EFFECT OF FERTILIZATION ON PASTURE GRASS

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THE EFFECT OF FERTILIZATION

ON THE NUTRITIVE VALUE OF PASTURE GRASS.

by

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The Effect of Fertilization on the Nutritive Value of Pasture Grass.

This paper reports a study of the nutritive value of fertilized vs. unfertilized pasture herbage, with particular reference to the quality of the protein, as measured by comparative feeding trials. A technique for the management of growing rabbits as experimental animals for such tests is also described.

Significant differences were found not only between certain pure species of grasses but also between the mixed herbage from fertilized and unfertilized pastures, which could not satisfactorily be explained on the basis of the usually advanced theories, viz., energy value, protein level and mineral (Ca. and P.) content of these feeds.

From the results obtained in these studies, the conclusions seem warranted that:

(1) quality of protein (amino acid balance) may be an important factor determining the nutritive value of pasture herbage and

(2) the improvement brought about through fertilization may be the result of a change in the constitution of its proteins.

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C O N T E N T S

ABSTRACT

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Significant differences were found not only between certain pure species of grasses but also between the mixed herbage of fertilized and unfertilized pastures, which could not satisfactorily be explained on the basis of the usually advanced theories viz., energy value, protein level and mineral (Ca. and P.) contents of these feeds.

From the results obtained in these studies, the conclusions seem warranted that:-

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THE EFFECT OF FERTILIZATION ON THE NUTRITIVE VALUE OF PASTURE GRASS.

1. INTRODUCTION.

Pasture improvement, through fertilization and improved management, is a problem that has been extensively studied during recent years. This is particularly true in countries where grasslands contribute the greater part of the foodstuffs for the raising of livestock. In Canada, possibly due to the short grazing season, making necessary the production of large quantities of roughages and grain concentrates for winter feeding, the pasture problem has received relatively little attention. In Quebec a considerable part of the pasture area consists of land that is too rough to cultivate and, in many cases, provides an inferior type of pasturage. Such pastures, if improved, would not only carry more livestock per acre but would provide a better quality of feed for our growing and milking stock which generally depend entirely on the pastures for their feed during the grazing season.

In view of the importance of this problem a committee was formed at Macdonald College in 1931, and investigational work was started, dealing with the effect of fertilizer treatment on yield, botanical and chemical composition, on permanent pastures of the Eastern Townships of Quebec. In 1932 the project was expanded to include a study of the nutritive value of herbage from fertilized and unfertilized areas. The results of the first two years of this latter phase of the work have been completed and form the basis of this thesis.

2. THE NUTRITIONAL PROBLEM IN PASTURES.

From the nutritional standpoint the botanical and chemical composition of the mixed pasture herbage are of special importance. Management, soil condition, climatic and seasonal variations are modifying factors which account for much of the variability in pasture studies.

Of the latter group, management is of outstanding importance. Jones (1933) reports that the management of a sward has an important influence on its botanical composition. Heavy grazing throughout the season, and particularly in the early spring, increases the clover content, while light grazing commencing late in the spring, promotes the growth of grass species.

The importance of management on the chemical composition, as effected through stage of growth, has been stressed by several workers. Hart, Guilbert and Goss (1932) conclude from analyses on pasture plants at various stages of growth, that the dry matter of the forage varies from a protein rich concentrate during early stages of growth to that of a poor roughage during later stages. Woodman et al. (1931) and Shutt (1929) also report stage of growth as an important factor in the chemical composition.

The importance of this work has been reflected in the vast amount of literature recently published on rotational grazing. Davies (1933), in discussing the subject, states, "the length of rest period and intensity of grazing are determined by the pasture type concerned and the demands of the species desired to encourage." Jones and Jones (1930) report that on natural pastures a four-week rest period provides greater carrying capacity and is more beneficial to the sward than a more drastic system. Woodman et al. (1931) arrived at a similar conclusion from an investigation on fertilizer treated pasture.

The importance of botanical composition in the nutritive value of pasturage is well recognized. The comparative richness of clovers in protein and calcium have long been known, and many farmers consider the percentage of clover in a pasture sward as indicative of its nutritive value. That this idea may not be entirely correct is pointed out in work reported by Hansson and Hagman (1932) in which they conclude that a white clover content of 15-20 per cent is sufficient to cover the protein requirements for heavy milking cows and that a clover content of 40-50 per cent. causes digestive disturbances in the grazing animals. Its importance in a mixed sward, however, and its high nutritive value as compared with other pasture species is strongly pointed out in the report by Stapledon (1933).

Similarly, grasses are commonly classified as desirable or undesirable species. In this connection

Stapledon (1933) reports that analyses show very little difference between species in respect to protein and fibre during early stages of growth, the distinguishing and important feature as between species and strains being the rapidity with which they fall in nutritive value as growth slackens. Similar results are reported by Woodman et al. (1926).

The digestibility of different pasture species has been reported by Sheehy (1932). His results show that there is no significant difference in the digestibility of the different food constituents in certain species of grasses and weeds.

Chemical studies on pasture herbage are divided into two distinct classes, those in which definite deficiency diseases occur in the grazing animals due to a deficiency in the herbage of certain mineral elements and those in which the mineral content of the herbage is sufficiently high to prevent the occurrence of such conditions.

The evidence in studies on mineral deficient herbage is clear cut. Definite proof that the deficiency of minerals is responsible for the low nutritive value of the herbage is established by the fact that the addition of these elements to the diet corrects the condition and induces growth. Similar results are obtained if the minerals are applied to the soil, thus increasing the mineral content of the herbage and thereby increasing its nutritive value.

The great majority of grasslands, however, cannot be classed as mineral deficient. Yet it is an established fact that there is considerable variability in the nutritive value of different pastures, and that, in many cases, the application of fertilizer increases the nutritive value of the herbage. The chemical changes generally reported as responsible for the increase in nutritive value are an increase in the percentage of protein, calcium and phosphorus and a decrease in the percentage of fibre. This assumes that natural pasture herbage, as the sole feed, does not satisfactorily meet the animal requirements from these nutrients. That such an assumption is open to question is evident from published literature. It may be well, therefore, to compare animal requirements for these nutrients with the quantities present in the herbage under natural conditions.

a. Mineral Requirements of Grazing Animals.

In experiments on the inorganic elements in nutrition Osborne and Mendel (1918) report that sodium, chlorine, magnesium and potassium, when present in the diet in traces are sufficient for growth. Calcium and phosphorus, however, were found to be required in considerable quantities.

Brown et al. (1932), working on the effects of various levels of calcium and phosphorus upon the production of rickets in rats, concludes that both the level and the ratio of calcium to phosphorus are necessary to adequately characterize the racketogenic properties of the diet. Bethke et al. (1932) reports a Calcium:Phosphorus ratio of 2:1 to 1:1 as the most favourable for growth, calcification and blood composition in the rat.

With regard to the quantities of calcium and phosphorus required by animals, Henderson and Weakley (1930) report, that for growing dairy animals, rations which contain less than .35 per cent of calcium and less than .20 per cent of phosphorus give rise to an abnormal bone condition. Orr, (1925) reports that dairy cows require 1.5 oz. of CaO and .8 oz. of P_2O_5 per 1,000 pounds live weight for maintenance and .55 oz. of CaO and .66 oz. of P_2O_5 per gallon of milk. This, expressed in per cent of an average ration,/ is approximately .5 per cent of calcium and .3 per cent of phosphorus. Archibald and Bennett (1933) report that in pasture herbage .15 per cent. of phosphorus is the lower limit of safety.

Orr (1929) reports, from analyses of herbage of various kinds of pastures, that good pastures will contain .7 per cent of calcium and .3 per cent of phosphorus.

Hart et al. (1923), in an experiment on the comparative efficiency of dry and green alfalfa in maintaining calcium and phosphorus equilibrium in milking cows reports that the question of whether positive or negative calcium balances prevail in liberally milking cows through the use of alfalfa hay as a carrier of calcium is determined by the quality of the hay. The term quality in this connection is used to designate the

(/ Average ration calculated from thumb rules of feeding - 1 lb. hay plus 3 lbs. silage per 100 lb. live weight, plus 1 lb. concentrate per 3 lbs. of milk).

degree of destruction in the curing process of the unknown factors affecting calcium assimilation. With positive calcium balances there were also positive phosphorus balances in these experiments.

Summarizing these results we find that while Orr's figures may represent an optimum calcium and phosphorus content in pasture herbage there is a lack of agreement among investigators as to the minimum quantities that will suit the requirements of animals. The evidence, however, indicates that the lower limits of safety are considerably below the optimum figures given by Orr. Also the properties of green plants which aid in the assimilation of calcium and the effect of sunlight are factors which may result in decreasing the importance of calcium and phosphorus balance in grazing animals. This may explain why the wide calcium-phosphorus ratio in clovers shows no detrimental effects in their nutritive value.

In Table I the chemical composition of the herbage of some representative natural pastures is given.

TABLE I. CHEMICAL COMPOSITION OF REPRESENTATIVE NATURAL PASTURES EXPRESSED IN PER CENT OF THE DRY MATTER - AVERAGE FOR SEASON.

Location of Pasture	Protein	Fibre	Ca.	P.
Masachusettes (1)	15.31	21.05	.57	.19
Vermont	19.05	17.87	.85	.27
England (cultivated)	17.65	23.00	.71	.32
Scotland (Natural)	15.63	24.56	.46	.29
" (Hill)	15.88	25.20	.40	.26
Montana (2)	17.60	24.30	.68	.29
Connecticut (3)	17.20	24.00	.60	.20
Quebec (4) (Unfertilized)	14.71	24.34	1.044	.37
" (Fertilized)	16.73	22.77	.922	.28

(1) Archibald & Bennett (1933).
(2) Graves et al. (1933).
(3) Brown. (1933).
(4) Herbage used in present investigation.

