AN INVESTIGATION OF DOGFISH OIL

AS A

SOURCE OF VITAMINS A AND D FOR POULTRY.

by

F. B. HUTT, B.S.A. (TORONTO), M.S. (WISCONSIN).

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Introduction

The dogfish is a source of trouble and loss in districts where fishing is an important industry. Unfotunately its flesh is valueless for food, and it has been difficult to find any marketing channel sufficiently profitable to warrant its destruction on a commercial scale.

With the increasing knowledge of vitamins and of their requirements by various animals, there has come into general use the practice of feeding cod liver oil to fowls and chicks kept in confinement. The advantages of so doing have been so well established that crude cod liver oil is sold by the carload in some of the commercial poultry sections in the United States and in rapidly increasing quantities in Canada.

It seemed possible that dogfish oil might serve in the same capacity as cod liver oil as a source of the desired vitamins. To determine if this were so the experiments described hereunder were carried out.

The nutritional value of cod liver oil, so far as is known at present lies in its content of vitamins A and D.

It has been shown by Emmett and Peacock (1923) and Hart, Steenbock, Lepkovsky and Halpin (1924) that the young chick requires vitamin A for normal development. Adult fowls starved of vitamin A for upwards of three months develop a disease described by Beach (1924) generally known as nutritional roup. Its diagnostic characters include inflammation of the conjunctiva, swelling of the eyelids, watery secretion from the eye and caseous pustules in the pharynx and oesophagus. The ophthalmia or the pustules may occur alone or together. Either is sufficiently recognizable to permit diagnosis of vitamin A deficiency.

Vitamin A can be obtained for poultry in yellow corn and several green feeds as well as in cod liver oil.

Vitamin D, the antirachitic factor, is necessary for the assimilation of calcium. Its importance for growing animals has been demonstrated by many recent writers, and Hart, Halpin and Steenbock (1922) have shown that the chick is particularly sensitive to rickets. On rations lacking Vitamin D, laying hens show their inability to assimilate calcium by laying thin shelled eggs, and often by leg weakness or complete paralysis. The greatest loss to the poultryman lies in the low hatching power of eggs from hens starved for Vitamin D. Needham (1925) has shown that from the 15th day of incubation on there is normally a rapid increase in the calcium content of the embryo and that this must come from the shell. The carbon dioxide given off by the embryo acts on the calcium carbonate of the shell to form calcium bicarbonate, which, being soluble, can be utilized by the embryo if vitamin D be present. Hess (1923) has shown that the egg possesses distinct antirachitic properties. However, if thehen has suffered from lack of vitamin D the egg will be low in the same substance and the developing embryo will be unable to assimilate the calcium bicarbonate. The result is either a weak chick or a dead one. The number of chicks dying during the last week of incubation is usually high early in the hatching season and represents one of the greatest sources of loss with which the poultryman has to contend. Though cod liver oil is high in vitamin D, it has not yet been definitely shown how the vitamin D content of eggs from hens fed cod liver oil compares with that of eggs from hens deprived of the antirachitic factor. Holmes (1926) and others have conclusively demonstrated, however, that feeding the oil results in much better hatches than are obtained without it.

In summer, the equivalent of vitamin D is supplied in abundance by the ultra-violet rays in sunlight. In the fall and winter months there is not only less sunlight but the amount of activating ultra-violet rays in winter sunlight is a doubtful quantity. In Canada, hens are usually confined during the winter and consequently, since the rays are filtered out by window glass, receive no ultra-violet light whatever. Some poultrymen use a quartz mercury vapor lamp to generate ultra-violet light for irradiating the hens, but the expense of such equipment is too great to permit its general adoption. Some green plants contain considerable amounts of vitamin D. Alfalfa is one of the best. Hughes, Titus and Witham (1925) found that even 10% of fresh green alfalfa had less antirachitic value than 0.5% of cod liver oil added to the same basal ration. Bethke, Kennard, and Kirk have shown that green red clover fed at a level of 18% (on a dry weight basis) delayed the onset of leg weakness but had less value than one-half hour daily of direct sunlight. The general practice adopted by poultry men has been to rely on cod liver oil as a source of vitamin D.

The discovery of the vitamins of cod liver oil was preceded by many years of general recognition of its medicinal value. There is no such history for dogfish oil. Holmes and Piggott (1925) found that rats made steady growth when their deficient ration was supplemented with 1 mg. of dogfish oil daily and very rapid growth on a supplement of 4 mg. daily. This indicates that the oil used contained vitamin A but is no guide to its content of vitamin D.

Experiments were carried out with mature stock and growing chicks.

1. Adult Stock

Experiment

On November 15th, 1926, 75 White Leghorn pullets were divided into five equal lots, housed in identical pens and fed the following basal ration.

Scratch Grain		
	Wheat	33.3 lbs.
	Oats	33.3 "
	Barley	33.3 "
Mash		
	Ground Oats	20 "
	Bran	20 "
	Middlings	20 "
	Ground Barley	20 "
	Meat Scrap	15 "
	Oyster Shell	3 "
	Bone Meal	3 "
	Salt	.5 "

This ration is lacking in vitamin D and very low in vitamin A. It is also to be criticized, because it does not contain enough kinds of protein to provide the best balance of amino acids. Such a ration would be expected to induce better egg production and distinctly better hatches if it were supplemented with skim milk or buttermilk. The former was not available and the latter was not used because it might contain sufficient vitamin A to influence the results.

Supplements to this ration were fed in the five pens as follows:

Pen 1. Crude cod liver oil (Vita Brand), 2% of the mash, providing vitamins A and D.

" 2. Crude dogfish oil, 2% of the mash.

" 3. Cabbage, one head every other day, providing vitamin A.

" 4. No supplement.

" 5. Cod liver oil, aerated at 100° C. for 20 hours, 2% of the mash providing vitamin D.

McCollum (1925) states that cod liver oil aerated at 100° C. for 12 to 20 hours loses its vitamin A content while its antirachitic value

is only slightly affected. It will be seen that the above mentioned supplements provided controls to indicate if the dogfish oil fed contained both A and D, A alone, D alone, or neither. The requirements of vitamins B and E were adequately met in all pens by the basal ration. The supply of cabbage available was exhausted by February 1st and thereafter Pen 3 received no supplement.

