Alpha-Tocopherol Content of Feedstuffs

R. H. Bunnell, J. P. Keating, and A. J. Quaresimo

To investigate the α-tocopherol content of a variety of animal feed products, an analytical method utilizing secondary magnesium phosphate was applied to a number of commercially available feedstuffs, with special attention to the accurate assay of alfalfa utilizing several methods of assay. The α-tocotrienol content of oats, barley, wheat, milo, and corn was also investigated; oats and barley contained significant amounts. With the values obtained in this study, the α-tocopherol content of a variety of animal rations was calculated to show the three- to four-fold range which can occur. The factors which should be considered in evaluating the vitamin E value of animal rations include natural variability, biological availability and value of α-tocopherol in feedstuffs, and environmental stresses on the animal.

A large number of tocopherol assays of foods and feedstuffs have been reported, using a variety of analytical techniques. Recently, Dicks (1965) made a comprehensive survey of the reported values for the tocopherol content of foods and feedstuffs. While the amount of data is impressive, this survey points up the lack of characterization of individual tocopherols in the majority of analyses cited. Our present knowledge of analytical methodology indicates the doubtful value of a total tocopherol analysis of a food or feedstuff, particularly in providing a reliable estimate of the biological vitamin E value.

Recent refinements and advances in tocopherol analysis have identified eight naturally occurring tocopherols and clarified their nomenclature and structure (Pennock *et al.*, 1964). The presence of tocopherols other than α-tocopherol, as well as the prevalence of nontocopherol-reducing substances in natural products, has led to analytical examination of these materials by techniques capable of separating and measuring α-tocopherol accurately: paper chromatography (Analytical Methods Committee, 1959); thin-layer chromatography (Bieri and Prival, 1965; Lambertsen *et al.*, 1962; Sturm *et al.*, 1966); gas-liquid chromatography (Bieri and Andrews, 1963; Ishikawa and Katsui, 1966; Nair and Turner, 1963; Slover *et al.*, 1967; Wilson *et al.*, 1962); and column chromatography (Bro-Rasmussen and Hjarde, 1957a). A complete compilation of vitamin E assay by chemical methods has been published by Bunnell (1967).

SELECTION OF FEED SAMPLES

Samples of feedstuffs selected on the basis of usage in the feed industry were obtained from feed suppliers in a number of localities in the United States. Samples of each type were collected and assayed during the same time periods. All corn samples, for example, were from the same year and had similar storage histories.

A wide geographical distribution of samples was obtained, where possible. Table I indicates the geographical origin and harvest year of the whole grains and millfeeds. The oilseed meals and animal products were selected from the same production year, generally 1965 and 1966.

Hoffmann-La Roche, Inc., Nutley, N.J. 07110

METHODS OF ASSAY

Basic Methodology. The method used was the secondary magnesium phosphate column chromatography technique of Bro-Rasmussen and Hjarde (1957b), which has been employed in the assay of liver tissue by Pudelkiewicz *et al.* (1960) and Dicks and Matterson (1961), of vegetable oils by Herting and Drury (1963), and of foods by Bunnell *et al.* (1965). It has yielded recoveries of 95 to 100% in separating α-tocopherol in natural products. The analysis entailed extraction of the sample, saponification without neutralization, re-extraction of the unsaponifiables in Skellysolve B, chromatography on Florex XXS, separation of α-tocopherol by column chromatography on secondary magnesium phosphate, and colorimetric measurement by the Emmerie-Engel method (Bunnell, 1967).

Because of the presence of large amounts of fat in some samples it was necessary to modify the saponification step by eliminating neutralization of the KOH. Rapid handling in cooling and extracting the saponified sample was necessary to avoid loss of tocopherol due to oxidation.

The use of Teflon stopcocks, etc., circumvented the need for stopcock grease, which caused loss of tocopherol separation on the magnesium phosphate column.

