THE EFFECT OF LOW CAROTENE FEED UPON SEMEN EVALUATION AND FERTILITY OF DAIRY BULLS

by

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INTRODUCTION

Carotene, the precursor of vitamin A, has long been considered of vital importance in both animal and human nutrition. The amount of carotene required in the ration depends largely on the ultimate utilization of the animal. Good dairy herd management requires a bull of high breeding fertility, and it would appear that a ration sufficiently high in carotene in maintaining a high level of fertility is one of the essential factors.

The majority of investigators reporting up to the present time have limited their studies to the dietary carotene level for normal reproduction in the cow, and in some instances the experiments reported were of short duration. Little, if any, attention has been given to the carotene requirements of the bull.

The purpose of this study was to determine the effect of a low carotene ration on semen production and quality as well as fertility of dairy bulls on a weekly semen collection schedule. This information would be of nutritional value to the dairyman, the purebred breeder and to bull stud personnel, who must maintain a high level of semen quality and the resultant high breeding efficiency of their bulls.

REVIEW OF LITERATURE

The role of vitamin A in both animal and human diets has been known for almost forty years, and this vitamin A ranks high in importance among the vitamins. The metabolism and functions of vitamin A and its precursors, the carotenes, has been under constant study by investigators since its discovery in 1913. Previous to 1926 physiological and nutritional investigations were conducted using small laboratory animals. Jones, Eckles and Palmer (16, p. 130) were the first group of investigators reporting on the role of vitamin A in the nutrition of dairy calves, which was a systematic study with large domestic animals. Earlier reports, however, had been published with reference to the particular difficulties in raising normal calves from cows that had been maintained on various types and qualities of hays.

Many investigators have studied the reproduction requirements of cows for vitamin A and up to the present time there have been a few studies using bulls. Guilbert and Hart, 1936, (11, p.417) stated the minimum intake of carotene necessary for normal reproduction in the cow is approximately 30 micrograms of carotene per kilogram of body weight daily. In 1939 Guilbert, Howell and Hart (12, pp. 91-103) reported 26 - 33 micrograms of carotene daily as the minimum with 130 - 165 micrograms of carotene daily during the last month of gestation. Davis and Madsen, 1941,

(7, pp.135-146) reported weak, blind and dead calves when the cows were receiving 45 micrograms of carotene per kilogram of body weight daily. At a 60 micrograms daily intake the calves were normal, and satisfactory reproduction was obtained with 132 micrograms of carotene per day. Hilton and Associates (14, pp.631-632) gave the vitamin A requirements for normal reproduction as 30,000 I.U. daily in 1944.

Later in 1949 Kuhlman and Gallup (19, p.688) reported the minimum carotene intake for normal reproduction was between 40 and 45 micrograms per pound of body weight daily with first calf heifers requiring an additional amount to take care of growth, maintenance, and reproduction. At 20 to 39 micrograms per pound of body weight 1.99 services per conception were required, while at 60 to 99 micrograms, 1.15 services were necessary. They also gave 40 to 45 micrograms of carotene per pound of body weight daily as a minimum figure for satisfactory reproduction in Jersey cows.

The National Research Council (24, p.9) recommendations are 6 milligrams of carotene per 100 pounds of body weight daily and an addition of 30 milligrams per head daily for the last two to three months before parturition.

Moore, et al, 1948 (22, pp.533-538) and Ronning, et al, 1951 (27, p.52) found significant breed differences in the carotene requirements necessary to prevent reproductive difficulties. Ronning, et al, (27, p.52) stated the

minimum safe level for reproduction in Guernseys to be 90 micrograms per pound of body weight daily, and that this level should be maintained throughout the gestation to insure no difficulties.

In 1942 Thorp (32, pp.27-31) reported that calves fed 6.7 micrograms of carotene per calf daily showed degenerative and inflammatory changes in the kidney, but these changes were not consistent. He also showed degeneration of the seminiferous tubules of the testicle. Jones, Haag and Dougherty in 1942 (18, p.689) found no relation between so-called low quality sperm and ration fed.

Sutton and Associates in 1940 (29, p.274) reported yearling dairy bulls on a low-carotene diet showed constriction of the optic nerve and degeneration of the germinal epithelium of the testes. Jones and Haag, 1944, (17, pp.632-633) found blindness and death in young Jersey bulls with blood plasma values of 0.3 p.p.m. carotene. Twenty-five micrograms per kilogram of body weight daily did not prevent blindness,but it was sufficient for normal growth and to maintain blood plasma carotene at 0.3 p.p.m. When 35 micrograms of carotene per kilogram of body weight was fed daily, the bulls grew normally even though one animal went blind. The blood plasma carotene content was reported as 0.46 p.p.m. Fertility was maintained at 35 micrograms, but at 25 micrograms one bull failed to settle cows to which he was bred.

Using the testicle biopsy technique in vitamin A studies, Erb and co-workers (8, p.769) fed bulls a low-carotene diet of beet pulp and grain. The bulls developed blindness, loss of co-ordination and gastro-intestinal disturbances. The testicle biopsies showed degeneration of the seminiferous tubules and almost complete disappearance of sperm. The quality of semen declined until a vitamin A supplement was fed. When the supplement was increased from 60,000 I.U. of vitamin A to 150,000 I.U. daily, semen quality improved. A second biopsy revealed the beginning of repair to the testicles.

Hodgson, et al,(15, p.669) found that bulls receiving 2 p.p.m. carotene in their hay failed to serve at the expected age of 10 - 12 months. When the carotene intake was increased to 10 p.p.m. the bulls began breeding. These investigations reported gross vitamin A deficiency symptoms appearing before impairment of breeding efficiency. Semen of the deficient bulls was found to be low in concentration, high in abnormal forms, high in pH and with low livability.

