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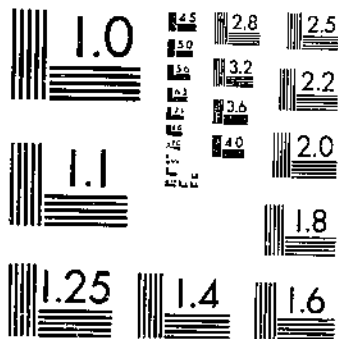
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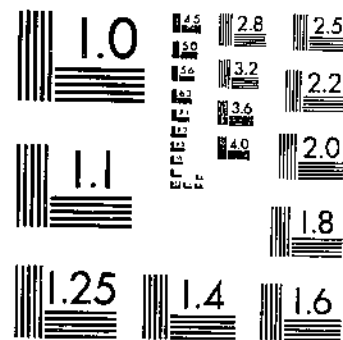
TB 632 (1939) USDA TECHNICAL BULLETINS  
LOSS DURING STORAGE OF VITAMIN A FROM ALFALFA LEAF MEALS FED TO  
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UNITED STATES DEPARTMENT OF AGRICULTURE  
WASHINGTON, D. C.

# LOSS, DURING STORAGE, OF VITAMIN A FROM ALFALFA LEAF MEALS FED TO CHICKENS<sup>1</sup>

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## INTRODUCTION

Although alfalfa leaf meals are commonly used as one of the sources of vitamin A in diets for poultry, little attention is ordinarily paid to the age of the meals used. However, the subject has received some scientific consideration. Fraps and Treichler<sup>2</sup> stated that the loss of vitamin A in alfalfa leaf meal stored at room temperature in mason jars for 11 months may be 50 percent of the vitamin A originally present. Smith<sup>3</sup> reported that leaves of baled alfalfa stored about 14 months in a hay barn contained 50 percent less vitamin A than the leaves of freshly baled alfalfa.

It seemed desirable to obtain data on the loss of vitamin A from alfalfa leaf meals during storage in paper-lined burlap bags, inasmuch as alfalfa leaf meals are now commonly packed and stored in such bags. Accordingly, a series of feeding trials with chicks was conducted at the Southwest Poultry Experiment Station of the Bureau at Glendale, Ariz., for the purpose of studying the effect of the length of the storage period on the relative vitamin A content of alfalfa leaf meal thus packed and stored.

## EXPERIMENTAL PROCEDURE

The first trial was begun on November 16, 1934, when the meals were about 1 month old. The remaining trials were begun when the meals were of about the following ages: Second trial, 4 months; third trial, 7 months; fourth trial, 14 months; fifth, and last, trial, 24 months.

<sup>1</sup> Submitted for publication July 6, 1938.

<sup>2</sup> FRAPS, G. S., and TREICHLER, RAY. EFFECT OF STORAGE ON VITAMIN A IN DRIED FOODS. *Indus. and Engin. Chem.* 25: 465-466. 1933.

<sup>3</sup> SMITH, MARGARET CAMMACK. THE EFFECT OF STORAGE UPON THE VITAMIN A CONTENT OF ALFALFA HAY. *Jour. Agr. Research* 53: 681-684. 1936.

Rhode Island Red chicks were used in all five trials. At the beginning of each trial there were 45 chicks on each diet. They were 1 day old when first fed the experimental diets. The chicks were weighed individually on the first and fourteenth days after hatching and at 14-day intervals thereafter until they were 70 days old. The quantities of feed consumed during the initial 13-day period and the subsequent 14-day periods were recorded. The chicks were confined in houses similar in all respects and were brooded under similar electric hovers.

The following basal diet known to be deficient in vitamin A but adequate in all the other known nutritive factors was used. This diet was employed by Heywang and Titus<sup>1</sup> in previous experiments with alfalfa leaf meal.

	Percent
Ground white corn.....	64
Wheat middlings.....	15
Commercial casein.....	10
Pure dried yeast.....	4
Ground limestone.....	3
Steamed bone meal.....	1
Common salt.....	1
Irradiated ergosterol in oil (2,000 international units of vitamin D per gram).....	2
Total.....	100

The following eight diets were fed in each of the five trials:

Diet 1—Basal diet only (negative control diet).

