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Some Factors Affecting the Stability of Carotene in Mixed Feeds

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Station Biochemistry
Department

AGRICULTURAL EXPERIMENT STATION
South Dakota State College of Agriculture and Mechanic Arts
Brookings, South Dakota



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Some Factors Affecting the Stability of Carotene In Mixed Feeds

A. W. HALVERSON AND C. M. HENDRICK¹

Introduction

The instability of carotene has been a matter of concern in the feeding industry for a number of years. Since green plant preparations such as alfalfa meal are among the most economical sources of provitamin A, they have received the most attention in carotene storage loss studies. Rather effective methods have been developed to reduce carotene losses in such products during their storage. However, carotene stability in mixed feeds or practical diets, an important phase of the problem, has received little attention until recently.

Sources other than dehydrated plant products are also used as carotene additives, and investigations should include these. The experiments reported here were directed toward obtaining information about the stability of carotene added in several forms and amounts to mixed diets designed primarily for young poultry. However, some ingredients that are more often used with other animals such as oats, wheat bran plus middlings, and especially urea, were also used in certain diets.

Review of Literature

In 1937 Fraps and Kemmerer (4) noted considerable loss of carotene from both alfalfa meal and oil sources during storage at normal temperatures. It was further reported that the stability of alfalfa meal carotene was decreased by mixing 1 part of alfalfa with 9 parts of cornstarch. In the study of oil supplements, these authors observed that the carotene was also unstable if mixed with corn meal

alone or corn meal with yeast, skim milk powder, or wheat gray shorts.

At about the same time, Bethke *et al.* (1) reported that the carotene of alfalfa meal was lost from a practical chick diet at a moderate rate and that the presence of meat scraps

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and dried skim milk in the diet increased the loss rate. Another study by Brunius and Hellstrom (2) showed that the carotene of alfalfa pelleted with 30 percent of soybean oil meal was considerably more stable than that of alfalfa meal alone. These workers also reported that a similar test with oatmeal produced no enhanced carotene stability.

Quite recently Wall and Kelley (11) studied the stability of several carotene oil preparations when mixed with three different carriers. They found that in general soybean oil meal and broccoli leaf meal (solvent extracted) had rather good stabilizing properties as compared to a chick mash. Different sources of the provitamin appeared to react somewhat differently to the stabilizing activity of various carriers, however.

The Kansas Agricultural Experiment Station investigators have done considerable work on the effect of carriers and feed ingredients upon the stability of the carotene of oil concentrates and of alfalfa meal in recent years. These workers observed that carotene losses for oil preparations were affected to some degree by the type of carrier employed, with cottonseed and soybean oil meals (expeller type) being much more stabilizing than glucose and sorghum starch (8). Then, in later work it was observed that unconverted rice bran was even better than cottonseed or soybean oil meals in stabilizing the carotene of an alfalfa extract (9).

With alfalfa meal the Kansas

workers (6) also found cottonseed and soybean oil meals (expeller type) and crude rice bran effective in improving carotene stability. The protective effect was greatest in feed mixtures of low alfalfa content. Tests further indicated that 50 percent additions of several common feed ingredients to alfalfa meal affected carotene stability noticeably with the loss rate increasing with some and decreasing with others. Another report (7) indicated that carotene of alfalfa added to practical diets at a 10 percent level was stabilized materially. In studies of the stabilizing effects of separate ingredients using an alfalfa-cerelese mixture, yellow corn and wheat bran were observed to be quite active.

A recent report by Kamstra *et al.* (5) of the South Dakota Experiment Station concerned studies of the effect of trace minerals and other dietary ingredients upon carotene stability in poultry diets. These workers added meat scraps plus limestone and then manganese salt alone and with iron, copper, and cobalt salts to mixed cereal-soybean diets which were supplemented with either carrot oil or alfalfa meal carotene sources. The data indicated that alfalfa carotene was quite unstable in the different mixed diets but was little affected by diet modification. Carrot oil carotene varied considerably in stability with losses increasing in the presence of added meat scraps and limestone and possibly with trace minerals with certain diets.

