EFFECT OF FURAZOLIDONE ON THE UTILIZATION OF CAROTENE BY LAYING HENS

by

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INTRODUCTION

It has been known for several years that beta carotene from dehydrated alfalfa is converted to vitamin A by the chicken (Bolin, Lampman and Berg, 1943) (Cheng and Deuel, 1950). However, its biological value, as a source of vitamin A for the chicken, has received much discussion both pro and con.

Some workers have found that carotene was as effective as the same weight of vitamin A in growth studies and production tests (Williams, Lampman and Bolin, 1938). However, there are numerous reports in the literature whereby a greater response was observed when some or all of the carotene was replaced with vitamin A (Camp et al., 1955). There is also the problem of incompatability of new products (drugs) in feeds and vitamin A utilization.

There are still many problems concerning the relative potencies of carotene and vitamin A. Therefore, a study was conducted in an effort to accomplish the following objectives: 1. To determine the feasibility of using carotene from alfalfa as the sole source of vitamin A activity for laying hens; 2. To determine what effect different levels of furazolidone[1] has upon the utilization of carotene from dehydrated alfalfa as a source of vitamin A. Criteria used for evaluation were egg production, feed conversion, maintenance of body weight, mortality, interior and exterior quality of eggs and percentage by weight changes in component parts of eggs.

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[1]Supplied as nf-180(R) which contains 50 grams of furazolidone per pound of supplement.

REVIEW OF LITERATURE

Carotene from alfalfa has served as a valuable source of vitamin A for the fowl. It was demonstrated by Emmett and Peacock (1923) that chickens required vitamin A. The fact that chickens could utilize carotene as a source of vitamin A was reported by Bolin, Lampman and Berg (1943). Laughland and Phillips (1955) reported that chicks could absorb vitamin A and convert carotene to vitamin A when less than two weeks of age. The work of Harvey et al. (1955) was in agreement with this; in fact, they reported that carotene from alfalfa ingested during the first 12 hours after hatching was converted to vitamin A by the chick. Chicks which had been fed a diet deficient in vitamin A and carotenoid pigments were given doses of carotene extracted from alfalfa. After dosage with carotene, increased liver stores of vitamin A and prolonged survival times were observed.

According to Glover, Goodwin and Morton (1948) the main site of conversion of carotene to vitamin A in the rat is the small intestine. Mattson (1948) was able to quantitatively measure vitamin A synthesized from carotene by the rat intestinal wall.

In the rat, two hours after a carotene meal, Thompson et al. (1950) found the primary site of conversion to be just proximal to the middle of the small intestine. No conversion was observed in that part of the intestine preceding the entrance of the common bile duct. Rosenberg and Sobel (1953) demonstrated carotene conversion to vitamin A in the isolated intestinal loop of the rat. Much of this work is summarized by Moore (1957).

It has been found that the small intestine is an important conversion site in the chick as well as in the rat. Cheng and Deuel (1950) placed day-old White Leghorn male chicks on a vitamin A low diet for three to four weeks

and then fasted them for 12 hours. The chicks then were given doses of beta carotene in cottonseed oil. Vitamin A first appeared in the intestinal wall after one hour and in the liver after three hours. The proportion of vitamin A was higher in the intestine up to six hours after feeding of the carotene. After absorption of carotene had ceased, concentration of vitamin A in the intestinal wall decreased while liver vitamin A increased. Through this experiment it was demonstrated that conversion of carotene takes place in the intestinal wall. After dosing with beta carotene, Thompson, Coates and Kon (1950) reported that vitamin A appeared in the intestinal wall within 0.5 hour; whereas, the liver stores did not increase appreciably till 3.5 hours later, thus indicating the small intestine is the site of conversion of carotene to vitamin A in the chick.

The duodenum has also been studied by Sibbald and Olsen (1958) as a possible site of carotene conversion. By injecting a carotene solution into the ligatured duodenum of six week old male Columbian Rock chicks, and then after four hours analyzing the duodenum for vitamin A, they found evidence of the conversion. There was, however, very little if any absorption of vitamin A.

Sibbald and Hutcheson (1959) undertook a study to determine if there was conversion of carotene to vitamin A in the ligatured crops of four week old, vitamin A depleted, male, White Leghorn chicks. They found no conversion of beta carotene after injecting into the crop one milliliter of two percent Tween 80 solution containing 25.5 micrograms of beta carotene per milliliter. By injecting bile with the carotene there was still no conversion to vitamin A, but carotene may have been absorbed by the crop in the presence of bile.

Harvey et al. (1955) found that the chick converted carotene to vitamin A as early as the first day of life. Feeding carotene to vitamin A deficient

chicks at one day of age prolonged survival time beyond that of the controls. They found that dietary vitamin A was used to support physiological functions involving vitamin A at least by the fifth day and probably earlier.

Williams, Lampman and Bolin (1938) were able to prevent development of deficiency lesions and promote normal egg production with carotene from dehydrated alfalfa. The dehydrated alfalfa was fed at a level of .25 milligram per bird per day. Later work by Rubin and Bird (1941) was in agreement with that of Williams and co-workers. They found carotene from alfalfa to be just as effective for vitamin A accumulation in the liver as when vitamin A was fed.

