FORAGE PLANT CELL WALLS FIBER COMPONENTS CONTENT AND DIGESTIBILITY OF NEW VARIETIES PERENNIAL GRASSES IN THE VEGETATION

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Forage perennial grass species are economically effective and high quality and healthy forage in ruminant nutrition. Lays and pastures presented about 70% from agricultural lands over the world; they are under influence of many peculiarities of climate changes, affecting productivity, botanical composition and quality and influence on animal production (**Humphreys et al.,** 2011). The forage plant cell walls fiber components, determining energy feeding value and enzyme *in vitro* digestibility as the main forage quality characteristics are variable in the vegetation. These parameters are determined for the standard Bulgarian perennial grass species: orchardgrass (*Dactylis glomerata* L.), tall fescue (*Festuca arundinacea* Schreb.), smooth bromegrass (*Bromus inermis* Leyss.) (**Naydenova,** 2012).

The new perennial ryegrass (*Lolium perenne* L), variety **IFK Harmoniya** is early diploid, high productive, ecologically stable (winter hardy and drought tolerant) and persistent. The variety is multifunctional, suitable for pasture, hay-pasture and amenity direction of use in pure stands or in mixtures with white clover for forage, or with red fescue for ornamental and sport use (**Katova,** 2011). The first Bulgarian crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.), variety **Svejina** is diploid, high productive, ecologically stable (winter hardy and drought resistant), resistant to leaves diseases and exceptionally long-lived and persistent. **Svejina** is multifunctional, suitable for pasture, hay pasture and amenity direction of use, for erosion control and landscape maintaining (**Katova,** 2012a). The first Bulgarian and European standard wheatgrass [*Agropyron desertorum* (Fisch.) Schultes], variety **Morava** is tetraploid, high productive, ecologically stable, winter hardy and very drought resistant, resistant to leaves diseases and exceptionally long-lived and persistent. **Morava** is multifunctional, suitable for hay, hay-pasture use, for erosion control and landscape maintaining (**Katova,** 2012b).

The aim of the study is to establish the changes in plant cell walls fiber components content – poliosides and lignin and enzyme *in vitro* degradability (digestibility) of new registered perennial grass varieties in OECD list, Official variety list of the Republic of Bulgaria as well as perennial grass breeding populations.

MATERIAL AND METHODS

The field crop experiments of three different species – 1. perennial ryegrass (*Lolium perenne* L.) var. **IFK Harmoniya -** diploid and breeding populations **NBG** and **SBG** tetraploids; 2. crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.) (AC), var. **Svejina -** diploid; 3. desert/standard wheatgrass [*Agropyron desertorum* (Fisch.) Schultes] (AD), var. **Morava** – tetraploid were established in 2011. The perennial grass forages were harvested during 2012 at five weekly periods, in 2013 at four (Table 1).

Plant sample preparation from the above ground part of the plants is effectuate by air ventilation at 65^0C till crumbly at previous fixing for 20 min at 105^0C and grinding till particle size 1,0 mm consecutively at laboratory mills QC 136 and QB 114, Labor Mim, Hungary and obligatory screen. Detergent analysis of Goering&Van Soest (1970) (EN ISO13906 2008) was performed as a standard systematic chemical analysis of plant cell walls fiber components. The following fiber fractions: *Neutral-detergent fiber (NDF)*; *Acid-detergent fiber (ADF)*, *Acid-detergent lignin (ADL) are determined*. Polyosides hemicellulose and cellulose as a cell walls components, contained in fiber fraction are presented emperically: *Hemicellulose = NDF – ADF; Cellulose = ADF – ADL. The degree of lignification* is presented as relation of ADL and NDF/100 (Akin&Chesson 1989). Enzyme *in vitro* digestibility of dry (СмСВ/IVDMD) and organic (СмОВ/IVOMD) matter is determined by two stage pepsin-cellulase enzyme method of Aufrere (Todorov et al. 2010). First step – previous attack with *pepsin* /200 FIB-U g^{-1}/,

Table 1. **Dates and phenological stages in the vegetation of perennial grasses**
Таблица 1. **Дати и фази на развитие през вегетацията на многогодишни житни треви**

