THE QUEENSLAND JOURNAL OF AGRICULTURAL SCIENCE

DECEMBER, 1957.

Vol. 14. - - No. 4.

VITAMIN A IN POULTRY.
Effects of Vitamin Deficiency.

By R. J. W. GARTNER, B.Sc. (Chemist, Biochemical Branch, Division of Animal Industry), and H. W. BURTON, H.D.A. (Assistant Adviser, Poultry Branch, Division of Animal Industry).

SUMMARY.

The results of two trials to determine the effects of vitamin A deficiency on fowls and on their progeny are presented.

In the 1955 pilot trial, hens receiving a ration containing no preformed vitamin A and no measurable quantity of carotene were not depleted of vitamin A in over seven months. This was due to their high initial reserves.

In the 1956 trial, pullets were used and artificial insemination and trap-nesting introduced.

The egg production of the vitamin A deficient groups was 23 per cent. lower than that of the vitamin A adequate controls when the deficiency was first established by liver analyses, 57 per cent. lower when lesions of the deficiency were first apparent by field examination, and finally production ceased. Egg production of pullets that were reversed after field evidence of vitamin A deficiency was established returned to normal two weeks after supplementation with vitamin A.

The hatchability of eggs of the vitamin A deficient groups was 40–50 per cent. when the deficiency was first established by liver analyses, 10–20 per cent. when field evidence of vitamin A deficiency was established, and finally nil. The average hatchability of the vitamin A adequate controls was 80 per cent. On reversal, the hatchability returned to normal one week after supplementation with vitamin A.

Vitamin A deficiency affects hatchability of eggs before it affects egg production.

On the vitamin A deficient ration, a decrease in egg production and hatchability was also associated with marginal liver vitamin A reserves.

When vitamin A deficiency was first confirmed, the eggs laid contained less than 8 μg. of vitamin A per yolk compared with 42 μg. from the controls.

There was no evidence of reduced hatchability due to vitamin A deficiency when total yolk-sac and liver reserves of vitamin A in day-old chickens were in excess of 8 μg.

Nodules on the oesophagus, although present on field examination, were not apparent in four pullets at autopsy; this was due to post-mortem decomposition. The disappearance of nodules after vitamin A supplementation may take up to eight weeks.

Pullets on a vitamin A deficient ration survived for a maximum period of 39½ weeks after the deficiency had been established by chemical analyses. Of the 24 deaths in the deficient groups, 10 were due to uncomplicated vitamin A deficiency, 5 to visceral gout, 4 to a rupture of the liver, 3 to egg peritonitis, 1 to myocardial degeneration and 1 pullet was too decomposed to show any lesions. No deaths occurred prior to the onset of vitamin A deficiency.

There was no difference in food consumption between groups, and pullets that succumbed to vitamin A deficiency were not emaciated.

Chickens were reared up to four weeks of age in four consecutive hatches from eggs of vitamin A sufficient and deficient pullets.

Rearing mortality was 71 per cent. in progeny from deficient pullets when reared on a deficient ration, 41 per cent. in progeny from deficient pullets when reared on a sufficient ration, 17 per cent. in progeny from deficient pullets when reared on a heavily supplemented ration (5x sufficient), 25 per cent. in progeny from sufficient pullets when reared on a deficient ration, and 4 per cent. in progeny from sufficient pullets when reared on a sufficient ration. Most deaths occurred towards the end of the first week of life.

I. INTRODUCTION.

The available literature contains numerous references to the effects of vitamin A deficiency on fowls of all ages. Early workers presented results which were not always in agreement and which were not usually supported by chemical analyses. Often carotene-containing feedstuffs were used as the sole source of vitamin A. Thus differences, attributed to vitamin A, between groups which were on a basal ration without green-feed and a basal ration plus green-feed could have been due at least partly to the effect of factors additional to carotene in the green-feed.

Polk and Sipe (1940), in their work on the effect of vitamin A deficiency on malposition of the chick embryo, used a depletion ration incorporating a small amount of carotene. Their figures, when subjected to further analysis, indicate that hatchability was unaffected by the level of vitamin A in the rations, being of the order of about 50 per cent. in both groups, with a high percentage of infertile eggs. Egg production was 27 per cent. less in the group on the depletion ration.

Rubin and Bird (1942) studied the progressive effects of vitamin A deficiency in laying fowls. Carotene was the sole source of vitamin A in the control group. They found that although egg production was decreased to almost zero, there was no appreciable effect on hatchability.

Temperton and Dudley (1946) placed four groups of fowls on a vitamin A depletion diet to study the effects of progressive vitamin A depletion in adult fowls. After one month some fowls developed clinical symptoms of vitamin A deficiency and all groups were then allowed access to runs of poor grass. The experiment did not yield clear-cut partitioning of the progressive stages of vitamin depletion. Fertility was unaffected by a deficiency in vitamin A, but a decline in hatchability occurred after the groups were taken off the grass runs and confined in pens. A decline in egg production was also apparent at this stage and was concurrent with clinical cases of vitamin A deficiency. Although these effects were probably due to vitamin A deficiency, the fall in hatchability could have been due to an inadequate intake of riboflavin after the fowls were taken off the grass runs. The ration used contributed only

1,185 micrograms riboflavin per lb. of feed, whereas Ewing (1947) recommended for satisfactory hatchability a minimum of 1,300 micrograms of riboflavin per lb. of feed for breeding hens.

Taylor, Stern, Russell and Jungherr (1947) conducted experiments to determine the provitamin A requirements of hens. They controlled the vitamin A potency of their rations by using different types and amounts of carotene-containing feeds. In comparing two groups for egg production, a drop in one was believed to be significant and to be due to a lower intake of carotene. The roosters in the respective groups were apparently maintained on the same rations as the hens, and a lowered hatchability in the group on the lowest carotene level was accentuated by an increased number of infertile eggs.

McClymont and Hart (1948) investigated the effect of an inadequate intake of vitamin A on egg production and hatchability. Their control ration contained green-feed throughout the experiment. Their deficient ration contained fish oil for the first six weeks to provide an adequate reserve of vitamin A in the fowls of this group. After six weeks, fish oil was omitted from their deficient ration. They found that vitamin A deficiency resulted in a cessation of egg production not preceded by a marked decline. There was no apparent effect on the fertility and hatchability of eggs. Some of the fowls on the deficient ration died.

Heywang (1952) obtained information on the level of vitamin A in the diet of laying and breeding fowls during hot weather. He used vitamin A *per se* to supplement the feed of six groups of pullets at varying levels. Data on egg production and hatchability at the commencement of the experiment and after 84 days indicated that the pullets on the unsupplemented ration laid fewer eggs, and these were of lower hatchability. No vitamin A analyses were presented.

A survey of the vitamin A reserves in fowls by Gartner and Ryley (1956) indicated that vitamin A deficiency is still a major cause of economic loss in commercial flocks in Queensland, but in this survey the vitamin A reserves of the fowls were related to diseases rather than to production. The object of the investigation reported here was to determine the effects of vitamin A deficiency in laying fowls and response from vitamin A supplementation once field evidence of vitamin A deficiency had been established. At various stages of the experiment chemical analyses were made on the livers of hens and their progeny, on eggs, on the feed and in one instance on faeces. Chemical data were obtained at certain stages to aid the evaluation of marginal vitamin A status in adult fowls in relation to egg production and hatchability and in chickens in relation to the vitamin A status of their dams. The results are presented in this paper.

II. METHODS.

(1) Husbandry in 1955 Pilot Trial.

A pilot trial was initiated on June 1, 1955, to determine whether feeding a vitamin A deficient ration to laying fowls would lower the hatchability of eggs and, if so, at what rate. In addition, data on the effectiveness of fertilisation by roosters rotated between pens was obtained.

The only hens available at the time were 18 months' old White Leghorns and White Leghorn–Australorp crossbreds. They were divided at random into two groups each containing 22 hens. These hens were housed in adjacent intensive pens, each pen measuring 14 ft. x 14 ft. They were run on deep litter consisting of pine shavings, which remained in the pens for the duration of the trial.

A male bird was placed in each pen. At first the males were interchanged twice a week to equalise their individual variations and their intake of vitamin A, but later were interchanged every second day owing to poor fertility in the group on the vitamin A deficient ration. Fertility did not improve in this group and for the last four hatches of the trial artificial insemination was carried out, using Australorp males.

