A B S T R A C T

DIGESTIBILITY OF CAROTENOIDS IN CATTLE

Submitted by

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In partial fulfillment of the requirements

for the Degree of Master of Science

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Agricultural and M[illegible] College

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ABSTRACT

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The objective of the research reported in this thesis was to investigate by the use of replicate balance trials, the digestibility of carotenoids in cattle receiving a maintenance ration of alfalfa hay separately, and with the addition of the following supplements: (a) rolled oats, (b) wheat germ oil, and (c) mixed tocopherols.

The available literature concerning carotene and vitamin A metabolism in cattle is related primarily to minimum requirements determined after depletion of the carotene and vitamin A reserves. The need for more complete information obtained under normal conditions of feeding and production has prompted a program of vitamin A research by the Colorado Station. The effect of different vitamin A sources on the growth and condition of Hereford steers and heifers has been investigated. Similar work on vitamin A nutrition in reporduction is now under way. Extensive carotenoid analyses of Colorado forages representing various conditions of maturity and handling have been completed. Obviously, however, recommended levels of feeding can have little meaning until reliable information that is pertinent to the digestibility of carotenoids becomes available. Diversity of results in reported work emphasizes the need for more thorough investigation. Many workers agree, however,

that small supplements of tocopherols or wheat germ oil enhance the utilization of carotene in rats. Valuable information might well be disclosed by feeding such substances as well as whole grain to cattle.

Ten digestion balance trials each of twenty days' duration were conducted, using at least five animals during each experimental period. The animals were heifers of the Hereford, Shorthorn, and Holstein breeds ranging in age from eight to thirty months during the course of the experiment. All animals were fed a maintenance ration of alfalfa hay during four periods. Replicate trials, using different control and test animals, were completed on alfalfa hay supplemented with one ounce of wheat germ oil per head daily; alfalfa hay supplemented with one pound of rolled oats per head per day; and alfalfa hay supplemented with 1.51 grams of mixed tocopherols per head per day.

Carotene digestibility was quite low and variable on all rations, averaging close to twenty per cent. No influence was observed for dietary carotenoid levels nor the xanthophyll to carotene ratio. Xanthophyll digestibility was also variable, but was appreciably higher than that for carotene for animals fed oats and wheat germ oil. Neither wheat germ oil nor tocopherols appeared to produce any effect on carotene digestibility. A small supplement of oats, likewise, did not enhance the digestibility of carotene, but appeared to cause an increase in xanthophyll retention together with reduction of serum vitamin A.

Carotenoids were apparently digested to a greater extent by pregnant animals, although the data in this case did not permit accurate interpretation of the influence of age in pregnant animals. Both carotenoid digestibility and serum levels of carotene and vitamin A appeared to be subject to seasonal variation, greater stability being observed in the gestating heifers.

The surprisingly high digestibility of xanthophyll reported in this thesis suggests the need for a more detailed and controlled study. The variability of results observed in this investigation as well as discrepancies reported in the literature show that a different approach to the problem will probably be necessary if the true picture is to be obtained.

Table 1.--CAROTENOID DIGESTIBILITY AND SERUM CAROTENE AND VITAMIN A.

Average apparent digestibility (Per cent)			Average blood values (per 100 milliliters of serum)	
Dry matter	Carotene	Xanthophyll	Carotene Micrograms	Vitamin A International Units
Maintenance ration of alfalfa hay (720 animal days)				
70.93	21.56	19.15	112.66	271.79
Alfalfa hay maintenance and rolled oats supplement (120 animal days)				
70.29	20.96	34.79	107.03	343.72
Alfalfa hay maintenance and wheat germ oil supplement (120 animal days)				
64.45	22.68	57.08	156.15	294.16
Alfalfa hay maintenance and mixed tocopherols supplement (120 animal days)				
68.13	21.92	18.03	88.59	323.23

T H E S I S

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY JAMES M. WING

ENTITLED DIGESTIBILITY OF CAROTENOIDS IN CATTLE

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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Chapter I

INTRODUCTION

The objective of the research reported in this thesis was to investigate by the use of replicate balance trials, the digestibility of carotenoids in cattle receiving a maintenance ration of alfalfa hay separately, and with the addition of the following supplements: (a) rolled oats, (b) wheat germ oil, and (c) mixed tocopherols.

The available literature concerning carotene and vitamin A metabolism in cattle is related primarily to minimum requirements determined after depletion of the carotene and vitamin A reserves. The need for more complete information obtained under normal conditions of feeding and production has prompted a program of vitamin A research by the Colorado Station. The effect of different vitamin A sources on the growth and condition of Hereford steers and heifers has been investigated. Similar work on vitamin A nutrition in reproduction is now under way. Extensive carotenoid analyses of Colorado forages representing various conditions of maturity and handling have been completed. Obviously, however, recommended levels of feeding can have little meaning until reliable information that is pertinent to the digestibility of caroten-

oids becomes available. Diversity of results in reported work emphasizes the need for more thorough investigation. Many workers agree, however, that small supplements of tocopherols and wheat germ oil enhance the utilization of carotene in rats. Valuable information might well be disclosed by feeding such substances as well as whole grain to cattle.

Chapter II

REVIEW OF LITERATURE

The literature reveals little information pertinent to the digestibility of carotenoids in cattle.

Evidence presented by With (75) indicated that the influence of many unidentified factors resulted in highly variable values for carotenoid digestibility in species other than cattle. Ereksen and Haygaard (20) found that the digestibility of carotene in man was one per cent for raw carrots, 19 per cent for cooked carrots, 45 per cent for raw spinach, and 58 per cent for cooked spinach. Wagner (71) observed that the apparent digestibility of beta carotene was 46 per cent in human subjects. Zeta carotene was reported to be inactive (48). Zeaxanthin was reported to have no vitamin A activity in rats and pigs, by Braude and co-workers (8). They also concluded that pigs used beta carotene less efficiently than did rats. In 1939 Kreula and Virtanen (37) found that human absorption of carotene from carrots varied from 4 to 36 per cent. Two years later they reported it was 20 per cent from raw carrots and 5 per cent from cooked carrots (69). A later report by Virtanen showed that carotene from carrots and powdered hay was absorbed by humans to

the extent of from one to 10 per cent. No influence was exerted by dietary lipides (68). In 1948 Kreula recorded carotene absorption from carrots in humans as 10 per cent when fed plain, and 50 to 80 per cent when mixed with oil (36). Work by Ahmad (2) likewise indicated a digestion coefficient of 10 per cent for carotene when carrots were fed plain, and a significant increase with the addition of oil. Ninety per cent of the carotene obtained from cooked spinach was absorbed by humans when fat was included in the diet, and 50 per cent was absorbed when the fat was withheld (74). Almost complete absorption of carotene fed with olive oil to rats was demonstrated by Basu (4); only 10 per cent absorption occurred when the olive oil was replaced by peanut oil. Dietary fat appeared to enhance the retention of carotene by hens (56). Carotene in alfalfa leaf meal was 35 per cent as effective for liver storage of vitamin A as carotene dissolved in linseed oil (23). Leonhardi (39) ascertained as the result of 20 experiments with 7 human subjects that carotene was absorbed to the extent of 8.5 per cent; addition of fat produced no significant increase. Rats absorbed carotene on a diet practically devoid of fat (43).

Carotenoid balance studies with sheep indicated an average retention of 20 per cent of the dietary carotene and 30 per cent of the xanthophyll (55). Human absorption of carotene amounted to only one per cent as reported by

Eckelen and Pannevis (19). Xanthophyll was absorbed to a greater extent than carotene.

Apparent digestibility of xanthophyll in human subjects was found to be 91.7 per cent (72). Kemmerer, Fraps, and Demotier (35) demonstrated a decrease in carotene utilization by dietary chlorophyll and xanthophyll. The xanthophyll content of butterfat was observed to remain fairly constant although feed varying in xanthophyll content was administered (33). The yellow color of the shank and skin of chicks, however, was found, by Hammond and Harshaw (27), to vary in intensity with the level of xanthophyll fed.

Carotene absorption has been observed to decline with the administration of mineral oil (11) (18) and (77); vitamin A, however, was unaffected. Plasma analysis verified the above conclusions (3).

Seshan and Sen (58) recorded negative carotene balances with cows and bullocks on low carotene diets. Retention occurred in all cases, however, when the feed was enriched with carotene. Fraps and Kemmerer (34) found that carotene digestibility in rats and chicks increased significantly as the dietary carotene was decreased. In a later report the Texas workers (22) reported that a higher percentage of carotene was digested at high levels of intake. Nediavoz (45) observed that an increase in the oral dosage of carotene to white mice

resulted in increased absorption. Absorption of carotene by rats varied, not only with the level fed, but also with the carotene content of the diet received before the experimental period (44). Forty per cent of the carotene from dried grass containing 145 gamma per gram was absorbed by chickens (38); however, fecal excretion amounted to only 10 to 13 per cent for rats fed a diet containing only 1 - 2 gamma of carotene (57).

