TB 1086 (1954) USDA TECHNICAL BULLETINS UPDATA
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MATRONE, G. ET AL 1 OF 1

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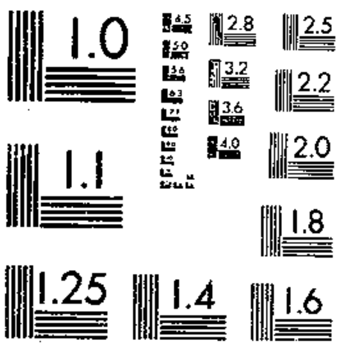

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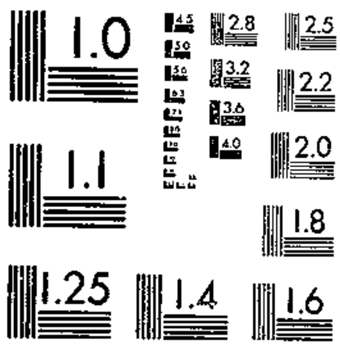

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Effects of Phosphate Fertilization on the Nutritive Value of Soybean Forage for Sheep and Rabbits

By GENNARD MATRONE, FRANK H. SMITH, VIRGINIA B. WELDON, W. W. WOODHOUSE, JR., W. J. PETERSON, and KENNETH C. BEESON

Technical Bulletin No. 1086
May 1954

UNITED STATES DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

In Cooperation With
North Carolina Agricultural Experiment Station and
North Carolina State Department of Agriculture

CONTENTS

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Effects of Phosphate Fertilization on the Nutritive Value of Soybean Forage for Sheep and Rabbits[1]

By Gennard Matrone,[2] Frank H. Smith, Virginia B. Weldon, W. W. Woodhouse, Jr., W. J. Peterson, and Kenneth C. Beeson[3]

INTRODUCTION

In recent years the criteria for evaluating fertility experiments have expanded from primarily a consideration of the yield of a crop to one including an assessment of its nutritive value. The change has been brought about by the thesis that—apart from yield—the nutritional quality of crops, and particularly of specific crops, is influenced by the fertility level of the soil. Thus the nutritive value of a crop is influenced by the various soil-management practices, which can either improve or impair this fertility. Under the stimulus of this broad concept a new line of investigation has opened up that depends on the animal for the burden of proof of nutritive value.

The soil, the plant, and the animal must all be considered together. In investigations of the influence of fertilization on the nutritive value of crops, the ultimate problem becomes one of evaluating a multitude of factors and the variability of their effects in a complex soil-plant-animal relationship. At present, however, many phases of this complex field of investigation are in controversy, because, in some cases, the ideas and objectives of experiments have not been clearly defined and because conclusions, in other cases, have been based on inadequately planned experiments with animals.

One source of confusion peculiar to soil-plant-animal investigations stems from the ambiguous use of the term "nutritive value." The term directly involves the animal. Plant composition as an index of nutritive value is not conclusive. The animal, unlike the plant, cannot subsist on simple elemental substances and needs numerous complex substances that in many cases are ill-defined or in some cases not defined at all, chemically. Moreover, the effects of nutrient

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[1] Submitted for publication August 18, 1953.

[2] Dr. Matrone became a member of the staff of the North Carolina Agricultural Experiment Station in February 1952.

[3] The following members of the staff of the North Carolina Agricultural Experiment Station assisted in the investigation: F. W. Sherwood and J. A. Weybrew gave helpful suggestions on the various phases of the investigation; H. L. Lucas took an active part in the design of experiments and in the statistical analyses of the data, particularly in the early stages of the work; H. M. Baxley made the blood analyses; and W. W. G. Smart, Jr., supplied the rabbit digestion trial data. Also, James L. Rea, Jr., in charge of the Tidewater Experiment Station farm supervised the growing of crops; C. D. Thomas, North Carolina State Department of Agriculture, and E. H. Hostetler, head of the Animal Husbandry Section of North Carolina State College of Agriculture, contributed facilities to the work.

interactions on the physiological processes of the animal are generally unknown. Further, just as the content of a mineral element in the soil is not a measure of its availability to the plant, similarly, the content of a nutrient in the plant is not a measure of its availability to the animal. These factors render uncertain the task of transposing common plant composition values into terms of overall nutritive value. The final proof of nutritional value, therefore, is still provided only by some response of the animal.

A second source of confusion stems from the lack of rigor in differentiating between the quantity of nutrients and the quality of nutrients. A sharp distinction is basic to the problem: it is essential for the formulation of clear experimental objectives and for the interpretation of experimental data. When tested by animal feeding, variations in the levels of soil fertility may give rise to at least three distinct possibilities, each of which involves a different concept.

1. The quantity of all nutrients of a crop is increased for any given soil by an increase in acre yield as the fertility level of the soil increases. When this change is brought about without a concurrent change in the quality of the nutrients (as availability or digestibility), the fertilizer effect is perfectly correlated with yield. In this case, the yield criterion alone is adequate for assessing the fertilizer effects.

2. The content of one or more of the nutrients in a plant varies without change in their quality. The fertilizer effects in this situation may be evaluated by chemical analysis of soil and plants. Moreover, when the nutrients are insufficient in the plants to meet the nutritional requirements of the animal and are derived from the soil, an improvement in soil fertility within the area affected should make a real contribution to the nutrition and health of the animal. For instance, most of the mineral deficiency diseases of grazing animals (cobalt, phosphorus, iodine, and copper) are reported to fall into this category.

3. A change in the nutritional quality of a specific nutrient with or without change in its quantity in the plants may be brought about by varying levels of soil fertility. The nutritional quality of a nutrient may be simply defined as the percentage of a nutrient that is absorbed from the gastrointestinal tract and utilized by the animal. Needless to point out, the nutritional quality of a nutrient or a food is difficult to assess, necessitating carefully designed and controlled animal experiments. Because of these difficulties, a change in the nutritive quality of food crops is more likely to go undetected than a change in quantity. If this exists in fact, the insidious effects of a deterioration in the nutritional quality of our crops on the health and nutrition of animals subsisting on them may be more far-reaching and damaging than the reduction in yield or in content of nutrients in the plant.

A third source of confusion peculiar to pasture and forage investigations is brought about by the change in botanical composition that often accompanies fertilization. Unless the botanical composition is adequately controlled or evaluated in forage-fertilization studies, the experimenter is primarily investigating the relative nutritive value of different mixtures of forage species. Because of the difficulty in interpretation, such investigations contribute little information to the general case and fundamental problem of whether it is possible to affect the intrinsic nutritive value of a specific forage species by varying the level of soil fertility.

REVIEW OF LITERATURE

In reviewing the literature no attempt is made to report on the extensive list of publications in which the effects of fertilization in relation to the nutritive content of crops are presented. Instead, the review is confined primarily to investigations that include an animal evaluation of the experimental treatments. It may be desirable at this point to summarize briefly the review by Browne (*11*),[4] Russell (*42*), and Beeson (*6, 8*) on the various phases of the soil-plant-animal relationship.

Browne (*11*) presents data showing a complex interrelationship between the mineral composition of plants and the type of soil, variety of crop, climate, water supply, fertilizers, and other cultural practices. Of these various factors, climate appears to be dominant. It is made plain, however, that the complexity of the interrelationship makes generalizations difficult.

Beeson (*8*) also concludes that in the light of existing data few generalizations can be made regarding the effect of fertilization of the soil on the nutritive value of plants as measured by mineral composition. He points out that in most plant species utilized for forages the concentration of phosphorus can be increased slightly by use of phosphate fertilizers, particularly when the original phosphorus concentration in the plants is low. In general the calcium concentration can be increased by liming, although the effect can be modified by soil and plant species factors. The effects of other fertilizer elements on the phosphorus and calcium concentrations in forage plants are too variable to permit generalization.

Mineral Deficiencies

Russell (*42*) concludes that mineral deficiencies in pastures are more important than vitamin deficiencies. The generalization is made that if the pasture contains all the inorganic nutrients needed for the health of the grazing animal and is sufficient in quantity, there is unlikely to be a deficiency of either vitamins or protein. This does not necessarily imply that a variation in the level of soil fertility may not affect vitamin content or protein quality, but it does emphasize that the apparent nutritive value of a crop may vary, depending upon the particular animal species used as the test subject. B vitamins and protein quality are reported to be of minor importance in the nutrition of cattle and sheep by virtue of the fact that these nutrients can be synthesized by the rumen bacteria.

The work of Theiler, Green, and DuToit (*52*) is one of the earliest systematic studies of the relation of phosphorus content of the soil to that of the forage grown thereon and to phosphorus deficiency in cattle. A similar relationship was investigated by Eckles, Gullickson, and Palmer (*15*) in their studies of conditions in various sections of Minnesota. More recently, Beeson (*7*) compiled data and mapped areas in the United States in which a number of mineral-deficiency diseases of animals have been recognized. The deficiencies include such elements as phosphorus, cobalt, copper, iron, calcium, and iodine. These investigations (*7*) show that the nutritional disorders reported

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[4] Italic numbers in parentheses refer to Literature Cited, p. 57.

arise mainly from a low concentration of a particular mineral element in the pasture forages. In other words, these deficiencies implicate the concept of quantity rather than the one of availability or quality of nutrients. It should be pointed out, however, that all the essential mineral elements of animal nutrition—with some reservations about sulfur—can be supplied to the animal in the inorganic form. Consequently, whether to apply the deficient mineral to the soil or supply it directly to the animal should be determined by the economics of the situation. It appears reasonable that such elements as calcium and phosphorus, which are essential to the nutrition of the plant, might well be applied to the soil; whereas, it might be more economical to add such elements as cobalt and iodine—which are not known to be limiting for plant growth—directly to the animal ration much as sodium chloride is added.

Effect of Fertilization on the Overall Nutritive Value of Pasture Forage

The overall effect of fertilization in pasture investigations is often measured in terms of weight gains or products of grazing animals (*16, 17, 20, 21*). The interpretation of these data in terms of the change in nutritive value of a particular plant is obscured, because modifying factors either have been disregarded or they have not entered into the objectives of the experiment. One difficulty is concerned with the methods of measuring the treatment effects. For example, Blaser and associates (*9*) reported an experiment in which the relative grazing value of heavily and lightly fertilized Napier grass was compared. The results for heavy and light fertilization, respectively, were (1) Number of animal grazing days per acre, 369 and 231; (2) gains per acre, 219 and 155 pounds; (3) average daily gain per animal, 1.61 and 1.41 pounds. The differences indicated by results (1) and (2) are in the direction one would expect, since the yield per acre was also higher in the heavily fertilized plot than in the lightly fertilized plot.

The third method of measuring the treatment effects is superior to the other two as a measure of nutritional value, because it is possible to make this measurement independently of yield per acre. But it is important to remember that the rate of grazing may profoundly affect the rate of gain (Gard and coworkers, *20*). For instance, if the pasture is overgrazed, the intake of each animal is necessarily restricted. Moreover, if the pasture is limited in yield of forage in one treatment and abundant in the other, the animals in the poor pasture may be forced to graze more of the less nutritious and fibrous parts of the forage plant than the animals on the abundant pasture. For example, Sotola (*51*), in a study with sheep, found that leaf protein of sweetclover hay was 45 percent more digestible than protein of the stems. Thus, the results would not reflect the effect of soil treatment only but rather would reflect that of pasture management.

For the average daily gain per animal to be an effective criterion of change in nutritive value, it is necessary that more than enough pasture be available for the grazing animals and that the experiment be designed with sufficient replications to estimate the true experimental error, which should include the subjective factors in pasture management.

As already indicated, a complicating factor in pasture investigations is the change in botanical composition that accompanies fertilization (*11, 16, 17, 20, 21, 45*). Gard and others (*20*) reported that lime and phosphate fertilizers increased fourfold the total yield from a mixed pasture sod. The yield of bluegrass from this sod was increased by the same fertilizer regime from 250 to 850 pounds per acre over a period of 3 years. The percentage of redtop and lespedeza also was increased in the treated plots. On the other hand, the percentage of weeds was much higher in the untreated plots. When the grazing results were expressed in terms of pasture days per acre and sheep gains per acre, there was more than a twofold advantage in favor of the treated pastures. The difficulties of appraising these results in terms of the yield difference, of the relative nutritive value of different forage mixtures, of the change in nutritional quality of a particular forage plant, or of a combination of these factors are plainly evident. A complete appraisal is necessary, however, if the objective is to evaluate the individual factors involved. A partial solution to the problem could be had by conducting digestion trials and making nutrient-availability determinations on the pasture forages concurrently with the grazing experiment. The data from such experimental methods will give a measure of total available nutrients independent of yield per acre.

As yet, there is little in the literature which shows that fertilization has changed significantly either the quality or availability of recognized nutrients or the quantity and kind of unknown nutritional factors in forages as measured by grazing animals.

Williams and coworkers (*56, 57*) claimed that the phosphorus in low-phosphorus hay is not so available to rats as that in high-phosphorus hay. They attributed their results to the fact that the low-phosphorus hays contained large amounts of substances such as iron and aluminum that might conceivably interfere with phosphorus assimilation by forming insoluble phosphates in the intestinal tract. The samples of hay, however, apparently were obtained from different locations without regard to climate or soil type. Another important consideration is that the work was conducted with the rat, which normally does not utilize roughage efficiently.

Eheart and Pratt (*17*) conducted a series of digestion and balance trials with dairy cows to determine the effect of fertilization of bluegrass pasture on the digestibility and utilization of its constituents, as well as on the quality and quantity of the resulting milk. They found that the grass from the unfertilized pasture contained more materials yielding energy, while the fertilized herbage was richer in digestible protein and minerals. In the interpretation of these results Eheart and Pratt (*17*) emphasized that their study dealt with the digestibility of total herbage produced on fertilized and unfertilized bluegrass sod and not with pure stands of fertilized and unfertilized bluegrass. They reported that although the unfertilized plot was typically a bluegrass pasture, it contained a higher percentage of weeds and undesirable grasses than did the fertilized plot. They concluded that there was no practical difference in the nutritive value of the fertilized and unfertilized herbage, since there were sufficient nutrients present in either to meet the needs of the cows for maintenance and milk production.

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In a study with sheep, Woodman and Evans (*59*) found that the digestibility of the organic matter of an herbage deficient in calcium and phosphorus was unchanged by the addition of adequate quantities of these elements to the ration.

Albrecht and associates (*1*, *2*, *49*) claimed that the nutrients, particularly protein, in plants grown on soils either deficient or unbalanced in plant nutrients are inferior to those contained in well-nourished plants. They stated further that this inferiority is caused both by a smaller content of the nutrient in the plant and, in the case of protein, by a poor quality. It is difficult, however, to find evidence in their published data to support these conclusions. The reader is referred to Beeson's review (*8*) for a critical appraisal of the work of Albrecht and coworkers (*2*, *26*, *32*, *49*).

Few investigations have been made in which the effect of the varying of levels of fertility has been measured in terms of animal reproductivity and longevity. This type of research, especially with large animals, is time-consuming. It is conceivable, however, that such a test might be a more critical measure of nutritive value than any other. Webb and others (*54*) reported in 1948 the results of a 4-year investigation in which they studied the effect of calcium and phosphate fertilizers upon the vegetation and upon the general health and reproduction of sheep subsisting on the vegetation. As a result of the fertilizer treatment, they found changes in botanical composition and yield of forage, but they found no positive evidence of any significant change in the chemical composition of the plants of the same species. The data on animal growth paralleled closely the plant data. The carrying capacity and gains per acre were greater for the fertilized pastures than for the untreated pastures, but no significant differences were found in the bone ash, serum phosphate, or reproductive performance of the experimental animals.

On the other hand, in experiments with small animals, Kendall and associates (*27*) found significant differences in the lactation response of rabbits fed lespedeza and soybean hays from differentially fertilized plots. The complete-fertilizer treatment produced crops with a higher nitrogen content in all cases. In turn, when these crops were compounded into rations, the carcasses of the young that were nourished on the milk of the does fed the rations from plots receiving complete fertilizer were significantly higher in quantity of dry matter, total nitrogen, and ash than the carcasses of the young that subsisted on the milk of the does fed the rations produced with potash and phosphate only. In the lespedeza comparison the protein content of the rations investigated was 10.8 and 13.5 percent for the incomplete fertilizer and the complete fertilizer, respectively, and in the soybean comparison, 12.8 and 15.5 percent. The lower levels of protein may be suboptimum for lactating does; in any case, the possibility cannot be ruled out that the disparity in protein content of the rations was responsible for the difference in nutritive value.

It is apparent that investigators have not obtained enough data covering a wide enough variety of conditions to permit generalizations. The composition of pasture plants may be altered by the application of various fertilizers, but the extent and characterization of the changes vary greatly and are related to soil type, climate, plant species, and cultural practices. The limited large animal data available indicate that the major results of differences in soil fertility may be their effect on yield that appears to be closely related to the pounds of gain or pounds of other animal products produced per unit of land. Such a direct relationship appears to be less probable for nonruminant animals.

Few of the animal experiments reviewed were designed to measure nutritional quality; the answer to this phase of the investigation awaits properly planned animal experiments. The data of a majority of the experiments reviewed were based on too few replications as to within-year replications and between-year replications. This robs the data of meaning even for local application. It is apparent from the foregoing review that to appraise the factors operative in the soil-plant-animal relationship the investigations must include control or measurement of biological variation arising from (1) gradients in soil fertility; (2) differences in forage composition due to the variability in composition of each species as well as variations in the botanical composition; and (3) differences in animal response.

OBJECTIVES AND EXPERIMENTAL METHODS

Scope of Investigation

The overall objective of this study was to determine the effect of applying phosphate fertilizer to a phosphorous-deficient soil on the nutritive value of the crops grown thereon in terms of the concentration and availability of certain recognized nutrients and in terms of unknown factors.

To accomplish this, a long-term experimental plan was designed and initiated in 1945. The work reported here covers a 5-year period that extends into 1949. The data presented were obtained from plant-composition studies, feeding experiments, digestion trials, and nutritional-balance studies with sheep and rabbits.

Field Layout for Hay and Grain Production

The crops were grown on a Bladen silt loam located on the Atlantic Coastal Plain near Plymouth, N. C. Most of the land was cleared of trees and brush for this investigation. None of the land used had had a fertilizer history for at least 25 years. Through soil analysis (table 1) and plant-growth responses, the soil was shown to be deficient in phosphorus and calcium.

TABLE 1.—*Analytical data on Bladen silt loam in 2 of the experimental plots, Plymouth, N. C.*

Field	Available phosphorus	pH	Base exchange capacity	Organic matter	Exchangeable calcium	Exchangeable magnesium	Exchangeable potassium
H-1:	*P. p. m.*		*Meq./100 gm.*	*Percent*	*Meq./100 gm.*	*Meq./100 gm.*	*Meq./100 gm.*
North	0	4.10	29.55	13.44	1.19	0.37	0.158
South	1	4.35	45.37	22.55	4.43	.62	.236
H-2:							
North	0	4.40	30.65	13.62	3.84	.68	.190
South	0	4.55	41.90	20.46	6.58	.60	.338

Soybeans (Ogden variety for all years except 1948, when Roanoke variety was grown) for both hay and seed, and corn (N. C. 27 hybrid) were planted to furnish a complete diet for lambs. The soybean hay was grown on 2 adjacent 7½-acre fields (H–1 and H–2, fig. 1) divided into a total of 12 plots. Six alternate plots received phosphate fertilizations and the other 6 none. Corn and soybeans for seed were grown in another field made up of similar soil, each crop being arranged in a randomized block design with 3 replications per crop.

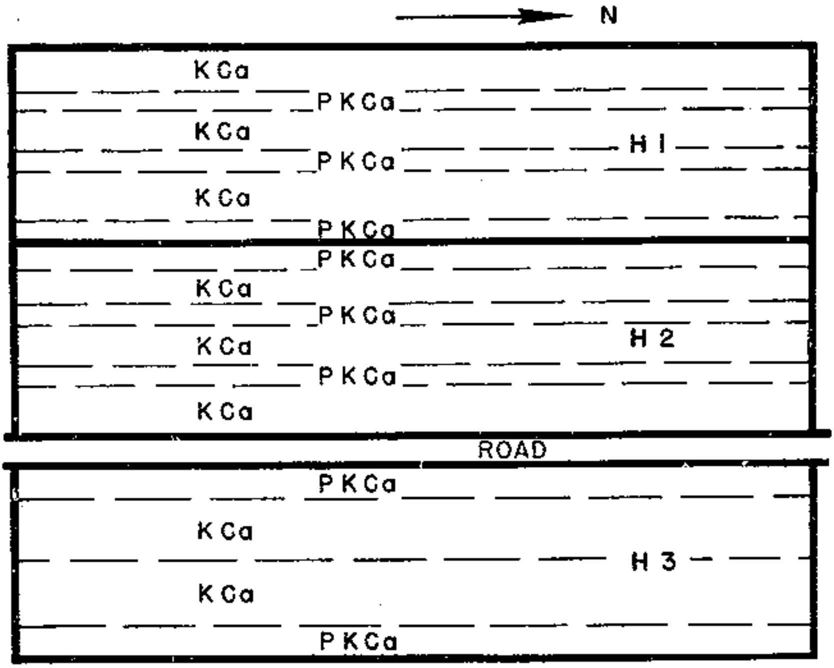

FIGURE 1.—Field plot layout at Tidewater Experiment Station, Plymouth, N. C. PKCa=plots fertilized with 40 pounds P_2O_5 per acre; KCa=plots with no phosphate applications. H–1 and H–2 fields are separated by a ditch; H–2 and H–3 fields by a road. Each field contains approximately 7½ acres.

All plots received 500 pounds limestone and the equivalent of 40 pounds K_2O per acre per year. Half of the plots received, in addition, the equivalent of 40 pounds of P_2O_5 per acre per year. The phosphate was applied in 1945 in the form of 18 percent superphosphate and the potash was applied in the form of KCl. In 1946, 1947, and 1948 the phosphate and potash were applied on the treated plots as 0–12–12 and in 1949 as a 0–14–14 mixture, whereas the potash was applied to the control plots in the form of KCl. The corn was also topdressed with 400 pounds of 16 percent nitrate of soda per acre. Each crop was planted in rows to permit weeding and cultivation.

A preliminary study indicated that a ratio of nonphosphated to phosphated acreage of two to one would be required for equivalent yields. Accordingly, all plots receiving phosphate were one-half the size of the nonphosphated plots.

Throughout this report, the fertilizer treatment applied to the phosphated plots will be referred to by the symbols PKCa and that applied to the nonphosphated plots by the symbols KCa.

Curing and Storing Soybean Hay

The soybean hay was field-cured in 1945 and 1946, and it was barn-cured with a fan-type hay drier in 1947 and 1948. The identity of the hay from the individual plots was maintained in the barn curing by assigning one-half of the drying area in the barn to the KCa hay and the other half to the PKCa hay and by isolating the hay from each plot with a layer of chicken wire.

After the hay was cured it was baled, labeled, and shipped to North Carolina State College for feeding tests.

Methods for Chemical Analyses

Throughout the investigation the methods of analysis of the Association of Official Agricultural Chemists (*4*) were used for the determination of moisture, nitrogen, crude fiber, ether extract, nitrogen-free extract, and ash in the plant material. Calcium was determined by the method of Halverson and Schulz (*22*) and phosphorus by the method of Koenig and Johnson (*29*). The calcium and phosphorus determinations on the leaves and stems of the 1947 soybean crop were made by the methods of Weybrew and associates (*55*) and of Simonsen and coworkers (*47*) as modified by Weybrew and Baxley.[5] Blood serum was analyzed for inorganic phosphorus by the method of Simonsen and others (*47*), for alkaline phosphatase by the method of Bodansky (*10*) modified to utilize the molybdivanadate color (*47*), for calcium by the method of Weybrew and associates (*55*), for magnesium by the method of Simonsen and coworkers (*48*), and for copper by the method of Parks and associates (*38*). The hemoglobin of the blood was determined by the method of Shenk and others (*44*). The soil analyses were determined by methods used in the Soils Laboratory of the North Carolina State College Department of Agronomy.

Sampling of Soybean Hay for Composition Studies

Duplicate field samples for chemical analysis were taken from each field plot. The samples of soybean hay were taken immediately after the soybeans were cut with a mowing machine at harvesting time. A sample was taken by crisscrossing the plot in a diagonal manner from end to end; the duplicate sample was taken in the same manner but in the reverse direction. In traversing the plot, a sample of several plants was taken at random at approximately equally spaced intervals. The samples were dried in a convection air oven at 55° to 65° C.

Statistical Analyses

All the data presented in this report were subjected to statistical analysis. The methods are described by Snedecor (*50*). Statements of significance are based on odds of at least 19 to 1.

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[5] Unpublished data.

COMPOSITION OF THE SOYBEAN PLANT AS AFFECTED BY SOIL FERTILIZATION

The composition of forages from the differentially fertilized plots for the 5-year experimental period are presented in tables 2 and 3. Plants from the phosphate-fertilized soils had a consistently higher concentration of phosphorus and calcium than the control plants throughout the period of investigation. The average concentration of phosphorus in the PKCa plants over the 5-year period was 33.5 percent higher than that of the KCa plants; the average concentration of calcium over a 4-year period was 17.4 percent higher in the PKCa plants than that of the KCa plants. These constituents were positively correlated with the change in yield per acre and plant size (tables 4 and 7).

