THE RELATION OF CERTAIN PELLETED FEEDS TO BOVINE HYPERKERATOSIS

by

Jack S. Copenhaver

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APPROVED:

Director of Graduate Studies

APPROVED:

Head of Department

Dean of School

Major Professor

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INTRODUCTION

Bovine hyperkeratosis first appeared in this country during the winter of 1939-1940 (Gibbons, et al, 1948) in a dairy herd in southwestern New York. Since this time, the disease has been reported in thirty-seven states and has caused considerable losses. It had reached such proportions by 1948 that a survey sponsored by the United States Department of Agriculture was made in five southeastern states in an effort to learn something of its nature. Twenty-six herds in twenty counties which had been affected with bovine hyperkeratosis were studied. These herds, which (normally) contained 4120 head of cattle, had 1295 head affected with bovine hyperkeratosis. This was thirty-one per cent of the total animals and, of the animals affected, 759 or about fifty-nine per cent died. The estimated total loss in these herds was $110,860 or about $4200 per herd.

Several materials, including certain lubricants, have been incriminated in different outbreaks of the disease. It was demonstrated that the lubricants capable of causing bovine hyperkeratosis contained highly chlorinated naphthalenes. In recent months, there have been numerous reports of outbreaks of the disease in herds in which pelleted feeds were being fed. The studies described in this thesis were conducted in an attempt to determine if enough lubricant, containing highly chlorinated naphthalenes, could gain entry into the feed during the pelleting process to cause hyperkeratosis in calves which consumed the pellets.

REVIEW OF LITERATURE

Bovine hyperkeratosis, first described by Olafson (1947), is an insidious, chronic disease of cattle, with a course of several weeks to three or more months. The first symptom generally noted is a watery discharge from the eyes and nose, followed by a loss of condition, poor appetite, depression, and a progressive thickening of the skin. The skin changes appear first over the withers and the sides of the neck and cheeks, and then extend back of the shoulders until the upper two-thirds of the body is involved. In most cases the hair is lost as the skin becomes thickened, dry, leathery, and creased. Diarrhea is sometimes present during the later stages of the disease, usually just before the animal dies. Very often the mouth shows papillary projections or lesions on the surfaces of the tongue, cheeks and palate.

At the autopsy of a typical case, papillary lesions are often found in the esophagus, and cyst-like lesions are present on the lining of the larger bile ducts and the mucosa of the gall bladder in most cases. Varying degrees of liver fibrosis and bile duct proliferations are present. The liver and kidneys are occasionally enlarged and pale in color. Pale streaks are often found in the cortex of the kidney. Flat ulcers are sometimes present in the abomasum, and the mucosa of the intestinal tract is occasionally thickened.

Since the first description of the disease, there have been reports by various investigators presenting evidence that it is caused by a

toxic substance or substances. Several seemingly unrelated substances have been incriminated in outbreaks, and in most cases, have been used to produce the disease experimentally. Olson, Cook and Brouse (1950) reported an outbreak in calves that had been fed, in addition to prairie hay, a particular lot of pelleted dehydrated alfalfa. Olson and Cook (1951) duplicated their work reported in 1950 by using pellets left over from the previous study. Olafson and McEntee (1951) experimentally produced bovine hyperkeratosis by feeding a processed concentrate. Later, McEntee, Hansel and Olafson (1951) produced the disease with the free fatty acid fraction of the ether extract of the processed concentrate.

Wagener (1951) reported the production of the disease with one lot of a wood preservative that was made and used in Germany. Miller, Bortree and Schook (1952) produced the disease by feeding a certain crop of timothy hay obtained from one pasture. They stated that the possibility existed that the hay was contaminated with a chemical agent similar to the wood preservative used by Wagener.

Bell (1952a) reported the production of the disease by the oral administration of a certain lubricant. In a second report, Bell (1952b) showed the causative agents, present in the lubricant previously used, to be highly chlorinated naphthalenes. Sikes and Bridges (1952) in a later report also described the production of bovine hyperkeratosis with chlorinated naphthalenes. In a recent report, Olafson, McEntee and Hansel (1952) reported that the German wood preservative contained two active fractions. One of these was identified as probably being

trichloronaphthalene and the other, although not positively identified, has some properties very similar to those of trichlorobenzene.

In a recent report, Bell (1953) described the relative toxicity of various chlorinated naphthalenes. He found that dichloronaphthalene and trichloronaphthalene were not toxic at the total dose of fourteen and twelve milligrams per pound body weight, respectively; that tetrachloronaphthalene was slightly toxic; and that pentachloronaphthalene, hexachloronaphthalene and heptachloronaphthalene were consistently toxic, with pentachloronaphthalene being less toxic than the other two. As little as 2.5 milligrams per pound body weight of hexachloronaphthalene and heptachloronaphthalene produced symptoms of the disease. Octachloronaphthalene was not toxic when given in a suspension, but 5.0 milligrams per pound body weight was toxic when given in solution in either mineral or vegetable oil.

Olafson, McEntee and Hansel (1952) reported that the plasma vitamin A levels decreased rapidly after the toxic agent in the processed concentrate was fed. The decline preceded the other symptoms of hyperkeratosis and continued at least a month beyond the period in which the causative agent was fed. When large amounts of vitamin A were fed to cattle with hyperkeratosis, plasma and liver vitamin A increased, but declined rapidly to their previous low levels, when feeding of the vitamin was stopped. Plasma carotene increased when large doses of carotene were fed, but plasma vitamin A did not, indicating the possibility of an anti-vitamin A factor which interferes with the conversion of carotene to vitamin A. Bell (1952c) stated that plasma

vitamin A levels dropped rapidly in calves that were given lubricants containing highly chlorinated naphthalenes or chlorinated naphthalenes in solution in mineral or vegetable oils.

EXPERIMENTAL

Objective

The object of this investigation was to determine if enough lubricant, containing highly chlorinated naphthalenes, could gain entry into the feed during the pelleting process, to cause bovine hyperkeratosis in calves which consumed the pellets.

Materials

Calves

Normal calves, two to four months of age, were obtained from the Virginia Polytechnic Institute's herds.

Feed

The feed consisted of a standard basal calf ration in pellet form, obtained from a local mill, and a limited amount of mixed orchard grass and alfalfa hay grown on the college farms. The formula of the basal calf ration was as follows:

Alfalfa Meal (17% D.P.)	300 pounds
Wheat Bran	600 pounds
Yellow Corn Meal	700 pounds
Corn Distiller's Solubles	400 pounds
Linseed Oil Meal	600 pounds
Ground Oats	600 pounds
Soybean Oil Meal (solvent 44% D.P.)	550 pounds
Molasses	200 pounds
Limestone (ground)	20 pounds

Phosphate	20 pounds
Salt	20 pounds
Trace Minerals (Tramin)	2 pounds
Vitamins (600 D, 1500 A Oil) (nonstabilized)	4 pounds
511 Irradiated Yeast (Premix)	1.5 pounds

Lubricants

The lubricants used in the pellet machine during the pelleting of the basal calf ration were furnished by the Sinclair Research Laboratories, Inc. They were identified as follows:

Lot I - a lubricant containing no chlorinated naphthalenes.

Lot II - a lubricant containing 3% chlorinated naphthalenes.

Chlorinated Naphthalenes

The chlorinated naphthalene that was administered to calves for comparison with the calves fed the pellets was furnished by the Sinclair Research Laboratories, Inc.

