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**BIOLOGICAL ASSAYS OF SOIL FERTILITY**

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## BIOLOGICAL ASSAYS OF SOIL FERTILITY<sup>1</sup>

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THE values of soil treatments, such as manure, phosphates, limestone, and other fertilizers, have usually been measured in terms of the increased yield of crops. Whenever the cost of the treatment is less than the sale value of the increased bulk, whether grain or forage, then the soil treatment is usually regarded as an acceptable farm practice. This assumption has been the basis of much of the experimental work with fertilizers and of many recommendations as to fertilizer use. Farmers, however, have been obliged to discount it for risks of weather, pests, crop diseases and prices. Hence with the commonly low and fluctuating monetary values from direct sale of field products, the margin of profit from treatments on many Missouri soils has frequently been small. This has not encouraged the additions of fertility to the soil as extensively as an approach to soil maintenance requires.

There has been only a little upward trend in fertilizer use despite the belief that fertilizer consumption in Missouri, for example, could be much increased with profitable results. In 1940, one and one-third million tons of limestone were used in the state, but even this amount is not sufficient to replace even the annual leaching loss of lime from the soil. With the decline of soil fertility recognized as the main cause of economic distress by only the few soil chemists, there is a serious need to translate soil fertility from a foreign language of chemical formulae and tonnage increase per acre, to one that speaks in terms of better animal growth and greater provision of more nourishing human food as antidotes for dangerous deficiency diseases that are ravaging and deforming both animal and human bodies.

Animal assays, or animal interpretations, of the value of soil treatments might encourage wider use of them for soil fertility maintenance and improvement.

### TREND TOWARD PASTORAL FARMING WITHOUT SOIL TREATMENT INVITES DISASTER

In many instances farmers on thin, eroded soil have not believed that the increased yields from

applications of fertilizers, including lime, particularly with weather and insect hazards, have been sufficient to warrant their use even on cash crops. This says nothing of their use on the pasture and hay crops of which the acre yields remain unmeasured and are not evaluated so commonly in immediate monetary returns. Since there is a rapidly gaining movement toward more livestock or toward the pastoral system of farming, this indifferent attitude toward soil maintenance is distressing. It will lead toward poorer yields, less profit, greater soil depletion and more devastating soil erosion. A continuation of this trend must result in a lowered economic status of farmers. It will make any future amelioration program all the more difficult. Any means, or any program, therefore, that may be used to demonstrate the feasibility of soil treatment in this state at this time is highly desirable.

It has long been known that the chemical composition of any single forage or hay crop may be influenced by the degree of maturity or time of cutting, and by the kind of soil on which it is grown. When soil treatments are used, the flora of a pasture sward may be modified so as to bring in plants of higher feeding value for animals. Research in pasture improvement shows that the concentrations of protein, minerals and carbohydrates in plants are changed by the application of different soil treatments. Such changes indicate that the use of fertilizers on farm crops may have effects beyond recognition as mere increase in weight of crops produced, and are effects that have therefore not been widely recognized. Perhaps, Crampton and Finlayson,<sup>3</sup> more than anyone else, have pointed to the value of bioassays of soil treatments.

From indications and suggestions by chemical studies of the plant behavior through refined control of nutrients offered on colloidal clay growth media, it is believed that there are important benefits from fertility additions to soils that cannot be measured in terms of bulk increase in yield. The hypothesis is ventured that hidden benefits from soil treatments can be demonstrated through assays with smaller animals. For detection of these benefits, experimental

<sup>1</sup>Contribution from the Department of Soils, Missouri Agricultural Experiment Station, Columbia, Mo. Journal Series. No. 788.

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<sup>3</sup>CRAMPTON, E. W. and FINLAYSON, D. A. The effect of fertilization on the nutritive value of pasture grasses. *Emp. Jour. Exp. Agr.*, 3:331-345. 1935.

attention may well go toward some new methods for evaluating soil treatments in terms of their biological assays.

#### BENEFITS OTHER THAN INCREASED YIELDS COME FROM SOIL TREATMENTS

Soil treatments show effects on the chemical composition of numerous crops grown in experimental trials in the field. Lespedeza, for example, has been consistent in its changes in composition as a response to various soil treatments. Table 1, giving the analyses of this crop grown with different soil treatments, is typical of these.