Rafsky and associates (51) suggested that vitamin A was not snythesized at a normal rate by aged humans. Carotene stores increased with age of cattle, however, as long as feed containing adequate carotene was accessible (25). Dienst and Bebber (17) reported that blood levels of carotene and vitamin A were unaffected by age.

Wide seasonal fluctuations were observed by Sutton and Soldner (65) in serum carotene and vitamin A in cattle. Carotene values reached a low level in June and a high level in October. Shifts in vitamin A values appeared to lag about one month following changes in carotene levels. Higher serum carotene levels were recorded for cows on pasture than for similar animals on a good winter ration containing more carotene than the pasture (73). One reason for the increased utilization of carotene from pasture was the large amount of tocopherol in pasture according to Cabel and Ellis (9) who computed the daily tocopherol intake of a 1000 pound cow on fresh pasture to

be 7.1 grams.

Adamstone (1) observed lesions of vitamin A deficiency in vitamin E deficient chicks, and concluded that synergism existed between the vitamins, or that tocopherols exerted a protective effect in relation to vitamin A. The vitamin A reserve of rats on rations deficient in vitamin E was depleted more quickly than those of controls receiving adequate tocopherol (41). Hickman, Kaley, and Harris (29) and Rao and co-workers (53) noted that carotene deficient diets became adequate when supplemented with tocopherol. The addition of tocopherol to a basal carrot diet doubled the value of carotene for rats (24). Sherman (60) found that growth of vitamin A deficient rats was stimulated by dietary wheat germ oil. Analysis of the feces showed that the difference was not due to an increased carotene digestibility. In a later report (59) the same author showed that alpha tocopherol inhibits the antagonism between carotene and the esters of linoleic and linolenic acids reported by Quackenbush, Cox and Steenbock (49). Several reports indicated that tocopherols improved the utilization of carotene by protecting it from oxidation in the intestinal tract (13) (46).

Different investigators agreed that the three tocopherols were equally effective in sparing action on vitamin A (30) (50). Deuel and co-workers (16) demonstrated that oral administration of shark liver oil in-

creased the vitamin A, but simultaneously depressed the carotene content of milk fat. Carotene depression was less severe when tocopherols were also included (28). When vitamin E was fed alone the carotene content increased significantly. Experiments at the Texas station (10) indicated that addition of whole grain to the ration of lactating cows had little effect on the vitamin A potency of butter fat. Jensen and Hickman (32) reported that the vitamin A enhancing property of soy bean phosphatide mixtures was due principally to the constituent tocopherols. Alpha tocopherol did not affect the utilization of carotene from spinach for liver storage of vitamin A (35). The growth rate of vitamin deficient rats receiving tocopherol and carotene was accelerated by dietary rice bran, which was shown to retard the oxidation of carotene in in vitro experiments (66).

Other experiments indicated increased in vitro stability of carotene when dissolved in unsaturated oils (21); oxidation occurred concurrently, however, with the accumulation of peroxides (5). The addition of tocopherols significantly increased carotene stability. Sullmann (63) reported that vitamin E delayed the oxidation of carotene in the presence of linseed or soy bean oils, or their unsaturated acids; lecithin delayed oxidation in the presence of the oils but not the acids. Alpha tocopherol acetate was not active. Biscegli (6) observed retardation

of the oxidation of vitamin A in an oil solution following addition of alpha tocopherol.

Chapter III

METHODS AND MATERIALS

Carotenoid digestion was studied by the use of a modification of the method described by Ritzman and Benedict (54). Feces and urine were separated by a sloping screen of hardware cloth of 3/8 inch mesh rather than by a continuously moving rubber belt. Balance trials were of 20 days duration with a mid-period break of several days, at which time the animals were removed to a corral for exercise. Body weights were determined for two consecutive days and an average was computed at the beginning, mid-period, and end of each trial. Animals were conditioned for this study by being placed in balance stalls for gradually increasing periods of time. No data were collected until all animals were well trained and content to remain in the stalls.

Three balance trials were completed with a Holstein and four Shorthorn heifers. A Hereford heifer was substituted for one of the Shorthorns during the three ensuing periods, after which all animals were replaced by six younger heifers, including five Herefords and a Shorthorn.

Hay of the same cutting and from the same fields was fed for as long as the supply lasted. When changes in feed occurred, all animals received the new feed during a preliminary period of at least seven days before being placed on balance trial. Sufficient feed to result in a slight gain in weight, but not enough to encourage excessive refusal of the coarser parts was provided at all times. Water was given twice daily. Salt and steamed bone meal were fed ad libitum in the corral, but restricted during the balance trials in order to obtain a dry, crumbly feces.

All animals were fed a maintenance ration of alfalfa hay during four periods. Replicate trials, using different control and test animals were completed on alfalfa hay plus one pound of rolled oats per head per day; alfalfa hay plus one ounce of wheat germ oil per head per day; and alfalfa hay supplemented with 18 grams per head per day of a mixture containing 84.0 milligrams of mixed tocopherols per gram.

During the balance studies, feed and refuse were weighed accurately to one gram. The wheat germ oil supplement was measured by volume in a graduated cylinder. The supplementary materials were placed on top of the hay at feeding to insure complete consumption. Samples of hay fed and refused were stored for analysis in tightly covered cans.

The feces was weighed, thoroughly mixed, and sampled daily. Aliquots representing 1/60-1/20 of the total daily excretion were placed in metal cans with tightly fitting covers and frozen. The size of aliquots remained constant for any given balance trial, but were decreased as the animals became larger, and daily excretion increased. At the end of each balance trial, after weighing for determination of moisture loss in storage, the composite feces samples were broken up, mixed, and sampled for carotenoid analysis as described by Petersen (47). The remaining moisture was removed from the feces by the use of a forced air dryer and finally, after grinding in a Wiley mill, in a drying oven by the method of the Association of Official Agricultural Chemists. All analyses were computed to a dry matter basis, and balances were determined for dry matter, carotene, and xanthophyll.

Blood samples taken at the beginning and end of each balance trial were analysed for carotene and vitamin A according to the method of Dann and Evelyn (12). Serum vitamin A values were corrected for the blue color produced by the reaction between carotene and Carr-Price reagent.

Aliquoting the feces offered a perplexing problem because of the tendency for part of the sample to stick to the container in which it was weighed. Finally it was decided to re-weigh the container after removing as

much of the feces as came out easily, and to make no attempt to remove it all. This did not interfere with sample sizes, but allowed for greater accuracy in moisture determinations.

Chapter IV

ANALYSIS OF DATA

General observations

After training, the animals willingly entered the balance stalls at the beginning of each balance trial. They were, however, quite nervous and difficult to handle at the time of removal, and for several days thereafter. Although most of the animals gained weight during the periods of confinement, they were usually thinner and rougher in appearance at the end of each period. After three or four days in the corral, normal appearance was regained. In almost every instance the animals weighed from 15 to 30 pounds more on the second day after removal from the balance stalls than on the first day. During the periods of coldest weather all animals except those in advanced stages of pregnancy lost weight during confinement in the balance stalls. The animals which were heavy with calf gained at this time.

The method of feces collection was satisfactory except during periods of extremely low temperature. At this time feces froze to the screens and could be removed only with much difficulty.

Carotene digestibility

The digestibility of carotene was quite low and extremely variable on all rations. The average carotene retention for 720 animal days on a maintenance alfalfa hay ration was 21.56 per cent. Variation from -21.67 to 55.96 per cent was encountered. Digestibility coefficients for carotene did not appear to be related in any way with the feed carotene content, nor with the ratio between xanthophyll and carotene in the ration.

Xanthophyll digestibility

The apparent digestibility of xanthophyll from a maintenance hay ration was subject to more variation than was carotene, ranging from a negative balance of -45.49 per cent to a retention of 85.72 per cent. Average digestibility was 19.15 per cent. No apparent influence was exerted by dietary levels of xanthophyll, nor the xanthophyll to carotene ratio.

Effect of rolled oats

Rolled oats supplemented to a maintenance alfalfa hay ration at the rate of one pound per head per day for a total of 120 animal days resulted in an average carotene digestibility of 20.96 per cent with a variation of from -14.61 to 43.80 per cent. Feeding of this supplement resulted in xanthophyll digestibility ranging from 4.51 to 59.93 per cent and averaging 34.79 per cent. Xantho-

phyll digestibility was at least 14 per cent higher in supplemented animals than in controls. This difference was greater than was observed under any other treatment in this study.

Effect of wheat germ oil

The average digestibility of carotene from a maintenance ration of alfalfa hay supplemented with 30 c.c. of wheat germ oil per head per day for 120 animal days was 22.68 per cent, ranging from 7.75 to 34.64 per cent. Average retention of xanthophyll in this case was 57.08 per cent with a range of from 24.44 to 88.32 per cent. There was little difference in xanthophyll digestibility between control animals and those receiving wheat germ oil.

Effect of mixed tocopherols

Supplementation of the basal hay ration with 1.51 grams of mixed tocopherols per head per day to six heifers for 20 days resulted in carotene digestibility ranging from 6.54 to 34.00 per cent with an average of 21.92 per cent. Dietary xanthophyll was retained at an average of 18.03 per cent under these conditions. The limits of variation were -22.00 to 35.44 per cent.

