FACTORS AFFECTING STORAGE STABILITY OF VITAMIN A, RIBOFLAVIN AND NIACIN IN A BROILER DIET PREMIX

by

QIANG ZHUGE

B. S., WUXI LIGHT INDUSTRY COLLEGE, CHINA, 1966

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Approved by:

Carol T. Kuyfenstra

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INTRODUCTION

Vitamins are necessary for biological catalytic activity. They represent a group of nutrients which is necessary for the normal health and development of animal organisms and which cannot be synthesized by the organisms requiring them. Usually, feed is fortified with vitamins to ensure animal health and improve animal growth efficiency, but vitamin stability is a problem. It is a matter of concern to both feed manufacturers and customers. If conditions in the feed promote the destruction of vitamins, the feed will be of little value, even though the diet should be adequately fortified.

Vitamin A is necessary for optimal growth, vision, epithelial cell health and reproductive performance of all animals. The chief sources of vitamin A are fish liver oils, alfalfa, carrots and corn. This vitamin is easily destroyed by light, air or other oxidizing agents, and synthetic vitamin A is added to nearly all livestock and poultry feeds.

Riboflavin, a B-vitamin, is also essential for growth and reproduction. Its requirement can be met by dairy products, liver meal, and alfalfa products. Ruminants have the capacity to synthesize riboflavin in the rumen, but dietary supplements are often added to monogastric diets, particularly those for poultry.

Niacin is also a member of the vitamin B-complex and is one of the more stable vitamins. Animals can synthesize part of their niacin requirements from the amino acid tryptophan. The dietary sources of niacin are liver, meat, fish, yeast, wheat germ, and alfalfa. The microbial flora of the rumen can also synthesize this

vitamin, but diets for non-ruminants are generally supplemented with niacin.

Many factors can decrease the stability of vitamins, the most important of which are heat, moisture, light, air and the presence of trace minerals which can act as catalysts enhancing chemical reactions. The degree and duration of adverse storage conditions is, of course, a significant factor in vitamin preservation in premixes.

Premixes are widely used as vehicles for the vitamin enrichment of feeds by the formula feed industry. The nutritional value of the final feed will be diminished if vitamins in the premixes are unstable. At present, it is not known how much the nature or the percent of diluent (carrier) affect premix stability under storage at different temperatures.

The objectives of this experiment were to compare the storage stability of vitamin A, riboflavin and niacin in a premix for a broiler diet:

1) with or without a complete mineral supplement added and stored at three different temperature ($1^{o}C$, $25\pm3^{o}C$ and $43^{o}C$).

2) with four different diluents (ground sorghum, ground corn, ground corn cobs and ground rice hulls).

3) at five different dilution levels (undiluted, 1:1, 1:4, 1:10, and 1:50).

REVIEW OF LITERATURE

IMPORTANCE OF VITAMINS

Vitamins are organic substances required by animals for normal life and functioning. They are similar to catalytic agents in that they are required in very small amounts (1). It is now well known that vitamins play a very important part in poultry nutrition. The discovery that vitamins are indispensable to the life and health of poultry has been of practical importance to poultry growers, as it is now possible to rear chicks at any time of the year regardless of climatic conditions (2).

Vitamins consist of various combinations and proportions of carbon, hydrogen, oxygen, nitrogen, with some vitamins also containing sulfur, and/or cobalt. In poultry all are necessary for health maintenance, growth, egg production and egg hatchability. Too much cannot be said about the necessity of seeing that adequate amounts of all of the essential factors, particularly the vitamins, are in mashes fed to poultry breeding flocks, for it depends a great deal upon the amounts included in the feed as to number and quality of the baby chicks produced (2). Vitamin requirements for broilers are very high because of their extremely high growth rates (3).

For the most part, vitamins serve as parts of enzyme systems which catalyze specific biochemical reactions occurring in different cells of the body. The animal body fails to function if it lacks any one of the required vitamins (4), and then deficiency symptoms will occur. Poultry are particularly susceptible to vitamin deficiencies, because they derive very little or no

benefit from microbial synthesis of vitamins in the gastro-intestinal tract (5).

Miller (6) has given a very complete early history of the important events resulting from the use of chickens as experimental animals in the study of the B-complex vitamins (6). Actually, chickens were the first animals to be used experimentally in vitamin B studies as far back as 1890 when Eijkman observed that chickens fed the scraps from a hospital kitchen developed paralysis similar to that which occured in beriberi patients (7). He also reported that rice polishings prevented this condition.

Schabile (4) claimed that more is known concerning the nutritive requirement of chicks than of adult chickens and that turkey poults have a slightly higher dietary requirement for most vitamins than do chicks. He suggested that Vitamin A and riboflavin levels require special attention in formulation of any poultry ration (4).

Vitamin A promotes growth and appetite, increases resistance to many infectious diseases and affects length of life. Deficiency of vitamin A results in retarded growth, poor vision, emaciation and weakness, ruffled feathers, low egg production and poor egg hatchability (2). The daily requirement of vitamin A for chickens (starter chicks of broiler strains) is 42 I.U. for a 250 g chick, increasing to 150 I.U. for a 1000 g bird (8).

Great interest has been shown in the last few years in the riboflavin requirement of poultry, not only because this vitamin is required for growth, survival, and reproduction, but also

because it is more expensive than other ingredients usually used in poultry rations. It is difficult to compose inexpensive rations for chicks adequate in this vitamin (2). Deficiency of riboflavin results in loss of vitality, loss of weight, broken feathers, low hatchability and egg production in layers.

The study of Hauge and Carrick (9) showed that the requirement of the growing chick for the growth-promoting factor now known as riboflavin is very great. Broiler chicks weighing less than 1500 g require about 3.6 mg of riboflavin per kilogram of feed (5). The requirement for larger broilers (2000 g) drops to 1.8 mg/kg of feed, but breeders' requirements are higher (3.7 mg riboflavin/kg feed) (5).

Niacin (nicotinic acid, or its amide form, nicotinamide) is required by all animals for the metabolic conversion of feedstuffs to energy. In chickens, deficiency of niacin will result in inflamed mouth, retarded growth, poor appetite, ragged feather development and scaly skin (2).

The requirement for niacin varies very much because nicotinic acid is synthesized in the animal body from the amino acid tryptophan. The niacin requirement, therefore, depends on the tryptophan content of the diet. Also, much of the niacin present in many of foods and feedstuffs is in a bound form that is not available to humans and animals. The vitamin can be released by treating the food materials with alkaline solutions, but such treatment might also destroy other vitamins present. Gries and Scott (5) obtained near maximum growth in chicks receiving 15 mg of niacin per kg of diet, when the diet contained 0.215% tryptophan. The National Research Council recommends a level of 27

mg niacin/kilogram of feed for broilers weighing less than 1500 g and 11 mg/kg for larger broilers. Breeding stock requires 11 mg of niacin/kg of feed (4).

VITAMIN STABILITY

While our knowledge of the vitamins in nutrition has grown steadily for many years, much remains to be learned about the factors affecting the stability of vitamins in different environments.

1. EFFECTS OF HEAT. Charles (10) reported that vitamins responded to various destructive forces such as the presence of oxidizing agents, reducing agents and the pH of the medium in which they were incorporated. The destructive forces were accelerated by heat and/or moisture. He found that most vitamins, both singly and in premixes, were unaffected when stored at winter temperatures in the southern part of the United States. However, deterioration began when the ambient temperature exceeded 24°C. When stored at 38°C, potency loss of 10% a month was not uncommon for many vitamins (11). This is not surprising, since in chemistry, a ten degree rise in temperature will approximately double oxidation-reduction reaction rates. Heat produced in the preparation of premixes and feeds could also contribute to vitamin destruction.

The effect of heat on vitamin loss was also shown by Wornick (11) who found that samples of feed products containing vitamins

and other additives were stable indefinitly in a deep-freeze, and at temperatures of -18 to 10°C potency losses were negligible. At the more common storage temperatures of 16-27°C, some losses in vitamins, occurred. The rate of loss approximately dcubled when the environmental temperature increased by about 14°C.

2. EFFECTS OF MOISTURE. Moisture acts in a number of ways to accelerate deterioration of feed nutrients. In combination with heat, bacterial and fungal growth is promoted when moisture content is high, and chemical reactions which follow result in a loss of potency. Most decomposition reactions occur in the liquid phase. Water is a major enemy of sensitive ingredients. In almost every case, rate cf potency loss increases as the moisture level rises (10,11).

Hygroscopic ingredients, such as choline chloride, can cause caking in a premix and the potential problem of this uptake of moisture must be recognized and dealt with through proper formulation (12).

3. EFFECTS OF MINERALS AND OXIDATION. Vitamin mixtures in combination with mineral salts and moisture are especially susceptible to decomposition. Many mineral salts are known to catalyze reactions leading to vitamin destruction. Since these reactions must generally occur in a liquid phase, the water-soluble mineral salts are the most troublesome (10).

