periments showed extensive destruction of methionine by these procedures, which might account for the inconclusive results obtained.

The results obtained with the nitrogenous components, and those with the meat fractionation procedure, strongly suggest that the major odor-forming reactions occurring during irradiation involve the water-soluble proteins in meat. The appearance of hydrogen sulfide in the irradiated dialyzate, E, is in agreement with the results obtained with irradiated glutathione solutions, because glutathione should appear in this fraction. However, another alternative must be considered—that this fraction is necessary in the water extract, A, in conjunction with other substances to produce the usual off-odor observed there. This would also be compatible with the previous suggestion that the off-odor results from a compound that breaks down on contact with the trapping reagents to give the products obtained.

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ALFALFA CAROTENE

Effect of Added Animal Fats and Vegetable Oils on Stability of Carotene in Dehydrated Alfalfa Meal

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The effect of adding animal fats or vegetable oil to dehydrated alfalfa meal both in the laboratory and at an alfalfa dehydrator was studied. Increasing amounts of fat or oil from 1 to 5% increased the stability of carotene, reduced dustiness, and gave a greener appearing meal, but had little effect on the stability of green color. The use of fat and oil containing 5, 15, and 40% of free fatty acid had essentially the same effect on carotene and color retention as materials which contained little of these acids. Added fat or oil may increase carotene stability by bringing carotene and naturally occurring antioxidants of alfalfa into mutual solution, thus allowing the stabilizers to operate more effectively.

LARGE SUPPLIES of low-priced animal fats have stimulated interest in their use in animal feeds. Nutritional studies with poultry and cattle have shown that incorporation of low-grade animal fats in feeds, up to 5% of the ration consumed, results in efficient use of the fat with no apparent ill effects (*4*, *8*). More recently, it has been shown that the stability of vitamin A added to feeds as fish liver oil was increased when 6% of stabilized animal fats were also added (*10*). Earlier studies by this laboratory demonstrated that the stability of crystalline carotene added to feed ingredients was greatly improved by the addition of unstabilized oils (*1*). Mitchell *et al.* (*5*) showed that carotene retention during storage is influenced by amount of oil used in application of antioxidant to a meal. The present report on carotene stabilization in dehydrated alfalfa indicates also that additions of animal or vegetable fat without added stabilizers enhance the stability of the carotene in the meal during storage at room temperatures.

Experimental Procedures

For laboratory studies, the oils or fats were incorporated into the alfalfa meal by dissolving them in a minimum amount of petroleum ether and spraying on the meal in a mixing chamber (*11*).

Preparation of Mixtures For plant scale studies, an oil-metering unit (*12*) was employed. The oil was added to the chopped dried alfalfa as it came from the dryer prior to grinding, thus assuring thorough mixing in the hammer mill (*12*).

Storage Tests For storage studies, 2-gram samples of the meals were weighed into open shell vials (20 × 70 mm.). Samples were stored at either 25° or 65° C. (77° or 149° F.) (*11*). A petroleum ether extract of alfalfa meal dissolved in oil solution and crystalline carotene in oil solution were employed in several experiments for comparison with the alfalfa meal samples. They were stored under the same conditions as were the meal samples in shell vials. Details of the preparation and storage of such carotene solutions have been presented (*2*).

Laboratory Scale Application

To study the relative effectiveness of several vegetable oils for stabilizing carotene in alfalfa meal, 5% by weight of oil was added as indicated above to each in a series of meal samples. The meals were stored in a constant-temperature room at 25° C. After 4 months of

storage, the meal without added oil has lost over half of its original carotene. All the oils tested in this series exhibited a marked stabilizing effect on the carotene (Table I). The preparation containing added mineral oil still retained 90% of its original carotene. Of the oils tested, the refined were more effective than the corresponding crude oils. Hydrogenated cottonseed oil shortening was more effective than refined cottonseed oil.