TABLE 2.—*Effect of phosphate fertilization on composition of the aboveground portion of the soybean plant, 1945–47; moisture-free basis*

Constituents	Fertilizer treatment	Composition [1] of soybean plant in—					Least significant difference [4] at the—	
		1945	1946 [2]	1947	1948 [3]	1949	5-percent level	1-percent level
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Crude protein	PKCa	21.91	19.85	18.89	14.34	18.81		
	KCa	21.06	18.72	15.47	19.21	17.10	1.08	1.45
Ether extract	PKCa			3.15				
	KCa			2.75				
Crude fiber	PKCa	26.13	28.86	35.37	34.11	34.40		
	KCa	27.48	28.09	34.88	31.51	32.84	1.85	2.47
Nitrogen-free extract	PKCa	44.39	35.51	38.35	43.08	38.60		
	KCa	44.98	37.18	40.06	40.99	40.71	1.68	2.24
Ash	PKCa	6.22	9.65	6.13	6.02	6.05		
	KCa	6.18	10.22	6.03	6.61	6.72	.77	1.03
Phosphorus	PKCa	.239	.151	.184	.144	.221		
	KCa	.202	.132	.132	.106	.136	.020	.027
Calcium	PKCa		.810	.834	.880	.886		
	KCa		.697	.725	.744	.739	.093	.125

[1] Each value is an average of samples from 6 plots.
[2] Composition of hay=approximately 90 percent soybeans and 10 percent bull paspalum grass (*Paspalum boscianum*).
[3] Roanoke variety in 1948 and Ogden variety all other years.
[4] For entries in same category.

The yearly response to phosphate fertilization in terms of pounds of P_2O_5 per acre taken up by the soybean plants increased somewhat after the first 2 years, but there was no definite trend indicated thereafter (table 5). The greatest response to phosphate fertilization in terms of uptake of P_2O_5 per acre, phosphorus concentration in the plant, and yield per acre was obtained with the 1949 crop. Apparently, seasonal variations in climatic factors had a marked influence on the response to phosphate fertilization from year to year.

TABLE 3.—*Influence of phosphate fertilization on composition of aboveground portion of the soybean plant, 5-year means, 1945–49; moisture-free basis*

Constituents	Composition of soybean plants with fertilizer treatment—		Least significant difference (PKCa/KCa) at—	
	PKCa	KCa	5-percent level	1-percent level
	Percent	*Percent*	*Percent*	*Percent*
Crude protein	18.76	18.31	0.51	0.68
Crude fiber	31.77	30.96	.85	1.13
Nitrogen-free extract	39.99	40.78	.75	1.00
Ash	6.81	7.15	.35	.46
Phosphorus	.187	.140	.008	.011
Calcium [1]	.852	.726	.046	.062

[1] Average of 4 years.

TABLE 4.—*Effect of phosphate fertilization on the yield of soybean hay,[1] 1945–49; 90 percent dry matter*

Crop year	Yield from—		Response to phosphate fertilization
	PKCa plots	KCa plots	
	Pounds per acre	*Pounds per acre*	*Pounds per acre*
1945	2,000	1,000	1,000
1946	2,000	1,000	1,000
1947	3,300	1,300	2,000
1948	2,800	1,200	1,600
1949	2,400	540	1,860

[1] Approximate yields.

On the average (table 5), 11.2 pounds of P_2O_5 was cropped annually from the PKCa plots, and 3.2 pounds of P_2O_5 per acre was cropped annually from the KCa plots. On the basis of these estimates, the treated plots were accumulating P_2O_5 at the rate of about 29 pounds per year, whereas the control plots were being depleted at the rate of 3 pounds or more per year. Despite the apparent accumulation of P_2O_5, analyses of soil samples taken in the spring of 1950 indicated that the available soil phosphorus in the experimental plots had undergone little change during the period of the investigation. This indicates that the accumulated phosphate reverted in the soil to an unavailable form.

The difference in the crude fiber content of the forages was less marked than was that of phosphorus and calcium. The most marked difference, 2.60 percent (P=0.01), was obtained in 1948. On the average, however, the plants from the PKCa plots were somewhat higher in crude fiber than those from the KCa plots (31.77 and 30.96 percent, respectively, P=0.056).

TABLE 5.—*Uptake of phosphorus by soybean forage in relation to phosphate fertilization, 1945–50*

Year	Phosphorus uptake by soybeans from—		Difference	Ratio PKCa/KCa
	PKCa[1] plots	KCa plots		
	Pounds per acre	*Pounds per acre*	*Pounds per acre*	
1945	10. 03	4. 36	5. 67	2. 30
1946	6. 93	3. 03	3. 90	2. 29
1947	13. 93	4. 00	9. 93	3. 48
1948	9. 25	2. 92	6. 33	3. 17
1949	14. 70	1. 42	13. 28	10. 35
1950	12. 20	3. 48	8. 72	3. 51
Mean, 1945–50	11. 17	3. 20	7. 96	3. 66

[1] Approximately 40 lb. P_2O_5 per acre added yearly to PKCa plots.

Observations made over the same period indicate that the protein content of the whole plant was dependent on an interrelationship involving the fertilizer treatment, the soybean variety, and leaf-to-stem ratio (tables 2, 3, and 7). Thus, in 1945 the difference in protein in the Ogden variety caused by fertilization was 0.85 percent. This difference became significant (at the 5-percent level) in 1946 and was highly significant (at the 1-percent level) in 1947 and 1949. The Roanoke variety planted in 1948 did not respond similarly. Although varietal and climatic differences cannot be ruled out, a factor that may have had a bearing on the results obtained in 1948 is that in the fall of 1947 oats were seeded in the experimental plots and taken off as hay in the spring. Since no nitrogen fertilizer was added, the experimental plots no doubt had been depleted in terms of soil nitrogen. If, in addition, it is assumed that the symbiotic nitrifying bacteria of the soybean legume were unable to make up the nitrogen deficit in 1948, then it can be postulated that the nitrogen content of the large plants from the PKCa plots was diluted.

An inverse relationship between phosphorus and nitrogen concentration of the plant has been reported by various workers (*12, 14, 30, 31, 37*) working with a number of species. Arnon and Hoagland (*3*), however, failed to note any change in nitrogen percentage in tomato leaves or fruit with a change in phosphorus supply in the medium. They attributed this lack of change to the fact that the medium contained a liberal supply of nitrogen. This interpretation is further substantiation of the postulation in the preceding paragraph.

The nitrogen-free-extract content of the plants from the PKCa plots followed a pattern inverse to that of protein (table 2). The differences in protein, phosphorus, and calcium observed in the whole plants also were characteristic of the parts of plants (table 6). The leaf-to-stem ratio changed from year to year, with the greater proportion of leaves in the plants from the KCa plots (table 7).

284131—54——3

Table 6.—*Effect of phosphate fertilization on the composition of leaves and stems of soybean plants, 1947–49; moisture-free basis* [1]

Constituents	Fertilizer treatment	Composition of leaves			L. S. D.[3] at—		Composition of stems			L. S. D.[3] at—	
		1947	1948 [2]	1949	5-percent level	1-percent level	1947	1948 [2]	1949	5-percent level	1-percent level
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Crude protein	PKCa	24.70	24.30	29.46			9.87	7.79	11.04		
	KCa	22.06	26.67	25.59	1.14	1.54	9.48	12.84	9.56	0.614	0.83
Crude fiber	PKCa	17.91	18.97	16.49			48.07	44.82	45.92		
	KCa	18.43	17.84	16.74	.59	.80	46.02	43.13	45.62	1.69	2.29
Nitrogen-free extract.	PKCa	45.33	45.57	40.58			36.04	41.95	37.09		
	KCa	47.72	44.31	44.38	1.86	2.52	37.35	38.23	38.46	2.18	2.95
Ash	PKCa	8.16	8.20	7.44			5.08	4.61	5.22		
	KCa	7.83	8.05	8.14	.52	.70	4.59	4.91	5.58	1.07	1.45
Phosphorus	PKCa	.224	.226	.283			.101	.090	.147		
	KCa	.166	.159	.186	.019	.026	.064	.061	.080	.0097	.013
Calcium	PKCa	1.179	1.302	1.194			.635	.626	.731	.0762	.103
	KCa	.983	.911	.914	.087	.120	.541	.601	.580		

[1] Each value is an average of samples from 6 plots.
[2] Roanoke variety in 1948 and Ogden variety in other years.
[3] For entries in the same category.

TABLE 7.—*Effect of phosphate fertilization on the weight of soybean plants and the proportion of leaves, stems, and pods, 1947–49*

Year	Fertilizer treatment	Plant part [1]			
		Whole	Leaves	Stem	Pods
		Grams	*Percent*	*Percent*	*Percent*
1947	PKCa	18.20	24.5	53.9	21.6
	KCa	7.60	27.1	54.5	18.4
Difference		10.60**	−2.6	−.6	3.2
1948	PKCa	19.11	40.5	59.1	0
	KCa	11.30	45.6	53.9	0
Difference		7.81**	−5.1**	5.2**	0
1949	PKCa	14.65	31.3	54.0	14.7
	KCa	3.34	39.9	49.3	10.8
Difference		11.31**	−8.6**	4.7**	3.9

[1] ** = Significant at the 1-percent level.

The relation of phosphate fertilization to the composition of the soybean plant from year to year was consistent with respect to the minerals—phosphorus and calcium—but was variable with respect to the organic constituents—crude protein and nitrogen-free extract. The increase in phosphorus and calcium concentration of the soybean plant can be related primarily to fertilization. The protein and nitrogen-free extract results present a more complex problem, since these constituents were probably affected by varietal and climatic factors as well as by the fertilizer treatment. For detailed presentation of these data the reader is referred to tables 42 to 57, inclusive, in the Appendix.

NUTRITIVE VALUE OF SOYBEAN HAY AS MEASURED BY ANIMAL ASSAY

1945 and 1946 Crops

The investigations carried out over a 2-year period, 1945–46, consisted of two feeding trials with lambs, each followed by a digestion trial and determination of nitrogen balance. Some of the data published by Matrone and associates (*33*) are discussed here, as they provide continuity to the subsequent data and a basis of experience in the modifications of later experiments.

The original plan called for growing soybean plants for hay, soybean seed for protein supplement, and corn for concentrate to furnish a complete ration for the experimental sheep. However, because of two abnormally wet seasons in 1945 and 1946, the corn crop for both years was a failure. The soybean hay crop for 1945 was badly contaminated with bull paspalum grass, because rains prevented proper cultivation.

More adequate cultivation in 1946 than in 1945 resulted in a much cleaner stand of soybean hay. The contamination in the 1945 crop was in a larger proportion in the KCa plots than in the PKCa plots. To obtain some measure of the effect of the bull paspalum contaminant, a careful botanical analysis of the soybean hay was made and a record of the orts, or feed refusals, was listed in both the feeding trials and the digestion trials for 1945 and 1946 (table 8).

The failure of the corn crops necessitated a change in the experimental ration, namely, the substitution of soybeans as the only concentrate in the animal experiments on the 1945 crops and of soybeans and Cerelose[6] in the animal experiments on the 1946 crops.

TABLE 8.—*Botanical composition,[1] at time of harvest, 1945 and 1946*

Forage species	Fertilizer treatment	Forage composition	
		1945	1946
		Percent	*Percent*
Soybeans	PKCa	58.8	91.3
	KCa	51.3	89.2
Bull paspalum grass	PKCa	41.2	8.7
	KCa	48.7	10.8

[1] Each value is an average of the data from 6 plots.

FEEDING TRIALS, LAMB EXPERIMENTS 1 AND 2

Experimental Methods and Design

In each of the 2 feeding trials, 30 lambs were divided into 6 groups; 3 groups, or pens, were fed the feeds from the PKCa plots and 3 groups, or pens, those from the KCa plots. Each pen was restricted to the hay produced from 2 of the 12 field plots. In this manner each pen received hay raised from different parts of the field. The hay was fed ad libitum in both feeding trials. In the first feeding trial (1945 crop), covering 70 days, the lambs were fed in addition 1 pound of soybeans per head per day. In the second feeding trial, an attempt was made to supplement the hay with a combination of soybeans and Cerelose (corn sugar). After 42 days of the second trial, the combination was discontinued, because the lambs went off feed frequently. For the next 63 days the hay was supplemented with Cerelose only, and these troubles were avoided.

For the control of parasites, the lambs were drenched with Cu-Nic (*13*), a copper sulfate and nicotine solution, at the beginning of each experiment.

Experimental Results

In the first feeding trial (1945 crop) the lambs fed the hay from the KCa plots gained on the average 1.64 pounds per lamb more than those on the hay from the PKCa plots; this difference, however, was not statistically significant (table 9 and Appendix, table 58).

[6] Crystalline glucose, Corn Products Sales Co., Norfolk, Va.

TABLE 9.—*Summary of feeding trials, lamb experiments 1 and 2, on 1945 and 1946 crops*

Items compared	Fertilizer treatment	1945	1946 [1]
Length of trial ... days		70	[2] 63
Pens (5 lambs per pen) ... number	PKCa	3	3
	KCa	3	[3] 3
Raw soybeans eaten per lamb ... pounds	PKCa	70.4	
	KCa	70.4	
Cerelose eaten per lamb ... do	PKCa		46.9
	KCa		46.4
Average hay intake per lamb ... do	PKCa	145.4	108.0
	KCa	146.7	110.3
Average gain per lamb ... do	PKCa	11.1	17.7*
	KCa	12.7	12.1
Standard deviation (*s*) ... do		4.3	3.4
Coefficient of variation (*v*) ... percent		36.0	28.8
s adjusted for hay intake ... pounds		3.53	2.17

[1] * = Significantly different at the 5-percent level.
[2] Period of second feeding trial when lambs received Cerelose only as a supplement to the hay.
[3] One lamb died of pneumonia day before beginning of period.

An analysis of variance of the gains made on the 1946 crop for the full period of the trial (105 days) indicated no significant difference in the hays from the two fertilizer treatments. The average daily gain per lamb was 0.198 pound for the PKCa treatment and 0.183 pound for the KCa treatment. However, for the 63-day period when the lambs received Cerelose only as a supplement to the hay, a significant increase in total gain of 5.6 pounds per lamb was obtained in favor of the lambs on hays from the PKCa plots (table 9). Moreover, the feed utilization was higher for the lambs on the hays from the PKCa plots than for those on the hays from the KCa plots.

Discussion of the Results

Clear-cut evidence that phosphate fertilization affected the nutritive value of soybean hay for lambs was not obtained from the two feeding experiments. The use of raw soybeans as the sole concentrate supplement in the first experiment may have covered up any inherent differences in the differentially fertilized hays, since soybeans are high in minerals, protein, and fat. The results obtained in the second experiment when Cerelose was used as the concentrate supplement suggest that phosphate fertilization increased the nutritive value of soybean hay for lambs. An interpretation of these data must recognize the difficulty in separating the residual effects of the first 42 days of the trial, when the combination of Cerelose and soybeans was fed, from the subsequent period when Cerelose alone was fed. Another criticism that can be leveled at the experiments is that there were orts. When this occurs, it is quite possible that the results do not reflect the effect of soil treatment.

DIGESTION TRIALS, LAMB EXPERIMENTS 1 AND 2

Experimental Methods and Design

In the digestion trial of the 1945 crops the ration fed approximated the feeding trial ration, i. e., hay plus 1 pound of soybean supplement per lamb. Following the feeding trial, 2 lambs were selected at random from each pen, making a total of 12 lambs, for a digestion trial and determination of nitrogen balance. A 7-day preliminary period followed by a 10-day collection period was used in these trials. Instead of compositing the hays from the several field plots within each treatment, a sample of hay from each of the 12 field plots was assigned to a lamb.

The design of the lamb digestion trial on the 1946 soybean hay crop was the same as that on the 1945 crop. In the 1946 digestion trial, however, the hays were processed in a hammer mill to minimize the selection of certain parts of the soybean plant by the lambs. Digestibility was determined for hay alone and for hay plus Cerelose in successive periods with the same lamb. Whether a lamb received hay or hay plus Cerelose in the first collection period was determined randomly. Each animal was fed from the same hay sample during both collection periods. The Cerelose was added at a constant ratio of 3 parts hay to 1 part Cerelose by weight. The digestion coefficients for the hay-plus-Cerelose rations were calculated on the assumption that the Cerelose was 100 percent digested.

Experimental Results, Lamb Experiment 1

An analysis of variance of the data showed a significant difference in the digestibility of the dry matter, crude fiber, and nitrogen-free extract between fertilizer treatments (Appendix, tables 59 and 60). In each case the difference was in favor of feed from the KCa plots. A study of the data from the replicated soil-to-animal experiment led to the view that the differences observed in the digestibility of these constituents might be related to the differences in the botanical composition of the hays from the respective plots. This point was pursued further by making a covariance analysis on the data, using bull paspalum and soybean hay consumed as the independent variables. The treatment means for the digestibility of dry matter, crude fiber, and nitrogen-free extract when adjusted to a common bull paspalum and soybean hay intake were not found to be significantly different between fertilizer treatments.

Since it had been ascertained that the soybean plants were lower in nitrogen-free extract and crude fiber than the bull paspalum plants, these results gave rise to a second question; namely, were the differences noted in the digestibility of the crude fiber and nitrogen-free extract also associated with the crude fiber composition of the rations? Accordingly, covariance analysis was made on each nutrient, using the percentage composition of the nutrient as the independent variable and the digestion coefficient of the nutrient as the dependent variable. The results obtained in this manner confirmed those obtained when the observed digestion coefficients were adjusted to a mean bull paspalum and soybean hay intake. Not only was the difference in the digestibility of crude fiber shown to be associated with the crude fiber content of the rations, but also the size of the experimental error was reduced to one-third (table 10). In other words, by making use of the concomitant measurement, percentage of crude fiber, the precision of the experiment was increased approximately threefold.

These analyses of the data indicated that the differences in the botanical composition of the rations were primarily responsible for the treatment differences observed in the digestibility of dry matter, crude fiber, and nitrogen-free extract.

TABLE 10.—*Comparison of observed crude fiber digestibilities adjusted for crude fiber content of the indicated rations for lambs, 1945*

Item compared	Rations from—		Difference [1]
	PKCa plots	KCa plots	
	Percent	*Percent*	*Percent*
Crude fiber [2]	20. 17	22. 94	−2. 77
Mean digestibility: [3]			
Observed	51. 70	59. 66	−7. 96**
Adjusted	55. 16	56. 20	−1. 04

[1] ** = Significant at the 1-percent level.
[2] Average of the data from 6 rations, each made up of hay from a different plot.
[3] Average of the data from 6 lambs; error variance for observed means=17.37; for adjusted means=5.70.

Experimental Results, Lamb Experiment 2

Of the apparent digestibility of the nutrients under study in experiment 2, only that of protein was significantly different between fertilizer treatments (Appendix, tables 61 and 62). On the average, the lambs on the hays from the PKCa plots digested the crude protein to a greater extent than did the lambs on the hays from the KCa plots. Inasmuch as the average crude protein content of the hays from the PKCa plots was also higher (table 2) than that from the KCa plots, there was a high probability that the two facts were related. This probability is particularly pertinent in that Mitchell (*34*) has shown that the apparent digestibility of protein is related to the level of protein fed.

With a view to testing this supposition, a covariance analysis was made on the data in which the apparent digestibilities of the hays were adjusted to a mean protein percentage. The treatment means are approximately of the same size after adjustment (table 11 and Appendix, table 62). They were not found to be significantly different. As a further check, the true digestibilities of the protein in the four rations were calculated, using the endogenous nitrogen and metabolic nitrogen constants (0.033 gm. per kilo body weight and 0.55 gm. per 100 gm. dry matter intake, respectively) ascertained by Harris and Mitchell (*24*) for sheep. An analysis of the calculated true digestibility values also showed no significant differences in digestibility of the protein among the four rations. This analysis of the data, therefore, indicated that the observed differences in the apparent digestibilities of the rations could be accounted for by the differences in the protein content of the rations.

Having arrived at a satisfactory explanation for the differences in apparent digestibility of protein, the writers still faced the equally pertinent question concerning the quality of protein in the respective hays. Some information on this point was expected from the nitrogen-balance determinations. Notwithstanding the fact that the mean nitrogen-balance values for the rations supplemented with Cerelose are greater than those not supplemented, an analysis of variance of the data indicated no significant differences among the treatment means (table 12 and Appendix, table 63).

TABLE 11.—*Comparison of observed protein digestibilities for lambs with adjusted protein digestibilities, adjusted for protein content, and with calculated true digestibilities of 4 rations, 1946 soybean crop*

Fertilizer treatment	Supplement to soybean hay	Protein, moisture-free basis	Digestibility of protein		
			Apparent, observed	Apparent, adjusted	True [1]
		Percent	*Percent*	*Percent*	*Percent*
PKCa	None	19.85	74.6	71.3	91.97
	Cerelose	15.09	69.3	71.0	92.42
KCa	None	18.60	71.3	69.2	88.14
	Cerelose	14.05	67.7	70.4	92.30

[1] Constants for endogenous and metabolic nitrogen published by Harris and Mitchell (*24*).

TABLE 12.—*Comparison of mean nitrogen-balance values, observed urinary nitrogen excretion, and urinary nitrogen excretion adjusted for nitrogen intake with coefficients of variation for lambs fed rations containing PKCa- and KCa-fertilized hay, 1946 soybean crop*

Item compared	Nitrogen values of—				Response to Cerelose [1]	Coefficient of variation
	Soybean hay from—		Cerelose plus soybean hay from—			
	PKCa plots	KCa plots	PKCa plots	KCa plots		
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*
Nitrogen intake	213	176	202	198	+11	------
Observed urinary nitrogen	145	124	119	106	−44**	11
Adjusted urinary nitrogen	137	138	116	106	−53**	6
Nitrogen balance	12.3	2.7	21.9	28.2	+35.1	265

[1] ** = Significant at 1-percent level.

If it is assumed, on the basis of the data presented in table 11, that the true digestibilities of the protein in these rations are the same, then the urinary nitrogen excretion can be taken as an inverse index of protein quality; e. g., the higher the urinary nitrogen excretion the lower the protein quality (assuming nitrogen intake is the same). With this in mind, the urinary nitrogen excretion values of the lambs were analyzed by means of (1) an analysis of variance and (2) a covariance analysis in which nitrogen excretion was adjusted to the mean nitrogen intake. Both analyses indicate that the lambs on the Cerelose-supplemented rations excreted a smaller amount of nitrogen than they did when on the nonsupplemented rations (table 12 and Appendix, table 64). A biological explanation of these facts may be that because of the submaintenance energy content of the hay rations the animals used part of the hay protein for energy purposes rather than for the repair and growth of body tissue. On the other hand, inasmuch as the animals during the period they were fed hay plus Cerelose received approximately the same amount of hay as when they were on hay alone, their energy intake was higher. Less of the hay protein therefore was used for energy purposes and more of the nitrogen was retained by the lambs during the period of Cerelose supplementation.

The nitrogen-balance data support the interpretations made from the urinary-nitrogen-excretion data. A partial explanation, at least, of why the foregoing effects were not found to be significant in the nitrogen-balance data is found in the large coefficient of variation associated with the nitrogen-balance determinations (table 12).

No evidence was found in either the urinary-nitrogen-excretion data or the nitrogen-balance values that phosphate fertilization of the soil affected the protein quality of the hay (table 12).

Additional information in the 1946 digestion trial data may have a bearing on the 1946 feeding trial results. At the end of the second collection period, blood was collected from the experimental lambs and determinations were made for serum inorganic phosphate. The serum phosphorus level of the group of lambs on the ration consisting of hay from the KCa plots plus Cerelose was significantly lower than that of the lambs in the other three groups (table 13). These same animals also had the highest excretion of ash in the feces, or the lowest net absorption of ash. Phosphorus-absorption measurements followed more or less the same pattern as those of ash, but the difference between treatments was not significant because of the great variation between lambs on the same treatment (Appendix, table 65). Furthermore, the percentage of phosphorus in the rations fed to the animals in this group was the lowest of the four diets (table 14). The low levels of inorganic phosphorus in the blood serum of the lambs on the KCa-hay ration supplemented with Cerelose, therefore, may have been a consequence of the phosphorus content and the availability of the phosphorus in the ration.

TABLE 13.—*Average inorganic phosphorus in the blood serum of lambs at the end of the second collection period of the 1946 digestion trial*

Fertilizer treatment	Phosphorus per 100 milliliters of serum when soybean hay was fed—	
	Without Cerelose	With Cerelose [1]
	Milligrams	*Milligrams*
PKCa plots	[2] 7.43	[3] 7.08
KCa plots	[3] 8.05	[2] 5.85**

[1] **=Interaction between phosphate fertilization and Cerelose supplement significant at the 1-percent level.
[2] 4 lambs in experiment.
[3] 2 lambs in experiment.

TABLE 14.—*Average* [1] *percentage of net absorption of ash, phosphorus, and calcium by lambs and the average phosphorus content of the rations employed, 1946; moisture-free basis*

Fertilizer treatment	Supplement to soybean hay	Phosphorus content of hay	Net absorption of—		
			Phosphorus	Ash	Calcium
		Percent	*Percent*	*Percent*	*Percent*
PKCa	None	0.165	7.7	42.7	32.2
	Cerelose	.111	14.3	41.8	24.1
KCa	None	.142	6.1	41.1	25.6
	Cerelose [2]	.095	3.3	35.7	18.9

[1] Each value is an average of data from 6 lambs.
[2] The probability of a significant interaction between phosphate fertilization and Cerelose supplement was $P=0.056$ for ash, and P at the 5-percent level for calcium.