Откос Harvest		Пасищен райграс *Lolium perenne* L.			*Гребенчат житняк Agropyron cristatum* Gaertn.	*Пустинен житняк Agropyron desertorum* Ficsh. Shultes
		ИФК Хармония **IFK Harmoniya**	Селекционна Популация **NBG**	Селекционна Популация **SBG**	**Svejina**	**Morava**
Week Седмица	Date Дата	Phenological stages/Фенологични фази				
2012						
4	19 April	Tillering	Tillering	Tillering	Tillering	Tillering
5	26 April	Early heading	Tillering	Early heading	Tillering	Tillering
6	03 May	Heading	Early heading	Early flowering	Early heading	Early heading
7	10 May	Early flowering	Heading	Flowering	Heading	Heading
8	17 May	Flowering	Early flowering	Full flowerng	Early flowering	Early flowering
2013						
5	24 April	Tillering	Tillering	Early heading	Tillering	Tillering
6	30 April	Early heading	Early heading	Heading	Early heading	Early heading
7	07 May	Heading	Heading	Early flowering	Heading	Heading
8	14 May	Flowering	Early flowering	Full flowerng	Early flowering	Early flowering

Merck 7190, Germany in *1 N* Hydrohloric acid for 24 hours. Second step – attack with cellulase *"Onozuka R-10"*, isolated from *Trichoderma viride* /Endo-1,4-β-glucanase; 1,4-(1,3:1,4)-β-D glucan - 4-glucanhydrolase/ with enzyme activity 1,2 $U.g^{-1}$, M 52 000, EC 3.2.1.4., Serva 16419, 1g l^{-1} in 0,05 M acetate buffer pH 4,6 for 24 hours at 40^0C.

RESULTS AND DISCUSSION

Changes in plant cell walls fiber composition in the vegetation

When establishing the efficiency and forage value of grasses it is of great importance to determine the content of principal chemical substances having relation to assimilation of grass biomass and the changes that occur in their concentration with the change of plant age. The plant cell walls fiber components are the main substances exerting substantial influence on plant quality and their nutritive value having in mind that fat quantity in grasses is in small quantity (approximately 2%) and nitrogen free extracts are computable character. When estimating the different species and particularly when proving the differences in the quality in different genotypes (clones) of one plant species or even variety the content of the individual structural plant cell wall fiber components has a substantial importance. They are directly related to plant morphological structure and cell walls composition and exert considerable influence on perennial grass digestibility and feeding value.

The plant cell walls fiber components content is presented on Table 2 and Table 3. The significance of fiber components for forage quality evaluation is optimal and relatively low content of NDF, ADF, ADL and Cellulose and high content of Hemicellulose as digestible poliosid. Plant cell walls fiber components of the forage plants contain from 300 to 800 g kg-1 of dry matter (30-80%) and they are main source of feeding energy for ruminats as less than 50 % of them are used (**Fahey & Hussein,** 1999). The Neutral detergent fibers determine the total content of fiber components of cell walls of lignin, cellulose and hemicallulose and

Table 2. **Plant cell walls fiber components content, degree of lignification and enzyme *in vitro* dry matter digestibility of new perennial grass species and varieties in the vegetation, 2012, % DM, coeff. lignification, % digestibility**
Таблица 2. **Състав, степен на лигнификация и ензимна *in vitro* смилаемост на нови видове и сортове многогодишни житни треви през вегетацията, 2012, % СВ, коеф. лигниф, % смилаемост**

