AN ABSTRACT OF THE THESIS OF

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Title: A Chick Growth Inhibitor in Alfalfa.

Abstract approved Redacted for privacy
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Ways and means of utilizing higher levels of alfalfa meal in chick rations were investigated. Growth values of regular sun-cured alfalfa (cut when one-fourth in bloom), early sun-cured alfalfa (cut when in the bud stage) and dehydrated alfalfa (cut when in the bud stage) all prepared from the same field were compared. A depressing effect on the growth of chicks was obtained with the three alfalfa meals when fed at the 20 per cent level. There was no difference between the growth of chicks on the rations containing dehydrated or early sun-cured alfalfa meal.

Growth of chicks on regular sun-cured alfalfa was less than on the other two meals. The same relative results were obtained when the alfalfa meals were compared on an ad libitum feeding program or an equalized feeding program. A ration containing 20 per cent alfalfa leaves depressed chick growth to a greater extent than one containing 20 per cent alfalfa stems. These results indicate that the major inhibiting effect of alfalfa should not be attributed to fiber, or unpalatability but to one or more growth inhibiting factors in alfalfa and that the factor(s) responsible for depressing chick growth is more highly concentrated in the leaves than in the stems of the plant.

Complete removal of the factor responsible for depressing chick growth is difficult and in this series of investigations was not accomplished. More of the inhibitor was removed by continuous extraction with water than by single or several successive batch extractions.

The growth inhibitor in alfalfa was counteracted by cholesterol or cholesterol plus 4 per cent cottonseed oil and to a lesser extent by lanolin and woolgrease plus 2 per cent cottonseed oil. Water treatment, irradiation, autoclaving, B-complex vitamins, glycerol, butanol, octanol and crude fish liver oil were ineffective in counteracting the inhibitor.

Saponins depress chick growth in a manner similar to alfalfa meal and may be the factor in alfalfa that is responsible for inhibiting chick growth.

From these preliminary studies it appears that some component of alfalfa meal soluble in water and fractionated by alcohol, possibly a saponin, is largely responsible for the growth depressing action of alfalfa meal when fed at high levels.

A CHICK GROWTH INHIBITOR IN ALFALFA

by

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TABLE OF CONTENTS

A CHICK GROWTH INHIBITOR IN ALFALFA

INTRODUCTION

Considerable information on the nutritional value of alfalfa is available. Alfalfa is comparatively high in protein which could well supplement the deficiencies of grains in certain amino acids. If alfalfa were used at a level to provide twenty per cent protein, it would more than meet requirements of the chick for arginine, lysine, cystine and tryptophan. It would fall short in supplying sufficient methionine and possibly glycine. When it is considered that these six amino acids are important in formulating practical poultry rations, one becomes impressed with the potential value of alfalfa meal.

Alfalfa meal is also an excellent source of vitamins. It contains many mineral elements, and good quality alfalfa will furnish between thirty and forty per cent as much total digestible nutrients as the common cereal grains.

In addition to its known and potential nutritive values, alfalfa is a feedstuff that is normally available in large quantities at prices below those of grains and other feeds.

Alfalfa meal is ordinarily fed at levels of about three to five per cent in chick rations. The following investigations deal with the possibility of a greater and more efficient utilization of this feedstuff in chick rations.

REVIEW OF LITERATURE

Sampson and Mussehl (24, p.306) were among the early workers to study the value of alfalfa in poultry rations. In 1936 they conducted feeding trials with White Plymouth Rock cockerels and obtained some interesting weight comparisons. At twelve weeks of age, males on 5 per cent alfalfa meal averaged fifty-seven grams (two ounces) heavier than similar birds on a 20 per cent alfalfa meal ration. After the birds were dressed the carcasses of the latter group averaged fifty grams more (approximately two ounces) than those fed a 5 per cent alfalfa meal ration. These results do not indicate any great adverse effect of alfalfa meal on growth.

Heywang (12, p.25) in 1938 reported that 4 per cent alfalfa leaf meal supplying 3600 units of vitamin A per pound of diet was the level beyond which no additional chick growth was attained. Autoclaving the alfalfa leaf meal improved its growth promoting property, and the explanation was offered that autoclaving possibly increased the digestibility of the fiber. The results indicated that 8 per cent was the highest level at which untreated alfalfa leaf meal should be included in a diet for growing chicks during the first eight weeks of life, but that autoclaved alfalfa leaf meal could be used at a level as high as 16 per cent of the total diet.

Clark, Rennels and Van Landingham (4, p.26) in 1944 found a variation in rate of growth between pullets and cockerels when alfalfa was incorporated in the ration. The results suggested that with cockerels, levels of 9 and 12 per cent alfalfa meal were less efficient for

growth than lower levels. No such relationship seemed to exist when the same rations were fed to pullets. They also found that a 5 per cent level of alfalfa meal gave best growth, but that a level up to 12 per cent of a good grade of alfalfa could be used without loss of feed efficiency. In 1946 Hart and Stuart (11, p.11) also conducted an experiment to determine the most desirable level of alfalfa meal in poultry mashes. Results from this work in which 0, 10, 15 and 20 per cent levels of alfalfa meal were fed indicated that the 10 per cent level was the most satisfactory.

Using from 7.4 per cent to 49.2 per cent alfalfa meal in chick rations, Alderson (3, p.6) found in 1947 that as the alfalfa level was increased, feed consumption per pound of net gain was increased and average net gain per chick decreased. Chicks fed the ration containing 49.2 per cent alfalfa had a mortality rate of sixty per cent. A satisfactory level of alfalfa intake appeared to lie between 7 and 15 per cent. Cooney, et al (5, p.830) in 1948 found that with each addition of 5 per cent alfalfa meal in a chick ration above the 5 per cent level there was a significant depressing effect on growth. When compared with chicks fed rations containing equivalent levels of fiber (Cellu Flour), it appeared that the results obtained with the various levels of alfalfa could not be entirely attributed to fiber. These studies indicated that there was an unidentified factor or factors in the alfalfa meal used which effectively reduced growth when fed at or above the 10 per cent level.

Draper (8, p.659; 9, p.18) working with various levels of field-cured and dehydrated alfalfa meal cut and prepared from the same plot

of ground found that the addition of some alfalfa exhibited a beneficial influence on rate of chick growth, and that as the amount of either field-cured or dehydrated alfalfa was increased from 5 to 15 per cent in the ration, the rate of gain tended to decrease.

On the contrary studies by Cooney and co-workers (6, p.2) with sun-cured and dehydrated alfalfa meal in chick rations have indicated that growth on 20 per cent sun-cured alfalfa meal was better than on an equal amount of dehydrated meal. Pelleting the different alfalfa meal rations reduced the growth depressing properties of both products. Jensen (15, p.14) has also observed that all groups of chicks fed pelleted rations containing various levels of alfalfa significantly outgained similar groups fed all-mash rations with corresponding levels of alfalfa. There was also a definite downward trend in growth rate as the level of alfalfa meal was increased.

Payne, et al (21, p.71) have reported that when alfalfa was cut in the prebud stage, bud stage and blossom stage and with alfalfa of each stage being dehydrated, shade-cured or sun-cured, the slowest growth was with the sun-cured meal from alfalfa cut at blossom stage.

Wilgus (25, pp.2,4) indicated that certain grades of alfalfa may actually be unpalatable to chicks and hence responsible for depressing growth on levels above 5 per cent in their rations. From further observations it appeared to Wilgus that the depressing effect upon chick growth may not be so much a matter of absolute level of fiber in the mash as bulkiness of the ration, that is, the volume of feed per unit weight. Later, in studying the growth promoting properties of eighty different samples of alfalfa leaf meal, Wilgus (26) reported that

different samples of alfalfa contained varying amounts of growth inhibitor when fed at a level of 20 per cent of the ration, and that dehydrated alfalfa leaf meal from the third cutting appeared to contain more of the growth inhibiting factor(s) than meal from earlier cuttings. In line with this Insko and Culton (14, p.769) reported that great differences existed in the growth promoting properties of different samples of alfalfa meal when fed to chicks and that these differences can probably be attributed to factors other than the fiber content.

Heywang (13, p.25) reported that when diets containing 0, 5, 10, 20 or 25 per cent dehydrated or sun-cured alfalfa meal were fed to different groups of White Leghorn pullets to twenty weeks of age, there was considerable variation in the growth depressing effects of the alfalfa meals. Growth depression was not accompanied by a decrease in feed consumption at whatever the level of dehydrated alfalfa meal. The 25 per cent level of sun-cured alfalfa meal did, however, result in a decrease in feed consumption.

Mussehl, Ackerson and Borchers (20, p.2) indicated in 1950 that there were limitations to the use of alfalfa because the nutrients are wrapped in fiber for which growing chicks have a relatively low utilization capacity. Experiments were carried on with high corn base rations carrying from 0 to 12 per cent of high quality dehydrated alfalfa meal. Parallel lots were fed pelleted rations containing from 0 to 15 per cent alfalfa. The authors concluded that there was no evidence of a growth inhibitor in the alfalfa products used in their

experiments.

Using two different samples of dehydrated alfalfa leaf meal, German and Couch (10, pp.844-845) reported that one sample depressed growth, whereas the other had little effect on the growth of chicks to ten weeks. There was a relation between the depression of growth and the level of alfalfa fed when 10, 20, 30 and 50 per cent of the inhibitory sample was included in the diet. This growth inhibition could not be overcome by feeding 3 mg. of copper per pound of feed. The feeding of a ration containing 50 per cent dehydrated alfalfa leaf meal resulted in 100 per cent mortality. The growth inhibitory substance in the alfalfa leaf meal was not extracted by 95 per cent hot ethanol.

It is interesting to note that Alder (1; 2, p.651) has reported that growing turkeys may be fed 35 to 40 per cent alfalfa leaf meal in the ration, if this is not included in the diet until after the eighth week. He pointed out, however, that the feeding of a ration containing 50 per cent alfalfa meal after the eighth week would reduce the growth rate.

The effect of fiber has been investigated by Davis and Briggs (7, pp.298-299) who found a significant increase in growth of chicks when 5, 10 and 15 per cent cellulose was added to a purified chick diet free of fiber but complete in all known nutrients. Additions of cellulose at levels ranging from 20 per cent through 50 per cent resulted in retarded growth but there was practically no mortality in these groups under the experimental conditions described. Lepp et al (17, p.374) showed that growth rate of young chickens on purified

diets containing 15 or 18 per cent casein was improved by the addition of cellulose up to a level of 10 per cent. The authors mentioned that cellulose may allow increased intestinal synthesis of the B group of vitamins.

Lepkovsky, Peterson and others at the University of California began a series of studies in 1946 on the growth inhibiting effect of high levels of alfalfa. Lepkovsky et al (16, p.217) reported that dehydrated alfalfa meal contained a naturally occurring substance, probably organic in nature, which depresses the growth of chicks. The storage of alfalfa meal at room temperatures or in the cold (16° F.) had little effect on the growth-depressing substance of alfalfa meal and the growth inhibitor was apparently stable to the existing methods of preparing alfalfa meal and to autoclaving in neutral, alkaline or acid medium. The inhibitor could be removed from alfalfa by repeated extraction with hot water. The vitamins of the B complex, in the amounts fed, had no effect on the inhibitor.