The apparent influence of age and pregnancy

A gradual increase in carotenoid digestion was observed with the advance of age. A rather sharp increase

in the per cent digested occurred shortly after breeding. At this time less variation in digestibility was noted.

Dry matter digestibility

The average dry matter digestibility was 70.93 per cent with a range of 50.25 to 86.08 per cent for the maintenance hay ration; 70.29 per cent, varying from 60.15 to 84.41 per cent for hay and rolled oats; 64.45 per cent, with a range of 61.76 to 70.77 per cent for the maintenance hay ration plus wheat germ oil; and 68.13 per cent, ranging from 59.12 to 78.15 per cent for alfalfa hay supplemented with tocopherols.

Blood serum carotene and vitamin A

Blood levels of carotene and vitamin A were variable, but did not change simultaneously and directly with changes in the digestibility of carotenoids. Serum carotene averaged 107.03 micrograms per 100 milliliters for six animals receiving one pound of rolled oats per head per day for 20 days. Vitamin A averaged 343.72 international units per 100 milliliters of serum. A reduction of serum vitamin A in contrast to a gain for controls is shown for the oats fed animals in appendix Tables 40 and 48. No definite trend in blood values was observed when tocopherols or wheat germ oil were supplemented.

Effect of season

Serum carotene was subject to seasonal variation, reaching the lowest values in June and July and the highest levels in January. Vitamin A, likewise, varied with the season, the lowest values occurring in May and the highest in January. These changes in blood serum values obtained with animals in the dry lot and digestion stalls were similar to variations noted during three years' study of both steers and heifers on range.

Effect of confinement to digestion balance stalls

Occasionally blood levels of carotene and vitamin A tended to rise during the period of a balance trial. In almost every instance, however, confinement appeared to depress serum values of both. After the animals were allowed the freedom of the corral for three or four days following a balance trial, serum carotene and vitamin A usually increased.

Table 1.--CAROTENOID DIGESTIBILITY AND SERUM CAROTENE AND VITAMIN A.

Average Apparent Digestibility (Per cent)			Average Blood Values (per 100 milliliters of serum)	
Dry matter	Carotene	Xanthophyll	Carotene Micrograms	Vitamin A International Units
Maintenance ration of alfalfa hay (720 animal days)				
70.93	21.56	19.15	112.66	271.79
Alfalfa hay maintenance and rolled oats supplement (120 animal days)				
70.29	20.96	34.79	107.03	343.72
Alfalfa hay maintenance and wheat germ oil supplement (120 animal days)				
64.45	22.68	57.08	156.15	294.16
Alfalfa hay maintenance and mixed tocopherols supplement (120 animal days)				
68.13	21.92	18.03	88.59	323.23

Table 1 contains only a summary of results. More complete information may be obtained from appendix Tables 2 through 80.

Chapter V

DISCUSSION

General discussion

Special attention must be given to the training and handling of animals for work of this type. The fact that a more thrifty appearance was attained by the animals after only a few days in the corral following a balance experiment indicated that confinement to the stalls for such a long period was indeed uncomfortable. That conditions of the experiment were not too rigorous to be endured, however, was evidenced by the willingness of the animals to enter the balance stalls at the beginning of each trial, as well as by the gains in body weight which usually occurred during confinement.

Because of the possibility of feed remaining in the rumen for long periods of time, cattle often present great variability in fecal dietary residues. The preliminary feeding periods, and the relatively long balance trials used in this work tended to eliminate most of the error caused by this aspect of rumen physiology. An average of the results for several animals receiving the same treatment, even when determinations were made simultaneously, tended to eliminate the errors of individual

values.

While apparent digestibility values did not reflect all the facts concerning true digestibility, they were, however, an accurate indication of the input and outgo of nutrient material. Negative balances showed that not all of the carotene appearing in the feces of animals in this experiment was the undigested portion of the carotene fed. It is probable that digestion occurred normally under these conditions, but for some as yet unexplained reason, carotenoids were demobilized and lost from body stores. Does this mean that the animals had less need for carotenoids at this time? If so, information concerning carotenoid requirements might be gained from a more complete study. If negative balances are related to the level of storage, feeding different levels of carotenoids to several groups of experimental animals before determination of balances should reveal significantly different digestibility figures. Interesting data might also be acquired by analysis of blood and feces of fasting animals.

Carotene digestibility

No logical explanation for the high variability of carotene digestion on the rations included in this study seems possible in the light of the data obtained. Some workers have reported relatively higher digestibility of carotene from high content feeds; others have found the opposite effect. It is obvious that if only part of

the data presented in this thesis were available, conclusions would be forthcoming in agreement with either school of thought.

Xanthophyll digestibility

While the importance of carotene has long been accepted, little or no consideration has been given to xanthophyll as a possible factor in animal nutrition. In view of the fact that in many cases xanthophyll was digested to a greater extent than carotene, it seems reasonable to assume that it does exert some influence within the animal body. If the body has no use for xanthophyll, why does it absorb so much of it, and what happens to it after absorption?

Effect of rolled oats

Sufficient data were not secured to determine why the feeding of oats resulted in an increase in xanthophyll digestibility. Perhaps something in the oats created a need for xanthophyll by the body. Carotene in small quantity was found in the oats fed, but xanthophyll was absent. Is there a factor in oats which destroys xanthophyll? If so, dietary oats might cause lower fecal xanthophyll, resulting in higher apparent digestibility while absorption remained unaffected.

Effect of wheat germ oil

Although wheat germ oil has previously been

shown to enhance the utilization of carotene in some species, it did not appear to influence apparent digestibility (46). Results presented in this thesis likewise show no significant difference in digestibility between wheat germ oil supplemented and control animals. If wheat germ oil enhanced carotene absorption and at the same time also retarded oxidation in the alimentary canal, fecal excretion would remain quite constant. Hence the effect of wheat germ oil could not be determined by carotene balance studies.

Effect of mixed tocopherols

The fact that mixed tocopherols did not influence apparent digestibility of carotenoids in cattle does not necessarily imply that such a supplement was not nutritionally important. The points discussed in the above paragraph apply to tocopherols as well as to wheat germ oil. The metabolism of carotene and vitamin A is probably related in some way to the tocopherols.

Apparent influence of age and pregnancy

Calves usually receive vitamin A as such in their mother's milk. After weaning, however, they receive no vitamin A as such but depend entirely upon vitamin A precursors. It is logical, therefore, that greater powers for digestion of carotene should develop with increasing age.

Pregnancy effects profoundly several physiological mechanisms which could well be involved with carotene metabolism. It is not surprising therefore that carotene was digested to a greater extent by gestating animals. Considering the importance of epithelial tissue in normal gestation and parturition, one wonders if nature has arranged for better utilization at this time of the vitamins concerned directly with the nutrition of such tissue. The fact that more of the dietary xanthophyll was retained by pregnant animals leads to speculation as to whether it also was needed for some special function during reproduction. One must not overlook the possible effect of age in interpreting the results obtained with pregnant animals.

Effect of season

Antagonism between thyroid activity and vitamin A metabolism has been indicated by experiments at this station and elsewhere. Since acceleration of thyroid activity is known to occur during the summer months, it is not surprising that carotenoid digestion as well as serum carotene and vitamin A were depressed at this time.

Chapter VI

SUMMARY AND CONCLUSIONS

Because of the need for information pertinent to the digestibility of carotenoids in cattle, the research presented herein was undertaken as a part of a rather extensive vitamin A study conducted by the Animal Investigations Section of the Colorado Experiment Station. Ten digestion balance trials each of twenty days duration were conducted, using at least five animals during each experimental period. The animals were heifers of the Hereford, Shorthorn, and Holstein breeds ranging in age from eight to thirty months during the course of the experiment. All animals were fed a maintenance ration of alfalfa hay during four periods. Replicate trials, using different control and test animals, were completed on alfalfa hay supplemented with one ounce of wheat germ oil per head daily; alfalfa hay supplemented with one pound of rolled oats per head per day; and alfalfa hay supplemented with 1.51 grams of mixed tocopherols per head per day.

Carotene digestibility was quite low and variable on all rations, averaging close to twenty per cent. No influence was observed for dietary carotenoid levels

nor the xanthophyll to carotene ratio. Xanthophyll digestibility was also variable, but was appreciably higher than that for carotene for animals fed oats and wheat germ oil. Neither wheat germ oil nor tocopherols appeared to produce any effect on carotene digestibility. A small supplement of oats, likewise, did not enhance the digestibility of carotene, but appeared to cause an increase in xanthophyll retention together with reduction of serum vitamin A. Carotenoids were apparently digested to a greater extent by pregnant animals, although the data in this case did not permit accurate interpretation of the influence of age in pregnant animals. Both carotenoid digestibility and serum levels of carotene and vitamin A appeared to be subject to seasonal variation, greater stability being observed in the gestating heifers.

The surprisingly high digestibility of xanthophyll reported in this thesis suggests the need for a more detailed and controlled study. The variability of results observed in this investigation as well as discrepancies reported in the literature show that a different approach to the problem will probably be necessary if the true picture is to be obtained.

