A STUDY OF THE CALCIUM IN LEGUME FORAGES

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Commonly, livestock are fed with all the forage they will consume, and then are fed in addition whatever grain or other concentrates they need to cover their specific requirements. The good forages are the foundation for efficient production of all classes of farm animal except swine and poultry.

The legume forages occupy a place of particular importance in the feeding of livestock, because of the several superiorities they have over all other forages. Morrison (1) gives a full detail of these superiorities in his book. One of them, the legume forages are the highest in calcium among all farm-grown feeds.

Several physiological studies (2-4) have been reported on the uptake and availability of calcium by the legume forages and numerous studies (5-7) have been carried out on the metabolism and availability of calcium in farm animals, but there are few noticeable studies (8) on the utilities of available forms of calcium contained in the legume forages, which are ingested by farm animals.

The work reported here consist of the calcium contents and its forms in the solubility of calcium in the legume forages, which are used in the investigations of the feeding trials by rabbit.

Materials and Methods

Preparation of legume forage hay: Eight kinds of legume forage, i. e., red clover, mammoth clover, Kenland clover, ladino clover, white clover, alfalfa, hairy vetch, and sweet clover used in this experiment, were seeded at each optimum season and harvested at the same time, late in July. The growth stages of these legume forages at the harvest time are shown in Table 1. Each forage was cut at 3 to 5 cm in height above the ground, and the leaves were immediately separated from stem, and the powdered hay was prepared after the weighing and the artificial drying with aeration at about 65°C.

Table 1. Growth condition of legume forages.

Kind of forage	Growth stage	Height (cm)
Red clover	pre-blooming	20-30
Mammoth clover	full-blooming	25-35
Kenland clover	blooming, early	20-30
Ladino clover	pre-blooming	15-25
White clover	blooming, early	10-15
Alfalfa	blooming, early	50-60
Hairy vetch	pre-blooming	20-40
Sweet clover	vegetative, late	20-30

Four kinds of wild white clover were collected on our campus and at a dike in Sendai, and three kinds of ladino clover were cultivated at the Miyagi Prefectural Agricultural Experiment Station (Table 3). All white clover are prepared to the powdered hay in the same way described above after the pre-drying in the sun for a few days, for the use of the feeding experiments with rabbit.

Preparation of Ca^{45}-labeled white clover: To study the transference of calcium in the digestive tract of animals, some forms of radioactive calcium compounds were used as tracer, and such as oral, intervenous and intramuscular doses were used. And it was expected that the oral administration of the Ca^{45}-labeled white clover is one of the more favorable methods for the investigation of the calcium utilizing aspects in animals. Three kinds of Ca^{45}-labeled white clover were prepared with the solution and soil culture methods for this purpose.

a) Solution culture: The white clover in good growth state was dug out and rinsed carefully by water not to harm the roots. Thereafter, the roots of the white clover were inserted into the hexagon wire screen with 2 cm diameter and then soaked into the culture solution filled in the Wagner pot (15 cm diameter, 14 cm height).

The Ca^{45}-labeled white clover No. R1 was cultured after the four days' pre-culture by tap-water only, by the solution containing 10 ppm of N, P_2O_5, and K_2O, and 2 ppm of MgO and Fe_2O_3, respectively, and $Ca^{45}Cl_2$ in weak hydrochloric acid, for five to 15 days with 30 minutes aeration twice daily, in August.

No. R3 white clover was cultured by well water added with Ca^{45} for 15 to 20 days without the pre-culture period and the aeration, in May.

b) Soil culture: The white clover was seeded on the fertile soil mixed with the rabbits feces containing Ca^{45} in the pot, October 1956. The $Ca^{45}Cl_2$ solution was added to the soil twice during the growth period of the white clover. At the 190 th (May, 1957) and 300 th day (September, 1957) after the

seeding, respectively, the Ca^{45}-labeled white clover was harvested. The mixture of both powdered hay was named No. R2.