Discussion of the Results

An analysis of the data from the replicated experiment conducted on the 1945 crop led to the view that the differences observed in the digestibility of dry matter, crude fiber, and nitrogen-free extract could be explained by the differences in the botanical composition of the respective hays. This interpretation was substantiated by the data from the digestion trial conducted on the soybean crop grown in 1946. In this crop, which was a much cleaner stand of soybean hay, no significant difference was found in either the bull paspalum content or in the digestibility of the dry matter, crude fiber, and nitrogen-free extract between the hays from the differentially fertilized plots. A higher apparent digestibility of the protein in the hays from the PKCa plots than in the KCa plots was found, but this was

284131—54——4

shown to be related to the difference in percentage of protein in the respective hays.

A study of the data from the 1946 digestion trial suggested the possibility that the phosphorus in the rations consisting of hay from the KCa plots plus Cerelose was less available than the phosphorus in the comparable rations containing hay from the PKCa plots. Inasmuch as the KCa hay-plus-Cerelose rations had also the lowest phosphorus concentration, it is possible that the superiority of the hay from the PKCa plots in the 1946 feeding trial, during the period when Cerelose was substituted as the supplement in place of raw soybeans, was primarily a reflection of a higher level of phosphorus nutrition. This hypothesis is strengthened by the fact that the animals on the hay from the KCa plots plus Cerelose had the lowest level of serum inorganic phosphorus.

1947 Crop

In several respects the soybean hay grown in 1947 was superior to the previous two crops as experimental material. Many of the difficulties encountered in growing the previous crops were circumvented, partly by means of improved techniques and partly by a more favorable growing season with less rainfall. Through frequent machine cultivation and hand weeding, the stand was maintained at approximately a 99-percent pure soybean forage. In contrast to the field method of curing the hay employed with the previous two crops, the 1947 crop was cured with a barn drier, resulting in a uniformly green, leafy hay of excellent quality. Moreover, the crop was harvested at a stage when the beans were just beginning to form in the pod in contrast to the well-formed beans in the previous years. The earlier stage of maturity was chosen to avoid the additional complicating variable encountered by the presence of beans in the pods.

As was shown in table 2, the soybean plants from the PKCa plots in 1947 were higher in protein, phosphorus, and calcium and lower in nitrogen-free extract than the plants from the KCa plots. An estimate of the changes brought about by barn curing of the soybean hay was made by comparing the composition of the barn-cured hay (table 15) with the composition of the soybean plants sampled at the time of harvest (table 2). The only marked differences in composition were in the crude fiber, which was higher in the barn-cured hay, and the nitrogen-free extract and ether extract, which were lower in the barn-cured hay. These differences were found for hays from both treatments. The changes in nitrogen-free extract and crude fiber might be expected since some loss in the sugars and other easily fermentable carbohydrates would occur during curing. On the other hand, since the ether extract of hays consists largely of pigments, the lower content in the barn-cured hay probably reflects losses from oxidation of plant pigments.

ANIMAL STUDIES

The animal experiments on the 1947 hay crop include two feeding trials with lambs, two feeding trials with rabbits, and a digestion and balance trial with rabbits. Lamb experiment 3 and 4 closely parallel rabbit experiments 1 and 2, respectively, both in terms of diets employed and experimental objectives. Part of the results of these experiments were reported by Matrone.[7]

TABLE 15.—*Composition of soybean forage after barn curing, 1947 crop*[1]

Constituent	Composition of soybean plants with fertilizer treatment		Difference[2]
	PKCa	KCa	PKCa-KCa
	Percent	*Percent*	*Percent*
Crude protein	17.37	15.47	+1.90**
Ether extract	1.86	1.91	−.05
Crude fiber	40.57	38.80	+1.77
Nitrogen-free extract	33.34	36.41	−3.07**
Ash	6.86	7.41	−.55
Calcium	.875	.814	+.61*
Phosphorus	.188	.134	+.054**

[1] Analyses made on cured hay as fed to lambs.
[2] *= Significant at the 5-percent level; **= significant at the 1-percent level.

FEEDING TRIALS, LAMB EXPERIMENT 3, FIRST PHASE

In most cases, the magnitude of the significant differences in composition noted in table 15 was small in a nutritional sense. The experimental sequence adopted, therefore, was (1) to determine if any significant differences in nutritive value would be manifested in growth trials, and (2) to follow up positive results with additional experiments designed to ascertain the causative factors.

At the beginning of each lamb experiment, the lambs were drenched with Cu-Nic (*13*) and were given free access to a salt mixture made up of 12 pounds of common salt to 1 pound of phenothiazine throughout the period of each experiment.

The lambs were fed ad libitum in individual pens. Fresh water in metal pails was kept before each animal at all times. Daily records of feed intake and biweekly records of body weight were made on each lamb. A mixture of pine shavings and sawdust was used for bedding.

The primary objective of this experiment was to determine the effect of phosphate fertilization on the nutritive value of the soybean plant as measured by lamb growth.

Preparation of the Rations

The hay was chopped in a hammer mill equipped with a ⅜-inch screen. Usually 12 bales (or a multiple thereof), one from each field plot, were processed at one time. A representative sample of chopped hay from each bale was retained and composited for each plot over the period of the experiment for chemical analysis. The processed hay from each plot was mixed with each of 2 supplements—corn grown under a fertilizer regime similar to the hay and Cerelose plus

[7] MATRONE, G. EFFECT OF PHOSPHATE FERTILIZATION ON THE NUTRITIVE VALUE OF THE SOYBEAN PLANT. 1950. [Unpublished doctor's thesis. Copy on file Library, N. C. State Col. Agr. and Engin., Raleigh.]

Jewel oil [8]—in the proportions indicated in table 16. The rations were prepared once a week.

TABLE 16.—*Rations fed in feeding trials, lamb experiment 3 and rabbit experiment 1, 1947 soybean crop*

Fertilizer treatment [1] of soybean hay	Ration ingredients (parts by weight)			
	Hay	Corn	Cerelose	Fat [2]
PKCa	100	100	0	0
KCa	100	100	0	0
PKCa	100	0	25	5
KCa	100	0	25	5

[1] All corn plots were also topdressed with 400 lb. of 16 percent nitrate of soda.
[2] Jewel oil (refined cottonseed oil, Swift & Co., Chicago, Ill.) in lamb experiment, or Spry (hydrogenated vegetable oil, Lever Bros., New York, N. Y.) in rabbit experiment.

Experimental Design

Twenty-three purebred Hampshire lambs obtained from two different sources were used in the feeding trial. Twelve lambs were obtained from the Tidewater Experiment Station, North Carolina; 6 of these lambs were offspring from dams subsisting solely on pastures and feed from phosphate-fertilized plots, and 6 were offspring from dams subsisting solely on pastures and crops from nonphosphated plots. These 2 groups of lambs were continued on the hays produced by the same fertilizer treatments during the feeding trial. Because the Tidewater farm was able to supply only 12 lambs, an additional 12 were obtained from the college flock near Raleigh, N. C. Inasmuch as the lambs were dropped over a period of several weeks, it was not possible to start all the lambs on the experiment at one time; therefore, the lambs were taken from their dams and started on the experiment as soon as they had attained at least 50 pounds in weight.

In the allotment of the lambs to the treatments, a balance was made on source of lambs and on sex across fertilizer treatments and supplements (Appendix, table 66). The average initial weight of the lambs on rations from the PKCa treatment was 52.7 pounds as compared to 52.8 pounds for the lambs on rations from the KCa treatment. The lambs were assigned at random to pens. They were fed as much as they would consume twice daily for an average experimental period of about 16 weeks. During the course of the experiment, the lambs were sheared in the latter part of June because of the warm weather. The experiment was terminated on September 10, 1948.

Experimental Results

Two lambs on the KCa-Cerelose ration died; one a few days before the end of the experiment and the other a week after. A wether lamb on the PKCa-corn ration died in a very emaciated condition halfway through the experiment, but this animal did not "go on feed," from the time it was placed on experiment until its death.

[8] Jewel oil, refined cottonseed oil, Swift and Co., Chicago, Ill.

TABLE 17.—*Summary of gains and feed intakes of lambs, feeding trial, lamb experiment 3, first phase, 1947 soybean hay and corn crop*

Fertilizer treatment for soybeans	Concentrate supplement [1]	Lambs	Length of trial	Average daily intake per lamb	Average daily gain [2] per lamb	Total gain [2] per lamb
		Number	*Days*	*Pounds*	*Pounds*	*Pounds*
PKCa	Corn	[3] 5	114	2. 013	0. 245	27. 6
KCa	do	6	113	1. 928	. 251	28. 2
Difference				. 085	—. 006	—. 6
PKCa	Cerelose	[3] 5	115	1. 474	. 109	12. 8
KCa	do	[4] 5	114	1. 234	—. 026	—18. 7
Difference				. 238	. 135*	31. 5*

[1] See table 16 for formulas of rations.
[2] Corn means significantly different from Cerelose means at the 1-percent level; *=significant at the 5-percent level.
[3] 1 lamb died before completion of experiment.
[4] Only 5 lambs were placed on experiment.

The gains and feed intake data are summarized in table 17 and in Appendix, table 67. An analysis of variance of the data showed that in the comparison in which the experimental hays were supplemented with Cerelose plus fat, the lambs on the PKCa rations made a significantly greater gain than the lambs on the KCa rations (table 17). In the comparison in which the experimental hays were supplemented with corn no significant difference between fertilizer treatments was obtained.

TABLE 18.—*Mean composition of rations [1] in feeding trials for rabbit experiment 1 and lamb experiment 3, 1947 crop*

Nutrient	Fertilizer treatment of soybeans	Hay plus supplement of—		Standard deviation of a mean
		Corn	Cerelose [2]	
		Percent	*Percent*	*Percent*
Crude protein	PKCa	11. 75	11. 47	
	KCa	11. 37	10. 65	0. 13
Ether extract	PKCa	2. 91	5. 11	
	KCa	3. 06	5. 17	. 10
Crude fiber	PKCa	18. 92	27. 70	
	KCa	18. 49	26. 70	. 38
Nitrogen-free extract	PKCa	54. 79	41. 84	
	KCa	55. 58	44. 29	. 34
Ash	PKCa	3. 66	4. 65	
	KCa	4. 02	5. 10	. 13
Calcium	PKCa	. 408	. 594	
	KCa	. 422	. 560	. 017
Phosphorus	PKCa	. 199	. 129	
	KCa	. 164	. 089	. 0024

[1] Rations made up of hay from 6 PKCa plots and 6 KCa plots.
[2] 100 gm. of ration contained 19.23 gm. of Cerelose and 3.85 gm. of added fat.

A probable explanation of these results (table 17) was sought in concomitant measurements. The composition of the rations (table 18) showed that the phosphorus content of the hay-plus-corn rations was adequate in terms of lamb requirements (*39*), but in the hay-plus-Cerelose rations the phosphorus content was marginal (0.129 percent (*35*)) for the lambs on the PKCa hay-plus-Cerelose rations and inadequate (0.089 percent P) for the lambs on the KCa hay-plus-Cerelose rations. These data suggested that the level of phosphorus nutrition was involved.

TABLE 19.—*Summary of analysis of blood from lambs on feeding trial, lamb experiment 3, first phase, 1947 soybean crop*

Blood constituent	Fertilizer treatment of soybeans	Hay with supplement [1] of—	
		Corn	Cerelose [2]
Hemoglobin____grams per 100 ml. blood__	PKCa________	11. 76	11. 15
	KCa_________	11. 14	9. 38
Cell volume____________________percent__	PKCa________	35. 30	33. 10
	KCa_________	32. 48	24. 38*
Inorganic phosphorus milligrams per 100 ml. of serum__	PKCa________	6. 59	5. 87
	KCa_________	5. 80	4. 59*
Phosphatase_____units per 100 ml. serum__	PKCa________	12. 05	10. 36
	KCa_________	12. 25	8. 16*
Calcium____milligrams per 100 ml. serum__	PKCa________	12. 44	13. 30
	KCa_________	11. 87	12. 18
Magnesium milligrams per 100 ml. serum__	PKCa________	3. 40	2. 88
	KCa_________	3. 55	[3] 2. 73
Copper_____________γ per 100 ml. serum__	PKCa________	71	65
	KCa_________	70	69

[1] Averages of analysis of blood samples taken from lambs at end of trial.
[2] KCa mean with Cerelose supplement is significantly different from other means; *= significant at the 5-percent level.
[3] Serum magnesium of lambs on Cerelose supplement is significantly different from that of lambs on corn supplement at the 5-percent level.

A chemical examination of the blood of lambs at the end of the trial gave corroborating evidence (table 19). An analysis of the blood data showed that hemoglobin, percentage of cell volume, serum inorganic phosphorus, serum calcium, serum magnesium, and serum copper of the lambs on the comparable hay-plus-corn ration were not significantly different among lambs, whereas the blood of the lambs on the hay-plus-Cerelose rations from the PKCa treatment were significantly higher in serum inorganic phosphorus, serum phosphatase, and percentage of cell volume than the blood of the lambs on the KCa hay-plus-Cerelose ration.

An interpretation of the differences in the phosphatase values of the blood is not clearly apparent. Although it is known that a higher serum phosphatase value is usually associated with rickets (*46*), it also has been observed that under normal conditions higher phosphatase values are also associated with the young rapidly growing animal (*25*). It is possible, therefore, that the higher phosphatase values in this experiment are a reflection of a superior rate of bone growth.

Another difference observed was that the serum magnesium level of the lambs on the corn supplement rations was higher (3.48 mg./100 ml. serum) than the serum magnesium level of the lambs on the Cerelose-supplemented rations (2.81 mg./100 ml. serum). Since both levels of serum magnesium are within the normal range, it is difficult to explain this difference. Perhaps the high Cerelose concentration of the diets depressed the gastric secretion of hydrochloric acid; whereas, corn might favor acid formation that might assist in magnesium solution and adsorption in the intestinal tract. The glucose (Cerelose) would be absorbed quickly; but the delayed absorption of the available carbohydrates of corn might favor acid fermentation in the intestinal tract. Indirect evidence that supports this interpretation is given by Rogoziński (*41*). He observed that the feces of sheep contained more intact chlorophyll when fed hay alone than when the hay was supplemented with corn. Rogoziński suggested that the available carbohydrate of corn favored the formation of acid, which degrades the chlorophyll molecule by removal of its magnesium. Another possibility is that the supplemental fat in the hay-plus-Cerelose ration formed insoluble magnesium soaps in the intestinal tract of the animal.

FEEDING TRIALS, LAMB EXPERIMENT 3, SECOND PHASE

Modification of First Phase Plan

Since the aforementioned data indicated that the differences observed in the lamb gains were influenced by the phosphorus content of the hays from the respective fertilizer treatments, the point was tested further. At the end of the 16-week feeding trial 8 lambs—4 on the PKCa hay-plus-Cerelose rations and 4 on the KCa hay-plus-Cerelose rations—were continued on experiment with dietary modifications for 64 days. The rations of half of the lambs on each treatment were supplemented with dicalcium phosphate and the other half of each group of lambs was continued as controls. Sufficient dicalcium phosphate was added to the dietary phosphorus-supplemented rations to raise the total phosphorus content to 0.25 percent. The dicalcium phosphate was mixed into the rations.

Experimental Results

The lambs on the dicalcium phosphate supplement gained approximately 3 times as much and consumed more ration than the lambs without dicalcium phosphate supplement, regardless of fertilizer treatment (table 20; Appendix, table 68). This indicated that the phosphorus levels were suboptimum in the unsupplemented rations.

During the course of the experiment, hemoglobin, percentage of cell volume, serum inorganic phosphorus, serum calcium, serum magnesium, and serum copper analyses of the blood of the lambs were made periodically. No significant change was observed in any of these constituents except serum phosphorus (table 21). An analysis of the data established that the level of this constituent in the blood serum of the lambs was significantly higher on the dietary phosphorus supplement than on the controls (Appendix, table 69). These data are in accord with the lamb gains (table 20).

TABLE 20.—*Summary of average daily gains and feed intakes and total gains of lambs, feeding trial, lamb experiment 3, second phase, dicalcium phosphate supplement, 64 days' duration, 1947 crop*

Fertilizer treatment of soybeans	$CaHPO_4$ supplement	Lambs	Daily feed intakes per lamb [1]	Daily gain per lamb	Total average gains [2]
		Number	*Pounds*	*Pounds*	*Pounds*
PKCa	+ [3]	2	2.284	0.204	13.0
KCa	+	2	2.101	.219	14.0
Difference			.183	−.015	−1.0
PKCa	0	2	1.745	.086	5.0
KCa	0	2	1.864	.063	4.0
Difference			−.119	.023	1.0

[1] Rations made up of hay plus Cerelose.
[2] Effect of $CaHPO_4$ supplement was significant at the 1-percent level.
[3] Total P content of rations adjusted to 0.25 percent.

TABLE 21.—*Summary of data on inorganic phosphorus of serum from lambs in feeding trial, lamb experiment 3, second phase, 1947 soybean crop*

Fertilizer treatment of soybeans	$CaHPO_4$ supplement	Lambs	Final inorganic P of serum [1]	Linear regression coefficient of serum inorganic phosphorus on time [2]
		Number	*Milligrams per 100 ml. serum*	*Milligrams per 100 ml. serum*
PKCa	+	2	7.35	+0.24
KCa	+	2	6.42	+.66
Difference			.93	
PKCa	0	2	5.88	+.12
KCa	0	2	4.70	−.39
Difference			1.19	

[1] Effect of $CaHPO_4$ supplement is significant at 5-percent level.
[2] KCa plots with $CaHPO_4$ supplement significantly different from KCa plots without supplement at 1-percent level.

An analysis of the trends indicated that during the course of the experiment there was a highly significant linear increase in the level of serum inorganic phosphorus in the blood of the lambs on the KCa rations supplemented with dietary phosphorus; whereas, there was a significant decrease indicated in the blood serum of the lambs on similar rations without dietary phosphorus. These data suggested that there was a deterioration in the phosphorus status of the lambs on the KCa rations as measured by inorganic phosphorus of the serum. In the comparison of PKCa rations with and without dietary phosphorus supplement, the trend of inorganic serum phosphorus of the lambs without supplement was slightly positive and lower than that of the lambs with supplement, indicating that the phosphorus status of the lambs fed PKCa rations did not undergo a pronounced change during the experiment.

FEEDING TRIALS, LAMB EXPERIMENT 4

The foregoing experiments directly associated the phosphorus concentrations of the experimental hays with the differences observed in their nutritive value. The next step was to determine if there were differences in nutritive value arising from factors other than phosphorus concentration. The composition data presented previously indicated that the differences in protein content of the hays might be one such factor. Another possibility was that the protein of the hays grown with and without phosphate fertilization also differed in quality. Moreover, it was conceivable that any response to protein might well be affected by the level of phosphorus in the rations. Accordingly, a fourth lamb feeding trial was undertaken in the spring of 1948 to study these factors.

Preparation of the Rations

The rations were prepared in a manner similar to those in experiment 3; however, the chopped hays from the PKCa plots were composited as were also the hays from the KCa plots. This was done because it was necessary to reduce the number of variables for the more detailed study.

Urea was used as a supplement in place of protein, because it is known that sheep can utilize this material as a substitute for part of their protein needs (*23*) and because a preformed protein might mask the quality of protein in the hays. Dupont urea (equivalent to 263 percent protein), containing 0.0345 percent phosphorus and 0.655 percent calcium, was used in the experiment. Because of the possible effects of differential supplements of urea on palatability, even though the PKCa hay rations were originally higher in protein, an equal amount of urea (approximately 0.96 lb.), which is equivalent to 2.5 percent protein per 100 pounds of ration, was added across the fertilization comparisons (table 22).

Dicalcium phosphate, containing 19.99 percent phosphorus and 25.01 percent calcium, supplied by the Tennessee Valley Authority, was used as the source of supplemental phosphorus. Enough dicalcium phosphate was added to the four rations receiving phosphorus supplement to raise the total phosphorus content of each to approximately 0.18 percent, a level that meets the recommended allowance for lambs (*30*).

The components of the eight rations are summarized in table 22. The basal rations were based on the hay-plus-Cerelose rations of the previous experiment. The basal ration made up of KCa hay contained 10.43 percent protein and 0.086 percent phosphorus; whereas, the basal ration made up of PKCa hay contained 11.64 percent protein and 0.129 phosphorus.

TABLE 22.—*Component parts and protein and phosphorus content of rations used in lamb experiment 4, 1947 soybean crop*

Fertilizer treatment of soybeans	Ration symbol	Parts by weight of ration ingredients					Protein [2]	Phosphorus [2]
		Hay	Cerelose	Fat [1]	Urea	$CaHPO_4$		
							Percent	*Percent*
KCa	(1)	100	25.00	5	0	0	10.43	0.086
PKCa	f	100	25.00	5	0	0	11.64	.129
KCa	p	100	24.40	5	0	.62	10.43	.180
PKCa	fp	100	24.68	5	0	.32	11.64	.180
KCa	u	100	23.75	5	1.25	0	13.22	.086
PKCa	fu	100	23.75	5	1.25	0	14.42	.129
KCa	up	100	23.15	5	1.25	.60	13.22	.180
PKCa	fup	100	23.43	5	1.25	.32	14.42	.180

[1] Wesson oil, cottonseed oil distributed by Wesson Oil & Snowdrift Sales Co., New Orleans, La.
[2] Protein and phosphorus values calculated from composition of ingredients, air-dry basis.

Experimental Design

A factorial experiment was designed to study the effects of phosphate fertilization, supplements of urea, supplements of dicalcium phosphate, and their interactions on the nutritive value of the rations. The three factors were employed at a zero level and at a plus level in all possible combinations, making a total of eight rations in a 2 by 2 by 2 factorial experiment.

Experimental Methods

Eighteen grade Hampshire lambs, nine wethers and nine ewes, were purchased from a farm in Wenona, N. C. The lambs were selected from a flock of approximately 35 on the basis of uniformity in appearance and weight. The lambs were allowed to adjust themselves to the environment of the experimental barn in Raleigh for a week. At the end of this period, 16 of the 18 lambs were selected and placed on experiment for 18 weeks.

The experiment was made up of 2 replications, one of wether lambs and the other of ewe lambs (table 70). The 8 lambs within each replication were divided, on the basis of weight, into 4 pairs that were randomized across the fertilizer comparisons and into individual pens. The initial weight range of the 16 lambs was 48 to 61 pounds with a mean weight of 55.5 pounds.

The lambs were fed individually as much as they would consume in 30 to 40 minutes twice daily. Because of the hot weather, the lambs were sheared twice—in the third week and in the third month of the experiments.

Experimental Results

The dicalcium phosphate supplement stimulated the feed intakes and gains of the lambs on the KCa-hay ration, but it did not significantly affect the gains or the intakes of the lambs on the PKCa-hay rations (table 23; Appendix, table 71). In contrast, the overall effect (table 23) of the urea supplement was to depress the intake and gains of the lambs on the PKCa-hay rations, but it did not depress the feed intakes or the gains of the lambs on the KCa-hay rations.

In the comparisons among the PKCa-hay rations, urea with and without dicalcium phosphate present had a depressing effect on gain; dicalcium phosphate alone had no effect.

In the comparisons among the KCa-hay rations, urea without dicalcium phosphate appeared to have a depressing effect; whereas, urea and dicalcium phosphate together appeared to exert an additive effect, giving the highest gains. It might be postulated from these data that phosphorus was the first limiting factor in the KCa-hay ration and that urea nitrogen was not utilized unless the phosphorus deficiency was corrected.

TABLE 23.—*Influence of phosphate fertilizer, dietary urea, and $CaHPO_4$ on the gains and feed intakes of lambs, 18 weeks' duration, lamb experiment 4, 1947 soybean hay crop*

Supplement	Total intake and gain per lamb [1] on soybean hay from—				Response to phosphate fertilization	
	PKCa plots		KCa plots			
	Intake	Gain	Intake	Gain	Intake	Gain
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
None	277.3	28.4	248.1	19.2	29.2	+9.2
Urea	237.9	19.5	244.2	14.3	-6.3	+5.2
$CaHPO_4$	281.9	27.7	266.2	24.2	+15.7	+3.5
Urea plus $CaHPO_4$	254.4	20.8	278.6	28.9	24.3	-8.1
Influence of urea: [2]						
No urea	280.5	28.1	257.2	21.7		
With urea	246.2	20.1	261.5	21.6		
Response	-34.3	-8.0*	+4.3	-0.1		
Influence of $CaHPO_4$: [2]						
No $CaHPO_4$	257.6	23.9	246.2	16.8		
With $CaHPO_4$	268.2	24.3	272.5	26.6		
Response	+10.6	+.4	+26.3	+9.8*		

[1] Each value is the mean of 2 lambs.
[2] Each value is the mean of 4 lambs. * = Significant at the 5-percent level.

In this experiment blood samples of the experimental lambs were also taken periodically and analyzed for hemoglobin, serum calcium, serum magnesium, and serum inorganic phosphorus (Appendix, table 72). As is shown in table 24, in the comparisons without $CaHPO_4$ supplement, the blood serum of the lambs on the PKCa-hay rations contained a significantly higher level of inorganic phosphorus than did the blood serum of the lambs on the KCa-hay rations. Dicalcium phosphate supplement increased significantly the serum inorganic phosphorus of the lambs on the KCa hays, but it did not appreciably increase the serum inorganic phosphorus of the lambs on the PKCa-hay rations. On the other hand, the level of this constituent in the blood of lambs receiving supplements of dicalcium phosphate decreased at a slower rate than it did in the blood of lambs receiving no supplement, regardless of the fertilizer treatment.