In 1947 Erb and associates (9, p.687) reported complete blindness in bulls receiving 2000 I.U. of vitamin A daily. After five months of therapy with 100,000 I.U. of vitamin A daily, only moderate sperm production was observed. These workers found that moderate prepubertal vitamin A deficiency did not completely inhibit sperm production,

however, fertility and reproductive capacity appeared to be seriously impaired. Likewise, in 1948 Bratton, et al. (3, p.779) found gross vitamin A deficiency symptoms appearing before any appreciable influence on semen quality was evident. There was, however, a degeneration of the seminiferous tubules of the testicles.

Byers in 1954 (5, pp.1-105) indicated that the influence of carotene intake on reproductive performance did not appear to be as critical with dairy bulls as with dairy cows. Semen quality deteriorated with advancing age regardless of an increasing carotene intake, showing that the length of exposure to a low carotene ration of the animal itself, or its ancestors influenced its reproductive performance. There was a significant difference in the breeding efficiency with the normal bulls far exceeding the vitamin A deficient bulls.

Studying the effects of pasture feeding on semen quality, Branton, et al. (2, pp.199-204) found that the addition of pasture to the ration of bulls did not affect body weight, sexual behavior, semen production or fertility.

Flipse and Almquist (10, pp.1123-1127) experimenting with mature dairy bulls in 1954, reported that the replacement of 2 pounds of grass-legume hay by 2 pounds of dehydrated young grass for a period of 4 months did not show any significant difference in semen quantity and quality or reproductive efficiency between the two groups of bulls,

the experimental and the controls.

Frequency of semen collection was studied by Bratton and associates (4, p.1444) using mature dairy bulls. The bulls were divided into two groups and an ajaculate was obtained at 6 day intervals for 360 days. The two groups of bulls were handled identically except that a second ejaculate was obtained from individuals in one of the groups. These investigators reported similar numbers of motile spermatozoa per ejaculate from the first ejaculate of both groups of bulls. The average 60 to 90 day non-return rate for first service cows was similar for both the one ejaculate and first ejaculate of the bulls which ejaculated twice. These percentages were 73.3 and 73.6 respectively, and the second ejaculate resulted in a 73.4 per cent non-return rate.

To determine the age at which puberty was reached, Baker, VanDemark and Salisbury (1, p.489) tested nine Holstein bull calves by weekly presenting them to a live mount. They first demonstrated sexual interest between six and eleven months of age and achieved their first ejaculates between nine and twelve months. For Milking Shorthorns, Olson (25, p.489) reported the first service, and this with much difficulty, of identical triplets at the age of 13.5 months. Magill (20, pp.1-60) studying two sets of identical twins found that the age at first service of the members of the Jersey twins was 35 and 36 weeks while that of

the Holstein X Ayrshire twins was 39 and 43 weeks.

Herman and Swanson (13, pp.1-82) examined 342 ejaculates from a total of 55 bulls. They reported wide variations in all semen properties studied in ejaculates from the same bull and in ejaculates from different bulls. Greatest variations were in length of time vigorous motility persisted and in the percentage of abnormal sperm per ejaculate. These same investigators (31, pp.321-331) later studied 256 semen samples from 10 bulls and found that the various semen properties varied among individuals. These workers found that volume and concentration of sperm were the two properties showing the greatest variation both between and among individual bulls. Initial motility varied the least, being either uniformly good or uniformly poor for each bull.

After a one year study of semen quality of five Holstein and five Guernsey bulls, Mercier (21, p.556) reported wide variations between bulls concerning volume, concentration, number of spermatozoa per ejaculate, methelene blue reduction time, and other spermatozoa abnormalities.

Working with a set of monozygotic Milking Shorthorn triplet bulls maintained under a uniform environment, Olson (25, p.489) reported that all bulls behaved similarly in stubbornness and lack of sexual interest. This study

indicated that identical triplet bulls are alike in semen production as well as other morphological and physical characteristics. Olsen (26, p.937) in a recent study using identical twin bulls concluded that under the same environmental conditions the performance of each member was similar to his mate or mates regarding growth, general appearance, behavior patterns and the quality and quantity of semen produced.

EXPERIMENTAL PROCEDURE

It was the purpose of this experiment to study the effect of carotene in the ration of dairy bulls as it affects the quality and fertility of semen. The properties of semen to be studied were volume of each ejaculate, livability and concentration of sperm and the percentage of live sperm. Semen from individual bulls was used to artificially inseminate cows from surrounding cooperating dairy herds and records made of the number of services per conception. A total of 19 bulls, 5 sets of identical twins and 9 unrelated animals, used in the study were divided into two groups. One group was placed on a high level of carotene intake and the other on a low level intake. The general procedure of the experiment was as follows:

1. Experimental Animals

According to their nutritional history prior to being placed on this experiment the 19 bulls were divided into groups.

a. Unrelated Bulls The unrelated bulls consisted of 5 Holsteins and 4 Jerseys and they were divided into two groups. In the first group there were 3 Jerseys and 2 Holsteins, and in the second group 1 Jersey and 3 Holsteins. The bulls in the former group were born to dams maintained at 50 micrograms of carotene per kilogram of body weight

daily for 2.3 to 9 months of the gestation period. For the purpose of this study these bulls were placed in the low carotene group. The second group of bulls were from dams receiving a daily carotene intake of 390 micrograms per kilogram of body weight. They were assigned to the high carotene group. All unrelated bulls and their dams were from the Oregon State College dairy herd.

b. Two Sets of Identical Twin Bulls Previously on Normal Ration One pair of twins, numbered TI_1 and TI_2 were born August 25, 1952 from a registered Ayrshire cow and a registered Holstein sire. The other pair, TJ_1 and TJ_2 were born September 18, 1952 sired by a registered Jersey bull and out of a purebred Jersey cow. Monozygocity in all twins was determined after careful visual observation and examination of their blood antigens. After weaning, the twins were supplied a normal ration satisfying 100 per cent of Morrison's Feeding Standard (23, p.1148) up to March 1, 1955, at which time they were placed on this experiment.

c. Three Sets of Identical Twin Bulls The first pair, numbered TR_1 and TR_2 were purebred Holsteins born April 26, 1954, the second pair, TV_1 and TV_2 were purebred Brown Swiss born February 6, 1955, and the third pair, TW_1 and TW_2 were Holstein X Jersey cross born March 2, 1955. These twin bulls received normal rations until 45 days after birth, at which time they were included in this study. All T_1 bulls were placed in the high level carotene group

and the T_2 bulls in the low level group.

