90. THE VITAMIN A, CAROTENE AND XANTHOPHYLL CONTENT OF THE YOLK OF HENS' EGGS

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IN the course of experiments with chickens carried on in this institute on the importance of vitamin A, carotene and xanthophyll in the diet of hens for their egg production, health, the hatchability of their eggs, the vitamin A in their liver and the viability of the chicks produced from these eggs, a large number of yolks were examined for their content of these three substances.

The results of these studies are the subject of the present paper.

Experimental

There were about 300 hens at our disposal—White Leghorns in their first production period. About 180 of these, i.e. groups A–H, were housed indoors in all-metal individual cages. This made it possible to keep a check on the food taken and the eggs laid by each hen. About 120 hens, i.e. groups V–VII, were housed in pens of which the exits were kept closed. None of the animals were given a chance to eat fresh grass or any other green food.

The diet given to the various birds differed only in respect of the constituents rich in the three above-named substances. Vitamin A was administered as dohyfral oil, which contains shark's oil and is also a source of vitamin D₃. Yellow maize and dehydrated alfalfa were used as sources of carotene. One group, namely group G, was given food in which all these constituents were lacking. In the groups without dohyfral in the diet vitamin D₃ was given instead. The other components of the hens' diet were very poor in the three substances under consideration.

The diet consisted of a mash, which was given dry, and grain, a mixture of unground maize, barley, wheat and oats (25 % of each) and in addition 2½ % of sunflower seeds.

The composition of the mash as originally given was:

	%
Maize	25
Wheat middlings	20
Barley flour	15
Oatmeal	10
Soya bean flour	10
Meat and bonemeal	10
Fish meal	6
Dried yeast	1
Mineral mixture	2.5
Dohyfral oil	0.1

This food was given to all the animals before experimentation began (8 December 1938). It was fed to groups C, D and VI throughout the whole experiment. The dohyfral oil contained about 1800 i.u. of vitamin A per g.

The pullets consumed about 110 g. of food daily. The quantity of the mash taken was as a rule not quite equal to the quantity of grain eaten. Most of the groups took rather more of the mash than of the grain. Nevertheless in the following we shall assume that the amounts were the same.

Where alfalfa was added to the mash, the amount of wheat middlings was correspondingly reduced.

The experiment took 9 months, from 2 February until 8 November 1939.

The following table shows the sources of carotene and vitamin A in the food of the different groups.

Group	A	B	C	D	E	F	G	H	V	VI	VII
Yellow maize 25 %	+	+	+	+	+	-	-	-	+	+	+
Alfalfa	+(5 %)	+(5 %)	-	-	-	+(5 %)	-	+(7.5 %)	+(5 %)	-	+(7.5 %)
Dohyfral 0.05 %	+	-	+	+	-	-	-	-	-	+	-

Groups A and C were formed on 2 February; B, D, V, VI and VII on 14 February; E and F on 17 March; and G and H on 4 April 1939.

In diets (groups F, G and H) from which yellow maize was omitted this was replaced by an equal quantity of white maize in the mash as well as in the grain mixture.

Vitamin A, carotene and xanthophyll were determined after saponification as recommended by Wolff [1938-9], by extraction with peroxide-free ether, after which Carr and Price's reaction was applied to determine the amount of vitamin A using the Pulfrich Stufenphotometer. To determine not only the vitamin A content but also the carotenoids the method had to be somewhat modified and amplified. The method described by Baumann *et al.* [1939] did not quite satisfy us in that it seemed rather complicated.

After discussing the matter with Mr Emmerie, whom we are glad to take the opportunity of thanking for the help he gave us and the interest he showed in our research, we decided to use the following method.

The analytical method

1. *Yolks.* After being weighed, the yolk is saponified with 125 ml. 10 % alcoholic KOH for 1½-2 hr. in a water bath using a reflux condenser. An equal volume of water is then added and the mixture, after being allowed to cool for 1 hr. in a refrigerator, is shaken three times with ether free from peroxide (75, 60 and 50 ml. respectively) in a 500 ml. separating funnel. The combined ether extracts are subsequently washed with water until they are entirely free from alcohol (generally three careful washings with 100 ml. H₂O are sufficient; the formation of emulsion may be prevented by means of a little alcohol). After being put to dry all night over anhydrous sodium sulphate, the ether is divided into two parts. The first part is evaporated in a stream of CO₂ and the residue dissolved in about 5-10 ml. CHCl₃, sometimes more, according to the amount of vitamin A expected to be present. With the antimony trichloride reagent the total blue coloration derived from the vitamin A, carotene and xanthophyll is determined using the Pulfrich Stufenphotometer, with filter S 61 and a 0.5 cm. cell. The Lovibond blue units in the half-yolk may then easily be read by the aid of the curve.

