A STUDY OF THE STABILITY OF VITAMIN A IN VARIOUS COD LIVER OIL - FEED MIXTURES

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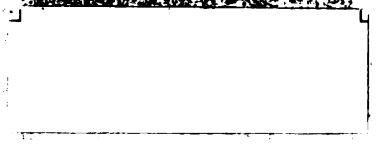

A STUDY OF THE STABILITY OF VITAMIN A IN VARIOUS COD LIVER OIL -- FEED MIXTURES

by

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Introduction

With the increase in the knowledge of vitamins during the past few years, greater emphasis has been placed on their importance in nutrition. In many cases vitamin supplements such as concentrates from natural sources or even synthetic preparations are added to rations to insure an adequate supply in the diet. As an example, cod liver oil is widely used to increase the vitamin A and D potency of mixed feeds. In the case of vitamin A, this type of fortification has not been entirely satisfactory because of the instability of vitamin A under practical conditions of use.

Several workers have shown that vitamin A is destroyed during storage. Holmes, Corbet, and Hartzler (9) found that the vitamin A of halibut liver oil was completely destroyed in 10 hours when kept at 96° under an atmosphere of oxygen. They also found that the vitamin A of halibut liver oil was completely destroyed in 21 weeks when 10 cc. of the oil was kept in a 40 cc. bottle in diffused light at room temperature. Under these same conditions, the vitamin A of cod liver oil was destroyed in 6 weeks. Lowen, Anderson, and Harrison (10) found that the vitamin A of salmon oil decreased from 269 BU/gm. to 160 BU/gm. or 40% in 35 days when 50 cc. samples were stored in 125 cc. Erlenmeyer flasks exposed to air at room temperature in diffuse winter light. The vitamin A of halibut liver oil under these conditions decreased 84% in 35 days. They also found that 30 cc.

samples of freshly prepared halibut liver oil stored in 50 cc. Erlenmeyer flasks exposed to air at room temperature in diffuse spring light lost 96% of the vitamin A in 31 days. But Ewe (5) found that samples of cod liver oil under conditions of commercial distribution did not lose any appreciable amount of vitamin A even after they had been stored 4 years.

Other workers have shown that vitamin A added to feeds is destroyed to a greater extent that the vitamin A which is present in the feed as precursors such as the carotenes. Dunn (4), in 1924, noted that when cod liver oil was mixed with starch and stored in stoppered bottles in the dark for 6 months, it lost its anti-xerophthalmic properties. Marcus (11) observed that when cod liver oil contentrate was mixed with finely ground compounds such as ferric oxide, magnesium oxide, calcium carbonate, lactose and others, the vitamin A was destroyed on standing. He also found that the vitamin A of a mixture of cod liver oil and the USP vitamin A deficient ration was destroyed to the extent of 85% in 10 days. Fraps and Kemmerer (6) found that when fish oils or their concentrates were added to feed mixtures, 79 - 100% of the vitamin A disappeared after 4 weeks of storage. Bethke, Record and Wilder (3) noted that not over 50% of the added vitamin A from cod liver oil was destroyed in a casein ration in 6 months. However, in a similar ration containing meat scraps and dried milk in the place of casein, approximately 75% of the vitamin A added was lost in 6 months. Holder and Ford (7) mixed cod liver oil with a vitamin A-deficient ration and stored it for 60 days. On eight-week tests with chicks, no loss of vitamin A was detected; however, there was an indication of some loss when the test period was extended to ten

weeks. Baird, Ringrose and MacMillan (2) reported that Vitamin A from fortified cod liver oil, when mixed in the ration to provide respectively 100, 150, 200, and 300 units of vitamin A per 100 grams ration, did not evidence complete destruction even when the rations were stored in burlap bags at summer temperatures for 25 weeks, although it underwent progressive destruction as the storage period advanced.

During some feeding experiments with a commercial dog food, it was observed that the vitamin A which had been added to the feed during its manufacture could not be accounted for several weeks later. Chemical tests of the feed showed that the vitamin A had been destroyed. In view of the fact that it could be expected that various ingredients of feed mixtures might show different effects toward the stability of vitamin A, investigation was carried out to determine, if possible, whether certain ones might be particularly well suited to preserve the vitamin A potency.

Experimental

The experimental procedure consisted of adding certain amounts of cod liver oil to different feeds. The oil and feed were mixed by hand and stored in cloth bags in laboratory lockers. At monthly intervals, samples were take out and the vitamin A determined by the following method:

1. Twenty grams of the sample was extracted with ethyl ether in a Soxhlet extractor for a minimum of three hours. Abernathy (1) reported that an extraction period of two hours was sufficient to extract all the vitamin A which was added to a feed. However, we found a minimum extraction period of 3 hours gave the most satisfactory results.