Bulls receiving a daily carotene intake of 130 micrograms or less per kilogram of body weight will be referred to as the "low carotene group" throughout this report while those receiving a daily carotene intake of higher than 130 micrograms as the "high carotene or carotene supplemented group."

2. Feeding and Management

All animals were fed a ration of grain and low carotene hay. When necessary to supplement nutritional requirements molasses dried beet pulp was added to the diet. Representative samples of all hays fed were obtained and the carotene content determined by chemical analysis. The individual bulls were weighed at bi-monthly intervals and rations calculated to meet Morrison's Feeding Standard (23, p.1148) for normal growth and reproduction. All feed stuffs were weighed to the animals and any refused feed weighed back and recorded. Hay refusal was held to a minimum by restricting the animals to the amount they would willingly clean up. When necessary, molasses dried beet pulp was added to the ration to meet total digestible nutrient requirements. Hay was fed twice daily and the grain and beet pulp when added were fed once daily. The carrot oil supplement was measured by using a glass syringe graduated in milliliters and then mixed with the grain.

The bulls were housed in individual tie stalls with mangers so constructed as to prevent feed exchange. Wood shavings were used as bedding and when exercised the animals had access to a paved corral. The only feed stuffs available to the bulls were those placed in the individual mangers.

The feeding regime as originally planned called for two groups of bulls on varying levels of carotene intake. The low carotene group consisted of 5 unrelated bulls, 200 B_1, 221 B_1, 252 B_1, 500 B_1 and 503 B_2, and 5 monozygotic twin bulls, TI_2, TJ_2, TR_2, TV_2 and TW_2. All T_2 twin bulls received a daily carotene intake ration of 50 micrograms per kilogram of body weight throughout the experiment. The 5 unrelated bulls received a daily carotene intake ration of varying levels for various periods of time throughout the experiment. Bull 200 B_1 was fed 50 micrograms of carotene per kilogram of body weight from 45 to 612 days of age, 90 micrograms from 613 to 1,007 days, and 130 micrograms from 1,008 to 1,338 days of age. Bull 221 B_1, 50 micrograms from 45 to 830 days, 90 micrograms from 831 to 1,225 days, and 130 micrograms from 1,226 to 1,437 days of age. Bull 252 B_1, 50 micrograms from 45 to 90 days, 90 micrograms from 91 to 485 days, and 130 micrograms from 486 to 1,028 days of age. Bull 500 B_1, 50 micrograms from 45 to 876 days, 90 micrograms from 877 to 1,271 days, and 130 micrograms from 1,272 to 1,481 days of age, and bull 503 B_2, 50 micrograms

from 45 to 582 days, 90 micrograms from 583 to 977 days, and 130 micrograms from 978 to 1,315 days of age.

Included in the high level or carotene supplemented group were 4 unrelated bulls, 264 B_2, 445 B_2, 493 B_3, and 490 B_1, and 5 twin bulls, TI_1, TJ_1, TR_1, TV_1 and TW_1. The 4 unrelated bulls after weaning were fed a daily carotene intake ration of 390 micrograms per kilogram of body weight throughout the entire experiment. The 5 T_2 members of the identical twin pairs received a daily ration containing 500 micrograms of carotene per kilogram of body weight over the entire feeding period.

3. Collection of Semen

The bulls were led individually from the tie stalls to the semen collection room adjoining the semen processing laboratory. The sheath and belly of each bull were clipped frequently to insure good washing and to avoid semen contamination by foreign matter. The sheath and belly of the bulls were washed using a brush and warm water containing Roccal solution. The washed area was then wiped dry with paper towels. After washing each bull was teased for about 5 minutes using the live mount.

As each bull approached the age of sexual maturity repeated attempts were made to collect semen samples. Age at sexual maturity was thus determined to be the date of the first ejaculate that produced semen with live sperm.

A standard artificial vagina was used to collect each ejaculate. The vagina consisted of a stiff rubber casing with a soft latex lining. Warm water at a temperature of 115° to 120°F was used to fill the vagina. A volumetric hard glass test tube attached to a rubber funnel on the end of the artificial vagina was used to catch the ejaculate.

4. Semen Processing

Immediately after ejaculation the artificial vagina was transferred to the processing laboratory adjoining the collection room and the glass tube containing semen was removed to record the volume of that ejaculate and to determine the initial motility and concentration. These were done without dilution of the semen using a low power microscope (100 magnification) with attached warming stage maintained at a temperature of 102° F.

Using a modified Blom's eosin differential stain (30, pp.981-987) the semen stains were prepared to determine the percentage of live and dead spermatozoa of each ejaculate. The stain preparation was made by placing a few drops of the staining liquid on the glass slide, then adding a very small drop of semen, mixing and drying at once on a warm plate maintained at 315° F.

To determine the concentration of spermatozoa, 0.1 ml. of semen was diluted in 9.9. ml. of water and this

dilution was kept under refrigeration up to the time to be observed. The remainder of the semen was diluted in egg yolk - citrate diluter at the rate of one part of semen to 10 parts of diluter. In preparing egg yolk - citrate diluter, one part of egg yolk and three parts of 2.9 per cent sodium citrate ($Na_3 C_6 H_5 O_7 \cdot 5\frac{1}{2} H_2O$) were mixed thoroughly with 500 units of aqueous penicillin and 500 milligrams of aqueous streptomycin per ml. of the diluter as a deterrent to bacterial growth. After dilution the diluted semen of each ejaculate was transferred into a small vial at the rate of three ml. each with a stopped cork, and all vials were kept surrounded by water in a beaker under refrigeration and maintained at a temperature of 40 to 44° F until the time of observation for the sperm motility in diluted semen.

