The effect of phenological stage and season on nutritive value, chemical composition and nutrient digestibility of lucerne (*Medicago sativa* L.) green forage in the alimentary tract of cattle

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ABSTRACT: Green forage of fourteen lucerne varieties grown at four Experimental Stations of the Research Centre for Cultivar Testing in Słupia Wielka was harvested in these stages: the first growth in the budding stage (cut I), re-growths in the pre-bloom stage of maturity (cut II) 35 days after the first cut, and the next cut, 42 days later (cut III). Three bulls equipped with rumen and duodenal cannulas were used to study rumen degradability by the nylon bag technique and intestinal digestibility by the mobile bag method. The nutritive value of lucerne green forage was estimated according to IZ-INRA (2001) feed evaluation system. The composition of the lucerne cuts differed in DM content ($P < 0.01$); the highest was found in cut III (226.9 g/kg), the lowest in cut II (182.0 g/kg). The crude protein content (CP) in DM of cuts I and II was similar ($P > 0.05$) but lower in cut III ($P < 0.05$). Cut II contained the highest ($P < 0.05$) levels of crude fibre, neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose (CE), and acid detergent lignin (ADL), and the lowest of non-structural carbohydrates (NFC) and soluble in neutral detergent cell content (CC), ($P < 0.01$). The differences between cut I and III in the contents of these components were not significant ($P > 0.05$). The highest DM digestibility in the total alimentary tract was found for cut I ($P < 0.05$). The DM of cuts II and III was digested at a similar rate ($P > 0.05$). There were no significant differences between the cuts in effective degradability, digestibility in the small intestine of the fraction not digested in the forestomachs and total digestibility of CP, CF, NDF, ADF, hemicellulose and cellulose ($P > 0.05$). The digestion of rumen undegraded CP in the post-rumen part of the alimentary tract was lower compared with the digestion of essential amino acids (81% and 98%; respectively). The first cut harvested in the spring in the budding stage contained about 5% more PDIA and PDIN, but 7% less LFU compared with both cuts of regrowth (cuts II and III) which contained on average: 58 g PDIA; 123 g PDIN and 0.93 kg LFU. All the cuts contained similar levels of PDIE, UFL per kg of DM (106 and 0.76 g, respectively).

Keywords: lucerne; phenological stage; season; nutrients; rumen degradability; intestinal digestibility

Lucerne (*Medicago sativa* L.), one of the most popular high-yielding plants harvested in 3–5 cuts per year, is a very important forage species from the aspect of cattle nutrition because of its high content of nutrients and their digestibility. The content of nutrients in feedstuffs varies, however, depending on the vegetation stage, season of harvesting and plant origin (Hoffman et al., 1993; Antoniewicz et al., 1995; Elizalde et al., 1999). Description of the content of nutrients, and their rumen and intestinal digestibility are essential for formulation of diets and prediction of their nutritive value for ruminants.

The aim of this study was to investigate the chemical composition, rumen degradability and intestinal digestibility of rumen undegraded nutrients of lucerne green forage harvested in two phenological stages (budding stage and pre-blooming stage of maturity) and lucerne at a similar stage of maturity but collected in two different seasons.

MATERIAL AND METHODS

Forage

Lucerne (*Medicago sativa* L.) was cultivated according to VCU methodology (1998) under the climatic conditions of north-western Poland. Samples of mixed fourteen varieties, all of them grown at four experimental stations of the Research Centre for Cultivar Testing in Słupia Wielka, were harvested at three commercial cutting times. The first growth in the budding stage was collected as cut I from 25 May to 7 June. The re-growths in the pre-blooming stage of maturity were collected 35 days after the previous cut (II, early summer, from 1 to 8 July), then 42 days later (III, late summer, 4 to 19 August). The fresh-cut material was dried at 50°C for 72 h and representative samples (on the DM basis of fourteen varieties from four experimental stations for each cut) were mixed and used for chemical analysis and estimation of nutrient degradability in the rumen and intestinal digestibility.