By fractionating a hot water extract of alfalfa meal Peterson (22, p.653) in 1950 obtained concentrates which when fed depressed the growth of chicks. The active principle was soluble in 50 and 80 per cent ethanol and was largely precipitated from an aqueous alcohol solution by the addition of acetone. The growth-depressing action of this material was to a great extent counteracted by the simultaneous feeding of cholesterol. The inhibitory concentrates had hemolytic properties which were destroyed by boiling the concentrates with cholesterol. The growth inhibiting factor present in alfalfa meal was

tentatively identified as a saponin. As the studies progressed (23, pp.600-605) it was found that the addition of both cholesterol and cottonseed oil to a diet containing 20 per cent alfalfa meal completely prevented the growth depression otherwise produced in chicks on such a diet. An identical effect was obtained with cottonseed oil and a phytosterol mixture prepared from soybeans. Preliminary tests with tallene esters obtained from tall oil, a by-product of the paper pulp industry, in the 20 per cent alfalfa diets gave good growth results. The growth depression produced by Quillaja saponin in the chick diet was also prevented by the addition of a mixture of cottonseed oil and cholesterol to the diet. Raising the chicks' blood level of cholesterol by treatment with diethylstilbestrol was ineffective as a means of preventing the growth depression brought about by a high level of alfalfa meal in the diet. Counteraction of the alfalfa growth inhibitor or inhibitors was not dependent upon an increase in the plasma level of sterols as determined by the Liebermann-Burchard reaction.

EXPERIMENTAL

PART I

Nutritional Studies on Alfalfa Meal

The animals used in this study were New Hampshire chicks. They were hatched from eggs laid by one of the Oregon Agricultural Experiment Station laying flocks. The chicks were individually wing-banded and the experimental lots consisted of chicks of comparable weight. All lots were managed alike in thermostatically-controlled,

electrically heated, wire floored battery brooders. Free access to water was provided. Individual body weights and lot feed consumption were determined at weekly intervals.

Basal mixture and control ration.

The basal mixture is shown in Table 1.

Table 1

Basal Mixture

Ingredients	Weight
Wheat	400 lbs.
Corn	500 lbs.
Oats	200 lbs.
Meat meal	100 lbs.
Fish meal (herring)	175 lbs.
Skimmilk, dried	100 lbs.
Whey, dried	70 lbs.
Oyster shell flour	40 lbs.
Salt	10 lbs.
Feeding oil 400 D, 3,000 A	5 lbs.
Manganese sulphate	6 ozs.
Total	1,600 lbs.-6 ozs.

In all instances except those to be indicated, the basal mixture constituted 80 per cent of the rations fed. The remaining 20 per cent consisted of various alfalfa products in the experimental ration and of millrun in the control ration.

The basal mixture, constituting 80 per cent of all rations, was formulated to meet the known requirements of the chick for the vitamins, essential amino acids and minerals. This basal mixture was used by Cooney et al (6, p.1).

Millrun was used in the control ration as a comparison for alfalfa. It is a by-product of the wheat milling industry. The crude fiber values of alfalfa and millrun are about 26 per cent and 8 per cent respectively and crude protein 19 per cent and 14 per cent. They both are bulky in form and are used in feeds by the poultry industry.

Alfalfa products.

In March, 1949, arrangements were made to obtain the following types of alfalfa meal which were taken from the same field and handled alike with the exception of treatments desired:

(1) Dehydrated alfalfa meal - alfalfa cut in the bud stage and artificially dehydrated. The temperature of the dehydrator was approximately 1500° F. at the entrance and 250° F. at the exit. The approximate time for passage of the alfalfa through the dehydrator was a minute and a half.

(2) Early sun-cured alfalfa meal - alfalfa cut at the same time as the dehydrated product but sun-cured.

(3) Normal sun-cured alfalfa meal - alfalfa cut when it was one-fourth in bloom and sun-cured.

The above products were prepared from successive cutting swaths. They were ground through the same size screen.

The crude protein values for the alfalfa products as determined from Kjeldahl nitrogen were: early sun-cured alfalfa, 16.5 per cent; dehydrated alfalfa, 19.7 per cent; and normal sun-cured alfalfa, 15.3

per cent. The millrun contained 13.7 per cent crude protein. These alfalfa meals and millrun were used in Experiments 1, 2 and 3.

For Experiment 4, alfalfa meal, alfalfa leaves, alfalfa stems and fresh alfalfa were prepared from alfalfa cut in the bud stage and full bloom stage. Each product except the fresh alfalfa was dehydrated at a temperature between 104° and 122° F. The fresh alfalfa was prepared by cutting the green plants and quickly freezing them between cold plates in a deep freeze at a temperature of approximately - 13° F. After the alfalfa plants were frozen, they were ground and stored at 10° F. Leaves and stems were separated manually from the dehydrated plants and finely ground. Crude protein values for the products tested were: alfalfa meal (bud stage) 17.5 per cent; alfalfa leaves (bud stage) 22.1 per cent; alfalfa stems (bud stage) 9.4 per cent; millrun - 16.1 per cent; alfalfa meal (full bloom stage) 12.7 per cent; alfalfa leaves (full bloom stage) 20.7 per cent; alfalfa stems (full bloom stage) 6.6 per cent. Moisture content as determined by air-drying for the fresh alfalfa was: fresh alfalfa (bud stage) 70 per cent; fresh alfalfa (full bloom stage) 58 per cent.

Experiment 1 - Comparison of sun-cured and dehydrated alfalfa meal.

According to one report (8, p.659), there appears to be no difference between the effects of field-cured or dehydrated alfalfa meals on the rate of chick growth. Another report (5), however, indicates that growth on 20 per cent sun-cured alfalfa meal was greater than on

an equal amount of dehydrated meal. The knowledge of any difference between the two types of alfalfa meal would be of economical value to the poultry industry. This experiment compares the effects of early sun-cured and dehydrated alfalfa meals on chick growth. Alfalfa meals were carefully prepared from the same field as described under alfalfa products so that any difference obtained would be attributed to the method of curing the alfalfa meal.

Three lots of ten chicks each were used in this experiment. Rations 1, 2 and 3 as described in Table 2 were fed ad libitum. Average weekly weights for each lot are presented in Figure 1. Feed consumption, feed utilization and average weight at eight weeks are presented in Table 2.

It will be seen from the growth curves in Figure 1 that chicks receiving either dehydrated or early sun-cured alfalfa meal (No. 2 and 3) grew at a slower rate than the control lot (No. 1). The typical inhibiting effect of alfalfa meal on growth is evident. There is essentially no difference in rate of growth between the rations containing dehydrated or early sun-cured alfalfa meal. Examination of the data in Table 2 shows that the chicks (Lot No. 1) with access to the ration containing millrun consumed more feed than those on diets containing dehydrated or early sun-cured alfalfa meal (Lots No. 2 and 3); average feed consumed per chick for Lots No. 1, 2 and 3 was 5.17 lbs., 4.01 lbs. and 3.85 lbs. respectively. It is of interest to note that feed efficiency for the three lots was practically the same. In previous work (6) the control ration has invariably given the best feed efficiency. From the standpoint of rate of gain, feed consumption

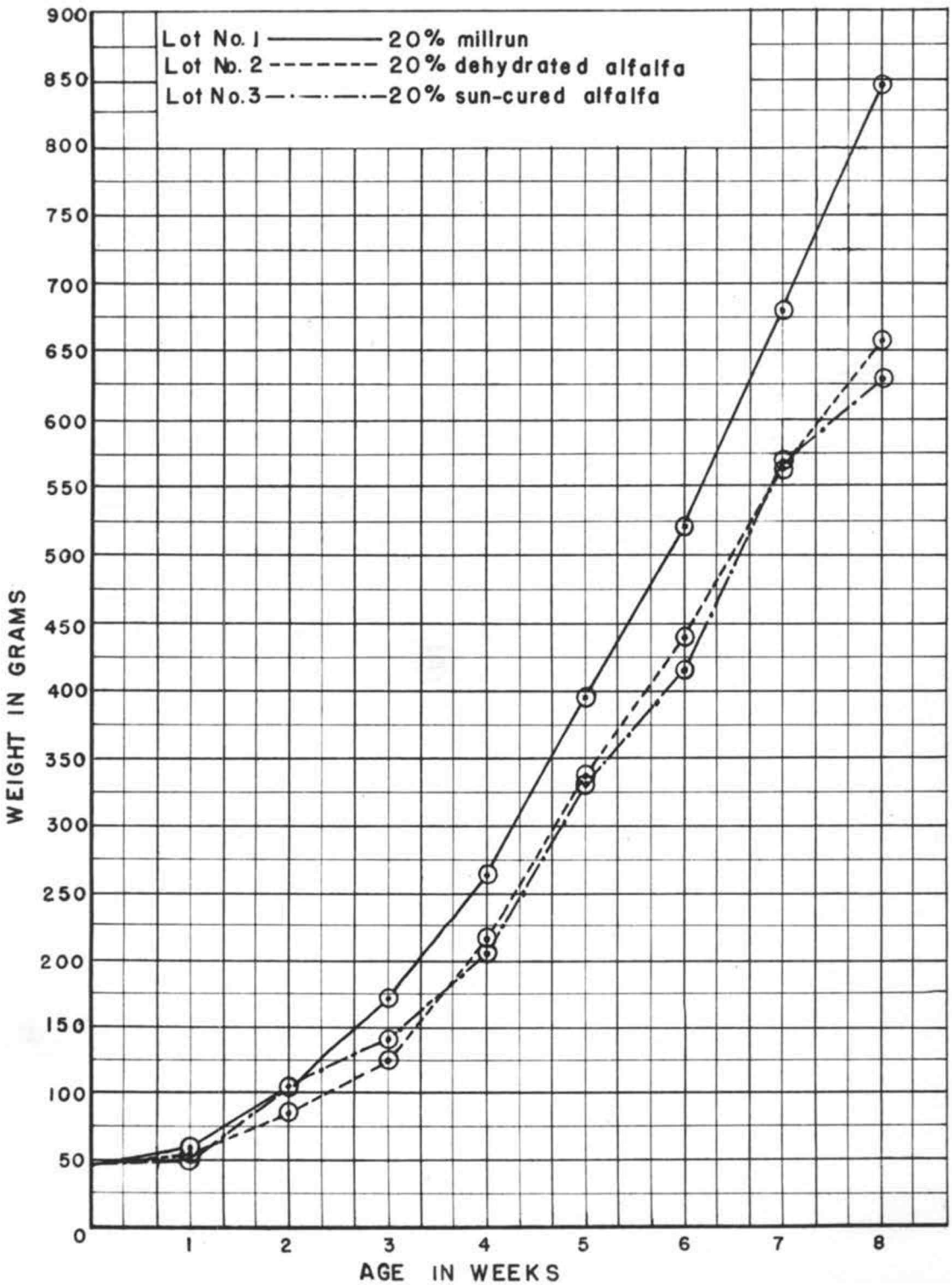

Figure 1. Growth rate of New Hampshire chicks on rations fed in Experiment No. 1.

and feed utilization, the dehydrated and early sun-cured alfalfa meals were about the same at the 20 per cent level.