From comparison with the animal requirements it is not indicated that the calcium and phosphorus content of the herbage from these pastures would be a limiting factor in its nutritive value. It would thus seem justifiable to conclude that an increase in the calcium and phosphorus contents of pastures such as these would be of little nutritional significance.

b. Protein Requirements of Grazing Animals.

The failure of the animal body to store protein so that it can be called upon for utilization in the body when not adequately supplied in the diet has resulted in simplifying the problem of determining the optimum quantities required. Consequently the requirements of animals for protein have been much more definitely established than is the case for minerals. The results of investigational work in this field have been condensed in feeding standards of which Morrison's (1929) is representative. This standard indicates that the requirements of milking and growing stock, for total crude protein do not exceed 15 per cent of the dry matter of the ration, while for fattening and resting stock, the requirements are considerably lower. Further evidence is given in the recent work of Slonaker (1931) who reports that diets that gave the best results for growth in rats contained just over 14 per cent of protein. Referring again to Table I (page 7), we find that the crude protein content of herbage under average conditions is sufficiently high to meet these requirements.

Further evidence to substantiate this conclusion is given in tables II and III. The higher protein of the young grass over the lower protein of the grass hay in the case of Graves' work

TABLE II. NUTRITIVE VALUE OF PASTURE GRASS FOR MILK PRODUCTION (DATA FROM GRAVES et al. (1933)).

	Clipped≠ Pasture Grass	Grass ≠≠ Hay
Average per cent. protein (in dry matter)	13.1-20.5	9.9-14.9
Feed eaten per day (lbs.)	136.6	44.1
Dry Matter eaten per day (lbs.)	32.5	36.7
Weight of Cow (lbs.)	1,368.0	1,332.0
Production - Milk (lbs.)	30.0	40.6
" Fat (%)	3.9	4.1
T.D.N. eaten per day (lbs.)	23.2	23.0
T.D.N. required per day (lbs.) (Savage Std.)	21.0	25.3
D.C.P. eaten per day (lbs.)	4.38	2.87
D.C.P. required per day (lbs.) (Savage Std.)	2.88	3.63

(≠ Average 30 days growth. ≠≠ average 45 days growth.)

or the constantly high protein of the nitrogen treated pasture as compared with the untreated grass in which the protein dropped markedly during part of the season, reported by Watson et al. (Table III).

TABLE III. RATE OF GAIN OF SHEEP AND PER CENT PROTEIN IN FEED (PASTURE HERBAGE)≠ ARRANGED FROM DATA FROM WATSON et al. (1932).

STOCK GRAZED	DATE	NITROGEN TREATED PASTURE		NATURAL PASTURE	
		Ave. Daily Gain.	% Protein in Grass	Ave. Daily Gain	% Protein in Grass
Sheep (Shear-lings).	April 29	.40 lbs.	22.	-	22.
	May 13	.60 "	21.	.75	16.
	May 28	.25 "	21.	.30	15.
	June 11	.20 "	22.	.20	15.
	July 25	.08 "	20.	.20	20.

(≠ Approximately the same amounts of feed eaten in each group.)

was not reflected in higher milk yield in the former case nor in more rapid growth in the latter case.

The evidence thus presented is strongly suggestive that a quantitive increase in the protein content of pasture herbage above that usually found in natural pasture grass (15-20 per cent, depending on season etc.) does not necessarily increase its nutritive value.

c. Energy Value of Pasture Herbage.

The question of energy value should be given some consideration in a discussion of the nutritive value of pasture herbage. Jones and Jones (1930) report from grazing studies that the rate of live weight increment decreased as the season advanced and that this was correlated with a reduction in carbohydrates and an increase in the protein, phosphate and lime contents of the herbage. (It might be well to point out that the results from this investigation do not conflict with those of Watson et al, table III. The grazing season in this experiment commenced in May and continued until September, while Watson's data were obtained in the part of the season from April until July.)

Energy value is closely related to the dry matter content. It may, however, vary considerably between feeds of similar dry matter content due to differences in digestibility. Fibre, as a rule, is comparatively low in digestibility and may be considered as a dilutent to other nutrients in the diet. Sheehy (1932) concludes that the dry matter content of pasture herbage is indicative of its nutritive value and his data substantiates this conclusion as far as grasses and weeds are concerned. His digestion trials, however, did not include clover and this together with the fact that clovers carry a lower percentage of fibre than the grasses as pointed out by Stapledon (1933) may account for Sheehy's conclusions, with regard to the nutritive value of clovers, being contrary to Stapledon's results.

The data in table IV taken from digestion trials on pasture herbage indicate that the organic matter of young grass is highly digestible.

TABLE IV. COEFFICIENTS OF DIGESTIBILITY OF ORGANIC MATTER IN PASTURE GRASS.

Reported By	Interval between cutting.	Digestibility of organic Matter.
Woodman et al. (1931).	1 week	79.2%
	2 weeks	79.6%
	3 "	79.5%
	4 "	76.4%
Watson et al. (1932)		
N - treated	3 "	79.4%
Natural	3 "	77.2%

That certain classes of stock, however, may be unable to consume sufficient of this bulky material to meet their nutritive requirements, on diets composed entirely of such herbage, is an important consideration.

In Table V the amounts of total digestible nutrients consumed by lambs (Watson et al. 1932) and by cows in milk (Graves et al. 1933) are compared with Morrison's feeding standard requirements. The protein figures are included in this table to show that the energy value and not the protein content of the herbage appeared to have been the limiting factor in the nutritive value of the diets. In

TABLE V. POUNDS OF DRY MATTER, TOTAL DIGESTIBLE NUTRIENTS AND PROTEIN CONSUMED BY LAMBS AND COWS AS COMPARED WITH MORRISON'S FEEDING STANDARD REQUIREMENTS.

STOCK	Dry Matter	T.D.N.	D.C.P.
Amount eaten by lambs 88 lb. (1)			
N-Treated Herbage	1.9	1.3	.28
Natural	1.8	1.2	.19
Amount required	2.5-2.7	1.8-2.0	.12-.15
Amount eaten by cow 1,368 lb. (2) yielding 30 lbs. 3.9% milk.	32.5	23.2	4.38
Amount required		20.68	2.75

(1) Watson et al. (1932). (2) Graves et al. (1933).

the case of the lambs the digestible nutrients consumed were slightly below requirements while the protein was in excess. The requirements of cows producing thirty or forty pounds of milk were satisfactorily met in Graves' work but, as pointed out by this investigation, heavily milking cows would be unable to obtain sufficient total nutrients from diets composed solely of herbage, and it is evident that the total digestible nutrients consumed, rather than the protein intake, would be the limiting factor in the nutritive value of such diets.

While this is an important consideration in the nutritive value of pasture herbage, it is doubtful if fertilizer treatment can be expected to increase the total digestible nutrient content of the herbage to any extent. Brown, (1933) reports the dry matter content of herbage to be highest on poor plots receiving no phosphorus and lowest on plots receiving phosphorus plus lime or nitrogen. Watson et al. (1932) reports a reduction in the percentage of organic matter in nitrogen treated pastures and, while the digestibility of the organic matter was increased by the treatment of nitrogen, his data on starch equivalents clearly indicates no difference in the energy value of the nitrogen treated and the natural pasture.

In the light of the evidence presented, it would appear that the increase in nutritive value of pasture herbage due to fertilizer treatment or the differences in nutritive value of different natural pastures cannot be fully explained on the basis of differences in the quantity of protein, minerals or total digestible nutrients.

3. EXPERIMENTAL.

The failure of previous studies to explain adequately observed differences in the nutritive value of herbage from different pastures and differences resulting from the application of fertilizers, led to the suggestion that quality of protein might be an influencing factor in the nutritive value of pasturage. As a result a series of feeding tests were planned to determine, if possible:-

(1) whether pasture herbage might be deficient in any one of the essential amino acids,

and

(2) whether, possibly, the often observed increase in nutrtive value due to fertilizer treatment might be the result of an improved amino acid balance.

a. General Plan of Experiment.

The experiment involved the feeding of grass clippings with, and without, supplements of purified amino-acids or mixtures of two or more amino acids.

The difficulty in obtaining grass clippings in large quantities and the extreme cost of the amino acid supplements necessitated the use of laboratory animals in this study. Rabbits were chosen as the most suitable substitute for larger farm animals on account of the similarity of the diets of domestic rabbits to those of farm ruminants.

(1) Rations to be Used.

The grass clippings were obtained from two farms in the Eastern Townships of Quebec cooperating in the Macdonald College pasture project. The particular areas were selected for their comparative smoothness, enabling the clipping to be done with a lawn-mower. These areas had been in grass for more than ten years. They had never received fertilizer treatment and consequently would be classed as natural pastures.

In the spring of 1933 one half of each of these areas received a treatment of potash and super-phosphate (potash 100 lbs. per acre, super-phosphate 500 lbs. per acre) and were fenced to permit controlled grazing. Three clippings were made during the season and after each clipping the cows were allowed to graze, there being portions that were too rough to clip. The first clipping was taken during the second week in June and represented the spring's growth of grass. The pastures were then grazed for about two weeks, after which the cows were taken off to allow the grass to grow for the second clipping which was made during the last week in July. The grass at this time was not plentiful because of the exceptionally dry summer. As a result no clipping was taken in August. The areas were grazed after the second clipping but were kept ungrazed about a month previous to the third clipping which was made during the last week in September.

