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A COMPARISON OF THE VITAMIN A POTENCY AND CAROTENE CONTENT OF DIFFERENT TYPES OF SILAGE¹

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The importance of vitamin A in the ration of the dairy cow has become increasingly evident in recent years and numerous assays have been made of the constituents of the ration. Feeding practices have changed in that prominence is being given to the use of artificially dehydrated hays and new types of silage instead of field cured hays, and consequently many studies have been carried out to determine the effect of these types of processing upon the vitamin A content of the crops. In much of this work a determination of the carotene content has been the method of assay since it has been shown that carotene is the main source of vitamin A in the feeding materials used.

REVIEW OF LITERATURE

It has been found in this laboratory (10) as well as in others (3, 6, 11) that the carotene content of the silage is often markedly higher than that of the fresh green plants from which the silage was prepared. However, no adequate explanation of this anomaly has been given. Peterson and co-workers (6) have advanced the theory that the action of acids which are present in silage, particularly in that prepared by the A.I.V. process, tended in some way to make the carotene present more readily extractable. Kane, Wiseman, Hartman and Cary (2) have reported that the acids present caused the formation, probably from xanthophyll, of a pigment which could not be readily separated from carotene by the usual partition methods, and which rendered inaccurate the spectrophotometric determination of carotene. In a recent investigation, Shinn, Kane, Wiseman and Cary (9) tested carotene extracts from fresh crops, hays, and silages. They found that, particularly in the last two materials, the carotene extract might contain from 20 to 40 per cent of a pigment which was not carotene.

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After the present work was completed, Quackenbush, Steenbock and Peterson (7) showed the presence of 5 new carotenoids in the extract from acidified alfalfa and from alfalfa silage. Three of these pigments are not differentiated from carotene by the usual methods of analysis and will cause false high values. A recent work by Wiseman, Kane, Shinn, and Cary (11) summarizes much of their work on hays and silages and states that the error from pigmented impurities varied from 11 to 32 per cent in the case of the hays and corn silage.

The question arose, therefore, whether the colorimetric determination of carotene is an accurate measure of the vitamin A potency of silages. In order to answer this question, samples of silage, representing several crops and preserved by different ensiling processes, were obtained; the carotene contents of each determined colorimetrically; and the vitamin A potency measured by biological assay. It was thought that, if some change did occur in the carotene fraction during ensiling and storage, this change might be greater in some samples than in others.

EXPERIMENTAL

In obtaining the silages, no attempt was made to sample the contents from any particular silo, but the material chosen was always of good quality and reasonably representative of that crop. All of the materials tested had been preserved in the usual tower type of silo. In each case, about 25 lbs. of silage was obtained, ground through a motor driven meat chopper, mixed thoroughly, and stored in tightly stoppered bottles in a refrigerator at $0^{\circ} \pm 5^{\circ}$ C. Most of the samples showed no signs of molding or other spoilage, but in the few cases where this did occur they were immediately discarded. Carotene analyses were made at the beginning, the end, and approximately the middle of the assay period according to the method of Russell, Taylor and Chichester (8), and each sample was fed to groups of 13 to 15 vitamin A deficient rats at a level calculated to furnish 1.0 microgram of carotene per day. The assay method was essentially that recommended by the U. S. Pharmacopoeia, using the four week assay period. Although only a single level of each silage was fed, because of the larger number of animals, the average responses are believed to have more significance than would be the case with the usual group of 10 animals. Because of the small amounts of relatively coarse material which were required daily, double supplements were administered on alternate days.

The materials were stored and fed in the wet condition, since previous work had shown that a rapid destruction of carotene occurred during drying and storage in vacuo (10), whereas the carotene in wet silage was fairly stable. However, not all of the samples used in the present experiment were found to have such stability with respect to their carotene contents. It had been planned to change the feeding level to correct any change in the caro-

tene content, but this practice did not prove feasible because of the time required in running the analyses. Therefore, the animals were fed at the original level of silage throughout the assays and the average carotene intakes were calculated from the results of the colorimetric determinations.

RESULTS AND DISCUSSION

The results of the carotene analyses are shown in Table 1. The storage

TABLE 1
Carotene content of silages

Material	Initial carotene content	First storage period	Carotene content end of first storage period	Second storage period	Carotene content end of second storage period	Average ¹ carotene content
	<i>microgm. per gm.</i>	<i>days</i>	<i>microgm. per gm.</i>	<i>days</i>	<i>microgm. per gm.</i>	<i>microgm. per gm.</i>
Regular corn silage #1 ...	19.3	19	16.3	22	20.3	18.1
Regular corn silage #2 ...	9.0	27	(17.8)	22	6.4	7.7
Molasses corn silage	15.9	21	11.8	30	11.8	12.6
Molasses oats silage	10.3	27	7.6	22	6.4	8.1
Molasses soybean silage ...	16.8	39	9.2	8	6.6	12.1
Molasses grass silage	28.4	21	25.7	22	23.1	25.7
A.I.V. soybean corn silage	26.1	49	25.8	8	33.2	26.4
A.I.V. alfalfa silage	25.4	21	13.6	30	8.8	14.6
A.I.V. corn silage (2 years old)	7.1	51	7.1	7.1

¹ Weighted average.

periods shown represent the time which elapsed between the 1st and 2nd, and the 2nd and 3rd analyses, which cover the total period of the bioassay in each case. The final column, which shows the average carotene content, has been weighted according to the number of days which elapsed between analyses. One value, shown in parentheses, was not used in the final average because it was badly out of line and believed to be in error.