Solubility of calcium in legume forage: To evidence the physiological role of calcium and the calcium contents of the various forms of calcium complexes in the life of legume forages, the determination of the solubility of calcium in leaf and stem of eight legume forages, and the white clover hay and the Ca^{45}-labeled white clover hay were made according to modifications of the method of Sveshnikov (4). Three grams of the powdered hay was taken in a 200 *ml* beaker and 60 *ml* distilled water was added, at the first step, and stirred by the magnetic stirrer, for one hour at the room temperature, and then the water soluble calcium was extracted. The supernatant obtained after the centrifuge at 3000 rpm for 10 minutes, was regarded as the fraction containing the water soluble calcium. The residue was extracted with 60 *ml* of the N-NaCl, N-AcOH, and N-HCl solutions successively, and each supernatant was regarded as the fraction containing the calcium which was soluble in each solution. The last residue may contain the insoluble calcium. Four supernatants and the last residue were taken in the porcelain evaporating-dish, and after the evaporation they were ashed at about 600°C. The ash was dissolved in 2N HCl, and made to volume with distilled water, usually 50 *ml* for four supernatants and 25 *ml* for the last residue, and an aliquots was used for the determination of calcium.

Total calcium and radioactive calcium determinations were made by the method of Comar *et al.* (9).

The proximate composition and phosphorus content of the white clover hays were determined according to the Methods of Analysis of the A.O.A.C. (10).

Results

The results of dry matter, calcium content of the dry matter and the solubility of calcium in leaf and stem of eight legume forages are presented in Table 2.

The dry matter content of leaf are higher than stem, except alfalfa which has the higher dry matter content of stem than leaf.

Calcium content of the dry matter of leaf is higher than stem in all legume forages. The maximum calcium content of leaf and the minimum content of stem occupied with alfalfa, and their contents are 33.0 mg and 7.7 mg, respectively. The calcium content in leaf of three kinds of clover, namely, red colver, mammoth clover, and Kenland clover belong to the red clover are even higher, being two to three times the values of stem. On the other hand, the calcium content in stem of ladino clover, white clover and hairy vetch are slightly lower than each value of leaf.

Table 2. Dry matter, calcium content and the solubility of calcium in leaf and stem of legume forages.

Legume forages	Red clover		Mammoth clover		Kenland clover		Ladino clover		White clover		Alfalfa		Hairy vetch		Sweet clover	
	leaf	stem	leaf	stem	leaf	stem	leaf	stem	leaf	stem	leaf	stem	leaf	stem	leaf	stem
Dry matter (%)	22.8	12.2	21.7	15.9	23.7	18.7	24.0	14.3	22.5	16.7	21.8	26.2	19.5	13.6	21.7	19.3
Calcium (mg/D.M.g)	22.58	10.63	22.05	8.63	20.61	7.89	14.68	11.35	16.02	12.33	33.00	7.71	10.13	8.02	29.14	10.65
Solubility of calcium (%)																
Water-soluble Ca (1)	36.5	20.6	28.5	17.0	30.5	18.6	27.7	15.9	29.8	19.1	45.9	33.2	19.1	13.8	50.6	29.0
N-NaCl soluble Ca (2)	29.1	31.6	28.1	33.7	27.8	33.1	25.5	30.1	26.3	28.4	25.8	25.0	21.8	17.7	26.9	29.8
N-AcOH soluble Ca	14.8	20.0	18.1	20.8	16.8	20.6	11.7	21.6	14.5	23.6	9.3	12.9	17.7	23.7	12.7	25.4
N-HCl soluble Ca	15.8	19.2	19.3	19.5	18.9	19.4	28.8	23.7	23.6	21.0	16.1	24.1	33.1	33.0	6.8	9.7
Insoluble Ca	3.8	8.6	6.0	9.0	6.0	8.3	6.3	8.7	5.8	7.9	2.9	4.8	8.3	11.8	3.0	6.1
N-NaCl soluble Ca (3)*	52.3	41.9	45.1	44.4	47.7	43.1	47.0	34.1	47.4	35.8	59.9	42.6	25.4	23.1	61.9	44.8
(3)/((1)+(2))	79.8	79.8	79.8	87.6	82.0	83.5	88.4	74.2	84.0	75.4	83.5	73.3	82.2	73.4	80.0	76.2

* Each sample was extracted only with N-NaCl solution.