The clippings were dried in the field until in a suitable condition to ship, after which they were forwarded to Macdonald College and drying completed by spreading in a thin layer on a barn floor. When dried the grass was ground in a hammer mill and stored in bags until required for feeding.

The amino acids involved as supplements in these experiments were those which it is considered, cannot be synthesized in the animal body. Sherman (1932) reports cystine, histidine, lysine and tryptophane as nutritionally essential and states that there is a lack of agreement among investigators as to whether or not proline, tyrosine and phenylalanine need be supplied in the diet. Plimmer (1931-32) reports cystine, histidine, lysine and tryptophane as indispensable and tyrosine and phenylalanine as being interrelated. Rose (1931-32) presents evidence to show that growth promoting proteins contain at least one essential dietary component other than the twenty known amino acids.

Consequently the amino acids that were considered in this investigation were cystine, histidine, lysine, tryptophane and tyrosine.

(2) Type of Experiment and Feeding Equipment.

Since an accurate record of individual feed consumption seemed essential in this study, individual feeding was necessary.

The equipment used in this investigation was designed in the Animal Husbandry Department of Macdonald College to meet the particular requirements of rabbit feeding. The manufacturing was done by the firm of Henderson and Barwick of Montreal. The cages were built in batteries of six. The frame work is of angle iron, the sides and tops of galvanized iron and the floors one-half-inch mesh wire. Each pen is equipped with a tray for the collection of excreta.

A difficult problem in the use of rabbits in experimental feeding is to prevent the wastage of feed. Rabbits have a habit of scratching over their feed and in this way large quantities are often scratched out of an ordinary feeder on to the floor or tray of the pen. Not only is it impossible to accurately recover such feed but such wastage may represent a selected, or rather a rejected, portion of the diet rather than a fair sample, so that any attempt to credit such feed is questionable practice. This led to the designing of the special feeder used, which almost entirely prevented the animals from scratching. It was found that by feeding the grass in a ground condition, in this type of feeder, the wastage did not exceed one per cent of the amount of feed eaten. This improved feeder, however, had not been perfected for use throughout the first feeding trial.

The details of the feeding pens and equipment are clearly shown in the accompanying photographs.

PHOTO I. BATTERY OF PENS USED IN EXPERIMENTAL FEEDING.

PHOTO II. CLOSE UP OF SINGLE PEN SHOWING FEEDER, DRINKING CUP AND TRAY.

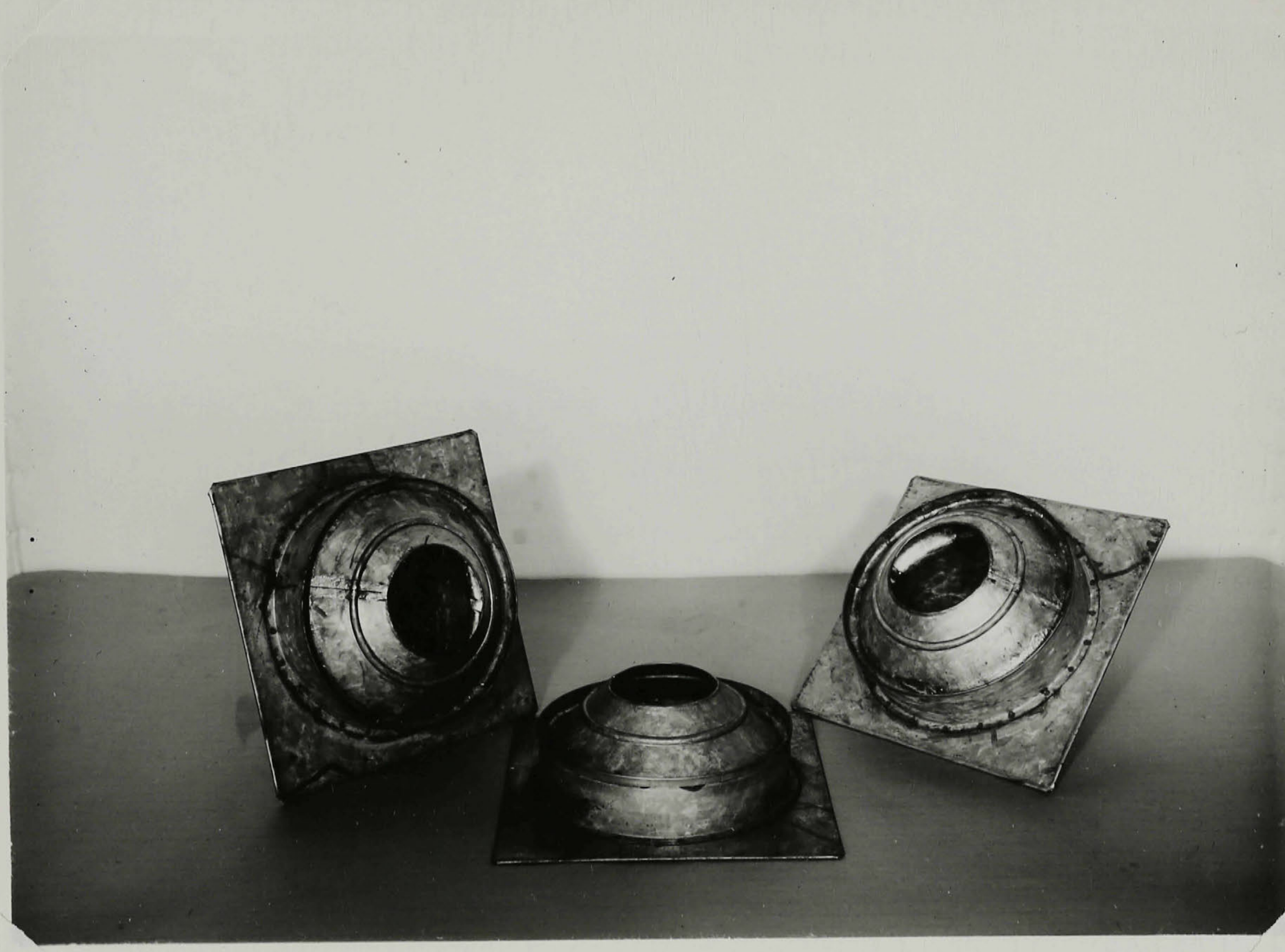

PHOTO. III. TYPE OF FEEDER DESIGNED FOR THE FEEDING OF GROUND GRASS CLIPPINGS.

4. FEEDING TRIAL NO. 1.

A Preliminary Test to Study the Use of Young Rabbits in Comparative Feeding Trials.

a. Object of Trial.

To study the adaptability of rabbits as experimental animals for use in comparative feeding tests on the nutritive value of pasture herbage.

b. Plan of Experiment.

The experiment was outlined to determine whether the nutritive value of reed canary grass could be increased by the addition of protein, carbohydrates, or minerals. It involved the feeding of seven groups of rabbits for a continuous period of forty days.

(1) Feeds used. The reed canary grass was clipped from pure pots at Macdonald College in the fall of 1932. At the time of cutting, the grass was about twelve inches high. It had reached a fairly advanced stage of maturity and had been slightly frozen so that it could not be considered a highly nutritious basic diet. It served, however, as a substitute for pasture herbage in this preliminary study.

The lactalbumin was a product of the Quinte Dairies Ltd., of Wellington, Ontario. Lactalbumin was chosen as a protein supplement on account of its well balanced amino acid content. Chemical analysis, however, showed this particular product to be rather crude with an ash content of over 23 per cent.

The dextrose was purchased from Merck and Co., of Montreal. The oats and minerals were obtained from the feed supply of the College stock farm.

(2) Rations fed. The details of the rations, together with their chemical analyses are given in tables VI, VIa and VIb.

TABLE VI. MIXTURES USED. FEEDING TRIAL NO. 1.

	Lot I	Lot II	Lot III	Lot IV	Lot V	Lot VI	Lot VII.
Reed Canary Grass	50	86.5	80.	96	100	98	95
Oats	50						
Dextrose		13.5	16.8				
Lactalbumin			3.2			2.	5.
Minerals				4			
Limestone 3 Bonechar 3 Salt 2							

TABLE VIa. ANALYSIS EXPRESSED ON AIR DRY BASIS

	Moisture	Crude Protein	Ether Extract	Crude Fibre	N-free Extract	Total Ash	Ca.	P.
Lot I	8.45	8.87	3.42	16.11	56.76	6.39	.235	.416
" II	7.08	6.45	2.36	18.79	57.10	8.22	.331	.286
" III	6.72	7.76	2.22	17.38	57.62	8.30	.553	.401
" IV	7.86	7.16	2.63	20.84	48.39		1.463	.529
" V	8.19	7.45	2.74	21.72	50.40	9.50	.383	.331
" VI	9.12	8.34	2.67	21.06	49.16	9.65	.525	.405
" VII	9.00	9.81	2.61	20.42	48.13	10.03	.745	.523

TABLE VIb. ANALYSIS EXPRESSED ON DRY MATTER BASIS.

	Crude≠ Protein	Ether≠ Extract	Crude≠ Fibre	N-free≠ Extract	Total≠ Ash	Ca.≠	P.≠≠
Lot I	9.69	3.73	17.60	62.00	6.98	.257	.454
" II	6.94	2.54	20.22	61.45	8.85	.356	.308
" III	8.32	2.38	18.63	61.77	8.90	.593	.430
" IV	7.77	2.85	22.62	52.52		1.588	.574
" V	8.12	2.98	23.65	54.90	10.35	.417	.360
" VI	9.18	2.95	23.17	54.08	10.62	.577	.445
" VII	10.78	2.87	22.44	52.89	11.02	.819	.575

≠ Official A.O.A.C. Analysis methods used.
≠≠ Fiske, Subbarow (1925) methods used.