The second part of the ether extract is also evaporated in an atmosphere of CO₂ (in a vacuum) and the residue dissolved in 75 ml. light petroleum (B.P. 60-80°) and then shaken in a separating funnel with 85 % alcohol until the latter has become totally colourless. In the case of not too strongly coloured yolks extraction with 75, 50, 50 and 40 ml. respectively is sufficient. The light petroleum fraction then contains the carotene while the xanthophyll is in the

combined alcoholic fractions.¹ The carotene solution is then washed twice with 50 ml. H₂O and left to dry all night in the refrigerator over anhydrous sodium sulphate. After evaporation (in a stream of CO₂ and in a vacuum) on the water bath, the residue is dissolved in light petroleum (B.P. 60–80°). The amount of light petroleum needed depends on the carotene content of the yolks. For light-coloured yolks we used 5–10 ml.; for strongly coloured ones up to 25 ml. By the use of filter S. 47 of the Stufenphotometer using a 0.5 cm. cell, and a curve constructed for pure β -carotene, the carotene content of the half-yolk may be determined.

The alcoholic solution of xanthophyll derived either from the whole yolk in the case of light-coloured yolks or else from a part, is diluted with at least five times its own volume of H₂O; then 50 ml. light petroleum are added, after which the mixture is well shaken and the water removed. Then the xanthophyll solution is again washed twice with 100 ml. H₂O and dried overnight in the refrigerator with anhydrous sodium sulphate. Like the carotene the xanthophyll derived from the half-yolk is then, after being evaporated in the water bath in a stream of CO₂ in a vacuum, dissolved in light petroleum, the amount of which varies from 5 to 25 ml. according to the quantity of xanthophyll present. By the aid of the Stufenphotometer and using a 0.5 cm. cell and filter S. 47, the calculation is made by means of a curve obtained for pure xanthophyll.

Since not only vitamin A but also carotene and xanthophyll give a blue coloration with antimony trichloride, it still remains to calculate the Lovibond blue units of the sum of the amounts of carotene and xanthophyll. This is done by means of a fourth curve which gives the blue value in photometer units. The value found on this curve can be converted into Lovibond units by the aid of the first curve. Subtraction of this number of Lovibond units from the number of Lovibond units previously found, gives the amount of vitamin A expressed in Lovibond units in the half-yolk. Multiplying this figure by 6.4 gives the same expressed as International Units. To find the vitamin A, carotene and xanthophyll contents of the whole yolk the above figures must be multiplied by 2.

When determining the vitamin A contents of the cod liver oil and dohyfral oil used, we kept strictly to the results given by Wolff [1938–9] for cod liver oil and assumed that the very light yellow colour of some extracts might be neglected.

2. Poultry feed. The method we followed in examining poultry feed was, in general, also the one indicated by Wolff [1938–9] and consisted of saponification and extraction with light petroleum and the subsequent determination of carotenoids. In analysing foodstuffs in which vitamin A might be expected as a result of adding cod liver oil, dohyfral oil or fish meal, we followed the same technique as in determining vitamin A, carotene and xanthophyll in yolks of eggs. This was permissible because the components of the feed could not be expected to contain any carotenoids (such as lycopene) other than carotene and xanthophyll, at least in more than traces.

From 5 to 15 g. of the feed to be examined were used, according to the amount of carotene that we expected to find, the sample having previously been ground so that it passed a 0.45 mm. sieve.

5 g. of finely ground maize for instance were saponified for 2 hr. with 75 ml. alcoholic 15 % KOH. After being cooled and diluted with an equal volume of water the liquid is put into a separating funnel and extracted with light petroleum (B.P. 60–80°) until no more yellow substance is transferred to the petroleum

¹ We call this fraction the xanthophyll fraction without wishing to imply that it contains only this carotenoid.

(generally four extractions with 60, 50, 40 and 40 ml. light petroleum are sufficient). The collected extractions are then washed with an equal volume of water. After this they are washed once with 50 ml. 0.5*N* KOH and then with H₂O until the petroleum is entirely free of alkali (generally three washings are sufficient). Then the carotene and xanthophyll are separated with 85 % alcohol, as described in the case of yolks. The further treatment corresponds to the one described above. For the calculation curves 2 and 3 are used.

In the case of the poultry feed containing carotenoids as well as vitamin A the light petroleum extract was divided into two equal parts and treated as described for yolks, or, if little of the active substance was to be expected in the feed, we made a separate analysis to determine the total vitamin A and carotenoids and a second one for the carotenoids alone, from which two analyses we were able to calculate the vitamin A content.

From the above it is clear that we did not use the biological method for determining the vitamin A and carotene contents of the yolks and feeds. At the end of the present paper we discuss the results of experiments in which the biological method was used, which in our opinion gave results concordant with ours. As far as we can see the physico-chemical method here employed gives results which, in the case of the materials we are dealing with, agree with those obtained biologically.

RESULTS

I. Vitamin A, carotene and xanthophyll contents of yolks of eggs laid by hens fed on a diet poor in these substances, and the effects of supplementing the diet

How low the content of these substances may be in the yolk of an egg is shown by examination of the yolks of group G, whose diet contained no constituents rich in carotene.