5. Semen Examination

The spermatozoa count on the smear stains of semen with a modified Blom's eosin differential stain was done under the microscope with exact count of 200 spermatozoa on each stained slide. Spermatozoa which absorbed the dye were classified as being dead while one not staining or partially staining, as being alive. Thus, the percentage of live and dead spermatozoa at the time of collection was obtained.

Using a Spencer hemacytometer, the concentration

of the spermatozoa was determined from the diluted semen at the rate of 0.1 ml. of semen to 9.9 ml. of water within 24 hours following the semen collection. As the volume of the hemacytometer dilution was known, the concentration of spermatozoa per ml. could be readily evaluated.

The diluted semen samples at the rate of one part of semen to 10 parts of egg holk - citrate diluter - were stored seven days after collection in a refrigerator maintained at 40 to 44° F. Then each sample was rated for the motility of the spermatozoa in diluted semen at 168 hours, using a high power microscope (440 magnification) with attached warming stage maintained at a temperature of 102° F. The rating given to each sample for the spermatozoal motility was from one to ten (representing 10 per cent to 100 per cent).

6. Fertility

The fertility of the bulls was determined by artificial insemination of a limited number of cows in cooperating herds, consisting of one to twenty-five cows and maintained under a variety of conditions of management. The semen used was no older than 72 hours. The condition of the cows bred was not taken into consideration in calculating the breeding efficiency of the bulls.

Some cows were bred up to eight times to as many as four different bulls, indicating that the difficulty in

conception might be with the cow. Nevertheless, these services were charged against the bulls.

RESULTS AND DISCUSSION

1. Identical Twin Bulls Previously Fed Normal Rations

Two pairs of identical twins were reared on a normal herd ration up to 2½ years of age and then placed on different levels of carotene in their rations. One pair of twins was Holstein X Ayrshire crossbred designated as TI_1 and TI_2. The other pair was purebred Jersey designated as TJ_1 and TJ_2. One animal of each pair, TI_1 and TJ_1 was placed on a ration containing a carotene supplement at the rate of 500 micrograms per kilogram of body weight daily. The other animal of each pair, TI_2 and TJ_2, was placed on a carotene-deficient ration at a rate of 50 micrograms of carotene per kilogram of body weight daily. The bulls were carried on the respective rations until slaughtered - a period of 14 months for TI_1 and TI_2, and 16 months for TJ_1 and TJ_2. Semen quality of the individuals after being placed on the carotene rations is shown in Table I.

Table I shows similarity in all properties of semen characteristics studied. The average volume per ejaculate of TI_1 and TJ_1 was 6.48 ml. and 4.77 ml. respectively in contrast with that of their mates, TI_2 and TJ_2 which produced 4.77 ml. and 2.46 ml. respectively. Another difference in favour of the carotene-supplemented group was the sperm motility in diluted semen at 168 hours; TJ_1 being 5.00, and TJ_2 being 4.48. All properties of semen charac-

Table I

Semen Production and Semen Quality
of Two Sets of Identical Twins Previously on Normal Ration

Bull No.	Born	Starting Collection Date *	Last Collection Date	No. of Collections	Volume (ml.)	Sperm Motility in Diluted Semen 0 Hr.	Sperm Motility in Diluted Semen 168 Hrs.	Sperm Count (Million/ ml.)	Live Sperm (%)
Carotene Supplemented									
TI_1	8-25-52	11-9-55	4-27-56	20	6.48	8.06	3.83	1125.83	91.84
TJ_2	9-18-52	11-9-55	6-2-56	30	3.58	8.65	5.00	1543.52	91.72
Average					5.03	8.36	4.42	1334.68	91.78
Low Carotene									
TI_2	8-25-52	11-9-55	4-27-56	21	4.77	8.56	3.89	1361.83	93.16
TJ_2	9-18-52	11-9-55	5-4-56	25	2.46	8.67	4.48	1842.42	92.18
Average					3.62	8.62	4.19	1552.13	92.67
t **					0.7634	-0.8491	0.6981	0.8401	-1.8031

* First collection date in this study

** Distribution of t at 5 percent level 2.7764

teristics in this study other than those formerly mentioned were in favour of the low-carotene group.

There was no significant difference in any of the properties studied when treated statistically. The study of the records of these two sets of identical twins revealed that they were on normal rations up to about 30 months of age before the beginning of this experiment. Also, semen collections had not been made for a period of about five months before the beginning of this study. It is conceivable that there may have been a carry-over of vitamin A from the normal ration causing the similarity of semen quality in this study. It is noted that TI_2 and TJ_2 on deficient carotene rations at the rate of 50 micrograms of carotene per head daily for about fourteen and sixteen months respectively did not show any adverse effects from the carotene deficiency. It did appear, however, that after six months on the experimental ration a slight difference in semen quality occurred in favour of the carotene supplemented animals. These results indicate a need for further study to determine how long the bull can be maintained on a low-carotene ration without affecting the semen quality.

2. Identical Twins Reared on Different Carotene Levels

Three pairs of identical twins were placed on carotene rations from time of weaning until they were

slaughtered. The twins consisted of one pair of Holsteins, TR_1 and TR_2; one pair of Brown Swiss, TV_1 and TV_2, and one pair of Holstein X Jersey cross-breds, TW_1 and TW_2. Number one of each pair received 500 micrograms per kilogram of body weight per day. Number two of each pair received fifty micrograms per kilogram of body weight per day. Table II shows the results of this study. It is noted that the low-carotene ration retarded sexual maturity by 10, 52, and 60 days respectively for each of the low-carotene animals. TR_1 and TR_2 were slaughtered after obtaining 13 and 16 weekly collections. The other pairs of twins are being continued on this study. The data up to approximately two years of age as shown in Table II reveals a slight advantage of the high-carotene group over the low-carotene group in sperm motility in diluted semen at 168 hours, total sperm count, and age of reaching sexual maturity. The motility of the spermatozoa in diluted semen at 168 hours of TV_1 and TW_1 were higher than those of TV_2 and TW_2, being 4.24 and 3.91 to 4.13 and 2.89 respectively. Total sperm per ml. of semen was 1211.65 millions for TV_1, 1085.72 millions for TW_1, 1042.32 millions for TV_2 and 826.31 millions for TW_2.

