Studies on chemical composition and feeding value in forage crops

XIII. Effect of the stage of maturity in spring and the time of repeated harvest in regrowth period on the chemical composition and feeding value of Ladino clover (*Trifolium repens latum*) grown in the warmer Setouchi district in Japan

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Ladino clover is one of the promising perennial leguminous forages as soiling and pasture crops in the warmer Setouchi district of Japan. There is, however, little information available concerning its feeding value. The objectives of this study were: (1) to determine the variation in feeding value of Ladino clover with advancing maturity in spring (the first growth period) and (2) to compare the feeding value among the first to fourth crops harvested at approximately the early bloom stage of growth.

Materials and Methods

The data on climatic conditions in the growing seasons of 1966 and 1967 are shown in Table 1. From this Table, it can be seen that the average temperature in June to mid-September of 1967 was slightly higher than that of the corresponding period of 1966. The total amount of precipitation was 411.6 mm during the period from June to September of 1967 which was 44 % less than the previous year (736.7 mm). Such climatic conditions in 1967 were unfavorable to the growth of the forage under study when compared with those in 1966.

The experiments were carried out at the experimental field of the Faculty of Agriculture, Okayama University in both 1966 and 1967.

Experiment in 1966. Oregon Ladino clover (*Trifolium repens latum*) was broadcasted at a rate of 500 g per 10 a in the fall of 1965. Prior to seeding 4 kg of N as ammonium sulfate, 4.8 kg of P_2O_5 as superphosphate, 5 kg of K_2O as muriate of potash and 30 kg of $Ca(OH)_2$ per 10 a, respectively, were applied to the field. In February of 1966, the clover stand was fertilized with 2 kg of N, 3.2 kg of P_2O_5 and 6.5 kg of K_2O per 10 a, respectively, as ammonium sulfate, superphosphate and muriate of potash. The clover was harvested at approximately the early bloom stage as shown in Table 2. The forages harvested at four cutting dates were assigned to two rabbits (about 2500 g of body weight) for each of the four consecutive trials starting in May of 1966. The rabbits were fed 600 g of fresh forage cut in small pieces (about 2 cm in length) per day in two equal portions. Water was available all day. Each digestion trial consisted of a 5-day preliminary period followed by a 5-day collection period in which total fecal collection was made. The animals were kept in individual metabolic cages. Feces were removed each day and dried at about 70°C in a forced air dryer for 7-8 hr. Feces collected for five days were then mixed thoroughly and ground finely in a hammer mill. Two hundred grams

Table 1. Outline of climatic condition for growing season of 1966 and 1967^{a)}

Year	Month	Precipitation (mm)				Temperature (°C)			Sunshine ^{b)} (hour)
		1 st decade	2 nd decade	3 rd decade	Total	Mean maximum	Mean minimum	Mean	
1966	April	43.5	20.4	44.3	108.2	18.3	7.5	12.8	176.4
	May	61.0	16.3	139.8	217.1	22.6	10.5	16.5	221.7
	June	133.4	46.0	45.1	224.5	25.5	17.2	20.9	168.5
	July	86.3	37.5	16.2	140.0	29.1	19.9	24.1	198.0
	August	46.6	23.8	5.2	75.6	32.1	24.0	27.6	215.4
	September	40.7	128.1	127.8	296.6	27.4	18.1	22.3	173.5
	October	5.6	34.8	22.9	62.3	22.4	10.9	16.2	170.0
	November	3.0	32.3	18.6	53.9	16.4	4.5	10.0	160.9
1967	April	97.9	104.2	27.8	229.9	18.8	9.1	13.5	150.8
	May	81.9	0.0	61.8	143.7	25.7	12.7	18.7	254.1
	June	12.2	0.0	75.7	87.9	28.4	17.3	22.4	226.5
	July	205.4	69.5	0.0	274.9	30.5	22.9	26.2	191.5
	August	2.4	3.7	20.5	26.6	33.8	23.6	28.0	248.5
	September	0.4	19.5	2.3	22.2	29.4	18.9	23.5	197.3
	October	27.6	19.0	83.1	129.7	22.1	11.4	16.1	169.6
	November	12.2	37.1	12.4	61.7	16.8	6.2	10.9	130.7

a) Cited from the records of the Okayama Meteorologic Observatory, some 300 m distant from the experimental field.