The results obtained from a study of this group also show what effect may be produced on the yolk in respect of its content of these substances by supplementing the diet of hens with suitable additions.

The mash fed to these hens during rather more than 2 months contained 1.5 μ g. carotene plus xanthophyll per g. Examination of the fish meal revealed that it contained about 25 I.U. of vitamin A per day's ration. The grain only contained 0.25 μ g. carotene per g.

The following table gives the contents of the yolks of eggs of hens from group G which were given from 14 June onwards the supplemented diet containing vitamin A or carotene. The figures found to the right of the line refer to eggs laid after the supplementary feeding began.

The table given below presents the vitamin A in I.U., the carotene and xanthophyll content as μ g. per yolk.

From this table it is evident how low the content of the yolks had become before 14 June and how much it rose even 2 weeks after the hens were given either alfalfa, cod liver oil (daily 4 ml. containing 740 I.U. vitamin A) or dohyfral oil (daily 0.42 ml. containing 739 I.U. vitamin A) or dehydrated alfalfa to the amount of 15 % of the mash, so that every day the hens consumed about 8 g. of alfalfa containing about 1160 μ g. carotene.¹

The average vitamin A content of the eggs of all the hens of group G was 58.5 in the first half of June; in May, 78 and in April, 137 I.U.²

¹ Before July the 1938 crop of lucerne was used which contained 104 μ g. carotene. The alfalfa used afterwards contained 141 μ g. per g.

² In calculating this average the eggs of one hen (No. 45) which differed markedly from all the others, were not taken into account.

Hen No. 29:		I. Effect of dohyfral oil						
Date ...	5. vi.	20. vi.	26. vi.	29. vi.-7. ix				
				Average of 4 yolks				
Vitamin A	52.2	99.9	173.3	185.3				
Carotene	6.8	6.1	6.8	12.0				
Xanthophyll	42.2	45.8	36.4	34.2				
Hen No. 38:		26. v.		16. vi.	19. vi.	20. vi.	22. vi.	28. vi.-7. ix.
	Date ...	16. v.						Average of 3 yolks
Vitamin A	89.6	103.3	70.7	77.3	128.0	171.5	177.6	
Carotene	3.0	4.4	6.7	6.8	4.0	3.4	8.6	
Xanthophyll	35.8	29.0	18.9	31.5	39.5	30.2	33.5	
Hen No. 39:		16. v.	25. v.	6. vi.	18. vi.	20. vi.	21. vi.	22. vi.
	Date ...							27. vi.-4. ix.
								Average of 3 yolks
Vitamin A	113.0	90.2	68.8	61.1	86.9	114.5	159.4	174.0
Carotene	2.0	9.4	9.2	5.7	5.8	6.0	7.2	7.7
Xanthophyll	40.2	20.4	22.8	40.4	38.4	37.6	38.0	40.2
Hen No. 12:		II. Effect of cod liver oil						
Date ...	23. iv.	5. v.	9. v.	26. v.	28. v.	30. vi.	1. vii.-6. ix.	
							Average of 3 yolks	
Vitamin A	105.0	51.2	32.0	46.9	18.5	108.8	145.5	
Carotene	7.6	8.6	3.8	7.8	6.9	5.2	6.9	
Xanthophyll	59.0	33.6	17.6	22.4	27.4	36.0	35.5	
Hen No. 17:		17. vi.	3. vii.	15. vii.	15. vii.-17. vii.			
	Date ...			Average of 2 yolks				
Vitamin A	72.4	59.5	131.8	162.8				
Carotene	8.4	5.4	13.8	10.7				
Xanthophyll	25.6	45.0	25.4	47.3				
Hen No. 30:		4. v.	9. v.	27. v.	29. vi.	30. vi.	8. vii.-4. ix.	
	Date ...						Average of 3 yolks	
Vitamin A	157.0	72.8	65.2	58.3	149.2	152.1	158.2	
Carotene	9.8	4.2	2.8	12.3	12.4	7.6	10.8	
Xanthophyll	41.6	24.0	32.0	32.8	58.8	53.0	43.7	
Hen No. 9:		III. Effect of dehydrated alfalfa						
Date ...	30. v.	26. vi.	30. vi.	6. vii.	9. vii.-6. ix.			
					Average of 4 yolks			
Vitamin A	14.7	151.2	153.6	211.2	207.0			
Carotene	3.4	22.0	15.8	11.8	27.8			
Xanthophyll	42.0	237.6	192.6	146.0	304.0			
Hen No. 3:		13. iv.	26. v.	5. vi.	22. vi.	26. vi.	2. vii.-7. vii.	
	Date ...						Average of 2 yolks	
Vitamin A	149.7	76.8	35.8	119.5	174.0	159.6	159.6	
Carotene	11.0	7.8	5.8	8.4	12.4	14.0	14.0	
Xanthophyll	88.6	24.8	32.0	155.6	153.2	184.0	184.0	
Hen No. 47:		18. v.	24. v.	26. v.	6. vi.	19. vi.	20. vi.	24. vi.
	Date ...							27. vi.-7. ix.
								Average of 4 yolks
Vitamin A	72.9	61.9	90.1	35.6	80.1	147.7	308.7	250.5
Carotene	2.8	3.6	7.6	6.5	15.0	9.4	18.0	38.9
Xanthophyll	28.6	44.2	37.4	31.4	66.5	133.8	324.8	310.5

* The dohyfral oil and cod liver oil were administered to the fowls separately.