A P P E N D I X

LIST OF APPENDIX TABLES

Table 1.--DESCRIPTION OF EXPERIMENTAL HEIFERS.

Animal number	Breed	Birth date
1	Red Shorthorn	August 13, 1945
2	Holstein	August, 1945
3	Roan Shorthorn	January 11, 1946
4	Red Shorthorn	January 14, 1946
5	Red Shorthorn	February 15, 1946
6	Hereford	January 28, 1946
7	Hereford	February 24, 1947
8	Hereford	August 8, 1947
9	Hereford	July 27, 1947
10	Hereford	July 19, 1947
11	Hereford	April 11, 1947
12	Shorthorn	March 12, 1947

BALANCE TRIAL NO. 1

(January 25 - February 21, 1947)

Table 2.--DRY MATTER CONTENT.

Description of sample	Animal number	Moisture loss in Storage per cent	Moisture loss in Dryer per cent	Moisture loss in Oven per cent	Analysed Moisture per cent	Analysed Dry matter per cent	Total Moisture per cent	Total Dry matter per cent
Feces	1	0.18	79.72	3.57	83.29	16.71	83.47	16.53
"	2	1.40	82.18	3.17	85.35	14.65	86.75	13.25
"	3	0.10	80.50	3.07	83.57	16.43	83.67	16.33
"	4	0.30	78.11	4.04	82.15	17.85	82.45	17.55
"	5	1.59	79.79	4.41	84.20	15.80	85.79	14.21
Hay				7.31	7.31	92.69	7.31	92.69
Refuse			2.24	3.28	3.28	96.72	5.52	94.48

Table 3.--ANALYSIS OF FEED AND FECES (MICROGRAMS PER GRAM).

Description of sample	Animal number	As analysed		Dry matter basis	
		Carotene	Xanthophyll	Carotene	Xanthophyll
Feces	1	17.80	64.70	106.52	387.19
"	2	14.11	54.20	96.31	369.97
"	3	14.70	52.90	89.47	321.97
"	4	17.20	64.40	96.36	360.78
"	5	15.80	54.40	100.00	344.30
Hay		35.40	124.30	38.19	134.10
Refuse		15.70	74.90	16.23	77.44

Table 4.--DRY MATTER BALANCE (GRAMS).

Animal number	Feed		Feces	Dry matter					Apparent digestibility
	Fed	Refused		Fed	Refused	Consumed	Feces	Balance	Per cent
1	6,291	83	11,506	5,831	78	5,753	1,902	3,851	66.94
2	6,604	30	14,018	6,121	28	6,093	1,857	4,236	69.52
3	5,591	114	10,672	5,182	108	5,074	1,743	3,331	65.65
4	5,191	58	8,750	4,812	55	4,757	1,536	3,221	67.71
5	5,203	38	10,080	4,823	36	4,787	1,432	3,355	70.09

Table 5.--CAROTENE BALANCE (MILLIGRAMS).

Animal number	Carotene					Apparent digestibility
	Fed	Refused	Consumed	Excreted	Digested	Per cent
1	222.69	1.27	221.42	202.60	18.82	8.50
2	233.76	0.45	233.31	178.85	54.46	23.34
3	197.90	1.75	196.15	155.95	40.20	20.49
4	183.77	0.89	182.88	148.01	34.87	19.07
5	184.19	0.58	183.61	143.20	40.41	22.01

Table 6.--XANTHOPHYLL BALANCE (MILLIGRAMS).

Animal number	Xanthophyll					Apparent digestibility
	Fed	Refused	Consumed	Excreted	Digested	Per cent
1	781.94	6.04	775.90	736.44	39.46	5.09
2	820.83	2.17	818.66	687.03	131.63	16.08
3	694.91	8.36	686.55	561.19	125.36	18.26
4	645.29	4.26	641.03	554.16	86.87	13.55
5	646.76	2.79	643.97	493.04	150.93	23.44

Table 7.--XANTHOPHYLL/CAROTENE RATIO.

Animal number	Consumed	Feces	Digested
1	3.50	3.63	2.10
2	3.51	3.84	2.42
3	3.50	3.60	3.12
4	3.51	3.74	2.49
5	3.51	3.44	3.73
Average	3.51	3.65	2.77

Table 8.--LIVE WEIGHT (POUNDS).

Animal number	Beginning	Mid-period	End	Gain or loss
1	712	791	745	33
2	582	663	668	86
3	551	634	630	79
4	527	591	587	60
5	487	555	549	62

BALANCE TRIAL NO. 2

(April 12 - May 5, 1947)

Table 9.--DRY MATTER CONTENT.

Description of sample	Animal number	Moisture loss			Analysed		Total	
		in Storage per cent	in Dryer per cent	in Oven per cent	Moisture per cent	Dry matter per cent	Moisture per cent	Dry matter per cent
Feces	1	0	78.01	5.57	83.58	16.40	83.58	16.40
"	2	0	81.87	5.88	87.75	12.25	87.75	12.25
"	3	.01	79.51	5.12	84.63	15.37	84.64	15.38
"	4	.01	79.60	5.26	84.86	15.14	84.87	15.13
"	5	0	79.70	5.72	85.44	14.56	85.48	14.56
Hay				13.07	13.07	86.93	13.07	86.93
Refuse				11.55	11.55	88.45	11.55	88.45

Table 10.--ANALYSIS OF FEED AND FECES (MICROGRAMS PER GRAM).

Description of sample	Animal number	As analysed		Dry matter basis	
		Carotene	Xanthophyll	Carotene	Xanthophyll
Feces	1	3.55	12.75	21.65	77.74
"	2	3.71	9.01	30.29	73.55
"	3	3.86	11.48	25.11	74.69
"	4	4.79	15.98	31.64	105.55
"	5	3.47	8.36	23.83	57.41
Hay		11.18	37.92	12.86	43.62
Refuse		9.20	34.25	10.40	38.72

Table 11.--DRY MATTER BALANCE (GRAMS).

Animal number	Feed		Feces	Dry matter					Apparent digestibility
	Fed	Refused		Fed	Refused	Consumed	Feces	Balance	Per cent
1	6,121	10	12,027	5,321	9	5,312	1,640	3,672	69.13
2	6,322	90	14,658	5,496	80	5,416	1,796	3,620	66.84
3	4,940	223	10,368	4,294	197	4,097	1,594	2,503	61.09
4	5,441	124	11,483	4,730	110	4,620	1,739	2,881	62.36
5	5,374	207	11,254	4,672	183	4,489	1,639	2,850	63.49

Table 12.--CAROTENE BALANCE (MILLIGRAMS).

Animal number	Carotene					Apparent digestibility
	Fed	Refused	Consumed	Excreted	Digested	Per cent
1	68.43	.01	68.42	35.50	32.92	48.11
2	70.68	.08	70.60	54.40	16.20	22.95
3	55.22	.20	55.02	40.02	15.00	27.26
4	60.83	.11	60.82	55.02	5.80	9.54
5	60.08	.19	59.89	39.06	20.83	34.78

Table 13.--XANTHOPHYLL BALANCE (MILLIGRAMS).

Animal number	Xanthophyll					Apparent digestibility
	Fed	Refused	Consumed	Excreted	Digested	Per cent
1	232.10	.03	232.07	127.49	104.58	45.06
2	239.74	.31	239.43	132.10	107.33	44.83
3	187.30	7.63	179.67	119.06	60.61	33.73
4	206.32	4.26	202.06	183.55	18.51	9.16
5	203.79	7.09	196.70	94.09	102.61	52.16

Table 14.--XANTHOPHYLL/CAROTENE RATIO.

Animal number	Consumed	Feces	Digested
1	3.39	3.59	3.19
2	3.39	2.43	6.63
3	3.27	2.98	4.04
4	3.32	3.34	3.19
5	3.28	2.41	4.93
Average	3.33	2.95	4.40

Table 15.--LIVE WEIGHT (POUNDS).

Animal number	Beginning	Mid-period	End	Gain or loss
1	820	825	814	- 6
2	709	730	740	31
3	683	693	645	-38
4	619	668	651	32
5	631	636	628	- 3

Table 16.--SERUM ANALYSIS.

Animal number	Carotene (Micrograms per 100 ml.) Beginning	Carotene (Micrograms per 100 ml.) End	Vitamin A (International units per 100 ml.) Beginning	Vitamin A (International units per 100 ml.) End
1	55.08	138.00		192.70
2	47.92	114.00		162.60
3	86.52	153.00		84.30
4	54.00	135.00		86.10
5	60.00	111.00		96.90

BALANCE TRIAL NO. 3

(June 22 - July 15, 1947)

Table 17.--DRY MATTER CONTENT.

