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FACTORS AFFECTING THE STABILITY OF THE VITAMIN A FROM COD LIVER OIL IN CEREAL FEEDS¹

A. W. HALVERSON² AND E. B. HART

*Department of Biochemistry, College of Agriculture,
University of Wisconsin, Madison*

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Numerous investigators have studied the losses of vitamin A in foods and feeds containing added cod liver oil. Marcus ('31) showed that vitamin A is unstable when added to finely divided solids. Approximately 85% of the vitamin, as measured by the Carr-Price reaction, was lost in 10 days when the unsaponifiable fraction was added to a vitamin A-free rat ration (U.S.P. basal diet) or to granulated lactose. No difference in vitamin A destruction was noted between storage under air or carbon dioxide. Fraps and Kemmerer ('37) reported that 79 to 100% of the vitamin A (cod liver oils) added to white corn meal was destroyed after 4 weeks' storage in the presence of air at either 7 or 28°C. They determined the vitamin A content of the stored samples by the spectrographic method.

Holder and Ford ('39) and Bethke et al. ('39) measured vitamin A losses, in a mixed ration containing added cod liver oil, by the chick assay method.³ The ration was stored in burlap sacks at room temperature (21 to 27°C.). Holder and Ford found no loss of vitamin A during storage periods of 8

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² Present address: Experiment Station Chemistry Department, South Dakota State College, Brookings.

³ Growth rate, deficiency symptoms and mortality records were used as criteria for comparison between groups of chicks.

weeks or less but were able to demonstrate a slight loss in a sample which was stored for 10 weeks. Bethke et al. observed that about 50% of the vitamin A activity of cod liver oil was still present in the ration after 6 months' storage. They further showed that the rate of destruction of vitamin A was approximately doubled by substituting meat scraps and dried skim milk for the casein component of the ration.

Thus, the data in the literature show ample proof of the instability of the vitamin A of cod liver oil in feeds and rations. However, the factors which retard or prevent the destruction noted have not been adequately investigated, and no explanations for the observed discrepancies are evident.

It was shown earlier in this laboratory that the carotene of dehydrated alfalfa can be effectively stabilized by sealing (in air) if the moisture content of the enclosed alfalfa is adjusted to 8 to 10% (Halverson and Hart, '48). Successful experiments with carotene added to mixed rations as alfalfa meal (15%) were also carried out. The observed stabilization was shown to be dependent upon the ability of the sealed alfalfa and cereal products to use up quickly the enclosed oxygen by oxidative reactions (Bailey and Gurjar, '20; Bailey, '40; Snow and Wright, '45; Halverson and Hart, '47).

Previous work in this laboratory has shown that the addition of free trace minerals (Fe, Cu, Co and Mn) to mixed rations containing alfalfa meal has no effect upon the observed carotene preservation that is obtained with feeds sealed with moisture contents of 8 to 10% (Halverson and Hart, '48). Also, the expected rancidity and bleaching changes which normally develop in such rations are completely prevented by sealing the stored feeds.

EXPERIMENTAL

Experiments were introduced to determine whether the vitamin A of cod liver oil is stabilized as effectively as is the carotene of alfalfa in feeds stored in gas-tight containers. Measured levels of cod liver oil were added to a mixed ration and to ground white corn samples which were stored under

sealed and unsealed conditions and at different moisture levels (3.2 to 15%). A commercial cod liver oil which contained approximately 290 μg of vitamin A alcohol per gram was employed throughout the studies. The percentage composition of the mixed ration follows: white corn, 30; wheat middlings, 20; wheat bran, 10; soybean meal, 20; oats, 16; CaCO_3 , 2; $\text{Ca}_3(\text{PO}_4)_2$, 1; and iodized salt, 1. Cod liver oil was added to this ration at levels of 0.5, 1 and 2%. All of the mixed ration samples were stored at 33 to 36°C. The effect of different storage temperatures upon vitamin A stability under sealed conditions and at different moisture levels was studied in an experiment with ground white corn which contained 1% of added cod liver oil. The white corn samples were prepared in duplicate to enable storage at two temperatures (22 to 25°C. and 33 to 36°C.). After 2.5 months or more of storage, the mixed ration and white corn samples were opened and analyzed for vitamin A and the percentage loss in the different samples was calculated.