Table 2 -- Effect of ration upon feed consumption, feed utilization and chick weight at eight weeks in Experiment No. 1.

Rations	Lot No.	No. of birds	Average pounds of feed per chick	Lbs. of feed per pound of gain	Average chick weight in grams	Standard deviation in grams
20% Millrun	1	10	5.17	2.94	848	125
20% Dehydrated alfalfa	2	10	4.01	2.97	659	85
20% Early sun-cured alfalfa	3	10	3.85	2.98	634	47

Experiment 2 - Comparison of early and normal sun-cured and dehydrated alfalfa meal.

Sun-cured and dehydrated alfalfa meals were compared again since the sample of normal sun-cured alfalfa was not available for Experiment 1. Ten lots of twenty chicks each were used. For the first week all chicks were fed the basal mixture. At the end of this period all chicks were weighed, rate of growth noted and the number reduced to eighteen; thus making all lots as nearly comparable as possible. The test rations shown in Table 3 were fed subsequently for seven weeks. Two methods of feeding were employed. Chicks in Lots 1 through 6 were allowed free access to their rations at all times with the exception of those in Lot 2. In this lot the level of feed intake was

restricted on a chick basis to approximately 80 per cent of that consumed by the chicks in Lot 3. The amount of feed consumed on each successive day by chicks in Lot 3 was used to determine the ration for the subsequent day in Lot 2.

Table 3 - Experimental rations for Experiment No. 2.

Ingredients	Lot numbers					
	1	2	3 & 7	4 & 8	5 & 9	6 & 10
			Control			
Basal mixture	100	100	80	80	80	80
Millrun			20			
Alfalfa dehydrated				20		
Alfalfa, early sun-cured					20	
Alfalfa, normal sun-cured						20
Total	100	100	100	100	100	100

In all previous experiments chicks fed the basal mixture plus 20 per cent millrun on an ad libitum feeding program consumed more feed per bird than those receiving the basal plus 20 per cent alfalfa meal. This had raised the question of palatability. To aid in determining the actual nutritional value of the test products, chicks in Lots 7 through 10 were fed an equal amount of feed (based on number of chicks). This was accomplished by restricting the subsequent daily feed intake of three of the four lots to that of the lot with the lowest feed intake. This "fourth" lot always had free access to its ration.

Average weekly weights for chicks in Lots 1 through 6 are plotted

in Figure 2. Similar weight curves for the chicks in Lots 7 through 10 are presented in Figure 3. Feed consumption, feed utilization and chick weights are presented in Table 4.

From the data presented in Table 4 and Figure 2 it will be noted that the same relative growth rates were obtained in this experiment as in Experiment No. 1 with respect to rations fed. In this experiment chicks allowed free access to the basal mixture grew at a slower rate than chicks fed the same basal mixture to which 20 per cent millrun had been added. A plausible explanation for this difference could lie in the greater food intake per chick on the latter ration; a difference of 0.49 lb. of feed per chick. Efficiency of feed utilization on these two rations was comparable.

Chicks in Lot 2 which were fed the basal mixture at a restricted level (approximately 80 per cent of the intake of Lot 3) exhibited an even slower rate of gain. The average feed intake per chick was 0.73 lb. less than similar chicks on an ad libitum feeding of the same ration and 1.12 lbs. less than the chicks receiving ad libitum a ration composed of the basal mixture plus 20 per cent millrun. The pounds of feed required to produce a pound of gain were similar to the other two lots. With the basal mixture used in this work the addition of 20 per cent bulky material such as millrun appeared to promote chick growth.

For the first three weeks that the birds were on the test rations there was little difference in rate of growth between the lots receiving the three types of alfalfa meal. After three weeks chicks fed the normal sun-cured and dehydrated meals began to fall behind those fed the early sun-cured meal. Part of these differences might

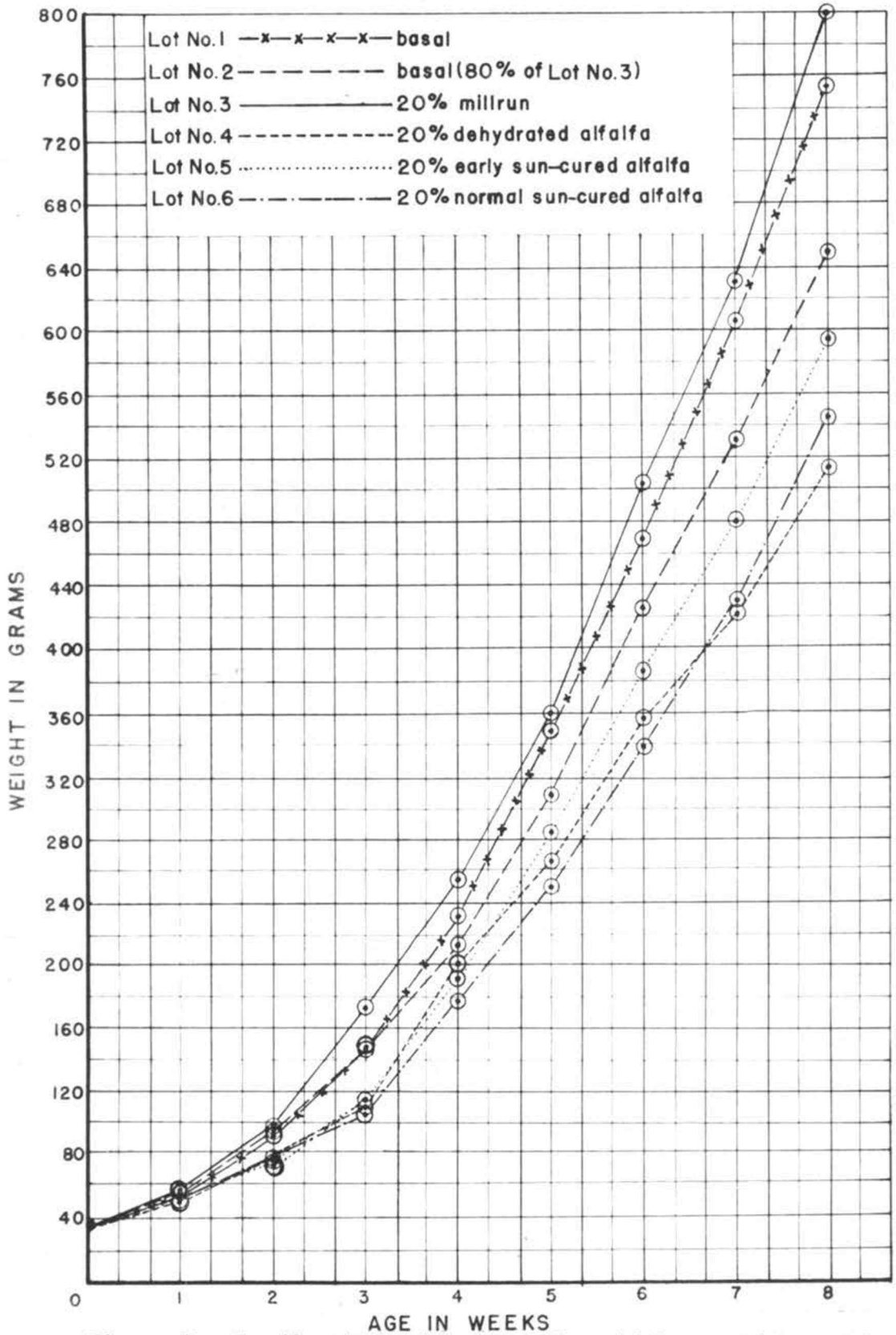

Figure 2. Growth rate of New Hampshire chicks on rations fed in Experiment No. 2.

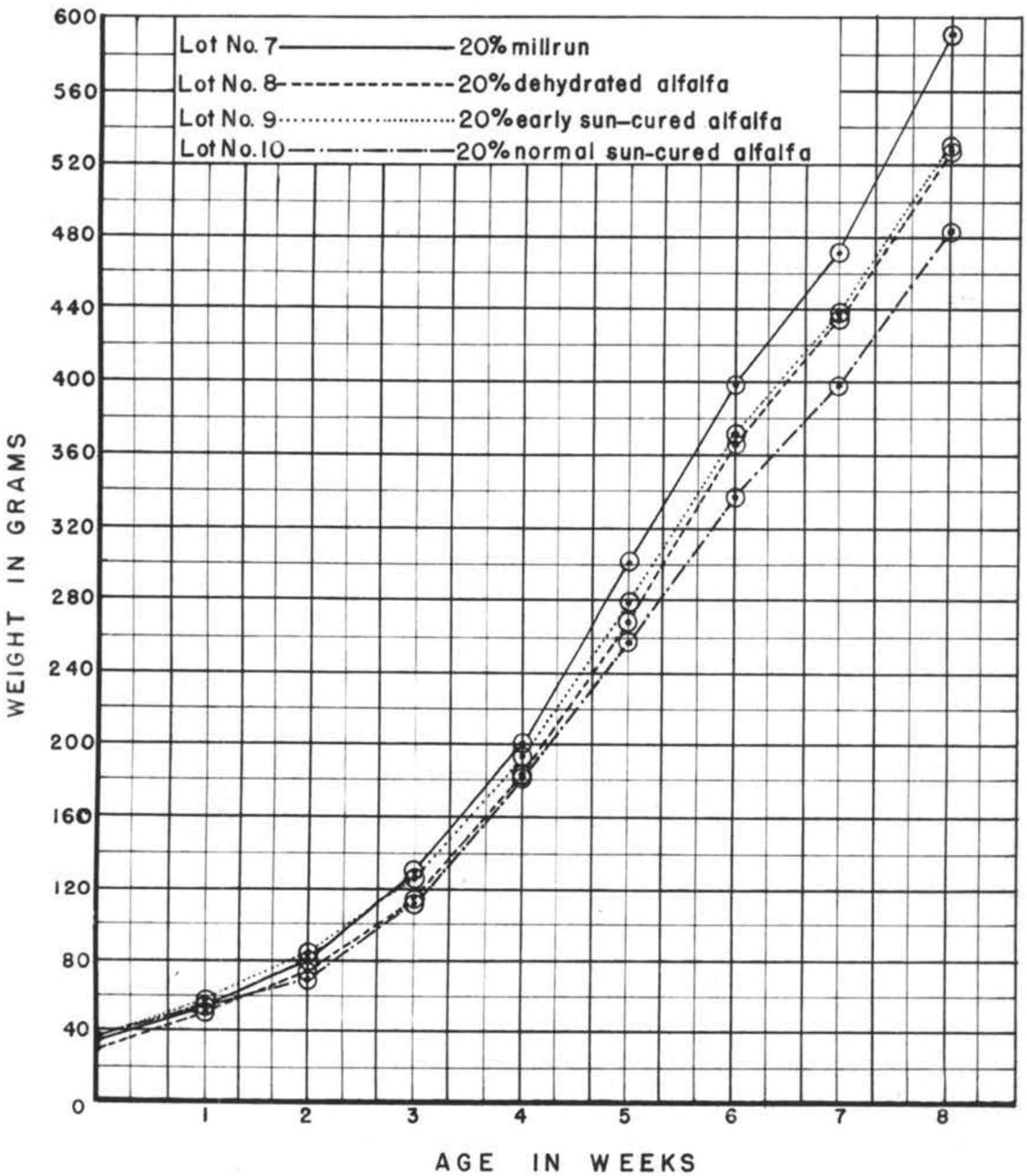

Figure 3. Growth rate of New Hampshire chicks fed equal amounts of the various rations in Experiment No. 2.