Description of sample	Animal number	Moisture loss in Storage per cent	Moisture loss in Dryer per cent	Moisture loss in Oven per cent	Analysed Moisture per cent	Analysed Dry matter per cent	Total Moisture per cent	Total Dry matter per cent
Feces	1	0	78.01	7.76	85.77	14.23	85.77	14.23
"	2	.04	79.04	8.57	87.61	12.39	88.01	11.99
"	3	.03	77.95	5.64	83.59	16.41	83.89	16.11
"	4	0	76.27	10.84	87.11	12.89	89.11	12.89
"	5	0	74.32	13.95	88.27	11.73	88.27	11.73
Hay				9.72	9.72	90.28	9.72	90.28
Refuse				9.84	9.84	90.16	9.84	90.16
Oats				8.11	8.11	91.89	8.11	91.89

Table 18.--ANALYSIS OF FEED AND FECES (MICROGRAMS PER GRAM).

Description of sample	Animal number	As analysed		Dry matter basis	
		Carotene	Xanthophyll	Carotene	Xanthophyll
Feces	1	4.52	22.68	31.76	159.38
"	2	5.25	26.13	42.37	210.90
"	3	4.97	33.98	30.29	207.06
"	4	6.21	29.74	48.18	230.72
"	5	6.11	35.59	52.09	303.41
Hay		11.54	64.51	12.78	73.48
Refuse		9.22	55.90	10.23	62.90
Oats		.03	0.00	.03	0.00

Table 19.--DRY MATTER BALANCE (GRAMS).

Animal number	Feed		Feces	Dry matter					Apparent digestibility
	Fed	Refused		Fed	Refused	Consumed	Feces	Balance	Per cent
1*	6,059	22	13,041	5,470	20	5,450	1,856	4,011	68.37
2*	5,979	7	13,343	5,398	6	5,391	1,600	4,208	72.45
3	5,439	21	11,335	4,911	19	4,892	1,826	3,066	62.67
4*	5,600	16	11,902	5,056	14	5,042	1,534	3,935	71.95
5	5,500	26	11,402	4,965	23	4,942	1,337	3,605	72.95

* Received 454 grams of rolled oats, which contained 417 grams of dry matter, therefore this amount was added to the above figures for dry matter consumed in computing apparent digestibility of dry matter.

Table 20.--CAROTENE BALANCE (MILLIGRAMS).

Animal number	Carotene					Apparent digestibility
	Fed	Refused	Consumed	Excreted	Digested	Per cent
1*	69.91	.20	69.71	58.95	11.09	15.91
2*	68.99	.06	68.93	67.79	1.37	1.99
3	62.76	.19	62.57	55.31	7.26	11.60
4*	64.62	.14	64.48	73.90	- 9.42	-14.61
5	63.45	.24	63.21	69.64	- 6.43	-10.17

* .13 milligrams carotene contained in oats and therefore added to the above figures for carotene consumed.

Table 21.--XANTHOPHYLL BALANCE (MILLIGRAMS).

Animal number	Xanthophyll					Apparent digestibility
	Fed	Refused	Consumed	Excreted	Digested	Per cent
1*	401.94	1.24	400.70	295.80	104.90	26.18
2*	396.65	.37	396.28	337.44	58.84	14.84
3	360.86	1.18	359.68	378.09	- 18.41	- 5.12
4*	371.51	.87	370.64	353.92	16.72	4.51
5	364.83	1.42	363.41	405.66	- 42.25	-11.62

* Received 1 pound of rolled oats per day.

Table 22.--XANTHOPHYLL/CAROTENE RATIO.

Animal number	Consumed	Feces	Digested
1	5.75	5.02	9.46
2	5.75	4.98	42.95
3	5.75	6.84	- 2.54
4	5.75	4.79	- 1.77
5	5.75	5.83	- 6.57
Average	5.75	5.49	8.31

Table 23.--LIVE WEIGHT (POUNDS).

Animal number	Beginning	Mid-period	End	Gain or loss
1	875	872	863	-12
2	795	745	793	- 2
3	717	690	728	11
4	687	680	708	21
5	664	645	680	16

Table 24.--SERUM ANALYSIS.

Animal number	Carotene (Micrograms per 100 ml.) Beginning	Carotene (Micrograms per 100 ml.) End	Vitamin A (International units per 100 ml.) Beginning	Vitamin A (International units per 100 ml.) End
1	81.60	114.00	728.40	176.10
2	70.80	82.80	72.60	173.20
3	60.00	144.60	87.00	248.20
4	153.00	56.70	160.50	112.20
5	42.00	72.00	35.10	121.60

BALANCE TRIAL NO. 4

(November 6 - November 30, 1947)

Table 25.--DRY MATTER CONTENT.

Description of sample	Animal number	Moisture loss			Analysed		Total	
		in Storage per cent	in Dryer per cent	in Oven per cent	Moisture per cent	Dry matter per cent	Moisture per cent	Dry matter per cent
Feces	2	1.87	80.06	10.46	90.52	9.48	92.39	7.61
"	3	0	74.62	14.58	89.20	10.80	89.20	10.80
"	4	.01	76.37	17.02	93.39	6.61	94.59	6.60
"	5	2.98	75.32	4.70	80.02	19.98	83.00	17.00
"	6	.08	77.70	7.78	85.48	14.52	85.56	14.44
Hay				12.15	12.15	87.85	12.15	87.85
Refuse				12.29	12.29	87.71	12.29	87.71
Oats				8.11	8.11	91.89	8.11	91.89

Table 26.--ANALYSIS OF FEED AND FECES (MICROGRAMS PER GRAM).

Description of sample	Animal number	As analysed		Dry matter basis	
		Carotene	Xanthophyll	Carotene	Xanthophyll
Feces	2	2.45	14.30	25.84	150.84
"	3	2.04	7.90	18.89	73.15
"	4	2.15	9.82	32.52	148.56
"	5	2.66	9.93	13.31	49.70
"	6	2.92	14.20	16.87	97.79
Hay		8.25	43.34	9.41	49.41
Refuse		5.43	27.24	6.18	31.00
Oats		.03	0.00	.03	0.00

Table 27.--DRY MATTER BALANCE (GRAMS).

Animal number	Feed		Feces	Dry matter					Apparent digestibility
	Fed	Refused		Fed	Refused	Consumed	Feces	Balance	Per cent
2	6,751	59	16,937	5,931	52	5,872	1,288	4,584	78.06
3*	6,221	65	15,274	5,456	57	5,399	1,650	3,749	64.46
4*	5,814	87	13,222	5,108	76	5,184	873	4,728	84.41
5*	6,374	7	14,733	5,600	6	5,593	2,229	3,364	60.15
6	6,594	49	14,705	5,793	43	5,750	2,123	3,627	63.08

* Received 454 grams of rolled oats which contained 417 grams of dry matter, therefore this amount was added to the above figures for dry matter consumed in computing apparent digestibility of dry matter.

Table 28.--CAROTENE BALANCE (MILLIGRAMS).

Animal number	Carotene					Apparent digestibility
	Fed	Refused	Consumed	Excreted	Digested	Per cent
2	55.81	.36	55.45	33.28	22.17	40.98
3*	51.34	.40	51.07	31.17	19.90	38.97
4*	48.06	.54	47.65	28.39	19.26	40.42
5*	52.70	.04	52.79	29.67	23.12	43.80
6	54.51	.27	54.24	35.82	18.42	33.96

* .13 milligrams of Carotene contained in oats and therefore added to the above figures for Carotene consumed.

Table 29.--XANTHOPHYLL BALANCE (MILLIGRAMS).

Animal number	Xanthophyll					Apparent digestibility
	Fed	Refused	Consumed	Excreted	Digested	Per cent
2	293.05	1.83	291.22	194.28	96.94	33.29
3*	269.58	2.02	267.56	120.70	146.86	54.88
4*	252.39	2.70	249.69	129.71	119.98	47.97
5*	275.70	.22	276.48	110.78	165.70	59.93
6	286.23	1.52	284.71	207.61	77.10	27.08

* Received 1 pound of rolled oats per day.

Table 30.--XANTHOPHYLL/CAROTENE RATIO.

Animal number	Consumed	Feces	Digested
2	5.25	5.84	4.37
3	5.24	3.87	7.38
4	5.24	4.57	6.23
5	5.24	3.40	7.17
6	5.25	5.80	4.19
Average	5.24	4.70	5.87

Table 31.--LIVE WEIGHT (POUNDS).

Animal number	Beginning	Mid-period	End	Gain or loss
2	929	925	935	6
3	785	770	800	15
4	780	775	785	5
5	760	765	760	0
6	998	990	1,015	17

BALANCE TRIAL NO. 5

(January 1 - January 31, 1948)

Table 32.--SERUM ANALYSIS.

Animal number	Carotene (Micrograms per 100 ml.) Beginning	Carotene (Micrograms per 100 ml.) End	Vitamin A (International units per 100 ml.) Beginning	Vitamin A (International units per 100 ml.) End
2	134.40	63.00	419.40	547.80
3	150.00	95.40	736.50	325.80
4	150.00	24.00	649.50	238.20
5	154.80	55.80	289.80	205.20
6	131.40	130.80	309.60	440.10

Table 33.--DRY MATTER CONTENT.