Седмици Weeks	Вид Species	НДВ NDF	КДВ ADF	КДЛ ADL	Хеми HEMI	Целу CELLU	Лигн Lignif	СмСВ IVDMD	СмОВ IVOMD
4	RyHarmo	51.74	27.45	2.60	24.29	24.85	5.0	78.54	79.21
5	Ry NBG	53.70	28.20	2.77	25.50	25.43	5.2	76.54	77.16
6	Ry SBG	55.40	30.65	3.25	24.75	27.40	5.9	67.94	67.55
7	AC	56.51	29.28	2.96	27.23	26.32	5.2	68.38	68.78
8	AD	55.40	28.32	2.62	27.08	25.70	4.7	73.45	74.52
4	RyHarmo	55.13	26.35	2.06	27.78	24.29	3.7	77.05	77.68
5	Ry NBG	47.93	23.98	1.39	23.95	22.59	2.9	80.58	80.92
6	Ry SBG	48.84	26.76	2.26	22.08	24.50	4.6	74.77	75.42
7	AC	54.97	29.39	3.15	25.58	26.24	5.7	67.89	68.88
8	AD	53.74	28.40	2.77	25.34	25.63	5.2	70.07	71.28
4	RyHarmo	54.09	28.98	4.07	25.11	24.91	7.5	72.15	73.26
5	Ry NBG	48.10	26.23	1.64	21.87	24.59	3.4	77.22	77.77
6	Ry SBG	51.55	30.30	3.14	21.25	27.16	6.1	69.02	69.25
7	AC	52.95	28.69	2.75	24.26	25.94	5.2	70.60	71.09
8	AD	55.16	30.46	3.22	24.70	27.24	5.8	67.12	67.97
4	RyHarmo	56.27	29.96	3.15	26.31	26.81	5.6	67.76	68.65
5	Ry NBG	52.89	29.93	3.31	22.96	26.62	6.2	72.22	72.15
6	Ry SBG	55.61	33.34	4.33	22.27	29.01	7.8	63.11	63.59
7	AC	64.30	35.96	5.24	28.38	30.68	8.1	58.26	59.15
8	AD	56.30	31.76	3.86	24.54	27.90	6.8	68.61	69.53
4	RyHarmo	60.00	34.22	4.07	25.78	30.15	6.8	61.02	61.86
5	Ry NBG	54.55	29.97	2.80	24.58	27.17	5.1	71.88	72.67
6	Ry SBG	56.08	32.67	3.89	23.41	28.78	6.9	62.96	63.97
7	AC	59.04	33.62	4.22	25.42	29.40	7.1	61.56	62.55
8	AD	60.79	34.00	4.43	26.79	29.57	7.3	60.38	61.56
Mean		54.84	29.95	3.20	24.85	26.76	5.8	69.52	70.72
SD		*3.76*	*2.87*	*0.90*	*1.86*	*2.03*	*1.3*	*6.00*	*5.61*

they are main characteristics of predicting animal intake at libitum by ruminats (**Casler & Vogel,** 1999).

In the first vegetative growth in growing process of the perennial grasses with the increase of their height parameters the plant cell walls components content increased. The lowest NDF content is measured for tetraploid populations perennial ryegrass **NBG** and **SBG** in the whole period investigated (Table 2 and Table 3). The low content of fiber components is resulted in high digestibility values and high forage quality. The Acid detergent fiber is the fraction contained lignin and cellulose of plant cell walls and determine forage digestibility by ruminant. Between forms perennial ryegrass the lowest ADF content is breeding population **NBG** in flowering stage in comparison of **SBG** and **IFK Harmoniya** while for the two varieties wheatgrass at the same development stage show closed values (Fig. 1).

Changes in degree of lignification and digestibility in the vegetation

Perennial grass species and varieties in spring growth during the 2012 reached pasture stage at 19 April and 26 April (Table. 2). The low degree of lignification is observed – coeff. 4.7 – 5.9. Comparing the three varieties of perennial ryegrass NBG has the low-

Table 3. **Plant cell walls fiber components content, degree of lignification and enzyme *in vitro* dry matter digestibility of new perennial grass species and varieties in the vegetation, 2013, % DM, coeff. lignification, % digestibility**

Таблица 3. **Състав, степен на лигнификация и ензимна *in vitro* смилаемост на нови видове и сортове многогодишни житни треви през вегетацията, 2013, % СВ, коеф. лигниф, % смилаемост**

