STUDIES ON

The Relationship Between Intake of Carotene and Vitamin A and their Content in Egg

A THESIS

Submitted to the Faculty of
Veterinary Science and Animal Husbandry
Magadh University
In Partial Fulfilment of the Requirements
for the Degree of
Master of Science (Animal Husbandry)

November, 1965

BY

A. K. Bose

B.Sc., B.V.Sc. & A.H.

Post-Graduate Department of Nutrition
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I certify that this Thesis has been prepared under my supervision by Sri A.K.Bose, a candidate for the degree of M.Sc.(A.N.) with Animal Nutrition as major subject, and that it incorporates the results of his independent study.

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A C K N O W L E D G E M E N T

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PATNA
November 1965.

A.K. Bose

C O N T E N T S

ILLUSTRATIONS

CHAPTER - I

INTRODUCTION

"Vitamins are organic compounds which are required for the normal growth and maintainance of life of animal including man, who as a rule are unable to synthesise these compounds by anabolic processes that are independent of environment other than air and which compounds are effective in small amounts, do not furnish energy and are not utilised as building units for the structure of the organism, but are essential for the transformation of energy and for the regulation of the metabolism of structural units."

The above definition of Vitamins given by Rosenberg (1942) closely differentiates a group of materials from all other food constituents required by animals including man.

In systematising the knowledge of the nutritional elements other than carbohydrates, fat, proteins, minerals and water, Funk (1912) called this new class of compounds "Vitamines", a term which was later changed to "Vitamins".

A generally recognised Vitamin is one that has been proved an essential dietary constituent for one or more species. Some Vitamins are metabolic essentials but not dietary essentials for certain species because they can be synthesised readily from other foods or metabolic constituents. Thus while Vitamin C has been proved a metabolic essential for many species, it is a dietary essential only in case of

man, guinea pig and monkeys because other animals are able to provide their needs through synthesis.

All animals require a dietary source of Vitamin A. The most important sources of Vitamin A in poultry are alfalfa leaf meal, Berseem leaf meal, silage and greens.

Carrots, Cabbage and spinach are especially rich in carotenes and are widely used in Poultry feeds. Yellow corn contains a considereble quantity of a special provitamin A known as Beta-hydroxy carotene or Cryptoxanthine, predominantly in the form of its ester.

The carotene and Vitamin A in most of the natural feed stuff are highly unstable because both carotene and Vitamin A are extremely sensitive to oxidation, auto-oxidation and light. They are quite stable however to heat in inert atmosphere. In order to ensure optimum stability, Vitamin A is now available in a starch coated matrix of gelatin and sugar so as to protect them from deleterious effects of light, air, moisture and minerals. They are marketted under various trade name by different Firms and are highly suitable as Vitamin A supplement in poultry mash. It is now recognised that there are four different carotenes which have Vitamin A activity namely, Alpha Carotene, Beta-Carotene, Gama-Carotene and Hydroxy-Beta Carotene(or cryptoxanthine).These carotenes occur in plants together with chlorophyll. They are generally

absent from the animal organism. In a remarkable contrast with carotenes, Vitamin A occur only in lipids of animal origin. It has not been found in plant kingdom.

The presence of carotene in Egg Yolk was first suggested by Willstatter and Escher (1911) being later confirmed by Kuhn and Brockman (1932) in several varieties of hens eggs. Whilst the detection of Vitamin A itself was claimed by Euler and Klussmen (1932, 1933). Both carotene and Vitamin A which are fat soluble Vitamins are found only in the lipid fraction of Egg Yolk and not in albumen (Black-1960).

The objective of undertaking the present study was primarily due to the fact that carotene and Vitamin A content of Egg Yolk are closely associated with the hatchability of eggs and livability of chicks, apart from forming an important dietary source of human nutrition.

VITAMIN A AND HATCHABILITY:-

It is now a firmly established fact that hatchability of egg is related to the Vitamin A potency of the egg.But there is conflicting evidence about the quantity required.This state of affair is due in part to the fact that different investigators have used different breeds of chickens and have measured the requirement in different units, as also due to differences in the source of dietary Vitamin A.

Mauch, Gondos, Bratu and Maxim (1958) observed with four groups each of 30 R.I.R. hens that hatchability percentage

is improved with higher Egg Yolk content of Vitamin A. His results are summarised in Table 1.1.

TABLE 1.1

TABLE SHOWING IMPROVED HATCHABILITY PERCENT WITH HIGHER VITAMIN A CONTENT OF EGG YOLK.
Mauch et al.(1958).

Number of Groups.	Vitamin A content in Microgram/gm.Yolk.	Hatchability percent.
1	7.3	65.2
2	8.0	74.8
3	8.1	71.4
4	10.1	79.00

Landau, Marcinke and Sprane (1957) experimented with three groups each of 50 hens, considering the fact that Vitamin A of egg yolk tended to increase as the dietary level of Vitamin A is increased. The first group received a protein mixture according to the usual norms without Vitamin A supplement.

The second group was given a supplement of young fodder cabbage which contained between 1000 to 2000 micrograms of Beta-Carotene per 100 grm.

The third group received a supplement of about 3000 I.U. of Vitamin A acetate in oil in the ration.

Hatchability in round numbers was in the first group 64%; in the second group 68% and in the third group 73% .

Dead embryos were in the same order 14,12 and 4%.

Chicks dead and removed from the experiment were 10, 9 and 6% .

A classical experiment was conducted by Baumann, Semb, Holmes and Halpin (1939) of the Wisconsin station to show the relationship between the Vitamin A content of the diet and its output in egg yolk. He further demonstrated that the livability and Vitamin A content of the chick is related to Vitamin A content of the egg from which it was hatched. These findings are of immense value to poultry breeders. Salient points of his observations are indicated in Table 1.2.

TABLE 1.2

TABLE SHOWING VARIATION IN VITAMIN A CONTENT OF EGG YOLK AT DIFFERENT LEVELS OF INTAKE.
Baumann et al.(1939).

Diet.	Numbers of eggs analysed.	Vitamin A per gm. yolk in micrograms.	Vitamin A per yolk in micrograms. Average.	Range.
Basal	8	6.6	112	89-133
Basal +1% Sardine Oil	8	5.4	96	56-154
Basal +5% Cod Liver Oil	8	7.8	146	105-195
Basal +2% Cod Liver Oil	8	9.2	170	136-235

The Vitamin A content of the eggs ranged from 5.4 microgram per gram of yolk on the Sardine Oil supplement to 9.2 microgram per gram yolk from hens receiving 2% Cod Liver

Oil. Variation within each group were wide.

TABLE 1.3

TABLE SHOWING VITAMIN A CONTENT OF CHICKS HATCHED FROM EGGS CONTAINING VARIOUS AMOUNTS OF VITAMIN A.

Baumann et al.(1939)

Diet in Hen.	Age of chicks in days	Weight of chicks in grams.	Numbers of chick analysed.	Vitamin A per chick in Blue Units.
Basal + 1% Sardine Oil-	1	37	5	23.6
Basal + .50 Codliver Oil-	1	35	5	58.00
Basal + 2% Codliver Oil-	1	33	5	156.00

The amount of Vitamin A in the newly hatched chick varied with the amount of Vitamin A in the Yolk from which it was hatched. Chicks from hens on the Sardine Oil (low Vitamin A) ration averaged 23 Blue Units per chick (1 Blue Unit=60 I.U.Vitamin A). Chicks from hens on 0.5% Cod Liver Oil averaged 58 Blue Units while those from hens on 2% Cod Liver Oil averaged 156 Blue Units. The difference between groups in the Vitamin A content of newly hatched chicks was much wider than the difference in the Vitamin A content of the eggs from which they were hatched.

Thus comparing the groups on Sardine Oil(low Vitamin A) and on 2% Cod Liver Oil the Vitamin A content of the Yolks averaged 96 and 170 micrograms respectively a variation less

than two fold. The Vitamin A content of the newly hatched chicks from these two groups was 23 and 156 Blue Units respectively, a variation of more than six fold.

Small variations in the Vitamin A content of the egg would therefore, appear to be of very considerable importance to the chick hatched there from unless adequate amounts of Vitamin A are available immediately after hatching.

TABLE 1.4

TABLE SHOWING THE EFFECT OF THE VITAMIN A CONTENT OF EGGS ON THE SURVIVAL OF CHICKS HATCHED THERE FROM.

Baumann et al.(1939)

Diet of Hen	Vitamin A in Egg Yolk in microgram.	Vitamin A in newly hatched chicks in Blue Units	No. of Chicks	Survival of chicks on a low Vitamin A diet(in days) Average.	Range.
Basal + 1% Sardine Oil-	96	23	34	~~21.2~~ 13.2	3-26
Basal + 0.5% Cod Liver Oil-	146	58	12	25.00	21-34
Basal + 2% Cod Liver Oil-	170	156	12	31.6	28-37

When the chicks from the various group were placed on a low Vitamin A diet, the periods of survival were roughly proportional to the amount of Vitamin A in the newly hatched chicks.

From the above data certain deductions in regard to Vitamin A metabolism in the fowl are possible. The daily requirement of the chick for Vitamin A can be calculated by comparing the survival of chicks from the various groups with the amount of Vitamin A in the yolk.

Chicks from the 0.5% Cod Liver Oil group survived 25 days, while those from 1% Sardine Oil group survived 13.2 days, on a low Vitamin A diet, a difference of 11.8 days. The Vitamin A content of the two groups of yolks was 146 and 96 micrograms, respectively. A difference of 50 micrograms of Vitamin A extended the lives of the chicks for 11.8 days or additional 4.2 micrograms were necessary per day.

Similarly comparing the chicks from the 2% Cod Liver Oil group with the Sardine Oil group the periods of survival are 31.6 and 13.2 days respectively, a difference of 18.4 days. The amounts of Vitamin A in the yolks are 170 and 96 micrograms respectively, a difference of 74 micrograms.

Thus 74 micrograms of Vitamin A prolonged the lives of the chicks for 18.4 days or additional 4 micrograms of Vitamin A were consumed per day. The two calculations are therefore in agreement.

A very important fact we have learned in recent years that breeding hens have a much higher requirement for certain Vitamins, particularly Vitamin A, B_{12} (or Cyano Cobalamin), riboflavin and Pantothenic acid than do hens

kept for commercial production. Breeding hens d not only have a definite requirement for Vitamins for body maintennance, high egg production and hatchability but they must also have an additional amount of storage and to be used as " Carry Over" through the egg yolk into the body of the chick at hatching time- for the baby chick to begin life with and to aid in the prevention of certain Vitamin- deficiency diseases during the first two or three weeks of live. Lack of carotene and Vitamin A can decrease livability and growth of chicks.

EFFECT OF "CARRY OVER" OF VITAMIN A FROM PARENT STOCK TO CHICKS:-

An outstanding example of showing what an adequate amount of Vitamin A fed to breeding hens will do in the way of increasing livability in their off-spring is shown in the following results obtained by Bearse, Gordon, Miller and Wyne (1937)

TABLE 1.5.

TABLE SHOWING EFFECT OF "CARRY OVER" OF VITAMIN A FROM PARENT STOCK TO CHICKS.

Beerse et al.(1937)

Vitamin A contents of the Breeders mash in U. S. P. Units per Lb.	Livability of chicks in days on a Vitamin-A deficient diet.
0	Hatchability- 0
795	7.3
1590	8.3
3180	19.2
6360	44.7

The lenthened survival of the above chicks from the higher Vitamin A level breeders ration show that the Vitamin A content of the egg yolk is responsible for the Vitamin A content of the chicks body weight at hatching time. It serves to supply the chick with Vitamin A which ensure growth and livability during the initial period of its life.

Though the main objective was to study the relationship between intake of carotene and Vitamin A and their content in egg yolk, some important side aspects

like digestibility of carotene with and without Vitamin A supplement, storage of Vitamin A in liver, level of Plasma Vitamin A and destruction of carotene in feeds due to storage have also been investigated.

CHAPTER -II

REVIEW OF LITERATURES

Differing from mammals, which nourish the embryo inside their bodies, give birth to living young and nurse them, birds produce eggs which contain sufficient nutrients for the embryo to develop outside the body and no preformed food is required after hatching. The extra nutrient in the egg for the embryo is supplied mainly through diet of the hen.

While the mineral content of eggs except for Iodine and manganese is not influenced by the nature of diet, there are marked effects in the case of several of the Vitamins. This is particularly true of Vitamin A, Vitamin D and riboflavin. The kind of ration which will result in the best production and hatchability is also the kind that provides eggs of the highest nutritive value for human consumption.

RELATIONSHIP BETWEEN INTAKE OF CAROTENE AND VITAMIN A AND THEIR CONTENT IN EGG YOLK:-

Baumann et al.(1939) demonstrated that Vitamin A content of egg yolk tended to increase from 5.4 micrograms to 9.2 micrograms per gram of yolk when the Vitamin A

intake was increased from 1% Sardine Oil supplement to 2% Cod Liver Oil.

A similar experiment was performed by Deuel, Hrubetz, Mattson, Morehouse and Richardson (1943), the results of which is summarised in Table 2.1.

TABLE 2.1

TABLE SHOWING EFFECT OF SEVERAL LEVELS OF VITAMIN A INTAKE ON CAROTENOID AND VITAMIN A CONTENT OF EGG YOLK.

Deuel et al.(1943)

Vitamin A supplement per lb. of food in microgram	Carotenoid in microgram per gram yolk.	Vitamin A in microgram per gram yolk.	Vitamin A ~~in~~ ~~I.U.~~ per gram of yolk.
0	32.2 ± 2.0	14.00	41.16 I.U.
333	29.4 ± 1.5	14.00	41.16 I.U.
666	33.0 ± 1.7	13.90	40.86 I.U.
4500	25.3 ± 0.7	14.5	42.63 I.U.
9000	21.7 ± 1.1	16.4	49.21 I.U.
18000	12.9 ± 0.8	18.7	54.97 I.U.
60000	8.4 ± 0.5	36.2	106.28 I.U.

Deuel, Halliday, Hallman and Miller (1941) and Deuel, Hallman, Johnston and Mattson (1942) demonstrated that when massive doses of Vitamin A were given to cows, a

marked suppression of the carotene content of butter fat occured. Fountaine and Bolin (1944) and Jensen, Boyer, Phillips, Rupel, and Lundquist (1942) confirmed the depressing action of massive doses of Vitamin A on the milk carotene in cows.

It has likewise been shown by Deuel et al. (1943) that the administration of massive doses of Vitamin A to chickens results in a pronounced decrease in the carotene content of egg yolk.

Hill, Scott, Norris and Heuser (1961) conducted an experiment to investigate the requirement of hens and their progeny for Vitamin A, using a stabilised source of Vitamin A. In the above experiment they observed egg production, hatchability, blood spot incidence, Vitamin A content of egg yolk and Vitamin A content of hen liver at the conclusion of the experiment. The relationship between dietary Vitamin A and the its content in egg yolk is brought about in Table 2.2.

TABLE 2.2

TABLE SHOWING EFFECT OF DIETARY VITAMIN A ON THE VITAMIN A CONTENT OF EGG YOLK.

Hill et al. (1961)

Dietary Vitamin A level in U.S.P. Units per Lb.	Vitamin A per gram yolk in U.S.P.Units.
800	0.7
1200	8.9
1600	-
2000	6.9
3600	10.6
5000	12.5
10000	16.3

Bandemer, Evans and Davidson (1958) demonstrated that there is a seasonal variation in the Vitamin A content of hen's egg. Five consecutive eggs were monthly analysed from 7 hens fed on a standard all mash ration containing 2000 U.S.P.of Vitamin A per Lb. He observed that Vitamin A content of eggs were highest in April and March for two months varying from 4.08 to 4.64 and 3.97 to 4.61 micrograms per gram of yolk and lowest in July 2.73 to 3.22 microgram per gram yolk.

Krieg (1961) estimated Vitamin A in 485 eggs of white leghorn hen kept intensively in battery cages over a period of 13 months. The hens had the same all mash feed with 3% Lucerne meal and 6500 I.U. Vitamin A per Kg. feed. The monthly average of Vitamin A content of egg varied from 6.50 microgram to 12.65 microgram per gram of yolk.

It is seen that Vitamin A content per gram of yolk estimated by the above workers are highly variable. While Deuel et al. (1943) has obtained highest value of Vitamin A of 41.16 I.U. per gram of yolk, that of Hill et al. (1961) is the lowest of 0.7 I.U. per gram of yolk. The Vitamin A content of egg estimated by other workers lie intermediate between them. The values of Hill et al. (1961) are in some agreement with that of Bandemer et al.(1958). Some of the probable reasons for such variation as mentioned above may be due to difference in such factors as the breed of birds, their level of carotene and Vitamin A intake and methods of estimations.

CAROTENE CONTENT OF EGG YOLK:-

Thudichum (1869) observed that the pigment in egg yolk was unsaponifiable and was exclusively soluble in fat solvents and gave it the name Lutein.

Schunk (1903) isolated the egg yolk pigment and showed by spectroscopic study that it was xanthophyll.