Results

All birds were weighed individually every two weeks, and trap nested for the duration of the experiment. The average body weight for each pen from November 15th to April 11th is shown in Chart 1. A number of the birds in some pens were victims of epidemics of infectious bronchitis, chickenpox and canker, which swept the college plant. For this reason the egg production was recorded for each two week period as the percentage of the possible production for the number of birds in the pen at the end of the two week period. This data is shown graphically in Chart.2.

It will be noted that changes in egg production closely paralleled changes in the body weight. In all lots except Pen 5, a decline in body weight and egg production is evident during the first month of the experiment. This can be attributed to the sudden change from their former ration to that of the experiment and in particular to their being deprived of the milk which they had formerly had. Pen 6 had a higher body weight and lower egg production than the other pens, and therefore was less affected by the change. A second decline in weight and egg production in the latter half of January was due to prolonged sub-zero weather, by which Leghorns, being smaller in weight, are more affected than heavy breeds. Following this, all lots gained in weight and egg production except Pen 3, which suffered more from canker and chickenpox than any other pen. The decline in egg production in Pen 4 in March was

Chart I

Average body weight of adult fowls

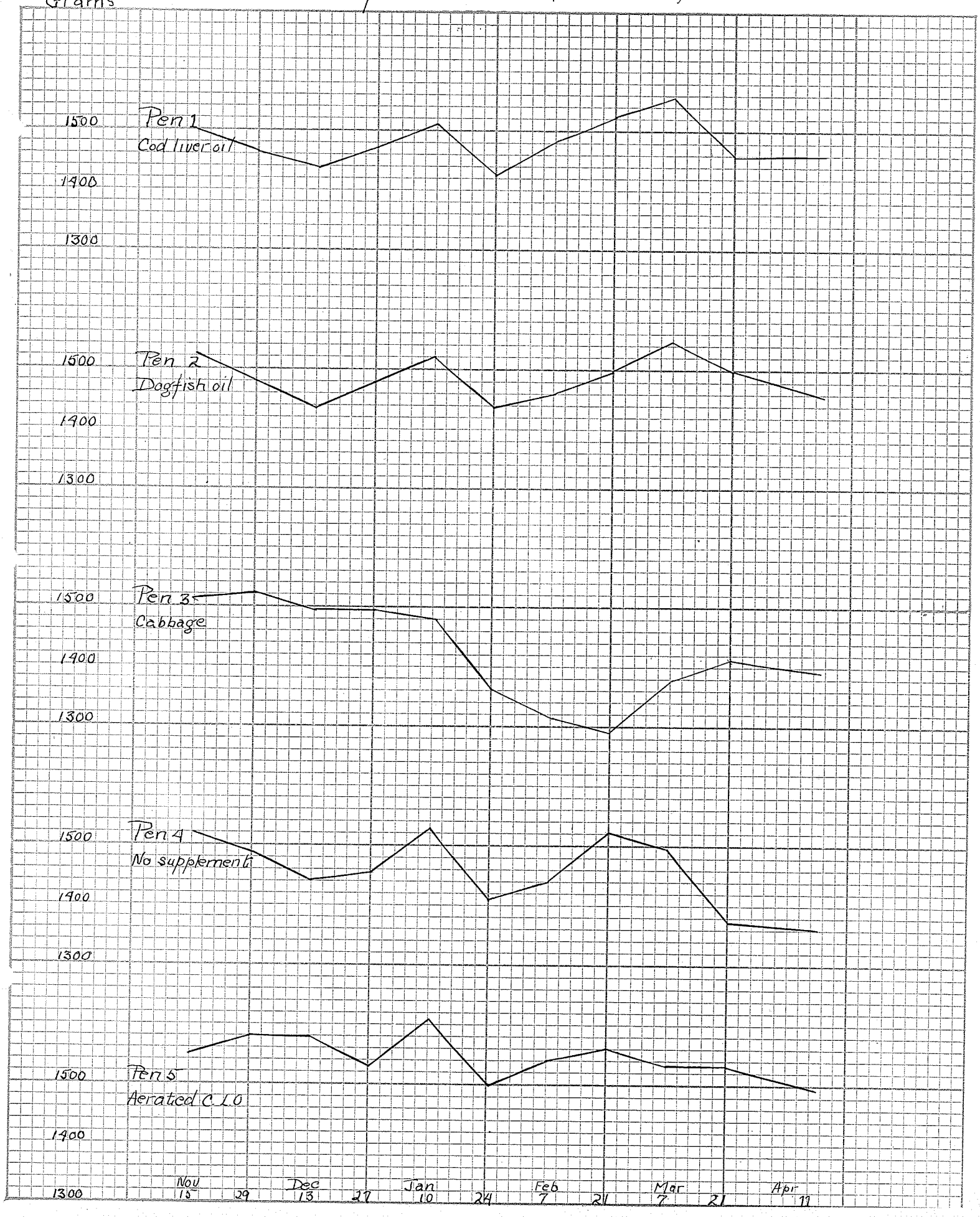
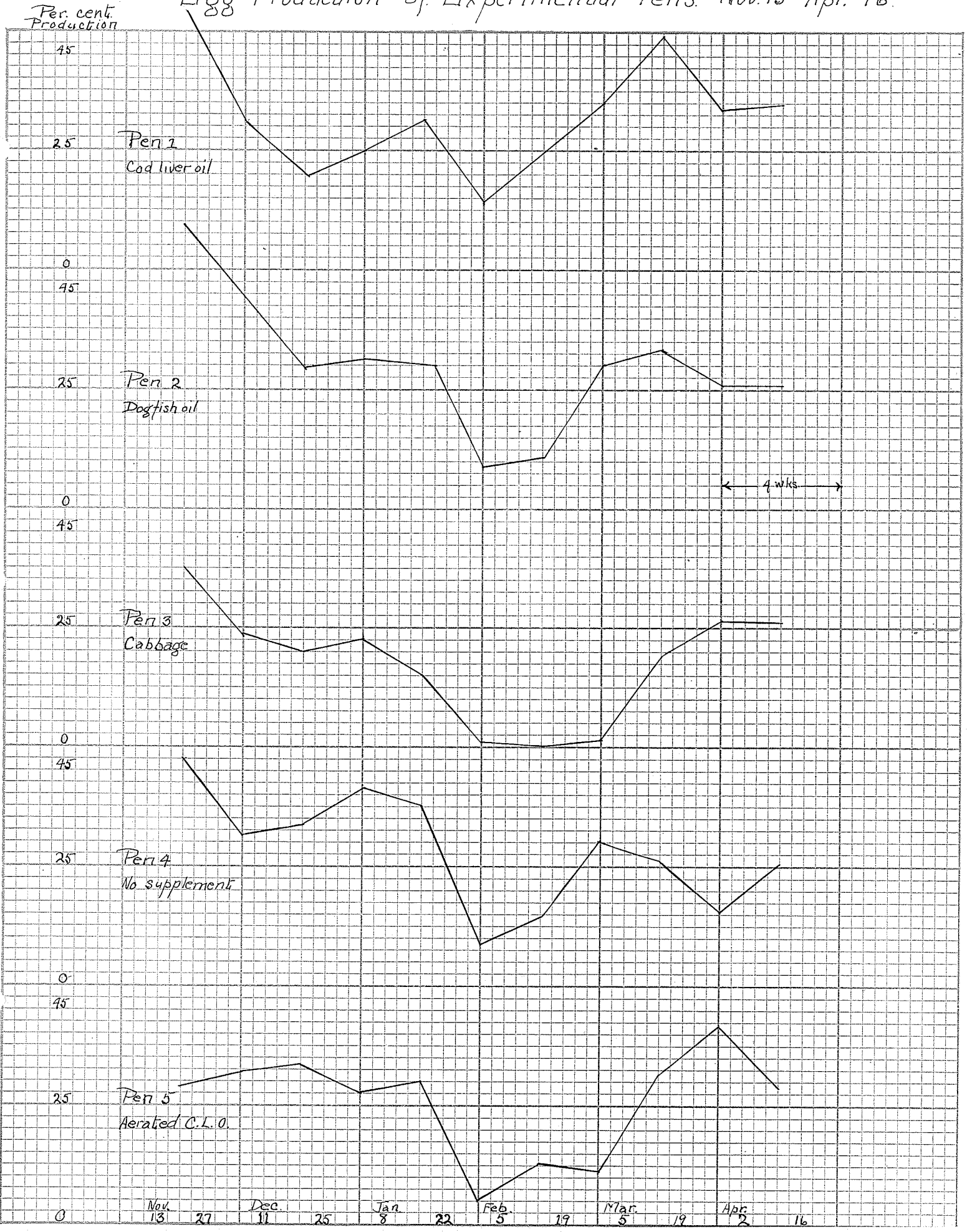


