

(*Dactylis glomerata* L.)

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Chemical composition, digestibility and feeding value of accessions orchardgrass (*Dactylis glomerata* L.)

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SUMMARY

Forage quality by chemical composition, digestibility, energy and protein feeding value of six accessions orchardgrass (*Dactylis glomerata* L.) grown in collection nurseries – Bulgarian and introduced varieties and ecotypes in spring, summer and autumn growths are evaluated in field trial in the Institute of Forage Crops, Pleven. The parameters of general chemical composition (Weende analysis), plant cell walls fiber components content by detergent analysis (Van Soest), enzyme *in vitro* digestibility of dry and organic matter (method d'Aufrere), potential energy and potential protein feeding value by different systems are determined. In the **first spring growth** the highest protein content 20,79, the lowest of fiber components CF 23,50%, NDF 51,87%, ADF 36,09 and the highest digestibility of dry matter 60,72% belongs to Var.25, followed by variants 30,26. The greatest variability is character to parameters ADL and degree of lignification: CV 31,8% 34,0% respectively. The highest Relative Feeding Value (RFV) is 109 and 102 rel.% for Var.25 and Var.27. Energy feeding value UFL-UFV 0,703-0,593 have

UFL-UFV 0,703-0,593
 .27,
 .25 – 160 g kg⁻¹
 .30 – 152 g kg⁻¹.

30
 .30
 68,89%
 (RFV) 106
 .%, 26, 25, 27, 30
 RFV .26 –
 115 .%.
 .30 UFL-UFV
 0,777-0,677.
 PBD-PDIN-
 PDIE:162,129,100 g kg⁻¹ (.26).

72,69%,
 74,07% (.30). RFV
 141 .%,
 .25 – 150 .%. .30

.27, 25, 30 –
 PBD-PDIN-PDIE: 196, 151, 112 g kg⁻¹

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 (*Dactylis glomerata* L.),

(*Dactylis glomerata*
 L.)
 (Santen and Sleeper,
 1961).
D. glomerata
Dactylis,

(Stewart and Ellison, 2011).

maximal values in Var.27 and maximal protein feeding value belong to Var.25 - 160 g kg⁻¹ dry matter and Var.30 – 152 g kg⁻¹. In the **second summer growth** all accessions demonstrate by mean values better forage quality – higher protein content, lower fiber components content and higher digestibility. The variants 25, 26 and 30 exceed mean protein and crude fiber values and Var.30 distinguish the highest digestibility 68,89%. The mean RFV is 106 rel.% as Variants 26, 25, 27, 30 exceed it. The maximal RFV belong to Var.26 – 115 rel.%. Energy net feeding value is the highest for Var.30 UFL-UFV 0,777-0,677. The maximal protein feeding value belongs to Var.26: PBD-PDIN-PDIE: 162, 129, 100 g kg⁻¹ respectively. The changes in orchardgrass forage digestibility from low to higher are consequently from first toward second and third growths. The mean digestibility of the accessions from **third autumn growth** is 72,69% and maximal 74,07% (Var.30). The mean RFV of the accessions is 141 rel.% and the highest is for Var.25 – 150 rel.%. The variant 30 demonstrates the highest net energy feeding value by different systems of evaluation and protein feeding value is high for the accessions Var.27, 25, 30 – PBD-PDIN-PDIE: 196, 151, 112 g kg⁻¹ dry matter.

Key words: orchardgrass (*Dactylis glomerata* L.), breeding, digestibility, energy and protein feeding value

INTRODUCTION

Orchardgrass (*Dactylis glomerata* L.) is an auto-tetraploid, out-crossing forage grass, and is one of the main perennial grasses used for grazing and hay production in temperate climates (Santen and Sleeper, 1961). Although *D. glomerata* is the sole species in the *Dactylis* genus, the species includes both Mediterranean and Continental ecogeographic subspecies that are diploid and tetraploid (Stewart and Ellison, 2011).

(*Medicago sativa* L.)
(Chamblee and Lovvorn, 1953; Santen and Sleeper, 1961; Brummer and Moore, 2000).

18
(Lumaret, 1988),
D. glomerata ssp.
glomerata
(Lindner et al., 2004)

(Peeters 2004).
200
50-
; 133
(Sanada et al., 2010a).

(
)
(Sanada et al.,
2010b),

(Dent and Aldrich 1963).

- Orchardgrass is highly preferred by livestock, exhibits early season growth, and is one of the most compatible perennial forage grasses when sown with perennial legumes, e.g., alfalfa (*Medicago sativa* L.) (Chamblee and Lovvorn, 1953; Santen and Sleeper, 1961; Brummer and Moore, 2000). It has a high and stable quality biomass yield with a large range of climate tolerance. It is frost-, shade-, heat- and drought tolerant with good regrowth ability.

- Among 18 subspecies with three ploidy levels (Lumaret 1988), tetraploid *D. glomerata* ssp. *glomerata* is the most common. It has high morphological variability (Lindner et al. 2004) and represents the major cocksfoot subspecies for forage production in Europe (Peeters 2004). More than 200 cultivars of cocksfoot have been bred since 1950s in the world; 133 of them are now maintained in Europe (Sanada et al. 2010a). Improving forage production and quality under abiotic stresses (tolerance to drought and cold) are objectives in orchardgrass breeding and genetics programs throughout the world (Sanada et al. 2010b) as also to increase disease resistance. The most important cocksfoot breeding criteria in Bulgaria, besides DMY and quality, persistency and rust resistance, is earliness or lateness, because it is a regular component of grass-legume mixtures. The optimal timing of cutting or ensiling (i.e., optimal balance between DMY and quality) for cocksfoot does not coincide with most other species but best suits with alfalfa.

- Cocksfoot can be classified as early to intermediate in maturity in comparison with other grasses and legumes (Dent and Aldrich 1963); At the same time, new material should be productive and adapted to local agro-ecological conditions. Very often required characteristics are available in natural populations, what refers them to direct

(Posselt and Willner, 2007).
 (Boller and Green, 2010, Sokolovic et al., 2016).
 (*Dactylis glomerata* L.)
 1966 . 1978 .
 (Katova, 2016).
 8 t/ha 0,5-0,6 t/ha
 (Naydenova, 2009; 2012; Naydenova et al., 1999; 2001).
 1978 ..
 1998 .
 (Tomov, 1979; 1987).
 25 ..
 (Santen and Sleeper, 1996)
 (Lacefield et al., 2003).
 (Dijk, 1959).

utilization in breeding (Posselt and Willner, 2007). Their value has long been recognized by plant breeders (Boller and Green, 2010; Sokolovic et al., 2016).

In Bulgaria cocksfoot breeding program started in 1966 and in 1978 the first and only variety Dabrava was registered and it's tetraploid (Katova, 2016). In pure sward provides over 8 t/ha of dry matter and 0,5-0,6 t/ha of seeds. Suitable for hay, grazing and silage. Resistant to drought, cold and leaf diseases. Forage quality characteristics, biochemical composition, digestibility and feeding value of variety are established (Naydenova, 2009; 2012; Naydenova et al., 1999; 2001). Dabrava was listed in the varieties list first in 1978, then in 1998 it was certified by the Patent Office of the Republic of Bulgaria (Tomov, 1979; 1987).

This is the oldest variety for our country and is maintained in the IFC - Pleven. The validity of the certificate lasts 25 years, necessitating a new selection program.

Breeding cocksfoot aims to create a highly productive variety with better palatability and forage quality. Cocksfoot starts growth early in spring and develops rapidly. It is tolerant enough of shade, drought, and heat (Santen and Sleeper, 1996), and can be grown in poor and shallow soils. At high rates of nitrogen, it is among the most productive of the cool-season grasses (Lacefield *et al.*, 2003).

However, its palatability and digestibility should be improved (Dijk, 1959). The main shortcoming of cocksfoot is its low palatability caused by harshness of the leaves through the presence of silicified dentations. The task of cocksfoot breeders is to create varieties with soft leaves better palatability at the same time maintaining the yield potential of the most productive cultivars. The aim of our studies was to select cocksfoot breeds with softer leaves and determine among

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(Sampoux et al., 2010).

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(*Dactylis glomerata* L.)

- them the most productive ones with good forage quality.

- Most breeding programmes had long not included bio-chemical traits related to feeding value as selection criteria. However, progress in the understanding of herbage feeding value has helped to define relevant bio-chemical criteria that can now be easily predicted by NIRS methods.

NIRS.

- Consequently, the use of these biochemical traits has become increasingly familiar in the last 10 years both to assess cultivar feeding value and to breed for improved feeding value (Sampoux et al., 2010).

- The aim of the study is to evaluate forage quality by chemical composition, digestibility, energy and protein feeding value of orchardgrass (*Dactylis glomerata* L.) accessions from collection nurseries as the initial breeding resource with a view to the breeding.

MATERIAL AND METHODS

- During the autumn of 2015, in the experimental field of the Institute of forage Crops - Pleven a collection nursery is based consisting of 6 accessions orchardgrass (3 varieties and 3 ecotypes of origin – Bulgarian – 4, Romanian – 2) with plants being individually arranged, through seedlings at a distance of 50/50 cm. Each accession is represented by 50 individual plants. In 2016 simultaneously to the assessment of the production potential of each growth and number, samples are taken for chemical analysis. The forage is obtained from three growths – spring, summer and autumn and its principal chemical composition and digestibility of dry matter are determined. Plant sample preparation from the above ground part of the plants is effectuate by air ventilation at 65⁰ till crumbly at

2015 .

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6 (3

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- 4, - 2)

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50/50 cm,

50

2016 .

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65⁰ -
 20 min 105⁰ ,
 1.0
 mm QC 136 QB 114, Labor Mim,
 () (2010).
 (Goering and Van Soest, 1970) (EN ISO13906 2008)
 fiber (/NDF)/,
 /Acid-detergent fiber (/ADF)/
 detergent lignin (/ADL) (Van Soest et al., 1991).
 =
 ; = - . -
 (/ x100) (Akin and Chesson, 1989).
in vitro (/IVDMD)
 (/IVOMD)
 Aufreere
 (1982) (Todorov et al., 2010).
 RFV;
 (Linn and Martin, 1991).
 (DDM%=88,9-(0,779xADF%);
 (DMI /% body weight/=120/NDF%)
 (RFV=DDMxDMI/1,29)
 :
 1.
 UFL-
 UFV (INRA, 1988),
 (- /FUM-FUG),

previous fixing for 20 min at 105⁰ and grinding till particle size 1,0 mm consequently at laboratory mill QC 136 and QB 114, Labor Mim, Hungary, and obligatory screening.

The forage principal chemical composition is determined by Weende systematic analytic procedure by the parameters crude protein (CP) and crude fiber (CF) (2010). Structural plant cell walls fiber components are analyzed by systematic detergent analysis (Goering and Van Soest, 1970) (EN ISO13906 2008) and presented as percent of dry matter. The following fiber fractions: Neutral-detergent fiber (NDF); Acid-detergent fiber (ADF), Acid-detergent lignin (ADL) are determined (Van Soest et al., 1991). Polyosides hemicellulose and cellulose as a cell walls components, contained in fiber fraction are presented: Hemicellulose = NDF – ADF; Cellulose = ADF – ADL.

The degree of lignification is presented by the coefficient as relation of ADL and NDF (ADL/NDFx100) (Akin&Chesson 1989). Enzyme *in vitro* digestibility of dry (IVDMD) and organic (IVOMD) matter is determined as percent by two stage pepsin-cellulase enzyme method of Aufreere (1982) (Todorov et al. 2010).

Evaluation of feeding value on the basis of fiber components is performed as Relative feeding value RFV; potential intake of digestible dry matter (Linn and Martin, 1991).

The Digestible Dry Matter (DDM%=88,9-(0,779xADF%); Dry Matter Intake (DMI /% body weight/=120/NDF%) and Relative Feeding Value (RFV=DDMxDMI/1,29) are estimated.

The feeding value estimation is performed as:

1. *The Energy feeding value* is calculated by French system: UFL-UFV (INRA, 1988), recalculated in Bulgarian by coefficients, followed by Todorov (1997).

Todorov (1997).
 () ;
 (AOAC 2010)
 dMO_{in vivo} Andrieu
 Demarquilly (1989)
 in vitro
 (UFL-UFV),
 (VEM-VEVI)
 2.
 (PDIN=PDIA+PDIMN PDIE=PDIA+PDIME)
 (INRA 1988)
 TDP/PBD – Total Digestible Protein/Protein
 Brute Digestible,
 PDIN,
 PDIN=PDIA+PDIMN PDIE –
 PDIE=PDIA+PDIMN g kg⁻¹

The following parameters are estimated:
 - Gross energy (GE), Metabolic energy
 (ME) on the basis of equations according
 - to experimental values of CP, CF (AOAC
 - 2010) and IVOMD. The coefficient of
 digestibility of organic matter dMO_{in vivo}
 (Andrieu and Demarquilly, 1989) is
 received by relationship on the basis of
 in vitro organic matter digestibility,
 - determined experimentally. Net energy is
 - determined according French (UFL-UFV),
 Bulgarian – Feed units for milk and Feed
 units for growth (FUM-FUG) and Dutch
 (VEM-VEVI) systems.

2. The potential Protein feeding value
 (PDIN=PDIA+PDIMN ; PDIE=PDIA+PDIME)
 is performed by French system (INRA
 1988). The parameters: TDP/PBD – Total
 Digestible Protein/Protein Brute Digestible
 and a really digestible protein in ruminant
 small intestine – PDIN (Protein digestible
 in intestine, depending on nitrogen)
 PDIN=PDIA+PDIMN and PDIE (Protein
 digestible in intestine depending on
 energy) PDIE=PDIA+PDIMN in g kg⁻¹ dry
 matter are established.

In comparative analysis in two
 vegetative stages and organic active
 products the individual and mean values
 and coefficients of variation of the
 parameters of forage feeding value are
 evaluated.

RESULTS AND DISCUSSION

The forage quality feed from 6
 accessions was studied in the first spring
 growth (Table 1). The average crude
 protein value is 18.21% and the maximum
 20.79% (var. 25) of the dry matter.

The number of accessions with a crude
 protein content above the average is 3.
 The average fiber content is 25.24% and
 3 samples have a lower content, which is
 the desired criterion – var. 25 - 23.50%
 with 2 % and vars. 27 and 29.

6
 (1).
 18,21%,
 20,79% (. 25)
 3.
 25,24%,
 3
 23,50% 2

27 29. -
. 25 27
57,73%. -
. 30 – 2,98%, -
29 27, 4,66%.
8,1 27, 29 30 -
-
59,67%. -
. 30 –
63,03%, . 27 – 61,36%
. 25 – 60,72%.

With the lowest values of total fiber components, NDF are also vars. 25 and 27 at mean NDF for all accessions of collection nursery 57.73%. ADL has the lowest value for Var. 30 - 2.98%, followed by accessions 29 and 27, at an average of 4.66%. The degree of lignification is on average 8.1, with variants 27, 29 and 30 having a lower degree of lignification. The average digestibility of the dry matter of the forage from cocksfoot accessions is 59.67%. Three of the accessions exceeded the average of the digestibility at the first growth, the highest being for var. 30 – 63.03%, followed by var. 27 - 61.36% and var. 25 - 60.72%.