In several cases the effects of the three supplementary foods showed very rapidly. A few examples will illustrate this point.

One of the dohyfral hens showed a rise of almost 50 units in 6 days; one of the alfalfa hens a rise of about 85 units in 8 days. The effects of cod liver oil showed up less quickly. Those that reacted most swiftly were the alfalfa hens. In this connexion we must not forget that the amount of carotene in lucerne is about $1\frac{1}{2}$ times that absorbed in the form of dohyfral and cod liver oil.

It should also be noted that 600 I.U. of vitamin A or an equivalent amount of carotene may be regarded as a fairly normal dose for a laying hen. Hence the amounts of dohyfral oil etc. here indicated should not be regarded as too high for these largely exhausted hens. This is also evident from the fact that the contents of these yolks did not equal those of the eggs derived from the best-fed groups even after the supplement was given for more than a month.

The vitamin A content of the eggs of the hens of group G without supplementary food was already in the middle of June considerably lower than that of eggs of the hens of the other groups that were regularly fed fairly well or very well.

Hens of group G that were given no supplementary food stopped laying when the yolks contained about 2 (or between 1 and 3) I.U. of vitamin A per g. The death rate among these fowls was high.

As the hens of group G consumed per day about 100 μ g. carotene and about 25 I.U. vitamin A (contained in about 3.3 g. fish meal) and laid about 1 egg in 3 days, it is easy to calculate that only a small percentage of the sources of vitamin A were transferred to the eggs. The percentage in group G is smaller than in some of the other groups, for instance group B, where the consumption of carotene was about 660 μ g. and of vitamin A about 50 I.U. Here the number of eggs is about 2 in 3 days, the yolks containing 320 I.U. of vitamin A and 31 μ g. carotene, so that about $\frac{1}{3}$ of the vitamin sources are transferred to the egg. This difference between groups G and B is not surprising if we assume that the organism itself needs a considerable amount of vitamin A per day. Supposing this is from 100 to 150 I.U., then group G has little or nothing left for secretion into the yolk, while in the case of the hens in group B there is enough left for the needs of the body even after a rich contribution has been made to the yolk.

One hen of group G (No. 45) went on laying longer than the others—until 31 July, in fact, on which date she was killed. From 6 April she had been fed on the group G diet and during that period had laid 68 eggs. The vitamin A content of these eggs gradually decreased to 70 I.U. or 3.6 per g. of yolk. Her liver proved to contain little vitamin A (5.93 I.U. per g. = 260 I.U. altogether).

The egg she was about to lay (its shell was already formed) still contained 54 I.U. of vitamin A, while the four largest yolks in the ovary contained a total of 73.5 I.U. of this vitamin, or 2.2 per g. Doubtless this hen, if it had been allowed to live, would only have laid a few more eggs at best. The body contained a great quantity—estimated at about 100–150 g.—of body fat of an intense yellow colour. Little vitamin A was found in this, only 3.8 I.U. per g. or a total of from 400 to 600 I.U.

To show that the results obtained from hen No. 45 are by no means unusual, we may report the following. In the 14 eggs examined before 13 July we found the average content to be 132 I.U. of vitamin A and only 9.4 μ g. of carotene. The 68 eggs laid during the experimental period must have contained a total of about 8500 I.U. of vitamin A and 600 μ g. of carotene. In the liver of the hens (at the beginning of the second laying) we repeatedly found great quantities of both substances, sometimes even more than 20,000 I.U. of vitamin A and more

than 1000 $\mu\text{g.}$ of carotene (the average quantity of vitamin A of 7 livers from group A was 14,443 I.U.). The animal consumed about 25 I.U. of vitamin A and 100 $\mu\text{g.}$ of carotene each day, that is in 4 months about 3000 I.U. and 12,000 $\mu\text{g.}$ of these substances respectively, in other words more than appeared in the eggs. Hence it may be that this hen's liver contained enough of both substances at the beginning of the laying to supply the yolks and in part its own needs.