Table III shows practically no difference in the average volume per ejaculate, being 3.92 ml. for the carotene-supplemented group and 3.88 for the low-carotene group. A slight difference of 0.04 was noted in the sperm motility

Table II

Semen Production and Semen Quality
of Three Sets of Identical Twins

Bull No.	Born	Age 1st Service Days	Last Collection Date	No.of Collections	Volume (ml.)	Sperm Motility in Diluted Semen 0 Hrs.	Sperm Motility in Diluted Semen 168Hrs.	Sperm Count (Million/ml.)	Live Sperm (%)
Carotene Supplemented (500 micrograms per kilogram of body weight)									
TR_1	4-26-54	574	3-2-56	13	3.99	6.55	3.82	965.62	89.81
TV_1	2-6-55	394	3-7-57	46	5.17	7.78	4.24	1211.65	87.21
TW_1	3-2-55	418	3-8-57	37	2.60	7.84	3.91	1085.72	89.33
Average		462			3.92	7.39	3.99	1087.66	88.78
Low Carotene (50 micrograms per kilogram of body weight)									
TR_2	4-26-54	584	3-15-56	16	4.01	6.17	3.25	1068.81	82.91
TV_2	2-6-55	446	3-7-57	35	5.21	8.00	4.13	1042.32	89.24
TW_2	3-2-55	478	3-8-57	32	2.41	7.22	2.89	826.31	86.01
Average		502.67			3.88	7.13	3.42	979.15	86.05
t *		-0.8197			0.0514	0.5435	2.0562	4.4010	1.9358

* Distribution of t at 5 per cent level 2.4469

Table III

Average Age at First Service, Semen Production and Semen Quality of Three Sets of Identical Twins

		Carotene Supplemented 500 Micrograms /Kilogram of Body Weight	Low Carotene 50 Micrograms /Kilogram of Body Weight
No. of bulls		3	3
Age at first service	days	462	502.67
No. of collections		96	83
Volume of ejaculate	ml.	3.92	3.88
Sperm motility in diluted semen at 0 hr.		7.39	7.13
Sperm motility in diluted semen at 168 hrs.		3.99	3.42 *
No. of sperm/ml. semen millions		1087.66	979.15**
Percentage of live sperm		88.78	86.05 *

* Approaching significance at 5 per cent level

** Significant at 5 per cent level

in diluted semen at zero hour in favour of the carotene-supplemented group. The differences in other semen properties were quite evident; the carotene supplemented group averaged 40.67 days earlier for the first service. Sperm motility of diluted semen at 168 hours was 3.99 and 3.42 for the high and low-carotene groups respectively. The percentage of live sperm averaged 88.78 and 86.05 for the high and low-carotene groups respectively. Statistically treated, the differences of the latter two properties were approaching significance at five per cent level of the t distribution. This indicates some evidence that the carotene deficiency has some effect on the livability of the spermatozoa. It first affects the concentration of the sperm as shown by the significant difference in sperm concentration, being 1087.66 millions per ml. of semen for the carotene-supplemented group and 979.15 millions for the low-carotene group.

3. Unrelated Bulls Fed Varying Low Carotene Levels

Bulls in the study included three Jerseys and two Holsteins. They were the progeny of dams receiving low-carotene intake during gestation as follows:

Experimental Animal	Carotene Intake of Dam During Gestation (Microgram/Kilogram of Body Weight)	Carotene Intake of Experimental Bulls	
		Level (Microgram/ Kilogram of Body Weight)	Period (days)
200 B_1	50- last 2.8 months	50	45- 612
		90	613-1,007
		130	1,008-1,338
221 B_1	50- last 7 months	50	45- 832
		90	833-1,225
		130	1,226-1,437
252 B_1	50-last 9 months	50	45- 90
		90	91- 485
		130	486-1,028
500 B_1	50- last 6 months	50	45- 876
		90	877-1,271
		130	1,272-1,481
503 B_2	50- last 3.3 months	50	45- 582
		90	583- 977
		130	978-1,315

The plan of this study was to continue the bulls on the same level of carotene intake as their dams were receiving. The bulls failed to develop normally so their carotene level was increased to 90 micrograms per kilogram of body weight daily. After approximately one year it became necessary to increase it to 130 micrograms to maintain the bulls in serviceable condition. The animals numbered 200 B_1, 221 B_1 and 252 B_1 were purebred Jersey bulls and numbered 500 B_1 and 503 B_2 were purebred Holstein bulls. The results of semen characteristics studied appear in Table IV through Table VIII.

Bull 200 B_1 from a dam receiving 50 micrograms of carotene daily per kilogram of body weight for the last 2.8 months of gestation was able to produce the first ejaculate at 387 days of age, which was 20 days later than that of bull 252 B_1 from a dam receiving 50 micrograms for approximately nine months of the gestation. Bull 221 B_1 from a dam of the same level of carotene intake as the dam of number 252 B_1 was able to produce the first ejaculate at 419 days of age. The data show that when all qualities of semen were studied there is an improvement at each increase of daily carotene intake. This indicates that bulls on long periods of carotene deficiency will respond to an increase in carotene intake readily but marked improvement appeared approximately two months after the increase. Maintaining bulls on comparatively low levels of carotene

Table IV

Semen Quality of the Jersey Bull 200 B_1
at Varying Levels of Carotene Intake at Different Ages

Carotene Intake (Microgram /Kilogram of Body Weight)	No. of Collections	Volume (ml.)	Sperm Motility in Diluted Semen Hr.	Hrs. 168	Sperm Count (Million /ml.)	Live Sperm (%)
Age at first service 387 days						
First collection to 18 months old						
50	29	2.62	6.32	3.05	911.67	69.49
18 - 24 months old						
90	9	4.37	8.56	3.78	1014.44	73.03
24 - 36 months old						
90	20	4.84	7.10	3.35	1006.89	77.67
130	9	3.10	6.44	2.38	1547.67	80.20
Over 36 months old						
130	30	4.34	7.32	2.44	1568.72	89.14