Methods

Preparation of the Pellets

The ration to be pelleted was mixed in a commercial feed mixer in the customary manner. It was mixed in two, two-ton lots and was delivered from the mixer, by elevator, directly to the pellet mill. After the feed went through the pellet mill, it was delivered to a cooler by elevators and from there by elevator to the bagging machine, where it was weighed and bagged. The bags were numbered in the order in which they came from the

bagging machine. The elevators, cooler, and bagging machine were cleaned after each lot of feed had been run through. After delivery, the feed was stored so that each ton lot was kept separate.

Lubrication of the Rolls of the Pellet Mill

Prior to the pelleting process, the rolls of the pellet mill were removed and thoroughly cleaned with gasoline. They were then weighed and packed with Lot I lubricant (containing no chlorinated naphthalenes) and again weighed. A one-pound, lever-type pressure grease gun was used to pack the lubricant into the rolls. The rolls were placed back on the machine after they had been packed (with lubricant), and one tone of feed was pelleted with no further lubrication of the roll. This ton of pellets was designated as Lot A. As soon as Lot A was pelleted, more of Lot I lubricant was pumped into the rolls, and additional lubricant was added at ten minute intervals during the pelleting of the second ton of feed, designated as Lot B. The only difference between Lot A and Lot B pellets was the difference in amount of Lot I lubricant each contained. Lot A pellets contained 3 oz. and Lot B contained 8 oz. When the pelleting of Lot B was completed, the rolls were removed and weighed, then cleaned and reweighed. They were then packed with Lot II lubricant (containing 3% chlorinated naphthalenes), weighed and placed back in the pellet mill. The third ton of feed was then pelleted. At the completion of the pelleting of the third ton, designated as Lot C, more of

Lot II lubricant was added to the rolls at the beginning and at ten minute intervals thereafter during the pelleting of the fourth ton of feed, designated as Lot D. The only difference between Lot C and Lot D pellets was the difference in amounts of Lot II lubricant each contained. Lot C contained 3 oz. and Lot D contained 13 oz.

The amounts of lubricant used during the pelleting of each lot of feed were determined by weighing the grease gun when full of lubricant and then weighing it after all of the lubricant used during the pelleting of each lot of feed had been pumped into the rolls. As a check, the rolls were weighed empty, full, and after the B and D lots had been run. The rolls were not removed or weighed between the running of A and B or between C and D lots. By simple calculation, the amount of lubricant in each lot of feed was determined.

Management of Calves on Pelleted Feed

The calves were weaned before being placed on the pellets. Prior to weaning, they were fed milk from the parent herd and a commercial grain supplement similar to that being fed to all calves in the parent herd.

The calves were weighed and assigned to individual stalls. Two calves were assigned to each lot of feed. Each pair of calves, receiving the same lot of pellets, was assigned one metal container for feed storage, one feed bucket in which feed was weighed and carried to the feed box, and one water bucket. The calves were

individually fed and watered twice daily. As soon as they had eaten, the calves receiving Lots A (calves 135 and 137) and B (calves 138 and 139) pellets were housed together in a large pen; and those receiving Lots C (calves 141 and 142) and D (calves 146 and 147) pellets were housed together in an adjoining pen.

All calves received a limited amount of mixed orchard grass-alfalfa hay in addition to their pellet ration. This hay was fed in the same feed box in which the pellets were fed. All feed was weighed and the weights recorded at the time of feeding.

The calves were observed daily and notes were made as to their appearance, appetite, health and general condition. They were weighed and a blood sample was taken from each calf at fourteen-day intervals. Carotene and vitamin A levels in the blood serum were determined according to the methods of Kimble (1939).

Management of Calves on Lubricants and Chlorinated Naphthalenes

Each lot of lubricant was given orally to each of two calves in order to demonstrate the effects of the lubricants. Two additional calves were given orally highly chlorinated naphthalenes for comparison with the calves receiving the lubricants and the calves fed the pellets. These calves were handled in the manner described for the calves that were fed the pellets. They were placed two to a stall and fed homegrown hay and a commercial grain supplement.

Two calves, 1555 and 1558, were each given orally in gelatin capsules a total of 50 grams of Lot I lubricant. This amount was divided into small doses and given during a period of 8 days. Calf 1560 was given 30.6 grams of Lot II lubricant, administered in divided doses over a period of seven days. This calf received a total of 918 mg. of chlorinated naphthalenes of which 550 mg. was hexachloronaphthalene, the remainder being di- and trichloronaphthalenes. The amount of hexachloronaphthalene given was at the level of 2.5 mg. per pound body weight. Calf 1561 was given 58.6 grams of Lot II lubricant, administered in divided doses over a period of sixteen days. This calf received a total of 1758 mg. of chlorinated naphthalenes of which 1055 mg. was hexachloronaphthalene, the remainder being dichloronaphthalene and trichloronaphthalene. The amount of hexachloronaphthalene given was at the level of 5.0 mg. per pound body weight. Calf 296 was given 17.6 grams of a 3 per cent solution of hexachloronaphthalene in corn oil in divided doses over a period of seven days. This calf received a total of 528 mg. of hexachloronaphthalene or 2.5 mg. per pound body weight. Calf 298 was given 28.5 grams of a 3 per cent solution of hexachloronaphthalene in corn oil in divided doses over a period of seven days. This calf received a total of 855 mg. of hexachloronaphthalene or 5.0 mg. per pound body weight. Calf 1531 served as a control. This calf was kept in the pen with the calves that received Lot II lubricant and was handled in the same manner, except that it did not receive any lubricant or chlorinated

naphthalenes. These calves were observed daily and notes made as in the case of the calves on pellets.

Symptoms, Gross and Microscopic Pathology

A post-mortem examination was performed on each calf either at the time it had become definitely ill or at the termination of the study. Careful search was made for the presence of gross lesions, and appropriate tissues were taken for the preparation of slides for microscopic study.

Results

Feed Consumption and Weight Gains of the Calves Fed Pellets

The feed consumption by weeks is shown in Table 1. The total and average daily feed consumption and the total and average daily weight gains are shown in Table 2.

There was a distinct difference between the amount of feed consumed and the weight gains of the calves that received Lots A and B pellets compared to those that received Lots C and D pellets. The calves (135 and 137) fed Lot A pellets and those (138 and 139) fed Lot B pellets had better appetites and consumed their feed more regularly than the calves (141 and 142) fed Lot C pellets or those (146 and 147) fed Lot D pellets. Little or no trouble was experienced in keeping the calves fed Lots A and B pellets on feed; however, the calves fed Lots C and D were erratic in their feed consumption and often refused the pellets. Calf No. 141 reached a peak in daily feed consumption during the second week

after which feed intake decreased until death on the 41st day. This calf consumed 83.75 pounds of pellets in the 41 days for an average daily feed consumption of 2.04 pounds. Calf No. 142 was very erratic in the amount of feed consumed. This calf consumed 111.5 pounds of pellets in 82 days for an average daily feed consumption of only 1.36 pounds. The calves fed Lot D pellets were also irregular in their daily feed consumption. Calf 146 had an irregular appetite from the 17th through the 75th day and again from the 121st through the 128th day. This calf consumed 771 pounds of feed in 135 days for an average daily feed consumption of 5.71 pounds. Calf 147 was also erratic in daily feed consumption throughout the feeding period. This calf consumed 637 pounds of pellets in 135 days for an average daily feed consumption of 4.72 pounds.

