EFFECTS OF FEEDING PROVITAMIN A, STABILIZED VITAMIN A, ARSANILIC ACID AND 1-2-DIHYDRO-6-ETHOXY-2,2,4 TRIMETHYLQUINOLINE ON PRODUCTION AND QUALITY OF EGGS

by

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B. Sc. (Vet.) Hon., University of Bombay, India, 1955

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Poultry Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1964

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INTRODUCTION

Phase 1.

Despite the fact that vitamin A is one of the first recognized vitamins, probably more confusion and conflicting claims exist regarding it than any other one. This can be ascribed basically to the variety of chemical entities possessing vitamin A potencies, to variations in susceptibility, to destruction among its different forms, to numerous factors influencing requirements, stability, availability, and to difficulties in assays (Wilgus, 1955).

A review of the voluminous work done on the vitamin A requirements for laying hens, leaves one baffled due to the lack of uniformity of techniques, diets, and sources of vitamin A activity used by different workers.

Thus it was deemed desirable to feed different levels of provitamin A and stabilized vitamin A to caged layers and to determine the differences between the two with regard to effect on egg production, exterior and interior quality of eggs, as well as liver stores and vitamin A content of blood serum.

Phase 2.

The use of arsenic compounds as stimulants, tonics and roborants is buried in antiquity. Morehouse and Mayfield (1946) gave a new turn to the use of arsenic acid compounds when they stumbled upon the discovery that growth stimulation occurs at sub-therapeutic levels.

Although painstaking research has given us plenty of information on the use and action of arsanilic acid (Pro-Gen 90)[1,2] in poultry, there is no evidence at present to support the use of arsenic compounds in the diet of laying birds. As no literature could be found giving information on the effect of arsanilic acid on interior egg quality, egg weight and production, it was interesting to perform a pilot experiment to see if there was any such effect.

Another problem confronting feed manufacturers and livestock raisers for many years was the stabilization against oxidation of fat soluble vitamins and pigments found in dehydrated forages and other feedstuffs during storage. An antioxidant, 1-2 dihydro-6-ethoxy-2,2,4 trimethyl-quinoline[3]--E.M.Q. for short--commercially known as Santoquin[4] has been very popular as an additive for preventing deterioration of vitamin A, but no reports could be found in the literature on its effect on egg production and interior quality. Thus the pilot experiment investigating the effects of arsanilic acid was made to include also the effects of Santoquin.

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[1]Hereafter referred to as arsanilic acid.

[2]Trade-mark of Abbott Laboratories, North Chicago, Illinois.

[3]Hereafter referred to as Santoquin.

[4]Trade-mark of Monsanto Chemical Company, St. Louis, Missouri.

REVIEW OF LITERATURE

Phase 1.

It was in 1906, that the classical work of Hopkins proved beyond doubt that no animal could live upon a mixture of pure protein, fat, and carbohydrates. Even when the necessary inorganic material was carefully supplied, the animals did not flourish. Although it was known that in rickets and scurvy dietetic factors were involved, the nature of such factors was obscure (Moore, 1957).

Osborne and Mendel (1913) reported that rats kept on an artificial diet suffered from keratomalacia, which could be cured by giving fractionated butter. This was confirmed by other workers who also found that vitamin A promoted growth. However, Funk and Macallum (1915) insisted that vitamin B from yeast was the only growth-promoting substance. Both were correct in their own beliefs, but the existence of vitamin A was thus recognized.

This was known to ancient Egyptians and the medical treatise called Eber's Papyrus in about 1500 B.C. recommended ox liver for correcting nightblindness (Moore, 1957).

Palmer and Kempster (1919) showed there was a relationship between the pigments of the carotenoid group and vitamin A, but they thought the relationship was merely apparent and the pigments per se, played no part in the vitamin metabolism.

Steenbock (1919) noted that vitamin A content of maize was parallel with the content of the yellow pigment and white maize was free from the vitamin. Rosenheim and Drummond (1920) confirmed that as a rule, foodstuffs containing lipochrome also contained vitamin A (Moore, 1957).

In 1922, a Japanese worker Takahashi, prepared concentrates from cod liver oil which were claimed to represent vitamin A in pure form. He also concluded that it contained only the elements carbon, hydrogen and oxygen, and that it was an unsaturated alcohol. However he gave the formula $C_{27}H_{24}O_2$, which was far removed from the pure vitamin $C_{20}H_{30}O$ (Moore, 1957).

Drummond et al. (1925) challenged Takahashi's claim, but it was found that only 5 micrograms of Takahashi's product could produce as much growth in rats as 50 micrograms of the Drummond concentrate. Although Takahashi's product was superior to that of Drummond et al. (1925), it was not quite pure.

Scientists hovered on the brink of crystallizing pure vitamin A, and although there was a growing suspicion that carotenoids had some sort of relation to vitamin A, many missing links still existed. When Hume et al. (1930) demonstrated that carotene could be destroyed by oxidation, and found a method to circumvent this, an even purer product was obtained.

Moore (1930) is to be credited with the last stretch of the race, in that he fed rats diets deficient in vitamin A till their livers were depleted. Then he fed them with carotene and found that vitamin A had reaccumulated in the liver.

That is how the mysterious relationship between carotene and vitamin A came to light, and it was confirmed that carotene could be converted to vitamin A only in the animal body.

It was Karrer who gave the correct formula to vitamin A, viz. $C_{20}H_{30}O$, which is half of the carotene molecule combined with one molecule of water so as to form a terminal hydroxyl group (Moore, 1959).

Although the correct formula was deduced, repeated attempts at crystallization were all unsuccessful. Holmes and Corbett (1937) crystallized the vitamin in the form of pale yellow needles, from liver oils obtained from three different species of fish.

In poultry rations, vitamin A is essential for growth, egg production, reproduction, efficient feed utilization, optimum vision and maintaining the integrity of mucous membranes. Provitamin A is the name given to four carotenoid pigments found in plants which can be converted in the animal body into pure vitamin A. They are called alpha- beta- gamma- and hydroxy beta-carotene or cryptoxanthine. All of these are reddish yellow crystalline compounds, very similar to each other in chemical structure.

The carotene content of natural feed ingredients is still used by research workers and the feed industry in calculating the total vitamin A level of poultry rations, even though numerous reports have shown significantly less biological activity than the accepted vitamin A equivalence based on rat experiments. These differences are due to the presence of less active isomers, less availability of the carotene found in the fibrous nature of the natural ingredients and unknown stability of the natural carotene compounds (Ewing, 1963).

Vitamin A is a general term, there being in all at least six isomers of vitamin A. They appear alike by the colorimetric assay method, but differ in biological activity and in spectrophotometric properties as follows:

Isomer	Biopotency
All-trans	100
13-cis (neo-a or 2-mono-cis)	75
9-cis (iso-a or 6-mono-cis)	21
9, 13-di-cis (iso-b or 2,6-di-cis)	24
11-cis (neo-b or 4-mono-cis)	24
11, 13-di-cis (neo-c or 2,4-di-cis)	15

The difference between "all-trans" vitamin A and "cis" vitamin A, primarily involves the different shapes of the vitamin A molecule. "All-trans" has a straight zigzag side chain while "cis" has a bent zigzag side chain (Ewing, 1963).