The ration fed to Lot I constituted what was considered to be a normal diet, consisting, as it did, of equal parts of oats and hay. Lot II received grass plus an addition of dextrose to increase its energy value to approximately that of Lot I. The ration of Lot III contained sufficient dextrose to make the energy value approximately similar to that of Lots I and II. In addition lactalbumin was added in sufficient quantities to raise the percentage of protein to that supplied in the grass diet of Lot V. Lot Iv. received grass plus a mineral supplement composed of three parts of ground limestone, three parts of bone char and two parts of salt. Lot V. received the grass without supplement. Lots VI and VII received grass plus 2 per cent and 5 per cent of lactalbumin respectively.

(3) Animals Used and Allotment. Thirty-five rabbits of approximately ten weeks of age were used in this experiment. The animals were raised at Macdonald College from white does (registered as sports of the Austrian Red Breed) mated to a White Flemmish Giant buck. Their allotment to feeding groups was based on weight, sex, condition and breeding.

c. The Data.

The data of this trial are incomplete as to feed intake, owing to the difficulty experienced with wastage. The response of the animals to the different treatments will, therefore, be only briefly mentioned, since the primary purpose of this trial was the development of a technique to enable the carrying out of further experiments.

d. Discussion of Results of Individual Lots.

Lot I. The animals in this lot made larger gains than the others in the experiment. Their gains and feed consumption were regular; they appeared healthy throughout the experiment and in each case finished the trial in excellent condition.

Lot II. The most outstanding feature in this lot was the development of muscle degeneration in the hind legs of four out of the five animals in the lot. In one of these animals the condition became so advanced as to cause paralysis of the hind quarters. The condition involved the muscles of the hind legs, and the nerves at the sacral region of the spinal column. In each case the heart was affected and some animals showed diseased adrenals. The fifth animal, on post-mortem examination, appeared normal.

While sufficient histological data are not available to draw definite conclusions concerning this pathological condition there seemed to be a close similarity between the condition resulting from the experimental diet in this lot and that reported by Madsen et al. (1933) in herbivora fed synthetic diets.

The gains and feed consumption in this lot were considerably smaller than those of Lot I. The animals, with the exception of the one that became paralyzed, finished the experiment in fair condition.

Lot III. The addition of lactalbumin to the grass-dextrose mixture had a beneficial effect. The animals in this lot made larger gains than those in Lot II, showed no signs of the degenerated muscle condition and were regular in their gains and feed consumption. At the close of the experiment they were in practically as good condition as those in Lot I.

Lot IV. The addition of minerals to the grass proved to be very unsatisfactory. This is, perhaps, explained by the excessively large quantity of calcium present in this diet. Only two of the five animals in this lot finished the experiment and even they lost weight and were in an emaciated condition after the forty days of feeding. The feed consumption was smaller than in the other lots and in most cases the animals showed a dislike for their feed, leaving the finer material in the bottom of the feeder.

Post-mortem examination showed each animal to be suffering from diseased kidneys. The urinary bladders were usually full and distended and in some cases degenerated kidney cells were present in the urine.

Lot V. The grass without supplement provided little more than a maintenance diet. Four out of the five animals completed the experiment. Their condition throughout the experiment, however, was poor and their gains were small and irregular. Post-mortem examination revealed no abnormalities in any of the animals.

Lot VI. The addition of lactalbumin at the rate of two per cent provided a slight improvement compared with Lot V. Two animals in this lot died from coccidiosis infestation. Post-mortem examination of the animals that completed the experiment showed no abnormalities.

Lot VII. The five per cent addition of lactalbumin improved the grass diet quite materially. It did not, however, make as satisfactory a diet as the lactalbumin-dextrose addition.

One animal in this lot lost weight consistently throughout the experiment and died immediately after the trial was completed. Post-mortem examination showed a constriction in the pyloric region of the stomach causing digestive disturbances on the bulky diet. The balance of this group were found to be normal on post-mortem examination.

e. Notes on Technique.

It was decided at the beginning of the experiment that if the grass could be fed in a ground condition it would greatly facilitate a thorough incorporation of the supplements in the mixtures. Some difficulty was encountered in getting the rabbits to eat the ground feed in cases when they had become accustomed to a diet of grass clippings or hay. It was found, however, that when young rabbits immediately after weaning were placed on rations of ground feed they quickly adapted themselves to the diet in this form.

A serious difficulty in this experiment was the prevention of feed wastage. Feed scratched out of the feeders became mixed with the excreta in the trays and could not be recovered and refed. The type of feeder used at this time was designed for the feeding of grass clippings. It consisted of a rack under which was a trough-like container for fine material. Although various modifications were made on these feeders it was found impossible to design a feeder, of this type, that would prevent the rabbits from scratching out feed and still allow them access to the feed. As a result of observations during this trial, the type of feeder shown in the photograph (page18c) was designed. These feeders were made with a bottom sufficiently large to prevent upsetting.

They have a capacity of about two hundred grams of ground grass which is more than sufficient for one day's feed. The hole in the top of the feeder is three inches in diameter and large enough to permit easy feeding but too small to allow the rabbits to get their feet into the feeder while they are feeding. This type of feeder has since given very satisfactory results, the only waste being feed that drops out of the rabbit's mouth while eating.

5. FEEDING TRIAL NO. 2.

The Nutritive Value of Timothy vs. Reed Canary Grass as Measured by Growth of Young Rabbits.

a. Object of Trial.

To study the relative nutritive values of reed canary grass and timothy.

b. Plan of Trial.

The experiment was planned to compare the nutritive value of immature timothy grass with that of immature reed canary grass; also to determine whether the addition of protein to these grasses increased their nutritive value. Besides the four rations necessary for these comparisons, a fifth, thought to be satisfactory for normal growth, was included as a check ration.

A sixty-day feeding period was used in this test. To follow the progress of the rabbits during the trial, live weights and feed consumption were checked at ten day intervals.

(1) Feeds Used. The reed canary grass and the timothy were cut from pure plots at Macdonald College in the early summer of 1933. The cutting was done when the grass was from four to six inches high. It was desired to obtain the grasses at the same stage of maturity. Consequently, the timothy, which grows less rapidly, was cut about a week later than the reed canary grass. The clippings were dried in the sun and then spread on a barn floor until ready for grinding.

Lactalbumin, obtained from the same source as that used in the previous experiment (see page19) was again used as a protein supplement. The oats, linseed meal and alfalfa meal were obtained from the feed supply of the College stock farm.

(2) Rations Fed. The details of the rations, together with their chemical analyses are given in Tables VII, VIIa, VIIb.

TABLE VII. RATIONS FED. FEEDING TRIAL NO. 2.

	Lot I.	Lot II.	Lot III.	Lot IV.	Lot V.
Reed Canary Grass	100		95		
Timothy		100		95	
Alfalfa Meal					50
Oats					40
Linseed Meal					10
Lactalbumin			5	5	

TABLE VIIa. ANALYSIS EXPRESSED ON AIR DRY BASIS.

	Moisture	Crude Protein	Ether Extract	Crude Fibre	N-free Extract	Total Ash	Ca.	P.
Lot I.	8.35	12.40	2.84	25.51	41.49	9.41	.382	.356
" II.	7.10	11.16	3.43	24.92	45.88	7.51	.370	.324
" III.	8.21	14.58	2.74	24.25	40.18	10.04	.401	.360
" IV.	7.01	13.40	3.30	23.69	44.35	8.25	.389	.329
" V.	6.88	14.16	3.43	21.40	49.42	4.71	.666	.361

TABLE VIIb. ANALYSIS EXPRESSED ON DRY MATTER BASIS.

	Crude Protein	Ether Extract	Crude Fibre	N-free Extract	Total Ash	Ca.	P.
Lot I.	13.53	3.10	27.83	45.27	10.27	.417	.388
" II.	12.01	3.69	26.82	49.39	8.09	.398	.349
" III.	15.88	2.99	26.42	43.77	10.94	.437	.392
" IV.	14.41	3.55	25.48	47.69	8.87	.418	.354
" V.	15.20	3.68	22.98	53.07	5.07	.715	.388

The animals were fed these rations ad lib. throughout the experiment.

(3) Animals Used and Allotment.

Twenty-five rabbits of approximately ten weeks of age were used in this experiment. The animals were cross-bred stock similar to those used in trial No. 1. They were alloted to five lots, taking into consideration, weight, sex and condition.

TABLE VIII. ALLOTMENT DATA. FEEDING TRIAL NO. 2.