The carotene content of the yolks of the eggs of group G

Since the hens of group G, as long as supplementary rations (particularly alfalfa) were withheld, absorbed little carotene, the content of the latter was naturally low. It was less than 15 $\mu\text{g.}$ per yolk. It is natural that a supplement in the form of shark's oil or cod liver oil produced very little increase. Of the carotene contained in the alfalfa a part was converted into vitamin A, another part found its way into the eggs. In the case of hens 9 and 47 this increased respectively from about 3 and 5 $\mu\text{g.}$ per yolk to 27.8 and 38.9 $\mu\text{g.}$ carotene. The carotene contents in yolks from the groups which were given during the whole experiment dehydrated alfalfa and yellow maize were generally of the same order.

The xanthophyll content of the yolks of group G was also very low on account of lack of yellow maize and lucerne. In May and June it was about 20 and 40 $\mu\text{g.}$ Giving the hens dohyfrol or cod liver oil produced no change worth mentioning, although the figures showed a certain increase in the case of a few of the birds. On the other hand alfalfa brought the content up to 300 $\mu\text{g.}$ per yolk in the case of 2 of the 3 hens and to 200 $\mu\text{g.}$ in the case of the third—values which correspond pretty well to those of the yolks from eggs belonging to various groups which were given yellow maize or lucerne throughout the whole experimental period.

From the results obtained in group G we may certainly conclude that yolks containing less than 60 I.U. of vitamin A per yolk (or about 3 I.U. per g.) are rare, because a diet as poor in carotene as that of group G will seldom be used, and furthermore the hens stop laying when the vitamin A content of the yolks is so much reduced. Hence these figures may be taken as the lowest limit for hens' eggs for sale on the market. For carotene this lowest figure is taken to be about 5 $\mu\text{g.}$ or about 0.25 $\mu\text{g.}$ per g. of yolk.

II. *Vitamin A, carotene and xanthophyll contents of yolks in the eggs of hens normally fed*

Turning from eggs laid by hens fed on unusual diets, we will now examine the results obtained in groups subsisting on rations used on different poultry farms.

We may class our groups under three heads:

- (a) Groups A, B, V and VII, the diet containing yellow maize and alfalfa.
- (b) Groups C, D, E and VI, the diet containing yellow maize but no alfalfa.
- (c) Groups F and H, the diet containing alfalfa but no yellow maize.

The carotene and xanthophyll contents of the different rations were approximately as follows:

The mash fed to A and B contained 10.2, D and E 3.1, 7.6 and H 12.5 $\mu\text{g.}$ carotene per g.

In the grain mixture containing yellow maize 1.8 $\mu\text{g.}$ carotene per g. were found, in that containing white maize there was 0.25 $\mu\text{g.}$ per g. Hence in 110 g. of the rations of A and B there were 561 + 99 or 660 $\mu\text{g.}$ carotene. In 110 g. of group H diet there were 687.5 + 13.75 = 701 $\mu\text{g.}$

The 3 g. of fish meal which the hens consumed daily contained about 25 I.U. of vitamin A.

In the first months we analysed single yolks, later on we mixed yolks of eggs laid by hens belonging to the same group.

The differences of vitamin A content in the yolks from the different groups come out most clearly from a comparison of results obtained with mixed yolks. For the mixture we always used 10 yolks of hens belonging to the same group. In this way the individual differences were greatly reduced. The yolks in question were from eggs laid between 26 July and 6 September, i.e. all at a time when the hens had already had the same rations for more than 5 or 6 months respectively.

To enable the reader to compute from the contents of whole yolks, those of 1 g. of yolk, we may mention that the average weight of the mixed yolks of one set of groups was 18.76, that of the other was 17.8 g.

(a) *Vitamin A contents of the mixed yolks of eggs laid by groups fed on yellow maize and lucerne (the mash fed to group A also contained dohyfral).*

Groups ...	A	B	V	VII
	332.2	338.8	259.7	276.8
	332.2	305.1	271.1	309.3
	304.8	307.1	267.7	293.0
	327.3	312.9	270.4	290.6
	310.8	333.3	250.3	284.0
Average	321.4	319.4	263.8	290.7

About 320 I.U. per yolk were found in groups A and B. For group VII this figure was less than 300, while for group V it was considerably lower still, namely about 264. A comparison of the vitamin A contents of yolks (both singly and mixed) belonging to groups A and B respectively showed that there was little difference in spite of the fact that group A's rations contained dohyfral (about 100 I.U. vitamin A in a day's rations), which was absent from the diet of group B.

In contrast to this is the effect of dohyfral in the case of the exhausted hens of group G, the yolks of which showed a considerable increase of vitamin A when about 740 I.U. per day were added to the rations in the form of dohyfral oil.

The livers of the hens of groups A and B showed a difference of vitamin A content amounting to 5349 I.U. per liver. This difference is small when the amount of dohyfral oil consumed in the course of 9 months (about 27,000 I.U.) is considered, but it is presumably connected with the consumption of this latter substance by group A.

Concerning the difference (55.6 I.U.) between the yolks of groups B and V it may be mentioned that the only variable factor in these groups was the housing.

