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Effect of plant growth stages on nutritional composition of promising Lucerne (*Medicago sativa* L.) genotypes

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Abstract
Nutritional composition of ten promising forage Lucerne genotypes was evaluated at pre-flowering, 50 per cent and post-flowering plant growth stages for dry matter, moisture, crude protein, cell wall constituents, *in vitro* dry matter and *in vitro* protein digestibility. Dry matter content at post-flowering stage was highest (23.27%) followed by 50 per cent (18.63%) and pre-flowering (16.91%) stages, respectively. Highest mean crude protein content was recorded at pre-flowering stage, followed by 50 per cent and post-flowering stages and the corresponding values were 20.82, 19.81 and 18.06 %, respectively. *In vitro* dry matter digestibility at pre-flowering stage was maximum (66.83%) followed by 50 per cent flowering with (64.76%) and post-flowering (60.78%) stages. *In vitro* protein digestibility at 50 per cent flowering stage was also maximum (85.97%) followed by the post-flowering (82.61%) and pre-flowering (80.11%) stages. Amongst the cuttings of forage lucerne genotypes at different plant growth stages, the cell wall constituents, *viz.,* neutral detergent fibre, acid detergent fibre, hemi-cellulose and acid detergent lignin were the lowest at pre-flowering stage and thereafter increased continuously with the advancement of growth stage.

Keywords: Dry matter, Fodder, Growth stages, Lucerne, Nutritional composition.

Abbreviations: ADF: Acid detergent fibre; **ADL:** Acid detergent lignin; **CP:** Crude protein; **DM:** dry matter; **IVDMD:** *In vitro* dry matter digestibility; **IVPD:** *In vitro* protein digestibility; **NDF:** Neutral detergent fibre.

Introduction

Lucerne (*Medicago sativa* L.) is an important cosmopolitan forage crop, and also known as "Queen of forage crops". Lucerne is one of the oldest among cultivated fodder crop. The crop is also called as alfalfa, which originated from Arabic language, means best fodder (Boltan *et al.,* 1972). It is grown as main fodder crop in many countries as its yield, protein content and palatability are better than other forage legumes (Lestingi *et al.,* 2009). It is grown on one million ha area in India and provides 1000 to 1200 quintals of green forage per ha with 10 to 12 cuttings per year. It is an important source of proteins, fibre and minerals in ruminant animals. Crude protein content, protein digestibility and dry matter digestibility are important criteria to determine the quality of forge crops. Lucerne contains 19.5 to 28.8 per cent crude protein, 36.5 to 46.2 per cent neutral detergent fibre, 20.4 to 28.4 per cent acid detergent fibre and 5.4 to 9.5 per cent acid detergent fibre lignin (Amrane and Michalet-Doreau, 1993). The chemical composition of lucerne dry matter varies with cuts. Crude protein tends to be lower in aged lucerne plants, while the crude fibre increases (Stanacev *et al.,* 2010). The digestibility of lucerne organic matter depends mostly on the proteins, cellulose and lignin content (Katic *et al.,* 2009). Lucerne forage is mainly harvested at 50% flowering stage. However, in the present study, the research work was undertaken to find out differences in the nutritional quality of lucerne forage harvested at different plant growth stages *viz.,* pre-flowering, 50% flowering and post- flowering.

Materials and Methods

Ten promising lucerne genotypes *viz.,* RLG-08-1, RLG-08-2, RLG-08-3, RLG-08-4, RLG-08-5, RLG-08-6, RLG-08-7, RLG-08-8, RL-88 and *Anand*-2 were grown on the research farm of All India Coordinated Research Project on Forage Crops, Mahatma Phule Krishi Viswavidyalaya, Rahuri. The plant material was harvested in triplicate at pre-(2 weeks), *i.e.* mid bud stage, 50 per cent flowering (3 weeks) and post-flowering (4 weeks), *i.e.* green pod growth stages. The collected plant samples were cut into small pieces, partially sun dried and completely dried in hot air oven at constant temperature of 60^0C till constant

weight. The sample was powdered using mechanical grinder, sieved and analysed for all nutritional constituents. The moisture, crude protein content and *In vitro* protein digestibility were estimated by A.O.A.C. (1990) method. *In vitro* dry matter digestibility was determined by the method of Tilley and Terry (1963). The cell wall fractions *viz*., ADF, NDF and ADL were separated by the procedure developed by Van Soest (1963) using Fibra Plus equipment. The hemicellulose content was calculated by subtracting ADF fraction from NDF. The data obtained was analyzed for statistical significance using Factorial Randomized Block Design (F-RBD) (Panse and Sukhatme, 1985).