The method proved accurate and efficient in the determination of α-tocopherol in the absence of α-tocotrienol, but the quantitative estimation of other tocopherols was unreliable. While recovery of total tocopherols of a standard charge was 100%, there was overlapping in the elution of the tocopherols other than α. The difficulty in obtaining an activated magnesium phosphate which could

Table I. Origin of Feedstuffs

Feedstuff	Crop Year	Geographical Origin
Whole yellow corn	1965	Delaware, Illinois, Iowa, Minnesota, Nebraska, New Jersey, Washington
Whole milo	1965	Arizona, Kansas, Missouri, Texas
Whole wheat	1965	Idaho, Illinois, Kansas, Maryland, Montana, Washington
Wheat mill feeds	1966	Idaho, Illinois, Kansas, Montana

quantitatively separate β- and γ-tocopherols (4% ether fraction) and δ-tocopherol (7% ether fraction) led to the decision to determine only the α-tocopherol content of feeds.

Special Methodology. ALFALFA. Initial attempts to apply these methods to alfalfa showed a variance between the total reductant level (nonchromatographed value), the total tocopherol level (sum of all fractions from magnesium phosphate chromatography), and the α-tocopherol level (2% ether fraction from magnesium phosphate chromatography) indicating the presence of reducing artifact and possibly other tocopherols. Earlier analyses using paper chromatography and secondary magnesium phosphate chromatography (Booth, 1963; Brown, 1952; Thafvelin and Oksanen, 1966) demonstrated only α-tocopherol present in forage grasses.

A more extensive investigation of the alfalfa products to substantiate the α-tocopherol levels determined by magnesium phosphate chromatography included application of paper and gas-liquid chromatography.

Paper Chromatography. The two-dimensional analysis method of the Analytical Methods Committee (1959) was used. The first dimension is absorption chromatography on zinc carbonate–coated papers. The second is reversed-phase partition chromatography.

Gas-Liquid Chromatography. The development of gas-liquid chromatography in the determination of tocopherol provided another means of examining alfalfa composition. Details concerning the determination of tocopherol by gas-liquid chromatography can be found in the review by Bunnell (1967). Gas-liquid chromatography, using conditions listed in Table II, was run on a number of fractions isolated from alfalfa samples.

Standard tocopherols were used in quantitation of the α-tocopherol in the sample. The column required saturation with the tocopherols to produce a constant detector response. In general, five to six 10-μl. injections of mixed standards—α-, γ-, and δ-tocopherols, 0.1% in analytical reagent grade carbon disulfide—sufficed.

Quantitative data were more reliable when the standard and sample tocopherols were in the same dose range. The direct calibration technique utilizing α-tocopherol standard was necessitated by the number and size of the peaks resolving prior to α-tocopherol in the alfalfa extract. This precluded the use of an internal standard, such as cholestane or octacosane. Judicious utilization of the standard tocopherol, for example, in bracketing the unknown analysis with standard chromatograms using the same attenuation, injection volume, and quantity of tocopherol produced accurate, reproducible results.

Table II. Conditions Used for Gas-Liquid Chromatography of Tocopherols

Instrument	F & M Model 500 with 1609 attachment
Detector	Hydrogen flame ionization
Column	6 foot copper, 1/4 inch O.D.
Stationary phase	2% SE-30 on Diatoport S, 80- to 100-mesh
Operating temperatures, ° C.	
Injector port	250
Detector	265
Column	210
Gas flow rates, ml./min.	
Carrier (helium)	70
Hydrogen	40
Purge gas (air)	300
Electrometer sensitivity Maximum 4×10^{-12}	8×10^{-11}
Scale deflection, %	50 to 60
Sample size	1 to 5 μg. in 0.005 ml. of carbon disulfide

Tocotrienols. Pennock *et al.* (1964) demonstrated the presence in nature of four methylated tocols and four related unsaturated tocotrienols. This has led to an increased interest in these tocotrienols in food and feed products. Slover *et al.* (1967) applied a gas chromatographic analysis to soybean oil, wheat germ oil, whole wheat flour, and corn germ meal.