The basal ration fed to one group contained no preformed vitamin A and no measurable quantity of carotene. The other group was fed this basal ration plus stabilized vitamin A (10,000 I.U./g.), incorporated to provide a ration containing about 2,800 I.U. of vitamin A per lb.

*Composition of Basal Ration.**

	lb.
Sorghum Meal	25
Wheatmeal	15
Bran	27
Pollard	23
Meatmeal	9½
Salt Premix +	½
	100
Vitamin D_3	280 I.U./lb.

+ Salt Premix consisted of 10 lb. salt to which were added 80 g. manganese sulphate and 2.6 g. synthetic riboflavin.

* The total riboflavin content of the ration was 2.8 mg./lb. feed.

The rations were fed *ad lib.* in open feed hoppers. Shellgrit and insoluble grit were made freely available. The ration was analysed for riboflavin initially and for vitamin A periodically. For the purpose of this paper, the basal ration will be referred to as the deficient ration and the basal ration plus added vitamin A (2,800 I.U./g.) as the sufficient ration.

Weekly egg production per group was recorded and suitable eggs incubated weekly to determine the hatchability. All eggs set were of good shell texture, were free from any cracks and had a minimum weight of 1¾ oz. Thin-shelled eggs and oversized eggs were not set. No consideration was paid to the shape of the egg. The eggs were set in a forced-draught incubator and the position of each tray was alternated weekly. Fumigation was carried out on the 18th day, when the eggs had been transferred to the hatching compartment.

At intervals, eggs and newly-hatched chickens were analysed for vitamin A. The livers of hens that died during the trial and of hens killed to maintain equal numbers in each group were also analysed. The trial was terminated on Jan. 20, 1956, and the livers of five hens from the control group and four from the group on the vitamin A deficient ration were analysed.

(2) Husbandry in 1956 Trial.

The second trial was planned on experience gained in the pilot trial. A group of Australorp pullets, 18 weeks of age, was taken. At five days of age they had been vaccinated with pigeon pox vaccine. From four to 10 weeks of age, sulphaquinoxaline (·0125 per cent.) had been incorporated in the mash as a preventive against coccidiosis.

Prior to being selected for the experiment, the pullets had been fed a diet high in vitamin A. Two pullets were killed and analyses confirmed that initial liver reserves of vitamin A were high. All the pullets were placed for 74 days on the vitamin A deficient ration used in the pilot trial. At the end of this time a further two were killed. These had liver reserves comparable with average levels encountered in practice. The pullets were divided into two groups at random on Apr. 17, 1956, and each group of 36 placed in separate but adjacent intensive pens. One group was maintained on the vitamin A deficient ration and will be referred to as the deficient group (D Group). The other group was placed on the vitamin A sufficient ration used in the pilot trial and will be referred to as the sufficient group (S Group).

The pullets were trap-nested and individual records on production and hatchability were kept. All the pullets were artificially inseminated twice a week for the first two weeks and then once weekly thereafter with pooled semen from four Australorp roosters fed a ration containing the same amount of stabilized vitamin A as was added to the sufficient ration.

Eggs from both groups were set five weeks after the pullets had been put onto their respective rations. A second hatch was set two weeks later and subsequently eggs were set every week.

After the first two hatching results the pullets were classified on the basis of fertility and hatchability of their eggs. Five pullets were classed as culls in each group. The culls were maintained in their respective groups but were not used in recording production and hatchability data. With regard to vitamin A reserves, it was considered that they would be representative of the group as their egg production and bodyweight were comparable with those of other pullets in the group. They were killed at intervals to determine vitamin A reserves, but with one exception in D Group, not until after the reversal of the trial.

At intervals before the reversal of the experiment, the two poorest producers in S Group and the four poorest producers in D Group were killed to determine liver vitamin A reserves. In addition, one cull from D Group was killed for the same purpose. The pullets in D Group were examined approximately twice a week for field evidence of vitamin A deficiency. The incidence of "pustules" in the oesophagus (henceforth referred to as nodules) was taken as field evidence of vitamin A deficiency.

The experiment was reversed after nodules on the oesophagus were detected in all pullets in D Group by field examination. S Group was divided into two equal groups of 14 pullets on the basis of their weights and prior egg production, and these are designated the SS and SD Groups. SS Group was maintained on the sufficient ration and SD Group was placed on the deficient ration. D Group was divided into two unequal groups—a DD Group of 10 pullets maintained on the deficient ration and a DS Group of 14 pullets placed on the sufficient ration. Thus SS and DS Groups were in one pen, and DD and SD Groups in the other. These four groups were treated separately in recording production and hatchability data.

This subdivision into four groups was an opportunity for the further culling of pullets from S and D Groups known to be abnormal. One additional cull was selected from each group. This made six culls from S Group, of which two were maintained in SS Group and four placed in SD Group. The four culls from D Group were all placed in DS Group. These culls were killed at intervals to assess liver vitamin A reserves. In addition, day-old chickens from SS, SD and DS Groups were analysed for total liver vitamin A reserves at hatching. Chickens from DD Group were not available, the hatchability in this group being nil at this stage.

All the pullets in Groups SS and DS were killed a week after the occurrence of nodules on the oesophagus in three pullets from SD Group. They were examined for parasite burden and their livers analysed for vitamin A. Survivors of Groups DD and SD were maintained on the deficient ration until the trial terminated on May 20, 1957.

(3) Chemical Analysis.

A chromatographic method was sought that could be used to separate vitamin A from the carotenoids present in yolks and in yolk-sacs and that at the same time could be used for the determination of vitamin A added to feeds. A method based on the "Report on Vitamin A in Mixed Feeds" (Cooley 1952) was tried. It was found to be unsuitable for our particular requirements and was modified as follows:

(a) Vitamin A in Yolks and Yolk-Sacs.

For each analysis the yolks from six hard-boiled eggs were mixed and 12 g. taken for saponification. Composite yolk-sac samples from four chickens were taken for saponification. The saponification and extraction procedure was that used for butterfat by McDowell (1949).

An aliquot of the sulphuric ether extract, normally 20 ml., was evaporated to dryness on a water bath in a 150 ml. Erlenmeyer flask. Two drops of pyridine and 0·5 ml. acetic acid anhydride were added and the acetylation carried out on a water bath. At all stages of evaporation and acetylation a gentle stream of carbon dioxide was directed over the solution. When most of the acetic acid anhydride had been driven off, a further 0·5 ml. was added together with two drops of pyridine and the acetylation continued. Most of the excess acetic acid anhydride, pyridine and by-products of acetylation were driven off with carbon dioxide. To render the product totally free from these polar substances, two lots of about 25 ml. petroleum ether (Shell X222, fraction boiling between 60 deg. and 80 deg. C.) were added after the second acetylation and evaporated off. The vitamin A was now in the form of vitamin A acetate and was taken up in 50 ml. petroleum ether. Acetylation in dilute solutions, or acetylation using only one lot of acetic acid anhydride and pyridine, gave incomplete conversion of vitamin A alcohol to vitamin A acetate.

The activation of the magnesia (Microbrand No. 2641) was the same as described by Cooley (1952). The chromatographic tube consisted of a reservoir 40 mm. in diameter and 100 mm. long sealed to tubing 18 mm. in inside diameter and 250 mm. long to which a capillary tube 75 mm. long with about a 1 mm. bore was sealed. The height of the magnesia plus hyflo-super-cel was 85 mm. and a further 10 mm. layer of anhydrous sodium sulphate was added. The column was washed with 50 ml. petroleum ether immediately prior to use. The 50 ml. acetylated extract was then passed through the column, and as soon as the liquid layer reached the top of the adsorbent a further 20 ml. petroleum ether was added to displace the remainder of the acetylated extract from the column.

Depending on the amount of vitamin A present in the original sample, either all the eluate and washings or an aliquot was taken for the Carr-Price reaction. A correction for 20 per cent. loss had to be made in this method.

About half of this was due to acetylation losses. The other half was due either to vitamin A adsorbed on the column in the form of the alcohol, through incomplete acetylation, or to destruction of vitamin A in the process of analysis.

For yolks containing normal amounts of vitamin A, the acetylation and chromatographic steps may be omitted without loss of accuracy. However, when the yolk contains little or no vitamin A, these steps are necessary to avoid interfering purple or green colours in the Carr-Price reaction due to the presence of carotenoids, mainly lutein. Presumably the blue colour due to vitamin A is developed before the other interfering colours. Chromatographed extracts of vitamin A deficient yolks or yolk-sacs still gave a slight galvanometer deflection with the Carr-Price reagent even though the solution remained colourless. For these samples, 1 I.U. was subtracted from the readings.