2. The ether was then evaporated from the extract by use of a warm water bath and reduced pressure.

3. The residue was saponified with 10-15 cc. of saturated alcoholic KOH on a steam bath. It was then cooled and extracted twice with 100 cc. portions of ethyl ether. The ether extract was washed with water until free from alkali when tested with phenolphthalein.

4. The ether extract was then dried overnight using anhydrous Na_2SO_4. The solution was filtered and the ether evaporated off as before.

5. The residue was taken up in a suitable volume of chloroform. Two-tenths cc. of the chloroform solution was mixed with 2 cc. of a saturated solution of $SbCl_3$ in $CHCl_3$ in a 1 cm. cell and the color determined immediately with a Lovibond tintometer. The results were calculated as Lovibond blue units per gram.

In order to test the dependability of the method, weighed amounts of cod liver oil were analysed by the above method and the results compared with those obtained by the direct determination of vitamin A in the chloroform solution of the oil. The results are given in Table I.

Table I

Dependability of Method for the Determination of Vitamin A

Sample	Clo.* gms.	BU/gm. **
C. l. o. analysed by above method	0.92	1195
C. l. o. in $CHCl_3$ (direct determination)	1.27	1117

* Clo. A commercial medicinal cod liver oil
** BU/gm. Lovibond Blue Units per gram of oil

The difference in the values obtained is probably within the experimental error, which indicates that the method is reliable.

In experiment 1, the ground Vita Cube dog food and six other feeds were mixed with cod liver oil. Kilogram quantities of the feed containing 5% oil were prepared and a sample was removed for an initial determination. The rest of the mixture was stored and samples taken out at monthly intervals for analysis. The results are given in Table II.

The vitamin A added to the Corn Flake feed was completely destroyed at the end of one month's storage. The vitamin A of the Vita Cube dog food underwent a gradual destruction and 75% was lost at the end of nine months. The other five feeds lost from 34 - 90+% in ten months.

Experiment 2 was carried out the same as experiment 1 with the exception that a different series of feeds was used. The results are given in Table III.

Higher vitamin A values were obtained because an oil was used which had a greater concentration of vitamin A than the oil used in experiment 1. Complete destruction of the vitamin A occurred in one month with Rice Krispie feed and Whole Wheat Biscuit feed. The amount lost with the other four feeds varied from 41-65% in seven months.

In experiment 3, two other feed ingredients, bone meal and wheat germ, were tested in a similar manner. These were mixed with 2 1/2% per cent oil instead of 5% as in the previous experiments. The results are given in Table IV.

The vitamin A was completely destroyed with the bone meal in one month and only a trace was left with the wheat germ at the end of five months.

TABLE II

STABILITY OF VITAMIN A OF COD LIVER OIL WHEN ADDED TO VARIOUS FEEDS (EXPERIMENT 1)

SAMPLE		Time (Months)										
		0	1	2	3	4	5	6	7	8	9	10
Vita Cube (ground)	*BU/gm	66.0	57.7	57.7	57.7	41.2	33.0	30.5	30.5	19.3	16.5	
	% lost		12.6	12.6	12.6	37.6	50.0	53.0	53.0	70.7	75.0	
Standard Middlings	BU/gm	57.7	47.8	42.0	45.3	49.5	45.3	39.6	49.5	25.6		38.0
	% lost		1.6	27.2	21.5	14.2	21.5	31.4	14.2	55.6		34.2
Corn Flake Feed	BU/gm	56.1	0.0									
	% lost		100.0									
Alfalfa Leaf Meal-13%	BU/gm	82.5	74.2	56.1	57.7	54.5	51.9	49.5	33.0	34.7	34.7	35.5
	% lost		10.1	32.0	30.0	34.0	37.1	40.0	60.0	58.0	58.0	57.0
Raw Bran	BU/gm	36.3	27.5	23.1	23.1	24.7	24.7	16.5	19.5	17.1	12.1	10.7
	% lost		24.2	36.4	36.4	32.2	32.2	54.6	46.3	52.9	66.6	70.6
Oatmeal Feed	BU/gm	70.1	61.8	28.8	33.0	33.0	13.5	27.0	26.4	22.0	20.9	12.7
	% lost		11.9	59.0	53.0	53.0	81.0	61.5	62.4	68.6	70.2	82.0
Pep Flake	BU/gm	57.7	57.7	36.3	43.7	38.5	24.8	18.7	T**	T	T	5.1
	% lost		0.0	37.1	24.3	33.3	57.0	67.6	99.0	99.0	99.0	91.2