However, a study by Gurcay, Boucher and Callenbach (1948) revealed that carotene was not as effective as vitamin A in either gain in body weight or liver storage. Vitamin A low diets were supplemented with increasing quantities of vitamin A and carotene ranging from 250 to 32,000 units of vitamin A activity per pound of feed. These diets were fed to White Holland turkey poults from hatching to eight weeks of age.

Results obtained by Johnson, Carrick and Hauge (1948) were not in agreement with those of Gurcay and co-workers when they compared fish liver oil and carotene from dehydrated alfalfa. When fed at levels up to 1200 units of vitamin A activity per 100 grams of feed, fish liver oil failed to promote the rate of growth and liver stores of vitamin A obtained when comparable levels of carotene, provided by dehydrated alfalfa leaf meal, were fed.

Pullets, receiving 2000 IU of vitamin A activity per pound of feed from fish liver oil, stored more vitamin A in their livers than pullets receiving dehydrated alfalfa; however, the pullets fed alfalfa laid eggs with the highest content of vitamin A and carotene, according to Frey and Wilgus (1949).

Alfalfa supplements were at least as effective as vitamin A for maintaining the blood level of total vitamin A potency.

In most cases Skoglund, Tomhave and Mumford (1949) found carotene from dehydrated vegetable waste in the form of a concentrate was just as efficient as vitamin A esters from fish liver oil in maintaining egg production, feed consumption and hatchability in laying pullets.

Camp et al. (1955) demonstrated a significant increase in growth and an improvement in feed efficiency when they substituted dry stabilized vitamin A for part of the vitamin A activity of alfalfa in chick diets. Harms, Reid and Couch (1955) observed a significant increase in vitamin A content of the liver when feeding stabilized vitamin A as compared to fish oil. Chick weight was increased also by feeding the stabilized vitamin A concentrate.

Gledhill and Smith (1955) conducted studies with Barred Plymouth Rock chicks, comparing a vitamin A dry carrier, vitamin A from fish oil and carotene from dehydrated alfalfa leaf meal at a level of 1000 IU per pound of feed. They observed higher average gains, increased feed efficiency and lower mortality when feeding the vitamin A on a dry carrier. In a second study birds receiving 500 IU of vitamin A from the dry vitamin A carrier stored 11 percent more of the vitamin than birds receiving 1000 IU of vitamin A from alfalfa or 500 IU of vitamin A from the fish oil. This does not agree with Johnson, Carrick and Hauge (1948). In production tests, birds receiving the dry vitamin A carrier laid more eggs than the birds receiving fish oil.

Olsen et al. (1959) checked the stability and utilization of vitamin A supplement using Columbian Rock chicks, the progeny of a flock of carotenoid-free vitamin A deficient hens. The vitamin A content of the hens' diet was

varied between 600 and 1800 IU per pound of feed. In all experiments, the greatest liver storage was given by the vitamin A in gelatin preparation, either as the palmitate or acetate. Using liver storage of vitamin A as a criteria, gelatin coated preparations were superior to preparations in which the vitamin was coated with wax or fat or was adsorbed by vegetable oil. Feeding oils and dehydrated cereal grasses were the poorest sources of vitamin A. All vitamin A sources had been assayed and were added according to potency.

Ely (1959) conducted tests using White Vantress cockerels to compare the utilization of carotene and pre-formed vitamin A. This was a four-week liver storage trial with all diets being fortified to 4800 IU of vitamin A activity per pound of feed. The materials tested were two samples of carotene, vitamin A derived from fish oil and vitamin A palmitate either in oil (adsorbed on soya flour) or as a wax-coated vitamin A in a dry carrier. Carotene derived from alfalfa meal and/or corn meal was poorly utilized up to 31 days. A purified beta carotene concentrate supplied in two different physical forms was less efficient in promoting liver storage than carotene derived from alfalfa meal or corn oil. Both fish liver oil and synthetic vitamin A palmitate in oil were substantially higher than the various carotene sources in promoting liver storage. The wax-coated vitamin A dry carrier was the most effective in promoting liver storage.

A growth depressing effect from alfalfa fed at levels from 5 to 50 percent of the diet has been observed (Cooney, Butts and Bacon, 1948), Lepkovsky et al.,1950) (Wilgus and Madsen, 1954) (Mangelson et al., 1949) (Kodras, Cooney and Butts, 1951). It was agreed the inhibitory factor was not fiber but some unidentified substance. Depressed growth and decreased feed

efficiency were observed when .10 percent or more saponin isolated from alfalfa was fed to growing chicks (Heywang and Bird, 1953) (Anderson, 1957).

German and Couch (1950) reported that different sources of alfalfa exhibit varying degrees of growth depression. Mussehl, Ackerson and Borchers (1950) reported no inhibition when the diets contained 15 percent high protein, high carotene and low fiber alfalfa.

Lower egg production has been observed when more than five percent dehydrated alfalfa or more than .26 percent saponin were added to the diet (Heywang, 1950) (Heywang, Thompson and Kemmerer, 1959) (Anderson, 1957). However, up to 30 percent alfalfa did not reduce egg production in turkeys, according to Kratzer and Davis (1957).