Another report from the South

Dakota Experiment Station (3) involved the comparison of carotene stability between feeds with high contents of soybean oil meal (expeller) or oats. Both were supplemented with carrot oil. Results indicated that carotene was much more stable in the soybean feed than in the oats feed. In addition, it was observed that mixed trace minerals (copper, cobalt, and manganese) increased carotene losses somewhat when added to these feeds.

Thus, the literature indicates that carotene stability varies considerably among feeds and supplements of various kinds. Evidently, diverse carotene stabilizing or destroying properties are apparent between different feed ingredients and among similar ones prepared differently. These variations indicate that carotene stability in mixed feed and diets is a complex phenomenon which will need much study to be completely understood.

Methods

Ration and Carotene Supplements

The basal diet used throughout this work was a corn-soybean type formulated as a chick starter ration. Its composition was as follows: white corn, 61.0 percent; solvent-extracted soybean oil meal, 35.0 percent; iodized salt, 0.5 percent; dicalcium phosphate (Analytical Reagent), 1.5 percent; and calcium carbonate (Analytical Reagent), 2.0 percent. The following amounts of vitamins and antibiotic were also included per 100 grams of diet: animal protein factor (28 mcg. vitamin B₁₂ and 4500 mcg. procaine penicillin per gram of product), 0.06 gm.; vitamin D₃ (Delsterol concentrate, 2000 AOAC units per gram of product), 0.05 gm.; riboflavin, 0.33 mg.; choline chloride, 0.044 gm.; calcium pantothenate, 0.66 mg.; and nicotinic acid, 0.66 mg. The soybean oil meal was freshly prepared in the laboratory for each experiment by grinding, extracting exhaustively with hexane, and autoclaving at 15

pounds pressure for 30 minutes. White corn was freshly ground for each experiment. The various changes in the basal diet will be described later.

The different carotene supplements used with the diets during the course of the studies included alfalfa meal (untreated), antioxidant-treated alfalfa meal, carotene feeding oil, and carotene-Wesson oil preparations. The alfalfa meal was high quality dehydrated material obtained from a commercial source, and the antioxidant-treated meal was of similar grade but also contained diphenyl - p - phenylenediamine added in soybean oil meal carrier. The carotene feeding oil was a commercial product consisting of carrot and wheat germ oil diluted with vegetable oil. The carotene-Wesson oil preparations were laboratory products which consisted of crystalline carotene and α -tocopherol dissolved in Wesson oil. Two different alfalfa meals (untreated) and several carotene-Wesson oil

preparations were used during the course of the studies. All carotene supplements were kept under refrigeration during the work.

Analytical Methods

The following method of analysis was used for the carotene determinations throughout the work. Samples (1-7 gms.) were weighed and transferred to 250 ml. glass stoppered bottles along with 30 ml. of a 3:7 mixture of acetone and n-hexane. After mild shaking the samples were placed in the dark and allowed to stand for 17 to 24 hours. Seventy ml. of n-hexane were then added per sample, and they were again kept in the dark for a 2 to 3 hour period during which the contents were shaken several times. After the contents had been allowed to settle, the clear supernatant extracts (about 85 ml. per sample) were decanted into graduated cylinders for volume measurement. Upon recording volumes, the extracts were transferred to 100 ml. volumetric flasks. A single rinse of the graduated cylinder with 10 to 15 ml. of 1:9 acetone and hexane solution followed the transfer step. The rinse was pooled with the extract in the volumetric flask. The sample extracts were then chromatographed.

The chromatographic procedure used was similar to one described by Quackenbush (10). In the procedure followed, the entire contents of each volumetric flask were passed through the adsorption column. The carotene was then eluted with 55 ml. of 1:9 mixture of acetone and hexane solution, about 5 ml. of which

was used for intermediate rinsing of the volumetric flask and column. Following chromatography, the eluate volumes were measured and absorption densities determined photometrically using the Evelyn colorimeter with 440 mu filter. Then the readings were calculated to carotene content by using a conversion constant based on β -carotene.

