THE QUEENSLAND JOURNAL OF AGRICULTURAL SCIENCE

VOL. 13. NO. 1.
MARCH, 1956.

VITAMIN A IN POULTRY.

A Survey of Liver Vitamin A Reserves in Fowls and their Relationship to Diseases.

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SUMMARY.

The results of a survey of the vitamin A reserves in fowls in Queensland are presented. The fowls were those from commercial flocks submitted for diagnosis of various diseases. Liver storage of vitamin A was used as the criterion.

Livers of 313 fowls from 162 flocks were analysed. The average level of vitamin A found was 82 micrograms per gram of liver, with a range of less than 0.5 to 820 μg./g. Liver levels between 0 and 2 μg./g. were recorded in 16 per cent. of the fowls; 10.5 per cent. had liver levels between 2+ and 10μg./g.; and the remaining 73.5 per cent. had levels that were considered normal.

All fowls with pustules in the oesophagus had liver levels between 0 and 2 μg./g.

In the age group greater than 6 weeks there were 32 fowls with liver levels between 0 and 2 μg./g., of which 18 had lesions of vitamin A deficiency. In the age group up to 6 weeks there were 19 chickens with liver levels of the same order, of which only two showed similar lesions. It is considered, therefore, that liver analyses are a valuable diagnostic aid in the latter group, where definite pathological lesions occur infrequently.

There appeared to be no correlation between any of the diseases encountered and liver storage levels, except that there was a high incidence of vitamin A deficiency associated with ascaridiasis.

Liver levels in chickens under two weeks of age were much lower (29 μg./g.) than the average for all ages in the survey.

Newly hatched, healthy chickens from 10 hatcheries, with adequate vitamin A in the breeders' ration and good hatchability, showed a mean liver storage level of 19.7 μg./g.

I. INTRODUCTION.

Vitamin A is an essential requirement for poultry at all stages of life. A moderate deficiency causes retarded growth in chickens, and in adults decreased egg production with a fall in hatchability and lowered resistance to infections. A severe deficiency causes loss of condition and finally death, associated usually with a specific pathological picture in the adult, such as pustules in the oesophagus, " frosted " kidneys and distended ureters. It has been shown experimentally that fowls fed rations deficient in vitamin A have decreased resistance to coccidiosis (Murphy, Hunter and Knandel 1938, Taylor and Russell 1947) and to *Ascaridia galli* infestation (Ackert, McIlvaine and Crawford 1931, Seifried 1933).

Relatively few surveys on liver vitamin A levels of healthy or diseased fowls from commercial flocks have been undertaken. Holland, Satterfield and Gauger (1941) examined 85 fowls of all ages suffering from specific diseases and under various managements. They concluded that, with the exception of avitaminosis A, it was not possible to correlate any specific avian disease or internal parasitism with the liver vitamin A stores. Chichester, Russell and Hudson (1938) examined the livers from 154 diseased fowls and compared the results with those obtained from 69 healthy fowls from an experimental flock. They concluded that liver levels of vitamin A in fowls that died from specific diseases showed no significant relationship to the causative agent, the mode of transmission of the disease or the physiological system involved. Rubin, Bird and DeVolt (1941) examined 83 fowls from 25 commercial flocks in which poor growth, general poor condition and in some cases symptoms and lesions suggestive of borderline vitamin A deficiency were evident. They presented evidence to indicate the extent to which poor quality alfalfa (lucerne) may be responsible for vitamin A deficiency. Jungherr (1945), using nasal histopathology as the criterion of vitamin A deficiency, conducted a survey to determine the degree of vitamin A deficiency in commercial poultry flocks. He examined 354 fowls from 252 batches and concluded that there was no correlation between his criterion of vitamin A deficiency and the breed, sex or season or the principal disorders.