In order to facilitate convenient adjustment of the moisture content of feeds, they were initially dried for approximately two hours at 95°C. prior to addition of the cod liver oil. Weighed portions of cod liver oil were then mixed into the dried feeds after they had been allowed to cool to room temperature. The feeds were then stored in glass chambers until moisture analyses could be made. The moisture content of portions of the feed samples containing added cod liver oil was then adjusted to definite levels and the samples were placed in pint-size ice cream cartons (3 \times 4 inches). The feeds were firmly packed into the containers by hand. The filled cartons, which contained 300 to 350 gm of feed apiece, were then thoroughly coated with a commercial wax⁴ by intermittent dipping in the melted wax.

The respiratory activity of the mixed ration containing no added trace minerals was determined at different moisture

⁴ The Flexowax employed in these studies was obtained from the Glyco Products Company, Inc., Brooklyn, New York. This wax melts at 52°C., is fairly flexible and does not readily crack.

levels (7.5 to 20%). The mixed ration employed was of the same composition as that used in the vitamin A stabilization studies except that no cod liver oil was added. One-hundred-gram samples of mixed ration (dry basis) were adjusted to the proper moisture content and then enclosed in gas-tight glass receptacles. The samples were stored in the dark for 5 days at 22 to 25°C. The changes in composition of the enclosed air were followed by determining the carbon dioxide and oxygen contents of the enclosed atmosphere at the end of the storage period. These contents were expressed as volume per cent of the enclosed gas. A detailed description of the apparatus and techniques employed in determining the effect of moisture level upon the respiratory activity of feed samples is given by Halverson ('49).

Since small amounts of both the trace minerals and vitamin A (fish liver oils) are commonly added to commercial poultry and certain livestock feeds, an investigation of the effect of these minerals upon the destruction of the added vitamin A in different feeds seemed desirable. A mixed ration containing added trace minerals and cod liver oil was stored under sealed and unsealed conditions and at different moisture levels for 10.5 months at 33 to 36°C. This ration was of the same composition as the mixed ration already described except for the presence of the added trace minerals. The following minerals were added in grams per 100 gm of ration: $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, 0.02; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.02; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.02; and $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.25. The minerals were finely ground in a mortar before being mixed into the ration.

An additional experiment was carried out to obtain further information about the effect of the trace minerals upon the rate of vitamin A destruction. In this instance ground white corn samples containing added cod liver oil were stored in contact with air (in paper sacks) and at 36 to 39°C. Vitamin A analyses were made at 0-, 3-, 6-, 12- and 24-day intervals and the percentage loss in the different samples was calculated.

In the same experiment an attempt was also made to find a convenient way to prevent the trace minerals from coming into

intimate contact with the feed as well as the added vitamin A. The idea of dissolving the minerals in gelatin seemed worthwhile; therefore, a gelatin water solution was prepared by mixing two parts of hot water containing the dissolved trace minerals with one part of gelatin flakes. After the heated solution was thoroughly mixed, it was allowed to cool and then dried in a vacuum desiccator at room temperature for 24 hours. After an additional drying period of 36 hours at 50°C. in a vacuum oven, the gelatin-mineral mixture was dry.

The same kind and amount of minerals were employed with the white corn as with the mixed ration experiment. The following amounts of the trace minerals in grams were dissolved in 2 gm of gelatin: $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, 0.02; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.02; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.02; and $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.25. Thus, the addition of the above amount of minerals to 100 gm of ground white corn involved the addition of 2 gm of carrier gelatin. The dried gelatin-mineral mix was ground in a Wiley mill, using a 2 mm sieve. In order to obtain uniform particles of different size, sieves were used to obtain two major fractions — one fraction passing through an 0.85 mm square mesh sieve and the other through a 1.5 mm round mesh but not through an 0.85 mm square mesh sieve. Equivalent amounts of gelatin were added to all of the corn-cod liver oil samples, whether the additions were made as the gelatin-mineral mix or merely as gelatin flakes and whether they were added to samples containing no added trace minerals or the trace minerals added in free form (unprotected).

