

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 <sup>a</sup>
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

<sup>a</sup> 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-

bled in a small rotary mixer (7). One half pound of meal could be treated effectively with this equipment. The amounts of antioxidant applied were 0, 0.02, and 0.1% of the meal, while the oils were used at rates of 16 and 80 pounds per ton. The Food and Drug Administration permits this antioxidant to be used at rates up to 0.015%. Larger amounts were used in this study because of the greater activity obtained (3). The samples were placed in 4-ounce bottles and stored at 37° C. Carotene determinations were made at intervals by the method of Silker, Schrenk, and King (5).

used. These data indicate that salmon body oil and acidulated cottonseed soap stock are the least desirable of the antioxidant agents studied, particularly at the higher level of application and in the absence of an antioxidant.

Although there were no other marked differences in the effects of the various oils, meal treated with choice white grease was somewhat more stable than meal treated with the vegetable oils when used at a rate of 80 pounds per ton. Inclusion of Santoquin tended to eliminate this slight advantage. Stabilizing the animal fat with 0.05% of a mixture of butylated hydroxyanisole,

in Kansas and Oklahoma to investigate possible variation in carotene stability. The meals were treated and placed in storage on the day following completion of collection. Thus, the meals were not more than 2 days old at the beginning of the storage period. A portion of each meal was placed in a 4-ounce bottle and stored without treatment. Other portions were treated with 16 pounds of Wesson oil per ton and with 0.02 and 0.05% of Santoquin. A portion of each oiled sample also was subjected to heat at 100° C. for 1 hour in an electric oven (3), to study the reported effect of enhancement of carotene stability by

**Table I. Carotene Loss in Alfalfa Meals Stored for 9 Weeks at 37° C. after Treatment with Santoquin and Antidust Agents**

Antidust Agent	% Antioxidant					
	0	0.02	0.1	0	0.02	0.1
	Expt. 1, 16-Lb. Oil			Expt. 2, 80-Lb. Oil		
None	63	39	29	61	..	..
Cottonseed oil	63	39	28	45	21	12
Soybean oil	64	40	28	45	22	14
Rice bran oil	61	40	26	41	21	14
Choice white grease, unstabilized	60	37	27	35	19	13
Choice white grease stabilized	60	34	25	35	17	12
Salmon body oil	70	39	30	60	26	17
Acidulated cottonseed soap stock	67	39	30	59	40	29

The data are presented in Table I. The same lot of meal was used for both experiments. Experiment 2 was started about 3 months after experiment 1. During this interval the bag of untreated meal was stored at -23° C. to minimize loss of carotene. At the start of experiment 1 the meal contained 20.1 mg. of carotene per 100 grams, while 3 months later it contained 19.9 mg. per 100 grams. The untreated samples lost 63 and 61% of the carotene in 9 weeks. Hence, though the experiments were not run concurrently, valid comparisons can be made of the data from the two experiments.

The antidust agents had no appreciable effect on carotene stability at the level of 16 pounds per ton in the absence of the antioxidant, although salmon body oil and acidulated cottonseed soap stock did appear to exert a slight deleterious effect when compared with the untreated meal. When 80 pounds of oil were used, meal treated with salmon body oil and acidulated cottonseed soap stock showed the same stability as the unoiled meal, whereas all the other oiled samples were considerably more stable. Addition of antioxidant improved carotene stability in all cases, and eliminated the slight deleterious effect of the salmon body oil and the soap stock noted at the level of 16 pounds per ton of antidust agent. At the rate of 80 pounds per ton, however, samples containing salmon body oil and cottonseed soap stock still were more unstable than the others, even when the high level of antioxidant was

propyl gallate, citric acid, and propylene glycol (Tenox-II) did not result in greater stability than was obtained when unstabilized fat was used. However, this antioxidant system is added commercially to animal fats primarily to protect the fat from autoxidation before use, and not for its direct preservative effect on the carotene of the alfalfa meal. The unstabilized fat used in this work was fresh. Had it been rancid, greater carotene destruction might have occurred, so that there might have been an advantage in using a stabilized product.

Although there may be a slight advantage in the use of animal fat over vegetable oils from the standpoint of carotene stability, at least at the higher rate of application, this advantage may be offset by the greater difficulty of applying the fat to the meal. Under plant conditions the fat is liquefied by heat and sprayed on the meal, after which it is distributed uniformly by means of a suitable mixer or blender. Good mixing is obtained if the meal is warm, but cold meal, such as is encountered in winter operations, causes difficulty due to congealing of the fat before it can be uniformly dispersed throughout the meal. This does not occur with vegetable oils.

**Behavior of Different Lots of Alfalfa Meal.** Some indication has been noted in this laboratory that alfalfa meals vary in their response to antioxidant, oil, and heat. A number of alfalfa samples were collected from dehydrators located

heat in conjunction with addition of oil. All samples were placed in storage at 37° C. Carotene destruction which occurred is shown in Table II, along with certain information concerning the samples.