In our survey liver vitamin A concentrations were used as the index of the vitamin A status in the fowls for the following reasons. Sherman and Baynton (1926), Moore (1931), McCoord and Luce-Clausen (1934), and Baumann, Riising and Steenbock (1934) showed that in the rat 95 per cent. of the total vitamin A body stores is concentrated in the liver. Moore (1953) stated that under normal nutritional conditions the liver appears invariably to be the main site of storage of vitamin A in mammals and birds. Popper (1941) studied the histological distribution of vitamin A in the human body by means of fluorescence microscopy, and found a homogenous distribution in the liver. Guilbert and Hinshaw (1934), Holmes, Tripp and Campbell (1936), and Baumann, Semb, Holmes and Halpin (1939) have shown that the liver stores of vitamin A in fowls vary with their consumption of the vitamin.

Vitamin A analyses were done on the livers from some of the live fowls with various diseases submitted for autopsy to the Animal Research Institute, Yeerongpilly, Queensland, during the years 1952, 1953 and 1954. The levels found were used for a survey of the incidence of vitamin A deficiency in commercial flocks in Queensland. The relationship, if any, between liver storage of vitamin A and specific diseases was examined. Attention was also paid to the possible value of liver analyses as an aid to diagnosis in young chickens where the symptoms and lesions of vitamin A deficiency are indefinite. In addition, groups of newly hatched, healthy cockerels were selected at random and autopsied in order to obtain normal figures for their liver storage of vitamin A at hatching. The results are presented in this paper.

II. METHODS.

(1) Examination of Fowls.

Live fowls from commercial flocks submitted to the Animal Research Institute for routine diagnosis were used. Initially all fowls submitted from a flock on any one day were examined, but later the number was limited to two fowls from any one flock.

The fowls were killed by dislocating the neck and a complete autopsy was done. The liver was removed and the gall bladder dissected from the liver tissue. Care was taken to prevent bile from contaminating the liver. Each liver was analysed separately.

(2) Chemical Analysis.

The whole liver was prepared for saponification by weighing into a tared flask after cutting into cubes of about one centimetre. Sufficient aqueous 10 per cent. potassium hydroxide was then added to cover the sample. It was not always possible to proceed with the analysis immediately. Moore (1937) and Jensen and With (1939) showed that no appreciable deterioration of vitamin A takes place when liver is stored for a few days under these conditions.

The liver was saponified on a hot plate under reflux until completely disintegrated, which usually took up to one hour. The extraction procedure chosen was an adaptation of that used by Pierce (1945) for blood vitamin A. The saponified liver was made to a definite volume with distilled water. An aliquot was pipetted into a 100 ml. centrifuge tube and made up to 20 ml. with distilled water. For small livers weighing up to 1 gram the total saponification liquor was made up to 20 ml. with water and used for the extraction. Then aldehyde-free ethyl alcohol (20 ml.) was added, followed by 30 ml. redistilled petroleum ether (Shell X222). The centrifuge tube was stoppered and shaken for one minute. At intervals of five minutes it was shaken for two further periods each of one minute. The tube was then centrifuged for five minutes at an R.C.F. value of 1700.

An aliquot of the petroleum ether was pipetted into a photometer test tube of 2-centimetre light path and evaporated over a hot plate under a stream of carbon dioxide gas. The residue was immediately taken up in 2 ml. dry chloroform and two drops of acetic acid anhydride added. Nine millilitres of Carr Price reagent was then added from a fast delivery burette, the intensity of the ensuing blue colour being determined by measuring the transmittance at 620 mμ using a Lumetron photo-electric colorimeter as a direct reading instrument with a high-sensitivity multiple-reflection type galvanometer. The value obtained was read from a graph drawn up on U.S.P. vitamin A reference standard distributed by the Board of Trustees of the United States Pharmacopoeial Convention.