Седмици Weeks	Вид Species	НДВ NDF	КДВ ADF	КДЛ ADL	Хеми HEMI	Целу CELLU	Лигн Lignif	СмСВ IVDMD	СмОВ IVOMD
5	Ry NBG	46.60	24.16	1.07	22.41	23.09	2.3	81.61	82.42
6	Ry SBG	44.27	24.10	1.20	20.17	22.90	2.7	81.66	82.75
7	AC	44.01	26.95	2.03	17.06	24.92	4.6	71.48	72.66
8	AD	49.14	33.67	2.88	15.47	30.79	5.9	78.99	80.07
5	Ry NBG	49.86	28.72	1.84	21.14	26.88	3.7	77.00	78.43
6	Ry SBG	49.62	25.00	2.07	24.63	22.92	4.2	66.40	67.02
7	AC	57.20	29.98	3.16	27.22	26.82	5.5	66.60	66.88
8	AD	55.36	31.73	2.81	23.63	28.92	5.1	69.17	70.00
5	Ry NBG	50.75	27.64	2.48	23.11	25.16	4.9	73.50	74.13
6	Ry SBG	40.42	35.92	3.96	4.50	31.96	9.8	57.36	58.12
7	AC	61.22	36.14	4.40	25.08	31.74	7.2	58.44	59.55
8	AD	63.23	36.75	3.90	26.48	32.85	6.2	60.96	62.32
5	Ry NBG	60.24	45.14	3.66	15.10	41.48	6.1	54.42	56.14
6	Ry SBG	60.90	37.00	4.49	23.91	32.50	7.4	52.63	54.28
7	AC	62.98	36.05	5.01	46.93	31.04	6.0	53.52	54.73
8	AD	64.12	38.05	5.97	26.07	32.08	9.3	49.21	50.67
Mean		53.74	31.68	3.18	22.68	29.13	5.7	65.80	66.88
SD		*7.84*	*6.09*	*1.39*	*8.67*	*4.94*	*2.07*	*10.90*	*10.74*

est lignification coeff. 2.9, **IFK Harmoniya** – intermediate; coeff. 3.7 and the highest for tetraploid ryegrass SBG – coeff. of lignification 4.6. The tetraploids usually have lower degree of lignification than the diploids, but the reason is that the ryegrass SBG is very early developing variety when is the stage of development is early heading. All plant forms of perennial ryegrass present lower lignification in comparison of crested wheatgrass and desert wheatgrass.

In the vegetation progress the degree of lignification increased at 10 May – seventh week, when the wheatgrass species reached the stage of early heading. For the two species/varieties wheatgrasses the degree of lignification state with closed values – coeff. 4.7 – 5.8 during the three weeks period, when the stems rest erected without lodging.

The crested wheatgrass at seventh week after spring growing – 10 May doubled the degree of lignification in comparison of the beginning of estimation – coeff. 8,1.

Perennial ryegrass variety and breeding populations have higher degree of lignification with comparison of wheatgrasses because of more rapid temp of growing and development. The tetraploid forms of perennial ryegrass present lower degree of lignification compared to diploid variety **IFK Harmoniya** during fourth, fifth and sixth weeks of development. Perennial ryegrass, **IFK Harmoniya** during the sixth week of vegetation and reaching the stage of heading present the highest degree of lignification in comparison of tetraploid forms **SBG** and **NBG.**

The enzyme *in vitro* digestibility of dry and organic forage matter of perennial ryegrass (all three forms) is higher in comparison of that of the wheatgrass species during whole experimental period in 2012 (Fig. 2). The highest value of digestibility was 80.58% reached at pasture stage for perennial ryegrass **NBG** during the