Diet 2—Basal diet except that the irradiated ergosterol in oil was replaced, weight for weight, by cod-liver oil (positive control diet).

Diet 3—99½ percent of the basal diet and one-half percent of dehydrated alfalfa leaf meal.

Diet 4—99½ percent of the basal diet and one-half percent of sun-cured alfalfa leaf meal.

Diet 5—99 percent of the basal diet and 1 percent of dehydrated alfalfa leaf meal.

Diet 6—99 percent of the basal diet and 1 percent of sun-cured alfalfa leaf meal.

Diet 7—98 percent of the basal diet and 2 percent of dehydrated alfalfa leaf meal.

Diet 8—98 percent of the basal diet and 2 percent of sun-cured alfalfa leaf meal.

The sun-cured and the dehydrated alfalfa leaf meals were prepared by a manufacturer in Van Nuys, Calif. Both meals were from the fifth cutting of alfalfa in October 1934, although the hays from which the meals were prepared had been grown in different fields and were not cut on the same day. The dehydrated meal was cured in an Arnold dryer operating at a temperature of approximately 1,100° F. The hay from which the sun-cured meal was made was cured 4 days, during which there was no rain or fog. Both meals were stored at room temperature.

The cod-liver oil used in the positive control diet was prepared for human consumption and was guaranteed to contain not less than 700 international units of vitamin A per gram. Fresh batches of the positive control diet were prepared every 6 or 7 days to guard against undue losses of the vitamin A supplied by the cod-liver oil.

<sup>1</sup> HEYWANG, BERT W., and TITUS, HARRY W. ALFALFA LEAF MEAL AS A SOURCE OF VITAMIN A FOR GROWING CHICKENS. Jour. Agr. Research 54: 559-569, illus. 1937.

## EXPERIMENTAL RESULTS AND DISCUSSION

Tables 1 to 5 give the results obtained in the series of trials. In the various groups of chicks there were a few deaths from accidents and other causes not attributable to the diets: Therefore, in order to have comparable data on the average number of chick days representing survival on each diet tested, the data shown in tables 1 and 2 were calculated for the 40 chicks that lived longest on each diet.

TABLE 1.—*Number of chickens living on the various diets at end of each trial of 70 days' duration*<sup>1</sup>

Diet No.	Supplement in diet	Chickens alive after consuming diets containing alfalfa leaf meal supplements of the following approximate ages (at beginning of trials) for a period of 70 days				
		1 month (trial 1)	4 months (trial 2)	7 months (trial 3)	14 months (trial 4)	24 months (trial 5)
		<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>
1	None (negative control).....	0	5	2	0	0
2	2 percent of cod-liver oil (positive control).....	40	40	40	40	40
3	1½ percent of dehydrated alfalfa leaf meal.....	40	40	37	0	0
4	½ percent of sun-cured alfalfa leaf meal.....	40	40	36	11	0
5	1 percent of dehydrated alfalfa leaf meal.....	40	40	40	18	0
6	1 percent of sun-cured alfalfa leaf meal.....	40	40	40	38	23
7	2 percent of dehydrated alfalfa leaf meal.....	(?)	(?)	40	32	0
8	2 percent of sun-cured alfalfa leaf meal.....	40	40	40	40	40

<sup>1</sup> Basis of 40 chicks on each diet at beginning of each trial.

<sup>2</sup> Data not used because of mortality from factors other than diet.