Ruminal and intestinal digestion

The rumen degradability of lucerne nutrients was estimated *in situ* by nylon bag techniques (Michaelet-Doreau et al., 1987). The intestinal digestibility of the components undegraded in the rumen was determined using the mobile bag technique according to Peyraud et al. (1988) on 3 bulls of 500 ± 25 kg body weight equipped with rumen and duodenal (about 10 cm after the abomasal pylorus) cannulas, fed meadow hay and concentrate (60:40 DM basis) twice daily. The concentrate contained 14.5% CP and consisted of (%): 50, ground barley; 10, ground wheat; 10, soybean meal; 28, wheat bran; and 2, mineral mixture. Samples of lucerne were ground to a particle size of 1.5 mm. Bags with a pore size of 50 (±15) μm (ANKOM Technology Corporation production) containing lucerne samples were incubated in the rumen for 2, 4, 8, 16, 24, 48, or 72 hours.

Chemical analyses

The approximate chemical composition of samples was determined by standard methods (AOAC, 1990); amino acids (AA) were estimated using an HPLC analyser (BECMAN 126 AA System Gold) after hydrolysis with 6 N HCl (110°C, 20 h), sulphur-containing amino acids were assayed after oxidation with performic acid. Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were measured according to Goering and Van Soest (1970).

Calculation

The coefficients of ruminal effective degradation (ED) of lucerne components and the rumen degradability constants were calculated at a rumen outflow rate of 0.06/h by using a function of Ørskov and McDonald (1979) and intestinal digestibility of the rumen undegraded components (*dsi*) according to Kowalski (1995). The degradation parameters were computed using the NEWAY. EXE procedure. For AA and CP, the ruminal disappearance (R_{16}), intestinal digestibility of rumen-undegraded fractions (dsi_{16}), and digestibility in the total alimentary tract (*TD*) were calculated according to Benchaar et al. (1994). The content of non-fibrous carbohydrates (NFC) in dry matter was calculated as 100 – (crude protein + ether extract + ash + NDF), cell contents (CC) as 100 – NDF, hemicelluloses (HE) as NDF – ADF, and cellulose (CE) as ADF – ADL, according to Cozzi et al. (2002). The nutritive value of lucerne forage was estimated according to the IZ-INRA (2001) feed evaluation system using INWAR software version 1.6 (2000). The results were analysed statistically (ANOWA) using Statistica software version 5.1 (1997). Differences were considered significant at $P \leq 0.05$.

RESULTS

The composition of lucerne differed in DM content ($P < 0.01$) between cuts; the highest content was found in cut III (226.9 g/kg), the lowest in cut II (182.0 g/kg; Table 1). The content of crude pro-

Table 1. Chemical composition of lucerne cuts (n = 14)

Ingredients		Cuts			$P <$	Means ± SD
		I	II	III		
Dry matter/kg fresh material		196.3^{A}	182.0^{B}	226.9^{C}	0.01	201.7 ± 21.0
Content (g/kg DM)	Organic matter	891.9^{A}	885.0^{B}	884.4^{B}	0.01	887.1 ± 7.4
	Crude protein	192.0^{Aa}	189.9^{ABa}	181.7^{Bb}	0.02	187.9 ± 10.3
	TAA	167.2^{a}	159.2^{b}	164.5^{a}	0.04	163.8 ± 8.8
	TEAA	85.9	88.5	84.6	0.07	86.3 ± 4.5
	Lys	11.2^{b}	11.1^{b}	11.9^{a}	0.04	11.4 ± 0.6
	Met	2.8	3.2	2.9	0.05	2.9 ± 0.2
	Ether extract	33.7	31.4	32.7	0.71	32.6 ± 7.3
	Crude fibre	320.3^{b}	355.9^{a}	325.1^{b}	0.02	333.7 ± 37.9
Carbohydrate fractions and cell content	NDF	462.9^{B}	501.1^{A}	444.3^{B}	0.01	469.4 ± 28.6
	ADF	374.2^{B}	408.2^{A}	374.2^{B}	0.01	385.5 ± 22.2
	NFC	203.2^{A}	162.6^{B}	202.4^{A}	0.01	189.4 ± 32.9
	CC	537.1^{A}	498.8^{B}	532.4^{A}	0.01	522.7 ± 28.6
	HE	88.7	92.9	93.3	0.84	91.7 ± 52.4
	CE	296.1^{B}	322.5^{A}	293.9^{B}	0.01	304.2 ± 26.5
	ADL	78.1^{B}	85.7 ^{A}	80.3^{B}	0.01	81.4 ± 5.8