LOT I.			LOT II.			LOT III.			LOT IV.			LOT V.		
No.	Weight (In grams)	Sex	No.	Weight (In grams)	Sex	No.	Weight (In grams)	Sex	No.	Weight (In grams)	Sex	No.	Weight (In grams)	Sex
6	1480	♀	7	1545	♀	31	1460	♀	12	1465	♀	11	1470	♀
5	1400	♀	33	1385	♂	15	1385	♂	32	1375	♂	17	1410	♂
13	1305	♀	14	1285	♀	8	1300	♀	10	1300	♀	3	1315	♂
27	1280	♂	24	1205	♀	4	1250	♀	9	1275	♂	26	1170	♀
29	1140	♂	23	1125	♀	22	1135	♀	19	1140	♀	21	1160	♀

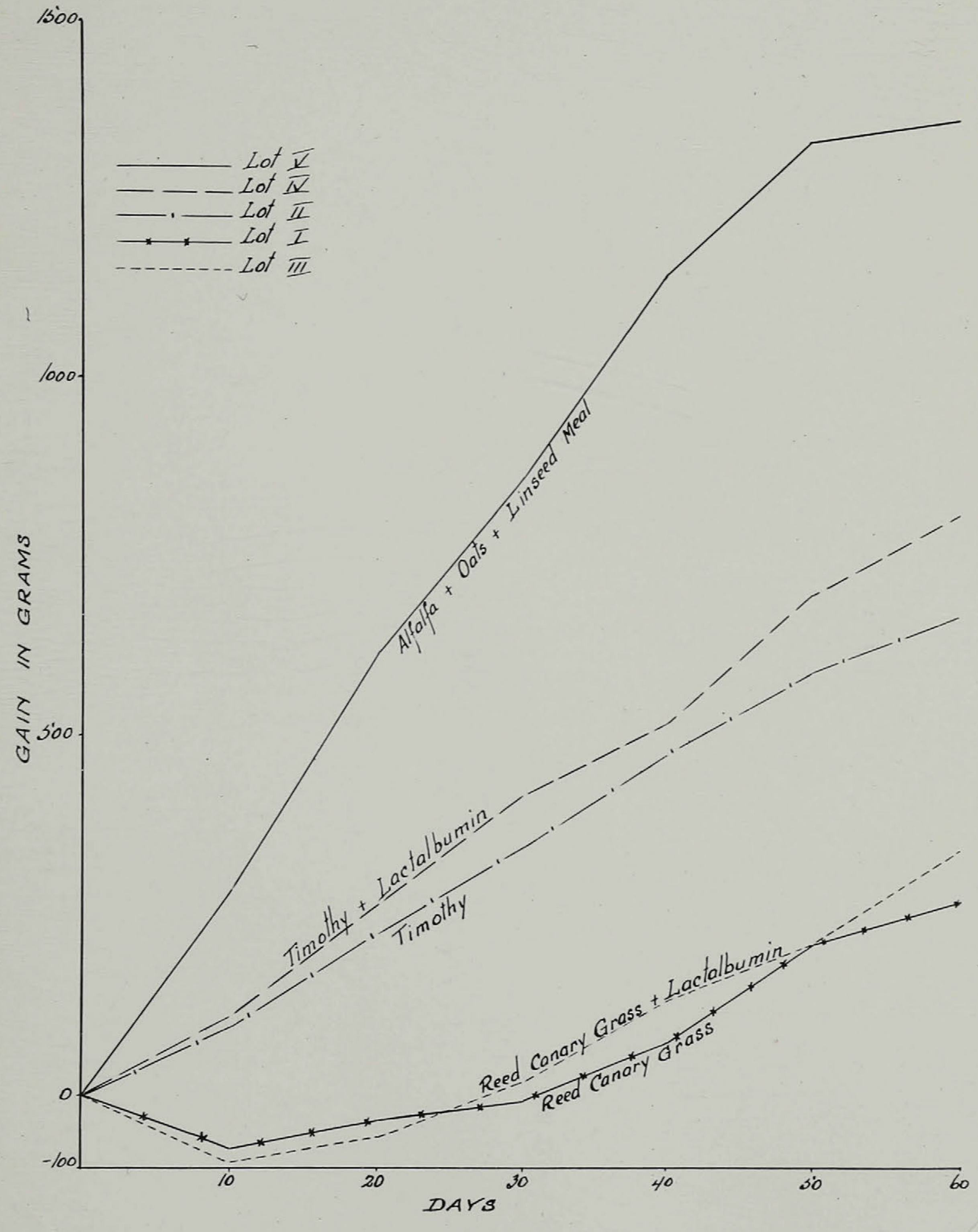

CHART I. MEAN LOT GAINS IN FEEDING TRIAL NO. 2.

c. Observations During Trial.

During the first ten day period a serious loss in weight and condition occurred in the animals on reed canary grass (Lot I) and reed canary grass plus lactalbumin (Lot III). One animal in each of these lots died during this period. Post-mortem examination showed both of these animals to have diseased kidneys and hearts. Consequently, they were replaced on the first weigh day by two extra rabbits which had been carried as spares. The animals receiving timothy (Lots II and IV) also lost their original bloom during the first period, though they continued to grow and actually gained in weight. The animals receiving the check ration (Lot V) showed no loss in condition and grew rapidly.

As shown in Chart I, (page 29b) all lots showed gains in weight after the first period. The gains, however, in Lots I and III were small and could only be accounted for by a certain amount of growth that took place and not by any improvement in condition. No consistent improvement could be noticed from the addition of protein to the reed canary grass in Lot III. During the third period, one animal in Lot I died. Post-mortem examination showed no abnormalities except a thin, brittle bone condition and a precipitate in the urine containing crystals of calcium oxalate. The prevalence of leg injury in the balance of the animals in Lot I was a peculiar feature in this group. Three out of the four animals were lame at various times during the trial. In no case was the injury serious and their recovery, without treatment, was complete before the end of the trial, but the fact that it occurred only in one lot is, perhaps, significant.

The timothy lots made consistent gains throughout the feeding period. They made fair growth and appeared healthy and in good condition. The addition of protein to the timothy diet produced a noticeable, though not a marked, improvement.

The check lot animals made normal gains. At the close of the experiment they were approaching maturity and their rate of growth began to slacken.

No post-mortem examinations were made at the end of this experiment.

d. The Data.

The details of initial weights, feed consumption and gains together with the statistical analysis of the gains are given in appendix Table 1. The gains in graph form, are given in Chart I. (page 29b).

It will be noticed from appendix Table I that the average initial weight for Lot V. was considerably higher than for the other lots. This is an expression of the difference in nutritive value of the diets, since all the rabbits were placed on their respective experimental diets for a preliminary period of five days after the allotment was made. The effect of this difference in initial weight on gains was taken out in the statistical analysis of the trial.

The consistency of the gains made by the animals on timothy, Lots II and Iv, is a noteworthy feature in these data, also the consistent but small increase in the gains of Lot Iv over Lot II from the addition of 5 per cent lactalbumin. The gains of the animals on reed canary grass (Lots I and III) showed more variability than found in other lots.

For purposes of analysis, the gains and feed consumption for rabbit No. 27, which died on the 16th day of the experiment, were calculated by the method of Yates (1933).

Statistical analysis was applied to the data, using the method of partial regression as described by Crampton (1934) by means of which the combined effects on gains of differences in initial weight and differences in feed intake of individual rabbits are removed, thus permitting an estimate of gains expected from a trial in which all animals were of the same weight at the start and consumed equal quantities of feed during the test. These corrected mean gains for each lot are as follows:-

Table IX. MEAN GAINS (to nearest whole gram)

FEEDING TRIAL NO. 2.

LOT I.	LOT II.	LOT III.	LOT IV.	LOT V.
Reed Canary Grass	Timothy	Reed Canary Grass and Lactalbumin	Timothy and Lactalbumin	Alfalfa Meal plus oats and Linseed Meal
287	641	390	870	1263

In this trial, and considering odds of 19 to 1 as significant, any difference between lot mean gains greater than 119 grams, may be taken as a real difference due to difference in the nutritive value of the rations. From this analysis it is evident that the difference between the mean gains of the animals on timothy (Lot II.) and those on reed canary grass (Lot I.) is highly significant. The addition of 5 per cent. lactalbumin to the reed canary grass (Lot III.) produced no significant increase over Lot I. In the case of the timothy, however, the addition of lactalbumin (Lot IV.) increased the gains significantly over those of Lot II. None of the rations were as satisfactory as the check ration as measured by gains in live weight of the animals.

a. Discussion of Results.

In the light of the foregoing results it seems evident that there is a marked difference in the nutritive value of immature timothy as compared with immature reed canary grass.

Observations during the trial substantiated by the data on feed consumption showed no difference in the palatability of the two grasses as measured by the quantity eaten by the animals.

The difference in the nutritive value of timothy as compared with reed canary grass cannot be explained on the basis of the ordinary chemical analysis of feeding stuffs. In this respect these grasses are very similar as shown in table VIIa (page 29). This strongly supports the conclusion drawn from the review of literature that differences in the quantity of protein, minerals

(Ca. & P.) or total digestible nutrients do not adequately account for the differences in the nutritive value of different grasses.

f. Conclusions.

(1) Immature timothy as fed at this stage of growth is superior in nutritive value to reed canary grass of a similar stage of maturity.

(2) The difference in nutritive value of these two grasses cannot be explained on the basis of quantitative differences in crude protein, calcium, phosphorus or total digestible nutrients.

6. FEEDING TRIAL NO. 3.

The Nutritive Value of Fertilized vs. Unfertilized Pasture Grass as Measured by Growth with Young Rabbits.

a. Object of Trial.

To measure the change in nutritive value of pasture herbage affected by treatment with mineral fertilizers.

b. Plan of Trial.

This experiment was planned to test the nutritive value of fertilized vs. unfertilized pasture grass by feeding the herbage unsupplemented, to young rabbits.

For this test a continuous feeding period of twenty-eight days was used, and live weights and feed consumption were recorded at seven day intervals.

(1) Feeds and Rations. The source and preparation of the herbage used in this experiment have already been described.

The rations together with their chemical analyses are given in tables X, Xa and Xb.

TABLE X. RATIONS FED - TRIAL NO. 3.

	LOT I.	LOT II.
Fertilized Grass	100	
Unfertilized Grass		100

TABLE Xa. ANALYSIS EXPRESSED ON AIR DRY BASIS.

	Dry Matter	Crude Protein	Ether Extract	Crude Fibre	N-free Extract	Total Ash	Ca.	P.
Lot I.	94.85	15.87	3.65	21.60	45.46	8.27	.990	.348
" II.	94.68	13.93	3.37	23.04	46.07	8.27	.873	.262

TABLE Xb. ANALYSIS EXPRESSED ON DRY MATTER BASIS.