Table 4 -- Effect of ration upon feed consumption, feed utilization and chick weights at eight weeks in Experiment No. 2.

Rations	Lot No.	Number of chicks		Average pounds of feed per chick	Lbs. of feed per pound of gain	Average chick weight in grams	Standard deviation in grams	Methods of feeding
		Started	Finished					
Basal mixture	1	18	15	4.64	2.94	753	94	ad lib.
Basal mixture (80% of Lot 3)	2	18	16	3.91	2.90	650	90	80% of Lot 3
Basal mixture & 20% Millrun	3	18	17	5.03	2.99	800	107	ad lib.
Basal mixture & 20% Deh. alf.	4	18	16	3.69	3.45	523	74	ad lib.
Basal mixture & 20% early sun-cured	5	18	14	4.10	3.33	596	104	ad lib.
Basal mixture & 20% normal sun-cured	6	18	15	3.80	3.39	544	48	ad lib.
Basal mixture & 20% Millrun	7	18	17	3.59	2.94	591	73	*Restricted
Basal mixture & 20% Deh. alf.	8	18	18	3.67	3.40	527	88	*Restricted
Basal mixture & 20% early sun-cured	9	18	16	3.57	3.31	528	74	*Restricted
Basal mixture & 20% normal sun-cured	10	18	15	3.61	3.68	482	92	*Restricted

*Restricted to smallest intake of groups 7, 8, 9, 10.

be explained on the basis of feed intake per chick. Chicks in Lot 4 (20 per cent dehydrated alfalfa meal) consumed 3.69 pounds of feed each. Those in Lot 6 (20 per cent normal sun-cured alfalfa meal) consumed 3.80 lbs. of feed each. The chicks in Lot 5 (20 per cent early sun-cured alfalfa meal) consumed 4.10 lbs. of feed each. These differences in feed consumption are small, but when feed intake was restricted to approximately the same level every day throughout the seven-week test period for all rations, rate of growth (see Figure 3) was the same for chicks receiving early sun-cured meal (Lot 9) and dehydrated meal (Lot 8). This is in agreement with results obtained in Experiment 1 where feeding was on a free choice basis with a difference in feed intake of only 0.16 lb. per chick.

As pointed out above, chicks fed the normal sun-cured alfalfa meal grew more slowly than those receiving early sun-cured meal or dehydrated meal.

Again in all instances when alfalfa meal was incorporated in chick rations at the 20 per cent level growth rate was significantly less than that obtained with chicks fed the straight basal mixture or the basal mixture plus 20 per cent millrun.

Experiment 3 - Contribution of alfalfa meal to the basal mixture.

Since the previous experiments with alfalfa at the 20 per cent level showed an inhibiting effect on chick growth, it was desirable to determine the amount of growth obtained from the alfalfa portion of the ration. This was carried out by allowing chicks access to the alfalfa ration and restricting a second group of comparable chicks to

the consumption of an amount of basal mixture equal to that in the alfalfa ration. Thus, both groups consumed the same amount of basal mixture but the alfalfa group consumed an additional 20 per cent of alfalfa meal.

Four lots of fifteen chicks each were used. Rations as shown for Lots 2, 3 and 4 in Table 5 were fed ad libitum. The ration shown under Lot 1 was fed ad libitum for the first week and thereafter was restricted daily on a per chick basis to approximately 80 per cent of the ration consumed by chicks in Lot 3. Average weekly weights for each lot are presented in Figure 4. Feed consumption, feed utilization and average chick weights at eight weeks are presented in Table 6.

Table 5 - Experimental rations for Experiment No. 3.

Ingredients	Lot numbers			
	1	2	3	4
Basal mixture	100	80	80	60
Millrun		20		40
Alfalfa, dehydrated			20	
Total	100	100	100	100

From data presented in Figure 4 and Table 6 it will be noted that the chicks in Lot 1, which received the basal mixture restricted to 80 per cent of the feed intake for Lot 3, grew slightly more than the chicks in Lot 3. Chicks in Lots 1 and 3 consumed the same amount of basal mixture but the Lot 3 chicks consumed an additional 20 per cent of dehydrated alfalfa meal. For this additional 20 per cent intake

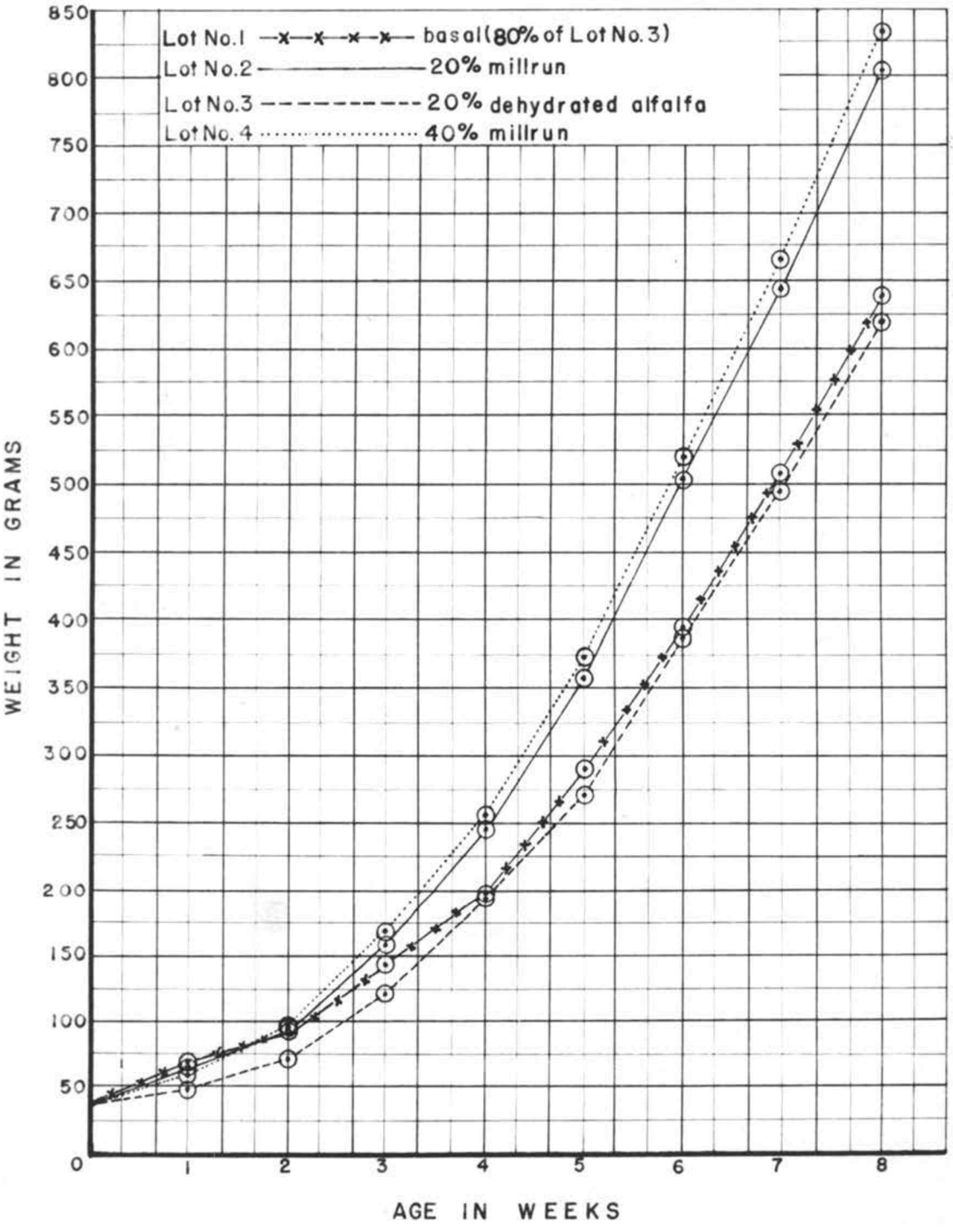

Figure 4. Growth rate of New Hampshire chicks on rations fed in Experiment No. 3.

Table 6 -- Effect of ration upon feed consumption, feed utilization and chick weights at eight weeks in Experiment No. 3.

Rations	Lot No.	Number of chicks		Average pounds of feed per chick	Pounds of feed per pound of gain	Average chick weights in grams	Standard deviation in grams
		Started	Finished				
Basal mixture (80% of Lot 3)	1	15	13	3.31	2.50	639	86
Basal mixture & 20% millrun	2	15	15	4.42	2.63	803	79
Basal mixture & 20% deh. alf.	3	15	14	4.12	3.22	621	97
Basal mixture & 40% millrun	4	15	12	5.59	3.21	831	92

of alfalfa, no increase in growth was obtained. In other words, the feeding value of the alfalfa in this experiment appeared to be zero or slightly negative. For comparison of similar chicks receiving the basal mixture ad libitum refer to Lot 1 of Experiment 2.

Chicks in Lot 2 grew at a normal rate. Chicks in Lot 4 which were fed a ration composed of 60 per cent basal mixture and 40 per cent millrun were equal to or slightly better than comparable chicks fed a ration composed of 80 per cent basal mixture and 20 per cent millrun. In view of the increased bulkiness of feed in the 40 per cent millrun lot, it appears that the basal mixture is adequate for good chick growth even when diluted as much as 40 per cent with millrun.

Experiment 4 - Effect of alfalfa meal, alfalfa leaves, alfalfa stems and fresh alfalfa on chick growth.

The growth-inhibiting effect of high levels of alfalfa has frequently been attributed to the crude fiber in the alfalfa. However, chicks (5, p.830) fed various levels of alfalfa grew less than chicks fed rations containing equivalent levels of fiber in the form of cellu flour. Also Experiment 3 showed that chicks restricted to the amount of basal mixture consumed by comparable chicks on a 20 per cent alfalfa ration grew more than the chicks on the 20 per cent alfalfa ration. This experiment was made to determine the effect of alfalfa stems which contain the major portion of the crude fiber in alfalfa, alfalfa leaves, alfalfa meal and fresh alfalfa on chick growth.

The alfalfa products were prepared as described under alfalfa products. Each product except the fresh alfalfa was incorporated in the test ration at the 20 per cent level. Each day throughout the experimental period portions of the frozen alfalfa were thawed and then mixed by hand into the basal mixture at a level equivalent to 20 per cent dry alfalfa meal. Twelve seven-day old unsexed chicks were used in each lot. Rations shown in Table 7 were fed ad libitum. Average weekly weights for each lot are presented in Figure 5. Average chick weights, feed consumption and feed utilization at five and eight weeks of age are presented in Table 7. Experimental lots fed the fresh alfalfa rations were discontinued at the end of four weeks. The different alfalfa products depressed chick growth. There was little difference between the products cut in the bud stage or full bloom stage. Alfalfa leaves at the 20 per cent level inhibited growth to a much greater extent than the stems; the final weights were 518 and 513 grams at eight weeks on leaves as compared to 713 and 700 grams on stems.