(b) Vitamin A in Feeds.

Saponification followed by extraction with petroleum ether was used for feed analyses in preference to a direct solvent extraction. Stabilized vitamin A added to poultry rations sometimes contains a protective coating insoluble in petroleum ether. Also, with a direct solvent extraction fat interferes with the Carr-Price reaction, particularly in samples low in vitamin A.

The saponification was carried out by refluxing 20 g. of feed for 10 min. with 5 ml. saturated potassium hydroxide solution and 50 ml. ethyl alcohol. The material was centrifuged and 25 ml. of the supernatant liquid extracted with 45 ml. petroleum ether and 20 ml. water. This was followed by a second extraction with 45 ml. petroleum ether. The extracts were combined and washed twice with about 100 ml. water. They were then made up to 100 ml. with petroleum ether and dried over anhydrous sodium sulphate. The solution was then acetylated and chromatographed as described above. Normally 50 ml. was taken for acetylation and after chromatography the eluate was made up to 50 ml., of which 25 ml. was taken for analysis. As in yolk analysis, 1 I.U. was subtracted from the readings in samples containing little or no vitamin A as seen by the absence of any blue colour, although small galvanometer deflections were still recorded.

III. RESULTS OF 1955 PILOT TRIAL.

(a) Egg Production.

There were no group differences in egg production throughout the trial.

(b) Hatchability.

The hatchability was calculated from the total number of chickens hatched and the total number of eggs set.

Eggs from both groups were set four weeks after the commencement of the trial, then at weekly intervals for 21 consecutive weeks. The hatchability fluctuated in the group on the sufficient ration from 78·79 per cent. to 45·16 per cent. and in the group on the deficient ration from 77·33 per cent. to 40·74 per cent. There was no regular pattern in the results, except that the group on the deficient ration tended to produce eggs of lower hatchability than the control group. This was neither constant nor significant and was due to a higher percentage of infertile eggs in this group (average 17·1 per cent., against 8·9 per cent. in the group on the sufficient ration). Artificial insemination of the hens from both groups in the last four hatches using Australorp males did not reduce the number of infertile eggs. Presumably some hens in both groups were sterile. It was decided to adopt trap-nesting in further work to enable infertile and unproductive hens to be identified at the commencement of the experiment.

(c) Liver Vitamin A Reserves of Hens.

The hens in this experiment had been used previously in a Random Sample Trial. The number was too small to permit any to be slaughtered to determine initial vitamin A reserves. A sample of the ration fed for the previous 18 months was analysed; it contained 12,300 I.U. of vitamin A per lb. This was over four times the amount of vitamin A added to the ration. An analysis of the liver meal used as a component of the ration (3½ per cent.) proved it was responsible for the additional vitamin A. The year the liver meal was produced and the year before that were periods of abundant rainfall in Queensland. This would result in pastures of high carotene content throughout the year and must have produced good reserves of vitamin A in the livers of grazing animals. In spite of processing, the liver meal was thus rich in vitamin A.

Table 1.

LIVER VITAMIN A RESERVES OF HENS ON VITAMIN A SUFFICIENT AND DEFICIENT RATIONS (1955).

Weeks From Commencement of Trial.	Liver Vitamin A (μg./g.).		Remarks.
	Sufficient Ration.	Deficient Ration.	
5	840	..	Killed
10	..	780	Died
11	828	..	Killed
11½	711	..	Died
20	..	400	Died
31	..	108	Died
33 (end of trial) ..	1,060	1,171	Killed
	822	566	Killed
	655	541	Killed
	477	479	Killed
	270	..	Killed

It was anticipated that the initial liver vitamin A reserves of these hens would be high, but depletion in three to six months was expected in the group on the deficient ration. Analytical data are presented in Table 1. The liver reserves were high in both groups when the trial was terminated after 33 weeks.

(d) Vitamin A Content of Egg Yolk.

As shown in Table 2, both groups produced eggs containing comparable amounts of vitamin A throughout the experiment. The eggs did not contain many pigments, for the ration was low in carotenoids. The carotenoid content ranged from 100 μg. per egg yolk at the beginning of the trial to less than 20 μg. at the end, of which about 7 per cent. was beta-carotene. Sjollema and Donath (1940) found that most of the carotenoid pigments in the yolk are in the form of xanthophylls, the usual ratio of carotenes to xanthophylls being 1:10.

Table 2.

VITAMIN A CONTENT OF YOLKS FROM HENS ON VITAMIN A SUFFICIENT AND DEFICIENT RATIONS (1955).

Weeks From Commencement of Trial.	Vitamin A in Yolks (μg.).	
	Sufficient Ration.	Deficient Ration.
6	118	112
11½	121	103
21½	72	85
32½	107	101

(e) Vitamin A Reserves of Chickens at Hatching.

Newly hatched chickens were analysed for vitamin A reserves on three occasions during the experiment. There were no differences between groups. The total vitamin A reserves at hatching averaged 34 μg., of which 12 μg. was in the liver and 22 μg. in the yolk-sac. Some of these chickens were hatched from eggs, the vitamin A contents of which were known by previous analyses of composite egg yolk samples. The average vitamin A reserves of newly hatched chickens was only one-third of the content of the unhatched eggs.

The distribution of vitamin A between yolk-sac and liver was examined in chickens from two hatches which were not given access to food. The experimental procedure involved slaughter of four chickens and the analysis of composite samples of yolk-sacs and livers. In one case chickens were killed two, three and four days after hatching. On the next occasion chickens were killed at hatching, one day later and two days later. There was no regular pattern in their total vitamin A reserves, but at hatching 65 per cent. of

the total vitamin A reserves was in the yolk-sac; the reserves dropped to 63, 48, 37 and 33 per cent. in successive days. Parrish, Williams, Hughes and Payne (1950) found that 60 per cent. of the vitamin A in the chicken at hatching was in the residual yolk.

IV. RESULTS OF 1956 TRIAL.

(1) Effects of Vitamin A Deficiency in Pullets.

(a) Egg Production.

Eggs laid by 31 pullets from each of Groups S and D were considered in calculating the number of eggs laid per pullet per week. The eggs laid by pullets noted as culls for infertility and poor hatchability were excluded.

At the end of the first stage of the trial, prior to separation into four groups, there were 29 pullets left in S Group and 25 in D Group, both excluding culls. At the end of the second stage of the trial, one week after the incidence of nodules on the oesophagus in SD Group, there were 14 pullets in SS Group, 13 in Groups DS and SD and five in DD Group.

The egg production over the whole period of the trial is illustrated in Fig. 1. The first stage comprised 18 weeks. For the first three weeks the egg production was higher in D Group. Thereafter it declined gradually, until at the reversal point, when field evidence of vitamin A deficiency was apparent in this group, the pullets were laying 1·38 eggs per week, compared with 4·66 eggs per week in S Group. The average weekly egg production in D Group over the whole period of 18 weeks was 3·46, compared with 4·69 in S Group. These differences, though significant, would have been greater if three of the poorest layers had not been in S Group and if four poor layers had not been taken from D Group for analysis against only two from S Group.

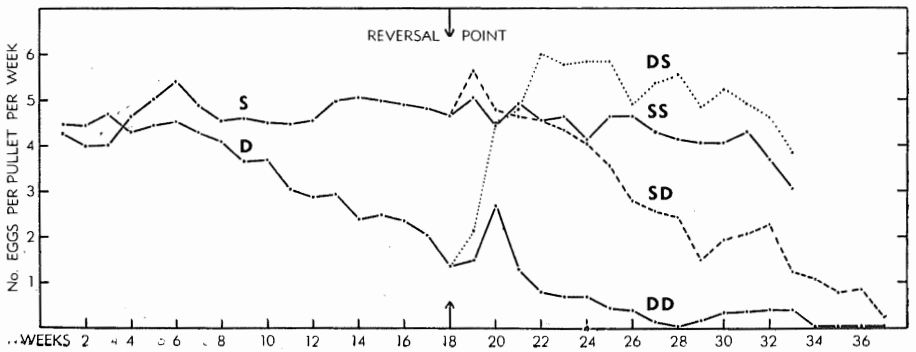

Fig. 1.

Egg Production Over the Period of the Trial.

