Availability to the Chick of the Carotene of Stabilized Alfalfa Meal

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The carotene of alfalfa meal was stabilized against oxidative deterioration during storage by adding 80 pounds of Wesson oil per ton and 0.02% of an antioxidant, and heating the meal at 100° C. for 1 hour. The heat treatment did not increase, measurably, the amount of isomerized carotene in the meal. With the chick as test animal, and the Association of Official Agricultural Chemists carotene method for determining feeding levels, the availability of the carotene of the stabilized and stored meals was similar to that of untreated meals whose initial carotene content was maintained by refrigeration.

THE STABILITY of the carotene in dehydrated alfalfa meal is improved appreciably by the addition of 4 or 5% of an animal fat or a vegetable oil to the meal (*5, 6, 8*). Further improvement of stability can be achieved by heating the oiled meal before placing it in storage. Thus, Mitchell, Beauchene, and Silker (*5*) observed a 49% loss of carotene in untreated meal stored at 25° C. for 3 months. Meal treated with Wesson oil at a rate of 80 pounds per ton lost 41% of its carotene under these conditions. Heating the oiled meal at 100° C. for an hour before storage reduced carotene loss to 29%, while the combined effect of 80 pounds of oil, 0.01% of antioxidant (Santoquin), and heat reduced carotene loss to 17%. Heat treatment probably caused greater penetration of the oil into the meal, resulting in more intimate contact between the native antioxidants and the carotene.

The effect of these treatments on carotene stability has been measured only by photometric means. However, high levels of oil in alfalfa meal were reported (*7*) to cause elution of noncarotene pigments during analysis by the Association of Official Agricultural Chemists (AOAC) method (*1*). Furthermore, carotenes undergo isomerization when heated. Also, colored oxidation products might be formed in stabilized meal which will contaminate the carotene fraction. Hence, the photometric determination of the carotene in stabilized alfalfa meal may not measure the quantity of carotene available for vitamin A synthesis by an animal. Therefore, chick growth experiments were performed to compare the biological availability of the carotene of stabilized and unstabilized alfalfa meals.

Preparation of Samples

Two samples of each meal were used for the comparison: untreated meal, and meal treated with antioxidant, oil and heat. When fed to the chicks, the untreated meals were to contain the original carotene content, as determined by the photometric method; the treated meals were to be reduced to about two thirds of the original potency by storage.

Alfalfa meals were treated with Santoquin (6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline) at the rate of 0.02% and the Wesson oil at the rate of 80 pounds per ton, using a technique described earlier (*2*). The treated meals were placed in wide-mouthed gallon jars, the jars were closed tightly, and placed in a circulating air oven at 100° C. Heat penetration was hastened by filling each jar about one-third full and rolling the jar frequently during the heating period. After an hour, the jars were removed from the oven and rolled frequently in a current of cool air to reduce the temperature quickly. Carotene losses resulting from the heat treatment were small, ranging from 0 to 3%. Portions of the original meals also were placed in jars and kept at −23° C. to prevent carotene loss. The samples of treated meal were stored at 37° C. and analyzed for carotene at intervals. When the carotene content (Table I) of the treated meals had decreased to about five sixths of the initial potency in the case of experiments I and II and to two thirds in experiments III and IV, the samples were transferred to cold storage (−23° C.).

β-Carotene is isomerized easily by heat. Therefore, a study was made to determine whether the experimental heat treatment had caused isomerization in addition to that which occurred when the fresh alfalfa was dehydrated. Stereoisomeric analyses were performed on the alfalfa meals used in experiments I and II by a slight modification of the method of Bickoff *et al.* (*3, 4*). The lime adsorbent available for this work failed to give clear-cut separation of the isomers when the bands were eluted successively, a difficulty also encountered by others (*7*). The procedure therefore was modified to the extent that the bands were retained on the column and were separated mechanically by extruding the adsorbent, carefully carving out each band, and eluting the separated portions with acetone. The stereoisomeric composition of the total carotene of the meals of experiments I and II is shown in Table II. Little, if any, additional isomerization occurred during the heat treatment.

Chick Feeding Experiments

Day-old Leghorn male chicks were inoculated with Newcastle virus vaccine, randomized into the desired number of groups, and placed in batteries, with wire screen floors, and kept in a controlled-temperature animal laboratory. Replicated groups were used for testing each feed. The chicks were fed a basal ration (Table III) to reduce their vitamin A stores. After 1 week, the chicks were weighed and placed on the exper-

Table I. Carotene Content of Alfalfa Meals at Time of Feeding

(Milligrams per 100 grams)

Expt.	Meal	Untreated Meal, Refrigerated	Treated Meal, Stored at 37° C.	Carotene Reduction by Storage, %
I and II[a]	A	22.3	18.5	17
	B	19.5	16.7	14
III	C	35.0	23.6	33
	D	18.9	11.7	38
IV	E	23.4	15.3	35
	F	30.7	21.7	29

[a] Meals were the same in experiments I and II.