b) Recorded by Jordan's recorder.

of forages were collected daily from the same forage fed to the animals during the collection period and then were treated by the same manner used in the case of the feces. The chemical compositions of the feces and forage samples for determination of digestibilities of dry matter, crude protein, crude fat, NFE and crude fiber were analysed by the conventional method¹⁾. Crude lignin, calcium and phosphorus were analysed according to the methods used in the previous report⁴⁾. Samples for leaf-stem separations were taken from the forage harvested on the third day of the collection period. Plant samples were separated into leaf, pedicel and flower fractions. The percentage of each was calculated from air-dry weight of the fractions. Plant height is shown as the average of 20 plants at the time of leaf-stem separation.

Experiment in 1967. In mid-February of 1967 the same area used in Experiment in 1966 was fertilized with 60 kg per 10 a of 7-11-7 (N-P-K) mixed fertilizer. As indicated in Table 2, the forage was harvested at the early vegetative, late vegetative, bud and early bloom stages of the first growth in spring and the second to fourth crops in the regrowth period were harvested at approximately the same as the early bloom stage in the first growth. Seven digestion trials were conducted from April to November of 1967 with the same feeding, collection, sampling and analytical procedures employed in Experiment in 1966.

Results and Discussion

Table 2 shows the fractions of the plant organs of the forage when harvested for the digestion trial. In spring of 1967, leaf content decreased sharply with advance in the growth stage from 71 % at the early vegetative to 36 % at the early bloom stage. Leaf content for each of the first to fourth crops harvested at approximately the early bloom stage was larger

Feeding value of Ladino clover

Table 2. Morphological character and yield of Ladino clover when harvested for digestion trial

Year	Stage of maturity	Date of digestion trial	Plant height	Fraction of organa ^{a)}			Yield per 10 a		
				Leaf	Pedicle	Flower	DM ^{b)}	DCP	TDN
1966	1 st crop	May 18-22	cm 43.4	% 33	% 66	% 1	kg 568	kg 121	kg 354
	2nd crop	June 20-24	35.6	41	57	2	184	42	130
	3 rd crop	July 23-27	44.0	44	54	2	216	43	141
	4 th crop	Oct. 20-24	39.9	48	52	0	201	47	141
1967	Early vegetative	April 2- 6	18.5	71	29	0	97	30	71
	Late vegetative	April 24-28	34.6	48	52	0	141	36	102
	Bud	May 10-14	52.0	40	60	0	197	46	147
	Early bloom	May 22-26	54.2	36	63	1	216	42	157
	Regrowth ^{c)} 2nd crop ^{d)}	June 18-22	32.6	48	49	3	138	26	92
	3 rd crop	July 13-17	31.2	49	47	4	81	21	60
	4 th crop	Nov. 15-19	24.2	61	39	0	77	19	61

a) Expressed on oven-dry basis.

b) Dry matter.

c) Harvested at the early bloom stage except 4 th crop.

d) The first cutting was made at the early bloom stage.

in 1967 than in 1966, particularly for the fourth crop. In general, the leaf content increased in both years with increase in the frequency of repeated harvest in the regrowth period.