PART II

CHEMICAL STUDIES ON ALFALFA MEAL

Extraction of Chick Growth Inhibitor in Alfalfa

The nutritional studies indicated that chick growth was depressed by high levels of alfalfa. Studies were then undertaken to determine possible methods of extracting the chick growth inhibitor from alfalfa.

Table 7 - Effect of ration upon chick weight, consumption and feed utilization at five and eight weeks of age in Experiment No. 4.

Rations	Lot No.	Number of chicks		Average chick weight in grams		Average Lbs. of feed per chick		Pounds of feed per lb. of gain	
		Start	Finish	5 wks.	8 wks.	5 wks.	8 wks.	5 wks.	8 wks.
Control									
20% Millrun	1	12	12	436	903	1.99	6.16	2.44	3.33
Alfalfa in bud-stage									
20% Fresh Alfalfa*	2	12	12	381	---	1.60	----	2.29	----
20% Deh. Alf. Meal	3	12	12	332	651	1.48	3.67	2.53	2.85
20% Deh. Alf. Leaves	4	12	12	247	518	1.15	3.13	2.88	3.14
20% Deh. Alf. Stems	5	12	12	362	713	1.82	4.58	2.19	3.20
Alfalfa in full-bloom stage									
20% Fresh Alfalfa*	6	12	12	348	---	1.54	----	2.47	----
20% Deh. Alf. Meal	7	12	12	311	644	1.50	4.03	2.77	3.15
20% Deh. Alf. Leaves	8	12	12	286	513	1.32	3.20	2.72	3.23
20% Deh. Alf. Stems	9	12	12	365	700	1.96	4.68	2.97	3.34

*Air-dry basis.

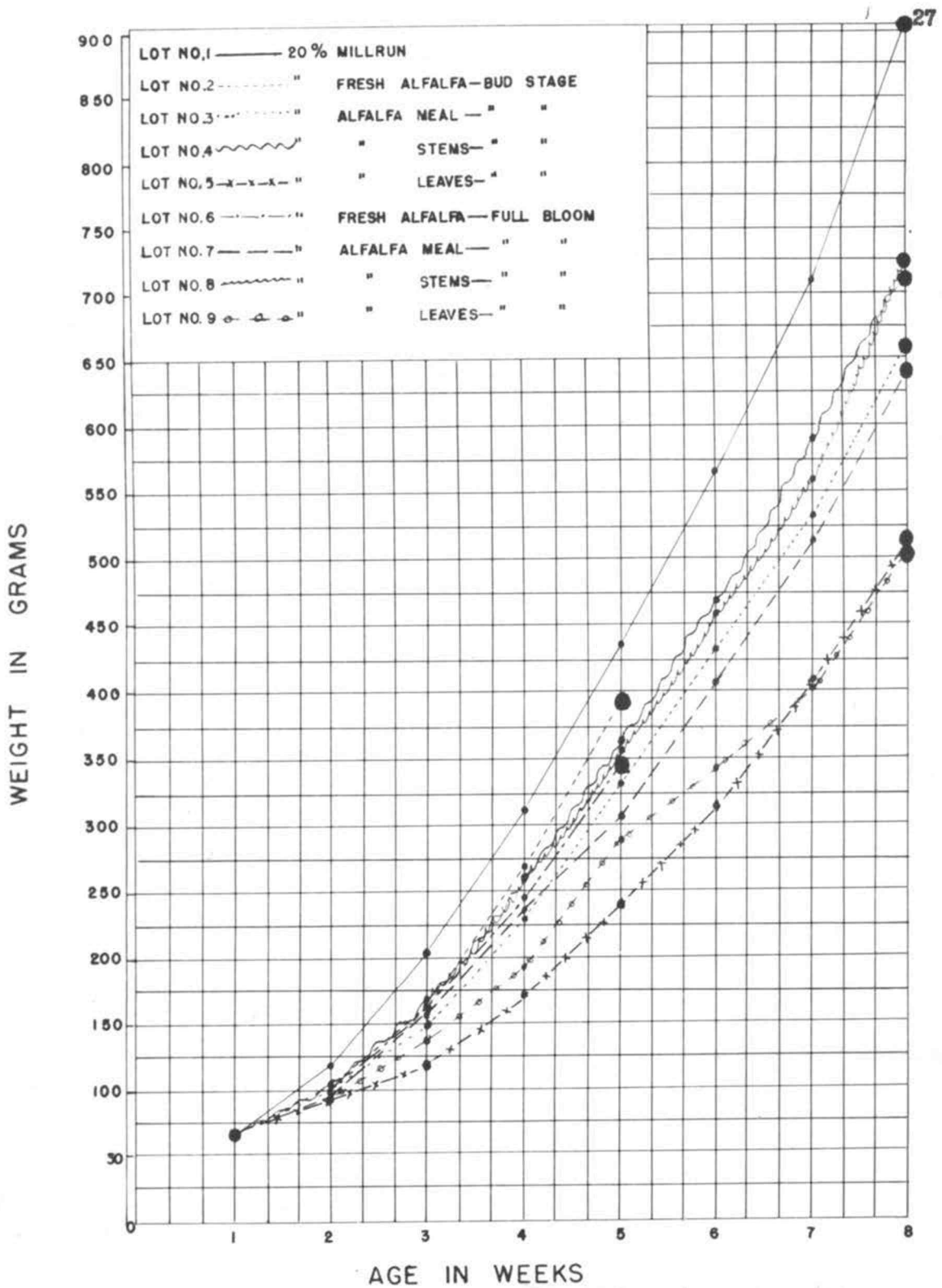

Figure 5. Growth rate of New Hampshire chicks on rations fed in Experiment No. 4.

Extraction.

Extraction of the growth inhibitor was first attempted with water. In trial 1 (see Table 8) alfalfa meal was extracted by allowing cold tap water to gently flow for twenty-four hours through the alfalfa meal which was enclosed in a fine sack and kept in a container. The alfalfa meal was then pressed as dry as possible in a filter press and placed in a drier at room temperature. A second batch of alfalfa meal was extracted in the same way using hot water (70° C.). A third batch of alfalfa meal was extracted with petroleum ether in a soxhlet-type of extractor for three days. A fourth batch was extracted with water for twelve hours at a pH of 3.9 using one gallon of water per pound of alfalfa.

In other trials, the general procedure employed in the extraction may be broadly outlined as follows: the alfalfa meal was extracted for twelve hours or longer using one gallon of water per pound of alfalfa. Extractions were carried out at room or higher temperatures. The water-alfalfa mixture was ladled in a fine sack and pressed in a filter press until the alfalfa was fairly dry. The extracted alfalfa was placed in a drier at room temperature. The water extracts were concentrated *in vacuo*. The extract was mixed with some millrun and dried. Batches of alfalfa meal were also extracted with ether or with different concentrations of ethanol.

One method of extraction was patterned after the procedure of Peterson (22, p.653). The alfalfa meal was extracted overnight at room temperature using one gallon of water per pound of alfalfa. Toluene

and chloroform were added to inhibit bacterial growth. The mixture was filtered and the residue washed three times with a large volume of water at 90° C.; the extract being pressed out each time in a filter press. The residue was dried at 40° C. The combined filtrates were concentrated *in vacuo* and 95 per cent ethanol added until a concentration of 80 per cent alcohol was reached. The resulting precipitate was filtered and mixed with millrun and dried.

In another case acetone was added with constant stirring to the alcohol filtrate until a syrupy mass separated out. The entire mass was dissolved in 50 per cent ethanol and mixed with millrun and dried. Results using these fractions are presented under trial 6 in Table 8.

Arrangements were made with the Western Regional Research Laboratory[1] to obtain saponin concentrates, sapogenin concentrates, flavone fractions, concentrated water extracts and water-extracted alfalfa from alfalfa meal. These alfalfa products were prepared in the following manner. The alfalfa meal was mixed with nearly boiling water, ladled into press cloths and drained by pressing in a cider press. Each batch of meal was treated in this fashion three successive times. Starting with sixty pounds of meal a total of 610 pounds of dilute extract solution was obtained. The extracted meal was dried under vacuum at 65° C.

[1] I am indebted to the United States Department of Agriculture, Western Regional Research Laboratory, Albany, California, for the preparation of these products. Dehydrated alfalfa meal designated as Wilgus #88 was used in these preparations.

The total dry weight of the extracted meal from 60 pounds of original meal was 39.75 pounds. Thus, 1 pound of extracted meal represented 1.5 pounds of original meal.

A portion of the dilute extract solution was vacuum concentrated to a total solids content of 62.24 per cent. This was designated as concentrated water extract.

Other portions of the dilute extract solution were vacuum concentrated and used in the preparation of fractions containing saponin, sapogenin and flavone.

The saponin concentrate was prepared by treating the concentrated water extract with 1-1 HNO_3 to pH 3, centrifuging down and discarding the precipitated protein and treating the liquor with saturated neutral lead acetate until precipitation was complete. The liquor was again treated with lead acetate and centrifuged. The combined lead precipitates were suspended in water and treated with H_2S to remove the lead as sulfide. The filtrate from the lead sulfide was concentrated and an equal volume of alcohol was added to precipitate pectin. The liquor freed from pectin was concentrated under reduced pressure and represented the saponin concentrate.

The sapogenin concentrate was prepared from the saponin concentrate. The saponin concentrate was treated with concentrated HCl and the mixture was boiled for twenty minutes. A gelatinous precipitate formed. It was centrifuged and dissolved in alcohol.

The extract fraction soluble in 80 per cent ethanol and insoluble in 85 per cent ethanol was prepared in the following way. Sufficient

alcohol was added to the concentrated water extract to raise the alcohol concentration to 80 per cent. The precipitate obtained upon filtering the mixture was dissolved in water and the procedure above was repeated twice. The combined solutions were freed of alcohol and the aqueous mixture was shaken thoroughly with chloroform. The aqueous solution was drawn off from the chloroform and mixed with ethanol whereupon a dark brown syrupy precipitate formed in the resulting 85 per cent ethanol solution. The precipitate was partially freed of ethanol by heating on a steam bath.

For the preparation of flavone and phenolic fractions the mother liquor from the precipitate just described was stirred with a 50-50 mixture of magnesium oxide and diatomaceous earth. Suction filtration of the mixture gave a bright yellow filter cake. This cake was first washed with 95 per cent alcohol, (washings were discarded) and then mixed with water and concentrated hydrochloric acid. The hot mixture was suction filtered. The filter cake was washed once with water and four times with 95 per cent ethanol. The alcoholic washings were combined and freed of alcohol. A slimy precipitate, which gave a positive sulfuric acid test for sapogenin, formed and was removed from the solution by centrifuging and decanting. The solution was extracted twice by shaking with isoamyl alcohol, centrifuging and siphoning off the alcohol layer. The isoamyl solution was washed five times by shaking with water, then partially dried with sodium sulfate and filtered. The isoamyl alcohol and water were removed from the solution by distillation which was finished under reduced pressure. The residue was dissolved in 95 per cent ethanol and designated as "flavone

fraction".