Table I. Effect of 5% of Added Oil on Stability of Carotene in Alfalfa Meal at 25° C.

Oiling Treatment	Carotene Retained after 4 Months, %
Control	47
Mineral oil	90
Hydrogenated cottonseed oil shortening	82
Cocoa butter	77
Refined rice oil	77
Cottonseed salad oil	76
Crude peanut oil	76
Refined sesame oil	74
Refined coconut oil	73
Crude cottonseed oil	72
Crude soybean oil	71
Crude sesame oil	68
Crude corn oil	64
Crude rice oil	62
Safflower oil	61
Lanolin	59

In another experiment, samples of a dehydrated alfalfa meal were treated in the laboratory with 1, 2, and 5% each of rice bran oil, white animal fat, and several yellow animal greases containing 5, 15, and 40% free fatty acid. All of these oils had about an equal preservative effect of the carotene (Table II). The free fatty acid content did not seem to be deleterious to the carotene within the limits of this experiment. Preservative effect of carotene increased with the amount of oil applied. The oil had no preservative effect on the color of the meal, although the greater the amount of oil applied, the darker green the samples appeared. High free fatty acid as such was not deleterious to the color of the meal.

Plant Scale Application

An experiment was performed at commercial dehydrator to determine the effectiveness of animal fat in stabilizing carotene in alfalfa meal. Melted tallow was metered continuously on the dried chopped hay just before it entered the hammer mill. Hammermilling thoroughly mixed the tallow with the meal and also heated the meal. Heating enhances retention of carotene (*5*), but heat as a factor could not be evaluated in the present experiment. Approximately 1 ton of meal was treated at each of three oil levels studied. The meal treated with 4.8% animal fat retained 62% of its carotene after 16 weeks of storage at 25° C. The comparable control sample retained only 38% after similar storage (Table II).

Effect of Natural Antioxidants

Dehydrated alfalfa meal is well supplied with natural antioxidants, which include tocopherol (*9*, *13*) as well as others (*3*). In the unoiled meal they may not be able to exert their maximum effect on the carotene, because part of the carotene is not in contact with the antioxidants. To determine the stabilizing effect that might be expected of the natural antioxidants under conditions which permit a more complete contact of carotene and stabilizers, the following experiment was performed.

An extract of alfalfa meal was prepared by soaking dehydrated meal in petroleum ether and filtering off the residue. This extract, which contained some of the natural antioxidants as well as carotene, xanthophyll, chlorophyll, and other fat-soluble constituents, was added to mineral oil and the solvent was removed in vacuo. The stability of the carotene in this extract was compared with that of crystalline carotene in mineral oil, carotene in alfalfa meal, and carotene in meal to which 15% of mineral oil had been added. The carotene in the extract was much more stable than the solution of crystalline carotene in mineral oil (Table III), and was also more stable than carotene in whole meal.

This suggested that the natural antioxidants of the alfalfa, although partially effective, were not being completely utilized in protection of the carotene in the meal. As has been observed in carrots (*7*, *14*), a portion of the carotene in dehydrated alfalfa may exist as discrete particles. Furthermore, the antioxidants probably exert their maximum effect only on that portion of the carotene with which they are mutually dissolved in the lipides of the meal. Therefore, a treatment such as oiling, which serves to bring the carotene and antioxidants into a common solution, should result in enhanced carotene retention. The meal sample that had been mixed with 15% of mineral oil retained about three and a half times as much carotene as the comparable unoiled sample (Table III). Perhaps a condition was approached wherein all the carotene and antioxidants were in solution. This would explain why the stability of carotene in this mixture was so similar to that of the crude concentrate in mineral oil (samples C and D, Table III).

The findings by Mitchell and Silker (*6*), that the carotene of mixtures of alfalfa meal and soybean meal was more stable with expeller meal than with solvent-extracted meal, can be explained on the basis described above, since expeller meal retains more oil, which acts as a mutual solvent for the carotene and natural antioxidants present.