The data of this experiment further confirmed the observations that the limiting factor in the hay from the KCa plots was primarily phosphorus. There was also some indication that the protein level in the rations containing the hay was suboptimal and that this deficiency was corrected with a supplement of urea nitrogen.

TABLE 24.—*Summary of data on inorganic phosphorus in the blood serum of lambs, lamb experiment 4, 1947 soybean crop*

Supplement	Inorganic phosphorus per 100 ml. of blood serum from lambs [1] fed soybean hay from—				Response to phosphate fertilization [3]	
	PKCa plots		KCa plots			
	Average final values	Linear regression [2]	Average final values	Linear regression [2]	Average final values	Linear regression [2]
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
No $CaHPO_4$	5.90	−0.232	4.74	−0.425	+1.16*	+0.193*
With $CaHPO_4$	6.60	−.052	6.96	−.106	−.36	+.054
Response to $CaHPO_4$ [3]	+.70	+.180*	+2.22**	+.319		

[1] Each value is the mean of 4 lambs.
[2] Linear regression of serum phosphorus on time.
[3] *=Significant at the 5-percent level; **=significant at the 1-percent level.

FEEDING AND DIGESTION TRIALS, RABBIT EXPERIMENT 1

Restrictions on the number of animals that may be utilized in large-animal research place limitations on the size and type of experiment that can be conducted. Because of these considerations, studies with rabbits were initiated with the 1947 crops. Chronologically, rabbit experiments 1 and 2 preceded lamb experiments 3 and 4, respectively. The experimental procedures were first carried out with the rabbits, and the experience gained thereby was utilized in the subsequent lamb experiments.

Rabbits of the Dutch breed from the colony maintained by the Nutrition Laboratory at North Carolina State College were used in this trial and all other rabbit experiments. Weanling rabbits within a day or two of the same age were used within experiments. A constant supply of water and rock salt was provided for each rabbit experiment.

The objective of the experiment was to evaluate the relative nutritive value of the crops under study as measured by rabbit growth. The basal diets were similar to those employed in lamb feeding trial 3 (table 16).

Experimental Methods and Design

The identity of each field replication of the soybean hays was kept intact in the rabbit experiment. The experiment was a randomized block design with 6 replications, 2 sources of supplements (corn and Cerelose), 2 fertilizer treatments, and 2 animals per experimental unit, making a total of 48 rabbits. The 2 animals that made up an experimental unit were placed in individual cages adjacent to each other on the battery. The 48 rabbits were divided into 6 outcome groups on the basis of weight, and the outcome groups were assigned at random to the 6 field replications of hay. The 4 diets within a replication were assigned to the experimental animal units, and the rabbits of an outcome group were assigned to positions within a battery replication at random.

The rabbits were placed on experiment at an average age of 7 weeks and were continued on experiment for a period of 10 weeks. During the early part of the experiment, three of the experimental rabbits died of what was suspected to be coccidiosis.

At the end of the feeding trial half of the rabbits (one from each experimental unit) were heart-punctured for blood samples. The other 24 were placed on a digestion-and-balance experiment to make an evaluation of the utilization of specific nutrients in the test rations, giving particular attention to the protein and phosphorus. The digestion trial was designed to have a 6-day preliminary period and a 5-day collection period. The urine was also collected in order to determine nitrogen, phosphorus, and calcium balances. The feed intakes of the rabbits during the digestion trials were equalized within replications.[9]

Feeding and Care of Animals

The rations, which were made up approximately once a week, were placed in 4-quart friction-top cans labeled with the number of the cage, the animal, and the diet. In this manner there was one can to correspond to each rabbit. The animals were fed once or twice daily (feed was kept before them at all times) from their allotted can. The difference in the weight of the ration can between the beginning of the week and the end of the week was taken as the measure of feed eaten during this period. Although precautions were taken to prevent the scattering of the feed by the rabbits, there was some loss.

Considerable difficulty was experienced in getting the rabbits on feed during the first 2 weeks, primarily because the soybean hays that were chopped into ½-inch pieces were coarse and did not mix thoroughly with the supplements. From the beginning of the third week to the end of the experiment, however, the chopped hays were processed further in a Wiley mill and the difficulties were overcome.

Inasmuch as the hays had not been sun cured, 2 drops of calciferol[10] per week was administered orally to each animal throughout the experiment. The rabbits were weighed at weekly intervals.

[9] SMART, W. W. G., JR. DIGESTION INVESTIGATIONS WITH RABBITS. 1949. [Unpublished master's thesis. Copy on file Library, N. C. State Col. Agr. and Engin., Raleigh.]

[10] Drisdol, brand of crystalline vitamin D_2 (calciferol) from ergosterol prepared by Winthrop-Stearns, Inc.; commercial product was diluted with Wesson oil to contain approximately 62 units per drop.

Experimental Results of Feeding Trials

In the corn-versus-Cerelose comparisons, the rabbits on the corn supplement consumed less and gained more than the animals on the Cerelose supplement (table 25; Appendix, tables 73 and 74). A comparison of the results of the fertilizer treatments shows that the rabbits on the PKCa-hay diets consumed and gained more than the rabbits on the KCa-hay diets, irrespective of the kind of supplement. These results were different from those obtained with lambs. Significant differences in feed consumption and gains of these animals were obtained only between the hays from the differentially fertilized plots supplemented with Cerelose (table 17).

TABLE 25.—*Average feed intake and gain per rabbit in a 10-week feeding trial, rabbit experiment 1, 1947 soybean crop*

Supplement to soybean hay	Total intake and gain per rabbit [1] on soybean hay from—				Response to phosphate fertilization [2]	
	PKCa plots		KCa plots			
	Intake	Gain	Intake	Gain	Intake	Gain
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Corn	2,900 (12)	590	2,589 (11)	479	+311	+111*
Cerelose	3,725 (10)	528	3,435 (12)	434	+290	+94*
Difference, corn-Cerelose [3]	−825	+62**	−846	+45**	----	---

[1] Numbers in parentheses = number of rabbits.
[2] * = Significant at the 5-percent level.
[3] ** = Significant at the 1-percent level.

TABLE 26.—*Regression coefficients of grams gain on grams feed intake and grams of feed per gram gain for diets of rabbits, feeding trial, rabbit experiment 1, 1947 soybean crop*

Fertilizer treatment	Supplement to soybean hay	Feed per 1 gram of gain	Regression coefficients of individual diets [1]
		Gm.	*Gm. gain per gm. of feed*
PKCa	Corn	4.9	0.303
KCa	do	5.4	.254
Difference		−.5	.049
PKCa	Cerelose	7.1	.147
KCa	do	7.9	.101
Difference		−.8	.046

[1] Corn regressions significantly different from Cerelose regressions at 1-percent level.

Since a record was made of the feed intake for each rabbit throughout the experiment, it was possible to evaluate the data further by running a covariance analysis (*50*) on feed intake and gain. This analysis showed that, irrespective of fertilizer treatment, the animals on the corn rations made a significantly greater gain per gram of food than did the rabbits on the Cerelose rations (table 26). Since the between-supplement regressions (table 26) were significantly different, a separate covariance analysis on the corn and on the Cerelose rations was made. When the gains were adjusted to a mean feed intake in this manner, the adjusted treatment means for the fertilization comparison within supplements were not significantly different in either case. From these results, it might be postulated that there was no great difference in the utilization of feed by rabbits fed the experimental hays.

As the blood obtained at the end of the feeding trial was limited in quantity, it was analyzed only for hemoglobin, serum inorganic phosphorus, and serum magnesium. Unlike the observations on the lambs, no significant correlation was found between these constituents and gains (table 27); nor was there a difference in serum magnesium in the blood of the rabbits on the corn rations as compared with those on the Cerelose rations.

TABLE 27.—*Mean concentrations of blood constituents*[1] *at end of feeding trial, rabbit experiment 1, 1947 soybean crop*

Fertilizer treatment	Supplement to soybean hay	Constituents in 100 ml. of blood		
		Hemoglobin [2]	Serum	
			Inorganic phosphorus	Magnesium
		Gm.	*Mg.*	*Mg.*
PKCa	Corn	9.32 (6)	10.00	4.73
KCa	do	8.37 (6)	9.16	4.21
PKCa	Cerelose	8.09 (4)	9.44	4.52
KCa	do	7.76 (5)	8.84	4.45

[1] Differences between means for any constituent were not significant at the 5-percent level.
[2] Numbers in parentheses designate number of animals.

Experimental Results of Digestion Trials

In the digestion phase of the rabbit study, explanations were sought for the differences observed in the nutritive value of the soybean hay. The level of phosphorus nutrition was not so exclusively involved in the weight gains of the rabbits as in the weight gains of the lambs. Differences in nutritive value were obtained in the rabbit experiment irrespective of the kind of supplement fed. The phosphorus content of the KCa hay-corn diets was 0.164±0.002 percent, a level almost adequate for lambs (*39*). The lack of a correlation between blood serum inorganic phosphorus and gains in the rabbit experiment strengthened this point of view. Nevertheless, the level and availability of phosphorus in the experimental diets could not be eliminated as factors on the basis of the feeding trial results. It was also possible that the level and utilization of the protein in the test hays were involved. Detailed data on the composition of the diet and nitrogen, phosphorus, and calcium balances are presented in the Appendix, tables 75, 76, 77, and 78.

The difference in gain per gram of feed (table 26) between the rabbits on the corn rations and those on the Cerelose rations may be explained on the basis of the total digestible nutrients of the respective diets (table 28). The corn diets contained an average total digestible nutrient content of 61 percent in contrast to the Cerelose diets, which contained 55 percent. The digestibility of dry matter followed a similar pattern (table 76). There was no significant difference in the total digestible nutrient content of corn rations in relation to the fertilizer treatments, but the Cerelose-supplemented rations with PKCa soybean hay contained more total digestible nutrients than those with the KCa hay (table 28).

The total digestibility and retention of nutrients in the corn and Cerelose-supplemented soybean hays produced under different fertilizer treatments differed significantly only with respect to their protein and phosphorus components (tables 29 and 30; and Appendix, tables 75 and 76).

TABLE 28.—*Total digestible nutrient content of ration, digestion trial, rabbit experiment 1, 1947 soybean crop*

Fertilizer treatment	Supplement to soybean hay	Average [1] total digestible nutrient content of each ration [2]
		Percent
PKCa	Corn	61.09
KCa	do	59.96
Difference		1.13
PKCa	Cerelose	56.13
KCa	do	53.13
Difference		3.00**

[1] Each value is an average of data obtained from 6 rabbits.
[2] Corn means significantly different from Cerelose means; **= significant at the 1-percent level.

The apparent digestibility of protein was higher in the PKCa-fertilized hay rations and also higher in the Cerelose rations in which corn and Cerelose were compared (table 29). Since the diets did not contain the same protein percentage, the protein composition and digestibility data were subjected to a covariance analysis, as described on page 17. The analysis of these data showed that the means of the apparent digestibilities of protein retention adjusted for the percentage of protein of the diets were not significantly different for the diets containing feed produced under different fertilizer treatments; but that in the corn-Cerelose ration comparison the adjusted treatment means were significantly different. This treatment of the data, therefore, gave no evidence that quality of the protein was superior in the PKCa rations; on the other hand, it indicated that the protein quality of the hay-plus-corn diets was inferior to the protein in the hay-plus-Cerelose diets for rabbits, irrespective of fertilizer treatment. The nitrogen-balance data substantiated these results (table 29; Appendix, table 77).

TABLE 29.—*Summary of nitrogen-balance data and apparent digestibility of protein, digestion trial, rabbit experiment 1, 1947 soybean crop*

Fertilizer treatment	Supplement to soybean hay	Protein [1]	Digestibility of protein [1]	Nitrogen		
				Intake	Balance [1]	Urine
		Percent	*Percent*	*Grams*	*Grams*	*Grams*
PKCa	Corn	12.25	65.96	5.413	1.388	2.154
KCa	do	10.98	61.42	4.862	1.227	1.821
Difference		1.27**	4.54**	.551	.161	.333
PKCa	Cerelose	12.65	71.54	6.431	2.102	2.490
KCa	do	11.12	66.68	5.569	2.090	1.637
Difference		1.53**	4.86**	.862	.012	.853

[1] Corn means significantly different from Cerelose means; **= significant at the 1-percent level.

TABLE 30.—*Summary of phosphorus-balance data from digestion trial, rabbit experiment 1, 1947 soybean crop*

Fertilizer treatment	Supplement to soybean hay	Phosphorus content of diet [1]	Phosphorus		Linear regression of phosphorus balance on phosphorus intake
			Intake	Balance [1]	
		Percent	*Grams*	*Grams*	*Grams*
PKCa	Corn	0.203	0.5707	0.4217	1.1890
KCa	do	.154	.4245	.2460	.9254
Difference		.049**	.1462	.1757*	.2636
PKCa	Cerelose	.129	.4171	.2688	1.0920
KCa	do	.084	.2677	.1731	1.0422
Difference		.045**	.1494	.0957*	.0498

[1] *= Significant at 5-percent level; **= significant at 1-percent level.

The nitrogen retained by the rabbits on the PKCa rations was not significantly different from that retained by the rabbits on the KCa rations. The animals on the corn-supplement rations, however, setained less nitrogen than the animals on the Cerelose-supplement rations.

284131—54——6

The phosphorus balance on every rabbit fed all four rations was positive. The animals on the PKCa-hay rations took in and retained more phosphorus than did the animals on the KCa-hay rations (table 30). Likewise, the rabbits on the corn-supplement rations took in and retained more phosphorus than did the rabbits on the Cerelose-supplement rations. From these results it is apparent that phosphate fertilization brought about an increase in the phosphorus concentration of the soybean hay and that the rabbits on the PKCa-hay rations retained more phosphorus than did the rabbits on the KCa-hay rations. Although this seems to be clearly brought out, there remains the question of whether the greater retention of phosphorus by the rabbits on the PKCa hay was a result alone of the larger intake, or, in addition, of a difference in availability of the phosphorus in the PKCa hays. To test the hypothesis implicit in this question, a covariance analysis of the data was made, and the mean phosphorus retention of each ration was adjusted to a mean phosphorus intake. The adjusted ration means were not found to be significantly different. Moreover, it was found that the rate of phosphorus retention per unit of phosphorus intake (see linear regression coefficients, table 30 and Appendix, table 78) within diets were much the same for all four rations.

These analyses of the data indicated that the greater retention of phosphorus by the rabbits on the PKCa rations as compared with the rabbits on the KCa rations was caused primarily by differences in phosphorus (feed) intake rather than to differences in the availability of the phosphorus.

Discussion of Results

Insofar as the rabbit feeding trial was concerned, the differences in gains observed between the PKCa-hay rations and the KCa-hay rations could be explained on the basis of the differences in food intake of the rabbits. This view was particularly tenable, as the gains per unit of intake of feed for the pair of diets within each of the two supplements were so similar (table 26). A choice of one of two hypotheses with which to explain the feeding trial results was possible: (1) Either the difference in feed intake resulted from a difference in palatability, or (2) the difference in feed intake resulted from a difference in one or more nutrient factors. The second hypothesis was investigated first. The results from the digestion trial indicated the desirability of starting with the protein and phosphorus nutrients. Accordingly, a second rabbit feeding trial was designed with these considerations in mind.

FEEDING TRIALS, RABBIT EXPERIMENT 2

The objective of the second rabbit feeding trial was to determine whether either protein or phosphorus, or both, were the nutritional factors responsible for the differences observed in the feed intakes and gains in the rabbit experiment 1. Accordingly, the second feeding trial was designed to study the effect of adding protein and phosphorus supplements to the hays from differentially fertilized plots. Protein supplementation and phosphorus supplementation were studied at a zero level, a plus level, and in combination across the two hays, making a total of eight diets. In addition to the two levels of protein employed in the foregoing diet combinations, three more levels of protein supplements as outlined below were used in the experiments with the two hays under study, thus, increasing the total number of diets to fourteen.

Since rabbits are unable to utilize dietary urea nitrogen to an appreciable extent, a preformed protein was employed. In formulating the rations, it was realized that an equalization of the protein content of the two hays with a preformed protein might mask the quality of the protein in the hays. It was decided, therefore, to employ five levels of protein supplements, starting with a very low level and building up to a high level, well beyond the protein requirement of the growing rabbit, which was estimated to be 15 percent of the air-dried ration (*58*). The hypothesis behind this, assuming the protein in the hay from the KCa plots to be the limiting factor, was that at some level of protein supplementation the growth curve of the rabbits on this hay would either approach or coincide with the growth curve of the rabbits on the PKCa hay. On the other hand, if protein were not the factor, the two growth curves would not approach each other at any level of protein supplementation.

Preparation of Rations

In this experiment the hays from the replicated field plots were combined within fertilizer treatments just as was done in the factorial lamb experiment 4.

The hays from the 1947 crop were ground and the rations were mixed in much the same manner as in the first rabbit experiment. The basal diets consisted of hay, Cerelose, and fat, as before, except that 7 parts (by weight) of Wesson oil[11] was used in this experiment instead of 5 parts of Spry (see table 16). The fat content of the basal diet was increased to widen the nutritive ratio, which in turn might put a further stress on the limiting factor of the diet for the rabbits.

Labco Vitamin Test Casein was used as the source of supplemental protein. The protein content of the diets studied ranged from 10.27 to 23.58 percent. In the diets of the animals receiving a mineral phosphorus supplement, the phosphorus content was adjusted to 0.5 percent with dicalcium phosphate (c. p. anhydrous). The components of the 14 diets are summarized in table 31.

Experimental Design

The experiment was carried out in a randomized block design with 5 replications. Two replications were made up of female rabbits and 3 of males, a total of 70 rabbits. Replications 1 (males) and 2 (females) were started November 1, 1948. Replications 3 (females) and 4 (males) were begun February 22, 1949, and replication 5 (males) was begun October 26, 1949. The experimental period for each replication was 8 weeks.

The 14 diets were classified into 7 ration groups to correspond to the 7 treatments applied across the 2 differentially phosphate-fertilized hays (table 31).

[11] Refined cottonseed oil, Wesson Oil and Snowdrift Sales Co., New Orleans, La.

TABLE 31.—*Component parts of diets used in feeding trial, rabbit experiment 2, 1947 soybean crop*

Diet group and symbol	Soybean fertilization	Ration ingredients						
		Hay	Cerelose	Wesson oil	Casein	$CaHPO_4$	Protein[1]	P[1]
		Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*
1	PKCa	100	25.00	7	0	0	11.46	0.127
	KCa	100	25.00	7	0	0	10.27	.085
2m	PKCa	100	22.83	7	0	2.17	11.46	.500
	KCa	100	22.59	7	0	2.41	10.27	.500
$3mc_1$	PKCa	100	20.84	7	2	2.16	12.98	.500
	KCa	100	20.60	7	2	2.40	11.79	.500
$4mc_2$	PKCa	100	18.85	7	4	2.15	14.49	.500
	KCa	100	18.60	7	4	2.40	13.31	.500
$5mc_3$	PKCa	100	14.86	7	8	2.14	17.52	.500
	KCa	100	14.61	7	8	2.39	16.33	.500
$6mc_4$	PKCa	100	6.89	7	16	2.11	23.58	.500
	KCa	100	6.84	7	16	2.36	22.39	.500
$7c_4$	PKCa	100	9.00	7	16	0	23.58	.238
	KCa	100	9.00	7	16	0	22.39	.195

[1] Protein and phosphorus values (air-dry basis) calculated from composition of ingredients.

The animals within a replication were grouped according to initial weight into seven pairs of rabbits. The rabbit pairs were assigned at random to the ration groups, and the rabbits within a pair were assigned at random to the two hays within a ration group. The rabbit pairs and rations were then assigned at random to the cages.

Experimental Results

The overall mean response to rations produced under phosphate fertilization was found to be highly significant at the 1-percent level. In this experiment, moreover, the rabbits on the PKCa-hay rations gained more and consumed somewhat less than the rabbits on the KCa-hay rations (table 32; Appendix, table 79). The premise, therefore, made in experiment 1 that the differences in feed intake were brought about by nutritional factors was substantiated; furthermore, the two nutritional factors implicated were protein and phosphorus.

The choice of casein as the protein supplement was unfortunate insofar as an evaluation of the response to phosphorus supplementation was concerned. Since casein is a phosphoprotein, the phosphorus and casein effects are confounded in this experiment. A comparison of the effect of phosphorus supplementation in diet groups 1 and 2 (table 32) is valid, however, since no casein supplement was employed in these diets—but the animals are too few for adequate evaluation. Hence, it is best to consider the significant effects caused by the combined use of casein and phosphorus supplementation.

TABLE 32.—*Effect of adding casein and $CaHPO_4$ supplements to soybean hay rations on mean feed intake and gains per rabbit, 8-week trial, 1947 soybean crop*

Diet[1] group and symbol	Supplement to hay ration		Total intake and gain per rabbit on soybean hay from—				Difference, PKCa−KCa	
			PKCa plots		KCa plots			
	$CaHPO_4$[1]	Casein[1]	Intake	Gain	Intake	Gain	Intake	Gain[2]
			Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
1	0	0	3, 790	471	4, 050	495	−860	−24
2m	+	0	4, 244	537	3, 960	431	+284	+106
3mc₁	+	2	4, 277	517	4, 321	517	−44	0
4mc₂	+	4	4, 076	627	4, 005	524	+71	+103**
5mc₃	+	8	4, 445	688	4, 363	539	+82	+149
6mc₄	+	16	4, 234	621	4, 255	535	−21	+86
7c₄	0	16	4, 047	662	4, 332	569	−280	+93
Means			4, 159	589	4, 184	516	−25	73**

[1] See table 31 for diet components.
[2] **= Significant at the 1-percent level.

A further breakdown of the data showed that the beneficial effect of casein (in presence of phosphorus supplementation) was most evident in the rations from PKCa hays. It was found, for example, that with increments in casein supplement for the rabbits on the PKCa-hay rations, there was an accompanying increment in gain. A similar trend was not so evident for the KCa-hay rations. This relation was further tested by determining the linear effect in the two sets of data (diet groups 1 and 2 were averaged for 0 increment of casein and diet groups 6 and 7 were averaged for the highest casein increment within each set of data). The results of this analysis showed a highly significant linear trend at the 1-percent level in the casein-supplemented PKCa series, but a nonsignificant linear trend in the KCa series (fig. 2).

The data on rabbit feeding trial 2 (table 32) demonstrated that there was a difference in nutritive value between the PKCa hay and the KCa hay beyond that caused by differences in protein, phosphorus, or feed intake.

1948 Crop

Although significant progress had been made in experiments with rabbits in ascertaining the factors involved in the differences in nutritive value observed in the experiment in the 1947 soybean hay crop rations, differences still remained. Despite this fact and because of the need for assaying subsequent crops, further work with the 1947 crop was discontinued and attention was given the 1948 soybean hay crops. Two rabbit experiments were conducted with these crops.

FEEDING TRIALS, RABBIT EXPERIMENT 3

Throughout the period of the investigations, several interested workers in the field of nutrition made repeated suggestions that

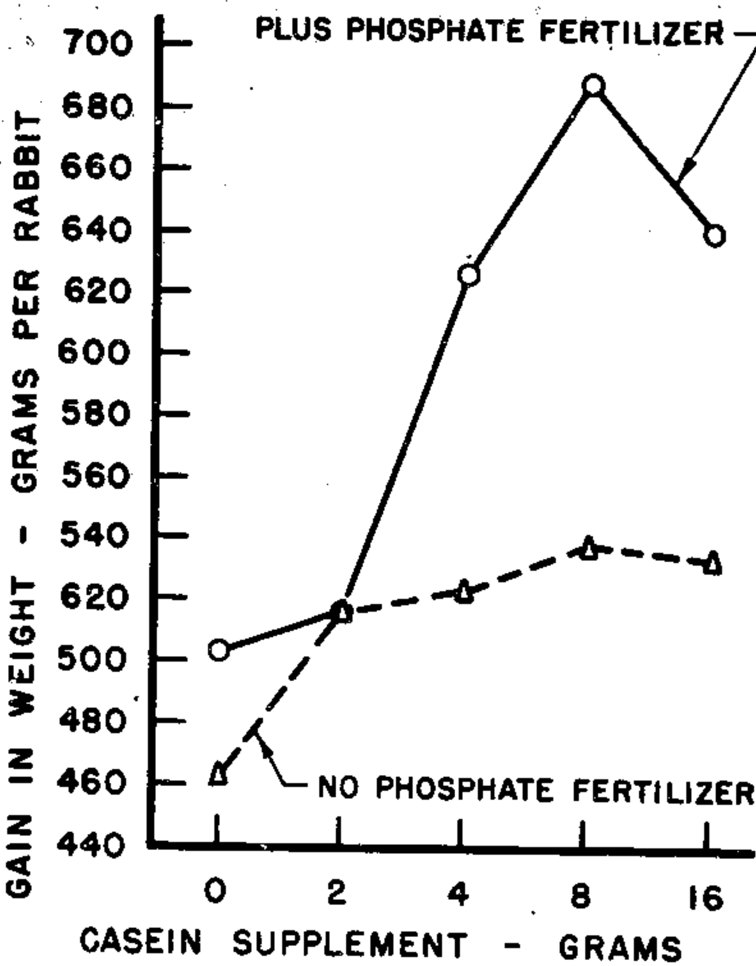

FIGURE 2.—Effect of casein supplement on the weight gains of rabbits fed hays from differentially fertilized plots. Feeding trials, rabbit experiment 2.

paired feeding trials be employed. The pros and cons of paired versus ad libitum feeding are too extensive to enumerate here. In any event, the writers felt that the best way to resolve the question insofar as this particular investigation was concerned was to conduct a paired feeding trial and an ad libitum feeding trial, together. The objectives of the feeding trial of rabbit experiment 3 were as follows: (1) To evaluate the relative nutritive value of the 1948 soybean hays from differentially phosphate-fertilized plots, as measured by rabbit growth, and (2) to make a comparison of the paired feeding method with the ad libitum feeding method.