TAA = content of amino acids

TEAA = content of essential amino acids

Values in the same row with different superscripts differ at $P < 0.05$ (small letter) and at $P < 0.01$ (capital letter)

tein in DM of cuts II and I was similar ($P > 0.05$), whereas cut III had a lower level ($P < 0.05$). The level of crude fibre, NDF, ADF, CE and ADL in the lucerne samples of cut II was the highest ($P < 0.05$) but that of NFC and CC ($P < 0.01$) was the lowest. The contents of those components found in cuts I and III did not differ ($P > 0.05$). The concentration of ash in cuts II and III was similar ($P > 0.05$) and significantly higher ($P < 0.01$) in comparison with cut I. The average daily temperature during the vegetation periods for cuts I, II and III was 10.1, 19.3, and 17.6°C, respectively.

DM digestibility in the total alimentary tract was highest for cut I ($P < 0.05$; Table 2). DM of cuts II and III was digested at a similar rate ($P > 0.05$). There were no significant differences between samples of cuts I, II, and III in estimated values of effective degradability, intestinal digestibility, and total digestive tract digestibility for CP, CF, NDF, ADF, HE, CE ($P > 0.05$). The content of the CP fraction readily soluble in the rumen, "*a*", was similar in lucerne cuts I and II but it was significantly higher than in cut III ($P < 0.05$). The content of fraction CC was similar in lucerne of cuts II and III but lower ($P < 0.05$) than in cut I.

The most effectively degraded fraction in the rumen compared with other nutrients was cell content, reaching 78% degradability, whereas the effective degradability of NDF was 25%, CF 17%, and ADF 16%. The digestibility of rumen undegraded fractions of lucerne (CF, NDF, ADF and CE) in the post-ruminal digestive tract was low, reaching an average value of 19% and ranging from 17 to 23%, the degradabilities of hemicellulose and cellulose in the rumen reached 44% and 22%, respectively. Over 60% of rumen-degraded HE and CE was composed mainly of the slowly degraded fraction (c = 4.5%/h). The digestibility of CF, NDF and ADF in the total digestive tract was higher than 30% (Table 2).

The essential amino acids of rumen-undegraded CP were digested in the post-ruminal part of the digestive tract at a high rate of 98%, whereas

Table 2. Nutrient effective degradability rate in the rumen and intestinal digestibility of different cuts of lucerne (n = 10)