The total gains and average daily gains of the calves that consumed Lots A and B pellets were significantly higher than the total gains and average daily gains of the calves that consumed Lots C and D pellets (Table 2). The calves (135 and 137) that received Lot A pellets made an average daily gain of 2.04 pounds for an average total gain of 275 pounds. The calves fed Lot C pellets had an average daily gain of -0.65 and 0.0 pounds, and a total gain of -27.0 and 0.0 pounds, respectively. Calf 141 gained in weight the first 14 days and then lost weight until death on the 41st day. Calf 142 had irregular gains throughout the period of feeding. The weight of this calf varied, and when sacrificed

on the 82nd day, the weight was exactly the same as it was at the beginning of the observation. The calves (146 and 147) that consumed Lot D pellets gained in weight, but the gains were less than those made by the calves fed Lots A and B pellets (Figure 5). Their average daily gains were 1.71 and 1.118 pounds, with a total gain of 232 and 159 pounds, respectively.

Serum Carotene and Vitamin A Levels

The results of the determination of the serum carotene and vitamin A levels of the calves fed the pellets are shown in Tables 3, 4, 5, and 6 and Figures 1, 2, 3, and 4. Calves on Lots A and B pellets were consistently above what would be considered a deficient level for vitamin A.

The calves fed Lot C pellets had a drop in carotene and vitamin A to a level that was indicative of a deficiency. Calf 141 showed a marked decrease in serum carotene level from the 17th to the 41st day, and at the time of death on the 41st day, the vitamin A level was only 4 micrograms per 100 ml. Calf 142 had a decline in serum carotene between the 27th and 41st days, a rise through the 69th day, and then a decline to the 82nd day. The vitamin A level of this calf dropped to an indeterminate level on the 69th day but had returned to 16.0 micrograms per 100 ml. at the time of autopsy on the 82nd day.

The calves (146 and 147) fed Lot D pellets had serum carotene values that were at the same levels as those of the calves on

Lots A and B pellets. The serum vitamin A levels, however, were consistently below the levels obtained for the calves on Lots A and B pellets. Calf 146 had a level below 5 micrograms per 100 ml. on the 48th and 55th days, while calf 147 had similar values on the 41st, 69th, and 125th days. These values are indicative of a vitamin A deficiency.

Table 1. Pounds of Feed Consumed per Week.

	Calf No.							
Weeks	135	137	138	139	141	142	146	147
1	12.2	19.0	18.5	15.2	16.0	9.8	19.0	15.0
2	21.8	30.2	29.5	27.0	20.0	5.5	24.5	22.0
3	35.5	38.0	37.5	26.2	18.8	12.5	25.2	32.2
4	40.0	28.0	40.0	27.8	16.2	7.5	28.8	27.0
5	42.0	39.0	37.8	34.8	7.8	7.0	30.5	28.5
6	43.0	42.5	42.2	42.2	5.0*	7.2	31.0	33.5
7	49.5	44.5	42.5	42.5		10.0	32.5	38.0
8	48.8	41.5	38.8	38.8		16.5	35.0	42.0
9	52.5	42.0	42.0	42.0		12.8	42.0	42.0
10	52.5	44.0	44.0	44.0		13.5	44.0	38.5
11	52.5	36.5	49.0	49.0		6.5	42.5	21.0
12	52.5	46.0	51.0	51.0		2.8**	45.0	25.5
13	52.5	52.5	52.5	52.5			52.5	34.0
14	52.5	52.5	52.5	52.5			52.5	42.5
15	52.5	52.5	52.5	52.5			52.5	36.5
16	56.0	56.0	56.0	56.0			55.5	39.0
17	56.0	56.0	56.0	56.0			56.0	45.5
18	56.0	56.0	56.0	56.0			47.5	40.8
19	56.0	56.0	56.0	56.0			54.5	34.0

* Calf 141 lived 41" days. Sixth week contains six days.
** Calf 142 lived 82" days. Twelfth week contains five days.

Table 2. Feed Consumption and Weight Gains.

Calf No.	Pellet Lot	Days on Feed	Total Gain	Ave. Daily Gain	Total Feed Consumed	Ave. Daily Feed Consumption
135	A	135	270	2.00	884.25	6.55
137	A	135	281	2.08	832.75	6.17
138	B	135	255	1.89	854.25	6.33
139	B	135	252	1.87	822.00	6.08
141	C	41	-27	-0.65	83.75	2.04
142	C	82	0	0.00	111.50	1.36
146	D	135	232	1.71	771.00	5.71
147	D	135	159	1.18	637.50	4.72

Table 3. Serum Carotene - Micrograms/100 Ml.

Calf No.	Pellet Lot	DAYS ON TEST										
		17	34	41	48	55	62	76	90	104	118	132
135	A	239	341		425		480	401	449	412	386	425
138	B	151	161		82		161	170	204	182	216	252
141	C	125	79	50								
146	D	173	179		121	187	221	142	216	287	264	297

Table 4. Serum Carotene - Micrograms/100 Ml

Calf No.	Pellet Lot	DAYS ON TEST 27	41	55	69	76	83	97	125	132
137	A	137	125	138	156		163	206	206	230
139	B	170	141	141	216		151	244	297	287
142	C	156	106	119	161	31	37*			
147	D	169	216	287	264	173	175	260	260	244

Table 5. Serum Vitamin A - Micrograms/100 Ml.

Calf No.	Pellet Lot	DAYS ON TEST										
		17	34	41	48	55	62	76	90	104	118	132
135	A	30	29		48		70	54	44	44	33	36
138	B	14	19		25		56	32	38	34	28	17
141	C	4	13	4								
146	D	12	20		0	1	23	14	19	9	14	19

Table 6. Serum Vitamin A - Micrograms/100 Ml.

Calf No.	Pellet Lot	DAYS ON TEST								
		27	41	55	69	76	83	97	125	132
137	A	24	47	28	29		51	28	30	
139	B	23	32	24	19		47	24	18	19
142	C	22	11	15	0	8	16*			
147	D	30	3	8	0	18	22	9	4	18