Wilson et al. (1936) stated that unit for unit carotene and vitamin A obtained from a fish oil concentrate were found to be utilized with equal efficiency by the chick.

Record et al. (1937) stated that the same number of international units had the same effect on chickens, whether fed as vitamin A or carotene. Maurisch et al. (1961), determined the vitamin A activity of pure beta-carotene stabilized in a gelatin beadlet against vitamin A, also fed in the same beadlet form. It was found that even pure beta carotene does not serve as an efficient source of vitamin A. This explained why poor response of carotene from natural sources occurred.

Carotene fed at 0.25 mg. per bird per day proved adequate to promote normal egg production, and to prevent development of deficiency lesions. On the other hand 0.1 mg. of carotene proved markedly inadequate; whereas, 0.5 mg. resulted in slight, but statistically significant increase in egg production and hatchability (Williams et al., 1939).

Almquist and Mecchi (1939) stated the minimum satisfactory value of vitamin A for all purposes was 1,800 I. U. per pound of feed per day when obtained from shark liver oil. Same levels of carotene (1,800 I. U. per

pound of feed per day) were inadequate for satisfactory hatchability, but egg production was unaffected. The survival time and liver reserves of the carotene fed birds were both lower than those given equivalent quantity of shark liver oil.

Bearse and Miller (1937) estimated that 2,270 I.U. of vitamin A per pound of diet should be adequate for health, production and hatchability. Russel et al. (1936) reported 2,200 I.U. per pound of diet as satisfactory for all purposes.

The average value of these reports figures out to be approximately 2,100 I.U. per pound of diet. Adding 400 I.U. to this figure, as an allowance for errors in estimation and deterioration during short periods of storage, it can be said that 2,500 I.U. per pound of diet is a minimum practical recommendation for poultry feeding (Almquist and Mecchi, 1939).

It certainly should not be too difficult to compound rations for laying hens which contain at least 2,500 I.U. per pound of diet. If the total ration includes 2.5 percent of dried alfalfa, containing 10 mg. of carotene per 100 gm. (only a fair grade of alfalfa), the vitamin A potency provided will amount to 1,890 I.U. per pound. Usually more than this amount of alfalfa is included. If the total ration also contains 25 percent yellow corn, the additional vitamin A potency will be approximately 800 I.U. per pound. The total, from these sources, is 2,690 I.U. per pound (Almquist and Mecchi, 1939).

Frey and Wilgus (1949) stated that pullets receiving 2,000 I.U. of vitamin A activity from dehydrated alfalfa laid eggs with the highest content of vitamin A and carotene, but those getting the same amount from fish liver oil stored more vitamin A in their livers.

In a similar study, Johnson et al. (1948) compared differences between fish liver oil and carotene from dehydrated alfalfa. Fed at levels of 120 units per gm. of feed, the 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.

Camp et al. (1955) demonstrated significant increase in growth, and an improvement in feed efficiency when they substituted dry, stabilized vitamin A for part of vitamin A activity of alfalfa in chick diets.

Harms et al. (1955) obtained significant increase in vitamin A content of the liver when feeding stabilized vitamin A compared to fish oil. Chick weight too, was increased by vitamin A concentrate.

Kemmerer and Fraps (1938) showed the percentage of carotene digested by rats and chickens depended on: (a) the quantity fed, (b) the nature of the material in which it was contained and (c) the kind of animal to which it was fed.

When carotene, in the form of dehydrated alfalfa, was fed at the level of 20 parts per million, rats digested 18 to 23 percent of it and chickens, 29 percent. When 1 part per million was fed, rats digested 43 percent and chickens 69 percent.

Russell et al. (1940) and Russell et al. (1942), studied the relation of fat in the diet to absorption of vitamin A. They found the quantity of carotene absorbed on a low-fat ration was definitely less than on a normal-fat ration. The hen could absorb 50 to 60 percent of carotene on a normal-fat ration, either in the free form or from plant tissues. Although the quantity of carotene absorbed increased with increasing dosage, there was a progressive decrease in the percentage absorbed. When the intake on the low-fat ration increased 3.5 times, the quantity absorbed

increased only 1.7 times, while under the same conditions, on the normal fat ration, the increase was five fold.

The conclusion drawn was that fat is necessary in the diet for satisfactory carotene absorption. The work indicated the fat requirement figure for satisfactory absorption of carotene lies somewhere between 0.07 and 3.83 percent, but the exact level was not determined. A normal amount of fat was found necessary for the retention of this vitamin in the liver, as one month after receiving large doses of vitamin A, 37 percent of it was recovered in the liver of birds fed 3.83 percent fat, but only 4.9 percent was recovered in the livers from birds fed a ration containing 0.07 percent fat.

The critical part that tocopherol must have played, and the possible role of fat as a source of tocopherol or vehicle for its absorption were not considered (Ewing, 1963).

Regarding losses of carotene from alfalfa, during storage, Wilder and Bethke (1941) found that machine dried alfalfa meal in burlap or paper bags, stored in a refrigerator at -23°C. to -26°C. lost 10 percent of its carotene in six months. At -10°C. to -15°C. the loss was about 14 percent in six months and 30 percent in one year. At 1 to 6°C. the loss was 50 percent in six months, and at room temperature, 60 to 72 percent in six months.

At 37°C. the meal lost 38 percent of its carotene in 16 days; at 60°C. the loss was 66 percent in 16 days, and at 80°C. the loss was 98 percent in 16 days. The rate of loss was the same in paper or burlap bags, and also in pellets and meal.

The use of dried or dehydrated alfalfa as a source of vitamin A has been going on since the early 1930's. The National Research Councils' Committee on Poultry Nutrition recognizes that equal numbers of units of vitamin A activity from carotene and from true vitamin A are equivalent as vitamin A sources for growing chickens (N.R.C., 1960). There has existed some confusion over the conversion ratio of carotene to vitamin A when dehydrated alfalfa is used. The generally accepted conversion for poultry and rats is on the basis of 0.6 microgram of beta-carotene being equivalent to 1.0 I.U. of vitamin A (Rutter, 1961).

Recent reports seem to indicate that carotene is not utilized efficiently by poultry as a vitamin A source. Some doubt that natural carotene carriers are satisfactory sources of vitamin A activity for poultry (Camp et al., 1955; Ely, 1959; Gledhill and Smith, 1955; Olsen et al., 1959; Williams, 1962).

Shellenberger et al. (1960) depleted newly-hatched chicks for one week, and then gave only carotene supplied by alfalfa meal as the sole vitamin A source throughout the period of growth plus one full year of egg production. This eye-opening research indicated that Leghorns could do well on carotene of alfalfa as the sole source of vitamin A, through the period of growth and a full year of production, even when feeds were not entirely consumed until one month after mixing.

Parrish and Sanford (1960) reported, hens fed a vitamin A deficient basal ration supplemented with 3,000 units of vitamin A per pound of feed supplied by alfalfa meal, averaged 4 percent higher production than those supplemented with 1,500 units of vitamin A.

Zimmerman et al. (1961) conducted a study to determine the value of carotene of alfalfa as the source of vitamin A activity, and also to determine the effect of furazolidone on the utilization of carotene of alfalfa by laying hens. A significantly higher rate of egg production was noted when 3,000 units of vitamin A activity per pound of feed, supplied as carotene of alfalfa, was fed as compared to 1,500 units.