Preparation of Rations

The experimental rations were constructed in a manner similar to those employed in rabbit feeding trial 1, e. g., 100 parts by weight of

KCa hay plus 25 parts of Cerelose and 5 parts of Wesson oil, and 100 parts by weight of PKCa hay plus 25 parts of Cerelose and 5 parts of Wesson oil.

Experimental Methods and Design

The identity of the field replications were kept intact in rabbit experiment 3 in a manner similar to that followed in rabbit experiment 1. The experiment was carried on in a randomized block design with 5 replications, 2 animals per replication per feeding method, making a total of 40 rabbits on experiment. Ten outcome groups consisting of 4 rabbits each were set up on the basis of weight and sex. The individuals of each quartet were further paired off on the basis of weight. Each pair was assigned to a field replication at random, and an individual of each pair was assigned to one of the 2 treatments at random. The experimental period was 8 weeks.

The feed intakes of the rabbits within a pair on the paired feeding method were adjusted daily. The age of the animals and particulars in the feeding and care of animals were similar to those used in rabbit experiment 1.

Experimental Results

The results (table 33; Appendix, tables 80 and 81) were negative. In 7 of the 10 pairs of animals on the paired feeding trial, the animal on the PKCa-hay ration refused feed more often than its counterpart on the KCa-hay ration. Nonetheless, under the ad libitum regimen, the rabbits on the PKCa-hay rations consumed slightly more of the rations than did the rabbits on the control rations. The animal variation encountered in the paired and the ad libitum feeding methods was not significantly different (see standard deviations, table 33).

TABLE 33.—*Average gains and feed intakes of rabbits fed soybean hay and a comparison of the paired feeding versus the ad libitum feeding methods (8-week trial), rabbit experiment 3, 1948 soybean crop*

Item compared	Fertilizer treatment of soybeans	Feeding method	
		Paired [1]	Ad libitum [1]
Intake per rabbit ____grams__	PKCa__________	2, 904	3, 319
	KCa__________	2, 904	3, 174
Gain per rabbit ________do__	PKCa__________	353	455
	KCa__________	342	438
Difference ________do		+11	+27
Standard deviation of gains ____do		90. 4	89. 0
Hemoglobin per 100 ml. of blood__________do	PKCa__________	11. 15	11. 80
	KCa__________	11. 06	11. 79

[1] 10 rabbits in each feeding method for each fertilizer treatment for hay.

These results (table 33) were not according to expectations. Differences in nutritive value could be predicted on the basis of the differences in phosphorus and protein composition of the experimental hays. The PKCa-hay diets contained approximately 11.1 percent protein and 0.111 percent phosphorus, whereas the KCa-hay diets contained approximately 14.8 percent protein and 0.082 percent phosphorus on a dry-matter basis. The phosphorus content of both types of diets was low, particularly in the KCa-hay diets. It is possible that the differences in protein content of the diets were canceled out by the differences in phosphorus content, which were in the reverse order.

These considerations suggested the possibility of adding adequate protein to study minerals, and an adequate supply of minerals to study protein in the test diets. The design of rabbit experiment 2 was in line with this concept. Another problem concerned the length of the experimental period. Was it too short to show the effects of a low phosphorus diet in the rabbit? Were the phosphorus stores of the weanling rabbits such that the young rabbit could sustain an adequate phosphorus nutrition under the conditions of the experiment? These questions and considerations led to a number of changes in the subsequent experiment on rabbits.

FEEDING TRIALS, RABBIT EXPERIMENT 4

The objective of this experiment was to ascertain if an adequate protein supplement to the diets based on hays from differentially phosphate-fertilized plots would reveal differences in nutritive value arising from the mineral component of the test diets.

In the earlier experiments reported, the young rabbits were weaned and placed on experiment at approximately 7 weeks of age, because animals younger than 7 weeks grew poorly on the high-fiber low-protein diets. It was determined, however, that rabbits 4 weeks old gained weight at a normal rate if additional egg albumin protein was added to the high-fiber diets. This information was used in rabbit experiment 4 as the basis for shortening the normal weaning period, since it was desirable to decrease the maternal influence on the phosphorus status of the weanling rabbits. The animals were weaned at 4 weeks of age and placed on a preliminary ration for a period of approximately 10 days. This ration consisted of 100 parts KCa hay, 13 parts Cerelose, 12 parts egg albumin, and 7 parts Wesson oil by weight. At the end of the preliminary period, the rabbits were placed on experiment.

Preparation of Rations

The hays from the replicated field plots were composited within fertilizer treatments. The PKCa-hay ration consisted of 100 parts hay, 17 parts Cerelose, 8 parts egg albumin, and 7 parts Wesson oil. The KCa-hay ration was similarly compounded. Egg albumin was used for the protein supplement, as it is low in ash and is especially low in phosphorus. The level of protein supplement employed was chosen on the basis of the results obtained in rabbit experiment 2. In other respects, the rations were prepared and handled as described under rabbit experiment 1.

Experimental Methods and Design

The experiment was part of a larger randomized block designed experiment. There were seven animals on each treatment. They were fed ad libitum. Other particulars were similar to those of rabbit experiment 1, except that in this experiment each rabbit was given 20 micrograms of biotin subcutaneously biweekly. The experimental period was 11 weeks.

Bone Measurements

Bone measurements were obtained in the following manner:

(1) Specific gravity was determined by the relationship:

$$\text{Specific gravity} = \frac{\text{weight in air}}{\text{weight in air} - \text{weight in water}}$$

Since the rabbit bones, procured after the feeding trial had ended, were porous and therefore absorbed water, the bones were dipped quickly into hot paraffin, covering the bone with a thin layer of the wax. This operation preceded the weighings. No correction was made for the paraffin.

(2) Breaking strength was determined by supporting the bone at both ends and hanging a 5-gallon empty solvent drum across the middle of the bone. Water was added to the can until the bone broke. The weight of the can plus that of the water was used as the measure of the breaking strength. The distance between the two supports of the bone was kept constant.

(3) Ash was determined on the fat-free bone.

Experimental Results

Three rabbits on the KCa-hay ration died during the 11-week experimental period; the first died at the close of the seventh week, the second in the eighth, and the third in the tenth. The animals were autopsied, but no gross abnormalities were observed. The carcasses of the first two rabbits were inadvertently disposed of before the humerus and femur were removed. The femur of the third rabbit was found to be in such a fragile condition that it was broken during its removal from the carcass. Fragile bones were found to be common among the femurs of the remaining rabbits on the KCa-hay ration when they were killed at the end of the experiment.

During the course of the experiment, it was observed that several animals on the KCa-hay ration went into nervous spasms when they were handled for the injection of biotin. All of the rabbits of this group appeared to be abnormally nervous and sensitive to handling. A summary of the various measurements taken in the experiment is presented in table 34. A graph of the accumulated gain curve is given in figure 3. Photographs of litter mates and of their femurs from the respective experimental diets are presented in figure 4.

The superiority of the PKCa-hay rations over that of the KCa-hay rations is plainly shown by the survival, the weight gain, the inorganic phosphorus content of the blood serum, and the measurements of bones of animals fed the rations. The phosphorus concentration of the rations indicate that the good results of the rations are the result of the level of phosphorus nutrition, since the calcium content of both rations is adequate (table 34).

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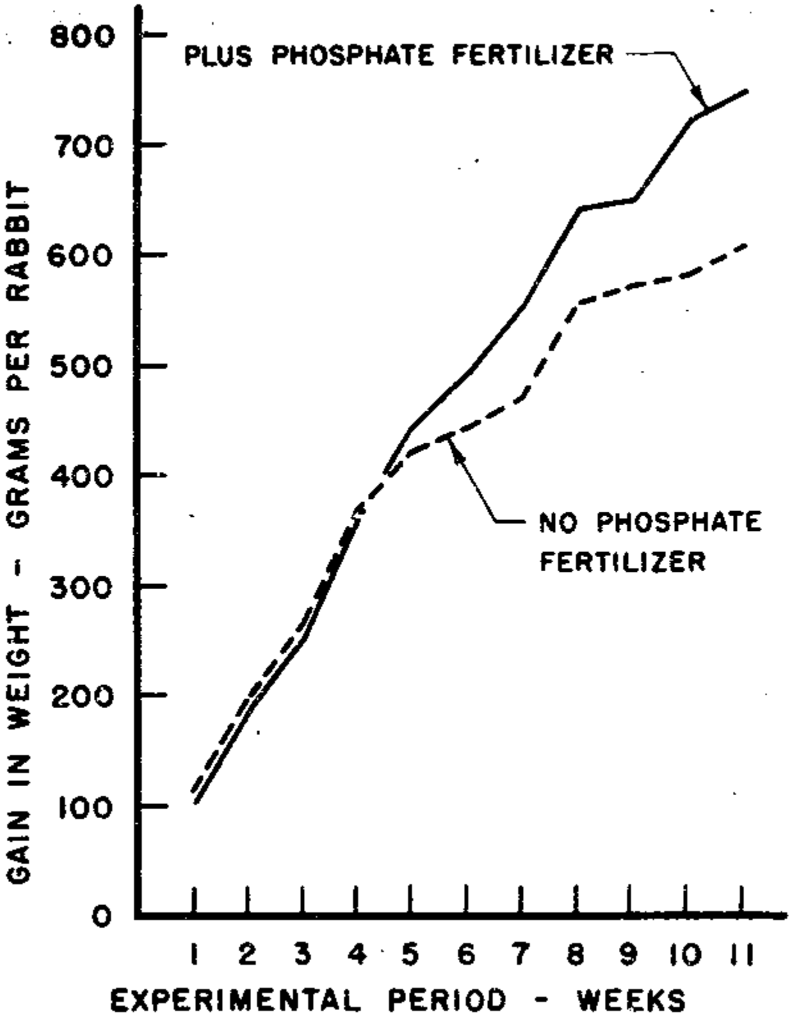

FIGURE 3.—Cumulative gains by rabbits on soybean hay from phosphated plots and nonphosphated plots, rabbit experiment 4.

The small difference in the ash content of both the femur and humerus bones between the contrasting groups of experimental rabbits and the marked differences in specific gravity and breaking strength suggest that the main difference in the bones was in their porosity. Apparently the rabbits on the KCa-hay ration either failed to deposit adequate minerals, thus forming a less compact bone, or mobilized the bone salts in order to help the animal overcome the deficiency of phosphorus in the diet. In either case these data (table 34) suggest a mechanism whereby growing rabbits can survive on low levels of phosphorus nutrition.

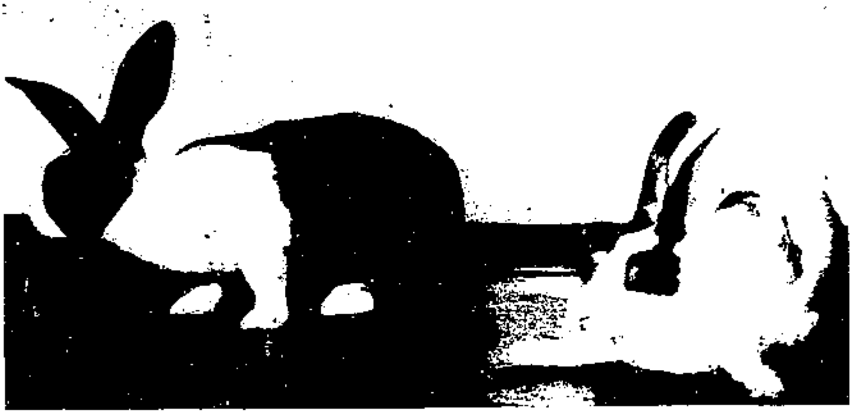

FIGURE 4.—*A*, Litter mates: Left, rabbit fed PKCa-hay ration; right, animal fed KCa-hay ration. *B*, Left femur: The one at right is from animal at right and shows evidence of fracture and subsequent healing.

TABLE 34.—*Summary of rabbit experiment 4, 11-week experimental period, 1948 soybean crop*

Item compared	Soybean hay from—		Difference, PKCa—KCa [1]
	PKCa plots	KCa plots	
Rabbits started .... number	7	7	
Rabbits survived .... do	7	4	
Average gain .... grams	771	607	164**
Average feed intake .... do	4, 698	4, 123	576
Blood analyses:			
Hemoglobin ....grams per 100 ml	10. 81	11. 09	—. 28
Inorganic phosphorus of serum grams per 100 ml	6. 05	4. 85	1. 20**
Serum calcium .... do	14. 21	14. 96	—. 75
Bone analyses:			
Femur .... specific gravity	1. 0057	. 8277	. 1780**
Humerus .... do	. 9451	. 8382	. 1069*
Breaking strength:			
Femur ... grams of water and can	8, 326	3, 855	4, 471**
Humerus .... do	6, 970	5, 040	1, 930*
Ash:			
Femur .... percent	56. 60	53. 92	2. 68
Humerus .... do	49. 63	49. 49	
Phosphorus content of diet ....do	. 109	. 081	. 028
Calcium content of diet ......do	. 672	. 560	. 112

[1] *= Significant at 5-percent level; **= significant at 1-percent level.

DISCUSSION OF STATISTICAL ESTIMATES AND EXPERIMENTAL DESIGN

Estimates of Components of Variance

Although the primary concern of the investigators was to design the experiments so that the experimental error included plot variation and animal variation rather than to estimate the components of the error variance, crude estimates of some of these components can be made from the experiments of this study. The paucity of information on this phase of soil-plant-animal investigations and the importance of this type of information in connection with the design of experiments are sufficient justification for the presentation and speculations that follow.

Estimates of Plot and Animal Variation in Feeding Trials

Of the lamb feeding trials conducted in this investigation only experiments 1 and 2 lend themselves to a breakdown of the error variance into component parts. In these experiments 6 pens of lambs consisting of 5 lambs per pen were each fed soybean hay from different plots. The analysis of variance with mean squares is presented in table 35. The last column of the upper part indicates the composition of the mean squares that are of interest. The estimates of the variance components given in the lower part of table 35 were computed by using the respective mean squares, as indicated by their algebraic compositions. The method of segregating mean squares into their component parts has been elucidated by Fisher (*18*), Tippett (*53*), and Snedecor (*50*).

TABLE 35.—*Analysis of variance of lamb gains obtained in lamb experiments 1 and 2, and estimates of variance components*

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean squares 1945	Mean squares 1946	Composition of mean squares
Treatments (T)	1	0.003921	0.060390	
Pens within treatments	4	.017175	.014558	sa^2+5sp^2
Lambs within pens	24	.007874	.012574	sa^2

ESTIMATES OF VARIANCE COMPONENTS

	1945	1946	
Pen variation sp^2	0.001860	0.000397	
Animal variation sa^2	.007874	.012574	

Unfortunately, the plot variation in the lamb feeding trials is confounded with the position of the pens in the barn. Nonetheless, it may be instructive to note the magnitude of the confounded effect which will be referred to simply as pen variation. It will be observed (table 35) that the pen variance component obtained in the 1945 feeding trial is larger than that obtained in the 1946 feeding trial. In the 1945 trial, moreover, the pen component of variance was roughly 24 percent as large as the animal component, whereas in 1946 it was only 3 percent as large. These data are insufficient to demonstrate that plot-to-plot variation was or was not manifested in the experimental error, but they do indicate that if it does exist its magnitude is of a low order.

Estimates of plot variation versus animal variation are considerably more conclusive in the rabbit-feeding trials than in the lamb trials. The means of the within-animal variation and the replication × fertilizer treatment interaction are presented in table 36. Estimates of the variance components are not presented, because it is obvious from a glance at the mean squares in the table that the variation encountered in each of the three rabbit experiments is the result of differences between animals.

The lamb digestion trial on the 1946 soybean hay crop is particularly suitable for variance components evaluation. The animal experiment was designed as a completely random experiment, 1 animal to each of the 12 plots. Two digestion trials were conducted with each lamb, one on the hay alone and the other on hay plus Cerelose. The experiment is of the split-plot type. The analysis of variance and estimates of the components of variance are presented in table 37. Error B is the subplot error and can be considered as essentially animal variation, whereas error A, the whole-plot error, includes plot variation as well as animal variation. There is evidence that the plot variation is a considerable part of the experimental error, particularly in the case of the digestibility of crude fiber, nitrogen-free extract, and net absorption of ash.

TABLE 36.—*Mean squares of rabbit gains obtained in trials from soybean hay rations of the 1947 and 1948 crop years*

Source of variation	Degrees of freedom		Mean squares			Composition of mean squares
	1947	1948	1947, ad libitum	1948, paired	1948, ad libitum	
Replications × treatment	5	4	9,722	5,783	750	sa^2+nsp^2
Animals within pairs	21	10	15,336	6,247	4,410	sa^2

TABLE 37.—*Analysis of variance and estimates of the variance components associated with digestion coefficients of indicated nutrients and net absorption of ash, lamb experiment 2, 1946 soybean crop* [1]

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean squares						Composition of mean squares
		Dry matter	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Absorption of ash	
Error A	10	5.317	7.622	3.994	33.075	14.890	82.448	sa^2+2sp^2
Error B	10	3.196	5.596	3.560	9.704	5.755	10.707	sa^2

ESTIMATES OF VARIANCE COMPONENTS

	Dry matter	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Absorption of ash
Plot variation sp^2	1.060	1.013	0.217	11.686	4.568	35.921
Animal variation sa^2	3.196	5.596	3.560	9.704	5.755	10.707
$\frac{sp^2}{sa^2}\times 100$	30	18	2	120	79	336

[1] Data from digestion trial 2 (table 61).

The experimental error associated with an animal measurement such as the digestion coefficient of a particular dietary nutrient may be a summation of (1) the variation arising from differences in the inherent digestive capacity of the experimental animals, (2) the variation arising from differences in the environment of the experimental animals, (3) the variation arising from analytical errors and digestion trial techniques, and (4) the variation arising from differences in the nutritive makeup of the diets. In the present case, it is assumed (although it is not strictly correct) that the variations enumerated in

(1) (2) and (3) are accounted for in the portion of variance herein given the term "animal variation," and the remaining variation (4), as synonymous with "plot variation."

The plot variation is assumed to arise from soil factors that vary or differ in a random fashion from plot to plot when receiving like fertilizer treatment. The point of interest here, however, is the data suggest that such animal measurements as the digestibility of single nutrients or nutrient categories are more sensitive to differences in soil factors than are overall measurements such as weight gains. On reflection this premise is logical, as weight gain or growth of an animal is an expression of the sum total of all the nutrients and their interactions, by means of which the effect of a variation in one nutrient of the feed may be obscured or counteracted by the variation in another nutrient in the feed. It follows, therefore, that digestibility studies and possibly balance studies may provide a sensitive biological means of screening out true soil-plant-animal relationships.

Experimental Design and Ancillary Measurements

Throughout the investigation, numerous ancillary measurements have been employed for the purpose of (1) reducing the experimental error and (2) interpreting the results. Some experimental designs were found better suited for ancillary measurements than others. The effectiveness of these procedures is evaluated as found in the present study.

TABLE 38.—*Reduction in experimental error of lamb and rabbit gains adjusted for feed intake*

Animal species and experiment No.	Experimental unit	Standard deviation: Unadjusted	Standard deviation: Adjusted	$\left[1-\frac{\text{adjusted}}{\text{unadjusted}}\right]\times 100$
TOTAL GAINS PER ANIMAL				
Lamb:		*Pounds*	*Pounds*	*Percent*
No. 1	Pen [1]	4.29	3.53	17.0
No. 2	do.[1]	3.40	2.17	36.2
Rabbit:		*Grams*	*Grams*	
No. 1	Individual	88.5	45.5	48.6
No. 2	do	100.8	95.0	5.7
No. 3	do	66.4	49.7	25.2
AVERAGE DAILY GAIN PER LAMB				
Lamb:		*Pounds*	*Pounds*	
No. 3	Individual [2]	0.0915	0.0632	30.8
No. 3	do.[3]	.0707	.0374	47.2
No. 4	do	.0358	.0222	37.9

[1] 5 lambs per pen.
[2] Ceretose supplement.
[3] Corn supplement.

FEEDING TRIALS

In table 38 data are presented showing the reduction in the standard deviation of gains when adjusted for feed intake by covariance analysis. A reduction in the standard deviation per experimental unit of approximately 30 percent on the average was obtained by adjustment of the gains for feed intake. This was true for both the rabbits and the lambs. It does not necessarily follow, however, that experimental control such as equalized feed intake will result in the same reduction in error obtained by the covariance analysis. Sherwood and Weldon [12] have shown that restrictions in feed intake of rats results in a lower experimental error. In the paired-feeding trial with rabbits on the 1948 soybean crop (table 33), however, the standard deviation of the paired-feeding trial was of the same magnitude as that of the ad libitum-feeding trial.

Covariance analysis of weight gain of animals adjusted for feed intake may often supply information concerning the animals' economy of food utilization. The analyses of errors of estimates from regression within ration groups were made on the data of the feeding trials of rabbit experiments 1 and 3 (table 39). From the analysis, it is apparent that the economy of food utilization was significantly different between diets. A further analysis of the variances encountered in rabbit experiment 1 showed that the hay-plus-corn rations were superior to the hay-plus-Cerelose rations (table 26). This interpretation was supported by the digestion trial data, which showed that the total-digestible-nutrient (T. D. N.) content of the hay-corn rations was higher than that of the hay-Cerelose rations. The analysis on rabbit trial 3 (table 39) was made to ascertain if the economy of food utilization of the paired-fed animals was different from that of the ad libitum-fed animals. It was found that the deviations within diets was of the same magnitude as that of the difference between diet regressions (table 39).

TABLE 39.—*Analysis of errors of estimates from average regression within diet groups, rabbit experiments 1 and 3*

Source of variation	Rabbit experiment 1		Rabbit experiment 3	
	Degrees of freedom	Mean squares [1]	Degrees of freedom	Mean squares
Deviation from average regression within diets	40	----	35	----
Deviation from individual diets	37	4, 671	32	3, 018
Difference between diet regression	3	127, 022**	3	3, 303

[1] **= Significant at the 1-percent level.

DIGESTION TRIALS

A similar examination of the digestion trial data would be repetitious, since the principles are the same. In its stead, therefore,

[12] Personal communication of unpublished data.

examples will be presented that will illustrate, it is hoped, additional concepts.

The choice of an ancillary measurement for a particular nutrient in digestion trials is not so obvious as is feed intake for gain. The relation between the apparent digestibility of protein and the percentage composition of protein in the diet has been characterized for ruminants by Mitchell (*34*). Unfortunately, less is known about other nutrients or nutrient categories. In cases where specific information is lacking, therefore, the writers have proceeded on the basis of two assumptions: (1) That the concentration of a nutrient in a diet is related to its digestibility, and (2) that the relationship over the range tested is linear.

The analysis of variance and covariance of the data on the digestibility of crude fiber taken from the lamb digestion trial on the 1945 soybean crop are shown in table 40. In this experiment there were 12 plots, 6 with and 6 without phosphate. The identity of the field plot was maintained in the digestion trial in which 1 lamb was fed with the hay from each plot.

It is apparent from an examination of table 40 that not only was the error reduced when crude-fiber composition of the diets was adjusted for digestibility, but that the treatment differences as well were accounted for by the variation in the crude-fiber concentration of the hays from the different plots. A more positive statement concerning the biological interpretation of these results cannot be made, because the relationship between crude-fiber concentration of the diet and the digestibility of crude fiber is not understood.

TABLE 40.—*Analysis of variance and of covariance of crude-fiber composition on crude-fiber digestibility of soybean hays, lamb experiment 1, 1945 soybean crop*

OVERALL STATISTICAL ANALYSIS

Source of variation	Analysis of variance		Covariance analysis	
	Degrees of freedom	Mean squares [1]	Degrees of freedom	Mean squares
Total	11			
Fertilization (F)	1	191.3605**	1	6.1606
Animals within plots	10	17.3684	9	5.6983

ANALYSIS OF ERRORS OF ESTIMATE FROM AVERAGE REGRESSION WITHIN FERTILIZER TREATMENTS

Source of variation	Degrees of freedom	Mean squares
Deviation from average regression within fertilizer treatments	9	
Deviation from individual fertilizer treatments	8	6.3183
Difference among fertilizer treatment regressions	1	.7371

[1] **= Significant at the 1-percent level.

Another example that illustrates the advantages of retaining the field identity in the digestion and balance studies with animals is given by the phosphorus balance data taken from the 1947 rabbit digestion trial. Balance determinations were conducted with 24 rabbits. The structure of the experiment was a split plot in a randomized block design. The hays from each of the 12 experimental plots were supplemented with corn in one ration and Cerelose in another. The analysis of variance of the balance data shows a significant effect for replications, fertilization, and supplements (table 41). In the covariance analysis these factors are not significant. In other words, the significant effects encountered could be explained by the variation in phosphorus intake. Thus, by keeping the plot variation in the balance experiment it was possible to make a comparison of the availability of the phosphorus in the hays. This would not have been possible had the hays from the plots within each fertilizer treatment been composited, since the average phosphorus concentration of the PKCa plants was higher than in the KCa plants. Regression cannot be discerned from two points.

