The effects of sources of Vitamin A and yeast upon utilization of Vitamin A by beef calves : the effect of feeding stilbestrol and antibiotics to cattle on a high barley fattening ration
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Abstract:
I. The first portion of the thesis presents the results of a study to determine the effects of sources of vitamin A, and yeast, upon utilization of vitamin A by beef calves. Two types of concentrated; vitamin A were studied: a corn-oil suspension of vitamin A (potency 1,600,000 I. U. per gram) and vitamin A palmitate in gelatin (potency 250,000 I.U. per gram). Yeast was added to the depletion rations of half the calves. The sixteen Hereford steer calves used in the trial were first depleted of vitamin A stores. Supplemental vitamin A was then fed via capsule at levels of 50,000 I. U. per steer daily for five or ten days, or 100,000 I. U. daily for ten days* Changes in blood plasma and liver vitamin A levels were used to determine utilization of vitamin A, There were no consistent' differences in the effects of the two types of vitamin A supplements used on plasma vitamin A content, or vitamin A storage in the liver. The depletion of vitamin A from the liver was faster when the steers received the corn-oil suspension of vitamin A. The addition of yeast to the ration had no apparent effect upon plasma-vitamin A values, but did tend to induce higher liver stores of vitamin A with slower subsequent depletion of liver stores® The level of 50,000 I.U. of vitamin A per steer daily for five days, was insufficient to initiate liver storage. When increased to ten days, 50,000 I. TL did initiate liver storage, and 100,000 I. U. per steer daily provided substantial liver storage. After vitamin A supplementation stopped, plasma and liver vitamin A content decreased rapidly at first, but tended to level off as the plasma and liver-vitamin A values decreased.

II. The second portion of the thesis presents the results of a cattle fattening trial designed to study the effects of adding stilbestrol and/or aureomycin to a high-barley fattening ration. Forty yearling Hereford steers were randomly divided into four lots of ten head each. The treatments used were: Lot 1, 10 milligrams of stilbestrol plus 75 milligrams of aureomycin per steer daily; Lot 2, 75 milligrams of aureomycin per steer daily; Lot 3, control; and Lot 4, 10 milligrams of stilbestrol per steer daily. The stilbestrol and aureomycin were incorporated into pelleted supplements which were group fed to each lot of steers at a rate of one pound per head daily. In addition to the pelleted supplements each lot was full-fed a basal ration of two-thirds barley and one-third beet pulp plus alfalfa hay free-choice. The animals fed stilbestrol gained 9.3 percent faster than the controls while the aureomycin or aureomycin-stilbestro1-fed animals gained 2.9 percent and 7 percent slower than the controls, respectively. The stilbestrol-fed and aureomycin-fed steers required 39 and 8 pounds less feed per hundredweight gain than the control steers, respectively, and the aureomycin-stilbestrol-fed steers required 57 pounds more. All four lots of steers lost money as follows: stilbestrol $12.18 per steer, aureomycin $14.67 per steer, aureomycin-stilbestrol $17.20 per steer; and control $l5.48 per steer.

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ABSTRACT

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I. THE EFFECTS OF SOURCES OF VITAMIN A AND YEAST UPON UTILIZATION OF VITAMIN A BY BEEF CALVES

INTRODUCTION

Vitamin A, required by all animals, is essential for maintenance, growth, reproduction, and milk production. One of the most important functions of vitamin A is to keep the epithelial structures of the body healthy and in proper functioning condition. Lack of the vitamin causes the proteins of the epithelial cells to be transformed into keratin, an insoluble protein. When keratinization progresses very far, the functions of the epithelial structures are impaired, and, in many cases, the tissues are so weakened that infections may get a foothold. Vitamin A is also necessary for the formation of visual purple which is required for vision in dim light, for the proper functioning of the nervous system, and for proper growth.

Although vitamin A does not occur as such in feeds of plant origin, green-leaved plants and certain other feeds of plant origin contain substances grouped under the term carotenoids. The most important of these carotenoids is Beta-carotene. Carotene is converted into vitamin A within the animal body and is, therefore, under natural conditions, the source of vitamin A for farm livestock.

When considering the nutrients required by farm livestock, vitamin A probably ranks near the first in importance, especially for ruminants in range areas. For, in these areas, livestock must subsist for considerable portions of each year on dry, mature range which has lost most, if not all, of its vitamin A potency. The possibilities of encountering a vitamin A deficiency are lessened considerably by the fact that animals have the

ability to store considerable amounts of vitamin A during periods of high vitamin A intake. However, the relatively dry climate in range areas, bringing about early maturing of the grass, seriously shortens the lush growing season when vitamin A storage is possible. Consequently, vitamin A storage is often insufficient to carry the animals through the winter months, especially after periods of drouth. To safeguard against possible vitamin A deficiencies, many range livestock producers have adopted the practice of fortifying winter supplemental livestock feed with vitamin A.

The trial reported herein was undertaken to study the utilization and storage of two types of vitamin A concentrate which might be used to fortify commercial feed mixtures. Changes in liver and blood plasma-vitamin A levels were used as yardsticks to determine vitamin A storage and/or utilization. The liver and blood plasma-vitamin A levels were determined by chemical analysis of blood and liver samples collected at regular intervals. It was also desired to determine levels of orally administered vitamin A (fed for only a short period of time), which would initiate liver storage.

During a mineral study with beef steer calves during the winter of 1953-54 (Thomas, 1954), routine blood plasma analysis indicated that yeast in the ration had a tendency to decrease plasma-vitamin A and carotene levels. To obtain further information on possible interrelationships between yeast and vitamin A, active dry yeast was incorporated into the rations of half the steers in the trial reported herein.