Table 1. Principal composition, fiber components content and digestibility of accessions Orchardgrass in collection nursery, first, second and third growths

Variant	DM	Ash	CP	CF	NDF	ADF	ADL	HEMI	CELLU	LIGNIF	IVDMD	IVOMD
/ First growth												
25	90,35	13,32	20,79	23,50	51,87	36,09	6,48	15,78	29,61	12,5	60,72	62,55
26	92,00	12,93	19,29	25,77	57,96	37,23	5,02	20,73	32,21	8,7	59,15	60,23
27	92,11	11,65	17,74	23,92	55,54	36,15	4,00	19,39	32,15	7,2	61,36	63,49
28	93,62	11,32	16,51	28,28	64,71	40,64	6,20	24,07	34,44	9,6	54,13	55,73
29	91,68	14,10	15,40	24,83	58,93	39,35	3,27	19,58	36,08	5,5	59,61	62,98
30	91,44	12,77	19,52	25,14	57,38	36,16	2,98	21,22	33,18	5,2	63,03	64,22
Mean	91,93	12,68	18,21	25,24	57,73	37,60	4,66	20,13	32,94	8,1	59,67	61,53
<i>SD</i>	<i>1,10</i>	<i>1,04</i>	<i>2,03</i>	<i>1,70</i>	<i>4,23</i>	<i>1,94</i>	<i>1,48</i>	<i>2,71</i>	<i>2,21</i>	<i>2,75</i>	<i>3,04</i>	<i>3,15</i>
CV	1,2	8,2	11,1	6,7	7,3	5,2	31,8	13,5	6,7	34,0	5,1	5,1
/ Second growth												
25	94,33	11,92	20,22	21,92	55,46	27,14	3,74	32,72	24,40	6,7	63,72	64,34
26	93,85	11,82	20,59	23,18	52,84	30,46	4,39	24,38	26,07	8,3	64,18	65,47
27	93,84	11,75	18,17	23,52	55,08	29,71	4,39	25,37	25,32	8,0	62,99	65,17
28	94,40	11,09	15,19	26,65	60,90	34,40	3,86	26,50	30,54	6,3	60,40	62,10
29	95,58	10,72	16,07	26,12	61,07	34,24	3,60	26,83	30,64	5,9	64,04	65,95
30	94,47	10,41	19,28	23,56	56,40	30,78	3,68	25,62	27,10	6,5	68,89	69,70
Mean	94,41	11,28	18,25	24,16	56,96	31,29	3,94	26,90	27,34	7,0	64,03	65,46
<i>SD</i>	<i>0,64</i>	<i>0,64</i>	<i>2,22</i>	<i>1,83</i>	<i>3,33</i>	<i>2,52</i>	<i>0,36</i>	<i>2,98</i>	<i>2,66</i>	<i>0,97</i>	<i>2,76</i>	<i>2,48</i>
CV	0,7	5,6	12,1	7,6	5,8	8,0	9,0	11,1	9,7	13,8	4,3	3,8
/ Third growth												
25	93,33	10,86	24,08	15,95	44,26	22,49	3,72	21,77	18,77	8,4	70,54	72,09
26	92,91	11,46	22,17	16,73	46,73	24,95	3,40	21,78	21,55	7,3	69,73	71,48
27	92,70	11,46	24,09	16,31	45,29	23,19	2,62	22,10	20,57	5,8	73,56	74,27
28	92,82	16,37	23,51	14,87	41,99	29,89	2,49	12,10	27,40	5,9	69,00	71,00
29	93,04	12,13	23,82	18,04	50,52	27,13	3,58	23,39	23,55	7,1	67,76	69,22
30	92,00	10,28	23,55	17,02	45,56	23,94	3,16	21,62	20,78	6,9	74,07	75,72
Mean	92,80	12,09	23,54	16,48	45,72	25,28	3,16	20,46	22,10	6,9	70,77	72,30
<i>SD</i>	<i>0,45</i>	<i>2,18</i>	<i>0,71</i>	<i>1,07</i>	<i>2,84</i>	<i>2,80</i>	<i>0,50</i>	<i>4,15</i>	<i>3,02</i>	<i>0,96</i>	<i>2,53</i>	<i>2,34</i>
CV	0,5	18,1	3,0	6,5	6,2	11,0	16,0	20,3	13,7	14,0	3,6	3,2

The digestibility of the organic matter of the feed of cocksfoot

27 30

2.

2.

corresponds to that of the dry matter. Variants 27 and 30 in the first growth are distinguished by the highest quality of the feed. Potential energy and protein nutritional value of the accessions of cocksfoot, the first growth is presented in Table 2.

Table 2. Energy and protein feeding value of accessions Orchardgrass in collection nursery, first, second and third growths

			UFL				UFV			VEM		VEVI		PBD		PDIN		PDIE	
Variant	DDM	DMI	RFV	GE	ME	UFL	UFV	FUM	FUG	VEM	VEVI	PBD	PDIN	PDIE					
/ First growth																			
25	60,79	2,31	109	11,80	5,82	0,680	0,564	0,564	0,461	856	1815	165	131	98					
26	59,90	2,07	96	11,72	5,70	0,659	0,542	0,546	0,443	834	1781	150	121	94					
27	60,74	2,16	102	11,64	5,85	0,703	0,593	0,583	0,484	861	1823	136	112	93					
28	57,26	1,85	82	11,58	5,50	0,627	0,609	0,520	0,416	796	1725	123	104	86					
29	58,25	2,04	92	11,55	5,62	0,672	0,560	0,557	0,457	826	1768	113	97	88					
30	60,73	2,09	98	11,73	5,88	0,698	0,586	0,579	0,479	866	1830	152	123	97					
Mean	59,61	2,08	96	11,67	5,73	0,673	0,576	0,558	0,457	840	1790	140	115	93					
<i>SD</i>	1,51	0,15	9	0,09	0,15	0,03	0,03	0,02	0,02	27	40	19	13	5					
CV	2,5	7,2	9,5	0,8	2,6	4,1	4,3	4,2	5,4	3,2	2,2	14	11	5					
/ Second growth																			
25	66,98	2,16	112	11,76	5,95	0,712	0,601	0,590	0,491	878	1849	159	127	98					
26	65,17	2,27	115	11,77	6,01	0,722	0,613	0,599	0,501	888	1866	162	129	100					
27	65,76	2,18	111	11,66	5,93	0,719	0,610	0,596	0,499	874	1844	139	114	95					
28	62,10	1,97	95	11,51	5,74	0,690	0,581	0,572	0,474	840	1791	110	95	87					
29	64,23	2,00	96	11,55	5,95	0,732	0,628	0,607	0,513	877	1848	118	101	91					
30	64,92	2,16	109	11,70	6,22	0,777	0,677	0,645	0,553	925	1823	146	121	100					
Mean	64,86	2,12	106	11,66	5,97	0,725	0,618	0,602	0,505	880	1854	139	114	95					
<i>SD</i>	1,64	0,12	8,6	0,11	0,15	0,03	0,03	0,02	0,03	27	42	21	14	5					
CV	2,5	5,4	8,1	0,9	2,6	4,0	5,3	4,0	5,3	3,1	2,3	15	12,2	5,5					
/ Third growth																			
25	71,38	2,71	150	11,92	6,45	0,809	0,709	0,671	0,549	968	1989	195	151	111					
26	69,46	2,57	138	11,84	6,33	0,795	0,693	0,659	0,567	948	1958	177	139	107					
27	70,83	2,65	146	11,93	6,51	0,824	0,726	0,683	0,593	979	2007	196	151	112					
28	65,62	2,86	145	11,97	6,08	0,740	0,629	0,614	0,514	911	1900	192	148	109					
29	67,77	2,38	125	11,92	6,25	0,764	0,657	0,633	0,537	933	1935	193	150	108					
30	70,25	2,63	143	11,89	6,62	0,851	0,758	0,706	0,619	998	2036	190	148	112					
Mean	69,22	2,62	141	11,91	6,37	0,797	0,695	0,661	0,563	956	1971	191	148	110					
<i>SD</i>	2,16	0,16	9	0,04	0,19	0,04	0,05	0,03	0,04	32	50	7	5	2					
CV	3,1	6,3	6,3	0,4	3,0	5,0	6,7	4,5	6,8	3,3	2,5	3,6	3,1	1,9					

30. (RFV) 96 %, - 25, 27 .25 - 109 .%. 25. .25 - 165 g kg⁻¹ . 30 - 152 g kg⁻¹.

The mean value of the relative nutritional value (RFV) is 96% and three accessions exceed 25, 27 and 30. The highest is var.25 - 109 ref. %. Energy nutrition is also highest for var. 25. With maximum protein nutrition, var. 25 - 165 g kg⁻¹ of dry matter and Var. 30 - 152 g kg⁻¹. Close to our results for crude protein (CP) content in general satisfactory, with ranged within 14.22-16.47 % for the 1st

14,22-16,47% 1-
 15,22-18,34% -
 Rancane et al. (2015),
 -
 -
 60% (61.17-64.99%).
 -
 2003).
 18,25%
 (21,86%), 26 30 -
 .25
 -
 51,87% .27 – 55,54%.
 -
 .25,
 .27, .27 -
 . 6,3 7,0.
 (1).
 -
 4
 68,89% .26 – 64,18%,
 64,03%.
 .30 -

cut and 15.22-18.34% for 2nd cut reported by Rancane et al. (2015), where CP content in general tend to be higher, although it varies by accessions, suggesting that the CP depends on the variety development stage and and the quantity of leaves.

Dry matter digestibility for all swards can be valued as good, it was above 60 % (61.17-64.99 %). For the highest quality hay, cocksfoot should be harvested in spring during late boot to early flowering stage.

Beyond this stage, digestibility decreases at the rate of about ½ % per day (Lacefield et al., 2003). Dry matter yield, digestibility and degree of lignification of cocksfoot were mostly influenced by mowing, while CP content – by the harvest year and variety.

In the second summer growth, cocksfoot had an average crude protein content of 18.25% (Table 1). Again, var. 25 (21.86%), 26 and 30 have the highest protein content and the lowest fiber content corresponding to it. Variant 25 contains 51.87% of NDF, and var. 27 – 55.54%.

The best quality of the feed – high protein and low in fiber is var.25, followed by Var.27. In second growth, Var. 27 has the lowest degree of lignification – coef. 6.3 at an average of 7.0.

In a second growth, the degree of lignification of the samples is less than that of the first, with the same protein content (Table 1). The digestibility of the accessions is higher in comparison to the first growth with 4 percentage points and is the highest for Var. 30-68.89% and Var. 26-64.18%, with an average of 64.03%. Two of the variants exceed the average for the digestibility of the feed.

The second growth has a higher average

106	30,	10	25, 26, 27
RFV		(2).
		.26 - 115	%. 2).
UFL-UFV		.30	.29,
30	0,732-0,628	0,777-0,677	.
		.29.	-
	.26,	PBD-PDIN-PDIE: 162,	
129,	100 g kg ⁻¹ .		
		1	
23,54%,			
.27 - 24,09%.		.28	
%,		14,87	
41,99%		16,48%.	
			45,72%.
		.27 -	.5,8
	6,9.		
74,07%.			.30 -
		141	%,
		.25 - 150	
%. (2).	27	-
196 g kg ⁻¹			
	191 g kg ⁻¹ .		
			(60,8% - 75.9%),

relative nutritional value compared to the first with 10 units and is 106 percent, with variants 25, 26, 27 and 30 exceeding it (Table 2).

With maximum RFV is Var.26 - 115%. The energy net nutritional value is the highest at var. 30 and var. 29, UFL-UFV, respectively, 0.0777-0.677 for Var. 30 and 0.732-0.628 for Var.29. The maximum protein feeding value of the forage has Var.26, PBD-PDIN-PDIE: 162, 129, 100 g kg⁻¹.

Table 1 presents the composition and digestibility of the cocksfoot accessions of a third regrowth that is uniquely distinguished by the highest quality and nutritional value. The average protein content is 23.54% highest, and the maximum value is of Var. 27-24.09%. On var. 28 corresponds to a lower fiber content of 14.87%, averaging 16.48%.

The content of the NDF is corresponding - 41.99% at an average of 45.72%. The contents of the ADF, ADL and degree of lignification are also consistent. The accessions have the lowest degree of lignification are 27 - coefficient 5.8 at an average coefficient of lignification 6,9. Highest digestible is the cocksfoot accession Var.30 - 74.07%.

Changes in the digestibility of cocksfoot from lower to high are in sequence from first to second and third crops.

The mean relative nutritional value of the accessions of cocksfoot of the third growth is 141% and the highest for Var. 25 to 150 percent (Table 2). Accession number 27 demonstrates the highest net energy nutritional value and protein nutritional value - total digestible protein 196 g kg⁻¹ dry matter at an average of 191 g kg⁻¹.

In accordance with our results, the values for the digestibility of dry matter (60.8% - 75.9%), CP (11.3% - 16.0%),

(11.3% - 16,0%), (54,4% - 61,4%), (28,8% - 36,1%) (59,5 % - 75,1%) (Robins et al, 2016; Sokolovi et al., 2016).

() () (Robins et al., 2015).

NDF (54.4% - 61.4% , 8% - 36.1%) and cell wall digestibility DNDF (59.5% - 75.1%) (Robins et al, 2016; Sokolovic et al., 2016).

- Negative correlation between dry mass and cell wall components (ADF and NDF) and a positive relationship between dry mass and digestibility (DMD and DNDF) were found (Robins et al., 2015). The accessions high in crude protein, low lignification and high digestibility are the subject of a selection for subsequent stages of the breeding program.

CONCLUSIONS

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⇒

⇒ : 25 (20,79%-24,08%), 27 (17,74%-24,09%), 30 (19,52-23,55%), (18,21-23,54%);

⇒ 25, 27, 29 30, - (5 7) , 8,1;

⇒ : 25, 27 30 - 74,07%, 59,67%-70,77%;

⇒

⇒

⇒ The forage quality evaluation accompanies the evaluation by productive potential in the breeding programs with cocksfoot in IFC – Pleven since the initial material in the collections.

⇒ There are significant variations in the parameters determining the forage quality and feeding value of the collection accessions.

⇒ The first breeding cycle has been performed and the accessions exceeded the mean value for the collection, Top 3, as follows:

⇒ By Crude protein content: 25 (20.79% - 24.08%), 27 (17.74%-24.09%), 30 (19.52-23.55%), at a mean value (18.21-23.54%);

⇒ By degree of lignification,: 25, 27, 29 and 30, with the lowest values for the parameter as a breeding criterion (5 to 7) at an average of 8.1;

⇒ By digestibility of dry matter: 25, 27 and 30 with the highest values of 74.07%, at an average of 59.67% - 70.77%;

⇒ By energy and protein feeding value, the results correspond to the principal biomass composition of the selected accessions.

