

NUTRITIVE VALUE OF LEAVES AND STEMS IN DIFFERENT LUCERNE VARIETIES

HRANJIVA VRIJEDNOST LIŠĆA I STABLJIKU U RAZLIČITIH VRSTA LUCERNE

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ABSTRACT

Chemical composition and nutritive value of stems and leaves of nine lucerne (*Medicago sativa*) varieties were investigated. Organic matter digestibility and net energy value were estimated on the basis of chemical composition and amount of gas produced at the incubation of samples with rumen liquor in vitro. It was found out that differences between varieties were due to differences in chemical composition of stems and leaves on one hand and on differences in their proportions on the other. Leaves represented on the average 43 % of dry matter yield and 61 % of net energy lactation (NEL) yield, respectively. Despite of the low proportion of leaves in total plant the variety Olimpik had the highest digestibility of organic matter and the highest net energy value. Varieties which were more resistant to diseases gave higher yields of forage, but with lower digestibility and net energy value.

Introduction and literature review

Lucerne is an interesting plant because of high crude protein concentration and high yields even in dry periods. Owing to high concentration of structural carbohydrates lucerne is suitable for ruminant nutrition, especially in cases of structural fibre deficiency in a diet.

Lucerne varieties differ in the concentrations of crude protein, structural carbohydrates and easily soluble carbohydrates (Puffe et al., 1984., Luthra et al., 1988). As a result of differences in chemical composition lucerne varieties differ also in digestibility.

Digestibility of roughage depends mainly on the proportion and digestibility of cell walls (Schoner and Pfeffer, 1985, Van Soest, 1982, Jones, 1970) since the cell content is nearly completely digestible. Digestibility of cell walls depends primarily on their chemical composition and on structural relationships between cellulose, hemicellulose and lignin (Jung, 1989, Kerley et al., 1988).

Diverse digestibility within different varieties of the same species can be due to the presence of some inhibitory substances which inhibit the growth and development of microorganisms in the rumen. These inhibitory substances represent a kind of plant protection against various diseases (Van Soest, 1982). It was established that the resistance of a variety to various diseases depended on the concentration and activity of saponins (Krzymanska and Waligora, 1983, Hanson et al., 1973).

The choice of lucerne for quality forage production relies on many criteria. It is important that the lucerne variety is highly yielding and resistant to diseases and to lodging and that it has a high organic matter digestibility as well as high protein concentration.

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In stems and leaves of some varieties the chemical composition and *in vitro* fermentation in the rumen liquor were determined in order to study their effects on the nutritive value of lucerne. The obtained data were used for the estimation of organic matter digestibility and net energy value. The relations between chemical composition of lucerne, *in vitro* fermentability and resistance to diseases were also investigated.

Material and methods

In nine lucerne varieties (Osječka 12, Osječka 10, Osječka 66, Osječka 11, Osječka 88, Osječka 89, Olimpik 84, Debarska, Prescott) the concentration of structural carbohydrates was determined by the detergent method (Goering and Van Soest, 1970). The samples of lucerne originated from the ecological variety experiment conducted at Jable (KIS, 1993). The experiment was designed as a random block in four repetitions. The basic plot measured 5 m². Lucerne was in the second year of growth. Samples from the second cutting were taken in the prebloom stage (July 3, 1992.). Samples of each variety were taken from three plots. For a proximate analysis and determination of structural carbohydrates all samples were combined while *in vitro* fermentability was performed on each sample. Leaves were separated from stems and buds and dried at 60 °C. They were ground through the 1 mm screen in the Wiley mill. *In vitro* fermentability was determined according to Menke et al. (1979). About 140 mg of a sample was weighed into syringes (100 ml) in 3 parallels and a mixture of rumen liquor and buffer (10 ml rumen liquor and 20 ml buffer) was added. Syringes were incubated in the water bath (39 °C) for 48 hours. The amount of gas production was measured several times during the experiment. Rumen liquor was taken from a fistulated cow which was fed a standard diet. A diet consisted of 20 % concentrates with minerals and vitamins and 80 % hay *ad libitum*. Hay contained 119 g/kg DM of crude protein (CP) and 308 g/kg DM of crude fibre (CF).