The chemical compositions of the forages are shown in Table 3. In Experiment of 1967, crude protein content decreased with advancing maturity in spring from 35.8 % at the early vegetative to 23.5 % at the early bloom stage and crude fat content decreased slightly, whereas crude fiber and crude lignin contents increased, especially at the early bloom stage the former increased to almost two times of that at the early vegetative stage. Phosphorus content decreased slightly as the plant developed, but calcium content decreased at the initial growth stages and increased slightly at the early bloom stage. KAMETAKA and HARUYAMA³⁾ have reported that the calcium content of wild white clover in bloom increases significantly. Such variations in chemical compositions may be due to changes in the fractions of plant organ with advancing maturity. As shown in Table 4, leaves contain more crude protein, calcium and phosphorus, and less crude fiber than pedicels. ИТОH et al.²⁾ have also reported that the calcium content in leaf of Ladino clover is higher than the value of pedicel. Moreover, the contents of crude protein and crude fiber in the leaves are almost unchanged with advancing maturity, whereas in the pedicels crude protein content decreases and crude fiber content increases. Therefore, it may be considered that an increase in the pedicel fraction with maturity was accompanied with lower crude protein and higher crude fiber in the whole plant.

The chemical compositions for the first to fourth crops harvested at approximately the early bloom stage in both years are given in Table 3. There is almost no difference in the average content of each component other than crude lignin in the first to fourth crops of both years but the average content of crude lignin was higher in 1966 than 1967. It appears that the higher content of crude lignin in 1966 is associated with lower leaf content of the forages in the corresponding year. While the fourth crop in 1967 had a higher leaf content than the

Table 3. Chemical composition of Ladino clover^{a)}

Year	Stage of Maturity	Moisture	Crude protein	Crude fat	NFE	Crude fiber	Crude ash	Crude lignin	Mineral	
									Ca	P
		%	%	%	%	%	%	%	%	%
1966	1 st crop	89.6	26.3	4.1	39.1	18.5	12.0	11.7	1.65	0.46
	2nd crop	88.0	26.9	4.0	42.3	15.2	11.6	8.5	1.68	0.43
	3 rd crop	88.6	25.5	4.1	43.0	16.5	10.9	11.9	1.87	0.40
	4 th crop	87.3	29.3	4.4	41.5	15.2	9.6	7.9	1.89	0.35
	Mean	88.4	27.0	4.2	41.4	16.4	11.0	10.0	1.77	0.41
	S.D. ^{b)}	0.8	1.4	0.1	3.1	1.3	0.9	1.8	0.11	0.04
1967	Early vegetative	91.4	35.8	4.2	35.6	9.0	15.4	5.7	1.78	0.70
	Late vegetative	89.7	29.5	3.2	42.0	13.7	13.4	6.1	1.54	0.63
	Bud	91.3	27.2	3.3	39.6	17.4	12.5	8.1	1.43	0.53
	Early bloom	88.3	23.5	4.0	43.0	17.6	11.9	8.8	1.54	0.50
	Regrowth	2 nd crop	79.9	23.3	4.3	45.0	16.2	11.2	1.87	0.36
		3 rd crop	89.2	30.3	3.9	38.8	15.7	11.3	1.51	0.52
		4 th crop	85.7	28.0	5.4	43.1	12.0	11.5	1.73	0.42
	Mean ^{c)}	85.8	26.3	4.4	42.4	15.4	11.5	7.2	1.66	0.44
	S.D. ^{b)}	3.6	3.0	0.6	3.1	2.1	0.3	1.2	0.18	0.06
	Mean ^{c,d)}	87.1	26.6	4.3	41.9	15.9	11.3	8.6	1.71	0.42
	S.D. ^{b)}	2.9	2.4	0.4	2.4	1.8	0.7	1.1	0.16	0.06

a) Expressed as a percentage of the dry matter except moisture.

b) Standard deviation.

c) Not include the early vegetative to bud stages.

d) Average of two years.