⇒ Accessions with the highest complex forage quality evaluation are varieties and ecotypes, which proves the native populations passes adaptability

- and high forage quality as a suitable genetic resource in cocksfoot breeding programs.

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Festuca

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Chemical composition, digestibility and feeding value of accessions from genus *Festuca*

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SUMMARY

Forage quality by chemical composition, digestibility, feeding value of seven accessions from genus *Festuca* – tall fescue (*Festuca arundinacea* Schreb.) Var.18, 19, 20, meadow fescue (*Festuca pratensis*) Var.21, red fescue (*Festuca rubra*) Var.22, 23, 24, in collection nursery – Bulgarian and introduced varieties and ecotypes in spring, summer and autumn growths are evaluated in field trial in the IFC-Pleven. The parameters of chemical composition (Weende analysis), fiber components content by detergent analysis (Van Soest), enzyme *in vitro* digestibility of dry and organic matter (method d'Aufrere), potential energy and protein feeding value by different systems are determined. In the **first spring growth** four accessions exceed mean protein value 16,28%. The highest protein content 20,88%, the lowest of fiber components CF 17,97%, NDF42,56%, ADF28,37, ADL 1,83%, the lowest degree of lignification coeff. 4,3 and the highest forage digestibility of dry matter 78,00% belongs to Var.20, followed by variants 21, 18, 19. The greatest variability is character to parameters ADL and degree of lignification: CV 33,0% and 20,8%, respectively. The

(RFV) 146 % .20.
 UFL-UFV 0,857-0,766
 .20,
 18: 0,812-0,717,
 131, 111 g kg⁻¹
 PBD-PDIN-PDIE: 166,
 .20.
 -
 .23
 .20 – 72,22%
 66,41%.
 (RFV) 122 %, .22 24
 149 132 .%.
 -
 .20
 UFL-UFV 0,815-0,720 .22 0,784-
 0,683.
 PBD-PDIN-
 PDIE: 193, 150, 106 g kg⁻¹ (.23).
Festuca
 -
 70,63%,
 72,11% (.19). RFV 146
 .%, - .19 21-
 152 .%. .18 21
 ,
 .18 19 – PBD-PDIN-PDIE: 174,
 137,108g kg⁻¹
 : *Festuca*,
 ,

highest Relative Feeding Value (RFV) is 146 for Var.20. Energy feeding value UFL-UFV 0,857-0,766 have maximal values in Var.20, followed by Var.18: 0,812-0,717 and maximal protein feeding value belongs also to Var.20 - PBD-PDIN-PDIE: 166, 131, 111 g kg⁻¹. In the **second summer growth** better forage quality has red fescue Var.24. The forage digestibility is suitable of those of first growth and is the highest also in Var.20 – 72,22% at mean value 66,41%.

The mean RFV is 122 rel. % as Var.22 and 24 exceed it, 149 and 132 rel.%, relatively. Energy net feeding value is the highest for Var.20 UFL-UFV: 0,815-0,720 and Var.22 – 0,784-0,683. The maximal protein feeding value is PBD-PDIN-PDIE: 193,150,106 g kg⁻¹ (Var.23) respectively.

The changes in fescue forage digestibility from low to higher are consequently from first toward second and third growths. The mean digestibility of the accessions from **third autumn growth** is 70,63% and maximal 72,11% (Var.19). The mean RFV is 146 rel. % and the highest is for Var.19, 21–152 rel. %. The variants 18 and 21 show the highest net energy feeding value by different systems of evaluation and protein feeding value is high for the accessions Var.18, 19 – PBD-PDIN-PDIE: 174, 137,108 g kg⁻¹ dry matter.

Key words: genus *Festuca*, breeding, digestibility, energy and protein feeding value

INTRODUCTION

Fescues are very diverse grasses which are important components of natural, permanent, and intensively managed grasslands, lawns, and turfs, and are used for conservation purposes. Fescue (*Festuca* spp.) species can be divided into two groups; the broad-leaved fescues meadow fescue (*F. pratensis* Huds.) and tall fescue (*F. arundinacea* Schreb.), and the fine-leaved fescues. Fine fescues are grouped into *Festuca rubra* (red fescue) and *Festuca ovina*

Festuca
 ,
 ,
 ,
 :
 (F. pratensis
 Huds.) (F.
 arundinacea Schreb.),
 -
 .
Festuca rubra

<p>() <i>Festuca ovina</i> () <i>Festuca</i> - , - (Jenkin, 1959). (<i>F. pratensis</i> Huds.) , (Borrill et al., 1976, Hultin and Fries, 1986).</p>	<p>(sheep fescue) complexes. The genus <i>Festuca</i> L. is distributed mostly in the temperate zones of both hemispheres; most abundant all around the Northern Hemisphere (Jenkin, 1959). Meadow fescue (<i>F. pratensis</i> Huds.) is a forage grass of high quality and yield potential considered native to Europe and Eurasia (Borrill et al., 1976, Hultin and Fries, 1986). Meadow fescue constitutes a significant component of species-rich permanent pastures and hay fields in alpine regions and in eastern Europe. It was probably introduced to Scandinavia from Europe and West Asia, and has since become naturalized, and it was also introduced to North America, Japan, Australia, and New Zealand. In North America the acreage of meadow fescue has been rather insignificant relative to the more drought tolerant tall fescue, however, Casler et al. (1998) concluded that meadow fescue may be more useful than tall fescue in intensive grazing management systems. Meadow fescue is also more prevalent at higher altitudes than tall fescue.</p>
<p>($2n = 2x = 14$) (S Z) (Lundqvist, 1962).</p>	<p>Meadow fescue is diploid ($2n = 2x = 14$) and obligate outbreeding with a two-locus (S and Z) gametophytic self-incompatibility system (Lundqvist, 1962).</p>
<p>, 1800 - 15 (Buckner et al., 1979). , N - ú</p>	<p>Tall fescue is an important cool-season perennial forage grass species throughout the temperate regions of the world. It was introduced in the USA in the 1800s, but was not planted widely until the middle of the twentieth century. It is one of the most popular pasture grasses and cultivated on about 15 million ha in the USA (Buckner et al., 1979). Superior growth, adaptability to a wide range of soils and climates, responsiveness to N fertilization, high tolerance to grazing, and availability of forage over much of the year are key reasons for its popularity over other forage grasses. Tall fescue demonstrates good shade</p>

tolerance and remains green all-year-round under irrigated conditions in cooler climates. Although tall fescue originates from Europe and northern Africa it is less widely accepted in the European market. Under intensive cultivation perennial ryegrass is in general preferred because of its better grazing persistence and palatability.

However, due to changing climatic conditions and the availability of improved cultivars the acceptance of tall fescue for forage purposes is increasing (Peeters, 2004). Apart from being used as forage, its uses extend to lawns, turf, and conservation purposes (Sleper and Buckner, 1995). Fine fescues are a group of cool season perennial grasses that are commercially and agronomically valued for forage, turf, landscape, and ornamental purposes. Fine fescues have very fine and narrow leaves that minimize the water loss through transpiration and give them good drought tolerance. Some of the important fine fescues include red fescue, Chewings fescue, sheep fescue, hard fescue, and blue fescue among many other species.

Fine fescues tolerate shade, drought, low pH (5.5-6.5), and low soil fertility (Hanson et al., 1969; Beard, 1973), and require little to no additional inputs of fertilizer or supplemental irrigation (Ruemmele et al., 1995).

There are two major tall fescue germplasm pools, Continental and Mediterranean. Genetic barriers are usually seen in crosses between Mediterranean and Continental types even with uniform ploidy levels with meiotic irregularities within progenies (Hunt and Sleper, 1981). There are two distinct functional tall fescue groups, i.e., forage and turf types. Forage-type germplasms are characterized by coarse leaves, upright growth habit, and tall plants.

(Meyer and Funk, 1989).

30-40
Festulolium

508

129

In Europe breeding for soft-leaved types has set new benchmarks and extended the usage of tall fescue. In soft-leaved types, the saw-like silicium teeth prevalent on leaf borders and ridges of coarse-leaved tall fescue are reduced to small rounded bulb-like structures.

Turf types have finer darkgreen leaves, short growth stature, and dense tillers.

Contrary to forage types, turf types can tolerate frequent and close mowing. Development of turf-type cultivars was mainly initiated in the 1970s. Red fescues have superior shade adaptation and generally have high shoot density, good uniformity, and fine textured medium to dark green leaves (Meyer and Funk, 1989).

The majority of the European meadow fescue cultivars have been developed in eastern/central and northern Europe. Meadow fescue germplasm and cultivars have also been used extensively during the last 30-40 years to develop *Festulolium* hybrids and introgression lines. Meadow fescue is a species with many positive attributes like tolerance toward abiotic and biotic stresses, good persistency and adaptation to grazing and frequent cutting, and high nutritive quality. This makes it highly appreciated in mixtures for conservation cuts and in pastures. It is hard to point at major breeding achievement. It seems that cultivars of meadow fescue are fairly long-lived and that breeding is characterized by a rather slow but continuous improvement; especially for important traits like winter survival and persistency.

Out of 508 cultivars listed in EU catalog, only 129 are forage type and the rest are turf type.

The largest European collection of cultivars with improved characteristics can be found on the French variety list that

31 cultivars with the oldest one being registered in 1986.

Forage quality is an important breeding goal as a slight increase in digestibility has been shown to have a major positive impact on animal performance (Casler and Vogel, 1999).

Traits such as forage and seed yield, *in vitro* dry matter digestibility (IVDMD), and detergent fiber parameters show promise with distinguished additive genetic variations (Nguyen et al., 1982, Nguyen and Sleper, 1985). Protocols have been developed to determine the *in vitro* dry matter digestibility (IVDMD).

In addition selection is performed on palatability since the correlation between digestibility, palatability, and animal performance is decreasing in elite material. Good cultivars for Europe should have a combination of good forage yield distribution throughout the year, persistency, high quality (defined by soft leaves, good digestibility, and high palatability), in combination with good disease resistance, fast establishment, and good seed yield.

As regards forage quality, methods for measuring *in vitro* dry matter digestibility are laborious and time consuming.

Molecular marker-assisted breeding has been initiated at the Noble Foundation to develop tall fescue cultivars with high digestibility (Saha et al., 2007). Transgenic tall fescue plants with lower lignin content and higher forage digestibility have been developed (Chen et al., 2004). Seasonal distribution of forage yield is as important as the total yield. Increased fall and winter forage yield is considered highly desirable in the USA. Increased concentration of Ca, P, and Mg was reported in selected populations (Sleper et al., 2002). Low mowing height and shading tolerance are always the

priority traits in turf-type tall fescue breeding.

Breeding goals in fine fescues depend on whether the focus is on forage or on turf use.

For example, turf breeders may want high fibre content because it improves wear tolerance whereas forage breeders want low fibre content as it is associated with improved digestibility (Vogel et al., 1989).

Ecotype selection was the earliest method used to develop fescue cultivars and is still considered an important breeding method (Fjellheim and Rognli, 2005a).

Germplasms from old pastures, roadsides, coastal areas, mining sites, seed fields, etc., were collected and tested in a number of environments; seed increased, and finally cultivar(s) released. Most of the earliest fescue cultivars were developed in this manner (Hopkins et al., 2007). The oldest Nordic, and possibly also European, cultivar, 'Svalofs Sena' from Sweden, dates back to 1917 and some of the newer cultivars are still developed from ecotypes (Fjellheim and Rognli, 2005a). Cultivars from ecotypes are either developed directly from multiplication of ecotypes or more commonly by phenotypic mass selection. Ecotype selection capitalizes on natural selection and together with the high levels of genetic variation present in the germplasm, can act to generate new populations in short periods of time (Valay and Van Santen, 1999).

As an example, the most recent Norwegian cultivar 'Norild' is a synthetic cultivar based on 11 clones selected among surviving plants of half-sib families (HSF) created from a local population tested for 3 years at a very northern location (70 N).

Recurrent phenotypic selection is a popular breeding technique to develop

priority traits in turf-type tall fescue breeding.

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Recurrent phenotypic selection is a popular breeding technique to develop

(7.2-10.5%) (Chen et al., 2003, Chen et al., 2004).

0,6-0,7 t/ha 9 t/ha

(*F. arundinacea* Schreb.)
1993
2005
(Katova, 2016).

(Naydenova, 2009, 2012; Naydenova et al., 1999, 2001).

improved cultivars. Recurrent selection appears to be a powerful means of accumulating favourable alleles in a population and is often the method of choice for improving traits with low heritability. It can give considerably uniform cultivars from diverse germplasm.

Transgenic plants with reduced lignin concentration, altered lignin composition, and increased dry matter digestibility (7.2–10.5%) have been developed (Chenet al., 2003, Chen et al., 2004). The only Bulgarian tall fescue, created in IFC, is Albena variety, which is hexaploid. In pure stand it provides over 9 t/ha of dry matter and 0,6-0,7 t/ha of seeds.

Suitable for hay, grazing and silage. Resistant to drought, cold, leaf diseases, acidic and salted soils. A variety of tall fescue Albena (*F. arundinacea* Schreb.) was entered in the varieties list in 1993 and has 2005 certificate of the Republic of Bulgaria (Katova, 2016). The qualitative characteristics of the feed, the chemical composition, the digestibility and the nutritional value of the variety have been established (Naydenova, 2009, 2012; Naydenova et al., 1999, 2001).

The aim of the study was to establish the chemical composition, digestibility and nutritional value of tall, meadow and red fescue and to make a selection of accessions with high forage quality for the next stages of the breeding programs.

MATERIAL AND METHODS

During the autumn of 2015, in the experimental field of the Institute of forage Crops - Pleven a collection nursery is based consisting of 7 accessions from genus *Fesuca* (3 tall fescue – *Festuca arundinacea* Schreb., 1 meadow fescue – *Festuca pratensis* L. and 3 red fescue *Festuca rubra* L.) varieties and ecotypes; by origin – Bulgarian – 4 (1 variety and 3 ecotypes), Romanian – 3 varieties), with

2015 .
-
7 (3
- *Festuca*
arundinacea Schreb., 1
- *Festuca pratensis* L. 3
Festucarubra L.)
;
- 4 (1 3)
- 3)

50/50 cm, 50 cm. Each accession is represented by 50 individual plants. In 2016 simultaneously to the assessment of the production potential of each growth and number, samples are taken for chemical analysis. The forage is obtained from three growths – spring, summer and autumn and its principal chemical composition and digestibility of dry matter are determined. Plant sample preparation from the above ground part of the plants is effectuate by air ventilation at 65° till crumbly at previous fixing for 20 min at 105° and grinding till particle size 1,0 mm consequently at laboratory mill QC 136 and QB 114, Labor Mim, Hungary, and obligatory screening.