SUMMARY

Efficient experimental designs and proper ancillary measurements can assist in the segregation, estimation, and interpretation of variation arising from each phase of the soil-plant-animal complex. Ancillary measurements can bring about a reduction in the experimental error, and in some cases they can help distinguish between differences involving quantity versus quality of nutrients.

TABLE 41.—*Analysis of variance and of covariance of phosphorus balance data taken from digestion trial, rabbit experiment 1, 1947 soybean crop*

Source of variation	Analysis of variance [1]		Covariance analysis [1]	
	Degrees of freedom	Mean squares [2]	Degrees of freedom	Mean squares
Replications (R)	5	0.080133*	5	0.010426
Fertilization (F)	1	.110541*	1	.007324
R×F (Error A)	5	.016009	4	.013191
Supplements (S)	1	.076433**	1	.008880
F×S	1	.009600	1	.008502
Error B	10	.009553	9	.005928

[1] Grams P intake=independent variable; grams P balance=dependent variable.
[2] *= Significant at the 5-percent level; **= significant at the 1-percent level.

GENERAL DISCUSSION OF RESULTS

The data from the series of lamb experiments reported herein support the conclusion that the differences in phosphorus content of the soybean hay from differentially phosphate-fertilized plots is the factor responsible for the differences observed in nutritive value. It was found that the lambs on the low-phosphorus rations made lower weight gains and had lower feed intakes and lower levels of serum inorganic phosphorus in the blood than those receiving higher phosphorus diets. Addition of a phosphorus containing mineral supplement to the KCa ration increased the feed intake, weight gains, and serum inorganic phosphorus of the blood serum (tables 23, 24). The data in the literature on phosphorus metabolism are in agreement with these results.

There is a marked agreement in the literature (*5, 15, 28, 40, 43, 52*) on the effects of suboptimum levels of phosphorus in the diet on appetite and the inorganic phosphorus of the blood. It has been suggested by Morris and Ray (*36*) that the appetite effect may be the result of a diminished secretion of digestive juices with a decreased phosphorus intake.

The results obtained with rabbits on the 1947 crops were different from those obtained with lambs in several respects. With lambs a difference in gains was obtained only when the experimental hays were supplemented with Cerelose; with rabbits a difference in gains was obtained when either Cerelose or corn was used as the supplement. A urea supplement had no significant effect on lamb gains, but the addition of casein increased the gains of the rabbits on the PKCa-hay diets.

A digestion trial on the rations fed during the first rabbit trial offered some explanation for the differences in response to the two hay-plus-Cerelose rations in that the total digestible nutrient content of the PKCa-hay diet was higher than that of the KCa-hay diet. From these data it might be assumed that the low phosphorus content of the poorer diet also affected the energy metabolism of the animals.

Data on the effect of suboptimum levels of phosphorus in the diet on the economy of feed utilization are not in complete agreement. In a study on sheep, Woodman and Evans (*59*) found that the digestibility of the ration was unchanged by either calcium or phosphorus deficiency; similar results with phosphorus deficiency were reported by Forbes (*19*) on rats and by Kleiber and associates (*28*) on steers. A diminished utilization of food energy has, however, been recorded by Theiler and coworkers (*52*), Kleiber and associates (*28*), and Riddell and others (*40*). Moreover, Morris and Ray (*36*) presented data that suggest that a low phosphorus intake may diminish the biological value of protein for sheep.

There is reason to believe, however, that differences in nutritional factors other than those relating to phosphorus were involved in the results with rabbits on the 1947 crops. A difference in gains was obtained between the two corn-supplemented diets, but the total digestible nutrient contents of these diets were not significantly different nor were the phosphorus levels of these rations suboptimum. More convincing evidence was obtained subsequently in rabbit experiment 2, a factorial experiment, in which both phosphorus and casein were added to the hay-plus-Cerelose rations; but the differences between the PKCa-hay rations and the KCa-hay rations became wider with increasing levels of casein. Feed intakes were not significantly different on the casein-supplemented rations. From a consideration of these data, it is apparent that neither phosphorus nor protein was the primary factor involved.

That the rabbits and lambs responded differently is not unexpected, since sheep, by virtue of their rumen, are for practical purposes independent of dietary B vitamins and can utilize nonprotein nitrogen for a considerable part of their dietary protein needs (*28*). On the other hand, the rabbit cannot utilize dietary urea nitrogen appreciably and probably is not as independent of dietary B vitamins as sheep. For this reason the rabbit may be a better experimental animal than the ruminant in investigations seeking to find differences in the nutritive value of forages resulting from fertilization practices, since it is no doubt more sensitive to limiting factors in the diet.

The contrasting results with rabbits on the 1947 soybean hay crops are as indicative of differences in experimental technique as year-to-year differences. For example, in feeding trial 1, 1947 crop, malformation of the bones and low serum inorganic phosphorus were not noted in the animals on the KCa hay-plus-Cerelose ration, despite the fact that it contained 0.086 percent P. Nevertheless, the rabbits on the 1948 KCa hay-plus-Cerelose diet, which contained 0.082 percent P and was supplemented with egg albumin, developed marked malformation of the bone and other phosphorus-deficiency symptoms. Two factors are offered as an explanation for the foregoing results: (1) The animals on the 1948 hay crop were placed on experiment at a younger age and left on experiment for a longer period; (2) the supplement of egg albumin, a high-quality protein that carries little phosphorus, brought about more rapid growth and put a stress on the limiting minerals in the diet. The addition of casein to the 1947 KCa hay-plus-Cerelose ration did not bring about symptoms of phosphorus deficiency, but this result was probably caused by the fact that casein is a phosphoprotein.

These studies suggest that in soil-plant-animal investigations the field replications should be composited for feeding trials, since the plot variation appears to be minor as compared to animal variation. Instead of basing the number of diets in the experiment on the number of field replications, it seems more logical to employ the factorial type of feeding experiment in which the suspected nutrients are supplemented and unsupplemented, singly and in combination. In this manner the investigator can determine whether differences in nutritive value exist and possibly determine some of the causative factors.

In contrast, it is suggested that in digestion and balance trials the animal experiments should be replicated to correspond to the field replicates, since the plot variation was appreciable as compared to animal variation. This procedure is recommended not only because it enables the investigator to segregate the variation associated with each component of the soil-plant-animal complex, but also because it assists him in evaluating the causative factors. When field replications are composited in digestion and balance studies, it is impossible to discern trends, because the investigator has only a few values with which to work.

The data also show that the use of purified ingredients in the rations may accentuate possible differences in the nutritive value of forages resulting from different fertilizer practices. This was illustrated in the lamb experiments in which a difference was obtained with a Cerelose supplement but not with a corn supplement. It was also shown in the rabbit results for the 1947 crop in which it was found that the differences in growth responses between experimental diets became wider when greater quantities of casein supplements were added to the rations. The rabbit data for the 1948 crop, in which the diets were supplemented with egg albumin, show that if the investigator is interested in studying the mineral content of a forage it is desirable to add a protein supplement to the rations composed of forages containing suboptimal levels of protein.

SUMMARY

An investigation concerned with the effect of phosphate fertilization on the nutritive value of soybean forage was carried on over a period of 5 years (1945–49). At extremely low levels of soil phosphorus, phosphate fertilization brought about numerous changes in the chemical composition of the soybean plant, with noticeable increases in the phosphorus, calcium, and protein concentration. Phosphate fertilization increased the yield of soybean hay by twofold or threefold. Phosphate fertilization increased the nutritive value of soybean hay as indicated by the growth of lambs and the growth and bone formation in rabbits fed phosphate-treated soybean hay.

The cause of the increased growth in sheep fed the phosphate-treated soybean hay appears to be the extra phosphorus in the phosphate-fertilized hay. In the 1947 crops, the protein and phosphorus factors were eliminated in the case of the rabbits, but differences in the nutritive value in favor of the forage grown on the phosphate-fertilized plots remained unexplained. No evidence was obtained that either the availability of the phosphorus or the quality of the protein of the soybean hays for either rabbits or lambs was altered by phosphate fertilization. Methods were illustrated and developed for an integrated attack on fertilization problems from the soil to the animals.

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APPENDIX

(Tables 42–81)

TABLE 42.—*Crude protein content of soybean plants; moisture-free basis*

Replication	Fertilizer treatment of plots	Crude protein content					
		1945	1946	1947	1948	1949	Mean
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	PKCa	22.77	18.37	17.87	13.95	19.08	18.41
	KCa	21.89	20.47	14.79	18.03	17.31	18.49
2	PKCa	22.22	20.17	16.80	14.49	19.04	18.54
	KCa	20.88	17.05	15.34	18.38	16.84	17.69
3	PKCa	23.44	20.69	17.74	14.89	19.77	19.31
	KCa	19.57	16.90	15.13	19.17	17.60	17.67
4	PKCa	21.00	19.38	16.46	14.26	18.41	17.90
	KCa	20.21	20.20	15.48	20.72	17.76	18.87
5	PKCa	21.27	18.41	17.67	14.00	18.30	17.93
	KCa	22.06	19.77	16.19	18.64	16.50	18.63
6	PKCa	20.77	22.08	16.77	14.42	18.26	18.46
	KCa	21.79	17.90	15.88	20.34	16.57	18.49
Mean	PKCa	21.91	19.85	18.89	14.34	18.81	18.42
	KCa	21.06	18.72	15.47	19.21	17.10	18.31

TABLE 43.—*Crude fiber content of soybean plants; moisture-free basis*

Replication	Fertilizer treatment of plots	Crude fiber content					
		1945	1946	1947	1948	1949	Mean
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	PKCa	28.09	31.19	35.74	33.28	34.70	32.60
	KCa	27.75	27.38	35.19	32.27	31.71	30.86
2	PKCa	29.49	27.66	35.26	34.22	35.11	32.35
	KCa	34.52	30.46	35.70	32.65	33.98	33.46
3	PKCa	25.92	28.23	35.75	35.44	34.72	32.01
	KCa	30.18	27.83	34.47	32.08	33.59	31.63
4	PKCa	24.83	30.31	34.94	33.60	35.39	31.81
	KCa	24.53	27.15	33.79	30.50	34.19	30.03
5	PKCa	23.54	27.35	34.78	33.76	33.96	30.68
	KCa	24.23	27.26	33.94	31.27	30.97	29.53
6	PKCa	24.89	28.44	35.74	34.35	32.50	31.18
	KCa	23.67	26.47	36.21	30.21	32.58	30.23
Mean	PKCa	26.13	28.86	35.37	34.11	34.40	31.77
	KCa	27.48	28.09	34.88	31.51	32.84	30.96

TABLE 44.—*Nitrogen-free extract content of soybean plants; moisture-free basis*

Replication	Fertilizer treatment of plots	Nitrogen-free extract content					
		1945	1946	1947	1948	1949	Mean
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	PKCa	42. 63	36. 06	37. 01	41. 03	38. 03	38. 95
	KCa	45. 76	35. 22	37. 01	41. 75	38. 47	39. 64
2	PKCa	42. 92	35. 94	39. 39	43. 60	37. 54	39. 88
	KCa	45. 53	37. 41	40. 15	41. 18	40. 10	40. 87
3	PKCa	42. 91	34. 74	38. 07	41. 48	36. 46	38. 73
	KCa	41. 56	36. 87	41. 74	40. 41	39. 03	39. 92
4	PKCa	45. 76	34. 62	39. 37	44. 40	38. 31	40. 49
	KCa	47. 70	36. 15	42. 03	40. 17	39. 96	41. 20
5	PKCa	45. 83	37. 72	38. 04	44. 66	39. 82	41. 21
	KCa	43. 58	37. 69	39. 69	42. 27	44. 00	41. 45
6	PKCa	46. 29	33. 96	38. 24	43. 31	41. 41	40. 64
	KCa	45. 73	39. 73	39. 71	40. 17	42. 71	41. 61
Mean	PKCa	44. 39	35. 51	38. 35	43. 08	38. 60	39. 99
	KCa	44. 98	37. 18	40. 06	40. 99	40. 71	40. 78

TABLE 45.—*Ash content of soybean plants; moisture-free basis*

Replication	Fertilizer treatment of plots	Ash content					
		1945	1946	1947	1948	1949	Mean
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	PKCa	6. 27	8. 14	6. 33	5. 78	5. 82	6. 47
	KCa	6. 38	10. 43	6. 33	7. 74	6. 88	7. 55
2	PKCa	6. 02	10. 05	5. 67	6. 20	6. 03	6. 75
	KCa	6. 68	9. 99	6. 11	6. 43	6. 67	7. 18
3	PKCa	7. 24	10. 44	6. 59	6. 44	6. 83	7. 51
	KCa	6. 57	13. 54	6. 02	6. 61	7. 16	7. 98
4	PKCa	6. 31	9. 52	5. 93	5. 94	5. 77	6. 69
	KCa	5. 55	10. 25	5. 77	6. 28	6. 74	6. 92
5	PKCa	5. 96	10. 63	6. 12	5. 74	6. 00	6. 89
	KCa	6. 19	9. 02	5. 94	5. 99	6. 56	6. 75
6	PKCa	5. 53	9. 12	6. 11	6. 00	5. 82	6. 52
	KCa	5. 70	8. 10	5. 98	6. 63	6. 32	6. 15
Mean	PKCa	6. 22	9. 65	6. 13	6. 02	6. 05	6. 81
	KCa	6. 18	10. 22	6. 03	6. 61	6. 72	7. 15

TABLE 46.—*Phosphorus content of soybean plants; moisture-free basis*

Replication	Fertilizer treatment of plots	Phosphorus content 1945	1946	1947	1948	1949	Mean
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	PKCa	0.236	0.140	0.178	0.132	0.225	0.182
	KCa	.169	.143	.112	.095	.137	.131
2	PKCa	.209	.135	.173	.165	.220	.180
	KCa	.161	.128	.122	.112	.125	.130
3	PKCa	.275	.145	.179	.157	.237	.199
	KCa	.186	.111	.118	.116	.147	.136
4	PKCa	.249	.172	.182	.141	.220	.193
	KCa	.209	.143	.129	.101	.145	.145
5	PKCa	.259	.149	.198	.129	.212	.189
	KCa	.237	.121	.129	.094	.120	.140
6	PKCa	.207	.167	.192	.137	.210	.183
	KCa	.249	.139	.144	.119	.140	.158
Mean	PKCa	.239	.151	.184	.144	.221	.188
	KCa	.202	.132	.132	.106	.136	.140

TABLE 47.—*Calcium content*[1] *of soybean plants; moisture-free basis*

Replication	Fertilizer treatment of plots	Calcium content 1946	1947	1948	1949	Mean
		Percent	*Percent*	*Percent*	*Percent*	*Percent*
1	PKCa	0.730	0.926	0.976	1.093	0.931
	KCa	.850	.764	.829	.841	.821
2	PKCa	.990	1.026	.882	.890	.947
	KCa	.680	.845	.802	.909	.809
3	PKCa	.870	.887	1.009	1.082	.962
	KCa	.720	.767	.796	.822	.776
4	PKCa	.670	.669	.791	.714	.711
	KCa	.610	.605	.655	.588	.614
5	PKCa	.660	.760	.809	.799	.757
	KCa	.770	.709	.714	.663	.714
6	PKCa	.940	.735	.815	.736	.807
	KCa	.550	.661	.667	.608	.622
Mean	PKCa	.810	.834	.880	.886	.852
	KCa	.697	.725	.744	.739	.726

[1] Data for calcium content are not available for the 1945 crop.

Table 48.—*Analysis of variance of constituents in soybean plants, 1945–49*

Source of variation	Degrees of freedom	Mean squares [1]					
		Protein	Crude fiber	Nitrogen-free extract	Ash	Phosphorus	Calcium [2]
Replications (R)	5	0.208	9.8549	7.9175	1.6874	0.000423	0.074172
Treatments (T)	1	52.170**	[3] 9.9633	9.5441*	1.7374	.034034**	.191774**
Years (Y)	4	.199	151.2802**	118.4173**	32.8878**	.016617**	.009711
T×Y	4	23.799**	6.4467	8.7708**	.4301	.001839**	.001018
R×T	5	2.372	2.9137	.2808	.4612	.000400	.006081
R×Y	20	.807	3.6794	2.5931	.4365	.000373	.004629
R×T×Y	20	.909	1.2870	2.0194	.4323	.000207	.008031
Pooled error	45	1.026	2.5310	2.0811	.4373	.000290	.006297

[1] *= Significant at 5-percent level; **= significant at 1-percent level.
[2] Data for calcium content are not available for the 1945 crop. The degrees of freedom for calcium should be adjusted accordingly.
[3] Significant at $P=0.056$.

TABLE 49.—*Crude protein content and analysis of variance of soybean plant (Ogden variety); moisture-free basis*

EXPERIMENTAL DATA

Replication	Fertilizer treatment of plots	Crude protein content				
		1945	1946	1947	1949	Mean
		Percent	*Percent*	*Percent*	*Percent*	*Percent*
1	PKCa	22.77	18.37	17.87	19.08	19.52
	KCa	21.89	20.47	14.79	17.31	18.62
2	PKCa	22.22	20.17	16.80	19.04	19.56
	KCa	20.88	17.05	15.34	16.84	17.53
3	PKCa	23.44	20.69	17.74	19.77	20.41
	KCa	19.57	16.90	15.13	17.60	17.30
4	PKCa	21.00	19.38	16.46	18.41	18.81
	KCa	20.21	20.20	15.48	17.76	18.41
5	PKCa	21.27	18.41	17.67	18.30	18.91
	KCa	22.06	19.77	16.19	16.50	18.63
6	PKCa	20.77	22.08	16.77	18.26	19.47
	KCa	21.79	17.90	15.88	16.57	18.03
Mean	PKCa	21.91	19.85	17.22	18.81	19.45
	KCa	21.06	18.72	15.47	17.10	18.09

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean squares [1]
Replications (R)	5	0.3023
Treatments (T)	1	22.2224**
Years (Y)	3	56.8486**
Y×T	3	5.924*
R×T	5	2.2968
R×Y	15	.5453
R×T×Y	15	1.2981
Pooled error	35	1.1182

[1] *=Significant at the 5-percent level; **=significant at the 1-percent level.

TABLE 50.—*Crude protein content of leaves and stems of the soybean plant; moisture-free basis*

Replication	Fertilizer treatment of plots	Crude protein content in—							
		Leaves				Stems			
		1947	1948	1949	Mean	1947	1948	1949	Mean
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	PKCa	25. 16	25. 24	28. 92	26. 44	10. 71	7. 93	11. 65	10. 10
	KCa	20. 67	25. 65	24. 78	23. 70	9. 81	12. 06	9. 89	10. 59
2	PKCa	24. 44	23. 33	29. 66	25. 81	9. 63	8. 35	11. 41	9. 80
	KCa	21. 71	26. 66	25. 67	24. 68	9. 57	12. 07	9. 76	10. 46
3	PKCa	24. 45	25. 02	31. 23	26. 90	10. 53	8. 16	12. 31	10. 33
	KCa	21. 20	26. 30	26. 85	24. 78	9. 48	13. 18	9. 77	10. 81
4	PKCa	24. 76	23. 44	29. 41	25. 87	9. 43	7. 68	10. 63	9. 25
	KCa	22. 58	28. 71	26. 83	26. 04	9. 17	13. 44	9. 73	10. 78
5	PKCa	24. 86	24. 38	29. 06	26. 10	9. 69	7. 21	10. 39	9. 10
	KCa	22. 89	25. 24	24. 46	24. 20	9. 77	12. 95	9. 31	10. 68
6	PKCa	24. 54	24. 38	28. 52	25. 81	9. 24	7. 42	9. 88	8. 85
	KCa	23. 35	27. 50	24. 96	25. 27	9. 10	13. 32	8. 92	10. 45
Mean	PKCa	24. 70	24. 30	29. 46	26. 15	9. 87	7. 79	11. 04	9. 57
	KCa	22. 06	26. 67	25. 59	24. 79	9. 48	12. 84	9. 56	10. 63

TABLE 51.—*Crude fiber content of leaves and stems of soybean plants; moisture-free basis*

Replication	Fertilizer treatment of plots	Crude fiber content in—							
		Leaves				Stems			
		1947	1948	1949	Mean	1947	1948	1949	Mean
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	PKCa	16. 94	18. 67	16. 47	17. 36	50. 18	45. 11	46. 56	47. 28
	KCa	18. 49	18. 01	16. 18	17. 56	44. 20	43. 45	45. 26	43. 30
2	PKCa	18. 17	18. 22	16. 60	17. 66	47. 68	45. 50	46. 79	46. 66
	KCa	17. 84	17. 65	17. 48	17. 66	48. 22	44. 06	45. 78	46. 02
3	PKCa	18. 70	19. 42	16. 08	18. 07	46. 71	46. 10	46. 02	46. 28
	KCa	18. 89	17. 93	17. 76	18. 19	45. 24	43. 94	45. 44	44. 87
4	PKCa	18. 29	19. 40	16. 79	18. 16	46. 71	43. 77	46. 91	45. 80
	KCa	19. 06	17. 42	16. 39	17. 62	44. 20	42. 44	48. 51	45. 05
5	PKCa	17. 95	18. 75	15. 99	17. 56	47. 87	43. 56	45. 62	45. 68
	KCa	18. 14	18. 04	15. 81	17. 33	45. 59	42. 68	43. 32	43. 86
6	PKCa	17. 43	19. 38	16. 99	17. 93	49. 24	41. 88	43. 60	45. 91
	KCa	18. 16	18. 00	16. 84	17. 67	48. 67	42. 19	45. 41	45. 42
Mean	PKCa	17. 91	18. 97	16. 49	17. 79	48. 07	44. 82	45. 92	46. 27
	KCa	18. 43	17. 84	16. 74	17. 67	46. 02	43. 13	45. 62	44. 92

TABLE 52.—*Nitrogen-free extract content of leaves and stems of soybean plants; moisture-free basis*

Replication	Fertilizer treatment of plots.	Nitrogen-free extract content of—							
		Leaves				Stems			
		1947	1948	1949	Mean	1947	1948	1949	Mean
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	PKCa	46.45	45.40	41.18	44.37	32.96	41.48	35.63	36.69
	KCa	49.73	45.79	45.91	47.14	38.60	38.57	37.85	38.34
2	PKCa	46.28	48.09	40.18	44.85	37.11	40.44	35.51	37.69
	KCa	48.67	45.48	43.21	45.79	36.46	37.91	37.56	37.31
3	PKCa	44.77	43.65	38.41	42.28	36.12	40.04	34.50	36.89
	KCa	48.74	44.80	40.78	44.77	39.26	36.75	37.14	37.72
4	PKCa	44.42	45.90	38.94	43.09	38.06	43.31	37.73	39.70
	KCa	46.40	41.91	42.83	43.71	40.33	38.59	37.29	38.70
5	PKCa	44.22	45.46	42.54	44.07	36.57	44.15	38.11	39.61
	KCa	46.47	45.88	47.24	46.53	33.51	39.17	41.19	37.96
6	PKCa	45.84	44.80	42.21	44.28	35.40	42.28	41.04	39.57
	KCa	46.28	41.99	46.33	44.87	35.92	38.48	39.70	38.03
Mean	PKCa	45.33	45.57	40.58	43.82	36.04	41.95	37.09	38.36
	KCa	47.72	44.31	44.38	45.47	37.35	38.23	38.46	38.02

TABLE 53.—*Ash content of leaves and stems of soybean plants; moisture-free basis*

Replication	Fertilizer treatment of plots	Ash content of—							
		Leaves				Stems			
		1947	1948	1949	Mean	1947	1948	1949	Mean
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	PKCa	7.77	8.18	7.59	7.85	5.39	4.72	5.21	5.11
	KCa	7.76	7.67	8.08	7.84	4.72	5.12	5.83	5.22
2	PKCa	7.28	7.99	7.24	7.50	4.54	4.93	5.34	4.94
	KCa	8.15	8.15	7.77	8.02	4.88	5.10	5.85	5.28
3	PKCa	8.36	8.70	7.99	8.35	5.60	4.93	6.27	5.60
	KCa	7.72	8.20	8.49	8.13	4.94	5.28	6.31	5.51
4	PKCa	7.74	8.14	7.15	7.68	4.89	4.36	4.92	4.72
	KCa	7.67	8.19	8.59	8.15	4.61	4.56	5.25	4.81
5	PKCa	7.96	8.06	7.41	7.81	4.96	4.22	4.95	4.71
	KCa	7.80	7.85	8.24	7.96	3.65	4.38	5.12	4.38
6	PKCa	9.87	8.10	7.24	8.40	5.09	4.51	4.60	4.73
	KCa	7.87	8.25	7.69	7.90	4.76	5.04	5.09	4.96
Mean	PKCa	8.16	8.20	7.44	7.93	5.08	4.61	5.22	4.97
	KCa	7.83	8.05	8.14	8.01	4.59	4.91	5.58	5.03

TABLE 54.—*Phosphorus content of leaves and stems of soybean plants; moisture-free basis*