Chart II

Egg Production of Experimental Pens. Nov. 15 - Apr. 16.



due to nutritional roup, which affected 10 out of the 12 birds remaining in the pen. This condition was gradually overcome by feeding two ounces of dogfish oil per day to the pen, and the egg production increased from 16% in the last two weeks in March to 25% in the first two weeks of April. Following a fairly good production in March, several birds in Pen 5 became affected with nutritional roup early in April, and the production of that pen declined. Only one case of nutritional roup was observed in the dogfish oil pen, and that in a smaller bird which was persistently chased away from the mash hopper by the rest of the flock.

A summary of egg production, mortality, and the occurrence of nutritional roup is given in Table 1.

Table 1. Summary of Egg Production, Mortality and Nutritional Roup November 15th to April 16th

Pen	Total Eggs	% Production	Total Mortality	Nutritional Roup			
				Opthalmia	Pustules	Mortality	Total Affected
1	595	31.29	8	0	0	0	0
2	652	31.22	3	1	0	0	1
3	400	19.15	3	1	2	0	3
4	602	28.14	3	8	7	1	10
5	512	25.68	6	4	8	2	9

It indicates clearly that both the cod liver oil and dogfish oil contained enough vitamin A to protect the hens against the nutritional roup by which the pens not receiving oil were affected. The smaller occurrence of the deficiency disease in Pen 3 is probably due to the fact that the birds had an amply supply of vitamin A in their cabbage till February 1st, and also had less drain on their reserve because of their lower egg production. The egg production of both cod liver oil and dogfish oil pens is significantly higher than in the other pens. The high mortality from canker and infectious bronchitis in the cod liver oil pen is difficult to explain, and may be due to chance.

One male bird was placed in each pen on February 15th, another was kept in reserve, and each was moved from one pen to the next every two days. Eggs from each pen were incubated in three separate lots set on March 9th, 16th and 27th. At each setting, all the eggs were put in one incubator so that the incubating conditions were identical for eggs from each pen. Data on the fertility and hatchability of the eggs is presented in Table 2.

Table 2. Data on Fertility and Hatchability of Eggs

	Pen 1 C.L.O.	Pen 2 Dogfish Oil	Pen 3 Cabbage	Pen 4 No supplement	Pen 5 Aerated C. L. O.
Eggs set	69	67	36	53	54
Infertile	0	10	0	3	1
% Fertility	100	86.3	100	94.1	98.1
Dead germs	27	32	15	22	21
Hatched	20	10	15	9	12
% hatch of total eggs	29.	14.9	41.7	17.	22.2
% hatch of fertile eggs	29.	17.5	41.7	18.	22.3
% hatch of eggs alive at 15th day	47.2	40.	71.4	32.1	37.5
Fully formed dead	3	4	2	5	6
Fully formed alive	4	1	1	8	3

Hatches from all lots except Pen 3 were low but quite in line with what could be expected from hens confined indoors and not receiving milk in their ration. The higher hatching power of eggs from Pen 3 was probably due to the fact that the egg production of the pen had been practically nil during February, and at the time the eggs were taken it was steadily increasing, a condition usually associated with strong germs. In all pens the number of dead germs occurring up to fifteen days of incubation was unduly high. Not enough is known of causes of embryonic mortality to give a definite reason for this, but it seems possible that

it may have been due to an inadequate balance of amino acids in the basal ration. Needham (loc.cit.) and Burke (1925) have shown that there is only a very slight increase in the calcium content of the embryo up to 14 days of incubation and that the most rapid increase comes thereafter. It is doubtful, therefore, if the larger number of dead germs before the 15th day can be attributed to any lack of vitamin D. The cod liver oil and dogfish pens did hatch a higher percentage of chicks from eggs with live embryos at 15 days than did Pen 4, which had no supplement, and Pen 5, which had aerated cod liver oil, but the numbers of eggs and the differences are too small to warrant any definite conclusion as to the vitamin D content of the dogfish oil.

In the latter part of March and the first week of April, from 25 to 43 eggs from each pen were weighed on the morning after they were laid. Dried empty shells of infertile eggs from each pen, taken from the incubators after one week's incubation, were also weighed. The records are given in Table 3.