The post-reversal stage of the trial was the period of 14 weeks from the 18th to the 32nd week. SS Group maintained an even egg production until just before they were killed, when hot weather caused a general drop in egg production. The egg production of DS Group was back to normal only two weeks after supplementation with vitamin A and thereafter was consistently higher than in SS Group. SD Group followed the same pattern as D Group, and when nodules were evident in this group at the 32nd week, the pullets were laying 2·28 eggs per week, against 3·71 in SS Group and 4·62 in DS Group.

From the individual egg production records, it was evident that in the depleted D and SD Groups the cessation of egg production was gradual in most instances. This is contrary to the findings of Taylor *et al.* (1947), who found on examination of individual records that death occurred about 12 days after the last egg was laid and that very little drop in production occurred prior to that time. McClymont and Hart (1948) reported that the rate of egg production was only slightly affected before cessation of production.

There was a slight and temporary increase in egg production in DD Group at the second week after reversal. The possibility that pullets in this group may have ingested small amounts of vitamin A from the faeces of SD Group, now in the same pen, was examined. Chemical analysis of faeces failed to show a measurable amount of vitamin A.

(b) Hatchability.

The number of pullets in the different groups from which eggs were set was the same as recorded for egg production. For the first hatch 135 eggs were set from both S and D Groups and equal numbers of eggs from each group were set for the next five hatches. For the last six hatches prior to reversing the groups, more eggs were set from S Group than from D Group. This was due to a marked fall in egg production in D group and a high requirement of chickens for other purposes. The last three hatchability values of D group resulted from 45, 52 and 27 eggs set. After reversing the groups, all suitable eggs laid were set.

The percentage hatchability over the whole period is illustrated in Fig. 2. For the 18 weeks' period prior to reversal, the hatchability of S Group maintained a normal level, while that of D Group dropped sharply. Individual hatchability and production data are not presented but examination of these records showed:

(1) In S Group two pullets laid eggs of much lower hatchability (58·14 and 37·50 per cent.) than the average for the group.

(2) In D Group, the eggs laid by two pullets accounted for 60 per cent. of the chickens hatched in the last five hatches. One of these pullets was the second highest producer in its group and the hatchability of its eggs

(average 85·19 per cent.) was only beginning to drop in the last batch of eggs set. Presumably these two pullets had greater vitamin A reserves at the commencement due to more efficient utilization.

(3) Vitamin A deficiency affects the percentage hatchability of eggs from individual pullets before it affects their egg production.

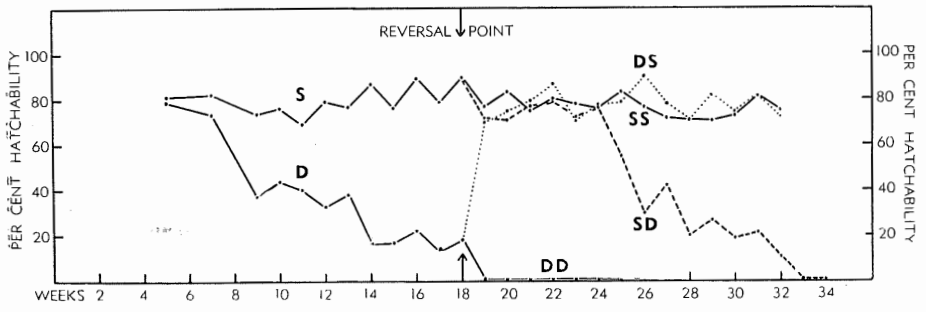

Fig. 2.

Hatchability Over the Period of the Trial.

Table 3 presents data on the time interval between a drop in hatchability and a drop in egg production from 20 pullets in D Group.

Table 3.

WEEKS INTERVENING BETWEEN DROP IN HATCHABILITY AND DROP IN EGG PRODUCTION FROM 20 PULLETS IN D GROUP.

Intervening Period (weeks)	−1.	0.	+2.	+3.	+4.	+5.	+7.
No. of Pullets ..	1	1	4	4	8*	1	1

*One pullet ceased egg production four weeks before the last hatch; in the last week it laid three eggs, from which three chickens hatched.

Table 4.

STAGE OF INCUBATION AT WHICH MORTALITY OCCURRED IN GROUPS S AND D.

Group.	Infertile Eggs.	Dead Embryos at :			Pipped.	Failed to Hatch.	Hatchability.
		5 days.	13 days.	18 days.			
	%	%	%	%	%	%	%
S	3·9	2·3	3·4	1·4	2·3	6·2	80·5
D	4·2	9·5	11·0	6·5	4·5	21·1	43·2

In Table 4 the stage of incubation at which mortality occurred in S and D Groups is presented.

The hatchability in DS Group was back to normal after only one week's access to a ration containing normal amounts of vitamin A. The hatchability in SS Group was comparable with that recorded for S Group. The hatchability in SD Group decreased in much the same way as the hatchability in D Group and was down to nil one week after the incidence of nodules on the oesophagus. The hatchability in DD Group was nil one week after the incidence of nodules on the oesophagus in D Group—i.e., one week after the reversal point—and remained at this level until egg production virtually ceased. The maximum number of eggs set in DD Group to give zero hatchability was 18, against 16 in SD Group.

Examination of individual records showed:—

(1) Two pullets in SS Group laid eggs of much lower hatchability (41·03 and 12·82 per cent.) than the average for the group. The one with the lowest percentage hatchability was also the lowest when it was in S Group. This was due to the production of infertile eggs. In DS Group two pullets also laid eggs of appreciably lower hatchability (51·28 and 47·54 per cent.) than the average for the group.

(2) In SD group the eggs laid by two pullets accounted for 65·8 per cent. of the chickens hatched in the last six hatches, one of these pullets being a good layer and the best producer in its group.

(3) Vitamin A deficiency affects the percentage hatchability of eggs from individual pullets before it affects their egg production. This is summarised for 11 pullets in Table 5.

Table 5.

WEEKS INTERVENING BETWEEN DROP IN HATCHABILITY AND DROP IN EGG PRODUCTION FROM 11 PULLETS IN SD GROUP.

Intervening Period (weeks)	+1.	+2.	+4.	+6.
No. of Pullets ..	5	3	2	1

The stage of incubation at which mortality occurred in SS, SD, DS and DD Groups is shown in Table 6

Table 6.

STAGE OF INCUBATION AT WHICH MORTALITY OCCURRED IN GROUPS SS, SD, DS AND DD.

Group.	Infertile Eggs.	Dead Embryos at :			Pipped.	Failed to Hatch.	Hatchability.
		5 days.	13 days.	18 days.			
	%	%	%	%	%	%	%
SS	4·0	3·7	9·2	1·8	0·8	3·6	76·9
SD	2·5	8·4	11·1	4·7	3·0	14·0	56·3
DS	2·6	4·1	6·5	2·5	0·5	5·5	78·3
DD	4·1	21·9	48·0	17·8	0·0	8·2	0·0

From Tables 4 and 6 it is evident that although vitamin A deficiency in the pullet affects the hatchability of eggs, it had no effect on the fertility. The range of infertile eggs for individual hatches in all groups was 0–9·8 per cent.

During the course of the trial 4,607 eggs were set and an overall fertility of 96·5 per cent. resulted. In a comparative test of artificial insemination *versus* natural mating over a hatching period of 300 days, Cooper (1955) obtained an overall fertility of 83·4 per cent. in his inseminated pens.

(c) Liver Vitamin A Reserves of Pullets.

Prior to separating the pullets into their respective groups at the commencement of the trial, four were killed to determine their liver vitamin A reserves. They were all normal (range 239–156 μg./g.). The liver vitamin A

Table 7.

LIVER VITAMIN A RESERVES OF PULLETS IN RELATION TO TIME FROM COMMENCEMENT OF TRIAL

Weeks From Commencement of Trial.	Liver Vitamin A (μg./g.)					Remarks.
	S.	D.	SS.	SD.	DS.	
1½	76	113	..	..	..	Killed
8½	220	6	..	..	..	Killed
9	..	2·5	..	..	..	Killed
10	132	1	..	..	..	Killed
11	..	<1	..	..	..	Killed (cull)
11½	..	<1	..	..	..	Killed
16	..	<1	..	..	..	Died
18	..	<1	..	..	..	Died
			REVERSAL.			
20½			..	..	4	Died
21			..	115	..	Killed (cull)
25½			26	<1	25	Killed (cull)
25½			..	<1	..	Killed (cull)
30			..	<1	34	Killed (cull)
30			..	..	9	Killed (cull)
31½			69	..	..	Killed (cull)
33½ (end of trial)			151	..	71	Killed
			143	..	64	Killed
			142	..	51	Killed
			140	..	47	Killed
			94	..	40	Killed
			91	..	36	Killed
			82	..	35	Killed
			75	..	35	Killed
			53	..	32	Killed
			46	..	23	Killed
			43	..	20	Killed
			35	..	18	Killed
			32	..	8	Killed
			15	..	4	Killed

reserves of all the pullets killed and of some that died during the experiment, and the time interval from commencement of the trial, are set out in Table 7. The egg production and hatchability of these periods, shown in Figs. 1 and 2, can thus be related to the vitamin A reserves in the pullets. The liver vitamin A levels were interpreted in the manner discussed by Gartner and Ryley (1956), in which levels between 0 and 2 μg./g. were considered indicative of vitamin A deficiency and from 2+ to 10 μg./g. were considered marginal.

