A NUTRITIONAL DISEASE OF CHICKS CAUSED BY FEEDING DRIED EGGS

by

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INTRODUCTION

Previous work with egg albumen has shown that when this was used as the sole or principal source of animal protein in rations for rats, chicks, and other animals, abnormal growth resulted. In some cases a pellagra-like syndrome appeared in the experimental animals. Limited work with dried egg fed to chicks has given somewhat comparable results. While the work on this subject has been rather extensive, certainly the problem has not been settled. Due to this and to the tremendous importance of the Poultry Industry which in 1931 was the second largest source of agricultural revenue, a continued detailed study of the biological value of eggs as a food is not only justified but of great economic importance.

For many years eggs, particularly in the dry form, and more often egg albumen, have been used as a source of animal protein in nutrition experiments. Until recently egg albumin, or egg white, was thought to be a complete protein except for glycine, an amino acid which many animals can synthesize so that it need not be a part of their ration. Recently, however, particularly since the components of vitamin B have been studied in so much detail, egg white appears to be lacking in some essential nutrient not previously recognized, or it may possibly contain some toxic factor. The purpose of this study was to try to verify previous work regarding this deficiency or toxicity and if possible to determine its nature and location in the egg. Chicks were used as experimental animals.

REVIEW OF LITERATURE

Linossier (24) studied the toxicity of eggs as early as 1905, having published an article on the subject in that year. He concluded in 1918 that there is some substance in eggs which has a toxic action on some predisposed human individuals. The toxic substance is destroyed by heat sufficient to coagulate all the albumin in the egg, in the yolk as well as in the white. He remarked that the reason cooked egg appeared harder to digest than raw egg was because it was digested in the stomach, while the raw egg is not hydrolized until it reaches the intestines.

References concerning the feeding of eggs, usually in the form of egg white or egg albumin, to experimental animals are numerous and cover a period of many years. Osborne and Mendel (29, 30, 31) reported that ovalbumin gave adequate growth in rats when it made up eighteen per cent of the total diet. In the first experiment however, ovalbumin was fed apparently to only two rats.

Bond (7) obtained somewhat similar results when twenty or thirty per cent of egg white was the chief source of protein. She admitted however, that "the results of feeding the lactating rat and her growing young on the egg white diet are not very conclusive" as the results were not satisfactory.

Boas (4) fed rats dried Chinese egg white at the rate of twenty per cent of the diet and found that it gave satisfactory growth for from four to six weeks, but later did not supply adequate nitrogen. Even though marmite was a part of the ration used, loss of hair occurred in the rats,

growth ceased for awhile, and then the weight fell. She concluded that egg white was inadequate as the sole source of nitrogen for rats, even when it amounted to twenty per cent of the total ration.

Later the same year Boas (5) reported that "rats fed on the whites from fresh English eggs grew normally and had good coats."

Considerably later Boas (6) reported rather extensive tests using 12.5 per cent egg white in various forms as the chief source of protein for young growing rats. Dried egg white produced severe lesions in the experimental rats unless the ration was supplemented with potato starch or arrowroot as sources of carbohydrate. Crude boiled egg white as the chief source of protein gave satisfactory results. When the egg white was coagulated by boiling before desiccation, it gave good growth results. Several feeds and supplements as raw potato, potato starch, dried yeast, fresh egg white, etc., when used in the diet with dried egg white prevented the appearance of the lesions and other characteristics of the pellagra-like symptoms encountered. The presence of a protective factor X in the above substances was postulated.

As early as 1918 Maignon (25) fed rats albumin in pellet form with sufficient bone ash, NaCl and $FeCO_3$ added to supply minerals, and $NaHCO_3$ to keep the urine faintly alkaline. He concluded that egg albumin would not sustain the life of, nor maintain a constant weight in white rats. In addition he studied the effect of egg albumin when it was fed at various seasons.

Goldberger and Lillie (16) also described a pellagra-like condition in experimental rats caused by a lack of his "P-P solid" prepared from auto-

claved yeast. None of the symptoms appeared until a varying time following the arrest of growth. The reader is referred to the original report for a complete description of the pellagra symptoms but some of these were as follows: A tendency for eyelids to adhere together, loss of fur varying from patches to "almost complete denudation of the head, neck, and trunk. With or without such loss of fur some of the animals have developed a dermatitis at one or more of the following sites: ears, front of neck and upper part of chest, forearms, backs of forepaws, shins, and the backs of the hindpaws." One symptom of particular interest, because it appears also in chicks with the pellagra syndrome is the linear fissuring or ulceration at the angles of the mouth. Goldberger and co-workers observed this in only a few of their experimental rats. Six per cent of their "P-P solid" included in the diet was followed, if the animals were still able to eat, by a clearing up of this condition and resumption of growth.

Later Goldberger and co-workers (17) as the result of extensive tests on the blacktongue preventive value of sixteen foodstuffs, found that hard boiled dried egg yolk did not exercise adequate blacktongue preventive action. However, it did seem to have a delaying or partially protective action as only two of the five experimental dogs developed definite signs of the disease, the third dog "presented very suggestive but transient indications of blacktongue," while the remaining two showed no signs of the disease. They found the cooked yolk was inferior in blacktongue preventive action to fresh beef, pork liver, and wheat germ.

Baglioni (3) fed white rats exclusively on egg albumin in unrestricted

amounts also supplying them daily 0.2 grams butter, 9 drops of lemon juice, and 0.2 grams of yeast. The animals were weighed regularly and chemical determinations, including nitrogen, urea, and ammonia, made on the urine and feces. The animals survived weeks and months although they showed noticeable disturbances consisting especially of hepatic and renal hyperfunction.

References concerning the effect of cooking or boiling on the toxicity of egg white are confused and contradictory. Scheunert and Wagner (39) have shown that the usual mode of cooking eggs does not diminish their nutritional value. They used rats as experimental animals.

On the other hand Friedberger and Seidenberg (14) studied the effect of a diet consisting solely of hens' eggs on the growth of rats and found that when whole eggs were used raw the rats gained 198 per cent in thirty days but with cooked whole eggs the gain was only 109 per cent. Stenqvist (42) made similar studies and came to an exactly opposite conclusion.

Findlay (13) has also reported the pellagra-like lesions in rats, ascribing these to a deficiency of vitamin B_2.

Guha (19) summarized the results of several workers on pellagra and showed that in spite of much work on the subject, it was not yet entirely acceptable that pellagra was the uncomplicated result of vitamin B_2 deficiency. In addition he found improbable the theory that pellagra was the result of a simple iron deficiency as vitamin B_2 in certain preparations could be inactivated by autoclaving in an alkaline medium. However, he recognized the possibility that pellagra might be associated with an iron deficiency. He further stated that "it is considered probable that pella-

gra is a complex syndrome arising from an association of various factors, in which the deficiency of an antipellagra factor (Goldberger's P-P factor), which is possibly, but not necessarily, identical with the growth promoting vitamin B_2, is the main but not the sole factor."

Considering the information available at present pellagra in human beings, skin lesions in rats, and the pellagra-like syndrome in chicks are in some way linked up with the vitamin B complex so that this must be considered necessarily in a study of the pellagra-like syndrome in chicks, which can be caused by feeding dried eggs in certain rations.

Hauge and Carrick (21) first reported in 1926 that vitamin B consisted of two parts and that yeast was poor in the antineuritic substance but rich in the growth factor. On the other hand corn was relatively antineuritic but poor in the growth factor.

Chick and Roscoe (8) in 1928 supplied further evidence to the data already published by several others of the dual nature of vitamin B. They described an improved animal technique for the study of vitamin B_2; suggesting in addition that many of the conflicting results obtained previously in studies of this complex might have been due to the use of caseinogen associated with varying fractions of vitamin B_2. They presented a method for the complete purification of caseinogen. Later the same authors (9) found fresh egg white to be rich in vitamin B_2, but devoid of vitamin B_1.

Aykroyd and Roscoe (2) reported that the vitamin B_2 value of wheat, maize, and dried peas was poor, while ox liver, yeast, and fresh whole milk were excellent sources of this vitamin. "Dried meat and egg yolk are

less good but richer than cereals." They further noted that the distribution of B_2, Goldberger's P-P factor, and the factor preventing black-tongue in dogs was identical.

Chick, Copping, and Roscoe (10) studied the egg white of hens' eggs and found that while this product was a relatively rich source of the antidermatitis vitamin B_2, it was lacking in vitamin B_1, and in this respect is unique among the foodstuffs hitherto examined. The egg white used was a concentrate made after the removal of the approximate ten per cent of the coagulable protein which the original contained. They found this concentrate would cure the dermatitis in rats associated with vitamin B_2 deficiency, as well as restore growth. The restoration of growth was not permanent however, which they believed due to a deficiency in the egg white of an unknown factor. They stated that "a daily dose of 2.5 to 5 grams of fresh, cooked, egg white (dry weight 0.3 to 0.6 grams), or of an extract -- equivalent to 5 to 10 grams -- will restore normal growth."