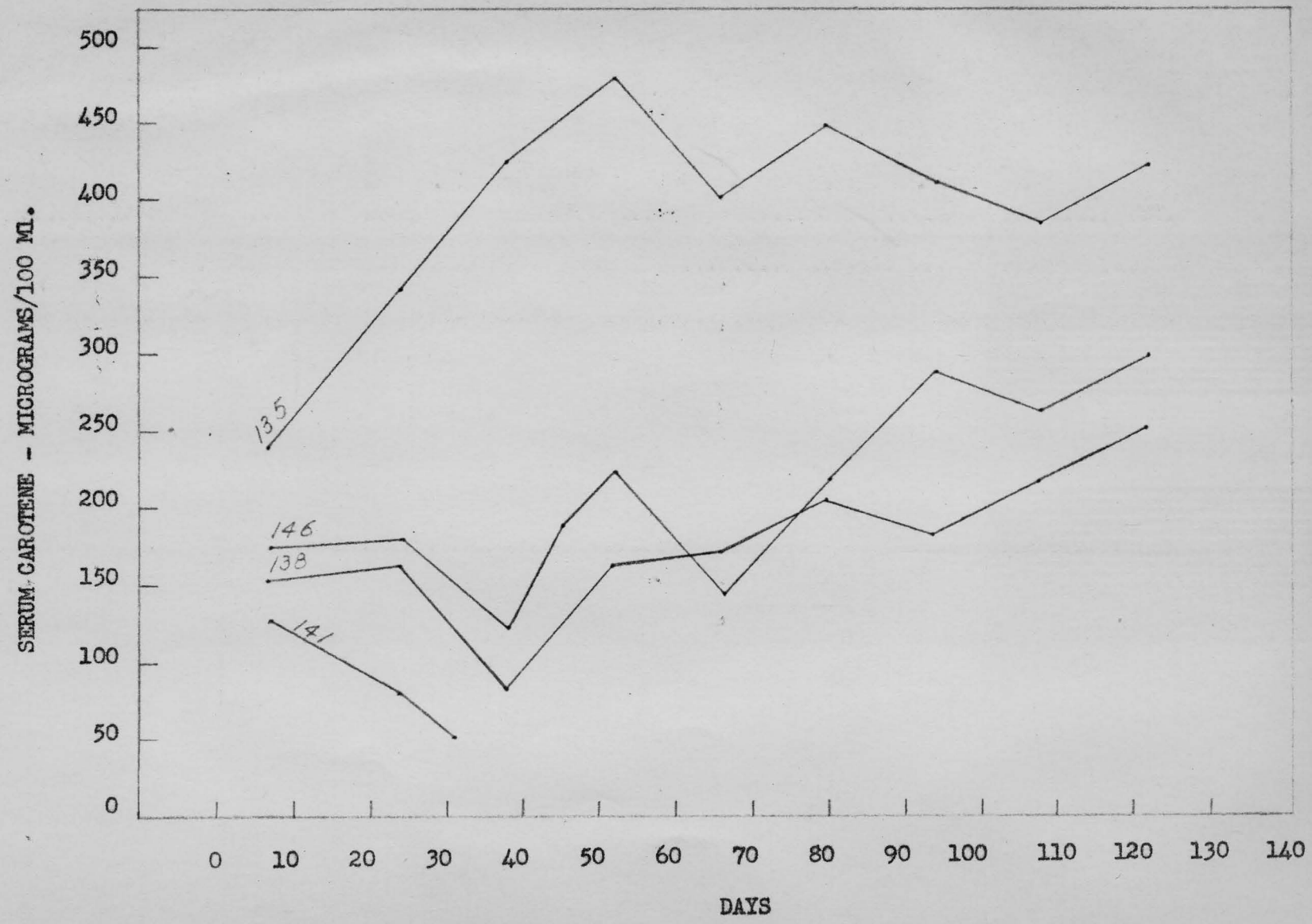

Figure 1 - Carotene Levels of Calves 135, 138, 141, and 146.

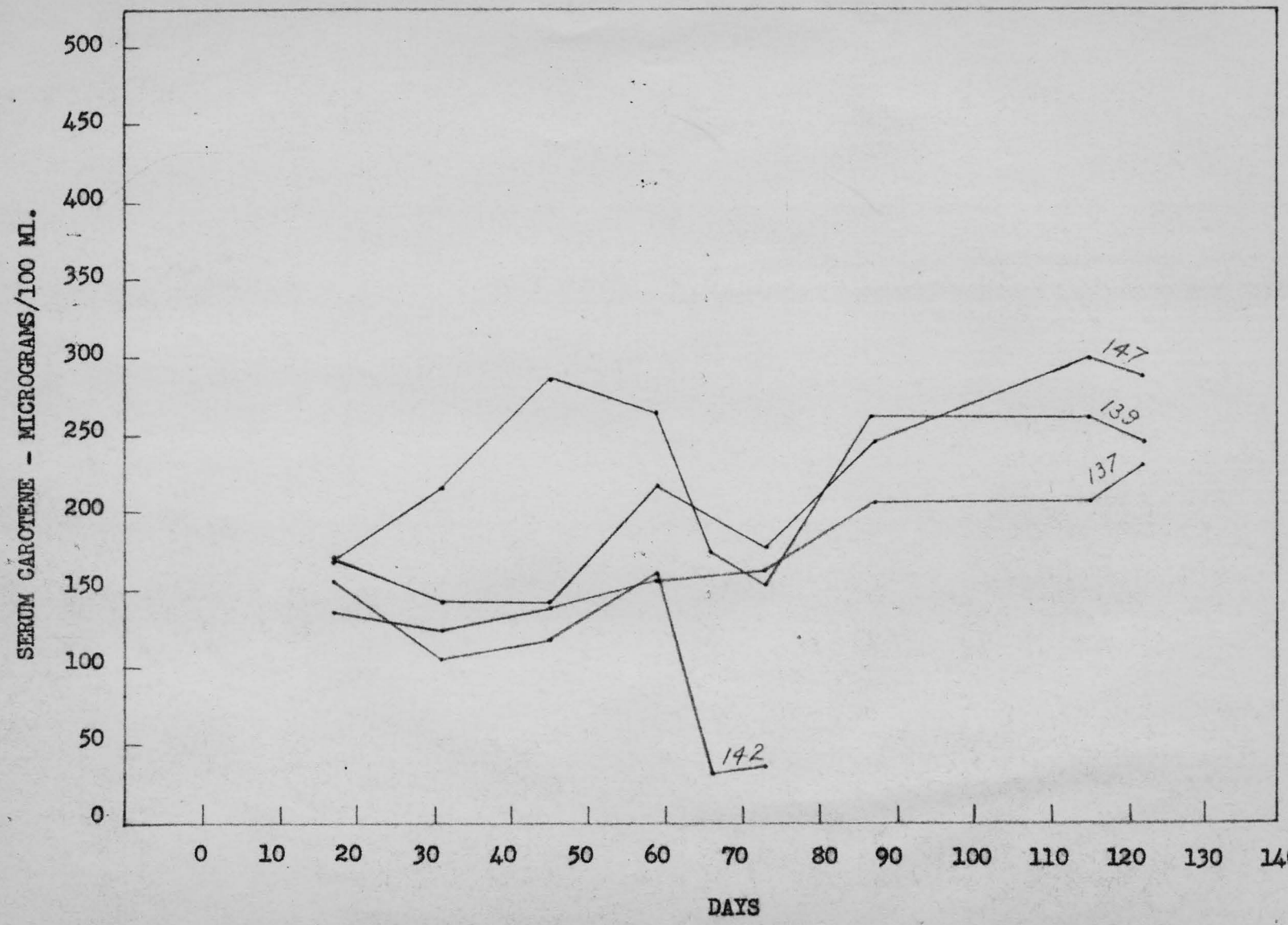

Figure 2 - Serum Carotene Levels of Calves 137, 139, 142, and 147.