These data show that the soil treatments demonstrated effects other than merely that of increasing the forage yields. When the percentage of protein and the increased forage yield as a result of the soil treatment of lime plus phosphate are considered, it is not beyond the imagination to consider that this type of forage should have some unlisted improvements and a greater feed value per unit of forage weight than would the forage receiving only phosphate. The data point to greater differences in the feed value per acre than would be indicated by a yield increase of only 594 pounds. The increases in delivery of phosphorus and calcium per acre are also items that must not be overlooked. Their significance, however, would seem small in comparison with the protein which gave differences both in concentration and in total yields. If the addition of lime will increase the protein content within the legume forages, as it has in the case of this lespedeza, it is not unreasonable to suppose that organic compounds other than proteins, not commonly determined analytically but yet highly essential in animal growth, may also be favorably influenced.

#### ANIMALS AS AN AID IN DETERMINING EFFECTS OF SOIL ON FORAGE VALUES

##### TRIALS WITH SHEEP, 1939-40

A preliminary experiment with sheep was conducted to ascertain some possible effects of lime on lespedeza as it gives values other than increased tonnage, increased protein, and increased minerals as commonly measured by chemical determinations.

Lespedeza hay was taken from an area which includes numerous phosphates comparably applied to wheat and barley on both limed and unlimed soil and grown to this legume. Before the baled hays were in shelter a heavy rain damaged them severely. The damage was greater to the hay from the limed land, probably because of its higher protein content,

TABLE 1.—Influence of limestone and phosphate on the composition and yields of lespedeza, Columbia, Mo., 1938.

Soil treatment	Yield lbs. per acre	N, %	P, %	Ca, %	Delivery, lbs. per acre		
					Protein	P	Ca
None.....	762	1.79	0.19	0.93	73.9	1.44	7.1
Phosphate.....	800	1.81	0.20	0.98	79.2	1.78	8.8
Phosphate and lime.....	1,394	2.09	0.19	0.94	182.0	2.53	13.2

so that when the better portions were saved the hay from the unlimed soil appeared to be of better quality. Because of much loss of hay, the supply carried the experiment only for 45 days, and was supplemented by other hay from two adjacent farms of similar soil. The analyses of these supplementary hays, as shown in Table 2, agreed well with those of the initial forage.

Experimental sheep were started on feed on September 28, 1939. They were given all the hay they would eat plus a daily supplement of  $\frac{1}{4}$  pound of oats and  $\frac{1}{4}$  pound of wheat bran per head per day. Table 2 gives the analyses of the hay. Table 3 gives the hay consumption, the gains by the lambs, and other details, during the 108-day feeding period from September 28, 1939, to January 13, 1940.

#### NUTRITIVE IMPROVEMENT GREATER THAN CHANGES IN CHEMICAL COMPOSITION AS COMMONLY MEASURED

The performance by the sheep as related to the composition of the hay shows clearly that the effectiveness in producing animal gains by the elements, or compounds, contained in the hay was much greater than was the increase in concentrations in the chemical composition of the forage because of the soil treatment. The analyses of the hay reveal an increase in protein concentration from 10.7% for the phosphated plots to 13.7% for lime addition with

TABLE 2.—Analyses and yields of lespedeza hay from Putnam silt loam with different soil treatments.

Soil treatment and location	Yields lbs. per acre		N, %	Protein, %	Ca, %	P, %
	Hay	Protein				
Phosphate (South Farm)...	2,190	235.4	1.72	10.7	0.866	0.170
Phosphate and lime (South Farm)...	2,652	363.3	2.20	13.7	0.944	0.175
No lime (South Farm)...	—	—	1.38	8.7	0.860	0.150
Lime (Bowling Farm)...	—	—	2.01	12.5	0.996	0.170

TABLE 3.—*Lespedeza* hay consumption as related to gains by lambs, 1939-40.\*

	Hay from untreated or phosphate treated soil	Hay from soil treated with phosphate and lime or lime only
Sheep days† . . . . .	1,382	1,437
Hay offered per pen, lbs. . . . .	3,864	3,987
Waste hay recovered, lbs. . . . .	882	906
Hay consumed, lbs. . . . .	2,982	3,081
Gain per head per day, lbs. . . . .	0.0735	0.1106
Hay consumed per head per day, lbs. . . . .	2.14	2.14
Hay per pound gain, lbs. . . . .	29.2	19.4
Protein per pound of gain, lbs.‡ . . . .	2.13	2.66
Gain per pound calcium, lbs.‡ . . . .	3.95	5.66
Gain per pound phosphorus, lbs.‡ . . . .	20.1	29.5
Gain per acre <i>lespedeza</i> , lbs. . . . .	74.9	136.5

\*Feeding period 108 days, Sept. 29, 1939, to Jan. 13, 1940.