*BU = Lovibond Blue Units

**T = Trace of Blue

TABLE III

STABILITY OF VITAMIN A OF COD LIVER OIL WHEN ADDED TO VARIOUS FEEDS (EXPERIMENT 2)

SAMPLE		(Time-Months) 0	1	2	3	4	5	6	7
Alfalfa Leaf Meal-17%	*BU/gm	160.5	153.4	112.5	107.3	82.5	63.3	60.5	55.0
	% lost		4.4	29.9	33.1	48.6	60.6	62.4	65.8
Hominy Feed	BU/gm	105.8	96.2	53.6	53.6	68.7	64.6	52.3	47.3
	% lost		9.1	50.3	50.3	35.0	39.0	50.6	55.3
Linseed Oil Meal	BU/gm	103.1	103.1	100.5	66.8	82.5	74.3	67.4	60.5
	% lost		0.0	2.5	44.8	20.0	27.9	34.6	41.3
Rice Krispie Feed	BU/gm	107.2	0.0						
	% lost		100.0						
Whole Wheat Biscuit Feed	BU/gm	96.2	0.0						
	% lost		100.0						
Wheat Krispie Feed	BU/gm	82.5	89.2	71.5	41.2	36.3	33.0	31.3	28.9
	% lost		0.0	13.3	50.0	56.0	60.0	62.1	65.0

*BU = Lovibond Blue Units

TABLE IV

STABILITY OF VITAMIN A OF COD LIVER OIL WHEN ADDED TO VARIOUS FEEDS (EXPERIMENT 3)

SAMPLE		Time (Months)					
		0	1	2	3	4	5
Bone Meal	*BU/gm	44.7	0.0				
	% lost		100.0				
Wheat Germ	BU/gm	42.9	43.7	30.5	39.6	28.6	T**
	% lost		0.0	29.0	9.2	33.3	99.0

* BU = Lovibond Blue Units
**T = Trace of Blue

It will be noted throughout the tests that there was considerable variation in the initial values for vitamin A. This was probably due to the inherent difficulty in obtaining uniform distribution of the cod liver oil in the feeds, since the mixing was done by hand.

In view of the fact that various cereal products gave variable results and even products from the same grains reacted differently, it was thought desirable to test the stability of vitamin A in mixtures of cod liver oil with the various unprocessed grains.

Five different grains were ground finely in a Hobart mill. A mixture of 500 grams containing 2 1/2% cod liver oil of each grain was prepared, stored and sampled as in the previous experiments. The results are given in Table V.

TABLE V

STABILITY OF VITAMIN A OF COD LIVER OIL
WHEN ADDED TO GROUND RAW GRAINS

SAMPLE		Time (Months)						
		0	1	2	3	4	5	6
Barley	*BU/gm	41.3	35.5	22.6	4.3			18.2
	% lost		14.1	45.3	0.0			55.9
Corn	BU/gm	49.5	41.3	27.5	47.9	35.5		27.5
	% lost		16.6	44.5	3.2	28.3		44.5
Oats	BU/gm	41.3	35.5		22.0	22.0		11.0
	% lost		14.1		46.8	46.8		73.4
Rice	BU/gm	42.9	0.0					
	% lost		100.0					
Wheat	BU/gm	41.3	33.0	19.3		25.9		22.0
	% lost		20.0	53.2		37.3		46.7

*BU = Lovibond Blue Units

The vitamin A was completely destroyed in the case of ground rice in one month. The oats mixture lost 73%, the barley, 56%, the wheat, 46%, and the corn, 44% of the vitamin A in 6 months.

Since considerable variations with the different feeds occurred with respect to the destruction of vitamin A, a number of mixtures of feeds with cod liver oil were tested. Linseed oil meal was mixed with oatmeal and also with standard middlings. Mixtures of cod liver oil with linseed oil meal and standard middlings had exhibited considerable stability, whereas that involving oatmeal was not particularly stable. The cod liver oil was pre-mixed with the linseed oil meal, since the latter absorbed the oil readily. Then the other feed was added and mixed thoroughly. Two hundred grams of the mixture was prepared containing 5% of the oil. Another group of mixtures was prepared in a similar way except that bone meal was added as an extra ingredient. The amount of bone meal added was equal to that of each of the other two feeds. The results are given in Table VI.