Nitrofurans have made their appearance in poultry management primarily in control of Salmonella organisms. In vitro studies by Mann (1958) demonstrated that growth of 2000 strains of Salmonella was inhibited by a concentration of from 12.5 to 25 micrograms of furazolidone per milliliter. More recent work has been undertaken to study their effect on egg production, feed efficiency, fertility, hatchability and growth rate.

At the Ohio Agricultural Experiment Station, Moore, Chamberlain and Carter (1954) fed furazolidone, a nitrofuran, continuously to turkeys during the breeding season. It had little effect on total egg production, fertility or hatchability.

When Cooper (1956) fed furazolidone to single comb White Leghorn pullets at levels of 0, 0.1, 0.15, and 0.20 percent of the diet during the period 14 to 26 weeks of age, there was no significant effect on body weight, food consumption or egg production. When Francis and Shaffner (1956) fed a diet containing 0.011 percent furazolidone or nitrofurazone to New Hampshire

pullets, there was no affect on total egg production, hatchability or egg shell quality.

Thayer (1956) obtained slightly different results when he fed five or 10 grams of furazolidone per ton of feed to single comb White Leghorn pullets. Furazolidone fed groups showed a lower rate of production than control lots and it took more feed to produce a dozen eggs, this being slight. Body weights were not maintained as well by those birds receiving furazolidone, but mortality was reduced by feeding the drug.

Carlson (1958) obtained greater egg production and feed conversion in most cases when single comb White Leghorn pullets and Commercial Hybrid pullets received a nitrofuran in the diet. The nitrofuran also reduced mortality of the Hybrid pullets. Furazolidone was fed at 25 grams per ton as a supplement to an all-mash laying ration containing four grams of penicillin per ton.

Lower mortality was also reported by Scott (1958) when 25 grams of furazolidone per ton of feed was fed to single comb White Leghorn pullets. Only a slight increase in egg production was noted. There was also evidence that the hens fed furazolidone layed a greater percent of larger eggs than hens fed the basal alone. Feed efficiency was also improved. A statistically significant improvement in egg production was obtained when New Hampshire hens on litter and White Wyandotte hens on wire were fed 50 grams of furazolidone per ton of basal, according to Dean and Stephenson (1958). Feed efficiency was improved in all phases of the test even when egg production was not increased. White Leghorn hens maintained in individual wire cages revealed that addition of furazolidone significantly increased egg production.

Smyth, Anderson and Fox (1959) found that feeding 0.011 percent furazolidone had no significant effect on egg production in turkeys.

Using a colorimetric method sensitive to 1 ppm of drug, Belloff, Buzard and Roberts (1958) demonstrated that eggs from hens receiving 100, 150 or 500 grams of furazolidone per ton of feed gave negative results when assayed for furazolidone. Furazolidone at these levels did not affect adversely egg production or quality of the egg shell.

Some dietary ingredients are known to enhance the absorption of carotene by the wall of the intestine. Russell et al. (1942) reported that the presence of fat in the diet improved the absorption of crystalline carotene by hens. The absorption of vitamin A was about the same on normal and low fat diets.

Almquist and Maurer (1955) found that inclusion of antibiotics (Chlorotetracycline-diamine penicillin mix) in a diet containing five percent dehydrated alfalfa (11,000 IU per kg of diet) increased the liver vitamin A slightly with greater increase at higher levels with a possible peak at the 55 mg level.

Neither choline or betaine increased the utilization of carotene, according to Edwards, Dunahoo and Fuller (1958) since an increased growth rate was not obtained at the suboptimal level of alfalfa leaf meal supplementation.

According to High (1955) rats that received 30 micrograms of carotene as a supplement, deposited in the liver and kidneys an average of 50 micrograms of vitamin A, while rats that received carotene plus aureomycin deposited an average of 63 micrograms of vitamin A in these two organs. There was no effect on vitamin A deposition when pre-formed vitamin A was fed

with aureomycin. Vitamin B_{12} also increased vitamin A deposition from carotene fed rats. Camp et al. (1955) demonstrated that choline chloride enhances the utilization of carotene furnished by dehydrated alfalfa. The addition of 400 mg of choline chloride per pound of feed to a diet containing alfalfa as the primary source of vitamin A activity also increased the average weight of the chicks.

When low levels of vitamin A are used an increase in blood spots may be encountered. The incidence of blood spots decreased with each increase in vitamin A, regardless of the source of vitamin A, according to Bearse, McClary and Saxena (1953). After two months on experiment, the group receiving 200 IU of vitamin A showed a higher incidence of blood spots than groups receiving 500, 800, and 2200 IU of vitamin A per 100 grams of feed. During the vitamin A depletion period, there was a significant increase in blood spots.

MATERIALS AND METHODS

This experiment was conducted in an open front straw loft poultry house at the Kansas State University Poultry Farm. The hens used were commercially obtained one-day-old Inbred-Crossbred[1] layers. They were reared in confinement at the Kansas State University Poultry Farm. All birds were wing banded for identification purposes. The vaccination program included intra-nasal vaccination for Newcastle and bronchitis at one day of age, fowl pox vaccination at 12 weeks and wing-web vaccination for Newcastle at 14 weeks.