Table V

Semen Quality of the Jersey Bull 221 B_1
at Varying Levels of Carotene Intake at Different Ages

Carotene Intake (Microgram /Kilogram of Body Weight)	No. of Collections	Volume (ml.)	Sperm Motility in Diluted Semen Hr. 0	Hrs. 168	Sperm Count (Million /ml.)	Live Sperm (%)
Age at first service 419 days						
First collection to 18 months old						
50 *						
18 - 24 months old						
50	20	2.67	7.00	3.60	646.86	57.73
24 - 36 months old						
50	11	5.00	5.60	1.86	288.36	63.53
90	21	5.20	7.00	2.40	654.71	69.81
Over 36 months old						
130	28	4.66	6.58	3.46	1075.61	83.23

* There were collections but not processed for all data.

Table VI

Semen Quality of the Jersey Bull 252 B_1
at Varying Levels of Carotene Intake at Different Ages

Carotene Intake (Microgram /Kilogram of Body Weight)	No. of Collections	Volume (ml.)	Sperm Motility in Diluted Semen Hr. 0	Hrs. 168	Sperm Count (Million /ml.)	Live Sperm (%)
Age at first service 367 days						
First collection to 18 months old						
90	4	2.83	4.75	3.00	728.00	78.67
130	9	2.08	7.00	2.88	1372.22	84.54
18 - 24 months old						
130	17	3.11	6.65	2.27	1000.62	85.07
24 - 36 months old						
130	35	3.96	5.56	1.71	859.56	75.40

Table VII

Semen Quality of the Holstein Bull 500 B_1
at Varying Levels of Carotene Intake at Different Ages

Carotene Intake (Microgram /Kilogram of Body Weight)	No. of Collections	Volume (ml.)	Sperm Motility in Diluted Semen Hr. 0	Hrs. 168	Sperm Count (Million /ml.)	Live Sperm (%)
Age at first service 294 days						
First collection to 18 months old						
50	37	4.24	6.39	3.19	841.50	-
18 - 24 months old						
50	23	5.01	5.57	2.43	262.75	-
24 - 36 months old						
50	21	5.41	4.00	1.20	526.84	56.94
90	21	7.29	4.29	1.30	706.88	73.53
Over 36 months old						
90	7	7.03	3.33	0.33	871.33	77.23
130	27	6.40	3.31	1.36	726.93	76.53

Table VIII

Semen Quality of the Holstein Bull 503 B_2
at Varying Levels of Carotene Intake at Different Ages

Carotene Intake (Microgram /Kilogram of Body Weight)	No. of Collections	Volume (ml.)	Sperm Motility in Diluted Semen Hr. 0	Sperm Motility in Diluted Semen Hrs. 168	Sperm Count (Million /ml.)	Live Sperm (%)
Age at first service 367 days						
First collection to 18 months old						
50	13	3.65	3.69	1.82	494.67	71.88
18 - 24 months old						
90	13	4.22	8.08	3.00	1265.19	69.42
24 - 36 months old						
90	22	6.69	7.95	3.33	1183.22	87.89
130	15	5.68	7.20	3.60	1523.63	74.82
Over 36 months old						
130	28	6.10	7.85	2.92	1506.81	86.37

for a long period of time (like bull 252 B_1 in Table VI) all semen properties studied dropped continuously but rather slowly. During the period of daily carotene intake of 130 micrograms per kilogram of body weight for about eighteen months the motility in diluted semen at zero hour, at 168 hours, the total sperm count in millions per ml. and the percentage of live sperm dropped from 7.00, 2.88, 1372.22 and 84.54 to 5.56, 1.71, 859,56 and 75.40 respectively.

There were only two Holsteins in the study, and their semen characteristics were similar to the Jerseys. Bull 500 B_1, Table VII, from a dam on a carotene deficiency for a long period of time during gestation, gave his first semen collection 73 days earlier than bull 503 B_2, Table VIII, from a dam of a shorter period on carotene-deficiency ration. Bull 500 B_1 was kept on the experiment until he was past four years of age to determine semen characteristics over a long period of time. He was maintained on a carotene level of 130 micrograms per kilogram of body weight daily for approximately 7.5 months. He was slaughtered after the last semen collection. The testicles, according to Byers, et al. (6, p.1560) showed marked reduction of the interstitial tissue, the cells of Leydig being reduced in number and showing evidence of nuclear degeneration. The basement membrane of the seminiferous tubules appeared thickened and the Sertoli cells showed

slight degenerative changes.

4. Unrelated Bulls Fed Different Carotene Levels

One purebred Jersey and three purebred Holstein bull calves from cows receiving a daily carotene intake ration of 390 micrograms per kilogram of body weight were placed on the experiment. Each of the calves received 390 micrograms per kilogram of body weight daily. This group was compared (in Table IX) to the five bulls previously described which received 50, 90, and 130 micrograms at varying ages. The animals in the former group were a Jersey, 264 B_2, and three Holsteins, 445 B_2, 493 B_3 and 490 B_1. The last Holstein listed (490 B_1) was on the experiment for only nine weeks and therefore was omitted from the averages in Table IX and Table X.