Growth assays.

The biological assays employed to detect the presence or absence of the growth-inhibiting factor were growth assays using chicks. These assays were conducted in the same manner as in the previous nutritional studies reported in Part I. Several of the studies on extraction, inactivation and determination of the growth inhibitor were included in one experiment. The extracted alfalfa meals were generally incorporated in the ration at 20 per cent level. The extracts were added to the control ration (basal plus 20 per cent millrun).

Growth of chicks on the various extracts and extracted alfalfa meals were compared with the growth of similar chicks on the 20 per cent alfalfa ration or the control ration.

Results are summarized in Table 8 and average weekly weights for each lot in trial 10 are presented in Figure 6. These data show that the growth inhibitor may be partially extracted with water. It will be noted that extraction of the inhibitor was not always successful even though the same procedure was followed. More of the inhibitor was removed by repeated batch extractions and by continuous extraction with tap water than by a single batch extraction. Also extraction with water at room temperature was better than at higher temperatures. Ethanol extraction did not appear to be very effective in removing the inhibitor.

Table 8 - Extraction of chick growth inhibitor in alfalfa using growth assays.

Trial	Ration and type of extraction	Equivalent per cent of alfalfa	Average chick weights in grams	Sex of chicks	Age of chicks started	No. of chicks started per No. of chicks finished	Duration of trial
1	85% Stock ration (5% alfalfa) plus 15% millrun.	4	326	Male	10 days	10/10	4 weeks
	87% Stock ration (5% alfalfa) plus 13% alfalfa extracted continuously with tap water for 24 hours.	19	298			10/10	
	87% Stock ration (5% alfalfa) plus 13% alfalfa extracted continuously with water (70° C.) for 24 hours.	19	302			10/10	
	87% Stock ration (5% alfalfa) plus 13% alfalfa extracted with water at pH 3.9 for 24 hours.	19	283			10/10	
	87% Stock ration (5% alfalfa) plus 13% alfalfa extracted in soxhlet-type extractor with petroleum ether for 3 days.	19	264			10/10	
2	20% Millrun (control)	0	237	Female	1 day	10/6	4 weeks
	20% Alfalfa	20	180			10/9	
	20% Alfalfa extracted with water for 12 hours using a stirrer.	23	191			10/6	
	20% Alfalfa extracted twice with water for 12 hours using a stirrer.	24	211			10/7	
	20% Alfalfa extracted with water for 24 hours using a stirrer.	24	218			10/8	
	Control plus 24-hour extract.	20	239			10/8	

Table 8 (continued).

Trial	Ration and type of extraction	Equivalent per cent of alfalfa	Average chick weights in grams	Sex of chicks	Age of chicks started	No. of chicks started per No. of chicks finished	Duration of trial
3	20% Millrun (control).	0	848	Male	1 day	10/9	8 weeks
	20% Dehydrated alfalfa.	20	659			10/10	
	20% Dehydrated alfalfa extracted with water for 12 hours.	22	735			10/10	
	20% Dehydrated alfalfa extracted with 100° C. water for 12 hours.	22	677			10/10	
	Control plus water extract of dehydrated alfalfa.	20	784			10/10	
	Control plus 100° C. water extract of dehydrated alfalfa.	20	808			10/10	
	20% Sun-cured alfalfa.	20	612			10/10	
	20% Sun-cured alfalfa extracted with water for 12 hours.	22	734			10/10	
	20% Sun-cured alfalfa extracted with 100° C. water for 12 hours.	22	678			10/10	
	Control plus water extract of sun-cured alfalfa.	20	860			10/8	
	Control plus 100° C. water extract of sun-cured alfalfa.	20	813			10/9	
4	20% Millrun (control)	0	245	Male	1 day	15/15	4 weeks
	20% Alfalfa	20	196			15/14	
	20% Alf. extracted with water for 24 hrs.	24	207			15/15	
	20% Alf. extracted with water for 48 hrs.	25	180			15/15	
	Control plus 48-hr. water extract of alf.	20	246			15/15	

Table 8 (continued).

Trial	Ration and type of extraction	Equivalent per cent of alfalfa	Average chick weights in grams	Sex of chicks	Age of chicks started	No. of chicks started per No. of chicks finished	Duration of trial
5	20% Millrun (control).	0	452	Male and female	8 days	18/18	4 weeks
	20% Alfalfa.	20	362			18/18	
	20% Alfalfa extracted with 50% ethanol for 12 hours.	21	354			18/17	
	20% Alfalfa treated with ether then extracted with water for 12 hours three successive times.	22	346			18/18	
	20% Alfalfa frozen at 12° F. and allowed to thaw three successive times then extracted with water for 12 hours.	22	309			18/18	
6	20% Millrun (control).	0	339	Male and female	7 days	20/19	4 weeks
	20% Alfalfa.	20	258			20/20	
	20% Alfalfa extracted with water[1].	22	257			20/20	
	20% Alfalfa extracted with water for 24 hours.	22	245			20/20	
	Control plus ethanol precipitate[1].	20	319			20/20	
	Control plus ethanol-acetone precipitate[1].	20	305			20/20	
	Control plus 24-hour water extract.	20	273			20/19	
7	20% Millrun (control).	0	476	Male and female	7 days	12/12	4 weeks
	20% Alfalfa.	20	399			12/12	
	20% Alfalfa extracted with 70% ethanol for 12 hours.	21	416			12/12	
	Control plus 70% ethanol extract.	40	373			12/12	

Table 8 (continued).

Trial	Ration and type of extraction	Equivalent per cent of alfalfa	Average chick weights in grams	Sex of chicks	Age of chicks started	No. of chicks started per No. of chicks finished	Duration of trial
8	20% Millrun (control).	0	708	Male and female	7 days	12/11	6 weeks
	20% Alfalfa.	20	569			12/12	
	20% Alfalfa extracted with water for 24 hours.	23	623			12/12	
	Control plus 24-hour water extract.	100	612			12/11	
9	20% Millrun (control).	0	413	Male and female	8 days	17/17	4 weeks
	20% Alfalfa.	20	281			17/19	
	20% Alfalfa extracted with water for 12 hours 3 successive times.	24	339			17/17	
	20% Alfalfa extracted continuously with tap water for 24 hours.	24	359			17/17	
	20% Alfalfa extracted with 95% ethanol for 12 hours.	21	300			17/15	
	Control plus water extract (12 hours 3 successive times).	20	389			17/17	
10	20% Millrun (control).	0	315	Male	8 days	15/15	24 days
	20% Millrun (control).	0	306			15/15	
	20% Alfalfa[2].	20	257			15/15	
	20% Alfalfa[2].	20	263			15/15	
	20% Alfalfa, extracted with water[2].	30	232			15/14	
	20% Alfalfa, extracted with water[2].	30	242			15/14	
	Control plus water extract[2].	20	303			15/14	
	Control plus water extract[2].	40	214			15/9	

[1]Patterned after the procedure of Peterson.

[2]Prepared by the United States Department of Agriculture, Western Regional Research Laboratory, Albany, California.

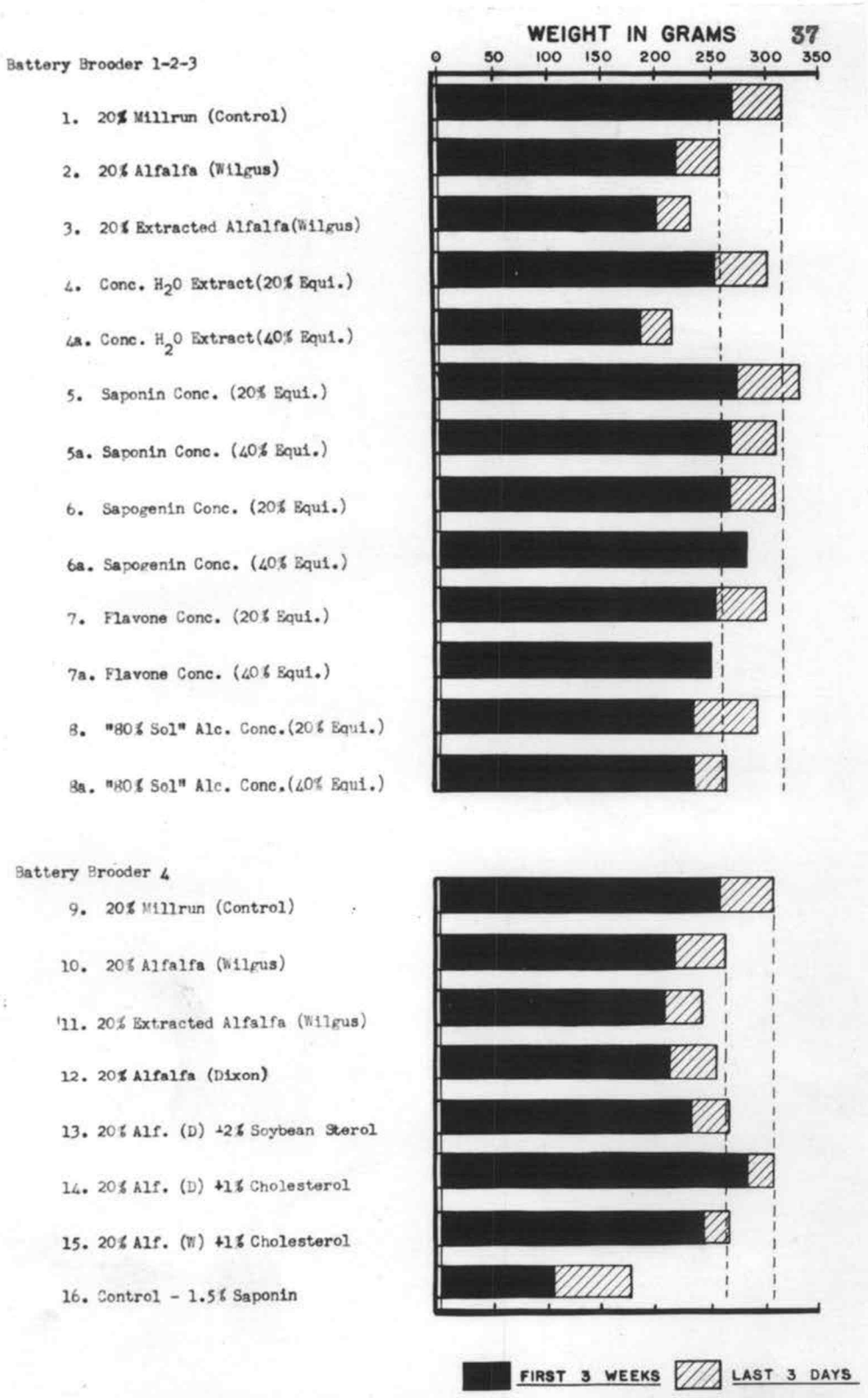

Figure 6. Effect of various rations used in chemical studies on chick growth.

Counteraction of chick growth inhibitor.