Hoagland and Snider (22) have studied vitamin G and found that beef, pork, and lamb contained approximately the same amounts. Beef and pork liver and beef kidney contained five to eight times as much as beef muscle. Approximately three per cent of any of the former provided ample vitamin G for growth in rats.

Apparently the vitamin B complex is well named as several references show that it is composed of even more than two factors. Reader (32) described a second heat labile factor in yeast which she named vitamin B_3. It was not possible to get a supply of B_3 free from B_1. In a later paper

the same author (33) supplied additional evidence of this heat labile vitamin B_3. She was able to concentrate this as it appeared in the mercuric sulphate precipitate of the Kinnersley and Peters (23) process for vitamin B_1. She considered vitamin B_2 to be the anti-pellagra vitamin of Goldberger et al (15) as interpreted by Hassan and Drummond (20), and the factor present in alkaline autoclaved yeast extract. "The term "anti-dermatitis" is probably preferable for vitamin B_2 since some evidence in this laboratory suggests that "pellagra" is due to a combined deficiency of vitamins B_2 and B_3." Later work by Roscoe (35) was not in agreement with this. She found in feeding rats daily doses of yeast extract, egg white filtrate or meat necessary to cure the dermatitis associated in young rats with vitamin B_2 deficiency no support for the theory that separate dietary factors were necessary to prevent and cure dermatitis and to promote growth.

Less work than on other phases has been done on the physiology of pellagra in experimental animals. Turner and Blanchard (45) working with pellagrins have reported that "there is a tendency to low serum albumin, which persists after symptoms of the disease have disappeared. The abnormality is one of disturbed digestion, resulting from difficulty in protein absorption and injury of the digestive system." They suggest that the low serum albumin which persists after external "diagnostic evidences of the disease have disappeared and in spite of adequate diet" may be due to a permanent injury to the digestive system. Sure, Kik, and Smith (43) have observed a distinct lowering of the red blood cell count in vitamin B_2 deficiency. The same observation has been made by Guha (19).

Sebrell (40) has studied the pellagra preventive value of many common foodstuffs and has prepared a table giving the results of his work, classing the materials as good, fair, slight, and of no value.

In addition to the work that has been done on dermatitis or pellagra in rats and which has been reported above, considerable other experimental work has been completed. Recently Salmon and Goodman (38) have reported extensive experiments on the raw egg white syndrome in rats. Additional references to the literature will be found in their work. They reported a positive harmful factor in raw egg white the results of which "are apparently not due to an anti-vitamin G action of the harmful factor although many of the symptoms are similar to those produced on certain vitamin G deficient diets and even more similar to those of pellagra."

Because the scope of the present planned experiments was confined to chicks, further reference to the work on rats seems unnecessary. Concerning the latter however, the many conflicting results are at times extremely difficult to understand and the suggestion may be made that the trouble, in part at least, may be due to the use of too few experimental animals.

Norris and Ringrose (27) and Ringrose, Norris, and Heuser (34) gave a detailed description of a pellagra-like syndrome in chicks where powdered egg albumin was the chief source of animal protein in the ration. However, they also encountered a similar condition when purified casein, commercial casein, and purified casein plus two and a half per cent of autoclaved yeast were the principal sources of animal protein. This would tend to indicate that vitamin G was involved. North (28) reported feeding boiled egg yolks to chicks which at six weeks of age averaged as much as a

check lot receiving a protein supplement of 12 per cent dried skimmilk plus six per cent of meat scrap. "Those receiving boiled egg white grew more slowly, but no indications of disease were visible." However, "chicks fed a ration in which 66 per cent of the dry matter was in the form of raw egg white developed a pellagra-like condition."

Tully and Franke (44) have described the same pellagra-like syndrome in chicks when 15 per cent of dried whole egg plus five per cent of dried buttermilk were the animal protein supplements used in a ration believed otherwise complete. Three or five per cent of Yeast-Foam Tablet Powder (dehydrated yeast cells not autoclaved), or five per cent similar yeast plus three per cent of meat and bone scraps did not prevent this trouble when used with the above ration. No signs of the trouble were found when the dried whole egg was replaced with meat and bone scraps.

It is evident from this review of the literature on the subject of the toxicity of or deficiency in dried eggs that the data are incomplete and some of them contradictory. Therefore additional work on this subject is justified.

EXPERIMENTAL

Objectives

The objectives of the experiments described herein were as follows:

1. To determine if the pellagra-like syndrome was due to a deficiency or toxicity in the eggs.
2. To determine whether the toxicity or deficiency was located in the yolk or in the albumen.
3. To discover methods of supplementing and treating the egg products to isolate the nature of the trouble.
4. To discover associated symptoms, including growth, mortality, appearance of the syndrome, blood hemoglobin, and serum albumin and globulin, in normal and affected chicks.

Method of Procedure

For the first three experiments day-old chicks were carefully sorted into lots of equal average weight at the beginning of each experiment, and banded at the time of weighing. Chicks were usually weighed at two week intervals throughout the six or eight weeks of the experiments. Feed was weighed in at the beginning of each experiment and carefully weighed back on each weigh-day; from these figures average feed consumption could be computed on a chick-day basis. Careful observations of growth conditions were made regularly and detailed results recorded on weigh-days. Particular care was taken to observe the incidence, severity, and duration of any

pellagra-like symptoms that appeared. The chicks in each lot were brooded in a six deck electric brooder for the first six weeks of any experiment. When experiments were continued for a greater length of time, each lot was equally divided to allow ample room and brooding conditions for as good growth as the rations and chicks allowed.

Results

Experiment 1

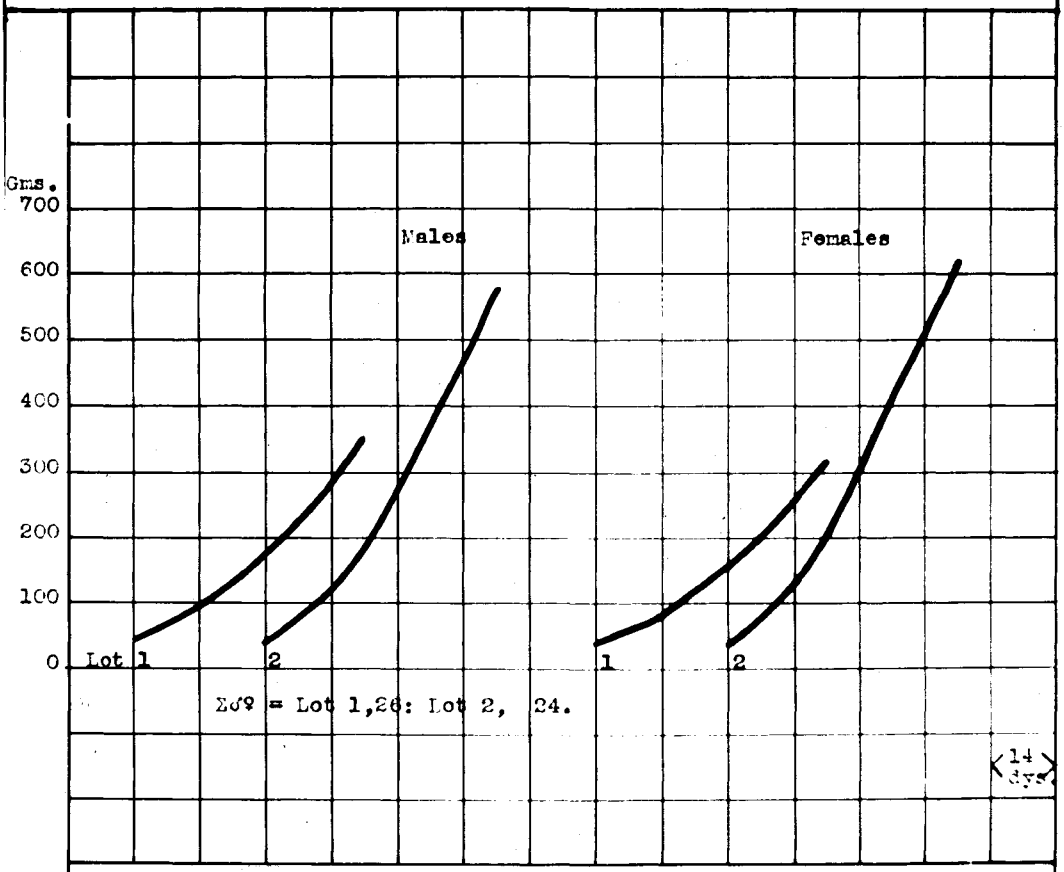
This trial was a study of the effect of adding dried whole egg to a ration low in total protein, but believed otherwise complete. Twenty-seven mixed heavy day-old chicks were used in each of two lots which were started November 2, 1934. Reference to Chart I gives the rations used, growth curves, and mortalities. The check Lot 1 was fed a ration containing ten per cent of dried buttermilk and five per cent of meat and bone scraps (hereafter referred to as meat scraps(as the only animal protein. This gave satisfactory growth though somewhat below what is considered normal or average. Lot 2 was fed a similar ration except that 15 per cent of dried egg replaced an equal amount of ground corn. The total protein in this lot was computed to be 22 per cent compared to 15.8 per cent in the check lot, and while such a wide variation as this was not the best practice, in this preliminary trial the interest was primarily in the probable toxic effect of the egg.