The birds were placed in the experimental house at seven months of age.

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[1]The Inbred-Crossbreds used were Hyline 934C.

They were randomly assigned to eight lots after being culled to remove birds with physical defects or showing signs of unthriftiness. The pullets remained in the house two weeks before the experiment started to allow adjustment to new surroundings.

The experiment was initiated on May 1, 1960, with all birds being placed on a vitamin A deficient carotenoid-free diet for three weeks to reduce body stores of vitamin A. During the experimental period, the birds were furnished feed and water ad lib. Body weights were taken initially and at 12, 24 and 32 weeks. Feed consumption, egg production and mortality records were maintained through the experimental period. Egg quality data were taken weekly from July 29 to September 2 and from November 18 to December 9.

The basal diet (Table 1) was mixed by the Department of Flour and Feed Milling, Kansas State University. The sorghum grain used was screened to eliminate contamination from yellow corn. The alfalfa and furazolidone were mixed into the basal diet at the poultry farm weekly and just prior to feeding.

The alfalfa which was procured locally was analyzed approximately every 30 days by the A.O.A.C.[1] method to determine the vitamin A activity present. It was then stored in sharp freeze to maintain that level of vitamin A potency until mixed in the basal. The alfalfa was precisely weighed out and added to the basal in quantities so that each pound of feed contained either 1500 or 3000 units of vitamin A activity (Table 2). The alfalfa used contained 20.94 percent protein and 22.77 percent fiber.

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[1]Association of Official Agricultural Chemists. Methods of Analysis, Carotene, 9th ed., pp. 654-655. 1960.

Table 1. Composition of the basal diet.

Ingredients	Amount per 100 pounds
Sorghum grain	73.5 lbs.
Wheat standard middlings	4.0
Soybean oil meal (44% solvent extracted)	13.0
Fish meal (Menhaden)	1.5
Brewer's dried yeast	1.5
Salt (NaCl)	.5
Ground limestone (calcium carbonate)	4.0
Steamed bone meal	2.0
$MnSO_4$	23.0 gms
Vitamin K (Klotogen F; 8 gm per lb.)	4.8
D-L Methionine	46.0
Vitamin D_3 (15,000 ICU per lb.)	5.00
Proferm$_{12}^{(R)}$ (Vitamin B_{12})	19.0
Merck 58A$^{(R)}$ (Vitamin premix)[1]	23.0
Choline Chloride (25% mix)	88.0

[1]Supplies: a. 3680 mg. of D-Pantothenic Acid per pound of suppl.
b. 6000 mg. of Niacin per pound of suppl.
c. 20,000 mg. of Choline Chloride per pound of suppl.
d. 2000 mg. of Riboflavin per pound of suppl.

For the egg quality studies, 10 eggs were obtained from each lot. The eggs were weighed on a Toledo balance and the AMS[1] method was used to determine albumen height and Haugh scores. After being dried in a thermostatistically controlled oven at 100 degrees centigrade for 24 hours, the egg

[1]U.S. Dept. of Agric., Agric. Mkt. Serv., Poultry Division AMS No. 246.

Table 2. Experimental design for eight month vitamin A study with laying pullets.

Lot	Diet
1	Basal - 1500 units of vitamin A activity per pound of feed - 0 grams of furazolidone[1] per ton of feed.
2	Basal - 1500 units of vitamin A activity per pound of feed - 25 grams of furazolidone per ton of feed.
3	Basal - 1500 units of vitamin A activity per pound of feed - 50 grams of furazolidone per ton of feed.
4	Basal - 1500 units of vitamin A activity per pound of feed - 125 grams of furazolidone per ton of feed.
5	Basal - 3000 units of vitamin A activity per pound of feed - 0 grams of furazolidone per ton of feed.
6	Basal - 3000 units of vitamin A activity per pound of feed - 25 grams of furazolidone per ton of feed.
7	Basal - 3000 units of vitamin A activity per pound of feed - 50 grams of furazolidone per ton of feed.
8	Basal - 3000 units of vitamin A activity per pound of feed - 125 grams of furazolidone per ton of feed.

[1] Manufacturer's recommended level: 25 grams of furazolidone per ton of feed.

shells were weighed on a Gram-Atic balance.

RESULTS AND DISCUSSION

All statistical analyses applied are described by Snedecor (1956).

Egg production declined as the experiment progressed, with all lots decreasing at approximately the same rate. Decline in egg production is a normal occurrence; therefore, this does not appear to be an effect from furazolidone. When the experiment was initiated, the birds were at their peak of 80 to 90 percent production.

Three thousand units of vitamin A activity maintained egg production at a significantly higher rate (Table 3) than did the 1500 units. This was significant at the 0.01 level. The increase was more than three percent (Appendix Table 1) with the mean percent production at the high and low level of vitamin A being 70.98 and 67.49, respectively.

Table 3. Analysis of variance of percent hen-day production.