Some investigators feel that oxidation is a more potent factor in vitamin A stability than is heat (13). Oxidizing agents such as iron salts in the ferric state are sometimes used in mineral

supplements. Where the concentrations of oxidizing and reducing compounds are high, it is possible for sensitive additives, such as vitamins, to be adversely affected (11).

4. EFFECTS OF pH. Certain micro-ingredients, including vitamins, are very sensitive to acidity or alkalinity. Most feed products exhibit a pH in the range of 5.5 to 6.5. Some ingredients, such as minerals and drugs, can cause major shifts in the pH. The possible effects of these shifts on stability should be investigated carefully (11). Generally, vitamin A is fairly stable to mild alkali, and is unstable in acidic solutions. Conversely, riboflavin is destroyed by alkali but is stable to dilute acid. Niacin is quite stable, being moderately resistant to destruction by both acids and bases.

5. EFFECTS OF LIGHT. That vitamin A and riboflavin are unstable to light is well known (11). Probably, the destructive action of ultraviolet rays is primarily the enhancement of oxidation processes, the mechanisms of which are not known (14).

VITAMIN A STUDIES. The effect of minerals on the destruction of vitamin A in feed was studied by Miller et al. (15) who added manganese sulfate (0.5%) to a wheat bran and cod liver oil mixture. Originally, the mixture contained 1870 U.S.P. units of vitamin A per gram, but they found no vitamin A remaining after storage for 56 days. The same ration with no manganese sulfate had 78% of vitamin A remaining under the same storage conditions. Creek et

al. (16) also found that vitamin A was destroyed when manganese sulfate was added to a complete ration.

Other research on retention of vitamin A in premixes with mineral supplements indicated that grinding premix samples and storing them in a freezer for one month had little effect on vitamin A, but there was a significant loss (about 10%) after storage for one month at uncontrolled room temperature (17).

The effect of metal catalysts (ferrous sulfate, zinc oxide and calcium carbonate), water activity, and temperature on the stability of vitamin A acetate was studied in a model food system containing no antioxidants. Kinetic data associated with the model system equilibrated to the lowest water activity indicated that all of the minerals tested had an effect on vitamin A acetate stability. Iron exibited the most significant effect. The stability of vitamin A acetate decreased with an increase in the water activity at a storage temperature of 45°C (18).

The effect of storage time and temperature as deteriorative factors for natural vitamin A was studied by Fraps and Treichler (19). The stability of vitamin A in various dried food products, such as breakfast cereals, was determined after storage for 19 months at room temperature. After 11 months of storage all samples showed more than a 50% decrease in vitamin A content. They concluded that the storage period was an important factor in the stability of vitamin A and suggested there might be decreased loss if products were placed at about 0°C. Fraps and Kemmerer (20) studied the stability of cod liver oil in feed mixture and found that storage at 6°C provided better stability of vitamin A than storage at higher temperatures.

The effect of antioxidants on vitamin A acetate stability has been studied by Patterson (21) at varying conditions of temperature and humidity. The antioxidants studied were Endox, Ethoxyquin and BHT, which were used in a poultry vitamin-mineral premix. Ethoxyquin (2.5%) was the most effective in preventing vitamin A loss. Humidity appeared to be a major factor in the deterioration of stabilized vitamin A acetate (21).

RIBOFLAVIN STUDIES. O.W.Charles (10) found riboflavin to be quite stable in feed mixtures. In general, riboflavin is stable to moderate heat and oxidation conditions, but may be destroyed by reducing agents and alkaline substances (12). Although sensitive to alkalies, riboflavin is stable to mineral acids in the dark (22).

When Robblee and Clandinin (23) prepared premixes using buttermilk powder, meat meal, fish meal, or wheat middlings as carriers and stored them in paper bags for up to 17 months at 43^{o}C, no appreciable loss of riboflavin occurred. Others have found that storage of riboflavin at -4.5 and 2.8 ^{o}C for up to 24 months caused no significant decomposition or loss of activity (24).

In a vitamin B-complex solution for parenteral feeding which contained riboflavin, thiamin and niacinamide, no composition change occurred during storage at -15, -1 or 7^{o}C for 24 hours (25).

Thiamin and riboflavin levels are normally quite high in Maitake mushrooms. In cold storage, the vitamin content decreased

slightly, but room temperature storage and boiling caused a greater decrease in both vitamins (26).

NIACIN STUDIES. Niacin has a relatively simple chemical structure, and, when kept dry, it is stable for long periods (27). Significant decomposition in feeds or premixes has not been reported.

PROPERTIES AND MEASUREMENT OF VITAMINS

Vitamin A is insoluble in water (23,28) or glycerol (23), and is soluble in absolute ethanol, methanol, chloroform, ether, fats and oils (23). Assay of vitamin A is usually begun by extracting the feed or feed material with one of these organic solvents. According to Drummond (29), vitamin A can be removed in small quantity from oils by cold extraction with alcohol.

For many years, vitamin A was determined primarily using colorimetric procedures. Vitamin A reacts with antimony trichloride-chloroform solution to yield a blue color that absorbs light at a wavelength of 620 nm. The color then fades and gives a new maximum at 580 nm. This was made the basis of a quantitative assay (30).

Fraps and associates (31) estimated vitamin A by spectrophotometry and by biological methods. They found the spectrophotometric method satisfactory for rapid preliminary testing of the oils.

In another antimony trichloride colorimetric method developed by Moore and Fritz (32) for the quantitative estimation of vitamin A in feed, the sample was first saponified, then extracted with a

mixture of ethanol and petroleum ether.

The American Association of Cereal Chemists' (AACC) method for vitamin A also uses saponification followed by extraction with ether, measuring the absorbence at 310, 325, and 340 nm on spectrophotometer, then making corrections to reduce the effect of interfering substances (32).

More recently, various high performance liquid chromatographic (HPLC) procedures have been reported for the determinations of vitamins A and E (34,35,36). Widicus and Kirk (37) have reported greater than 90% recovery of vitamin A from whole wheat, corn, oats, wheat and rice cereals using HPLC analysis of methanol: chloroform extracts of the cereals without previous saponification of the samples. Hexane and chloroform (85:15), or methanol and water (90:10) were generally used as solvents, and the vitamin was detected by measuring its absorbance at wavelengths in the ultraviolet range.

Sullivan and Norris (38) studied the chemical determination of riboflavin in milk products. A fluorometric method was proposed by Supplee et al. (39) who determined riboflavin by converting it to lumiflavin and extracting it with chloroform.

Riboflavin is classified as a water soluble vitamin, but it dissolves even more readily in aqueous sodium chloride or dilute alkali solution (23). It is much less soluble in alcohol and other organic solvents than in water.

In the AACC method for riboflavin, 0.1N sulfuric acid is used to extract the vitamin. After the pH is adjusted to about 4.5 and impurities oxidized by potassium permanganate, concentration of

the vitamin is determined fluorometrically (40).

Niacin is soluble in both water and alcohol (23). Gyorgy and Pearson (41) described a method to determine niacin and its derivatives by paper chromatography.

The AACC method for niacin is quite complicated and requires the toxic reagent, cyanogen bromide. It involves the use of water and heating to extract the niacin from the sample after which the concentration is determined colorimetrically (42).

High performance liquid chromatography has now become an effective method to analyze B-vitamins. Wills et al. (43) measured 8 water soluble vitamins in a single analysis by HPLC. They described the effect of varying proportions of water/methanol as the eluting solvent and the addition of various salts, buffer solutions and paired ion chromatography (PIC) reagents on retention times of the vitamins. They showed that paired ion chromatography, combined with ultraviolet and fluorescence spectroscopy, allowed for highly selective and sensitive detection of the vitamins (43).

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

PART 1. EFFECT OF MINERALS AND TEMPERATURE ON PREMIX VITAMIN STABILITY

The scaled-down formula for the broiler diet premix used throughout this experiment is given in Table 1. All vitamins used in the premix were purchased from Hoffmann-La Roche Inc., Nutley, New Jersey, and were the same as those normally used in feed preparation in the pilot feed mill of the Department of Grain Science and Industry at Kansas State University. The gelatin coated vitamin A acetate was diluted with ground corn from 65000 I.U. to 10000 I.U. per gram. The riboflavin supplied by Hoffmann-La Roche contained about 50% riboflavin compounded with maltodextrin, calcium sulfate dihydrate and calcium silicate. Niacin used was in the form of 99.5% nicotinic acid with no diluent added by the manufacturer. The vitamin B-complex mixture used in preparing the vitamin mixture for this experiment (Table 1) contained riboflavin, niacin, choline chloride, calcium pantothenate and ground corn and was also prepared in the pilot feed mill. No antioxidant was added to any of the premix samples in this experiment.