The use of the single extraction procedure with petroleum ether offers a more rapid method than the triple extraction and washing technique with sulphuric ether. Yudkin (1941) showed that petroleum ether gave incomplete extractions of vitamin A from saponified matter as compared with sulphuric ether. This is due to the concentration of fat present in the sample, and Gallup and Hoeffer (1946), working on vitamin A in sheep livers, found that petroleum ether is a suitable solvent provided that a soap equivalent of no more than 0·05 g. of oil is present in the 50 per cent. alcoholic solution to be extracted.

III. RESULTS.

(1) Survey of Commercial Flocks.

(a) General.

In most cases where two or more livers from fowls in the same age group and flock were analysed, the levels were found to be comparable whether deficient, marginal or normal. This is shown in the following cases :—

Flock.	Diagnosis.	Liver vitamin A μg./g.		
A	Vitamin A deficiency ..	<1	1·8	1·4
B	Nephritis	35	30	33
C	Respiratory disease ..	110	95	88
D	Rickets	500	600	400

It is thus considered that generally the analysis of livers from two fowls gives a reliable indication of the vitamin A status in any particular age group. There were, however, some exceptions : (a) where vitamin A supplementation had recently commenced after a deficiency had been diagnosed in the field ; (b) where grossly inadequate feeding space was provided ; and (c) where large numbers of *Ascaridia galli* were found in some fowls and not in others, the former having low and the latter normal levels. In a few cases no explanation was found for individual differences in the levels.

During the survey, livers of 313 fowls from 162 flocks were analysed. The average level of vitamin A found was 82 micrograms per gram of liver. The levels found in individual fowls varied from less than 0·5 μg./g. to 820 μg./g.

The grouping of results is shown in Fig. 1. These show that 16 per cent. of the fowls had liver levels between 0 and 2 μg./g., which we considered indicative of vitamin A deficiency. A further 10·5 per cent. had levels considered marginal (2 + to 10 μg./g.), and the remaining 73·5 per cent. had levels that were regarded as normal.

