

MODIFYING VITAMIN COMPOSITION OF EGGS: A REVIEW^{1,2}

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Primary Audience: Egg Producers, Nutritionists, Quality Control Personnel, Hatchery Managers

SUMMARY

Egg vitamin composition is variable and dependant primarily on the vitamin content of the diet of laying and breeding hens. Vitamin A content of eggs responds slowly to dietary vitamin A changes whereas, riboflavin content of eggs responds rapidly to similar changes. Riboflavin reaches a natural maximum content in eggs, while the egg content of vitamin D, pantothenic acid, folacin, biotin, and vitamin B₁₂ responds greatly to increases in the diet level of these vitamins. At one to two times the National Research Council diet requirement levels, transfer efficiency to the egg is very high for vitamin A; it is high for riboflavin, pantothenic acid, biotin, and vitamin B₁₂; it is medium for vitamin D₃ and vitamin E; and low for vitamin K₁, thiamine, and folacin. This information can assist growers in formulating diets for laying and breeding hens that will enrich egg vitamin content. At the same time, growers can eliminate much of the variability that has been observed in egg vitamin composition.

Key words: Efficiency, egg vitamin content, transfer from diet, variability

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INTRODUCTION

Poultry scientists have been interested in eggs as food for many years and have found that the quality of eggs is variable and dependent upon a number of physical, chemical, and biological factors. The variability in wholesomeness of eggs has been shown to be related to cleanliness, freshness, physical characteristics, bacterial contamination, foreign chemical residues, and appropriate nutrient content. Recently there has been a rebirth

of interest in the nutritive composition of eggs. The purpose of this review is to examine the variability in vitamin content of eggs, to assess the factors that may influence egg vitamin content, to look at the patterns of vitamin deposition in eggs, and to calculate indices of vitamin transfer by the hen from her diet to the egg.

In the past, poultry nutritionists have been interested in establishing dietary levels of vitamins that supported maximum levels of egg production and/or hatchability of eggs. Thus, the establishment of maximum performance

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of the hen through dietary manipulation resulted in concentrations of critical vitamins in the eggs. To put it another way, the normal egg concentration of vitamins tended to be associated with either high rates of egg production in layers or high rates of egg hatchability in breeders. Egg vitamin content tended to be a function of biological performance.

Today the public's interest in the nutritive value of foods has increased markedly. These concerns have brought about a greater awareness about the nutrient composition of foods. Among these concerns are the concentration and variability in levels of nutrients in foods. For the egg this has meant concerns about too much cholesterol and the nature of fatty acids, and, to a lesser extent, concerns about protein, vitamins, and minerals in eggs. As a consequence, the interest in modifying egg vitamin composition now extends beyond production considerations to designing a high quality food for human consumption that is of consistent nutrient composition.

FACTORS INFLUENCING EGG VITAMIN COMPOSITION

There is some evidence that genetics plays a role in the utilization and deposition of vitamins in eggs. Maw [1] first described riboflavin deficiency in eggs that inherited in a recessive gene carried by a strain of White Leghorns. Scrimshaw *et al.* [2] reported that the thiamine content of eggs from White Leghorns is greater than that found in eggs from Rhode Island Reds and Barred Plymouth Rocks fed the same diet. Mayfield *et al.* [3] showed that White Leghorn hens laid eggs higher in riboflavin and thiamine content than those from New Hampshires fed the same diets. Howes and Hutt [4] found no differences between thiamine content of eggs associated with the presence of the gene for rapid feathering and that of eggs associated with slow feathering in crossbred hens. More recently Leeson *et al.* [5] reported that White Leghorn and Rhode Island Red hens respond differently to deficiencies of vitamins in egg production and hatchability of eggs. While some breed differences in vitamin utilization exist, no differences have been described in major strains of White Leghorns used as the basis for modern egg production.

The riboflavin content of eggs has been shown to decline with increasing age of White Leghorn-type hens both in experimental studies by Squires and Naber [6] and in commercial breeding flocks by Naber and Squires [7]. Robel [8] reported significant changes in the vitamin content of turkey eggs based on the age of the breeder hens. Pyridoxine and vitamin B₁₂ levels decreased with age, while biotin levels increased with the age of the hen.