(b) *Vitamin A contents of yolks of eggs laid by groups fed on yellow maize and no alfalfa (the food of D and VI contained dohyfral as well).*

Groups ...	D	VI	E
	132.3	139.2	138.7
	152.9	146.3	149.7
	156.2	135.1	149.7
	152.0	147.4	146.3
	165.9	140.7	147.4
Average	151.9	141.7	146.4

As the figures of these tables show, a diet including yellow maize and alfalfa produced yolks containing twice as much vitamin A as rations without alfalfa.

Only once was a mixed yolk of group C analysed. It proved to contain 142.5 i.u. per yolk. Groups C, D and VI were fed alike. Group E differed from these three in that the hens received no dohyfral oil. The results showed that this difference did not affect the vitamin A content of the yolks.

(c) *The vitamin A contents of the yolks of the eggs from the groups fed on alfalfa and no yellow maize (the food of these groups contained no dohyfral).*

Groups ...	F	H
	157.1	254.8
	144.8	281.6
	159.0	267.0
	168.3	282.9
	170.6	259.9
Average	160.0	269.2

The difference between groups F and H in regard to diet lay in the fact that alfalfa constituted 10 % of the mash of group F and 15 % of that of group H, or about 5 and 7.5 % of the whole rations of these two groups respectively.

While the average content of group F corresponded with that of the groups that were given yellow maize but no lucerne, the content of the yolks obtained from group H was considerably higher than the latter, though distinctly lower than the content of those whose rations contained both yellow maize and lucerne. Hence 5 % lucerne in the mash or 2.5 % in the total daily diet given to group H over and above the quantity fed to group F had not quite the same effect as the 25 % maize in the case of group B, in spite of the fact that it contains more carotene. From a comparison of groups VII and H we may conclude that it is inadvisable to give hens maize in addition to 15 % of lucerne in the mash on account of the small difference effected in the vitamin A content of the yolks (290.7-269.2 = 21.5 i.u.).

In regard to the question whether 15 % alfalfa in the mash is not rather too much for other reasons, i.e. on account of the high percentage of crude fibre, we will only remark that in our opinion this is not unlikely.

The vitamin A contents of single yolks examined from the beginning of the experiment till about the end of July or the beginning of August were as follows.

When both alfalfa and yellow maize were given the content of vitamin A was somewhat more than 200 i.u. at the beginning of the experiment (groups A and B), which figure rose to a good 300 by the beginning of August (some yolks did not reach more than about 250-270¹); groups V and VII did not rise to more than 230 and 250 i.u. respectively.

On a diet including yellow maize but no lucerne the yolks produced during May and June (groups C, D and VI) contained 150 i.u. of vitamin A. This amount corresponds with that of the mixed yolks of these groups.

No yolks from group E (yellow maize, no alfalfa or dohyfral) were examined in June and July. At the beginning of May they showed respectively 98.4, 123.1 and 122.8 i.u.

In group F given alfalfa without yellow maize or dohyfral the content of the single yolks (yolks of 4 hens were examined) rose to 224 i.u. before August (namely to 212.4, 247.4, 225.7 and 209.9). The average content of the August mixed yolks was 160 i.u.

In the case of 3 hens in group H the content of the yolks rose towards August respectively to 258.3, 243.8 and 261.2 i.u., which means that they reached about the same height as the mixed yolks in August.

¹ As the hens had not had any alfalfa before, a gradual increase was to be expected.

From the analyses of mixed yolks and single yolks we see that the vitamin A content of these yolks varies very much according to the food given to the hen. A diet that was very good from this point of view produced yolks with a content 5 or 6 times as high as that of the yolks with the lowest content; moderate rations produced yolks containing 3 times as much. The minimum contents were about 50 to 60 i.u. per g. of yolk, the maximum about 330. It appeared that they vary greatly with the amount of carotene in the food. A comparison of groups E and G proves that the carotene of yellow maize¹ (without lucerne and without dohyfral) increases the vitamin A content of the eggs. It also improves the egg production and health of the hens. A comparison of F with G shows the same effect of alfalfa (without dohyfral and without yellow maize). This effect is a little greater than that produced by yellow maize for, as we saw, the average of group F was 160, of group E 146.4 i.u. The carotene content of the food of group F was higher than that of group E.

The carotene and xanthophyll contents of mixed yolks in $\mu\text{g.}$ per yolk

(a) *In the yolks of groups given a diet containing both yellow maize and dehydrated alfalfa the following amounts of carotene and xanthophyll ($\mu\text{g.}$) were found (the rations of group A also included dohyfral).*

	A		B		V		VII	
	Car.	Xan.	Car.	Xan.	Car.	Xan.	Car.	Xan.
	35.6	438.6	29.8	412.2	28.4	417.0	35.8	335.4
	44.4	524.6	32.2	394.4	31.1	341.0	34.4	360.0
	33.2	474.2	38.2	390.0	27.5	340.4	33.9	451.2
	38.2	535.6	32.0	388.0	23.2	262.0	36.0	471.1
	40.6	558.1	24.8	402.3	29.0	295.0	37.2	462.8
Average	38.4	506.2	31.4	397.4	27.8	351.0	35.4	461.1

Comparing the vitamin A content with the carotene content it may be seen that the number of i.u. of vitamin A was nearly 10 times the number of $\mu\text{g.}$ of carotene. If we assume that 1 i.u. of vitamin A is equivalent to 0.6 $\mu\text{g.}$ carotene, the ratio of the biological values is about 5 : 1.