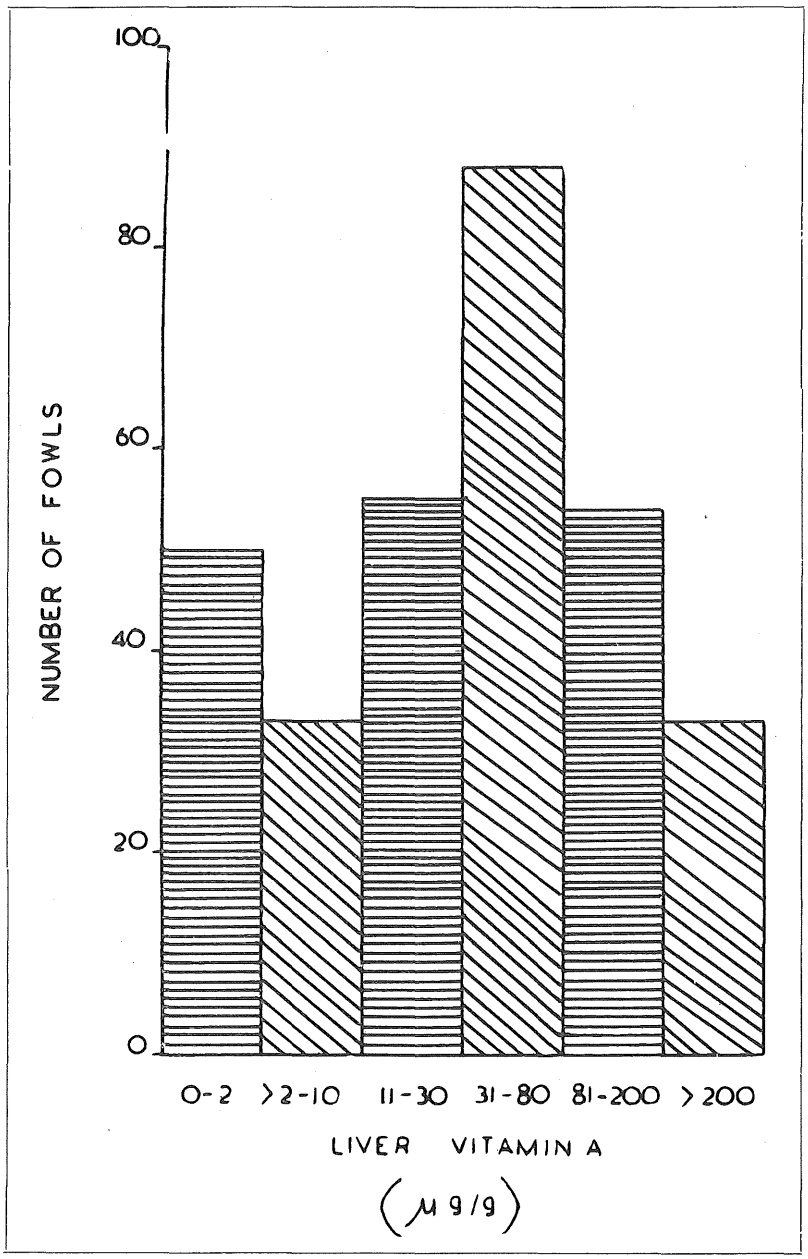

Fig. 1.

Distribution of liver vitamin A levels in the 313 fowls examined from commercial poultry flocks.

The liver vitamin A levels found in chickens up to 6 weeks of age are shown in Table 1. Of the 137 chickens, 19 had deficient vitamin A levels and of these only one 6-week-old chicken showed pustules in the oesophagus and one other kidney lesions. The liver vitamin A levels found in chickens under two weeks of age are lower than the average for the survey.

Table 1.

LIVER VITAMIN A LEVELS AND AGE OF CHICKENS.

Age in Weeks.	Number of Chickens.	Number of Flocks.	Liver Vitamin A (μg./g.).			Number With Levels <10 μg./g.
			Minimum.	Maximum.	Mean.	
0–1	21	11	3	100	21	5
1–2	17	9	0·5	87	37	5
2–3	32	16	< 1	600	99	5
3–4	20	9	< 1	544	113	1
4–5	18	11	< 1	820	88	10
5–6	30	16	< 1	740	124	8

The age group of 0–6 weeks accounts for 44 per cent. of the total number of fowls analysed and for 38 per cent. of the incidence of vitamin A deficiency. The age group of $4\frac{1}{2}$–6 weeks accounts for 12 per cent. of the total number analysed and for 26 per cent. of the incidence of vitamin A deficiency. Jungherr (1945) found that the age group of 5–16 weeks showed a significantly higher incidence of vitamin A deficiency than older fowls.

(b) Correlation of Analyses with Pathological Findings.

All of the 15 fowls showing pustules in the oesophagus had liver vitamin A levels between 0 and 2 μg./g. Five other fowls with kidney lesions, which were likewise considered to be typical of vitamin A deficiency, also had liver levels of this order. There were, however, 31 fowls with levels between 0 and 2 μg./g. not showing any definite lesions of vitamin A deficiency. In some of these fowls the feeding history or the symptoms observed in the flock suggested that vitamin A deficiency might have been a contributing factor in causing deaths. In others a deficiency might have remained undiagnosed without the assistance of chemical analyses. This applied particularly to chickens under 6 weeks of age. In fact, 17 of the 31 fowls not showing definite pathological findings were less than 6 weeks old.

(c) Liver Vitamin A Levels in Specific Avian Diseases.

Liver vitamin A levels found in fowls with specific diseases are shown in Table 2. Diseases such as spirochaetosis, visceral leucosis, histomoniasis, egg peritonitis and tumours are included, but they are not discussed because of the limited number of fowls involved.

Table 2.

LIVER VITAMIN A LEVELS FOUND IN SPECIFIC AVIAN DISEASES.