Three batches of the premix were prepared with trace minerals and Premix A (Table 1) omitted. One batch was stored at 43°C (constant temperature incubator), one at 1°C (refrigerator), and the third at room temperature (25±3°C). Three more batches of the premix were prepared with trace minerals added. One batch of the

mineral-fortified premix was also stored at each of the three temperatures. Components of each batch were individully mixed by hand in a large mixing bowl. Each premix sample was stored in a 350 ml styrofoam cup with a snug-fitting, snap-on lid. Vitamin A, riboflavin and niacin content were measured every two weeks for 16 weeks. Riboflavin and niacin levels were also measured after 27 weeks of storage.

PART 2. EFFECT OF DILUENT TYPE ON VITAMIN STABILITY

Three batches of the complete, scaled-down broiler premix were prepared as in Part 1 except that one batch contained yellow corn which had been ground in a hammermill with a 1/8" screen as a diluent instead of ground sorghum. In the second batch ground corn cobs (from Cob Division, The Andersons, Maumee, Ohio) were substituted for sorghum, and in the third batch ground rice hulls (Riceland Foods, Stuttgart, Arkansas) were the diluent. All samples for this part of the experiment were stored at room temperature in styrofoam cups with lids, and samples were taken as in Part 1.

PART 3. EFFECT OF DEGREE OF DILUTION ON VITAMIN STABILITY IN THE PREMIX

Four batches of the complete premix were prepared as described in Part 1, except that samples were mixed in a twin-shell mixer. The batches were diluted with ground sorghum as follows:

1. one part premix to one part sorghum.
2. one part premix to four parts sorghum.
3. one part premix to ten parts sorghum.
4. one part premix to fifty parts sorghum.

Samples were taken every two weeks using an electric divider.

VITAMIN EXTRACTION AND ASSAY

Because of the high vitamin concentrations and low amounts of interfering materials in the broiler premix, vitamin A, riboflavin and niacin were measured by high performance liquid chromatography (HPLC), which is a much simpler and faster procedure than other common methods of vitamin analysis. The chromatograph used was a Varian Model 5000 equipped with a Varichrome variable wavelength detector. Each vitamin was measured at its wavelength of maximum absorption (A_{max}) when dissolved in its extraction and eluting solvent. A_{max} for vitamin A was 326 nm and all vitamin A determinations were done at that wavelength. A_{max} for niacin was 262 nm, and that for riboflavin was 267 nm. Since the peak of riboflavin was smaller than that of niacin, the wavelength of 267 nm was used to measure concentrations of both vitamins on the same chromatogram. Niacin still absorbed very strongly at 267 nm.

Pure vitamin standards (vitamin A acetate from ICN Biochemicals, riboflavin from United States Biochemical Corporation, and niacin from Sigma Chemical Company) were used to determine retention times of the vitamins and to prepare all standard curves, which were repeated previous to each set of determinations for each sampling time. Correlation coefficients for the linear regression lines of the standard curves for the

three vitamins were all above 0.99. Chromatogram peak areas were calculated by multiplying peak height by peak width at half height.

Extracts of broiler diet premixes containing all ingredients except vitamin A, riboflavin or niacin were analyzed to insure that no other compound in the extract interfered with the determination of each vitamin at the chosen wavelength. When pure vitamin standards were added to extracts of the complete diet premix, areas of peaks corresponding to vitamin A, riboflavin or niacin were proportionately increased.

Extracts were prepared fresh shortly before HPLC analysis and their pH values were measured. Samples were taken by hand-quartering for the undiluted samples (Parts 1 and 2) or by electric divider for the diluted samples (Part 3). Extraction was carried out by placing the samples in volumetric flasks and stirring with the appropriate solvent for 20-30 minutes. After filtering the mixtures through Whatman No.42 filter paper, and 0.042 micron microfilters, 10 microliter aliquots of the extracts were injected into the chromatograph. All vitamin extracts were shielded from exposure to light as much as possible, either by using low actinic glassware or covering flasks with aluminum foil and by working in semi-darkened areas.

VITAMIN A DETERMINATION. Several organic lipid solvents (methanol, chloroform and hexane) were tried to determine which would be the best extractant for vitamin A. The best separation and recovery of this vitamin was obtained using 100% methanol

(Fisher HPLC grade) as the extraction solvent and as the HPLC mobile phase. When samples were extracted with chloroform, it was not possible to get complete separation of vitamin A from other components of the mixture. No vitamin A peak appeared on the chromatograms when hexane was the extraction solvent. Retention time of vitamin A was 2.8 minutes using a 20 x 0.46 cm, 5-micron octadecylsilyl (ODS) column (Alltech) with 100% methanol as the extracting and eluting solvent (See Figure 1). There were two peaks on the vitamin A chromatograms of feed extracts. All non-vitamin A substances eluted first, followed by the vitamin A peak.

The analytical column was protected with an 8 x 0.46 cm guard column packed with pellicular reverse phase packing material (Alltech). Retention time was longer and resolution poorer when a 30 x 0.46 cm, 10-micron Alltech ODS column was used. Recovery of vitamin A from the premix was 84.0$\pm$3.7% using this HPLC method. Vitamin A could not be detected by this method when the extracts concentration was lower than 1.2×10^{-4} mg/microliter.

RIBOFLAVIN AND NIACIN DETERMINATIONS. Various combinations of methanol and water have been used to measure B-vitamins by HPLC (42). In this case the optimum mobile phase yielding the best separation of niacin and riboflavin in the shortest time was found to be 30% methanol : 70% water. All water used in preparation of the mobile phase was purified by reverse osmosis followed by distillation in an all-glass still. Thirty percent aqueous methanol was also used to extract the B-vitamins from the broiler premix. The 10-micron, Alltech ODS column was found to give the best separation for these vitamins, and the same guard column was

used as in the vitamin A determinations. Figure 2 shows the separation of riboflavin and niacin under the conditions of this experiment. There were 3 peaks for completely formulated samples on B-vitamin chromatograms. Injecting a sodium chloride solution produced as peak at the some place on the chromatogram. There were 2 peaks on the chromatograms for those samples without minerals: niacin was eluted first, and followed by riboflavin. All concentration of niacin in this experiment could be detected on HPLC instrument, but riboflavin could not be detected when the extract concentration was lower than 4.5×10^{-6} mg/microliter. samples and 0.05 for diluted ones. Recovery of niacin from the premix was $98 \pm 2.7\%$ and that for riboflavin, $99 \pm 1.5\%$.

RESULTS AND DISCUSSION

PART 1. THE EFFECTS OF STORAGE TEMPERATURE AND MINERALS ON PREMIX VITAMIN STABILITY

1) VITAMIN A

Data for this part of the experiment are given in Table 3. To simplify comparisons, linear regression lines with slopes representing the rate of disappearance of the vitamin from the premix were calculated for each treatment. Slopes of the lines were compared using the analysis of variance procedure with Duncan's test (see Table 6). It is apparent from the increasingly steeper slopes that the rate of vitamin A disappearance from the premix increased as the storage temperature increased. This was true whether or not the premix contained minerals, and is in general agreement with results of earlier investigators (10, 11, 17, 19, 20). While it was, therefore, not surprising to find higher losses of vitamin A with storage at 25 (R.T.) or 43°C (HEAT), it was unexpected to find so high a rate of loss in samples stored at 1°C (COLD). Samples without minerals stored in the cold for 16 weeks lost about 14% of their vitamin A per month, and by the end of the storage period those samples had lost 56% of their vitamin A content. Others have found little loss of vitamin A when samples were stored in a freezer, but significant losses (10% in a month) at room temperature storage (17). Wornick (11) reported negligible losses in vitamin potency. Data from the present experiment indicate that storage at a temperature of 1°C does delay the destruction of vitamin A in the premix, but

certainly does not prevent it, even when the vitamin A acetate is protected by a gelatin coat. When Fraps and Treichler (19) stored breakfast cereals at room temperature for 11 months, they noted a 50% decrease in vitamin A content. From Table 4 it is apparent that vitamin A in the premix samples in this experimment had decreased more than 50% in only 4 months.

While it appears that the rate of vitamin A disappearence was faster for samples without minerals stored at room temperature and in the cold than it was for samples prepared with minerals, analysis of variance with Duncan's test showed the differences were not significant at alpha = 0.05 (Table 6). Although the slope of the regression line for the sample with minerals stored in the cold is the least, the correlation coefficient for that line is also the lowest, indicating that the data fit that line least well.

It should be noted (Table 6) that while differences between batches stored with minerals and those stored without minerals at three different temperatures were not significant, differences between vitamin A loss at the three temperatures were significant when minerals were in the mix, but were not significant in the batches stored without minerals. Apparently, there is an interaction between the presence of minerals in the mix and storage temperature.

Miller et al. (15) found manganese sulfate to have a negative effect on vitamin A stability. Iron and copper salts have also been implicated in vitamin A destruction (11). Since the premix used in this experiment contained those minerals, it is possible that they contributed to vitamin A loss.