The rate of egg output can also influence egg vitamin content. Koenig *et al.* [9] showed that White Leghorn pullets exhibiting low rates of egg output produced eggs of vitamin A content similar to eggs of pullets exhibiting high egg output rates after four months of lay. Following the first year of egg production, however, the hens with low rates of egg output produced eggs with higher vitamin A content than did those showing high rates of egg output. Because most pullets enter the laying cycle with considerable liver stores of vitamin A, egg vitamin A can be maintained for several months [10] before egg output of the vitamin changes. In general, high rates of egg production limit egg vitamin content since egg output is not completely compensated for by the increased dietary intake of vitamins.

Among the factors influencing egg vitamin composition, the most important one is feed vitamin content. This has been shown in detail by recent investigations [6, 10, 11]. However, changes in the patterns of vitamin concentration in eggs, as a response to dietary concentration, vary with and are a function of individual vitamins. Egg riboflavin level in yolk and albumen responded rapidly to the dietary level of this vitamin [6]. The data also show that there is a ceiling on the amount of riboflavin that the laying hen can transfer to the egg when fed between two and four times the 1984 NRC requirement level [12].

On the other hand, egg vitamin A content in response to dietary vitamin A level was delayed for 8 to 12 weeks due because of the well known modulating effect and storage capacity of the liver [10]. After equilibrium is established, there is some relationship between egg vitamin A content to diet level. But at four times the NRC level of requirement egg content responded only partially to diet content of the vitamin. The egg content of vitamin B₁₂ was almost exactly proportional to diet content over one to four times the NRC

requirement level for the breeding hen [11]. For vitamin B₁₂ some time was required to reach egg equilibrium content because the pullets had high storage levels of the vitamin at the outset of the study. It seems fair to conclude that feed vitamin content has the greatest and most widespread influence on egg vitamin content.

VARIABILITY IN EGG VITAMIN COMPOSITION

While variability in egg nutrient composition has been reported in the past [13], some of this variability has resulted from the methods of analysis employed. In recent years analytical methods have been improved and standardized. In 1988, the Egg Nutrition Center organized a nationwide study on the nutrient content of commercial eggs with analytical services provided by Hazelton Laboratories America [14]. The samples analyzed in this study were on composites of eggs. Each composite was made from an average of ten commercial flocks obtained from two to six states in the United States. Fifteen composite samples of whole eggs were analyzed in the study.

The mean value for each vitamin and the range of values for each vitamin expressed as a percent of the mean was calculated and is presented in Table 1. From this data it is obvious that the range of vitamin contents from the mean value was a low of 22% for vitamin A and

a high of 110% for vitamin B₁₂. The mean range for all vitamins was 50%. This variability in vitamin content of eggs from the Egg Nutrition Center study probably underestimates the true variability between commercial flocks because the analyses for this study were conducted on large composite samples.

Recent analyses on sixteen Ohio individual flocks [7] for three vitamins also appear in Table 1. The results show a greater range in egg content of vitamin B₁₂ (166%) and vitamin A (48%), but a lower range for riboflavin (36%). However, a close examination of the data from the Egg Nutrition Center [13] showed that one composite of eggs had a very high riboflavin content. This high value for riboflavin in the one sample was beyond related studies showing a ceiling on egg riboflavin levels in response to diet content [6, 15] and was responsible for the larger range of riboflavin values in the Egg Nutrition Center study. The lower variability in vitamin A egg content from the Egg Nutrition Center data may result from the modulating effect of the liver on vitamin A transfer to the egg previously mentioned. The high range in vitamin B₁₂ content of eggs may confirm the previous observation that there is no ceiling on B₁₂ transfer to the egg at up to four times the National Research Council dietary requirement level. This variability in egg vitamin content is of concern when consistency of food composition is desired, particularly when one considers the nutrient labeling of foods.