Item		ED	*a*	*b*	*c*	*dsi*	*TD*
DM	I	0.49[a]	29	47	4.9	0.39[a]	0.69[a]
	II	0.47[b]	25	44	6.0	0.36[b]	0.66[b]
	III	0.46[b]	27	42	6.5	0.33[b]	0.66[b]
	Means ± SD	0.48 ± 0.01	27 ± 4.2	44 ± 4.7	5.8 ± 0.01	0.36 ± 0.01	0.66 ± 0.02
CP	I	0.65	44[b]	45	5.3[c]	0.82	0.94
	II	0.65	40[b]	47	6.3[b]	0.79	0.93
	III	0.64	36[a]	53	7.1[a]	0.81	0.93
	Means ± SD	0.65 ± 0.02	40 ± 1.8	48 ± 5.2	6.2 ± 0.02	0.81 ± 0.02	0.93 ± 0.02
CC	I	0.78	58[a]	33	8.9[b]	0.81[a]	0.96
	II	0.77	50[b]	39	13.3[a]	0.75[b]	0.94
	III	0.79	53[b]	36	12.4[a]	0.77[b]	0.95
	Means ± SD	0.78 ± 0.02	54 ± 2.8	36 ± 5.6	11.5 ± 0.03	0.77 ± 0.06	0.95 ± 2.2
CF	I	0.17	–	67	2.8	0.20	0.34
	II	0.18	–	51	3.9	0.18	0.32
	III	0.15	–	51	3.0	0.18	0.31
	Means ± SD	0.17	–	56 ± 6.9	3.2 ± 0.07	0.19 ± 0.04	0.32 ± 0.04
NDF	I	0.25	5	58	3.1	0.16	0.37
	II	0.24	5	51	3.6	0.18	0.38
	III	0.25	7	49	3.6	0.18	0.36
	Means ± SD	0.25 ± 0.04	6 ± 0.08	51 ± 4.3	3.4 ± 0.2	0.17 ± 0.03	0.37 ± 0.06
ADF	I	0.13	1.5	45	2.8	0.25	0.34
	II	0.19	2.5	46	3.5	0.19	0.34
	III	0.16	2.2	51	2.3	0.17	0.30
	Means ± SD	0.16 ± 0.05	2.1 ± 0.03	474.2	2.5 ± 0.3	0.20 ± 0.05	0.33 ± 0.04
NFC	I	0.67	65[b]	24[a]	11.4	0.67[ab]	1.00
	II	0.66	72[a]	15[b]	14.6	0.48[b]	0.99
	III	0.71	72[a]	17[ab]	18.5	0.72[a]	0.99
	Means ± SD	0.68 ± 0.10	70 ± 3.9	19 ± 0.6	14.8 ± 2.6	0.62 ± 0.2	0.99 ± 0.01
Item		ED	*a*	*b*	*c*	*dsi*	*TD*
HE	I	0.44	6	62	7.2	0.10	0.48
	II	0.49	5	68	3.7	0.10	0.54)
	III	0.38	4	64	4.9	0.08	0.43
	Means ± SD	0.44 ± 0.08	5 ± 0.02	66 ± 3.1	5.3 ± 1.6	0.09 ± 0.01	0.48 ± 0.03
CE	I	0.22	–	70	3.5	0.26	0.41
	II	0.24	–	59	4.5	0.20	0.39
	III	0.21	–	56	3.0	0.24	0.37
	Means ± SD	0.22 ± 0.04	–	62 ± 4.1	4.5 ± 1.9	0.23 ± 0.03	0.39 ± 0.02

ED – effective rumen degradation of components estimated according to Ørskov and McDonald (1979) at an outflow rate of 0.06/h

a – immediately rumen-soluble fraction of rumen degradability components (%); *b* – slowly rumen-soluble fraction of rumen degradability components (%); *c* – rate of degradation fraction *b* (%*b*/*h*)

dsi – coefficient of intestinal digestibility of rumen undegraded components

TD – coefficient of the component digestibility in the total digestive tract

Table 3. Rumen, post-rumen and total alimentary tract (n = 5) digestibility of essential amino acids from lucerne cuts

Item	R_{16}				dsi_{16}				TD			
	I	II	III	Means ± SD	I	II	III	Means ± SD	I	II	III	Means ± SD
CP	0.64	0.65	0.67	0.65 ± 0.02	0.83	0.78	0.81	0.81 ± 0.02	0.94	0.92	0.94	0.93 ± 0.02
TAA	0.61	0.57	0.62	0.61 ± 0.02	0.98	0.98	0.98	0.98 ± 0.02	0.99	0.99	0.99	0.99 ± 0.02
EAA	0.64	0.61	0.65	0.63 ± 0.02	0.98	0.97	0.98	0.98 ± 0.02	0.99	0.99	0.99	0.99 ± 0.04
Arg	0.64	0.59	0.66	0.63 ± 0.02	0.99	0.99	0.99	0.99 ± 0.02	1.00	0.99	1.00	1.00 ± 0.02
His	0.61	0.74	0.78	0.71 ± 0.05	0.99	0.97	0.98	0.98 ± 0.03	0.99	0.99	0.99	0.99 ± 0.01
Ile	0.58	0.56	0.60	0.58 ± 0.06	0.98	0.98	0.98	0.98 ± 0.04	0.99	0.99	0.99	0.99 ± 0.02
Leu	0.56	0.57	0.62	0.58 ± 0.06	0.98	0.98	0.98	0.98 ± 0.04	0.99	0.99	0.99	0.99 ± 0.02
Lys	0.61	0.60	0.63	0.61 ± 0.03	0.98	0.97	0.98	0.98 ± 0.01	0.99	0.99	0.99	0.99 ± 0.02
Met	0.69	0.67	0.69	0.68 ± 0.03	0.98	0.97	0.98	0.98 ± 0.01	0.99	0.99	0.99	0.99 ± 0.02
Cys	0.73	0.71	0.77	0.74 ± 0.07	0.98	0.94	0.98	0.96 ± 0.02	0.99	0.98	0.99	0.99 ± 0.02
Phe	0.89	0.98	0.98	0.95 ± 0.04	0.96	0.98	0.98	0.97 ± 0.03	0.98	0.97	0.98	0.98 ± 0.02
Thr	0.60	0.59	0.63	0.61 ± 0.04	0.98	0.98	0.98	0.98 ± 0.01	0.99	0.99	0.89	0.96 ± 0.06
Val	0.60	0.59	0.65	0.61 ± 0.02	0.98	0.98	0.98	0.98 ± 0.02	0.99	0.99	0.99	0.99 ± 0.02