() () (AOAC, 2010). (Goering and VanSoest, 1970) (ENISO13906 2008)

: /Neutral-detergentfiber (/NDF), /Acid-detergentfiber (/ADF)/ /Acid-detergent lignin (/ADL) (Van Soest et al., 1991).

= - ; =

(/ x100) (Akin and Chesson, 1990). *in vitro*

(/IVDMD)

(/IVOMD)

Aufreere (1982) (Todorov et al., 2010).

plants being individually arranged, through seedlings at a distance of 50/50 cm. Each accession is represented by 50 individual plants. In 2016 simultaneously to the assessment of the production potential of each growth and number, samples are taken for chemical analysis. The forage is obtained from three growths – spring, summer and autumn and its principal chemical composition and digestibility of dry matter are determined. Plant sample preparation from the above ground part of the plants is effectuate by air ventilation at 65° till crumbly at previous fixing for 20 min at 105° and grinding till particle size 1,0 mm consequently at laboratory mill QC 136 and QB 114, Labor Mim, Hungary, and obligatory screening.

The forage principal chemical composition is determined by Weende systematic analytic procedure by the parameters crude protein (CP) and crude fiber (CF) (2010). Structural plant cell walls fiber components are analyzed by systematic detergent analysis (Goering and Van Soest, 1970) (EN ISO13906 2008) and presented as percent of dry matter.

The following fiber fractions: Neutral-detergent fiber (NDF); Acid-detergent fiber (ADF), Acid-detergent lignin (ADL) are determined (Van Soest et al., 1991).

Polyosides hemicellulose and cellulose as a cell walls components, contained in fiber fraction are presented empirically: Hemicellulose = NDF – ADF; Cellulose = ADF – ADL. The degree of lignification is presented as relation of ADL and NDF/100 (Akin and Chesson, 1989).

Enzyme *in vitro* digestibility of dry (IVDMD) and organic (IVOMD) matter is determined by two stage pepsin-cellulase enzyme method of Aufreere (1982) (Todorov et al., 2010).

RFV;
 (Linn and Martin, 1991).
 $(DDM\% = 88,9 - (0,779 \times ADF\%));$
 $(DMI / \% \text{ bodyweight} = 120 / NDF\%)$
 $(RFV = DDM \times DMI / 1,29)$

UFL-UFV (INRA, 1988),
 $(- / FUM - FUG),$

Todorov (1997).
 $()$
 (AOAC 2010)

$dMO_{in vivo}$ Demarquilly (1989) *invitro* Andrieu
 $(UFL - UFV), (-)$
 $(VEM - VEVI)$

2. $(PDIN = PDIA + PDIMN)$
 $PDIE = PDIA + PDIME)$
 (INRA 1988)
 TDP/PBD – Total Digestible Protein /Protein Brute Digestible, PDIN,

PDIE –

Evaluation of feeding value on the basis of fiber components is performed as Relative feeding value RFV; potential intake of digestible dry matter (Linn and Martin, 1991). The Digestible Dry Matter ($DDM\% = 88,9 - (0,779 \times ADF\%);$ Dry Matter Intake ($DMI / \% \text{ body weight} = 120 / NDF\%$) and Relative Feeding Value ($RFV = DDM \times DMI / 1,29$) are estimated.

The feeding value estimation – energy and protein is performed as: 1. The Energy feeding value is calculated by French system: UFL-UFV (INRA, 1988), recalculated in Bulgarian by coefficients, followed by Todorov (1997).

The following parameters are estimated: Gross energy /GE/, Metabolic energy /ME/ on the basis of equations according to experimental values of CP, CF (AOAC 2010) and IVOMD.

The coefficient of digestibility of organic matter $dMO_{in vivo}$ (Andrieu and Demarquilly, 1989) is received by relationship on the basis of *in vitro* organic matter digestibility, determined experimentally. Net energy is determined according French (UFL-UFV), Bulgarian – Feed units for milk and Feed units for growth (FUM-FUG) and Dutch (VEM-VEVI) systems.

2. *The potential Protein feeding value is performed by French system (INRA 1988). The parameters: TDP/PBD - Total Digestible Protein/Protein Brute Digestible and a really digestible protein in ruminant small intestine – PDIN (Protein digestible in intestine, depending on nitrogen) $PDIN = PDIA + PDIMN$ and PDIE (Protein digestible in intestine depending on energy) $PDIE = PDIA + PDIMN$ in $g \text{ kg}^{-1}$ dry matter are established.*

In comparative analysis in two vegetative stages and organic active products the individual and mean values and coefficients of variation of the parameters of forage feeding value are evaluated.

RESULTS AND DISCUSSION

7
Festuca
(1).
16,78%,
20,88% (. 20 -
)
4.
21,14%, 4 -
20 - 17,79% 3 -
. 19, 18 ()
21 (). -
. 20, 18,
52,14%.
. 20 -
1,83%, 18 21,
3,17%.
6,0
20, 21 18
67,05%.
. 20 - 77,99%,
10 %- . 21 -
71,29% . 18 - 71,17%. -
20 21
- -
33,0% - CV
23,7%. -

The forage quality from 7 fescue accessions in Collection nursery was studied in *the first spring growth* (Table 1). The average crude protein value is 16.78% and the maximum 20.88% (Var. 20 – tall fescue) of the dry matter.

The number of accessions with a crude protein content above the average is 4. The average fiber content is 21.14% and 4 accessions have a lower content, which is the desired criterion – var. 20 - 17.79% with 3% and Vars. 19 and 18 (tall fescue) and 21 (meadow fescue).

With the lowest values of total fiber components, NDF are also Vars. 20, 18, 19 and 21 at mean NDF for all accessions of collection nursery 52.14%. ADL has the lowest value for Var. 20 – 1.83%, followed by accessions 18 and 21, at an average of 3.17%.

The degree of lignification is on average 6.0, with variants 20, 21 and 18 having a lower degree of lignification. The average from fescue accessions is 67.05%.

Four of the accessions exceeded the average of the digestibility at the first growth, the highest being for Var. 20 – 77.99%, 10% units over, followed by Var. 21 – 71.29% and Var. 18 – 71.17%. The digestibility of the organic matter of the feed of fescues corresponds digestibility of the dry matter of the forage to that of the dry matter. Variants 20 and 21 in the first growth are distinguished by the highest quality of the feed. The most variable are characteristics ADL – CV 33.0% and degree of lignification – 23.7%.

1.

Table 1. Principal composition, fiber components content and digestibility of accessions Tall fescue, Meadow fescue and Red fescue in collection nursery, first, second and third growths

Variant	DM	Ash	CP	CF	NDF	ADF	ADL	HEMI	CELLU	LIGNIF	IVDMD	IVOMD
/ First growth												
18	92,66	12,56	17,88	19,84	46,34	29,32	2,28	17,02	27,04	4,9	71,17	75,11
19	92,27	13,10	17,57	18,91	51,47	28,62	3,16	22,85	25,46	6,1	67,94	69,78
20	90,92	14,87	20,88	17,97	42,56	28,37	1,83	14,19	26,54	4,3	77,99	81,94
21	92,02	12,60	19,51	20,81	53,26	31,11	2,45	22,15	28,66	4,6	71,29	72,53
22	93,10	10,34	14,84	22,43	53,34	30,69	3,96	22,65	26,73	7,4	62,49	64,59
23	93,75	9,55	15,01	23,26	59,50	32,44	4,75	27,06	27,69	8,0	56,51	59,42
24	92,88	7,41	11,80	24,78	58,51	33,37	3,76	25,14	30,01	6,4	61,99	64,88
Mean	92,51	11,49	16,78	21,14	52,14	30,56	3,17	21,58	27,45	6,0	67,05	69,75
<i>SD</i>	0,90	2,52	3,10	2,46	6,10	1,91	1,0	4,49	1,50	1,42	7,22	7,55
CV	1,0	21,9	18,5	11,6	11,7	6,2	33,0	20,8	5,5	23,7	10,8	10,8
/ Second growth												
18	94,26	11,32	19,65	20,70	52,39	31,37	3,46	21,02	27,91	6,6	64,55	66,22
19	94,12	9,00	14,78	27,04	58,50	31,76	4,30	26,74	27,46	7,4	59,33	59,76
20	94,19	11,09	18,87	20,60	53,97	26,94	3,85	27,03	23,09	7,1	72,22	73,78
22	92,54	12,61	19,83	14,96	43,28	25,23	3,33	18,05	21,90	7,7	68,70	71,53
23	92,96	10,83	23,89	17,87	53,63	26,69	3,10	26,94	23,59	5,8	62,39	65,59
24	92,20	10,42	20,05	19,04	48,30	26,11	3,04	24,19	23,07	6,3	71,28	73,66
Mean	93,38	10,88	19,51	20,04	51,68	28,02	3,51	24,00	24,50	6,8	66,41	68,42
<i>SD</i>	0,92	1,18	2,91	4,03	5,25	2,81	0,48	3,73	2,53	0,71	5,15	5,54
CV	1,0	10,8	14,9	20,1	10,2	10,0	13,7	15,6	10,3	10,5	7,8	8,1
/ Third growth												
18	93,05	12,21	21,81	15,28	44,26	25,12	2,00	19,14	23,12	4,5	71,52	73,58
19	92,57	12,44	21,33	14,87	42,58	24,59	2,61	17,99	21,98	6,1	72,11	73,53
20	92,58	11,21	20,05	16,31	48,42	25,93	2,86	22,49	23,07	5,9	67,19	68,04
21	92,78	14,53	19,23	15,11	42,17	25,73	2,95	16,44	22,78	7,0	71,71	73,39
Mean	92,75	12,60	20,60	15,43	44,36	25,34	2,60	19,02	22,74	5,9	70,63	72,14
<i>SD</i>	0,22	1,39	1,18	0,77	2,85	0,61	0,43	2,57	0,53	1,03	2,31	2,73
CV	0,2	11,1	5,7	5,0	6,4	2,4	16,5	13,5	2,3	17,6	3,3	3,8

2.

(RFV) 118 %, - 20, 18 19. .20 - 146 . %.

20.

166 g kg⁻¹ .20 -

152 g kg⁻¹ .21 -

()

15,50-16,80% F.

arundinacea 16,80-17,70% F.

Potential energy and protein nutritional value of the accessions of fescue, the first growth is presented in Table 2. The mean value of the relative nutritional value (RFV) is 118% and three accessions exceed 20.18 and 19.

The highest is Var.20 - 146 ref. %. Energy nutrition is also highest for Var. 20. With maximum protein nutrition, Var. 20 - 166 g kg⁻¹ of dry matter and Var. 21 - 152 g kg⁻¹. Close to our results for crude protein (CP) content in general satisfactory, with ranged within 15.50-16.80 % for *Festuca arundinacea* and 16.80-17.70% for *Festuca pratensis* reported by Singh (2007), where CP

pratensis Singh (2007),
(56.51 - 77.99%).
60%
½ % (Lacefield
et al., 2003).

content in general tend to be higher, although it varies by accessions, suggesting that the CP depends on the variety development stage and the quantity of leaves. Dry matter digestibility for all accessions can be valued as good, it was above 60 % (56.51 - 77.99%). For the highest quality hay, cocksfoot should be harvested in spring during late boot to early flowering stage. Beyond this stage, digestibility decreases at the rate of about ½ % per day (Lacefield et al., 2003). Dry matter yield, digestibility and degree of lignification of fescue were mostly influenced by mowing, while CP content – by the growth and genetic diversity (accessions).

2.

Table 2. Energy and protein feeding value of accessions Tall fescue, Meadow fescue and Red fescue in collection nursery, first, second and third growths

	UFL		UFV		VEM		VEVI		PBD		PDIN		PDIE	
Variant	DDM	DMI	RFV	GE	ME	UFL	UFV	FUM	FUG	VEM	VEVI	PBD	PDIN	PDIE
/ First growth														
18	66,06	2,59	133	11,65	6,28	0,812	0,717	0,673	0,586	943	1950	136	112	101
19	66,61	2,33	120	11,64	6,02	0,755	0,652	0,626	0,532	897	1879	134	110	96
20	66,80	2,82	146	11,82	6,52	0,857	0,766	0,710	0,626	990	2023	166	131	111
21	64,67	2,25	113	11,73	6,22	0,786	0,685	0,651	0,560	931	1931	152	122	102
22	64,99	2,25	113	11,49	5,87	0,726	0,622	0,602	0,508	865	1829	106	93	88
23	63,63	2,02	100	11,49	5,70	0,682	0,572	0,566	0,467	832	1778	108	94	84
24	62,59	2,05	100	11,33	5,94	0,754	0,656	0,625	0,536	874	1843	76	74	82
Mean	65,05	2,33	118	11,59	6,08	0,767	0,667	0,636	0,545	904	1890	125	105	95
<i>SD</i>	1,57	0,29	17	0,17	0,28	0,06	0,06	0,05	0,05	54	83	31	19	11
CV	2,4	12,3	14,3	1,4	4,6	7,5	9,5	7,4	9,5	5,9	4,4	25	18	11
/ Second growth														
18	64,46	2,29	115	11,72	6,04	0,737	0,630	0,611	0,515	894	1875	153	123	98
19	64,16	2,05	102	11,48	5,73	0,686	0,577	0,569	0,471	836	1785	105	93	84
20	67,91	2,22	117	11,68	6,34	0,815	0,720	0,676	0,588	950	1962	145	118	102
22	69,25	2,77	149	11,74	6,19	0,784	0,683	0,650	0,558	927	1926	155	124	102
23	68,11	2,24	118	11,91	6,17	0,742	0,632	0,615	0,516	916	1909	193	150	106
24	68,56	2,48	132	11,73	6,41	0,742	0,729	0,683	0,596	961	1980	156	126	104
Mean	67,08	2,34	122	11,71	6,15	0,767	0,662	0,634	0,541	931	1891	151	122	99
<i>SD</i>	2,19	0,25	16	0,14	0,24	0,06	0,06	0,04	0,05	24	67	28	18	8
CV	3,3	10,7	13,3	1,2	3,9	7,4	9,1	6,9	8,9	2,6	3,5	19	15	8,0
/ Third growth														
18	69,33	2,71	146	11,83	6,36	0,809	0,710	0,671	0,580	956	1971	174	137	108
19	69,74	2,82	152	11,81	6,33	0,806	0,708	0,669	0,578	952	1964	170	134	106
20	68,70	2,48	132	11,74	6,13	0,762	0,658	0,632	0,538	913	1904	157	126	100
21	68,86	2,85	152	11,74	6,14	0,780	0,679	0,647	0,555	922	1917	150	121	102
Mean	69,16	2,67	146	11,78	6,27	0,797	0,697	0,661	0,570	941	1948	163	130	104
<i>SD</i>	0,47	0,18	9	0,05	0,10	0,02	0,03	0,02	0,02	19	30	11	7	4
CV	0,7	6,7	6,5	0,4	1,6	2,9	3,7	2,9	3,7	2,1	1,6	6,8	5,6	3,5

(1).
 24 22 - 19,51%
 .23 (23,89%),
 - -
 . 20,04 %, - . 22
 14,96% . 5 % - . 23
 24, . . - -
 .23, .24.
 , . 23 -
 - . 5,8
 6,8. -
 - -
 , - -
 (1). -
 72,22% .24 - 71,28%,
 66,41%. .20 -
 -
 .%, 6 122
 RFV (2). 22 24
 .22 - 149 .%.
 . 20 . 22, UFL-UFV
 0,815-0,720 . 20 0,784-
 0,683 .22.
 PBD-PDIN-PDIE: 193, 150, 106 g kg⁻¹.
 1 .23,
 ,
 -
 -
 -
 . 20,60%,
 . 18 -
 21,81%. . 19 -
 14,87%, 15,43%.