Resistance to diseases was estimated visually and marked from 0 to 5. 0 meant that plants were very much affected by diseases, and 5 meant that no sign of a disease was noticed. The most often noticed diseases were *Uromyces striatus* and *Pseudopezzia medicaginis*.

The following exponential equation (Orskov and McDonald, 1979) was used to describe the dynamics of gas production at *in vitro* fermentation of forage (Menke et al., 1979):

$$p = a + b(1 - e^{-ct}) \quad /eq 1/$$

Where a, b, c, are constants calculated for each

sample. p represents the amount of gas production in time t.

The effective gas production (EGP) was calculated by equation No. 2. The theoretical rumen outflow rate k = 0.05 h⁻¹ was considered.

$$EGP = a + \frac{bc}{c + k} \quad /eq 2/$$

Organic matter digestibility (OMD in %) was estimated on the basis of gas production (GP in ml/200 mg DM), crude protein concentration (CP in g/kg DM) and ash (ASH in g/kg DM) by the following equation (Menke and Huss, 1987):

$$OMD \% = 0.889 GP + 0.0651 CP + 0.0651 ASH + 14.88 \quad /eq 3/$$

Net energy value (NEL in MJ/kg DM) was estimated on the basis of gas production (GP in ml/200 mg DM) and crude protein concentration (CP in g/kg DM), ether extract (EE in g/kg DM) and N-free extract (NFE in g/kg DM) by the following equation (Kirchgessner, 1988):

$$NEL (MJ/kg DM) = 0.075 GP + 0.0087 CP + 0.00161 EE + 0.0056 NFE - 2.422 \quad /eq 4/$$

The results of fermentation test were processed by the analysis of variance (computer programme LSMLMW, Harvey, 1987).

Results and discussion

Tables 1 and 2 show the results of chemical analysis and nutritive value of single lucerne variety. Considering the prebloom stage the average values show a relatively low crude protein concentration (181 g/kg DM), and high concentrations of crude fibre (317 g/kg DM), neutral detergent fibre (428 g NDF/kg DM) acid detergent fibre (356 g ADF/kg DM) and cellulose (276 g/kg DM). These data are comparable with the data for early bloom stage (DLG, 1982, Puffe et al., 1984). Differences between varieties in crude protein and crude fibre concentrations were similar to those determined by Puffe et al. (1984). Lucerne is especially noted for low concentrations of hemicellulose (92 g/kg DM) and high concentrations of cellulose (276 g/kg DM).

The highest differences between varieties were established for the concentrations of crude fibre (CV = 7.9 %) and hemicellulose (CV = 20.3 %), but other nutrients were interesting as well. Differences between varieties

Table 1: Results of chemical analysis in different lucerne varieties in g/kg dry matter
Tablica 1: Rezultati kemijskih analiza kod različitih sorata lucerne u g/kg ST

Variety Sorta	Dry matter Suha tvar	Crude protein sir. bjelančev.	Crude fibre Sirova vlaknina	Ash Sirovi pepeo	NDF NDF	ADF ADF	ADL ADL	Cellulose Celuloza	Hemi- cellulose Hemi- celuloza
Osječka-12	265	171	310	89	434	379	83	296	55
Osječka-11	267	181	373	86	451	371	83	288	80
Osječka-10	259	186	309	91	428	338	75	263	90
Osječka-88	261	180	283	89	418	335	74	261	83
Osječka-89	260	195	311	91	434	349	81	268	85
Debarska	266	166	312	86	433	353	79	274	80
Olimpik	250	188	332	83	432	363	83	280	69
Prescot	266	180	321	83	413	362	84	278	51
Osječka-66	267	184	299	84	409	352	80	272	57
Average Prosjek	260	181	317	87	428	356	80	276	72
Maximum Najviše	267	195	373	91	451	379	84	296	90
Minimum Najmanje	250	166	283	83	409	335	74	261	51
CV %	2.1	4.8	7.9	3.7	3.0	4.1	4.5	4.1	20.3

CV % - coefficient of variability - koeficijent variabilnosti

in concentrations of nutrients, organic matter digestibility and net energy were the consequence of different chemical composition on the one hand and leaves: stems ratio on the other. Some varieties differed significantly in the proportion of leaves ($p < 0.05$). The proportion of leaves in dry matter yield ranged from 40 % to 46 % DM, which is in agreement with the results presented by Puffe et al. (1984).

