, G., Mihailova, G., Girginov, D., Penkov, D., Shindarska, Z., Naydenova, Y., Nedjalkov, K., & Tchobanova, S. (2010). Practicum in animal nutrition. Ed: N.Todorov N., East-West, Sofia, Bulgaria, ISBN 978-954-321-733-5, 463.

Vasilev, E., (2009). Chemical composition of subclovers forage (*Tr. subterraneum* L.) and crude protein yield in pasture mixtures with grasses. *Journal of Mountain Agriculture on the Balkans*, (12), 2: 329-341.

Vasilev, E., (2006). Productivity of subterranean clover (*Tr. subterraneum* L.) in pasture mixtures with some perennial grasses for the conditions of Central North Bulgaria. *Plant Sciences*, Sofia, (43), 149-152 (In Bulgarian).

Vasileva, V. (2015). Botanical composition of swards with participation of subterranean clover (*Trifolium subterraneum* L.). *Bulletin of the Union of Scientists* – Rousse, Agriculture and Veterinary Medicine Sciences, (7), 160-165 (in Bulgarian).

Vasileva, V., & Vasilev, E., (2012). Study on Productivity of some Legume Crops in Pure Cultivation and Mixtures. *Agriculturae Conspectus Scientificus*, (77), 2, 91-94.

Vasileva, V., Vasilev, E., & Athar, M., (2011). Nodulation and root establishment of two clover species grown in pasture mixtures with wheatgrass. *FUUAST Journal of Biology*, 1, (1): 1-4.

Vasileva, V., & Vasilev E., (2012). Dry mass yield from some pasture mixtures with subterranean clover (*Trifolium subterraneum* L.). *Journal of Mountain Agriculture on the Balkans*, (15), 1024-1033.

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. Netherlands Journal of Agricultural Science, 39, 31-48.

Yakimova, Y., & Yancheva, H., (1986). Phytocenological and ecological characteristics of some annual clovers in the Strandja region. *Plant Sciences*, (23), 47-53 (in Bulgarian).