In our previous work (44) five per cent of dried buttermilk had always been used with the 15 per cent of egg, although later other additional

27 chicks to each lot.

Mixed heavy chicks.
started 11/2/34.

	Lot 1 %	Lot 2 %
ground yellow corn	51	36
wheat bran	10	10
wheat middlings (standard)	20	20
dried whole egg (commercial)	--	15
dried buttermilk	10	10
meat and bone scraps	5	5
ground limestone	2	2
salt	1	1
cod liver oil	1	1
Totals	100	100
per cent crude protein	13.46	18.00
Ca:P=	1.4:.94	1.55:.9



♂♀ refers to number of chicks, male and female, living through the seven weeks of the experiment. 2 in Lot 2 had severe slipped tendons.

Chart I. Rations, growth curves, and mortalities in Experiment I.

supplements were used, and such a ration produced the pellagra-like syndrome in a very severe form. In this experiment twice as much dried buttermilk and five per cent of meat scraps were used. At four weeks of age 11 chicks showed only very slight indications of the syndrome, as evidenced by trifling scabbiness at the beak junctions and some roughening of the bottoms of the feet. In some cases only one toe on each foot would be affected and in no case were feet cracked or bleeding. At six weeks only four chicks showed noticeable though very slight indications of trouble.

It would seem from this one trial that this method of increasing the milk to ten per cent and adding five per cent of meat scraps almost completely prevented the appearance of the syndrome, even though 15 per cent of dried whole egg was used. This seems to indicate a deficiency rather than a toxicity in the eggs.

Growth in this trial was of great interest. At seven weeks the males in Lot 1 averaged 355 $\pm$ 13 grams and in Lot 2, 575 $\pm$ 20. The mean difference was 220 grams while the standard deviation of this was 36. There was therefore a highly significant difference between the males of the two lots. At the same time the females in Lot 1 averaged 315 $\pm$ 13 grams and those in Lot 2 618 $\pm$ 13. The mean difference was 303 and the SMD 27 grams. This is also a highly significant difference. This demonstrated the efficiency of the proteins or some other factor in the dried egg. As it was thought that in two lots of chicks showing such a mean difference in weight at seven weeks, differences in hemoglobin might be found, this was determined on all of the chicks which finished the experiment. Hemoglobin was determined in chicks of Lot 1 when these were 48 days of age, and in Lot 2

when they were 49 days old. It was not thought that the one day difference in time of determination would affect results although this has not been studied in chicks. Determinations were made with the "Improved Newcomer Model" hemoglobinometer using the Newcomer (26) method, but making corrections for excess turbidity of acid hematin solutions of birds' blood first described by Dukes and Schwarte (11).

The mean hemoglobin content of blood of chicks from Lot 1 was 7.8 ± 0.109 and in Lot 2, 8.48 ± 0.131 grams per 100 c.c. When the results were analyzed by variance (41) the difference was found to be significant but not highly significant.

Experiment 2

Further Studies with Dried Egg, With and Without Supplements

This trial consisting of five pens of 25 Single Comb White Leghorn chicks.Each was started December 10, 1934. Lot 3 was a check lot in which 20 per cent of dried buttermilk was the only animal protein supplement used. The crude protein in this ration was 13.78 per cent, rather low for optimum growth. Lots 4 and 5 were fed combinations of animal protein, the only animal protein variant being the commercial dried egg. This was to study the effect of two different levels of egg and its effect on the chicks where it was used in combination with equal amounts of dried buttermilk and meat scraps. Lot 6 was fed 12.5 per cent of dried egg as the sole source of animal protein, this percentage being used to give the same total crude protein in this ration as in Lot 3. Lot 7 was the same as Lot 6, ex-

cept that six per cent of dried yeast (not autoclaved) was added at the expense of an equal amount of ground yellow corn to study the value of added vitamin G. Reference to Chart II gives rations in more detail, growth curves, and numbers of chicks living through the experiment. Table 1 gives additional information concerning this experiment.

Table 1*

Protein and minerals in rations, and growth in Experiment 2

Lot Number	3	4	5	6	7
	Percent	Percent	Percent	Percent	Percent
Protein in ration	13.78	15.6	17.76	13.84	16.58
Ca in ration	2.07	1.8	1.77	2.08	1.85
P in ration	0.96	0.88	0.88	0.86	0.95
Ca:P	2.16:1	2.05:1	2.01:1	2.42:1	1.93:1
Mean weight of males at 6 weeks	277 (10)**	369 (11)	447 (14)	360 (14)	451 (14)
do females	261 (14)	380 (14)	418 (8)	365 (7)	453 (8)

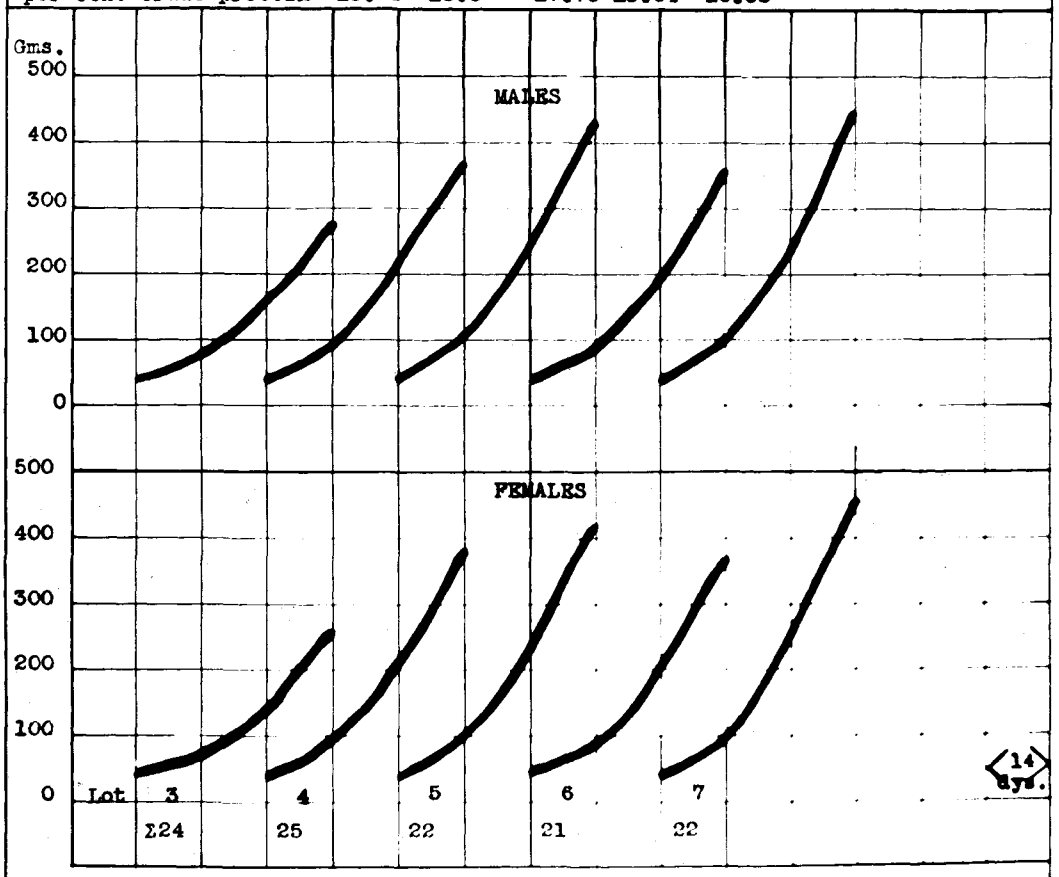
**Refers to numbers of chicks averaged.
*Analyses done under supervision of B. H. Thomas, Head, Animal Chemistry (and Nutrition Subsection.

In this experiment there were no evidences whatever of the pellagra syndrome in any of the lots fed commercial dried egg. Whether this was due to the Leghorn chicks used, to the brand of dried egg as it was different from that used in our previous work (44), or to some other factor, could not be determined from these experiments.