S Group.—As would be expected, the pullets in this group had normal liver vitamin A reserves.

D Group.—The liver vitamin A reserve of a pullet was marginal 8½ weeks after commencement of the trial. At this stage the egg production of the group had been falling gradually and was less than the egg production of S Group. At the same stage there was an even greater difference in hatchability, D Group's being of the order of less than 50 per cent. Pullets killed subsequently showed decreasing reserves until they reached levels indicative of vitamin A deficiency.

SS Group.—The culls that were killed had normal reserves of vitamin A. At the end of the trial all the pullets were killed to determine the level and distribution of vitamin A in the group. The average level (82 μg./g.) was the same as found by Gartner and Ryley (1956) in their survey of commercial flocks in Queensland.

SD Group.—The liver vitamin A level of a pullet in this group was deficient 7½ weeks after reversal. As in D Group, egg production and hatchability had declined at this stage.

DS Group.—A pullet that died 2½ weeks after reversal had a marginal liver vitamin A level. The liver reserves were normal in another pullet 7½ weeks after reversal. The average reserve at the end of the trial was 35 μg./g.; two pullets had marginal reserves; the rest were normal.

DD Group.—Livers from pullets that died in this group were not analysed for vitamin A, as extremely low reserves of vitamin A in D Group had been established prior to reversal.

(d) Vitamin A Content of Egg Yolks.

Six eggs were selected at random from each group and the yolks bulked for analysis. When vitamin A deficiency was first confirmed in D Group by liver analysis, the eggs laid by this group contained less than 8 μg. of vitamin A per yolk, against 42 μg. per yolk in S Group. Two further analyses, each at fortnightly intervals, revealed that the vitamin A content of eggs from D Group decreased still further until the exact amount present, if any, was indeterminable by the chemical method employed. The vitamin A content of eggs from S Group was 62 and 48 μg. per egg over the same period. These levels in S Group were lower than those obtained in eggs during the 1955 trial (121–72 μg./yolk). No eggs were analysed after reversal.

(e) Vitamin A Reserves of Chickens at Hatching.

The vitamin A reserve in day-old chickens from SS, SD and DS Groups was determined (Table 8). Each analysis represents composite liver and yolk-sac samples from four chickens.

Table 8.

VITAMIN A RESERVES IN THE LIVER AND YOLK-SAC OF CHICKENS AT HATCHING.

Weeks After Reversal.	Liver Vitamin A (μg.).			Yolk-sac Vitamin A (μg.).			Total Vitamin A (μg.).		
	SS.	SD.	DS.	SS.	SD.	DS.	SS.	SD.	DS.
2	13	22	13	11	16	11	24	38	24
3	20	8	12	10	3	4	30	11	16
4	19	6	14	12	2	8	31	8	22

Reference to the hatchability data (Fig. 2) shows that the period, four weeks after reversal, was three weeks before the hatchability in SD Group began to fall. At this stage the total vitamin A reserve in the SD chickens was 8 μg., compared with 11 and 38 μg. in the preceding weeks. It is apparent that there was a marked fall in the vitamin A reserves of chickens at hatching prior to the effect of a vitamin A deficient ration on the hatchability of eggs.

(f) Field Examination for Lesions of Vitamin A Deficiency.

Depleted pullets were examined approximately twice a week for nodules on the oesophagus. This was done prior to artificial insemination and thus did not entail extra handling. Feed was removed at least two hours before examination. This was desirable, as in our experience food particles can be confused with small nodules.

In D Group nodules were first detected in four pullets and three days later in the remainder. In SD Group there was a lag of 11 days from the time nodules were detected in three pullets until the last two in the group exhibited lesions.

The disappearance of nodules after vitamin A supplementation may be a slow process. In DS Group, although nodules could not be detected in three pullets at four days after supplementation, it took eight weeks for nodules to disappear in the last three pullets.

(g) Principal Autopsy Findings.

Table 9 sets out the principal autopsy findings of all the pullets that died during the trial. After field evidence of vitamin A deficiency had been established, nodules on the oesophagus were found in eight of the 10 pullets that died in DD Group and in eight of the 10 pullets that died in SD Group. The pullets not exhibiting these lesions were all in an advanced stage of decomposition when autopsied. Yet by field examination nodules were detected in them. at the most, a week prior to death.

Table 9.

PRINCIPAL AUTOPSY FINDINGS OF PULLETS THAT DIED DURING THE TRIAL.

Group.	Weeks Deficient as Determined by		Principal Autopsy Findings.
	Chemical Evidence.	Field Evidence.	
D	2	..	Died during treatment with carbon tetrachloride ; kidneys friable, suggestive of cloudy swelling ; liver extremely friable
D	6	..	Myocardial degeneration ; kidneys extensively mottled ; liver swollen and soft
D	7	..	Large haematoma of right lobe of liver ; liver enlarged, very friable and soft ; kidneys enlarged, mottled
D	8	0	Liver extremely friable with sub-capsular haemorrhage ; 12 early nodules on oesophagus ; kidneys cloudy swelling
DS	..	..	Peritonitis due to abrasion of cloaca when being artificially inseminated ; few nodules left on oesophagus
DD	14½	6½	Egg peritonitis ; nodules on oesophagus ; kidneys swollen, suggestive of cloudy swelling ; liver friable
DD	16	8	Nodules on oesophagus ; kidneys pale, with slight frosting
DD	18½	10½	Egg peritonitis ; nodules on oesophagus ; kidneys swollen
DD	19	11	Nodules on oesophagus ; kidneys swollen, uniformly frosted; ureters distended with urates
DD	21	13	Massive sub-capsular haemorrhage of liver ; no nodules on oesophagus ; advanced stage of decomposition
SD	6	..	Egg peritonitis ; kidneys coarse mottling ; ureters slightly distended with urates ; fatty degeneration of liver
DD	25	17	No nodules on oesophagus ; advanced stage of decomposition
SD	13½	6½	Large blood clot on both sides of liver ; liver friable ; pale kidneys ; no nodules on oesophagus ; decomposed
SD	15	8	Visceral gout ; deposit of urates on kidneys, epicardium and pericardium ; ureters distended with urates ; no nodules on oesophagus ; decomposed
SD	15½	8½	Visceral gout ; nodules on oesophagus ; kidneys pale ; thick deposit of urates over liver, heart ; ureters distended with urates
SD	16	9	Nodules (difficult to detect) on oesophagus ; kidneys white ; urates on epicardium ; ureters distended with urates ; decomposed
DD	31	23	Visceral gout ; nodules on oesophagus ; deposit of urates on kidneys, liver, epicardium and intestines ; ureters distended with urates
DD	32½	24½	Nodules on oesophagus ; kidneys frosted ; ureters distended with urates
SD	17½	10½	Visceral gout ; nodules on oesophagus ; kidneys white ; thick layer of urates on kidneys, liver, pericardium and epicardium
SD	17½	10½	Nodules on oesophagus ; liver enlarged, with slight, patchy haemorrhages
SD	17½	10½	Nodules on oesophagus
SD	18½	11½	Few nodules (on pharynx only) ; decomposed
SD	19	12	Nodules (on pharynx only) ; urates in ureters and in kidneys ; decomposed.
SD	21	14	Visceral gout ; few nodules (difficult to detect) on oesophagus; kidneys markedly pale ; urates in kidneys and ureters ; deposit of urates on liver and lung ; decomposed
DD	37½	29½	Mouth to crop heavily studded with nodules ; kidneys pale, swollen and enlarged ; ureters distended with urates
DD	39½	31½	Nodules on oesophagus, pharynx, tongue ; kidneys pale, with deposition of urates ; ureters grossly distended with urates

Of the 24 deaths in the deficient groups (excluding the one that died after treatment with carbon tetrachloride), 10 were due to uncomplicated vitamin A deficiency and five to visceral gout. Hungerford (1951) stated that visceral gout is a condition seen as a symptom of ureamia and in acute vitamin A deficiency. Four deaths were due to either a partial rupture of the liver with sub-capsular haemorrhage or a complete rupture of the liver with intra-abdominal haemorrhage, three to egg peritonitis, one to myocardial degeneration and one pullet was too decomposed to enable a diagnosis. It must be remembered that vitamin A deficiency was probably a contributing factor in all deaths and certainly was the only cause of death in 15 pullets.