The crude protein concentration in leaves was high (232 - 282 g/kg DM) while the concentration of crude fibre was low (98 - 114 g/kg DM). The stems contained 85 - 108 g/kg DM of crude protein and 420 - 462 g/kg DM of crude fibre. Similar concentrations and differences were found out by Puffe et al. (1984).

Organic matter digestibility and concentrations of crude protein, crude fibre, crude ash and net energy in leaves and stems were not related (negligibly low and nonsignificant correlations). As expected from the data on chemical composition the organic matter digestibility and net energy value in leaves were higher than in stems. In spite of the fact that leaves represented only 43 % of total dry matter yield they represented 61 % of produced net energy lactation. Mechanical losses during drying in the field could be quite high and ranged

from 15 - 20 % (Mrhar, 1987). If drying conditions are bad the losses can be doubled. Most mechanical losses are due to crushing of the leaves which get dried very quickly. Due to high nutritive value of leaves even the lowest loss means considerably lower digestibility and energy value of the product.

Organic matter digestibility and net energy value of leaves and stems were the highest in the variety Olimpik. Because of the relatively low proportion of leaves the nutritive value and organic matter digestibility of a total plant were not markedly higher than in other varieties. Stems of the variety Olimpik contained a considerably higher concentration of protein and a lower concentration of crude fibre. Due to diverse distribution of nutrients between stems and leaves the variety Olimpik is suitable for hay production. It can be expected that the effect of crushing on the nutritive value of hay will be lower than in other varieties.

The variety Osječka 88 was outstanding because of its high leaves: stems ratio. Consequently it has high organic matter digestibility and net energy value, low concentrations of crude fibre, ADL, cellulose and ADF, and an average crude protein concentration.

Tables 3 and 4 show the results of in vitro fermenta-

Table 2. Chemical composition and nutritive value of leaves and stems, their proportion in total plant dry matter yield, nutritive value and air dry matter yield of total plant in different lucerne varieties**Tablica 2. Kemijski sastav i hranjiva vrijednost listova i stabljike, njeni dijelovi u prinosu suhe tvari i biljke, hranjiva vrijednost cijele biljke i prinos zračno suhe tvari u različitim sorta lucerne**

Sorta Variety	Leaves - Lišće						Stems - Stabljika						Total plant - Cijela biljka		
	PDM	CP	CF	ASH	OMD	NEL	PDM	CP	CF	ASH	OMD	NEL	OMD ¹	NEL ¹	ADMY
	%	g/kg DM g/kg ST			%	MJ/kg DM MJ/kg ST	%	g/kg DM g/kg ST		%	MJ/kg DM MJ/kg ST	%	MJ/kg DM MJ/kg ST	t/ha	
Osječka-12	41.8 ^{abc}	258	101	93	72.3	6.62	58.2	98	443	59	50.7	3.26	59.7	4.66	16.14
Osječka-11	42.5 ^{abc}	262	110	100	73.8	6.62	57.5	85	462	59	49.4	3.05	59.8	4.57	15.56
Osječka-10	40.4 ^a	282	107	103	73.5	6.56	59.6	88	457	59	49.9	3.12	59.4	4.51	16.77
Osječka-88	46.2 ^b	246	98	91	73.8	6.79	53.8	91	437	60	49.0	3.14	60.5	4.83	14.42
Osječka-89	44.1 ^{abc}	262	109	102	74.2	6.63	55.9	90	447	61	50.1	3.17	60.7	4.69	16.51
Debarska	41.6 ^{ac}	243	105	94	73.7	6.71	58.4	94	431	56	50.8	3.37	60.3	4.76	14.95
Olimpik	41.9 ^{ac}	255	101	94	75.1	6.84	58.1	107	420	60	52.7	3.54	62.1	4.93	13.76
Prescot	45.7 ^{bc}	263	114	90	72.2	6.56	54.3	108	430	59	52.2	3.45	61.3	4.87	12.72
Osječka-66	43.3 ^a	232	109	94	71.8	6.53	56.7	93	432	55	51.6	3.45	60.3	4.78	10.91
Average-Prosjeak	43.1	256	106	96	73.0	6.65	57.0	95	440	59	51.0	3.28	60.0	4.73	14.64
Maximum-Najviše	46.2	282	114	103	75.1	6.84	59.6	108	462	61	52.7	3.54	62.1	4.93	16.77
Minimum-Najmanje	40.4	232	98	90	71.8	6.53	53.8	85	420	55	49.0	3.05	59.4	4.51	10.91
CV %	4.5	5.6	4.9	5.0	1.5	1.6	3.4	8.5	3.1	3.3	2.5	5.3	1.4	2.9	13.2

a) - values with the same superscript do not differ significantly ($p > 0.05$) - jednako označene vrijednosti se međusobno ne razlikuju značajno ($p > 0.05$)