Replication	Fertilizer treatment of plots	Phosphorus content of—							
		Leaves				Stems			
		1947	1948	1949	Mean	1947	1948	1949	Mean
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	PKCa	0.200	0.225	0.282	0.236	0.103	0.083	0.157	0.114
	KCa	.146	.144	.185	.158	.057	.057	.079	.064
2	PKCa	.223	.258	.291	.257	.093	.100	.141	.111
	KCa	.166	.185	.179	.177	.062	.056	.074	.062
3	PKCa	.219	.256	.290	.255	.090	.091	.164	.115
	KCa	.158	.185	.198	.180	.062	.058	.087	.069
4	PKCa	.230	.206	.289	.242	.101	.095	.149	.115
	KCa	.170	.143	.197	.170	.065	.063	.088	.072
5	PKCa	.239	.205	.271	.238	.112	.079	.140	.110
	KCa	.163	.131	.166	.158	.063	.061	.070	.064
6	PKCa	.231	.205	.272	.236	.106	.089	.132	.109
	KCa	.192	.168	.193	.184	.072	.070	.079	.074
Mean	PKCa	.224	.220	.283	.244	.101	.090	.147	.113
	KCa	.166	.159	.186	.171	.064	.061	.080	.068

TABLE 55.—*Calcium content of leaves and stems of soybean plants; moisture-free basis*

Replication	Fertilizer treatment of plots	Calcium content of—							
		Leaves				Stems			
		1947	1948	1949	Mean	1947	1948	1949	Mean
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	PKCa	1.360	1.474	1.448	1.427	0.760	0.746	0.959	0.822
	KCa	1.054	1.003	1.024	1.027	.547	.692	.673	.637
2	PKCa	1.173	1.274	1.243	1.230	.629	.606	.729	.655
	KCa	1.119	.968	1.100	1.062	.647	.676	.753	.692
3	PKCa	1.302	1.493	1.495	1.430	.731	.687	.934	.784
	KCa	1.072	.951	1.020	1.014	.568	.667	.644	.626
4	PKCa	1.022	1.121	.918	1.020	.512	.654	.563	.576
	KCa	.859	.813	.751	.808	.433	.511	.435	.460
5	PKCa	1.137	1.240	1.103	1.160	.590	.527	.629	.582
	KCa	.908	.898	.821	.876	.548	.556	.515	.540
6	PKCa	1.082	1.209	.959	1.083	.587	.538	.574	.566
	KCa	.883	.830	.766	.826	.503	.501	.462	.489
Mean	PKCa	1.179	1.302	1.194	1.225	.635	.626	731	664
	KCa	.983	.911	.914	.936	.541	.601	.580	.574

TABLE 56.—*Analyses of variance of constituents in soybean leaves and stems, 1947–49*

LEAVES

Source of variation	Degrees of freedom	Mean squares [1]					
		Protein	Crude fiber	Nitrogen-free extract	Ash	Phosphorus	Calcium
Replications (R)	5	0. 8310	0. 4189	5. 7388	0. 2209	0. 000539	0. 112000
Treatments (T)	1	17. 0707**	. 1284	24. 3543**	. 0521	. 048621**	. 754871**
Years (Y)	2	51. 5469**	11. 3838**	49. 7838**	. 3394	. 006656	. 008167
Y×T	2	32. 8877**	2. 3561*	20. 4601**	1. 0055*	. 001212**	. 028553**
R×T	5	1. 7693	. 1152	1. 7759	. 2240	. 000204	. 015009**
R×Y	10	. 9594	. 2656	4. 3871	. 2050	. 000517	. 005054*
R×T×Y	10	. 4458	. 3041	. 8342	. 1586	. 000027	. 001462
Pooled error	25	. 911932	. 2509	2. 4437	. 1902	. 0002584	. 005608

STEMS

Source of variation	Degrees of freedom	Protein	Crude fiber	Nitrogen-free extract	Ash	Phosphorus	Calcium
Replications (R)	5	0. 6485	1. 5621	4. 2106	0. 7564	0. 000036	0. 053452
Treatments (T)	1	10. 0807**	16. 2812**	1. 0541	. 0312	. 017867**	. 073261**
Years (Y)	2	1. 5963*	28. 5306**	36. 3554**	1. 4362	. 003303**	. 014125
Y×T	2	36. 6569**	2. 5652	25. 4462**	. 6682	. 000839	. 011779
R×T	5	. 5810	1. 3513	2. 6651	. 0849	. 000038	. 009848
R×Y	10	. 2937	3. 0420	5. 0245	. 1110	. 000102	. 005400
R×T×Y	10	. 1306	1. 3573	2. 0562	. 0501	. 0000453	. 001255
Pooled error	25	. 26589	2. 02996	3. 3653	. 81388	. 0000664	. 004631

[1] *= Significant at the 5-percent level; **= significant at the 1-percent level.

TABLE 57.—*Average weight and analysis of variance of soybean plants; air-dried basis*

EXPERIMENTAL DATA

Replication	Fertilizer treatment of plots	Average weight of plant			
		1947	1948	1949	Mean
		Grams	*Grams*	*Grams*	*Grams*
1	PKCa	23.20	25.12	13.06	20.46
	KCa	6.95	17.65	2.82	9.14
2	PKCa	14.45	27.25	17.88	19.86
	KCa	8.60	13.21	4.02	8.61
3	PKCa	17.60	23.30	18.83	19.91
	KCa	6.20	8.45	3.28	5.98
4	PKCa	16.95	19.09	16.04	17.36
	KCa	7.10	8.82	2.66	6.19
5	PKCa	17.60	19.91	10.88	16.13
	KCa	8.90	9.30	2.86	7.02
6	PKCa	19.40	21.70	11.23	17.44
	KCa	7.85	10.38	4.39	7.54
Mean	PKCa	18.20	19.11	14.65	18.53
	KCa	7.60	11.30	3.34	7.41

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean squares [1]
Replications (R)	5	10.2585
Treatments (T)	1	1,111.6673**
Years (Y)	2	192.9655**
T×Y	2	.6033
R×T	5	4.0481
R×Y	10	9.0937
R×T×Y	10	5.8327
Pooled error	25	6.7802

[1] **= Significant at the 1-percent level

TABLE 58.—*Average daily gain per lamb and analysis of variance for experiments 1 and 2*

70-DAY FEEDING TRIAL, LAMB EXPERIMENT 1, 1945 SOYBEAN CROP

Daily gain from fertilizer treatment					
PKCa plots			KCa plots		
Pen 1	Pen 2	Pen 4	Pen 3	Pen 5	Pen 6
Pound	*Pound*	*Pound*	*Pound*	*Pound*	*Pound*
0.257	0.229	0.114	0.186	0.129	0.129
.129	.243	.129	.214	.000	.100
.314	−.071	.000	.300	.100	.229
.100	.243	.171	.371	.229	.129
.171	.229	.129	.229	.171	.214
.194	.174	.109	.260	.126	.160

63-DAY FEEDING TRIAL, LAMB EXPERIMENT 2, 1946 SOYBEAN CROP

Pen 3	Pen 4	Pen 5	Pen 1	Pen 2	Pen 6
0.190	0.286	0.175	0.302	0.159	[1] 0.226
.492	.095	.556	.127	.127	.286
.175	.238	.302	.079	.111	.254
.349	.222	.381	.190	.222	.270
.111	.317	.333	.349	.079	.095
.263	.232	.349	.209	.139	.226

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean squares	
		Experiment 1	Experiment 2
Pens	5		
Treatments (T)	1	0.003921	0.060390
Pens within treatments	4	.017175	.014558
Animals within pens	24	.007874	.012574

[1] Missing plot value.

TABLE 59.—*Composition of rations, dry-weight basis, feed intake by lambs, and digestion coefficients, lamb experiment 1, 1945 soybean crop*

HAY FROM PKCA-FERTILIZED PLOTS

Lamb No.	Proximate composition of rations					Feed intake		Digestion coefficients						
	Crude protein	Ether extract	Crude fiber	N. f. e.[1]	Ash	Hay	Soy-beans	Dry matter	Crude protein	Ether extract	Crude fiber	N. f. e.[1]	T.D.N.[2]	Ash
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	26. 43	10. 25	20. 53	36. 99	5. 81	6, 348	4, 536	65. 09	82. 80	89. 34	54. 44	57. 71	75. 01	26. 29
4	24. 25	9. 10	22. 03	36. 95	7. 66	8, 559	4, 536	61. 58	80. 45	87. 10	52. 95	53. 96	68. 95	33. 09
10	28. 02	11. 57	20. 11	33. 02	7. 28	7, 472	4, 536	61. 41	80. 85	88. 76	49. 24	50. 33	72. 28	27. 08
16	24. 79	9. 63	22. 00	36. 31	7. 27	9, 025	4, 536	64. 67	81. 52	89. 45	59. 61	54. 21	72. 39	41. 77
29	30. 58	11. 99	17. 39	33. 67	6. 39	6, 169	4, 536	63. 30	82. 37	88. 83	46. 87	54. 02	75. 49	17. 79
34	28. 09	12. 63	18. 94	34. 37	5. 97	5, 686	4, 536	59. 70	81. 71	88. 49	46. 91	44. 09	72. 14	25. 66
Mean	27. 03	10. 86	20. 17	35. 21	6. 73	7, 210	4, 536	62. 63	81. 62	88. 66	51. 67	52. 39	72. 71	28. 61

HAY FROM KCA-FERTILIZED PLOTS

Lamb No.	Crude protein	Ether extract	Crude fiber	N. f. e.[1]	Ash	Hay	Soy-beans	Dry matter	Crude protein	Ether extract	Crude fiber	N. f. e.[1]	T.D.N.[2]	Ash
13	24. 71	10. 63	20. 97	37. 60	6. 09	7, 507	4, 536	68. 64	83. 79	91. 13	56. 29	63. 29	78. 10	43. 52
18	22. 78	9. 28	22. 52	37. 99	7. 43	9, 263	4, 536	63. 79	81. 72	89. 03	56. 47	55. 93	71. 17	39. 75
22	21. 20	8. 61	24. 80	39. 86	5. 53	8, 854	4, 536	65. 47	80. 16	88. 99	64. 80	58. 51	73. 63	25. 76
25	22. 29	8. 50	24. 09	38. 39	6. 73	9, 042	4, 536	64. 81	80. 58	87. 16	60. 98	57. 18	71. 27	41. 54
28	23. 03	9. 56	23. 25	38. 21	5. 95	6, 105	4, 536	63. 31	80. 21	88. 75	59. 82	54. 40	72. 26	27. 89
30	24. 23	9. 10	22. 00	38. 58	6. 09	7, 945	4, 536	67. 19	82. 75	91. 99	59. 58	61. 11	75. 57	34. 22
Mean	23. 04	9. 28	22. 94	38. 44	6. 30	8, 119	4, 536	65. 52	81. 54	89. 51	59. 66	58. 40	73. 67	35. 45

[1] Nitrogen-free extract.

[2] Total digestible nutrients.

TABLE 60.—*Analysis of variance of the digestion trial data, lamb experiment 1, 1945 soybean crop*

UNADJUSTED ANALYSIS

Source of variation	Degrees of freedom	Mean squares [1]					
		Crude protein	Ether extract	Crude fiber	N. f. e.[2]	T. D. N.[3]	Ash
Treatment	1	0.020	2.150	191.36**	108.60*	2.746	140.08
Error	10	1.509	1.896	17.37	16.47	6.500	60.00
Standard deviation (s)		1.25	1.38	4.16	4.06	2.55	7.75

ADJUSTED ANALYSIS [4]

Source of variation	Degrees of freedom	Crude protein	Ether extract	Crude fiber	N. f. e.	T. D. N.	Ash
Treatment	1	1.898	4.491	5.896	35.35	11.836	191.35*
Error	8	1.437	2.018	12.456	19.42	5.847	24.39
Standard deviation (s)		1.20	1.42	3.53	4.41	2.42	4.99

[1] *=Significant at the 5-percent level; **=significant at the 1-percent level.
[2] Nitrogen-free extract.
[3] Total digestible nutrients.
[4] Adjusted to the mean soybean intakes and mean bull paspalum grass hay intakes.

TABLE 61.—*Coefficients of apparent digestibility of indicated nutrients,*[1] *digestion trial, lamb experiment 2, 1946 soybean crop*

Lamb No.	Feed intake[2]		Dry matter	Crude protein	Ether extract	Crude fiber	N. f. e.[3]	T. D. N.[4]	Ash
	Hay	Cerelose							
	Grams	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
HAY FROM PKCA-FERTILIZED PLOTS									
2	7, 500	------	55. 50	73. 53	81. 68	43. 30	54. 55	53. 42	45. 70
	7, 000	2, 340	55. 12	68. 53	81. 79	44. 64	56. 33	53. 56	39. 20
3	[5]7, 500	------	60. 32	76. 39	81. 72	48. 02	62. 02	57. 00	42. 69
	7, 000	2, 340	53. 27	70. 95	79. 47	36. 63	54. 75	50. 44	42. 16
4	8, 000	------	58. 74	78. 09	82. 14	44. 49	57. 55	56. 19	44. 33
	7, 500	2, 500	51. 96	69. 34	80. 40	34. 17	51. 23	49. 53	48. 13
7	8, 000	------	58. 27	73. 47	80. 17	42. 93	62. 76	54. 77	43. 44
	7, 500	2, 500	54. 37	69. 19	77. 90	36. 88	59. 13	50. 99	43. 78
9	4, 801	------	56. 67	73. 16	79. 23	42. 64	57. 92	53. 09	48. 93
	4, 692	1, 660	54. 78	66. 83	79. 78	42. 53	56. 40	51. 53	46. 01
10	8, 000	------	53. 42	72. 78	83. 72	39. 63	56. 33	52. 18	25. 96
	7, 500	2, 500	49. 48	70. 76	78. 91	35. 98	46. 47	47. 23	37. 18
HAY FROM KCA-FERTILIZED PLOTS									
1	5, 500	------	51. 91	69. 16	82. 17	33. 91	54. 68	50. 47	37. 04
	7, 500	2, 500	52. 36	70. 87	81. 03	37. 87	53. 40	51. 23	32. 68
5	8, 000	------	56. 94	74. 64	79. 29	36. 59	60. 70	53. 68	48. 47
	7, 000	2, 340	53. 05	71. 16	77. 83	32. 13	56. 39	50. 27	45. 49
6	4, 000	------	55. 24	67. 55	77. 68	41. 20	60. 79	49. 63	44. 99
	6, 000	2, 000	51. 36	64. 84	75. 11	38. 01	57. 11	46. 88	36. 87
8	8, 000	------	58. 81	76. 10	81. 63	39. 92	62. 75	55. 49	48. 00
	7, 500	2, 500	52. 26	65. 61	78. 06	36. 45	55. 84	49. 92	39. 46
11	8, 000	------	57. 87	71. 14	80. 87	46. 21	61. 62	55. 98	34. 92
	7, 500	2, 500	52. 34	65. 63	77. 51	42. 54	54. 04	50. 95	30. 96
12	4, 393	------	56. 07	68. 92	80. 10	48. 91	58. 65	53. 13	33. 02
	7, 500	2, 500	53. 11	67. 95	77. 19	47. 13	54. 45	50. 73	28. 73

[1] For composition of hay in digestion trial rations, see tables 42, 43, 44, 45; for analyses of variance, see table 37.

[2] Two collection periods—with hay alone and with hay plus Cerelose.

[3] Nitrogen-free extract.

[4] Total digestible nutrients.

[5] 2×3,750, intake for 5-day collection, all other feed intake values based on 10-day collection period.

TABLE 62.—*Protein content and coefficients of apparent digestibility of protein in soybean hays and statistical analysis data, digestion trial, lamb experiment 2, 1946 soybean crop*

HAY FROM PKCA-FERTILIZED PLOTS

Lamb No.	No hay supplement		Cerelose supplement [1]	
	Crude protein	Digestible protein	Crude protein	Digestible protein
	Percent	*Percent*	*Percent*	*Percent*
2	18. 37	73. 53	13. 78	68. 53
3	20. 17	76. 39	15. 55	70. 95
4	22. 08	78. 09	16. 58	69. 34
7	18. 42	73. 47	13. 84	69. 19
9	19. 38	73. 16	15. 25	66. 83
10	20. 69	72. 78	15. 54	70. 76

HAY FROM KCA-FERTILIZED PLOTS

Lamb No.	Crude protein	Digestible protein	Crude protein	Digestible protein
1	20. 47	69. 16	15. 39	70. 87
5	19. 06	74. 64	14. 82	71. 16
6	16. 90	67. 55	12. 69	64. 84
8	20. 20	76. 10	15. 17	65. 61
11	17. 90	71. 14	13. 45	65. 63
12	17. 05	68. 92	12. 79	67. 95
PKCa mean	19. 85	74. 57	15. 09	69. 27
KCa mean	18. 60	71. 25	14. 05	67. 68

STATISTICAL ANALYSIS

Source of variation	Analysis of variance		Covariance analysis	
	Degrees of freedom	Mean squares [2]	Degrees of freedom	Mean squares
Total	23		22	
Fertilizers (F)	1	36. 140*	1	7. 575
Lambs between plots	10	7. 622	9	4. 179
Supplements (S)	1	118. 240**	1	1. 0152
F×S	1	4. 480	1	
Within lambs	10	5. 596	9	6. 087

[1] Composition values calculated from hay protein values.
[2] *=Significant at the 5-percent level; **=significant at the 1-percent level.

TABLE 63.—*Nitrogen balance of 12 lambs on soybean hay and analysis of variance, digestion trial, lamb experiment 2, 1946 soybean crop*

HAY FROM PKCA-FERTILIZED PLOTS

Lamb No.	No supplement	Cerelose supplement
	Grams	*Grams*
2	+4.3	+36.5
3	+9.3	+19.8
4	+30.0	+25.6
7	+23.3	+19.5
9	−14.4	+15.6
10	+21.2	+14.1

HAY FROM KCA-FERTILIZED PLOTS

Lamb No.	No supplement	Cerelose supplement
1	−7.9	+46.9
5	+27.4	+15.6
6	−18.9	+25.3
8	+15.4	+30.3
11	+14.5	+26.7
12	−14.1	+24.4
PKCa mean	12.3	21.9
KCa mean	2.7	28.2

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean squares [1]
Total	23	-------
Fertilization (F)	1	15.36
Lambs between plots	10	162.67
Supplements (S)	1	1 841.00*
F×S	1	379.21
Within lambs	10	231.99

[1] *=Significant at the 5-percent level.

TABLE 64.—*Nitrogen intake and urinary nitrogen excretion by 12 lambs on soybean hay and statistical analysis data, digestion trial, lamb experiment 2, 1946 soybean crop*

HAY FROM PKCA-FERTILIZED PLOTS

Lamb No.	No supplement		Cerelose supplement	
	N intake	Urine N	N intake	Urine N
	Grams	*Grams*	*Grams*	*Grams*
2	202. 5	146. 6	189. 0	93. 2
3	221. 3	150. 4	206. 6	126. 8
4	259. 2	172. 4	250. 5	150. 4
7	216. 8	136. 0	203. 3	121. 2
9	135. 4	113. 4	133. 7	73. 8
10	243. 2	155. 8	228. 0	147. 2

HAY FROM KCA-FERTILIZED PLOTS

Lamb No.	N intake	Urine N	N intake	Urine N
1	166. 1	122. 8	226. 5	113. 6
5	231. 2	145. 2	202. 3	128. 4
6	99. 2	85. 8	149. 4	71. 8
8	237. 6	165. 4	222. 8	115. 8
11	210. 4	135. 2	197. 3	102. 8
12	110. 1	90. 0	188. 3	103. 6
PKCa mean	213. 1	145. 4	201. 9	118. 8
KCa mean	175. 8	124. 1	197. 8	106. 0

STATISTICAL ANALYSIS

Source of variation	Analysis of variation		Covariance analysis	
	Degrees of freedom	Mean squares [1]	Degrees of freedom	Mean squares [1]
Total	23		22	
Fertilization (F)	1	1, 747. 62	1	152. 819
Lambs between plots	10	1, 148. 87	9	38. 270
Supplements (S)	1	3, 001. 61**	1	3,838. 701**
F × S	1	110. 94	1	661. 688**
Within lambs	10	178. 84	9	56. 56

[1] ** = Significant at the 1-percent level.

TABLE 65.—*Phosphorus intake and phosphorus absorption of 12 lambs on soybean hay and statistical analysis data, digestion trial, lamb experiment 2, 1946 soybean crop*

HAY FROM PKCA-FERTILIZED PLOTS

Lamb No.	No supplement		Cerelose supplement	
	P intake	P absorbed	P intake	P absorbed
	Grams	*Grams*	*Grams*	*Grams*
2	10.500	1.687	9.770	0.573
3	10.126	3.270	9.450	2.781
4	13.520	.027	12.525	2.861
7	11.920	−2.363	11.175	2.449
9	8.234	.698	8.178	.093
10	11.600	1.750	10.875	.139

HAY FROM KCA-FERTILIZED PLOTS

Lamb No.	P intake	P absorbed	P intake	P absorbed
1	7.865	−1.960	10.725	0.861
5	9.680	1.316	8.470	1.139
6	3.840	−.835	5.760	−1.670
8	11.440	2.494	10.725	−.210
11	11.120	1.538	10.425	1.079
12	5.640	.485	9.960	.674
PKCa mean	10.983	.845	10.329	1.483
KCa mean	8.264	.506	9.344	.312

STATISTICAL ANALYSIS

Source of variation	Analysis of variance		Covariance analysis	
	Degrees of freedom	Mean squares	Degrees of freedom	Mean squares
Total	23	2.24703	22	----------
Fertilization (F)	1	3.4156	1	0.4798
Lambs between plots	10	2.23297	9	1.9826
Supplements (S)	1	.2952	1	----------
F × S	1	1.0384	1	2.3527
Within lambs	10	2.46029	9	2.5470

TABLE 66.—*Outline of feeding trial, lamb experiment 3, first phase, 1947 soybean crop*

Source of lambs	Ration supplement	PKCa plot No.[1]	Sex [2]	KCa plot No.[1]	Sex [2]
Tidewater station, N. C.	Corn	1+	W	1–0	E
		2+	W	2–0	W
		5+	E	5–0	E
	Cerelose	1+	E	1–0	E
		2+	W	2–0	W
		5+	E	5–0	E
Raleigh, N. C.	Corn	3+	E	3–0	W
		4+	W	4–0	W
		6+	E	6–0	E
	Cerelose	3+	W	3–0	W
		4+	W	4–0	W
		6+	[3] E	6–0	E

[1] Plots 1+ and 1–0, 2+ and 2–0, etc., are adjacent plots.
[2] W=Wether lamb; E=ewe lamb.
[3] This ewe lamb was not placed on experiment, because it made abnormally low gains while with its dam.

TABLE 67.—*Average daily feed intakes and average daily gains, and statistical analysis, feeding trial (averaging 16 weeks), lamb experiment 3, first phase, 1947 soybean crop*

EXPERIMENTAL DATA

Replications	Hay from PKCa-fertilized plots				Hay from KCa-fertilized plots			
	Cerelose supplement		Corn supplement		Cerelose supplement		Corn supplement	
	Intake	Gains	Intake	Gains	Intake	Gains	Intake	Gains
	Pounds	*Pound*	*Pounds*	*Pound*	*Pounds*	*Pound*	*Pounds*	*Pound*
1	1.463	0.139	1.673	0.195	1.283	0.031	2.086	0.353
2	1.056	.001			1.046	-.170	2.038	.263
3	2.074	.191	2.382	.304			2.299	.270
4	1.294	.021	1.781	.186	1.287	-.004	2.165	.285
5	1.485	.194	2.202	.298	.921	-.052	1.164	.127
6			2.028	.241	1.631	.058	1.816	.185
Mean	1.474	.109	2.013	.245	1.234	-.026	1.928	.251

OVERALL STATISTICAL ANALYSIS

Source of variation	Analysis of variance	
	Degrees of freedom	Mean squares [1]
Total	20	0.14213
Fertilization (F)	1	.014213
Supplement (S)	1	.222735**
F×S	1	.031439*
Error	17	.006579

STATISTICAL ANALYSIS BY SUPPLEMENTS

Source of variation	Analysis of variance				Covariance analysis			
	Cerelose supplement		Corn supplement		Cerelose supplement		Corn supplement	
	Degrees of freedom	Mean squares [1]	Degrees of freedom	Mean squares	Degrees of freedom	Mean squares	Degrees of freedom	Mean squares
Total	9		10		8		9	
Fertilization	1	0.0455*	1	0.0101	1	0.0149	1	0.0010
Error	8	.0084	9	.0050	7	.0040	8	.0014

[1] *= Significant at 5-percent level; **= significant at 1-percent level.

TABLE 68.—*Average daily feed intakes and daily gains per lamb with and without dicalcium phosphate supplement, feeding trial (64-day duration), lamb experiment 3, second phase, 1947 soybean crop; and statistical analysis data*

EXPERIMENTAL DATA

Field replication No.	Fertilizer treatment of plot	$CaHPO_4$ supplement	Average daily feed intake	Average daily gain
			Pounds	*Pounds*
1	PKCa	0	1.850	0.063
4	do	0	1.659	.109
Mean			1.745	.086
5	KCa	0	1.730	.109
6	do	0	1.998	.016
Mean			1.864	.063
2	PKCa	[1] +	1.678	.188
3	do	+	2.890	.219
Mean			2.284	.204
1	KCa	+	2.389	.219
4	do	+	1.813	.219
Mean			2.101	.219

STATISTICAL ANALYSIS

Source of variation	Analysis of variance		Covariance analysis	
	Degrees of freedom	Mean squares [2]	Degrees of freedom	Mean squares [2]
Total	7			
Fertilization (F)	1	0.000032		
$CaHPO_4$ supplement (S)	1	.037538**	1	0.028377*
F×S	1	.000760		
Error	4	.001466	3	.0019353

[1] Total P content of rations adjusted to 0.25 percent.
[2] *= Significant at the 5-percent level; **= significant at the 1-percent level.