Kuhn, Winterstein and Lederer (1931) reported in contrast to Schunk (1903) that the pigment of the egg yolk was not a single carotenoid but that the egg yolk contained zeaxanthin in addition to the lutein.

Palmer and Kempster (1919) later demonstrated that the carotenoids which are present in egg yolks depended on the carotenoids fed.

Brown (1938) and Mann (1946) have subsequently demonstrated that in general hens deposit in the egg yolk at least part of any carotenoids consumed.

Gillam and Heilbron (1935) have evaluated quantitatively the pigments in several batches of eggs. In each batch the hens were maintained on a different diet. His observations have been summarised in the Table 2.3.

TABLE 2.3

TABLE SHOWING QUANTITATIVE EVALUATION OF CAROTENE, CRYPTOXANTHINE AND TOTAL CAROTENOIDS IN EGG YOLK.

Gillam and Heilbron(1935)

Diets of Hen.	Numbers of eggs analysed.	Total carotenoids in milligram per 100 gram yolk.	Cryptoxanthine in milligram per 100 gm.yolk.	Carotene in milligram per 100gm.yolk
Heavy maize.	60	2.00	0.19	0.015
Grass.	17	4.20	0.14	0.02
No grass.	24	4.40	0.013	0.013
Grass.	24	11.00	0.17	-

It is evident from Table 2.3 that carotene and cryptoxanthine of the egg is slightly variable with the diet and its concentration is higher in heavy maize diet than in the diet containing no green roughage.

Vodarig (1958) reported that on normal diet hens produced yolks containing 2 to 10 microgram Betacarotene. According to Ferrando (1963)Beta carotene varies from 41.2 to 119 microgram per egg.

Jensen (1963) observed when sea weed meal which contains notable amount of Betacarotene when fed

to laying hens at 10 and 15 percent level with a basal ration containing 27 percent yellow corn egg yolk contained 157 and 173 micrograms of carotene Plus cryptoxanthin per 100 gram of yolk.

YOLK COLOUR AS INDEX OF VITAMIN A POTENCY:-

The popular belief that Yolk colour serves as an index of Vitamin A potency of eggs is without basis. The colour of yolk is almost entirely a matter of feeding, though it appears to be influenced somewhat by the rate of laying of an individual hen.

The liberal use of xanthophyll bearing feeds such as fresh or dried green roughage and yellow corn will result in the production of deep yellow yolks, whereas feeding of rations in which these materials are restricted will cause the production of pale yolks, According to Parkhurst (1937) the extent to which any particular feed colours the egg yolk depends primarily upon it a content of xanthophylls and certain plant pigments which are not converted into Vitamin A, a pale white crystalline solid.

Payne, Schumacher, Peterson and Hughes (1942) studied the factors influencing Egg Yolk colour, They observed seven of the nine laying hens that were fed pigments extracted from grass silage produced olive

coloured yolks. The pigment responsible for off coloured eggs was found in the Ether soluble fraction of the alcohol extract of grass silage and appears to be a decomposition product of chlorophyll.

According to current views relative light yolks can be produced if the sole pigment is not over 20.30 percent corn or 5 percent alfalfa meal.

Rauch (1961) observed that upto 30 mg. Beta carotene per kg. feed did not affect yolk colour.

CAROTENE AND VITAMIN A REQUIREMENT FOR LAYING HENS:-

As it is scientifically established now that there is a positive correlation between intake of carotene and Vitamin A in feeds and their output in eggs, it becomes essential to review here some of the literatures dealing with the carotene and Vitamin A requirement of laying hens.

The literatures available on the above subject is again highly varied because the requirements of Vitamin A and carotene are greatly influenced by the stability of these substances in the ration, the number of eggs produced and the environment of the hen.

Most of the experimental work has been done for

the purpose of determining the minimum rather than the optimum requirements of carotene and Vitamin A for egg production and hatchability. From the point of Practical Poultry Husbandry the optimum requirement is more important than minimum requirement.

Record, Bethke, Wilder, and Chamberlain (1937) reported the results of two trials with white lehorn pullets and estimated that the Vitamin A requirements for good egg production and hatchability are from 350 to 400 I.U.of Vitamin A per 100 grams of feed. In another experiment these workers found that a minimum of approximately 400 micrograms of carotene from alfalfa meals (667 I.U. of Vitamin A) per 100 grams of feed was required for good egg production and hatchability.

Russel, Platt, Taylor and Chichester(1936) found that 2200 I.U. of Vitamin A per Lb. of feed gave as satisfactory results for egg production as did larger amounts of Vitamin A.

Sherwood and Fraps (1935) estimated that about 300 Sherman-Munsell Units (429 I.U.)of Vitamin A per 100 grams of feed is required to maintain hens in good health and high egg production. They estimated that it required 750 Sherman-Munsell Units (1071 I.U)

of Vitamin A per 100 grams of feed to allow the hens to produce eggs, the yolks of which would contain a satisfactory amount of Vitamin A.

Beerse and Miller (1937) reported that 500 Sherman-Munsell Units (720 I.U.) of Vitamin A per 100 grams of feed in a breeding ration supplied sufficient Vitamin A for maximum hatchability.

McClary, Miller and Beerse (1942) reported on completion of the experimental work inaugurated several years previously to determine the Vitamin A requirements of growing pullets, laying hens and breeding birds. These requirements were determined to be 250,500 and 700 U.S.P. Units respectively.

Titus (1939) Senior Biological Chemist, Incharge of Poultry Nutrition Investigations, Bureau of Animal Industry, stated that he and his associates from their experience and from other observations have tentatively suggested the following practical standards for the Vitamin A content of poultry feeds:-

For growing chicks 320 I.U.per 100 grams of feed.
For laying hens 700 I.U.per 100 grams of feed.
For breeding stock 1040 I.U.per 100 grams of feed.

He also stated that the minimum requirements

of Vitamin A were undoubtedly very much less than these amounts suggested as practical for feeding.

The Vitamin A requirements for laying hens recommended by different workers have been summerised in the Table 2.4.

TABLE 2.4

TABLE SHOWING VITAMIN A REQUIREMENTS FOR LAYING HENS*

Date.	Investigators.	Units/100 grams.	Units/Lb.	Remarks.
1937-	Record, Bethke, Wilder & Chamberlain	350-400	1590- 1810	
....	-do-	667	3000	From alfalfa leaf meal.
1936-	Russell, Platt, Taylor and Chichester	485	2200	Good health and egg production.
1936-	Sherwood and Fraps	429	1950	
1936-	-do-	1071	4850	Egg of satisfactory Vitamin A content.
....	Titus	700	3165	Tentative-Practical.
....	University of California	550	2500	Minimum practical recommendation.
....	McClary, Bearse and Miller	More than 400	1810	.....
1940-	Sherwood & Fraps	750	3400	Minimum for good egg production.
1940-	Sherwood & Fraps	833	3780	Recommended for safety.
	VITAMIN A REQUIREMENTS OF BREEDERS.			
1937-	Record, Bethke, Wilder & Chamberlain	350-400	1590- 1810	
....	-do-	667	3000	From alfalfa leaf meal.
1937-	Bearse & Miller	714	3225	Maximum hatchability.
1939-	Titus	1040	4720	Tentative practical.
1940-	Sherwood & Fraps	920	4160	Required for hatchability and high Vitamin A marked eggs.

* Quoted from Poultry Nutrition-By Ray Ewing
(Fourth Revised Edition PP-778)

Almquist and Mecchi (1938) considering the above results along with the findings of other investigators came to the conclusion that above 2500 I.U. of Vitamin A per pound of feed may be treated as a minimum practical recommendation for Poultry feeding.

CAROTENE REQUIREMENTS OF LAYING HEN:-

There are only limited number of works where the Vitamin A requirement of the laying hen has been met entirely from Carotene.

Lampman, Williams and Bolin (1939) conducted a series of trials in which laying hens received a basal diet low in Vitamin A but adequate in other known dietary factors, supplemented with alfalfa meal at such rates as to provide 0.1, 0.2, 0.25, 0.3, 0.4 and 0.5 milligram of carotene per bird daily.

Birds receiving 0.2 milligram of carotene per day equivalent 333 U.S.P.Units of Vitamin A maintained good body weight and fair egg production with good hatchability and failed to develop deficiency lesions in the throat, whereas 0.1 milligram per bird daily was definitely insufficient to prevent deficiency symptoms.

Increasing the alfalfa meal intake to provide

0.5 milligram of carotene per bird daily slightly increased egg production but otherwise did not seem to benefit the hen.The higher level resulted in a higher Vitamin A potency of the eggs, better livability of the chicks and a greater storage of Vitamin A in the livers of the hens.

It cannot be said for certain that 0.2 milligram of carotene is definitely the lowest level on which laying hens can live and do well, but it is doubtless very close to the minimum.

Birds were carried on through their second laying year on levels of 0.2, 0.3 and 0.5 mg. of carotene per bird per day. It was thought that if the low level was marginal, it should become apparent during the extended period. The results obtained, however, again have demonstrated the 0.2 level (approximately 333 U.S.P. Units of Vitamin A) to be adequate as measured by egg production absence of lesions, mortality and hatchability.

Wight, Stern, Russel and Jungherr (1947) found that a feeding level of 540 I.U. of Pro-Vitamin A per pound of feed, laying hens developed a serious Vitamin A deficiency in four months, often accompanied by a severe eye infection. At 1170 I.U.per pound results were normal for 6 months, after which egg

production dropped noticeably. A level of 1600 I.U. gave nearly as good results as did 3070 Units except for a slight drop in egg production.

N.R.C.REQUIREMENT OF VITAMINS FOR CHICKENS:-

On the basis of the available evidence from quantitative studies the committee on Animal Nutrition of the National Research Council(1954) has estimated the Vitamins requirement of the Poultry. The requirements are stated in Table 2.5.

TABLE 2.5

TABLE SHOWING VITAMIN REQUIREMENTS OF POULTRY*

Name of Vitamins.	Starting chickens	Growing chicken	Laying hens	Breeding hens.
Vitamin A activity (U.S.P.Units)	1200	1200	2000	2000
Vitamin D_3(I.C.U.)	90	90	225	225
Thiamine(Mg.)	00.8	?	?	?
Riboflavin(Mg.)	1.3	00.8	1	1.7
Pantothenic Acid(Mg.)	4.2	4.2	2.1	4.2
Pyridoxin(Mg.)	1.3	?	1.3	1.3
Biotin (Mg.)	00.04	?	?	?
Cholin(Mg.)	600.00	?	?	?
Folacin(Mg.)	00.25	?	00.11	00.16

*N.R.C.Requirements(1954).

The N.R.C. requirements of Vitamin A is 1200 U.S.P. Units per Lb. of diet for growing chickens and 2000 U.S.P. Units per pound of diet for laying and breeding hens.

There is a growing consensus now among the Poultry nutritionists to revise the N.R.C. requirement of Vitamin A because o the results of individual studies on which the N. R. C. requirement is based have varied widely.

The source of Vitamin A activity used in the various studies on Vitamin A requirement were fish oil, alfalfa meals or yellow corn, all of which were unstable to some degree, especially after mixture in the experimental diets. Even with the precaution of frequent diet mixing, the instability of the Vitamin or its precursors introduced considerable uncertainty into the quantitative estimates of requirements.

Hill, Scott, Norris and Heuser (1961) conducted an experiment for reinvestigation of the Vitamin A requirements of hens and their progeny using one of the stabilised Vitamin A preparations. It was considered appropriate because of the uncertainties concerned in earlier work and the wide variations in estimates derived from the different experiments. They conducted two experiments in successive years to determine the Vitamin A requirements of single comb white leghorn hens. The Vitamin A used in the experimental diet was a commercially prepared product based

on synthetic Vitamin A Palmitate in a beaded gelatin carrier containing an anti-oxidant mixture bf further enhance stability.

According to them the minimum requirement of laying hens for maximum egg production, maintainence of body weight and health, and minimum incidence of blood spotting defect was ~~12~~ 1200-1600 U. S. P. Units of Vitamin A per pound of diet. The lower level of Vitamin A appeared marginal for these functions, but was not improved by the incorporation of an additional anti-oxidant in the diet. The minimum requirement for normal, healthy progeny of hens fed adequate Vitamin A is approximately 600 U.S.P. Units of Vitamin A per pound of diet using a stabilised Vitamin A product.

STORAGE OF VITAMIN A IN LIVER:-

Some Vitamins are stored in the body in large amounts; others to only a very limited extent. Lever is a large store house of Vitamin A in animal body.

Guilbert and Hart (1934) have demonstrated that the total storage of Carotene and Vitamin A in the liver and depot fat of cows which had access to a carotene rich ration throughout life was estimated to be 0.6 to 0.7 gram for the younger animals and upto 3.6 grams in

aged cows. From 67 to 93 percent of the storage was in the liver.

Davies and Moore (1935) in studies with rats have shown that the adult is able to store with massive doses, enough Vitamin A in its liver to supply its theoritical requirements for a century, but that these superfluous stores are eliminated at a very rapid rate until a state of stable storage is reached.

Deuel *et al.* (1943) conducted an experiment on Chickens in which he fed them Vitamin A at different levels and then analysed their liver for Vitamin A storage. Their findings have been recorded in Table 2.6.

TABLE 2.6

TABLE SHOWING EFFECT OF SEVERAL LEVELS OF VITAMIN A INTAKE AND ITS CONTENT IN LIVER OF CHICKEN.

Deuel et al. (1943)

Vitamin A supplement per lb. of food in microgram	Vitamin A in microgram per gram of liver.	Vitamin A in I. U. per gram of liver.
0	111	326.34
333	200	588.00
666	-	-
4500	-	-
9000	1378	4151.32
18000	2082	6121.08
30000	1396	4104.24
60000	1388	4080.72

Hill et al. (1961) performed two similar experiments in two strains of chickens. Their estimation of Vitamin A content per gram of liver tissue is given in Table 2.7.

TABLE -2.7

TABLE SHOWING EFFECT OF DIETARY VITAMIN A ON THE VITAMIN A CONTENT OF THE LIVER OF THE HEN.

Hill et al.(1961)

Vitamin A in USP Units per lb. of diet.	Vitamin A in USP Units per gm.of liver in experiment-1	Vitamin A in USP Units per gram of liver in experiment-2
800	No detectable Vitamin A.	16
1200	17	18
1600	70	-
2000	21	9
3600	-	330
5000	1540	600
10000	-	3100

The data in Table 2.7 show that relatively low levels of Vitamin A were present in the livers of hens fed 2000 U. S. P. Units of Vitamin A per pound or less. The Vitamin A concentration in the liver was markedly increased with higher levels of Vitamin A.

According to them at the lower levels of Vitamin A there was no close relationship between the Vitamin A content of the liver and the productive performance

of the hen or the ability to transfer Vitamin A to the egg yolk. The lower levels of Vitamin A which were adequate for normal productive performance were evidently sufficient to meet tissue requirements but not sufficient to produce high liver storage levels.

One outstanding difference between the two experiments of Deuel et al. and Hill et al., is relatively higher Vitamin A storage per gram of liver tissue at all levels of intake in case of former. The probable reason may be dependent upon the form of Vitamin A fed as well as upon the vehicle used to administer the Vitamin A.

Kurnick et al.(1961) observed that liver storage of pullets ranged from 3.5 ± 10.45 I.U. of Vitamin A per liver when 500 I.U. of Vitamin A per lb. was fed to 336 ± 44 I. U. per liver at 3000 I. U. per lb.

Marusich and Bauernfeind (1963) observed that Vitamin A storage in the liver is cumulative and bore a marked relationship to the amount of dietary Vitamin A and the length of period on that diet. Their findings are indicated in Table 2.8 and 2.9.

TABLE -2.8

TABLE SHOWING STORAGE OF VITAMIN A IN LIVER IN 7 WEEKS AT DIFFERENT LEVELS OF FEEDING.

Marusich et al.(1963)

Supplements	I.U.of Vitamin A per lb. of feed.	I.U.of Vitamin A consumed in 7 weeks.	I.U.of Vit A found in liver.	P.C.of Vit. A stored in liver.
Dry Vitamin A beadlets-	2500	18.310	5150	27.8
Dry Vitamin A beadlets-	5000	36,200	16910	46.8

TABLE -2.9

TABLE SHOWING STORAGE OF VITAMINA A IN LIVER IN FOUR WEEKS AT DIFFERENT LEVEL OF FEEDING.

Marusich et al.(1963)

Supplements	I.U.of Vit.A per lb. of feed.	I.U.of Vit.A consumed in 4 weeks.	I.U.of Vit A found in liver.	P.C.of Vit. A stored in liver.
Dry Vitamin A beadlets-	500	1210	30	2.6
-do-	1000	2380	200	8.3
-do-	2500	5880	1480	25.2
-do-	5000	12060	4320	35.8

The above findings are in accord with the observations made by Guilbert and Hinsew (1934) , Harem, Reid and Couch (1955), Bramus, Scott and Levine (1960) and Kurnick, Reid, Kemmerer Vevich and Heywang (1961).