In the second summer growth, fescues had an average crude protein content of 19.51% (Table 1). Variants 23 (23.89%), 24 and 22 have the highest protein content and the lowest fiber content corresponding to it.

The average crude fiber content is 20.04% but preferable are those with the lowest content - Var. 22 contains 14.96% CF, with 5 % units lower content followed by Var. 23 and 24 red fescue. The best quality of the feed - high protein and low in fiber - is Var. 23, followed by Var.24.

In second growth, Var. 23 has the lowest degree of lignification - coef. 5.8 at an average of 6.8. In a second growth, the degree of lignification of the accessions is higher than that of the first, with higher protein content (Table 1).

The digestibility of the accessions is close to the first growth with and is the highest for Var.20 - 72.22% and Var.24 - 71.28%, with an average of 66.41%. Three of the variants exceed the average for the digestibility of the feed. The second growth has a higher average relative nutritional value compared to the first with 6 units and is 122 rel.%, with Variants 22 and 24 exceeding it (Table 2).

With maximum RFV is Var.22 - 149%. The energy net nutritional value is the highest at Var. 20 and Var. 22, UFL-UFV, respectively, 0.815-0.720 for Var. 20 and 0.784-0.683 for Var.22. The maximum protein feeding value of the forage has Var.23, PBD-PDIN-PDIE:193, 150, 106 g kg⁻¹.

Table 1 presents the composition and digestibility of the fescue accessions of a third autumn regrowth that is uniquely distinguished by the highest quality and nutritional value. The average protein content is 20.60% highest, and the maximum value is of Var. 18 - 21.81%. On Var.19 corresponds to a lower fiber content of 14.87%, averaging 15.43%.

15,43%.
 42,58% 44,36%.
 18 – 4,5
 5,9.
 19 – 72,11%.

146 .%,
 19 – 152 .% (2).
 18
 174 g kg⁻¹
 - 163 g kg⁻¹.

(7.2% - 9.5 % -),
 (Cheng et al., 2003). Poblaciones et al.
 (2008)

Festuca, CP (%) 15.64 (10.1 – 22.3);
 ADF (%)29.69 (25.2 – 33.4), NDF (%)
 49.34 (39.3 – 63.8), ADL (%) 4.56 (2.47 –
 8.74), 64.43 (51.5 – 73.3),
 Leme ien et al. (2011), De
 Boever and De Campeneere (2016).

⇒

Festuca

The content of the NDF is corresponding – 42.58% at an average of 44.36%. The contents of the ADF, ADL and degree of lignification are also consistent. The accessions have the lowest degree of lignification are Var.18 – coefficient 4.5 at an average coefficient of lignification 5.9.

Highest digestible is the fescue accession Var.19 – 72.11%. Changes in the digestibility of fescue from lower to high are in sequence from first to second and third growths.

The mean relative nutritional value of the accessions of fescue of the third growth is 146% and the highest for Var. 19 to 152 rel.%, (Table 2). Accession number 18 demonstrates the highest net energy nutritional value and protein nutritional value – total digestible protein 174 g kg⁻¹ dry matter at an average of 163 g kg⁻¹.

In accordance with our results, the values for the digestibility of dry matter with variation between tall fescue genotypes are obtained (7.2% - 9.5%), (Cheng et al., 2003). Poblaciones et al. (2008) reported close to our results average and margins for forage quality characteristics for *Festuca*, CP%, 15.64 (10.1% - 22.3%), ADF, % 29.69 (25.2 - 33.4), NDF % 49.34 (39.3 - 63.8) , ADL % 4.56 (2.47 - 8.74) and digestibility DMD, % 64.43 (51.5% - 73.3) as also Lemeziene et al. (2011), De Boever and De Campeneere (2016).

The accessions high in crude protein, low lignification and high digestibility are the subject of a selection for subsequent stages of the breeding program.

CONCLUSIONS

⇒ The forage quality evaluation accompanies the evaluation by productive potential in the breeding programs with genus *Festuca* in IFC - Pleven since the initial material in the collections.

- ⇒ There are significant variations in the parameters determining the forage quality and feeding value of the collection accessions by species and intraspecies.
- ⇒ The first breeding cycle has been performed and the accessions exceeded the mean value for the collection, Top 3, as follows:
 - ⇒ By Crude protein content: 20 (20.05% - 20.88%), 23 (15.01% - 23.89%), 18 (17.88 - 21.81%), at a mean value (16.78 - 20.60%);
 - ⇒ By degree of lignification,; 20, 21, 18 and 23, with the lowest values for the parameter as a breeding criterion (4.5 to 6) at an average of 6.2;
 - ⇒ By digestibility of dry matter: 20, 21 and 19 with the highest values of 77.99%, at an average of 66.41% - 70.63%;
- ⇒ By energy and protein feeding value, the results correspond to the principal biomass composition of the selected accessions.
- ⇒ Accessions with the highest complex forage quality evaluation are varieties and ecotypes, which proves the native populations possess adaptability and high forage quality as a suitable genetic resource in tall fescue, meadow fescue and red fescue breeding programs.

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Self-seeding of subterranean clover in degraded seed production stands

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SUMMARY

The possibility degraded white clover seed production stands to be under sown with self-seeding species was studied in field trial carried out at the Institute of Forage Crops, Pleven, Bulgaria. The under sowing was performed during the fourth year of usage of white clover stands 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"). It was found that the subterranean clover effectively used autumn-winter soil moisture, formed a sufficient number of seeds for self-seeding and germinated plants occupied the sites of the dropped white clover plants. The best potential for self-seeding showed *Trifolium subterraneum* ssp. *brachycalicinum*, the total number of emerged self-seeded plants was 188 number of plants/m² vs. *Trifolium subterraneum* ssp. *yaninicum* – 153 /m² and *Trifolium subterraneum* ssp. *subterraneum* – 105 /m². The self-seeding ability allows subterranean clover be used

for under sowing of degraded seed production white clover stands, thus to prolonge their durability and the stands could be used for forage.

Key words: self-seeding, subterranean clover, degraded stands, white clover

(Luscher et al., 2014).

(Dimitrova, 1986; Vasilev, 2004, Sulas et al., 2006; Petkova et al., 2015; Churkova and Bozhanska, 2016).

(Carneiro, 1999; Naydenova et al., 2013; Bartholomew, 2014).

(*Trifolium subterraneum* L.) (Yakimova and Yancheva, 1986; Piano et al., 1996; Howieson et al., 2008).

(McCaskill et al., 2016).

(Porqueddu et al., 2003).

(Frame et al., 1998).

for under sowing of degraded seed production white clover stands, thus to prolonge their durability and the stands could be used for forage.

Key words: self-seeding, subterranean clover, degraded stands, white clover

INTRODUCTION

Legumes are becoming increasingly important in the system of sustainable agriculture (Luscher et al., 2014). However, their productivity with advancing the age of crops (for perennial legumes) decreased due to loss of plants, the swards diluted and free spaces are occupied by weeds (Dimitrova, 1986; Vasilev, 2004, Sulas et al., 2006; Petkova et al., 2015; Churkova and Bozhanska, 2016).

Legumes species that can provide self-sowing become of practical importance, due to their possibility of prolonged presence in the sward. In one season they could provide productivity of forage as well seeds for propagation (Carneiro, 1999; Naydenova et al., 2013; Bartholomew, 2014).

Subterranean clover (*Trifolium subterraneum* L.) is speices with self-seeding ability (Yakimova and Yancheva, 1986; Piano et al., 1996; Howieson et al., 2008). It has a low widespread habitat and occupies open spaces between other plants from the lowest floor of the sward, as well coexists well with perennial grasses and legumes (McCaskill et al., 2016). It grows up early in the spring and forms a dense sward (Porqueddu et al., 2003).

Reproductive organs are formed in early May and the seeds ripen before the end of the spring in hedgehog-shaped heads that remain on the soil surface (Frame et al., 1998). Substantial part of the formed seeds is hard and germinates after two-three years. This biological specificity

and Piano, 1994).
 (Pecetti
 (Vasilev, 2006).
 (Nichols et al.,
 2012; Lucas et al., 2015).
 (Assyov et a., 2012).
 (Ilieva and Vasileva, 2011;
 Vasileva et al., 2011; Ilieva et al., 2015;
 Naydenova and Vasileva, 2015, 2016;
 Kirilov and Vasileva, 2016; Vasileva et al.,
 2016).
Trifolium subterraneum
ssp. brachycalicinum (Vasileva, 2015).

turns the superficial soil layer into an original seed bank (Pecetti and Piano, 1994). The precipitations during the late summer contribute to emergence of new self-sown plants (Vasilev, 2006).

Subterranean clover is a widespread component in the pastures and other grasslands of the temperate areas of Central and Northern Europe and America (Nichols et al., 2012; Lucas et al., 2015). For Bulgaria it is relatively new crop and is found in open dry grasslands in the plains and lowlands (Assyov et al., 2012). The studies with subterranean clover in recent years showed that it has practical applicability for the climatic conditions of Bulgaria (Ilieva and Vasileva, 2011; Vasileva et al., 2011; Ilieva et al., 2015; Naydenova and Vasileva, 2015, 2016; Kirilov and Vasileva, 2016; Vasileva et al., 2016). Some of studies involved the use of the species for direct under sowing of degraded perennial seed production stands and the self-seeding capacity of subclover in these stands. Such studies were done for alfalfa, where the best self-seeding capacity showed *Trifolium subterraneum ssp. brachycalicinum* (Vasileva, 2015).

In this study we aim to investigate the self-seeding capacity of three subclover subspecies through under sowing of white clover seed production stands.

MATERIAL AND METHODS

The experimental work was carried out on the experimental field of Institute of Forage Crops, Pleven, Bulgaria on slight leached chernozem soil subtype without irrigation. Seed production white clover stands was sown in 2007 and cared according with accepted technology. Long plots method was used, plot size of 5 m² and 4 replications of the treatments. During the autumn of fourth year from the use of the swards, across the rows, under

(2010)

Trifolium subterraneum ssp. *brachycalycinum* ("Antas"), *Trifolium subterraneum* ssp. *yananicum* ("Trikkala") and *Trifolium subterraneum* ssp. *subterraneum* ("Denmark").

400 /m²
12 cm.

:

() - ;
+ *Trifolium subterraneum* ssp. *brachycalycinum*;
+ *Trifolium subterraneum* ssp. *yananicum*;
+ *Trifolium subterraneum* ssp. *subterraneum*.

0.25 m²

m².

SPSS 2012.

- sowing with three subterranean clover subspecies was performed, i.e. *Trifolium subterraneum* ssp. *brachycalycinum* (cv. Antas), *Trifolium subterraneum* ssp. *yananicum* (cv. Trikkala) and *Trifolium subterraneum* ssp. *subterraneum* (cv. Denmark).

- Under sowing was done with 400 germinated seeds/m² and between rows spacing of 12 cm. Treatments were as follows: white clover (without under sowing) - control; white clover + *Trifolium subterraneum* ssp. *brachycalycinum*; white clover + *Trifolium subterraneum* ssp. *yananicum*; white clover + *Trifolium subterraneum* ssp. *subterraneum*.

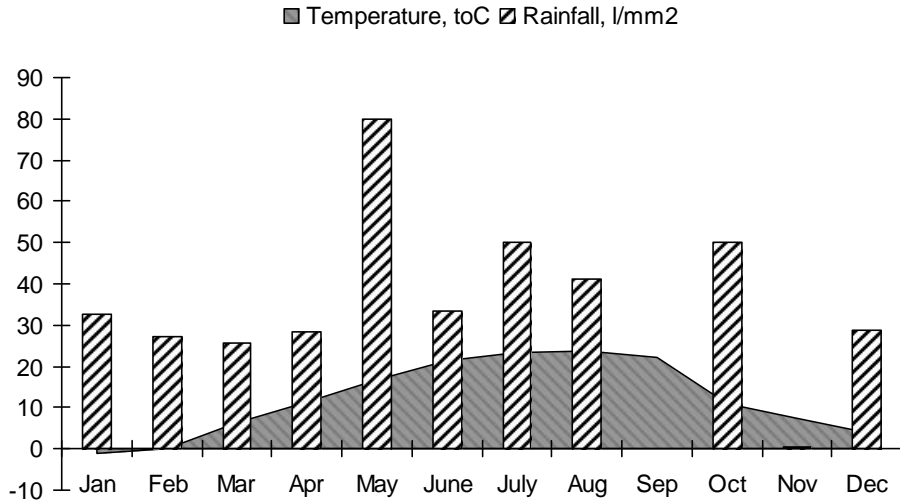
- In the year after under sowing the number of germinated self-seeded subclover plants was recorded from 0.25 m² and equated to the number of m². They were done at the beginning of August and in early November, respectively in cotyledons, the first not true leaves and first true leaves stages. Total number of germinated self-seeded plants was calculated. Experimental data were processed statistically using a software product SPSS 2012.

RESULTS AND DISCUSSION

- Agrometeorological conditions, particularly the amount and distribution of rainfall are important for self-seeding of subterranean clover and they could be defined as unfavourable during the period of study (Figure 1).

1).

- In the year after under sowing long dry period with extremely high temperatures occurs. Rainfall during the second half of the growing season (after mid-July) was below the average norm and not conducive to germination of self-seeded plants. Subterranean clover was found successfully self-seeded by mid to late growing season despite the unfavorable conditions.



1.
Fig. 1. Agrometeorological conditions in the first year after under sowing

The number of germinated self-seeded plants in the first true leaves stage was found 52 plants/m² for *Trifolium subterraneum* ssp. *yaninicum* and 56 plants/m² for *Trifolium subterraneum* ssp. *brachycalycinum*, and less – 39 plants/m² for *Trifolium subterraneum* ssp. *subterraneum* in the first recording done in August (Table 1). Fewer number of germinated plants of *Trifolium subterraneum* ssp. *subterraneum* is related to the weaker competitiveness of this subspecies recognized by Lucas et al. (2015).