Results and Discussion

***Dry matter*:** Dry matter content at post-flowering stage was the highest (23.27%) followed by 50 per cent flowering (18.63%) and pre-flowering (16.91%) stages. The data on interaction effects between genotypes and growth stages on dry matter percentage revealed significantly superior dry matter content of 19.53% in *Anand*-2 at pre flowering stage, whereas genotype RLG-08-5 was superior with 20.55 and 27.43% at 50% flowering and post-flowering stages, respectively. The data reported in present study were in agreement with Amrane and Michalet-Doreau (1993).

***Crude protein*:** Crude protein content was highest (20.82%) at pre-flowering stage, followed by 50 per cent flowering (19.81%) and post-flowering (18.06%) stages. Katic *et al.* (2006) also reported crude protein content of 18 to 25% in lucerne forage. The interaction effect between genotypes and growth stages showed highest crude protein content of 21.87% in both genotypes, RLG-08-8 and *Anand*-2 at pre-flowering stage.

***In vitro dry matter digestibility*:** IVDMD values at pre-flowering stage were highest (66.83%), followed by 50 per cent flowering (64.76%) and post-flowering stage (60.78%), respectively. The data on effect of interaction between genotypes and growth stages on IVDMD percentage revealed the highest IVDMD value of 70.48% in genotype RL-88 at pre-flowering stage was at par with same genotype at 50 per cent flowering stage. The IVDMD of legume and non-legume forage crops were in the range of 67.17 to 69.89 and 59.07 to 67.72%, respectively as reported earlier by Gupta and Pradhan (1975).

***In vitro protein digestibility*:** Highest IVPD was recorded at 50 per cent flowering stage followed by the post- and pre-flowering stages with corresponding values of 85.97, 82.61 and 80.11%, respectively. The effect of interaction between genotypes and growth stages on IVPD percentage showed significantly highest IVPD of 88.63% in the genotype *Anand*-2, followed by the genotype RLG-08-6 with 88.37% at 50 per cent flowering stage.

***Neutral detergent fibre*:** NDF content at pre-flowering stage was lowest (41.37%), followed by 50 per cent flowering (46.95%) and post-flowering stage (48.52%). Gupta and Pradhan (1975) reported NDF content of 43.92% in lucerne. Ahuja *et al.* (1974) found that NDF content of six different fodder crops ranged from 39.3 to 62.8%. The data on effect of interaction between genotypes and growth stages on NDF percentage revealed lowest NDF of 40.20% in genotype RL-88 which was at par with the genotype RLG-08-5 with 40.60% at pre-flowering stage. At post flowering stage, the lowest NDF of 47.30% was observed in *Anand*-2.

***Acid detergent fibre*:** ADF content at pre-flowering stage was lowest (32.54%), followed by 50 per cent flowering (35.25%) and post-flowering stage (35.99%). The data on effect of interaction between genotypes and growth stages on ADF percentage showed lowest ADF content of 31.50% in the genotype RL-88 which was statistically at par with genotype RLG-08-3 at pre-flowering stage. The genotype RL-88 was also recorded at par ADF (35.50%) at post flowering stage with the lowest recording genotype RLG-08-5 with 35.10% ADF. Stivers *et al.* (1983) reported the ADF content of alfalfa forage was <30, 30-35, 35-40 and >40% at bud (no flowers), initial flowering, mid bloom and full bloom (pod) stage, respectively. Vasiljevic *et al.* (2009) observed that maturity of lucerne led to an increase in the acid detergent fibre contents. The values were 32.09, 40.16 and 40.93% for harvest at 1, 2 and 3 cut, respectively.