As the total crude protein was the same in Lots 3 and 6, growth could be compared on an equal protein basis only in these two lots. When the

25 chicks to a lot started 12/10/1934. S.C.W.Leghorns

	Lot 3	4	5	6	7
	%	%	%	%	%
ground yellow corn	44	46	41	51	45
wheat bran	10	10	10	10	10
wheat middlings	20	20	20	20	20
dried buttermilk	20	10	10	--	--
dried egg (commercial)	--	5	10	12.5	12.5
meat and bone scrap	--	5	5	--	--
dried yeast	--	--	--	--	6
steamed bonemeal	2	--	--	2	2
ground limestone	2	2	2	2.5	2.5
salt	1	1	1	1	1
cod liver oil	1	1	1	1	1
totals	100	100	100	100	100
per cent crude protein	13.78	15.6	17.76	15.84	16.58



Σ Refers to number of chicks, male and female, living through the six weeks of the experiment.

Chart II. Rations, growth curves, and mortalities in Experiment 2.

weights at six weeks of males, and of females, in these two lots were compared by analysis of variance a highly significant difference was found. Based on this one experiment the protein of dried egg is superior to that of dried buttermilk.

In Lot 7 the growth was better than in Lot 6, as there was a highly significant difference between the two lots. However, as this experiment does not show what factor in the yeast was responsible for this great difference, opportunity exists here for further study.

Individual hemoglobin determinations were made on five representative males and females in each lot when the chicks were 44 days old. The mean hemoglobin values of the ten chicks in each lot from Lot 3 to Lot 7 were respectively as follows: 4.9, 4.9, 5.1, 5.3, and 5.4 grams per 100 c.c. By analysis of variance there was no significant difference among the lots. While the results of hemoglobin determinations in this experiment were considerably lower than the 7.8 and 8.9 grams found in Experiment 1, the results are not comparable as the chicks were of slightly different ages and also not of the same variety.

Owing to the paucity of work on the serum albumin and globulin of chicks' blood, and also its relationship to the pellagra-like syndrome, it was decided to make a study of this. Anding and Sinnani (1) have shown among other things that in pellagra there is a tendency to a slight decrease in the serum or plasma proteins. The work reported here was done on chick blood serum following the method of Greenberg (18) with the slight modification that it was necessary to use only 0.4 mgms. of standard tyrosine solution instead of 0.8. This was because of the lower serum albumin

and globulin in the first chicks used for this work as compared to the same proteins of dog blood.

Blood analyses in this work were made with blood from four chicks, one male and three females. The mean percentage albumin was 1.65 and globulin 0.6. As these results were so decidedly different from those reported by other workers for dog blood it was thought at first our procedure was faulty. Consequently one sample of dog blood serum was divided and seven determinations were made for serum albumin and globulin using the same method except that the standard tyrosine solution contained the usual 0.8 mgms. for both analyses. The mean of these seven determinations was 3.97 per cent albumin and 1.53 per cent globulin; these results agreed with those of other workers closely enough to show that our analytical procedure was not at fault.

The results of analysis of chicks' blood serum in Experiment 2 are given in Table 2.

Table 2

Percentages of serum albumin and globulin in blood from Single Comb White Leghorn chicks in Experiment 2

Lot No.	1 mean percent albumin	2 mean percent globulin	Number of Chicks Averaged for 1 and 2	
3	2.0	0.7	6	6
4	2.1	0.7	6	6
5	2.1	0.6	6	6
6	2.3	0.5	6	6
7	2.2	0.8	5	5

Due to the labor involved it was not possible in this first study to analyze all the samples the first day. Lots 5 and 7 were analyzed when the

chicks were 45 days old; Lots 4 and 6 at 52 days, and Lot 3 at 55 days. The lots were kept on their experimental rations until after blood samples were taken by the heart probe method.

When the results were interpreted by analysis of variance there were no significant differences in either serum albumin or globulin among the lots.

Experiment 3

In neither trials 1 nor 2 could the severe pellagra-like syndrome found in our previous experiments (44) be duplicated. While it was true the rations used in this present study were not exactly the same as those of our former work, it was thought the source of dried egg might be responsible. Accordingly this subject was investigated.

In most of our previous work (44) the eggs used for drying were strictly fresh and dried in incubators in the Poultry Department. For later experiments there, a commercial dried egg was used. According to the manufacturers this was dried whole egg. It may have been made from second grade eggs where the yolk and white could not be separated. Very little of this product is manufactured. In trials 1 and 2 it was found that the product used was likely a Chinese reconstituted whole egg. Reconstituted egg is made up, according to the manufacturers, of 75 per cent dried egg yolk and 25 per cent dried egg albumen. On the basis of egg analyses the mixture should be made up to consist of 65.6 per cent dried yolk and the rest albumen. As the method of making up the commercial dried egg used in our former work, as well as the different commercial dried egg used in Experiments

1 and 2 was not investigated until after the experiments had been completed, the jobbers of both samples could not positively state as to the method of manufacture.

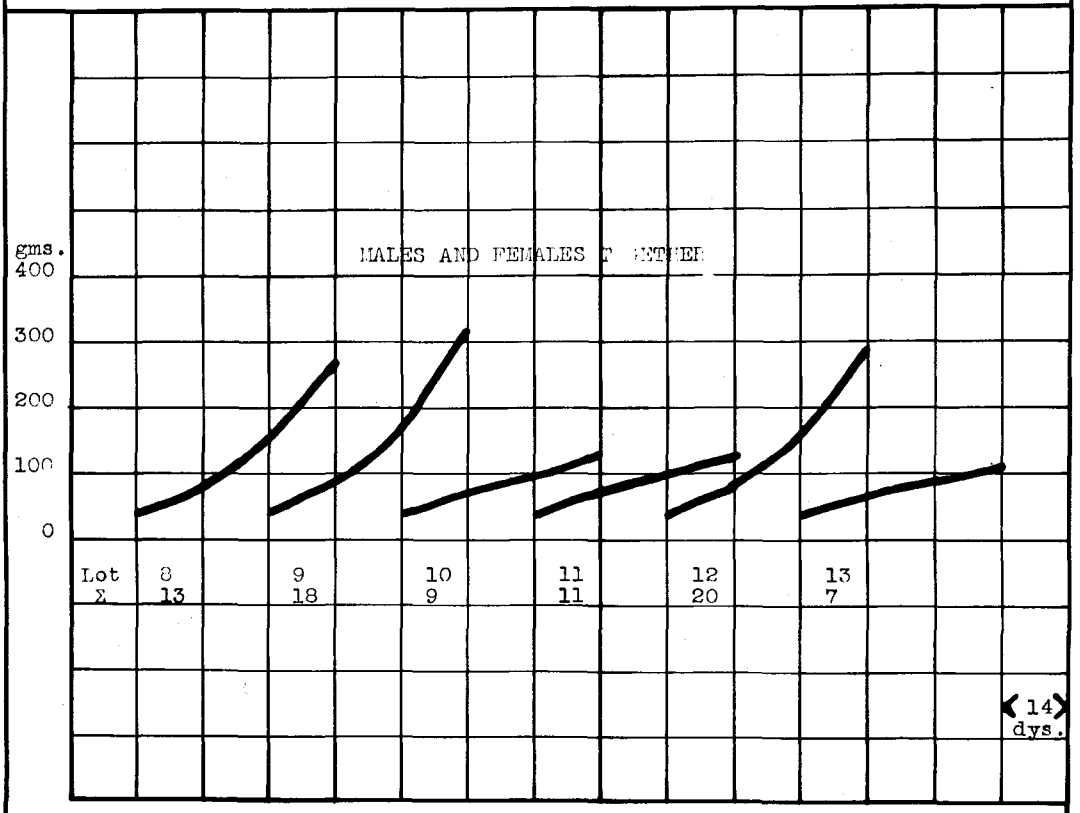
Experiment 3, consisting of six lots of 25 White Rock chicks each, was designed to study these two samples of commercial dried egg, as well as dried egg albumen. Through the courtesy of the Director of the Agricultural Experiment Station in South Dakota, the commercial dried egg left over from our work there was brought to the Iowa Agricultural Experiment Station. It was dried whole egg and called Sample 1. Fifteen per cent of this and five per cent of dried buttermilk were the only animal protein supplements used in Lot 8. This was exactly the same formula as had given such severe signs of the pellagra syndrome in our previous work (44). Lot 9 was the same except that reconstituted egg, called Sample 2, was used. Lot 12 was the same as 9 except that the five per cent of dried buttermilk was omitted and the same amount of ground yellow corn added.

Lots 10, 11, and 13 were fed 8.6 per cent dried egg albumen to supply the same level of crude protein as was obtained from the 15 per cent of dried egg. Lot 10 was fed albumen plus five per cent dried buttermilk; Lot 11 was the same except that six per cent dried yeast (not autoclaved) was added, replacing an equal amount of corn; in Lot 13, 8.6 per cent albumen was the sole source of animal protein. Reference to Chart III gives rations in greater detail, growth curves, and mortality.