The determination of α-tocopherol in natural products by secondary magnesium phosphate column chromatography does not separate α-tocopherol from α-tocotrienol. To assure a more definitive value, feedstuffs reported to contain tocotrienols were analyzed by two-dimensional, thin-layer chromatography (Whittle and Pennock, 1967). This chromatography was performed on Eastman Chromatogram sheets (No. 6060, silica gel with fluorescent indicator) and provided a rapid and effective qualitative determination of the tocopherols present in the product. Where a significant amount of α-tocotrienol (5,7,8-trimethyl tocotrienol) was found, further quantitative assays were performed by two-dimensional paper chromatography employing the method described by Dicks-Bushnell and Davis (1967).

RESULTS

The α-tocopherol contents of the feedstuffs assayed are presented in Table III. Using these values, the α-tocopherol content of a variety of typical animal rations (compositions of these rations may be obtained by writing to the authors) were calculated (Table IV). To calculate vitamin E content in International Units, the National Formulary chemical equivalence of 1 mg. of *d*-α-tocopherol ≅ 1.49 I.U. was used.

Special studies confirmed that only α-tocopherol was found in alfalfa. Paper chromatographic studies of alfalfa extracts, carried through all stages of purification except magnesium phosphate chromatography, revealed a spot which had an R_f value similar to γ-tocopherol in the first dimension but did not migrate in the second dimension. This spot gave both positive Emmerie-Engel and *o*-dianisidine reactions. Similar paper chromatography of the 2% ether eluate from the magnesium phosphate chromatography of alfalfa extracts revealed the presence of α-tocopherol only, with no other reducing spots. Paper chromatography of the 4% ether eluate, which normally contains β- and γ-tocopherol, revealed the absence of tocopherols but the presence of the nontocopherol spot described above. Recently Herting and Drury (1967) also noted the presence of an unidentified reducing

Table III. Alpha-Tocopherol Content of Feedstuffs

Feed	Number of Samples	High Value, Mg./100 G.	Low Value, Mg./100 G.	Mg./100 G. Average of All Samples
Alfalfa				
Dehydrated 17%	6	12.10	2.80	7.27
Dehydrated 20%	6	14.10	3.78	8.90
Sun-cured 13%	4	6.11	1.76	4.07
Alfalfa hay	2	5.29	5.24	5.27
Barley, whole[a]	6	4.28	2.17	3.63
Brewer's grains, dried	5	4.77	1.74	2.68
Brewer's yeast, dried	1	0.20	...	...
Corn, whole yellow	17	3.50	1.14	1.99
Corn gluten feed	5	2.62	0.69	1.48
Corn gluten meal	5	3.91	1.25	2.59
Cottonseed meal	6	1.61	0.25	0.92
Distiller's dried grains	4	3.99	1.72	3.05
Distiller's dried corn solubles	2	5.67	5.49	5.58
Fat, animal	7	1.59	0.23	0.79
Fat, hydrolyzed animal and vegetable	3	6.90	4.91	5.68
Fat, poultry	2	2.39	1.39	1.89
Fish meals				
Herring	3	3.14	0.81	1.68
Menhaden	3	1.27	0.11	0.57
Peruvian	2	0.33	0.09	0.21
Others	3	0.89	0.33	0.56
Grape pomace, fermented	1	7.73	...	...
Linseed meal	6	1.04	0.32	0.77
Malt sprouts	1	0.42	...	0.42
Meat and bone meal	7	0.16	0.07	0.08
Milo, whole	12	1.59	1.02	1.22
Molasses, cane	3	0.87	0.34	0.54
Oatmeal, feeding[a]	2	2.46	2.25	2.36
Oats, whole[a]	4	2.36	1.78	2.05
Peanut meal	1	0.29	...	0.29
Poultry by-products meal	3	0.41	0.11	0.22
Rice, brown	2	1.42	1.28	1.35
Rice bran	2	8.72	3.43	6.08
Rice hulls	1	0.74	...	0.74
Rice polish	1	8.95	...	8.95
Safflower meal	1	0.08	...	0.08
Soybean hull flakes	1	0.66	...	0.66
Soybean meal, solvent process				
44% protein	8	0.49	0.15	0.30
50% protein	9	0.52	0.13	0.33
Unspecified	4	0.32	0.13	0.22
Wheat, whole	4	1.47	0.32	1.11
Wheat bran	2	1.90	1.52	1.71
Wheat middlings	4	3.40	1.93	2.68
Wheat mill run	4	4.40	2.11	3.17
Wheat Red Dog	1	3.55	...	3.55

[a] Values include α-tocotrienol, see Table VI.