CONCENTRATION OF CAROTENE AND VITAMIN A IN BLOOD:-

Little work has been done to determine the carotene and Vitamin A content of blood in the field of Poultry nutrition. Vitamin A is not found free in blood but always in association with blood protein as "Vitamin A-Protein Complex". The extensive work which has been carried out in this aspect in the field of human nutrition is briefly discussed in Table 2.10.

TABLE -2.10

TABLE SHOWING CAROTENE AND VITAMIN A CONTENT OF HUMAN BLOOD SERUM ESTIMATED BY VARIOUS WORKERS*

Sl. No.	Name of worker with year	Average carotene content in microgram per 100 c.c.serum.	Average Vitamin A content in I. U. per 100 c.c. serum.
1.	Kimble (1939)	166	127
2.	Murill, Horton, Lefberman and Newburgh (1941)	213 ± 72	93 ± 15
3.	Ralli, Bauman & Roberts (1941)	123	139.56
4.	Harris, Hickman, Jensen & Spies (1946)	210 ± 31	-
5.	Yarbrough (1941)	183 ± 124	71 ± 3.3.
6.	Abels, Gorham, Pack and Rhoads (1941)	210	170
7.	Sinclair (1948)	100	70

*Quoted from "The Lipids" by Harry J. Deuel.

According to some of the above mentioned workers there exists a difference in carotene and Vitamin A content of blood between male and female. The level is higher in male

than in females.

According to Clausen, Baum, Mccoord, Rydeen, and Breese (1940) and Maddock, Wolback and Maddock (1949) serum Vitamin A is normally quite high in the dog. Maddock et al. reported values varying between 173 and 355 microgram per-cent for the Vitamin A value in the serum of normal dogs.

Braun (1945) has recorded in the case of cows, blood serum values as high as 1520 microgram percent carotene, when abundant carotene are available in the diet, although figures as low as 140 microgram percent are also recorded during periods when there are no green feeds. Rasmussen, Cole and Miller (1944) reported that the serum carotene level in the horse is 97 ± 7.8 microgram percent, while the plasma Vitamin A content is only 12.5 ± 3.5 microgram percent. It is believed that the horse converts carotene into Vitamin A quite inefficiently.

Deuel et al. (1943) studied the carotene and Vitamin A content of blood serum in chickens with different dietary level of Vitamin A. His observations are summerised in the Table 2.11.

TABLE-2.11

TABLE SHOWING CAROTENE AND VITAMIN A CONTENT OF BLOOD SERUM IN CHICKEN AT DIFFERENT DIETARY LEVEL OF VITAMIN-A.

Deuel et al.(1943)

Vitamin A supplement in microgram per lb. of food.	Carotene in microgram per 100 c.c. serum.	Vitamin A in microgram per 100 c. c. serum.
0	143.2	139
333	218.0	166
9000	111.4	134
18000	106.8	174
30000	62.6	133
60000	37.0	207

It is evident from the above data that the administration of massive doses of Vitamin A results in a pronounced decrease in the carotene level of plasma. The rise in Vitamin A content of plasma does not follow any definite pattern.

Taylor, Wight, Stem, Russel, Walter and Junghert (1947) recorded that the blood plasma of laying hens receiving 3070 I.U.provitamin A per lb. of feed contains

about 150 I.U. of Vitamin A per 100 c.c. blood serum with wide individual variations. The level of Vitamin A in the plasma is primarily a function of body storage. A drop in the average plasma Vitamin A values to 75 I.U. per 100 ml. indicates marked depletion of the body reserves, while the an average value below 30 I.U. indicates almost total depletion and imminent serious effects.

Squibb (1961) estimated the Vitamin A content in blood serum of white Leghorn hen maintained on a diet containing 3000 I.U. of Vitamin A per lb. of feed. The Vitamin A content per 100 c.c. serum was 41.26 microgram or 120.30 I.U. withwide individual variations.

According to some authors in newly hatched chick serum Vitamin A content was 49.6 ± 3.9 microgram per 100 c.c. of serum.

He observed that the Vitamin A content of blood serum of a flock of white leghorn hen with a history of a borderline Vitamin A deficiency was 30.42 microgram or 39.43 I. U. per 100 c.c. serum, while the results of Taylor et al. and Squibb are in fair agreement with each other, the estimations of Deuel et al. is comparatively high and indicates that the blood serum tend to resist any marked change in Vitamin A concentration higher level of intake.

Further more, Almquist (1952) noted that in

chicks and turkeys, plasma Vitamin A shows an essentially linear relation to the log of the concentration of Vitamin A in the liver.

According to Taylor et al. (1947), Almquist (1952), Squibb (1961) and Barter (1963) the concentration of Vitamin A in the plasma is a fair index of Vitamin A nutrition in Poultry.

DIGESTIBILITY OF FOOD CAROTENES:-

It has been known for some years that carotene has Vitamin A potency when it is fed to animals, still little work has been done on the digestibility of carotene. The amount of carotene which disappears in the passage of feed through the digestive tract is considered to be the amount of carotene digested. It does not take into consideration the amount of carotene destroyed during the process of digestion.

Whitnah, Peterson.Atkinson and Cave (1937) have reported that a large amount of carotene was excreted in the faeces of cows and that from 79 to 210 p.c. of the carotene consumed was recovered in the faeces. The author made no explanation of the excessively high amount of carotene recovered from faeces but it is probable that the supposed carotene determined in the faces was contaminated with other yellow pigments.

Wilson, Dasgupta and Ahmad (1937) reported that carotene eaten by humans in raw or cooked carrots or cooked spinach was about 90% absorbed when the diet contained fat and only 30% absorbed when the fat was omitted.

Van-Eikelen and Pennevis (1938) also report that fat aided the absorption of carotene.

Kemmerer and Fraps (1938) conducted a trial to determine the digestibility of carotene by rats and chickens. He observed yellow pigments in the excrement of rats and chickens which had received feed containing practically no carotene. These colouring materials could not be separated from the carotene by the usual chemical procedure for carotene.

To eliminate this error, he conducted one control experiment in which the feed did not contain any yellow pigment. He subtracted the amount of yellow pigment excreted by the control bird from that of experimental bird. He observed that when carotene in the form of dehydrated alfalfa leaf meal was fed at the level of 20 parts per million or 20 microgram per gram of feed the rats digested 18 to 23 percent of it and the chickens 29 percent. When 1 part per million or one microgram carotene per gram feed was fed the rats digested 43 percent and the chickens 69 percent.

FACTORS AFFECTING DIGESTION AND ABSORPTION OF CAROTENES:-

The extent of digestibility of carotene as well as

its absorption is influenced by a number of extraneous factors.

(1) AMOUNT OF CAROTENE ADMINISTERED:- In the case of rats, Fraps and Meinke (1945) reported that the digestibility of alfa-carotene ranged from 47% in rats receiving 20 microgram daily to 33% for those receiving 60 microgram.

Kemmerer and Fraps (1938) found that the digestibility of Beta carotene in rats varied with the amount fed and with the vehicle. When it was fed to rats in dehydrated alfalfa at levels 1, 10.5 and 20 parts per million the digestibility co-efficients were 43,22 and 18 to 23 respectively.

(2) THE EFFECTS OF THE FOOD GIVEN SIMULTANEOUSLY WITH CAROTENE:-Digestibility co-efficients were much higher for carotene when the provitamin is given in oil.

Thus Kemmerer and Fraps (1938) reported a co-efficient of digestibility of 51 when carotene was given in oil to rats and of 22 when it was fed as dehydrated alfalfa without added fat.

Wilson and Coworkers (1937) observed digestibility of 90% for carotene when it was given in raw or cooked carrots or cooked spinach with fat but the co-efficient of digestibility was only 50 when it was given without fat.

(4)SURFACE AREA AND THE ABSORPTION OF BETA-CAROTENE:- Shaw and Deuel (1944) have shown that the absorption of Beta

Carotene fed in cottonseed oil to rats is a function of surface area. After the feeding of massive doses of approximately 4 mg. (4000 microgram) in a single dose, Beta Carotene was absorbed at the rate of approximately 180 microgram per 100 sq. cm. per hour. After 12 hours, less than 20% of the carotenoid could be isolated from the lumen of the gut; however, at twelve and eighteen hours approximately 50% of the administered dose could be recovered from the wall of the intestine. After 42 hours almost 20% of the carotenoid fed could still be extracted from the gut wall. It seems probable that these conditions may likewise be applicable to the absorption of other carotenoids.

(5)THE EFFECT OF THYROXINE AND THIOURACIL:-Not only is the rate of absorption of carotene controlled by Thyroxine and Thiouracil, but it has also been found that the digestibility of this pigment is a function of the thyroid secretion.

Chanda, Clapham, McNaught and Owen (1951) demonstrated conclusively that the digestibility of carotene was higher during the administration of thyroxine and lower during the feeding of Thiouracil.

According to Chanda et al.(1951) goats were able to digest carotene more effectively than were cows. This difference in digestibility is consistent with the suggestion

of Schultze and Turner (1945) that the thyroid gland is more active in the goat than in the cow.

(6)THE EFFECT OF SUBSTANCES FED CONCOMITANTLY WITH BETA CAROTENE ON ITS ABSORPTION:- The beneficial effects of fats and oils on the absorption of carotene have already been discussed. Other substances when present in the gut simultaneously may likewise help or hinder the absorption of the provitamin A.

(a) Effect of Mineral Oil:- Curtis and Kline (1939) and Alexander, Lorenzen, Hoffman and Garfinkel (1947) reported that plasma carotene values and the Vitamin A in the liver were increased to a lesser degree after the ingestion of carotene in the presence of mineral oil than when the latter substance was absent. These results have been confirmed by a number of workers.

(b) The Effect of Tocopherols:-According to Jackson (1934) the tocopherols are known to enhance the Vitamin A effect of both carotene and Vitamin A.

Major and Watts (1948) were unable to demonstrate any improvement in the utilisation of carotene or any increased deposition of Vitamin A in the liver of rabbits onhigh tocopherol diets as compared with results obtained with feeds low in this Vitamin.

(c) The Effect of Xanthophyll:-The question as to

whether or not the Xanthophyll increase or decrease the utilization of Beta carotene is still uncertain.

Sherman (1947) reported that the addition of Xanthophyll to the diet of rats apparently decreased the destruction of carotene, Vitamin A alcohol and Vitamin A acetate in the gastro-intestinal tract.

However, Kemmerer, Fraps and Mottier (1947) in the same year found Xanthophyll and Chlorophylls when fed with carotene dissolved in cottonseed oil, decreased the effectiveness of carotene for liver storage of Vitamin A by about 20% .

Kelley and Day (1950), High and Day (1950), Vavich and Kemmerer (1950), Collison, Hallman, Martin and Orent-Keiles (1951) are all in agreement that any effect which the Xanthophylls may exert on the Vitamin A storage is concerned with the absorptive rather than with the metabolic phases.

CONVERSION OF CAROTENES TO VITAMIN A:-

Until quite recently it has been generally assumed that the liver is the organ in which Beta-carotene is changed into Vitamin A. Sexton, Mehl and Deuel (1946) were the first to suggest that the intestine is the site of the transformation of carotene to Vitamin A.They stated that" the conversion of carotene to Vitamin A may be an extra-hepatic function in the

rat. The wall of intestine is suggested as a possible site of such transformation". There are several arguments in favour of this concept. In the first place, it was shown that no appreciable amounts of Beta-Carotene are present in the livers of rats, regardless of how large an amount of carotene is given in thediet. Moreoever, it was found that carotene injected intra-splenically, could not be utilised as a source of Vitamin A. Rats died presenting typical symptoms of avitaminoeis A inspite of the fact that large amounts of carotene were present in the livers of the animals at the time of death.

Steenbock and Baumann (1941- 42) likewise have demonstrated that injected carotene was in-effective as a source of Vitamin A. Mattson, Mehl and Deuel (1947) demonstrated that Vitamin A could be detected in the intestinal walls of Vitamin A deficient rats shortly after the feeding of Beta-carotene. Vitamin A appeared in the intestinal wall earlier than in the liver and remained at higher levels there for four hours. The intestinal wall has since been shown to be the site of transformation of Beta Carotene to Vitamin A in the rat by Glover, Goodwin and Morton (1947- 48) and Mc Coord and Clausen (1946); in the chicken by Thompson, Coates and Kon (1950) and Cheng and Deuel (1950); in pig by Thompson, Ganguly and KON (1947, 1949) and in sheep, goats and rabbits by Goodwin

Dewar and Gregory (1946, 1948).

Davies (1952) noted that the Vitamin A storage in avian coccidiosis is decreased. It is suggested that the invasion of the intestinal wall by coccidia may result in impairment of the conversion of carotene to Vitamin A.

EFFECT OF THYROID ON THE CONVERSION OF CAROTENE TO VITAMIN A:-

In addition to playing an important role in the absorption of carotene from the gastro-intestinal tract, there seems to be some evidence that the Thyroid gland in some way regulates the transformation of carotene to Vitamin A.

Munde (1926) noted the appearance of Xeropthalmia in rabbits, eight to twenty months after total thyroidectomy.

Fasold and Heidemann (1933) likewise made the interesting observation that the carotene content of goat milk following thyroidectomy, coincided with a decrease in the Vitamin A content.

Von Euler and Klussman (1933) also pointed out that an antagonism exists between carotene and thyroxine.

Drill and Truant (1947) likewise reported that thyroidectomy in rats is followed by a depression in the carotene-Vitamin A reaction, since it was found to be impossible to relieve ocular symptoms caused by Vitamin A deficiency by means of daily doses of Beta-carotene amounting to as much as 10 microgram.

to as much as 10 microgram.

Goodwin (1948) demonstrated that in the hyperthyroid rat, the converse of the above occurs, namely, that carotene is converted to Vitamin A more efficiently than in the animal which possesses a normal thyroid function.

There are a number of reports in the literature on the effect of hypothyroidism on carotene metabolism when the dificiency has been produced by the administration of thiouracil or thiourea.

Johnson and Baumann (1947) reported that very little storage of Vitamin A occurred in the livers of rats treated with Thiourea or thiouracil followed by administration of carotene; thyroxine increased the ability of these animals to convert carotene to Vitamin A.

Kelley and Day (1948) confirmed the fact that Vitamin A deposition in the liver after the feeding of carotene was decreased in thiouracil-treated rats and increased when thyroid was given.

Although all the above reports are in agreement in supporting the hypothesis that the carotene-Vitamin A change is regulated by the thyroid gland, several reports are in disagreement.

Weise, Deuel and Mehl (1947) were unable to demonstrate any differences in the liver storage of Vitamin A after the administration of 348 micrograms of Beta-carotene irrespective of whether or not the rats had previously been treated with thiouracil.

Morgan and Arnich (1953) also concluded that the normally functioning thyroid gland is not essential for carotene utilisation in rats and dogs.

Although bulk of evidence would seem to support the hypothesis that the thyroid secretion regulates the carotene-Vitamin A reaction, more proof is needed before this theory can be accpted unequivocally.

ABSORPTION OF VITAMIN A FROM THE INTESTINE:-

Vitamin A is much more readily absorbed from the gastro-intestinal tract than is its provitamins-Carotene. In contradistinction to carotene, Vitamin A is as well utilised when given by the parentral route as it is when administered orally.

Sobel, Rosenburg and Engel (1952) reported that when Vitamin A was administered to cows intravenously as an aqueous dispersion, the increased excretion of Vitamin A in the milk was fifteen times as great as when the same

quantity of Vitamin A was given orally dissolved in oil.

(a) Hydrolysis of Vitamin A Esters as a Preliminary to Absorption:- On the basis of the experiments of Gray, Morgareidge and Cawley (1940) it is now generally accepted that Vitamin A esters must first be hydrolysed to the alcohol form before they are absorbed from the gastrointestinal tract. It is believed that an esterase in the intestinal juice is responsible for this hydrolysis.

Gray et al. (1940) found that when the naturally occurring ester was given to rats, a small but steady increase in the amount of free alcohol in the intestinal contents resulted as absorption proceeded.

However, the decesive finding which indicated that hydrolysis of the ester must precede absorption was the proof that the Vitamin A present in the intestinal wall was largely in the form of the free alcohol.

In the samples of gut wall obtained from rats in the three to six hour period after the feeding of Vitamin A ester 82% of the total Vitamin A in the tissues was found to be in the form of the free alcohol.

Eden and Sellers (1950) extended the observation of this phenomenon to calves and sheep. When the animals were slaughtered 4 hours after the administration of Vitamin A

acetate, almost complete hydrolysis had obtained in the contents of the intestinal lumen, in some cases and only a partial hydrolysis in other cases.

When Vitamin A ester was fed, the ester fraction in the mucosa was 73% of the total Vitamin A in the case of calves and 56% the case of the sheep.

On the other hand, the corresponding values for the ester fraction in the intestinal mucosa of the calves and sheep respectively after the administration of Vitamin A alcohol were 82 and 77%. This indicates that esterification of the Vitamin A alcohol occurs after it has been absorbed into the mucosa.

