Amino acid contents and intestinal digestibility of lucerne in ruminants as influenced by growth stage

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ABSTRACT: Lucerne (*Medicago sativa* L. var. Palava), harvested at four successive dates over a 30-day period, was evaluated for chemical composition (dry matter, organic matter, crude protein, ether extract, crude fibre, nitrogen-free extract, neutral detergent fibre, acid detergent fibre, acid detergent lignin), amino acid contents and intestinal digestibility in dairy cows. Dry matter (r = 0.78), organic matter (r = 0.95), crude fibre (r = 0.91), neutral detergent fibre (r = 0.94), acid detergent fibre (r = 0.79) and acid detergent lignin (r = 0.48) presented positive linear correlation coefficients (r) with growth stage, whereas crude protein (r = –0.96), ether extract (r = –0.86) and nitrogen-free extract (r = –0.70) showed negative relationships. Total essential amino acid content decreased (r = –0.94) from 84.1 to 55.3 g/kg of dry matter with maturity, with r-values higher than –0.90 obtained between growth stage and contents of lysine, methionine, threonine and valine. With the exception of tyrosine (r = –0.68), r-values between growth stage and individual non-essential amino acids were all higher than –0.90. Total amino acid (r = –0.98) and nitrogen (r = –0.99) contents presented comparable tendencies with successive sampling times. Whereas no definite trends were detected for the amino acid composition of rumen incubated (16 hours) lucerne samples, the intestinal digestibility of total essential (r = –0.78), total non-essential (r = –0.58) and total (r = –0.69) amino acids as well as nitrogen (r = –0.99) decreased with growth. However, due to a small sample size (n = 4), most linear relationships between constituents and growth stage were insignificant. It can be concluded that, although limited in the sample size, this report presents information on the decrease in amino acid contents and intestinal amino acid digestibility as growth proceeds in lucerne (var. Palava) produced in the Czech Republic, which could be utilized in the feeding of ruminants.

Keywords: lucerne; amino acids; intestinal digestibility

Lucerne (*Medicago sativa* L.) is the main forage crop for ruminants, especially in temperate countries (González et al., 2001). However, its protein value might be reduced by nitrogen losses that occur during extensive rumen degradation (Broderick, 1995; Dhiman and Satter, 1997; Elizalde et al., 1999a; González et al., 2001; Broderick, 2006; Pozdíšek and Vaculová, 2008). Furthermore, the protein value of forages is related to the stage of maturity (Písaříková et al., 2007; Jančík et al., 2008) due to its influence on microbial synthesis and site of digestion (González et al., 2001), with ruminal protein digestibility decreasing as lucerne matures. This phenomenon is found with both fresh lucerne (Balde et al., 1993;

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Elizalde et al., 1999b) and lucerne hay (Kawas et al., 1990).

Estimations of intestinal digestibility of rumen undegraded protein of feeds are critical in the application of protein evaluation systems for ruminants. The mobile nylon bag technique, based on the incubation of rumen degraded feed samples, presents a simple and adaptable method to study digestion between the duodenum and ileum or anus (Faría-Mármol et al., 2002).

A preliminary study was conducted to evaluate the influence of stage of growth on the chemical composition (dry matter, organic matter, crude protein, ether extract, crude fibre, nitrogen-free extract, neutral detergent fibre, acid detergent fibre, acid detergent lignin), amino acid contents and intestinal digestibility of lucerne grown in the Czech Republic.

MATERIAL AND METHODS

Samples

Lucerne (*Medicago sativa* L. var. Palava) from the same field was harvested at four successive dates, representing different stages of growth. Each harvest day is represented by one lucerne sample. The first harvest was at a height of 60 cm (end of May; indicated in this study as day 0), with successive harvests in 6 (second harvest, height of 65 cm), 20 (third harvest, early budding stage), and 30 (fourth harvest, beginning of flowering stage) days later. After freeze-drying, harvested samples were milled to pass through a four-millimetre sieve for a mobile bag technique and through a one-millimetre sieve for chemical analyses.

The average annual temperature was 8.9°C, with annual rainfall totalling to 626 mm. The altitude of the field was 240 m. For soil fertilization 40 kg/ha P_2O_5 and 60 kg/ha K_2O were applied per year.

Intestinal digestibility of lucerne amino acids

The mobile bag technique (Frydrych, 1992; Homolka et al., 2007) was used to determine intestinal digestibility of lucerne in three dry Black Pied cows fitted with permanent large ruminal cannulas (120 mm internal diameter) and a T-piece cannula in the proximal duodenum.