On the solubility of calcium in leaf and stem of each legume forage, in general, the water-soluble calcium content of leaf is comparatively quite higher than the value of stem, and the other four calcium fractions are low. The insoluble calcium content in leaf and stem of all legume forages are lower than the soluble calcium content in the other four fractions, particularly being lowest in leaf.

The water-soluble and N-NaCl soluble calcium content in leaf of three kinds of clover belong to the red clover, except the leaf of red clover containning 36.5 percent water-soluble calcium, occupied approximately 30 percent, and the N-AcOH and N-HCl soluble calcium contained 15 to 20 percent, respectively. In stem, the N-NaCl soluble calcium content are higher than the value of leaf, and they occupied over 30 percent, and the other soluble calcium fractions occupied about 20 percent.

The water-soluble calcium content of alfalfa leaf occupied 46 percent, and the level of the N-AcOH soluble calcium are low and that of insoluble calcium are the lowest (2.9 percent), comparing with the insoluble calcium content in leaf and stem of the other legume forages.

The water-soluble calcium content in leaf and stem of hairy vetch are lower than the other forages, and the N-HCl soluble fraction and insoluble calcium are the highest of any other forages.

The water-soluble calcium content in leaf of sweet clover occupied about a half of the total calcium, and the N-HCl soluble content of both leaf and stem are shown to be the lowest as compared with the other forages.

In all samples, when they were extracted with N-NaCl solution at first, the N-NaCl soluble calcium accounted for about 80 percent of the total calcium content of water-soluble and N-NaCl soluble fractions, and it appears that the quite large amounts of calcium in leaf and stem of the legume forages are replaceable by sodium.

The results of the chemical analyses (proximate composition, calcium and phosphorus contents and the solubility of calcium) of the white clover hay are presented in Table 3.

In the proximate composition, the crude protein and crude fat of both white and ladino clover hay were higher and the crude fiber was lower in content, comparing with the values of Morrison (1). On the contrary, it showed the lower contents of crude protein and fat, and the higher amount of crude fiber content in comparison with the values of Kametaka and Haruyama (11). The crude fat contents of No. W 2 and WL 7 clover hay which were harvested in July, and the crude fiber content of No. L3 clover hay harvested in November are comparatively quite high. The ash and calcium content of all hay showed a high content as compared with the values of Morrison (1) and Kametaka and Haruyama (12).

Table 3. Chemical composition and the solubility of calcium in white clover hays.

Clover hay No.	W 1	W 2	L 3	W 4	W 5	L 6	WL 7	L 8
Kind of clover	White clover	White clover	Ladino clover	White clover	White clover	Ladino clover	W5 + L6	Ladino clover
Date of collection	May 1955	July 1955	Nov. 1955	May 1956	July 1957	July 1957		May 1958
Area of collection	A	A	C	B	B	C		C
Constituent								
Dry matter (%)	91.45	90.93	90.43	88.21	89.14	90.05	90.21	89.79
Calcium (mg/D.M.g)	8.59	12.20	13.25	15.98	15.56	15.84	15.75	16.43
Phosphorus (mg/D.M.g.)	1.94	2.36	2.55	3.30	3.05	2.93	2.94	3.12
Proximate composition (% on the dry basis)								
Crude protein	27.15	25.89	26.96	27.44			26.65	29.69
Crude fat	3.96	4.86	4.66	3.48			5.33	3.54
Crude fiber	11.62	16.22	19.06	15.43			14.43	16.49
N.F.E.	45.55	40.54	38.84	40.92			40.65	37.95
Ash	11.72	12.49	10.48	12.73			12.94	12.33
Solubility of calcium (%)								
Water-soluble Ca	26.4	28.2	30.1	30.0	28.8	22.9	25.5	29.0
N-NaCl soluble Ca	29.6	31.0	31.0	28.8	27.5	27.8	27.6	29.6
N-AcOH soluble Ca	16.0	18.1	17.7	16.2	16.3	22.2	19.4	17.1
N-HCl soluble Ca	25.0	18.7	16.4	21.3	20.6	19.6	20.0	18.8
Insoluble Ca	3.0	4.0	4.8	3.7	6.8	7.5	7.5	5.5

A. Our campus
B. A dike in Sendai
C. The Miyagi Prefectural Agricultural Experiment Station

The calcium content of each fraction has shown the similar amount of all white clover hay generally. The water-soluble calcium of No. L6 is slightly low and the N-AcOH soluble calcium is high, and the N-HCl soluble calcium of No. W 1 is comparatively high as compared with the calcium content in each fraction of the other hays, respectively.