Table 3 gives other results of this experiment. Because sex determinations of chicks at six weeks of age in Lots 8, 9, and 12 were unavoidably inaccurate, and undiscernible in Lots 10, 11, and 13 without destroying the

Chart III
25 chicks to a lot started 1/26/35. White Rock chicks.

Lot	8	9	10	11	12	13
	%	%	%	%	%	%
ground yellow corn	27	27	33.4	27.4	32	38.4
wheat bran	15	15	15	15	15	15
wheat middlings	15	15	15	15	15	15
pulverised whole oats	20	20	20	20	20	20
dried whole egg sample 1	15	--	--	--	--	--
dried whole egg sample 2	--	15	--	--	15	--
dried egg albumen	--	--	8.6	8.6	--	8.6
dried buttermilk	5	5	5	5	--	--
ground limestone	1	1	1	1	1	1
salt	1	1	1	1	1	1
cod liver oil	1	1	1	1	1	1
[illegible]	--	--	--	6	--	--
	100	100	100	100	100	100



Σ Refers to number of chicks, male and female together, living through the six weeks of the experiment.

Chart III. Rations, growth curves, and mortalities, in Experiment 3.

chicks, the sexes were grouped together in computing the growth means.

Table 3

Partial data of Experiment 3, chicks 6 weeks of age

Lot Number	8	9	10	11	12	13
Mean weight at six weeks	269	322	128	121	268	105
Per cent mortality	48	28	64	56	20	72
No pellagra syndrome, number of chicks	4	7	0	0	12	0
Slightly rough feet, number of chicks	2	6	0	0	4	0
Slight pellagra syndrome, number of chicks	5	5	1	6	2	0
Severe pellagra syndrome, number of chicks	2	0	8	5	2	7

A glance at Chart III shows that the crude protein from 8.6 per cent dried egg albumen, even when supplemented with dried buttermilk, or with milk and yeast, produced markedly inferior growth to that secured from the same amount of protein from dried egg. Egg yolk not only contains better protein or some growth factor which markedly accelerates growth, but also a factor tending to prevent the pellagra syndrome. When analysed by variance there were no significant differences in the growth of Lots 10, 11, and 13.

Weights of Lots 8, 9, and 12 when analyzed by variance showed a significant difference. There was no significant difference between Lots 8 and 12, or between Lots 9 and 12. The significant difference was found to be between Lots 8 and 9. The better growth of the latter lot, which was fed

reconstituted dried egg and dried buttermilk, probably was due to the larger percentage of dried egg yolk in the reconstituted egg as compared to the whole dried egg or Sample 1.

The pellagra syndrome in Lots 8 and 9 was less severe than in our previous experiments (44) even though the ration fed Lot 8 was the same as used in that work.

Serum albumin and globulin were determined in five chicks in Lot 11 and four in Lot 13 when they were 44 days of age; and in nine chicks in Lot 9 when they were 47 days old. Due to the labor involved it was impossible to run all the chemical determinations of the three lots on the same day, but chicks were always kept on their proper experimental rations until after the blood samples were taken. Table 4 gives the results of these determinations. The chicks in Lots 11 and 13 were so small that it was necessary to pool blood in some few cases to get the required quantity. In Lot 11 two chicks were used for one pooled sample and a duplicate of this determined. This meant that while five chicks were used two of the samples were pooled and duplicates. In Lot 13 three chicks were used for one pooled sample, this procedure was duplicated for a second. From only one chick was sufficient blood obtained to yield another blood sample of sufficient size. While too few chicks were used in Lots 11 and 13 for dependable results as measured by blood determinations, such results offer suggestions for further study. When Lots 9, 11, and 13 were analyzed statistically there were no significant differences in the albumins. In the Globulins differences were all significant except between Lots 9 and 13.

Table 4

Serum albumin and globulin in three lots of Experiment 3

Lot Number	9	11	13
Number of chicks	9	5	4
Number of samples	9	5	3
Mean percent albumin	3.5	3.0	3.0
Mean percent globulin	0.7	0.5	0.6

The percentages of globulin found in this experiment were close to those of Experiment 2, but the albumin was considerably higher. As up to this time serum albumin and globulin had not been determined on any "normal" chicks, except possibly those of Lot 3, no comparisons could be made except with blood from chicks in the second trial.

Experiment 4

This experiment was primarily to secure "normal" chicks for serum albumin and globulin determinations. However, as 110 Single Comb White Leghorn chicks were available, it was decided to divide them into two lots to study the value of adding two per cent of sea sand to one of the rations, as both of these were relatively high in fibre. Reference to Chart IV shows rations, growth curves, and mortalities for the six weeks of the experiment.

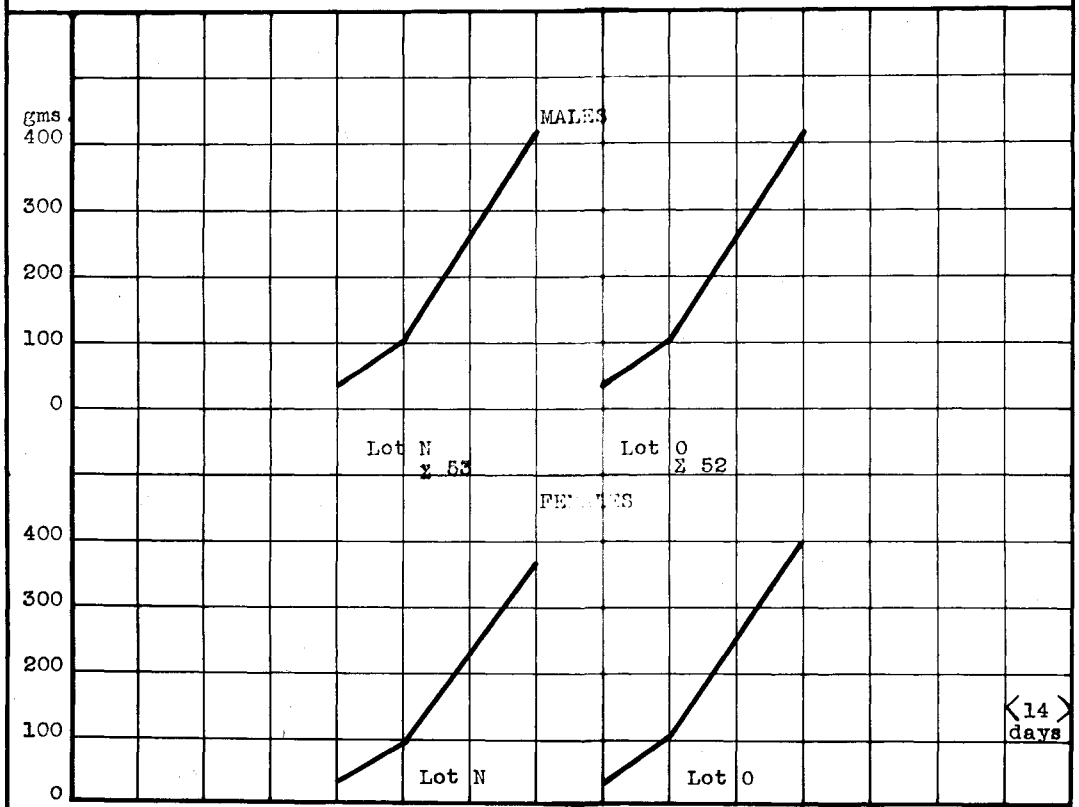
Lot 14 was made up of 56 chicks at the start and Lot 15, 54 chicks. As Upp (46) has shown that day-old chick weight is an unreliable index of

56 chicks in Lot N: 54 chicks in Lot O. started 2/14/35. S.C.W.L. chicks.

Chart IV

	Lot 14N %	15-O %
ground yellow corn	31	29
wheat bran	15	15
wheat middlings	15	15
ground whole oats	20	20
meat and bone scrap	12	12
dried buttermilk	5	5
salt	1	1
white sea sand	0	2
cod liver oil	1	1
Totals	100	100

As well as supplying "normal"chicks for serum albumin and globulin determinations, this trial is a study of the effect of sand for grit in rations containing large amounts of fibre (20 % ground oats, etc.) for battery brooded chicks.



Σ Refers to numbers of chicks, male and female together, living through the six weeks of the experiment.

Chart IV. Rations, growth curves, and mortalities, in Experiment 4.

weight at two, four, or twelve weeks, the chicks in this experiment were not weighed at the start, but sorted at random into two lots. The chicks were weighed at two weeks of age and wing-banded then. They were weighed only once more, at six weeks of age. At this time males in Lot 14 averaged 418 grams and those in Lot 15, 415 grams. There was of course, no significant difference between these two means. Females in Lot 14 averaged 362 grams and those in Lot 15, 400 grams. There was a statistically significant difference between the means of the females. Additional work would be necessary before conclusions could be drawn as to the value of adding sand to such a ration. This was not followed up as it was not primarily a part of this study.