Cows were fed twice a day (6 a.m. and 4 p.m.) with a daily ration consisting of 4 kg lucerne hay, 10 kg maize silage, 1 kg barley meal, and a vitamin and mineral supplement. Freeze-dried harvested samples were milled to pass through a four-millimetre sieve. For ruminal incubation were weight for each harvest day 2 g of lucerne into 30 nylon bags (10 bags per cow; bag size 5 × 15 cm; pore size 42 μm; together for all harvest days 120 bags), with the weight of 15 mg dry matter of the feed sample corresponding to 1 cm^2 of the active surface area of the bag (Uhelon 130 T, Silk and Progress Moravská Chrastová, Czech Republic). After rumen incubation for 16 h bags were washed in water for 30 minutes. Then the nylon bag residues of each cow (10 bags per cow) were quantitatively filtrated on filter paper and weighed after 24 h drying at 50°C. This *in sacco* method can be used for the determination of differences between feeds and element release in the ruminal environment (Čerešňáková et al., 2007).

The residues of rumen incubated samples were weighed into 30 nylon bags for each harvested day (weight of 0.5 g per bag; bag size 4 × 4.6 cm; pore size 42 μm; together for all harvest days 120 bags). Bags were sealed and incubated in a solution of pepsin and 0.01N HCl (hydrochloric acid) for 2.5 hours at 39°C, whereafter 10 bags per cow were inserted through the cannula into the cow's duodenum. Bags found within 24 h in the faeces were washed in water and subsequently freeze-dried. The intestinal digestibility of dry matter and amino acids were calculated as the amount of dry matter and individual amino acids in the mobile bags recovered in the faeces as a proportion of the amount of dry matter and individual amino acids in rumen degraded samples, respectively.

Chemical analyses

Dry matter, ash, crude protein (Kjeldal nitrogen × 6.25), ether extract, crude fibre, neutral detergent fibre, acid detergent fibre, and acid detergent lignin were analysed in the original samples according to the AOAC (1990). Nitrogen-free extract was calculated as the difference between dry matter and the sum of crude protein, ether extract, ash and crude fibre.

Individual amino acids were determined in the harvested samples (n = 4), residues of rumen incubated (16 h) samples, and residues of samples after

24 h of intestinal digestion. Amino acid analysis was performed with a T 339 Amino Acid Analyzer (INGOS Ltd., Prague, Czech Republic) after samples were hydrolysed in 6M HCl. For determination of sulphur amino acids (cystine and methionine) the samples were treated with performic acid prior to hydrolysis.

Statistical analysis

Linear correlation coefficients (Snedecor and Cochran, 1991) were calculated between the stage of growth (days of sampling) and individual constituents. Statistical analysis was performed using the statistical programme SAS (SAS, 2003).

RESULTS AND DISCUSSION

As indicated by linear correlation coefficients (r) presented in Table 1, contents of crude protein, ether extract and nitrogen-free extract decreased as the time of sampling proceeded, with dry matter, organic matter, crude fibre and neutral detergent fibre following the opposite trend. However, an unexpected decline in acid detergent fibre content was found between the second (Day 6) and the third (Day 20) harvest time, followed by an increase at the fourth (Day 30) sampling. Acid detergent lignin content presented an opposite trend. This phenomenon could partly be attributed to sampling error, as supported and suggested by several studies (Bal et al., 2000; Yang and Beauchemin, 2006). Due to a small sample size (n = 4) the r-values, except for crude protein, showed as insignificant at $P < 0.05$.

The above results were as expected, and in accordance with findings reported by Elizalde et al. (1999b), Aufrère et al. (2000), González et al. (2001), Yu et al. (2003), Niwińska et al. (2005) and Christodoulou et al. (2007). The crude protein values 226.0 and 212.0 g/kg of dry matter in lucerne at the late vegetative and early bud stages, respectively, with the corresponding values 884.0 and 883.0 g/kg of dry matter for organic matter, reported by Elizalde et al. (1999b), are in agreement with present results. Balde et al. (1993) and Hoffman et al. (1993) reported the higher crude protein values 269.0 and 252.0 g/kg of dry matter at the late vegetative and early bud stages, respectively, whereas Niwińska et al. (2005) found the value 192.0 g/kg of dry matter in the bud stage. The latter authors reported the value 320.0 g/kg of dry matter for crude fibre, which increased to 356.0 g/kg of dry matter in the pre-blooming stage. Most of the above studies concluded that maturity caused a reduction in the cell content to cell wall, and leaf to stem ratios (González et al., 2001).