The outline of the preparation of the Ca^{45}-labeled clover and its calcium content and the amount of calcium and radioactivity in each fraction are presented in Table 4.

Table 4. Calcium content and the solubility of calcium in the Ca^{45}-labeled white clover hay.

Clover hay No.	R 1*		R 2		R 3	
Culture method	Solution culture		Soil culture		Solution culture	
Culture period (day)	5-15		190, 300		15-20	
Calcium (mg/dry matter g)	8.86		14.95		12.15	
Solubility of calcium (%)						
	Ca	Ca^{45}	Ca	Ca^{45}	Ca	Ca^{45}
Water-soluble Ca	12.7	14.1	34.3	34.6	20.3	21.2
N-NaCl soluble Ca	33.2	39.3	30.1	30.4	28.8	34.4
N-AcOH soluble Ca	22.6	24.4	16.7	16.8	24.9	19.9
N-HCl soluble Ca	23.3	17.0	13.9	13.4	19.8	19.6
Insoluble Ca	8.2	5.2	5.0	4.8	6.2	4.9

* Included the top and root

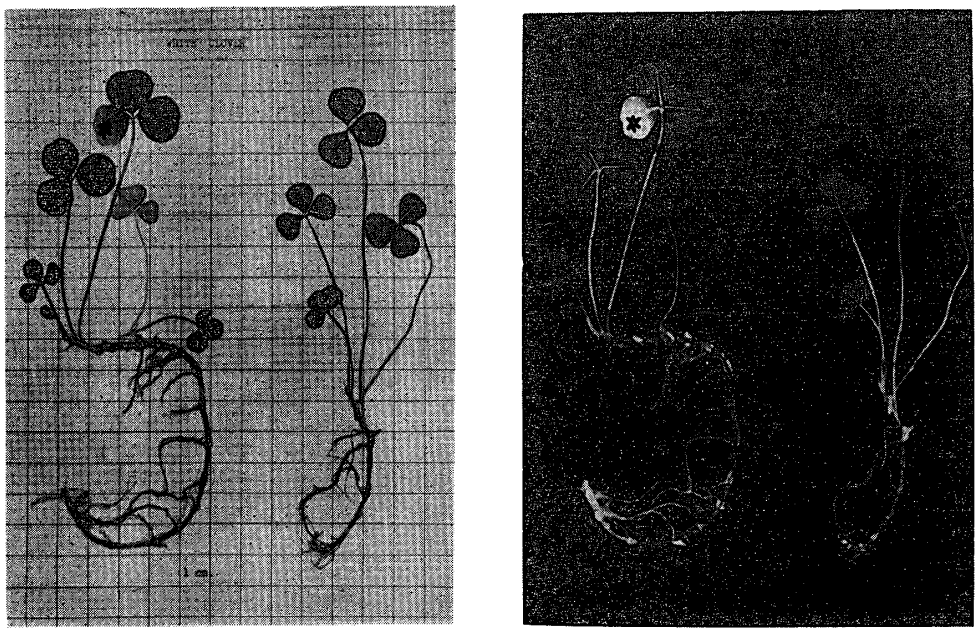
Table 5. Calcium content and the solubility of calcium in the top and root of white clover.

Part of white clover	Top	Root
Raw matter (g)	205	210
Dry matter (g)	25.5	35.0
Calcium (mg/dry matter g)	11.10	6.08
Solubility of calcium (%)		
Water-soluble Ca	23.5	13.4
N-NaCl soluble Ca	29.5	34.8
N-AcOH soluble Ca	20.4	25.7
N-HCl soluble Ca	18.3	23.9
Insoluble Ca	8.3	2.2

The Ca^{45}-labeled white clover No. R1 which was water cultured in August inside of a glass house, was checked for the growth and the absorption of Ca^{45} from the culture solution by means of the decomposition of the culture

solution under the high temperature. As to total administered Ca^{45} in the culture solution, only 12 percent in leaf and stem, and 6 percent in root have been absorbed, respectively.