To get so-called normal serum albumin and globulin values in Single Comb White Leghorn chicks at eight weeks of age determinations for these proteins were made on ten chicks from Lot 14. These results were needed to compare with blood proteins from chicks affected with the pellagra syndrome. By using the ratio of the standard deviation to the significant mean difference it had been found that ten chicks supplied an ample number of observations. While growth records of Lot 14 were not kept after the chicks were six weeks old, the chicks were fed their usual ration until after blood samples had been taken. Table 5 shows results of this work.

These results are decidedly different from those obtained in Experiment 2, where the means of the albumin in the five lots tested ranged from 2 to 2.3 per cent. While the results are not strictly comparable due to the slightly different ages of the chicks from which blood samples were

Table 5

Serum albumin and globulin in normal eight week Leghorn chicks from Lot 14

Chick Number	Percent Albumin	Percent Globulin
25	3.28	0.33
6	3.41	0.34
4	3.75	0.34
14	3.64	0.54
22	4.58	0.49
13	4.43	0.39
5	5.90	0.99
16	4.28	0.34
23	4.28	0.24
30	4.21	0.45
Mean + P.E.	4.18 + 0.5	0.44 + 0.14

obtained they are suggestive that the ration does have a marked effect in altering the blood serum albumin.

To compare the serum albumin and globulin of blood of eight week Leghorn chicks with that of Leghorn hens, the globulin was determined in the serum of nine hens, and the albumin in eight of these. The hens used were all one or more years old and probably not laying. The mean percentage albumin was 2.2 and the globulin 1.7. From these results it is obvious that great changes occur in these two blood proteins due to age as well as to feed and this subject might be investigated much further.

Experiment 5

This experiment was a brief trial based on the results of Sabry (36, 37) as applied to chicks. He has reported pellagra in human beings as a toxemia due to his theorized dioxyphenylalanine. Ten c.c. daily of a ten

per cent solution of sodium thiosulphate when given to pellagrins intravenously for from 20 to 60 days showed remarkable results in treating this disease.

Sixteen Single Comb White Leghorn chicks were started February 23, and were fed the ration given to Lot 13 which had produced such poor growth and severe pellagra. In addition to this ration chicks were fed sodium thiosulphate, $Na_2S_2O_3 5H_2O$, throughout the experiment in their drinking water. The thiosulphate was fed on a body weight basis, using one-tenth as much as had been injected in man, in an attempt to prevent pellagra or the pellagra syndrome in the chicks. One c.c. of the ten per cent solution was fed daily per chick. While measurements were accurate, intake varied to some extent due to variation in the amount of the drink used.

Reference to Chart V gives the rations used and shows the growth results of the six chicks which were all that lived to six weeks, as compared to normal Leghorn growth. All of the surviving six chicks showed the pellagra syndrome in severe form so the method of using sodium thiosulphate as practiced in this brief trial had no effect in preventing this trouble. None of the heavy mortality occurred before the seventeenth day so that it was probably all due to the ration.

Experiment 6

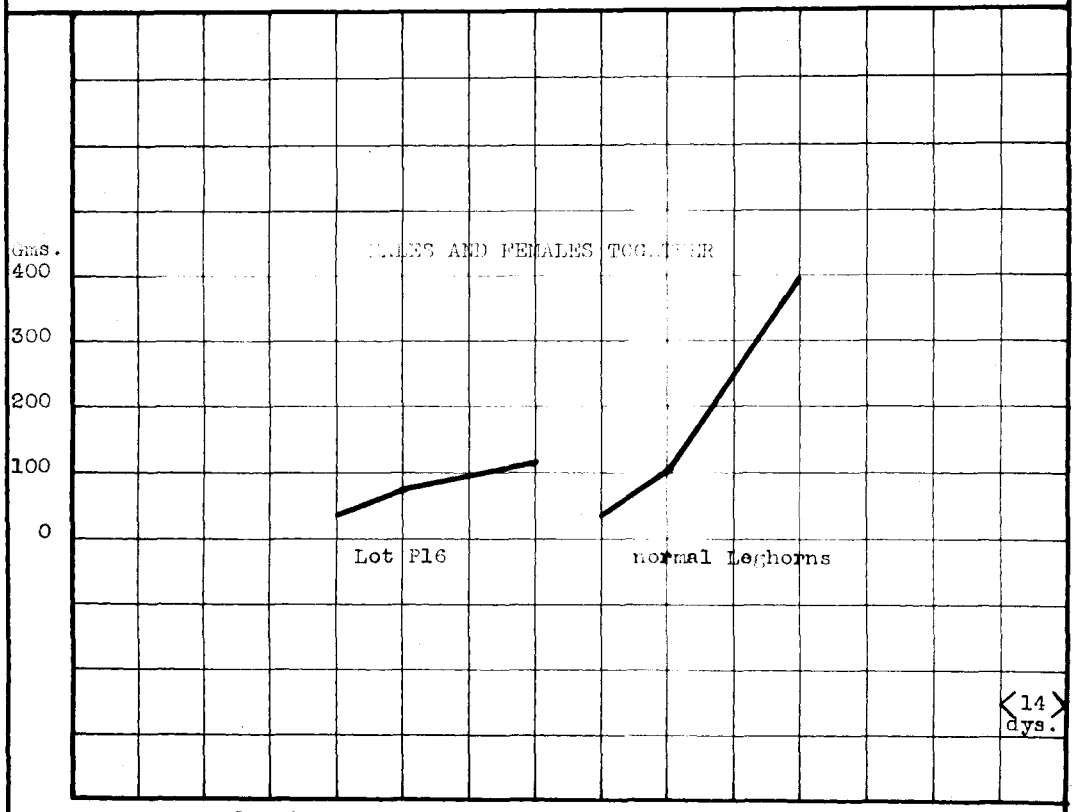
Studies with Dried, Dried Heated, and Liquid Egg Albumen

This experiment in which 21 White Rock chicks were used in each of four lots was a further study of the feeding value of commercial dried

Chart V.
13 chicks to this lot, started 2/23/35. S.C.W.L. chicks.

	Lot P 16 %
ground yellow corn	38.4
wheat bran	15
wheat middlings	15
pulverized oats	20
dried egg albumen	8.6
ground limestone	1
salt	1
cod liver oil	1
Total	100

1 c.c. of 10% solution of sodium thiosulphate daily per chick was fed in the drinking water throughout the experiment.



Only six of the 13 chicks started in this lot lived to six weeks. All of these had severe pellagra.

Chart V. Ration and mortality of Experiment 5. Growth in this Lot 16 as compared to growth of "average" Leghorn chicks.

egg albumen as well as of liquid egg albumen.

Lot 17 was a repetition of Lot 13 where 8.6 per cent dried egg albumen was the sole source of animal protein. As the total calcium and phosphorus was low in these rations, for Lot 18 two per cent of steamed bonemeal was added, at the expense of yellow corn, to see if this addition would be of any value.

Lot 19 was fed liquid egg albumen from strictly fresh eggs. In separating the yolks from the whites extreme care was taken that the albumen was never contaminated by even the slightest amount of yolk. Sixty-two per cent of liquid albumen supplied the same protein as that obtained from 8.6 per cent of the dried product. The liquid albumen was mixed fresh twice daily with 38 per cent of the basal ration. The latter was the same as that fed to Lot 18 except that the dried egg albumen had been omitted.

Lot 20 was fed the same ration as Lot 18 except that the commercial dried egg albumen used had been heated for 16 hours at 106 degrees C.

Reference to Chart VI shows rations used, computed calcium and phosphorus in the rations, growth curves, and mortalities for the experiment.