It was concluded that from the standpoint of egg production, mortality and feed conversion, laying hens could utilize carotene from alfalfa as their sole source of vitamin A.

Parrish and Sanford (1962) studied the relative value of the vitamin A activity of carotene of alfalfa meal, and of stabilized vitamin A during the laying period. Average egg production was higher when stabilized vitamin A was fed, but production from all the groups was good. The groups were: (1) basal diet plus 2,000 I.U. per pound of vitamin A activity supplied as carotene of alfalfa, (2) basal diet plus 3,000 I.U. per pound of vitamin A activity also given by carotene of alfalfa and (3) 2,000 I.U. of stabilized vitamin A. Feed conversion was optimum in pullets fed the ration supplemented with stabilized vitamin A. There were no significant differences in egg weights, shell weights, percent shell or Haugh units.

Phase 2.

Ever since Morehouse and Mayfield (1946) discovered that arsonic acid at sub-therapeutic levels promotes growth, a new vista has been developed for the use of arsonic compounds. Feed manufacturers have been quick to grasp and exploit this fact, and quite a lot of research has been conducted to derive the utmost benefit from this accidental finding.

Even as early as 1786, Fowler described the therapeutic use of 1 percent As_2O_3, and many modern veterinary practitioners still make frequent use of it. Inorganic arsenic (along with some other elements like copper, cobalt and zinc) is peculiar in that it possesses an oligodynamic pharmacology. Only low concentrations show stimulatory effect, but as concentrations get higher and higher, inhibition or frank toxicity develops. Arsenic acid derivatives have held their place in medicine since olden times and are still considered to be the fountainhead of modern chemotherapy. Certain of the arsenic acid derivatives are among the best drugs for the control of deadly diseases such as trypanosomiasis and amebiasis in human beings. In poultry diseases, coccidiosis, histomoniasis, avian monocytosis, nonspecific enteritis and spirochaetosis are all within the range of its activity. Arsenic has been the Dr. Jekyll and Mr. Hyde of the elements to the layman. While its good effects are little known, it has come as a godsend to impoverished writers of detective fiction. The arsonic compounds are distinctly different from arsenic itself, but even here, Dr. Jekyll still has more than a shadow of Mr. Hyde (Frost, 1952).

Arsanilic acid is actually p-aminophenyl-arsonic acid, and is the primary intermediate used in the manufacture of most other arsenicals such as 3-nitro-4 hydroxyphenyl arsonic acid, acetarsone, tryparsamide, arsphenamine, etc. It occurs as an odorless, pure, creamy-white powder, which is free-flowing and blends easily with other ingredients in feeds. Its sodium salt is much more soluble in water and is commonly tabletted for veterinary use (Frost, 1953).

Abbot et al. (1954) found arsanilic acid and penicillin to be equally effective in stimulating growth, under various experimental conditions.

Even at the level of 500 mg. per Kg. of ration, arsanilic acid did not supress growth or produce any other evidence of toxicity. The lowest level which did supress growth, slightly, was 1,000 mg. per Kg. of feed, which is more than 10 times the level permitted by the Food and Drug Administration for use in commercial feeds. At levels of 1,500 mg./Kg. of ration, symptoms similar to thiamine deficiency were noticed. At levels of 2,000 mg./Kg., or above, excessive mortality occurred.

A similar study made by Frost and Spruth (1953) concurs that arsanilic acid is well tolerated by White Leghorns up to 12 weeks, at 0.1 percent of the ration, i.e. 10 times the recommended feeding level. The difference between arsanilic acid and antibiotics is that the former is unable to spare the requirement for vitamin B_{12} and pantothenate as antibiotics do. At present, the arsenic compounds are being used commercially mainly for improvement of feathering and increase of pigmentation of shanks, skin, comb and wattles, as this is the only consistent effect seen. The effect on growth, when added to a diet already containing an antibiotic is inconsistent. There is no evidence, at present, to support the use of arsenic compounds in the diet of laying birds.

The mode of action of phenylarsanic acids is not known. It is speculated, their capacity to eliminate or control many harmful organisms could account for at least a part of their favorably effect on growth and feed efficiency. The effect of these compounds on comb, wattles and feathers has led to the suggestion they may affect hormonal balance in some way. They appear to act at a different disease level than antibiotics, and in some cases show an additional complimentary plus effect with antibiotics (Bird, 1952).

The optimum concentration limit of 3-nitro-4-hydroxyphenylarsonic acid in drinking water of chickens, for growth stimulation, is approximately 0.00025 percent. In the feed, the same effect is obtained by approximately 0.009 percent, which is also the most effective level for arsanilic acid. Such treatment is naturally more effective during the early part of the growing period than during the latter part (Morehouse, 1949).

At the recommended level of arsanilic acid (90 gm. per ton), the amount of arsenic found in the liver and kidneys, even on the last day of feeding, was much less than the allowance for arsenic residue on fruit (3.5 p.p.m. of As_2O_3). The amount of arsenic in tissues was directly proportional to the level of the arsenic compound in the diet. Arsenic deposition occurs greatest in the liver and kidneys and least in muscles and skin. The level of arsenic in the muscles is actually less than that found naturally in fish. One would have to consume fantastic amounts of such tissue to approach therapeutic dose levels of arsenic (Frost, 1952).

With reasonable care in handling and feeding, no concern need be felt in the use of these compounds. Arsanilic acid and sodium arsanilate is toxic to poultry, only if fed above 0.03 percent (270 gm. per ton of feed), and the 3-nitro compound is toxic if fed continuously at the level of 0.01 percent (90 gm. per ton of feed)(Patrias, 1952). Pullets receiving the 3-nitro compound came into production, on an average 15.1 days, earlier than the controls, and yet there was no adverse effect on egg weights (Morehouse, 1949).

Yates and Schaible (1961) did not get improved performance when arsanilic acid was added to a 16 percent protein ration.

Stabilization against oxidation of fat soluble vitamins and pigments in feedstuffs. Forages and poultry feeds cannot be produced fresh all year round. The feed and fodders have to be stored and used when required. This process causes deterioration of fat-soluble vitamins and all too often the vitamin content is less than half the expected amount. Screening tests at Western Regional Research Laboratory, U.S.D.A., Albany, California (where over 400 antioxidants that are effective in rubber, plastics, foods, etc. were screened) showed that 6-alkoxy substituted dihydroquinolines were more active than other compounds for preserving provitamin A in dehydrated alfalfa meal. One of these, 1-2 dihydro-6-ethoxy-2,2,4 trimethylquinoline (E.M.Q. for short) commercially known as Santoquin[1] has almost complete miscibility with oily vehicles and is available in quantity (Gassnet et al., 1960). Chronic toxicity studies with graded levels up to 0.075 percent of antioxidant in the diet, revealed no significant effect on growth, feed consumption, livability, egg production, fertility, and hatchability of eggs. Progeny from birds raised on the antioxidant-containing diets also showed no effect of treatment on growth and livability. Histological examination of liver, spleen, kidney, ovary, oviduct and thyroid of hens showed no changes correlated with treatment. Tissues of cockerels showed neither gross nor micro-pathology that could be ascribed to the treatment. However, the testes of birds on higher levels of antioxidant were larger than those of the control birds, while the thyroids were somewhat smaller than those of untreated birds (Gassnet et al., 1960). Wilson et al. (1959) made a detailed study of excretion and metabolism of

[1] Trade-mark of Monsanto Chemical Company, St. Louis, Missouri.