TABLE 1. Variability in the vitamin content of eggs from commercial flocks

VITAMIN	EGG NUTRITION CENTER STUDY, 1988		OHIO FLOCKS (Naber and Squires, 1993)	
	Mean ^A	Range	Mean ^B	Range
Thiamine	0.063 mg	64%		
Riboflavin	0.501 mg	52%	0.405 mg	36%
Niacin	0.08 mg	38%		
Pantothenic Acid	1.170 mg	26%		
Pyridoxine	0.144 mg	35%		
Folic Acid	0.0436 mg	36%		
Vitamin B ₁₂	0.833 μg	110%	3.09 μg	166%
Vitamin A	624 IU	22%	3400 IU	48%
Vitamin E	1.22 IU	65%		

^AUnits per 100 g of whole egg composites from 15 regions of the United States

^BUnits per 100 g of egg albumen for riboflavin, per 100 g egg yolk for vitamin B₁₂, and vitamin A from 16 individual flocks.

RELATIONSHIP OF DIETARY VITAMIN CONTENT TO EGG VITAMIN CONTENT

A major part of this review is an attempt to look at the relationship of dietary vitamin levels to egg vitamin contents. One way to view this problem is to compare specific diet vitamin levels to levels of the vitamin found in eggs. Then one can calculate a proportionality index based on setting the egg level of the vitamin obtained by feeding the lowest diet level equal to 100. With such a calculation, higher egg levels, obtained by feeding higher diet levels, would have an index of 100 only if egg level increased in direct proportion to the diet level. Calculations of this type were made from research data in the literature for studies with White-Leghorn type hens fed either practical or purified diets. Data were used only from those papers where both diet and egg contents of the vitamin were published or could be calculated from the data presented. Egg content data for these calculations was selected

from time periods after equilibrium to diet level of the vitamin had been reached. The pooled results of these calculations appear in Table 2.

In almost every case, egg vitamin content increased as the diet level of the vitamin increased (Table 2). While more vitamin was transferred to the egg with increased diet level, the calculated proportionality index shows that egg levels usually fell short of keeping pace with increasing diet content. In the case of vitamin E, feeding ten times the amount resulted in an increase of five times the vitamin E in the egg, a proportionality index of 50 [16]. In this case the vitamin E was given in ten daily doses of $\frac{1}{10}$ the amount shown as the comparable diet level. Egg levels of vitamin A and D increase considerably in response to dietary level increases but not in direct proportion to diet levels [10, 17, 18]. While no data on vitamin K₁ eggs levels in response to change in diet level exists, Griminger [19] was able to demonstrate a marked reduction in the prothrombin times in plasma from chicks hatching from eggs laid by hens given ten times more

TABLE 2. The relationship of dietary vitamin level to egg vitamin content

VITAMIN	REFERENCE	DIET LEVEL	EGG LEVEL OF VITAMIN	PROPORTIONALITY INDEX
		(units/kg)	(units/g)	
A	Squires and Naber, 1993	4,000 IU	10.3 IU	100
		8,000 IU	16.9 IU	82
		16,000 IU	24.0 IU	58
	Hill et al., 1961	2,600 IU	3.7 IU	100
		3,500 IU	4.2 IU	84
		4,400 IU	6.3 IU	100
		11,000 IU	12.7 IU	81
D	Bethke et al., 1936	540 IU	0.9 IU	100
		5,400 IU	6.0 IU	67
		54,000 IU	57.0 IU	63
E	Dju et al., 1950 (as α -tocopherol)	100 mg ^A	0.07 mg	100
		200 mg	0.12 mg	86
		300 mg	0.15 mg	71
		400 mg	0.24 mg	86
		600 mg	0.28 mg	67
		1,000 mg	0.35 mg	50
K ₁	Griminger, 1964	1 mg	54 min ^B	-
		10 mg	15 min	-

Product Components Symposium

NABER

389

TABLE 2. (cont'd)