Table 3. **Weights of Eggs**

Pen 1. C. L. O.			Pen 2. DOGFISH OIL			Pen 3. CABBAGE			Pen 4. NO SUPPLEMENT			Pen 5. ABRATED C. L. O.		
Hen	Eggs	Ave.wt. grams	Hen	Eggs	Ave.wt. grams	Hen	Eggs	Ave.wt. grams	Hen	Eggs	Ave.wt. grams	Hen	Eggs	Ave.wt. grams
184	1	43.25	191	9	52.85	206	3	52.83	222	4	53.98	236	6	56.43
183	3	48.30	192	4	49.60	207	6	44.11	223	4	55.12	237	1	57.00
185	8	53.04	193	4	52.94	208	3	50.72	226	1	49.60	238	3	47.33
187	4	55.60	194	2	55.50	209	9	48.41	229	3	50.78	239	8	55.35
190	7	56.47	195	4	50.30	214	4	51.79	231	2	47.90	242	5	55.41
178	1	60.20	196	5	45.61	215	2	48.17	232	3	52.10	244	4	47.80
Floor	1	41.35	197	6	49.02	216	5	51.85	233	7	55.73	250	8	54.76
			201	4	48.79	219	5	45.34				249	2	47.35
			203	4	54.64	220	6	45.03				Floor	3	47.18
Six hens		52.81	Ave. 9 hens		51.028	Ave. 9 hens		48.694	Ave. 7 hens		52.604			

Weights of Shells

178	3	4.70	185	2	5.60	207	3	3.18	229	1	3.65	248	1	4.85
181	2	3.22	191	2	4.45	208	2	3.82	223	1	5.25	249	1	3.75
183	3	4.47	193	1	4.40	215	1	3.20	225	1	5.65			
Ave.	3 hens	4.13	194	3	4.08	220	1	4.40	230	2	3.20			
			195	1	4.80	Ave.	4 hens	3.65	231	1	3.65			
			196	1	4.00				234	5	4.12			
			201	1	2.85				227	2	4.08	Ave.	2 hens	4.30
			197	2	3.70				Ave.	7 hens	4.23			
			Ave.	9 hens	4.22									

The smaller weights of eggs and shells from Pen 4 are in all probability due to the lower egg production of that pen. The weight of egg gradually increases as the laying cycle advances, and the other pens had therefore an advantage over Pen 3. Hart and co-workers (1925) reported that the weights of shells of eggs from irradiated hens was distinctly higher than from non-irradiated hens. In this experiment, the data does not show any significant difference either in weight of whole egg or of egg shell between the different pens.

Chicks hatched from each pen were killed, cut open, dried at 80° C. for 48 hours, burned under a hood and ashed in an electric furnace at 550° C. for 12 hours. The data obtained is recorded in Table 4.

Table 4. Analyses of Chicks

Chick	Weight grams	Dried Weight grams	Ash grams	% Ash of Dry Weight
		Pen 1. Cod Liver Oil		
2599	34.5	10.233	.5859	5.73
2600	33.9	8.575	.5212	6.08
2601	34.3		.5855	
2602	35.7	10.308	.6115	5.93
2603	33.1	8.863	.4979	5.60
2604	32.6	9.866	.5608	5.68
2652	35.25	9.590	.5087	5.20
2655	34.80	11.815	.6180	5.23
2656	31.85	9.646	.5475	5.67
Average	34.00	9.862	.5596	5.69
		Pen 2. Dogfish Oil		
2612	37.6	9.680	.6099	6.30
2614	31.2		.5058	
2615	36.1		.6605	
2616	35.0		.5674	
2617	36.3	10.135	.4695	4.63
2618	38.2	9.943	.5651	5.68
2669	30.7	8.064	.4915	6.09
Average	35.01	9.455	.5528	5.67

Table 4 - Con. Analyses of Chicks

Chick	Weight grams	Dried Weight grams	Ash grams	% Ash of Dry Weight
		Pen 4. No Supplement		
2627	30.8		.4898	
2628	31.8	9.469	.4836	5.10
2629	33.0	8.612	.4839	5.62
2630	33.9		.5535	
2631	33.4	8.475	.5329	6.28
2667	33.3	9.890	.4630	4.67
Average	32.7	9.111	.5011	5.42
		Pen 3. Cabbage		
2621	35.0	9.203	.5614	6.10
2622	31.2		.5322	
2623	37.2	9.862	.5525	5.60
2624	32.4		.5483	
2625	32.7	9.142	.4972	5.44
2626	27.4		.4644	
2658	32.9	11.632	.5184	4.46
2663	38.35	10.700	.5448	5.09
2664	30.25	8.422	.4957	5.89
2665	31.35	9.052	.4785	5.27
2666	30.15	8.860	.5190	5.86
Average	34.62	9.609	.5193	5.46
		Pen 5. Aerated C. L.O.		
2632	35.2	8.861	.5230	6.08
2633	33.8		.5391	
2634	34.2		.5264	
2635	33.7		.5537	
2636	36.3		.5972	
2653	38.9	10.624	.5756	5.42
2660	33.6	8.621	.4931	5.72
2661	35.9	10.595	.5287	4.99
2662	35.5	10.640	.5777	5.43
2668	31.8	9.550	.4387	4.59
Average	34.89	9.815	.5353	5.36

These records show that the chicks from the Pens 1, 2, and 5, (receiving oil) were heavier than chicks from pens not receiving oil. The chicks from Pen 3 would be expected to be small because the eggs were small. The eggs from Pen 4 weighed slightly less than those from Pen 1, but more than those from Pen 2, and one would therefore expect the chicks to weigh as well. It is possible that better development of the chicks from the oil-fed pens was due to the vitamins supplied but the number of chicks is rather small to permit a definite conclusion. The ash content of the chicks does not show the difference between oil-fed pens and controls that would be expected. Where the antirachitic factor is present, bone formation in the embryo progresses normally and the ash content of the chick is distinctly higher than in chicks from eggs low in the anti-rachitic factor. Hart and co-workers (1925) found that the ash of chicks from irradiated hens averaged 0.531 grams and the ash of non-irradiated hens only 0.310 grams. The data recorded in this experiment shows no difference as great as this. The ash of chicks from cod liver oil and dogfish oil pens averaged 0.559 and 0.552 grams respectively. The ash from Pen 5 was 0.535 grams per chick and from those not receiving oil 0.501 and 0.519 grams. On the basis of Hart's figures all the chicks were normal.