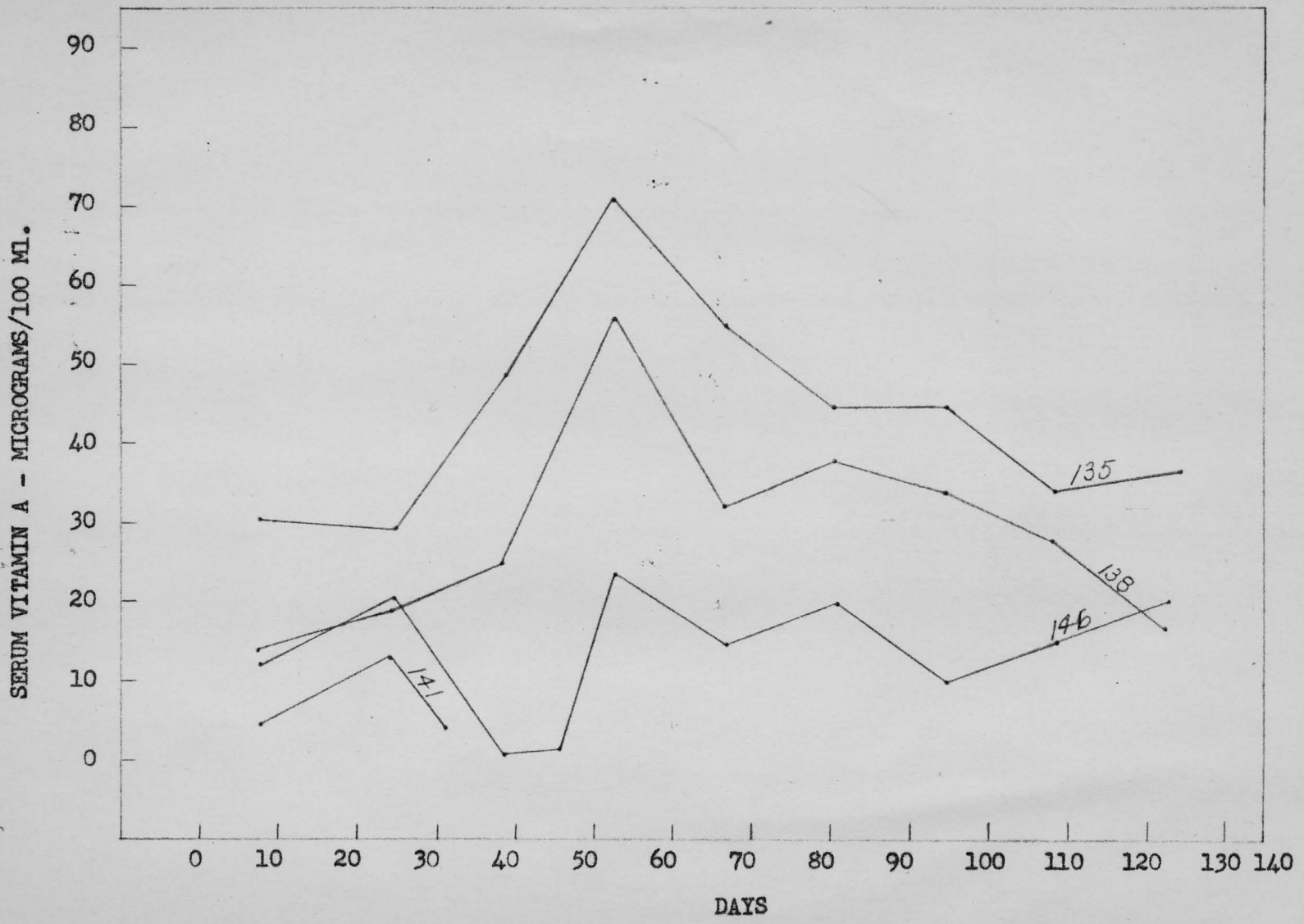

Figure 3 - Vitamin A Levels of Calves Number 135, 138, 141, and 146.

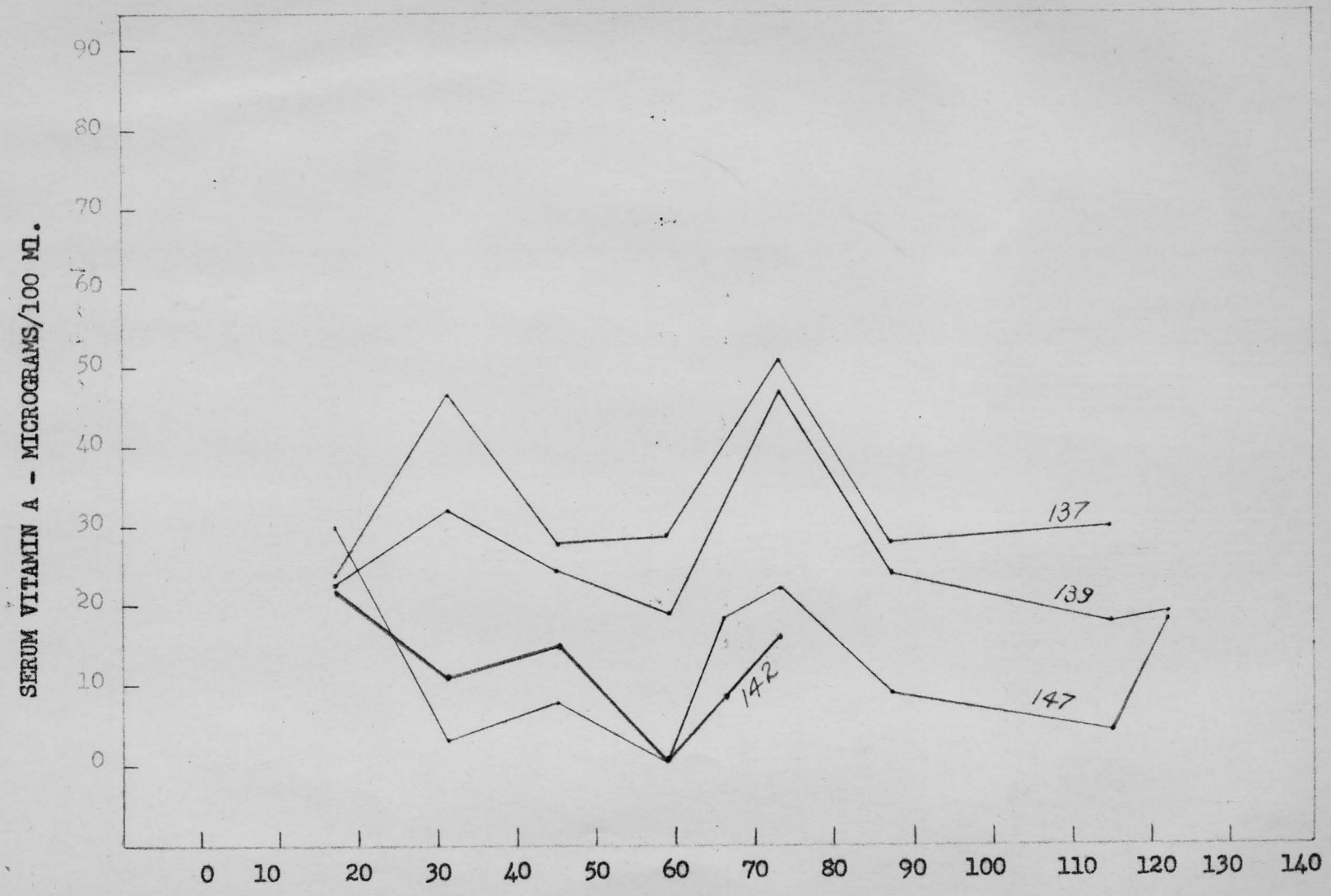

Figure 4 - Vitamin A Levels of Calves Number 137, 139, 142, and 147.

Symptoms, Gross and Microscopic Pathology

Calves 135 and 137, fed Lot A pellets, and calves 138 and 139, fed Lot B pellets, showed no symptoms of bovine hyperkeratosis during the 135 days of feeding. No evidence of gross pathology was present at autopsy and no microscopic changes similar to those occurring in hyperkeratosis were found.

Calves 141 and 142, fed Lot C pellets, developed symptoms that have been associated with bovine hyperkeratosis.