The method described was found satisfactory with a number of feed types tested. Comparing the method described with one using a different extraction procedure (Soxhlet), comparable carotene values were obtained with several feeds containing different carotene sources and concentrations. It was concluded that the method used was well adapted for measurement of both real and relative differences in carotene stability among diets.

Carotene analyses were made in duplicate on all samples and content was calculated on an air dry basis. All samples were kept under refrigeration (-17.5° C.) during the analysis intervals. The maximum variation observed in the duplicate analyses amounted to less than ± 3 percent of mean values.

Storage Procedure

The carotene stability test periods involved storage of all samples for 30 days at $37 \pm 0.5^{\circ}$ C. in a small constant temperature oven. Insect infestation of diets during the storage tests was minimized by use of a small amount of naphthalene in the oven. Samples concerned with a carotene supplement in each experiment were stored simultaneously.

Results and Discussion

Effect of Single Modifications of the Basal Diet Upon Carotene Stability

This experiment was undertaken to determine the effect of the separate addition of several feed ingredients (see Table 1) upon the stability of different carotene supplements with a practical diet. The various ingredients were added to the basal diet either by replacement of corn or by direct addition. Three carotene supplements—alfalfa meal, carotene feeding oil, and carotene-Wesson oil preparation (0.1 percent added *a*-tocopherol) were used. The alfalfa and feeding oil supplements supplied similar and adequate amounts of carotene (about 6 mcg. per gram of diet) and the Wesson oil supplement furnished a lesser amount (about 1 mcg. per gram).

Most of the ingredients used for modification were obtained from commercial feed sources. The trace minerals, however, were made up in the laboratory as finely ground mixtures of mineral and starch at about a 1:3 dilution. Preparation of the trace minerals in such form facilitated addition and mixing with the diets. Urea was added as the pure compound in the studies concerned. Of the ingredients used for modification, all except minerals and urea were refrigerated throughout the investigation.

The carotene losses in the various diets following storage at 37° C. for 30 days are shown in Table 1. The data show that alfalfa carotene losses were but little affected by the diet modifications used although the re-

placement of corn with oats tended to increase the loss to some extent. Losses from all alfalfa diets were rather high, however, amounting to about one-third of the original carotene.

Results for oil supplemented diets differed from those with alfalfa in several respects. Losses of carotene from the basal diet with oil supplements were less than half those observed for the alfalfa supplemented basal. They were increased to a noticeable extent by most of the modifications. In some instances the losses became about as great as with alfalfa (when a high trace mineral level or fish meal or meat scraps were present). Ingredients such as oats and wheat bran-middlings also caused definite increases in losses especially with the carotene-Wesson oil supplement, but all other substitutions or additions resulted in only small increases. It was notable that urea had no effect upon carotene losses when added either alone or with trace minerals. Thus, the data showed that alfalfa and oil carotene supplements vary considerably in their sensitivity to diet modifications of the type used. However, oil supplements of different composition and carotene content appear quite similar.

Effect of Variation of the Content of Alfalfa in Certain Mixtures on Carotene Stability

The effect of variation in the alfalfa content of two mixtures on carotene stability was studied. Various

Table 1. Carotene Losses During Storage in Diets Containing Different Carotene Supplements. (Storage at 37° C. for 30 Days)