E.M.Q. (Santoquin) tagged with carbon-14 in the heterocyclic ring. The most outstanding finding concerning the fate of oral E.M.Q. was its rapid absorption and the rapid and nearly complete excretion of its metabolites. The portion of administered material eliminated in feces was modified, perhaps by digestive juices or the microflora to some form which was not readily absorbed. Material in the urine and feces accounted for almost all of the ingested E.M.Q. The material recovered in the urine was not E.M.Q. This was clearly demonstrated by paper chromatography after substantial doses of untagged E.M.Q. and small doses of E.M.Q. C^{14} were administered. The material was not extensively degraded, however, as it still fluoresced strongly. This was confirmed in the respiratory study. Had the heterocyclic ring been broken, a considerably increased excretion of $C^{14}O_2$ could have been anticipated. A very small portion of the administered E.M.Q. was found as respired CO_2, or temporarily stored in body tissues, or excreted in milk, and was incorporated at least in part into normal body constituents. Solubility characteristics again indicated the stored material was not unchanged E.M.Q. There is a possibility this material was not E.M.Q. at all, as approximately 5 percent of the radioactivity of tagged E.M.Q. was an impurity (Wilson et al., 1959).

MATERIALS AND METHODS

Phase 1.

For the first phase of the experiment, 36 seven-month old Inbred-Crossbred pullets[1] reared at the University Poultry Farm, under normal poultry management practices were randomly distributed into six lots of six birds each, and were kept in two identical Bussey Hen Batteries. The birds had all been vaccinated, debeaked, and were ready for use. All birds were weighed and numbered prior to commencement of the experiment.

The birds were put in the batteries on January 1, 1963 and 20 days were allowed to elapse before taking observations to permit adjustment to confinement in cages, and new environmental conditions. Also during this pre-experimental period, the birds were given only the colorless basal, provitamin A-free diet to deplete body stores of previously acquired vitamin A.

The necessary addition of the requisite amounts of pro and stabilized vitamin A was made on January 21, and the experiment started.

The basal diet (composition given in Table II) was supplemented as indicated in Table I. Feed and water were supplied to the birds ad lib. at all times.

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[1] Hy-Line 934 H.

TABLE I

EXPERIMENTAL DESIGN TO STUDY EFFECTS OF FEEDING DIFFERENT LEVELS OF PROVITAMIN A AND STABILIZED VITAMIN A TO CAGED LAYERS

LOT	DIET FED
1	Basal + 6,000 I.U. stabilized vitamin A per Kg.
2	Basal + 6,000 I.U. provitamin A per Kg.
3	Basal + 3,000 I.U. stabilized vitamin A per Kg.
4	Basal + 3,000 I.U. provitamin A per Kg.
5	Basal + 9,000 I.U. stabilized vitamin A per Kg.
6	Basal + 9,000 I.U. provitamin A per Kg.

All diets were maintained iso-caloric and iso-nitrogenous throughout the experiment.

TABLE II

COMPOSITION OF THE BASAL DIET USED IN THE EXPERIMENT

INGREDIENTS	AMOUNT PER 45.5 KG.
Sorghum grain (screened and ground)	33.4 Kg.
Wheat standard midlings	1.8 Kg.
Soybean oil meal (44% solv. extr.)	6.0 Kg.
Fish meal (Menhaden)	0.7 Kg.
Brewer's dried yeast	0.7 Kg.
Salt (NaCl)[1]	0.2 Kg.
Ground limestone[1]	1.8 Kg.
Dicalcium phosphate[1]	0.9 Kg.
Total	45.5 Kg.
Added per 45.5 Kg. of ration	
Manganese sulphate[1]	23.0 gm.
Vitamin K (Klotogen 17.6 gm. per Kg.)[2]	4.8 gm.
D-L methionine[2]	46.0 gm.
Vitamin D_3 (330,000 I.C.U. per Kg.)[2]	5.0 gm.
Vitamin B_{12} (26.4 mg. per Kg.)[2]	19.0 gm.
B-complex vitamin mix[2,3]	23.0 gm.
Choline chloride (25% mix)[2]	88.0 gm.

[1]Mineral pre-mix.

[2]Additive pre-mix.

[3]Supplies in mg./Kg. D-pantothenic acid 8096; niacin 13,200; choline chloride 440,000 and riboflavin 44,000.

The basal diet was mixed in the feed building of the University Poultry Farm. Sorghum grain was screened before grinding in a hammer mill. The macro-nutrients were weighed on a large Toledo balance while the micro-nutrients including manganese sulphate were weighed on a Toledo computogram balance.

The mineral and vitamin premixes were prepared separately in carriers of about seven Kg. of ground sorghum grain each, and then each were re-mixed with about 45.5 Kg. sorghum grain in a Hobart mixer. The mixing

in both mixers was continued for five minutes, timed with an interval timer alarm clock.

All the ingredients were then mixed in a large horizontal mixer, for five minutes, and stored in burlap sacks containing 45.5 Kgs. each. The basal was prepared every two weeks, and the supplements were mixed in every week. Seven Kg. of basal, including the specific amount and type of vitamin A, were each weighed into six feed cans for the six different treatments. The cans were labeled and utmost care was taken to prevent any error.

The provitamin A was obtained from dehydrated alfalfa meal, procured locally, weighed and analyzed monthly by the A.O.A.C.[1] method to determine vitamin A activity before using. It was stored in a sharp freeze, and utilized when necessary.

At the end of each period, the feed left over, if any, was weighed and the actual feed consumed for each period was determined.

Ventilation was of the forced-draft type, and 14 hours of artificial light per day was given throughout the experiment by three rows of five 60-watt electric bulbs hanging from the ceiling, on either side of the batteries. The lights had connection with a time clock, and supplied light from 6 a.m. to 8 p.m. daily.

The experiment was conducted for five periods of 20 days each. Every 20 days, eggs were collected and numbered for three successive days. The breakout was performed at the and of the 3-day period, till which time the eggs were stored at about 50°F.

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

All measurements of egg quality, including weight, were individually recorded at the same time for the entire three-day sample.

The egg weights were taken on a Mettler balance. The albumen height and Haugh units were determined according to the method described in AMS No. 246 of U. S. Department of Agriculture (Kilpatrick et al., 1960). After the shells were dried in a thermostatically controlled oven at 100°C. for 24 hours, shell weights were determined on a Gram-Atic balance. The shells were allowed to cool off for five minutes before weighing.

Yolk weights were taken on a Mettler balance at breakout time. The chalaza was all removed carefully with a forceps, and the yolk was rolled on an absorbant paper to remove any traces of albumen sticking to it before weighing. The percent yolk and percent shell were arrived at by calculation.

At the end of the fifth and last period, 50 percent of the birds in each of the six lots were sacrificed, and their blood and livers were analyzed for carotenoids and vitamin A in the serum and vitamin A content of the liver. Eggs collected a week before the birds were sacrificed, were also analyzed for I.U. of vitamin A per gm. and also I.U. of vitamin A per yolk.

Phase 2.