LITERATURE REVIEW

The presence of the water soluble vitamins B and C in certain food materials was ascertained primarily from data gathered from observations on disease. In contrast, the conception of fat-soluble vitamin A arose from the failure to secure normal growth in experimental animals for a long period of time on purified diets which furnished adequate proteins, fats, carbohydrates, and salts. Hopkins in 1906 and Stepp in 1909 both presented data showing that certain fat-soluble substances were indispensable for growth in mice and rats. It was not until 1913, however, that the existence of vitamin A was first clearly recognized. In work with rats that appeared almost simultaneously in that year, McCollum and Davis, and Osborne and Mendel, ascertained the presence of a growth factor, "fat-soluble A", differentiated from other compounds in cod liver oil and in butter. In 1931, Karrer obtained a highly concentrated vitamin A preparation and determined the structure of the vitamin and in 1933 he and associates established its chemical nature. In 1937, Kuhn and Marrion announced the synthesis of vitamin A and Holmes and Corbet obtained vitamin A for the first time in crystalline form. Carotene was isolated from carrots by Wachenroder in 1831 and Willstatter established the emperical formula in 1906. The vitamin A activity of the carotenoids was discovered by Stennbock in 1919. (Sherman and Smith, 1931, and Rosenberg, 1945).

Guilbert et al (1935, 1937, 1940) did much of the early work that established the minimum requirements of vitamin A for farm animals. Their work was based upon nightblindness as a criterion supplemented by checks on vitamin A storage. Evidence was presented which indicated that the amounts

of vitamin A or carotene that just prevent nightblindness represent a physiological minimum. Based on this criteria their stated requirements for cattle, sheep, swine and horses all fell within the same relative ranges. The minimum carotene requirement was 25-33 micrograms per kilogram of body weight daily, and the minimum vitamin A requirement was 5 to 8 micrograms per kilogram of body weight daily. Excellent growth occurred at these levels yet storage after extended periods was meager. Studies of storage at different levels of intake indicated that levels of at least five times the minimums would be a desirable minimum for practical purposes of significant storage and reproduction. The requirements per unit of body weight were about the same for young and old animals, but the young were more susceptible to pathological manifestations during privation. Evidence was also advanced that indicated vitamin A requirements are related to body weights rather than energy requirements as was previously supposed. If vitamin A requirements were related to energy requirements, the rat, with its higher metabolic rate, should have a requirement of about five times that of farm animals when based on unit of body weight. The data indicated that in actuality the requirements were very nearly the same per unit of weight. The data also showed a tendency for the rate of depletion of vitamin A stores to decrease as depletion advanced and reserves became smaller. Moore (1939), using nyctalopia and papillary edema as criteria substantiated the requirements established by Guilbert and co-workers. Lewis and Wilson (1945), using growth, blood levels, and liver storage as criteria also indicated that about eight micrograms was the minimum requirement for calves. Moore et al (1943) presented data which indicated that the measure-

ment of spinal fluid pressure is a fairly critical index of adequacy or inadequacy of carotene intake. Using this as a criteria they determined that a carotene intake of 66 micrograms per kilogram body weight was a daily winter minimum requirement for dairy calves.

Riefman et al (1943) in working with rats found the rate of absorption of vitamin A to be proportional to the concentration of the administered material. There was no relationship found between the rate of absorption of neutral fat and vitamin A. Barrick et al (1948) studied the absorption of carotene and vitamin A from the gastrointestinal tract of the sheep. They found that relatively large amounts of vitamin A were required to cause a noticeable rise of vitamin A in the blood plasma. The change in the vitamin A content of the blood was much slower and less pronounced following the administration of carotene than in the case of vitamin A. No absorption was observed as a result of administering either vitamin A or carotene into the colon. Esh et al (1948) reported that, in dairy animals, there was an indication that lecithin enhanced absorption of vitamin A. Lecithin added to vitamin A increased the transmission of colostral vitamin A.

Although most textbooks state that the conversion of carotene into vitamin A occurs chiefly in the liver, considerable data is accumulating to indicate the intestinal wall is the primary site of conversion. However, conflicting observations occur in the literature and it is probable that conversion can occur at more than one site in the body. Kon and Thompson (1951), on the basis of evidence presented in an extensive literature review, concluded that carotene is transformed to vitamin A mainly in the intestinal wall and that it is carried thence by the lymphatics to the blood stream and

finally to the liver. Alexander and Goodwin (1950) demonstrated conversion of carotene to vitamin A in rats in either the intestine or the intestinal wall. Oral administration of carotene to rats with the intestinal lymphatic vessel cannulated resulted in a marked increase in the vitamin A content of the lymph. No carotene was observed in the lymph. Thompson et al (1950) made extensive studies of intestinal conversion in rats and pigs. They found that very little conversion took place until after the bile and pancreatic juice entered the intestine with the peak of conversion just proximal to the middle of the intestine. When the intestinal contents were washed out from the living intestine, vitamin A appeared almost exclusively in the wall of the intestine, indicating conversion in the wall rather than in the contents of the digestive tract. The efficiency of conversion of carotene increased with the state of dispersion. Evidence also indicated that vitamin A is transported to the liver by the lymph.