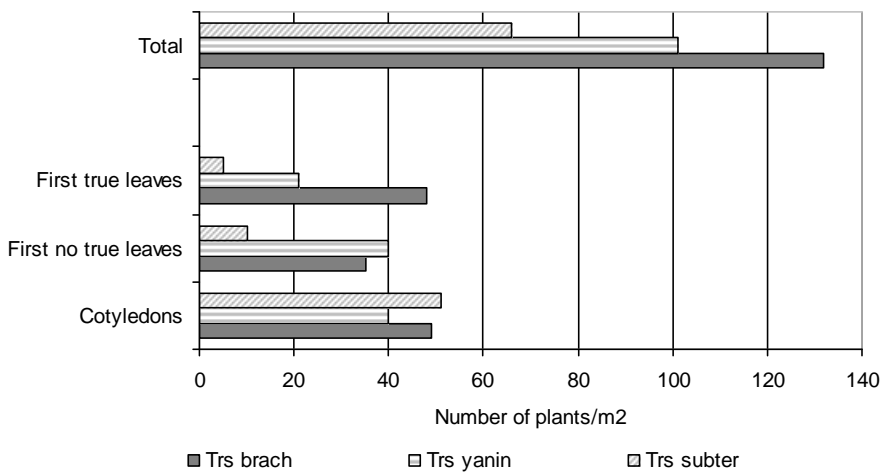
1.
Table 1. Number of germinated self-seeded subterranean plants in under sowed degraded seed production white clover stands (early August, first year after under sowing)

/Treatments	/August number of self-seeded plants/m ²
White clover + <i>Tr. subterraneum</i> ssp. <i>brachycalycinum</i>	56±2.0
White clover + <i>Tr. subterraneum</i> ssp. <i>yaninicum</i>	52±3.2
White clover + <i>Tr. subterraneum</i> ssp. <i>subterraneum</i>	39±5.6
/Average	49±3.5
SE (P=0.05)	5.1

±, STDEV

With the advancing of vegetation agrometeorological conditions were exceptionally bad. In September, the average daily temperature was high (22 °) and rainfall lacked (0.0 l/m²). After the precipitations (50.2 l/m²) fallen in the second ten days of October new self-seeded and germinated plants was recorded at the beginning of next month. The data are shown in Figure 2. The number of germinated plants in stage cotyledons for *Trifolium subterraneum* ssp. *brachycalycinum* and *Trifolium subterraneum* ssp. *subterraneum* was closed (49 and 51 the number of plants/m²) and 40 the number of plants/m² for *Trifolium subterraneum* ssp. *yaninicum*. The number of germinated plants in first not true leaves stage varied in wide limits (from 10 to 40 number of plants/m²). The same trend was observed for the number of germinated plants in the first true leaves stage (from 5 to 48 number of plants/m²).

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Trs brach, *Trifolium subterraneum* ssp. *brachycalycinum*; Trs yanin, *Trifolium subterraneum* ssp. *yaninicum*; Trs subter, *Trifolium subterraneum* ssp. *subterraneum*

. 2.

(

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Fig. 2. Number of germinated self-seeded subterranean plants in under sowed degraded seed production white clover stands (early November first year after under sowing)

(Pecetti and Piano, 1994).

Trifolium subterraneum ssp. *brachycalycinum* (132 /m²), *Trifolium subterraneum* ssp. *yaninicum* (101 /m²) *Trifolium subterraneum* ssp. *subterraneum* (66 /m²). *Trifolium subterraneum* ssp. *brachycalycinum*

Trifolium subterraneum ssp. *brachycalycinum*.

(Pecetti and Piano, 1994; Carneiro, 1999; Lemus, 2013).

(Loi et al., 2005).

Trifolium subterraneum ssp. *yaninicum* *Trifolium subterraneum* ssp. *subterraneum*

(Vasileva, 2015).

Obviously, the subterranean clover used effectively autumn-winter soil moisture and formed seeds successfully. In the conditions of our study subterranean clover formed sufficient number of seeds, which in case of favorable conditions are self-seeded. This confirms the high ecological plasticity of the species found by other authors (Pecetti and Piano, 1994).

The total number of germinated self-seeded plants in early November was biggest for *Trifolium subterraneum* ssp. *brachycalycinum* (132 number of plants/m²), followed by *Trifolium subterraneum* ssp. *yaninicum* (101 number of plants/m²) and *Trifolium subterraneum* ssp. *subterraneum* (66 number of plants/m²). We found that *Trifolium subterraneum* ssp. *brachycalycinum* showed best potential for self-seeding when was used for under sowing of degraded white clover seed production stands. We assume that this is probably due to the higher germination of seeds of *Trifolium subterraneum* ssp. *brachycalycinum*. In the Mediterranean area high germination rate and low hard seeds of subterranean clover is not seen as a good characteristics. Species and varieties with a higher percentage of hard seeds are appreciated, due to the possibility of their later activation and greater reliability of the stands (Pecetti and Piano, 1994; Carneiro, 1999; Lemus, 2013). For areas with a Mediterranean climate the selection of subterranean clover for hardiness of the seeds is leading, which allows its gradual emergence and thus self-supporting in the stands for several years, although it is an annual type (Loi et al., 2005).

For the conditions of the experiment, *Trifolium subterraneum* ssp. *yaninicum* and *Trifolium subterraneum* ssp. *subterraneum* showed weaker ability for self-seeding and germination in degraded stands. Analogous results we received when alfalfa seed production stands were under sowed with for this subspecies subterranean clover (Vasileva, 2015).

Trifolium subterraneum ssp. *brachycalycinum* (188 /m²) vs. *Trifolium subterraneum* ssp. *yananicum* (153 /m²) and *Trifolium subterraneum* ssp. *subterraneum* (105 /m²) (Vasileva, 2017).

The trend *Trifolium subterraneum* ssp. *brachycalycinum* to show the best self-seeding ability and emergence was confirmed by the total number of germinated self-seeded plants, i.e. *Trifolium subterraneum* ssp. *brachycalycinum* (188 number of plants/m²) vs. *Trifolium subterraneum* ssp. *yananicum* (153 the number of plants/m²) and *Trifolium subterraneum* ssp. *subterraneum* (105 number of plants/m²) (Table 2).

After botanical composition analyses high percent of participation of this subspecies was found (Vasileva, 2017).

2.

Table 2. Total number of germinated self-seeded subterranean plants in under sowed degraded seed production white clover stands in the year after under sowing

Treatments	Total number of self-seeded plants/m ²
White clover + <i>Tr. subterraneum</i> ssp. <i>brachycalycinum</i>	188±63.5
White clover + <i>Tr. subterraneum</i> ssp. <i>yananicum</i>	153±52.4
White clover + <i>Tr. subterraneum</i> ssp. <i>subterraneum</i>	105±40.2
/Average	149
SE (P=0.05)	24.0

±, STDEV

So, due to the self-seeding capacity, subterranean clover could be successfully used for under sowing of degraded seed production stands. The degradation may result from various factors – short duration of the species, less adaptability to over use, adverse soil and climatic conditions, ect.

Under sowing prolongs the durability of the stands and enhance the productivity and quality of the forage as Springer (1997) found. In addition, self-seeding ability of subterranean clover and possibility degraded perennial stands be under sowed with subterranean clover allows more efficient use of resources - one of the challenges of modern sustainable agriculture.

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CONCLUSIONS

Trifolium subterraneum ssp. *brachycalycinum*,
188
/m² vs. *Trifolium subterraneum*
ssp. *yananicum* (153 /m²)
Trifolium subterraneum ssp. *subterraneum*
(105 /m²).

Subterranean clover used for under sowing of white clover seed production stands effectively used autumn-winter soil moisture, formed sufficient number of seeds for self-seeding and germinated self-seeded plants occupied the places from dropouts white clover plants. *Trifolium subterraneum* ssp. *brachycalycinum* showed the best potential for self-seeding – the total number of germinated self-seeded plants was 188 number of plants/m² vs. *Trifolium subterraneum* ssp. *yananicum* (153 number of plants/m²) and *Trifolium subterraneum* ssp. *subterraneum* (105 number of plants/m²). The self-seeding ability allows subterranean clover be used for under sowing of degraded seed production white clover stands, thus to prolonge their durability and the stands could be used for forage.

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(*Medicago sativa* L.)

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Forage quality and energy feeding value estimation of alfalfa (*Medicago sativa* L.) in integrated pest management

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SUMMARY

045 The after-effect of the synthetic insecticide Eforia 043 ZK, applied alone and in combination with reduced doses of mineral oil Acarzine on chemical composition and enzyme in vitro digestibility, as well as on energy feeding value of the dry mass of alfalfa was studied. The field experiment was conducted in the period 2014-2015 at the Institute of Forage Crops, Pleven, Bulgaria. The treatments were carried out at the beginning of the flowering stage in the second undergrowth (59-60) for control of economically important pests as a method of integrated pest system. The results demonstrate that application of studied synthetic active compounds had a positive impact on composition, digestibility and energy feeding value of alfalfa.

2014-2015.
(59-60)

The treatment increased crude protein content and decrease plant cell walls fiber components content - acid detergent

61.52%,

5.4%

(UFL - 0,701; UFV - 0,589; PBD - 153; PDIN - 124 PDIE - 95).

043

- lignin, acid detergent fiber, neutral detergent fiber and hemicellulase. An optimal combination of expressed decrease in plant cell walls fiber components content with a considerable increase of digestibility was established after applying of Acarzine.

- Digestibility reaches 61.52%, respectively with an increase from 5.4%. The highest energy feeding value was found after used of mineral oil Acarzine (UFL - 0,701; UFV - 0,589; PBD - 153; PDIN - 124 PDIE - 95). The synthetic product Eforia led to an increase of parameters which characterize the forage energy feeding value, in a degree similar to that of Acarzine.

Key words: chemical composition, enzyme *in vitro* digestibility, energy feeding value, Eforia 043 ZK, Akarzin, alfalfa

INTRODUCTION

- The importance of feed quality as a complex concept in animal nutrition encompasses the nutrient balance, decreasing the solubility of the protein, increasing the degradability of the fiber components and optimizing the availability of secondary plant metabolites (Milne, 2002). Evaluating the quality of the alfalfa obtained as an essential stage in the forage production helps to establish forages with an increased nutritional value and digestibility in organic and integrated production.

- Factors that affect forage quality include different species, leaf -to -stem ratio, stage of growth, soil agents, climate, harvesting, diseases, and pests (Arzani et al., 2001).

- Insect pests can lower forage quality, particularly if they cause significant leaf loss. Dellinger et al. (2006) found few significant differences detected in dry matter yields or forage quality between untreated and treated with

(Milne, 2002).

(Arzani et al., 2001).

. Dellinger et al. (2006)

(Naydenova et al., 2010; Naydenova et al., 2011; Georgieva et al., 2016; Nikolova et al., 2016),

insecticides alfalfa cultivars which showed that insect pests influence not only the quantity but also the quality of the forage.

The content of fiber components of plant cell walls determining the energy nutrition value and forage digestibility depending on various insecticidal products, applied in the integrated pest management system for perennial leguminous forage crops in Bulgaria are limited. Studies related to the assessment of the energy nutritional value of fodder crops are limited too as well as determining the quality of the forage and its nutritional value (Naydenova et al., 2010; Naydenova et al., 2011; Georgieva et al., 2016; Nikolova et al., 2016). That is a prerequisite for continuing and expanding the research work in this field.

043 The aim of present study was to determine the after-effect of synthetic product Eforia 034 ZK used for alone and in combination with mineral oil Akarzin on chemical composition and enzyme in vitro digestibility, as well as on energy feeding value of the dry mass of alfalfa.

MATERIAL AND METHODS

045 The after-effect of the synthetic insecticide Eforia 043 ZK, applied alone and in combination with reduced doses of mineral oil Acarzine on chemical composition and enzyme in vitro digestibility, as well as on energy feeding value of the dry mass of alfalfa was studied (the test variants are given in Table 1). The field experiment was conducted in the period 2014-2015 at the Institute of Forage Crops, Pleven, Bulgaria. The treatments were carried out at the beginning of the flowering stage in the second undergrowth (59-60) for control of economically important pests as a method of integrated pest system.

The after-effect of the synthetic insecticide Eforia 043 ZK, applied alone and in combination with reduced doses of mineral oil Acarzine on chemical composition and enzyme in vitro digestibility, as well as on energy feeding value of the dry mass of alfalfa was studied (the test variants are given in Table 1). The field experiment was conducted in the period 2014-2015 at the Institute of Forage Crops, Pleven, Bulgaria. The treatments were carried out at the beginning of the flowering stage in the second undergrowth (59-60) for control of economically important pests as a method of integrated pest system.

1.

Table 1. Characteristic of products

Commercial product	Active substance	Dose, ml da ⁻¹	Application
1. / Control	/ Untreated	-	-
2. 045 3	30 g l ⁻¹ + 15 g l ⁻¹	125	
2.Eforia 045 3	30 g l ⁻¹ thiamethoxam + 15 g l ⁻¹ lambda-cyhalothrin		Vegetative
3. Acarzine	85% - + 15%	400	Vegetative
	85% mineral oil - paraffin type + 15% emulsifier		
4. 045 3 +	30 g l ⁻¹ + 15 g l ⁻¹ +	100 + 400	Vegetative
	85%+ + 15%		
4.Eforia 045 3 +	30 g l ⁻¹ thiamethoxam + 15 g l ⁻¹ lambda-cyhalothrin +		
Acarzine	85% mineral oil - paraffin type + 15% emulsifier		
5. 045 3 +	30 g l ⁻¹ + 15 g l ⁻¹ +	75 + 400	Vegetative
	85%+ + 15%		
5.Eforia 045 3 +	30 g l ⁻¹ thiamethoxam + 15 g l ⁻¹ lambda-cyhalothrin +		
Acarzine	85% mineral oil - paraffin type + 15% emulsifier		

protein (/CP), Keldahl (N x 6,25) crude fiber (/CF) (AOAC 2001). (/NDF), (/ADF) (/ADL) Goering & Van Soest (1970), = / 100. / in vitro (/IVDMD) (Todorov, 2010).

It was used the split plot method with three replications and natural background of soil supply with the major nutrients.

The main feeding characteristics were studied – chemical composition and *in vitro* degradability of dry matter for forage quality evaluation.

The main composition of alfalfa forage was determined as crude protein (CP), by Keldahl (N x 6.25) and crude fiber (CF) by Weende system (AOAC 2001). The plant cell walls fiber components content or neutral-detergent fiber (NDF), acid-detergent fiber (ADF) and acid-detergent lignin (ADL) were determined followed the systematic detergent analysis of Goering & Van Soest (1970), and degree of lignification - coefficient = ADL/NDFx100.

The enzyme degradability/digestibility *in vitro* of dry matter (IVDMD) was determined as percent by two-stage pepsin-cellulase method of Aufrere, 1982 (Todorov, 2010).

In comparative analysis of forage quality between variants of forage treatment the individual and mean values were evaluated according to their significance for forage quality.