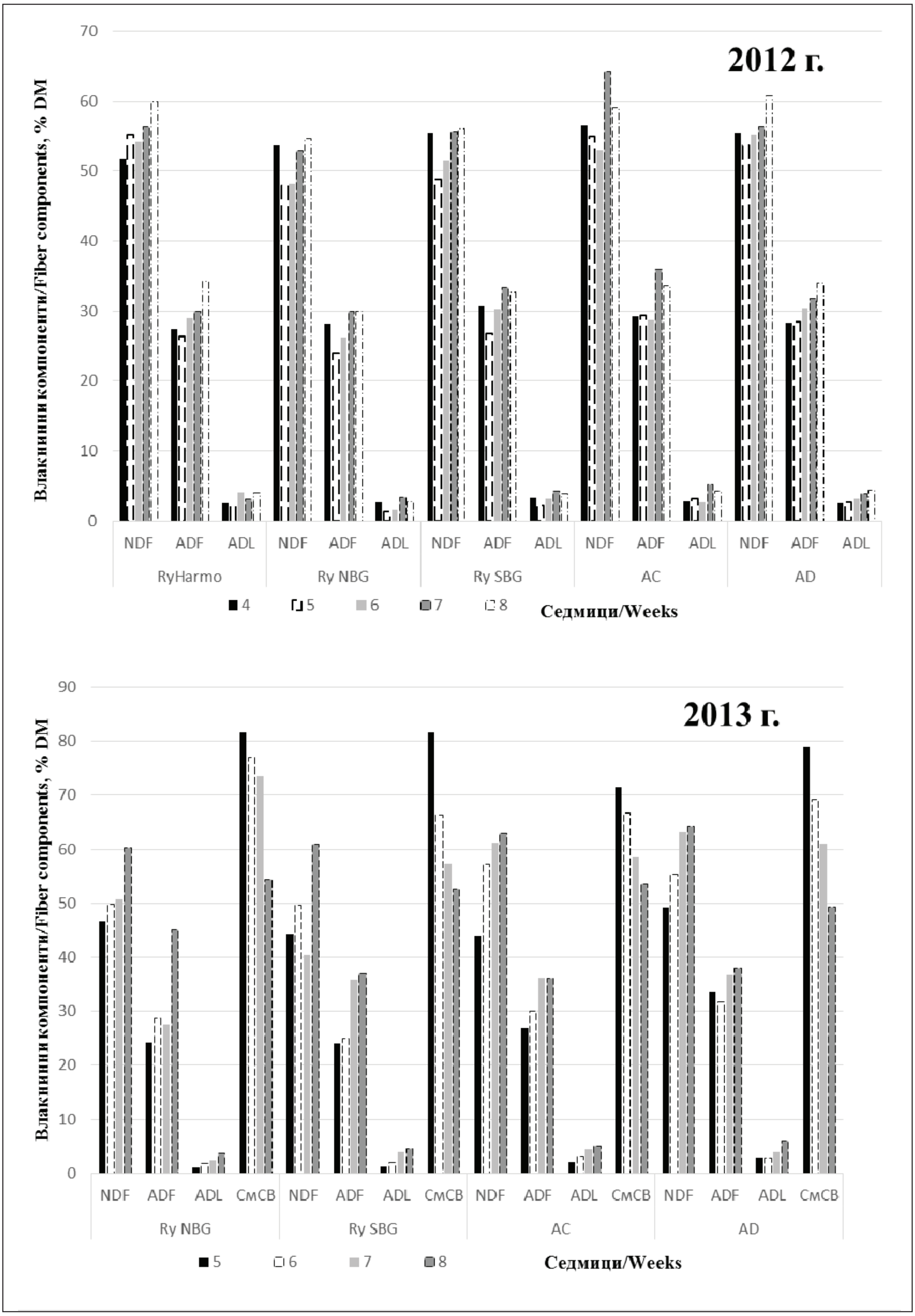

Fig. 1. **Plant cell walls fiber components and digestibility of perennial grass species and varieties**
Фиг **1. Влакнинни компоненти и смилаемост на видове и сортове житни треви**