Perhaps the most significant fact to be derived from these figures is that the samples varied rather widely with respect to the stability of the carotene. In certain cases the behavior appeared somewhat erratic with small losses or gains. These differences are believed to be due mainly to the difficulty of adequately sampling such heterogeneous material. In general, there was a loss of carotene during storage, this loss being most pronounced with the A.I.V. alfalfa and the molasses soybean silages. However, in no case was the loss as rapid as that which occurred when silage was dried at room temperature in a vacuum desiccator over sulfuric acid and stored in vacuo at $0^{\circ} \pm 5^{\circ}$ C. The figures emphasize the fact that the vitamin content of a test material

may change during the assay period so that the results may be subject to errors.

The levels of carotene present are not intended to represent the exact levels which may be expected in the crops described since no particular attempt was made to secure representative samples. It is of interest to note that one crop, corn, gave values ranging from 7.7 to 18.1 micrograms of carotene per gram of silage. It seems probable that similar variations might be found with different samples of the other crops.

The results of the bioassays are shown in Table 2. All the assays except

TABLE 2
Vitamin A content of silages

Material	Daily supplement of wet silage	Average carotene content of supplement	Average growth response	Vitamin A in supplement	Vitamin A per 0.6 microgm. of carotene
	<i>mg.</i>	<i>microgm.</i>	<i>gm.</i>	<i>U.S.P. units</i>	<i>U.S.P. units</i>
A.I.V. corn silage (2 yrs. old)	140.0	0.98	41.8 ± 2.3	3.80	2.33
A.I.V. alfalfa silage	39.4	0.56	12.7 ± 2.3	2.03	2.18
Molasses corn silage	62.7	0.79	25.4 ± 2.2	2.66	2.02
Regular corn silage #1	51.0	0.93	28.5 ± 2.9	2.85	1.84
A.I.V. soybean-corn silage	42.7	1.13	36.8 ± 2.1	3.42	1.82
Regular corn silage #2	110.0	0.86	24.2 ± 3.3	2.57	1.79
Molasses oats silage	98.0	0.79	20.4 ± 1.8	2.36	1.79
Molasses grass silage	35.2	0.90	20.5 ± 1.6	2.38	1.59
Molasses soybean silage	59.6	0.72	10.4 ± 1.9	1.90	1.58
International standard carotene	a	1.20	32.6 ± 2.1	3.13	1.57

* Fed in 0.1 ml. of corn oil.

that on the A.I.V. corn silage were run on a single large group of animals and the results should therefore be strictly comparable. The A.I.V. corn silage was not available at the same time as the other samples and had to be assayed at a later date. The molasses soybean and A.I.V. alfalfa silages, which in Table 1 showed the greatest loss of carotene, and therefore were fed at the lowest levels of carotene intake, gave low average growth responses. In this colony such a rate of growth is too low to give reliable results. Therefore, the most reliable values may be considered to be those of the regular corn Nos. 1 and 2, molasses corn, molasses oats, molasses grass, and A.I.V. soybean-corn silages.

In Table 2, the next to the last column shows the vitamin A content, in U.S.P. units, of the supplement fed. These values were obtained by reference to a growth-response-dosage curve for the U.S.P. reference cod liver oil. In order to compare the different supplements on a uniform basis, values were calculated which show the number of U.S.P. units per 0.6 micro-

gram of carotene in the supplement. This figure was chosen as a basis for comparison since it is the value of the International Unit of vitamin A. The figures in the last column therefore show the ratios of U.S.P. units of vitamin A, as determined by biological assay, to International Units of carotene, determined colorimetrically.

An examination of these values shows, on the whole, good agreement, with the variation among the different groups probably being little more than may be expected in this type of work. If all the values are used the average is 1.88 ± 0.06 with a coefficient of variation of 13 per cent. If the 2 year old A.I.V. corn silage is not considered, since the data on this sample were obtained with a different group of rats, the average is 1.83 ± 0.05 with a coefficient of variation of 10 per cent. And if the results on the molasses soybean and A.I.V. alfalfa silages, which are considered less reliable because of the low growth response, are also dropped the values become 1.81 ± 0.04 and 7 per cent. All these averages are in close agreement and it may be concluded that one International Unit of carotene is equivalent to 1.8 U.S.P. units of vitamin A, or that 1 microgram of carotene is equivalent to 3 U.S.P. units.