The energy feeding value

: 1.
 -
 RFV; (Linn and Martin, 1991).
 (0.779 x ADF%); (DMI / % NDF%)
 (RFV = DDM x DMI / 1.29). 2.
 UFL-UFV (INRA 1988),
 Todorov (1997) - /FUM-FUG) (VEM-VEVI).
 (PDIN=PDIA+PDIMN PDIE=PDIA+PDIME) (INRA 1988)
 TDP/PBD - Total Digestible Protein/Protein Brute Digestible, PDIN,
 PDIE –

estimation was performed as 1. Evaluation of feeding value based on fiber components – Relative feeding value RFV; potential intake of digestible dry matter (Linn and Martin, 1991). The Digestible Dry Matter (DDM% = 88.9 - (0.779 x ADF%); Dry Matter Intake (DMI / % body weight / = 120 / NDF%) and Relative Feeding Value (RFV= DDM x DMI / 1.29) were estimated. 2. The Energy feeding value was calculated by French system: UFL-UFV (INRA, 1988), recalculated in Bulgarian - Feed units for milk and Feed units for growth (FUM-FUG) by coefficients, followed by Todorov (1997) and Dutch (VEM-VEVI) systems. The Protein feeding value (PDIN=PDIA+PDIMN and PDIE=PDIA+PDIME in the g kg⁻¹ dry matter were established) was performed by French system (INRA 1988) by the parameters: TDP/PBD - Total Digestible Protein/Protein Brute Digestible and a really digestible protein in ruminant small intestine, PDIN (Protein digestible in the intestine, depending on nitrogen) and PDIE (Protein digestible in intestine depending on energy).

RESULTS AND DISCUSSION

Forage quality is a pooled indicator defined by different, interrelated indicators. It is influenced by a number of factors, including the agrometeorological conditions for growth and development, growing technology, harvesting stages and others (Buxton, 2003). The application of plant protection products if necessary to protect crops from an attack of various types of weeds, diseases, and pests is one of the key points for achieving optimal yields and high-quality forage.

The results in regard to the main chemical composition of forage – content of crude protein and crude fibers as plant cell wall fiber components content, a degree of lignification and enzyme in vitro

in vitro - degradability/digestibility of dry matter under influence of applied products were affected positively in different level (Table 2).

2.

2012-2014

Table 2. Principal composition, fiber components content and digestibility of dry alfalfa biomass in an integrated protection system, 2012-2014

	DM	Ash	CP	CF	NDF	ADF	ADL	HEMI	CELLU	LIGNIF	IVDMD	DIVOMD
1	96.0	8.9	180.12	78.53	97.93	40.77	8.9	57.2	258.5	19.8	58.23	57.48
2	95.9	9.0	189.72	71.54	17.13	34.17	7.3	83.0	256.8	18.5	60.85	59
3	95.0	9.0	197.92	48.53	98.03	20.97	1.2	77.1	241.2	22.2	61.52	60.72
4	95.2	8.8	189.82	51.73	99.43	29.37	3.0	70.1	252.6	18.3	58.95	57.62
5	94.4	8.6	182.72	71.04	07.33	29.27	2.1	78.1	252.7	17.7	60.28	59.12
Min	94.36	8.56	180.12	48.53	99.43	20.97	1.2	57.2	241.2	17.7	58.23	57.48
Max	95.97	9.04	197.92	78.54	17.13	40.77	8.9	83.0	258.5	22.2	61.52	60.72
Mean	95.27	8.85	188.02	64.24	03.93	30.87	4.5	73.1	252.4	19.3	59.97	58.79
SD	0.66	0.19	0.70	1.32	0.83	0.73	0.34	1.00	0.67	1.8	1.35	1.32
CV	0.7	2.1	3.7	5.0	2.0	2.2	4.5	13.7	2.6	9.3	2.2	2.2

Legend: CP- /Crude protein, g kg⁻¹; CF- /Crude fiber; HEMI - /Hemicellulose; CELLU- /Cellulose; LIGNIF- /Degree of lignification = ADL/NDFx100; NDF- /Neutral-detergent fiber; ADF - /Acid-detergent fiber; ADL - /Acid-detergent lignin; % dry matter; IVDMD- / *In vitro* dry matter digestibility; %

The treatment with the study product, input single or in combination led to increase of the protein level with mean value 5.5% in comparison of control (Table 2). At the single application of Eforia and Acarzine was formed forage with increased content of protein with 5.3 and 9.9% respectively (189.7 and 197.9 g kg⁻¹) while at the combine used, the increase was from 1.4 to 5.4%. Maximal value in the crude protein content was established in the application with mineral oil Acarzine.

It is known that existed negative correlation between crude protein content and those of crude fiber content (Pavlov, 1996), that is confirmed by the investigation (r=-0.848).

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(45.1%), - 22.6% - 4)
 (100 ml da⁻¹) + .

((100 ml da⁻¹) + 3)
 6.7 2.3% 4)

(, .)
 6.6 10.6%.

58.23 61.52%
 , -
 (61.52%),
 - 5)
 da⁻¹) + (60.28%) (75 ml

3.
 a,

UFL-UFV: 0.701-0.589,
 FUM-FUG: 0.581-0.482 kg⁻¹

da⁻¹) + [5] (75 ml
): 0.568 - 0.469.

(VEM-VEVI)

lowest by 22.6% - in the variant 4) Eforia (100 ml da-1) + Acarzine.

The content of the cellulose was reduced again at the above mention two variants 3) Acarzine and 4) Eforia (100 ml da-1) + Acarzine (a decrease of 6.7 and 2.3%, respectively).

The degree of lignification followed relationships showed for ADL. It was not substantially affected by the action of the products, as there was a decrease in the values of the indicator from 6.6 to 10.6% with the exception of mineral oil (which was an increase in the content).

Increased digestibility is an indicator of high-quality forage. The digestibility of forage dry matter from alfalfa was in limits 58.23 – 61.52% and was an indicator influenced poorly by the effects of the applied products. At the single application the digestibility was higher at Acarzine treatment (61.52%), and at combined application – 5) Eforia (75 ml da-1) + Acarzine (60.28%).

Energy feeding value

Changes in potential net energy feeding value of forage assessed in French, Bulgarian and Dutch energy systems are presented in Table 3. The use of synthetic products determined increased energy nutrition in all variants as compared to the control. A maximum mean value is obtained by treatment with the mineral oil Acarzine: UFL-UFV: 0.701-0.589, while with respect to FUM-FUG: 0.581-0.482 kg-1 dry matter. It was following the combination treatment with the more pronounced reduced dose of the insecticide [5] Eforia (75 ml da⁻¹) + Akarzin]: 0568-0469.

The estimated average energy nutrition under the Dutch system (VEM-VEVI) followed those relationships under the influence of single and combination treatment with products.

3.

, 2014-2015

Table 3. Energy feeding value and protein potential feeding value of dry alfalfa biomass in an integrated protection system, 2014-2015

	VEM/VEVI													
	DDM	DMI	RFV	GE	ME	UFL	UFV	FUM	FUG	VEM/VEVI	PBD	PDIN	PDIE	
1	62.36	3.02	145.8	11.62	5.74	0.66	60.55	10.55	20.45	1836	1784	136	113	89
2	62.87	2.88	140.2	11.67	5.83	0.68	10.56	70.56	40.46	851	1808	146	119	92
3	63.90	3.02	149.4	11.70	5.94	0.70	10.58	90.58	10.48	870	1837	153	124	95
4	63.25	3.00	147.3	11.67	5.79	0.67	10.55	70.55	70.45	843	1796	145	119	91
5	63.26	2.95	144.5	11.63	5.84	0.68	60.57	40.56	80.46	852	1810	139	115	90
Min	62.36	2.88	140.2	11.62	5.74	0.66	60.55	10.55	20.45	836	1784	136	113	89
Max	63.90	3.02	149.4	11.70	5.84	0.70	10.58	90.58	10.48	870	1837	153	124	95
Mean	63.13	2.97	145.4	11.66	5.83	0.68	10.56	70.56	40.46	850	1807	144	118	91
<i>SD</i>	0.56	0.06	3.45	0.03	0.07	0.04	0.01	0.10	0.01	12	20	6.6	4.2	2.3
CV	0.9	2.0	2.4	0.3	1.3	2.0	2.6	2.0	1.2	1.5	1.1	4.6	3.5	2.5

Legend: DDM – , %/Digestible Dry Matter, %; DMI – , %/Dry matter intake, % of body weight; RFV – O . , %/Relative Feeding Value, relative %; UFL (Fr) FUM, VEM (Dutch) – /Feed units for milk; UFV (Fr) FUG, VEVI (Dutch) – g kg⁻¹/Feed units for growth, g kg⁻¹; PBD (TDP) – ()/Protein brute digestible (Total digestive protein), PDIN, PDIE in g kg⁻¹

136 153 g kg⁻¹ (PBD) (3).
 4.1%
 (53 g kg⁻¹).
 1.4%
 (1.8%).
 PDIN
 PDIE.
 PDIN PDIE 124 95 g kg⁻¹
 (9.7 6.7%
).

Protein feeding value
 The total digestible protein in the context of that study ranged from 136 to 153 g kg⁻¹ (PBD) (Table 3). The application of all preparations viewed as an average value tended to increase as the highest increase of 4.1% was observed in the use of Acarazine (n increase of 53 g kg⁻¹). As a result of the combined use of mineral oil with insecticidal product in reduced doses was formed on average of 1.4% more total digestible protein. Similar was the increase of the contents after the single use of Eforia (1.8%).
 A similar upward trend depending on the type and mode of application of the products was found in terms of values of PDIN and PDIE. Maximum mean PDIN and PDIE values of 124 and 95 g kg⁻¹ were found after Acarazine use (an increase of 9.7 and 6.7%, respectively). High values of total digestible protein had and Eforia single treatment as the increase in compared to the control was

5.3 3.4%
(PDIN PDIE).

PDIN PDIE
1.8 – 5.3% 1.1 – 2.4%

5.3 and 3.4%, respectively (PDIN and PDIE). In the other variants, the PDIN and PDIE values increased in the range 1.8 - 5.3% and 1.1 - 2.4% respectively.

An optimal combination of expressed decrease in plant cell walls fiber components content with a considerable increase of digestibility was established after applying of Acarzine, followed by the single use of Eforia.

CONCLUSIONS

Self-contained application and combined treatment of alfalfa with synthetic products Acarzine (mineral oil) and Eforia (insecticide) applied in reduced doses had a positive effect on the composition, digestibility, and nergy feeding value of alfalfa.

The treatment increased crude protein content and decrease plant cell walls fiber components content – acid detergent lignin, acid detergent fiber, neutral detergent fiber and hemicellulase.

An optimal combination of expressed decrease in plant cell walls fiber components content with a considerable increase of digestibility was established after applying of Acarzine. Digestibility reaches 61.52%, respectively with an increase from 5.4%.

The highest energy feeding value was found after used of mineral oil Acarzine (UFL - 0,701; UFV - 0,589; PBD - 153; PDIN - 124 PDIE - 95). The synthetic product Eforia led to an increase of parameters which characterize the forage energy feeding value, in a degree similar to that of Acarzine.

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5.4%

61.52%,

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(Ryabtseva, 2009).
 (Tyutyunnikov and Fadeev, 1984).
 (*Pisum sativum* L.) (*Vicia sativa* L.)
 150 (Unkovich and Pate, 2000) 125 kg N/ha (Brady, 2000; Haas et al., 2007),
 (*Vicia faba* L.),
 120-180 kg/ha,
 (Kostov and Pavlov, 2000).

they had considerably lower productive potential. The arithmetic sum of ranks of the main biochemical parameters (crude protein, crude fiber, fats, ash) of the seeds and aboveground biomass determined the highest quality of the forage of soybean, followed by white lupine, vetch, broad bean, chickpea and peas. On the basis of the comparative assessment of annual leguminous crops, it is recommended, along with the traditionally grown soybeans, peas and vetches, in the structure of protein crops to be incorporated the species as white lupine, broad bean and chickpea.

Key words: assessment, lupine, broad bean, chickpea, soybeans, peas, vetch

INTRODUCTION

In the recent years, one of the main directions in the development of Bulgarian agriculture is the organic production, in which legumes are of key importance. The annual legume crops are preferred in crop rotation with a positive impact on subsequent crops (Ryabtseva, 2009). An important additional source of nitrogen is the biological nitrogen accumulated in the soil resulting from the living activity of nitrogen-fixing bacteria that absorb the molecular nitrogen from the air. In this way, during the period of their vegetation, the fodder crops in symbiosis with tuberos bacteria enrich the soil with nitrogen (Tyutyunnikov and Fadeev, 1984). Traditionally grown peas (*Pisumsativum*L.) and vetch (*Vicia sativa* L.) under favorable conditions can fix up to 150 (Unkovich and Pate, 2000) and 125 kg N/ha (Brady, 2000; Haas et al., 2007) respectively, and they are determined as good predecessors. No less valuable in this respect is the broad bean (*Vicia faba* L.), which enriches the soil with nitrogen up to 120-180 kg/ha, while at the same time it distinguishes with high productive potential and good nutritional value (Kostov and Pavlov, 2000).

(*Glycine max* (L.) Merr.)

(*Cicer arietinum* L.) –

(Kolev et al., 1999).
Petrova and Angelova (2013)

(*Lupinus albus* L.).

2000-2014 .
130% (Faostat, 2014),
ù

ù
162 kg N/ha (Sparrow et al., 1995; Kurlovich et al., 2002).

Soybeans (*Glycine max* (L.) Merr.) is characterized by a high nutritional value, especially of the seeds, which have a high content of protein and fat, and chickpea (*Cicer arietinum* L.) - with drought resistance and cultivation in low-energy inputs production systems (Kolev et al., 1999). According to Petrova and Angelova (2013), the interest in chickpea growing in Bulgaria increases in recent years, which is related to the market search for various and healthy products. According to the authors, the broad bean and chickpea occupy limited areas in modern Bulgarian agriculture, but they could be included in the structure of protein crops, especially in areas which are inappropriate for traditionally grown peas, beans and soybeans.

From annual legume crops, at the global level, there is an increasing interest in the cultivation of white lupine (*Lupinus albus* L.). Only in the countries of the European Union, the area of these crops has increased in the period 2000-2014 by 130% (Faostat, 2014), determinative by the favorable chemical composition combined with high productivity. Lupine is suitable for green fertilization as in this respect surpasses the other legumes. It is drought-tolerant and resistant to acidic soils. After its harvesting, up to 162 kg N/ha are accumulated in the soil (Sparrow et al., 1995; Kurlovich et al., 2002).

The purpose of this study was to make a comparative agrobiological characteristic of annual legume crops peas, vetch, white lupine, broad bean, soybeans and chickpea.

MATERIAL AND METHODS

2015-2016 .