Depending on the particular type and amounts of minerals in a premix or feed, the pH can very widely. As can be seen in Table 2, the pH of the premix used in this experiment ranged from 6.5 without minerals added to about 3.5 with minerals. Vitamin A is considerably more stable under basic conditions than in acid media, but apparently, the pH difference between batches with or without minerals in this experiment was not enough to affect vitamin A stability.

When the data from the various treatments in this part of the experiment were compared using second order regression, correlation coefficients were higher than with first order (linear) regression. A plot of the second order equations for samples with minerals is shown in Figure 3. All vitamin A data from this experiment showed the same type of second order relationship, indicating that vitamin A disappearence did not occur at a constant rate throughout the storage period, but that the vitamin was destroyed slowly at first, with the rate increasing as time went on. It is possible that during the storage period the gelatin coat on the vitamin A acetate was breaking down allowing the vitamin to be more susceptible to oxidizing conditions in the mixes.

2) RIBOFLAVIN

Table 5 contains the data for this part of the experiment, and in Table 6 are shown the slopes of the linear regression lines, their correlation coefficients, Duncan's grouping and percent of riboflavin loss after 27 weeks of storage. It is apparent that

riboflavin was destroyed fastest in the sample containing minerals stored at 43°C, where, after 27 weeks of storage more than 75% of that vitamin had been destroyed. For the batch without minerals stored at the high temperature, loss was 58%. This was still significantly higher than for batches without minerals stored at room temperature or below. As with vitamin A loss, presence of minerals in the mix seemed to accentuate the loss resulting from increased storage temperature. In this experiment the best conditions for maintaining riboflavin potency were the absence of minerals in the premix with storage at 1°C.

Under the conditions of this experiment riboflavin appears to be a more stable vitamin than vitamin A, since slopes of the linear regression lines for riboflavin were less steep than those of the lines for vitamin A. This could be partly expressed by the fact that, opposed to the case of vitamin A, riboflavin tends to be more stable in acid than in alkaline conditions. Since the pH of the premix in this experiment was acidic, destruction of riboflavin by minerals was probably less severe than in premixes where more alkaline conditions prevail.

It has been reported that riboflavin is relatively heat stable (12), but this experiment indicates that loss of riboflavin can be accelerated at high storage temperatures.

Both riboflavin and vitamin A are susceptible to destruction by light. In this experiment, samples were protected as much as possible from light, so it was not considered a factor here.

As with vitamin A, non-linear (second order) regression also seemed to fit the riboflavin data better than first order regression. Figure 5 is a plot of the second order equations for

riboflavin loss from mineral supplemented premix, which suggested that riboflavin was destroyed faster earlier in the storage period that it was later on.

3) NIACIN

Results of the niacin determinations are in Table 5. Slopes of linear regression lines for niacin are much flatter than those for either vitamin A or riboflavin, indicating that niacin was the most stable of the three vitamins (Table 8). This is in agreement with reports in the literature concerning this vitamin. However, there was significantly more niacin lost from samples containing minerals than from those without minerals at all three storage temperatures. Storage of batches at 43°C led to higher loss of niacin than storage at room or refrigerator temperatures. Highest loss (18%) of niacin was from samples with minerals stored at 43 °C. Least loss occurred in the batch without minerals stored at 1°C.

According to much of the literature, niacin is a quite stable vitamin (10,25). This could be due, in part, to its simple chemical structure. As our data indicate, however, when preparations containing niacin are stored at warm temperatures losses will occur, especially if the mix contains a mineral supplement. Losses of 30% in niacin content have been noted during food cooking, indicating that niacin is not absolutely stable to heat (45).

As is often the case when slopes of lines are nearly horizontal, the correlation coefficients for the niacin regression

lines are generally not very high. The slopes for mineral-supplemented batches stored at room or refrigerator temperatures are close to zero. Data is dispersed in a cloudlike way around the line, and those correlation coefficients were very low. When the data were subjected to second order regression, correlation coefficients were about the same as they were for first order regression for any of the treatments, indicating that niacin probably did disappear from the mixes in a linear way.

PART 2. THE EFFECTS OF DILUENT TYPE ON PREMIX VITAMIN STABILITY

1) VITAMIN A

As can be seen in Table 9, diluent type had a significant effect on vitamin A stability. The slopes of the linear regression lines show that vitamin A disappears significantly faster from premix diluted with sorghum than with ground corn, corn cobs, or rice hulls. Ground rice hulls or corn cobs appeared to be a somewhat better diluent than ground corn, but the difference was not statistically significant ($p = 0.05$).

A two-way analysis of variance with storage time and type of diluent as sources of variance shows the effects of both variables to be highly significant at $p = 0.0001$ (Table 9). The effects of storage time, and the interaction between diluent type and storage time (W * D) are significant also.

As previously discussed, the data for the disappearance of vitamin A from premix samples fits second order regression curves better than that first order ones. From Table 9, it appears that rice hulls or corn cobs should be better diluents for mixes containing vitamin A, with ground sorghum being the poorest diluent. At present, sorghum grain is commonly used as a diluent for mixes containing vitamin A. Data from this experiment show that for maximum retention of vitamin A in the mix, corn cobs or rice hulls would be a better choice.

The pH values for water suspensions of the complete premixes with the various diluents are all between pH 4 and 5, so variation in pH was probably not the reason for the observed differences in stability.

Sorghum is known for its high concentration of phenolic compounds, which might bind or destroy vitamin A when that grain is used as a diluent.

Browning (12) stated that destruction of vitamin A can be brought about by peroxides present in rancid fat. It is not hard to imagine, then, that ingredients with higher fat content (particularly unsaturated fat) could be detrimental to vitamin A stability. Rice hulls and corn cobs generally contain less than 1% ether extract. Fat content of the sorghum and corn is in the range of 2 to 5%. This could possibly have been a factor in the more rapid disappearence of vitamin A when those diluents were used. Addition of antioxidants such as Endox, Ethoxyquin, and BHT to a poultry vitamin and mineral premix was shown to be effective in preventing vitamin A loss (21).

2) RIBOFLAVIN

The rate of loss of riboflavin was significantly lower when corn cobs were used as a diluent than when sorghum, corn, or rice hulls were used (Table 10). There was no significant difference among the latter three diluents. The two-way analysis of variance using storage time and type of diluent as sources of variance shows that the effects of both were highly significant. The nature of the diluent appears to affect vitamin A stability more than that of riboflavin, since the comparable F-value was much larger for vitamin A than for riboflavin.

Figure 6 shows the plots of the second order equations for the disappearance of riboflavin from the various premixes. Again, riboflavin appears to be more stable when corn cobs were the

diluent used, and there was not much difference between the other three diluents. Corn cobs and rice hulls are both composed mainly of crude fiber, with very little protein and fat. The ash content, however, is about 20% which is considerably higher in rice hulls than in corn cobs. It is possible that some mineral component of the rice hulls was detrimental to the stability of riboflavin in this study.

3) NIACIN

As with riboflavin, slopes of the linear regression lines indicate that corn cobs are the best diluent for use in this premix (Table 11). After 27 weeks of storage of the complete vitamin-mineral premix at room temperature there appeared to be no loss of niacin when corn cobs were used as diluent. Contrary to our findings with vitamin A and riboflavin, sorghum appeared to be a better diluent than corn or rice hulls as far as niacin stability was concerned. Although loss of niacin was small, the two-way analysis of variance (Table 11) showed the effects of diluents and storage time to be significant at $p = 0.0001$.

PART 3. THE EFFECTS OF DEGREE OF DILUTION ON PREMIX VITAMIN STABILITY

1) VITAMIN A

Table 12 shows the linear regression equations for the disappearance of vitamin A from the diluted premixes. Ground sorghum grain was used as the diluent in all cases. It is apparent that slopes of these lines tend to become less steep as the degree of dilution increases. It is also apparent from the intercepts of these lines that the range of initial concentrations of vitamin A in the mixes was quite wide. For this reason, and because of some missing data due to problems with the HPLC instrument, no valid statistical analysis could be performed using these data. However, we can have some confidence that the data represent the actual situation, because correlation coefficients for the linear regression lines are high, indicating a good fit of the data to the line. Except that the slopes for the 1:4 and 1:10 dilutions were nearly the same, the general trend was to flatter slopes on increasing degree of dilution, which tends to strengthen the results.

As the degree of dilution of the premixes increased, pH values also increased, from 3.94 for the undiluted premix to 5.54 for the 1:50 dilution. Vitamin A is stable in basic solutions. Actually, conventional methods for vitamin A determination involve saponification with concentrated potassium hydroxide. The increase in pH noted here might have contributed to its increased stability in the more dilute premixes.

In Part 1 of this experiment we noted that minerals did not

significantly affect the storage stability of vitamin A. So apparently the increase in storage stability in the more dilute mixes was not caused by the greater separation of vitamin and mineral components in the mix. The more highly diluted mixes had less surface area exposed to the air which could have decreased the amount of vitamin A oxidation by atmospheric oxygen.