Calf 141 developed profuse lacrimation, anorexia, and some depression on the 15th day. By the 22nd day the gums were red and a nasal discharge had appeared. The symptoms persisted, the calf became extremely weak, and died on the 41st day (Figure 6). The gross lesions at autopsy included: enlarged, pale kidneys; enlarged liver, with several pale areas on surface that extended into the parenchyma; distended gall bladder, wall slightly thickened; extensive wart-like proliferations on roof of mouth and dental pad; several proliferative lesions on tongue; some inflammation of abomasum and small intestine; and some pneumonia in lower portion of anterior lobes of lungs. Microscopic changes included: fibrosis and some bile duct proliferation in the liver; dilatation of the collecting tubules of the kidney and some fibrosis (Figure 10); squamous metaplasia of seminal vesicles (Figure 11) and epididymis; and some keratinization of the hair follicles.

Calf 142 was lacrimating on the 24th day. This symptom was followed by depression, nasal discharge, and partial anorexia. These symptoms persisted and on the 82nd day depression was marked,

anorexia was complete, and the calf was extremely weak and thin (Figure 7). The calf was sacrificed at this time. The kidneys had many white areas, up to one-half inch in diameter, on the surface. These areas extended into the cortex. The liver was normal in size but there were several yellow areas on its surface that extended into the parenchyma. Three or four cyst-like structures were found on the floor of the vagina near the anterior end. Several ulcers were present in the abomasum near the pylorus. The hooves were indented about one inch below coronary band and horn growth was retarded. Microscopic examination of tissues revealed: degeneration of liver cells and bile duct proliferation; squamous metaplasia of the ducts of the parotid gland; slight keratinization of the hair follicles; dilatation of kidney tubules; and squamous metaplasia of the epithelium of Gartner's ducts (Figure 12).

Calves 146 and 147, fed Lot D pellets, developed some of the symptoms associated with bovine hyperkeratosis.

Calf 146 began to refuse the pellets on the 17th day and the daily feed consumption was irregular for the remainder of the feeding period. Red areas appeared on the oral mucosa, gums, and lips on the 33rd day. Considerable water was consumed and polyuria was evident. The calf was sacrificed on the 135th day. The kidneys were slightly enlarged, with numerous pale areas that extended into the cortex. Some bile duct proliferation was present and the liver was firm and tough on sectioning. The hooves

were indented and horn growth considerably retarded. Microscopic examination of tissues revealed some dilatation of the collecting tubules and fibrosis of kidney; slight bile duct proliferation (Figure 13); and squamous metaplasia of epididymis and parotid gland (Figure 14).

Calf 147 refused the pellets on the 17th day. The feed consumption was extremely irregular throughout the remainder of the feeding period. Some lacrimation was present at times and the calf was constantly depressed. When autopsied on the 135th day the hooves were indented, and horn growth markedly retarded (Figure 8). The kidneys were slightly enlarged and pale areas, that extended into the cortex, were present. The liver was normal in appearance, but there was evidence of bile duct proliferation and it was firm and tough on sectioning. Several ulcers were in the abomasum near the pylorus. Microscopic examination of the tissue revealed: dilatation of kidney tubules, degeneration of tubule cells, and fibrosis of the kidney; degeneration of liver cells and bile duct proliferation; dilatation of mucous glands of gall bladder; and squamous metaplasia of the parotid gland and seminal vesicles.

Calves 1555 and 1558, given oral administrations of Lot I lubricant, and the control calf (1531) did not develop clinical symptoms, or gross and microscopic lesions of bovine hyperkeratosis.

Calves 1560 and 1561, given oral doses of Lot II lubricant, developed symptoms of bovine hyperkeratosis. These calves showed

marked lacrimation and depression on the fourth day. Wrinkling of the skin and proliferations around the nose and in the mouth appeared later. The symptoms continued to progress until the time of autopsy on the 45th day. The gross lesions found at autopsy included: proliferations in the right and left nostrils, on the lips, dental pads and gums; marked hyperkeratosis (Figure 9) of skin on neck, withers, sides and back; enlargement of the kidneys with striated cortex; epididymis enlarged and hard; cyst-like nodules on mucosa of gall bladder; evidence of bile duct proliferation; and several flat ulcers in abomasum. Microscopic changes found in tissues obtained at autopsy included: dilatation of kidney tubules; bile duct proliferation; squamous metaplasia of epididymides; dilatation of mucous glands of the gall bladder; and, an accumulation of keratinized material on the skin and keratinization of the hair follicles.

Calves 296 and 298 that were given hexachloronaphthalene developed typical symptoms and lesions of bovine hyperkeratosis as described by Bell (1952b, 1953).

Figure 5. Differences in growth of calf 147 (left) and calf 137 (right).

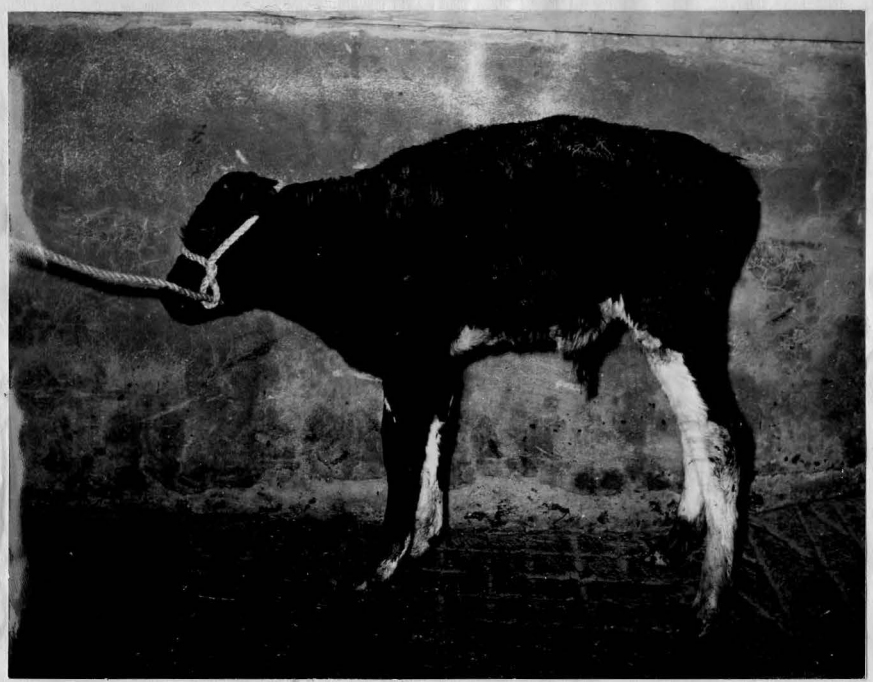

Figure 6. Depression, lacrimation, and wrinkling of skin shown by calf 141 after 41 days on Lot C pellets.

Figure 7. Depression, rough hair coat, and failure to gain shown by calf 142 after 68 days on Lot C pellets.

Figure 8. Normal horn growth in calf 137 (above) and retarded horn growth in calf 147 (below).

Figure 9. Hyperkeratosis in calf 1560 30 days after first administration of Lot II Lubricant.

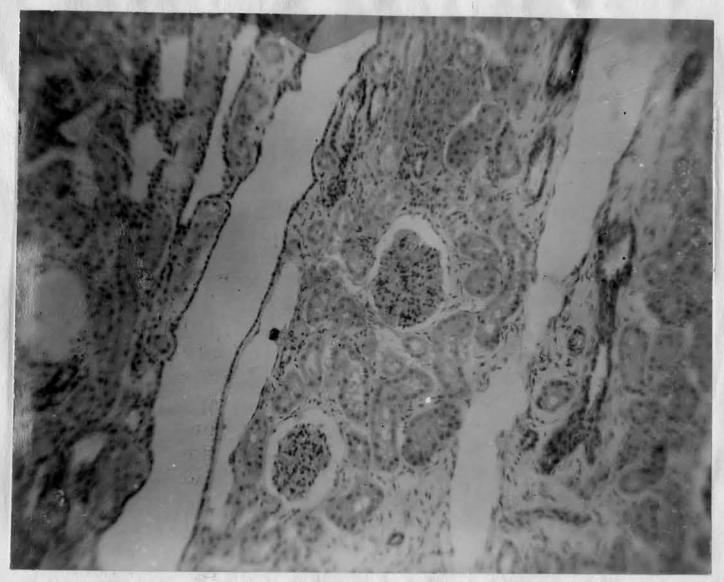

Figure 10. Dilatation of kidney tubules of calf 141.