The second phase of the study was conducted in a semi-monitor type poultry house, with three compartments, at the University Poultry Farm. The house was cleaned and fresh new litter put in. The seven-month old Inbred-Crossbred pullets[1], which had been previously vaccinated and de-beaked were individually weighed, wing-badged and randomly distributed into three lots of 60 birds each. The assignment of pens was also done randomly. The control lot was kept in pen 18 A, the Santoquin supplemented

[1]Hy-Line 934 H.

lot was assigned to pen 18 B, and the arsanilic acid supplemented lot was confined to pen 18 C.

The birds were put in the pens on October 6, 1962, and were fed the basal diet, containing no vitamin A activity for 20 days to deplete body reserves of stored vitamin A. On October 26, 1962, 3,000 I.U. of vitamin A activity per Kg. of basal ration, supplied by dehydrated alfalfa meal, were added to the basal of 18 A. The same amount of dehydrated alfalfa meal plus Santoquin (antioxident E.M.Q.), at the recommended rate of 0.015 percent, was added to the basal diet of 18 B. The same amount of dehydrated alfalfa meal plus 23 gm. of arsanilic acid per 45.5 Kg. of feed was added to the basal diet of 18 C.

The same basal as listed in Table II was used for these birds also. Feed and water were supplied to the birds ad lib. The procedure of preparing the basal, and addition of the supplements was identical to the description given in Phase 1 of the experiment. Environment was natural.

The experiment was conducted for eight periods of 23 days each (with the exception of the last period which was 29 days). All the birds were trap-nested at the end of each period for three successive days. All the eggs so collected were weighed, but only 16 eggs randomly selected from each lot, each day, were used for breakout studies. The eggs were stored in a refrigerated room at about 50°F. till the end of the three-day period. All egg quality measurements were recorded at the same time for the three-day sample of 48 eggs in each lot.

The rest of the procedure followed was identical to that of Phase 1 except that the birds were not sacrificed at the end of the experiment for a study of the tissues.

The data obtained in both phases of the experiment were analyzed for analysis of variance and least significant differences (Snedecor, 1956).

RESULTS AND DISCUSSION

Phase 1.

Percent production. The percent production was calculated on a hen-day basis for five 20-day periods. The analysis of variance of percent production was run on the total number of eggs laid by all six lots during the experimental period only. There were no significant differences between the treatments; however, there were significant differences between periods.

TABLE III

ANALYSIS OF VARIANCE OF PERCENT PRODUCTION DURING THE EXPERIMENTAL PERIOD

SOURCE OF VARIATION	d.f.	S.S.	M.S.	F-VALUE
Treatments	5	292.0	58.4	1.33 n.s.
Periods	4	606.1	151.5	3.45*
Residual	20	877.5	43.9	
Total	29			

n.s. Non-significant.

*Significant < 0.05

Egg weights. The analysis of variance of egg weight data revealed significant differences ($P < .01$) in treatments, periods, and treatments x periods (Table IV).

The least significant difference (hereafter referred to as L.S.D.) between treatments, indicated that the diet containing 3,000 I.U. of stabilized vitamin A caused the production of eggs significantly heavier

than 3,000 I.U. of provitamin A. Similarly, 9,000 I.U. of stabilized vitamin A caused the production of eggs significantly heavier than 9,000 I.U. of provitamin A. However, 6,000 I.U. of provitamin A caused the production of eggs significantly heavier than 6,000 I.U. of stabilized vitamin A, which seems strange and cannot be explained.

Supplementation with 3,000 I.U. of stabilized vitamin A caused the production of the heaviest eggs--significantly heavier than all other lots. There were no significant differences in egg weights between 3,000 I.U. of provitamin A, 6,000 I.U. of provitamin A, and 9,000 I.U. of stabilized vitamin A.

TABLE IV

ANALYSIS OF VARIANCE OF EGG WEIGHTS

SOURCE OF VARIATION	d.f.	S.S.	M.S.	F-VALUE
Treatments	5	995.60	199.12	41.06**
Periods	4	253.97	63.49	13.09**
Treatment x periods	20	45.63	45.63	9.41**
Within (error)	389	4.83	4.83	
Total	418			

Ranked lots based on Fisher's L.S.D. method for egg weight[1]

	Ranked lots					
	1	6	2	5	4	3
Treatment means	58.62	60.79	61.93	62.18	62.34	63.78

**Significant < 0.01

[1]L.S.D. 0.87 for 0.05 level. Any two lots not underscored by the same line are significantly different at 0.05 level of probability.

Shell weights. The analysis of variance of shell weight data revealed no significant differences between treatments, but there were significant differences between periods, and between the interaction of treatments and periods.

TABLE V

ANALYSIS OF VARIANCE OF SHELL WEIGHTS

SOURCE OF VARIATION	d.f.	S.S.	M.S.	F-VALUE
Treatments	5	1.5199	0.304	1.68 n.s.
Periods	4	3.1624	0.791	4.37**
Treatments x periods	20	6.9449	0.347	1.92**
Within (error)	389	70.2183	0.181	
Total	418			

n.s. Non-significant.

**Significant <0.01.

Percent shell. The analysis of variance of the percent shell data showed significant variations between the treatments as well as between the periods; however, the interaction between treatments and periods was non-significant. Supplementation with 6,000 I.U. of stabilized vitamin A caused the production of significantly greater percent shell than any other treatment. The other treatments showed no significant differences between each other.

TABLE VI

ANALYSIS OF VARIANCE OF PERCENT SHELL

SOURCE OF VARIATION	d.f.	S.S.	M.S.	F-VALUE
Treatments	5	7.60	1.52	3.62**
Periods	4	11.89	2.97	7.08**
Treatments x periods	20	9.03	0.45	0.11 n.s.
Within (error)	389	163.52	0.42	
Total	418			

Ranked lots based on Fisher's L.S.D. for percent shell[1]

	Ranked lots					
	3	4	6	2	5	1
Treatment means	8.32	8.44	8.46	8.50	8.56	8.77

**Significant 0.01

n.s. Non-significant.

[1]L.S.D. 0.26 for 0.05 level. Any two lots not underscored by the same lines are significantly different at .05 level of probability.

Albumen height. The analysis of variance of the albumen height data revealed significant variations between treatments, and also between periods, but not between interaction of treatments and periods. The greatest albumen height was obtained by supplementation of the diet with 3,000 I.U. of provitamin A and 3,000 I.U. of stabilized vitamin A. The lowest albumen height was produced by the birds in the lot supplemented with 9,000 I.U. of stabilized vitamin A.

TABLE VII

ANALYSIS OF VARIANCE OF ALBUMEN HEIGHT

SOURCE OF VARIATION	d.f.	S.S.	M.S.	F-VALUE
Treatments	5	82.26	16.45	16.74**
Periods	4	13.99	3.50	3.56**
Treatments x periods	20	0.97	0.49	0.49 n.s.
Within (error)	389	382.46	0.98	
Total	418			

Ranked lots based on Fisher's L.S.D. method for albumen height[1]

	Ranked lots					
	5	1	2	6	3	4
Treatment means	5.8	6.0	6.3	6.4	6.8	6.9

**Significant < 0.01

n.s. Non-significant.

[1]L.S.D. 0.4 for .05 level. Any two lots not underscored by the same line are significantly different at .05 level of probability.