VITAMIN	REFERENCE	DIET LEVEL	EGG LEVEL OF VITAMIN	PROPORTIONALITY INDEX
		(units/kg)	(units/g)	
Riboflavin	Squires and Naber, 1993	2.2 mg	yolk-3.1 μ g	100
		4.4 mg	yolk-5.6 μ g	90
		8.8 mg	yolk-5.7 μ g	46
		2.2 mg	alb.-1.6 μ g	100
		4.4 mg	alb.-3.3 μ g	103
		8.8 mg	alb.-3.4 μ g	53
	Petersen et al., 1947	2.5 mg	2.4 μ g	100
		3.6 mg	4.6 μ g	133
		5.1 mg	5.4 μ g	110
		7.5 mg	5.5 μ g	76
10.0 mg		6.1 μ g	63	
Pantothenic Acid	Snell et al., 1941	4.4 mg	3.6 μ g	100
		19.0 mg	17.3 μ g	110
		42.6 mg	35.1 μ g	101
Folacin	Sunde et al., 1950 (yolk only)	0.25 mg	29 ng	100
		0.50 mg	42 ng	72
		0.75 mg	57 ng	66
		1.00 mg	116 ng	100
		1.25 mg	107 ng	74
		1.50 mg	206 ng	118
		2.00 mg	155 ng	67
		3.00 mg	259 ng	74
	Couch and German, 1950 (yolk only)	0.22 mg	330 ng	100
		0.42 mg	380 ng	52
0.82 mg		880 ng	72	
Biotin	Buenrostro and Kratzer, 1984 (yolk only)	25 μ g	180 ng	100
		75 μ g	330 ng	61
		150 μ g	560 ng	52
		300 μ g	770 ng	36
Vitamin B12	Denton et al., 1954	30 μ g	41 ng	100
		50 μ g	88 ng	128
		100 μ g	154 ng	112
		250 μ g	221 ng	65
	Squires and Naber, 1992 (yolk only)	4.0 μ g	13 ng	100
		8.0 μ g	26 ng	100
		16.0 μ g	48 ng	92
^A Oral administration daily over 10 days ^B Plasma prothrombin times in chicks				

vitamin K₁ in their diets. This is indirect proof that more vitamin K₁ transferred to the egg when hens were fed more of the vitamin. Egg riboflavin content increases markedly with diet increases but drops off sharply at high diet levels [6, 15]. Pantothenic acid content of eggs appears to increase in direct proportion to diet level [20]. While the folacin content of eggs increases with more dietary folacin, the relationships on proportionality are not very consistent and may have been influenced by variations in assay of the vitamin and gut synthesis of folacin [21, 22]. Biotin level in egg yolk shows an increasing level in response to elevation of diet content, but the proportionality index declines [23]. Finally, with vitamin B₁₂, large increases in egg content are noted with increasing diet levels; proportionality is maintained over a 25-fold increase in diet content [11, 24].

It may be concluded from the data presented in Table 2 that egg levels of vitamins are generally responsive to diet contents. However, the pattern of egg level in response to diet level is a function of individual vitamins with riboflavin exhibiting upper limits in egg content, while most vitamins show some response in egg content and others appear to increase in egg in a manner directly related to diet content (pantothenic acid and vitamin B₁₂).

VITAMIN TRANSFER EFFICIENCY TO THE EGG

Another way to look at diet to egg vitamin relationships is to calculate the efficiency of vitamin output in eggs as a function of dietary intake. Data required for such calculations are somewhat different from those used for the previous section. Transfer efficiency of a vitamin depends on diet level and diet intake on one hand and on egg output considering egg weight and rate of egg production on the other. Only data from research papers using White

Leghorn-type hens fed practical diets under conditions where normal rates of egg production were maintained were used for these calculations. Where data on feed intake, egg weight, and egg production were presented, these were used in transfer efficiency calculations. The data are presented in Table 3.