Clausen (1943), Popper and Volk (1944) have further confirmed the hydrolysis of Vitamin A esters prior to absorption.

FACTORS ALTERING THE RATE OF ABSORPTION OF VITAMIN A:-

1) Effect of Age:- Sobel, Boeman and Kramer (1950) reported that Vitamin A was absorbed at a markedly diminish rate in the new born baby as compared with children over one year of age or with that of adults when it was administered either in an oily medium or in an aqueous dispension.

Clausen (1933) reported that the maximum level of Vitamin A in the blood was attained three to five hours after its oral administration in older children.

Refsky and Newman (1948) reported that the absorption of Vitamin A proceeds relatively slowly in the aged.

2) The Effect of Concentration of Administered Vitamin A:- It has been shown that the concentration of the Vitamin A administered is an important factor which influences the rate of utilization in this species.

In the experiment of Reifman, Hallman and Deuel(1943) a direct proportionality was found over a wide range between the concentration of the material fed and the rate of absorption. Their results are summerised in the table 2.12.

TABLE -2.12

TABLE SHOWING THE AVERAGE ABSORPTION OF VITAMIN A (CALCULATED AS I.U. PER 100 SQ. CM.OF BODY SURFACE PER HOUR) FOR THE DIFFERENT DOSAGE OF VITAMIN A.FED.

Reifman et al. (1943)

Dosage of Vitamin A in I.U.	Absorption of Vitamin A in I.U.
100	4.2- 6.5
1000	28.5
10000	369.00
100000	2108.00
1000000	10140.00

(3) Schmidt and Schmidt (1930) reported that Vitamin A can still be efficiently absorbed by Choledotomised Vitamin A deficient rat, i.e., the importance of bile in the absorption of Vitamin A is much less than in the case of carotene.

(4) The Effects of Fat Feeding:-Fat plays a less important role in the absorption of Vitamin A from the gastrointestinal tract than it does in the case of carotenoids.

This fact has been confirmed by De (1937) and Reifmann et al. (1943).

Russel, Taylor, Walker and Polskin (1942)also fund that fat is not essential for the absorption of Vitamin A in the fowl.

(5) The Effect of Mineral Oil:-The presence of mineral oil in the intestine may likewise modify the absorption of Vitamin A but there also the effects are less serious than in the case of Provitamin A.

Moness and Christiansen (1929) as well as Alexander and Coworkers (1947) failed to demonstrate any deleterious effect what-so-ever on the part of mineral oil on the utilisation of Vitamin A.

(6) A Comparison of the Utilisation of Vitamin A Alcohol and Vitamin A Esters:-According to McCoord, Katsampes, Lavender, Martin, Ulstrom and Tully (1948) as well as Sobel,

Bauman and Kramer (1949) the absorption of Vitamin A is believed to proceed equally well in normal subjects when given as the ester or as the alcohol. However, since the hydrolysis of the Vitamin A ester to the alcohol must precede its absorption, any conditions which retard or inhibit the hydrolysis of the ester should at the same time reduce the effectiveness of the ester form. Accepting this as a working hypothesis Week and Savigne (1949) compared the biological responses to Vitamin A given in the form of natural esters, acetate or free alcohol when fed to chickens in jojoba seed oil, ethyl laurate, sharkliver oil, cottonseed oil, corn oil, castor oil or mineral oil. In all cases the Vitamin A deposition is lowest when the natural ester is fed. In general, Vitamin A acetate elicits a slightly lower response than does the free alcohol, but this condition is revised if the quantity of oil given with the Vitamin is reduced to a minimum.

(7) The effect of Emulsifying Agents:-Lecithin has been shown to enhance the absorption of Vitamin A.

Koh and Sutton (1948) as well as Scharf (1947) noted that Vitamin A is better utilised when given with Soybean lecithin than when this phospholipid is absent.

(8) The Effect of Thyroxine:-Reciprocal relationships exist between the metabolism of Vitamin A and that of Thyroxine.

Belasco and Murlin (1940), Shadhu and Brody (1947) and Schultze and Hundhausen (1939) have reported that large

amount of Vitamin A produce an antithyroid action.

THE STABILITY OF VITAMIN A IN THE INTESTINE:-

Baumann, Rising and Steenbock (1934) found that Vitamin A may be largely destroyed in the gastrointestinal tract.

Reifman and Coworkers (1943) were unable to demonstrate any appreciable destruction in three hour test.

Geiger (1952) also reported no carotene or Vitamin A is destroyed by intestinal bacteria over a twenty four hour period.

DESTRUCTION OF CAROTENE AND VITAMIN A OF FEED ON STORAGE:-

In a study of the effect of various factors on the destruction of carotene in plant material Seshan and Sen (1942- 43) found that oxygen plays the predominant part in the destruction of carotene, and heat, light, moisture and enzymes are accelerating agents. The carotene could be destroyed by the Photo-chemical action of ultra-violet light alone was also recognised. Under Indian climatic conditions even specially cured hay containing large amounts of carotene lost its Vitamin A value on storage.

Baird, Ringrose, and MacMillan (1939) reporting on the stability of Vitamin A showed that there is a substantial loss of this factor in chick rations and feed ingredients when these are stored under ordinary conditions for various lengths of time. However, there is considerable disagreement as to the amount of destruction or how rapidly such destruction takes

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Fraps and Treichler (1933) reported losses in Vitamin A potency of 30-80 percent in 6 to 19 months in alfalfa leaf meal, dried peas, yellow corn (whole or ground) and powdered whole milk.

Kick and Bethke (1929) reported very little if any destruction of carotene in yellow corn when stored for one year.

Record, Bethke and Wilder (1937) stated that there was a loss of Vitamin A from Cod Liver Oil and carotene from alfalfa meal when mixed in a ration, but the destruction was not complete even after a storage period of six months in a heated well lighted room.

Fraps and Kemmerer (1936) reported that the Vitamin A in Cod Liver Oil when mixed with feed was entirely destroyed in two weeks when stored at temperatures as low as 7° - 9° C.

Fraps and Kemmerer (1937) also reported that fish liver Oils ~~or~~ concentrates when added to finely divided feeds such as white corn meal, white corn meal and yeast, white corn meal and soybean oil meal lost their Vitamin A potency to the extent of 70 to 100 percent when stored at room temperature and as low as 6° C. for four weeks.

Baird, Ringrose and MacMillan (1939) conducted seven experiments covering respectively 0,4,8,12,16,21 and 25 weeks of storage of the ration to determine the stability of Vitamin A in mixed feed ingredients. When Vitamin A in the

form of fortified codliver oil was mixed in the ration to provide 100, 150, 200 and 300 I.U. of Vitamin A per 100 grams ration, the Vitamin A did not evidence complete destruction even when the rations were stored in burlap bags at summer temperature for 25 weeks, although it underwent progressive destruction as the storage period advanced.

According to Seshan and Sen (1942-43) under Indian climatic conditions even specially cured hay containing large amounts of carotene lost its Vitamin A value on storage, most probably due to a slow spontaneous oxidation of the pigment. The complete disappearance of carotene was merely a matter of time, usually taking from 10 to 15 weeks. Their results are given in table 2.13.

TABLE-2.13

TABLE SHOWING EFFECTS OF STORAGE ON THE CAROTENE CONTENT OF HERBAGE*

Sample Number	Carotene content of dried sample mg/kg.	Period of storage in weeks.	Carotene content after storage mg/kg.	Loss of carotene percentage.
1.	225.2	6	53.6	76.2
2.	215.3	8	26.8	87.5
3.	174.2	10	20.6	88.2
4.	218.9	13	7.4	96.6
5.	103.1	15	5.5	94.7

*Quoted from Animal Nutrition Research in India By K. C. Sen- Page 258 .

Lease, Weber and Steenbock (1938) found that a pronounced destruction of Vitamin A occurs with rancid fats. This is one of the most important reasons for destruction of Vitamin A potency of food stuffs when mixed with fish liver oil.

The variations observed by different workers in the Vitamin A potency of feeds on storage are dependent on various factors like interval of time elapsed after mixing

the food ingredients, presence of ingredients rich in fat content and bad storage conditions.

In order to minimise the destruction of Vitamin A potency various stabilisers have been recommended.

The tocopherolas are now recognised to be the most important stabilising agents for Vitamin A and carotene.

Bacharach (1940), Moore (1940) and Davies and Moore (1941) were the first to demonstrate the synergistic action of tocopherolas on the deposition of Vitamin A in the tissue.

Hickman, Harris and Woodside (1942), Hickman, Kaley and Harris (1944) demonstrated that the growth promoting effect of Vitamin A alcohol, Vitamin A acetate and U. S. P. Vitamin A reference Oil was enhanced when natural Vitamin E preparations were present.

This "sparing" or "synergistic" action of the tocopherols was termed "Co-Vitamin E" activity by Hickman and Coworkers.

The "Co-Vitamin E" action on Vitamin A has been confirmed by Guggenheim (1944) Sanders and Coworkers (1944), Gridgeman (1945), Lenley and coworkers (1947) and Galeone and coworkers(1948).

CHAPTER-III

MATERIALS AND METHODS

To study the relationship between intake of Carotene and Vitamin A and their excretion in eggs, two separate sets of experiments were conducted-- the first one designated as uncontrolled feeding experiment and the second as controlled feeding experiment. The methods adopted for each of them has been stated separately.

I) UNCONTROLLED FEEDING EXPERIMENT:-

In the first experiment Carotene and Vitamin A was estimated weekly in eggs of four groups of hens maintained on four experimental meshes having different Vitamin A potency per kg. of feed.

SOURCE OF EXPERIMENTAL EGGS:-

The birds under experiment belonged to "Cheap poultry ration scheme" of Live Stock Research Station, Patna and they were of white Leghorn breed of the same hatch.

They were maintained in Poultry pens situated in the premises of Government Cattle Breeding and Dairy Farm, Patna. All the pens received the same management.

The birds were divided into four experimental groups each consisting of 25 hens. Each group was again divided into three subgroups of 8, 8 and 9 hens

respectively and were kept in separate pens.

EXPERIMENTAL MASH:-

Each group of hens were maintained on a different experimental mash evolved under the Cheap poultry ration Scheme. The composition of the experimental mashes are noted in Table 3.1.

TABLE -3.1

TABLE SHOWING THE PERCENTAGE COMPOSITION OF DIFFERENT EXPERIMENTAL MASHES.

Name of ingredient.	Experimental mash-I.	Experimental mash-II.	Experimental mash-III.	Experimental mash-IV.
1.Yellow Corn-	20	10	5	5
2.Barley-	10	5	5	5
3.Wheat bran-	20	24	25	-
4.Groundnut Cake-	31	28	16	-
5.Berseem meal-	5	5	5	5
6.Fish meal-	10	-	10	-
7.Silkworm pupe meal-	-	7	-	13
8.Rice polishings-		17	10	35
9.Penicillin waste-		-	20	33
10.Bone meal-	1	1	1	1
11.Common Salt-	0.5	0.5	0.5	0.5
Crude Protein content-	23.42	23.23	23.23	22.36

1.32 grams of Rovimix A- 325 was supplemented with 100 Kilograms of each experimental mash. Rovimix A-325 contained 3,25000 I.U. of Vitamin A per gram.

SELECTION OF EGGS:-

For estimation of Carotene and Vitamin A content of eggs, three eggs were selected as representative sample of each group (one egg from each sub-group) on the basis of random selection on every Tuesday evening between 3-4 P.M.

Immediately after collection the eggs were preserved in refrigerator at 0°C. till they were estimated for Carotene and Vitamin A content. The Carotene and Vitamin A content of three eggs were daily estimated for the next four consecutive days after collection.

PERIOD OF THE EXPERIMENT:-

The experiment was started in the first week of May, 1966 and continued till the second week of July for a period of 11 weeks. It was then discontinued for four weeks for the rainy season to be established fully and taken up again for another two weeks to study the effect if any of the rainy season on Carotene and Vitamin A content of the eggs.

Room temperature was recorded daily throughout the duration of the experiment.

In the later part of this experiment variation in Carotene and Vitamin A content of eggs stored at room temperature for 7, 14 and 21 days were also studied.

These eggs of each group was collected on the basis of random selection as stated above and they were stored at room temperature for 7, 14 and 21 days in a well ventilated room on a enamelled tray. Eggs were estimated for Carotene and Vitamin A content on 0, 7, 14th and 21st day.

II) CONTROLLED FEEDING EXPERIMENT:-

The second experiment was also conducted to study the relationship between intake of Carotene and Vitamin A and their output in eggs but at a controlled level of feeding.

EXPERIMENTAL BIRDS:-

The experiment was performed on four hens of approximately same age and body weight of white leghorn breed purchased from Central Poultry Farm, Patna.

FEEDING OF BIRDS:-

The birds were maintained throughout the experiment on a basal mash, the composition of which is recorded in Table 3.2.

TABLE-3.2

TABLE SHOWING THE PERCENTAGE COMPOSITION OF THE BASAL MASH.

Serial Number.	Name of the ingredient.	Percent of the ingredient
1.	Yellow Corn	50
2.	Groundnut cake	25
3.	Wheat Bran	12.50
4.	Fish meal	7.50
5.	Mineral mixture	3.00
6.	Bone meal	2.00

The hens were maintained for four weeks on a basal mash without any Vitamin A supplement for depletion of body storage of Vitamin A, if any due to previous feeding. During the depletion period eggs were not collected for analysis.

The hens were kept in separate cages for individual collection of eggs and the mash was offered daily in the morning at about 8 A. M.

GROUPING OF BIRDS:-

On completion of four weeks of depletion period, the hens were divided into two groups.

Hen number 1 and 2 formed the experimental group while hen number 3 and 4 formed the control group based on

orm of Vitamin A palmitat
te" manufactured by Roche
in a dilu**t**ed form for
te was a standardised pro
ne million I.U.per gram.
as accurately weighed in
volume was made upto the
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l of the body to be
supplementation

random selection.

On termination of depletion period of four weeks, eggs were collected for two weeks and were estimated for Carotene and Vitamin A content of the eggs.

During the second week of the egg collection period, a digestibility study of Carotene was made on the experimental hens.

The food consumption and the feaces voided by the hen number 1 and 2 of the experimental group were daily recorded for seven days.

The Carotene content of the feaces were daily estimated. The estimation of Carotene from the pooled sample of feaces collected during the week could not be done at the end of the experiment as it was likely to be oxidised on storage.

The total amount of Carotene ingested during the seven days were calculated from the total food consumption. The carotene content of the feed was estimated at the beginning of the digestibility trial.

VITAMIN A SUPPLEMENTATION:-

On expiry of two weeks of egg collection period (without Vitamin A supplementation) Vitamin A supplementation at controlled level was started in the two experimental hens.

A concentrated form of Vitamin A palmitate known as "Vitamin A Concentrate" manufactured by Roche Private Ltd., India was used in a diluted form for supplementation. The concentrate was a standardised product in ground nut oil containing one million I.U.per gram. One gram of Vitamin A concentrate was accurately weighed in a 50 ml. volumetric flask and the volume was made upto the mark with ground nut oil. The content was thoroughly mixed by Shaking.

0.1 ml. of this ground nut oil would therefore contain 2000 I. U. of Vitamin A.

2000 I. U. of Vitamin A was daily administered for three weeks to each of the experimental hen in 0.1 ml. of ground nut oil by means of a graduated one ml. pipette. Separate pipettes were used for the two hens. The pipettes were rinsed with 0.2 ml. of ordinary ground nut oil after supplementation and the rinsings were also administered to each hen.

The volumetric flask containing the diluted "Vitamin A Concentrate" was kept in the refrigerator wrapped in a black cloth.

COLLECTION OF EXPERIMENTAL EGGS:-

To allow the Vitamin A level of the body to be stabilised, during the first week of the supplementation

period no eggs were collected for Carotene and Vitamin A estimation. During the next two weeks eggs were collected from the experimental as well as controll group of hens and their Carotene and Vitamin A content were estimated.

DIGESTIBILITY OF CAROTENE WITH VITAMIN A SUPPLEMENTATION:-

During the last week of the Vitamin A supplementation period a digestibility trial for Carotene was again run for a week with the two experimental hens. The digestibility trial was conducted in the same pattern as stated previously.

ESTIMATION OF VITAMIN A LEVEL IN LIVER AND BLOOD SERUM:-

On completion of 3 weeks of Vitamin A supplementation period- about 20 c.c. of blood was collected by heart puncture from all the four hens for estimation of Vitamin A in serum and then the hens were sacrificed and their liver collected for estimation of Vitamin A.

DESTRUCTION OF CAROTENE IN FEEDS ON STORAGE:-

The destruction of Carotene on storage in the basal mash used in controlled feeding experiment was also investigated.

From the pooled sample of the feed, Carotene was estimated at the beginning and end of the experiment and the percentage of destruction of Carotene due to storage was

calculated.

ESTIMATION OF CAROTENE AND VITAMIN A:-

Carotene was estimated in the biological materials, like feeds, feaces and egg yolk by Bacharach's method (1950) modified by Majumder and Gupta (1960) and Vitamin A was estimated by the method of Oser, Melnick and Pader (1943).