†Number of sheep times the days on feed. Some lambs were removed before the end of the period.

‡When the total gain is calculated as though produced by the hay only and no value given the supplement. Supplement consisted of  $\frac{1}{2}$  pound daily of mixture of equal parts of oats and wheat bran. Lambs weighed 70 pounds each as mean per pen at the outset.

the phosphate. This was an improvement in protein composition of nearly 30%. When combined with better tonnage yield it amounted to 128 pounds more protein per acre.

The gains made by the lambs were fairly uniform per pen throughout the duration of the experiment, but the figures show that those fed the phosphated hay gained 0.073 pound per day, while those fed the phosphated and limed hay gained 0.110 pound per day. This represented an improvement in animal growth of slightly more than 50% from a soil treatment which improved the chemical composition of the hay by only 30%.

Since the two pens of sheep consumed exactly the same amount of hay per head per day, namely, 2.1 pounds, it appears that the sheep were consuming the maximum and that the gain beyond the equivalents in improvement in chemical composition must have been due to some variations in the hays not detected by the common methods of feed analyses. Had no supplement been fed, this difference might even have been greater since the supplement probably covered some of the nutrient deficiencies in the hay grown on soil given phosphate but no limestone. The animal assay revealed values beyond those commonly assigned when measured by chemical assay.

According to the data in Table 3, 29.2 pounds of the hay from the phosphated plots were required for each pound of animal gain. Only 19.4 pounds of the hay from the soil with the treatment of lime and phosphate were necessary for the same gain. From such results it not only appears that the hays differ

in feeding value, but it also suggests forcefully that the nutritive ingredients in the hays are significantly different. If the supplement, which was a constant for both pens, is disregarded, then from the phosphated hay it required 3.13 pounds of protein to produce a pound of gain, while in the hay from the phosphated and limed soil a pound of gain was produced from only 2.66 pounds of protein. This suggests a 17% greater efficiency in the use of the protein in the better hay.

When the calcium in the hay is considered, while that in the supplement is disregarded, then a pound of this element consumed in the phosphated hay produced only 3.95 pounds of animal gain, while the same quantity of calcium in the hay from soil treated with both phosphate and lime produced 5.66 pounds of gain. This greater efficiency in the calcium delivered to the animal amounted to more than 43% when reflected as body gain. With similar consideration of the phosphorus, the hay receiving only phosphate as a soil treatment produced 20.1 pounds of animal gain for each pound of phosphorus supplied in the hay, while the hay from the limed soil produced 29.5 pounds of gain for each pound of phosphorus consumed. Here again is a difference as large as 47%, or a much greater efficiency in the conversion of crude soil fertility nutrients into high-priced, readily salable animal products.

#### ECONOMY PER ACRE WIDELY DIFFERENT

Converting these differences to the farm-acre basis, an acre of the *lespedeza* hay grown on phosphated soil would have produced 74.9 pounds of lamb gain, while an acre of the soil given the fertility addition of both lime and phosphate would have produced 136.5 pounds. This final difference in animal increase per acre is the summation of the significance of soil treatments. Through the simple addition of limestone to phosphate as a soil treatment there was an increase of 21% in the yields of hay, an increase of 30% in the protein content of the forage, an increase of 50% in the animal gain per unit weight of forage fed, a 17% greater efficiency in the use of protein, a 43% greater efficiency in the use of calcium, a 47% greater efficiency in the use of phosphorus, and an 80% greater efficiency in the use of the land as a means of converting a small part of its fertility into marketable products of higher values for human sustenance. Thus, through this long chain of effects, the importance of soil fertility restoration in the form of soil treatments brings itself more nearly to the significance it deserves.

## TRIALS WITH SHEEP 1940-41

Because the hay used in the first attempt at a biological assay of effects by soil treatments was not produced under constant soil conditions throughout the trial, the experiment was repeated using the hays grown on soils of the station experiment plots. Lambs were again obtained from the source used previously and the experiment followed the same plan as that of 1939-40. The hays were harvested and stored in good condition. They were from adjacent ranges and all operations had been carried out at the same time and by identical procedures.

The crop yields and the analyses of the forages are given in Table 4. With them is a summary of the feeding data compiled for 98 days of the experiment.