In addition, an autopsy was done on most of the pullets killed for liver vitamin A assay. In SD Group two pullets showed fatty degeneration of the liver and in another pullet from the same group the abdomen contained fluid from a ruptured yolk.

(h) Field Observations.

Eggs laid by the five culls in both S and D Groups were lighter in colour throughout the trial than the eggs laid by the other pullets. Shell texture of all eggs was good throughout, even in the vitamin A deficient groups.

There was a marked difference in appearance between the chickens of pullets on sufficient and deficient rations after hatchability in the deficient groups had started to decline. The chickens from the sufficient groups dried out well and were first-class by the 22nd day, whereas the chickens from the deficient groups were still sticky and on appearance would be culled in accordance with commercial hatchery practice.

The feeding habits of both groups were similar. When pullets became vitamin A deficient, they frequented their nests just as often, even after laying had virtually ceased.

Apart from the incidence of nodules on the oesophagus, a casual inspection of the respective groups would not enable the deficient pullets to be singled out. Closer observation revealed that vitamin A deficient pullets became listless after their egg production dropped. They did not work the litter and their droppings, accumulating under the perches, had to be cleared out weekly and the litter stirred. The vitamin A sufficient pullets worked their litter as normal, healthy fowls do.

None of the pullets that succumbed to vitamin A deficiency was emaciated. In fact, the longest lived survivor from DD Group was still in good condition when it died 39½ weeks after vitamin A deficiency had been established in this group.

(i) Food Consumption.

Food consumption was recorded in S and D Groups over the last 39 days of the first part of the trial after D Group had been found to be vitamin A deficient by chemical analysis. Over this period S Group consumed 5·13 oz. of feed per pullet per day, against 4·89 oz. by D Group. This difference of 0·24 oz. per fowl per day is not considered significent. No records were kept after reversal, since there were then two groups in each pen.

(j) Weights of Pullets.

The pullets were individually weighed at the commencement of the trial, once prior to reversal, at reversal, and then at fortnightly intervals. The last weighing was when all survivors in SS and DS Groups were killed. There was no difference in the average weight gains (Table 10) between S and D Groups. The weights in SD Group were comparable with those in SS Group up to the time nodules were detected in the former group, but a week later the average weight in SD Group dropped by 5 oz. The heaviest pullet was in DD Group; it weighed 9 lb. 8 oz. at death. However, the average weight of the five survivors was considerably less than in DS Group.

Table 10.

AVERAGE WEIGHT OF PULLETS FROM EACH GROUP AT CERTAIN PERIODS OF THE TRIAL

Period.	S.	D.	SS.	SD.	DS.	DD.
	Lb. oz.	Lb. oz.	Lb. oz.	Lb. oz.	Lb. oz.	Lb. oz.
Commencement of Trial	5 3½	5 5½				
At Reversal	6 1	6 3				
Difference	+ 13½	+ 13½				
Commencement of Reversal			6 1	6 1	6 4	6 2
Week After Nodules in SD Group ..			6 5	5 13*	6 13*	5 9†
Difference			+ 4	− 4	+ 9	− 9

*13 survivors out of 14.
†5 survivors out of 14.

(k) Ascaridia galli Infestation.

In the first part of the trial, there were indications that a heavier infestation of *Ascaridia galli* was present in D Group than in S Group. Nine weeks before reversal a pullet killed in D Group had a few mature worms. The pullets in both groups were then dosed with 1 ml. of carbon tetrachloride to control the parasites. Two weeks after treatment another pullet killed in D Group had 20 adult *Ascaridia galli*. The treatment with carbon tetrachloride was repeated, this time after a starvation period of 12 hours. One of the pullets in D Group, which died during treatment, harboured 85 worms, of which 10 per cent. were fourth-stage. About six hours after treatment a

composite faecal sample collected from 12 pullets in each group revealed 30–60 eggs per gram of faeces in S Group and 300–400 e.p.g. in D Group. A week later a further egg count showed no eggs in S Group and 100 e.p.g. in D Group

Thereafter a proprietary piperazine compound was used to avoid the danger of liver damage from repetitive and frequent dosing with carbon tetrachloride. Five weeks before reversal the pullets were starved overnight and the anthelmintic incorporated in the ration for one day. The treatment apparently removed all the adult worms, the egg counts being negative, but $1\frac{1}{2}$ weeks later 100 e.p.g. were again present in a composite sample from D Group. A further treatment eradicated the infestation and no eggs were voided by either group until three weeks after reversal—i.e., for $8\frac{1}{2}$ weeks.

After reversal, SS and DS Groups were in one pen and SD and DD Groups in another. For this stage of the trial, correlation of *Ascaridia galli* infestation with the vitamin A status was less satisfactory. From the 5th to the 10th week after reversal egg counts were done at weekly intervals on bulked faeces. With one exception, SS and DS Groups showed fewer eggs per gram than SD and DD Groups. At the 15th week after reversal all the pullets in SS and DS Groups, having been killed, were examined for *Ascaridia galli*. The average number of worms was 1·9 in pullets from SS Group and 9·6 in pullets from DS Group. At the same time SD and DD Groups were treated with a piperazine compound and a count made of all the worms voided. The average figures were 3·6 worms in pullets from SD Group and 5·0 worms in pullets from DD Group. These are not considered pathogenic burdens. Individual variation within groups makes these results not statistically significant.

(1) Survival Time after Incidence of Vitamin A Deficiency.

The survival time after the incidence of vitamin A deficiency as diagnosed by chemical analysis is shown for DD and SD Groups in Fig. 3. In DD Group the range was $14\frac{1}{2}$ to $39\frac{1}{2}$ weeks with a mean survival time of $25 \cdot 50 \pm 9 \cdot 09$ weeks. In SD Group the range was 6 to 32+ weeks, there being one survivor when the trial was terminated. Taylor *et al.* (1947) found that in one experiment, at a feeding level of 540 I.U. of vitamin A per lb. of feed, over 80 per cent. of the pullets died within six months of the time vitamin A deficiency was first observed. McClymont and Hart (1948) found that the time between cessation of egg production and death from vitamin A deficiency was $44 \cdot 7 \pm 17 \cdot 3$ days. In DD Group the time between cessation of egg production and death from vitamin A deficiency was $64 \cdot 0 \pm 38 \cdot 4$ days.

The mean weekly maximum temperature and the mean weekly relative humidity are shown graphically in Fig. 3. For any particular week, the mean values for temperature and humidity are recorded for the preceding week, since if these factors affect mortality rate, it would be due to their influence in the days preceding death.

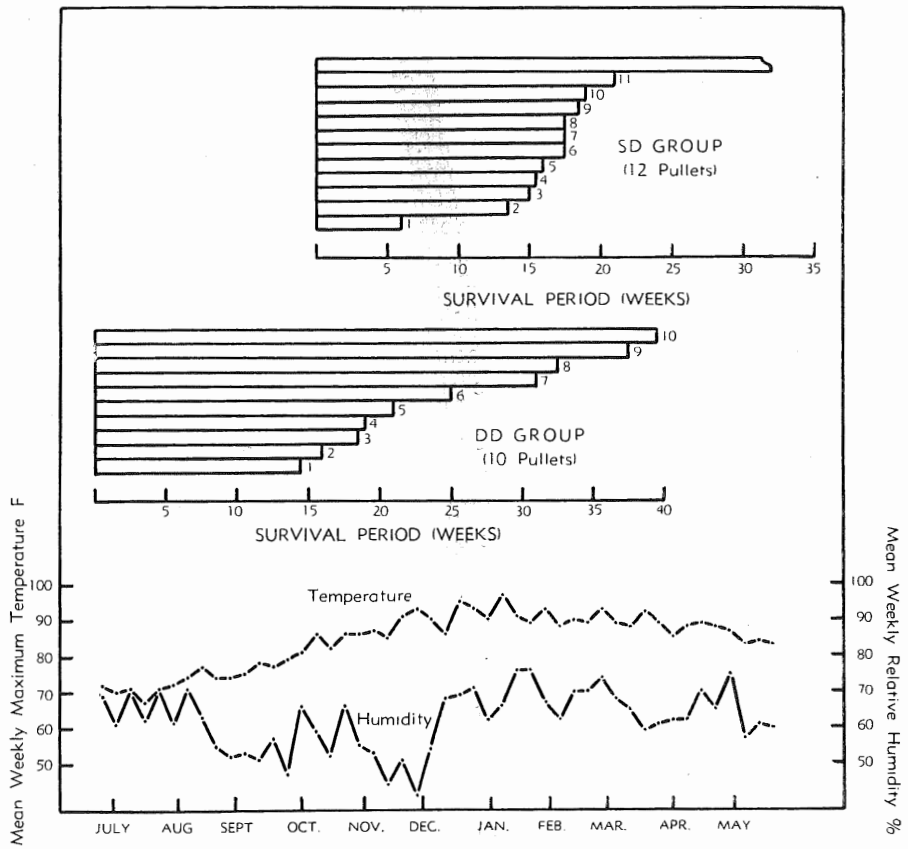

Fig. 3.