TABLE 2.—*Total number of chick-days lived on the various diets*

[2,800 possible chick days]

Diet No.	Supplement in diet	Chick-days lived by the birds after consuming, for 70 days, diets containing alfalfa leaf meal supplements of the following approximate ages (at beginning of trials)				
		1 month (trial 1)	4 months (trial 2)	7 months (trial 3)	14 months (trial 4)	24 months (trial 5)
		<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>
1	None (negative control).....	1,218	1,718	1,420	944	1,255
2	2 percent of cod-liver oil (positive control).....	2,800	2,800	2,800	2,800	2,800
3	1½ percent of dehydrated alfalfa leaf meal.....	2,800	2,800	2,778	1,282	1,369
4	½ percent of sun-cured alfalfa leaf meal.....	2,800	2,800	2,760	2,210	2,070
5	1 percent of dehydrated alfalfa leaf meal.....	2,800	2,800	2,800	2,060	1,570
6	1 percent of sun-cured alfalfa leaf meal.....	2,800	2,800	2,800	2,763	2,653
7	2 percent of dehydrated alfalfa leaf meal.....	(?)	(?)	2,800	2,695	2,030
8	2 percent of sun-cured alfalfa leaf meal.....	2,800	2,800	2,800	2,800	2,800

<sup>1</sup> Data not used because of mortality from factors other than diet.

The total number of chick days for the negative control diet in the several trials was variable but was nearly the same in trials 1 and 5. Some of this variability was probably caused by differences in the quantity of vitamin A stored in the chicks when they were hatched, which, in turn, is attributable to differences in the vitamin A intake of the parent stock.

Inasmuch as the chicks in the several trials were not from the same parent stock and were not hatched at the same season of the year, only the results obtained in the same trial are strictly comparable. To compare the relative ability of the diets to maintain life in any one trial, an index of the ability of each diet to maintain life was computed in the following manner:

$$\frac{(\text{Chick days lived on diet for which index is being computed}) - (\text{Chick days lived on negative control diet})}{(\text{Chick days lived on positive control diet}) - (\text{Chick days lived on negative control diet})} = \text{Index of ability to maintain life.}$$

As an example, the index for diet 3 in trial 5 (table 3) was computed as follows:

$$\frac{1,369 - 1,255}{2,800 - 1,255} = \frac{114}{1,545} = 0.074$$

According to this method of computing the index, if a diet contained an adequate quantity of vitamin A, the numerical value of the index would be 1. If the vitamin A content was inadequate, the index would be less than 1.

TABLE 3.—*Indices of ability of the diets to maintain life*

Diet No.	Supplement in diet	Indices of ability of diets to maintain life after the birds had consumed, for 70 days, diets containing alfalfa leaf meal supplements of the following approximate ages (at beginning of trials)				
		1 month (trial 1)	4 months (trial 2)	7 months (trial 3)	14 months (trial 4)	24 months (trial 5)
1	None (negative control)	0.0	0.0	0.0	0.0	0.0
2	2 percent of cod-liver oil (positive control)	1.000	1.000	1.000	1.000	1.000
3	½ percent of dehydrated alfalfa leaf meal	1.000	1.000	.984	.171	.074
4	¼ percent of sun-cured alfalfa leaf meal	1.000	1.000	.971	.687	.528
5	1 percent of dehydrated alfalfa leaf meal	1.000	1.000	1.000	.601	.204
6	1 percent of sun-cured alfalfa leaf meal	1.000	1.000	1.000	.980	.905
7	2 percent of dehydrated alfalfa leaf meal	( <sup>1</sup> )	( <sup>1</sup> )	1.000	.945	.502
8	2 percent of sun-cured alfalfa leaf meal	1.000	1.000	1.000	1.000	1.000

<sup>1</sup> Data not used because of mortality from factors other than diet.

The data in tables 1, 2, and 3 indicate that both meals, even when fed at a level of only one-half of 1 percent of the diet, supplied enough vitamin A to maintain life until those meals were about 6½ months old. By the time they were 9½ months old (end of trial 3) a slight loss in vitamin A content became apparent, but 1 percent of both meals was still sufficient to maintain life. However, by the time the meals were 16½ months old (end of trial 4) the loss of vitamin A became more marked, and by the time the meals were 26½ months old (end of trial 5)

the loss was very pronounced. The data in these three tables also show that the dehydrated meal lost its vitamin A potency more rapidly than the sun-cured meal.