Before proceeding with the actual estimation of Carotene and Vitamin A in the biological materials, to test the accuracy of the method recovery test was conducted with known amounts of added Carotene and Vitamin A respectively. The results of the recovery test experiment have been incorporated in the Chapters on Results and Discussion in Table 4.1 and 4.2.

ESTIMATION OF CAROTENE:-

Observations from various Laboratories have since shown and confirmed that the application of heat or the use of alkali in the extraction process was prejudicial to an accurate determination of Catorene in Biological material, atempts have therefore been made from time to time to refine the technique for Carotene estimation incorporating the advantages of this knowledge.

The method adopted for estimation of Carotene in feeds, feaces and egg yolk is practically the same with minor changes

ESTIMATION OF CAROTENE IN EGG YOLK:-

The waring blend or homogenisation suggested by

Majumder and Gupta (1960) has been found to be very convenient for extraction of Carotene in all types of biological material. This method was therefore adopted for estimation of Carotene in Egg-yolk.

Before proceeding with the actual estimation the weight of each egg was recorded by a sensitive balance.

By breaking the egg shell the yolk was separated out from albumen and was collected in a petridish. To remove the last trace of albumen, the yolk was thoroughly washed with distilled water and then dried over a filter paper. The yolk was finally weighed in a balance.

4 grams of the yolk was accurately weighed in a 50 ml. beaker. 25 ml., 1:1 Acetone and Petroleum ether (boiling point 40-60^{0} C) was added to it along with a pinch of hydroquinone as antioxidant and thoroughly mixed with a glass rod. After mixing the entire material was run in a waring blendor and homogenised for a minute. The extraction was repeated thrice each time adding 25 c.c. of 1:1 Acetone and Petroleum ether. The extract was then filtered through glass wool to remove the suspended materials. The glass wool was further washed with 10 c.c. of 1:1 Acetone petroleum ether mixture to remove the last trace of Carotene with the filtrate. Acetone being miscible with water is removed from the filtrate by washing with water in a separating funnel. Four washings

are considered enough. The residual petroleum ether extract was then collected in a 100 ml. volumetric flask to which was added 10 grams of anhydrous sodium sulphate to absorb the last trace of water. For separation of xanthophylls and other non carotenoid pigments from carotene powder chromatography as suggested by Majumder and Gupta (1960) was adopted. Petroleum ether extract free from traces of Acetone and water was shaken with 7 grams of bonemeal powder in a beaker. Xanthophylls and other non-carotenoid pigments were adsorbed in the bone-meal. The pure Carotene extract was then allowed to stand for half an hour and filtered through a Whatman No.40 filter paper.

Before taking the final reading in the photo-electric colorimeter, the volume was made constant at 10 ml. The reading for Carotene was taken at 440 millimicron wave length using filter No.42. The reading of theunknown was taken against the blank set at zero.

The concentration of the unknown is calculated from the following formula:-

Concentration of unknown=Reading of unknown x Calibration factor.

Where calibration factor = $\frac{\text{Concentration of Standard}}{\text{Reading of Standard.}}$

PREPARATION OF STANDARDS:-

10 milligram of Crystalline Beta-Carotene was dissolved in 100 ml. of Petroleum Ether(b.P 40-60° C).

Therefore 1 ml. of Petroleum Ether contained 100 micrograms of Beta-Carotene.

.05 ml., 0.1 ml., 0.2 ml. and 0.4 ml. of stock solution of Beta-Carotene were taken in different test tubes and the final volume was made 1 ml. with Petroleum ether. Hence, 1 ml. of each standard contained 5, 10, 20 and 40 micrograms of Beta-Carotene respectively. For each concentration of standard, calibration factor was determined in duplicate. From their average value, calibration factor used in the estimation of carotene was finally determined. The calibration factor used in this experiment for estimation of Carotene was 0.12.

PROCESSING OF BONE MEAL FOR USE AS CHROMATOGRAM:-

For the purification of bone meal for use as chromatogram the bone meal was carefully sifted from any adhering tissues, then washed thoroughly with hot tap water and dried. It is then boiled in absolute alcohol under reflex for four hours. After drying it was left in Petroleum ether overnight in a refrigerator and finally dried in the oven. This processing was necessary to remove the fats and pigments if any in it.

ESTIMATION OF CAROTENE IN FEEDS:-

The procedure adopted for the estimation of Carotene in feeds was the same as stated above. The feeds

were finely grinded in a pestle and morter before Carotene was estimated.

In each estimation feeds were extracted four times with 1:1 Acetone-Petroleum Ether Mixture instead of three as it was stated previously for egg yolk. 2 grams of pooled sample of feed was used in each estimation.

ESTIMATION OF CAROTENE IN FEACES:-

Feaces were daily collected quantitatively during the digestibility trial of Carotene and estimation of Carotene was also conducted from day to day to avoid further oxidation.5 grams of representative sample of fences was used for each estimation.

ESTIMATION OF VITAMIN A:-

Vitamin A was estimated in egg yolk, liver and Poultry mashes by the method of Carr, Melnick and Pader (1943) and in blood plasma by the method of Dann and Evelyn (1938).

ESTIMATION OF VITAMIN A IN EGG-YOLK:-

2 grams of egg yolk was accurately weighed in a 50 ml. conical flask. It was then saponified by refleXting on a boiling water bath for 30 minutes with freshly prepared 0.5 N alcoholic Potassium hydroxide using 15 ml. for each gram of sample(Vitamin A is present in the unsaponifiable parts of the animal lipid). It was then cooled and transferred to a separating funnel adding an

equal volume of water as a wash. Extracted four times with 15 ml. of annesthetic ether each time and the aqueous phase was discarded. The ether extract was washed once with 50 ml. water and once with 25 ml. of 0.5 N aqueous Potassium hydroxide. The ether extract was then repeatedly washed with water till the last washing give no pink colour with Phenolpthalien. The last trace of water was removed by adding 10 grams of anhydrous Sodium Sulphate. The ether extract free from all traces of water was evaporated to dryness on a water bath in a 50 ml. beaker. The residue was dissolved immediately in 0.5 ml. of Chloroform and transferred to the cuvette of the photo-electric colorimeter adding an equal volume of chloroform as a wash. 9 ml. of 25 percent Antimony Trichloride solution is then added rapidly to 1 ml. Chloroform containing extracted Vitamin A and a fleeting blue colour is developed which is measured at 620 millimicron wave length using filter No.62, within 4 seconds of the colour development. The reading of the unknown was taken against the blank set at zero.

The concentration of the unknown is calculated from the following formula:-

Concentration of the unknown=Reading of unknown x Calibration factor.

PREPARATION OF STANDARDS:-

10 milligram of Crystalline Vitamin A Acetate was

dissolved in 100 ml. of analytical grade Chloroform in a 100 ml. volumetric flask. Therefore each ml. of Chloroform contained 100 micrograms of Crystalline Vitamin A Acetate.

.05 ml., 0.1 ml., 0.2 ml. and 0.4 ml. of the stock solution of Vitamin A was taken in different test tubes and the volume was made 1 ml. with Chloroform.

Hence, each ml. of the standards contained 5,10, 20 and 40 micrograms of Vitamin A respectively. For each concentration of the standard, readings were taken in a Klett Summerson Photo-electric Colorimeter at 620 millimicron wave length using filter No.62.

For each concentration of the standard calibration factor was determined in duplicate and from their average value calibration factor used in the estimation of Vitamin A , in this experiment was determined. The Calibration factor used in the estimation of Vitamin A in this experiment was 0.267.

25 percent of Antimony trichloride solution was prepared by dissolving 25 grams of Antimonytrichloride in 100 ml. of Chloroform. Antimonytrichloride solution was prepared every week and stored at room temperature.

CORRECTION FOR CAROTENE IN VITAMIN A ESTIMATION:-

Estimation of Vitamin A in presence of Carotene present some difficulty because Carotene which is also

extracted with Vitamin A contributes to the development of blue colour in Carr-price reaction between Vitamin A and Antimony trichloride. It is therefore necessary to make allowance for that part of the blue colour caused by the Carotene-Antimony trichloride reaction. The simplest method is to estimate the carotene separately and apply a correction factor to the value of Vitamin A obtained.

Determination of the correction factor for Carotene was based on the line suggested by Pettand Lepage (1940).

To 1 ml. of Chloroform containing 50 micrograms of Carotene 9 ml. of 25 percent Antimonytrichloride solution was added- it produced a blue colour equivalent to 14.68 micrograms of Vitamin A or one microgram of Carotene produced a blue colour equivalent to 0.29 micrograms of Vitamin A.

In order to apply the correction factor amount of carotene found in any sample was multiplied by 0.29 which gave the value of Vitamin A equivalent for the amount of carotene present in that biological sample and this value substracted from the estimated amount of Vitamin A gave the actual Vitamin A content in the sample.

ESTIMATION OF VITAMIN A IN LIVER:-

The method for estimation of Vitamin A in liver tissue is the same as stated above except that one gm of liver tissue was first triturated in a pestle and morter with 30 ml. of .5 N alcoholic Potassiumhydroxide and then saponified following the same procedure as above.

ESTIMATION OF VITAMIN A IN BLOOD SERUM:-

About 20 c.c. of blood was collected from each hen by heart puncture for estimation of Vitamin A in blood serum.

Vitamin A is not found free in blood. It is always in association with blood protein as "Vitamin A protein complex". "Vitamin A protein complex" in blood is easily broken either by adding alcohol or by saponification.

In the present method (Dann and Evelyn,1938) Vitamin A has been estimated in serum without saponification.

PROCEDURE:-

5 ml. serum was taken in a centrifuge tube of about 25 ml. capacity. While shaking slowly an equal volume 95 percent ethanol was added to it followed by two volumes of Petroleum ether(b.P. 40-60). The centrifuge tube was then stoppered and shaken for 10 minutes. It was then centrifuged for one minute at 1500 revolution per minute. From the supernatant layer 5 ml.

of Petroleum ether was pipetted out and put in the cuvett of the Klett-summerson photoelectric colorimeter. The petroleum ether was evaporated in a water bath and the residue was dissolved in 1 ml. of Chloroform. The blue colour was finally developed by adding 9 ml.of 25 percent Antimonytrichloride solution and the reading of the unknown was taken against the blank set at zero.

The Vitamin A content of the serum was calculated from the formula:-

Concentration of the unknown=Reading of the unknown x Calibration factor.

UNITS OF VITAMIN A ACTIVITY:-

In various experimental work that has been revieved different units of Vitamin A have been used by different workers. In order to make the work comperable all these units, have been converted to International Unit, which is the recognised standard to-day:-

One international unit of Vitamin A is estimated to be equivalent to=.34 microgram of crystallin Vitamin A Acetate.

=.30 microgram of Vitamin A alcohol.

=0.7 Sherman Munsell units.

=0.6 microgram of Beta-carotene.

=1 U.S.P.Unit.

60 I.U.of Vitamin A =1 Blue Unit.

C H A P T E R - IV

RESULTS AND DISCUSSION.

In order to study the relationship between intake of Carotene and Vitamin A and their excretion in eggs, two separate experiments were conducted.

In the first experiment Carotene and Vitamin A was estimated weekly in eggs of four groups of hens maintained on four experimental mashes having different Vitamin A potency per Kg. of feed.

The experimental hens belonged to "Cheap Poultry Ration Scheme" of Livestock Research Station, Patna.

In the later part of the first experiment variation in Carotene and Vitamin A content of eggs stored at room temperature for 7, 14 and 21 days were also investigated.

The experiment started in the first week of May, 1965 and continued till middle of July, 1965. It was then discontinued for four weeks for the rainy season to be established fully and taken up again to study the effect if any of the rainy season on carotene and Vitamin A content of eggs.

The composition of the experimental mashes used in this experiment has been mentioned in Table 3.1 ~~and 3.2~~ in the Chapter on Materials and Methods.

TEST FOR THE ACCURACY OF THE METHODS ADOPTED FOR ESTIMATION OF CAROTENE AND VITAMIN A:-

Before proceeding with the actual estimation of Carotene and Vitamin A in the biological materials accuracy of the methods of estimation adopted was tested by recovery test experiments. The results are recorded in Table 4.1, 4.2, 4.3 and 4.4.

TABLE- 4.1

TABLE SHOWING THE AGREEMENT BETWEEN DUPLICATE ESTIMATION OF CAROTENE IN EGG YOLK.

Number of observation	Source.	Total Carotene content in micrograms.	Average Carotene content in micrograms.
1.	3 grams of egg- yolk	4.65	4.77
2.	-do-	4.89	

TABLE- 4.2

TABLE SHOWING PERCENT OF CAROTENE RECOVERED FROM EGG YOLK AFTER ADDITION OF KNOWN AMOUNT OF CAROTENE.

Source	Total Carotene content in micrograms.	Amount of Carotene recovered	Percentage of Carotene recovered after addition of known amount of Carotene.
3 Grams egg yolk-	4.77	-	-
3 Grams egg yolk+ 10 micrograms Beta Carotene-	14.77	13.93	94.31

TABLE-4.3

TABLE SHOWING THE AGREEMENT BETWEEN DUPLICATE ESTIMATION OF VITAMIN A IN EGG YOLK.

Number of Obser-vations.	Source	Total Vitamin A con-tent in micrograms.	Average Vitamin A con-tent in micrograms.
1.	3 grams of egg yolk.	12.39	12.26
2.	-do-	12.13	

TABLE-4.4

TABLE SHOWING PERCENT OF VITAMIN A RECOVERED FROM EGG YOLK ON ADDITION OF KNOWN AMOUNT OF VITAMIN-A.

Source	Total Vitamin A content in micrograms.	Amount of Vitamin A recovered.	Percentage of Vitamin A recovered after addition of known amount of Vitamin A
3 grams egg yolk-	12.26	-	-
3 grams of egg yolk+ 10 micrograms Vitamin A	22.26	19.33	86.83

Though there is a fair agreement between duplicate estimation of Carotene and Vitamin A, hundred percent recovery of Carotene and Vitamin A could not be obtained may be because some of the Carotene and Vitamin A were oxidised during the process of extraction.

ESTIMATION OF CAROTENE IN EXPERIMENTAL MASHES:-

From the pooled sample of each feed, Carotene content of the four experimental mashes were estimated which is recorded in Table 4.5

TABLE 4.5

TABLE SHOWING CAROTENE CONTENT OF THE DIFFERENT EXPERIMENTAL MASHES.

Experimental mashes.	Carotene content in milligram per kg of mash.	Carotene content converted to Vitamin A in I.U. per Kg. mash.
Experimental Mash-I	2.25	3735
Experimental Mash-II	2.74	4548
Experimental Mash-III	1.88	3120
Experimental Mash-IV	3.42	5677

The sources causing variation in the Carotene content of the mashes may be the various ingredients used in the experimental mashes.

Barley, wheat bran, ground nut cake and rice polishings cannot perceptibly affect the Carotene content of the different mashes is concluded from the fact that they are extremely poor sources of Carotene.

Berseem meal which is present at 5% level in all the experimental mashes is supposed to contribute equally

for carotene content in all the mashes.

Yellow corn at 20, 10, 5 and 5 percent level in the experimental mash I, II, III and IV respectively are likely to affect slightly the Carotene content of the mashes.

The above facts lead to the inevitable conclusion that fish meal, silk worm pupa meal and Penicillin waste the three ingredients used in the mashes are the contributory factors for variation in Carotene contents of feed.

In order to pin point the exact source causing the variation in Carotene content of feeds, the fish meal, silk worm pupa meal and Penicillin waste the ingredients used in different experimental mashes were analysed for Carotene and Vitamin A content and the results are indicated in Table 4.6.

TABLE 4.6

TABLE SHOWING CAROTENE AND VITAMIN A CONTENT OF FISH MEAL, SILK WORM PUPA MEAL AND PENICILLIN WASTE.

Sl. No.	Name of the ingredient.	Carotene content in milligram per kg.	Vitamin A in I. U. per Kg.
1.	Fish meal-	trace	Nil.
2.	Silk worm pupa meal-	11.81	Nil.
3.	Penicillin waste-	Nil.	Nil.

From perusal of Table 4.6, it is evident that silk worm pupa meal is the main contributory factor causing the variation in Carotene content of the mashes.

Lampman *et al.* (1939) conducted a series of experiments to find thet Carotene requirement of laying birds. He observed that 0.2 mg. of Carotene per bird per day to be adequate as measured by egg production, absence of lesion, mortality and hatchability. This requirement of Carotene for the laying birds can be met from all the experimental mashes. Considering the fact that a laying bird on an average eats 4 ounces (112 grams) of mash daily.

The N. R. C. requirement of 2000 I. U. of Vitamin A activity per Lb. of feed can only be met from the Carotene content of the experimental mash II and IV.

It is also interesting to note here that none of the animal protein sources in the feeds contained any preformed Vitamin A though animal protein sources are generally supposed to possess some pre-formed Vitamin A, which of course depends upon their industrial method of preparation.