Haugh units. The analysis of variance of the Haugh units data revealed significant differences between treatments, periods, and also between interactions of treatments with periods. The highest Haugh units were obtained by the birds fed the diet supplemented with 3,000 I.U. of provitamin A and 3,000 I.U. of stabilized vitamin A. The lowest Haugh units were produced by the lot of birds supplemented with 9,000 I.U. of stabilized vitamin A.

TABLE VIII

ANALYSIS OF VARIANCE OF HAUGH UNITS

SOURCE OF VARIATION	d.f.	S.S.	M.S.	F-VALUE
Treatments	5	3215.77	643.15	13.71**
Periods	4	1116.24	279.06	5.95**
Treatments x periods	20	6412.92	320.65	6.84**
Within (error)	390	18292.68	46.90	
Total	419			

Ranked lots based on Fisher's L.S.D. method for Haugh units[1]

	Ranked lots					
	5	1	2	6	3	4
Treatment means	73	76	77	79	80	82

**Significant < 0.01

[1]L.S.D. 3.0 for .05 level. Any two lots not underscored by the same line are significantly different at 0.05 level of probability.

Yolk weights. The analysis of variance of the data for yolk weights showed no significant variation between treatments or between periods, but there was a significant difference between the interaction of treatments and periods.

TABLE IX

ANALYSIS OF VARIANCE OF YOLK WEIGHTS

SOURCE OF VARIATION	d.f.	S.S.	M.S.	F-VALUE
Treatments	5	175.47	35.09	1.98 n.s.
Periods	4	133.04	33.26	1.87 n.s.
Treatment x periods	20	933.27	46.66	2.63*
Within (error)	389	6906.06	17.75	
Total	418			

*Significant <0.05

n.s. Non-significant.

Percent yolk. The analysis of variance of percent yolk data revealed significant differences between treatments, and also between periods; however, the reaction of treatments and periods was non-significant. The greatest percent yolk was produced by the lot of birds fed 6,000 I.U. of stabilized vitamin A. All the other treatments showed no significant differences between each other.

TABLE X

ANALYSIS OF VARIANCE OF PERCENT YOLK

SOURCE OF VARIATION	d.f.	S.S.	M.S.	F-VALUE
Treatments	5	103.49	20.70	11.31**
Periods	4	90.59	22.65	12.38**
Treatments x periods	20	35.73	1.79	0.98 n.s.
Within (error)	369	674.23	1.83	
Total	398			

Ranked lots based on Fisher's L.S.D. method for percent yolk[1]

	Ranked lots					
	2	3	6	4	5	1
Treatment means	28.59	28.86	28.87	28.93	28.96	30.25

**Significant < 0.01

n.s. Non-significant.

[1]L.S.D. 0.47 for .05 level. Any two lots not underscored by the same line are significantly different at 0.05 level of probability.

TABLE XI

SUMMARY OF EXPERIMENTAL TREATMENTS, PHASE 1

	RANKED LOTS[1]					
	1	2	3	4	5	6
Egg weight	3,000[2]	3,000	9,000[2]	6,000	9,000	6,000[2]
Shell weight	No significant differences					
Percent shell	6,000[2]	9,000[2]	6,000	9,000	3,000	3,000[2]
Albumen height	3,000	3,000[2]	9,000	6,000	6,000[2]	9,000[2]
Haugh units	3,000	3,000[2]	9,000	6,000	6,000[2]	9,000[2]
Yolk weight	No significant differences					
Percent yolk	6,000[2]	9,000[2]	3,000	9,000	3,000[2]	6,000

[1]Any two lots not underscored by the same line are significantly different.

[2]Stabilized vitamin A.

The feed efficiency. The feed efficiency (pounds of feed consumed to produce a dozen eggs) in all the lots was good. The best feed efficiency was obtained in the lot supplemented with 9,000 I.U. of stabilized vitamin A. The poorest feed efficiency was observed in the lot supplemented with 3,000 I.U. of provitamin A. Birds in the lots supplemented with 3,000 I.U. and 9,000 I.U. of stabilized vitamin A showed better feed efficiency than those fed provitamin A. Birds supplemented with 6,000 I.U. of provitamin A showed slightly better feed efficiency than stabilized vitamin A fed birds. The feed efficiency was calculated for the experimental period only.

TABLE XII

SHOWING FEED EFFICIENCY AND PERCENT PRODUCTION

	UNITS PER KG.					
	3,000 Pro	3,000 Stabl.	6,000 Pro	6,000 Stabl.	9,000 Pro	9,000 Stabl.
Feed consumed	172.1	163.2	163.2	145.0	153.6	149.5
Eggs laid	489	477	493	435	475	473
Feed efficiency	4.22	4.11	3.97	4.00	3.88	3.79
Percent production	80.12	78.14	80.76	71.14	77.84	77.38

Vitamin A content of serum, liver and eggs. Three birds (50 percent) were randomly selected from each lot and were sacrificed at the end of the experiment (May 2, 1963), to determine the vitamin A content in the serum and liver. Eggs for vitamin A analysis were saved for a period of one week prior to the termination of the experiment.

TABLE XIII

SHOWING VITAMIN A CONTENT OF SERUM, LIVER AND EGGS

LOT	TREATMENT	SERUM[1] CAROTENOIDS mmg./100 ml.	SERUM[1] VITAMIN A I.U./100 ml.	LIVER[1] VITAMIN A I.U./gm.	EGG YOLK[2] VITAMIN A Units/gm.
1	3,000 I.U. Stabilized-A	23.0	181	17.2	12.6
2	3,000 I.U. Pro-A	56.8	58	5.4	5.6
3	6,000 I.U. Stabilized-A	23.2	218	178	18.2
4	6,000 I.U. Pro-A	85.8	104	4.6	9.1
5	9,000 I.U. Stabilized-A	29.6	265	376	20.7
6	9,000 I.U. Pro-A	135.7	145	25	12.4

[1]Three samples in each average.

[2]Eggs collected a week before birds were sacrificed.

An evaluation of the data tabulated in Table XII reveals that the vitamin A content of serum increased progressively as the units of vitamin A in the diet were increased, but when provitamin A was fed, the units of vitamin A of the serum were only about half as many as was observed when stabilized vitamin A was fed. On the other hand the birds fed provitamin A showed more carotenoids in their serum than those fed stabilized vitamin A.

Liver stores of vitamin A exhibited a similar trend to blood serum content. The higher the levels of stabilized vitamin A in the diet the higher the content of stored vitamin A. Supplementation of the diet with 6,000 I.U. of provitamin A produced atypical results in that the livers had actually less vitamin A content than supplementation with 3,000 I.U. of provitamin A. However, the 9,000 I.U. had an increase of about five times above the level of 3,000 I.U. Stabilized vitamin A at the same level resulted in much higher vitamin A reserves in the liver than provitamin A.

The vitamin A content of egg yolks exhibited progressive increments as the level of vitamin A increased in the diet. Stabilized vitamin A gave much higher levels of vitamin A in the yolk than provitamin A. The levels of vitamin A in egg yolk obtained when 3,000 I.U. of provitamin A were added to the diet were 64 percent less than those obtained by Haleem (1955). This was probably due to seasonal variation as his birds were sacrificed in October while these birds were sacrificed in May.

Phase 2.

Egg weights. The data for egg weights showed a significant tendency to increase in all three pens from the first trap-nesting period to the last.