In Parts 1 and 2 of this experiment, where our solvent extract to sample ratio was very large and fairly constant, we could be reasonably sure that we were extracting a constant percentage of the vitamin A from the mixes. However, in this part of the experiment the solvent to sample ratio was much smaller because concentrations of vitamin A were so low that sample needed to be large. Using larger volumes of solvent, and then concentrating the extract would have concentrated compounds from the plant material that would interfere with the chromatographic analysis. It is also possible that when the concentrations were very low, a higher percent of the vitamin was extracted. Those factors could have influenced results of the dilution experiment.

2) RIBOFLAVIN

From the slopes of the linear regression equations in Table 13 it appears that dilution of the premix greatly improved riboflavin stability. Slopes of lines for the 1:1, 1:4, and 1:10 dilutions are not much different, but at 1:50 dilution level, the line was nearly horizontal and very little riboflavin was lost.

As we noted in Part 1 of this experiment, the presence of minerals in the mix appeared to affect riboflavin stability more than vitamin A stability. The higher dilution level, by reducing

the possibility of direct contact between riboflavin and mineral molecules, made their potential for interaction less, thereby protecting the vitamin. Riboflavin is more stable under acidic than basic conditions (2). Since all mixes in this experiment had pH values less than 7, pH of the mixes was probably not a factor in riboflavin destruction. As with vitamin A dilution, different solvent to sample ratios and widely different concentations of riboflavin in the mix could have influenced these results.

3) NIACIN

Niacin is known to be a very stable vitamin, being resistant to destruction by heat, light, acid, alkali, and oxidation (11), so it is not surprising that increasing the dilution of the premix had less effect on the stability of this vitamin than it did for vitamin A or riboflavin (See Table 14).

At the 1:50 dilution level there was a large interfering peak on the niacin chromatograms caused by the plant material (ground sorghum) in the mix. Various conditions of flow rate and mobile phase composition were tried, without success, to separate it from the niacin peak, nor was pre-column clean-up with Sep pak C18 cartridges able to remove it, so data from that premix batch are not available.

Although niacin is quite stable in this premix, dilution of the mix by a factor of 1:4 or 1:10 with sorghum appeared to slow its rate of destruction.

SUMMARY AND CONCLUSIONS

The effects of minerals, temperature, and nature and degree of diluents on the stabilities of vitamin A, riboflavin and niacin in a broiler premix were studied over a 16 to 27-week storage period.

Three storage temperatures were used: uncontrolled room temperature (about 25°C), 1°C (refrigerator) and 43°C (incubator). Ground sorghum was the diluent for the control and ground corn cobs, ground corn, ground rice hulls were the alternatives. Degree of dilution with ground sorghum were 1:1, 1:4, 1:10 and 1:50.

Vitamin concentration determinations were measured by high performance liquid chromatography (HPLC). Methanol (100%) was used to extract vitamin A and as the mobile phase for HPLC; 30% methanol : 70% water was used to extract riboflavin and niacin and also was used as the mobile phase.

Vitamin A was measured during a 16-week period. It was the least stable among the three vitamins. Presence or absence of minerals did not make a significant difference on the stability of vitamin A in the broiler premix. Higher storage temperatures increased the rate of vitamin A destruction. Destruction of vitamin A was greatest when ground sorghum was used as a diluent and least when the diluent was ground rice hulls. Stability of vitamin A in the premix appeared to increase as the degree of dilution increased.

After 27 weeks, riboflavin data indicated that the presence of trace minerals in the premix adversely affected riboflavin stability. Higher storage temperatures accelerated riboflavin loss. Type of diluent used appears to be less significant in

riboflavin stability than with vitamin A. Corn cobs were the best diluent for riboflavin, with no significant difference among ground corn, ground sorghum and rice hulls. The vitamin appeared to be most stable in the most highly diluted premix.

Niacin was considerably more stable than riboflavin or vitamin A, but the presence of minerals and high temperature did significantly affect stability of niacin. At room temperature or lower there was no loss of niacin in samples prepared without minerals and less than 10% loss in samples prepared with minerals even after 27 weeks. Ground corn cobs were the best diluent with respect to niacin stability, which was also better at higher dilution level.

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TABLE 1. BROILER RATION

PREMIX A	
LIMESTONE	4.54 GRAMS
DICALCIUM PHOSPHATE	4.54
SODIUM CHLORIDE	2.27
PREMIX B	
VITAMIN A (10000 I.U./g)	0.200
VITAMIN D_3	0.080
VITAMIN B_{12}	0.100
* VITAMIN B-COMPLEX	0.45
D-L METHIONINE	0.35
CHOLINE CHLORIDE	0.40
** TRACE MINERALS	0.23
*** DILUENT	2.73
TOTAL	15.89 GRAMS

* The composition of the vitamin B-complex mixture was: niacin, 5.31%; riboflavin (50%), 3.52%; choline chloride (50%), 17.625%; corn, 67.43%; calcium pantothenate, 7.875%.

** The composition of the trace mineral mix was : cobalt (0.10%), calcium (4.00-6.00%), copper (1.00%), iron (10.00%), manganese (10.00%), zinc (10.00%), iodine (0.30%).

Actual ingredients were: calcium carbonate, manganese sulfate, iron sulfate, zinc sulfate, iron carbonate, zinc oxide, copper oxide, mineral oil, potassium iodine, cobalt carbonate.

*** Ground sorghum, ground corn, ground corn cobs, or ground rice hulls.

TABLE 2

pH VALUES OF VITAMIN EXTRACTS

	SOLVENT		
	100% METHANOL	70% WATER : 30% METHANOL	100% WATER
COMPLETE PREMIX			
HEAT	3.62	3.88	4.68
ROOM	3.13	3.94	4.56
COLD	3.58	3.98	4.42
WITHOUT MINERALS			
HEAT	6.54	5.86	6.12
ROOM	6.43	5.71	6.04
COLD	6.43	6.10	6.21
CORN COBS	3.06	4.00	4.92
GROUND CORN	3.04	3.98	4.43
RICE HULLS	2.85	4.07	4.63
DILUTION			
1:1	4.40	4.10	
1:4	5.47	4.50	
1:10	5.83	5.07	
1:50	5.98	5.54	5.58
ALL MINERALS	4.09	4.29	5.46
100% METHANOL	7.43		
30% METHANOL + 70% WATER		6.40	
DISTILLED WATER			5.82

TABLE 3

DATA SUMMARY ON VITAMIN A (MICROGRAMS/PER GRAM PREMIX)

	WEEK							
BATCH	0	2	4	6	8	12	14	16
I-H	28.38	27.18	29.60	28.35	20.88	11.10	10.83	12.68
S.D	1.10	0.05	0.75	1.50	0.70	0.25	0.40	0.94
I-R	25.98	27.50	24.48	27.13	21.83	15.55	12.58	13.83
S.D	0.80	0.03	0.03	1.50	0.58	0.65	0.25	0.20
I-C	29.30	31.15	29.98	31.53	28.93	22.23	21.78	16.28
S.D	0.58	1.00	0.33	1.71	0.30	2.52	1.37	0.20
II-H	25.53	24.86	24.93	20.50	22.45	14.58	9.48	7.63
S.D	0.29	0.40	0.80	1.76	0.25	0.70	0.90	0.85
II-R	25.20	27.38	25.38	22.03	20.35	20.33	15.13	8.30
S.D	1.03	0.43	0.03	2.08	1.15	1.00	0.84	0.45
II-C	27.45	25.35	25.38	22.03	20.35	20.33	15.13	8.30
S.D	1.03	0.18	0.43	1.99	0.43	1.35	0.03	2.05
C-C	28.20	28.33	30.23	25.80	28.38	23.13	19.50	15.20
S.D	0.35	0.43	0.43	1.75	1.40	2.60	2.50	0.70
G-C	24.25	26.23	24.10	23.05	18.48	18.48	15.50	9.30
S.D	0.35	0.43	0.10	1.45	1.70	1.50	1.29	1.05
R-H	25.10	23.33	25.25	26.93	22.83	19.28	18.98	10.10
S.D	0.35	0.03	0.55	2.58	1.65	0.55	1.00	0.70
1:1	13.71	11.95	13.69	11.66	9.60	9.03	5.73	4.36
S.D	0.05	0.01	0.03	0.08	0.01	0.03	0.03	0.05
1:4	4.29	4.33	3.91	3.36	3.35	1.46	1.31	1.07
S.D	0.05	0.01	0.02	0.02	0.02	0.01	0.02	0.05
1:10	3.08	2.46	1.55	1.55	1.15			
S.D	0.02	0.03	0.04	0.02	0.02			
1:50	0.72	0.50	0.56	0.36	0.27			
S.D	0.05	0.02	0.02	0.03	0.03			

I = Complete formula, II = Without minerals, H = Heat, R = Room temperature, C = Cold, C-C = Corn cobs, G-C = Ground corn, R-H = Rice hulls, S.D = Standard Deviation.