The xanthophyll content was more than 10 times the carotene content. Hence the colour of the yolks produced on yellow maize and lucerne—and undoubtedly in general on green food—is chiefly due to xanthophyll.

To conclude from this that there is no reason to prefer eggs with highly coloured yolks to others is however not correct, for the intense colour is a fairly sure proof that sufficient green food was consumed by the hen and this green food is, as we saw, a good source of vitamin A in yolks.

An egg gets its value as a source of vitamin A almost exclusively from the vitamin itself.² We noted that this is also true of eggs of hens fed on a diet poor in vitamin A and carotene, as in the case of group G.

¹ The only difference between the foods of these two groups was that group G was given white and group E yellow maize.

² In saying this we take for granted that xanthophyll is of no value for the human or animal organism as a source of vitamin A.

(b) *In the yolks of eggs produced on a diet including yellow maize but no alfalfa the following amounts of carotene and xanthophyll ($\mu\text{g.}$) were found (the diet of groups D and VI contained also dohyfral).*

	D		VI		E	
	Car.	Xan.	Car.	Xan.	Car.	Xan.
	14.4	140.0	22.2	315.0	21.0	287.7
	17.4	177.6	24.0	298.0	18.2	280.0
	22.6	190.6	17.8	229.0	25.0	258.5
	22.8	149.1	16.8	277.2	25.2	291.0
	24.2	202.0	21.4	334.6	26.0	320.0
Average	20.3	171.8	20.4	290.7	23.1	287.4

As was to be expected the carotene content is lower here than in groups given both maize and lucerne. Here too, as in groups given both alfalfa and maize, the carotene content is small as compared with that of vitamin A; the ratio between the two is not quite the same as that found in the other groups; for in D, VI and E about 20 $\mu\text{g.}$ of carotene were found, while each yolk was found to contain about 147 i.u. of vitamin A. Hence the ratio was about 1 : 7. In the case of groups A, B, V and VII it was nearly 1 : 10.

These yolks, too, are rich in xanthophyll. In groups VI and E the xanthophyll contents were respectively 14 and 12.5 times as large as the carotene content. Why group D eggs contain so much less xanthophyll per yolk cannot be explained; the rations of D and VI were identical, moreover these yolks derive their colour chiefly from xanthophyll. That maize can produce yolks with a high xanthophyll content is obvious from the figures of group E (without alfalfa or dohyfral).

(c) *Carotene and xanthophyll contents ($\mu\text{g.}$) of yolks from groups with rations containing alfalfa but no yellow maize (there was no dohyfral in these diets).*

	F		H	
	Car.	Xan.	Car.	Xan.
	18.4	240.0	22.2	327.0
	14.0	226.0	22.4	277.6
	18.2	268.0	25.6	242.0
	20.0	256.8	21.0	265.0
	15.0	280.8	19.6	316.4
Average	17.1	256.1	22.1	258.6

The carotene content in H is higher than in F, a fact which is not to be wondered at considering that the mash fed to H contained 15 % of lucerne, while that fed to F contained only 10 %. Maximal contents were not reached in these groups.

It appears that the carotene in yellow maize is a better source of vitamin A and carotene in yolks than that contained in lucerne. The xanthophyll contents in groups F and H are somewhat less than in groups E and VI.

Our results agree with those obtained by the biological method (experiments with young rats). Bethke *et al.* [1927] reported experiments in which the vitamin A of yolks was determined with young rats. It was found that: (1) vegetable food (e.g. blue grass) gave the same growth results as cod liver oil, and (2) the growth of the rats was satisfactory if they were given at least 0.1 ml. of yolk of egg, provided that the latter was laid by a hen fed cod liver oil, alfalfa or blue grass.

If by "satisfactory" was meant the growth accepted by Sherman-Munsell as the basis for the rate of growth, then we may conclude that the whole yolk

contained 180 Sh.M. units. But presumably Bethke and co-workers required a rate of growth greater than "satisfactory". If 1.4 I.U. were taken as equivalent to 1 Sh.M. unit (as is often assumed) then Bethke's yolks must have contained at least 250 I.U.

A third conclusion reached by Bethke was that when the animals were given a satisfactory diet the biological action of the yolks was five times as great as when the diet contained insufficient vitamin A and carotene.