In addition to natural Carotene content ofthe feeds to meet the N.R.C. requirement of Vitamin A of laying hen, each experimental mash was supplemented with 4290 I.U.of Vitamin A per Kg. of mash in the form of Rovimix A 325, a stabilised form of Vitamin A, manufactured

by Roche Private Ltd., India.

CAROTENE AND VITAMIN A CONTENT OF EGG YOLK IN DIFFERENT EXPERIMENTAL GROUP OF HENS:-

The carotene and Vitamin A content in the egg yolk of different experimental group of hen have been recorded in Table 4.7, 4.8 and 4.9

TABLE-4.7

Table showing weekly average (of 3 eggs) carotene content of egg yolk of different experimental groups:

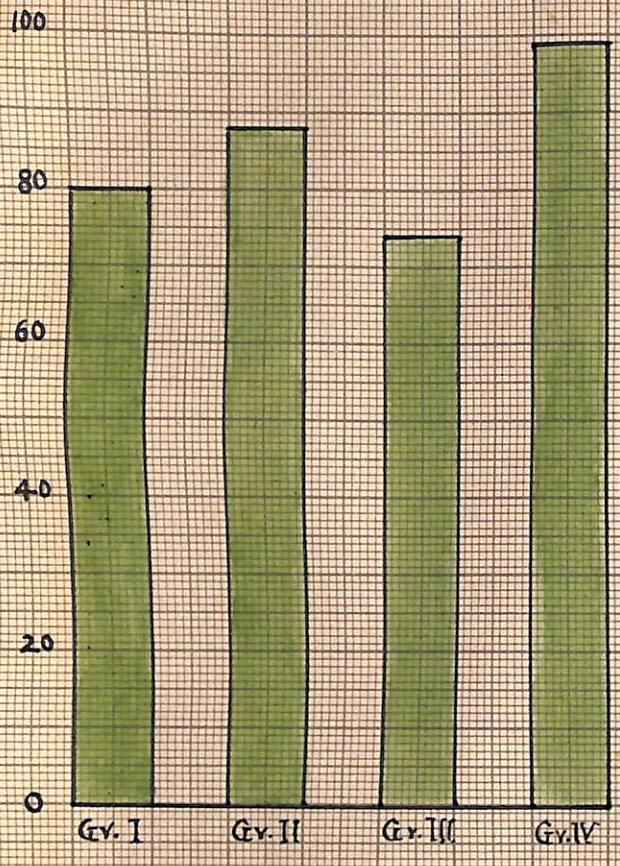
Date of collection of eggs of	Weekly average Carotene content in micrograms per gram of egg-yolk			
	Experimental Group-I	Experimental Group-II	Experimental Group-III	Experimental Group-IV
4.5.65	1.63	1.59	1.46	1.84
11.5.65	1.55	1.57	1.46	1.85
18.5.65	1.54	1.58	1.44	1.82
25.5.65	1.49	1.55	1.43	1.78
1.6.65	1.45	1.53	1.40	1.69
8.6.65	1.42	1.49	1.39	1.63
15.6.65	1.36	1.40	1.36	1.57
22.6.65	1.43	1.51	1.40	1.60
29.6.65	1.49	1.52	1.46	1.63
6.7.65	1.57	1.58	1.49	1.66
13.7.65	1.59	1.59	1.48	1.68
10.8.65	1.57	1.63	1.52	1.69
17.8.65	1.60	1.58	1.52	1.65

TABLE-4.8

Table showing weekly average (of 3 eggs in each group) Vitamin A content of egg yolk of different experimental groups:

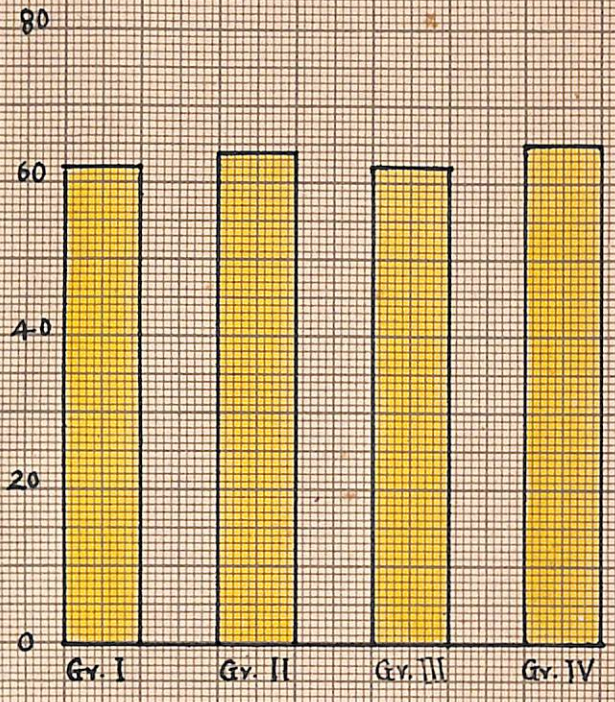
Date of collection of eggs.	Weekly average Vitamin A content in micrograms per gram of egg yolk.			
	Experimental Group-I	Experimental Group-II	Experimental Group-III	Experimental Group-IV
4.5.65	4.58	4.67	4.56	4.76
11.5.65	4.56	4.63	4.53	4.71
18.5.65	4.56	4.62	4.54	4.67
25.5.65	4.53	4.60	4.49	4.64
1.6.65	4.47	4.54	4.47	4.60
8.6.65	4.40	4.43	4.34	4.47
15.6.65	4.34	4.38	4.27	4.34
22.6.65	4.43	4.54	4.39	4.51
29.6.65	4.52	4.60	4.52	4.67
6.7.65	4.60	4.73	4.52	4.71
13.7.65	4.56	4.71	4.54	4.67
10.8.65	4.56	4.74	4.47	4.78
17.8.65	4.56	4.67	4.46	4.72

The relative Vitamin A potency per kg. of mash and its content per gram of egg yolk of the four experimental group is shown in the Histogram-I.



1 Small Sq. = 100 I.U.

FEED



4 Small Sq. = 1 I.U.

EGG YOLK

TABLE-4.9

Table showing average carotene and Vitamin A content of egg yolk during the experimental period.

Experimental Group.	Carotene content in milligram per Kg. of experimental mash.	Carotene content in microgram per gram of yolk.	Vit.A content in microgram per gram of yolk.	Vit.A potency in I.U. per gram of yolk.
Experimental Group-I	2.25	1.51	4.51	15.75
Experimental Group-II	2.74	1.55	4.60	16.09
Experimental Group-III	1.88	1.45	4.46	15.51
Experimental Group-IV	3.42	1.70	4.63	16.43

From perusal of Table 4.7, 4.8 and 4.9 it is evident that in all the four experimental groups carotene and vitamin A content of the egg yolk is dependent on the carotene content of the meshes (vitamin A supplementation was the same in all groups).

The carotene and Vitamin A content of Egg yolk in experimental group IV is the highest having maximum Vitamin A potency per kilogram of feed while that of experimental Group III is the lowest having the minimum Vitamin A potency per kilogram of feed. The relative Vitamin A potency per Kg. of mash and its content per gram of yolk of the four experimental group is shown in the Histogram-I.

CAROTENE CONTENT OF EGG YOLK:-

The carotene content of the egg yolk in experimental group I, II, III and IV are 1.51, 1.55, 1.45 and 1.70 micrograms respectively.

The carotene content of the experimental mash 1 and 2 are very close to each other and so also the carotene content of the eggs of these two experimental groups.

According to Gillam and Heilbron (1935) the quantities of carotene, cryptoxanthene and xanthophylls are in the order of 0.015, 0.019 and 1.79 milligram per 100 gram of yolk.

According to Vodering (1958) and Ferrando (1963) the egg yolk contains 2 to 10 and 41.2 to 119 micrograms of Beta-Carotene per egg respectively.

Our result though in agreement with that of Ferrando (1963), it is a bit high in comparison to that of Gillan and Heilbron (1935) and Vodering (1958).

On review of work of Ferrando (1963) it appears that he got 157 and 173 micrograms of carotene including Cryptoxanthene per 100 grams of sea weed meal which contains notable amount of carotene was fed to laying hens at 10 and 15 p.c. level in basal ration containing 27% yellow corn.

Hence the appearance of carotene content of eggs observed in our study may be on account of contamination of carotene with crypto-xanthene that could not be separated completely from carotene by chromatography method, which is a rapid chromatography than column chromatography.

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Hence the apparently high carotene content of eggs observed in our study may be due to contamination of carotene with crypto-xanthene, which could not be separated completely from carotene by Powder Chromatography method, which is a rapid chromatographic process than column chromatography.

VITAMIN A CONTENT OF THE EGG:-

It is evident from Table 4.9 that Vitamin A content of the egg yolk in the experimental group I, II, III and IV are 4.51, 4.60, 4.46 and 4.63 micrograms per gram of yolk respectively, i.e. higher the carotene content of the experimental mash, greater the Vitamin A content of the egg.

Baumann et al (1939) demonstrated that Vitamin A content of egg yolk tended to increase from 5.4 micrograms to 9.2 micrograms per gram of yolk when the Vitamin A intake was increased from 1% Sardine Oil (Low Vitamin A) supplement to 2% Cod Liver Oil (High Vitamin A).

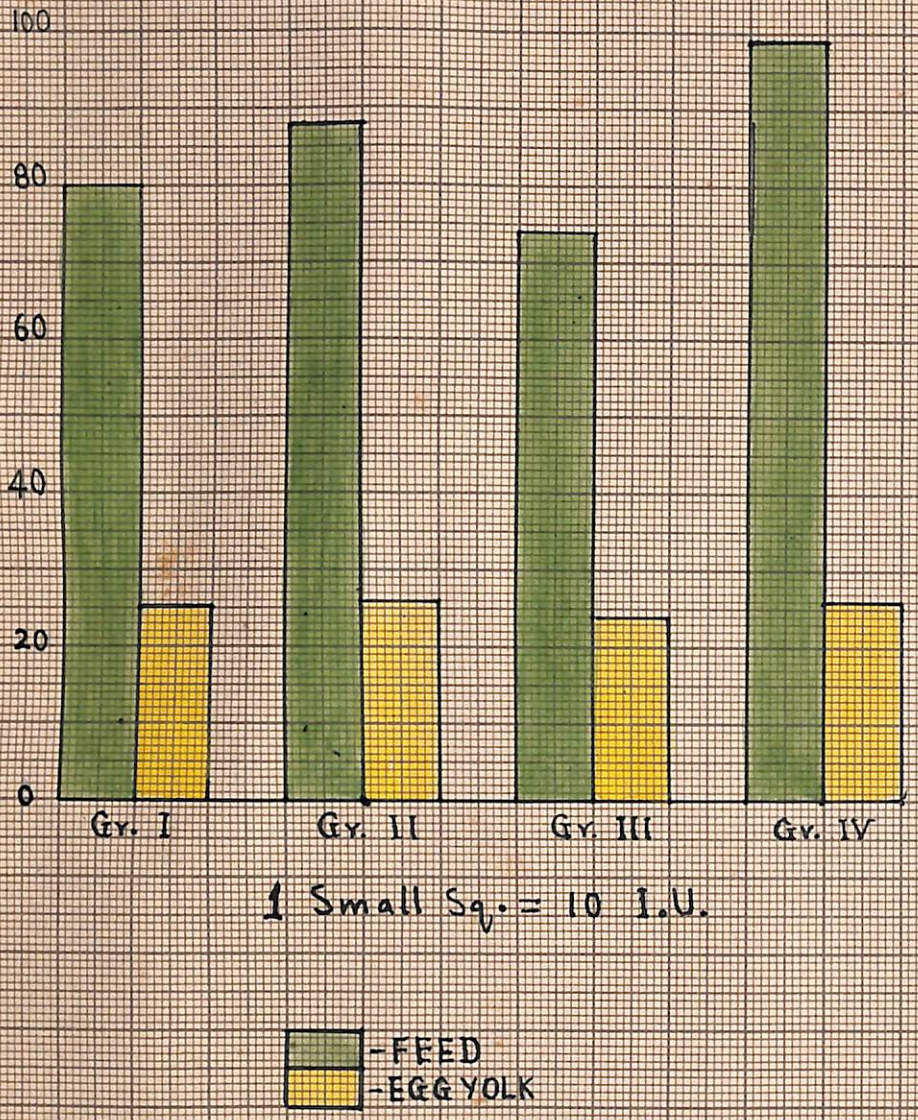
Deuel et al (1943) observed that Vitamin A content of egg yolk rose from 14 to 36.2 micrograms per gram of yolk when Vitamin A supplementation per Lb of food was 0 and 60,000 micrograms respectively.

Hill et al (1961) recorded that Vitamin A content of egg yolk increased from 0.7 to 16.3 I.U. per gram of yolk when dietary Vitamin A level was 800 and 10,000 USP units per Lb respectively.

Our result supports the findings of Baumann et al (1939); Deuel et al (1943) and Hill et al (1961) that Vitamin A content of egg tends to increase with higher dietary level of Vitamin A.

The Vitamin A content of egg yolk estimated by various workers comparitively at the same dietary level have varied widely.

The relative Vitamin A potency between intake (considering 100 grams as average daily food consumption) and its content in an average egg yolk (16 grams weight) of the four experimental group is shown in Histogram-II.



Deuel et al (1943) observed that on feeding 4500 microgram of Vitamin A (13230 I.U. approximately) per Lb of feed the egg yolk contained 14.5 microgram of Vitamin A (42.63 I.U.) per gram of yolk.

Hill et al (1961) obtained a value of 16.3 U.S.P. of Vitamin A per gram of yolk when dietary Vitamin A level was 20,000 (ten thousand) U.S.P. Units per Lb.

In our result the experimental group IV which had a Vitamin A potency of about 5,000 I.U. per Lb of feed, the average content of Vitamin A is 16.43 I.U. per gram of yolk.

Some of the probable reasons for variation in the Vitamin A content of egg yolk observed by the above mentioned workers may be due to difference in such factors as the breed of birds, sources of Vitamin A supplement and methods of estimation.

The relative vitamin A potency between intake (considering 100 grams as average daily food consumption) and its content in an average egg yolk (16 grams weight) of the four experimental group is shown in Histogram-II.

STATISTICAL ANALYSIS:-

Statistical test was conducted to test the effect of different treatments on the Vitamin A and carotene content of egg yolks.

Analysis of variance (Snedecor-1957) which was run to test the variation between treatments, between weeks and between animals is indicated in Table 4.10

TABLE-4.10

Analysis of variance showing the effect of different feeds on carotene and Vitamin A content of egg yolk.

Sources of variation.	Carotene D.F.	Carotene M.S.	Vitamin A. D.F.	Vitamin A. M.S.
Between treatments-	3	0.4524**	3	0.1127**
Between weeks-	12	0.425 **	12	0.2334**
Between animals-	11	0.1267**	11	0.0833**
Error-	129	0.0056	129	0.007
Total-	155		155	

** Significant at 1% level.

On perusal of table 4.10 it is evident that variation between treatments, between weeks and between animals each is highly significant for both Vitamin A as well as carotene of egg yolk.

TABLE-4.11

Table showing the weekly average maximum room temperature with date of collection of eggs in each week.

Sl. No. of week	Date of collection of eggs during the week.	Average weekly maximum temperature in (^{o}C.).
1.	4-6-65	40.15^{o}C.
2.	11-6-65	40.07^{o}C.
3.	18-6-65	42.47^{o}C.
4.	25-6-65	42.60^{o}C.
5.	1-6-65	42.30^{o}C.
6.	8-6-65	38.18^{o}C.
7.	15-6-65	35.37^{o}C.
8.	22-6-65	36.66^{o}C.
9.	29-6-65	30.07^{o}C.
10.	6-7-65	34.06^{o}C.
11.	13-7-65	33.60^{o}C.
12.	10-8-65	31.78^{o}C.
13.	17-8-65	30.94^{o}C.

EFFECT OF HIGH ENVIRONMENTAL TEMPERATURE ON CAROTENE AND VITAMIN A CONTENT OF EGGS :-

That high environmental temperature appears to have an adverse effect on the carotene and Vitamin A content of eggs is evident from Table 4.7, 4.8 and 4.11.

In the first week of the experiment the average Vitamin A potency was 16, 16.35, 15.82 and 17.04 I.U. per gram of yolk in the experimental group, I,II, III and IV respectively.

The carotene and Vitamin content of egg yolk decreased to a minimum value with increasing environmental temperature. The Vitamin A potency was lowest on the seventh week of the experiment being 15, 15.19, 15.12 and 15.40 I.U. per gram of yolk in the experimental group I,II,III and IV respectively.This finding is further supported by the fact that the analysis of variance between weeks was also highly significant (Table 4.10).

With the on set of rains on the 3rd of week of June the temperature fell and the carotene and Vitamin A content of egg tended to return to their normal value.

On the 13th week of the experiment the average Vitamin A potency was 16.05, 16.34, 15.163 and 16.60 I.U. per gram of yolk in experimental group I, II, III and IV respectively, almost at the same level as in the first week

of May, whether the fall of carotene and Vitamin A content of eggs is due to lower intake of carotene and Vitamin A on account of lower food intake at high environmental or due to higher Vitamin A requirement of the hen during the period of stress caused by high environmental temperature is not definitely known.