Description of sample	Animal number	Moisture loss in Storage per cent	Moisture loss in Dryer per cent	Moisture loss in Oven per cent	Analysed Moisture per	Analysed Dry matter per cent	Total Moisture per cent	Total Dry matter per cent
Feces	2	.03	75.45	12.41	87.86	12.14	88.19	11.81
"	3	0	78.66	5.82	84.48	15.52	84.48	15.52
"	4	0	78.54	4.78	83.32	16.68	83.32	16.68
"	5	0	79.46	4.10	83.56	16.44	83.56	16.44
"	6	0	73.08	11.73	84.81	15.19	84.81	15.19
Hay				18.70	18.70	81.30	18.70	81.30
Refuse				34.65	34.65	65.35	34.65	65.35

Table 34.--ANALYSIS OF FEED AND FECES (MICROGRAMS PER GRAM).

Description of sample	Animal number	As analysed		Dry matter basis	
		Carotene	Xanthophyll	Carotene	Xanthophyll
Feces	2	10.31	38.22	84.92	314.82
"	3	8.88	23.06	57.22	148.58
"	4	9.79	32.72	58.69	196.16
"	5	11.62	34.62	70.68	210.58
"	6	6.55	36.46	43.12	240.02
Hay		25.89	48.53	31.85	59.69
Refuse		14.51	32.67	22.20	49.99

Table 35.--DRY MATTER BALANCE (GRAMS).

Animal number	Feed		Feces	Dry matter					Apparent digestibility
	Fed	Refused		Fed	Refused	Consumed	Feces	Balance	Per cent
2*	8,232	95	18,267	6,692	62	6,630	1,945	4,709	70.77
3	7,717	13	15,496	6,274	08	6,266	2,405	3,861	61.61
4*	7,865	20	13,589	6,394	13	6,381	2,267	4,141	64.62
5	7,862	13	15,714	6,392	08	6,384	2,583	3,801	59.54
6*	8,133	53	16,620	6,612	35	6,577	2,524	4,077	61.76

* 24 grams of wheat germ oil were supplemented and this amount was added to the above figures for dry matter consumed in computing the balance.

Table 36.--CAROTENE BALANCE (MILLIGRAMS).

Animal number	Carotene					Apparent digestibility
	Fed	Refused	Consumed	Excreted	Digested	Per cent
2*	213.14	.21	212.93	165.17	47.22	7.75
3	199.82	.02	199.80	137.61	62.19	31.12
4*	203.65	.06	203.59	133.05	70.54	34.64
5	203.59	.02	203.57	182.56	21.01	10.32
6*	210.59	.12	210.47	108.83	101.64	48.29

* Received 24 grams of wheat germ oil per day.

Table 37.--XANTHOPHYLL BALANCE (MILLIGRAMS).

Animal number	Xanthophyll					Apparent digestibility
	Fed	Refused	Consumed	Excreted	Digested	Per cent
2*	399.45	4.75	394.70	61.23	333.47	84.48
3	374.50	.64	373.86	35.73	338.13	90.44
4*	381.65	.99	380.66	44.47	336.19	88.32
5	381.54	.64	380.90	54.39	326.51	85.72
6*	394.67	2.64	392.03	60.58	331.45	84.54

* Received 24 grams of wheat germ oil per day.

Table 38.--XANTHOPHYLL/CAROTENE RATIO.

Animal number	Consumed	Feces	Digested
2	1.88	.37	7.07
3	1.88	.26	5.50
4	1.88	.33	4.82
5	1.88	.30	16.17
6	1.18	.56	3.30
Average	1.74	.36	7.37

Table 39.--LIVE WEIGHT (POUNDS).

Animal number	Beginning	Mid-period	End	Gain or loss
2	935	925	885	-50
3	851	842	815	-36
4	733	755	813	80
5	734	745	825	91
6	1,030	985	980	-50

Table 40.--SERUM ANALYSIS.

Animal number	Carotene (Micrograms per 100 ml.) Beginning	Carotene (Micrograms per 100 ml.) End	Vitamin A (International units per 100 ml.) Beginning	Vitamin A (International units per 100 ml.) End
2	232.40	72.00	765.90	150.60
3	207.30	102.60	557.10	290.10
4	183.60	168.00	640.50	329.40
5	222.00	171.00	931.10	566.90
6	222.00	115.20	1,064.10	164.70

BALANCE TRIAL NO. 6

(February 6 - February 28, 1948)

Table 41.--DRY MATTER CONTENT.

Description of sample	Animal number	Moisture loss in Storage per cent	Moisture loss in Dryer per cent	Moisture loss in Oven per cent	Analysed Moisture per cent	Analysed Dry matter per cent	Total Moisture per cent	Total Dry matter per cent
Feces	2	.51	79.98	5.27	85.25	14.75	85.76	14.24
"	3	.15	80.24	5.16	85.40	14.60	85.65	14.35
"	4	.00	85.31	5.08	90.39	9.61	90.39	9.61
"	5	.13	79.79	5.21	85.00	15.00	85.13	14.87
"	6	.77	79.25	5.37	84.62	15.38	85.39	14.61
Hay				11.76	11.76	88.24	11.76	88.24
Refuse			44.67	4.92	49.59	50.41	49.59	50.41

Table 42.--ANALYSIS OF FEED AND FECES (MICROGRAMS PER GRAM).

Description of sample	Animal number	As analysed		Dry matter basis	
		Carotene	Xanthophyll	Carotene	Xanthophyll
Feces	2	1.93	8.76	13.08	59.39
"	3	2.40	14.07	16.44	96.37
"	4	2.09	14.38	21.75	149.64
"	5	2.38	12.38	15.87	82.53
"	6	2.17	13.33	14.11	86.67
Hay		5.89	39.77	6.67	45.07
Refuse		2.48	18.91	4.92	37.51

Table 43.--DRY MATTER BALANCE (GRAMS).

Animal number	Feed		Feces	Dry matter					Apparent digestibility
	Fed	Refused		Fed	Refused	Consumed	Feces	Balance	Per cent
2	7,385	4	15,801	6,517	2	6,515	2,250	4,261	65.40
3*	7,335	61	15,872	6,472	31	6,441	2,278	4,163	64.24
4	7,015	28	14,034	6,190	14	6,176	1,349	4,827	78.16
5*	7,165	18	15,852	6,322	9	6,313	2,357	3,956	62.43
6*	7,355	17	16,367	6,490	9	6,481	2,391	4,090	62.87

* 24 grams of wheat germ oil were supplemented and this amount was added to the above figures for dry matter consumed in computing the balance.

Table 44.--CAROTENE BALANCE (MILLIGRAMS).

Animal number	Carotene					Apparent digestibility
	Fed	Refused	Consumed	Excreted	Digested	Per cent
2	43.47	.01	43.46	29.46	14.00	32.21
3*	43.17	.30	42.87	37.45	5.42	12.64
4	41.29	.14	41.15	29.34	11.81	28.69
5*	42.17	.23	41.94	37.41	4.53	10.80
6*	43.29	.08	43.21	33.74	9.47	21.96

* Received 24 grams of wheat germ oil per day.

Table 45.--XANTHOPHYLL BALANCE (MILLIGRAMS).

Animal number	Xanthophyll					Apparent digestibility
	Fed	Refused	Consumed	Excreted	Digested	Per cent
2	293.72	.07	293.65	133.63	160.02	54.49
3*	291.69	1.16	290.53	219.53	71.00	24.44
4	278.98	.53	278.45	201.86	76.59	27.51
5*	284.93	.34	284.59	194.52	90.07	31.65
6*	292.50	.34	292.16	207.23	84.93	29.07

* Received 24 grams of wheat germ oil per day.

Table 46.--XANTHOPHYLL/CAROTENE RATIO.

Animal number	Consumed	Feces	Digested
2	6.76	4.53	11.43
3	6.78	5.86	13.09
4	6.77	6.88	6.49
5	6.79	5.20	19.88
6	6.76	6.14	8.97
Average	6.77	5.72	11.97

Table 47.--LIVE WEIGHT (POUNDS).

Animal number	Beginning	Mid-period	End	Gain or loss
2	880	867	895	15
3	845	862	852	7
4	837	835	847	10
5	827	825	835	8
6	997	992	990	- 7

Table 48.--SERUM ANALYSIS.

Animal number	Carotene (Micrograms per 100 ml.) Beginning	Carotene (Micrograms per 100 ml.) End	Vitamin A (International units per 100 ml.) Beginning	Vitamin A (International units per 100 ml.) End
2	130.80	118.20	291.60	213.90
3	150.00	171.00	429.30	183.90
4	165.60	172.20	286.60	138.90
5	271.50	174.00	265.80	118.50
6	156.00	128.40	171.60	208.65

BALANCE TRIAL NO. 7

(April 19 - May 12, 1948)

Table 49.--DRY MATTER CONTENT.