During the culture period, the radioactivity in the intact clover had been estimated by the use of the survey-meter, and it was shown that the young leaves contained the higher amount of radioactivity, followed by stem, being lowest in old leaves. The autoradiogram which is presented in Figure 1 demonstrates that more radiocalcium deposits in all portions along the vessels.



Photograph Autoradiogram

* This part is soaked in the culture solution.

Fig. 1. Photograph and Ca^{45} autoradiogram of the Ca^{45}-labeled white clover by solution culture.

The results of the calcium content and the solubility of calcium of the top and the root of the white clover are shown in Table 5. The calcium content in the top, leaf and stem, was higher approximately two times than that in the root of white clover, and the water-soluble and residual insoluble calcium content in leaf and stem are higher than the root, and the other three soluble calcium contents are lower. Therefore, the calcium content and particularly, the water-soluble calcium percentage of the Ca^{45}-labeled clover No. R1 which is included the root is lower than the other two labeled clovers. In comparison with the contents of the stable and radioactive calcium in each fraction, the Ca^{45} contents in the N-NaCl fraction are higher than the stable one, and in the N-HCl fraction are lower.

The Ca^{45}-labeled white clover No. R 3 are water cultured immediately in well water with administered $Ca^{45}Cl_2$ solution, without the preculture period, and it has well grown, owing to the optimum temperature.

In this white clover, about 25 percent of the total administered radiocalcium has been absorbed in leaf and stem.

The amount of Ca^{45} (%) in the water-soluble and N-HCl soluble fractions are the same as that of the stable calcium, and Ca^{45} in the N-NaCl soluble fraction is higher than the stable one, and in the N-AcOH soluble fraction is lower. The Ca^{45}-labeled white clover hay No. R2 has been cultured in soil containing the radiocalcium ever since the seedtime, and consequently, the contents (%) of the stable and radioactive calcium are almost the same in all fractions.

Discussion

It is assumed that the similar forms of the calcium complexes has been contained in the water-soluble and N-NaCl soluble fractions of the legume forages as follows. In leaf of the legume forages, except hairy vetch which contains many insoluble calcium, the water-soluble calcium content is higher than the N-NaCl soluble one, and on the contrary, in stem of the forages with the exception of alfalfa containing the higher dry matter content of stem than leaf, the N-NaCl soluble calcium content is higher than the water-soluble calcium.

Therefore, it is supposed that it has been caused by the differences of the cell structures between leaf and stem, and then, in general, the larger parts of several same forms of calcium complexes in the water-soluble and N-NaCl soluble fractions in leaf consists of the comparative soft tissues, have been extracted with water, but in stem which has relatively hard tissues, major parts of the same forms of calcium in both fractions, are extracted with N-NaCl solution, and which are caused by the differences of its osmotic pressure.

Kametaka and Haruyama (12) have reported that the calcium content of the wild white clover in bloom increases significantly. As to eight white clover hays, the calcium content of No. W2 which was collected in full bloom in July are higher than that of No. W1 before bloom at May, and the seasonal alterations of the calcium content was not recognized in the other clover hay.

Generally, the many differences on the solubility of calcium in all white clover hay has never been observed. The leaf content of the ladino clover hay No. L 3 is higher than the stem, and No. L 6 ladino clover hay contains less in the leaf than the stem at the harvest time, and the water-soluble fraction of the ladino clover hay No. L3 are higher than that of No. L6 and the N-AcOH soluble fraction occupies less in No. L 3 than No. L 6. These

estimations are in accord with the results obtained from the solubility of calcium in leaf and stem of ladino clover, that is, the water-soluble fraction of leaf are higher than that of stem, and leaf contains a lower N-AcOH soluble fraction than stem.