Squibb, Braham and Arroyave (1958) showed in short time experiments that a temperature of 36°C. depresses serum Vitamin A levels in rats but not in hens and arrived at the conclusion that the hens do not appear to be very sensitive to moderate increase in environmental temperature.

Adams, Andrews, Gardiner, Fontaine and Carrick (1962) showed that increasing the Vitamin A in the diet from 1000 to 1[illegible] I.U. per 100 grams did not improve the performance of [illegible]hicks kept at 90°C.

Asca[illegible]nd Bartov (1963) studied the Vitamin A requi[illegible] of chicks at moderately high temperature. T[illegible]ments were carried out to test the influence [illegible]peratures one varying between 21°-25°C. in a[illegible]tioned room and the other at 28°C. with arr[illegible] extra heating for about 6-7 hours a day to [illegible]C. in order to simulate variations in [illegible]ased V[illegible]ng hot summer days.

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[illegible]st week o[illegible]

[illegible]s high as [illegible]

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Ascarelli and Bartov (1963) studied the Vitamin A requirement of chicks at moderately high temperature. The experiments were carried out to test the influence of two temperatures one varying between 21°-25°C. in an air conditioned room and the other at 28°C. with arrangement for extra heating for about 6-7 hours a day to reach 33°-34°C. in order to simulate variations in temperature during hot summer days.

The birds were kept at 3 levels of Vitamin A 75,150 and 300 I.U./100 grams feed. All the usual criteria of Vitamin A activity (growth, plasma level, liver storage and survival) were investigated.

He observed the response to increase in levels of Vitamin A was similar with both environments and his findings did not support the hypothesis of increased Vitamin A requirement at high environmental temperature.

Rurnic, Reid, Kemmerer, Vevich and Heywang (1961) demonstrated that white leghorn pullets show greater Vitamin A storage during winter. The probable reason for greater storage observed by these workers may be higher intake of food during the cooler months of the year.

Bernion and Warren (1933), Bruckner (1936), Lee Hamilton and Callahan (1937) observed that food intake is reduced at high environmental temperature.

Heywang (1952) observed that the low performance of laying hens in hot weather is due to a reduced Vitamin intake concomitant to the reduced feed intake and not to any Vitamin A requirement.

The reduced Vitamin A intake due to lower food intake at high environmental temperature though seems to be fairly well established, nothing can be definitely said as yet about increased Vitamin A requirement during the period of high environmental temperature.

In the first week of May when the environmental temperature was as high as 40°C. the carotene and Vitamin A

content was comperatively high in all groups than on 7th week, when the environmental temperature has dropped down to 35.37°C. No abrupt change in carotene and Vitamin A content of the egg was observed at any stage of the experiment with rise and fall of environmental temperature.

On the contrary the very gradual decrease in the carotene and Vitamin A content of the egg during the summer months as seen from the comparison of Table 4.7 and 4.8 with Table 4.11 apparently indicates that probably it is the cumulative effect of reduced food intake which is reflected through the reduced carotene and Vitamin A content of the eggs during the high environmental temperature rather than increased Vitamin A requirement of the hen at high environmental temperature.

VARIATION OF CARCTENE AND VITAMIN A CONTENT OF EGGS STORED AT ROOM TEMPERATURE FOR 7, 14 and 21 DAYS :-

3 eggs of each experimental group I, II, III and IV of Cheap Poultry Ration Scheme were stored for 7, 14 and 21 days at room temperature in a well ventilated room on a enamelled tray.

The carotene and Vitamin A content of the egg yolk were estimated on 0, 7th, 14th and 21st day and the results are recorded in Table 4.12 and 4.13.

TABLE 4.12

Table showing variation of Vitamin A content of egg yolk on storage at room temperature(Average of 3 eggs each).

Experimental Group.	Vitamin A content in micrograms per gram of yolk.				Percent of loss of Vitamin A content between 0 day & 21st day
	0 Day.	7th day.	14th day.	21st day.	
Experimental Group-I	4.56	4.52	4.34	3.93	13.81
Experimental Group-II	4.74	4.60	4.43	4.07	14.13
Experimental Group-III	4.49	4.27	4.00	3.77	16.03
Experimental Group-IV	4.78	4.67	4.47	3.97	16.94

TABLE 4.13

Table showing variation of carotene content of egg yolk on storage at room temperature(Averege of 3 eggs each).

Experimental Group	Carotene content in micrograms per gram of yolk.				Percent of loss of Carotene content of egg yolk between 0 & 21st day.
	0 day	7th day	14th day	21st day	
Experimental Group- I	1.69	1.57	1.49	1.36	14.46
Experimental Group- II	1.58	1.63	1.49	1.40	11.39
Experimental Group- III	1.49	1.46	1.40	1.36	9.55
Experimental Group- IV	1.85	1.82	1.65	1.59	14.05

On perusal of Table 4.12 and 4.13, it appears that the variation in carotene and Vitamin A content of egg yolk between 0 and 7th day is not appreciable in any of the experimental group.

From 7th to 21st day there is a decline both in carotene and Vitamin A content of egg yolk in all the groups.

It was further observed that between 14th and 21st day the albumen became watery in all the experimental groups. But none of the eggs were found to have decomposed which is very frequent in fertile eggs due to formation of embryo. All eggs used in this experiment were infertile.

Bandemer, Evans and Davidson (1958) conducted an experiment to study the Vitamin A content of fresh and stored shell eggs.

Vitamin A was estimated in the fresh eggs and after storage at $0^{o}C$. for 4, 8 and 12 months.

The Vitamin A content per grem of yolk in the above order were 3.52, 3.57, 2.69 and 2.81 microgram.

He suggested that development of rancidity in the oil of the yolk to be responsible for the loss of Vitamin A.

The other probable reasons for loss in carotene and Vitamin A content of egg yolk on storage at room temperature may be due to passage of water from albumen to yolk and thereby diluting the yolk content.

According to Smith (1959) yolk lipid passes to

albumen on storage, hence another probable reason for decline in carotene and Vitamin A content of eggs on storage may be that some of carotene and Vitamin A content of yolk is carried away with the lipid fraction of yolk to albumen.

II Controlled Feeding experiment:- The controlled feeding experiment was conducted with 4 hens for a comparative study of carotene and Vitamin A content of egg yolk with and without Vitamin A supplementation.

The digestibility of carotene as well as Vitamin A content of liver and plasma in the hens with and without Vitamin A supplementation was also investigated.

The hens were kept for four weeks on a basal mash containing no Vitamin A to deplete their body storage of Vitamin A if any due to previous feeding.

After four weeks of depletion period the hens were divided into two groups.

Hen number 1 and 2 formed the experimental group while hen number 3 and 4 formed the control group based on random selection.

The carotene content of the basal mash was estimated at the commencement of the controlled feeding experiment and is recorded in Table 4.14

TABLE 4.14

Table showing the carotene and Vitamin A content of the basal mash.

Sl.No. & item.	Carotene content in milligram per kg. of mash.	Vitamin A content in I.U. per kg. of mash	Vitamin A potency per kg. of mash.
1.Basal mash	1.76	Nil	2921

The basal mash did not contain any Vitamin A supplement in form of fish liver oil or stabilised Vitamin A.

Assuming that a hen eats on an average 112 grams (4 ounces approximately) of feed per day the Vitamin A requirement of the hen can be met from the carotene content of the above mentioned basal mash, which according to Lampman et al (1939) is 0.2 milligram per hen per day or 200 micrograms per 100 grams of feed.

On termination of depletion period of 4 weeks, eggs were collected for two weeks and were estimated for carotene and Vitamin A content. The results are recorded in Table 4.15.

TABLE 4.15

Table showing the average carotene and Vitamin A content of the eggs in all the four hens without Vitamin A supplementation.

Hen Number.	No.of eggs analysed.	Carotene content in microgram per gram of yolk.	Vitamin A content in micrograms per gram of yolk.
Hen No.1	3	1.45	3.13
Hen No.2	3	1.36	3.27
Hen No.3	5	1.61	3.07
Hen No.4	5	1.41	2.93

The average carotene and Vitamin A content (without Vitamin A supplementation) was 1.44 and 3.10 micrograms per gram of yolk respiectively. The average Vitamin A potency was 11.60 I.U. per gram of yolk.

On termination of two weeks of egg collection period without Vitamin A supplementation, administration of Vitamin A was started in Hen Number 1 and 2. Each hen was supplemented orally with 2000 I.U. of Vitamin A in ground nut oil menstrum daily for three weeks.

After one week of Vitamin A supplementation eggs were collected for three weeks and were estimated for carotene and Vitamin A content. The results are given in Table 4.16.

TABLE-4.16

Table showing the Carotene and Vitamin A content of the eggs in experimental group after Vitamin A supplementation.

Hen Number	No. of eggs analysed.	Carotene content in microgram per gram of yolk.	Vitamin A content in microgram per gram of yolk.
Hen No.1	3	1.14	6.18
Hen No.2	4	1.02	6.37

The average carotene and Vitamin A content per gram of yolk with Vitamin A supplementation was 1.08 and 6.27 micrograms per gram respectively and Vitamin A potency 20.22 I.U. per gram of yolk.

The carotene and Vitamin A content of eggs in Hen No.1 and 2 at varying period of Vitamin A supplementation is given in Table-4.17 .

TABLE-4.17

Table showing carotene and Vitamin A content of egg yolk at varying periods of Vitamin A supplementation in Hen No.1 and 2

Hen No.1			Hen No.2		
Period of supplementation.	Carotene content in microgram per gram yolk.	Vit. A content in microgram per gram yolk.	Period of supplementation.	Carotene content in micro-grams per gram yolk.	Vit. A content in micro-grams per gram yolk.
Without Supplementation.	1.46	3.11	Without Supplementation.	1.37	3.27
" "	1.46	3.18	" "	1.37	3.27
" "	1.43	3.11	" "	1.37	3.27
With Supplementation.			With Supplementation.		
12th day	1.17	6.07	10th day	1.08	6.40
13th day	1.14	6.27	12th day	1.05	6.40
18th day	1.11	6.20	13th day	0.99	6.34
			16th day	0.99	6.34

On perusal of Table 4.17 it is evident that at the high level of Vitamin A supplementation, the Vitamin A content in Egg Yolk almost became double, while the carotene content per gram of yolk was depressed in both hens.

Deuel et al (1943) observed that carotene content was depressed from 32.2 ± 20 to 8.4 ± 0.5 micrograms per gram of yolk when the Vitamin A supplementation per Lb. of food was 0 and 60000 micrograms respectively.

Deuel et al (1941-42) demonstrated that when massive doses of Vitamin A were given to cows, a marked suppression of carotene content of butter fat occurred.

Jensen et al (1942) and Fountaine and Bolin (1944) confirmed the depressing action of massive doses of Vitamin A on the milk.

Thus our results also confirm the findings of Deuel et al (1943).

The N.R.C. requirement of Vitamin A is 2000 I.U. per Lb. of mash for laying hens, which comes to 500 I.U. of Vitamin A per hen per day based on calculation that a hen eats on an average 4 ounces of mash daily. It has already been stated above that the average Vitamin A potency was 11.80 I.U. per gram of yolk when the Vitamin A supplementation was nil.

On Vitamin A supplementation which was four times the N.R.C. requirement of laying hen, the average Vitamin A potency rose to 20.22 I.U. per gram of yolk, less than double the amount in comparison to zero Vitamin A supplementation period.

The excess Vitamin A is stored to a great extent in liver as shown later probably also in other storage organ of the body which was not studied in the present experiment.

Thus it is seen the increase in Vitamin A content of egg is relatively small in comparison to level of feeding

and hence production of eggs of higher nutritive value for human consumption may not be an economic proposition.

DIGESTIBILITY OF CAROTENE:-

Digestibility of carotene was also studied in hen no.1 and 2 during the last week of egg collection period with and without Vitamin A supplementation.

The results of digestibility study on carotene with and without Vitamin A supplementation are recorded in Table 4.18 and 4.19 .

TABLE-4.18

Table showing the total intake and excretion of carotene feeds and faeces of Hen No.1 and 2 and the average digestibility of carotene for the period of observation.

No. of observation	Hen No.	Total intake of carotene in microgram during one week period.	Total excretion of carotene in micrograms during one week period.	Average digestibility of carotene.
1.	1	1166.12	285.24	75.51
2	2	1091.22	270.20	75.24

From perusal of Table 4.18, it appears that the average digestibility of carotene without Vitamin A supplementation was 75.37%.

TABLE-4.19

Table showing the total intake and excretion of carotene in feeds and faeces of Hen No.1 and 2 and the average digestibility of carotene with Vitamin A supplementation.

No. of observation.	Hen No.	Total intake of carotene in microgram during 1 week period with Vitamin A supplementation.	Total excretion of carotene in microgram during 1 week period with Vitamin A supplementation.	Average digestibility of carotene.
1.	1	1135.20	428.92	62.21
2.	2	1101.74	406.91	63.06

From Table-4.19 it is seen that the average digestibility of carotene with Vitamin A supplementation was 62.63%.

Thus from Table 4.18 and 4.19 it is evident that the digestibility of carotene changes with Vitamin A supplementation.

Kemmerer and Fraps (1938) observed that when carotene in the form of dehydrated alfalfa leaf meal was fed to chickens at the level of 20 microgramms per gram of feed the digestibility of carotene was 29% and 1 microgram per gram of feed was fed the digestibility was 69%.

Our basal mash contained 1.76 micrograms of carotene per gram of mash and the average digestibility was 75.37%. Our result though in agreement with that of

Kemmerer and Fraps (1938) appears to be apparently high which may be due to presence of yellow pigments in the feaces which cannot be separated from the carotene by usual chemical procedure (Kemmerer and Fraps-1938).

It is interesting to note that it was observed in our study that the average digestibility of carotene is depressed when 2000 I.U. of Vitamin A. Palmitate was orally administered in a ground nut oil menstrum daily to each hen No.1 and 2.

Although no direct reference in this respect is available, indirect support of this point can be derived from the works of Deuel et al (1941-42). They demonstrated that when massive doses of Vitamin A were given to cows, a marked depression of butter fat occurred.

Jenson et al (1942) and Fountain and Bolin (1944) confirmed the depressing action of massive doses of Vitamin A on the milk carotene.

It has likewise been shown by Deuel et al (1943) that the administration of massive doses of Vitamin A to chicken results in a pronounced decrease in the carotene content of the egg yolk. One of the probable reasons for reduced digestibility of carotene at high level of Vitamin A supplementation may be that carotene is not stored as such in the body.

It is converted to Vitamin A in the walls of intestine (Sexton et al-1946; Clover et al-1947, 48; Thompson et al-1950 and Dewar and Gregory-1946,48) before absorption.

As such the animal system might have a selective affinity for preformed Vitamin A to its precursor carotene when both are present in the food.

Hence the lower digestibility of carotene at high level of Vitamin A supplementation may be a contributory factor for depressing carotene content in Egg Yolk and milk observed by the above workers.

EXTENT OF APPARENT ABSORPTION OF VITAMIN A:-

The extent of apparent absorption of Vitamin A was studied in Hen No.1 and 2 from the total intake and excretion of Vitamin A during the last week of Vitamin A supplementation period. The results are indicated in Table-4.20 .