Vitamin A analysis

The method of vitamin A analysis employed was essentially that described by The Association of Vitamin Chemists, Inc. ('47). The vitamin A was extracted from the samples with ether. After saponification the washed ether extract was freed of excess water by shaking with portions of saturated aqueous NaCl solution, rather than by treatment with anhydrous Na_2SO_4 . The blue color of the Carr-Price reaction was meas-

ured with the Evelyn colorimeter and calculations were made following calibration of the instrument with the U.S.P. Vitamin A Reference Standard, 1948.

During the latter phases of the study, a chromatographic purification was included in the analytical procedure to remove further the colored pigments present in the saponified extract. In the mixed feed analysis, a high blank reading was always obtained with samples containing no added vitamin A. The error introduced by blank corrections was not too great, but the possibility that foreign substances or pigments might be inhibiting color development in the antimony trichloride reaction was always apparent. Any procedure capable of reducing the blank reading and, thereby, removing impurities would thus give additional information to substantiate or disprove the results.

The chromatographic column employed consisted of a mixture of 5 parts of Hyflo Super-Cel⁵ and 3 parts of lime.⁶ The column, which was 19.4 mm in diameter by 10 to 11 cm long, was firmly packed under partial vacuum and covered with a 1- to 2-cm layer of anhydrous Na₂SO₄. This column gave recoveries for pure vitamin A (saponified) of 95 to 97% and for vitamin A added to feed extracts of 90 to 95% under the conditions employed in the experiments. Thus, it was concluded that any inhibitory substances that might originally have been present were removed by the column, since good recoveries were obtained even in the presence of extracts from the mixed ration. Since the blank readings were also reduced to negligible values, it was possible to make the calculations with reasonable confidence that the blank corrections were not introducing a significant degree of error.

The procedure employed for the chromatographic determinations was as follows. The purified unsaponifiable extract was dissolved in petroleum ether (distilled Skelly A) by evaporating off the ether solvent and replacing it with Skelly A. After the adsorption column was completely wet with Skelly

⁵ Johns-Manville, Chicago, Ill.

⁶ Grand Prize Hydrated Finishing Lime, United States Gypsum Co.

A, the vitamin A extract was poured onto the column and suction was applied. The adsorbed vitamin A was eluted immediately with 200 ml of a solution of 3% acetone in Skelly A. The entire eluant, together with the extract solvent, was collected in a single fraction. After the Skelly A solvent was evaporated off, the chromatographed residue was dissolved in a measured volume of dry distilled chloroform, and then colorimetric determinations (Carr-Price) were made by the standard procedure.

The mixed ration analyses were performed by the standard Carr-Price method as described by The Association of Vitamin Chemists, Inc. ('47), as well as by the extended chromatographic procedure. The two methods gave values which agreed with surprising uniformity, the reported values being obtained by the standard method. The samples reported in tables 2 and 4 were analyzed only by the extended chromatographic procedure. All samples were analyzed in duplicate and the reported values represent the average of the two analyses. Good agreement was obtained between the duplicate analyses, for the average variation between different analyses of the same sample was generally not greater than 10%.

RESULTS

The data show that sealing to prevent the access of oxygen as well as to permit control of the moisture level had little effect upon the retention of the vitamin A of cod liver oil added to a mixed ration or ground white corn samples. The losses for the mixed ration are shown in table 1 and those for the white corn in table 2. Samples sealed with high moisture levels (10 to 15%) showed no greater preservation during storage than those at lower moisture levels (3.3 to 5%) or even unsealed samples. Thus, it is obvious that the absence of oxygen as induced by respiratory action (table 3) at the higher moisture levels was not the primary factor in vitamin A preservation in the samples studied.

Significant losses of vitamin A occurred during storage under sealed and unsealed conditions and at different moisture

levels. While slightly greater losses were apparent in the unsealed than in the sealed mixed ration samples when the storage period was 9 to 10.5 months at 33 to 36°C., no definite

TABLE 1

Vitamin A losses in a mixed ration sealed at different moisture¹ levels and with and without added trace minerals. (Storage temperature 33-36°C.)