Diet Modifications	Carotene Losses with Different Supplements		
	5% Alfalfa Meal Diet*	0.5% Carotene Feeding Oil Diet*	0.5% Carotene-Wesson Oil Diet*
1. Corn-soybean basal diet† 35.7	35.7	9.5	16.5
Modified basal containing any one of the following feeds by replacement‡:			
2. 15% Oats 44.8	44.8	17.5	35.8
3. 15% Wheat bran and middlings (1:1) 39.3	39.3	14.1	31.6
4. 15% Alfalfa meal 37.6	37.6
5. 15% Dried buttermilk 32.9	32.9	15.2	23.6
6. 15% Meat scraps 38.7	38.7	28.5	35.1
7. 15% Fish meal 40.3	40.3	36.8	32.1
Modified basal containing any one of the following mineral feeds and compounds per 100 grams of diet:			
8. Steamed bone meal, 1.0 gm. 34.3	34.3	17.3	20.6
9. Limestone,			
(a) 1.0 gm. 35.7	35.7	14.7	21.2
(b) 4.0 gm. 39.7	39.7	17.6	20.0
10. Manganese salt,§			
(a) 0.02 gm. 33.4	33.4	17.7	16.8
(b) 0.08 gm. 33.3	33.3	15.7	17.6
11. Trace Mineral Salts,			
Manganese Iron Copper Cobalt			
(a) 0.02 gm. 0.01 gm. 0.001 gm. 0.00001 gm. 34.8	34.8	23.0	20.6
(b) 0.08 gm. 0.04 gm. 0.004 gm. 0.00004 gm. 39.0	39.0	38.1	38.6
12. Urea, 2.0 gm. 32.9	32.9	10.7	17.3
13. Urea, 2.0 gm. + Trace mineral salts,			
Manganese Iron Copper Cobalt			
0.08 gm. 0.04 gm. 0.004 gm. 0.00004 gm. 36.6	36.6	40.5	37.5

*Initial carotene contents of diets were 6.2, 6.8, and 1.0 mcg. of carotene per gram of diet for alfalfa meal, carotene feeding oil, and carotene-Wesson oil diets, respectively. Alfalfa was added to diets at the expense of corn, and oil supplements were added without correction.

†The basal diet, a chick starter ration, consisted of white corn (61.0%), soybean oil meal (35.0%), iodized salt (0.5%), dicalcium phosphate (1.5%), and calcium carbonate (2.0%) plus vitamin and antibiotic supplements (see text).

‡Feeds added to diet at the expense of corn.

§MnSO₄ · H₂O.

||MnSO₄ · H₂O, FeSO₄ · 7H₂O, CuSO₄ · 5H₂O and CoSO₄ · 6H₂O.

combinations of alfalfa meal and basal diet and of alfalfa meal and meat scraps were prepared and then stored as in the previous experiment. The basal diet, as such, was mixed with various proportions of alfalfa and no ingredient replacements were involved. Two types of alfalfa meal, untreated (as in the previous experiment) and antioxidant-treated were used. The alfalfa meal was

from the same batch as used previously while the meat scraps were not.

The data plotted in Fig. 1 show that carotene stability varied markedly both between and within mixtures. The carotene of untreated alfalfa was much decreased in stability when present at low level in the meat scraps mixtures but was increased in stability in basal diet mix-