R_{16} – coefficient of rumen degradation during 16 h of incubation
dsi_{16} – coefficient of intestinal digestibility of undegraded protein in the rumen
TD – coefficient of total alimentary tract digestibility of EAA

the post-ruminal digestion of rumen-undegraded CP was only 81% (Table 3).

Cut I of first-growth lucerne harvested in the spring at the budding stage contained about 5% more PDIA and PDIN but 7% less of LFU than both cuts of regrowth (cuts II and III), which contained on average: 58 g PDIA, 123 g PDIN, and 0.93 kg LFU (Table 4). All the cuts contained similar levels of PDIE, UFL per kg of DM (106 and 0.76 g, respectively).

DISCUSSION

The chemical composition of the cuts of lucerne green forage investigated in this study indicated a lower level of crude protein and cell content, but a higher level of crude fibre and cell walls in comparison with the data presented by Faría-Mármol et al. (2002) and IZ-INRA (2001). These data, however, refer to lucerne cultivated under the climatic and agronomic conditions of Western and Southern Europe, demonstrating that the environment influences the chemical composition of forages (Żebrowska et al., 1995).

In our experiment, lucerne harvested in the budding stage was characterised by a higher level of organic matter, non-fibre carbohydrates, and cell contents but a lower level of crude fibre, NDF, ADF, CE and ADL in comparison with lucerne harvested in the pre-bloom stage of maturity. The obtained results are in line with those presented by Hoffman et al. (1993) and Elizalde et al. (1999), who showed that CF, NDF and ADF concentrations increase as lucerne matures. In our investigation, the content of these fibre fractions was higher in lucerne of cut II than cut I, and surprisingly in cut III. Cuts II and III were harvested in the same phenological stage, but the environmental conditions differed. During the growing period of lucerne collected in cut II, the average daily temperature was 19.3°C; this is 1.7°C higher than determined during the vegetation period of cut III. The temperature differences during vegetation may have influenced the chemical composition of lucerne. According to the results of Juan et al. (1993) and Hall et al. (1998) differences in environmental conditions affected the chemical composition of forage, especially by modifying plant morphology (leaf:stem ratio). This is in keeping with the results of Wilson et al. (1991), who found that higher temperatures promoted higher production of NDF and lignin in Bermuda grass stem.

The digestibility in the total alimentary tract shows that the dry matter digestion of lucerne cut in the budding stage was higher than when it was collected in the pre-bloom stage of maturity, confirming the results of Elizalde et al. (1999), who found that *in situ* DM degradation decreased with the maturity stage as a result of increased levels of poorly digested ingredients. The content of crude fibre, NDF, ADF, CE and ADL in our experiment was also associated with lucerne maturity. Our results showed that about 50% of lucerne DM of all cuts was effectively degraded in the rumen, about one-third was estimated as a readily rumen-soluble fraction, and the content of the DM fraction slowly degradable in the rumen was on average 44% and the rate of degradation was 6%/h. Faría-Mármol et al. (2002) reported that about 38% of lucerne rumen-degraded DM was slowly soluble in the rumen fraction degraded at a rate of 8.5%/h. Their lucerne