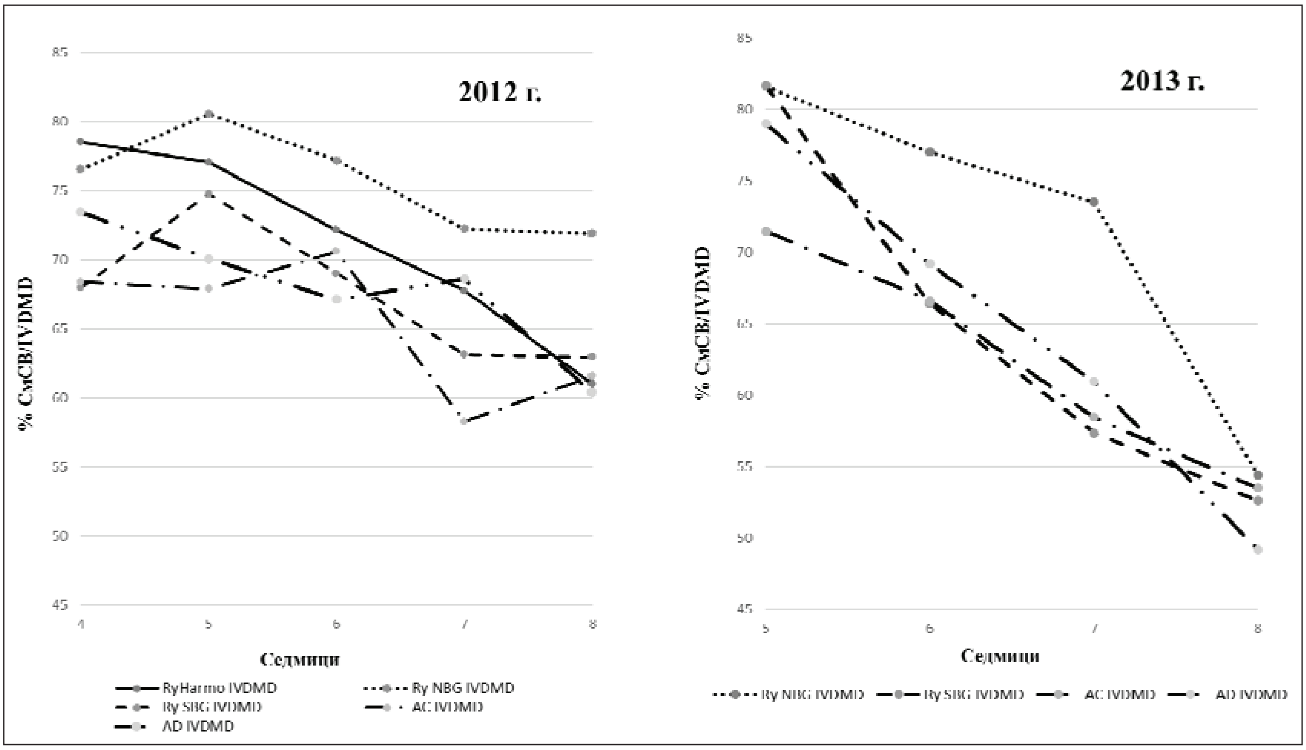

Fig. 2. **Enzyme *in vitro* digestibility of dry matter of new species and varieties perennial grasses**
Фиг. 2. **Ензимна смилаемост на сухото вещество на нови видове и сортове житни треви**

fifth week of development. The digestibility state very high up to 17 May – last eight week of investigation – 71.88%, after that slowly decreased. Distinguishing the perennial ryegrass breeding forms concerning digestibility is found during the sixth week when the digestibility is exceptionally high for **NBG** – 77.22%, high for variety **IFK Harmoniya** – 72.15%, while for the **SBG** the digestibility is comparatively lower – 69.02%. The reason of lower digestibility for **SBG** is it earliness of maturity.

Higher digestibility is established for the tetraploid standard wheatgrass *Agropyron desertorum* - **Morava** in comparison with diploid crested wheatgrass *Agropyron cristatum* (L.) – **Svejina** for each week studied (from fourth to seventh week). The two species and varieties of wheatgrass reached stage of heading and that is why the digestibility decreased: 61.56% for crested wheatgrass and 60.38% for standard wheatgrass.

During the 2013 year the observations continue for the tetraploid breeding populations of perennial ryegrass **NBG** and **SBG** and for crested and desert wheatgrass varieties from the fifth to eighth week of plant development. The plant spring growth starts later comparing to 2012 in reason of lower air temperatures in April. The lowest degree of lignification is observed coeff 2.3 – 2.7 in the fifth week for the ryegrass **NBG** and **SBG** respectively. The degree of lignification during the vegetation is progressively increased. The breeding population of perennial ryegrass **NBG** rest with the lowest degree of lignification in the whole period investigated. The other breeding population **SBG** after the seventh week triple the lignification degree value very fast because of early type of maturity (Table 3). Principally the wheatgrass varieties in the third year of plant development show higher degree of lignification than perennial ryegrass populations. From the beginning of spring growing of the wheatgrass varieties doublt the degree of lignification till the flowering stage. Comparing the two wheatgrass species and varieties *Agropyron desertorum* (Fisch.) Schultes **Morava** is higher lignified coff. 9.3.