Disease.	Number of Flocks.	Number of Fowls.	Liver Levels 0 — 2 μg./g.	Liver Levels 2 + — 10 μg./g.	Liver Levels >10 μg./g.
Salmonellosis	4	7	0	2	5
Encephalitis	5	10	0	0	10
Nutritional encephalomalacia ..	16	29	3*	6	20
Rickets	6	12	0	0	12
Caecal coccidiosis	4	6	0	0	6
Intestinal coccidiosis—					
E. necatrix	8	16	1*	0	15
E. maxima	3	6	3	0	3
E. acervulina	3	6	2†	0	4
Fowl pox	9	19	1	4‡	14
Respiratory disease	26	61	10	3‡	48
Fowl cholera	3	5	0	0	5
Bluecomb	5	7	0	0	7
Nephritis (uraemia)	4	6	0	1‡	5
Leucosis	6	7	0	1‡	6
Ascaridiasis§	7	9	6	0	3
Avitaminosis A	12	16	16	0	0
Other diseases—					
Spirochaetosis	1	2	0	0	2
Visceral leucosis	1	1	0	1‡	0
Histomoniasis	3	3	0	0	3
Egg peritonitis	3	3	0	0	3
Tumours	5	6	2†	0	4

* Green feed as the only source of vitamin A supplement.

† Green feed as the only source of vitamin A supplement ; no supplement in the other.

‡ No data on the ration.

§ Ascaridiasis was diagnosed only when more than 25 *A. galli* were present.

Salmonellosis.—Of the seven chickens with this disease, two from the one flock had liver vitamin A levels of less than 10 μg./g. The other five had normal levels.

Encephalomyelitis.—This disease, as seen by us, is similar histopathologically to infectious avian encephalomyelitis, though not yet confirmed. All the liver vitamin A levels in this group were normal.

Nutritional encephalomalacia.—Normal liver vitamin A levels were found in 20 chickens (69 per cent.) in this group. Three chickens (10 per cent.) from one flock, where a carotene-containing foodstuff (grass or lucerne chaff) provided the sole source of vitamin A, had deficient levels. Six chickens (21 per cent.) from four other flocks had marginal levels.

Rickets.—All the 12 fowls in this group had rickets caused by a lack of calcium. The rations appeared adequate in vitamins A and D_3 and the liver vitamin A levels were satisfactory. Although rickets associated with a lack of vitamin D_3 sometimes occurs in Queensland, none was encountered in the survey.

Coccidiosis.—In Table 2 the disease has been divided into sections according to the principal species of coccidia found in the lesions. It was considered that, as beta-carotene is converted into vitamin A in the mucosa of the small intestine (Kon and Thompson 1951), the damage to the mucosa caused by the intestinal parasites might interfere with this conversion in cases where green feed was the main source of vitamin A.

The liver vitamin A levels found in the six chickens with caecal coccidiosis were all normal.

The levels found in cases of intestinal coccidiosis due to *Eimeria necatrix* varied. Of the 16 fowls in this sub-group only two had green feed as the sole vitamin A supplement and one of these was vitamin A deficient. All the 14 other fowls had access to fish oil supplements and their liver levels were normal.

Three of the six fowls with *E. maxima* had deficient vitamin A levels and were all from the same flock. No pustules or kidney lesions were found and the owner claimed they were fed recommended amounts of vitamin A concentrates in addition to green feed.

Of the six fowls with *E. acervulina* two were deficient—one had green feed as the sole source of vitamin A and the other no vitamin A supplement at all.

Fowl pox.—The 19 fowls in this group had the cutaneous form of this disease. Only one was vitamin A deficient and the feeding of the four fowls with marginal liver vitamin A reserves was questionable. The remaining 74 per cent. had normal liver levels.