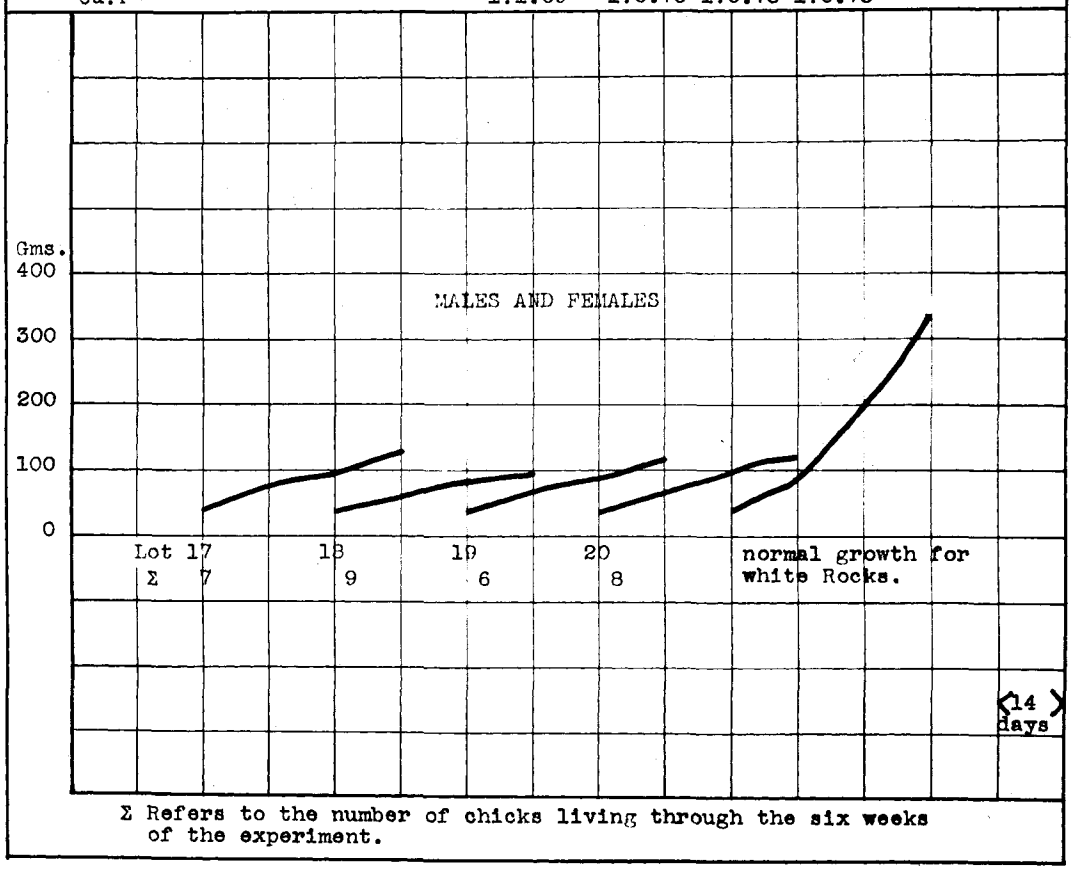
At six weeks of age the mean weights of the very limited number of chicks which survived the experiment did not differ significantly. Merely a glance at Chart VI shows the markedly inferior growth of all lots. Growth was so poor it was impossible to distinguish the sex of the chicks at six weeks without making post mortem examinations.

In Lot 17 the seven surviving chicks all had severe to very severe pellagra-like symptoms. Often the chicks' feet showed such severe emaciation that it appeared as though nothing but skin and bones were present. This

21 chicks to a lot started 3/29/1935 White Rocks

Chart VI

	Lot 17 %	18 %	19 %	20 %
ground yellow corn	38.4	36.4	36.4	36.4
wheat bran	15	15	15	15
wheat middlings	15	15	15	15
pulverised whole oats	20	20	20	20
dried egg albumen	8.6	8.6	--	--
dried egg albumen heated	--	--	--	8.6
liquid egg albumen	--	--	62	--
ground limestone	1	1	1	1
steamed bonemeal	--	2	2	2
salt	1	1	1	1
cod liver oil	1	1	1	1
Totals	100	100	100	100
per cent total calcium	0.44	1.04	1.04	1.04
per cent total phosphorus	0.48	0.76	0.76	0.76
Ca:P=	1:1.09	1:0.73	1:0.73	1:0.73



Σ Refers to the number of chicks living through the six weeks of the experiment.

Chart VI. Rations used, computed calcium and phosphorus in the rations, growth curves, and mortalities in Experiment 6.

manifestation was often apparent without any evidence of cracking of the bottoms of the feet. In Lot 18, the nine surviving chicks did not show quite as severe symptoms. This may have been due to the change in the mineral content of the ration, as compared to Lot 17, but additional work would be necessary to check this as so few chicks survived.

In Lot 19, of the six surviving chicks, two showed no signs of the syndrome, the other four showed slight to more severe indications of the trouble. In Lot 20, where eight chicks survived, the syndrome was very severe indicating that heating the commercial dried egg albumen exerted no influence in improving its nutritional value.

It is believed evident from this and previous experiments that raw egg albumen from fresh eggs, as well as commercial dried albumen, either heated or as sold, is decidedly deficient in protein or in some unknown growth factor.

To determine if chicks from the above experiment were permanently injured 25 of these were fed a complete ration using 12 per cent meat scraps and six per cent dried buttermilk as sources of animal protein. All but two of these chicks showed noticeable to very severe indications of the syndrome. The average weight of these 25 chicks at six weeks of age when they were started on the complete ration was only 112 grams. At eight weeks of age they averaged 255 grams, six showed no signs of the syndrome, seven more were almost healed and the rest rapidly healing. At ten weeks there were no signs of the pellagra syndrome whatever and the 25 chicks had an average weight of 531 grams.

It seems evident judging from this one trial that chicks affected with the pellagra syndrome can recover when they are fed a complete ration and that they are not permanently injured as evidenced by growth and external appearances.

Experiment 7

Trials with Coagulated and with Coagulated Dried Albumen

As several investigators previously referred to have shown that cooking egg albumen reduced its toxicity as measured by rat feeding experiments it was decided to try to duplicate these results using chicks as experimental animals.

Fifteen Single Comb White Leghorn chicks were used in each of two lots for this experiment which was started April 3, 1935. Lot 21 was fed 62 per cent of coagulated egg albumen, made by boiling either fresh eggs, or infertile eggs from incubators. The coagulated albumen was carefully separated from the yolk and mixed with 38 per cent of the basal ration. This ration was the same as used for Lot 19. Sixty-two parts of such albumen were designed to supply the same protein as 8.6 parts of dried albumen. The ration fed to Lot 21 was mixed twice daily for the first four weeks of the experiment, and three times daily for the remaining two weeks.

The coagulated dried albumen fed as the only animal protein to Lot 22 was prepared as follows: Fresh or infertile eggs were boiled hard and the albumen very carefully separated from the yolk. The coagulated albumen, after it was minced into rather small pieces, was then thoroughly dried at 100 degrees C. This resulted in a dark brown very hard product. Only with

some difficulty was this finely powdered for use. Eight and six-tenths parts of this coagulated, dried, powdered albumen were made up to 100 with basal ration 19. This ration was kept in front of the chicks of Lot 22 at all times.

In Lot 21, 62 parts of coagulated albumen, which contained 53.4 parts of water, were made up to 100 with the basal ration. This resulted in a ration containing 8.6 per cent of coagulated dried albumen, exclusive of water. Both Lots 21 and 22 therefore contained 8.6 per cent of coagulated egg albumen, on a dry weight basis.

Reference to Chart VII shows rations, growth results, and mortalities for this trial. At six weeks males in Lot 21 averaged 194 grams while those in Lot 22 had a mean of 339 grams. There was a highly significant difference between the two lots of males. Similarly where the average weights of females at six weeks in Lots 21 and 22 were 197 and 311 grams respectively, there was a highly significant difference between these means. Based on the protein content of the two rations the superior growth of chicks in Lot 22 cannot be explained from this experiment although it is possible that Lot 21 did not get sufficient feed, particularly for the first four weeks. While an effort was made to have feed in front of these chicks at all times this was not always possible because of the unusually great feed wastage. In addition the high moisture content of this ration possibly precluded optimum feed consumption.

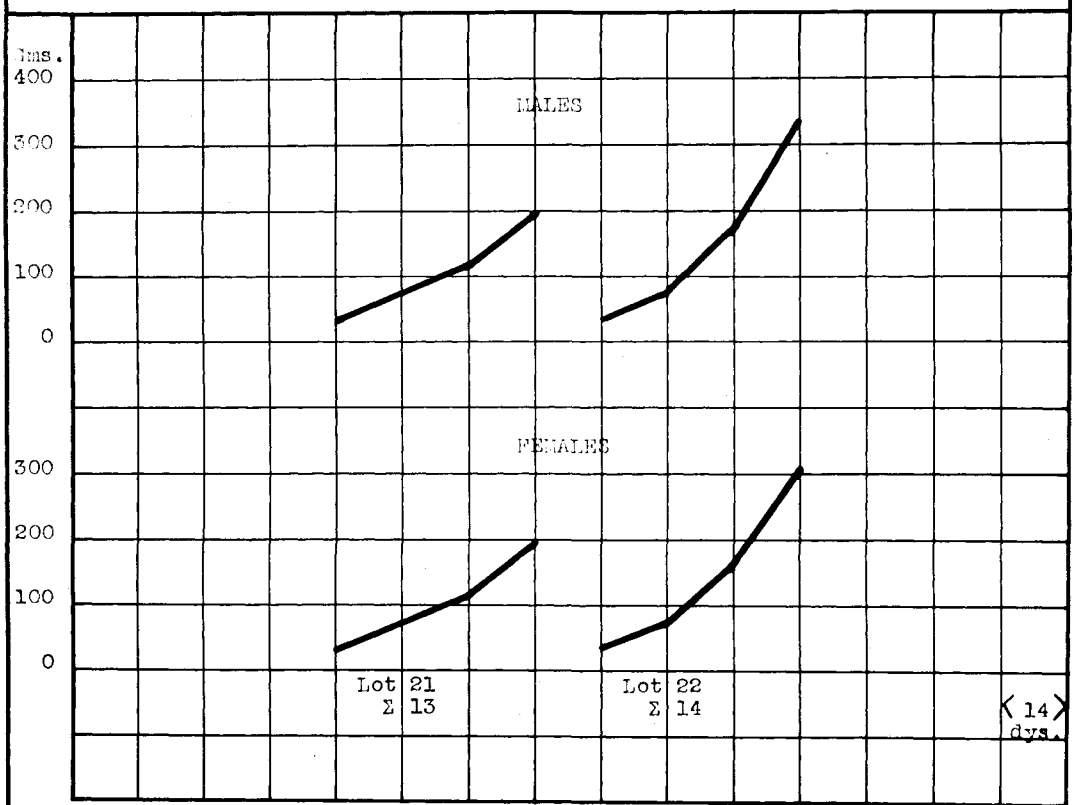
The chief interest in this trial, however, was the occurrence of the pellagra syndrome. None of the chicks in either of the lots showed any evidence of this trouble whatever. This substantiated the work of others with

Chart VII
15 chicks to a lot, started 4/3/35.