Table 4. Nutritive values of lucerne cuts estimated according to the IZ-INRA (2001) system (in kg of DM)

Cuts	Net energy units		Fill values (kg)			Protein values (g)				
	UFL	UFV	LFU	SFU	CFU	PDIA	PDIN	PDIE	Lys (% PDIE)	Met (% PDIE)
I	0.76	0.68	0.87	0.87	0.87	61.91	129.50	107.36	6.86	1.71
II	0.78	0.70	0.93	0.88	0.88	58.28	125.13	105.16	6.82	1.69
III	0.75	0.67	0.93	0.88	0.88	58.81	121.61	106.69	6.77	1.67

UFL – net energy value for milk production; UFV – net energy value for meat production; LFU – fill unit for lactating dairy cows; SFU – fill unit for sheep; CFU – fill unit for cattle other than dairy cows; PDIA – protein truly digested in the small intestine originating from rumen-undegraded dietary protein; PDIN – the sum of PDIA and microbial protein digested in the small intestine synthesised from the rumen-degraded dietary protein when energy and other nutrients are not a limiting factor; PDIE – the sum of PDIA and microbial protein digested in the small intestine synthesised from the rumen-degraded dietary protein when the degraded N and other nutrients are not a limiting factor

contained 488 g NDF/kg DM, which was composed of 75% ADF and 15% ADL. In our experiment, the mean content of NDF in lucerne (502.8 g/kg DM) was similar, however, it contained 81% ADF and 18% ADL, indicating that a higher level of slowly degraded NDF components decreases DM digestibility. Moreover, on average about 80% of ADF was cellulose, which requires a longer rumen retention time and was degraded to a lower extent than hemicellulose. According to the results of Cozzi et al. (2002) cellulose, a component of NDF, is degraded in the rumen faster than total NDF, however, in our experiment the rates of cellulose and NDF digestion were equal. A possible reason for lower rumen degradation of CE estimated in our study was poorer development of cellulolytic microorganisms in the rumen since the diet for animals in our experiment contained less forage than in the experiment of Cozzi et al. (2002), 60% and 75% on DM basis, respectively. Intestinal digestibility of DM and CC of lucerne harvested at the budding stage was higher than that of lucerne collected at pre-bloom maturity, which was a consequence of the difference in the level of NDF, ADF and ADL contents. The mean intestinal digestibility of NDF and ADF estimated in our experiment was 18% and it was similar to that determined in the experiment of Siciliano-Jones and Murphy (1989), who found that the respective values ranged from 6.4% to 16%. Michalet-Doreau et al. (2002) reported that those fractions were not digested by intestinal enzymes but they were degraded by microbial enzymes in the caecum to a different extent.

The results obtained in the present experiment indicate high, 98%, intestinal digestibility of the lucerne amino acids not digested in the rumen during 16 h incubation, whereas the digestibility of crude protein amounted only to 80%. This means that apart from easily digestible amino acids, the fraction of crude protein not degraded in the rumen also contains other nitrogen compounds of low digestibility. Schröder et al. (1997) reported similar results for SBM and fish meal. It seems that these differences in the rate of intestinal digestibility of amino acids and crude protein may be related to fibre-bound nitrogen, which is resistant to decomposition by microbial and mammalian enzymes (Huhtanen and Hristov, 2001). The estimated nutritive value of lucerne cultivars grown in Poland presented in this study differs from the respective values published in official feed tables IZ-INRA (2001). A similar observation was reported by Żebrowska et al. (1995), who concluded that the plant variety and the environmental conditions influenced the chemical composition, ruminal and intestinal digestion and, consequently, the nutritive value of forage.

CONCLUSIONS

Phenological stage and temperature during vegetation influenced the content of dry matter, organic matter, cell contents, carbohydrate fractions and lignin in the analysed lucerne green forage. The stage of maturity of the harvested lucerne influenced the rate of digestibility of DM and CC along the alimentary tract, but this effect was not significant for CP, CF, NDF, ADF, HE and CE. The differences in nutritive values of different lucerne cuts result from the differences in chemical composition and digestion processes in the alimentary tract of ruminants.

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