Table IV. Calculated Alpha-Tocopherol Content of Typical Animal Rations

Ration	α-Tocopherol, Mg./Lb.			Vitamin E, I.U./Lb.[a]		
	High	Low	Av.	High	Low	Av.
Chicken laying	12.46	4.17	7.27	18.57	6.22	10.83
Chick breeder	10.60	4.08	6.80	15.79	6.08	10.13
Broiler starter	11.05	3.83	6.63	16.47	5.70	9.88
Broiler grower	11.93	4.23	7.09	17.78	6.30	10.57
20% chick starter	12.11	3.97	7.11	18.05	5.91	10.59
17% chick grower	13.77	4.73	5.72	20.52	7.05	8.53
14% pullet grower	15.03	5.40	9.25	22.39	8.05	13.78
Turkey starter	9.77	2.98	5.79	14.56	4.44	8.62
Pig prestarter	5.29	1.74	3.05	7.88	2.60	4.54
Swine growing & finishing	12.95	4.19	7.36	19.29	6.25	10.97
Swine lactation 1	17.90	5.46	10.59	26.67	8.14	15.78
Swine lactation 2	14.94	5.38	9.18	22.26	8.02	13.68
Dairy cattle 1, 18% protein	10.94	5.08	7.51	16.30	7.57	11.19
Dairy cattle 2, 15% protein	7.84	4.81	6.07	11.68	7.17	9.05
Beef cattle, 9% protein	7.77	4.66	5.93	11.58	6.95	8.83

[a] Calculated values based on chemical equivalency of 1 mg. of *d*-α-tocopherol $\cong$ 1.49 I.U.

substance in alfalfa which chromatographed like α-tocopherol on alumina-coated sheets and could therefore result in an overestimate of the amount of α-tocopherol present.

Gas chromatography of the 2% ether eluate from the magnesium phosphate chromatography confirmed the presence of only α-tocopherol, in quantitative agreement with the Emmerie-Engel assay (Table V). A peak occurs in the gas chromatogram of alfalfa extracts, which has the same retention time as γ-tocopherol, but this fraction is an artifact with no reducing properties.

Qualitative two-dimensional paper chromatographic analyses of milo, corn, whole wheat flour, barley, and oats revealed the presence of considerable quantities of α-tocotrienol in oats and barley, a small amount in whole wheat flour, and none in milo or corn (Figure 1). An authentic sample of synthetic *dl*-α-tocotrienol was used as a reference standard. Using gas-liquid chromatography, Slover *et al.* (1967) reported significant amounts of α-tocotrienol in corn meal. We were unable to detect α-tocotrienol in any of the whole corn samples in this study. The quantity of α-tocotrienol in the wheat flour sample was too small for quantitative paper chromatography. The oat and barley samples were quantitatively assayed by two-dimensional paper chromatography of the 2% ether eluate of the magnesium phosphate column. Since this eluate contains only α-tocopherol and α-tocotrienol, the quantitative estimation was simplified. The relative amounts of α-tocopherol and α-tocotrienol in single samples of whole oats and barley are shown in Table VI.