The yields of hay harvested in 1940 averaged somewhat larger than those in 1939, probably due to heavier summer rainfall. From two phosphate ranges the yield for 1940 was 3,000 pounds per acre; that for the two areas treated with both phosphate and limestone 3,812 pounds per acre. The difference in yield, namely, 27%, was greater than that obtained in 1939 when liming resulted in an increase of 21% in the forage yield.

## ANIMAL INCREASES AS INDICES OF FORAGE EFFICIENCY

The data in Table 4 show the 98-day feeding results obtained in 1940-41. The figures for the gains and the hay consumed are comparable to those obtained in 1939. Both pens consumed about the same amount of hay, namely, 2.0 pounds per head per day as compared to 2.1 pounds for the preceding year. The lambs receiving phosphated hay gained an average of 13.8 pounds per head in this 98-day period, an average of 0.1408 pound per day. Those given hay grown on land receiving both phosphate and lime gained as an average, a total of 16.1 pounds per head during this period, an average of 0.1644 pound per head per day. This difference in rate of gain is more than 16%. Further calculations in a manner similar to those in the first trial with sheep show that the sheep receiving phosphated hay produced a pound of gain for each 14 pounds of hay consumed, while those receiving the limed and phosphated hay produced a pound of gain from only 12.6 pounds of hay. Accordingly, the phosphated hay would produce 210 pounds of animal gain per acre, while that grown on the phosphated and limed land would produce 301 pounds per acre. When considered for hay only, these figures are high because part of the gain comes

TABLE 4.—Hay yields and consumption as related to gains by lambs, September 28, 1940, to January 4, 1941, 98 days.\*

	Hay from phosphate-treated soils (8 lambs)	Hay from soil treated with lime and phosphate (9 lambs)
Yield per acre, lbs. . . . .	3,000	3,812
Nitrogen, % . . . . .	1.95	2.05
Calcium, % . . . . .	1.13	1.31
Phosphorus, % . . . . .	0.226	0.220
Sheep days . . . . .	784	882
Hay offered per pen, lbs. . . . .	3,146.8	3,324.6
Waste recovered, lbs. . . . .	1,568	1,496.7
Hay consumed, lbs. . . . .	1,578.8	1,827.9
Gain per pen, lbs. . . . .	111	145
Gain per head per day, lbs. . . . .	0.1408	0.1644
Hay consumed per head per day, lbs. . . . .	2.013	2.072
Hay per pound gain, lbs. . . . .	14.22	12.6
Protein per pound of gain, lbs. . . . .	1.74	1.61
Gain per pound calcium, lbs. . . . .	6.23	6.06
Gain per pound phosphorus, lbs. . . . .	31.1	36.1
Gain per acre lespedeza, lbs. . . . .	210.9	301.5
Difference in yield of hay, % . . . . .		27.0
Difference in rate of gain, % . . . . .		16.6
Difference in gain per acre, % . . . . .		43.1
Difference in concentration of protein, % . . . . .		4.9

\*Calculations of data in this trial were made in the same manner as for Table 3.

from the supplement fed, but, since the quantity of supplement supplied was constant, the figures are comparable. This is a difference in animal production per acre of more than 43% because of soil treatments as contrasted to differences of only 27% in the tonnage yields of the two hays because of soil treatments.

The following facts stand out prominently regarding soil treatments as they are reflected by animal behaviors: Soil treatments in the field may modify the chemical composition of forages; animal gains made from these different forages reflect differences much greater than are the differences in chemical composition as commonly evaluated, according to methods of feed analyses; the nutritive efficiencies of elements contributed by the soil and of the compounds formed by the plants apparently are much improved by soil treatments; the tonnage yields per acre of forage are not the complete measure of the values to be derived from soil treatments; some other elements, compounds, or complexes in addition to increased mineral content in the forage derived through soil treatments seem responsible for these improvements in animal gain, differences in the calcium and phosphorus contents alone apparently being too small to account directly for the wide differences in animal gain; finally, if soil treatments are to be

measured for their fullest value in better agriculture, their measure in the form of a biological assay with animals seems necessary.

#### LABORATORY ANIMALS AND ASSAY OF SOIL FERTILITY TREATMENTS

As a support for the results obtained from the experiments with sheep, more detailed data were gathered by feeding lespedeza hay from the same source to rabbits. Two pens each containing three young female chinchilla rabbits were fed the hay used for sheep in 1939. The rabbits received all the hay they would eat along with a daily supplement of 20 grams of oats per head per day while they were small and 30 grams later. They were fed in screen-floored pens. Both the feces and the urine were collected to permit a chemical balance of the output against the intake in the ration of hay and oats. Complete records were kept of the hay given and of the waste removed. Tables 5 and 6 give the results of feed consumption and of animal growth for a period of 100 days, extending from March 6 to June 13. The digestibility and other evaluations are given in Table 6.