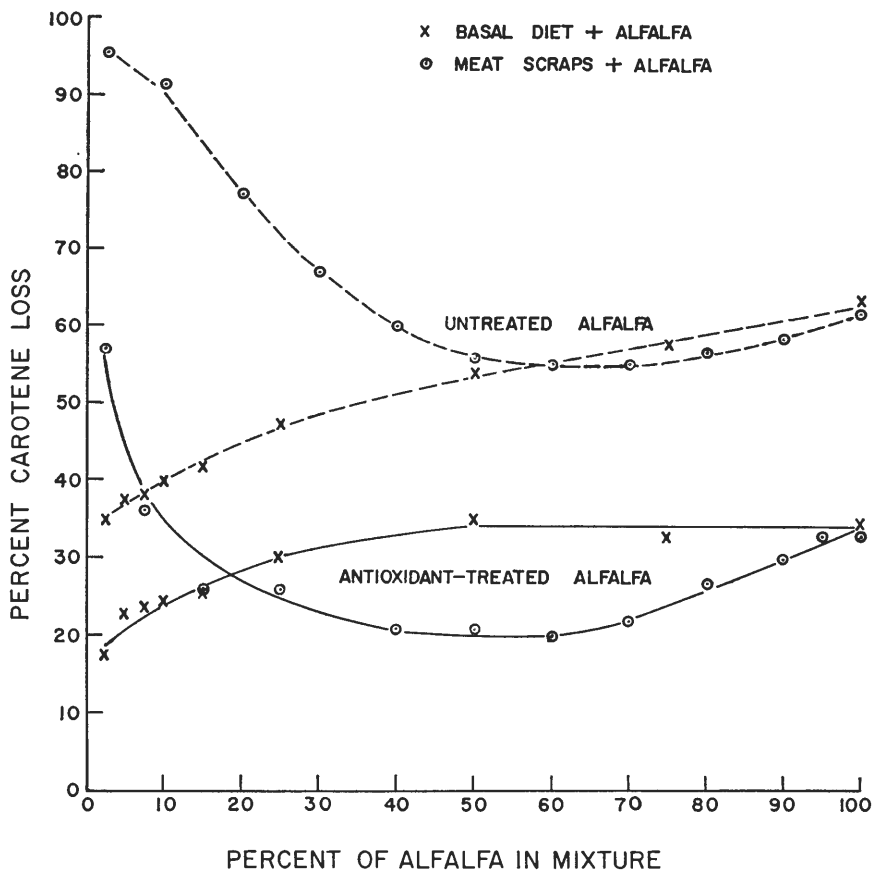


Fig. 1. Effect of feed additive and content in alfalfa mixtures upon carotene losses during a 30-day, 37 °C. storage period. Initial carotene contents were 101 and 225 mcg. per gram for untreated and antioxidant-treated pure alfalfa meal, respectively.

tures at a similar level. However, the carotene destroying and stabilizing effects from these two diluent feeds decreased rather rapidly as alfalfa content increased until carotene losses became quite similar with the two mixtures or even somewhat less for meat scraps mixtures at high alfalfa levels (60 percent and higher).

With antioxidant-treated alfalfa meal mixtures, the same general stability patterns were evident but the carotene had greater stability and was slightly less affected by dilution changes. In addition carotene was noticeably stabilized by meat scraps throughout the intermediate range of the dilution curve (25-90 percent alfalfa), thereby resulting in meat scraps mixtures having lower carotene loss rates than did basal diet mixtures over much of the dilution range.

Additional experiments of a similar nature have verified the results shown in Fig. 1 for meat scraps plus alfalfa, but no studies have been made which will explain the nature of the curves (the carotene destroying and stabilizing properties of meat scraps at different concentrations). Thus, clear evidence was obtained that the stability of alfalfa carotene is materially affected by the type and content of feed ingredients in mixtures as well as by the kind of alfalfa product.

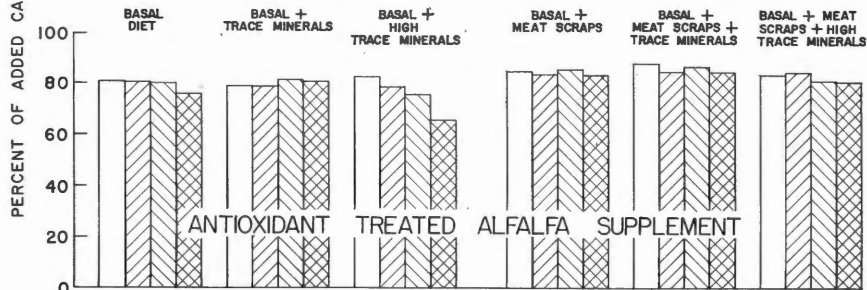
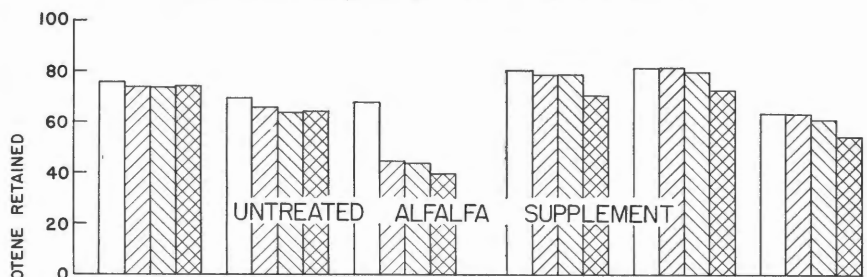
Study of Diet Age as a Factor in Carotene Stability