	Crude Protein	Ether Extract	Crude Fibre	N-free Extract	Total Ash	Ca.	P.
Lot I.	16.73	3.85	22.77	47.93	8.72	1.044	.367
" II.	14.71	3.56	24.34	48.66	8.73	.922	.277

As shown by these analyses the fertilized herbage, as compared with the unfertilized, is considerably higher in its protein and phosphorus content.

(2) Animals Used and Allotment. Animals from the regular breeding unit were not available for this test but it was found possible to secure from another department chinchilla rabbits of approximately ten weeks of age. They were not only much smaller than those used in other tests but were less uniform in size. They were alloted by pairs to two groups taking into consideration weight, sex and condition.

TABLE XI. ALLOTMENT DATA. FEEDING TRIAL NO. 3.

LOT I.			LOT II.		
Rabbit No.	Weight (In grams)	Sex.	Rabbit No.	Weight (In grams)	Sex
1	920	♂	2	960	♀
3	910	♀	4	855	♀
5	785	♂	6	760	♀
7≠≠	635	♂	8≠	725	♀
9≠	575	♀	10≠	520	♂
11≠	450	♀	12≠≠	500	♂

≠ Died during trial.
≠≠ Paired after re-allotment (see text).

c. Observations During Trial.

As above mentioned, animals used in this experiment were smaller than those used in previous work (See table VIII, page 29a, for comparison). It was also realized that chinchillas were often found more delicate than the larger breed regularly used. We were not greatly surprised, therefore, that during the first week of the experiment two of the smaller animals in each group died. Post-mortem examination revealed a diseased liver in one rabbit. This condition, however, could not have developed as a result of the experimental diet. No abnormalities were found in the other three animals. The eight rabbits remaining, which for the most part were larger animals, adapted themselves to the diets satisfactorily. The results of the animals that died were, therefore, discarded and the two (one

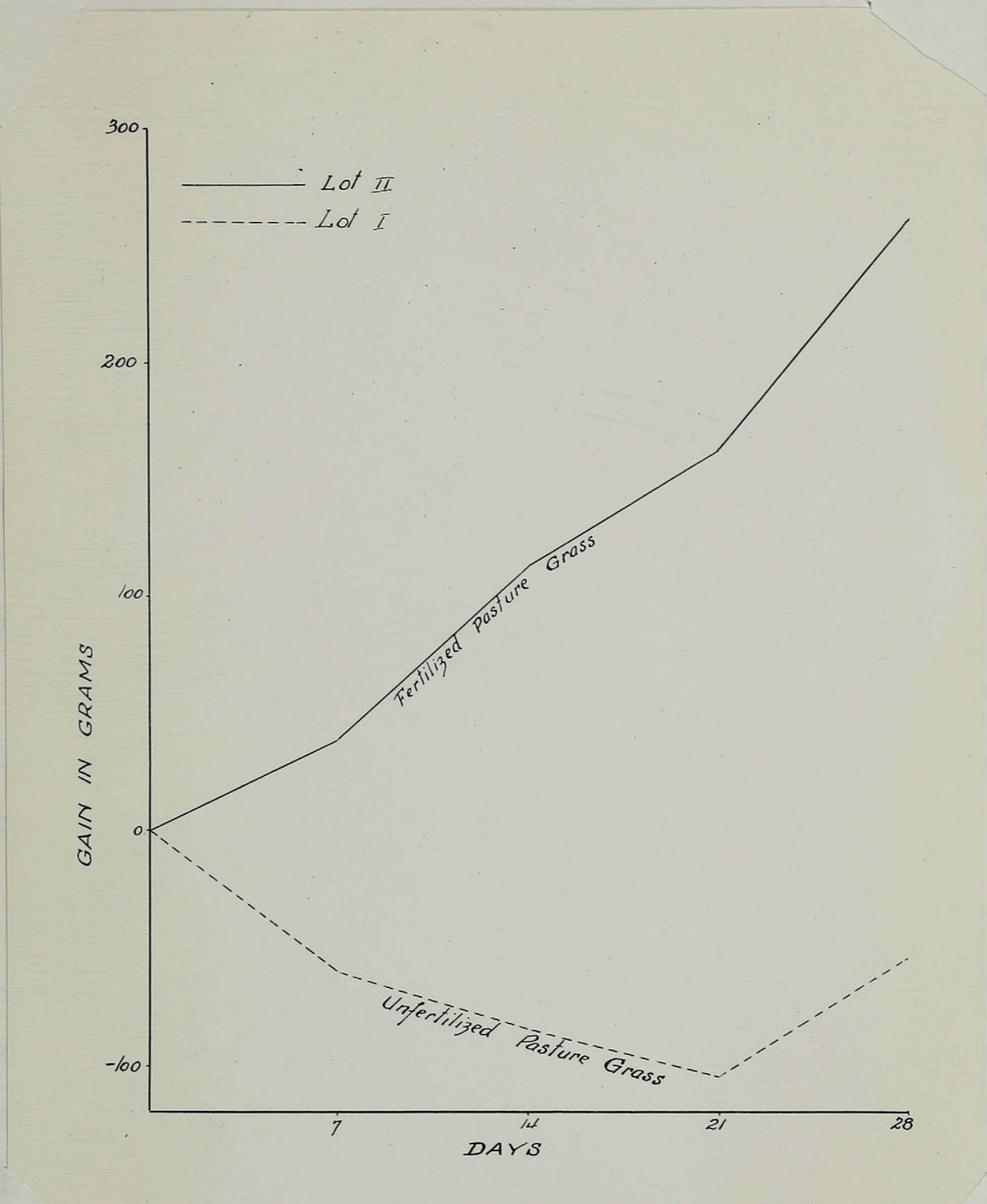

CHART II. MEAN LOT GAINS IN FEEDING TRIAL NO. 3.

in each lot) whose pair-mates had died became the fourth pair. (Nos. 7 and 12.)

A marked difference was noticeable throughout the experiment in the condition of the animals in the two lots. Those on the fertilized grass (Lot I) were in good condition throughout the trial and made fairly rapid growth. The animals on unfertilized grass (Lot II) began to fail in condition immediately after the trial commenced. This loss in weight and condition continued for the first three weeks of the experiment, until, in most cases, the animals were in a state of emaciation. During the last period, however, every animal in the lot showed a slight increase in weight.

A post-mortem examination was made on representative animals after the experiment finished. The animals that were examined appeared normal.

d. The Data.

The details of the initial weight, feed consumption and gains, together with the statistical analysis of the gains are given in appendix Table II. The gains, in graph form, are shown on Chart II (page 37a).

The marked variability of the gains in the animals receiving the unfertilized grass (Lot II) together with the small number of animals per lot greatly decreases the significance of the average results. The difference in the mean gains of these lots, however, is extremely marked and statistical analysis of the gains showed this difference to be significant.

This analysis did not take into account differences in initial weight and feed consumption. It is realized that the average daily feed consumption was about 4.5 per cent higher for Lot I. than for Lot II. but it does not seem likely that this small difference in feed consumption was responsible for the marked difference in gains.

e. Conclusions.

In the light of the results of this experiment it is strongly indicated that there is a marked difference in the nutritive value of herbage from pastures treated with mineral fertilizers as compared with herbage from similar unfertilized pastures.

7. FEEDING TRIAL NO. 4.

The Effect of Fertilization on the Protein Content of of Pasture Grass.

a. Object of Trial.

To study the effect of the application of mineral fertilizers on the quality of the protein in the herbage.

b. Plan of Trial.

Tables XII, XIIa, and XIIb, give the details of the rations used, together with their chemical analyses.

TABLE XII. RATIONS FED - FEEDING TRIAL NO. 4.

	LOT I.	LOT II.	LOT III.	LOT IV.	LOT V.	LOT VII.
Fertil-ized Grass	92.6		90.0		55.5	
Unfertil-ized Grass		99.0		96.0		59.5
Casein					5.00	5.0
Cystine			.40	.40	.13	.13
Oat Hulls	3.2		4.3	1.3	19.185	17.185
Sucrose	3.2		4.3	1.3	19.185	17.185
Salt	1.0	1.0	1.0	1.0	1.0	1.0

TABLE XIIa. ANALYSIS EXPRESSED ON AIR DRY BASIS.

	Moisture	Crude Protein	Ether Extract	Crude Fibre	N-free Extract	Total Ash	Ca.	P.
Lot I.	4.98	14.88	3.46	21.00	46.84	8.84	.921	.329
" II.	5.27	13.79	3.34	22.81	45.61	9.18	.864	.259
" III.	4.92	14.95	3.39	20.77	47.29	8.68	.949	.322
" IV.	5.20	13.85	3.27	22.52	46.16	9.00	.855	.254
" V.	4.42	14.10	2.60	17.96	54.08	6.84	.616	.268
" VI.	4.60	13.46	2.52	19.06	53.30	7.06	.583	.226

TABLE XIIb. ANALYSIS EXPRESSED ON DRY MATTER BASIS.

	Crude Protein	Ether Extract	Crude Fibre	N-free Extract	Total Ash	Ca.	P.
Lot I.	15.66	3.65	22.09	49.29	9.31	.969	.346
" II.	14.56	3.53	24.08	48.14	9.69	.912	.273
" III.	15.72	3.57	21.84	49.74	9.13	.998	.339
" IV.	14.61	3.45	23.76	48.68	9.50	.902	.268
" V.	14.75	2.72	18.79	56.57	7.16	.644	.280
" VI.	14.12	2.65	19.97	55.86	7.40	.611	.237

From Table XII, it will be noticed that a mixture of equal parts of oat hulls and sugar was used to adjust the protein and energy values of the several diets to approximately those of Lot II (unfertilized grass plus salt.). With the addition of the 5 per cent casein in Lots V and VI, a rather large allowance of this mixture was necessary to maintain the protein level wanted with the result that the nitrogen-free extract was somewhat above, and the fibre somewhat below those of the other lots. This may partly explain the more rapid growth made on these latter diets.