TABLE VI

STABILITY OF VITAMIN A WHEN ADDED TO VARIOUS FEED MIXTURES

SAMPLE		Time (Months) 0	1	2	3	4	5
Linseed Oil Meal + Oatmeal	*BU/gm	75.6	53.6	47.9	49.5	49.5	49.5
	% lost		29.1	36.7	34.5	34.5	34.5
Linseed Oil Meal + Standard Middlings	BU/gm	78.4	51.2	47.9	53.6	49.5	49.5
	% lost		34.7	39.0	31.7	36.9	36.9
Linseed Oil Meal + Oatmeal + Bone Meal	BU/gm	77.6	0.0				
			100.0				
Linseed Oil Meal + Standard Middlings + Bone Meal	BU/gm	82.5	0.0				
			100.0				

*BU = Lovibond Blue Units

The difference in the amount of vitamin A lost with the first two mixtures in five months is perhaps not significant. However, when bone meal was added, the vitamin A was completely destroyed in one month. Although the oil was mixed with the linseed oil meal and the bone meal added as an extra ingredient, the presence of the latter was obviously responsible for the destruction of vitamin A.

Further experiments were carried out with those feeds in which the vitamin A was destroyed in one month. Bone meal, ground rice, Rice Krispie feed and Corn Flake feed were each extracted with ethyl ether in Soxhlet extractors for 3-4 hours. The extracted feeds were then mixed with 5% cod liver oil and determinations carried out as in the previous experiments. After the feeds were mixed with the oil, they stood overnight before samples were removed for an initial determination, of the vitamin A. These results are given in Table VII. The vitamin A was completely destroyed with the bone meal overnight and in the case of all the other samples within one week. This shows that the ether extract of the feed does not contain anything which might accelerate the destruction of vitamin A.

Another experiment was carried out in which linseed oil meal was extracted with ethyl ether and the oil mixed with the extracted meal as in the preceding experiment. In this case, the vitamin A was completely destroyed in one month. It may have been destroyed in much less time, but the sample was not tested until it had been in storage for one month.

Discussion

The results of this investigation show that when cod liver oil is mixed with different feeds, marked variations in the rate of destruction of vitamin A occur. The results shown in Tables II-V inclusive represent

TABLE VII

STABILITY OF VITAMIN A WHEN ADDED TO FEEDS PREVIOUSLY EXTRACTED WITH ETHYL ETHER

SAMPLE	**0 Week *BU/gm	1 Week BU/gm
Bone Meal	0.0	0.0
Rice (ground	57.8	0.0
Rice Krispies	57.8	0.0
Corn Flakes	57.8	0.0

*BU = Lovibond Blue Units
** Samples stood overnight before first determination.

mixtures of a single ingredient with cod liver oil, except in the case of the Vita Cube dog food. Variations were observed ranging from a loss of 31% in 6 months to complete destruction in less than one month.

The feeds in which the least destruction occurred were standard middlings and linseed oil meal. Those which showed the greatest destruction were bone meal, Corn Flake feed, Rice Krispie feed, ground rice, and Whole Wheat Biscuit feed.

Although the vitamin A was destroyed readily when cod liver oil was mixed with Corn Flakes and Whole Wheat Biscuit feed, no such instability was observed in the case of the ground corn and wheat. It is interesting that considerable stability was noted with several other wheat products. However, in the case of the ground rice the vitamin A was destroyed as rapidly as in the Rice Krispie feed.

No significant difference in the destruction of vitamin A was observed between the addition of oatmeal to linseed oil meal and the addition of standard middlings to linseed oil meal when the oil was first mixed with the linseed oil meal. This is interesting in view of the fact that vitamin A was more readily destroyed when mixed with oatmeal than with standard middlings.

When bone meal was added to the mixtures in which the cod liver oil had been incorporated with linseed oil meal, the vitamin A was destroyed very rapidly. Since the oil was mixed with the linseed oil meal and at least a large amount of it was absorbed by the linseed oil meal, it would appear that the bone meal catalysed the destruction of vitamin A. Holmes, and Corbet (8) reported that several compounds, which were used in adsorption columns, caused a catalytic destruction of vitamin A. The results obtained with bone meal are in agreement with the work of Marcus (11) who reported very rapid destruction of vitamin A when cod liver oil

was mixed with various inorganic salts.

It was observed that the ether extract of bone meal, Corn Flakes, rice and Rice Krispies did not contain anything which might accelerate the destruction of vitamin A.