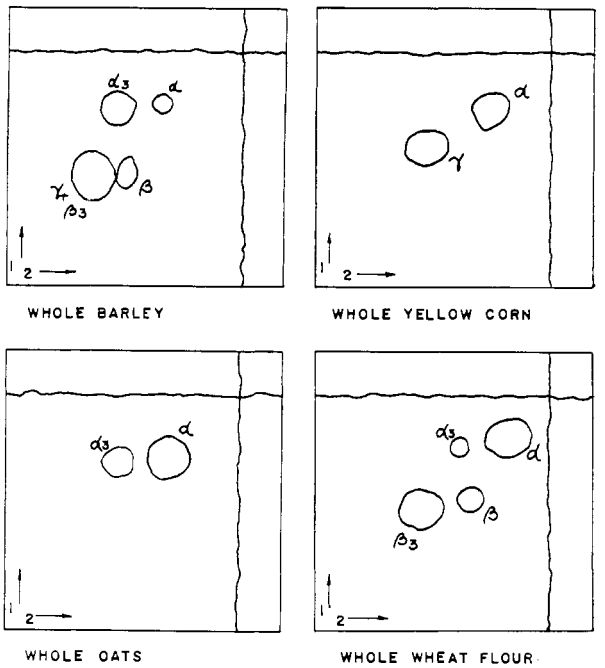

Figure 1. Two-dimensional thin layer chromatography of four feedstuffs

1 = 1st dimension
2 = 2nd dimension

Subscript 3 indicates tocotrienol

DISCUSSION

While there are many values for the tocopherol content of feedstuffs in the literature (Dicks, 1965), the data presented here provide comparative information on the α-tocopherol content of many common feedstuffs as determined by a single method of assay of accepted reliability. The considerable variation in the α-tocopherol content of many feedstuffs can result in a three- to fourfold difference in α-tocopherol content in typical animal rations (Table IV).

Other observations, revealed during the course of this study, were the importance of modifying the procedures to suit each particular feedstuff and verifying results by cross-checking with other methods. Constant vigilance in the latter respect is important when employing secondary magnesium phosphate column chromatography to ensure that the 2% ether eluate contains only α-tocopherol.

Table V. Alfalfa Assays

Sample	Total Reductants,[a] Mg./100 G.	Total "Tocopherols,"[b] Mg./100 G.	α-Tocopherol, Mg./100 G.	
			Column chrom.	GLC
1	30.9	24.4	10.0	9.9
2	47.4	34.2	15.2	14.5
3	26.0	24.9	10.5	11.2

[a] Nonchromatographed value.
[b] Sum of all fractions from magnesium phosphate chromatography.

Table VI. Alpha-Tocotrienol Content of Oats and Barley

	α-Tocopherol + α-Tocotrienol, Mg./100 G.	α-Tocopherol, %	α-Tocotrienol, %
Whole oats	4.12	64	36
Whole barley	4.08	30	70

The importance of this is illustrated in the case of alfalfa (Table V), where the total tocopherol value is meaningless because of the presence of artifacts. The α-tocopherol value is also subject to error due to leakage of reducing artifacts if a column of improper activity is used.

Special attention was given to alfalfa to ensure accurate assays, because this material is potentially the richest source of α-tocopherol of common feedstuffs. In view of the difficulties encountered, literature values for the tocopherol content of alfalfa should be critically evaluated in the light of the analytical techniques used. Unfortunately, the α-tocopherol content of dehydrated alfalfa meal can range widely. In this study, a range of 2.80 to 14.10 mg. of α-tocopherol was found for 12 samples of 17 and 20% dehydrated alfalfa meal. This variation is probably due to stage of cutting, length of time between cutting and dehydration, conditions and length of storage, etc. The importance of minimizing the length of time between cutting and dehydration was demonstrated by Van der Veen and Olcott (1967), who showed the rapid change of fatty acids in alfalfa lipids after cutting. In a similar study with field-cured hay, Thafvelin and Oksanen

(1966) showed the differences in linolenic acid and tocopherol content in sun-cured, low-moisture hay *vs.* slower cured, higher moisture hay. The former lost 50% of its vitamin E during a 1-month storage, but there was no change in linolenic acid content. Hay dried in swaths under moist conditions lost 60% of its vitamin E and 50% of its linolenic acid within 4 days of cutting. Such hay has been shown to be capable of producing nutritional muscular degeneration when fed to ruminants.

In addition to losses on drying, Brown (1953) has shown that the tocopherol content of timothy hay can vary depending on age at harvest; timothy grass contained as much as 1.51 mg. % of tocopherol on June 17, but not more than 0.55 mg. % on July 7. Thafvelin and Oksanen (1966) showed similar results. On the other hand, Booth (1964) has shown that the tocopherol content of lucerne can increase the first day after cutting, followed by a decrease. Harvesting in bright sunshine or bruising of the leaves, however, can reduce or eliminate this short-lived, postharvest increase of tocopherol content.