Complete removal of the factor in alfalfa responsible for depressing chick growth appears to be extremely difficult and in this series of investigations was not accomplished. Other studies were conducted to determine possible methods of counteracting the inhibitor with chemical agents and by chemical treatments.

Various grades and types of commercially available sterols were added to the 20 per cent alfalfa meal ration for the purpose of testing their counteraction of the growth inhibitor. These included soybean and cottonseed soapstock, lanolin, wool grease, crude fish liver oil, crude soybean sterols and pure cholesterol. Soybean oil, corn oil (Mazola Oil) and cottonseed oil (Wesson Oil) were incorporated along with the sterols in the experimental rations. Other substances tested were: glycerol, octanol, butanol, the B-complex vitamins and a commercial APF concentrate. Some batches of alfalfa meal were soaked in water for a period of twelve hours and then dried. Other batches of alfalfa were autoclaved for periods of one-half hour, one hour and four hours. Another batch of alfalfa was spread in thin layers and irradiated with a quartz-mercury vapor lamp for a period of 48 hours. The meal was periodically mixed during this latter treatment to reduce excessive bleaching. Experimental rations contained 20 per cent of the treated alfalfa meal or 20 per cent alfalfa plus the chemical substance to be tested. Growth assays with chicks were conducted on the experimental rations in the same manner as in the studies on extraction. Results obtained in these studies are

summarized in Table 9. Average weekly weights for each lot in trial 7, Table 9 are presented in Figure 7.

The growth inhibitor in alfalfa was counteracted by cholesterol or cholesterol plus 4 per cent cottonseed oil and to a lesser extent by lanolin plus 2 per cent cottonseed oil and wool grease plus 2 per cent cottonseed oil. The other treatments and chemical substances did not counteract the growth depressing effect of alfalfa meal.

During this same period of time Peterson (22) noted that there was a considerable amount of foaming in the aqueous solution as fractionation was taking place. This phenomenon suggested the presence of saponins, thus a possible explanation for the toxicity of alfalfa meal in chick rations. Studies which followed indicated that the growth depressing properties of alfalfa could be counteracted by cholesterol.

Determination of chick growth inhibitor in alfalfa by growth assays.

To assist in the identification of the factor or factors present in alfalfa, the water extract was fractionated and the properties of these fractions studied. The effects upon chick growth of cholesterol and saponin, both separate and in combination were studied.

Saponin, sapogenin and flavone concentrates and a precipitate soluble in 80 per cent and insoluble in 85 per cent ethanol were prepared as described under extraction in the extraction studies. These concentrates were incorporated in the control ration at levels equivalent to 20 and 40 per cent alfalfa. Two saponin concentrates and cholesterol with saponin were incorporated in the control ration at

Table 9 - Chemical counteraction of chick growth inhibitor in alfalfa using growth assays.

Trial	Ration and treatment	Average chick weights in grams	Sex of chicks	Age of chicks started	No. of chicks started per No. of chicks finished	Duration of trial
1	Control	302	Female	7 days	10/10	4 weeks
	20% Alfalfa	240			10/10	
	20% Alfalfa, irradiated with ultra-violet light	231			10/10	
2	Control	453	Male and female	1 day	10/10	5 weeks
	20% Alfalfa	394			10/10	
	20% Alfalfa, 5 liters of water added, dried at 50° C.	412			10/10	
	20% Alfalfa, 8 liters of water added, dried at 50° C.	395			10/10	
3	Station mash (5% alfalfa) plus 15% millrun	326	Male	10 days	10/10	4 weeks
	Station mash (5% alfalfa) plus 15% alfalfa autoclaved one-half hour.	241			10/10	
	Station mash (5% alfalfa) plus 15% alfalfa autoclaved one hour.	231			10/10	
4	20% Millrun (control)	245	Male	1 day	15/15	4 weeks
	20% Alfalfa	196			15/14	
	20% Alfalfa, water added, then dried at 50° C.	198			15/13	
	20% Alfalfa, autoclaved for 4 hours.	203			15/13	
5	20% Millrun (control)	848	Male	1 day	10/9	8 weeks
	20% Alfalfa	659			10/10	
	20% Alfalfa plus vitamins (5X)[1]	663			10/10	

Table 9 (continued).

Trial	Ration and treatment	Average chick weights in grams	Sex of chicks	Age of chicks started	No. of chicks started per No. of chicks finished	Duration of trial
6	20% Millrun (control)	903	Male and female	7 days	10/11	8 weeks
	20% Alfalfa	712			10/12	
	20% Alfalfa, plus 4% crude fish liver oil	617			10/12	
7	20% Millrun (control)	339	Male and female	7 days	20/19	4 weeks
	20% Alfalfa	258			20/20	
	20% Alfalfa plus 1% cholesterol	331			20/20	
	20% Alfalfa plus 2% glycerol	245			20/20	
	20% Alfalfa plus 10% glycerol	241			20/20	
	20% Alfalfa plus 2% butanol	253			20/20	
	20% Alfalfa plus 2% octanol	248			20/19	
8	20% Millrun (control)	945	Male and female	7 days	12/12	7 weeks
	20% Alfalfa	822			12/12	
	20% Alfalfa plus 3% cottonseed soapstock plus 3% soybean oil.	688			12/11	
	20% Alfalfa plus 3% soybean soapstock plus 3% soybean oil.	696			12/12	
	20% Alfalfa plus 3% lanolin[2].	766			12/11	
	20% Alfalfa plus 3% woolgrease[2].	796			12/12	

Table 9 (continued).

Trial	Ration and treatment	Average chick weights in grams	Sex of chicks	Age of chicks started	No. of chicks started per No. of chicks finished	Duration of trial
9	20% Millrun (control)	413	Male and female	17 days	17/17	4 weeks
	20% Alfalfa[3]	281			17/15	
	20% Alfalfa[3] plus animal protein factor (5# per ton)	329			17/17	
	20% Alfalfa[3] plus 1% cholesterol	390			17/17	
	20% Alfalfa[3] plus 1% cholesterol plus 4% cottonseed oil	410			17/16	
	20% Alfalfa[3] plus 3% lanolin plus 2% cottonseed oil	302			17/17	
	20% Alfalfa[3] plus 3% woolgrease plus 2% cottonseed oil	346			17/16	
10	20% Millrun (control)	452	Male and female	8 days	18/18	4 weeks
	20% Alfalfa	362			18/18	
	20% Alfalfa plus animal protein factor (25# per ton)	387			18/18	
	20% Alfalfa plus 4% woolgrease plus 2% corn oil	357			18/16	
	20% Alfalfa plus 6% woolgrease	349			18/18	
11	20% Millrun (control)	306	Male	8 days	15/15	24 days
	20% Alfalfa (D)[3]	255			15/14	
	20% Alfalfa (W)[4]	263			15/15	
	2% Soybean sterols[5] plus 20% alfalfa (D)[3]	267			15/15	
	1% Cholesterol plus 20% alfalfa (D)[3]	307			15/14	
	1% Cholesterol plus 20% alfalfa (W)[4]	266			15/15	

Table 9 (continued).

[1]Supplemented with five times the recommended allowance for vitamin A, thiamin, riboflavin, vitamin D, pantothenic acid, pyridoxin, niacin, choline, folic acid and alpha-tocopherol.

[2]Chicks changed from 20% alfalfa to this ration at second week.

[3]Alfalfa prepared by the Dixon Dryer Company, Dixon, California.

[4]Alfalfa prepared by the Western Regional Research Laboratory, U.S.D.A., Albany, California and designated as Wilgus #88.

[5]Supplied by distillation Products, Inc.

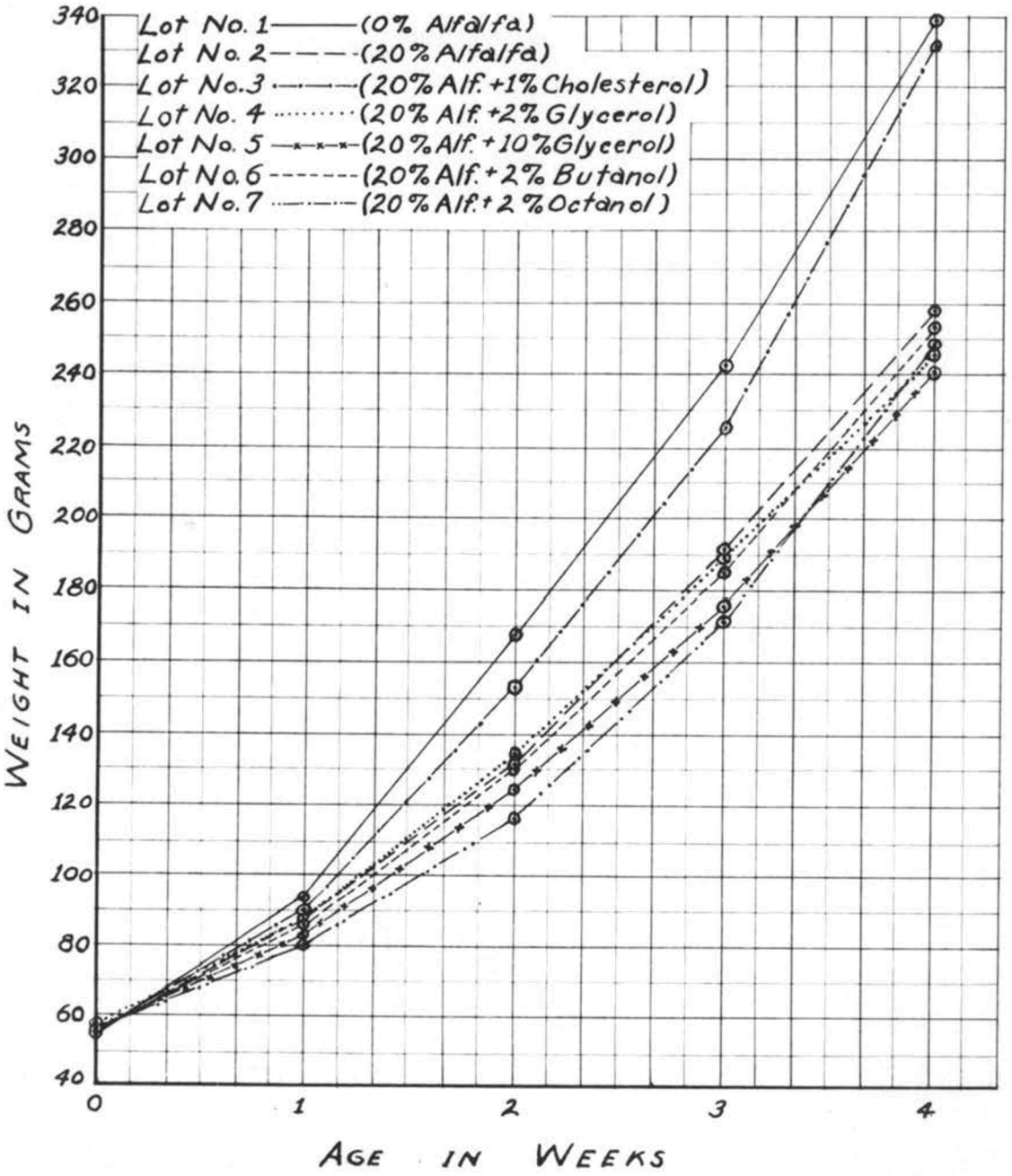

Figure 7. Growth rate of New Hampshire chicks on rations fed in trial 7, Table 9.

various levels. Cholesterol was added to the alfalfa ration. All experimental rations were tested through chick growth studies as described under the extraction studies.