Table 4. Chemical composition of two plant fractions of Ladino clover

Stage of Maturity	Leaf fraction					Pedicel fraction					
	Crude protein	Crude fiber	Crude ash	Mineral		Crude protein	Crude fiber	Crude ash	Mineral		
				Ca	P				Ca	P	
Early vegetative	% 37.6	% 7.9	% 11.2	% 1.80	% 0.70	% 23.4	% 14.5	% 14.4	% 1.70	% 0.59	
Late vegetative	40.0	8.5	10.8	1.63	0.68	20.9	13.7	13.8	1.50	0.58	
Bud	38.7	8.9	9.8	1.50	0.58	19.4	21.5	13.9	1.40	0.50	
Early bloom	38.8	9.7	10.0	1.70	0.50	14.8	23.4	12.4	1.51	0.40	
Regrowth	2 nd crop	33.4	9.0	9.8	1.71	0.38	15.7	21.5	11.9	1.60	0.30
	3 rd crop	37.2	10.2	8.4	1.62	0.49	21.4	21.7	11.4	1.52	0.48
	4 th crop	35.4	7.4	9.7	1.86	0.45	13.1	13.0	11.9	1.56	0.40
Mean ^{a)}	37.4	8.8	9.9	1.72	0.54	19.4	18.5	12.8	1.54	0.46	
S.D. ^{b)}	2.1	0.9	0.8	0.13	0.11	3.6	4.2	1.1	0.26	0.03	

a) Average of the 1st, 2nd, 3rd and 4th crops harvested at approximately the early bloom stage.

b) Standard deviation.

Values expressed as a percentage on a dry matter basis.

third crop, its crude protein content was lower. This may be due to the lower content of crude protein of the fourth crop in both leaves and pedicels when compared with that of the third crop as shown in Table 4.

Feeding value of Ladino clover

As the frequency of repeated harvest in the regrowth period increases, a sharp decrease is indicated in the contents of crude fiber and lignin in both years, but there was no distinct tendency of either increase or decrease in the other feed constituents.

When the chemical composition was compared with the alfalfa of the previous report⁴⁾, the Ladino clover was richer in crude protein, crude ash, calcium and phosphorus but the content of crude fiber was lower through the first growth and regrowth periods.

The apparent digestibility of each component of the forage is summarized in Table 5. Almost no difference was observed in the digestibilities of dry matter, organic matter, crude fat and NFE among four stages in spring of 1967 but those of crude protein and crude fiber remained almost constant to the bud stage and then declined about four and five percents, respectively, at the early bloom stage.

The average digestibility of each component, especially crude fat and crude fiber, for the first to fourth crops harvested at approximately the early bloom stage was much higher in 1967 than in 1966. These results may be due to the fact that the forages harvested in 1967 contained an average of 2.8 % less crude lignin than those harvested in 1966. The reason for this remarkable difference in the crude fat digestibility between both years is not known.

During the first growth and regrowth periods in both years, the forages were more digestible with respect to all feed constituents than the alfalfa cited above.

Table 5. Apparent digestibility and digestible nutrients of Ladino clover

Year	Stage of Maturity	Dry matter	Organic matter	Crude protein	Crude fat	NFE	Crude fiber	DCP ^{a)}	TDN ^{a)}	
		%	%	%	%	%	%	%	%	
1966	1 st crop	68.7	68.6	78.5	38.7	75.1	47.5	20.6	62.3	
	2nd crop	72.1	73.4	85.1	41.9	86.1	48.8	22.9	70.5	
	3 rd crop	71.1	72.1	79.0	44.0	80.1	47.8	20.1	65.5	
	4 th crop	76.1	76.4	80.3	32.8	87.0	48.7	23.5	70.2	
	Mean	72.0	72.6	80.7	39.3	82.1	48.2	21.8	67.1	
	S.D. ^{b)}	2.7	2.8	2.6	4.2	4.8	0.6	1.3	3.5	
1967	Early vegetative	80.7	82.7	86.6	61.6	84.9	67.8	31.0	73.1	
	Late vegetative	80.4	81.7	87.2	56.6	83.7	69.6	25.7	72.8	
	Bud	82.4	83.6	86.4	58.8	89.4	67.7	23.5	74.8	
	Early bloom	80.3	80.4	82.7	61.8	85.4	62.2	19.5	72.6	
	Regrowth	2 nd crop	73.8	73.6	79.8	52.5	77.4	49.2	18.6	66.4
		3 rd crop	79.5	80.2	85.5	60.0	85.9	60.7	25.9	74.0
		4 th crop	85.7	86.5	89.1	75.2	85.2	75.0	25.0	79.7
		Mean ^{c)}	79.8	80.2	84.3	62.4	83.5	61.8	22.2	73.2
	S.D. ^{b)}	3.1	4.5	3.4	8.2	3.5	9.1	3.2	3.1	
	Mean ^{c,d)}		75.8	76.4	82.5	50.9	82.5	55.0	22.0	70.1
S.D. ^{b)}		4.7	5.3	3.5	13.2	4.3	9.4	2.5	4.3	