The optimal dietary content of amino acids to animals has been intensively studied for many years (Plitzner et al., 2007). Amino acid contents in lucerne at harvest (g/kg of dry matter) reflected variations caused by different times of harvest (Table 2). Relative to the linear relationship between growth stage and total essential amino acid content (r = –0.94), the values for arginine (r = –0.15), histidine (r = –0.45) and phenylalanine (r = –0.35) were low (Table 2). However, the linear correlations between growth stage and individual non-essential

Table 1. Chemical composition of lucerne as influenced by stage of growth (g/kg DM)

Day of harvest	DM (g/kg)	OM	CP	EE	CF	NFE	NDF	ADF	ADL
Day 0[1] (n = 2)	143.7	882.6	230.3	17.5	254.9	379.9	351.0	268.5	92.7
Day 6[2] (n = 2)	150.8	880.2	212.4	19.0	296.6	352.2	411.2	318.1	89.8
Day 20[3] (n = 2)	145.5	889.5	163.7	16.7	401.8	307.3	430.3	288.9	113.1
Day 30[4] (n = 2)	205.6	896.8	163.1	13.3	381.0	339.4	533.2	424.1	96.4
r	0.78	0.95	–0.96*	–0.86	0.91	–0.70	0.94	0.79	0.48

[1]first harvest (height of 60 cm; end of May); [2]second harvest (height of 65 cm); [3]third harvest (early budding stage); [4]fourth harvest (beginning of flowering stage)

DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; CF = crude fibre; NFE = nitrogen-free extract; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; r = linear correlation coefficient; *$P < 0.05$

Chemical composition was determined from double chemical measurements (n = 2)

Table 2. The influence of stage of growth on amino acid contents of harvested and rumen incubated (16 hours) lucerne samples and intestinal digestibility

Day of harvest	Essential amino acids										Non-essential amino acids										
	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Valine	TEAA	Alanine	Aspartate	Cysteine	Glutamate	Glycine	Proline	Serine	Tyrosine	TNEAA	TAA	Nitrogen
Harvested lucerne composition (g/kg of dry matter)																					
Day 0[1]	11.6	9.4	6.7	19.0	11.8	3.2	5.5	7.0	9.9	84.1	9.1	23.8	3.0	18.9	8.4	11.4	9.1	6.2	89.9	174.0	36.8
Day 6[2]	8.4	8.4	5.8	15.4	10.1	2.7	9.6	7.7	9.7	77.8	10.3	24.0	2.5	17.8	8.4	11.2	8.8	7.1	90.1	167.9	34.0
Day 20[3]	9.7	3.3	2.6	5.3	10.1	2.2	7.9	5.9	6.8	53.8	7.5	15.0	2.0	17.3	6.2	8.0	7.7	4.3	68.0	121.8	26.2
Day 30[4]	10.2	8.1	4.1	8.3	7.9	2.0	4.6	5.1	5.0	55.3	5.5	13.3	1.9	12.9	4.3	4.9	4.7	5.3	52.8	108.1	26.1
r	–0.15	–0.45	–0.80	–0.88	–0.91	–0.97*	–0.35	–0.91	–0.99*	–0.94	–0.91	–0.96*	–0.95*	–0.90	–0.98*	–0.98*	–0.93	–0.68	–0.94	–0.98*	–0.96*
Composition of residues after 16 hours rumen incubation (g/kg of dry matter)																					
Day 0[1]	4.2	2.2	3.4	5.9	6.1	1.5	4.7	2.8	4.2	34.9	3.2	6.9	1.3	5.8	3.6	2.3	1.9	4.4	29.3	64.2	25.1
Day 6[2]	8.5	8.6	5.9	15.3	8.5	2.7	9.8	7.8	9.1	85.2	9.0	18.4	2.6	17.6	8.5	9.9	9.0	3.5	78.5	154.7	29.2
Day 20[3]	3.1	3.2	2.6	4.5	7.9	1.4	6.8	4.0	3.9	37.4	4.7	8.9	1.2	8.9	4.6	5.1	5.2	3.6	42.3	79.7	15.9
Day 30[4]	10.2	3.1	5.0	9.4	8.3	2.1	6.1	3.8	6.5	54.6	5.9	10.7	2.0	11.2	5.5	5.8	3.3	3.6	48.0	102.6	17.0
r	0.40	–0.26	0.01	–0.14	0.60	–0.02	–0.07	–0.19	–0.04	–0.08	0.06	–0.09	0.01	0.05	–0.03	0.09	–0.12	–0.61	–0.02	–0.01	–0.83
Intestinal digestibility of lucerne																					
Day 0[1]	0.765	0.665	0.670	0.685	0.666	0.832	0.661	0.630	0.706	0.698	0.716	0.635	0.804	0.712	0.724	0.546	0.674	0.720	0.691	0.695	0.867
Day 6[2]	0.826	0.820	0.760	0.824	0.676	0.877	0.897	0.887	0.829	0.822	0.872	0.879	0.853	0.900	0.834	0.886	0.872	0.653	0.844	0.833	0.846
Day 20[3]	0.785	0.815	0.538	0.521	0.606	0.787	0.740	0.726	0.663	0.687	0.764	0.785	0.811	0.835	0.807	0.769	0.830	0.674	0.784	0.736	0.773
Day 30[4]	0.571	nd	0.405	0.524	0.321	0.559	nd	0.346	0.867	0.513	0.639	0.478	0.574	0.608	0.646	0.687	0.131	nd	0.538	0.525	0.699
r	–0.75	0.71	–0.91	–0.79	–0.88	–0.87	0.11	–0.65	0.34	–0.78	–0.55	–0.50	–0.79	–0.46	–0.47	0.15	–0.67	–0.49	–0.58	–0.69	