Previous observations made during the course of work at this laboratory led to the belief that the age of diet ingredients affected the degree

to which they caused or prevented carotene losses in feed mixtures. Therefore, an experiment was undertaken to determine the effect of aging diets prior to carotene addition on subsequent losses of the provitamin during storage. The basal diet and several modifications were prepared by grinding the corn, mixing the diet constituents (without carotene supplementation), and then storing for periods of 2, 4, and 6 months at room temperature in open bottles within cardboard containers. The corn ingredient was freshly ground for each diet preparation while single refrigerated batches of soybean oil meal and of meat scraps were used throughout the experiment. At the termination of the aging periods, the various diets together with freshly mixed ones were supplemented with different carotene sources and stored as usual for 30 days at 37° C.

The diet modifications used included trace mineral additions at two levels to the basal diet and to a similar diet modified by addition of 15 percent of meat scraps. The meat scraps modification was at the partial expense of the corn ingredient. The trace mineral salts and their levels were similar to those already described (see Table 1 footnotes), the lower level contained 0.02, 0.01, 0.001, and 0.00001 percent of manganese, iron, copper, and cobalt salts, respectively, and the higher level four times these amounts.

Fig. 2. Effect of diet age upon retention of added carotene in modified diets following storage for 30 days at 37° C.



AGE OF DIETS BEFORE SUPPLEMENTATION

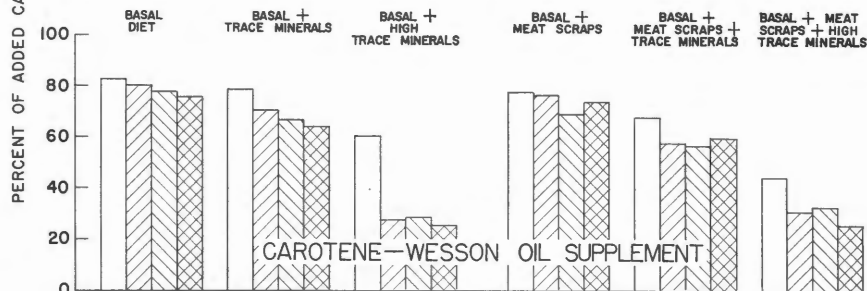
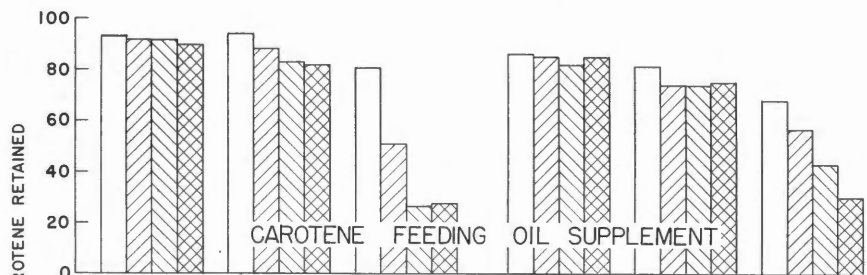


Table 2. Carotene Losses During Storage for a Corn-Soybean Diet as Affected by Aging of the Diet or its Modifications at Room Temperature for Various Periods Prior to Carotene Addition.* (Storage at 37° C. for 30 Days After Carotene Supplementation)