Bearse & Miller [1937] concluded from their experiments with young rats that yolks contain 7.5-40 Sh.M. units per g., or per whole yolk 135-720. A higher content was obtained by means of a sufficient amount of dehydrated alfalfa.

Chicks hatched from eggs laid by hens which had consumed little carotene died sooner on a diet poor in vitamin A than chicks from better eggs. We found that the amount of vitamin A present in the liver of newly hatched chicks ran parallel with the content of the yolks. This result agrees with that found by Baumann *et al.* [1939], i.e. that the vitamin A content of the bodies of newly hatched chicks ran parallel with the number of units found spectrophotometrically in the yolks. The number of days these little chicks could be kept alive on a diet poor in vitamin A was also dependent on the amount of this substance found in the yolks by the non-biological method.

Hence we may conclude that the results obtained by the biological method correspond very well with our own, which fact is significant in establishing the value of our results, in view of the uncertainty which still exists as to whether physico-chemical estimation gives the same results as the biological method. Both methods may be used for the materials we are dealing with. We estimated the amounts of vitamin A and carotene in a large number of livers of our hens and young chicks: (a) from hens belonging to our 11 groups, (b) from chicks hatched from eggs laid by hens of groups V, VI, and VII and (c) from young cockerels fed on various diets. The results of these estimations corresponded with what we expected on the ground of our researches into the relation between yolks and diets.

It may be expected that hen's eggs on the market will contain about 300 I.U. of vitamin A. Poultry farmers are well aware that eggs for marketing are often not very good for hatching. The rations which poultry breeders usually give their hens are usually better than those used by farmers whose aim is to produce eggs for consumption.

It should be noted that the low content found in the case of group G is seldom met with, because the egg production of hens given this diet decreases too much and the death-rate is too high.

We may conclude that in eggs, as in milk and butter, the vitamin A activity depends very largely on the amount of green food consumed, whether fresh or conserved in such a way as to allow a considerable part of the carotene to be retained. The vitamin A activity of butter is of the same order of magnitude as that of yolks.

Where poultry have access to pasture they eat enough grass to bring up the vitamin A content of the yolks to a maximum. This undoubtedly is often not the case with poultry kept to produce eggs for consumption.

As we saw, feeding 25 % yellow maize as the only source of carotene produced a vitamin A activity in the eggs of our experiments only half as great as feeding both maize and green fodder (dehydrated alfalfa). In actual practice it is unlikely that poultry will often be given sufficient maize to produce eggs with 300 I.U. of vitamin A.

SUMMARY

The object of this research was to determine the vitamin A, carotene and xanthophyll contents of the yolks of hens' eggs of groups fed on different diets. After preliminary treatments both the yellow colour derived from carotene and xanthophyll and the blue colour (derived from vitamin A according to Carr and Price) were determined by means of the Stufenphotometer.

We used 300 hens for our experiments which lasted 9 months.

Although storage of vitamin A in the liver before laying begins, and in general individual capacities of the hens, may give rise to marked differences, it appeared that after the birds had been in production for some time the amount of the three above-named substances found in the yolks depends chiefly on feeding, i.e. on the vitamin A potency of the hens' rations. Hence it is this factor which determines whether a yolk contains 300 or 150 or only 50–60 i.u. of vitamin A. In the latter case the content could be distinctly increased within a few days by supplementing the diet with dohyfral (containing shark oil), cod liver oil or alfalfa.

Yellow maize and green fodder, either fresh or dried, are the most important sources of the vitamin A and carotene of the yolk. With the help of dehydrated alfalfa, cod liver oil or dohyfral oil, hens which had previously been reduced to a state of vitamin A exhaustion were brought into a condition where they laid eggs the yolks of which contained twice to four times as much vitamin A as before.

The highest values were found in eggs from hens fed on a diet containing both yellow maize and dehydrated alfalfa. Yellow maize alone—constituting 25 % of the rations—produced yolks with a vitamin A content of 150 i.u. per yolk; with 7.5 % alfalfa containing 140 μ g. carotene per g. in the ration but without yellow maize it was about 270 μ g. per yolk.

Yolks with the highest vitamin A content—produced on rations containing yellow maize plus lucerne—had a carotene content of almost 40 μ g.; the poorest eggs fell to less than 10 μ g. per yolk.

Hence a yolk derives its vitamin A potency mainly from the vitamin itself. The main source of the latter is, however, the carotene contained in the food.

There is much more xanthophyll than carotene in yolks. The yellow colour is therefore chiefly due to the former. Nevertheless strongly coloured eggs are generally also rich in vitamin A and carotene, because the xanthophyll in them is certainly in part derived from those components of the diet which also contain a great deal of carotene.

Green fodder plays a very important part in connexion with the vitamin A potency of eggs, as it does in the case of milk and butter.

Our results are in good agreement with those obtained by the biological method.

On several poultry farms the vitamin A potency of the ration is not sufficient for the production of eggs containing more than 150–200 i.u. of vitamin A.

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