Table II. Stereoisomeric Composition of the Carotene of Alfalfa Meals Used in Experiment I

Meal	Neo-β-carotene B, %	β-Carotene, %	Neo-β-carotene U, %
A untreated	24	59	17
A treated	23	59	18
B untreated	25	55	20
B treated	22	54	24

Table III. Basal Diet

	Pounds	Grams
White corn	59	
Soybean oil meal (44% solvent)	31	
Nonfat dry milk solids	2	
Brewer's yeast	2	
Steam bone meal	2	
Calcium carbonate	1	
Iodized salt	0.5	
Vitamin premix[a]		800
Wesson oil		300
Manganese sulfate		25

[a] Vitamin premix. Vitamin B_{12}, 1 mg.; Delsterol, 40 grams; riboflavin, 0.4 gram; niacin, 2 grams; calcium pantothenate, 1 gram; menadione, 50 mg.; choline chloride, 50 grams; and wheat shorts to make to 800 grams.

imental rations. The latter consisted of the basal ration to which the proper amount of an alfalfa meal was added to supply 400 units of vitamin A potency per pound of ration. The quantities of the meals needed to supply this amount were calculated from their carotene contents as determined by the AOAC photometric method. Four hundred units of vitamin A potency per pound of feed was used in order to maintain a sufficiently low vitamin A intake to cause a nutritional stress. In this way, it was believed differences in response would be more readily apparent.

Negative and positive controls were included in each feeding trial. Chicks in the negative control groups were fed the basal ration, while those in the positive control groups received the basal, plus sufficient untreated alfalfa, to contribute 3000 units of vitamin A potency per pound of ration. All rations were freshly prepared at weekly intervals, at which time the unconsumed portion of the previous mix was weighed for feed consumption records and discarded. Thus, the carotene content of feeds was close to the calculated level throughout the feeding trials. The alfalfa meals were kept in the freezing compartment of a refrigerator during the trials to prevent carotene loss. Carotene determinations were made at various times during the feeding period to verify the potency of the meals.

The chicks were weighed at 2-week intervals over a period of 8 weeks. Observations were made of deaths, eye condition, muscle incoordination, and other vitamin A deficiency symptoms. Part of these data are presented in Table IV.

Average weights of birds fed the treated meals were, in general, as good or slightly better than weights of those fed the untreated meals. Feed conversion (feed consumed/weight gain) of the birds fed the two types of meals were similar. Mortalities were no greater in the groups fed the rations containing the treated meals. In experiment II, several chicks did poorly, indicating presence of a low-level infection, but there appeared to be no differences in this respect due to type of meal used. Birds getting 3000 units of vitamin A per pound (positive control) grew somewhat better than the other birds in each experiment, but in experiment IV they did not grow markedly better than some of the other groups. Almost all of the chicks which received the basal ration only (negative controls) died in less than 28 days.

Incoordination due to vitamin A deficiency was not observed in chicks receiving 400 units of vitamin A potency from alfalfa meal. In only one of the four experiments were eye lesions noted in groups receiving the alfalfa meals. The condition was pronounced in 5% of the chicks, while in an additional 25% it consisted of only a slight watering of the eyes. There were no more cases in groups receiving treated meal than in those receiving untreated alfalfa.

With a possible exception in the case of positive controls, the alfalfa added to the ration is believed not to contribute an important amount of an essential nutrient except vitamin A. The various experimental treated and untreated rations contained only 0.2 to 0.4% of alfalfa.

These data indicate that use of oil, antioxidant, and heat to improve stability of carotene in alfalfa meal, as measured by photometric means, does not adversely affect the utilization of the carotene by chicks.

Literature Cited

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Table IV. Growth, Feed Conversion, and Mortality of Chicks Fed Treated and Untreated Alfalfa Meals as Sources of Vitamin A Activity

Expt.[a]	Item	Untreated 1[b]	Untreated 2	Treated 1	Treated 2	Untreated 1	Untreated 2	Treated 1	Treated 2	Positive Control
		Sample A				Sample B				
I	Wt., grams, 8 wk.	820	850	905	825	883	870	891	947	1072
	Feed conversion	3.2	3.1	2.9	3.1	3.0	3.0	2.8	3.0	2.7
	Mortality	1	0	2	0	0	0	0	0	0
II	Wt., grams, 8 wk.	786	799	801	828	747	815	799	784	946
	Feed conversion	3.4	3.2	3.3	3.3	3.4	3.2	3.2	3.3	3.2
	Mortality	0	3	1	1	2	0	0	0	0
		Sample C				Sample D				
III	Wt., grams, 8 wk.	896	895	910	885	889	911	854	931	997
	Feed conversion	2.8	2.8	2.9	2.9	3.1	2.8	2.9	2.8	2.7
	Mortality	1	0	1	0	1	1	0	1	0
		Sample E				Sample F				
IV	Wt., grams, 8 wk.	1014	1008	1011	987	988	999	1042	1025	1069
	Feed conversion	2.7	2.7	2.7	2.8	2.8	2.8	2.7	2.7	2.7
	Mortality	0	0	0	0	0	0	1	1	0

[a] 15 white Leghorn males per group in experiments I, II, and III; 16 heavy strain high-line Leghorn males per group in experiment IV. Experimental period was 8 weeks following 1-week depletion period.
[b] Numbers 1 and 2 designate replicated groups in each experiment.