The experiments of Heywang and Titus<sup>5</sup> demonstrated that appreciably more vitamin A is required to maintain a normal rate of growth than is required only to maintain life. This fact is also shown by the data presented in this bulletin. Although one-half percent of either of the two meals, when they were about 6½ months old, supplied enough vitamin A to maintain life, 2 percent did not supply enough to maintain a normal rate of growth when the meals were only 1 to 2½ months old (table 4).

TABLE 4.—Average weight of chickens on last day of each trial (seventieth day)

MALES						
Diet No.	Supplement in diet	Average weight of chickens after consuming, for 70 days, diets containing alfalfa leaf meal supplements of the following approximate ages (at beginning of trials)				
		1 month (trial 1)	4 months (trial 2)	7 months (trial 3)	14 months (trial 4)	24 months (trial 5)
		Grams (1)	Grams (2)	Grams (3)	Grams (4)	Grams (5)
1	None (negative control).....					
2	2 percent of cod-liver oil (positive control).....	828	907	721	972	986
3	½ percent of dehydrated alfalfa leaf meal.....	682	705	586	(1)	(1)
4	½ percent of sun-cured alfalfa leaf meal.....	770	781	645	357	(1)
5	1 percent of dehydrated alfalfa leaf meal.....	737	769	627	356	(1)
6	1 percent of sun-cured alfalfa leaf meal.....	783	852	714	720	504
7	2 percent of dehydrated alfalfa leaf meal.....	(1)	(1)	701	595	(1)
8	2 percent of sun-cured alfalfa leaf meal.....	759	809	702	770	660

FEMALES					
1	None (negative control).....	(1)	(1)	(1)	(1)
2	2 percent of cod-liver oil (positive control).....	752	826	641	856
3	½ percent of dehydrated alfalfa leaf meal.....	824	682	549	(1)
4	½ percent of sun-cured alfalfa leaf meal.....	716	745	565	410
5	1 percent of dehydrated alfalfa leaf meal.....	668	690	570	495
6	1 percent of sun-cured alfalfa leaf meal.....	671	750	617	764
7	2 percent dehydrated alfalfa leaf meal.....	(1)	(1)	630	541
8	2 percent of sun-cured alfalfa leaf meal.....	669	742	635	693

<sup>1</sup> No survivors.

<sup>2</sup> Insufficient survivors for comparison.

<sup>3</sup> Data not used because of mortality from factors other than diet.

Lambert, Ellis, Black, and Titus<sup>6</sup> have suggested the use of a method developed by Titus and associates at the Agricultural Research Center, Beltsville, Md., for determining the efficiency of feed utilization. This method is dependent on the fact that efficiency of feed utilization ( $E$ ) is a linear function of live weight ( $W$ ), and the equation  $E = C - kW$  is an expression of the relationship involved.

<sup>5</sup> HEYWANG, B. W., and TITUS, H. W. See footnote 4.

<sup>6</sup> LAMBERT, W. V., ELLIS, N. R., BLACK, W. H., and TITUS, H. W. THE ROLE OF NUTRITION IN GENETIC RESEARCH. Amer. Soc. Anim. Prod. Proc. 29: 236-243, 1935.



After one has obtained the numerical values of the constants  $C$  and  $k$  by properly fitting the above-mentioned equation to the experimental data, he can readily compute the efficiency of feed utilization at any given live weight, the average efficiency between any two live weights, or the average efficiency from time of hatching to any given live weight.

In the present series of experiments this method was used for comparing the efficiencies of the several diets. Since the positive-control diet contained an adequate supply of vitamin A, it was possible to obtain a relative measure of the adequacy of the vitamin A content of any of the other diets and, thus, to compare the other diets among themselves.

The efficiencies of the diets when the birds had reached a live weight of 400 g are shown in table 5. The efficiency of the diet containing 2 percent of cod-liver oil (diet 2) was considered as 100 percent in calculating the relative efficiencies of the other diets. The data for diets 6 and 8 are of particular interest because of the relatively high indices of ability to maintain life on those diets, as shown in table 3. At the end of trial 5, the relative efficiency of feed utilization when the birds had reached a live weight of 400 g on the diet containing 1 percent of sun-cured meal (diet 6) was approximately 44 percent less when it was 26½ months old (end of trial 5) than when it was 3½ months old (end of trial 1). The index of ability to maintain life had decreased only 9.5 percent during the same period. The efficiency of feed utilization when a live weight of 400 g had been reached on the diet containing 2 percent of sun-cured meal (diet 8) had decreased about 28 percent by the time the meal was 26½ months old, whereas the index of ability to maintain life remained the same.