Klosterman et al (1949) injected carotene suspended in water or carotene in cottonseed oil into the blood stream of sheep. They found that injected carotene was very rapidly removed from the blood stream. However, apparently this carotene was not converted to vitamin A as no increase of the vitamin in blood or storage in the liver was noted. Lambs given carotene by mouth or allowed green grass for a short period showed an increase of vitamin A in the blood serum. These observations, coupled with the fact that no measurable amount of carotene is found in the blood of normal sheep, suggest that carotene is converted to vitamin A by sheep during digestion and/or absorption rather than by the liver. Swick et al (1952) reported that when large doses of carotene were given pigs six to seven hours before

slaughter, there was a marked increase (up to 20 fold) in the concentration of vitamin A in the mesenteric lymph with a smaller rise in the blood plasma and intestinal wall.

Church et al (1954) studied the utilization of intravenously administered carotene by sheep and cattle. The carotene used was an aqueous preparation. In wethers, increases in plasma-vitamin A after carotene injections were highly significant. In calves, on the other hand, carotene injection caused no significant differences in plasma-vitamin A values or liver storage. In addition, advanced vitamin A deficiency symptoms present in some of the calves were not relieved and appeared to progress during the ten days of the experiment. Eaton et al (1951) reported a very limited conversion of carotene to vitamin A in the blood of vitamin A deficient dairy calves given intravenous injections of aqueous suspensions of carotene. Warner and Maynard (1952) reported that intravenously injected carotene in coconut oil gave no beneficial effects when administered to vitamin A deficient dairy calves. However, in a second trial with aqueous colloidal carotene administered intravenously, a significant increase in plasma-vitamin A was obtained.

Parrish et al (1951) studied the relative values of vitamin A and carotene in swine rations during gestation and lactation. Although there was no positive evidence that the carotene supplemented gilts or their pigs suffered from a vitamin A deficiency, the data clearly showed that, unit for unit, carotene is less effective than preformed vitamin A as a vitamin A supplement for swine during gestation and early lactation. Hentges et al (1952) studied the effects of carotene administered orally, intramuscularly and intravenously on vitamin A deficiency in pigs. It was found that, with

aqueous preparations of carotene, all three methods afforded complete recovery from vitamin A deficiency symptoms and normal plasma-vitamin A levels. Orally administered carotene was converted most rapidly while intravenous injections were utilized before intramuscular injections. Carotene in cottonseed oil administered intramuscularly remained at the site of injection and was ineffective in relieving avitaminosis A. Bieri and Pollard (1953) demonstrated rapid conversion of carotene to vitamin A in rats, using intravenous injections of carotene dispersed in water.

During periods when the carotene or vitamin A intake is high animals have the ability to store vitamin A and carotene. In studies with rats, Davies and Moore (1935) showed that the adult rat is able to store, with massive doses, enough vitamin A in its liver to supply its theoretical requirements for a century, but that these superfluous stores are eliminated at a very rapid rate until a state of stable storage is reached. Guilbert and Hart (1934) reported that the liver tissue of mature beef cows, reared under favorable conditions, was found to have a concentration of vitamin A approximating that of high potency cod liver oil.

There is some evidence that in storage, as in absorption, an aqueous medium may be superior to an oily medium. Sobel et al (1948) in experiments with rats demonstrated that vitamin A was more effective (as measured by liver storage) when dispersed in aqueous media than in oily solutions. Sobel and Rosenberg (1950) reported that, in rats, orally ingested vitamin A in an aqueous dispersion was more effectively transferred to milk and stored in the suckling offspring than vitamin A in oil solution (stores were four times as great). Johnson and Baumann (1949), in studying depletion

and utilization of vitamin A in rats, found that the retention of vitamin A is improved when the intake of food is restricted. Also, they reported that in normally growing rats, a decrease in the hepatic reserves of vitamin A was accompanied by an increase in the amount and concentration of the vitamin in the kidney. No such kidney increase occurred in rats whose growth was restricted during depletion.

Frey and Jensen (1946) reported that, in cattle, the rate of depletion of the hepatic reserves of vitamin A and carotene decreased as the hepatic reserves of the two constituents decreased. The data indicated that hepatic reserves of carotene are maintained in cattle in direct proportion to the carotene intake, and that an increasing rate of loss of hepatic reserves of vitamin A occurred with decreased carotene intake. Braun (1945) presented data which suggested that utilization of stored vitamin A first forces available carotenoid stores to be converted into vitamin A, thus decreasing the carotenoid level without decreasing the vitamin A level at first. There was no correlation between carotenoid and vitamin A values of the liver and those of the blood under normal conditions. Only upon rapid depletion and below normal storage was low vitamin A storage reflected by low blood levels. Watkins and Knox (1954) reported on a study of supplemental feeding of range breeding cows during the precalving and calving period. The data indicated that even without supplemental feeding of carotene, there were no major deficiencies over an eight year period, even in the most critical season.

Riggs (1940) reported wide variations in the time required for depletion of vitamin A reserves, particularly in older animals. Apparently the

condition of the range plays a large part. A range in depletion times, for 260 range cattle, was from three to sixteen months with shorter times in dry years with limited green grass. Also, he reported one steer which remained on a deficient ration for 381 days without becoming completely night blind, but which showed numerous other symptoms of deficiency, including swelling of the joints, suggesting a possible difference between individuals in the ability to utilize carotene or vitamin A for a given physiological function. Guilbert and Hart (1934) in experiments with steers, heifers, and mature cows, reported that the carotene in the adipose tissue, which constitutes a part of the vitamin A reserve, may be withdrawn during vitamin A privation without coincidental withdrawing of depot fat.