Another factor which should be taken into account, particularly for poultry, is the availability of tocopherol from alfalfa. Bunnell (1957) first noted that only about one third of the tocopherol in alfalfa appeared to be utilized by the chick, as measured by liver storage. This observation has been confirmed by more extensive studies of Pudelkiewicz and Matterson (1960). Recently, Olson *et al.* (1966) isolated a compound from alfalfa lipids that inhibited tocopherol deposition in chick tissues. Aside from alfalfa, certain legumes—kidney beans (Desai, 1966; Hintz and Hogue, 1964) and peas (Sanyol, 1953)—have been reported to have an antivitamin E or E-absorption inhibiting effect. There have been no reports, to the authors' knowledge, of inhibition of absorption of vitamin E from grains or grasses.

Since corn and milo are important grains in the United States, a large number of samples were assayed. Corn appears to be a better source of α-tocopherol than milo. Corn is variable in α-tocopherol content, but can be an important source for feeds, particularly poultry feeds which often contain high percentages of corn. Herting and Drury (1963) reported that corn oil can be very variable in both total tocopherol and the ratio of α- to γ-tocopherol. The percentage of α-tocopherol can range from 5 to 47%, so that it is difficult to predict the vitamin E value of a particular corn sample. Although an average value can be used for calculating the vitamin E content of a ration, this value can be subject to wider variation than that of most other grains.

Wheat, particularly wheat by-products such as middlings and mill run, can contribute to the vitamin E content of a ration, but these by-products are not generally used in as high a concentration as corn. Oats contain somewhat less α-tocopherol and a considerable amount of α-tocotrienol. Barley also contains considerable amounts of α-tocotrienol and is higher in tocopherol than oats. The protein meals cannot be regarded as important sources of α-tocopherol, with the possible exception of corn gluten meal.

Other factors, besides the actual calculated vitamin E content, must be taken into consideration in deciding upon the adequacy of a particular ration with respect to vitamin E. Allowance should be made for the fact that the natural unesterified α-tocopherol is prone to oxidative destruction, which can be accelerated by the grinding of grains, by stresses such as added minerals and fats in mixed feeds, and by pelleting. When the vitamin E content of a ration is calculated in International Units, using the value of 1.49 I.U. per mg. of *d*-α-tocopherol, this represents a theoretical vitamin E value based on chemical equivalency to the corresponding acetate ester. While the biological value of *d*- and *dl*-α-tocopheryl acetates has been well established, the full theoretical, biological value of unesterified α-tocopherol may be more difficult to achieve under practical feeding conditions. This was demonstrated by Harris and Ludwig (1949), who reported that, under certain conditions of testing, *d*-α-tocopherol had a potency of 0.92 I.U. in the rat resorption bioassay in contrast to a theoretical potency of 1.49 I.U. This apparently can be variable between species, because Week *et al.* (1952) showed that *d*-α-tocopherol was somewhat more efficiently utilized by man than *d*-α-tocopheryl acetate. These differences may be related to gastrointestinal conditions in the animal.

Calculations of the α-tocopherol content of typical animal rations (Table IV) reveal that deficiency states could result under certain conditions of stress because of borderline vitamin E content. While calculating the vitamin E potency of a feedstuff or ration based on the α-tocopherol results in a variable underestimate of the actual vitamin E potency, the contribution of the other tocopherols on a practical feeding basis is difficult to assess. The monomethylated tocopherols, such as δ-tocopherol and δ-tocotrienol, can be ignored because their contribution is negligible. While the other tocopherols and tocotrienols show some activity by classical bioassay procedures, the results are more variable than for α-tocopherol. This fact, coupled with the difficulty of assaying accurately for all eight tocopherols in feedstuffs, makes it impractical to quantitate their contribution to the vitamin E potency of a feedstuff. Some form of bioassay, such as liver storage, on the complete ration would be easier to carry out. More work of this type should be done to determine the degree of correlation between the chemical and biological assay of a complete ration, taking into account the contribution of the non-α-tocopherols and revealing any incomplete assimilation of the tocopherols from the ration.