***Hemi-cellulose*:** Hemicellulose content at pre-flowering stage was lowest (8.83%), followed by 50 per cent flowering (11.75%) and post-flowering stage (12.53%). Gupta and Pradhan (1975) showed that hemi-cellulose content in lucerne fodder was 9.02%. The data on effect of interaction between genotypes and growth stages on hemi-cellulose percentage revealed lowest hemi-cellulose of 8.20% in the genotype RLG-08-1 which was statistically at par with genotypes RL-88, RLG-08-2, RLG-08-3, RLG-08-5, RLG-08-6, RLG-08-7 and RLG-08-8 at pre-flowering stage.

Table 1. Dry matter, crude protein, IVDMD and IVPD at different plant growth stages in promising forage lucerne genotypes

Lucerne genotypes	Dry matter (%)			Crude protein (%)		
	Pre-flowering stage	50 % flowering	Post-flowering stage	Pre-flowering stage	50 % flowering	Post-flowering stage
RLG-08-1	16.79	17.43	21.71	21.00	19.68	16.62
RLG-08-2	15.84	16.18	17.24	20.12	19.25	16.62
RLG-08-3	13.57	18.02	21.33	19.68	18.37	17.93
RLG-08-4	16.54	18.87	26.40	21.43	21.00	19.25
RLG-08-5	17.16	20.55	27.43	19.68	20.56	18.37
RLG-08-6	18.41	19.33	23.05	21.00	20.56	18.81
RLG-08-7	18.02	19.63	23.51	20.56	20.12	17.93
RLG-08-8	16.57	17.79	24.52	21.87	18.37	17.50
RL-88	16.72	18.74	23.77	21.00	19.25	18.37
Anand-2	19.53	19.82	23.74	21.87	21.00	19.25
Mean	16.91	18.63	23.27	20.82	19.81	18.06
Range	13.57-19.53	16.18-20.55	17.24-27.43	19.68-21.87	18.37-21.00	16.62-19.25
	Growth stages	Genotypes	Interaction	Growth stages	Genotypes	Interaction
S.E. ±	0.01	0.02	0.04	0.01	0.02	0.03
CD at 5 %	0.04	0.07	0.13	0.03	0.06	0.10

Lucerne genotypes	IVDMD (%)			IVPD (%)		
	Pre-flowering stage	50 % flowering	Post-flowering stage	Pre-flowering stage	50 % flowering	Post-flowering stage
RLG-08-1	68.40	65.60	60.40	75.08	86.66	84.21
RLG-08-2	69.30	66.40	62.10	83.13	84.09	81.57
RLG-08-3	67.80	64.00	61.00	80.00	87.80	78.57
RLG-08-4	65.40	65.00	63.20	83.67	83.33	81.81
RLG-08-5	66.20	65.60	60.40	82.22	85.71	85.10
RLG-08-6	62.30	61.60	61.60	81.25	88.37	82.97
RLG-08-7	64.20	62.20	58.20	76.59	85.37	83.13
RLG-08-8	65.70	62.80	58.40	80.00	85.71	80.00
RL-88	70.48	70.31	62.20	79.16	84.09	83.33
Anand-2	68.60	64.10	61.30	80.00	88.63	85.41
Mean	66.83	64.76	60.78	80.11	85.97	82.61
Range	62.30-70.48	61.60-70.31	58.20-63.20	75.08-83.67	83.33-88.63	78.57-85.41
	Growth stages	Genotypes	Interaction	Growth stages	Genotypes	Interaction
S.E. ±	0.02	0.05	0.08	0.01	0.03	0.06
CD at 5 %	0.08	0.14	0.25	0.05	0.09	0.17

***Acid detergent lignin*:** ADL content at pre-flowering stage was lowest (5.47%), followed by the 50 per cent flowering with 6.39% and post flowering stage with 7.64%. The data on effect of interaction between genotypes and growth stages on ADL percentage showed the lowest ADL content of 4.80% in the genotype RLG-08-2 which was statistically at par with the genotypes RLG-08-1 and RLG-08-8 at pre-flowering stage. Gonzalez *et al*. (2001) observed ADL contents in lucerne samples at vegetative (2 weeks), bud (4 week), full flowering (6 week) and early pod setting stage (8 week) were 6.05, 7.17, 7.86 and 7.21%, respectively. Aufrere *et al.* (2000) reported ADL contents in lucerne forage crop at vegetative and bud stage were 6.5 and 7.9% and in hay 8.2%.