It will also be noted that, based on requirements for larger animals there does not appear to be a deficiency of minerals (Ca. or P.) in any of these diets.

Results reported by Miller and Chibnall (1932) and by Evans (1931) suggest that perhaps pasture grass is deficient in cystine. From an amino acid analysis of timothy, conducted at this Institution, Dyck and McKibbin (1933) reported a notably low cystine content.

It was, therefore, decided to test the effect of adding purified cystine to the basal diets of both fertilized and unfertilized grass. Cystine was thus included in the rations of Lots III and IV at the rate of .4 per cent. This was equivalent to about 3. per cent of the protein which was the rate at which this amino acid was included, and found to give satisfactory results, in the experimental diets of Mitchell and Smuts (1932).

A mixed amino acid supplement was also included in the tests. For this purpose casein, which contains all the essential amino acids in liberal quantities except cystine, was fortified with cystine to make up the deficiency and used as the mixed supplement. The cystine was added to the casein at the rate of 2.6 per cent, thus bringing the cystine content of casein to about 3.54 per cent. (Figures for cystine content of casein taken as 0.94 per cent, Sherman (1932)). The cystine fortified casein constituted 5.13 per cent of the diets of Lots V and VI. Assuming the essential amino acids to represent 20.1 per cent of the casein, (Sherman (1932)), the addition was equivalent to substituting the diet with a 1.14 per cent mixture of these five amino acids.

The feed consumption in this experiment was controlled, the amounts allowed daily per rabbit being approximately 80 per cent of what previous trials had indicated constituted full feeding. For the first seven days this allowance was 100 grams air dry feed. For the next fourteen days this was increased to 125 grams. During the last period it was necessary, because of an error in a shipment of cystine, to reduce the feed allowance to 115 grams per rabbit per day. This reduction during the last period is clearly reflected in the growth curve (see Chart III, page 46a.)

A twenty-eight day feeding period was used in the conducting of these tests. To obtain data as to the response of the animals to the different treatments, live weights were checked at seven-day intervals.

(1) Feeds used. The source and preparation of the herbage used as the basis of these rations have already been described (see page 19). The cystine was purchased from the Eastman Kodak Company, Rochester, N.Y. A good quality lactic acid casein was obtained from the Champlain Milk Products Co., Inc.,

Stanbridge, Quebec. The oat hulls used in the adjustment of the protein and energy values of the rations were purchased from the Quaker Oats Company. The salt and sugar were commercial products.

(2) Animals Used and Allotment. Thirty eleven week old rabbits were used in this experiment. The animals were obtained from the regular breeding unit and were similar cross-bred stock to that used in trials Nos. I and II. They were alloted to six groups, taking into consideration weight, sex and condition.

TABLE XIII. ALLOTMENT DATA. FEEDING TRIAL NO. 4.

LOT I.			LOT II.			LOT III.			LOT IV.			LOT V.			LOT VI.		
No.	Weight (In grams)	Sex	No.	Weight (In gr.)	Sex	No.	Weight (In gr.)	Sex	No.	Weight (In gr.)	Sex	No.	Weight (In gr.)	Sex	No.	Weight (In gr.)	Sex
1	1735	♀	2	1725	♂	11	1675	♀	28	1645	♂	29	1815	♂	30	1785	♀
18	1525	♂	16	1605	♀	32	1645	♂	8	1645	♂	6	1520	♀	7	1520	♂
19	1450	♀	17	1455	♀	31	1455	♀	5	1495	♂	20	1500	♀	3	1470	♂
4	1435	♂	21	1375	♂	12	1410	♂	26	1385	♀	13	1435	♀	9	1395	♂
10	1325	♂	27	1280	♂	14	1245	♂	22	1255	♂	23	1175	♀	24	1260	♂

c. Observations During Trial.

Marked differences in the response of the animals to the different treatments were evident immediately after the trial had started. Series A animals showed no loss in condition and continued to grow fairly rapidly. Those in Lot V, receiving the mixed amino acid supplement showed a considerable improvement over the other two lots. The cystine addition in Lot III produced no noticeable immediate effect.

In Series B, more pronounced differences occurred. The animals receiving the straight grass diet (Lot II) and those receiving the addition of cystine (Lot IV) lost condition. The addition of cystine to the unfertilized grass (Lot IV), however, produced a noticeable improvement over Lot II, the loss in condition during the first period being less severe. The addition of the mixed supplement in lot VI resulted in a marked improvement over the other lots of this series. Those animals, with the exception of one, compared favourably with those receiving the fertilized grass plus the same supplement (Lot V).

Nine days after the experiment had started, one animal in Lot II died. This rabbit was in an extremely emaciated condition. No abnormalities were found on post-mortem examination, consequently it appeared that the death was due to the unsatisfactory diet.

During the balance of the experimental period there was no noticeable difference between Lots I and III. These animals were in good condition throughout the experiment and made fairly rapid growth.

Lots V and VI, receiving the mixed supplement, were in excellent condition and made rapid growth. These animals showed a greater liking for their diet than did the other animals. They cleared up their alloted quantities of feed more rapidly and always seemed hungry. One animal (No. 30) in Lot VI was an exception as far as gains and condition were concerned. At the start of the experiment it was one of the largest animals in the lot. The bulky diet, however, seemed to be unsatisfactory as it showed little or no change in weight during the trial. Post-mortem examination showed this rabbit to be definitely abnormal. The pyloric opening in the stomach was partially blocked causing the accumulation of a packed mass of material in the stomach that could not pass through into the intestine. The data from this animal were discarded, therefore, in the analysis of the trial.

In Lot IV, the beneficial effect of the cystine addition became less pronounced towards the end of the experiment. The animals in Lot II lost condition rapidly at first, and while their condition did not improve, gains in weight were recorded from the amount of growth that took place. The animals in Lot IV did not become poor and emaciated as rapidly as those in Lot II. While their loss in condition was more gradual it did, however occur, and at the close of the experiment their condition was not greatly different from the animals receiving no supplement.

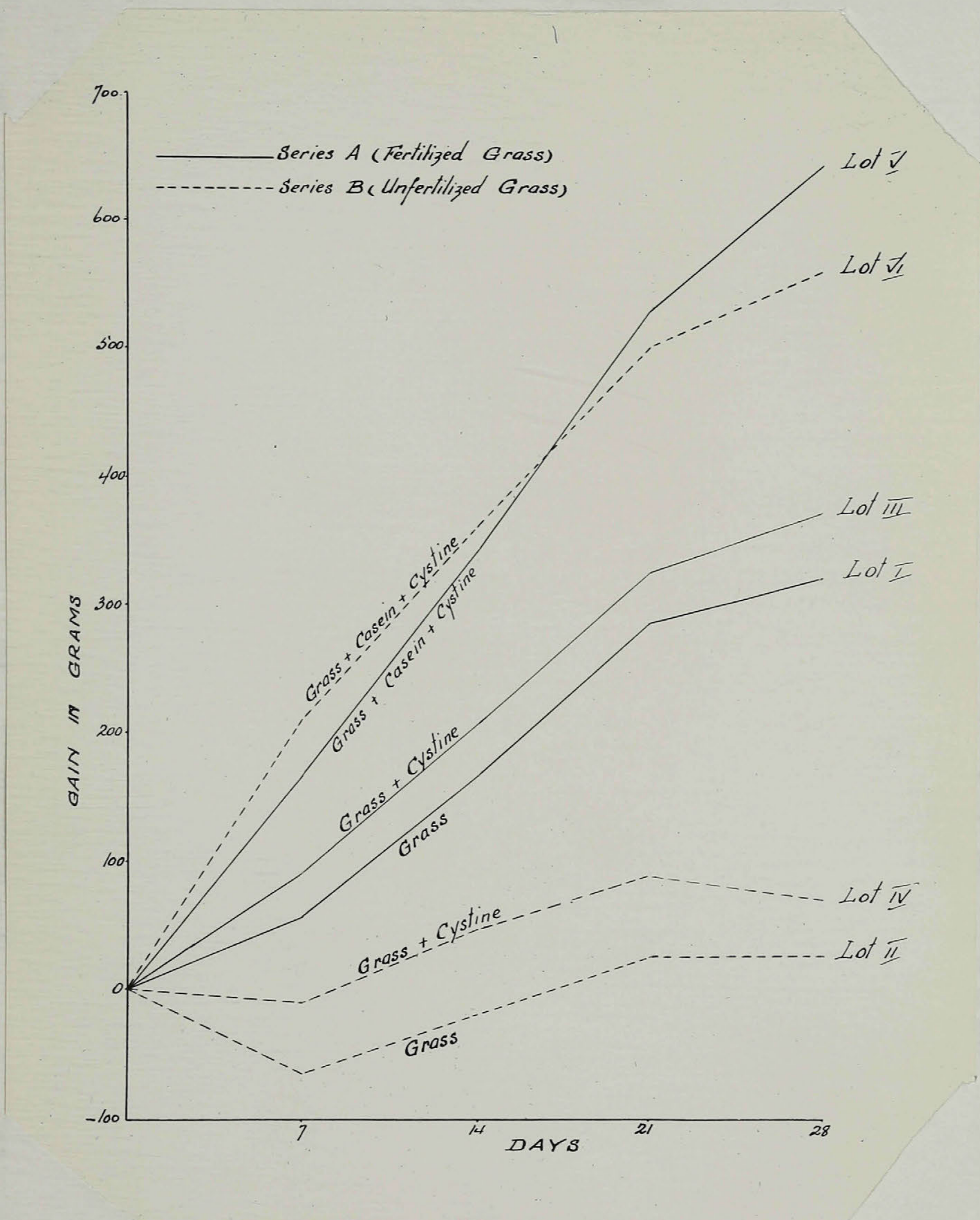

CHART III. MEAN LOT GAINS ON EQUAL FEED CONSUMPTION FEEDING TRIAL NO. 4.

d. The Data.