* The data at 10 weeks and those for dilutions 1:10 and 1:50 at 12 - 16 weeks are not available because of instrument problems.

TABLE 4

DATA SUMMARY ON RIBOFLAVIN (MG/PER GRAM PREMIX)

	WEEK									
BATCH	0	3	5	7	9	11	13	15	17	27
I-H	0.625	0.630	0.613	0.440	0.373	0.403	0.365	0.375	0.300	0.165
S.D	0.012	0.001	0.007	0.007	0.010	0.008	0.008	0.010	0.005	0.014
I-R	0.738	0.583	0.605	0.590	0.385	0.413	0.440	0.365	0.378	0.395
S.D	0.003	0.003	0.005	0.005	0.010	0.013	0.010	0.008	0.003	0.003
I-C	0.708	0.590	0.600	0.608	0.433	0.535	0.535	0.393	0.458	0.423
S.D	0.018	0.022	0.003	0.008	0.008	0.008	0.008	0.005	0.013	0.003
II-H	0.733	0.605	0.563	0.455	0.470	0.480	0.430	0.388	0.310	0.360
S.D	0.018	0.003	0.008	0.013	0.003	0.005	0.015	0.005	0.005	0.013
II-R	0.583	0.543	0.538	0.465	0.460	0.360	0.315	0.308	0.285	0.313
S.D	0.020	0.043	0.003	0.007	0.003	0.010	0.018	0.003	0.013	0.003
II-C	0.665	0.648	0.465	0.443	0.495	0.413	0.370	0.393	0.398	0.360
S.D	0.015	0.023	0.005	0.018	0.008	0.003	0.003	0.008	0.013	0.005
C-C	0.615	0.543	0.543	0.453	0.438	0.403	0.438	0.360	0.358	0.355
S.D	0.013	0.023	0.010	0.017	0.012	0.008	0.015	0.005	0.003	0.010
G-C	0.703	0.598	0.610	0.580	0.400	0.435	0.433	0.365	0.338	0.329
S.D	0.017	0.043	0.015	0.005	0.007	0.007	0.003	0.005	0.007	0.003
R-H	0.728	0.710	0.620	0.590	0.443	0.478	0.450	0.413	0.360	0.385
S.D	0.010	0.030	0.013	0.010	0.013	0.003	0.008	0.003	0.003	0.003
1:1	0.340	0.275	0.256	0.271	0.251	0.241	0.209	0.191	0.211	0.178
S.D	0.008	0.014	0.002	0.004	0.003	0.003	0.001	0.001	0.001	0.004
1:4	0.144	0.113	0.068	0.096	0.095	0.089	0.091	0.081	0.080	
S.D	0.003	0.006	0.001	0.003	0.002	0.001	0.001	0.002	0.001	
1:10	0.113	0.114	0.081	0.106	0.105	0.068	0.046	0.045	0.038	
S.D	0.002	0.006	0.001	0.003	0.005	0.006	0.004	0.002	0.004	
1:50	0.011	0.011	0.010	0.010		0.011		0.011	0.009	0.009
S.D	0.001	0.002	0.001	0.001		0.001		0.001	0.002	0.001

I = Complete Formula, II = Without minerals, H = Heat, R.T. = Room Temperature, C = Cold, C-C = Corn Cobs, G-C = Ground Corn, R-H = Rice Hulls, S.D = Standard Deviation.

TABLE 5

DATA SUMMARY ON NIACIN (MG/PER GRAM PREMIX)

	WEEK									
BATCH	0	3	5	7	9	11	13	15	17	27
I-H	1.490	1.495	1.458	1.458	1.450	1.483	1.378	1.300	1.323	1.255
S.D	0.013	0.015	0.035	0.013	0.003	0.017	0.068	0.015	0.015	0.055
I-R	1.410	1.470	1.458	1.350	1.410	1.420	1.403	1.273	1.323	1.345
S.D	0.017	0.003	0.015	0.003	0.003	0.013	0.015	0.005	0.015	0.003
I-C	1.515	1.528	1.508	1.435	1.450	1.495	1.540	1.300	1.465	1.310
S.D	0.003	0.007	0.005	0.013	0.003	0.041	0.045	0.008	0.010	0.017
II-H	1.398	1.458	1.438	1.350	1.380	1.475	1.405	1.300	1.318	1.323
S.D	0.008	0.008	0.010	0.003	0.008	0.013	0.003	0.017	0.035	0.010
II-R	1.350	1.450	1.415	1.280	1.255	1.345	1.468	1.290	1.270	1.355
S.D	0.008	0.005	0.017	0.008	0.005	0.043	0.010	0.043	0.003	0.012
II-C	1.280	1.370	1.360	1.475	1.455	1.405	1.355	1.283	1.428	1.343
S.D	0.015	0.028	0.035	0.005	0.020	0.025	0.017	0.022	0.008	0.003
C-C	1.290	1.390	1.410	1.485	1.340	1.420	1.440	1.280	1.398	1.310
S.D	0.025	0.003	0.028	0.017	0.003	0.020	0.035	0.010	0.008	0.022
G-C	1.523	1.580	1.500	1.488	1.345	1.310	1.458	1.390	1.435	1.320
S.D	0.008	0.010	0.020	0.003	0.003	0.025	0.017	0.005	0.008	0.055
R-H	1.465	1.506	1.453	1.580	1.325	1.473	1.595	1.465	1.473	1.285
S.D	0.003	0.003	0.042	0.005	0.005	0.025	0.003	0.038	0.008	0.008
1:1	0.804	0.842	0.841	0.873	0.781	0.715	0.709	0.793	0.726	0.728
S.D	0.005	0.004	0.012	0.008	0.002	0.011	0.005	0.018	0.010	0.006
1:4	0.492	0.525	0.500	0.520	0.497	0.535	0.529			
S.D	0.003	0.002	0.002	0.001	0.001	0.013	0.008			
1:10	0.552	0.576	0.605	0.578	0.511	0.540	0.606	0.586	0.543	
S.D	0.008	0.007	0.015	0.001	0.006	0.001	0.018	0.003	0.031	

I = Complete Formula, II = Without Minerals, H = Heat,
R = Room Temperature, C = Cold, C-C = Corn Cobs,
G-C = Ground Corn, R-H = Rice Hulls, S.D = Standard Deviation.

TABLE 6

EFFECTS OF STORAGE TEMPERATURE AND MINERALS ON PREMIX VITAMIN A STABILITY

SLOPES OF LINEAR REGRESSION LINES		DUNCAN'S GROUPING[1]	PERCENT LOSS (16 WEEKS)	CORRELATION COEFFICIENT
BATCHES WITH MINERALS:				
HEAT[2]	-1.3088	E	67	0.917
R.T.[3]	-1.0572	C	55	0.931
COLD[4]	-0.9013	AB	42	0.891
BATCHES WITHOUT MINERALS:				
HEAT	-1.2059	DE	69	0.956
R.T.	-1.0855	CD	56	0.914
COLD	-0.9539	BC	55	0.915

1. Groupings containing the same letter are not significantly different at alpha = 0.05.
2. Samples were stored at 43° in a constant temperature incubator.
3. Samples were stored at room temperature (25 ± 3°C).
4. Samples were stored in a refrigerator at 1°C.

TABLE 7

EFFECTS OF STORAGE TEMPERATURE AND MINERALS ON PREMIX RIBOFLAVIN STABILITY

	SLOPES OF LINEAR REGRESSION LINES	DUNCAN'S GROUPING[1]	PERCENT LOSS (27 WEEKS)	CORRELATION COEFFICIENT
BATCHES WITH MINERALS:				
HEAT[2]	-0.0184	H	75.5	0.942
R.T.[3]	-0.0129	FG	53.8	0.785
COLD[4]	-0.0102	DE	42.6	0.799
BATCHES WITHOUT MINERALS:				
HEAT	-0.0138	G	58.3	0.857
R.T.	-0.0111	EF	54.3	0.866
COLD	-0.0112	EF	50.7	0.805

1. Groupings containing the same letter are not significantly different at alpha = 0.05.
2. Samples were stored at 43°C in a constant temperature incubator.
3. Samples were stored at room temperature (25 $\pm$ 3°).
4. Samples were stored in a refrigerator at 1°.