Source of variation	df	ss	F-test
Weeks	31	12090.56	22.20**
Level of vitamin A	1	779.35	44.36**
Level of furazolidone	3	490.23	9.30**
Furazolidone x vitamin A	3	648.14	12.30**
Residual	217	3811.82	

**Significant at the 0.01 level.

Vitamin A deficiency symptoms were not apparent at any time during the experiment. Since these pullets had been depleted of their body reserves of vitamin A initially, it is apparent that, not only can chickens convert carotene from dehydrated alfalfa to vitamin A, but 1500 units of vitamin A activity will maintain the birds in good health. This is in agreement with Williams, Lampman and Bolin (1938).

Even though 1500 units of vitamin A activity prevented deficiency symptoms, it was not enough for maximum egg production as 3000 units of vitamin A activity in the diet maintained egg production at a significantly higher rate. The optimum requirement for vitamin A is probably between these two figures.

Twenty-five grams of furazolidone per ton of feed was just as effective

as 50 or 125 grams of furazolidone in maintaining egg production when fed with 3000 units of vitamin A activity. All three levels maintained egg production at a significantly higher rate than the zero level of furazolidone. This is in agreement with the work of Carlson (1958); however, it is not in agreement with the work of Thayer (1956). When 1500 units of vitamin A activity were fed, 125 grams of furazolidone significantly increased egg production over all other levels of the drug. At this same level of vitamin A, zero and 50 grams of furazolidone maintained egg production at a significantly higher rate than did 25 grams of the drug with no difference in rate of production with zero and 50 gram levels. The mechanism through which furazolidone acts to increase egg production was not studied in this experiment.

Egg production was affected by an interaction between vitamin A and furazolidone. Greater production resulted when a combination of 3000 units of vitamin A activity was fed with either 25 or 50 grams of furazolidone. This interaction observed with egg production loses its significance as the increase in production was noted at the high level of vitamin A activity.

Feed conversion (Appendix Table 1) appeared to be a little better at the 3000 units of vitamin A; however, this difference was not significant.

As the level of furazolidone increased, feed conversion was significantly improved (Table 4). This confirms the results of Carlson (1958) and Dean and Stephenson (1958). This increased feed conversion does not appear to be due to improved utilization of carotene. If that were the case, we would not expect the response observed at the high level of vitamin A activity. Therefore, the observed effect may be similar to that observed with antibiotics, that is improved intestinal environment for more efficient absorption of nutrients.

Table 4. Analysis of variance of feed conversion.

Source of variation	df	ss	F-test
Level of vitamin A	1	.05	5.00 n.s.
Level of furazolidone	3	.37	12.00 *
Residual	3	.03	

n.s. - Not significant at the 0.05 level.
* - Significant at the 0.05 level.

Neither furazolidone nor vitamin A had an effect on the mortality. Mortality was low for all lots (Appendix Table 1). Deaths that did occur were from prolapse of the uterus, avian leukosis and unspecified causes. Since care was taken to precisely add the vitamin A activity, mortality related to vitamin A was not expected. Reduction in mortality by furazolidone is expected only when there are disease outbreaks or severe stresses on the birds, neither of which occurred during this experiment.

None of the variables had any effect on body weights over the 32 week experimental period (Appendix Table 3). This is not in agreement with Thayer (1956) who found that body weight was not maintained as well by those birds receiving furazolidone (Appendix Table 1).

The weekly differences observed in egg weights during collection period I can be attributed to the fact that these pullets were still young and egg size was still increasing. There are also marked seasonal variations in egg weight, according to Cunningham, Cotterill and Funk (1960). There was no difference in egg size attributed to vitamin A levels during either collection period (Table 5).

Furazolidone fed at the rate of 25, 50, or 125 grams per ton of feed

Table 5. Analysis of variance of egg weights.

Source of variation	Collection period I[1]			Collection period II[2]		
	df	ss	F-test	df	ss	F-test
Weeks	5	18.61	2.78*	3	9.93	1.95 n.s.
Level of vitamin A	1	.14	.10 n.s.	1	3.23	1.90 n.s.
Level of furazolidone	3	146.48	36.44 **	3	184.34	36.15 **
Furazolidone X vitamin A	3	5.32	1.32 n.s.	3	7.30	1.43 n.s.
Residual	35	46.89	-	21	35.71	-

[1]Collection period I for egg quality studies was from July 29 to September 2, 1960.

[2]Collection period II for egg quality studies was from November 18 to December 9, 1960.

n.s. - Nonsignificant at the 0.05 level.
** - Significant at the 0.01 level.

reduced egg size (Appendix Table 2) at both levels of vitamin A activity during collection period I. The reduction in egg size was significant at the 0.01 level (Table 5). This does not agree with the results obtained by Scott (1958). The 125 grams of furazolidone also reduced egg size below the 25 and 50 gram levels of the drug. There was no difference between egg weights when 25 and 50 grams of furazolidone were fed at either level of vitamin A activity.

This reduction in egg size appears to stem from the reduction in yolk size which was observed also. This agrees with the work of Jull (1924) who reported a correlation ($r = 0.820 \pm .011$) between yolk weight and egg weight.

During the second collection period the effect of furazolidone on egg weights was again most severe at 125 grams per ton with 25 and 50 grams per ton also reducing egg size at the 3000 unit level of vitamin A activity. However, at the 1500 unit level of vitamin A activity only the 125 gram level

of furazolidone reduced egg size. Again there was no difference between the 25 and 50 gram levels of the drug at either level of vitamin A activity.