Table 2. NDF, ADF, Hemi-cellulose and ADL contents at different plant growth stages in promising forage lucerne genotypes

Lucerne genotypes	Neutral detergent fibre (%)			Acid detergent fibre (%)		
	Pre-flowering stage	50 % flowering	Post-flowering stage	Pre-flowering stage	50 % flowering	Post-flowering stage
RLG-08-1	41.10	47.10	48.80	32.90	36.90	37.50
RLG-08-2	42.10	46.80	48.30	33.50	36.10	35.90
RLG-08-3	40.80	47.30	47.90	31.80	35.40	35.10
RLG-08-4	41.70	47.50	48.90	32.50	36.00	36.50
RLG-08-5	40.60	46.90	48.50	32.10	35.10	35.30
RLG-08-6	42.20	47.20	49.30	33.30	33.90	35.90
RLG-08-7	41.50	46.10	48.70	32.60	33.70	35.60
RLG-08-8	41.70	46.30	48.10	32.70	35.00	36.60
RL-88	40.20	47.80	49.40	31.50	34.90	35.50
Anand-2	41.80	46.50	47.30	32.50	35.50	36.00
Mean	41.37	46.95	48.52	32.54	35.25	35.99
Range	40.20-42.20 Growth stages	46.10-47.80 Genotypes	47.30-49.40 Interaction	31.50-33.50 Growth stages	33.70-36.90 Genotypes	35.10-37.50 Interaction
S.E. ±	0.05	0.10	0.17	0.06	0.11	0.20
CD at 5 %	0.16	0.29	0.50	0.18	0.33	0.57

Lucerne genotypes	Hemicellulose (%)			Acid detergent lignin (%)		
	Pre-flowering stage	50 % flowering	Post-flowering stage	Pre-flowering stage	50 % flowering	Post-flowering stage
RLG-08-1	8.20	10.20	11.30	5.00	6.60	8.20
RLG-08-2	8.60	10.70	12.40	4.80	6.80	7.60
RLG-08-3	9.00	11.90	12.80	5.10	5.30	6.80
RLG-08-4	9.20	11.50	12.40	5.40	5.60	6.80
RLG-08-5	8.50	11.80	13.20	5.70	5.80	8.00
RLG-08-6	8.90	13.30	13.40	6.00	7.80	8.00
RLG-08-7	8.90	12.40	13.10	6.20	7.00	7.80
RLG-08-8	9.00	11.86	11.50	5.00	5.80	8.00
RL-88	8.70	12.90	13.90	6.10	7.20	8.60
Anand-2	9.30	11.00	11.30	5.40	6.00	6.60
Mean	8.83	11.75	12.53	5.47	6.39	7.64
Range	8.20-9.30 Growth stages	10.20-13.30 Genotypes	11.30-13.90 Interaction	4.80-6.20 Growth stages	5.30-7.80 Genotypes	6.60-8.60 Interaction
S.E. ±	0.09	0.17	0.29	0.02	0.05	0.08
CD at 5 %	0.26	0.48	0.83	0.08	0.14	0.25

Conclusion

The nutritional composition of ten lucerne genotypes evaluated at different plant growth stages showed higher crude protein, IVDMD and lowest NDF, ADF, ADL contents at pre-flowering stage and highest IVPD at 50 per cent flowering stage, while DM contents were higher at post-flowering stage. Considering CP, IVDMD and IVPD the cultivars *Anand*-2 and RL-88 were better followed by two promising genotypes, RLG-08-5 and RLG-08-1.

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