: *Pisum*
sativum L. (4), *Vicia*
sativa L (666), *Glycine*

The field experiment was conducted during the period 2015-2016, at the Institute of Forage Crops (Pleven). Six annual leguminous crops were objects of this experiment: peas *Pisum sativum* L. (Pleven 4 variety), vetch *Vicia sativa* L. (Obrazets 666 variety), soybeans *Glycine*

max (L.) Merr. (),
Lupinus albus L. (),
Cicer arietinum L. (),
Vicia faba L. ().
 8),
 (),
 20 cm,
 50
 (),
 (- ,
 () , cm;
 1 , cm;
 cm/day;
 ;
 g/plant;
 1000 , g).
 Plaisted and Peterson (1959)
 Wricke (1965) – PP W^2 .
 ()
 (g/kg DM):
 () – Kjeldahl;
 () – Weende
 ; () () -
 Sandev (1979); () -
 (AOAC, 2010).

max (L.) Merr. (Galena variety), white
 lupine *Lupinus albus* L. (Garant variety),
 chickpea *Cicer arietinum* L. (Plovdiv 8
 variety), broad bean *Vicia faba* L. (Hioska
 variety). The soil type was slightly leached
 chernozem. The sowing was done at the
 one and same calendar time (the third
 decade of March), at a distance between
 rows of 20 cm as for each species was
 sown 50 seeds. During the vegetation
 period, the plants were grown under
 organic conditions (without the use of
 fertilizers and pesticides), phenological
 observations (sowing-germination,
 germination-beginning of flowering,
 beginning of flowering-full maturity) and
 biometric measurements (plant height,
 cm; height to 1 pod, cm; growth rate,
 cm/day; number of pods and seeds per
 plant, aboveground mass, g/plant, seed
 weight, g/plant; mass of 1000 seeds, g)
 were conducted. The ecological stability
 was estimated through application of the
 methods of Plaisted and Peterson (1959)
 and Wricke (1965) – respectively the
 parameter PP and ecovalence W^2 .

Biochemical analysis of
 aboveground mass (at the stage of
 flowering) and seeds included the
 following determinations (g/kg DM): crude
 protein (CP) by the *Kjeldahl* method;
 crude fiber (CF) – by *Weende* method;
 calcium (Ca) and phosphorus (P) by
 Sandev (1979); crude fats and ash
 (AOAC, 2010).

RESULTS AND DISCUSSION

The meteorological conditions
 during vegetation period of the two
 experimental years were characterized by
 great variability (Table 1). The rainfall in
 April (73.1 mm) and May (76.3 mm) of
 2016 were about twice more to the same
 period of 2015, while in June 2016, the
 amount of rainfall was less (45.8 mm)
 compared to the previous two months
 which influenced the general growth and
 development of plants. Regarding the
 temperature, it can be noted that the

(-) 2015 .
 2016 .
 16.8 17.6 °C.

period of active vegetation (April-June) in
 - 2015 took place under conditions of
 - higher average daily temperatures
 - compared to 2016. In general, the
 average daily temperature during the
 vegetation period of the first and the
 second experimental year was
 respectively 16.8 and 17.6 °C.

1.

Table 1. Meteorological characteristic of the study period

/Years	/Months				
	III	IV	V	VI	VII
	/Average daily temperature, °C				
2015	6.7	12.2	18.8	20.7	25.8
2016	8.5	15.3	16.4	23.0	24.6
	/ Rainfalls, mm				
2015	68.4	43.6	30.6	95.7	21.5
2016	76.6	73.1	76.5	45.8	7.8

(2).
 (14-16
 , (18)

The phenological observations
 - showed considerable differences among
 - the species, some more at the phase of
 germination (Table 2). This phase occurred
 - at the earliest in the varieties of white lupine
 and broad bean (14-16 days after sowing),
 - followed by peas, vetch and chickpea (18
 days).

2.

Table 2. Duration of main interstage periods in annual leguminous species, temperature sums and rainfalls

Species	Sowing-germination, days	Germination-beginning of flowering, days	Beginning of flowering-full maturity, days	Vegetation period, days	Active temperatures for the vegetation period, °C	Rainfalls for the vegetation period, mm
/White lupine	14	39	57	95	1867	179
/Peas	18	44	39	83	1589	176
/Soybeans	25	43	68	111	2384	169
/Vetch	18	50	34	84	1616	176
/Chickpea	18	52	52	103	2115	187
/Broad bean	16	39	35	74	1732	177

(25).

Soybean plants germinated last (25
 - days). The development of annual
 legume crops of white lupine and broad
 - bean during the next period “germination-
 - flowering” went with an intensity

corresponding to the development during the previous phenological period. A longer period, respectively, more days were necessary for the occurrence of the flowering phase in peas and soybeans (43-44 days), vetch and chickpea (50-52 days).

The studied species also differed in the duration of the period "beginning of flowering-full maturity". This period was the shortest for the vetch (34 days), followed by broad bean (35 days) and peas (39 days), and the longest – for soybeans (68 days). In ascending order, the succession with regard to the longevity of vegetation period was as follows: broad bean – 74 days, peas - 83 days, vetch – 84 days, white lupine - 95 days, chickpea – 103 days and soybeans – 111 days. In annual legume crops, most of which were not tolerant to drought and high temperatures, especially during flowering, the short vegetation period had an essential meaning. For the harvesting of the annual legume species, subject to the present study, total temperatures of 1589 to 2384 ° C and rainfalls of 169 to 187 mm were required.

Plant height, growth rate and the quantity of aboveground biomass are parameters that are important for the conditions of organic production. High values of these indicators determine greater competition ability and correlates negatively with the weed biomass formation (Uhr et al., 2014). In terms of height and growth rate, maximum values showed peas (respectively 110.0 cm and 1.32 cm / day⁻¹), followed by chickpea and broad bean with considerably lower values, and with regard to aboveground mass – soybeans (57.96 g / plant), followed again by chickpea and broad bean (Table 3).

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3.

Table 3. Vegetative characteristics of annual leguminous crops

/Species	Height cm	Growth rate m/day	Aboveground mass g/plant
/White lupine	51.08	0.52	26.10
/Peas	110.00	1.32	23.38
/Soybeans	70.00	0.62	57.96
/Vetch	59.33	0.70	4.33
/Chickpea	71.61	0.69	43.00
/Broad bean	60.35	0.88	38.63
LSD _{0.05}	9.20	0.11	8.31

The study of the elements in the yield structure and the relation between the individual and total productivity of the varieties was a means of identifying the most appropriate variety for the particular agro-climatic conditions. The data obtained from the comparative study showed essential variation in the main components of productivity (Table 4). The high enough disposition of the first pod was an important requirement that helped harvesting plants without losses.

The varieties Pleven 4 and Plovdiv 8, respectively in the species of peas and chickpea, forming longer stems, set the first pod at a higher height. With the lowest disposition of the first pod was the soybeans, variety Galena, but the same variety exceeded significantly the other varieties by number of pods (52) and number of seeds (122) per plant. With a favorable combination of number of pods and seeds was the white lupine (respectively 13 and 50), as well chickpea (23 and 20) which despite the greater number of pods did not form more than 20 seeds per plant. Vetch and bean were characterized by low values of these traits, respectively 5-7 numbers of pods and 17-22 seeds per plant.

No significant differences were established regarding the indicated traits in the most species (varieties). The biological peculiarities of each variety and the different species belonging to the studied accessions did not suggest obligatory that a relatively higher number of seeds

17 g.
(16.52 g)

and of pods will determine a higher seed weight per plant. In this trait, the broad bean represented by variety Chioska, formed a small number of seeds, but with mass higher than 17 g. In soybeans, variety Galena, the seed weight per plant was lower (16.52 g) despite the higher number of seeds per plant. The lowest values for these traits were reported in vetch.

4.

Table 4. Biometric characteristic of annual leguminous crops

Species	1 Height to 1 pod, cm	Number of pods per plant	Number of seeds per plant	Number of seeds per pod	Seed weight g/plant	1000 a Mass of 1000 seeds, g
White lupine	28.5	13	50	3.78	13.82	278.72
/Peas	51.9	8	27	3.46	3.25	119.27
/Soybeans	12.0	52	122	2.37	16.52	135.07
/Vetch	35.2	5	17	3.25	0.94	54.60
/Chickpea	34.0	23	20	0.86	13.94	206.08
/Broad bean	29.2	7	22	3.18	17.52	801.10
LSD _{0.05}	3.91	3.65	9.20	0.55	4.75	35.91

et al., 2014).

(Regulation /EU/ 505, 2012),

(Uhr et al., 2014; Uhr and Ivanov, 2015)

W₂ PP,

(1).

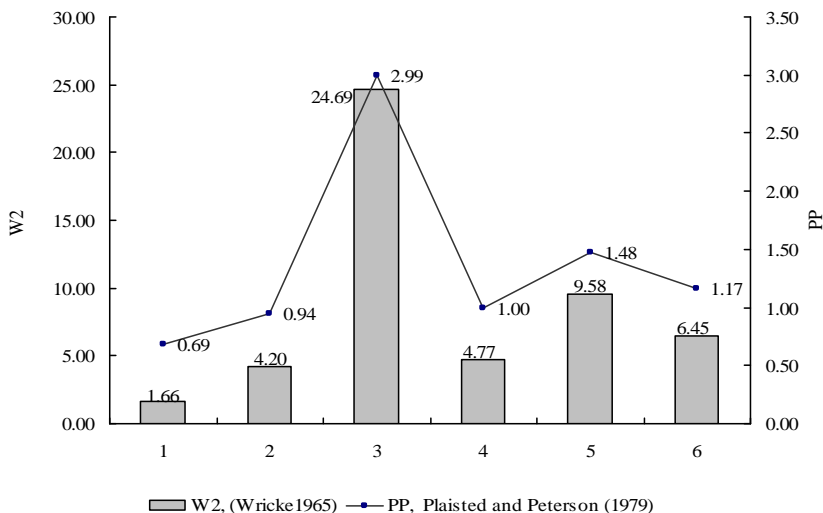
18.8%,

1

Productivity was an integral indicator for the complex of qualities that a variety owns, including resistance to stress factors. Suitable for organic production were species and varieties grown with minimal use of non-renewable and external resources (Regulation / EU / No 505, 2012), which also distinguished with high productive potential and stability (Uhr et al., 2014; Uhr and Ivanov, 2015) under the specific soil and climatic conditions of the region. Over the two-year experimental period, the highest productivity was demonstrated by broad bean and soybeans, but at values of stability parameters W₂ and PP, which defined them as ecologically unstable (Figure 1). Compared to the indicated species, the productivity of white lupine was lower by 18.8% but with stability parameters close to 1 determining it as medium-stable, on the base of that, it was a suitable compromise for organic production conditions.

As medium stable species may also be

pointed peas and vetch, but they showed the lowest productivity for the experimental period.



1 - White lupine; 2 - Peas; 3 - Soybeans; 4 - Vetch; 5 - Chickpea; 6 - Broad bean

1. “W₂” (Wricke, 1965) “PP” (Plaisted and Peterson, 1959)
Fig. 1. Stability parameters “W₂” (Wricke, 1965) and “PP” (Plaisted and Peterson, 1959) of the trait “seed productivity”

(5) 125.4
 g/kg DM 187.7 218.6 g/kg
 DM
 (172.2 g/kg DM)
 (302.5 290.5 g/kg DM).

In terms of the biochemical composition of the aboveground mass and seeds, the studied crops manifested essential differences. The crude protein content (Table 5) in the aboveground mass was in the range of 125.4 g/kg DM in chickpea to 187.7 and 218.6 g/kg DM, respectively in white lupine and soybeans. Similar was the dependence regarding the mineral composition of the biomass. The data on the crude fiber content at flowering-pod formation stage showed a strong variation with a minimum value in soybeans (172.2 g / kg DM) and maximum values for peas and chickpea (302.5 and 290.5 g/kg DM).

Table 5. Biochemical composition of the aboveground mass of annual leguminous crops, g/kg DM

Species	Crude protein	Crude fibers	Ash	Arithmetic sum of the ranks for CP, CF and ash	Rank
/White lupine	187.7	202.1	156.0	6	2
/Peas	168.8	302.5	89.5	13	4
/Soybeans	218.6	172.2	126.0	4	1
/Vetch	170.4	287.3	75.2	13	5
/Chickpea	125.4	290.5	81.0	16	4
/Broad bean	165.1	185.0	82.4	11	3

6). , , (316.39, 189.75) 50.66 g/kg D () 160.95 g/kg D () 16.90 g/kg

As regards seed quality, on average for the study period, soybeans and white lupine varieties outperformed significantly the other accessions, because they showed the highest values of the main tested indicators (Table 6). Soybeans, variety Galena, which demonstrated very good productivity (g/plant), also realized the highest content of crude protein, crude fat and ash in the seeds (respectively 316.39, 189.75 and 50.66 g/kg DM). With such a good combination of productivity and crude protein, crude fat and ash content, was characterized also white lupine, variety Garant. The abovementioned varieties had also the highest content of calcium and phosphorus.

The crude fiber content of seeds influenced the nutritional value and the digestibility of the forage. The data showed strong variation, in limits from 16.90 g/kg DM (chickpea) to 160.95 g/kg DM (white lupine). In chickpea low values were established in almost all biochemical parameters studied.

The arithmetic sum of the ranks of parameters from the biochemical analysis of seeds determined the rank 1, respectively the highest quality of the soybean forage, followed by white lupine, vetch, broad bean, chickpea and peas.

6.
, g/kg DM

Table 6. Biochemical composition of seeds of annual leguminous crops, g/kg DM

Species	Crude protein	Crude fibers	Crude fats	Ash			Arithmetic sum of the ranks	Rank
White lupine	282.52	160.95	109.25	41.01	3.68	5.28	15	2
/Peas	228.20	62.63	13.49	33.02	1.31	4.11	31	6
/Soybeans	316.39	119.64	189.75	50.66	2.23	5.72	11	1
/Vetch	280.14	43.53	18.26	34.56	2.07	4.43	21	3
/Chickpea	204.38	16.90	57.61	33.03	1.31	4.48	24	5
/Broad bean	248.52	64.88	14.89	35.81	1.52	5.11	23	4

CONCLUSIONS

The comparative agrobiological assessment of annual legume crops of peas, vetch, lupine, broad bean, soybeans and chickpea in the organic production conditions showed a strong variation of the quantitative and qualitative parameters, which suggested wide possibilities for their use. Broad bean (variety Hioska) and peas (variety Pleven 4) were characterized by a short vegetation period, a greater height and a fast growth, and soybeans (variety Galena) and chickpea (variety Plovdiv 8) were distinguished with the accumulation of considerable quantities of aboveground biomass.

A high productivity during the experimental period showed broad bean and soybeans, but they were ecologically unstable. Compared to the indicated species, the productivity of white lupine was lower by 18.8%, but it was ecologically stable, which determined it as a suitable compromise for organic production conditions. As species with medium stability may be pointed peas (Pleven 4) and vetch (Obrazets 666), but they characterized with considerably lower productivity.

The arithmetic sum of the ranks of parameters from the biochemical analysis of seeds determined the rank 1, respectively the highest quality of the soybean forage, followed by white lupine, vetch, broad bean, chickpea and peas.

- On the basis of the comparative
- assessment of annual leguminous crops, it is recommended, along with the traditionally grown soybeans, peas and vetches, in the structure of protein crops to be incorporated the species of white lupine, broad bean and chickpea.

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