Carotene loss ranged from 63 to 77% in the untreated samples. Treatment with 0.02% of Santoquin sharply reduced carotene loss, and a greater decrease occurred when 0.05% of the antioxidant was added. The meals which were the more unstable in the untreated condition, in general were also the more unstable in the presence of oil and antioxidant. This indicates that meals having poor storage characteristics probably will not respond so well to the action of an antioxidant as meals of inherently better keeping quality.

The effect of heat on meals treated with antioxidant and oil was variable. With samples 1, 5, and 6 heating resulted in an appreciable further increase in stability. Some response was obtained with samples 3 and 8, while the effect was negligible with 4 and 7. When response to heating was obtained, the effect was of similar magnitude with both levels of antioxidant. Thus, the results indicate that alfalfa meals may differ both in inherent carotene stability and in the extent to which this stability will be influenced by antioxidants and heat.

**Effect on Carotene Stability of Pelleting Oiled Meals.** In view of the improvement in carotene stability ob-

**Table II. Variation in Carotene Loss of Alfalfa Meals Treated with 16 Pounds of Wesson Oil per Ton and with Santoquin at Two Levels**

(Storage at 37° C. for 9 weeks)

No.	Obtained at	Variety	Cutting	Stage of Growth	Initial Carotene, Mg./100 G.	% Carotene Lost				
						No treatment	0.02% Santoquin		0.05% Santoquin	
						No heat	Heat	No heat	Heat	
1.	Dewey, Okla.	Unknown	1	Unknown	17.2	72	46	34	39	27
2.	Eureka, Kan.	Kansas Common	1	1/3 bloom	19.4	64	34	30	25	24
3.	Independence, Kan.	Kansas Common	1	1/2 bloom	18.1	63	26	22	20	16
4.	Neodesha, Kan.	Kansas Common	1	1/3 bloom	20.4	69	35	36	27	25
5.	Topeka, Kan.	Buffalo	2	Early bud	24.5	69	48	39	38	31
6.	St. Marys, Kan.	Kansas Common	2	Early bud	23.9	77	58	48	48	39
7.	Belvue, Kan.	Kansas Common	2	Early bud	22.7	75	39	39	29	26
8.	Wamego, Kan.	Kansas Common	2	Early bud	24.5	72	38	35	29	23

tained by heating oiled meal under laboratory conditions, it is of interest to know if a similar response can be expected under commercial conditions. As considerable heat is developed during the pelleting of meal, an experiment was conducted to determine if the heat so produced is sufficient to alter carotene stability when oil is applied to the meal prior to pelleting.

Alfalfa meal was treated with Santoquin at a rate of 0.05% and with Wesson oil at levels of 16 and 80 pounds per ton, as described above. Because the spraying and mixing equipment was designed for small batches, the operation was repeated until approximately 25 pounds of meal had been subjected to each treatment. Samples were removed for storage of the loose meal, and the remainder of each lot was passed through a California pellet mill which is part of the production facilities of a local milling company. The pellets (3/16-inch diameter) were too hot to be held in the hand immediately after pelleting. After standing for 2 hours in paper bags, the temperature at the center of the bags was 70° C.

The carotene stability of the meals and pellets during storage at 37° C. is shown in Table III. When the meal was oiled with 16 pounds of oil and treated with antioxidant, carotene loss in the unpelleted sample was 31% in 10 weeks, while its pelleted counterpart lost 21%. At 80 pounds per ton, the loose meal lost

20% and the pelleted sample lost 15% of the carotene.

In order to eliminate compactness of the pellets as a factor in carotene stability in the above experiment, some of the pellets were passed through a Wiley mill equipped with a 20-mesh screen. The ground pellets were stored and analyzed concurrently with the other samples. It will be seen in Table III that the ground pellets lost carotene at essentially the same rate as did the pellets. It had been demonstrated previously that pelleting untreated alfalfa meal had no stabilizing effect on carotene (4, 7). Hence, the stabilizing effect of the pelleting operation as observed in this study was not due to exclusion of air from the surfaces of the alfalfa particles. These data indicate that enough heat was generated during the pelleting operation to cause an increase in stability similar to the increase obtained by heating oiled meal under laboratory conditions. Perhaps commercial oiling and pelleting would produce a different magnitude of response than was obtained in this study, as the oil would be introduced prior to hammermilling, thus subjecting the meal to heat from both the milling and pelleting operations.

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**Table III. Effect of Pelleting on Carotene Stability of Alfalfa Meal Treated with Wesson Oil and Antioxidant**

(Storage at 37° C. for 10 weeks)

Treatment	Stored as	% Carotene Loss
Untreated meal	Meal	69
	Meal	31
	Pellets	21
16-lb. oil + 0.05% Santoquin	Ground pellets	24
	Meal	20
	Pellets	15
	Ground pellets	16