There is still much to be learned about the practical importance and the actual extent of vitamin E deficiency among farm animals under field conditions. Ill-defined, apparent vitamin E-deficiency conditions in commercial poultry and turkey flocks and dairy herds have shown rather dramatic responses to high level vitamin E supplementation. Unfortunately, these studies are usually uncontrolled and unpublished. In many cases, however, a probable vitamin E deficiency has been partially confirmed by the finding of very low plasma tocopherol levels in the affected animals. Often, chemical assay of the ration being fed these animals reveals an apparently adequate level of vitamin E. These field conditions may also be complicated by low selenium levels in the rations, which could result in a higher requirement for vitamin E (Scott and Thompson, 1967).

Published reports are beginning to appear showing significant growth responses of beef cattle to supplemental vitamin E on rations which would normally be considered adequate in vitamin E, based on chemically determined tocopherol. Chapman *et al.* (1964) reported increased performance when vitamin E was fed at the rate of 50 I.U. daily to cattle on limited and full-fed concentrate rations. Recently, Dyer (1967) demonstrated improvements in average daily gain of steers on hay-concentrate rations with supplemental vitamin E. Interestingly, steers on a 100% alfalfa ration did not respond to vitamin E. Increasing responses to supplemental vitamin E were obtained on rations containing increasing proportions of wheat in the ration. For example, steers receiving 100% alfalfa with supplemental vitamin E showed an average daily gain of 1.49 pounds, while steers receiving a 40% alfalfa–60% wheat ration with supplemental vitamin E showed an average daily gain of 1.92 pounds. The fact that lowering the alfalfa content enabled the animals to show a response to supplemental vitamin E again raises the question of inhibitory factors in alfalfa which limit vitamin E absorption. Dyer's statement that "the need for vitamin E by cattle fed rations containing concentrates has been demonstrated many times. It is no longer a question as to whether or not vitamin E is beneficial for cattle but rather how much should be fed and what feeding regimes may accentuate or depress the effects of vitamin E," again emphasizes the need for more biological studies to correlate the chemically determined vitamin E content of a ration with the vitamin E actually utilized physiologically by the animal from practical commercial rations.

Although a bioassay, such as liver storage, is a measure of absorption of tocopherol, other biological parameters are needed to equate liver and blood tocopherol levels with the optimum physiological needs of the animal under practical commercial farm conditions, because the effect of different stresses on vitamin E requirements of farm animals on practical feeding regimes is not well known, with the exception of the well-documented stress of unsaturated fatty acids in increasing vitamin E requirements. The effect of other stresses will undoubtedly be learned in the future. An example of this is the demonstration by O'Dell *et al.* (1960) that nitrite can cause a vitamin E deficiency in rats even though normal levels are present in the feed.

The general practice in modern animal husbandry appears to place less dependence on the natural vitamin content of a ration as the sole source, but to supplement on a reasonable basis as nutritional insurance, particularly in the beef cattle industry where routine supplementation with vitamin A has been established as economically sound. A similar trend is beginning to be evident with vitamin E, where the use of the commercial *d-* or *dl-*α-tocopheryl acetate form renders supplementation simple and free from stability problems, since the acetate ester, unlike the free tocopherol form, is not subject to oxidation.

CONCLUSIONS

In the long range, an intelligent reliance on the natural vitamin E content of a ration based on reliable knowledge of both true content and biological value, coupled with sound supplementation practices, is probably the best approach for the animal industry. More knowledge is needed, however, of not only the accurate vitamin E content of feeds as determined by chemical assay, but also the full biological significance of this value as measured by practical biological parameters.

ACKNOWLEDGMENT

The authors are indebted to H. S. Wilgus of Hoffmann-La Roche, Inc., for assistance in locating sources of feedstuffs and to the many feed manufacturers who supplied the samples.