S.C.W.L. chicks

	Lot 21	22
	%	%
ground yellow corn	36.4	36.4
wheat bran	15	15
wheat middlings	15	15
pulverized oats	20	20
coagulated egg albumen	62	8.6 dried at 100 deg. C.
ground limestone	1	1
steamed bonemeal	2	2
salt	1	1
cod liver oil	1	1
	100	100

62 % coagulated egg albumen contained the same protein as 8.6 % coagulated dried egg albumen.



Σ Refers to the number of chicks, male and female together, living through the six weeks of the experiment.

Chart VII. Rations, growth curves, and mortalities, for Experiment 7.

rats, that coagulating the egg albumen before feeding destroyed some toxic property which undoubtedly exists in raw egg albumen, either fresh or dried.

DISCUSSION

This experimental work opens up a new field in that it shows the need for a much more detailed study of the nutritional value of eggs. Previous work has been confined for the most part to a study of the albumen. While a study of the albumen is important because of the increasing use of this product, as well as dried yolk, in bakeries, a study of the whole egg should be more extensively pursued.

As only a very small part of the eggs produced in this country are used for drying it does not seem reasonable to emphasize too much the deficiency of only one part, namely the albumen. This study definitely shows that dried egg is nutritionally a much more complete product than dried albumen and therefore the suggestion is made that dried eggs should be used as an entity and not separately in the form of dried yolk and dried albumen.

However, this involves a problem concerned in the manufacture of dried eggs. The dried egg business in this country is relatively new, consequently changes have been made and are to be expected. Egg yolk is usually dried by the spray process at a temperature of 160 degrees Fahrenheit. Egg albumen is dried in shallow pans at 130 degrees, as this product has not yet been dried successfully by the spray process. When an order for dried egg is obtained, because little of this is used as compared to yolk and albumen separately, it is reconstituted by mixing 75 per cent of yolk and 25 per cent albumen. As mentioned before these proportions are not correct to represent whole dried egg. It may be that later eggs can be dried successfully without separation of the two constituents and the resulting product

more generally used.

Some of the results obtained in this work may be masked due to the use of different varieties of chicks in some of the experiments. Undoubtedly it would have been better to use the same variety for all trials but at certain seasons of the year, because of the scarcity of hatching eggs, this is often difficult.

In these experiments two different lots of dried egg were used and it is probable that they were prepared by different methods. However, it was only because of unforseen results in some of the work herein reported that the two different samples were used. This procedure, which might be questioned, in this case resulted in findings of value that might not have been otherwise uncovered.

The value of hemoglobin determinations in a study of this kind possibly might be questioned due to the many factors which are believed to affect the results. Only very meager data are available concerning this blood constituent in chicks and as yet some of the results may be questioned due to the methods which have been used. That there is opportunity for much further work here is demonstrated by the markedly different results obtained in Experiments 1 and 2 of this study. However, it was not felt that further work on this subject was justified in this thesis as no significant differences had been found among the different lots of Experiment 2.

No studies of the values for serum albumin and globulin in chicks' blood were found. While Dyer and Roe (12) have made a detailed study of the blood of hens, their work on albumin and globulin was done on blood plasma. It was not possible therefore, to compare our findings with theirs.

Results secured in the work herein reported indicate that the ration has a marked effect on these two blood proteins. It is suggested that for further work plenty of assistance should be available so that the necessary blood samples from all lots of any experiment can be obtained and the determinations made the same day. One of the difficulties which will need to be overcome is that of obtaining sufficient blood from some chicks which exhibit the syndrome in a severe form and which are much below normal in size.

CONCLUSIONS

1. Fifteen per cent of dried egg added to a ration containing ten per cent of dried milk and five per cent of meat scraps markedly increased growth in chicks and produced only very slight indications of a pellagra syndrome.

2. While no significant differences were found among the means of the hemoglobin in Experiment 2, these results were quite different from those of the first trial.

3. Based on one experiment a ration containing dried egg as the only source of animal protein gave highly significantly better growth than one containing dried buttermilk fed at the same protein level.

4. Six per cent of dried yeast increased growth highly significantly when used to supplement a ration containing 12.5 per cent dried egg as the principal source of animal protein.

5. There is a possibility that Leghorn chicks may not exhibit the pellagra syndrome as do other varieties of chicks.

6. Different samples of dried egg gave significantly different results.

7. The crude protein from 8.6 per cent dried egg albumen, even when supplemented with dried buttermilk, or with milk and yeast, produced markedly inferior results, measured by growth and mortality, in comparison with those produced by the same amount of protein from dried egg.

8. Egg yolk not only contains some factor which markedly accelerates growth, but also a factor tending to prevent the pellagra syndrome.

9. Serum albumin and globulin in chicks and hens varied with the age of

the birds and the rations used. The relation between the pellagra syndrome and these blood constituents was not conclusive.

10. The mean percentage serum albumin of normal eight week Leghorn chicks was 4.18 - 0.5, the serum globulin 0.44 - 0.14.

11. Sodium thiosulphate as used in this experiment was of no value in preventing the pellagra syndrome.

12. Raw egg albumen from fresh eggs was just as deficient or toxic as commercial dried egg albumen whether the latter was used as sold or after heat treatment.

13. Egg albumen coagulated by boiling, either without or with additional heat treatment, when fed to chicks possessed no toxic properties.

14. Chicks showing the pellagra syndrome and stunted in growth showed complete recovery in from two to four weeks when they were fed a complete ration.

SUMMARY

A review of the literature on the feeding values of dried egg* and of egg albumen is given.

Experiments are reported with 28 lots of chicks fed commercial dried egg, and egg albumen or egg white, where these products were used both as the only source of animal protein and with various supplements. The effects of egg feeding were measured by growth, the appearance of the pellagra syndrome, and in some of the experiments by hemoglobin and blood serum albumin and globulin determinations.

When a ration containing 15 per cent of commercial dried egg is supplemented with ten per cent of dried buttermilk and five per cent of meat scraps the incidence of the pellagra syndrome is very slight and growth is excellent.

Dried egg fed to growing chicks as the only source of animal protein resulted in highly significantly better growth than a ration containing the same amount of protein from dried buttermilk. It is probable that this increased growth was not due altogether to the protein; however, the addition of yeast to the dried egg protein ration still further increased growth to a highly significant extent.

Commercial dried egg is not all prepared by the same method and different samples give different results in feeding experiments.

Dried egg albumen even when supplemented with yeast, milk, or combinations of these, in rations believed otherwise complete failed to produce

*Dried egg is used to mean whole eggs, without shell and shell membranes, dried.

good growth and chicks exhibited a very severe pellagra syndrome.

Poor growth and the pellagra syndrome in an equally severe form resulted from the feeding of either raw egg albumen or commercial dried albumen in rations otherwise complete. Heating the dried albumen at a high temperature had no effect on its nutritional value. However, when dried egg was used at the same protein level, growth was incomparably better and the pellagra syndrome almost absent. Vitamin A of the egg yolk probably was the cause of the increased growth, but there was undoubtedly a factor apart from vitamin A which tended to prevent this nutritional disorder.

The work of other investigators is substantiated in that coagulating egg albumen in some way markedly improves its nutritional value.

Hemoglobin studies are reported for chicks from seven of the lots. The range of the means of the lots was from 4.9 to 8.9 grams per 100 cc.

The first report on the serum albumin and globulin content of chick blood is given. In normal chicks the percentage serum albumin is similar to that of dogs but the globulin is considerably lower. These blood constituents vary to quite an extent with the ration and the age of the chicks but the relation between these proteins and the pellagra syndrome is not clear cut.

Chicks much stunted in growth and showing the pellagra syndrome when fed a complete ration for a short time grow at a very rapid rate and all external signs of the nutritional disease disappear.

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