Sheep apparently have a slower depletion rate than cattle, and under normal conditions the possibility of a vitamin A deficiency in sheep is probably rather remote. Cunha et al (1946) reported that when ewes had grazed on green mountain feed during the summer, low quality roughages during the winter did not affect reproduction. Blood plasma-vitamin A determinations of the ewes and lambs showed very little difference, regardless of roughage fed. Weir et al (1949), on the other hand, found that the quality of winter roughage fed to ewes which had been on pasture during the summer did influence the blood plasma-vitamin A levels and the liver vitamin A reserves of ewes and their lambs. However, the ability of sheep to withstand extended periods on a low carotene ration was demonstrated by ewes which were on a straw ration for two successive winters. While these ewes did not maintain as high a blood level or liver storage, they did maintain levels above the critical levels. Pierce (1954) in a study covering seven

years and five reproductive cycles also found that depletion of vitamin A reserves in sheep was slow. It took at least 16 to 19 months to lower the plasma-vitamin A value from about 30 micrograms per hundred milliliters down to about 20 micrograms per hundred milliliters.

Davis and Madsen (1941) reported on an extensive study of vitamin A and carotene in cattle blood plasma. The data indicated that long continued inadequate carotene intake and vitamin A deficiency in cattle can be determined by blood analysis. The critical level of carotene in the plasma was found to be 20 to 25 micrograms per hundred milliliters and for vitamin A in the same sample about 16 micrograms per hundred milliliters. Cattle having carotene and vitamin A values at these levels or above usually did not show the characteristic clinical symptoms of vitamin A deficiency. The data also indicated that after cattle have been depleted of carotene reserves the blood carotene level is dependent upon carotene intake, and the vitamin A content of blood plasma is closely related to its carotene content. However, vitamin A values tend to reach a stable level and do not increase proportionally with increasing carotene levels (above 0.9 milligrams per hundred milliliters, also reported by Braun in 1945). Heifers receiving 20 to 45 micrograms of carotene daily produced deficient calves although the mothers themselves remained apparently normal. When heifers received 60 micrograms of carotene daily, apparently normal calves were born. Payne and Kingman (1947) reported that, to support normal gestation, first calf, range Hereford heifers must have considerably higher plasma-carotene levels than aged Hereford cows. The data indicated that the level for first calf heifers must be at least 110 micrograms per hundred

milliliters to support normal gestation whereas aged cows with levels as low as 83 micrograms per hundred milliliters had no abortions or deficiency symptoms over a two year period. Braun (1945) and Sutton et al (1945) reported that vitamin A and carotene decrease markedly in blood plasma of cattle at the time of parturition and beginning lactation. The maximum decrease in blood plasma-carotene was reached one week following parturition and amounted to 46 percent of the three week prepartum level. For vitamin A it was three days and 52 percent, respectively. Parrish et al (1951) stated that, in swine, vitamin A concentrations in the blood serum of the dams decreased as parturition approached and increased during the days immediately following parturition. Rasmussen et al (1944) reported that the horse has normal plasma values considerably below those of the cow, and apparently is inefficient in the conversion of carotene to vitamin A.

Jackson (1931) in studies with rats showed that mineral oil causes a considerable loss of vitamin A if it is mixed with the source of vitamin A prior to ingestion. Separate administration only slightly affected vitamin A utilization. Dutcher et al (1934) reported that the presence of mineral oil had no adverse effect upon the vitamin A potency of cod liver oil. However, relatively small amounts of mineral oil had a marked effect in lowering vitamin A potency from carotene. The carotene absorbed in unassimilated mineral oil was voided in the feces in almost direct proportion to the amount of carotene fed, indicating that the utilization of carotene was almost completely prevented by the mineral oil.

Lease and Steenbock (1939) presented data showing that the rate of loss of vitamin A from the liver of the rat was not affected greatly, if at all,

by the amount of fat in the diet, by feeding of rancid fats, or by the rapid depletion of fat from the liver as effected by the administration of choline. Muelder and Kelly (1942) found, with rats, that adding ten percent fat to a basal diet aided absorption of vitamin A sufficiently to produce statistically significant gains in weight over a basal diet containing no fat, but not over a basal diet containing five percent fat. Brown and Bloor (1945) reported that the nature of the fat being absorbed seemed to be of some importance in the utilization of carotene from carrot by the rat. Liver storage was higher when the rats were fed liquid acid diets than when fed solid acid diets. Parham et al (1950) studied the influence of solvent extracted versus hydraulic processed cottonseed meals in beef cows. They found that although the difference in levels of blood fat was statistically significant, this difference appeared to have little relationship to levels of carotene and vitamin A in the blood. Raper (1950), in work with beef calves, found that when the fat content of the ration was raised from two to five and one-half percent, the plasma-carotene level rose significantly. Plasma-vitamin A, and liver-vitamin A and carotene were not affected. Thomas et al (1949) found that when young calves (under 90 days) had been on a high vitamin A intake, the elimination of carotene and vitamin A did not immediately change the plasma-vitamin A concentration whereas elimination of fat as well resulted in a marked increase in plasma-vitamin A.

Ross and Gallup (1949) reported on studies with beef cattle with indicated an inverse relationship between the level of plasma-inorganic-phosphorus and plasma-carotene. Cattle with low plasma-phosphorus levels had been observed to have higher plasma carotene levels than cattle with normal

or above normal levels of plasma-phosphorus. Klosterman et al (1952) found that, in rats given equal amounts of carotene or vitamin A, liver stores of vitamin A were greater in the animals fed low phosphorus than in those fed high phosphorus rations. They also reported a highly significant negative correlation between plasma-inorganic-phosphorus and vitamin A in lambs fed low and adequate phosphorus rations deficient in carotene. Gallup et al (1953) reported similar results with steers and lambs. In steers which had been depleted in phosphorus and vitamin A reserves and then given daily carotene supplements (half also received phosphorus), the plasma-carotene levels were consistently higher in the steers on the low-phosphorus ration, while the plasma-vitamin A values were lower. There was decreased liver vitamin A storage in the phosphorus deficient steers. The results obtained with lambs in a similar experiment showed a different trend to that noted with cattle. Plasma-vitamin A values were higher in the phosphorus deficient lambs and the liver vitamin A stores were greater in the phosphorus deficient lambs. Thomas et al (1953) reported similar results in beef cows with plasma-carotene values generally higher in the phosphorus deficient cows than in those fed adequate phosphorus. Milk from the phosphorus deficient cows contained more carotene but less vitamin A than that from the cows fed adequate phosphorus.