This can be attributed to the fact that the birds had just come into production, and egg size was gradually increasing. Another reason is the seasonal variation normally seen in eggs (Cunningham et al., 1960). There were no significant differences between treatments, and there were no significant differences between the interaction of treatment and periods. This is illustrated in Table XIV below:

TABLE XIV

ANALYSIS OF VARIANCE OF EGG WEIGHTS

SOURCE OF VARIATION	d.f.	S.S.	M.S.	F-VALUE
Treatments	2	2.95	1.47	0.51 n.s.
Periods	7	160.45	22.92	8.10**
Treatments x periods	14	50.98	3.64	1.29 n.s.
Within (error)	48	135.78	2.83	
Total	71			

n.s. Non-significant.

**Significant $\leq$ 0.01

Shell weights. The data for shell weights did not show any significant differences in the treatments nor in the treatment x period interaction, but significant differences were obtained between periods. This can be attributed to seasonal factors (Pope et al., 1960).

TABLE XV

ANALYSIS OF VARIANCE OF SHELL WEIGHTS

SOURCE OF VARIATION	d.f.	S.S.	M.S.	F-VALUE
Treatments	2	0.68	0.34	1.95 n.s.
Periods	7	3.14	0.45	2.57*
Treatments x periods	14	0.42	0.30	1.73 n.s.
Within (error)	48	0.83	0.17	
Total	71			

n.s. Non-significant.

*Significant < 0.05

Percent shell. The percent shell data were affected by the treatments. The L.S.D. shows that the untreated controls had significantly lighter egg shells than the treated birds. Although there was not quite a significant difference between the eggs from birds fed diets supplemented with Santoquin and those fed diets supplemented with arsanilic acid. The former did have heavier egg shells than the latter, and the difference approached significance. The significant differences in the periods are due to environmental factors (Pope et al., 1960).

TABLE XVI

ANALYSIS OF VARIANCE OF PERCENT SHELL

SOURCE OF VARIATION	d.f.	S.S.	M.S.	F-VALUE
Treatments	2	1.04	0.52	7.43**
Periods	7	13.54	1.93	27.57**
Treatments x periods	14	0.57	0.04	0.57 n.s.
Within (error)	48	3.11	0.07	
Total	71			

Ranked lots based on Fisher's L.S.D. method for percent shell[1]

	Ranked lots		
	A	B	C
Treatment means	8.571	8.761	8.859

**Significant ≤ 0.05

n.s. Non-significant.

[1]L.S.D. 0.155 for 0.05 level. Any two lots not underscored by the same line are significantly different at 0.05 level of probability.

Albumen height. The data for albumen height showed a progressive decrease from period to period, and the differences were significant, but no significant differences could be obtained between the treatments, nor between treatments x periods.

TABLE XVII

ANALYSIS OF VARIANCE OF ALBUMEN HEIGHT

SOURCE OF VARIATION	d.f.	S.S.	M.S.	F-VALUE
Treatments	2	0.59	0.30	0.33 n.s.
Periods	7	29.96	4.28	4.65**
Treatments x periods	14	1.06	0.08	0.08 n.s.
Within (error)	48	4.40	0.92	
Total	71			

n.s. Non-significant.

**Significant < 0.05

Haugh units. The average Haugh units in all the lots were more than 72, which is the minimum for AA grade of U.S.D.A. standards. Since significant differences in the data were obtained between the periods for egg weight and albumen height, it is not surprising that significant differences were obtained between periods in Haugh units. There were no significant differences in the treatments, or interaction between treatments and periods.

TABLE XVIII

ANALYSIS OF VARIANCE OF HAUGH UNITS

SOURCE OF VARIATION	d.f.	S.S.	M.S.	F-VALUE
Treatments	2	17.53	8.77	1.61 n.s.
Periods	7	1868.54	266.93	49.07**
Treatments x periods	14	60.92	4.35	0.80 n.s.
Within (error)	48	261.33	5.44	
Total	71			

n.s. Non-significant.

**Significant < 0.01

Yolk weights. Analysis of the data for yolk weights showed significant differences in treatments, periods and interaction between the two. The L.S.D. showed that the Santoquin treated lot had the least yolk weight, the untreated controls ranked next and the arsanilic acid supplemented lot had maximum yolk weights.

TABLE XIX

ANALYSIS OF VARIANCE OF YOLK WEIGHTS

SOURCE OF VARIATION	d.f.	S.S.	M.S.	F-VALUE
Treatments	2	0.350	0.175	19.44**
Periods	7	49.831	7.119	791.00**
Treatments x periods	14	6.066	0.433	48.11**
Within (error)	48	4.349	0.009	
Total	71			

Ranked lots based on Fisher's L.S.D. method for yolk weights[1]

	Ranked lots		
	B	A	C
Treatment means	17.22	17.26	17.38

**Significant < 0.01

[1]L.S.D. 0.04 for 0.05 level. Any two lots not underscored by the same line are significantly different at 0.05 level of probability.

Percent yolk. The analysis of variance of the data for percent yolk showed significant differences in treatments and periods, but not in the interaction between treatments and periods. The Santoquin supplemented lot had the least percent yolk. The arsanilic acid lot ranked next, and the untreated birds had the highest percent yolk.

TABLE XX

ANALYSIS OF VARIANCE OF PERCENT YOLK

SOURCE OF VARIATION	d.f.	S.S.	M.S.	F-VALUE
Treatments	2	7.58	3.79	15.79**
Periods	7	44.12	6.30	26.25**
Treatments x periods	14	3.77	0.27	1.13 n.s.
Within (error)	48	11.54	0.24	
Total	71			

Ranked lots based on Fisher's L.S.D. method for percent yolk[1]

	Ranked lots		
	B	C	A
Treatment means	28.24	28.92	28.93

**Significant < 0.01

n.s. Non-significant.

[1]L.S.D. 0.28 for 0.05 level. Any two lots not underscored by the same line are significantly different at 0.05 level of probability.

Percent production. Analysis of variation of the data for percent production revealed significant differences in treatments and in periods, but not between the interaction of treatments and periods. The highest percent production was obtained in the non-supplemented control lot, and in the Santoquin supplemented lot. There were no significant differences between the two. The data for the arsanilic acid supplemented lot showed significantly less percent production.

TABLE XXI

ANALYSIS OF VARIANCE OF PERCENT EGG PRODUCTION

SOURCE OF VARIATION	d.f.	S.S.	M.S.	F-VALUE
Treatments	2	311.84	155.92	8.36**
Periods	7	2876.85	410.98	20.90**
Residual	14	275.30	19.66	
Total	23			

Ranked lots based on Fisher's L.S.D. method for percent egg production[1]

	Ranked lots		
	18C	18B	18A
Treatment means	62.27	69.81	70.02

**Significant < 0.01

[1]L.S.D. 0.28 for 0.05 level. Any two lots not underscored by the same line are significantly different at 0.05 level of probability.

Feed efficiency. The feed efficiency in all the three lots was very poor. The non-supplemented controls showed a feed efficiency of 4.59 (pounds of feed to produce a dozen eggs). The Santoquin supplemented birds showed a feed efficiency of 5.19, and the arsanilic acid supplemented birds showed a feed efficiency of 5.27.

No analysis of variance was run on feed efficiency, as it was felt that the high population of rodents contributed to such high feed requirements, and that the rodents were of unequal population in the three pens.