a) Expressed as a percentage on a dry matter basis.

b) Standard deviation.

c) Not include the early vegetative to bud stages.

d) Average of two years.

The feeding values of the forages expressed as DCP and TDN are presented in Table 5. It was found from Experiment in 1967 that DCP content declined sharply with advancing maturity from 31.0 % at the early vegetative to 19.5 % at the early bloom stage. A sharp decline of

DCP content from the vegetative to the bud stage undoubtedly is due to a decrease in crude protein content with advancing maturity because the digestibility of corresponding component remained almost unchanged to the bud stage. The rate of decline in DCP was about 0.26 % per day as compared with about 0.7 % for the alfalfa cited above. There was no appreciable difference in TDN content among the forages harvested at the four stages in spring of 1967.

The average DCP contents of the first to fourth crops harvested at approximately the early bloom stage were 21.8 % and 22.2 %, respectively, in 1966 and in 1967. The average TDN contents of the corresponding crops were 67.1 % in 1966 and 73.2 % in 1967. This remarkable difference in TDN may be due to the difference in the digestibilities of feed constituents, particularly those of crude fat and crude fiber, since there are no appreciable difference in the average contents of chemical compositions between both years as shown in Table 3.

In the comparison of DCP and TDN contents among the first to fourth crops harvested at approximately the early bloom stage, there was no large difference in DCP content in 1966, but in 1967 the third and fourth crops showed a much higher content of DCP as compared with the others.

Dry matter and nutrient yields per 10 a are give in Table 2. Dry matter yield per 10 a in spring of 1967 increased rapidly from 97 kg at the early vegetative to 216 kg at the early bloom stage. Each yield of the first to fourth crops harvested at approximately the early bloom stage in 1967 was extremely low in comparison with those in 1966. This decline in dry matter yield in 1967 is mainly due to reduction in density of the sward with drought as well as with the lapse of the year. Yields of DCP and TDN also increased with advancing maturity and the maximum yield of DCP in spring was attained in the bud stage and that of TDN in the early bloom stage. The growth stages of maximum yields of both digestible nutrients were the same as the alfalfa cited above.

The yields of DCP and TDN for each of the second to the fourth crops were markedly inferior to those of the first crop at the early bloom stage.

Relationships between leaf content and chemical constituents, such as crude protein and crude lignin, of the forages harvested in both years and DCP and TDN contents are shown in

Table 6. Correlation and regression of leaf content and chemical constituents with digestible nutrients of Ladino clover

X	Y	r	Regression equation	Standard error of the estimate
Leaf content	Crude protein	0.741**	—	
	Crude fiber	-0.942***	—	
	DCP	0.776**	—	
	TDN	0.488		
Crude protein	DCP	0.967***	$Y = -4.62 + 1.003X$	0.87
	TDN	0.424		
Crude lignin	DCP	-0.716**	—	2.53
	TDN	-0.786**	$X = 83.25 - 1.599X$	
Crude fiber	DCP	-0.830**	—	
	TDN	-0.448		

Chemical constituents and digestible nutrients expressed on a dry matter basis.

***(**) Statistically significant at the 0.1% (1%) level.

— These equations were not computed.