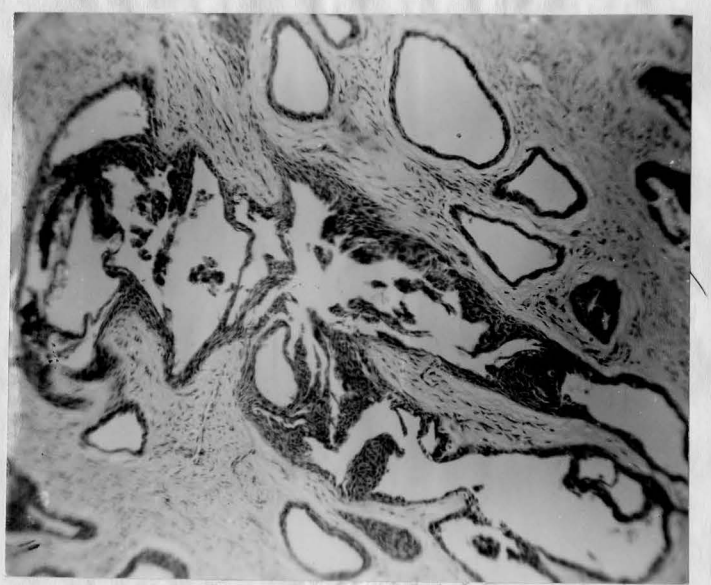

Figure 11. Squamous metaplasia of Seminal Vesicles of calf 141.

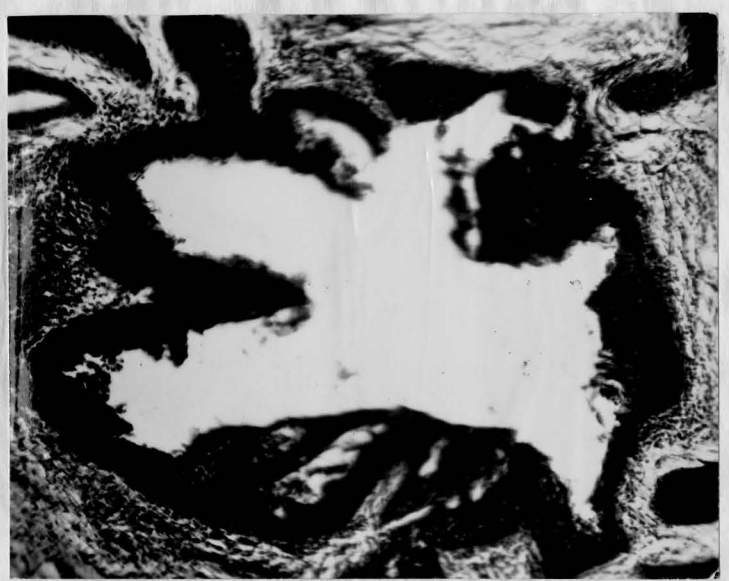

Figure 12. Squamous metaplasia of Gartner's duct of calf 142.

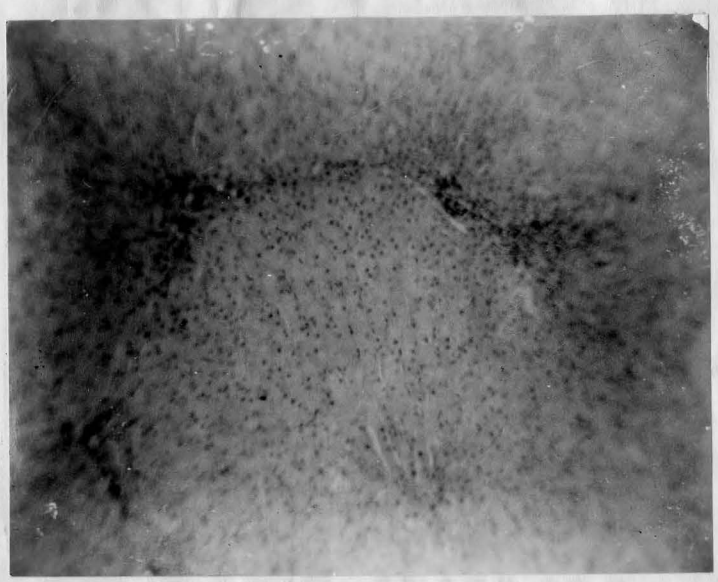

Figure 13. Bile duct proliferation in liver of calf 146.

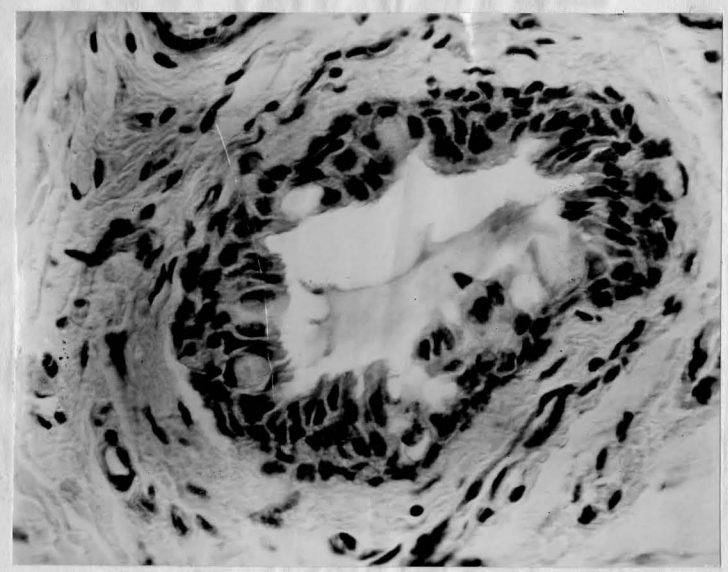

Figure 14. Squamous metaplasia of parotid gland of calf 146.

DISCUSSION

Calves 135 and 137, fed Lot A pellets, and calves 138 and 139, fed Lot B pellets, developed no symptoms of bovine hyperkeratosis at any time during the 135 days they were fed. Their appetites remained good and they had average daily gains of 1.87 to 2.08 pounds (Tables 1, 2). These gains are within the normal range for calves of this age under similar conditions. The serum carotene and vitamin A values (Fig. 1, 2, 3, 4) of these calves remained well above the levels seen in calves affected with bovine hyperkeratosis (Olafson et al, 1952). No gross evidence of bovine hyperkeratosis was present at autopsy on the 135th day, and microscopic examination of tissues revealed no lesions similar to those encountered in this disease. The data clearly indicate that the pellets, made with the rolls of the pellet mill lubricated with Lot I lubricant (containing no highly chlorinated naphthalenes) were not capable of causing bovine hyperkeratosis. These pellets were not toxic, although three ounces of lubricant passed from the rolls into Lot A and eight ounces passed into Lot B.