When linseed oil meal was extracted with ethyl ether before the cod liver oil was added, the vitamin A was destroyed in one month and probably in less time. This shows the presence of a stabilizing agent in the ether soluble fraction of the oil meal. In view of the observations of Holmes, Corbet and Hartzler (9) that lecithin acts as an antioxidant for vitamin A, it is more than likely that this or some other fat soluble antioxidant is present in considerable amounts in linseed oil meal and perhaps to a much lesser extent in certain other feeds. This would account at least in part for the variations in the rate of destruction of vitamin A in the various feeds.

These findings are in general agreement with the observations of Bethke et al. (3) who reported a loss of 50% and 75% of the vitamin A in 6 months with two different rations. However, the results of this study show less rapid destruction of vitamin A than those observed by Fraps and Kemmerer (6), but more rapid destruction than reported by Holder and Ford (7) and by Baird et al. (2).

This investigation emphasizes the importance of the fact that the addition of vitamin A to a ration during its manufacture does not insure its presence when the ration is used. If cod liver oil is added to a feed mixture, it should be added to an ingredient which would tend to preserve the vitamin A. Moreover, the feed should be used within a reasonable time after the addition of the oil.

Summary

The results of this study show that vitamin A of cod liver oil is unstable during storage when incorporated in feed mixtures. Wide variations in the rate of destruction of vitamin A were observed with the different feeds. In the case of Corn Flakes, Rice Krispies, Whole Wheat Biscuit, bone meal and ground rice, the vitamin A was completely destroyed in one week or less. In other feeds, there was a progressive destruction of the vitamin A as the storage period increased.

The rate of destruction observed with some of the cereal products differed from that of the unprocessed grain. An exception to this was ground rice in which the destruction of vitamin A was the same as that of Rice Krispie feed.

When bone meal was added to a feed mixture to which cod liver oil had been added, the vitamin A was completely destroyed within one month. This fact indicates that bone meal acts as a catalyst in the destruction of vitamin A.

The ether extract of certain feeds did not contain anything which might accelerate the destruction of vitamin A. However, ether extract of linseed oil meal contained some substance which acted as a stabilizing agent for vitamin A. Apparently, this stabilizer is present to a greater extent in some feeds than others, which may account in part for the variations in the rate of destruction of vitamin A which was observed with the various feeds.

From a practical standpoint, when cod liver oil is to be added to a feed mixture, it would be best to add it to an ingredient in which the vitamin A will not be readily destroyed.

References

(1) Abernathy, N. W. The Use of the Antimony Trichloride Method for the Determination of Vitamin A in Feeds, Milk and Butter. Thesis for M.S. at Michigan State College (1938)

(2) Baird, F. D., Ringrose, A. T., and MacMillan, M. J. The Stability of Vitamins A and D in Mixed Feed Ingredients. Part II - Vitamin A. Poul. Sci. 18:441 (1939)

(3) Bethke, R. M., Record, P. R., and Wilder, V. H. M. The Stability of Carotene and Vitamin A in a Mixed Ration. Poul. Sci. 18:179 (1939)

(4) Dunn, L. C. The Effect of Cod Liver Oil in Various Amounts and Forms on the Growth of Young Chickens. J. Biol. Chem. 61:129 (1924)

(5) Ewe, Geo. E. Cod Liver Oil - Stability of Vitamin A Content under Conditions of Commercial Distribution. J. Am. Pharm. Assoc. 22:1085-6 (1934)

(6) Fraps, G. S. and Kemmerer, A. R. Losses of Vitamin A and Carotene from Feeds During Storage. Texas Agr. Expt. Sta. Bull. No. 557 (1937)

(7) Holder, R. C. and Ford, S. K. The Stability of Vitamin A from Cod Liver Oil in Mixed Feeds. Poul. Sci. 18:345 (1935)

(8) Holmes, H. N. and Corbet, R. E. Catalytic Effects of Porous Powders on Pure Vitamin A. J. Biol. Chem. 127:449 (1939)

(9) Holmes, H. N., Corbet, R. C., and Hartzler, E. R. Lecithin and Hydroquinone as Antioxidants for Vitamin A. Ind. and Eng. Chem. 28:133 (1936)

(10) Lowen, L., Anderson, L., and Harrison, R. W. Cereal Flours as Antioxidants for Fishery Products. Ind. and Eng. Chem. 29:151 (1937)

(11) Marcus, J. K. The Destructive Action of Finely Divided Solids on Vitamin A. J. Biol. Chem. 90:507 (1931)