Table 6. There was a significant positive correlation between the leaf content and the contents of crude protein and DCP and a significant negative correlation between the leaf content and the crude fiber content, and their correlation coefficients were 0.741 ($P < 0.01$), 0.776 ($P < 0.01$) and -0.942 ($P < 0.001$), respectively. The crude protein content was highly correlated with DCP content, its correlation coefficient being 0.967 ($P < 0.001$) as compared with 0.995 ($P < 0.001$) for the alfalfa cited above. Relationships existed between the crude lignin content and the contents of DCP and TDN, and their correlation coefficients were -0.761 ($P < 0.01$) and -0.786 ($P < 0.01$), respectively. The crude lignin content was more closely correlated with the TDN content in the alfalfa cited above than in the the Ladino clover in these experiments. This is probably due to the fact that the TDN content for the Ladino clover varied little with the date of harvest as compared with the alfalfa.

It is clear from the data in Table 6 that the crude protein (CP) and the crude lignin (CL) are the prominent constituents for the purpose of predicting DCP and TDN, respectively, and the following equations are given: $DCP = -4.62 + 1.003 \text{ CP}$ (S.E. ± 0.87) and $TDN = 83.25 - 1.599 \text{ CL}$ (S.E. ± 2.53) where crude protein, crude lignin, DCP and TDN contents are shown in percentage of dry matter.

Summary

Effects of the time of repeated harvest in regrowth period as well as the stage of maturity in spring on the feeding value of Ladino clover produced in the warmer Setouchi district of Japan have been determined in 1966 and 1967.

1. As the plant matured in spring the contents of crude fiber and crude lignin increased considerably whereas that of crude protein decreased markedly. Moreover, the content of phosphorus also decreased but not very much. The calcium content increased slightly after a decrease in the initial stages. The DCP content declined sharply at a rate of 0.26 % units per day from the early vegetative to the early bloom stage but the TDN content remained almost unchanged.

2. An appreciable difference in the average content of DCP for the first-fourth crops harvested at approximately the early bloom stage was not found between both years but the average TDN content was higher in 1967 than in 1966. The DCP and TDN contents for the fourth crops harvested in the fall of both years showed a tendency of being higher than those of the first to third crops.

3. Crude protein (CP) and crude lignin (CL) are the prominent constituents for the prediction of DCP and TDN, respectively, and the following equations are given: $DCP = -4.26 + 1.003 \text{ CP}$ (S.E. ± 0.87) and $TDN = 83.25 - 1.599 \text{ CL}$ (S.E. ± 2.53).

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飼料作物の化学的成分と飼料価値に関する研究

XIII. 瀬戸内地帯で生産されたラジノクローバーの化学的成分と飼料価値におよぼす生育段階と刈取回次の影響について

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瀬戸内地帯（岡山大学農学部附属農場）で生産されたラジノクローバーの飼料価値におよぼす1番刈草の生育段階と再生草の刈取回次の影響について1966年と1967年の両年にわたって検討した。

1番刈草において生育がすすむにつれて、葉量、粗蛋白質、リン含量が減少し、粗繊維と粗リグニン含量が増加した。カルシウム含量は生育の初期にわずかに減少し、その後開花初期にわずかながら増加の傾向がみられた。DCP含量もまた1日あたり0.26%ずつ低下したが、TDN含量はほとんど変化しなかった。

開花初期頃に収穫した1～4番刈草の飼料成分含量は

両年間に大差がなかった。そして刈取回数が増すにつれて粗繊維と粗リグニン含量が著しく減少した。1～4番刈草を含めた両年の平均DCP含量は $22.0 \pm 2.5\%$ 、TDN含量は $70.1 \pm 4.3\%$ 、カルシウム含量は $1.71 \pm 0.16\%$ 、リン含量は $0.42 \pm 0.06\%$ であった。

粗蛋白質(CP)と粗リグニン(CL)がDCPとTDN含量のそれぞれを推定するためのすぐれた成分で次の方程式を得た。DCP $= -4.62 + 1.003$ CP (推定値の標準誤差0.87), TDN $= 83.25 - 1.599$ CL (推定値の標準誤差2.53), 各成分含量は乾物中の%で示す。