The calves (141 and 142) fed Lot C pellets developed symptoms, gross, and microscopic lesions (Fig. 10, 11, 12) of bovine hyperkeratosis. The symptoms, which included depression, lacrimation, anorexia, and loss of weight (Fig. 6, 7), were those described by Olafson (1947) and Bell (1952a, 1953) as being indicative of the disease. The gross and microscopic pathology was also similar to that described by these authors. The vitamin A values (Fig. 3, 4) of

both calves dropped to levels that were indicative of deficiency and were similar to the levels described by Olafson, McEntee and Hansel (1952) as occurring in bovine hyperkeratosis. The results demonstrate that pellets of Lot C were toxic and that they produced symptoms and lesions of bovine hyperkeratosis. The effect of the Lot C pellets on calves indicate that they were different in some manner from Lots A and B. This difference can be ascribed to the entry of Lot II lubricant (containing highly chlorinated naphthalenes) into the pellets during the pelleting process. Three ounces of the lubricant passed from the rolls into one ton of pellets that were designated as Lot C. The fact that the calves developed symptoms rapidly indicates that the lubricant (containing highly chlorinated naphthalenes) passed from the pellet mill very rapidly and was distributed largely in the first few bags of this lot.

Calves 146 and 147, that received Lot D pellets, developed mild symptoms and some gross and microscopic lesions (Fig. 13, 14) associated with bovine hyperkeratosis. The symptoms, especially irregular feed consumption, low daily weight gains, retarded horn growth, depression, and occasional lacrimation (Fig. 5, 8) were more pronounced in 147. Vitamin A levels (Fig. 3, 4) of both calves fell to values less than 5 micrograms per 100 ml. of serum on two different occasions. This depression of vitamin A has been described as an important symptom in bovine hyperkeratosis (Olafson et al 1952). The symptoms, lesions, and vitamin A levels indicate that the pellets were toxic and that they differed from Lots A and B which were non-toxic.

The toxicity of Lot D pellets can be attributed to the presence of lubricant (containing highly chlorinated naphthalene) in the pellets as the result of it passing from the rolls into the pellets. It is also evident that Lot D pellets were less toxic than those of Lot C. This difference existed in spite of the fact that 13 oz. of Lot II lubricant (containing highly chlorinated naphthalenes) passed into the pellets during pellet formation. This apparent discrepency can be attributed to the difference in the method of lubrication of the rolls of the pellet mill during the pelleting of Lots C and D. In the former case the roll was removed and packed at the beginning of the operation, whereas in the latter case approximately 1 3/8 ounces of lubricant were introduced every ten minutes. It became apparent at the end of the pelleting operation that the rolls were empty at the start of the pelleting of Lot D. The lubricant introduced at 10 minute intervals into the rolls, passed into the pellets shortly after each lubrication. Calves 146 and 147 therefore received toxic pellets at intervals but not in concentrations in any interval that would produce marked symptoms. The vitamin A levels which dropped to values indicative of deficiency and of the ingestion of a hyperkeratogenic substance (Olafson et al), on two occasions indicate that there was intermittent ingestion of toxic pellets.

Calves 1555 and 1558, which received Lot I lubricant (containing no highly chlorinated naphthalenes), remained normal. Calves 1560 and 1561, which received Lot II lubricant (containing highly chlorinated naphthalenes), developed typical symptoms and lesions of bovine

hyperkeratosis. These results with the two lubricants are in agreement with those reported by Bell (1950a, 1952b) who demonstrated that lubricants containing highly chlorinated naphthalenes were capable of producing the disease. The results further demonstrate the non-toxic properties of Lot I and the marked toxicity of Lot II. It is evidenced that Lot II lubricant, used in the pellet mill during the pelleting of Lots C and D, was capable of producing toxic effects when it gained entry into the feed.

The calves, 296 and 298, that were given oral administration of hexachloronaphthalenes, developed symptoms and lesions of bovine hyperkeratosis similar to those described by Bell (1952b, 1953). The response of these calves to the chlorinated naphthalenes was similar to the response of calves 1560 and 1561 to Lot II lubricant. It is apparent, therefore, that the toxicity of Lot II lubricant was due to highly chlorinated naphthalenes.

The control calf, 1531, remained free of symptoms and had high levels of vitamin A throughout the study, furnishing evidence that no contagious disease or nutritional deficiency due to diet existed.

CONCLUSIONS

1. Pellets made with the pellet mill rolls lubricated with Lot I lubricant (containing no highly chlorinated naphthalenes) were not toxic and did not produce symptoms of bovine hyperkeratosis in calves.

2. Pellets made with the pellet mill rolls lubricated with Lot II lubricant (containing highly chlorinated naphthalenes) were toxic to calves. Symptoms and lesions of bovine hyperkeratosis were produced in calves that were fed the pellets.

3. Lubricants can pass from the pellet mill rolls into pellets during the pelleting process. If the lubricant contains highly chlorinated naphthalenes, the possibility that the pellets will be toxic to cattle exists.

SUMMARY

Four tons of feed were mixed and then pelleted in one-ton lots. Two of the tons, Lots A and B, were pelleted with the rolls of the pellet mill lubricated with a lubricant (Lot I) that contained no chlorinated naphthalenes. Two calves were assigned to each lot of pellets and individually fed the amount of pellets they would eat twice daily. These calves remained normal throughout 135 days of feeding. There was no evidence of toxicity of these pellets.

The other two tons of pellets, Lots C and D, were pelleted with the rolls of the pellet mill lubricated with a lubricant (Lot II) that contained 3% of highly chlorinated naphthalenes. Two calves were assigned to each of these lots of pellets and were individually fed the amount of pellets they would eat twice daily. All of the calves developed clinical symptoms and gross and microscopic lesions of bovine hyperkeratosis. Lots C and D pellets were toxic when fed to calves under the conditions of this experiment.

Two calves were given oral administrations of the lubricant (Lot I) that contained no chlorinated naphthalenes. They each received a total of 50 grams of lubricant in 10 gram doses given in a period of eight days. These calves remained normal throughout the 56-day feeding period. This demonstrated that Lot I lubricant which was used in pelleting of Lots A and B pellets was not toxic when fed to calves.

Two other calves were given oral administrations of Lot II lubricant. One of these calves was given enough lubricant so that

he received a total of 2.5 milligrams per pound body weight of hexachloronaphthalene. The other calf was given enough lubricant so that he received a total of 5 milligrams per pound body weight of hexachloronaphthalene. These calves developed symptoms and gross and microscopic lesions of bovine hyperkeratosis very rapidly. This indicated that Lot II lubricant which was used in the pelleting of Lot C and D pellets was toxic when administered to calves.

Two calves received oral administrations of a 3% solution of hexachloronaphthalene in corn oil. One calf received 2.5 milligrams of hexachloronaphthalene per pound body weight, and the other received 5.0 milligrams per pound body weight. Both developed symptoms of bovine hyperkeratosis very rapidly. The symptoms and lesions produced by the hexachloronaphthalene were very similar to those produced by the oral administration of Lot II lubricant and also those produced when Lot C and D pellets were fed. Vitamin A values dropped to levels indicative of deficiency; the calves became depressed, thin, and microscopic lesions were present in the kidney, liver, epididymis, seminal vesicles and parotid gland. This indicated that the toxic agent in Lot II lubricant was highly chlorinated naphthalenes.

It was demonstrated that a lubricant passed from the pellet mill rolls into pellets during the pelleting process, and that when this lubricant contained highly chlorinated naphthalenes, the pellets were toxic to cattle.

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