King et al (1940) reported that, in cattle, a reduced plasma-vitamin C level occurs shortly after the sumptoms of avitaminosis A appear. The subcutaneous injection of crystaline ascorbic acid seemed to alleviate several symptoms associated with a lack of vitamin A, namely, a noticeable improvement in the rough scaly condition of the hair and skin and an attenuating

effect upon retinal hemorrhages. Boyer et al (1942) also reported a definite indication of an interrelationship between blood plasma-ascorbic acid and vitamin A in dairy calves, particularly when vitamin A values fell below 10 micrograms per hundred milliters. It was also found that in rats, when vitamin A deficiency develops, the excretion of vitamin C is greatly reduced, indicating that the lowered blood and tissue vitamin C is the result of impaired synthesis. Mayer and Krehl (1948) found that one of the first symptoms of the vitamin A deficiency syndrome in the rat was a depletion of the animal's vitamin C reserves, evidenced by symptoms resembling scurvy and curable by ascorbic acid as well as by a decrease in the ascorbic acid content of the liver, blood, and adrenals.

Davies and Moore (1941) and Hickman et al (1942) reported a pronounced synergistic effect between vitamin A and vitamin E. It was noticed that vitamin E deficient rats had much lower vitamin A reserves than rats receiving equal amounts of vitamin A plus supplements of vitamin E. The data indicated that the effect of the vitamin E deficiency does not lie entirely in the inability of the liver to absorb vitamin A but also in a decreased power of retention after absorption. Prolonged deficiency of vitamin E led to a secondary deficiency of vitamin A as indicated by the disappearance of vitamin A from the liver. Also, a vitamin A deficiency was prevented by the administration of vitamin E to low vitamin A rations.

McGillwray (1951) published a report on the apparent intestinal synthesis of carotene by sheep. Carotene-lignin ratios were studied and measured at different points of the digestive tract, ingested food, and voided feces. The lignin recovery in the feces was 96 percent indicating negligi-

ble digestibility. The ratios remained relatively stable through the stomachs. In the intestine the carotene-lignin ratios decreased through the upper portion of the small intestine, increased through the ileum reaching a maximum in the caecum, and decreased slightly through the colon and rectum. Some animals actually showed a negative carotene balance. The author stated that synthesis of carotene by intestinal micro-organisms had been demonstrated on an agar medium innoculated with caecal contents.

PROCEDURE

General

The experiment consisted of two experimental periods of vitamin A supplementation. Previous to each of the two periods of vitamin A supplementation, the steers were fed rations low in vitamin A until the blood plasma-vitamin A levels dropped to approximately 12 micrograms of vitamin A per hundred milliliters of plasma. Two forms of vitamin A concentrate--a corn-oil vitamin A suspension with a potency of 1,600,000 I. U. of vitamin A per gram and vitamin A palmitate in gelatin with a potency of 250,000 I. U. of vitamin A per gram---were used as supplemental vitamin A sources during the two experimental periods.

On December 23, 1954, sixteen Hereford steer calves were divided into two lots of eight calves each and placed in separate, but adjoining, pens. Each pen contained eight individual feeding stalls in a shed, where the steers were individually fed, and an outside exercise area. A common watering tank was located inside the shed. During the period from December 23, 1954 to January 10, 1955, the calves were individually fed two pounds per head daily of a grain supplement (about 17 percent crude protein) and approximately six pounds of low quality hay per head daily. On January 6, one steer died of urinary calculi, and was replaced by a healthy animal on January 10.

First Depletion Period

On January 11, the calves were weighed and initial blood samples were taken from the jugular vein. The calves were then fed vitamin A depletion rations consisting of approximately seven pounds of wheat straw per animal

daily plus three pounds of a grain supplement containing about 30 percent crude protein. The grain ration for half the calves (four from each pen) contained 3.5 percent active dried yeast, which provided an intake of approximately 0.1 pound of yeast per steer daily. The composition of the supplemental rations is presented in Table I.

The steers remained on vitamin A depletion rations for a period of 127 days, until May 18. For the first three months of thie period, weights and blood samples were taken every 28 days, except the third period which was delayed a week (to 35 days) because of excessive snow. After April 12, weights and blood samples were taken every 14 days. On April 12, the intake of concentrates was increased to four pounds per head daily (yeast was decreased to 2.5 percent of the ration to hold intake steady at approximately 0.1 pound daily). By this time, some of the steers were beginning to show deficiency symptoms such as incoordination and impaired vision.