TABLE 69.—*Inorganic phosphorus and analysis of variance in the blood serum of lambs, feeding trial, lamb experiment 3, second phase, 1947 soybean crop*

EXPERIMENTAL DATA

Treatment No.	Fertilizer treatment of plots	$CaHPO_4$ supplement [1]	Inorganic phosphorus per 100 ml. of serum when collected on—					
			9/10/48	9/23/48	10/7/48	10/22/48	11/4/48	Mean
			Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
1	PKCa	+	7. 94	7. 02	11. 07	7. 46	8. 06	8. 32
	PKCa	+	4. 83	6. 49	7. 24	6. 60	6. 78	6. 39
2	KCa	+	5. 18	5. 40	6. 93	7. 56	6. 83	6. 38
	KCa	+	4. 12	6. 86	6. 70	6. 49	8. 17	6. 47
3	PKCa	0	5. 49	5. 84	7. 01	5. 99	5. 36	5. 94
	PKCa	0	5. 10	4. 95	6. 96	6. 38	5. 67	5. 81
4	KCa	0	4. 13	4. 81	3. 91	2. 83	3. 20	3. 78
	KCa	0	6. 40	5. 78	6. 62	3. 84	5. 43	5. 61

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean squares [2]
Total	39	----------
Treatment (T)	3	12. 29
Fertilization (F)	1	11. 08
P supplement (P)	1	25. 65*
F×P	1	. 16
Periods (Pds)	4	2. 99
Lambs within (error A)	4	4. 44
T×P	12	1. 43
Treatment 1:		
Linear	1	1. 10
Quadratic	1	2. 87*
Treatment 2:		
Linear	1	8. 70**
Quadratic	1	. 882
Treatment 3:		
Linear	1	. 303
Quadratic	1	2. 21
Treatment 4:		
Linear	1	2. 98*
Quadratic	1	----------
Remainder (error B)	16	. 575

[1] Total P content of ration raised to 0.25 percent.

[2] *= Significant at the 5-percent level; **= significant at the 1-percent level.

TABLE 70.—*Outline of feeding trial, lamb experiment 4, 1947 soybean crop*

Lamb and replication pair No.	Ration symbol [1]	Pen No.	Initial weight
Replication 1: [2]			*Pounds*
4	up	1	59
4	fup	2	58
2	fp	3	48
2	p	4	52
1	(1)	5	55
1	f	6	53
3	fu	7	56
3	u	8	55
Replication 2: [3]			
2	fp	9	61
2	p	10	61
1	(1)	11	55
1	f	12	55
3	u	13	58
3	fu	14	58
4	fup	15	54
4	up	16	51

[1] See table 22 for explanation of symbols.
[2] Ewe lambs.
[3] Wether lambs.

TABLE 71.—*Total feed intakes and gains per lamb, feeding trial (18-week duration), lamb experiment 4, 1947 soybean crop; and statistical analysis data*

EXPERIMENTAL DATA

Ration symbol [1]	Replication 1, ewe lambs		Replication 2, wether lambs		Means	
	Intake	Gain	Intake	Gain	Intake	Gain
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
(1)	209.3	11.2	286.9	27.2	248.1	19.2
f	252.4	26.1	302.1	30.6	277.3	28.4
p	259.1	24.4	273.2	24.0	266.2	24.2
fp	287.5	31.0	276.3	24.4	281.9	27.7
u	241.5	13.2	246.8	15.4	244.2	14.3
fu	201.1	18.8	274.6	20.1	237.9	19.5
up	286.6	27.9	270.7	29.9	278.6	28.9
fup	255.7	20.8	253.0	20.8	254.4	20.8

STATISTICAL ANALYSIS

Source of variation	Analysis of variance		Covariance analysis	
	Degrees of freedom	Mean squares [2]	Degrees of freedom	Mean squares [2]
Total	15	36.934	14	14.98
Replications (R)	1	22.57		
Treatments (T)	7	55.54		
f	1	23.52	1	16.00
p	1	103.02*	1	27.14
u	1	64.00	1	18.18
f×p	1	89.30	1	55.46
KCa comparisons p	1	192.08*	1	71.43*
PKCa comparisons u	1	124.82*	1	22.28
R×T (error)	7	20.38	6	12.60

[1] See table 22 for explanation of symbols.
[2] *=Significant at the 5-percent level.

TABLE 72.—*Inorganic phosphorus in the blood serum of lambs fed different rations and analysis of variance, lamb experiment 4, 1947 soybean crop*

EXPERIMENTAL DATA

Ration symbol [1]	Replication [2]	Inorganic phosphorus per 100 ml. of serum when collected on—				Treatment mean	Treatment component		
		4/20/49	5/20/49	7/6/49	8/12/49		Linear (TL)	Quadratic (TQ)	Cubic (TC)
		Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*			
(1)	W	7.67	5.68	5.20	6.25	5.50	−4.74	+3.04	+0.02
	E	6.38	4.59	4.12	4.14		−7.19	+1.81	−0.83
f	W	8.68	6.70	6.24	5.49	6.52	−10.03	+1.23	−1.81
	E	6.86	6.76	6.09	5.30		−5.35	−0.69	+0.45
p	W	6.91	6.64	6.85	7.13	6.86	+0.87	+0.55	−0.41
	E	8.15	5.92	6.77	6.50		−4.10	+1.96	−4.20
fp	W	7.10	7.56	6.36	7.20	7.34	−0.90	+0.38	+3.70
	E	7.54	7.36	8.22	7.39		+0.42	−0.64	−2.76
u	W	10.65	6.43	9.53	5.04	6.55	−13.73	−0.27	−14.91
	E	6.93	4.17	6.11	3.51		−8.32	+0.16	−9.24
fu	W	7.76	5.63	7.81	7.05	6.59	+0.05	+1.37	−7.25
	E	7.00	5.60	6.51	5.75		−3.20	+1.00	−2.90
pu	W	8.81	6.76	5.11	7.17	6.83	−6.57	+4.11	+3.31
	E	6.78	6.55	6.46	7.01		+0.60	+0.78	+0.50
fpu	W	6.27	4.91	6.64	6.25	6.62	+1.67	+0.97	−5.21
	E	7.21	7.79	8.36	5.53		−4.47	−3.41	−3.39

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean squares [3]
Total	63	
Replications (R)	1	4.2487
Treatments (T)	7	2.1490
Phosphorus supplement (P)	1	6.2060
R×T (Error A)	7	2.9382
Periods (Pds)	3	7.2969*
Linear (L)	1	13.2000**
Quadratic (Q)	1	2.3850
Cubic (C)	1	6.3080*
R×Pds (Error B)	3	.5677
Pds×T trends	21	1.4063*
TL	7	1.4360*
PL	1	5.0130**
PC	1	2.9520*
UC (U=urea effect)	1	3.4550*
Remainder	11	.7420
R×Pds×T (Error C)	21	.5160

[1] See table 22 for explanation of symbols.
[2] W=Wether replication; E=ewe replication.
[3] *=Significant at the 5-percent level; **=significant at the 1-percent level.

TABLE 73.—*Total feed intake and gains of rabbits, feeding trials (10-week duration), rabbit experiment 1, 1947 soybean crops; and statistical analysis data*

EXPERIMENTAL DATA

Replications	Hay from PKCa-fertilized plots				Hay from KCa-fertilized plots			
	Cerelose supplement		Corn supplement		Cerelose supplement		Corn supplement	
	Intake	Gains	Intake	Gains	Intake	Gains	Intake	Gains
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
1	3, 610	638	3, 281	772	2, 879	239	2, 846	546
	3, 866	524	2, 176	443	3, 226	451	3, 039	574
2	4, 045	580	3, 662	826	3, 571	462	2, 588	538
	[1] 4, 045	[1] 580	2, 865	605	3, 180	367	2, 153	413
3	3, 613	387	2, 761	481	3, 325	429	2, 739	360
	3, 894	564	2, 953	614	4, 038	478	3, 332	704
4	3, 300	450	2, 337	445	3, 379	398	1, 580	201
	[1] 3, 300	[1] 450	2, 383	362	3, 282	381	2, 171	390
5	3, 589	507	3, 010	600	3, 816	410	[1] 2, 680	[1] 473
	4, 090	546	3, 026	652	3, 364	451	2, 680	473
6	3, 653	516	3, 724	815	3, 764	549	3, 086	654
	3, 695	566	2, 618	463	3, 396	508	2, 243	419
Mean	3, 725	528	2, 900	590	3, 435	434	2, 589	470

STATISTICAL ANALYSIS

Source of variation	Analysis of variance		Covariance analysis	
	Degrees of freedom	Mean squares [2]	Degrees of freedom	Mean squares [2]
Total	44			
Replications (R)	5	34, 114		
Fertilization (F)	1	121, 807*	1	7, 286
R×F (Error A)	5	9, 722	4	4, 163
Supplements (S)	1	34, 770	1	82, 330**
F×S	1	1, 281	1	907
Error B	10	7, 837	9	2, 473
Rabbits within (Error C)	21	15, 336		
Pooled Error (ABC)	36	12, 474	35	4, 070

[1] Animal died during experiment.

[2] *=Significant at the 5-percent level; **=significant at the 1-percent level.

TABLE 74.—*Statistical analysis of corn supplement and Cerelose supplement data, feeding trial, rabbit experiment 1, 1947 soybean crop*

CORN SUPPLEMENT ANALYSIS

Source of variation	Analysis of variance		Covariance analysis	
	Degrees of freedom	Mean squares	Degrees of freedom	Mean squares
Total	23			
Replications (R)	5	34, 768		
Fertilization (F)	1	74, 037	1	3, 598
R×F (Error A)	5	6, 328		
Rabbits within (Error B)	11	22, 248		
Pooled error (AB)	16	18, 663	15	2, 951

CERELOSE SUPPLEMENT ANALYSIS

Source of variation	Degrees of freedom	Mean squares	Degrees of freedom	Mean squares
Total	21			
Replications (R)	5	8, 654		
Fertilization (F)	1	49, 051	1	7, 502
R×F (Error A)	5	9, 760		
Rabbits within (Error B)	10	4, 590	9	
Pooled error (AB)	15	6, 926	14	4, 909

TABLE 75.—*Nutrient composition of rations as consumed, digestion trial, rabbit experiment 1, 1947 soybean crop, air-dry basis*

CORN-SUPPLEMENTED RATIONS

Replication	Fertilizer treatment of plot	Dry matter	Crude protein	Ether extract	Crude fiber	Ash	Nitrogen-free extract	Phosphorus
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	PKCa	90. 90	12. 44	3. 35	18. 94	3. 87	52. 30	0. 236
	KCa	93. 84	11. 19	3. 15	18. 29	3. 96	55. 22	. 170
2	PKCa	90. 78	12. 31	3. 54	18. 18	3. 79	52. 96	. 248
	KCa	91. 01	11. 63	3. 24	17. 71	3. 95	54. 48	. 178
3	PKCa	91. 30	12. 35	3. 35	19. 60	4. 05	51. 95	. 165
	KCa	90. 73	10. 98	3. 20	18. 87	3. 76	53. 92	. 110
4	PKCa	91. 88	11. 94	3. 86	18. 84	3. 67	53. 57	. 197
	KCa	93. 16	10. 44	3. 10	18. 25	3. 79	57. 58	. 186
5	PKCa	93. 06	12. 44	3. 10	19. 02	3. 81	54. 69	. 198
	KCa	91. 74	11. 00	2. 95	18. 70	3. 89	55. 20	. 140
6	PKCa	90. 92	12. 04	3. 27	19. 12	3. 57	52. 92	. 176
	KCa	93. 39	10. 66	3. 16	18. 36	4. 41	56. 84	. 137
Mean	PKCa	91. 47	12. 25	3. 41	18. 95	3. 79	53. 07	. 203
	KCa	92. 36	10. 98	3. 14	18. 36	3. 96	55. 87	. 154

CERELOSE-SUPPLEMENTED RATIONS

Replication	Fertilizer treatment of plot	Dry matter	Crude protein	Ether extract	Crude fiber	Ash	Nitrogen-free extract	Phosphorus
1	PKCa	91. 47	12. 88	5. 25	27. 74	5. 19	40. 41	0. 154
	KCa	91. 81	10. 38	4. 37	27. 00	5. 35	44. 71	. 107
2	PKCa	91. 17	11. 63	5. 85	28. 38	4. 64	40. 67	. 164
	KCa	93. 17	11. 63	5. 41	26. 86	5. 09	44. 18	. 120
3	PKCa	93. 15	12. 54	5. 55	28. 22	4. 96	41. 88	. 102
	KCa	90. 66	10. 98	5. 52	28. 16	5. 23	40. 72	. 053
4	PKCa	92. 34	12. 06	6. 14	27. 67	4. 63	41. 84	. 097
	KCa	92. 59	11. 38	5. 41	26. 23	5. 95	43. 62	. 069
5	PKCa	94. 43	14. 31	5. 38	28. 72	5. 40	40. 62	. 147
	KCa	93. 27	11. 81	5. 24	26. 69	5. 31	44. 22	. 068
6	PKCa	91. 59	12. 48	6. 03	28. 25	4. 79	40. 04	. 108
	KCa	93. 82	10. 54	5. 61	27. 14	5. 82	44. 71	. 085
Mean	PKCa	92. 36	12. 65	5. 70	28. 16	4. 94	40. 91	. 129
	KCa	92. 54	11. 12	5. 26	27. 01	5. 46	43. 69	. 084

TABLE 76.—*Coefficients of apparent digestibility of nutrients in soybean hay, with feed intakes, digestion trial, rabbit experiment 1, 1947 soybean crop; and analysis of variance data*

CORN-SUPPLEMENTED RATIONS

Replication	Fertilizer treatment of plots	Feed intake	Total digestible nutrients	Dry matter	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Ash
		Grams	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	PKCa	330	60.41	48.02	62.69	73.00	10.45	75.76	52.36
	KCa	326	55.48	54.60	51.16	77.14	7.32	71.60	53.17
2	PKCa	320	60.65	59.41	62.42	79.87	12.65	73.10	66.44
	KCa	320	54.36	62.78	64.72	87.80	12.52	77.24	68.52
3	PKCa	266	57.76	53.27	63.17	77.92	4.09	67.67	58.67
	KCa	267	59.91	58.96	59.61	79.92	7.77	75.28	58.08
4	PKCa	206	64.66	62.83	71.41	82.51	13.60	76.32	70.24
	KCa	207	63.99	62.81	60.06	80.79	16.21	77.71	54.66
5	PKCa	298	57.06	57.92	66.64	76.68	8.74	69.11	66.04
	KCa	299	59.38	61.11	61.82	79.09	12.16	72.73	63.30
6	PKCa	237	66.00	64.74	69.40	84.01	24.57	77.05	63.33
	KCa	238	66.66	66.36	71.16	85.11	17.82	80.27	63.64
Mean	PKCa		61.09	57.70	65.96	79.01	12.35	73.17	63.45
	KCa		59.96	61.10	61.42	81.49	12.30	75.81	60.23

CERELOSE-SUPPLEMENTED RATIONS

Replication	Fertilizer treatment of plots	Feed intake	Total digestible nutrients	Dry matter	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Ash
1	PKCa	329	50.31	48.37	69.94	70.79	5.96	64.17	67.53
	KCa	315	49.31	50.69	65.78	83.16	7.20	63.63	66.72
2	PKCa	381	58.21	54.99	69.60	83.14	31.05	59.92	67.60
	KCa	384	57.02	54.71	71.18	82.76	16.78	68.52	67.52
3	PKCa	320	55.89	53.35	69.48	82.26	16.80	67.66	68.27
	KCa	324	51.12	53.61	62.45	82.93	19.93	68.80	67.02
4	PKCa	265	58.05	54.60	74.42	83.70	15.31	68.90	73.78
	KCa	269	51.64	49.13	67.54	81.64	5.93	65.65	54.46
5	PKCa	298	54.32	52.41	72.78	83.92	13.48	66.10	71.81
	KCa	299	52.30	49.80	67.62	79.22	8.11	66.24	45.39
6	PKCa	318	59.99	56.24	73.00	85.53	23.99	68.56	65.80
	KCa	291	58.73	56.30	65.50	84.48	22.56	70.26	62.06
Mean	PKCa		56.13	53.33	71.54	83.06	18.27	65.89	69.13
	KCa		53.35	53.87	66.68	82.37	13.42	67.18	60.53

TABLE 76.—*Coefficients of apparent digestibility of nutrients in soybean hay, with feed intakes, digestion trial, rabbit experiment 1, 1947 soybean crop; and analysis of variance data*—Continued

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean squares for nutrients						
		Total digestible nutrients	Dry matter	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Ash
Replications (R)	5	40. 57	33. 05	31. 70	23. 00	118. 74	18. 41	25. 60
Fertilization (F)	1	22. 83	23. 42	132. 30	5. 63	35. 97	23. 23	188. 89
R×F (Error A)	5	2. 86	15. 25	15. 46	5. 81	16. 27	10. 49	66. 60
Supplements (S)	1	200. 86	201. 90	176. 20	34. 08	74. 20	379. 45	65. 04
F×S	1	4. 08	12. 26	. 17	16. 53	34. 51	2. 69	53. 73
Error B	10	8. 84	14. 88	11. 81	5. 18	37. 93	7. 46	36. 92

TABLE 77.—*Nitrogen balance, digestion trial, rabbit experiment 1, 1947 soybean crop; and analysis of variance data*

EXPERIMENTAL DATA

Replication	Corn-supplemented ration		Cerelose-supplemented ration	
	PKCa plots	KCa plots	PKCa plots	KCa plots
	Grams	*Grams*	*Grams*	*Grams*
1	1. 750	1. 575	2. 673	2. 858
2	1. 643	2. 691	2. 991	3. 203
3	. 468	. 514	2. 077	1. 268
4	1. 223	. 510	1. 146	1. 483
5	1. 450	1. 271	2. 006	2. 174
6	1. 792	. 801	1. 719	1. 548
Mean	1. 388	1. 227	2. 102	2. 090

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean squares [1]
Replications (R)	5	1. 5539**
Fertilization (F)	1	. 0452
R×F (Error A)	5	. 1740
Supplement (S)	1	3. 7272**
F×S	1	. 0327
Error B	10	. 1428
Error (A+B)	15	. 1532

[1] **= Significant at the 1-percent level.

TABLE 78.—*Phosphorus intakes and balances, digestion trial, rabbit experiment 1, 1947 soybean crop; and statistical analysis data*

CORN-SUPPLEMENTED RATIONS

Replication	KCa-fertilized plots		PKCa-fertilized plots	
	Intake	Balance	Intake	Balance
	Gram	*Gram*	*Gram*	*Gram*
1	0.5542	0.2054	0.7788	0.7646
2	.5696	.4869	.7936	.6096
3	.2937	.0662	.4389	.2189
4	.3850	.1916	.4058	.2489
5	.4186	.3463	.5900	.4202
6	.3261	.1794	.4171	.2680
Mean	.4245	.2460	.5707	.4217

CERELOSE-SUPPLEMENTED RATIONS

Replication	Intake	Balance	Intake	Balance
1	0.3371	0.1717	0.5067	0.3715
2	.4608	.4177	.6248	.5520
3	.1717	.1315	.3325	.2533
4	.1856	.0978	.2571	.1284
5	.2033	.0510	.4381	.1561
6	.2474	.1689	.3434	.1517
Mean	.2677	.1731	.4171	.2688

STATISTICAL ANALYSIS

Source of variation	Analysis of variance		Covariance analysis	
	Degrees of freedom	Mean squares [1]	Degrees of freedom	Mean squares
Replications (R)	5	0.08013298*	5	0.01042562
Fertilization (F)	1	.11054123*	1	.00732438
R×F (Error A)	5	.01600937	4	.01319139
Supplements (S)	1	.07643331**	1	.00887966
F×S	1	.00960000	1	.00850158
Error B	10	.00955263	9	.00592847
Error A+B	15	.01170488	14	.00490015

[1] *= Significant at the 5-percent level; **= significant at the 1-percent level.

TABLE 79.—*Total feed intake and gains of rabbits, feeding trial (8-week duration), rabbit experiment 2, 1947 soybean crop; and statistical analysis data*

EXPERIMENTAL DATA

Diet group [1]	Fertilizer treatment	Replication										Mean	
		1 (males)		4 (males)		2 (females)		3 (females)		5 (males)			
		Feed intake	Gains	Feed intake	Gains	Feed intake	Gains	Feed intake	Gains	Feed intake	Gains	Feed intake	Gains
		Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
1	PKCa	3, 874	474	3, 692	595	3, 730	595	3, 924	446	3, 729	337	3, 790	471
	KCa	4, 466	550	4, 110	559	4, 102	557	4, 085	477	3, 488	330	4, 050	495
2m	PKCa	4, 415	599	3, 897	479	4, 564	524	4, 436	684	3, 810	400	4, 244	537
	KCa	4, 149	487	3, 635	419	4, 299	472	4, 252	534	3, 466	245	3, 960	431
$3mc_1$	PKCa	4, 410	559	4, 140	598	4, 804	597	4, 149	340	3, 820	489	4, 277	517
	KCa	4, 028	647	4, 654	510	4, 890	616	4, 355	583	3, 679	229	4, 321	517
$4mc_2$	PKCa	4, 945	681	3, 787	641	4, 318	681	3, 690	580	3, 641	554	4, 076	627
	KCa	4, 139	443	4, 016	607	4, 122	595	3, 980	520	3, 768	457	4, 005	524
$5mc_3$	PKCa	4, 401	688	4, 714	822	4, 895	706	4, 331	755	3, 886	467	4, 445	688
	KCa	4, 762	703	4, 673	693	4, 855	684	3, 632	549	3, 893	64	4, 363	539
$6mc_4$	PKCa	4, 448	726	3, 499	528	4, 730	710	4, 005	629	4, 488	511	4, 234	621
	KCa	4, 367	338	4, 249	717	4, 551	691	4, 169	557	3, 940	371	4, 255	535
$7c_4$	PKCa	4, 571	760	3, 610	604	4, 396	656	4, 035	628	3, 623	660	4, 047	662
	KCa	4, 662	594	4, 040	599	4, 898	552	4, 393	751	3, 668	347	4, 332	569

STATISTICAL ANALYSIS

Source of variation	Analysis of variance		Covariance analysis	
	Degrees of freedom	Mean squares [2]	Degrees of freedom	Mean squares [2]
Total	69	----	----	----
Replications (R)	4	86,703**	4	70,822**
Treatments (T)	13	36,188**	----	----
Fertilization	1	94,062**	1	103,498**
Casein	1	127,238**	1	68,064**
R×T (Error)	52	10,179	51	9,021

[1] See table 31 for makeup of diet.
[2] **= Significant at the 1-percent level.

TABLE 80.—*Feed intake and gains of rabbits, feeding trial, rabbit experiment 3, paired feeding method, 1948 Roanoke soybean crop; and statistical analysis data*

EXPERIMENTAL DATA

Field replication	PKCa-fertilized plots		KCa-fertilized plots	
	Intake	Gains	Intake	Gains
	Grams	*Grams*	*Grams*	*Grams*
2	3,347 2,975	521 360	3,347 2,979	344 295
3	3,016 2,232	391 205	3,016 2,232	347 246
4	3,134 2,850	343 422	3,134 2,842	440 283
5	2,618 2,576	195 326	2,618 2,576	340 367
6	3,541 2,753	405 365	3,541 2,753	398 360
Mean	2,904	353	2,904	342

STATISTICAL ANALYSIS

Source of variation	Analysis of variance		Covariance analysis	
	Degrees of freedom	Mean squares	Degrees of freedom	Mean squares
Total	19			
Replications (R)	4	7,012		
Fertilization (F)	1	639	1	6,747
R×F	4	5,783	3	7,297
Remainder	10	6,247	9	4,172

TABLE 81.—*Feed intake and gains of rabbits, feeding trial, rabbit experiment 3, ad libitum feeding method, 1948 Roanoke soybean crop; and statistical analysis data*

EXPERIMENTAL DATA

Field replication	PKCa-fertilized plots		KCa-fertilized plots	
	Intake	Gains	Intake	Gains
	Grams	*Grams*	*Grams*	*Grams*
2	3, 287	479	3, 195	492
	3, 238	509	3, 430	444
3	3, 885	485	2, 914	477
	3, 207	451	3, 155	494
4	3, 652	500	3, 384	470
	3, 132	380	3, 288	357
5	3, 681	432	2, 993	369
	2, 973	468	3, 304	424
6	3, 588	525	3, 375	479
	2, 543	324	2, 690	371
Mean	3, 319	455	3, 174	438

STATISTICAL ANALYSIS

Source of variation	Analysis of variance		Covariance analysis	
	Degrees of freedom	Mean squares	Degrees of freedom	Mean squares
Total	19			
Replications (R)	4	3, 509		
Fertilization (F)	1	1, 549	1	2, 296
R×F	4	750	3	750
Remainder	10	4, 410	9	2, 474

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