Description of sample	Animal number	Moisture loss			Analysed		Total	
		in Storage per cent	in Dryer per cent	in Oven per cent	Moisture per cent	Dry matter per cent	Moisture per cent	Dry matter per cent
Feces	7	.05	80.61	4.96	85.57	14.43	85.62	14.38
"	8	.04	82.56	4.67	87.23	12.77	87.27	12.73
"	9	.06	76.95	5.26	82.21	17.79	82.27	17.73
"	10	.74	78.81	5.13	83.94	16.06	84.68	15.32
"	11	.71	76.08	4.94	81.02	18.98	81.73	18.27
"	12	3.53	78.57	4.92	83.49	16.51	87.02	12.98
Hay				8.16	8.16	91.84	8.16	91.84
Refuse				17.18	17.18	82.82	17.18	82.82

Table 50.--ANALYSIS OF FEED AND FECES (MICROGRAMS PER GRAM).

Description of sample	Animal number	As analysed		Dry matter basis	
		Carotene	Xanthophyll	Carotene	Xanthophyll
Feces	7	4.60	14.27	31.88	98.89
"	8	3.54	11.40	27.72	89.27
"	9	5.97	15.56	33.56	87.46
"	10	6.27	12.61	39.04	78.51
"	11	6.63	10.02	34.93	52.79
"	12	4.19	12.10	25.38	73.28
Hay		7.78	22.80	8.47	24.83
Refuse		6.16	35.53	7.44	42.90

Table 51.--DRY MATTER BALANCE (GRAMS).

Animal number	Feed		Feces	Dry matter					Apparent digestibility
	Fed	Refused		Fed	Refused	Consumed	Feces	Balance	Per cent
7	4,109	31	7,874	3,774	25	3,749	1,132	2,617	69.81
8	3,557	11	7,187	3,267	9	3,258	915	2,343	71.92
9	6,039	26	6,926	5,546	22	5,524	1,227	4,297	77.79
10	3,893	20	6,131	3,575	16	3,559	939	2,620	73.61
11	4,495	31	6,415	4,128	25	4,103	1,172	2,931	71.44
12	5,416	11	9,441	4,974	9	4,965	1,225	3,740	75.33

Table 52.--CAROTENE BALANCE (MILLIGRAMS).

Animal number	Carotene					Apparent digestibility
	Fed	Refused	Consumed	Excreted	Digested	Per cent
7	31.96	.23	31.73	36.09	- 4.36	-13.74
8	27.67	.08	27.59	25.36	2.23	8.08
9	46.97	.19	46.78	41.18	5.60	11.97
10	30.28	.15	30.13	36.66	- 6.53	-21.67
11	34.96	.23	34.73	40.94	- 6.21	-17.88
12	42.13	.08	42.05	31.09	10.96	26.06

Table 53.--XANTHOPHYLL BALANCE (MILLIGRAMS).

Animal number	Xanthophyll					Apparent digestibility
	Fed	Refused	Consumed	Excreted	Digested	Per cent
7	93.70	1.33	93.37	111.94	-18.57	-19.89
8	81.12	.47	80.65	81.68	- 1.03	- 1.28
9	137.71	1.12	137.59	107.31	30.28	22.00
10	88.77	.85	87.92	73.72	14.20	16.15
11	102.50	1.33	101.17	61.87	39.30	38.85
12	123.50	.47	123.03	89.77	33.26	27.03

Table 54.--XANTHOPHYLL/CAROTENE RATIO.

Animal number	Consumed	Feces	Digested
7	2.94	3.10	.43
8	2.92	3.22	- .16
9	2.93	2.61	1.84
10	2.92	2.01	- .75
11	2.91	1.51	-2.17
12	2.93	2.89	1.04
Average	2.93	2.56	.04

Table 55.--LIVE WEIGHT (POUNDS).

Animal number	Beginning	Mid-period	End	Gain or loss
7	345	358	370	25
8	295	298	307	12
9	463	463	475	12
10	388	395	385	- 3
11	448	452	465	17
12	543	553	558	15

Table 56.--SERUM ANALYSIS.

Animal number	Carotene (Micrograms per 100 ml.) Beginning	Carotene (Micrograms per 100 ml.) End	Vitamin A (International units per 100 ml.) Beginning	Vitamin A (International units per 100 ml.) End
7	271.20	49.50	213.00	168.90
8	318.00	75.00	138.90	117.30
9	303.00	51.00	134.50	87.90
10	207.00	34.50	143.70	66.30
11	171.00	52.50	1,017.90	16.20
12	255.00	36.00	1,229.10	91.80

BALANCE TRIAL NO. 8

(May 16 - June 11, 1948)

Table 57.--DRY MATTER CONTENT.

Description of sample	Animal number	Moisture loss			Analysed		Total	
		in Storage per cent	in Dryer per cent	in Oven per cent	Moisture per cent	Dry matter per cent	Moisture per cent	Dry matter per cent
Feces	7	0	77.67	11.33	89.00	11.00	89.00	11.00
"	8	0	77.61	16.70	94.31	5.69	94.31	5.69
"	9	.03	74.29	13.25	87.54	12.55	87.57	12.52
"	10	.07	78.33	6.53	84.86	15.14	84.93	15.07
"	11	.18	75.50	5.30	80.80	19.20	80.98	19.02
"	12	0	80.02	5.39	85.41	14.59	85.41	14.59
Hay				10.00	10.00	90.00	10.00	90.00
Refuse				17.43	17.43	82.57	17.43	82.57

Table 58.--ANALYSIS OF FEED AND FECES (MICROGRAMS PER GRAM).

Description of sample	Animal number	As analysed		Dry matter basis	
		Carotene	Xanthophyll	Carotene	Xanthophyll
Feces	7	3.05	4.74	27.73	43.09
"	8	2.50	4.21	43.93	73.99
"	9	3.45	8.03	27.49	63.98
"	10	2.79	9.05	18.43	59.78
"	11	2.69	8.79	14.01	45.78
"	12	2.72	6.64	18.64	45.51
Hay		9.04	12.32	10.04	13.69
Refuse		6.98	9.41	8.45	11.40

Table 59.--DRY MATTER BALANCE (GRAMS).

Animal number	Feed		Feces	Dry matter					Apparent digestibility
	Fed	Refused		Fed	Refused	Consumed	Feces	Balance	Per cent
7	4,570	16	9,214	4,113	14	4,099	1,014	3,085	75.26
8	4,462	10	9,800	4,016	8	4,008	558	3,450	86.08
9	5,029	14	9,366	4,526	11	4,515	1,173	3,342	74.02
10	4,905	12	9,752	4,415	9	4,406	1,470	2,936	66.64
11	5,096	11	7,593	4,586	9	4,577	1,444	3,143	68.52
12	5,798	7	10,768	5,218	6	5,212	1,571	3,641	69.86

Table 60.--CAROTENE BALANCE (MILLIGRAMS).

Animal number	Carotene					Apparent digestibility
	Fed	Refused	Consumed	Excreted	Digested	Per cent
7	41.29	.14	42.15	28.12	14.03	33.29
8	40.32	.08	40.24	24.51	15.73	39.09
9	45.44	.12	45.32	32.25	13.07	28.84
10	44.33	.10	44.23	27.09	17.14	38.75
11	46.04	.10	45.94	20.23	25.71	55.96
12	52.39	.06	52.33	29.28	23.05	44.05

Table 61.--XANTHOPHYLL BALANCE (MILLIGRAMS).

Animal number	Xanthophyll					Apparent digestibility
	Fed	Refused	Consumed	Excreted	Digested	Per cent
7	56.30	.15	56.15	43.69	12.46	22.19
8	54.98	.09	54.89	41.28	13.61	24.80
9	61.96	.12	61.84	75.05	-13.21	-21.36
10	60.46	.10	60.36	87.88	-27.52	-45.59
11	62.78	.10	62.68	61.06	1.62	2.58
12	71.43	.06	71.37	71.50	- .13	.00

Table 62.--XANTHOPHYLL/CAROTENE RATIO.

Animal number	Consumed	Feces	Digested
7	1.33	1.55	.90
8	1.36	1.68	.87
9	1.36	2.33	-1.01
10	1.37	3.24	-1.61
11	1.36	3.02	.10
12	1.36	2.44	.06
Average	1.36	2.38	- .12

Table 63.--LIVE WEIGHT (POUNDS).

Animal number	Beginning	Mid-period	End	Gain or loss
7	363	368	405	42
8	307	323	363	56
9	460	478	510	50
10	365	413	445	80
11	468	470	488	20
12	558	583	478	20

Table 64.--SERUM ANALYSIS.

Animal number	Carotene (Micrograms per 100 ml.) Beginning	Carotene (Micrograms per 100 ml.) End	Vitamin A (International units per 100 ml.) Beginning	Vitamin A (International units per 100 ml.) End
7	9.00	38.10	175.80	172.30
8	9.75	35.40	211.50	173.80
9	40.50	36.50	267.10	254.40
10	17.25	79.20	100.20	417.00
11	11.25	28.50	102.80	263.10
12	40.50	60.90	207.60	309.00

BALANCE TRIAL NO. 9

(July 13 - August 13, 1948)

Table 65.--DRY MATTER CONTENT.