After April 12, seven steers which had plasma-vitamin A levels of less than 15 micrograms per hundred milliliters were given a daily supplement of approximately 2500 I. U. of vitamin A to stop further depletion of vitamin A stores until the remaining steers became sufficiently depleted. A commercial source of vitamin A (potency: 10,000 I. U. per gram) was used for this purpose and was mixed with soybean oil meal so that the final mixture would contain 2500 I. U. of vitamin A in one tablespoon of the soybean oil meal. The desired amount of soybean oil meal was then added to the daily grain ration of the steers requiring vitamin A supplementation. On May 2, supplementation of vitamin A was adjusted, with some calves being reduced to 1250 I. U. per day and others added to the supplemental list, at a level of

Table I. Depletion Rations Used

Ration No.	1	2
Ingredients:	%	%
Barley	28.75	25.25
Beet Pulp, D.M.	20.00	20.00
Soybean Oil Meal	25.00	25.00
Linseed Oil Meal	10.00	10.00
Urea (262)	3.00	3.00
Wheat Bran	10.00	10.00
Bonemeal	2.00	2.00
Trace Minerals	0.25	0.25
Salt	1.00	1.00
Active Dry Yeast	0.00	3.50 1/

1/ Decreased to 2.5% after April 12th.

Table II. Average Weights Per Steer During Depletion Periods

Ration No. Treatment	1 No Yeast	2 Yeast 1/
Date:	Average weight per steer (Lbs.)	
December 22	497	507
January 11	475	486
February 8	465	483
March 8	500	514
April 12	552	559
April 24	551	560
May 10	552	554
June 14	573	580
Total Gain Per Steer	76	73
Daily Gain Per Steer to June 14	0.43	0.42

1/ Yeast was not included in the ration until January 11.

either 1250 I. U. or 2500 I. U. daily, depending upon the plasma-vitamin A levels. By May 10, the average plasma-vitamin A levels were dowm to 12 micrograms per hundred milliliters of plasma.

First Experimental Period

On May 18 liver samples (1 to 2 grams in weight) were collected from each steer by biopsy technique (Seghetti and Marsh, 1953). Each steer was given one capsule, containing 50,000 I. U. of vitamin A, each day for five consecutive days beginning on May 19. The two vitamin A concentrates, vitamin A in oil and vitamin A palmitate in gelatin, were administered so that four calves on each of the two depletion rations received one type. Blood samples were taken on May 18 and on the 3rd, 6th, 8th, and 10th days thereafter. On the 10th day, a second liver sample was obtained to determine vitamin A storage in the liver.

Second Depletion Period

Following the first ten-day experimental period, which ended May 28, the calves were continued on the depletion rations previously used. Blood samples were taken on June 14 and July 12 to check the rate of depletion of plasma-vitamin A. By July 12, several of the calves had plasma-vitamin A levels of less than 15 micrograms per hundred milliliters. On July 18, four calves with plasma-vitamin A levels between 10 and 15 micrograms per hundred milliliters were placed on a daily supplement of 1250 I. U. of vitamin A per day. Seven other calves with plasma levels of less than 10 micrograms per hundred milliliters were provided a daily supplement of 2500 I. U. of vitamin A daily. On July 26, the yeast was removed from the ration.

Second Experimental Period

The second experimental period began on July 27, when the first liver samples were collected, and continued for thirty days. The steers were given concentrated vitamin A, via capsule, daily for ten days beginning July 27. Because the results of the first experimental period revealed that the levels of supplementation were too low to produce definite patterns of liver storage, the level of vitamin A supplementation and/or the length of the supplemental period were increased during the second experimental period. The steers which had received vitamin A in oil and vitamin A palmitate in gelatin, respectively, during the first experimental period were randomly divided into two groups each. One group received 50,000 I. U. and the other group 100,000 I. U. daily of its respective concentrate, so that the steers were divided into four groups according to treatment as follows:

(1) Four calves, each receiving 50,000 units of vitamin A in oil daily for ten days.

(2) Four calves, each receiving 100,000 units of vitamin A in oil daily for ten days.

(3) Four calves, each receiving 50,000 units of vitamin A palmitate in gelatin daily for ten days.

(4) Four calves, each receiving 100,000 units of vitamin A palmitate in gelatin daily for ten days.

One calf in group 2 died on July 31 of acute peritonitis resulting from a duodenal puncture during the liver biopsy on July 27.

Blood samples were taken from the jugular vein on the 1st, 2nd, 3rd,

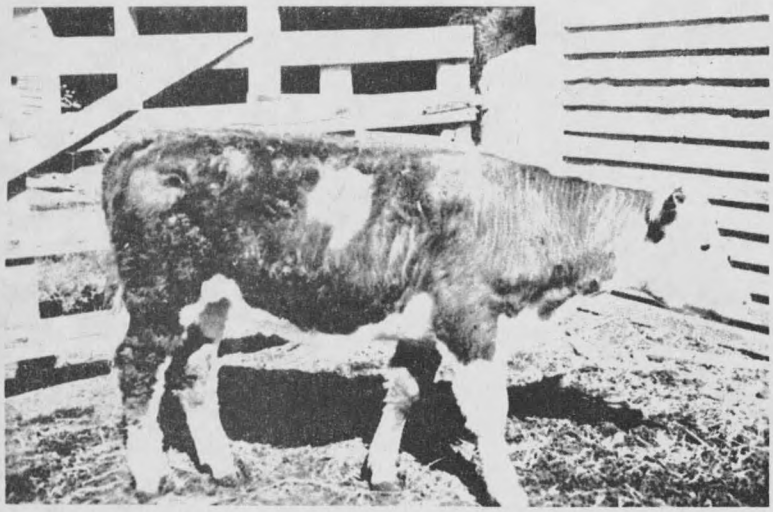

Figure 1. Typical vitamin A deficient steer with shaved area showing where liver biopsies were taken.