TABLE 8

EFFECTS OF STORAGE TEMPERATURE AND MINERALS ON PREMIX NIACIN STABILITY

	SLOPES OF LINEAR REGRESSION LINES	DUNCAN'S GROUPING[1]	PERCENT LOSS (27 WEEKS)	CORRELATION COEFFICIENT
BATCHES WITH MINERALS:				
HEAT[2]	-0.0102	DE	18.0	0.906
R.T.[3]	-0.0041	B	9.0	0.623
COLD[4]	-0.0076	C	13.3	0.692
BATCHES WITHOUT MINERALS:				
HEAT	-0.0047	B	8.8	0.601
R.T.	-0.0019	A	3.7	0.037
COLD	-0.0003	A	0.0	0.006

1. Groupings containing the same letter are not significantly different at alpha = 0.05.
2. Samples were stored at 43°C in a constant temperature incubator.
3. Samples were stored at room temperature (25 ± 3°).
4. Samples were stored in a refrigerator at 1°C.

TABLE 9

EFFECTS OF DILUENT TYPE ON
PREMIX VITAMIN A STABILITY

SLOPES OF LINEAR REGRESSION LINES		DUNCAN'S GROUPING[1]	PERCENT LOSS (16 WEEKS)	CORRELATION COEFFECIENT
SORGHUM	-1.057	C	56.0	0.931
CORN COBS	-0.791	A	39.8	0.865
CORN	-0.930	AB	52.1	0.930
RICE HULLS	-0.769	A	43.6	0.828

1. Groupings containing the same letter are not significantly different at alpha = 0.05.

TWO-WAY ANALYSIS OF VARIANCE
WITH STORAGE TIME (W) AND TYPE OF DILUENT (D)
AS SOURCES OF VARIANCE IN VITAMIN A STABILITY

SV	DF	SS	F	P-value
WEEKS	7	2480.83	328.75	0.0001
DILUENTS	3	323.88	100.15	0.0001
W * D	21	249.30	11.01	0.0001
ERROR	64	68.99		

TABLE 10

EFFECTS OF DILUENT TYPE ON
PREMIX RIBOFLAVIN STABILITY

SLOPES OF LINEAR REGRESSION LINES		DUNCAN'S GROUPING[1]	PERCENT LOSS (27 WEEKS)	CORRELATION COEFFICIENT
SORGHUM	-0.0129	G	53.8	0.785
CORN COBS	-0.0080	CD	37.8	0.878
CORN	-0.0140	G	58.7	0.884
RICE HULLS	-0.0144	G	56.3	0.870

1. Groupings containing the same letter are not significantly different at alpha = 0.05.

TWO-WAY ANALYSIS OF VARIANCE
WITH STORAGE TIME (W) AND TYPE OF DILUENTS (D)
AS SOURCES OF VARIANCE IN RIBOFLAVIN STABILITY

SV	DF	SS	F	P-value
WEEKS	9	1.479	151.66	0.0001
DILUENTS	3	0.0367	11.29	0.0001
W * D	27	0.168	5.75	0.0001
ERROR	80	0.0867		

TABLE 11

EFFECTS OF DILUENT TYPE ON
PREMIX NIACIN STABILITY

SLOPES OF LINEAR REGRESSION LINES		DUNCAN'S GROUPING[1]	PERCENT LOSS (27 WEEKS)	CORRELATION COEFFICIENT
SORGHUM	-0.0041	B	9.0	0.623
CORN COBS	-0.0017	A	3.3	0.190
CORN	-0.0081	CD	14.2	0.701
RICE HULLS	-0.0063	BC	11.0	0.443

1. Groupings containing the same letter are not significantly different at alpha = 0.05.

TWO-WAY ANALYSIS OF VARIANCE
WITH STORAGE TIME (W) AND TYPE OF DILUENT (D)
AS SOURCES OF VARIANCE IN NIACIN STABILITY

SV	DF	SS	F	P-value
WEEKS	9	0.3670	30.15	0.0001
DILUENTS	3	0.1555	38.33	0.0001
W * D	27	0.3516	9.63	0.0001
ERROR	80	0.1082		

TABLE 12

EFFECTS OF DEGREE OF DILUTION
ON PREMIX VITAMIN A STABILITY

DEGREE OF DILUTION[1]	LINEAR REGRESSION EQUATIONS	CORRELATION COEFFICIENT
1:0	-1.057X + 30.21	0.931
1:1	-0.567X + 14.38	0.947
1:4	-0.232X + 9.65	0.974
1:10	-0.239X + 3.20	0.955
1:50	-0.052X + 0.69	0.938

1. Prepared on the basic of 1 part of complete premix to 0, 1, 4, 10, or 50 part of diluent.

TABLE 13

EFFECTS OF DEGREE OF DILUTION
ON PREMIX RIBOFLAVIN STABILITY

DEGREE OF DILUTION[1]	LINEAR REGRESSION EQUATIONS	CORRELATION COEFFICIENT
1:0	-0.0129X + 0.647	0.785
1:1	-0.0055X + 0.301	0.899
1:4	-0.0042X + 0.320	0.711
1:10	-0.0058X + 0.066	0.890
1:50	-0.000067X+0.011	0.689

1. Prepared on the basic of 1 part of complete premix to 0, 1, 4, 10, or 50.

TABLE 14

EFFECTS OF DEGREE OF DILUTION
ON PREMIX NIACIN STABILITY

DEGREE OF DILUTION[1]	LINEAR REGRESSION EQUATIONS	CORRELATION COEFFICIENT
1:0	-0.0041X + 1.44	0.623
1:1	-0.0049X + 0.83	0.654
1:4	-0.0022X + 0.50	0.587
1:10	-0.0003X + 0.57	0.048