The semen characteristics studied showed slightly higher average quality of semen from the bulls receiving 390 micrograms of carotene daily per kilogram of body weight as compared to the bulls which received varied amounts of carotene. Averages of semen characters of each bull is shown in Table X. The low-carotene group, Table X, was equal to the high-carotene group in percentage of live sperm but rated considerably lower in total sperm. Statistical analysis showed no significant difference in any of the semen properties studied. That may be due to the small number of collections made from the high-carotene

Table IX

Semen Production and Semen Quality of Unrelated Bulls

Bull No.	Born	Age 1st Service Days	Last Collection Date	No. of Collections	Volume (ml.)	Sperm Motility in Diluted Semen 0 Hrs.	Sperm Motility in Diluted Semen 168 Hrs.	Sperm Count (Million/ml.)	Live Sperm (%)
Carotene Supplemented (390 micrograms per kilogram of body weight)									
264 B_2	1-1-50	372	2-24-53	82	3.65	6.84	2.43	832.80	73.86
445 B_2	5-20-50	406	10-28-52	20	5.62	6.90	2.67	853.63	78.94
493 B_3	8-2-50	325	2-6-54	28	5.11	6.93	3.08	789.71	77.45
490 B_1	*3-2-50	312	5-13-51	9	1.74	3.78	0.60	625.50	77.60
Total		1103		130	14.38	20.67	8.18	2476.14	230.25
Average		367.67			4.79	6.89	2.73	825.38	76.75
Low Carotene (50,90, and 130 micrograms per kilogram of body weight at varying ages)									
200 B_1	12-24-49	387	8-7-53	97	3.81	7.01	2.95	1217.41	79.10
221 B_1	5-21-49	419	4-24-53	80	4.32	6.77	3.05	771.38	74.05
252 B_1	6-2-51	367	3-13-54	63	3.42	6.02	2.09	971.38	79.48
500 B_1	4-15-49	294	5-1-53	136	5.59	4.56	1.86	667.25	71.18
503 B_2	1-24-50	367	8-28-53	89	5.54	7.19	2.96	131.07	81.02
Total		1951		465	22.68	31.55	12.91	3758.49	384.83
Average		390.20			4.54	6.31	2.58	751.70	76.97
t **					0.6050	1.8080	0.7463	0.6075	-0.1475

* Not included in the average insufficient data

** Distribution of T at 5 per cent level 2.3060

Table X

Average Age at First Service,
Semen Production and Semen Quality of Unrelated Bulls

	Carotene Supplemented 390 Micrograms /Kilogram of Body Weight	Low Carotene 50, 90 and 130 Micrograms /Kilogram of Body Weight
No. of bulls	3	5
Age at first service	367.67	390.20
No. of collections	130	465
Volume of ejaculate ml.	4.79	4.54
Sperm motility in diluted semen at 0 hrs.	6.89	6.31
Sperm motility in diluted semen at 168 hrs.	2.73	2.58
No. of sperm/ml. semen millions	825.38	751.70
Percentage of live sperm	76.75	76.97

group.

5. Comparison of Performances of All Carotene Supplemented Bulls to All Low Carotene Bulls

All previous discussions in this report deal with segments of the experiment to determine the effect of a low carotene ration upon the semen quality of dairy bulls. Table XI shows a comparison of all the bulls receiving 130 or more micrograms of carotene daily per kilogram of body weight to all the bulls which received less than 130 micrograms daily. The former is designated as the high-carotene or the carotene-supplemented group while the latter is designated as the low-carotene group. The TI_2 and TJ_2 bulls were excluded from this comparison because they had previously been receiving a normal ration and were not on a low-carotene ration long enough to deplete, to any great extent, the vitamin A storage in their bodies. Bull 490 B_1 receiving a carotene-supplement ration was not on experiment long enough to obtain a fair mean average which included only nine ejaculates.

Table XII shows the averages of each property of semen characteristics in this study for comparison between these two groups of bulls. The differences in volume per ejaculate and in percentage of live sperm were not marked being 0.24 ml. and 4.65 per cent respectively in favour of the carotene-supplemented group. There was no difference

Table XI

Semen Production and Semen Quality of Unrelated and Identical Twin Bulls

Bull No.	Born	Age 1st Service Days	Last Collection Date	No.of Collections	Volume (ml.)	Sperm Motility in Diluted Semen 0 Hrs.	Sperm Motility in Diluted Semen 168 Hrs.	Sperm Count (Million/ml.)	Live Sperm (%)
Carotene Supplemented (390 and 500 micrograms per kilogram of body weight)									
264 B_2	1-1-50	372	2-24-53	82	3.65	6.84	2.43	832.80	73.86
445 B_2	5-20-50	406	10-28-52	20	5.62	6.90	2.67	853.63	78.94
493 B_3	8-2-50	325	2-6-54	28	5.11	6.93	3.08	789.71	77.45
490 B_1*	3-2-50	312	5-13-51	9	1.74	3.78	0.60	626.50	77.60
TI_1	8-25-52		4-27-56	20	6.48	8.06	3.83	1125.83	91.84
TJ_1	9-18-52		6-2-56	30	3.58	8.65	5.00	1543.52	91.72
TR_1	4-26-54	574	3-2-56	13	3.99	6.55	3.82	965.62	89.81
TV_1	2-6-55	394	3-7-57	46	5.17	7.78	4.24	1211.65	87.21
TW_1	3-2-55	418	3-8-57	37	2.60	7.84	3.91	1085.72	89.33
Total				276	36.20	59.55	28.98	8408.48	680.16
Average					4.53	7.44	3.62	1051.06	85.02
Low Carotene (50, 90, and 130 micrograms per kilogram of body weight)									
200 B_1	12-24-49	387	8-7-53	97	3.81	7.01	2.95	1217.41	79.10
221 B_1	5-21-49	419	4-24-53	80	4.32	6.77	3.05	771.38	74.05
252 B_1	6-2-51	367	3-13-54	65	3.42	6.02	2.09	971.38	79.48
500 B_1	4-15-49	294	5-1-53	136	5.59	4.56	1.86	667.25	71.18
503 B_2	1-24-50	367	8-28-53	89	5.54	7.19	2.96	131.07	81.02
TR_2	4-26-54	584	3-15-56	16	4.01	6.17	3.25	1068.81	82.91
TV_2	2-6-55	446	3-7-57	35	5.21	8.00	4.13	1042.32	89.24
TW_2	3-2-55	478	3-8-57	32	2.41	7.22	2.89	826.31	86.01
Total				548	34.31	52.94	23.18	6695.93	642.99
Average					4.29	6.62	2.90	836.99	80.37
t **					1.0415	4.8460	4.9320	3.8254	3.7533

* Not included in the average insufficient data. ** Distribution of t at 5 per cent level 2.1199.