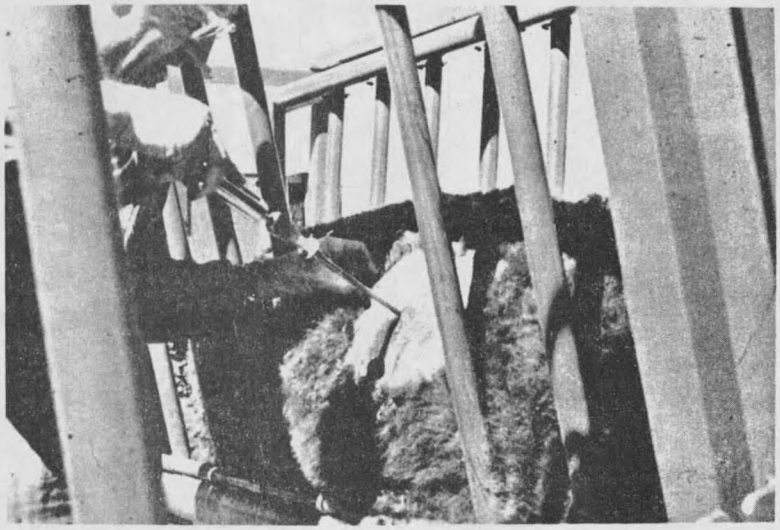

Figure 2. Performing a liver biopsy.

4th, 6th, 8th, 10th, 15th, 20th, 25th, and 30th days of the experimental period (July 27 considered the 1st day). Liver samples were obtained on the 1st, 10th, 20th, and 30th days.

All blood samples collected during the trial were analyzed for plasma-vitamin A and plasma-carotene content (Boyer et al, 1953). The liver samples were analyzed for vitamin A content (modified method of Gallup and Hoefer, 1946).

The combined scales and bleeding chute, used during the first depletion period, was situated approximately one hundred yards from the dry-lot pens. After grass began to grow in the Spring it became difficult to move the steers from the dry-lot pens to the scale house without some of the steers consuming green grass. Therefore, since any carotene ingested while the steers were being moved to and from the weighing pen would affect the blood levels of both carotene and vitamin A, the steers were not weighed after May 10, with the exception of one weight on June 14, after the end of the first experimental period. A bleeding chute was constructed within one of the dry-lot pens and was used for the collection of the blood samples after May 10. The steers were trucked a distance of about five miles for the collection of the liver samples, which were taken at the Veterinary Research Laboratory at Montana State College.

RESULTS AND DISCUSSION

The average weights by period, total gains per steer and daily gains per steer are presented in Table II. During the preliminary period, when the steers received low quality hay plus a grain supplement (approximately 17 percent crude protein), steers in both groups lost weight, 22 and 23 pounds per steer for group 1 and 2, respectively. Yeast was not incorporated into the supplemental ration until January 11, when the crude protein content of the supplement was increased to approximately 30 percent. At this time, the steers were fed about seven pounds of wheat straw per head daily. There was a further decrease in weight from January 11 to February 8, 10 and 3 pounds per steer for groups 1 and 2, respectively. After February 8, the steers made gains of approximately 1.25 pounds per head daily until April 12. After April 12, however, when vitamin A deficiency symptoms became apparent, growth ceased until May 10, when the last weights were taken before the first period of vitamin A supplementation. The average weights on June 14, twenty-two days after administration of supplemental vitamin A had stopped, showed that further gains had been made. Except for minor period to period variation, the average losses and gains of the steers were remarkably uniform.

Very good results were obtained with the depletion rations used in the experiment, as indicated by the plasma-vitamin A levels presented in Table III; and the plasma-carotene levels presented in Table IV. During the first experimental period in particular, the decrease in plasma-vitamin A was fairly consistent throughout the 127-day depletion period. While the vitamin A depletion of the steers receiving yeast was slower during the first

part of the period, the depletion during the latter part of the period was accelerated. By April 12, there was only a slight advantage in the average plasma-vitamin A levels from the yeast-fed steers.

Table III. Blood Plasma-Vitamin A Levels During Depletion Periods

Treatment	Yeast	No Yeast
	Micrograms per 100 milliliters (Average)	
Date:		
January 11	28.9	29.9
February 8	29.6	25.0
March 8	26.1	19.1
April 12	17.8	16.0
April 26	12.9	13.3
May 10	11.6	12.0
May 18	13.3	16.3
May 24	25.9	24.4
May 26	20.8	20.8
May 28	14.3	15.4
June 14	24.5	17.3
July 12	12.1	11.0
July 27	12.5	12.0

Statistical analysis (Ostle, 1954) showed the depletion in plasma-vitamin A content (to April 12), for each group of steers, to be a simple linear regression with a highly significant ($P \leq .01$) correlation between the length of time the steers were fed vitamin A depletion rations and the decrease in plasma-vitamin A content.

After April 12, it became necessary to provide supplemental vitamin A to some steers on both rations to prevent the plasma-vitamin A levels of these steers from becoming dangerously low before the rest of the animals reached a satisfactory stage of depletion. It was believed that a level of 2500 I. U. of supplemental vitamin A per day would maintain the plasma-vitamin A level and prevent a further depletion. However, it was found that

Table IV. Blood Plasma-Carotene Levels

Ration	Yeast	No Yeast
	Micrograms per 100 milliliters (Average)	
Date:		
January 11	152.6	155.1
February 8	46.6	41.4
March 8	26.4	23.6
April 12	13.5	13.9
April 26	9.8	11.0
May 10	8.0	10.5
May 18	8.0	7.9
May 21	11.9	12.0
May 24	11.6	12.1
May 26	6.4	6.6
May 28	9.5	9.9
June 14	16.3	18.8
July 12	27.8	15.8
July 27	23.8	14.1
July 28	23.0	12.3
July 29	25.3	16.8
July 30	26.0	18.8
August 1	23.1	19.1
August 3	18.4	12.0
August 5	17.6	11.1
August 10	19.1	15.6
August 15	17.3	13.4
August 20	16.4	12.0
August 25	14.1	11.7

even this relatively low level of vitamin A intake frequently caused the blood plasma-vitamin A levels to rise, indicating a difference in the efficiency with which individual animals utilize vitamin A. Accordingly, the level of supplementation was decreased to 1250 I. U. daily for several steers. The slight rise in average plasma-vitamin A between May 10 and May 18 was undoubtedly due to the addition of this supplemental vitamin A to the rations of some of the steers.