#### RABBIT GAINS AS INDICES OF DIFFERENCES IN EFFICIENCY OF FORAGE AS FEED

The figures obtained in the experiments with rabbits (Table 5) show results in close agreement with those obtained with sheep. The rabbits fed the hay from limed soil ate only about 3% more than those fed the phosphated hay, but made 38% more gain. The quantity of hay necessary to produce a unit of gain was also in agreement with the results obtained with sheep. While 9.69 grams of phosphated hay were required for each gram of gain, only 7.24 grams of the hay that was phosphated and limed were necessary for the same gain. This is a difference of nearly

TABLE 5.—Hay consumed and rabbit gains during 100 days, March 6 to June 13, 1940, average of three rabbits in each pen.

	Lespedeza hay from soil treated with phosphate (pen 1)	Lespedeza hay from soil treated with phosphate and lime (pen 2)
Hay consumed, grams	22,531	23,297
Hay consumed per head per day, grams	75.1	77.6
Rabbit gain per pen, grams	2,325	3,214
Grams hay per gram gain, grams*	9.69	7.24
Average gain per rabbit per 100 days, grams	775	1,071

\*When the gain is calculated as though produced by the hay only.

TABLE 6.—Digestibility data for lespedeza hays from different soils.

	From soil treated with phosphate	From soil treated with phosphate and lime
Gain per gram of hay, grams*	0.103	0.138
Gain per gram of oats, grams*	0.353	0.487
Gain per gram oats and hay, grams	0.0797	0.1075
Digestibility of dry matter, %	64.8	61.4
Gain per gram nitrogen fed, grams	5.15	5.22
Digestibility of nitrogen, %	55.5	57.6
Gain per gram nitrogen retained, grams	13.4	14.0
Gain per gram calcium fed, grams	10.94	13.07
Digestibility of calcium, %	62.9	56.5
Digestibility of phosphorus, %	40.3	28.8
Calcium voided in urine per gram calcium fed, grams	0.169	0.084
Gain per gram calcium retained, grams	23.3	27.7
Gain per gram phosphorus fed, grams	48.7	49.4
Phosphorus voided in urine per gram phosphorus fed, grams	0.0259	0.0158
Gain per gram phosphorus retained, grams	124	180
Gain per gram feces, grams	0.227	0.279

\*When the entire ration, grain and hay, is considered and the gain divided by the weight of oats, or of hay, consumed.

34%. That all of the gains made by the rabbits should be in such close agreement with those obtained with the sheep suggests the possibility of using these smaller animals as bioassay agents.

The digestibility of the two different hays was determined from the analyses of all materials fed and from the analyses of the urine and feces. Only a small portion of the results from the calculations are included in Table 6, but these figures give additional information regarding the efficiency of the two hays. The hay from soil receiving both phosphate and lime contained a greater quantity of nitrogen, phosphorus, and calcium than the hay grown on soil receiving only phosphate. However, the differences in digestibility and retention of the different elements for which analyses were made were much greater than these analyses indicate.

Despite the greater gains made by the animals on the hay from the limed and phosphated soil, they digested only 61% of the bulk ingested, while the animals fed hay from the soil which received only phosphate digested 65% of the bulk ingested. Similarly, the calcium in the phosphated and limed hay was digested to the extent of 56% and to 63% in the phosphated hay. For the phosphorus in the hays the corresponding figures were 29% and 42% digestible. In spite of the higher digestibility of the

bulk, of the calcium, and of the phosphorus for the hay given only the single soil treatment, the gains per gram of calcium and per gram of phosphorus fed were higher for the hay on soil treated with both lime and phosphate.

The rabbits fed the limed and phosphated hay produced 5.22 grams gain for each gram of nitrogen fed, while the phosphated hay produced 5.15 grams gain for each gram of nitrogen fed. Calculations of the amount of nitrogen fed and voided show that for each gram of nitrogen retained in the animal body, those animals fed the phosphated hay made 13.4 grams of gain, while those receiving hay from the limed and phosphated land used this same unit of protein to build a body gain of 14.0 grams. This may seem to be a small difference, but it points to either a better balanced protein or a more efficient utilization of the protein when offered in combination with other items delivered in the feed.