LITERATURE CITED

Analytical Methods Committee, *Analyst* **84,** 356 (1959).
Bieri, J. G., Andrews, E. L., *Iowa State J. Sci.* **38,** 3 (1963).
Bieri, J. G., Prival, E. L., *Proc. Soc. Exptl. Biol. Med.* **120,** 554 (1965).
Booth, V. H., *Analyst* **88,** 627 (1963).
Booth, V. H., *Phytochemistry* **3,** 273 (1964).
Bro-Rasmussen, F., Hjarde, W., *Acta Chem. Scand.* **11,** 34 (1957a).
Bro-Rasmussen, F., Hjarde, W., *Acta Chem. Scand.* **11,** 44 (1957b).
Brown, F., *Biochem. J.* **51,** 237 (1952).
Brown, F., *J. Sci. Food Agr.* **4,** 161 (1953).
Bunnell, R. H., *Poultry Sci.* **36,** 413 (1957).
Bunnell, R. H., "The Vitamins," 2nd ed., Vol. **VI,** Paul György and W. N. Pearson, Eds., p. 261, Academic Press, New York, 1967.
Bunnell, R. H., Keating, J., Quaresimo, A., Parman, G. K., *Am. J. Clin. Nutr.* **17,** 1 (1965).
Chapman, H. L., Jr., Shirley, R. L., Palmer, A. Z., Haines, C. E., Carpenter, J. W., Cunha, T. J., *J. Animal Sci.*, **23,** 669 (1964).
Desai, I. D., *Nature* **209,** 810 (1966).
Dicks, M. W., *Wyoming Agr. Expt. Sta., University of Wyoming Bull.* **435,** 1965.
Dicks, M. W., Matterson, L. D., *J. Nutr.* **75,** 165 (1961).
Dicks-Bushnell, M. W., Davis, K. C., *Am. J. Clin. Nutr.* **20,** 262 (1967).
Dyer, I. A., *Feedstuffs* **39,** 20 (March 25, 1967).
Harris, P. L., Ludwig, M. I., *J. Biol. Chem.* **181,** 611 (1949).
Herting, D. C., Drury, E. E., *J. Chromatog.* **30,** 502 (1967).
Herting, D. C., Drury, E. E., *J. Nutr.* **81,** 335 (1963).
Hintz, H. F., Hogue, D. E., *J. Nutr.* **84,** 3 (1964).
Ishikawa, S., Katsui, G., *J. Vitaminol.* **12,** 106 (1966).
Lambertsen, G., Myklestad, H., Braekken, O. R., *J. Sci. Food Agr.* **13,** 617 (1962).
Nair, P. P., Turner, D. A., *J. Am. Oil Chemists' Soc.* **40,** 353 (1963).
O'Dell, B. L., Erch, Z., Flynn, L., Garner, G. B., Muhrer, M. E., *J. Animal Sci.* **19,** 1280 (1960).
Olson, G., Pudelkiewicz, W. J., Matterson, L. D., *J. Nutr.* **90,** 199 (1966).
Pennock, J. F., Hemming, F. W., Kerr, J. D., *Biochem. Biophys. Res. Commun.* **17,** 542 (1964).
Pudelkiewicz, W. J., Matterson, L. D., *J. Nutr.* **71,** 143 (1960).
Pudelkiewicz, W. J., Matterson, L. D., Potter, L. M., Webster, L., Singsen, E. P., *J. Nutr.* **71,** 115 (1960).
Sanyol, S. N., *Calcutta Med. J.* **50,** 409 (1953).
Scott, M. L., Thompson, J. N., *Feedstuffs* **39,** 20 (December 23, 1967).
Slover, H. T., Shelley, L. M., Burks, T. L., *J. Am. Oil Chemists' Soc.* **44,** 161 (1967).
Sturm, P. A., Parkhurst, R. M., Skinner, W. A., *Anal. Chem.* **38,** 9 (1966).
Thafvelin, B., Oksanen, H. E., *J. Dairy Sci.* **49,** 282 (1966).
Van der Veen, J., Olcott, H. S., J. AGR. FOOD CHEM. **15,** 682 (1967).
Week, E. F., Sevigne, F. J., Ellis, M. E., *J. Nutr.* **46,** 353 (1952).
Whittle, K. J., Pennock, J. F., *Analyst* **92,** 423 (1967).
Wilson, P. W., Kodicek, E., Booth, V. H., *Biochem. J.* **84,** 524 (1962).

Received for review February 21, 1968. Accepted May 10, 1968.