Height of the thick albumen, as measured by a micrometer, was not effected by different levels of vitamin A activity or furazolidone (Appendix Table 4). However, there was an interaction between vitamin A and furazolidone during the second collection period. A combination of 3000 units of vitamin A activity and the zero level of furazolidone resulted in a significantly thicker albumen than the low level of vitamin A activity with the zero level of furazolidone. This interaction noted with albumen must be discounted as the stimulus was received at the high level of vitamin A activity. Higher levels of the drug did not depress albumen height at either level of vitamin A activity.

Haugh scores used as a measure of interior quality were not affected by experimental variables (Appendix Table 5). All lots, during both collection periods, averaged more than 72 Haugh units which is the minimum for AA grade for USDA standards (data not shown). Weekly variations in Haugh scores were not accounted for.

When 1500 or 3000 units of vitamin A activity per pound of feed were fed, 25, 50 and 125 grams of furazolidone per ton of feed significantly reduced yolk weight except for 50 grams of furazolidone per ton at the low level of vitamin A activity (Table 6). The difference between 25 and 50 grams of the drug was not significant. One hundred twenty five grams of furazolidone per ton of feed reduced yolk weight significantly below 25 and 50 grams of the drug. Average yolk weights are shown in Appendix Table 2.

The effect of furazolidone on yolk weight followed the same pattern as that of egg size (Appendix Table 2). It has been accepted by most workers that

Table 6. Analysis of variance of yolk weights.

Source of variation	df	ss	F-test
Weeks	3	1.17	1.77 n.s.
Level of vitamin A	1	.09	.41 n.s.
Level of furazolidone	3	24.11	36.55 **
Furazolidone X vitamin A	3	.21	.32 n.s.
Residual	21	4.52	

n.s. - Not significant at the 0.05 level.
** - Significant at the 0.01 level.

a reduction in yolk weight is due to premature ovulation and not a reduction in rate of growth of the ovum (Warren and Conrad, 1939). How furazolidone functions to reduce egg weight and yolk weight was not undertaken in this experiment.

Yolk made up a significantly greater percentage of the whole egg when the pullets received 1500 units of vitamin A activity in the diet. The mean yolk weight at the low level of vitamin A activity was 28.82 grams and at the high level 28.30 grams (Appendix Table 2). This difference was significant at the 0.01 level (Table 7).

Furazolidone at 125 grams per ton of feed greatly reduced percent yolk at the low level of vitamin A activity. Other results, even though significantly different, did not follow any trend and cannot be interpreted as being an effect from furazolidone.

An interaction observed, with zero and 50 grams of furazolidone in combination with 1500 units of vitamin A activity, produced a greater percentage of yolk than the same levels of drug at 3000 units of vitamin A activity. Since one interaction was at the zero level, it was not due to furazolidone

interacting with vitamin A, but some unknown factor. Because of the interaction where no drug was involved, it would put in question the cause of the interaction at the 50 gram level of furazolidone.

Percent albumen (Appendix Table 2) was significantly increased when the pullets received 3000 units of vitamin A activity (Appendix Table 6). This was just reverse of that found with percent yolk. The correlation between percent yolk and percent albumen was not studied in this experiment. A furazolidone-vitamin A interaction observed must be discounted as the response was at the high level of vitamin A.

Shell weight was significantly reduced by 50 and 125 grams of furazolidone per ton of feed at the low level of vitamin A activity (Appendix Table 7), during collection period I. At the high level of vitamin A activity the 125 gram level of the drug significantly reduced shell weight below all other levels. However, 25 grams of furazolidone per ton of feed gave significantly lighter shells than did the zero level of the drug. None of the variables affected shell weight during the second collection period.

Percent shell was not affected by level of vitamin A. There were weekly differences but this may have been caused by environmental factors, which is not uncommon (Pope et al., 1960). Furazolidone at the 125 gram level significantly increased percent shell even though shell weight was reduced. This is in accord with work of Asmundson and Baker (1940) who found that as egg size decreases percentage shell increases. There was a tendency for the 25 and 50 gram levels of furazolidone to increase percent shell also (Appendix Table 2). No explanation is found in the data for the interaction observed between 25 grams of furazolidone and the low level of vitamin A. Further study is necessary to determine its importance.

During the second collection period, there appeared an extremely large number of blood spots. No scoring system was devised to indicate relative amounts of blood so this data was not analyzed statistically. However, this did not appear to be an effect from furazolidone as the number of inedible eggs (Appendix Table 2) was just as high for the zero level of the drug as for the 125 grams per ton level of furazolidone.

At the high level of vitamin A activity, the number of blood spots was reduced almost 40 percent below the number appearing in those groups receiving 1500 units of vitamin A activity. This is in agreement with the results reported by Bearse, McClary and Saxena (1960). They reported blood spots decreased with increases in vitamin A levels in the diet. The vitamin A was not the cause of the blood spots, but the reduction in number of blood spots can be attributed to the increase in vitamin A level in the diet. The cause of the increase in blood spots was not determined.