The chicks used in this work were only those that had successfully emerged from the shell without assistance. They were killed when they had been out of the shell 24 hours. Hart's chicks were taken at 1 to 2 hours after hatching and his low ash determinations were mostly made on chicks that remained alive to 21 days, but were unable to get out of the shell. The excellent hatching power of the eggs from Pen 3 tends to show that hens in this pen had not yet exhausted their reserve of vitamin D. This would be expected from their period of low production

in the winter and would lead one to expect a fairly normal ash content in the chicks. However, eggs from Pen 4 had the lowest hatchability of any pen, and chicks from this pen would be expected to be low in ash. The average ash of these chicks was lowest of any pen. The average ash of chicks from the cod liver oil flock was 11.6% higher, from the dogfish oil flock 10.5% higher, and from the aerated clod liver oil flock 6.1% higher than from this pen. These differences are much smaller than Hart's, but do indicate a higher ash in chicks from all the oil-fed pens such as would be expected if the antirachitic factor were in the ration. It is possible that had the ash analyses been taken on chicks which were unable to get out of the shell, (as were Hart's) that more definite results would have been obtained.

Biometrical analysis of the above data gives the following statistical constants.

Pen	No. Chicks	Mean ash grams	Probable Error of Mean	Standard Deviation
1. C.L.O.	9	.5596	± .0087	± .039
2. Dogfish	7	.5528	± .0156	± .0629
3. Cabbage	11	.5193	± .0062	± .0305
4. No Supplement	6	.5011	± .0086	± .0314
5. Aerated C.L.O.	10	.5353	± .0092	± .0435

The difference between the means of pens 1 and 4 is .0585 ± .0122. Since this difference is 4.79 times its probable error the odds are over 700 to 1 against its being due to chance (Pearl, 1925) and it is therefore distinctly significant.

The difference between the means of pens 2 and 4 is .0517 ± .0178. This difference is only 2.9 times its probable error and hence the odds are only 19 to 1 against its being due to chance. This result is hardly significant. This is due to the greater range in this group making its standard deviation and hence the probable error of its mean higher than in the other group. A larger number of chicks should give a better result.

The difference between the cod liver oil and dogfish oil pens is not significant.

2. Chicks

Experiment

One hundred and four chicks two days old were divided on February 8th, into two lots of twenty and two lots of thirty-two. They came from a cross of Rhode Island Red males with Barred Rock females, and hence could be separated into cockerels and pullets at hatching. By putting an equal number of male and female chicks in each pen, any variation in results due to different rates of growth in the sexes was eliminated. Lots 1, 2 and 3 received the following basal ration:

Ground white corn	82 lbs.
Beef Scrap	15 "
Ground Oyster Shell	2 "
Sodium Chloride	2 "

Lot 4 received the following basal ration:

Ground Yellow Corn	82 lbs.
Beef Scrap	15 "
Ground Oyster Shell	2 "
Sodium Chloride	1 "

All pens received only water to drink. The original plan was to use Hart's rachitic ration, consisting of 97 parts white corn, 2 of calcium carbonate, 1 of sodium chloride, and skimmed milk ad libitum. Skim milk was not available and buttermilk was considered to contain too much vitamin A, therefore, it was necessary to provide protein in the form of beef scrap. Supplements to these rations were supplied as follows:

Lot 1 (20 chicks)	= 1% crude cod liver oil.
Lot 2 (20 ")	= 1% " dogfish "
Lot 3 (32 ")	= No supplement.
Lot 4 (32 ")	= " "

Yellow corn is known to contain an abundance of vitamin A. It was expected that growth curves of the chicks on the white corn rations coupled with examination for rickets would indicate if the dogfish oil contained as much of either vitamin A or D as the cod liver oil.

After three weeks on the basal ration, seven of the remaining twenty chicks in Lot 4 (yellow corn ration) were penned separately (Lot 5) and fed as before with the addition of 1% cod liver oil. Six others were put in a separate run (Lot 6) and fed 1% dogfish oil in their ration. It was expected that all these chicks would make normal growth because their vitamin A requirement was adequately met by the yellow corn but that at six or seven weeks' of age, rickets would develop in the control pen and in the others unless vitamin D were in the oils fed.

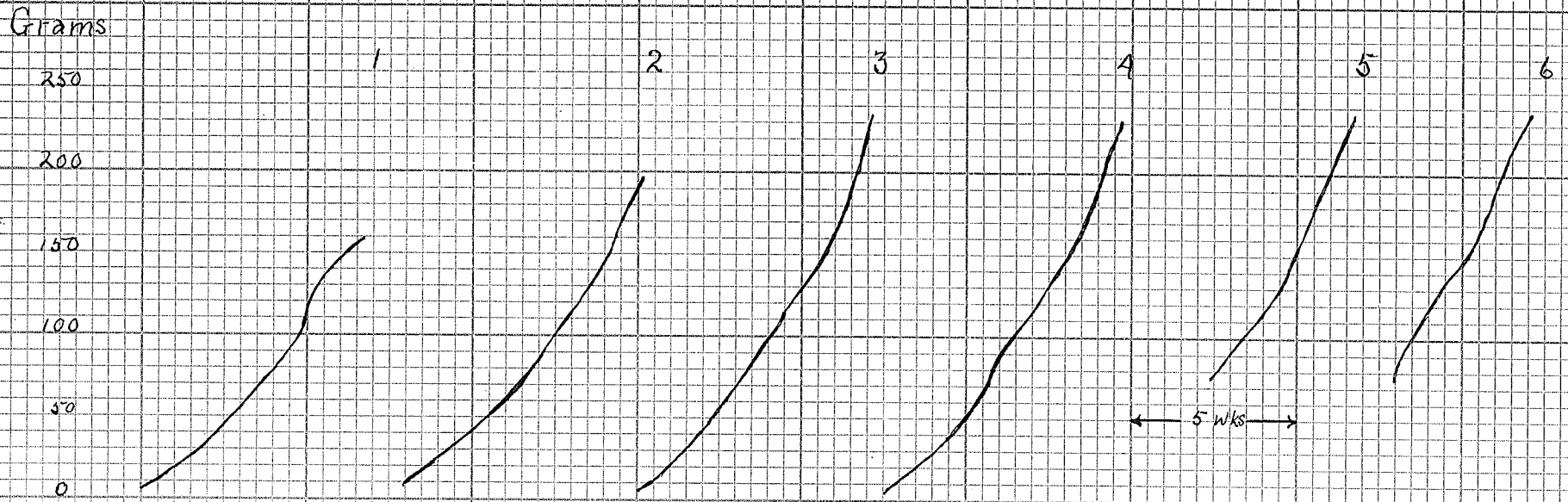
The chicks were brooded under electric hovers in the basement of the poultry building at the Agricultural College. They received no direct sunlight. Brooding conditions were not ideal in that the room was difficult to ventilate and often became too stuffy for the best health of the chicks. The chicks had shavings for litter. Their mash was fed for the first three weeks in hoppers left before the chicks continuously. After that, a moist mash was given thrice daily and the hoppers removed.