The details of the initial weights and gains together with the statistical analysis of the gains are given in appendix Table III. The gains in graph form, are given in Chart III. page 46a).

For purposes of statistical analysis the gains for rabbit No. 17 (Lot II), which died on the ninth day of the experiment, and those for rabbit No. 30 (Lot VI) found abnormal on post-mortem examination after the trial, were calculated according to the method of Yates (1933).

The mean lot gains are shown in the table following:-

TABLE XIV. MEAN GAINS. FEEDING TRIAL NO. 4.

(Figures in grams).

LOT I.	LOT II.	LOT III.	LOT IV.	LOT V.	LOT VI.
Fertilized Grass.	Unfertilized Grass.	Fertilized Grass and Cystine.	Unfertilized grass plus cystine	Fertilized grass plus cystine plus casein.	Unfertilized grass plus cystine plus casein.
321	25	370	70	643	553

Statistical analysis (variance method) indicated that the difference between the mean gains of the animals on fertilized herbage (Lot I) and those on unfertilized herbage (Lot II) is highly significant. The increase in mean gains of Lots III and IV over Lots I and II respectively, as a result of the addition of cystine, however, is not significant. The addition of mixed supplement in Lots V and VI resulted in a highly signi-icant increase over Lots I and III and II and Iv respectively. The difference between Lots V and VI, however, is not significant.

e. Results and Discussion.

In the light of the foregoing results it is evident, that aside from any difference in the total protein content, there is a marked difference in the nutritive value of fertilized as compared with unfertilized herbage. The chemical analyses of these rations (Lots I and II) shows no significant differences in the constituents except in the case of phosphorus. That the animals in Lot VI made satisfactory gains on a still lower percentage of phosphorus is evidence that this element was not a limiting factor in Lots I and II. The difference, then, in the nutritive value of these two rations cannot be explained by any difference in the chemical analysis shown in table XIIa (page 41).

The failure of the addition of cystine to increase significantly the nutritive value of the herbage in either series is evidence that, in the case of these pastures, cystine is not deficient or else that still another amino acid is involved with cystine in limiting the feeding value of the herbage.

The marked increase in the growth of the rabbits as a result of the addition to their diets of the mixed amino acid supplement is the important feature of this experiment. It is possible, of course, that a part of this increase was due to the slightly higher content of nitrogen-free extract in these diets. This theory, however, fails entirely to account for the markedly greater increase in the unfertilized as compared with the fertilized grass diet.

The conclusion seems warranted, therefore, that the addition of the mixture of the essential amino acids was responsible for the improved feeding value of these latter diets.

f. Conclusions.

(1) The increase in the protein content of pasture herbage resulting from the application of mineral fertilizers does not satisfactorily account for the increase in the nutritive value of fertilized vs. unfertilized herbage.

(2) The addition of cystine to the herbage indicates either that cystine is not a limiting factor in its nutritive value, or that one or more other amino acids are involved with cystine in the deficiency, thereby masking the effect of the single amino acid addition.

(3) The evidence from this experiment supports the conclusion that the quality of the protein in pasture herbage is an influencing factor in its nutritive value.

(4) It is also indicated that the application of mineral fertilizers to pastures brings about a change in the protein quality of the herbage.

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A P P E N D I X

TABLE I. INITIAL WEIGHT, FEED EATEN AND GAINS (EXPRESSED IN GRAMS). DATA FROM FEEDING TRIAL no.2.

	LOT I.				LOT II.				LOT III.				LOT IV.				LOT V.		
	REED CANARY GRASS 100%				TIMOTHY 100%				REED CANARY GRASS 95% LACTALBUMIN 5%				TIMOTHY 95% LACTALBUMIN 5%				ALFALFA MEAL 50% OATS 40% LINSEED MEAL 10%		
Rabbit No.	Initial Weight	Total Feed	Gain	Rabbit No.	Initial Weight	Total Feed	Gain	Rabbit No.	Initial Weight	Total Feed	Gain	Rabbit No.	Initial Weight	Total Feed	Gain	Rabbit No.	Initial Weight	Total Feed	Gain
6	1430	8160	385	7	1385	8430	760	31	1375	7810	460	12	1500	8005	820	11	1750	10285	1355
5	1425	8330	205	33	1385	7605	670	15	1450	7430	165	32	1450	8120	735	17	1525	7750	935
13	1340	7150	210	14	1275	8060	620	8	1275	7560	300	10	1335	7570	685	3	1500	9675	1365
27	1200	8220	340	24	1250	7715	540	4	1275	8950	545	9	1330	7585	830	26	1475	9960	1525
1	1350	8135	225	23	1250	8305	735	16	1450	7645	235	19	1225	7965	1000	21	1425	10200	1615
Av.	1349	7999	273		1309	8023	665		1365	7879	341		1368	7849	814		1535	9574	1359
orrected Mean Gains			287.56				641.14				389.78				870.58				1262.87

TABLE IA. ANALYSIS OF VARIANCE AND COVARIANCE OF GAINS. FEEDING TRIAL NO. 2.

Due to	Degrees of Freedom	Uncorrected $S(Y)^2$	Degrees of freedom	Corrected by Regression $S(Y)^2$	Variance	Standard Error.
Treatment	4	3796256.00	4			
Replicate	4	193576. 00	4			
Error	16	296314. 00	13+	98938.77	7610.67	87.239
Total	24	4286146.00	24			

+Three degrees of freedom lost through calculation of two regression values and estimation of one missing value.

TABLE II. - INITIAL WEIGHTS, FEED EATEN AND GAINS (expressed in grams). FEEDING TRIAL No. 3.

LOT I.							
FERTILIZED GRASS 100%				UNFERTILIZED GRASS 100%			
Rabbit No.	Initial Weight	Total Feed	Gains	Rabbit No.	Initial Weight	Total Feed	Gains.
1	920	2775.	305	2	960	2620	-245
3	910	2765	250	4	855	2985	- 55
5	785	2900	330	6	760	2545	65
7	635	1985	160	12	500	1810	15
Av.	812.50	2606.25	261.25		768.75	2490.00	- 55.00

TABLE IIa. VARIANCE ANALYSIS OF GAINS. FEEDING TRIAL No. 3.

DUE TO	Degrees of Freedom	S X^2	VARIANCE	Standard Error
Treatment	1	20028.12	200028.12	
Replicate	3	29059.37	9686.46	
Error	3	43359.38	14451.26	120.20
Total	7	272446.87	38920.98	

TABLE III. INITIAL WEIGHT, FEED EATEN AND GAINS (EXPRESSED IN GRAMS) FEEDING TRIAL No.4.

LOT I.				LOT II.				LOT III.				LOT IV.				LOT V.				LOT VI.		
Fertilized Grass 92.6% Oat Hulls 3.2% Sucrose 3.2% Salt 1.0%				Unfertilized Grass 99.0% Salt 1.0%				Fertilized Grass 90.0% Cystine .4% Oat Hulls 4.3% Sucrose 4.3% Salt 1.0%				Unfertilized Grass 96.0% Cystine .4% Oat Hulls 1.3% Sucrose 1.3% Salt 1.0%				Fertilized Grass 55.5% Casein 5.0% Cystine .13% Oat Hulls 19.19% Sucrose 19.18% Salt 1.00%				Unfertilized Grass 59.50% Casein 5.0% Cystine .13% Oat Hulls 17.19% Sucrose 17.18% Salt 1.00%		
Initial Weight	Total Feed	Gain	Rabbit No.	Initial Weight	Total Feed	Gain	Rabbit No.	Initial Weight	Total Feed	Gain	Rabbit No.	Initial Weight	Total Feed	Gain	Rabbit No.	Initial Weight	Total Feed	Gain	Rabbit No.	Initial Weight	Total Feed	Gain.
1615	3255	300	2	1550	3255	190	11	1610	3255	315	28	1455	3255	30	29	1635	3255	625	30	1635	3255	560
1450	3255	310	16	1435	3255	-335	32	1575	3255	480	8	1475	3255	-110	6	1450	3255	645	7	1450	3255	545
1355	3255	245	17	1215	3255	15	31	1300	3255	290	5	1340	3255	145	20	1330	3255	655	3	1315	3255	570
1300	3255	400	21	1305	3255	30	12	1300	3255	240	26	1300	3255	40	13	1440	3255	520	9	1300	3255	455
1160	3255	350	27	1090	3255	225	14	1090	3255	525	22	1195	3255	245	23	1225	3255	770	24	1210	3255	635
1376	3255	321		1319	3255	25		1375	3255	370		1353	3255	70		1416	3255	643		1382	3255	553

TABLE IIIA. VARIANCE ANALYSIS OF GAINS. FEEDING TRIAL NO. 4.

Due to	Degrees of Freedom	$S(Y)^2$	Variance	Standard Error
Treatment	5	1550016.80	310003.30	
Replicate	4	147188. 33	36797.08	
Error	20 (18)+	245241. 90	13624.55	116.73
Total	29	1942446.99	66980.93	

+ Two degrees of freedom lost from estimation of two missing values.