The specific activity (Ca^{45}%/ total Ca%) of each calcium fraction in the Ca^{45}-labeled white clover No. R 1 and R 3 prepared with solution culture, are estimated to be larger than one in the water and N-NaCl soluble calcium fractions. Accordingly, it is assumed that the calcium is absorbed and consisted in plant as the calcium complexes of the relatively soluble forms after the duration of two to three weeks, and at this period, the calcium amount converting the calcium complexes of the N-AcOH soluble forms from the N-NaCl soluble calcium, have been increased and or the increase of N-AcOH soluble calcium itself occurs. On the other hand, the insoluble radiocalcium has been counted to be 4.9 percent as compared with 6.2 percent of the stable calcium during the short culture period, and then it is suspected that the insoluble calcium is constituted in plant during this period, but many days have been required for the complete formation of the insoluble calcium by the fact that the stable and radioactive calcium occupy the same ratios in the insoluble fraction.

As it has been required that the radioactive calcium consists in the same ratios of the stable calcium content in the Ca^{45}-labeled white clover for the use of the feeding trials and physiological experiments of the animals, it is desirable to use the soil cultured Ca^{45}-labeled clover, although it needs many days for the preparation.

It is supposed that the presumption of the chemical forms of calcium complexes in each fraction used in this experiment have many interests. At first, by the researches on the solubility of the inorganic calcium compounds and low molecular organic calcium compounds mainly organic acids bound calcium with water and diluted acids, the larger parts of the water-soluble inorganic calcium compounds and organic acids complexes should be extracted in the form of calcium ion. The N-NaCl soluble calcium content is associated with the cell-structure of the organs in plant and the osmotic pressure as described above, so that the ladino clover hay No. L 8 was extracted with the various concentrations of NaCl solution following the water extraction and the results of them expressed in Table 6. Only two thirds of the calcium content in the N-NaCl soluble fraction were extracted with 0.1 N to 0.5 N concentrations of NaCl solution, and a half content of calcium in 0.1 N NaCl solution were eluted with under 0.02 N concentration, as same as the values by water extraction repeatedly. Judging from these points of view, the forms of calcium complexes in the N-NaCl soluble fraction may be almost the same one of the water-soluble fraction.

Table 6. Comparison of the solubility of calcium in white clover hay* by the various concentrations of NaCl solution.

Normality of NaCl solution	1	0.5	0.25	0.1	0.02	0.01	0
Solubility of calcium (%)							
Water-soluble Ca	29.0	29.8	29.4	30.1	29.5	28.0	28.7
NaCl soluble Ca	29.6	21.2	21.1	20.0	10.1	10.9	11.0
N-AcOH soluble Ca	17.1	19.2	18.9	19.8	23.9	23.1	23.4
N-HCl soluble Ca	18.8	21.4	23.5	23.6	25.8	25.9	26.9
Insoluble Ca	5.5	6.4	7.1	6.5	10.7	12.1	10.0

* White clover hay No. L 8

To evidence the fractions containing the Ca-proteinate, the total nitrogen, protein nitrogen content and radioactivity in protein nitrogen in each calcium fraction of the Ca^{45}-labeled clover hays was estimated, and the results of them are presented in Table 7. The nitrogen contents of each calcium fraction occupied about one fourth in the water fraction, one half in the insoluble fraction and only one fourth in the other three soluble fractions. The insoluble fraction contains the highest amount of protein nitrogen, followed by the water soluble fraction, the lowest fraction being in the other three. Nevertheless, the percentage of Ca^{45} in protein nitrogen of each fraction to total radioactivity in each fraction is higher in the N-NaCl and N-AcOH soluble fractions than the others, and it is supposed that the main parts of Ca-proteinate may be included in the N-NaCl and N-AcOH soluble fractions.

Table 7. Total N, protein N and radiocalcium in protein N in the solubility of calcium in white clover hay.

Clover hay No.	L 3	WL 7	R 3	R 3
Total nitrogen (%)	3.90	3.85	3.70	
Solubility of calcium	Nitrogen distribution (%)			Ca^{45} content in protein N (%)*
Water-soluble Ca	24.2	27.8	25.3 (8.0)	1.1
N-NaCl soluble Ca	6.9	8.8	5.2 (0.4)	3.2
N-AcOH soluble Ca	2.5	3.5	4.4 (0.5)	5.6
N-HCl soluble Ca	1.9	3.5	3.7 (0.3)	0.4
Insoluble Ca	64.5	56.4	62.4 (40.5)	0.8

The figures in parentheses are the value of protein N.