At the conclusion of the experimental period, three eggs from each lot were analyzed by the method (modified) of Neff et al. (1949) for carotenoid pigments and vitamin A content of the yolks. There was considerable variation from egg to egg within each lot as the eggs were selected without regard to rate of lay. Since there was a large degree of variation within lots, and only a small number of eggs were analyzed, these data were not subjected to statistical analysis. The data are presented in Appendix Table 9. There was a tendency, however, for yolk vitamin A to increase as the level of furazolidone increased.

SUMMARY

An experiment was conducted with laying pullets to determine the effect of furazolidone, a nitrofuran, upon the utilization of carotene, as a source of vitamin A, from high quality dehydrated alfalfa. The following criteria were used to detect any effect: percent production, feed conversion, mortality, maintenance of body weight, internal and external quality of eggs and percentage by weight changes in component parts of eggs. Vitamin A activity was added at 1500 and 3000 units per pound of feed and furazolidone was added at 0, 25, 50 and 125 gram levels per ton of feed.

Egg production was maintained at a significantly higher level by 3000 units of vitamin A activity per pound of feed as compared to 1500 units of vitamin A activity per pound of feed.

Furazolidone improved feed conversion at both levels of vitamin A activity. This effect was observed with each increase in the drug level. The drug also helped maintain egg production at a higher rate when fed at levels above 25 grams per ton of feed together with 3000 units of vitamin A activity. At the low level of vitamin A activity a higher rate of egg production was observed only when more than 50 grams of furazolidone per ton of feed was added.

Egg size and yolk size were both reduced by furazolidone at 25 grams per ton of feed or higher levels. The reason for this reduction was not determined; therefore, more exploration is necessary.

Vitamin A deficiency symptoms were not observed at any time, mortality was low and egg production was considered normal for the conditions of the experiment. Since this experiment ran for eight months and the birds had been depleted of their body stores of vitamin A initially, it can be concluded that

laying pullets can utilize carotene from alfalfa as their sole source of vitamin A.

The interactions between furazolidone and vitamin A were very limited and in most cases questionable. Thus it appears that under the conditions of this experiment furazolidone has no effect upon the utilization of carotene from dehydrated alfalfa.

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APPENDIX

Table 1. Percent production, percent mortality, feed conversion, and gain in body weight of laying hens on an eight-month vitamin A study.

Lot No.	Diet: Basal plus	% production[1]	% mortality	Feed conversion[2]	Gain in body weight (lbs.)
1	1500 units vitamin A 0 grams furazolidone	68.07	4	4.62	0.2
2	1500 units vitamin A 25 grams furazolidone	64.57	2	4.40	0.3
3	1500 units vitamin A 50 grams furazolidone	66.82	4	4.37	0.2
4	1500 units vitamin A 125 grams furazolidone	70.49	0	3.91	0.3
5	3000 units vitamin A 0 grams furazolidone	67.39	4	4.40	0.1
6	3000 units vitamin A 25 grams furazolidone	72.26	6	4.24	0.2
7	3000 units vitamin A 50 grams furazolidone	71.96	4	4.08	0.4
8	3000 units vitamin A 125 grams furazolidone	72.31	6	3.91	0.3

[1]Percent production on a hen-day basis.
[2]Pounds of feed per dozen eggs.

Table 2. Summary of data collected for egg quality studies.

Lot No.	Av. egg weights collection period I	Av. egg weights collection period II	Av. yolk weight collection period II	% yolk collection period II	% albumen collection period II	% shell		Inedible[1] eggs due to blood spots
						Collection period I	Collection period II	
1	60.82	64.13	18.75	29.43	62.14	8.43	8.43	11
2	59.33	62.97	18.01	28.66	62.84	8.71	8.50	15
3	58.62	63.29	18.48	29.24	62.36	8.44	8.40	14
4	56.62	59.45	16.49	27.95	63.18	8.69	8.87	14
5	60.95	66.29	18.65	28.08	63.29	8.43	8.63	8
6	59.23	63.25	18.14	28.67	63.11	8.44	8.22	11
7	59.30	63.82	18.15	28.45	62.54	8.51	9.01	4
8	55.47	58.49	16.37	28.01	62.90	8.78	9.09	10

[1]Number of eggs containing blood spots out of a total of 40 eggs.

Table 3. Analysis of variance of body weights.

Source of variation	df	ss	F-test
Weeks	3	.99	.00 n.s.
Level of vitamin A	1	.00	.00 n.s.
Level of furazolidone	3	.01	.00 n.s.
Furazolidone X vitamin A	3	.01	.00 n.s.
Residual	21	.00	.00 n.s.

n.s. - Not significant at the 0.05 level.

Table 4. Analysis of variance of albumen height.

	Collection period I			Collection period II		
Source of variation	df	ss	F-test	df	ss	F-test
Weeks	5	3.08	5.17 **	3	1.24	8.20 **
Level of vitamin A	1	.05	.42 n.s.	1	.00	.00 n.s.
Level of furazolidone	3	.07	.17 n.s.	3	.13	.80 n.s.
Furazolidone X vitamin A	3	.47	1.33 n.s.	3	.55	3.60 *
Residual	35	4.19	--	21	1.00	--

n.s. - Not significant at the 0.05 level.
* - Significant at the 0.05 level.
** - Significant at the 0.01 level.