Results

Growth curves representing the average weights of each lot taken at weekly intervals up to seven weeks are given in Chart 3. They indicate that up to that time the only noticeable difference between the pens was that the cod liver oil chicks averaged 193 grams, the dogfish oil chicks 234 grams, and the remaining lots between 260 and 270 grams. The white corn chicks receiving no oil supplement showed no lack of vitamin A compared with the yellow corn chicks at seven weeks. It is difficult to see where enough vitamin A could be found in the basal ration of the white corn chicks to maintain normal growth indefinitely. It is possible that a longer time than seven weeks may be necessary to use up the storage of vitamin A received by the chick

Chart III

Growth curves of chicks



1. White corn cod liver oil
2. White corn dogfish oil
3. White corn
4. Yellow corn
5. Yellow corn cod liver oil
6. Yellow corn dogfish oil

in the egg although Hart, Steenbock, etal (1924) and Heuser and Norris (1926) found growth of chicks on diets deficient in A to be checked at five to eight weeks. The slower growth made by the cod liver oil and dogfish oil pen is probably due in greater part to their quarters being more cramped than those of the other lots. It could not be observed that the oils fed affected the appetites of the chicks in any lot.

Chick mortality was heavy in all lots. Some chicks in all lots showed signs of leg weakness after three weeks on the experimental diet. At four weeks of age there were two in Lots 1 and 2, and one in each of Lots 4, 5 and 6. The symptoms were more those of polyneuritis than of rickets and since it had occurred in all pens but one, it was considered probably that the basal ration was too low in vitamin B. Accordingly yeast was fed to the affected chicks and 20% of wheat middlings was added to the ration of all chicks. This did not bring about recoveries and several further cases occurred. It is possible that lack of ventilation and cramped space may have been contributory factors to this condition. At seven weeks of age the condition of the different pens was as follows:

Lot 1	-	(white corn, C.L.O.)	7	chicks,	4	with leg	weakness
Lot 2	-	(" " Dogfish)	7	"	1	" "	"
Lot 3	-	(white corn)	19	"	6	" "	"
Lot 4	-	(yellow ")	7	"	1	" "	"
Lot 5	-	(" " , C.L.O.)	6	"	1	" "	"
Lot 6	-	(" " , Dogfish)	6	"	1	" "	"

Under the circumstances, and since cod liver oil chicks were affected as well as the others, it was felt that leg weakness was not of itself an accurate guide to the antirachitic potencies of the oils used. At 47 days of age, five chicks were taken from each lot for analyses of the bones. Both tibio-tarsi of each chick were dissected out. One was analyzed for ash content, the other fixed in 10% formalin and kept for silver nitrate test.

The bones ashed were dried at 110° C. for 48 hours, crushed, extracted in 85% alcohol at 60° C. for 30 hours, dried 12 hours at 110° C., weighed, and ashed in an electric furnace at 700° C. for 12 hours. The data obtained is shown in Table 5.

Table 5. Analyses of Bones of Chicks

Chick	Ration	Weight grams	Wt. of Tibia grams	Ash grams	% Ash	Condition of chick
		Lots 1 and 5 - Cod Liver Oil.				
2218	White corn	326	1.371	.4990	35.86	Leg weakness
2216	" "	262	1.198	.4454	37.10	" "
2219	" "	175	.799	.3307	41.37	" "
2203	" "	318	1.369	.5193	36.68	" "
2202	" "	220	1.049	.3992	38.15	Normal
2212	" "	156	.669	.2566	38.35	"
2273	Yellow "	244	1.404	.5440	38.74	Leg weakness
2296	" "	237	1.178	.4472	37.74	Normal
2291	" "	255	1.237	.4202	42.06	"
2289	" "	287	1.503	.5745	38.22	"
Average		248	1.177	.4436	38.42	
		Lots 2 and 6 - Dogfish Oil.				
2221	White corn	273	#	#	#	Normal
2226	" "	248	1.043	.4827	46.28	"
2228	" "	276	1.375	.4868	35.40	
2233	" "	291	1.610	.6375	39.59	Leg weakness
2235	" "	216	.998	.3678	36.08	" "
2282	Yellow "	283	1.594	.5951	37.50	Normal
2287	" "	291	1.165	.4579	39.30	"
2293	" "	248	1.241	.4759	38.35	"
2298	" "	340	1.441	.5829	40.45	"
2301	" "	213	1.235	.4342	35.16	Leg weakness
Average		267.9	1.300	.5023	38.68	

Error in determination.

Table 5. - Con.

Chick	Ration	Weight grams	Wt. of Tibia grams	Ash grams	% Ash	Condition of chick
		Lots 3 and 4 - Controls.				
2242	White corn	319	1.411	.4700	33.31	Leg weakness
2252	" "	337	1.999	.7958	39.79	Normal
2253	" "	184	.885	.2982	33.68	"
2267	" "	294	1.348	.5022	37.25	"
2260	" "	255	1.297	.3908	30.14	Leg weakness
2278	Yellow "	280	1.561	.6099	39.07	Normal
2279	" "	358	1.614	.5878	36.42	Leg weakness
2281	" "	322	1.350	.4999	37.03	Normal
2294	" "	237	.910	.3343	36.74	"
2297	" "	174	.730	.2563	35.48	"
Average		276	1.310	.4735	35.89	

The cod liver oil chicks had 7.05% more, and the dogfish oil chicks 7.77% more ash than the controls. The differences are not great but indicate that calcification processes had been better in both lots of oil fed chicks and suggest that vitamin D was provided in the oils but lacking in the controls. The dogfish oil was evidently every bit as rich in vitamin D as the cod liver oil.