Description of sample	Animal number	Moisture loss in Storage per cent	Moisture loss in Dryer per cent	Moisture loss in Oven per cent	Analysed Moisture per	Analysed Dry matter per cent	Total Moisture per cent	Total Dry matter per cent
Feces	7	0	77.68	11.87	89.55	10.45	89.55	10.45
"	8	0	79.05	10.76	89.81	10.19	89.81	10.19
"	9	1.21	81.21	4.57	85.78	14.22	86.99	13.01
"	10	.48	78.92	4.15	83.07	16.93	83.55	16.45
"	11	0	76.34	3.99	80.33	19.67	80.33	19.67
"	12	0	78.61	4.44	83.05	16.95	83.05	16.95
Hay				7.09	7.09	92.91	7.09	92.91
Tocopherol				5.55	5.55	94.45	5.55	94.45

Table 66.--ANALYSIS OF FEED AND FECES (MICROGRAMS PER GRAM).

Description of sample	Animal number	As analysed		Dry matter basis	
		Carotene	Xanthophyll	Carotene	Xanthophyll
Feces	7	3.69	26.84	35.31	256.84
"	8	4.71	31.03	46.22	304.51
"	9	5.63	35.27	39.59	248.03
"	10	5.16	28.94	30.48	170.94
"	11	6.77	34.31	34.42	174.43
"	12	5.88	35.97	34.69	212.21
Hay		12.39	48.76	13.34	52.48

Table 67.--DRY MATTER BALANCE (GRAMS).

Animal number	Feed		Feces	Dry matter					Apparent digestibility
	Fed	Refused		Fed	Refused	Consumed	Feces	Balance	Per cent
7*	3,867	-	8,571	3,593	-	3,593	896	2,713	75.18
8*	3,680	-	7,364	3,419	-	3,419	750	2,684	78.15
9*	4,104	-	7,337	3,813	-	3,813	955	287	75.06
10	3,748	-	6,831	3,482	-	3,482	1,124	2,358	67.73
11	4,065	-	6,010	3,777	-	3,777	1,182	2,595	68.70
12	4,461	-	7,891	4,145	-	4,145	1,338	2,807	67.73

* Received 18 grams of tocopherol mixture containing 15.20 grams of dry matter.

Table 68.--CAROTENE BALANCE (MILLIGRAMS).

Animal number	Carotene					Apparent digestibility
	Fed	Refused	Consumed	Excreted	Digested	Per cent
7*	47.93	-	47.93	31.63	16.30	34.00
8*	45.61	-	45.61	34.68	10.93	23.96
9*	50.87	-	50.87	37.79	13.08	25.71
10	46.45	-	46.45	34.25	12.20	26.26
11	50.39	-	50.39	41.00	9.39	18.63
12	55.29	-	55.29	46.40	8.89	16.08

* Received 1.51 grams of mixed tocopherols per day.

Table 69.--XANTHOPHYLL BALANCE (MILLIGRAMS).

Animal number	Xanthophyll				Apparent digestibility
	Fed	Refused	Consumed	Excreted	Digested	Per cent
7*	188.56	-	188.56	230.04	-41.48	-22.00
8*	179.43	-	179.43	228.50	-49.07	-27.35
9*	200.11	-	200.11	236.75	-36.64	-18.31
10	182.74	-	182.74	192.09	- 9.35	- 5.12
11	198.22	-	198.22	206.20	- 7.98	- 4.02
12	217.53	-	217.53	283.84	-66.31	-30.48

* Received 1.51 grams of mixed tocopherols per day.

Table 70.--XANTHOPHYLL/CAROTENE RATIO.

Animal number	Consumed	Feces	Digested
7	3.93	7.27	-2.54
8	3.93	6.59	-4.49
9	3.93	6.26	-2.80
10	3.93	5.61	- .77
11	3.93	5.03	- .85
12	3.93	6.12	-7.46
Average	3.93	6.15	-3.15

Table 71.--LIVE WEIGHT (POUNDS).

Animal number	Beginning	Mid-period	End	Gain or loss
7	405	415	418	13
8	365	370	362	- 3
9	510	513	520	10
10	450	458	445	- 5
11	490	497	500	10
12	590	595	605	15

Table 72.--SERUM ANALYSIS.

Animal number	Carotene (Micrograms per 100 ml.) Beginning	Carotene (Micrograms per 100 ml.) End	Vitamin A (International units per 100 ml.) Beginning	Vitamin A (International units per 100 ml.) End
7		108.90		281.40
8		135.00		395.10
9		147.00		222.00
10		201.00		230.40
11		91.20		295.50
12		99.00		243.30

BALANCE TRIAL NO. 10

(August 18 - September 8, 1948)

Table 73.--DRY MATTER CONTENT.

Description of sample	Animal number	Moisture loss			Analysed		Total	
		in Storage per cent	in Dryer per cent	in Oven per cent	Moisture per cent	Dry matter per cent	Moisture per cent	Dry matter per cent
Feces	7	.28	77.90	3.64	81.54	18.46	81.82	18.18
"	8	.34	80.00	3.39	83.39	16.61	83.73	16.27
"	9	.00	73.32	2.39	75.71	24.29	75.71	24.29
"	10	.00	74.32	3.42	77.74	22.26	77.74	22.26
"	11	.15	74.66	3.16	77.82	22.18	77.97	22.03
"	12	.00	77.45	2.80	80.25	19.75	80.25	19.75
Hay				7.21	7.21	92.79	7.21	92.79
Tocopherol				5.55	5.55	94.45	5.55	94.45

Table 74.--ANALYSIS OF FEED AND FECES (MICROGRAMS PER GRAM).

Description of sample	Animal number	As analysed		Dry matter basis	
		Carotene	Xanthophyll	Carotene	Xanthophyll
Feces	7	1.34	11.94	7.26	64.68
"	8	1.09	10.62	6.56	63.94
"	9	1.67	13.58	6.88	55.91
"	10	1.27	12.55	5.71	56.38
"	11	1.57	13.73	7.08	61.90
"	12	1.77	12.35	8.96	62.53
Hay		3.45	34.87	3.72	37.58

Table 75.--DRY MATTER BALANCE (GRAMS).

Animal number	Feed		Feces	Dry matter					Apparent digestibility
	Fed	Refused		Fed	Refused	Consumed	Feces	Balance	Per cent
7*	3,211	-	6,782	2,979	-	2,979	1,233	1,761	59.12
8	3,111	-	6,589	2,887	-	2,887	1,072	1,815	62.87
9	3,266	-	6,209	3,031	-	3,031	1,508	1,523	50.25
10	3,124	-	6,304	2,899	-	2,899	1,403	1,496	51.60
11*	3,186	-	5,497	2,956	-	2,956	1,211	1,760	59.55
12*	3,367	-	6,138	3,124	-	3,124	1,212	1,927	61.69

* Received in addition 18 grams of a tocopherol mixture which contained 15.20 grams of dry matter.

Table 76.--CAROTENE BALANCE (MILLIGRAMS).

Animal number	Carotene					Apparent digestibility
	Fed	Refused	Consumed	Excreted	Digested	Per cent
7*	11.08	-	11.08	8.95	2.13	19.22
8	10.74	-	10.74	7.03	3.71	34.54
9	11.28	-	11.28	10.38	.90	7.98
10	10.78	-	10.78	8.01	2.77	25.70
11*	11.00	-	11.00	8.57	2.43	22.09
12*	11.62	-	11.62	10.86	.76	6.54

* Received 1.51 grams of mixed tocopherols per day.

Table 77.--XANTHOPHYLL BALANCE (MILLIGRAMS).

Animal number	Xanthophyll				Apparent digestibility
	Fed	Refused	Consumed	Excreted	Digested	Per cent
7*	111.95	-	111.95	79.75	32.20	29.76
8	108.49	-	108.49	68.54	39.95	36.82
9	113.90	-	113.90	84.31	29.59	25.98
10	108.94	-	108.94	79.10	29.84	27.39
11*	111.09	-	111.09	74.96	36.13	32.52
12*	117.40	-	117.40	75.79	41.61	35.44

* Received 1.51 grams of mixed tocopherols per day.

Table 78.--XANTHOPHYLL/CAROTENE RATIO.

Animal number	Consumed	Feces	Digested
7	10.10	8.91	15.12
8	10.10	9.74	10.77
9	10.10	8.12	32.86
10	10.11	9.88	10.77
11	10.10	8.75	14.87
12	10.10	6.98	54.75
Average	10.10	8.73	23.19

Table 79.--LIVE WEIGHT (POUNDS).

Animal number	Beginning	Mid-period	End	Gain or loss
7	450	445	451	1
8	345	340	350	5
9	560	550	563	3
10	455	460	453	-2
11	510	505	517	7
12	605	595	607	2

Table 80.--SERUM ANALYSIS.

Animal number	Carotene (Micrograms per 100 ml.) Beginning	Carotene (Micrograms per 100 ml.) End	Vitamin A (International units per 100 ml.) Beginning	Vitamin A (International units per 100 ml.) End
7	75.30	38.25	287.10	453.30
8	76.50	42.00	233.40	341.10
9	69.00	50.25	237.60	205.40
10	83.40	43.50	332.70	609.30
11	63.00	43.50	338.70	385.50
12	70.50	33.00	161.10	456.00

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