The tendency of higher digestibility of perennial ryegrass forms in comparison of wheatgrass varieties remains the same during the 2013 than 2012. The highest digestibility is estimated during pasture stage in fifth week for perennial ryegrass forms **NBG** and **SBG.**

In the vegetation progress the digestibility decreased for all species and varieties investigated. In the sixth and seventh weeks the digestibility is relatively high and the highest value is observed for perennial ryegrass **NBG** 78.43% and 74.13% respectively. More demonstrative is decreasing of digestibility for the perennial ryegrass **SBG** in the same period – 67.02% and 58.12%. From the fifth to seventh weeks the digestibility for the **Morava** and **Svejina** wheatgrass new and the first Bulgarian varieties remains comparatively high (from 80.07% to 62.32%). In the last week observed all species and varieties decreased digestibility stronger for desert wheatgrass **Morava** 49.21%.

CONCLUSIONS

The perennial grass species and varieties present high forage quality in pasture development stage. Changes in forage quality appeared forward vegetation process but they have different temp.

Tetraploid perennial ryegrass populations remains high quality forage for the longest period in the first spring growth which allow enlarged pasture period near to a month.

The tetraploid perennial ryegrass breeding population **NBG** demonstrates the best forage quality characteristics in comparison with other studied species and varieties. *Pasture stage* forage fiber components, lignification and digestibility are presented as follow: 2012: NDF 53.70%, ADF 28.2%, ADL 2.77%, Lignification 5.2 and IVDMD 76.54 and for 2013 respectivelly: 46.60%, 24.16%, 1.07%, coeff. 2.3, 81.61%. *Flowering stage* forage fiber components, lignification and digestibility are presented as follow: 2012: NDF 54.5%, ADF 29.97%, ADL 2.8%, Lignification 5.1 and IVDMD 71.88% and for 2013 respectivelly: 60.24%, 45.14%, 3.66%, coeff. 6.1, 54.42%.

The tetraploid perennial ryegrass breeding population **SBG** demonstrates faster changes in forage quality characteristics because it is the earliest in maturity. Pasture stage forage fiber components, lignification and digestibility are presented as follow: 2012: NDF 55.4%, ADF 30.65%, ADL 3.25%, Lignification 5.9 and IVDMD 67.94% and for 2013 respectivelly: 44.27%, 24.1%, 1.2%, coeff. 2.7, 81.66%. *Flowering stage* forage fiber components, lignification and digestibility are presented as follow: 2012: NDF 56.08%, ADF 32.67%, ADL 3.89%, Lignification 6.9 and IVDMD 62.96% and for

2013 respectivelly: 60.90%, 37.00%, 4.49%, coeff. 7.4, 52.63%.

Forage quality for two wheatgrass varieties has higher values of plant cell walls fiber components content and lower digestibility in comparison with perennial ryegrass.

Desert wheatgrass **Morava** in the vegetation process present higher values of plant cell walls fiber components content and lower digestibility in comparison with diploid crested wheatgrass **Svejina.**

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SUMMARY

The forage plant cell walls fiber components, determining energy feeding value and enzyme *in vitro* digestibility as the main forage quality characteristics are variable in the vegetation. The aim of the study is to establish the changes in plant cell walls fiber components content – poliosides and lignin and enzyme *in vitro* degradability (digestibility) of new registered perennial grass varieties in OECD list, Official variety list of the Republic of Bulgaria as well as perennial grass breeding populations. The perennial grass species and varieties present high forage quality in pasture development stage. Changes in forage quality appeared forward vegetation process but they have different temp. Tetraploid perennial ryegrass populations remains high quality forage for the longest period in the first spring growth which allow enlarged pasture period near to a month.

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Forage quality for two wheatgrass varieties has higher values of plant cell walls fiber components content and lower digestibility in comparison with perennial ryegrass. Desert wheatgrass **Morava** in the vegetation process present higher values of plant cell walls fiber components content and lower digestibility in comparison with diploid crested wheatgrass **Svejina.**

Key words: *fibers, in vitro digestibility, forage quality, new perennial grass species and varieties*

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ЗАДЪЛЖИТЕЛНИ ИЗИСКВАНИЯ КЪМ АВТОРИТЕ

1. Авторът представя статията за рецензиране от Редакционната колегия на списанието, задължително придружена с протокол от научната секция, напълно комплектувана и записана на електронен носител (диск, флаш памет), ако не е изпратена по електронна поща.

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