Respiratory diseases.—This group includes fowls with infectious laryngotracheitis, respiratory fowl cholera, infectious coryza, a disease resembling chronic respiratory disease, and other fowls with respiratory symptoms in which the exact etiology was not determined. Livers of 61 fowls from 26 flocks were examined. Of these, 10 fowls (16 per cent.) from seven flocks were deficient in vitamin A.

Fowl cholera.—The five fowls in this group had the septicaemic form of the disease. The vitamin A levels found in the livers of all these fowls were normal.

Bluecomb.—All the seven fowls examined had normal liver vitamin A levels.

Nephritis.—This condition of unknown etiology has been described by Hungerford (1951) as uraemia and is characterised by swollen, pale kidneys difficult to distinguish from the " frosted " kidneys seen in vitamin A deficiency. However, the history of the outbreak, the symptoms and a lack of distension of the ureters aid in the diagnosis. One of the six fowls in this group had a marginal liver vitamin A level.

Leucosis.—The levels in the six fowls with neural leucosis only were all normal. The seventh fowl with both neural and visceral leucosis (not involving the liver) had a liver vitamin A level of 4 μg./g. No fowls with uncomplicated visceral leucosis were included in the survey. Gordon (1953) found that the majority of fowls affected with leucosis showed sub-normal vitamin A liver levels.

Ascaridiasis.—The nine fowls in Table 2 all had more than 25 *Ascaridia galli* and only three of these had normal liver vitamin A levels. Of three fowls from one flock, one with 50 *A. galli* was deficient in vitamin A, while the other two were free from worms of this species and had normal vitamin A stores.

(2) Liver Vitamin A Levels in Healthy Chickens at Hatching.

Lowered hatchability has been associated with low vitamin A levels in the ration fed to the breeding hens (Polk and Sipe 1940). To obtain normal figures for the liver storage of vitamin A at hatching, cockerels hatched in the one incubator from eggs obtained from 10 different hatcheries were selected at random and autopsied before they had eaten. Twelve cockerels from each hatchery were used and their livers bulked in groups of three, so that four analyses were done on each group. The vitamin A in the rations of the breeding hens from these hatcheries appeared adequate and the hatchability of the total number of eggs set averaged 75·4 per cent. (range, 68·1 – 82·6).

Table 3.

LIVER VITAMIN A LEVELS AT HATCHING IN CHICKENS FROM FLOCKS WITH GOOD FEEDING HISTORIES.

Group.	Breed.	Liver Vitamin A (μg./g.).		
		Min.	Max.	Mean.
A	White Leghorn	17	23	21
B	White Leghorn	21	24	22
C	Australorp	18	24	21
D	White Leghorn	13	18	16
E	Australorp	14	21	18
F	Australorp	17	23	20
G	White Leghorn	21	27	24
H	Australorp	15	18	16
I	Australorp	13	20	17
J	Australorp-White Leghorn Cross	22	24	23

The results obtained in newly hatched, healthy chickens from these flocks with histories of good feeding are shown in Table 3. The results gave no evidence of any relationship between the liver vitamin A concentrations found and the hatchability. It will be noted that the levels found in all the 10 groups are of the same order, with a minimum of 13 μg./g., a maximum of 27 μg./g. and a mean of 19·7 μg./g.

These figures are comparable with those given by Castano, Baucher and Callenbach (1951) for chickens at hatching, namely 65 I.U./g. and 73 I.U./g. (21·7 μg./g. and 24·3 μg./g.)

IV. DISCUSSION.

In fowls, liver vitamin A levels between 0 and 2 μg./g. were considered indicative of vitamin A deficiency, these levels being largely supported by pathological findings attributed to avitaminosis A. Rubin, Bird and DeVolt (1941) chose 15 blue units per gram of liver (8·3 μg./g.) as the "low" point of vitamin A storage, this figure representing approximately half of the lowest concentration found in normal fowls. Arbitrarily, liver vitamin A levels between 2 + and 10 μg./g. have been chosen as marginal levels for this survey.