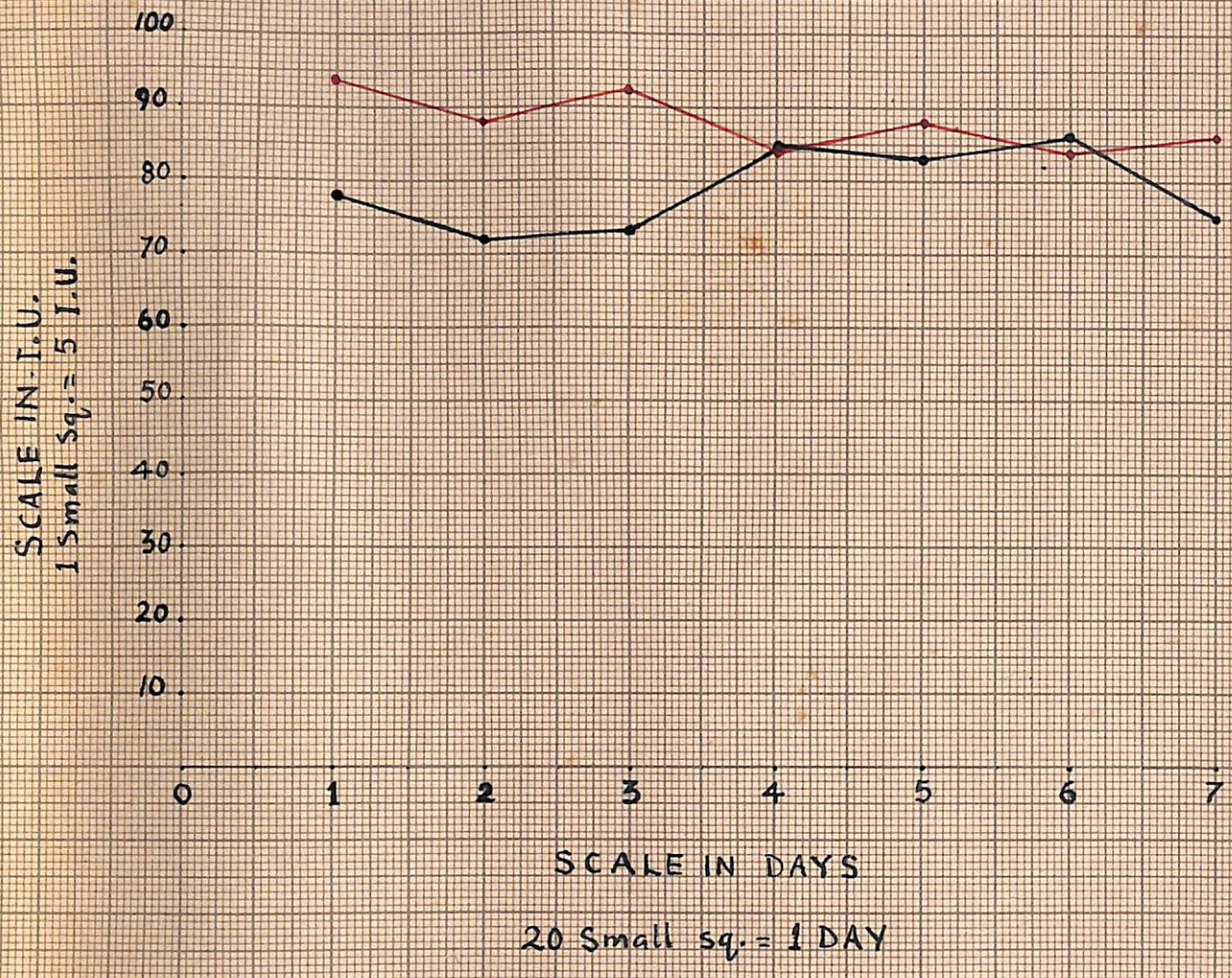
TABLE-4.20

Table showing the daily intake and excretion of Vitamin A in Hen No.1 and 2 during the last week of Vitamin A supplementation.

Sl. No. of days.	Hen No.	Daily intake of Vitamin A in I.U.(approx)	Daily excretion of Vitamin A in I.U. in faeces	Apparent absorption of Vitamin A
1.	1	2000	391.68	
2.	"	"	364.90	
3.	"	"	368.92	
4.	"	"	429.58	80.07%
5.	"	"	419.76	
6.	"	"	434.75	
7.	"	"	379.76	
1.	2	2000	471.90	
2.	"	"	443.70	
3.	"	"	463.68	
4.	"	"	427.70	77.83%
5.	"	"	444.80	
6.	"	"	424.84	
7.	"	"	435.96	

It is seen from Table-4.20 that the apparent absorption of Vitamin A on the third week of supplementation period was 78.95%.

The daily excretion of Vitamin A in feaces in hen No.1 and 2 during Vitamin A supplementation is graphically shown in Figure-I



It is interesting to observe from Table-4.20 that the extent of apparent absorption of Vitamin A was practically the same both on the 1st and last day of the digestibility trial experiment. This tends to indicate that the system was not saturated with Vitamin A even after a total administration of about 42000 I.U. of Vitamin A. This is further corroborated by the fact that Hill et al (1961) obtained as high a concentration as 3100 U.S.P. units of Vitamin A per gram of liver as against only 748.55 I.U. of Vitamin A per gram of liver found by us.

The daily excretion of Vitamin A in faeces in Hen No. 1 and 2 during Vitamin A supplementation is shown in Figure-I.

VITAMIN A STORAGE IN LIVER AND PLASMA:-

All the four hens were sacrificed at the end of the controlled feeding experiment with a view to ascertain the fate of the absorbed Vitamin A due to supplementation, its level in liver and plasma was estimated.

The results are recorded in Table-4.21

TABLE-4.21

Table showing Vitamin A content of liver in Vitamin A supplemented and unsupplemented hens.

No. of observation.	Hen No.	Extent of total Vitamin A supplementation in I.U.	Vit. A in I.U. per gram liver tissue.	Vit. A in I.U. per liver.	Average Vit. A in I.U. gram liver tissue.
1.	3	Nil	10.23	208.99	9.38
2.	4	Nil	8.54	160.39	
3.	1	42000	750.80	15766	746.55
4.	2	42000	746.30	14552	

TABLE-4.22

Table showing percent of Vitamin A stored in the liver of hens supplemented with Vitamin A.

No. of observation.	Hen No.	Total amount of Vit. A in I.U. administered in three weeks.	Total storage of Vit. A in I.U. in liver.	P.C. of Vitamin A stored in liver
1.	1	42000	15766	36.83%
2.	2	42000	14552	33.99%

From perusal of Table-4.21 and 4.22 it is clearly seen that with no Vitamin A supplementation the liver storage was as low as 9.38 I.U. per gram of liver tissue.

The level of Vitamin A rose as high as 748.55 I.U. per gram of liver tissue when 2000 I.U. of Vitamin A was daily supplemented to each hen no.1 and 2 and the average storage in liver during three weeks was 35.42% of the total intake.

Hence the liver has aptly been called the store house of Vitamin A in body.

The Vitamin A stored in the liver of unsupplemented hens may be due to previous storage or to storage from current carotene intake.

Hill *et al* (1963) reported that 16, 18 and 9 USP Unites of Vitamin A per gram of liver tissue of the hens maintained on 800, 1200 and 1600 I.U. of Vitamin A per Lb. respectively. The Vitamin A content of the liver tissue increased to 330, 600 and 3100 U.S.P. units per gram on feeding 3600, 5000 and 10000 U.S.P. units of Vitimin A per Lb.

This led him to the conclusion that relatively low levels of Vitamin A were present in the livers of hens feed 2000 U.S.P. units of Vitamin A per pound or less.

The Vitamin A concentration in the liver was markedly increased with higher level of Vitamin A supplementation.

Hence, our result confirms the finding of Hill *et al* (1963).

Marusich and Coworker (1963) observed that Vitamin A storage in the liver is cumulative and bore a marked relationship to the amount of dietary Vitamin A and the length of time maintained on that diet.

He has demonstrated that Vitamin A level of the liver rose from 25.2 to 27.85 in chicks when 2500 I.U. of Vitamin A per Lb. of diet was fed for 4 weeks and 7 weeks respectively.

VITAMIN A CONCENTRATION OF PLASMA:-

According to Taylor et al (1947), Almquist (1952), Squibb (1961) and Ascarelli and Bartov (1963) an important index of Vitamin A nutrition in Poultry is the Vitamin A concentration of plasma.

The Vitamin A content of the serum was estimated in the Vitamin A supplemented and the unsupplemented Hens and the results are recorded in Table-4.23 .

TABLE-4.23

Table showing Vitamin A content of serum in Vitamin A supplemented and unsupplemented hens.

No. of observation	Hen No.	Extent of Vit. A in I.U. supplementation in 3 weeks.	Vit.A microgram per 100 c.c. serum.	Vit.A in I.U.per 100 c.c. serum.	Average Vit. A in I.U.100 c.c. serum.
1.	3	Nil	42.51	124.89	112.43
2.	4	Nil.	33.33	97.99	
3.	1	42,000	92.47	271.86	227.56
4.	2	42,000	63.34	183.27	

It is evident from Table 4.23 that the Vitamin A content of the serum in supplemented group and unsupplemented group was 227.56 and 112.43 I.U. per 100 c.c. of serum respectively.

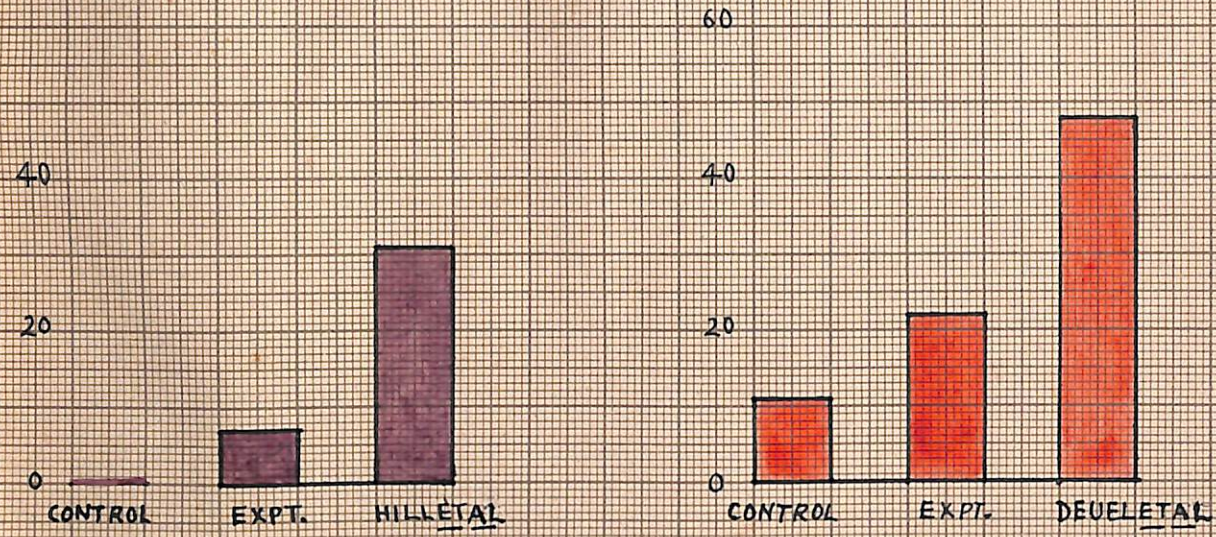
RELATIONSHIP BETWEEN PLASMA AND LIVER LEVELS OF VITAMIN A:-

Lewis, Bodansky, Falk and McGuire (1942) were of the opinion that plasma tends to resist any changes in Vitamin A concentration, despite wide variations in the amount of Vitamin A stored in the liver. This view point has been supported to some extent by the works of Brenner, Brookes and Roberts (1942) and Krause (1949).

Deuel et al (1943) has also demonstrated that Vitamin A content in plasma was 166 and 174 micrograms

Relation between level of Vitamin A stored in the liver, its level in serum and its content in eggyolk in control and experimental group of hen along with the values obtained by Deuel et al (1943) and Hill et al (1961) are schematically represented in Histogram-III.

2 Small Sq. = 1 I.U.



1 Small Sq. = 100 I.U.

LIVER

1 Small Sq. = 10 I.U.

SERUM

per 100 c.c. of plasma when the Vitamin A content was 200 and 2082 microgram respectively per gram of liver.

From our result it can also be seen that Vitamin A content per gram of liver tissue rose from 9.38 I.U. to 748.56 I.U. almost 75 times with ~~and without~~ Vitamin A supplementation, while the change in serum level was hardly doubled from 112.43 to 227.56 I.U. per 100 c.c. serum a change hardly double.

Hence, from the Vitamin A content of plasma the liver storage of Vitamin A cannot be assessed.

Relation between level of Vitamin A stored in the liver, its level in serum and its content in egg yolk in control and experimental group of hen along with the values obtained by Deuel et al (1943) for egg yolk and serum and Hill et al (1961) for liver schematically represented in histogram-III.

DESTRUCTION OF CAROTENE CONTENT OF FEEDS ON STORAGE:-

There is destruction in carotene content of feeds on storage has been reported by numerous workers. In order to study destruction of carotene in feeds, the basal mash which was used in the controlled feeding experiment was estimated for its carotene content at the beginning and end of the experiment. The results are recorded in Table-4.24 .

TABLE-4.24

Table showing percentage of carotene lost in the feed during a storage period of ten weeks.

Source	Carotene content in milligram per Kg. feed at the start of the exp.	Carotene content in milligram per Kg. feed at the end of experiment	Loss of carotene content due to storage in percent.
Basal Mash	1.76	1.58	10.22

It is seen from Table 4.24 that 10.22% of carotene content of feed was lost during a storage period of ten weeks.

The probable reasons for the loss of carotene content of the feed may be oxygen of the air, heat, light, moisture and enzyme.

Kick and Bethke (1929) reported very little if any destruction of carotene in yellow corn when stored for one year.

Guilbert (1935) demonstrated that there was 30% loss of carotene in alfalfa meals stored in the dark in paper sample containers fore period of 8 weeks at room temperature of 68 to 86°F.

Sesan and Sen (1942-43) reported 76.2% loss of carotene from herbage during a storage period of six weeks and 94.7% during a period of 15 weeks.

From review of literature it appears the loss of carotene from green forages and herbage is more in comparison to other food ingredients, especially from those which have a hard coating.

T E R - V

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CHAPTER-V

SUMMARY

To study the relationship between intake of carotene and Vitamin A and their content in egg carotene and Vitamin A content were investigated in the eggs of four groups of hen maintained on four experimental cheap poultry meshes.

The experimental meshes I, II, III and IV contained 2.25, 2.74, 1.88 and 3.42 milligram of carotene per kilogram of feed. Silk worm pupa meal containing a high level of 11.81 milligram carotene per kilogram of meal was identified as the main source of variation for the carotene content of the different experimental meshes.

The N.R.C. requirment of 2000 I.U. of Vitamin A activity per pound of feed for the laying hen was fully met from the natural carotene content of the feed only in experimental group II and IV.

To meet the N.R.C. requirment of Vitamin A in the remaining two group as well as due to unstable and uncertain nature of the feed carotene, each experimental mash was, however, supplemented with 1.32 grams of Rovimix A-325 per 100 Kg. of mash, which is

equivalent to 4290 I.U. of Vitamin A per kilogram of mash.

There eggs of each experimental group I, II, III, and IV were analysed weekly for their carotene and Vitamin A content. It was observed that the group I having the highest Vitamin A potency per kilogram of mash had also the maximum carotene and Vitamin A per egg, where as the experimental group III having the lowest Vitamin A potency per kilogram of mash had the minimum carotene and Vitamin A content per gram of yolk. The average Vitamin A potency per gram of yolk in the experimental group I, II, III and IV were 15.75, 16.09, 15.51 and 16.43 I.U. respectively.

Thus it is seen that the Vitamin A potency in egg tends to increase with the higher Vitamin A content of feed.

Effect of high environmental temperature on the carotene and Vitamin A content of the egg was also investigated. It was observed that the apparently low but statistically significant change in the carotene and Vitamin A content of egg occured at high environmental temperature in the region of 40°C. and above.

Towards the later part of this experiment variation in the carotene and Vitamin A content of the egg due to storage at room temperature for 7, 14 and 21 days was also investigated.

It was observed that the variation in carotene and Vitamin A content between 0 day and 7th day was not

appreciable in any of the experimental group. From 7th to 21st day there was a decline both in carotene and Vitamin A content of egg in all the groups. The average loss in carotene and Vitamin A during the three weeks of storage was 12.36 and 15.21 percent respectively.

The inference drawn from the uncontrolled feeding experiment that the carotene and Vitamin A content of the egg is correlated with their level of intake was further confirmed from the observations of controlled feeding experiment.

It was seen that when the two experimental hens of the controlled feeding experiment were supplemented with 2000 I.U. of Vitamin A in a ground nut oil menstrum daily for three weeks the average Vitamin A content increased almost twice to a value of 6.27 micrograms from 3.20 micrograms per gram of yolk. The carotene content was however appreciably depressed from 1.44 micrograms to 1.08 micrograms per gram of yolk.

From the digestibility study of the carotene with and without Vitamin A supplementation it was observed that the digestibility of the carotene was perceptibly reduced from 75.37 percent to 62.63%, when 2000 I.U. of Vitamin A was daily administered to the experimental hens. The probable reason for this may be a selective affinity

of the animal system for preformed Vitamin A than for its precursor carotene when both are present in the food.

The average apparent absorption of Vitamin A during the Vitamin A supplementation period was found to be 77.90 percent and was practically the same on the first and the last day of the digestibility trial.

This tends to indicate that the system was not saturated with Vitamin A even after a total administration of about 42000 I.U. of Vitamin A over a period of three weeks. This is further corroborated from the fact that the concentration of Vitamin A in the liver of Vitamin A supplemented hen was only 748.55 I.U. per gram of liver while Hill et al (1961) obtained as high as 3100 U.S.P. units per gram of liver comparitively at the same level of feeding as ours.

The level of Vitamin A storage in liver of the control and experimental group was 9.38 and 748.55 I.U. per gram respectively an increase of almost seventyfive times due to Vitamin A supplementation , where as the Vitamin A content per 100 ml. of serum was only 112.43 and 227.56 I.U. in the control and experimental group respectively.

Thus it appears that the blood serum tends to resist any changes in the Vitamin A concentration despite the wide variations in the amount of Vitamin A stored in the liver.

The destruction of carotene in feeds due to storage was also investigated during the controlled feeding experiment.

The basal mash which was used in the controlled feeding experiment lost about 10.22 percent of carotene during a storage period of ten weeks.

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