Carotene Supplements and Diet Modifications†	Carotene Losses With Diets Aged Different Periods Before Supplementation			
	0 mo.	2 mo.	4 mo.	6 mo.
	%	%	%	%
5% Untreated Alfalfa Meal‡				
a. Corn-soybean basal diet	24.4	26.2	26.8	26.0
b. (a) + trace minerals	30.8	34.2	36.2	35.8
c. (a) + high trace minerals	32.1	55.2	56.3	60.3
d. Corn-soybean diet + 15% meat scraps	19.4	21.4	21.3	29.3
e. (d) + trace minerals	18.8	18.5	20.0	27.3
f. (d) + high trace minerals	36.3	36.6	38.8	45.5
5% Antioxidant-Treated Alfalfa Meal§				
a. Corn-soybean basal diet	19.5	19.9	20.0	24.1
b. (a) + trace minerals	21.1	21.3	18.7	19.3
c. (a) + high trace minerals	17.6	21.7	26.4	34.4
d. Corn-soybean diet + 15% meat scraps	15.4	16.4	14.6	17.0
e. (d) + trace minerals	12.0	15.2	13.3	15.6
f. (d) + high trace minerals	16.4	15.7	18.8	20.2
0.5% Carotene Feeding Oil				
a. Corn-soybean basal diet	7.0	8.5	8.6	10.1
b. (a) + trace minerals	6.4	12.1	17.6	18.7
c. (a) + high trace minerals	19.8	49.5	74.0	72.7
d. Corn-soybean diet + 15% meat scraps	14.2	15.2	18.9	15.7
e. (d) + trace minerals	19.0	26.3	26.3	25.4
f. (d) + high trace minerals	32.6	43.7	57.1	70.5
0.5% Carotene-Wesson Oil Preparation				
a. Corn-soybean basal diet	17.3	19.9	22.3	24.4
b. (a) + trace minerals	21.4	29.8	33.2	36.0
c. (a) + high trace minerals	39.9	72.5	71.6	74.4
d. Corn-soybean diet + 15% meat scraps	22.6	23.8	31.0	26.1
e. (d) + trace minerals	32.4	42.3	43.6	40.6
f. (d) + high trace minerals	56.0	69.6	67.5	74.8

*Diet age was measured from the time of grinding (corn) and mixing of ingredients to when carotene supplements were added. (See Table 1 footnote for the basal diet composition.)

†Respective supplements supplied approximately 9.2, 10.2, 6.8, and 33.5 mcg. of carotene per gram of diet. Alfalfa was added to diets at the expense of corn, and oil supplements were added without correction.

‡Carotene loss with the pure meal amounted to 44.3 percent.

§Carotene loss with the pure meal amounted to 22.0 percent.

Four different carotene supplements were used—alfalfa meal (untreated), antioxidant-treated alfalfa meal, carotene feeding oil, and carotene-Wesson oil preparation (1 percent added α -tocopherol). The untreated alfalfa was of a new batch which had not been used previously. Samples of alfalfa meal alone were stored along with supplemented diets for control purposes.

Carotene loss data obtained with

the alfalfa and oil supplemented diets of various composition and age background are shown in Table 2. The data have also been graphed in terms of percent of carotene retained (Fig. 2). In general, the results indicated that diet age does have an effect upon carotene stability in certain diets.

Results with the untreated alfalfa meal supplement using modifications which promoted only slight

changes in carotene retention in freshly mixed diets showed that losses increased markedly in aged diets containing a high trace mineral level. These extra losses induced in high trace mineral diets were about as great with 2 as with 6 months of storage before the test period. This indicates that the carotene stabilizing property of the diet deteriorated to almost maximum degree during the shortest aging period (2 months). Losses in similar diets containing no trace minerals or a normal level were little affected by diet age before supplementation. When meat scraps were included in the diets, normal losses tended to be reduced slightly, and losses induced by aging with high trace mineral diets were considerably reduced.

Examination of the results for the antioxidant-treated alfalfa meal supplemented diets indicated that losses were less than those observed with untreated alfalfa and also were less affected by modification and aging. Indeed, the losses induced by high trace minerals in aged diets were minor. Also meat scraps additions improved carotene stability in a manner similar to that observed with untreated alfalfa meal. The fact that carotene loss rate in the diets was similar to that with pure alfalfa meal was further indication that the carotene of antioxidant-treated meal was almost unaffected by the diet treatments used.