SUMMARY AND CONCLUSIONS

Phase 1.

The first phase of the experiment was devoted to studying the effect of feeding 3,000, 6,000 and 9,000 I.U. of stabilized vitamin A and provitamin A to six lots of six hens each, housed in two identical Bussey Hen Batteries. The basal ration consisted of vitamin A and carotene free constituents, to which the type and level of vitamin A desired was added.

Eggs were collected for analysis for three successive days at intervals of 20 days each. Five such collection periods were utilized. Percent production, feed efficiency, egg weight, shell weight, percent shell, albumen height, Haugh units, yolk weight and percent yolk were the criteria used for judging the efficiency of the type and level of vitamin A in the diet. The following conclusions were drawn from this experiment:

1. The percent production showed no significant differences in the different treatments.

2. The feed efficiency was better when 3,000 I.U. and 9,000 I.U. of stabilized vitamin A were fed than when the same levels of provitamin A were used. There was no difference in the 6,000 I.U. level of vitamin A.

3. Egg weights were significantly greater when stabilized vitamin A was fed at 3,000 I.U. and 9,000 I.U. levels, but 6,000 I.U. of stabilized vitamin A caused significant lowering of the egg weights.

4. The percent shell was significantly greater when 6,000 I.U. of stabilized vitamin A was fed, but there were no significant differences in the other treatments.

5. Albumen height was best when 3,000 I.U. of vitamin A was fed either as provitamin A or as stabilized vitamin A. 9,000 I.U. stabilized vitamin A and 6,000 I.U. stabilized vitamin A gave significantly lower albumen heights.

6. Haugh units were best when 3,000 I.U. of stabilized or provitamin A or 9,000 I.U. of provitamin A were fed. The level of 9,000 I.U. of stabilized vitamin A gave significantly lower Haugh units.

7. Percent yolk was significantly highest in the lot fed 6,000 I.U. of stabilized vitamin A. There was no significant difference between the other lots.

8. Thus feeding 3,000 I.U. of stabilized vitamin A gave the best feed efficiency, and egg weights; feeding 3,000 I.U. of provitamin A gave the best albumen height and Haugh units; feeding 6,000 I.U. of stabilized vitamin A gave the best percent shell and percent yolk.

Phase 2.

The second phase of the experiment involved the feeding of the antioxidant Santoquin and arsanilic acid to hens kept on litter. Three lots of 60 hens each were housed in a semi-monitor type house. The first, was the control lot getting only a basal diet with 3,000 I.U. of vitamin A activity per 45.5 Kg. derived from analyzed, dehydrated alfalfa. The second lot received the same diet plus Santoquin at the recommended rate of 0.015 percent. The third lot received the same diet as the controls plus 23 gms. of arsanilic acid per 45.5 Kg. of basal.

Eggs were collected by trap-nesting the birds, every 23 days, for three successive days for analysis. Eight such studies were made in all. The same criteria were used as in Phase 1.

The following conclusions were drawn:

1. Significant differences were obtained only in the percent shell, yolk weights, percent yolk and percent production.

2. The percent shell was significantly less in the controls than in the supplemented birds, but there were no significant differences between the two treatments.

3. The birds supplemented with arsanilic acid showed significantly higher yolk weights, than the controls. The controls showed significantly higher yolk weights than the Santoquin treated birds.

4. The controls and the arsanilic acid fed birds showed significantly higher percent yolk than the Santoquin treated birds.

5. Percent production was significantly higher in the controls, and the Santoquin supplemented birds than in the ones supplemented with arsanilic acid.

6. Thus supplementing the diet with arsanilic acid decreased percent production, but increased the yolk weight, percent yolk and percent shell; supplementation of the diet with Santoquin decreased yolk weight and percent yolk, but increased the percent shell. Percent production was not significantly affected.

ACKNOWLEDGMENTS

It is a great pleasure for the author to express hearfelt appreciation for Dr. Paul E. Sanford, major professor, who worked shoulder to shoulder in all phases of the experiment, constantly giving encouragement and guidance.

Thanks and gratitude are also extended to Professor Thomas B. Avery, Head, Department of Poultry Science, for giving full use of facilities for work at all times, and for his kindness and encouragement.

The author is indebted to Dr. D. B. Parrish, Department of Biochemistry, for analysis and packaging the alfalfa meal and storing it in the sharp freeze throughout the duration of the experiment.

Thanks are due to Dr. C. V. Reddy who extended valuable advice in analysis of the data.

Gratitude and sincere appreciation must be expressed to the United States Agency for International Development for financing my trip and education, and for furnishing generous allowance for the period of my stay in the U. S.

Thanks are due to all members of the Poultry Department for their extreme friendliness and assistance whenever required.

Sincere appreciation must be expressed to my wife, Perviz, who worked and maintained our family back home, without whom my coming to the U.S.A. would have been impossible.

Lastly, gratitude is due to Dr. N. C. Buch, Professor and Research Officer in Physiology of Reproduction, Institute of Agriculture, Anand, India, who gave innumerable sacrifices to enable me to come to U.S.A. to get higher education.

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EFFECTS OF FEEDING PROVITAMIN A, STABILIZED VITAMIN A, ARSANILIC ACID AND 1-2-DIHYDRO-6-ETHOXY-2,2,4 TRIMETHYLQUINOLINE ON PRODUCTION AND QUALITY OF EGGS

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Poultry Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1964

The experiment was divided into two phases. The first phase studied the effect on percent production, egg weights, shell weights, percent shell, albumen height, Haugh units, yolk weight and percent yolk of feeding 3,000; 6,000 and 9,000 I.U. of stabilized vitamin A and provitamin A to six lots of six hens each. The same basal diet containing no vitamin A activity was fed to all the lots.

A three day sample of all the eggs laid by each of the lots was taken every 20 days for egg quality measurements. Five such periods were used.

Conclusions drawn were:

(a) Feed efficiency was better when stabilized vitamin A was fed at 3,000 I.U. and 9,000 I.U. levels than when same levels of provitamin A were fed.

(b) Feeding 3,000 I.U. of stabilized vitamin A gave the best feed efficiency and the best egg weights.

(c) Feeding 3,000 I.U. of provitamin A gave the best albumen heights and best Haugh units.

(d) Feeding 6,000 I.U. of stabilized vitamin A gave the best percent shell and best percent yolk.

The second phase of the experiment was performed to see the effects of the antioxidant, Santoquin, and arsanilic acid (Pro-Gen 90) on egg production, egg weight, shell weight, percent shell, albumen height, Haugh units, percent yolk and yolk weight of hens housed in floor pens on deep litter.

The 180 hens were divided into three lots of 60 each. The controls were fed basal diet containing 3,000 I.U. of vitamin A activity per 45.5 Kg. of basal. The second lot was given the same diet plus the antioxidant Santoquin at the recommended rate of 0.015 percent. The third lot was given the same diet as the controls plus 23 gms. of arsanilic acid per 45.5 Kg. of basal.

A three day sample of eggs was taken every 23 days for eight such periods. The following conclusions were drawn:

(a) Supplementing the diet with arsanilic acid decreased the percent production, but increased the yolk weight, percent yolk, and percent shell.

(b) Supplementing the diet with Santoquin decreased yolk weight and percent yolk, but increased percent shell. Percent production was not significantly affected.