The transfer efficiency of vitamin A was very high when vitamin A was fed at one or two times the 1984 NRC requirement level, but dropped markedly at four times the NRC requirement level [10]. While egg vitamin A transfer did not respond to four times the NRC requirement level, the liver content of vitamin A continued to increase. Vitamin D transfer efficiency averaged about 20%, but there was only a small reduction in transfer efficiency over a 100-fold range in diet level of this vitamin [18]. The efficiency of transfer for vitamin E in three different studies [16, 25, 26] was somewhat variable but in the same range as vitamin D. For vitamin K₁ transfer efficiency was low based on limited data [27]. Thiamine transfer to the egg also exhibits low efficiency [3, 4]. The transfer of riboflavin was relatively good at one and two times the NRC laying hen requirement level, but like vitamin A was sharply reduced at higher diet levels [6, 15]. Both research papers dealing with pantothenic acid [20, 28] show a consistently high rate of transfer to the egg (similar to riboflavin at the lower diet levels). Folacin transfer to eggs showed a low efficiency between 7 and 10% [29, 30] and did not seem to be affected by the presence of antibiotic in the hen diet. Biotin transfer to eggs calculated from three papers [23, 29, 31] was uniformly good and had results like those for riboflavin and pantothenic acid. The transfer efficiency for vitamin B₁₂ was also like that for riboflavin, pantothenic acid, and biotin [11, 32] but unlike riboflavin, transfer efficiency continued to be high at 40 times the NRC requirement level for the breeding hen. Clearly, extensive fortification of eggs with vitamin B₁₂ is possible [11].

Product Components Symposium

NABER

391

TABLE 3. Vitamin transfer to the egg by laying hens fed practical diets

VITAMIN	REFERENCE	DIET LEVEL	OUTPUT IN EGG	TRANSFER EFFICIENCY
		(units/kg)		(%)
A	Naber and Squires, 1993	4,000 IU ^A	153 IU ^B	77
		8,000 IU	244 IU	80
		16,000 IU	250 IU	39
D	Bethke <i>et al.</i> , 1936 (from cod liver oil)	540 IU ^A	13 IU ^B	24
		5,400 IU	90 IU	17
		54,000 IU	850 IU	16
E	Dju <i>et al.</i> , 1950	30 mg	580 µg ^B	19
	Bartov <i>et al.</i> , 1965	12.6 mg ^C	205 µg ^B	16
	Nobile and Irving, 1966	22.2 mg	870 µg ^B	39
K ₁	Griminger and Brubacher, 1966	5.2 mg ^D equivalent	25 µg ^B	5
Thiamine	Mayfield <i>et al.</i> , 1955	4.6 mg	57 µg	12
	Howes and Hutt, 1956	4.2 mg	43 µg ^B	10
Riboflavin	Naber and Squires, 1993	2.2 mg ^A	105 µg	43 ^E
		4.4 mg ^B	221 µg	46 ^E
		8.8 mg	231 µg	24 ^E
	Petersen <i>et al.</i> , 1947	2.5 mg ^A	124 µg	43
		3.6 mg ^C	229 µg	52
		5.1 mg	265 µg	47
		7.5 mg	279 µg	31
		10.0 mg	280 µg	23
Pantothenic Acid	Snell <i>et al.</i> , 1941	19.0 mg	935 µg	49
	Evans <i>et al.</i> , 1952	12.7 mg ^C	608 µg	48
Folacin	Waibel <i>et al.</i> , 1952	0.70 mg	5.1 µg ^B	7
		0.70 mg + antibiotic	5.7 µg	8
	Terri <i>et al.</i> , 1959	0.37 mg ^C	3.6 µg ^C	10
Biotin	Sunde <i>et al.</i> , 1950	0.16 mg ^C	5.9 µg	37
	Waibel <i>et al.</i> , 1952	0.16 mg ^C	4.8 µg ^B	30
	Buenrostro and Kratzer, 1984	0.17 mg ^C	8.0 µg ^B	48
Vitamin B ₁₂	Naber and Squires, 1993	4.0 µg ^C	0.22 µg ^B	44
		8.0 µg	0.42 µg	42
		16.0 µg	0.78 µg	39
	Halick <i>et al.</i> , 1953	120 µg equivalent ^F	4.60 µg	38

^AAt or near NRC requirement level for the laying hen
^BEgg yolk output only
^CAt or near NRC requirement level for the breeding hen
^DDaily intake of 0.52 mg for 10 days
^ECorrected for egg size and rate of egg production
^FDaily intake of 12 µg per hen