TABLE 5.—Efficiency of the diets when the birds had reached a live weight of 400 g<sup>1</sup>

Diet No.	Supplement in diet	Efficiency of diets after the birds had consumed, for 70 days, diets containing alfalfa leaf meal supplements of the following approximate ages (at beginning of trials)									
		1 month (trial 1)		4 months (trial 2)		7 months (trial 3)		14 months (trial 4)		24 months (trial 5)	
		Calculated efficiency	Percent of diet 2	Calculated efficiency	Percent of diet 2	Calculated efficiency	Percent of diet 2	Calculated efficiency	Percent of diet 2	Calculated efficiency	Percent of diet 2
2	2 percent of cod-liver oil (positive control).....	0.3523	100.0	0.3705	100.0	0.3812	100.0	0.3605	100.0	0.4025	100.0
3	½ percent of dehydrated alfalfa leaf meal.....	.3119	88.5	.3327	87.7	.2911	76.4	(?)	-----	(?)	-----
4	½ percent of sun-cured alfalfa leaf meal.....	.3299	93.6	.3418	90.1	.3223	84.5	.1014	28.1	(?)	-----
5	1 percent of dehydrated alfalfa leaf meal.....	.3050	86.6	.3254	85.7	.3334	87.5	.1823	50.6	(?)	-----
6	1 percent of sun-cured alfalfa leaf meal.....	.3365	95.5	.3307	87.1	.3457	90.9	.3181	88.2	.2171	53.9
8	2 percent of sun-cured alfalfa leaf meal.....	.3520	99.9	.3333	93.2	.3020	85.0	.3235	89.7	.2905	72.2

<sup>1</sup> Data are not given for diet 1 because no chicks survived, nor for diet 7 because in the first 2 trials there was considerable mortality not attributable to the diet, and in the fifth trial none of the chicks survived to the end.

<sup>2</sup> No survivors.

The relative efficiencies of feed utilization, when a live weight of 800 g has been reached, were calculated for diets 6 and 8 and found to be approximately 99 percent for both diets at the end of trial 1. Similar calculations showed that at the end of trial 5 the chicks fed diet 6 would have ceased to grow at a live weight of about 666 g, and that the relative efficiency of feed utilization of the chicks on diet 8 at a live weight of 800 g was only 3.6 percent. Furthermore, the indices of ability to maintain life and the data on survival of the chicks, the total number of chick days lived, and the live weights at 70 days, as well as the data on the efficiency of feed utilization, all clearly indicate that the vitamin A content of the dehydrated meal decreased at such a rate that, when it had been stored for 14 months, it contained no more vitamin A than did the sun-cured alfalfa leaf meal after it had been stored for 24 months.

### SUMMARY

Dehydrated and sun-cured alfalfa leaf meals were stored in paper-lined burlap bags for use in five feeding experiments with growing chickens of the Rhode Island Red breed. The work was carried on at the Southwest Poultry Experiment Station of the Bureau of Animal Industry at Glendale, Ariz., and was begun in 1934. The experiments were of 70 days' duration and were initiated when the meals were about 1, 4, 7, 14, and 24 months old, respectively. Viability, growth, and efficiency of feed utilization were used as relative measures of the loss in vitamin A potencies of the meals.

Evidence was obtained that there was only a slight decrease in the vitamin A content of the meals after they had been stored 7 to 9½ months. However, the decrease in vitamin A content became more marked when the meals were 16½ months old and was very pronounced when they were 24 to 26½ months old.

The vitamin A content of alfalfa leaf meal dehydrated at 1,100°F. decreased at such a rate that, when it had been stored for 14 months, it contained no more vitamin A than did the specially sun-cured alfalfa leaf meal after it had been stored for 24 months.

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