* Percent of Ca^{45} in protein N to total Ca^{45} in each fraction.

The intercellular substance, middle lamella in the tissues of plant consists largely of a pectic compound which appears to be calcium pectate, and it has been shown by Molisch (13) cited by Bonner (14) that the middle lamella does contain abundant calcium. Hashimoto (15) reported that 2 percent acetic

acid soluble calcium in soybean plant were considered to be combined with pectic substances and to play the mechanical role in the plant body. Therefore, it is presumed that the most effective metabolic calcium compounds must be present in the N-AcOH soluble fraction.

The calcium in the N-HCl soluble fraction mainly consists of calcium oxalate and one part of calcium phosphates, according to the solubility of the calcium compounds.

It may be regarded that the insoluble calcium content shows the comparative high amount with the accumulation of the soluble calcium by the treatment of this experiment, although it has been known that it consists of a very small amount of the insoluble calcium in plant.

In summary, by these results and discussions, the presumed form of the calcium complexes in each fraction by the treatment in this report are presented in Table 8.

Table 8. Calcium complexes presumed in the solubility of calcium in legume forage (white clover hay).

Solubility of calcium	Calcium complexes presumed
Water-soluble Ca	Ca ion, Main part of inorganic Ca compounds, Organic acid bound Ca (except of Ca oxalate)
N-NaCl soluble Ca	Residual part of water soluble Ca, Ca proteinate
N-AcOH soluble Ca	Ca proteinate, Ca pectate, $CaCO_3$, One part of Ca phosphate
N-HCl soluble Ca	Ca oxalate, One part of Ca phosphate
Insoluble Ca	$CaSiO_3$-β, etc.

It appears that there is no stable organic complexes of calcium and or the most parts of calcium present in the state of adsorption in plant, since all of it may be extracted by each solution. There is no reason that if calcium does not form the certain complexes in plant, it is impossible to perform the normal actions needed in plant.

Summary

The legume forages are the highest in calcium content among all farm-grown feeds, and it occupies an important place in the feeding of livestock as the calcium source of the rations too.

On eight legume forages, eight white clover hay and three kinds of the Ca^{45}-labeled white clover hay, the chemical compositions were estimated, particularly calcium content and its forms in the soluble fractions as the preliminary report of the studies on the calcium metabolism and the feeding trials of rabbit, using these legume forages.

The calcium content and the solubility of calcium in leaf and stem of eight legume forages, namely, red clover, mammoth clover, Kenland clover, ladino clover, white clover, alfalfa, hairy vetch, and sweet clover were estimated. The calcium content of the dry matter of leaf are higher than stem in all legume forages. The determination of the solubility of calcium in the legume forages were made according to modifications of Sveshnikov's method, that is, the extraction of calcium with water, N-NaCl, N-AcOH, and N-HCl solution, successively. In general, the water-soluble calcium content of leaf are comparatively higher than that of stem.

The proximate composition, calcium and phosphorus contents, and the solubility of calcium in eight white clover hays are determined. The calcium content of the dry matter consists of 1.2 to 1.6 percent. The solubility of calcium in all white clover hay gave almost the same values.

Three kinds of the Ca^{45}-labeled white clover hay are prepared with the solution and soil culture methods. It could be prepared with the Ca^{45}-labeled clover hay containing the radioactive calcium in the same ratios of the stable calcium content with the soil culture method, although it required many days for the preparation.

It is assumed that the similar forms of the calcium complexes are contained in the water-soluble and N-NaCl soluble fractions of the legume forages. It is supposed that the main parts of Ca-proteinate may be included in the N-NaCl and N-AcOH soluble fractions by the estimation of Ca^{45} in protein nitrogen of each fraction in the Ca^{45}-labeled white clover hay. It appears that there is no stable organic complexes of calcium in plant.

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