Hart, Steenbock and Lepkovsky (1925) found the ash content of tibia from control chicks to average at 42 days 38.8% ash in one lot and 40.1% in another. Tibiae from their chicks fed cod liver oil averaged from 44.8% to 47.6% ash in five lots, and one lot had an average of 50.1%. These bones were extracted in "hot 95% alcohol" and would have had a more complete extraction of fat than did the bones used in this experiment. This would account for the higher percentages of ash obtained in their experiment in both normal and control chicks than were found by the writer. Hughes, Titus and Witham (loc.cit.) showed that the ether extract of bones from rachitic chicks was less than half of that from normal chicks. This means that if the fat be not completely extracted the difference in ash content between bones of normal and rachitic chicks

will be less sharply defined, and would account for the fact that in this experiment the difference between the percentage of ash in the bones of oil fed and control chicks was not quite so great as was reported by Hart, Steenbock and Lepkovsky.

Heuser and Norris (1927) found the ash content of fat-free bones of control chicks to be 33.5% and of bones from chicks receiving cod liver oil to be from 35.02% to 44.42%, varying with the grade of oil used.

It was considered possible that a more complete fat extraction would make the difference between ash content of bones from oil-fed and control chicks more distinct. The only bones available were the shafts of tibiae from which the distal and proximal ends had been removed for silver nitrate test and microscopical examination. Seventeen of these were extracted with ether for 36 hours and ashed in an electric furnace at 700° C. The results are presented in Table 6.

Table 6. Analyses of Ether Extracted Bones

Chick	Bone grams	Ash grams	% Ash	
		Cod liver oil.		
2291	.4940	.2965	60.02	
2296	.4326	.2701	62.43	
2218	.5554	.3271	58.89	Average ash Content
2216	.5155	.2979	57.79	
2202	.3847	.2215	57.57	59.61%
2212	.2442	.1490	61.01	
		Dogfish Oil.		
2287	.4771	.2722	58.10	
2226	.2306	.1425	61.79	Average ash Content
2221	.4757	.2510	52.76	
2228	.5588	.3136	56.12	57.17%
2298	.7012	.4004	57.10	

Table 6. - Con.

Chick	Bone grams	Ash grams	% Ash	
		Control		
2260	.6500	.2041	31.23	
2294	.2600	.1438	55.31	Average ash Content
2252	1.1171	.6232	55.76	
2281	.5508	.3100	52.65	
2279	.7892	.4051	51.33	of 6 - 50.14%
2267	.5117	.2793	54.58	of 5 - 53.95%

The ash content of the shafts of the bones was much higher as was to be expected when the ends, which contain most of the cartilage of the bone were removed. One chick in the control lot had an extremely low ash content but even if this one be not considered, the average ash content of the remaining five samples was only 53.92% which is significantly lower than the average for the other two lots.

Biometrical analysis of the two sets of data obtained from the ash analyses of the bones yields the statistical constants recorded in Table 7.

Table 7. Analysis of Data on the Ash Content of Bones

Ration	Number of Chicks	Standard Deviation	Mean Ash per cent.	Difference from Control	Difference / P.E. Diff.
			Alcohol-extracted Bones.		
Cod liver oil	10	± 1.84	38.42 ± .39	2.53 ± .70	3.6
Dogfish oil	9	± 3.22	38.68 ± .72	2.79 ± .92	3.3
Control	10	± 2.72	35.89 ± .58		
			Other Extracted Bones.		
Cod liver oil	6	± 1.73	59.61 ± .47	5.69 ± .69	8.2
Dogfish oil	5	± 2.92	57.17 ± .88	3.25 ± 1.01	3.2
Control	5	± 1.64	53.92 ± .49		

Since the differences are not less than 3.2 times their probable errors they may safely be considered as significant. It is therefore reasonably certain that vitamin D was present in the dogfish oil used as well as in the cod liver oil. The more significant difference between the cod liver oil lots and the controls, coupled with the greater variability in the dogfish oil lots tends to indicate that so far as the samples used were concerned, the cod liver oil was slightly superior to the dogfish oil.

For the silver nitrate test, bones were fixed in 10% formalin for several days, and washed in water. The distal ends were split and put for one minute in 1.5% silver nitrate solution. They were then removed to light Left under water, and examined. Ten bones from each lot were classified as follows:

Cod liver oil lots	8 good	1 fair	1 poor
Dogfish oil lots	7 "	3 "	
Control lots	2 "	2 "	6 "

This classification can only be made in an arbitrary way. Thos considered poor had not a sharply defined zone of calcification and had areas of proliferating cartilage greater than three millimetres in width. The good bones had sharply defined zones of calcification and narrow zones of proliferating cartilage less than two millimetres in width.

For microscopical examination the proximal ends of two tibiae from each lot were cut from the shafts, after fixing in formalin, washed, split open and decalcified in a 10% solution of nitric acid in 70° alcohol, changed daily for seven days. Following this the bones were debydrated in the usual way, cleared in cedar oil and imbedded in paraffin. Sections cut at 10 microns were stained with Ehrlich's haematoxylin and eosin. Examination showed that the zone of calcification was distinctly marked in the bones from chicks fed cod liver oil, well marked in the

bone from one dogfish oil chick and not in the other, but poorly defined in the controls. The latter did not have as wide an area of proliferating cartilage as might be expected in badly rachitic chicks, but the absence of a distinct zone of calcification and the presence of osteoid tissue invading the cartilage made them easily recognizable as abnormal.

Summary

1. Both cod liver oil and dogfish oil, when fed to mature fowls at the rate of 2% of the mash, contained enough vitamin A to prevent nutritional roup which occurred in control pens.

2. The ash content of chicks from hens fed cod liver oil was 11.6% higher than that of controls and was statistically significant.

3. The ash content of chicks from hens fed dogfish oil was 10.5% higher than that of controls and was barely significant.

4. Bones of chicks fed cod liver oil and from chicks fed dogfish oil were significantly higher in ash than bones of controls.

5. Silver nitrate tests and microscopical examination showed that bone formation had been better in both lots of oil fed chicks than in controls.

Conclusion

The numbers of experimental animals involved were smaller than might be desired but from all the evidence it seems reasonable to conclude that dogfish oil contains both vitamin A and vitamin D. The vitamin D content of the particular sample of dogfish oil used was slightly less than that of the cod liver oil used.