The U. S. Pharmacopoeia Unit of vitamin A is considered to be biologically equivalent to the International Unit of carotene and the reference cod liver oil has been thus standardized. However, the growth responses from the feeding of the various silages, when translated into U.S.P. units by means of the growth-response-dosage curve for the reference oil, gave values ranging from 1.58 to 2.33 U.S.P. units per International Unit of carotene fed. Furthermore, a sample of the International Standard Carotene Preparation, fed at a single level, gave a growth response equivalent to 1.57 U.S.P. units per 0.6 microgram (1 International Unit) of carotene. The fact that all these supplements showed a relatively high potency in terms of U.S.P. units would seem to indicate that the carotene analyses have not given values which were too high, due to the inclusion in the carotene fraction of pigments not having provitamin A properties. These results are at variance with what might be expected on the basis of the work of Shinn and coworkers (9, 11) and of Quackenbush and associates (7). The reason is not known. It may be that the samples of reference cod liver oil used in preparing the dosage-growth-response curve were below the stated potency. To some extent the high results may be due to the natural variations which may be found to occur among groups of experimental animals. Munsell (5), for example, has reported that, over a period of 1 year, 8 groups of rats receiving identical vitamin A supplements in the form of the U.S.P. reference cod liver oil showed average growth responses in 5 weeks ranging from 16.4 to 33.5 grams. It is also possible that these differences can be explained by the work of Dyer, Key and Coward (1) and of Lathbury and Greenwood (4) which showed that different oils or even different lots of the same kind of oil, when used as diluents for vitamin A supplements, may influence the results.

The results obtained on feeding the A.I.V. corn silage are of particular interest in view of the purpose of this work. This material had remained in the silo for a period of approximately 18 months instead of the usual period of 6 to 10 months; furthermore, it was acid-treated silage. Consequently, if acid could cause the formation of pigments which would markedly raise the apparent carotene content, one might expect a correspondingly low biological value per unit of carotene fed. However, just the opposite was found to be the case. The ratio of U.S.P. to International Units, 2.33, was higher for this sample than was the case with any of the other silages, even though levels of reference cod liver oil fed at the same time as the A.I.V. corn silage gave less growth than did similar feedings made while the remainder of the assays were being run. This fact, as well as the fact that all of the other silages gave a higher growth response per unit of carotene than did the International Standard carotene, is believed to be further evidence that the formation of new carotenoids by the action of acid on green plant tissue does not interfere with the accuracy of the colorimetric carotene determination as a measure of vitamin A potency of silages.

SUMMARY

Carotene analyses and vitamin A bioassays have been carried out on 9 samples of silage, including regular corn silage, crops ensiled with molasses, and by the A.I.V. procedure. In every case the bioassays showed a higher vitamin A potency per unit of carotene than was obtained on a sample of International Standard Carotene, but the differences were of such magnitude that the carotene analysis may be taken as a reliable index of vitamin A potency. One International Unit (0.6 microgm.) of carotene in silage was found to be equivalent to 1.8 U.S.P. units of vitamin A.

REFERENCES

- (1) DYER, F. J., KEY, K. M., AND COWARD, K. H. The influence of the solvent on the vitamin A activity of (a) carotene and (b) cod-liver oil. *Biochem. J.* **28**: 875. 1934.
- (2) KANE, E. A., WISEMAN, H. G., HARTMAN, A. H., AND CARY, C. A. Spectrophotometric data bearing on the character of the pigments obtained in routine determinations of carotene in hays, silages, and freshly cut plant materials. *J. DAIRY SC.* **19**: 466. 1936.
- (3) KRAUSS, W. E., AND WASHBURN, R. G. Studies on A.I.V. silage. III. Carotene preservation and biological properties of the milk. *J. DAIRY SC.* **19**: 454. 1936.
- (4) LATHBURY, K. C., AND GREENWOOD, G. N. The influence of the solvent on the biological effect of carotene and vitamin A. *Biochem. J.* **28**: 1665. 1934.
- (5) MUNSELL, H. E. Vitamin A. Methods of assay and sources in foods. *J. Am. M. Ass.* **111**: 245. 1938.
- (6) PETERSON, W. H., BOHSTEDT, G., BIRD, H. R., AND BEESON, W. M. The preparation and nutritive value of A.I.V. silage for dairy cows. *J. DAIRY SC.* **18**: 63. 1935.
- (7) QUACKENBUSH, F. W., STEENBOCK, H., AND PETERSON, W. H. The effects of acids upon carotenoids. *J. Biol. Chem.* **123**: xviii. 1938.

- (8) RUSSELL, W. C., TAYLOR, M. W., AND CHICESTER, D. F. Colorimetric determination of carotene in plant tissue. *Plant Physiol.* **10**: 325. 1935.
- (9) SHINN, L. A., KANE, E. A., WISEMAN, H. G., AND CARY, C. A. The accuracy of routine carotene determinations as a measure of vitamin A potency. *J. Biol. Chem.* **119**: lxxxix. 1937.
- (10) TAYLOR, M. W., AND RUSSELL, W. C. The stability of carotene in plant tissues. *J. Nutrit.* **16**: 1. 1938.
- (11) WISEMAN, H. G., KANE, E. A., SHINN, L. A., AND CARY, C. A. The carotene content of market hays and corn silage. *J. Agr. Res.* **57**: 635. 1938.