Table 5. Analysis of variance of Haugh scores.

Source of variation	Collection period I			Collection period II		
	df	ss	F-test	df	ss	F-test
Weeks	5	162.57	4.87 **	3	71.59	7.43 **
Level of vitamin A	1	6.09	.91 n.s.	1	.00	.00 n.s.
Level of furazolidone	3	10.78	.54 n.s.	3	11.97	1.24 n.s.
Furazolidone X vitamin A	3	33.23	1.66 n.s.	3	21.82	2.26 n.s.
Residual	35	233.54	--	21	67.32	--

** - Significant at the 0.01 level.
n.s. - Not significant at the 0.05 level.

Table 6. Analysis of variance of percent albumen.

Source of variation	df	ss	F-test
Weeks	3	.18	.30 n.s.
Level of vitamin A	1	.87	4.35 *
Level of furazolidone	3	1.74	2.90 n.s.
Furazolidone X vitamin A	3	2.18	3.65 *
Residual	21	4.27	--

n.s. - Not significant at the 0.05 level.
* - Significant at the 0.05 level.

Table 7. Analysis of variance of shell weight.

Source of variation	Collection period I			Collection period II		
	df	ss	F-test	df	ss	F-test
Weeks	5	.124	1.40 n.s.	3	.104	.45 n.s.
Level of vitamin A	1	.008	.46 n.s.	1	.201	2.61 n.s.
Level of furazolidone	3	.373	7.07 **	3	.633	2.74 n.s.
Furazolidone X vitamin A	3	.129	2.44 n.s.	3	.440	1.91 n.s.
Residual	35	.614	--	21	1.612	--

n.s. - Not significant at the 0.05 level.
** - Significant at the 0.01 level.

Table 8. Analysis of variance of percent shell.

Source of variation	Collection period I			Collection period II		
	df	ss	F-test	df	ss	F-test
Weeks	5	.42	3.27 *	3	.14	.03 n.s.
Level of vitamin A	1	.01	.34 n.s.	1	.28	1.87 n.s.
Level of furazolidone	3	.64	8.33 **	3	1.69	3.73 *
Furazolidone X vitamin A	3	.25	3.21 *	3	.83	1.87 n.s.
Residual	35	.90	--	21	3.21	--

n.s. - Not significant at the 0.05 level.
* - Significant at the 0.05 level.
** - Significant at the 0.01 level.

Table 9. Vitamin A and carotenoid pigment content of egg yolk.

Lot	Carotenoids		Vitamin A	
	Micrograms per gram	Micrograms per yolk	Units per gram	Units per yolk
1	5.84±1.21*	116.51	4.85±1.41	96.82
2	6.59±1.50	121.48	5.71±2.74	106.39
3	6.27±0.58	106.22	5.15±2.20	87.42
4	5.91±1.15	97.44	6.73±1.55	111.02
5	8.09±2.84	150.69	7.10±2.65	132.39
6	8.07±0.48	161.64	7.15±0.94	143.33
7	9.09±2.49	174.61	9.07±4.77	176.25
8	10.28±0.57	186.55	10.29±0.36	187.51

* Standard deviation from the lot mean.

EFFECT OF FURAZOLIDONE ON THE UTILIZATION OF CAROTENE BY LAYING HENS

by

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A study was conducted to determine the effect of furazolidone on the utilization of carotene by laying hens. The hens used were confinement reared Inbred-Crossbred layers. The hens were vaccinated for Newcastle, bronchitis and fowl pox. At eight months of age they were randomly assigned to eight lots and after a two week adjustment period they were placed on a vitamin A deficient-carotenoid free diet to deplete body reserves of vitamin A. After three weeks on the depletion diet, carotene in alfalfa was added at levels of either 1500 or 3000 units of vitamin A activity per pound of feed. Furazolidone was added at the time the hens were placed on the depletion diet at levels of 0, 25, 50 and 125 grams per ton of feed at each level of vitamin A.

Egg production was maintained at a significantly higher level by 3000 units of vitamin A activity compared to 1500 units. Furazolidone at 25 grams or higher levels per ton of feed with 3000 units of vitamin A and 50 grams per ton or higher levels with 1500 units of vitamin A also maintained a higher level of egg production.

Feed conversion was significantly improved with each increase in furazolidone level at both levels of vitamin A. Level of vitamin A had no effect on feed conversion.

Mortality was not affected by either level of vitamin A or furazolidone. No deaths were diagnosed as vitamin A symptoms. Mortality was very low, ranging from 0 to 6 percent.

Egg size and yolk size were markedly reduced by 125 grams of furazolidone per ton of feed. Other levels of the drug also reduced egg size below the zero level of the drug. Percent shell was increased by the high level of the drug, even though egg weight was reduced. Interior quality was not affected by vitamin A or the drug.

It is concluded, because of the limited and questionable interactions, that furazolidone has no effect on carotene utilization by laying hens. Further, that carotene from alfalfa can be utilized satisfactorily as a sole source of vitamin A by laying hens when precise levels are added and feed is mixed at least weekly.