1) - values for total plant are calculated on the basis of results for leaves and stems considering their proportions

- vrijednosti za cijelu biljku su izračunate na osnovi rezultata za lišće i stabljike uzimajući u obzir njihove udjele

Legend: - Legenda:

PDM - proportion in dry matter yield - udjel u prinosu suhe tvari

CP - crude protein - sirove bjelancevine

CF - crude fibre - sirova vlaknina

ASH - ash - sirovi pepeo

OMD - organic matter digestibility - probavljivost organske tvari

NEL - net energy-lactation - neto energija-laktacija

ADMY - air dry matter yield - prinos zračno suhe tvari

bility in the rumen liquor. Gas production, regardless of the part of the plant, was the highest in the variety Olimpik at all incubations. Stems of the varieties Olimpik, Prescott and Osječka 66 gave a higher amount of gas than any other variety ($p < 0.05$).

Negative correlation ($r = -0.82$; $p < 0.01$) exists between the variety resistance to diseases and in vitro fermentation of forage (amount of gas production after 6 hours). In varieties which are more resistant to diseases lower gas production and a lower energy value were determined (Table 5). The results of our research can be

partly compared with those obtained by Luthra et al. (1988). The authors mentioned found out that the lucerne varieties which were more resistant to *Peronospora* contained less reducing sugars and, to a degree, less structural carbohydrates. It should be taken into consideration that in the first six hours of incubation gas production depends on the concentration of easily soluble carbohydrates. Diverse amount of gas production might be affected by diverse presence of inhibitory substances.

Hanson et al. (1973) reported that active saponins

Table 3: Gas production at in vitro incubation of lucerne leaves in rumen liquor, coefficients of exponential equations (a,b,c) and effective gas production (EGP) using rumen outflow rate $k = 0.05 \text{ h}^{-1}$ in ml/g dry matter

Tablica 3: Količina plina pri in vitro inkubaciji lucerninog lišća u soku buraga, koeficijenti eksponentnih funkcija (a,b,c) te efektivna količina plina (EGP) pri izlasku iz buraga $k = 0.05 \text{ h}^{-1}$ u ml/g ST

Sorta Variety	Incubation time in hours Vrijeme inkubacije u satima				a	b	c h ⁻¹	EGP
	4	10	24	48				
Osječka-12	111 ^a	188 ^a	224 ^{ab}	244 ^{ab}	3.1 ¹⁾	236.7	0.1341	175.5
Osječka-11	111 ^a	187 ^a	229 ^{ac}	249 ^{ac}	5.5	239.5	0.1283	177.8
Osječka-10	106 ^b	180 ^b	221 ^{bd}	240 ^{bd}	6.1	231.1	0.1271	172.0
Osječka-88	111 ^{ab}	193 ^{ac}	236 ^{cef}	259 ^{efe}	4.5	251.7	0.1219	182.9
Osječka-89	109 ^{ab}	187 ^{ab}	230 ^{adeg}	251 ^{adfg}	4.4	241.3	0.1296	178.5
Debarska	110 ^{ab}	192 ^{ad}	235 ^{cphi}	258 ^{cphi}	5.6	248.9	0.1221	182.2
Olimpik	116 ^a	198 ^{ad}	240 ^{fh}	262 ^{eh}	5.6	251.9	0.1287	187.0
Prescot	111 ^{ab}	186 ^{ab}	223 ^{ad}	243 ^{ad}	4.4	234.6	0.1319	174.5
Osječka-66	109 ^{ab}	187 ^{ab}	227 ^{adi}	247 ^{adi}	5.6	237.9	0.1279	176.6
Average Prosječno	110	189	229	250	5.0	241.5	0.1280	178.6
Maximum Najviše	116	198	240	262	6.1	251.9	0.1341	187.0
Minimum Najmanje	106	180	221	240	3.1	231.1	0.1219	171.9
CV %	2.4	2.7	2.8	3.1	18.9	3.1	3.1	2.6

a) values with the same superscripts within the incubation time do not differ significantly ($p > 0.05$).