Results are summarized in Table 10. The growth inhibition of alfalfa was counteracted by cholesterol as previously reported under chemical counteraction studies. One saponin preparation depressed chick growth at the 1.5 per cent level but not at the 0.5 per cent level. Another sample of a saponin concentrate from the same source when fed at the 1.5 per cent level depressed rate of growth to about one-half that obtained with similar chicks fed the control ration. The other saponin preparation fed at 0.3 per cent level depressed growth more than 20 per cent alfalfa meal.

DISCUSSION

Nutritional studies reported here show that chick growth is inhibited by alfalfa meal when incorporated in the ration at the 20 per cent level. This was true for all samples of alfalfa meal tested. There was no difference in rate of growth of chicks on a ration containing dehydrated or early sun-cured alfalfa meal.

The alfalfa meals varied in their growth depression properties at the 20 per cent levels. Chicks fed normal sun-cured alfalfa meal grew more slowly than those receiving early sun-cured or dehydrated meal. This was true with both ad libitum and restricted feeding. In a previous trial (6, p.2) sun-cured alfalfa did not depress chick growth as did the dehydrated meal. German and Couch (10, pp.844-845) reported

Table 10. Determination of chick growth inhibitor in alfalfa by growth assays.

Trial	Ration	Equivalent per cent of alfalfa	Average chick weights in grams	Sex of chicks	Age of chicks started	No. of chicks started per No. of chicks finished	Duration of trial
1	20% Millrun (control).		339	Male and female	7 days	20/19	4 weeks
	20% Alfalfa.		258			20/20	
	20% Alfalfa plus 1% cholesterol.		331			20/20	
2	20% Millrun (control).		413	Male and female	8 days	17/17	4 weeks
	20% Alfalfa.		281			17/15	
	20% Alfalfa plus 1% cholesterol.		390			17/17	
	Control plus 0.5% saponin[1].		389			17/17	
	Control plus 0.5% saponin[1] plus 1% cholesterol.		388			17/17	
3	20% Millrun (control).		452	Male and female	8 days	18/18	4 weeks
	20% Alfalfa.		362			18/18	
	Control plus 0.3% saponin[2].		294			18/18	
	Control plus 1.5% saponin[1].		292			18/18	
4	20% Millrun (control).		306	Male	8 days	15/15	24 days
	20% Alfalfa.		255			15/14	
	20% Alfalfa plus 1% cholesterol.		307			15/14	
	Control plus 1.5% saponin[3].		176			15/9	

Table 10 (continued).

Trial	Ration	Equivalent per cent of alfalfa	Average chick weights in grams	Sex of chicks	Age of chicks started	No. of chicks started per No. of chicks finished	Duration of trial
	20% Millrun (control).		315			15/15	24 days
	20% Alfalfa.		257			15/15	24 "
5	Control plus saponin concentrate[4].	20	331	Male	8 days	15/15	24 "
	Control plus saponin concentrate[4].	40	310			15/10	24 "
	Control plus sapogenin concentrate[4].	20	308			15/15	24 "
	Control plus sapogenin concentrate[4].	40	282			15/10	21 "
	Control plus flavone concentrate[4].	20	299			15/15	24 "
	Control plus flavone concentrate[4].	40	250			15/10	21 "
	Control plus precipitate (soluble in 80% and insoluble in 85% ethanol)[4].	20	292			15/15	24 "
	Control plus precipitate soluble in 80% and in 85% ethanol[4].	40	263			15/10	24 "

[1] Baker's saponin purified.

[2] Merck's saponin - pure.

[3] A different sample of Baker's saponin purified.

[4] Prepared from alfalfa (Wilgus #88) by the Western Regional Research Laboratory, U.S.D.A., Albany, California.

that one sample of dehydrated alfalfa depressed growth whereas the other had little effect. Payne, et al (21, p.71) have reported that of the alfalfa cut in the prebud stage, bud stage and in the blossom stage and with each stage in turn being dehydrated, shade-cured and sun-cured, the smallest growth was with sun-cured meal cut at blossom stage. The studies of Wilgus (26; 14, p.769) also indicate that different samples of alfalfa meal contain varying amounts of the growth inhibitor.

From available evidence it would appear that the age of alfalfa plants at harvesting time is related to the amount of inhibition obtained in chick growth when such plants are fed. Marker and Lopez (19) have made a study of the seasonal variation of steroidal sapogenins in plants. They showed that after fruiting the plants contained no monohydroxy steroids, but only the complex polyhydroxy steroids. As the flowering and fruiting season approached these polyhydroxy steroids were changed progressively to the simpler steroids and localized in the fruit or flower stem of the plant. Although the factor or factors present in alfalfa meal have not been conclusively identified, it is possible that the seasonal variation of sapogenins in plants may be responsible for the differences in growth depression by alfalfa cut at various stages. As previously pointed out, Peterson (22, p.653) has tentatively ascribed the chick growth depressing property of alfalfa meal to a saponin.

Fiber level or unpalatability do not appear to be responsible for the depressing effect of alfalfa on chick growth. When feed intake was restricted to the same level, the rate of growth for chicks receiving a

basal ration containing 20 per cent millrun and no alfalfa was greater than for chicks fed the same basal plus either 20 per cent sun-cured or dehydrated alfalfa. Also chicks which received as a ration only the basal mixture grew slightly more than chicks which received the same amount of basal mixture plus an additional 20 per cent feed intake of dehydrated alfalfa. A bulky ration containing 40 per cent millrun and no alfalfa showed no growth inhibiting effect. The results obtained with the alfalfa stems and alfalfa leaves further support the hypothesis that fiber is not the factor responsible for the inhibition of chick growth and indicate that the inhibitor may be concentrated in the leaf portion of the alfalfa plant. It has been shown by Lepkovsky et al. (16, p.217) and by this study on extraction that an inhibiting material can be removed from alfalfa by repeated extraction with water and that the extracted alfalfa inhibited growth less. With this evidence the major effects of alfalfa should not be attributed to fiber level, or unpalatability but to one or more growth inhibiting factors.

Complete removal of the inhibitor in alfalfa is apparently difficult and in this series of investigations was not accomplished. More of the inhibitor was removed by continuous extraction with water (Trial 1 and 9, Table 8) than by single or several successive batch extractions.

Extraction with water appeared to be better in most trials (trial 1, 3, 6 in Table 8) at room temperature than at higher temperature. Partial extraction of the inhibitor with ethanol (trial 5, 7, 9 in Table 8) was successful only at a 70 per cent concentration. No

relation appeared to exist between the time of extraction and the amount of inhibitor removed. In many trials the extracted alfalfa depressed growth more than unextracted alfalfa (trial 4, 5, 6 and 10, Table 8). It may be possible that short treatments with water or other liquids helped to expose the inhibitor in the alfalfa plant and hence bring about a greater depression in chick growth when the treated meals were fed.

Water and other solvent extracts did not depress chick growth when incorporated in the ration at a level equivalent to 20 per cent alfalfa. Water extracts at the 40 and 100 per cent equivalent levels did depress growth of chicks (trial 6, 7, 8 and 10, Table 8).

Growth depression produced by alfalfa at high levels was completely counteracted in all cases but one by the addition of cholesterol with or without cottonseed oil. The one instance in which cholesterol did not counteract the chick growth inhibitor in alfalfa was when 1 per cent cholesterol was added to a ration containing 20 per cent "Wilgus" alfalfa meal (trial 11, Table 9; Figure 6). Complete counteraction with cholesterol agrees with results obtained by Peterson (22). However, the use of cholesterol is not a practical solution to the problem of bringing about a greater and more efficient utilization of alfalfa meals in chick rations because of its present cost. Attention was directed towards testings other sterols which were commercially available at low cost. The inhibitor was counteracted to a lesser extent by the addition of cottonseed oil with lanolin or wool grease. Their use in a ration is of questionable value at the levels tested.

Results obtained with 2 per cent soybean sterols plus 2 per cent alfalfa do not agree with those of Peterson (23) who obtained complete counteraction using soybean sterol plus cottonseed oil. Glycerol, butanol, octanol, APF, B-complex vitamins, cottonseed and soybean soapstock and crude fish liver oil were ineffective in counteracting the inhibitor. The inhibitor appeared stable to water treatment, irradiation and autoclaving.

The effects of cholesterol, saponin and water extract fractions on chick growth were studied to assist in the identification of the inhibitor. Feeding chicks a control ration containing saponin at various levels invariably depressed chick growth. The effects of saponin and alfalfa meal on chick growth appeared very similar. Baker's saponin fed in a control ration at the 1.5 per cent level depressed rate of gain to one-half the rate obtained with similar chicks on a control ration. The prepared sapogenin, saponin and flavone concentrates did not appear to have any significant depressing effect at either the 20 or 40 per cent equivalent level. The water extract soluble in 80 per cent and insoluble in 85 per cent alcohol had a depressing effect at the 40 per cent equivalent level.

Saponins are known to react with sterols to form addition compounds which no longer possess toxic properties. It has been suggested by Peterson (23) that an insoluble sterol-saponin compound is formed in the digestive tract of the chick when fed a ration containing alfalfa and cholesterol. The mode of action of cholesterol on the inhibitor is unknown at present.

From these preliminary studies it appears that some component of alfalfa meal soluble in water and fractionated by alcohol, possibly a saponin, is largely responsible for the growth depressing action of alfalfa meal when fed at high levels.

SUMMARY

Ways and means of utilizing higher levels of alfalfa meal in chick rations were investigated.

1. Chick growth is inhibited by alfalfa meal when incorporated in the ration at the 20 per cent level.
2. There was no difference in the rate of growth between chicks fed a ration containing dehydrated or early sun-cured alfalfa meal. Chicks fed normal sun-cured alfalfa grew less than those receiving early sun-cured or dehydrated alfalfa meal. The same relative results were obtained when compared on an ad libitum feeding program or an equalized feeding program.
3. The major depressing effect of alfalfa upon rate of growth of chicks should not be attributed to fiber level, or unpalatability but to one or more growth inhibiting factors in the alfalfa.
4. Complete removal of the factor(s) responsible for depressing chick growth is apparently difficult and in this series of investigations was not accomplished. The inhibitor was soluble in water and could be fractionated with ethanol.
5. The growth inhibitor in alfalfa was counteracted by cholesterol or cholesterol plus 4 per cent cottonseed oil and to a

lesser extent by lanolin and wool grease plus 2 per cent cotton-seed oil. Water treatment, irradiation, autoclaving, vitamin supplementation, glycerol, butanol, octanol and crude fish liver oil were ineffective in counteracting the inhibitor.

6. Saponins depress chick growth in a manner similar to alfalfa meal and may be the factor in alfalfa that is responsible for inhibition.

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