Of the 313 fowls examined from 162 commercial flocks, 16 per cent. were vitamin A deficient. This high percentage indicates that vitamin A deficiency is still a major cause of economic loss in commercial flocks in Queensland.

In cases of ascaridiasis there was a high incidence of vitamin A deficiency, where six out of the nine fowls examined showed deficient liver vitamin A levels. Seifried (1933) found that vitamin A deficiency increased susceptibility of fowls to internal parasitism. Ackert, McIlvaine and Crawford (1931) showed that fowls on a low intake of vitamin A were more susceptible to *Ascaridia galli* than those receiving an adequate amount. Our findings do not necessarily indicate inadequate vitamin A in the ration, as ascaridiasis is a condition which might be expected to interfere with the absorption and/or utilization of the vitamin. To support this, 50 *A. galli* were present in one of three fowls from a flock on adequate vitamin A supplements, but, whereas the two non-infested fowls had normal liver vitamin A levels, the one with ascaridiasis was deficient.

Seifried (1938) believed that vitamin A deficiency in fowls may favour increased susceptibility to respiratory diseases. The incidence of vitamin A deficiency in the 61 cases of respiratory diseases examined was 16 per cent., this being of the same order as found in the whole survey. Nevertheless, our findings do not necessarily disagree with those of Seifried, as many of the fowls with respiratory disease were examined after an outbreak had been in progress for some time and they may have been supplemented with vitamin A in an attempt to control the outbreak.

Davies (1952), examining 39 fowls infected with caecal or intestinal coccidiosis which were submitted for diagnosis, recorded liver vitamin A levels of 0–128 I.U./g. (0–42 μg./g.) with a mean of 6·9 I.U./g. (2·3 μg./g.). Ten experimentally infected fowls had levels of 2–38 I.U./g. (0·7–12·7 μg./g.) with a mean of 20·8 I.U./g. (6·9 μg./g.). Ten unaffected fowls from the same flock had normal reserves with a mean of 292 I.U./g. (97·3 μg./g.). In our survey there was no correlation between coccidiosis and vitamin A deficiency.

In fowls with intestinal coccidiosis, reliable data regarding green feed supplements as the only source of vitamin A are required to determine whether the lesions caused by the various species of coccidia inhibit the conversion of carotene to vitamin A through damage to the mucosa of the small intestine. These data are often difficult to obtain from owners. In Queensland most commercial mashes also contain some vitamin A concentrate. Therefore, in the limited number of fowls examined, no interpretation could be made of the relationship between intestinal coccidiosis and liver vitamin A levels where green feed was the only source of the vitamin.

Deficient liver vitamin A levels occured in all fowls where lesions, considered to be typical of vitamin A deficiency, were found at autopsy. It was mainly in the age group under 6 weeks that low liver vitamin A levels were found without a corresponding pathological picture. Thus liver analyses of chickens in this age group is a valuable aid in diagnosing vitamin A deficiency, which occurs as frequently in this group as in adults.

Livers of newly hatched chickens from 10 flocks with good hatchability and histories of good feeding had a mean value of 19·7 μg./g. Levels of this order in the livers of chickens at hatching would therefore appear to indicate adequate amounts of vitamin A in the breeder's ration.

The liver vitamin A levels of chickens at hatching are not as high as in the average adult fowl. Only after two weeks of age do their liver vitamin A concentrations build up to levels comparable with the average found in this survey. Further experimental work under controlled conditions is necessary before precise interpretations can be made of the vitamin A status in these chickens from their liver levels. The relationship of hatchability to the vitamin A concentration in the breeders' ration and to the vitamin A reserves in newly hatched chickens may be studied simultaneously.

V. ACKNOWLEDGEMENT.

Our thanks are due to officers of the Poultry Branch, Queensland Department of Agriculture and Stock, who submitted most of the fowls included in the survey and provided information on their feeding.

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(Received for publication Dec. 7, 1955).