TABLE 4. Classification of vitamins by relative transfer efficiency to the egg by the hen from her diet

TRANSFER EFFICIENCY ^A	VITAMIN
Low (5 to 10%)	Vitamin K ₁
	Thiamine
	Folacin
Medium (15 to 25%)	Vitamin D ₃
	Vitamin E
High (40 to 50%)	Riboflavin
	Pantothenic Acid
	Biotin
	Vitamin B ₁₂
Very High (60 to 80%)	Vitamin A

^AWhen the vitamin is fed at one or two times the NRC dietary requirement level and after egg equilibrium to diet content of the vitamin has been reached

CONCLUSIONS AND APPLICATIONS

1. After examination of the data calculated for transfer efficiency of the vitamins, it may be concluded that eggs can be enriched in their vitamin content over certain ranges of diet fortification and with varying efficiency of vitamin transfer. If vitamin enrichment of eggs were to be seriously considered, vitamin transfer efficiency as well as vitamin costs would enter into such considerations.
2. Table 4 shows a classification of vitamins by relative transfer efficiency of the hen from her diet to the egg. In the future, use of this data could help eliminate much of the variability in egg vitamin content from commercial flocks as demonstrated earlier in this review.
3. It is a challenge to nutritionists to consider this information in the formulation of diets not only to maximize egg output in layers and hatchability in breeders but also to extend their concern to the uniformity of egg content of vitamins as food for human consumption.

REFERENCES AND NOTES

1. Maw, A.J.G., 1954. Inherited riboflavin deficiency in chicken eggs. *Poultry Sci.* 33:216-217.
2. Scrimshaw, N.S., F.B. Hutt, M.W. Scrimshaw, and C.R. Sullivan, 1945. The effect of genetic variation in the fowl on the thiamine content of the egg. *J. Nutr.* 30:375-383.
3. Mayfield, H.L., R.R. Roehm, and A.F. Beeckler, 1955. Riboflavin and thiamine content of eggs from New Hampshire and White Leghorn hens fed diets containing condensed fish or dried whale solubles. *Poultry Sci.* 34:1106-1111.
4. Howes, C.E. and F.B. Hutt, 1956. Genetic variation in efficiency of thiamine utilization by the domestic fowl. *Poultry Sci.* 35:1223-1229.
5. Leeson, S., B.S. Reinhart, and J.D. Summers, 1979. Response of White Leghorn and Rhode Island Red breeder hens to dietary deficiencies of synthetic vitamins. 2. Egg production hatchability and chick growth. *Can. J. Anim. Sci.* 59:561-567.
6. Squires, M.W. and E.C. Naber, 1993. Vitamin profiles of eggs as indicators of nutritional status in the laying hen: Riboflavin study. *Poultry Sci.* 72:483-494.
7. Naber, E.C. and M.W. Squires, 1993. Vitamin profiles of eggs as indicators of nutritional status in the laying hen: Diet to egg transfer and commercial flock survey. *Poultry Sci.* 72:1046-1053.
8. Robel, E., 1983. The effect of age of breeder hen on the levels of vitamins and minerals in turkey eggs. *Poultry Sci.* 62:1751-1756.
9. Koenig, M.C., M.M. Kramer, and L.F. Payne, 1935. Vitamin A content of eggs as related to rate of production. *Poultry Sci.* 14:178-182.
10. Squires, M.W. and E.C. Naber, 1993. Vitamin profiles of eggs as indicators of nutritional status in the laying hen: Vitamin A study. *Poultry Sci.* 72:154-164.
11. Squires, M.W. and E.C. Naber, 1992. Vitamin profiles of eggs as indicators of nutritional status in the laying hen: Vitamin B₁₂ study. *Poultry Sci.* 71:2075-2082.
12. National Research Council, 1984. Nutrient requirements of poultry. 8th rev. ed. National Academy Press, Washington, DC.
13. Naber, E.C., 1979. The effect of nutrition on the composition of eggs. *Poultry Sci.* 58:518-528.