Survival Following Chemical Diagnosis of Vitamin A Deficiency in Groups DD and SD, together with Temperature and Humidity Records. The numbers alongside the histograms represent total number of deaths. Since the submission of this paper the 12th pullet from SD group died at 35 weeks.

As seen in Fig. 3, both temperature and humidity were higher in the initial period of deficiency in SD Group than in the initial period of deficiency in DD Group. There were only $5\frac{1}{2}$ weeks intervening between the second and 10th deaths in SD Group. High temperatures and high humidity may have furnished additional stress, thereby contributing to the mortality. In DD Group there were only four survivors at the beginning of this period and two of them died before its end.

(2) Effects of Vitamin A Deficiency in Chickens.

(a) General.

Experimental facilities limited the amount of investigational work on chickens. Chickens were reared up to four weeks of age from four consecutive hatches of eggs from S and D Groups. The first lot was hatched from eggs

laid 10 weeks after commencement of the trial, when egg production and hatchability of D Group had fallen and there was chemical evidence of vitamin A deficiency. The eggs laid by D Group at the time were low in vitamin A and the few day-old chickens analysed had no liver vitamin A reserves. Thus the day-old chickens from D Group were considered vitamin A deficient, while those from S Group were considered vitamin A sufficient. The day-old chickens were divided into four groups:—

ds Group: deficient chickens on a vitamin A sufficient ration.

dd Group: deficient chickens on a vitamin A deficient ration.

ss Group: sufficient chickens on a vitamin A sufficient ration.

sd Group: sufficient chickens on a vitamin A deficient ration.

The ds Group was omitted from the last two rearing experiments. Instead, a dss Group was included in which deficient chickens were reared on a ration containing five times (14,000 I.U./lb.) the amount of vitamin A usually used.

Because the chickens were to be reared for only one month, the chickens were fed, instead of a starter ration, the same ration as used for the adult fowls. The calcium intake was thus lower than normal. This may have been responsible for a disorder, resembling rickets, in some chickens in sd and dd Groups at the first rearing trial. However, chickens in the subsequent rearing trials did not show this disorder.

There was the same number of chickens in the different groups in each of four rearing experiments, these being 14, 13, 12 and 12 respectively. This number was limited in each instance to the number of chickens hatched from D Group. For convenience, the results are presented as representative of 51 chickens in dd, ss and sd Groups and of 27 chickens in ds and dss Groups.

(b) Rearing Mortality.

The rearing mortality up to four weeks was:—

dd Group: 71 per cent.
ds Group: 41 per cent.
dss Group: 17 per cent.
sd Group: 25 per cent.
ss Group: 4 per cent.

The heaviest mortality occurred in ds Group 4–6 days after hatching, in dd Group 3–7 days after hatching, and in sd Group 5–6 days after hatching.

(c) Liver Vitamin A Reserves of Chickens.

Three chickens from each group were analysed individually at four weeks of age. As shown in Table 11, the liver vitamin A reserves of normal chickens were exhausted within four weeks of the chickens being placed on a vitamin A deficient ration (sd Group).

Table 11.

LIVER VITAMIN A RESERVES IN FOUR-WEEKS-OLD CHICKENS.

Group.	ds.		dss.		dd.		ss.		sd.	
	μg./g.	μg./ liver.	μg./g.	μg./ liver.	μg./g.	μg./ liver.*	μg./g.	μg./ liver.	μg./g.	μg., liver.*
Experiment 1 ..	32	166	..	..	<0·5	—	33	168	<0·5	—
	25	227	..	..	<0·5	—	11	59	<0·5	—
	13	72	..	..	<0·5	—	11	62	<0·5	—
Experiment 2 ..	22	137	..	..	<0·5	—	18	148	<0·5	—
	21	143	..	..	<0·5	—	15	103	<0·5	—
	16	115	..	..	<0·5	—	13	111	<0·5	—
Experiment 3 ..	..	..	178	984	..	..	21	125	<0·5	—
	..	..	161	1,189	..	..	21	107	<0·5	—
	..	..	134	938	..	..	18	132	<0·5	—
Experiment 4 ..	..	..	188	1,345	..	..	29	230	<0·5	—
	..	..	115	865	..	..	21	129	<0·5	—
	..	..	96	625	..	..	13	64	<0·5	—

* As levels below 0.5 μg. of vitamin A cannot be determined accurately, total liver vitamin A reserves are not given.

There were more deaths in ds Group than in sd Group, although at four weeks of age chickens in ds Group had normal liver vitamin A reserves, while those in sd Group were deficient. These greater losses in ds Group were presumably due to low initial reserves of vitamin A and the time required to build up reserves in the group. From the mortality data it appears that the chicken is more affected by vitamin A deficiency towards the end of the first week of life. There were fewer losses in dss Group than in ds Group. Presumably the high concentration of vitamin A in the ration enabled a more rapid build-up of vitamin A reserves within the first week of life. Their liver reserves of vitamin A after four weeks were certainly greater. Harvey, Parrish, Sanford and Hughes (1955), in their work on the utilization of carotene and vitamin A during the first week after hatching, established that chickens develop soon after hatching the biological systems necessary to use vitamin A.

(d) Autopsy Findings.

Five chickens in the groups on the vitamin A deficient rations had kidney lesions typical of vitamin A deficiency. There were no other lesions suggestive of vitamin A deficiency. This was not unexpected, as previous work (Gartner and Ryley 1956) showed that lesions of vitamin A deficiency are normally not apparent in chickens under six weeks of age.

(e) Weight Gains.

Chickens were weighed at hatching and at weekly intervals. Examination of weight data did not show conclusive results. This was due to the high

mortality in some groups as well as to variations in the initial weights of day-old chickens in each rearing trial. The trend was for the best weight gains to be in dss Group, followed by ss, ds, sd and dd Groups.

V. DISCUSSION.

When vitamin A is restricted in the diet, the time taken for fowls to develop clinical symptoms of vitamin A deficiency must be related to their body reserves. At an unexpectedly early stage of their experiment, Temperton and Dudley (1946) produced vitamin A deficiency in pullets after only one month on a diet supplying the equivalent of 454 I.U. of vitamin A per lb. of feed. Taylor *et al.* (1947) induced vitamin A deficiency in laying hens after four months at a feeding level of 540 I.U. of provitamin A per lb. of feed. In our 1955 pilot trial, we were unable to deplete laying hens after over seven months on a vitamin A deficient ration. In the 1956 trial, pullets on the same ration were depleted in 4½ months and again in less than two months when groups were reversed. Chemical analyses confirmed that the differences in depletion time were related to the initial liver vitamin A reserves.

The amount of vitamin A deposited in the egg must also be a function of the body reserves in the laying hen—more so than the amount of vitamin A ingested daily. After more than seven months on the deficient ration, the hens in 1955 still laid eggs containing vitamin A in amounts equivalent to that of eggs laid by hens on the vitamin A sufficient ration. If a comparison can be drawn between the hens and the pullets in the successive experiments, the vitamin A content of the eggs from the hens on the deficient ration was higher than that present in eggs from the pullets on the sufficient ration. The liver vitamin A reserves of these hens were likewise appreciably greater than the reserves of the pullets.