#### EFFICIENCY OF CALCIUM

When the growth and digestion figures are calculated on the basis of the calcium, the results are more striking with even a higher efficiency than that for protein. Even though the difference in the calcium concentration within the hays was relatively small, yet the difference in gain per unit of calcium fed and retained is large. The pen of rabbits fed the phosphated hay made a gain of 10.9 grams for each gram of calcium supplied and a gain of 23.2 grams for each gram retained in the body, whereas the corresponding figures for the rabbits fed the hay from the phosphated and limed soils show gains of 13.7 grams and 27.1 grams, respectively.

It is significant that the total amount of calcium retained by both pens was almost the same, namely, 53.1% and 52.9%, yet the rabbits on the limed hay made much more rapid gains per unit of calcium retained. This would indicate that neither of these hays was failing to deliver an ample amount of calcium, but the presence of the calcium in the soil influenced the physiology of the plants so that they provided a forage of which the calcium content could be more efficiently utilized by the animals.

#### EFFICIENCY OF PHOSPHORUS

The phosphorus digestion and retention also show striking differences according to soil treatment. For each gram of phosphorus given, the animals fed on the phosphated hay produced 47.7 grams of gain, while on the phosphated and limed hay the gain was 49.4 grams. If the gains are computed on the basis of the phosphate retained in the body, then the phos-

phated hay produced 123 grams of gain, whereas the hay from the phosphate and lime treated plots produced 180 grams gain for each gram retained. This is a difference of over 46%. This is again significant since the phosphorus treatment on both hays was constant. This also indicates that the addition of the limestone produced physiological differences in the plants which had a pronounced effect on the utilization and efficiency of the phosphorus. Although the rabbits which were fed the hay grown on the phosphated soil retained a slightly larger percentage of the total amount of nitrogen, calcium, and phosphorus given them, the gain made per unit of these materials retained in the body was much greater where the plants were grown on soil well supplied with lime.

It is a striking fact that although the rabbits were fed the greater quantities of calcium and phosphorus in the limed hay, yet they excreted less of both of these elements in the urine. This tends further to suggest that when these elements were digested out of the feed, the hay which received only phosphate must have been deficient in something and thus prevented the calcium and phosphorus from being effectively utilized.

Regarding the composition of these hays there is the further fact that the animals fed the phosphated hay produced 0.228 gram of gain for each gram of feces excreted, while on the limed and phosphated hay the corresponding gain was 0.279 gram, an increase of 23%. The animals fed the phosphated hay excreted 23% more waste to make the same amount of gain as did those fed on hay receiving both phosphate and lime.

#### SUMMARY AND DISCUSSION

From chemical analyses and digestion measurements made on sheep and rabbits, it is evident that the increase in tonnage per acre is an insufficient measure of the value of a soil treatment. Lespedeza grown on soil receiving both lime and phosphate produced from 20 to 26% higher yields than where phosphate alone was applied. However, when the same hays were fed to experimental animals the difference in meat as pounds became 60 to 80%. There were no great differences in the concentrations of calcium or phosphorus in these hays, yet in terms of animal nutrition it is forcefully suggested that the presence of the lime must have altered the physiology of the lespedeza plants during growth. This limed hay apparently contained certain substances essential for animal growth and mineral uti-

lization which was not determined by the usual chemical analyses, measuring the mineral elements and protein. Though the increased quantity of protein is in approximate agreement with better animal growth, yet the gains were greater per unit of the protein supplied in the limed hay. This indicates delivery of other substances necessary for animal metabolism by this soil treatment and that analyses for the common feed constituents as now practiced are not sufficient to measure the value of soil treatments. It seems that some type of biological assay is the only means by which these improvements can be measured.

The correlation between the results obtained from sheep and rabbits is so close that it appears possible to eliminate the larger and more expensive animals, and use laboratory animals in the experiments, then interpret the results in equivalents of farm animals.

If a definite correlation between the nutritive behavior of rabbits and larger animals can be established, it will be possible to use many more soil areas under treatment and obtain biological assays with laboratory animals when with sheep or cattle such assay would be impossible, or limited to few expensive and time-consuming trials.

The foregoing report has dealt only with forages grown on soils of different calcium treatment. If calcium can bring about such marked differences, it is logical to believe that other fertility elements which play an important role in the growth of plants might also influence a plant's composition sufficiently to be reflected by the test animals. It is essential that thought be given to the use of this refined measure by means of animal assays for determining the influences by other soil treatments whose effects are not yet detectable in terms of tonnage increases.