Table XII

Average Semen Production and Semen Quality of Unrelated and Identical Twin Bulls

	Carotene Supplemented 390 and 500 Micrograms /Kilogram Body Weight	Low Carotene 50, 90 and 130 Micrograms /Kilogram of Body Weight
No. of bulls	8	8
No. of Collections	276	548
Volume of ejaculate ml.	4.53	4.29
Sperm motility in diluted semen at 0 hrs.	7.44	6.62
Sperm motility in diluted semen at 168 hrs.	3.62	2.90
No. of sperm/ml. semen millions	1051.06	836.99
Percentage of live sperm	85.02	80.37

statistically in the volume per ejaculate, but the difference of 4.65 per cent in live sperm was statistically significant at the five per cent level. Sperm motility in diluted semen at zero hour and 168 hours of the high-carotene group and the low-carotene group was 7.44, 3.62 and 6.62, 2.90 respectively. The differences in these two respects were highly significant at the five per cent level when treated statistically. The difference in the total number of sperm per ml. of semen was pronounced with 1051.06 and 836.99 millions per ml. for the carotene-supplemented group and the low-carotene group respectively. Statistically, the difference in semen concentration was significantly higher than that of the percentage of live sperm. All properties of semen characteristics studied in this work were in favour of the high-carotene group.

6. Fertility

A limited number of cows was used to evaluate the breeding performance of the bulls. To check the breeding efficiency, artificial insemination was practiced on the cows regardless of their condition. Most of them were in farm herds. No semen used for inseminating was older than seventy-two hours. The data available for determining the breeding performance of the bulls as shown in Table XIII were too limited to draw definite conclusions.

According to Table XIII, the breeding efficiency

Table XIII

Breeding Efficiency of Bulls

	Bull (no.)	Cows Bred (no.)	Services (no.)	Pregnancy (no.)	Efficiency (%)
Carotene Supplemented (390 micrograms per kilogram of body weight)					
	264 B_2	-	18	8	44.4
	493 B_3	-	47	25	53.1
Total	2		65	33	50.77
Low Carotene (50, 90, and 130 micrograms per kilogram of body weight)					
	200 B_1	31	45	14	31.1
	221 B_1	29	42	11	26.2
	252 B_1	19	21	9	42.8
	500 B_1	19	30	6	20.0
	503 B_2	48	52	25	48.1
Total	5	146	190	65	34.21

of two bulls fed the carotene-supplemented ration was 50.77 per cent whereas that of five bulls fed the low-carotene ration was only 34.21 per cent. Although the percentage of the carotene-supplemented group was low, there was a highly significant difference in this respect among the two groups of bulls. Among the low-carotene group, bull 500 B_1 had the lowest percentage of conceptions in relation to services. Only 20.0 per cent of the services with his semen resulted in pregnancies. His daily carotene intake was 50 micrograms per kilogram of body weight from 45 to 876 days of age, 90 micrograms from 877 to 1,271 days of age and 130 micrograms from 1,272 to 1,481 days of age. When slaughtered, this bull's testicle showed a severe degeneration as the result of this low-carotene intake.

SUMMARY

The dairy bulls in this study consisted of four Jerseys and five Holsteins forming an unrelated bull group, and five sets of identical twins. The twins consisted of one pair of purebred Jerseys, one pair of purebred Holsteins, one pair of purebred Brown Swiss, one pair of Holstein X Ayrshire crossbreds, and one pair of Holstein X Jersey crossbreds. Semen collections were made at weekly intervals.

The bulls of the identical Holstein X Ayrshire twins, fed 500 and 50 micrograms of carotene respectively per day for each kilogram of body weight, produced semen of nearly equal quality. Semen quality was determined by sperm motility of the diluted semen at zero and 168 hours, sperm count per milliliter of semen, percentage of live sperm at time of collection, and percentage of normal appearing sperm. The identical purebred Jersey bulls fed the same levels of carotene as the Holstein X Ayrshire twins also produced semen of nearly equal quality. Each member of the pairs was fed a normal herd ration for a period of approximately thirty months before the experiment started. The results indicate that dairy bulls have the ability to maintain sufficient vitamin A in their bodies to produce high quality semen for a period of fourteen to sixteen months after being placed on a low-carotene ration contain-

ing 50 micrograms per kilogram of body weight.

Of the other three pairs of identical twins, one member each was placed on the 500 microgram level of carotene, and the other members of the group on the 50 microgram level at weaning time. Semen collection was made from one pair for only four months. The other two pairs were collected from for nine months. Semen collections were made from all the bulls for comparatively short periods of time. Semen analysis showed greater livability of the sperm, a higher sperm count, and a higher percentage of live sperm from the bulls which were fed 500 micrograms of carotene than those which were fed 50 micrograms. The greatest difference was in the total sperm count per milliliter of semen with an average of 1087.66 and 979.15 millions for the two rations respectively.

Bulls which received low-level carotene rations born from dams which received low-level carotene rations produced semen with greater variation in quality than bulls on a high-carotene ration born from dams which also received high-level carotene rations.

When data of all bulls with daily low-carotene intakes of 50, 90, and 130 micrograms per kilogram of body weight are compared with high-carotene group bulls with 390 and 500 micrograms, the difference is significant in all properties of semen characteristics studied with only one exception; the volume per ejaculate. The effect of low-

carotene intake was more evident in the sperm motility in diluted semen at zero and 168 hours, the percentage of live sperm, and the concentration of the sperm.

The breeding efficiency of these two categories of bulls was determined with cows from cooperating farm herds, and by the use of artificial insemination. The performance of the high-carotene group far exceeded that of the low-carotene group with 50.77 and 34.21 per cent respectively. The low percentages may be accounted for because only two bulls were available and all of the pregnancy data could not be obtained from the cooperating farmers.

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