[1]first harvest (height of 60 cm; end of May); [2]second harvest (height of 65 cm); [3]third harvest (early budding stage); [4]fourth harvest (beginning of flowering stage)
TEAA = total essential amino acids; TNEAA = total non-essential amino acids; TAA = total amino acids; *r* = linear correlation coefficient; *$P < 0.05$

amino acid contents were in agreement with the value for total non-essential amino acid content (r = –0.94). Good agreement was obtained between r-values for nitrogen (r = –0.96) and total amino acid content (r = –0.98). The amino acid composition of rumen incubated (16 hours) samples did not present any notable relationships with growth stage (Table 2).

Information on the amino acid composition of lucerne as influenced by growth stage is limited. Balde et al. (1993) found that the contents of individual amino acids, as a fraction of crude protein content, decreased with increasing stage of maturity. However, relative proportions were not influenced. In agreement with present results, the values 167.0 and 86.0 g/kg of dry matter for total and total essential amino acid contents, respectively, for lucerne in the bud stage were reported by Niwińska et al. (2005), with the values 11.0 and 3.0 g/kg of dry matter for lysine and methionine contents, respectively. Contents of NDF, ADF and ADL in relation to individual amino acids had a wide range of insignificant correlation coefficients (not tabulated data). However, these findings should be interpreted with caution for a small sample size (n = 4).

The intestinal digestibility of dry matter (not tabulated) was found to be 0.402, 0.357, 0.308 and 0.257 at days 0, 6, 20, and 30, respectively, presenting a linear correlation coefficient –0.99 (P < 0.05). The intestinal dry matter digestibility was considerably lower than the intestinal disappearance of nitrogen (Table 2), in accordance with results obtained by Taghizadeh et al. (2005) with lucerne hay.

Despite some exceptions (histidine, phenylalanine, valine, proline), the intestinal digestibility of amino acids decreased as the stage of growth proceeded (Table 2). In agreement with the relationship between growth stage and nitrogen content, intestinal nitrogen digestibility was highly negatively related to growth stage, in accordance with results presented by González et al. (2001). This shows that the use of a constant value for the intestinal nitrogen digestibility of fresh lucerne is inappropriate.

Intestinal digestibility varied between 0.513 and 0.822 for total essential amino acids, 0.538 and 0.844 for total non-essential amino acids, and 0.525 and 0.833 for total amino acids. With the exception of day 20, the intestinal digestibility of total essential and total non-essential amino acids was comparable to the values derived for total amino acid digestibility at each growth stage. The intestinal digestibility of total essential (r = –0.78) and total non-essential (r = –0.58) amino acids was related to a similar magnitude to harvest time like the intestinal digestibility of total amino acids (r = –0.69). This indicates that intestinal digestibility was not substantially different among individual amino acids within a growth stage.

Comparable to the present results, Ballet et al. (1998) reported the intestinal digestibility values (uncorrected for microbial contamination) 0.878, 0.854 and 0.885 for arginine, methionine and total amino acids, respectively, for lucerne harvested at the bud stage (33.6 g/kg of dry matter for nitrogen, 232.0 g/kg of dry matter for crude fibre) and evaluated in sheep. These authors found the values 0.885 and 0.630 for lysine and nitrogen, respectively. Elizalde et al. (1999a) presented the values 0.658, 0.637, and 0.647 for disappearance of total essential, total non-essential and total amino acids from fresh lucerne (32.6 g/kg of dry matter for nitrogen) in the small intestine of steers. The corresponding values for intestinal digestibility of lucerne hay with a nitrogen content of 13.4 g/kg of dry matter, and evaluated in steers, were 0.830, 0.220 and 0.500, respectively (Taghizadeh et al., 2005). Niwińska et al. (2005) found the intestinal digestibility of individual amino acids in lucerne harvested at three commercial cutting times in Poland to vary between 0.940 (cysteine) and 0.990 (arginine), with a mean value of 0.810 for crude protein. Lower crude protein than amino acid digestibility, which was not found in the present study, indicates that the crude protein fraction undegraded in the rumen contains other low digestible nitrogen compounds (Niwińska et al., 2005).

This study, although hampered by a small sample size, illustrated that the amino acid contents and intestinal digestibility of lucerne (var. Palava) grown in the Czech Republic are influenced by growth stage, even over a period as short as 30 days. It has important practical implications in that the above parameters cannot be treated as a constant, but have to be established for each growth stage in further studies.

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