DESCRIPTION OF SAMPLE	WATER CONTENT	NO ADDED TRACE MINERALS			ADDED TRACE MINERALS
		2.5 mos. ¹	3 mos. ²	9 mos. ³	10.5 mos. ⁴
	%	% loss	% loss	% loss	% loss
Unsealed	5.1	44	38	92	100
Sealed	3.3	34	36	54	90
Sealed	5.0	34	36	66	92
Sealed	7.5	44	44	73	91
Sealed	10.0	36	37	73	90
Sealed	12.5	41	33	..	91
Sealed	15.0	31	47	..	89

¹ One per cent of cod liver oil in ration (290 µg of vitamin A alcohol per gram of cod liver oil).

² Five-tenths per cent of cod liver oil in ration.

³ Two per cent of cod liver oil in ration.

⁴ Five-tenths per cent of cod liver oil in ration containing the following percentages of trace minerals: FeCl₂·4H₂O, 0.02; CuSO₄·5H₂O, 0.02; CoCl₂·6H₂O, 0.02; and MnSO₄·H₂O, 0.25.

TABLE 2

Effect of different storage temperatures upon vitamin A losses in stored white corn sealed at different moisture levels¹ (8-month storage period)

DESCRIPTION OF SAMPLE	WATER CONTENT	PER CENT OF LOSS AT	
		22-25°C.	33-36°C.
	%		
Unsealed	7.1, 5.0	26	39
Sealed	3.2	20	22
Sealed	5.0	20	26
Sealed	7.5	17	28
Sealed	10.0	18	26
Sealed	12.5	25	39
Sealed	15.0	30	46

¹ One per cent of cod liver oil added to the ground white corn samples (290 µg of vitamin A alcohol per gram of cod liver oil).

differences in preservation could be credited to sealing with either the white corn samples after 8 months' storage at two different temperatures or the mixed ration samples after 2.5 to 3 months' storage at 33 to 36°C. Vitamin A losses were greater in the sealed corn samples which contained 12.5 and 15% of moisture than in those sealed with lower water content.

The extent of the vitamin A losses appeared to be dependent upon the sample composition as well as the length and temperature of storage, rather than upon sealing and control of the moisture level of the sealed samples. Losses with the mixed ration containing added cod liver oil were more rapid than

TABLE 3

Effects of moisture content upon carbon dioxide production and oxygen consumption by the mixed ration containing no added trace minerals.¹ (Stored for 5 days at 22 to 25°C.)

MOISTURE CONTENT OF SAMPLE	CO ₂	O ₂
%	%	%
7.5	0.2	19.5
10.0	1.0	18.9
12.5	2.5	14.7
15.0	4.3	5.4
20.0	11.8	0.2

¹ All samples initially sealed in air.

those observed with white corn; slightly greater losses resulted with the former in 2.5 to 3 months than with the latter in 8 months' storage. Larger losses of vitamin A were also observed in mixed ration samples stored for 9 months than were found after 2.5 to 3 months of storage under either sealed or unsealed conditions (table 1). The higher storage temperature employed in the white corn studies shown in table 2 similarly resulted in an accelerated rate of vitamin A destruction in both the sealed and unsealed samples.

Most of the vitamin A was destroyed in the mixed ration during 9 to 10.5 months' storage under sealed and unsealed conditions, and with and without the added trace minerals

(table 1). Because the loss was so extensive in the sample without added trace minerals (Fe, Cu, Co and Mn) when the storage period was 9 months, it is obvious that a shorter period of storage is essential for studying the assumed capacity of the trace minerals to accelerate vitamin A destruction in sealed samples.

The ability of the trace minerals to accelerate the destruction of vitamin A in unsealed samples was demonstrated by the results shown in table 4. Almost complete vitamin A de-

TABLE 4
Vitamin A losses in stored white corn (unsealed) containing the trace minerals added in free form and in a dried gelatin-mineral preparation.¹
(Storage temperature 36–39°C.)