a) - jednako označene vrijednosti u vrijeme inkubacije se međusobno ne razlikuju značajno ($p > 0.05$)

1) - coefficients of exponential equation and EGP were not statistically processed

1) - koeficijenti eksponentne jednadžbe i EKP nisu statistički obrađeni

Table 4: Gas production at in vitro incubation of lucerne stems in rumen liquor, coefficients of exponential equations (a,b,c) and effective gas production (EGP) using rumen outflow rate $k = 0.05 \text{ h}^{-1}$ in ml/g dry matter

Tablica 4: Količina plina pri in vitro inkubaciji lucerninih stabljika u soku buraga, koeficijenti eksponentnih funkcija (a, b, c) te efektivna količina plina (EGP) pri izlasku iz buraga $k = 0.05 \text{ h}^{-1}$ u ml/g ST

Sorta Variety	Incubation time in hours Vrijeme inkubacije u satima				a	b	c h ⁻¹	EGP
	4	10	24	48				
Osječka-12	63 ^{ab}	117 ^{ab}	155 ^{ab}	172 ^{ab}	3.1 ¹⁾	168.5	0.1083	118.4
Osječka-11	58 ^c	112 ^a	151 ^a	169 ^a	2.7	166.7	0.1021	114.6
Osječka-10	59 ^{ac}	114 ^a	153 ^a	170 ^a	1.3	168.8	0.1056	115.9
Osječka-88	61 ^{acd}	116 ^{ac}	147 ^{ac}	174 ^{ac}	2.3	169.6	0.1044	116.9
Osječka-89	60 ^{ac}	115 ^a	153 ^{acd}	173 ^{acd}	2.4	169.6	0.1033	116.7
Debarska	63 ^{acf}	118 ^{adf}	158 ^{acfg}	176 ^{aefg}	2.9	171.7	0.1069	119.9
Olimpik	66 ^g	123 ^e	164 ^{eh}	181 ^{eh}	2.3	177.5	0.1121	125.1
Prescot	65 ^{bdeg}	121 ^{bcde}	161 ^{bcfh}	180 ^{bcfh}	3.8	174.8	0.1079	123.2
Osječka-66	65 ^{fg}	124 ^{fe}	163 ^{dgh}	181 ^{dgh}	2.6	178.9	0.1073	124.6
Average Prosječno	62	118	156	175	2.6	171.8	0.1064	119.5
Maximum Najviše	66	124	164	181	3.8	178.9	0.1121	125.1
Minimum Najmanje	58	112	147	169	1.3	166.7	0.1021	114.6
CV %	4.6	3.5	3.7	2.7	26.2	2.5	2.8	63.3

a) values with the same superscripts within the incubation time do not differ significantly ($p > 0.05$).

a) - jednako označene vrijednosti u vrijeme inkubacije se međusobno ne razlikuju značajno ($p > 0.05$)

1) - coefficients of exponential equation and EGP were not statistically processed

1) - koeficijenti eksponentne jednadžbe i EKP nisu statistički obrađeni

Table 5. Correlation coefficients between gas production after 6 (GP₆) and 24 (GP₂₄) hours of incubation, effective gas production (EGP), organic matter digestibility (OMD), net energy value (NEL), air dry matter yield (ADMY) and resistance to diseases (RD) in different lucerne varieties.

Tablica 5: Korelacijski koeficijenti među proizvodnjom plina poslije 6-og (GP₆) i 24-og (GP₂₄) sata inkubacije, efektivnom količinom plina (EGP), probavljivošću organske tvari (OMD), neto energetsom vrijednošću (NEL), prinosom zračno suhe tvari (ADMY) i otpornosti na bolesti (RD) u različitim sortama lucerne.