14. **Egg Nutrition Center**, 1988. Final draft report on cholesterol, fatty acid, and nutrient analysis of large shell eggs. For Egg Nutrition Center, Washington, DC. Prepared by Hazelton Laboratories America, Inc., Madison, WI.
15. **Petersen, C.F., C.E. Lampman, and O.E. Stamberg**, 1947. Effect of riboflavin intake on egg production and riboflavin content of eggs. *Poultry Sci.* 26:180-186.
16. **Dju, M.Y., M.L. Quaife, and P.L. Harris**, 1950. Utilization of pure α , β and δ -tocopherols by laying hens. *Am. J. Physiol.* 160:259-263.
17. **Hill, F.W., M.L. Scott, L.C. Norris, and G.F. Heuser**, 1961. Reinvestigation of the vitamin A requirement of laying and breeding hens and their progeny. *Poultry Sci.* 40:1245-1254.
18. **Bethke, R.M., P.R. Record, O.H. M. Wilder, and C.H. Kick**, 1936. Effect of different sources of vitamin D on the laying bird. II. Storage of vitamin D in the egg and chick and mineral composition of the mature embryo. *Poultry Sci.* 15:336-344.
19. **Griminger, P.**, 1964. Effect of vitamin K nutrition of the dam on hatchability and prothrombin levels in the offspring. *Poultry Sci.* 43:1289-1290.
20. **Snell, E.E., E. Aline, J.R. Couch, and P.B. Pearson**, 1941. The effect of diet on the pantothenic acid content of eggs. *J. Nutr.* 21:201-205.
21. **Sunde, M.L., W.W. Cravens, H.W. Brvins, C.A. Elvehjem, and J.G. Halpin**, 1950. The pteroylglutamic acid requirement of laying and breeding hens. *Poultry Sci.* 29:220-226.
22. **Couch, J.R. and H.L. German**, 1950. Pteroylglutamic acid studies with the mature fowl. *Poultry Sci.* 29:539-544.
23. **Buenrostro, J.L. and F.H. Kratzer**, 1984. Use of plasma and egg yolk biotin of White Leghorn hens to assess biotin availability from feedstuffs. *Poultry Sci.* 63:1563-1570.
24. **Denton, C.A., W.L. Kellogg, J.R. Sizemore, and R.J. Lillie**, 1954. Effect of injecting and feeding vitamin B₁₂ to hens on content of the vitamin in the egg and blood. *J. Nutr.* 54:571-577.
25. **Bartov, I., P. Budowski, and S. Bornstein**, 1965. The relation between α -tocopherol content of the breeder diet and that of the newly hatched chick. *Poultry Sci.* 44:1489-1494.
26. **Nobile, S. and E.A. Irving**, 1966. Relationship of α -tocopherol in the feed to total tocopherol in the egg. *Vet. Rec.* 78:113-114.
27. **Griminger, P. and G. Brubacher**, 1966. The transfer of vitamin K₁ and menadione from the hen to the egg. *Poultry Sci.* 45:512-519.
28. **Evans, R.J., J.A. Davidson, and H.A. Butts**, 1952. The pantothenic acid content of fresh and stored shell eggs. *Poultry Sci.* 31:777-780.
29. **Waibel, P.E., M.L. Sunde, and W.W. Cravens**, 1952. Effect of addition of penicillin to the hen's ration on biotin and folic acid content of eggs. *Poultry Sci.* 31:621-624.
30. **Terri, A.E., P.M. Gannis, and R.C. Ringrose**, 1959. The B-vitamin content of eggs as influenced by antibiotic in the laying ration. *Poultry Sci.* 38:360-362.
31. **Sunde, M.L., W.W. Cravens, C.A. Elvehjem, and J.G. Halpin**, 1950. Effect of diet and cecectomy on the intestinal synthesis of biotin in mature fowl. *Poultry Sci.* 29:10-14.
32. **Halick, J.V., B.L. Reid, C.L. Brown, and J.R. Couch**, 1953. The vitamin B₁₂ content of egg yolks as influenced by oral and parenteral administration of the vitamin. *J. Nutr.* 50:331-340.