The second trial clearly established the effects of vitamin A deficiency in pullets. Contrary to the findings of Rubin and Bird (1942) and McClymont and Hart (1948) and in agreement with those of Temperton and Dudley (1946) and Heywang (1952), it was found that hatchability is affected by vitamin A deficiency. When chemical evidence of vitamin A deficiency was first established in the periods before and after reversal, the hatchability of the depleted pullets was 40–50 per cent., against about 80 per cent. in the controls. When field evidence of vitamin A deficiency was established, the hatchability was 10–20 per cent. As a further stage of depletion the hatchability reached 0 per cent. The egg production of the depleted pullets was also affected by vitamin A deficiency, though the time required was greater. It was 23 per cent. lower than the controls when the deficiency was first established, 57 per cent. lower when lesions of the deficiency were first apparent, and finally ceased.

The advanced stage of vitamin A deficiency in fowls is comparatively easy to detect by field examination. Administration of vitamin A in appropriate amounts will alleviate the condition and restore egg production and hatchability to normal. Incipient vitamin A deficiency is not easily recognized. Here a fall in egg production may be attributed to a seasonal decline rather than to a nutritional deficiency. As shown by the data on egg production, this decline prior to the appearance of any pathological lesions of vitamin A deficiency is appreciable and thus of considerable economic importance. The marked lowering of hatchability with incipient vitamin A deficiency may be proportionally of even greater economic importance in the case of hatcheries.

Field observations by one of us (H. W. Burton) has indicated that the egg production of pullets is less affected by any disturbance in their early stage of laying than at a later period. In D Group, there was an average of 2–4 weeks intervening between a drop in hatchability and a drop in egg production. At a later stage of laying, the intervening period averaged only 1–2 weeks in SD Group. These differences, as shown by comparison of Tables 3 and 5, may be related to those field observations.

Further observations by one of us (H. W. Burton) in connection with other poultry husbandry trials have indicated that any group of fowls has an egg-laying potential for a laying season and that when any factor which depresses production is removed, the production is thereafter enhanced in an endeavour to maintain this potential. Egg production was depressed in D Group through a deficiency of vitamin A. After reversal, DS Group consistently laid more eggs per pullet per week than SS Group. This is in keeping with the above observations.

It was postulated from the 1955 trial that the deposition of vitamin A in the egg and therefore the hatchability are functions of the liver reserves rather than of the amount of vitamin A in the diet. This appears likely for fowls with adequate reserves of vitamin A. In the 1956 trial, marginal levels of liver vitamin A were encountered in pullets on a deficient ration when the egg production and hatchability had dropped. At the end of the trial, two pullets in DS Group also had marginal liver levels, although they were on a sufficient ration and their egg production and hatchability were normal. Evidently some fowls take longer than others to build up their reserves after a depletion. Thus a knowledge of the vitamin A content of the ration is necessary for the interpretation of marginal liver levels (2 + to 10 μg./g.) in relation to production and hatchability.

From the limited amount of analytical work done on chickens at hatching, it appears that reserves of vitamin A in day-old chickens must be less than 8 μg. before the effect of the deficiency becomes apparent as the reduced hatchability.

Ewing (1947) stated that the depletion of liver vitamin A takes place at least two weeks before any pathological symptoms can be observed. From our findings, this time interval can be as great as eight weeks. This emphasizes

the usefulness of chemical analyses when no clinical symptoms are apparent in the fowls but production and husbandry data indicate a possible vitamin A deficiency. This time interval of eight weeks may be shorter and approach Ewing's estimate if the fowls are under some additional stress at the time. Increased temperatures might provide the additional stress, although the temperatures after reversal were higher than before, yet the time interval between the depletion of liver reserves and the incidence of pathological lesions was still seven weeks.

Of the 24 natural deaths in the deficient groups, four were due to partial or complete rupture of the liver. The liver has a close relationship to the venous vascular system and is formed of cells of glandular type of epithelium. It is accepted that vitamin A plays some role in the development of epithelial cells. Should vitamin A deficiency affect these cells, or cells lining the liver sinusoids, then the haemorrhages could have been due to a generalised fragmentation of these cells and spontaneous rupture. Alternatively, the fragility of the liver, as observed in eight of the 25 pullets that died in the deficient groups and in one pullet in DS Group that died 2½ weeks after reversal, would cause it to rupture when subjected to a trauma of any description. Keratinization of the epithelia is usually associated with vitamin A deficiency. However, epithelial cells with more complex physiological roles and with power to divide, such as liver cells, are not replaced by keratinizing epithelium (Wolbach 1954). Thus in the case of liver cells, keratinization would not be a mechanical hindrance to haemorrhage.

A further three deaths were due to egg peritonitis. In the ova, the follicular cells are responsible for the development of the vitelline membrane, which in turn maintains the yolk in its normal shape. Keratin is one of the components of the vitelline membrane (Romanoff and Romanoff 1949). As mentioned, keratinization of the epithelium can take place in the event of vitamin A deficiency. Should similar changes occur in the vitelline membrane and the amount of keratin be increased, it might result in the loss of elasticity and increased liability for the yolk to rupture. This could have been the cause of some of the mortality from egg peritonitis.

It appears that nodules on the oesophagus, although present prior to death, were either difficult or impossible to detect once post-mortem decomposition had set in. To examine this, a pullet from SD Group, showing nodules on the oesophagus by field examination, was killed by injecting about 10 ml. of air intravenously. It was then left for two days in the pen. Numerous nodules were seen in the pharyngeal area prior to killing it. Examination of the pharynx at autopsy revealed only three recognizable yellow nodules. The remainder of the oesophageal mucosa showed very prominent submucosal glands. These contained no yellow material, but appeared as "craters" approximately 1 mm. in diameter. Removal of the yellow material from typical nodules left similar "craters". It seems not improbable that many of the "craters"

contained nodules at death which were detached together with the general oesophageal debris by bacterial action some time after death. Thus the absence of nodules on the oesophagus in fowls showing marked post-mortem decomposition does not eliminate the possibility of vitamin A deficiency.

The data on the relationship between *Ascaridia galli* infestation and vitamin A deficiency indicate a possible relationship in the first part of the trial. However, no significant differences were found in the parasite burden after the reversal stage of the trial.

VI. ACKNOWLEDGEMENTS.

Our thanks are due to Dr. J. M. Harvey for his help in the preparation of this paper; Mr. L. G. Newton, B.V.Sc., and the veterinary pathology staff of the Animal Research Institute, Yeerongpilly, for their autopsy examinations; Mr. P. J. O'Sullivan, B.Agr.Sc., for the parasitological examinations; Mr. P. B. McGovern, M.A., B.Sc., for his assistance in the planning of the experiment; and Nicholas Pty. Ltd., who supplied the activated magnesia No. 2641 used in the chemical procedure and the vitamin D_3 used in the experimental ration.

REFERENCES.

COOLEY, M. L. 1952. J. Ass. Off. Agric. Chem. Wash. 35: 706.

COOPER, D. M. 1955. Vet. Record. 67: 461.

EWING, W. R. 1947. Poultry Nutrition. Ewing, California.

GARTNER, R. J. W., and RYLEY, J. W. 1956. Qd J. Agric. Sci. 13: 1.

HARVEY, J. D., PARRISH, D. B., SANFORD, P. E., and HUGHES, J. S. 1955. Poult. Sci. 34: 1348.

HEYWANG, B. W. 1952. Poult. Sci. 31: 294.

HUNGERFORD, T. G. 1951. Diseases of Poultry. Angus and Robertson, Sydney.

MCCLYMONT, G. L., and HART, L. 1948. Aust. Vet. J. 24: 5.

MCDOWELL, A. K. R. 1949. J. Dairy Res. 16: 348.

PARRISH, D. B., WILLIAMS, R. N., HUGHES, J. S., and PAYNE, L. F. 1950. Arch. Biochem. 29: 1.

POLK, H. D., and SIPE, G. R. 1940. Poult. Sci. 19: 396.

ROMANOFF, A. L., and ROMANOFF, A. J. 1949. The Avian Egg. Wiley and Sons, New York.

RUBIN, M., and BIRD, H. R. 1942. Maryland Sta. Bul. A12: 339.

SJOLLEMA, B., and DONATH, W. F. 1940. Biochem. J. 34: 736.

TAYLOR M. W., STERN, J. R., RUSSELL, W. C., and JUNGHERR, E. 1947. Poult. Sci. 26: 243.

TEMPERTON, H., and DUDLEY, F. J. 1946. Harper Adams Util. Poult. J. 31: 61.

WOLBACH, S. B. 1954. The Vitamins. Vol. 1. Academic Press, New York.