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PHOTOGRAPHS

Fig. 1. Nutritional roup in Pen 4 (no supplement) 18 weeks after the experiment started.

Fig. 2. Two hens from Pen 4 showing ophthalmia due to vitamin A deficiency.

Fig. 3. Same. Other hens.

Fig. 4. Silver nitrate test on bones from 4 chicks fed cod liver oil.

Fig. 5. Silver nitrate test on bones from 4 chicks fed dogfish oil.

Fig. 6. Silver nitrate test on bones from 4 control chicks.

Figs. 7, 8, 9, 10 & 11.

Photomicrographs of sections of proximal ends of tibiae. Magnification 8.5 diameters.

7. No. 2291 - Cod liver oil.

8. No. 2226 - Dogfish oil.

9. No. 2221 - Dogfish oil.

10. No. 2242 - Control.

11. No. 2253 - Control.

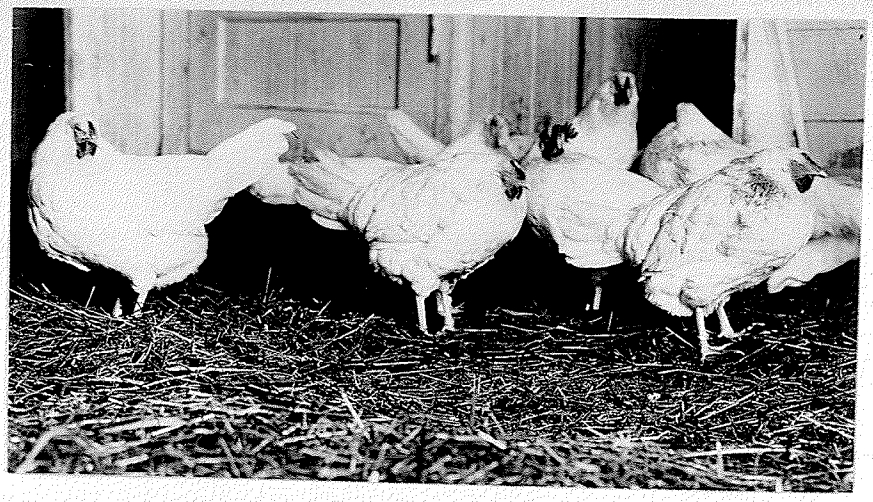

Fig. 1

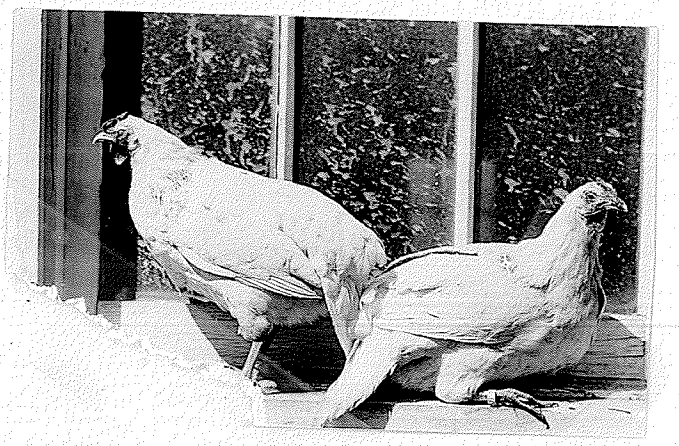

Fig. 2

Fig. 3

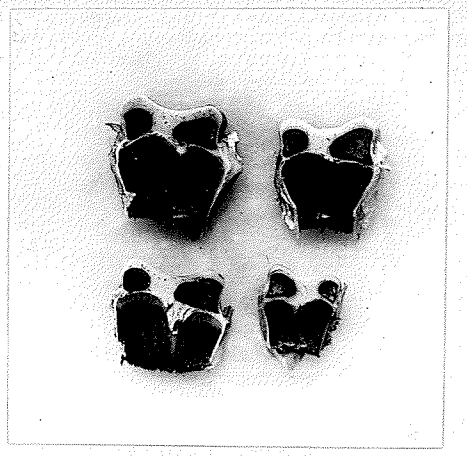

Fig. 4

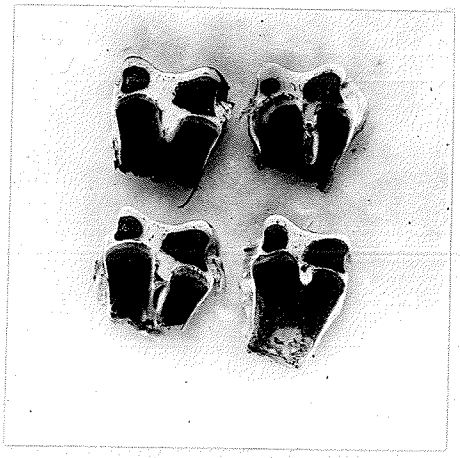

Fig. 5

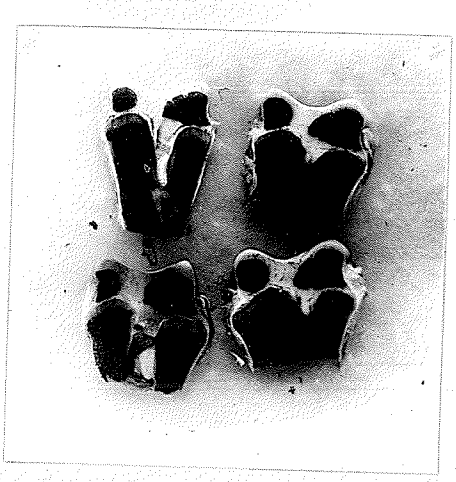

Fig. 6

Fig. 7

Fig. 8

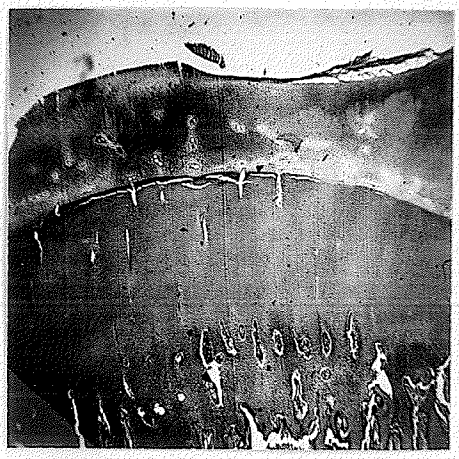

Fig. 9

Fig 10

Fig 11