1. Prepared on the basic of 1 part of complete premix to 0, 1, 4, 10, or 50.

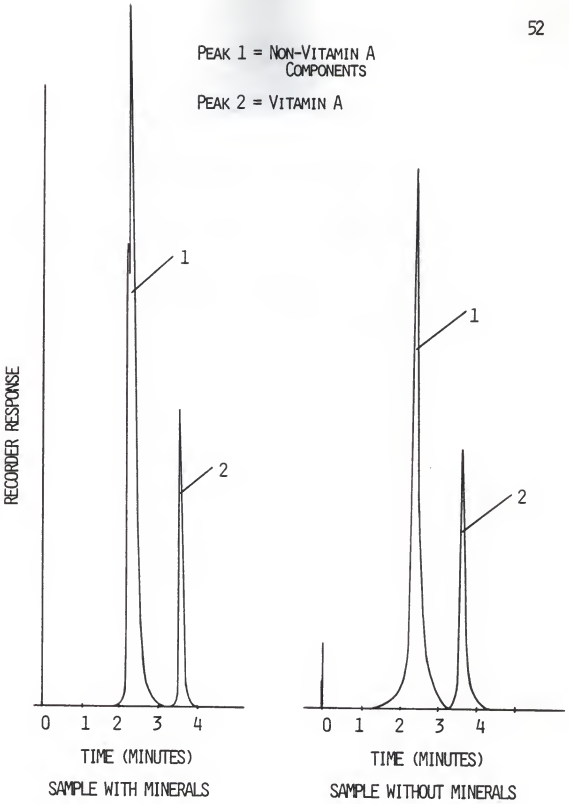

FIGURE 1. VITAMIN A CHROMATOGRAMS

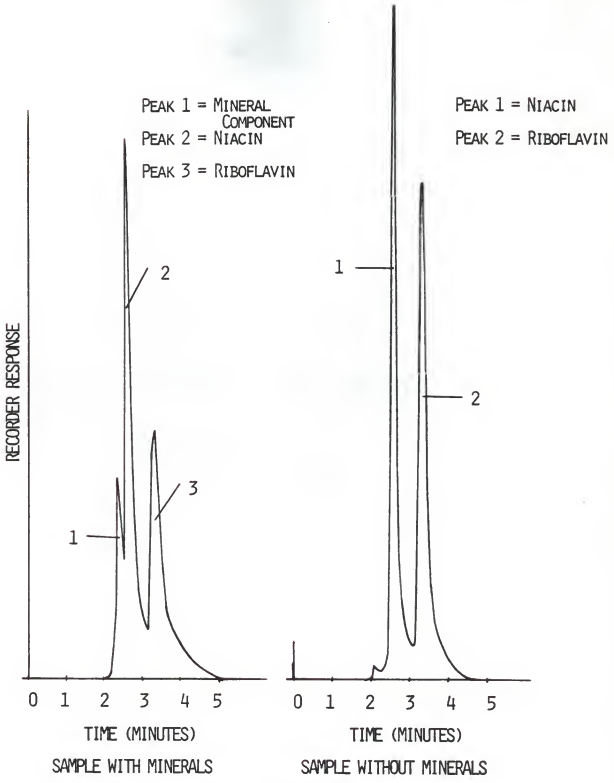

FIGURE 2. RIBOFLAVIN AND NIACIN CHROMATOGRAMS

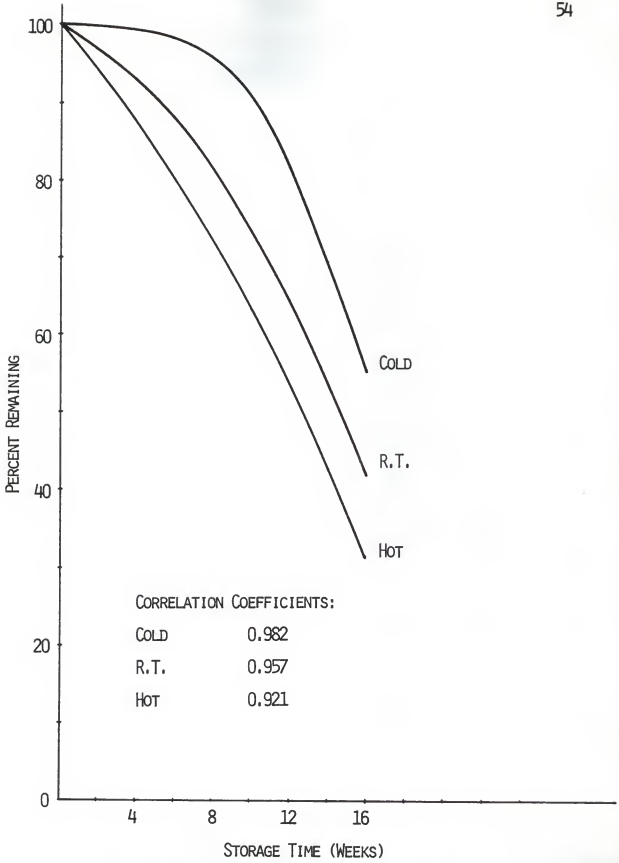

FIGURE 3. SECOND ORDER REGRESSION LINES OF THE EFFECTS OF STORAGE TEMPERATURE AND MINERALS ON VITAMIN A

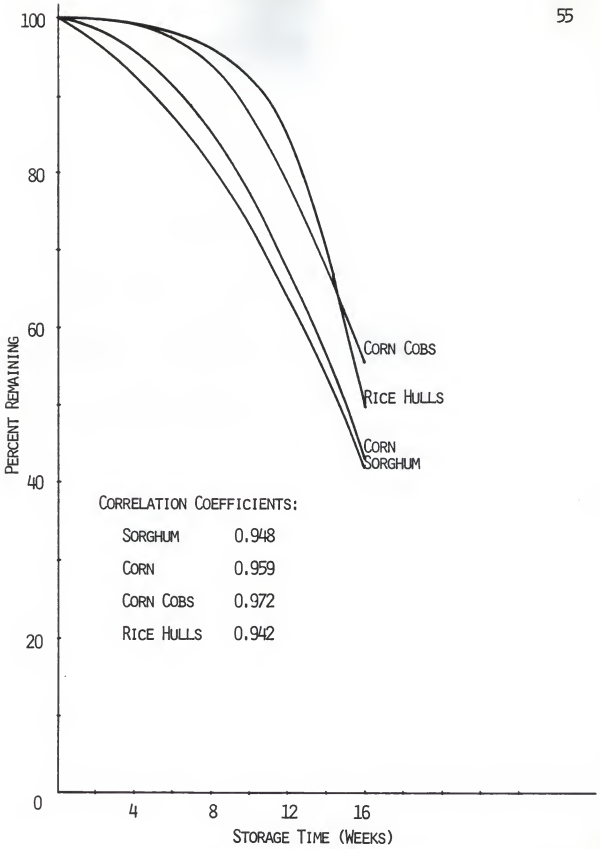

FIGURE 4. SECOND ORDER REGRESSION LINES OF THE EFFECTS OF DILUENT TYPE ON VITAMIN A

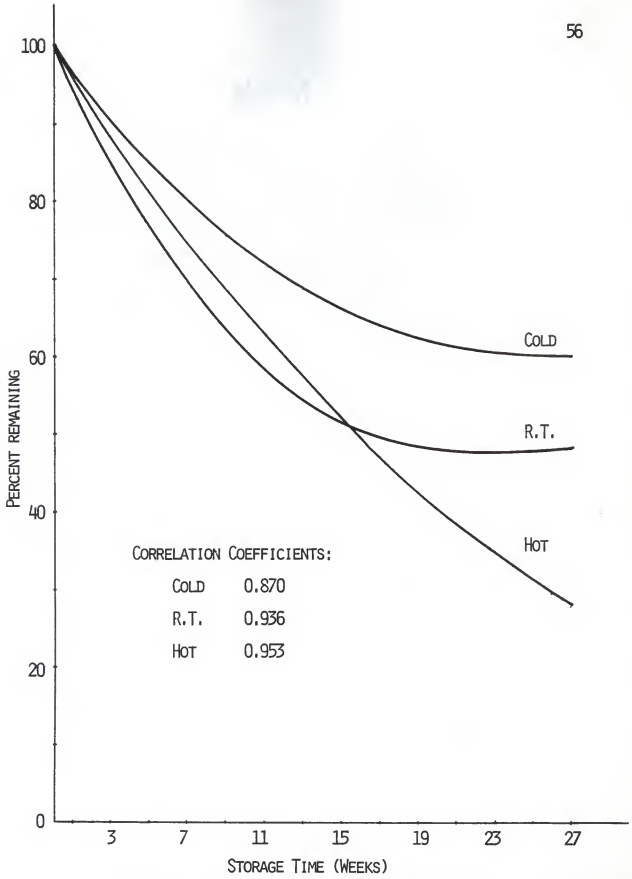

FIGURE 5. SECOND ORDER REGRESSION LINES OF THE EFFECTS OF STORAGE TEMPERATURE AND MINERALS ON RIBOFLAVIN

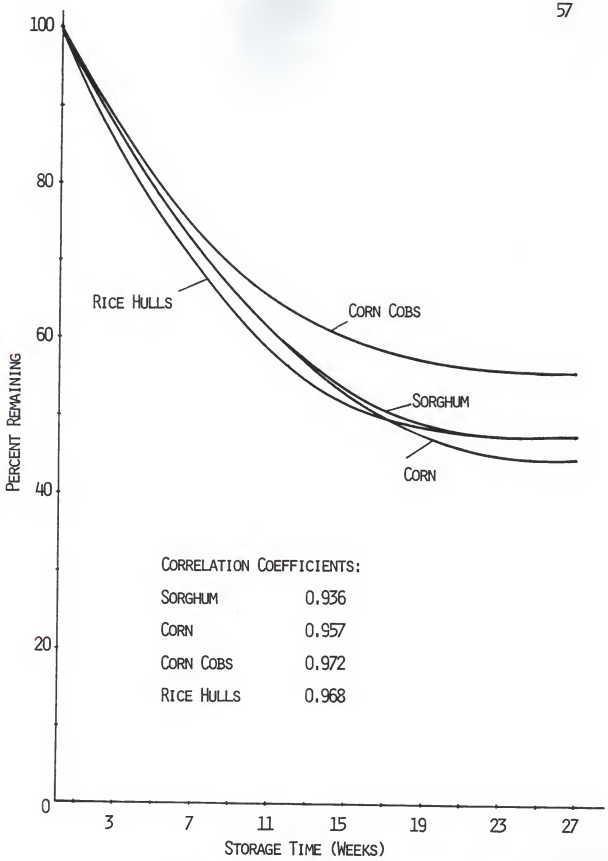

FIGURE 6. SECOND ORDER REGRESSION LINES OF THE EFFECTS OF DILUENT TYPE ON RIBOFLAVIN

FACTORS AFFECTING STORAGE STABILITY OF VITAMIN A, RIBOFLAVIN AND NIACIN IN A BROILER DIET PREMIX

by

QIANG ZHUGE

B. S., WUXI LIGHT INDUSTRY COLLEGE, CHINA, 1966

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The effects of minerals, temperature, and nature and degree of diluents on the stabilities of vitamin A, riboflavin and niacin in a broiler premix were studied over a 16 to 27-week storage period.

Vitamin A was measured during a 16-week period. It was the least stable among the three vitamins. Presence or absence of minerals did not make a significant difference on the stability of vitamin A in the broiler premix. Higher storage temperatures increased the rate of vitamin A destruction. Destruction of vitamin A was greatest when ground sorghum was used as a diluent and least when the diluent was ground rice hulls. Stability of vitamin A in the premix appeared to increase as the degree of dilution increased.

After 27 weeks, Riboflavin data indicated that the presence of trace minerals in the premix adversely affected riboflavin stability. Higher storage temperatures accelerated riboflavin loss. Type of diluent used appears to be less significant in riboflavin stability than with vitamin A. Corn cobs were the best diluent for riboflavin, with no significant difference among ground corn, ground sorghum and rice hulls. The vitamin appeared to be most stable in the most highly diluted premix.

Niacin was considerably more stable than riboflavin or vitamin A, but the presence of minerals and high temperature did significantly affect stability of niacin. At room temperature or lower there was no loss of niacin in samples prepared without minerals even after 27 weeks. Ground corn cobs were the best diluent with respect to niacin stability, which was also better at higher dilution level.