The effects obtained with the carotene feeding oil and the carotene-Wesson oil supplements were quite similar notwithstanding different compositions and carotene

contents. However, in most instances a slightly greater carotene stability was obtained with the feeding oil. The carotene of oil supplements was shown to be affected by high trace mineral modification to a greater extent than observed with alfalfa, especially in those diets aged before supplementation. However, as with alfalfa, diets which contained no added trace minerals or a normal level showed little effect from aging upon carotene stability. The addition of meat scraps to oil supplemented diets did not change the losses materially although slight changes such as increased loss in freshly mixed diets and reduction of some age-induced losses were noted.

Comparison of results with the four carotene supplements indicated considerable similarity in loss patterns in spite of certain differences. The effect of addition of the lower trace mineral level and of meat scraps separately and in combinations was rather minor as was the effect of aging of such diets with the various supplements. Likewise, the large increase in carotene losses observed with aging of the high trace mineral diets (without added meat scraps) was characteristic of all supplements although the antioxidant-treated alfalfa supplement showed a lesser response. The effect of meat scraps upon the aging response as well as upon losses in general appeared to differ slightly between alfalfa and oil supplements. Addition of that ingredient to alfalfa diets reduced normal losses slightly and also retarded age-

induced losses. Similar addition to oil supplemented diets increased the loss rate some in the fresh ones but caused little change in losses induced by aging with high trace minerals.

While the effects observed from aging of high trace mineral diets were notable, of equal or greater importance were data showing the

lack of effect of the age factor upon carotene losses in diets containing ordinary trace mineral levels (lower level) or meat scraps or both. Indeed the constancy of carotene stability observed with such treatments indicates that mixed diets retain their carotene stabilizing properties for long periods under normal conditions.

Summary

Carotene storage losses were observed in a corn-soybean diet during a 30-day, 37 °C. storage period using various diet modifications as well as several carotene supplements.

Study of diet modification by separate addition of common feed ingredients to the diet—15 percent of oats, wheat bran plus middlings, dried buttermilk, meat scraps or fish meal, 1 and 4 percent of ground limestone, 1 percent of steamed bone meal, 2 percent of urea or trace minerals at two levels—indicated that such modifications caused carotene losses to increase in many instances. The losses increased to a greater extent with oil supplements than with alfalfa, which showed only slight effects from the diet changes. However, since oil carotene was more stable than that of alfalfa in unmodified diets, the extra losses induced in oil supplemented diets by modification caused mainly an equalization of losses among supplements.

Study of modification by variation in the level of alfalfa used in feed mixtures demonstrated an im-

portant effect upon carotene stability from change in the kind and amount of feed added as well as the type of alfalfa. These conclusions, based upon data using basal diet-alfalfa mixtures and, in addition, meat scraps-alfalfa mixtures, showed carotene stability to increase with increase in basal diet content or to decrease with increase in meat scraps content of the different mixtures. However, meat scraps mixtures were also observed to impart stability to alfalfa carotene at low meat scraps levels. Similar data obtained with antioxidant-treated alfalfa showed a greater degree of stability and somewhat less variation in losses with different mixtures than was observed with untreated alfalfa.

Study of modification by aging of diets prior to carotene supplementation indicated that both alfalfa and oil sources are affected quite similarly by this variable. Comparison of losses among freshly mixed diets and those aged 2, 4, and 6 months before the stability test period showed that losses were not greatly affected by aging in the

basal diet or when meat scraps or a normal trace mineral level or both were present although losses differed some among diets and supplements.

However, losses increased markedly in the high trace mineral diets following aging. The age-induced loss generally approached maximum in diets subjected to only the 2-month aging treatment. Since losses induced by aging were larger with oil than with alfalfa supple-

ments, total losses were greater with oil diets in spite of initial greater stability. When meat scraps were present in the diets; losses with alfalfa supplements were reduced, and especially when aging effects were involved. Meat scraps affected the stability of oil type carotene to lesser extent so the net result was a further increase in the superiority of alfalfa over oil when meat scraps were present in high trace mineral, aged diets.

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