	GP ₆	GP ₂₄	EGP	OMD	NEL	RD
	ml/g DM ml/g ST	ml/g DM ml/g ST	ml/g DM ml/g ST	%	MJ/kg DM MJ/kg ST	(0-5)
RD (0-5)	-0.82**	-0.85**	-0.80**	-0.59	-0.78*	/
ADMY(t/ha)	-0.69*	-0.67	-0.69*	-0.48	-0.68*	0.74*

* p < 0.05, ** p < 0.01

had a negative influence on the growth and development of some microorganisms.

The positive correlation ($r = 0.74$, $p < 0.05$) between yield and variety resistance to diseases is also interesting. It is very difficult to estimate the effect of resistance on yield. Willis et al. (1969) found out that by the use of fungicides the yield could be augmented for 9 % to 27 % of dry matter. They confirmed that yield can be restricted by diseases. In the present study more resistant varieties showed a lower energy value. Antagonism between yield and resistance on one hand and nutritive value on the other was found out.

No statistically significant correlations were found between the structural carbohydrates concentration and resistance to diseases. Nevertheless, the fact that structural carbohydrates have an important role in the resistance of varieties to lodging should be considered.

Our results show that more criteria should be taken into account simultaneously when selecting and growing new lucerne varieties. The most important are: high DM yields, high digestibility and net energy value, resistance to diseases and to lodging. Despite the fact that varieties which are more resistant to diseases have a lower nutritive value, the use of pesticides should be avoided in future thus new varieties more resistant to diseases should be selected.

Conclusion

Diverse lucerne varieties differ in chemical composition, organic matter digestibility and net energy value. The most significant differences appear in the concentrations of crude fibre and hemicellulose. These differences can be the result of variable chemical composition and leaves: stems ratio.

Lucerne leaves represented on the average 43 % of

dry matter yield. Due to their high net energy value they represented 61 % net energy lactation (NEL) yield.

The variety Olimpik would be the most suitable for quality hay production. It shows the highest organic matter digestibility and net energy value despite the low proportion of leaves. Because of relatively high proportion of net energy concentrated in the stems of Olimpik the loss of nutritive value, caused by crushing of leaves during drying, is considered to be lower than in other varieties.

Relatively high correlations were determined between the resistance to diseases, in vitro fermentation of forage, net energy value and air dry matter yield. More resistant varieties gave better yields but had a lower energy value.

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SAŽETAK

Istraživan je kemijski sastav i hranjiva vrijednost stabljika i lišća devet vrsta lucerne (*Medicago sativa*). Ocijenjena je probavljivost organske tvari i vrijednost netto energije na temelju kemijskog sastava i količine plina proizvedenog pri in vitro inkubaciji uzoraka u soku buraga. Nađeno je da se razlike među vrstama mogu pripisati razlikama kemijskog sastava stabljika i lišća s jedne strane te razlikama omjera stabljika i lišća u prinosu cijele biljke s druge strane. Lišće lucerne predstavljalo je prosječno 43 % suhe tvari, odnosno 61 % proizvedene netto energije laktacije (NEL). Unatoč niskom udjelu lišća u prinosu cijele biljke vrsta Olimpik imala je najvišu probavljivost organske tvari i najvišu vrijednost netto energije. Vrste otpornije na bolesti dale su najviše prinose krmiva, ali sa slabijom probavljivošću i nižom netto energijom.

IZVLEČEK

HRANILNA VREDNOST LISTOV IN STEBEL PRI RAZLIČNIH SORTAH LUCERNE

Pri devetih sortah lucerne (*Medicago sativa*) smo raziskovali kemično sestavo in hranilno vrednost listov in stebel. Prebavljivost organske snovi in neto energijsko vrednost smo ocenjevali na osnovi kemične sestave vzorca in količine plina pri in vitro inkubaciji vzorca v vampnem soku. Ugotovili smo, da so medsortne razlike v kvaliteti posledica različne kemične sestave listov in stebel na eni ter razlik v razmerju listov in stebel v pridelku cele rastline na drugi strani. Lucernini listi so v povprečju predstavljali 43 % celotnega pridelka suhe snovi oziroma 61 % pridelane neto energije laktacije (NEL). Kljub nizkemu deležu listov v pridelku cele rastline, je imela sorta olimpik najvišjo prebavljivost organske snovi in neto energijsko vrednost. Sorte odpornejše na boleznj so boljše po pridelku, slabše pa po prebavljivosti in neto energijski vrednosti.