DESCRIPTION OF SAMPLE	% LOSS DURING STORAGE PERIODS OF			
	3 days	6 days	12 days	24 days
Sample (corn + vitamin A) + gelatin flakes	0	0	24	37
Sample + coarse gelatin- mineral mix	1.2	13	25	40
Sample + fine gelatin- mineral mix	1.6	14	39	56
Sample + gelatin flakes + free trace minerals	76	92	95	98

¹ One per cent of cod liver oil added to the ground white corn samples (290 µg of vitamin A alcohol per gram of cod liver oil).

struction occurred in 6 days in a white corn-cod liver oil sample which contained added trace minerals. The samples reported in table 4 were stored in the presence of air (in paper sacks) at 36 to 39°C. The stabilizing effect of adding the minerals (Fe, Cu, Co and Mn) in the form of a dried gelatin preparation was apparent. The vitamin A loss for the sample containing the coarse gelatin-mineral mix was not significantly greater than for the sample which contained no added trace minerals. While the observed loss in the sample containing the fine gelatin-mineral mix was more rapid than in the coarse gelatin-mineral sample, the preservation obtained with the

former was still very pronounced when compared with the sample which contained the trace minerals in free form (unprotected).

The white corn sample containing the trace minerals in free form developed a rancid odor as well as a yellow gold color within a few days of storage at 36 to 39°C. No changes in odor or color were evident in the corn plus added vitamin A samples which contained either no added trace minerals or else the minerals added as the coarse gelatin-mineral mix. Very slight rancidity and color change were evident in the corn sample containing the fine gelatin-mineral mix after 24 days' storage.

DISCUSSION

The studies reported indicate that vitamin A losses in a mixed ration and white corn samples containing added cod liver oil are not affected by the presence of gaseous oxygen. The fact that the type of feed employed (mixed ration versus white corn) affected the rate of vitamin A loss, while sealing did not, indicates that the presence or absence of undefined constituents in a feed has more effect upon vitamin A destruction than does the presence of oxygen. It is interesting that sealing in gas-tight containers prevented the development of rancidity (color and odor changes) and yet had no effect upon vitamin A losses.

It was notable that the rapid vitamin A destruction and rancidity development in the white corn sample containing added free trace minerals (Fe, Cu, Co and Mn) were largely prevented when the minerals were added in the form of a gelatin-mineral mix rather than in free form. Thus it is apparent that such minerals can be added to rations in an available form by which the usual destructive catalysis of fats, vitamin A and other similar compounds is almost completely prevented. The ratio of gelatin to minerals employed in the study was about 6.5 to 1.

The results with the mixed ration (unsealed and without added trace minerals) are in essential agreement with those obtained by Holder and Ford ('39) and Bethke et al. ('39), who measured storage losses of vitamin A in a mixed ration

by the chick assay method. These investigators observed slow losses of vitamin A during the extended storage (in open sacks) of mixed rations which contained no added trace minerals. They were unable to obtain the rapid losses noted by Fraps and Kemmerer ('37) in spectrographic studies with white corn containing added cod liver oil.

SUMMARY

1. The vitamin A in a mixed ration and ground white corn samples containing added cod liver oil was not stabilized by storage in sealed containers (gas-tight) at different moisture levels. The removal of enclosed oxygen by respiration of the feed samples at higher moisture levels (10 to 15%) showed no demonstrable effect upon vitamin A preservation. Comparable vitamin A losses occurred in sealed and unsealed samples under similar storage conditions. Vitamin A losses in both sealed and unsealed samples increased with temperature and length of storage.

2. Significant amounts of the added vitamin A in the mixed ration and white corn samples (without added trace minerals) were retained after several months' storage. Approximately 60% of the initial vitamin A content of the mixed ration was still present in samples stored in sealed and unsealed containers for 2.5 to 3 months at 33 to 36°C. The vitamin A of cod liver oil was more stable in ground white corn than in the mixed ration, under sealed and unsealed conditions and at similar storage temperatures.

3. The extremely rapid destruction of vitamin A induced by adding trace minerals (Fe, Cu, Co and Mn) to an unsealed white corn sample containing added cod liver oil was largely prevented by adding the minerals in a dried gelatin-mineral mixture rather than in free form. The experiments did not determine whether the free trace minerals are capable of accelerating vitamin A destruction in feeds stored in an atmosphere free of oxygen.

4. The effective prevention of rancidity and vitamin A destruction in practical poultry rations and certain livestock

feeds which commonly contain small amounts of added (free) trace minerals is an important problem. The present study indicates that a practical solution of the problem depends upon the addition of the trace minerals in a form which limits their ability to come in contact and react with other constituents of the ration.

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