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Table II. Effect of Added Animal Fats on Stability of Carotene at 25° C.

(In laboratory tests and in commercial alfalfa meal dehydrator)

Oil Applied, %	Animal Fat, 2.5% FFA	Yellow Grease 5% FFA	Yellow Grease 15% FFA	Yellow Grease 40% FFA	Rice Bran Oil (for Comparison)
Carotene retained after 40 weeks, laboratory tests at 25° C. Control, 32% retention					
1	39	38	40	37	38
2	43	49	41	40	46
5	58	41	48	47	50
Carotene retained after 16 weeks, added in commercial dehydrator. Control, 38% retention					
0.4	46	..	..	..	..
1.4	47	..	..	..	..
4.8	62	..	..	..	..

Table III. Relative Stability of Carotene in Alfalfa Meal and in Oil Solution

Nature of Sample	Carotene Retained after 2 Weeks at 65° C. (149° F.)
A. Crystalline carotene in mineral oil solution	0[a]
B. Dehydrated alfalfa meal	21
C. Dehydrated meal plus 15% added mineral oil	76
D. Petroleum ether extractables from alfalfa in mineral oil solution	77

[a] Carotene completely disappeared in 1 day.

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ALFALFA CAROTENE

Effects of Antidust Oils on Stability of Carotene in Dehydrated Alfalfa Meal

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Various oils and fatty materials were used as antidust agents for alfalfa meal. Salmon body oil and acidulated cottonseed soap stock at a rate of 16 pounds per ton of meal reduced carotene stability slightly when an antioxidant was not added. Addition of an antioxidant eliminated this effect. At a rate of 80 pounds per ton and in the absence of an antioxidant, cottonseed oil, soybean oil, rice bran oil, and choice white grease appreciably improved carotene stability, while salmon body oil and acidulated cottonseed soap stock had no effect. Use of choice white grease resulted in the greatest improvement in carotene stability, although addition of an antioxidant with the antidust agents eliminated this advantage. There was considerable variation in the response of different lots of meal to oil, antioxidant, and heat. Sufficient heat was developed during pelleting of oiled meal to improve carotene stability.

THE USE OF ANTIOXIDANTS for stabilizing the carotene of alfalfa meal has been of growing interest since the issuance of a patent to Kephart (*2*) in 1949 covering the use of *N,N'*-diphenyl-*p*-phenylenediamine for this purpose. The antioxidants are applied by dissolving or suspending them in a triglyceride oil and spraying the mixture on the meal. However, one of the early reasons for adding oil to alfalfa meal was not as a carrier for antioxidants, but as a means of reducing dustiness of the meal and enhancing the color of the product. A considerable amount of meal still is oiled solely for control of dustiness. Oils have been used for this purpose at rates of 1% or less. Such levels do not give adequate control of dustiness, however, and the trend is toward use of up to 5% of oil.

Among the triglycerides which have been used are various vegetable oils, animal fats, and fish oils. Little is known of the comparative effect of such oils on carotene stability when added to alfalfa meal. Mitchell *et al.* (*3*) reported that meal treated with Wesson oil at a rate of 80 pounds per ton of meal showed considerably greater carotene stability than meal treated at a rate of 16 pounds per ton. Van Atta *et al.* (*6*) found a similar effect with coconut oil. They also reported seven vegetable oils having widely different iodine values did not differ in their effect on carotene stability when used for oiling alfalfa meal. The data reported herein are concerned with the effect on carotene stability of high and low levels of various antidust agents and with the comparative effectiveness of an antioxidant when applied with the dust-control agents.

Experimental

Alfalfa meal was treated with oil and antioxidant (6-ethoxy-1, 2-dihydro-2,2,4-trimethylquinoline, available under the name Santoquin for use on alfalfa) by dissolving them in a mixture of acetone and Skellysolve B and applying the solution to the meal by means of a nasal atomizer while the meal was being tum-