The vitamin A depletion of the steers during the second depletion period was more erratic. It is believed that this was due primarily to the difficulty in keeping the steers from consuming any grass during the lush growing season. The fence lines surrounding the two lots were soaked with oil to prevent the growth of grass, and any grass observed was physically removed from the pens. In spite of these precautionary measures, the plasma-carotene values presented in Table IV indicate the steers undoubtedly obtained some grass.

The design of the experiment, showing how the steers were subdivided into the various treatments, is presented in Table V. The average blood plasma-vitamin A levels of the steers during the two experimental periods of supplementation are presented in Tables VI and VII. The average liver vitamin A levels for the two experimental periods are presented in Tables VIII and IX.

The feeding of 50,000 I. U. of vitamin A per steer daily resulted in a substantial rise in plasma-vitamin A levels during the first experimental period (May 18 to May 28). This rise in plasma-vitamin A values was to be expected, since the amount of vitamin A supplemented daily (50,000 I. U.)

Table V. Design of the Experiment, Showing Steers Receiving Various Treatments.

Treatment:	Vitamin A in Gelatin	Vitamin A in Oil	Yeast	No Yeast
Steer No.				
61		X	X	
62		X*	X	
63	X			X
64	X*			X
65		X*	X	
66	X		X	
67		X		X
68	X*			X
71	X*		X	
72	X*		X	
73		X*		X
74	X			X
75		X	X	
76	X		X	
77		X*		X
78		X		X

*Received 100,000 I. U. per steer daily during the second supplementary period.

Table VI. Blood Plasma-Vitamin A Levels During Supplementary Periods.

Treatment:	Vitamin A in Gelatin	Vitamin A in Oil	Yeast	No Yeast
	Micrograms per 100 Milliliters (Average)			
Date:				
May 18	14.6	14.9	13.3	16.3
May 21	15.0	16.9	17.3	14.6
May 24	26.3	24.0	25.9	24.4
May 26	21.0	19.5	20.8	20.8
May 28	15.0	14.6	14.3	15.4
July 27	14.9	9.4 1/	12.5	12.0 1/
July 28	29.1	22.7	17.1	33.1
July 29	30.9	22.6	19.6	32.5
July 30	27.1	25.9	26.4	26.7
August 1	33.5	33.7	34.0	33.1
August 3	34.8	33.4	33.9	34.4
August 5	32.4	29.7	32.5	30.4
August 10	26.6	24.9	24.5	27.3
August 15	19.5	19.7	20.6	18.4
August 20	19.8	18.9	20.1	18.4
August 25	15.3	13.3	15.3	13.3

1/ One steer died as result of biopsy; figures in these groups are averages of seven steers. (second period only)

Table VII. Blood Plasma-Vitamin A Levels For Second Experimental Period Comparing Levels of Supplemental Vitamin A.

	Vitamin A in Oil		Vitamin A in Gelatin	
	50,000 I.U.	100,000 I.U.	50,000 I.U.	100,000 I.U.
	Micrograms per 100 Milliliters (Average)			
Date:				
July 27	9.5	9.3 1/	17.5	12.3
July 28	28.3	15.3	22.3	36.0
July 29	22.5	22.7	24.3	37.5
July 30	23.5	29.0	26.8	27.5
August 1	32.3	35.7	30.5	36.5
August 3	32.8	34.3	32.0	37.5
August 5	30.4	29.0	29.8	35.0
August 10	22.3	28.3	25.0	28.3
August 15	20.0	19.3	16.8	22.3
August 20	18.0	20.0	16.3	23.3
August 25	11.3	16.0	12.5	18.0

1/ One steer died as result of biopsy; figures in this group are averages of three steers.

Table VIII. Liver Vitamin A Levels Comparing Types of Supplemental Vitamin A.

Type of Supplement:	Vitamin A in Oil		Vitamin A in Gelatin	
Level of Supplementation:	50,000 I.U.	100,000 I.U.	50,000 I.U.	100,000 I.U.
Date:	Micrograms per gram (Average)			
May 18 1/	2.10		2.26	
May 28	1.91		2.16	
July 27	0.88	0.90 2/	3.88	0.95 3/
August 5	2.93	6.80	4.05	6.80
August 15	1.73 3/	4.25	2.78	4.63
August 25	1.13 3/	2.30	2.15	4.05

1/ Failed to obtain a liver sample from one steer in each group; Figures for the first period are averages of seven steers each.
2/ One steer died as result of biopsy; figures in this group are averages of three steers.
3/ Failed to obtain liver samples from one steer on these dates; Figures are averages of three steers.

Table IX. Liver Vitamin A Levels Comparing Yeast in the Ration and No Yeast in the Ration

Ration:	Yeast	No Yeast
Date:	Micrograms per gram (Average)	
May 18	2.31	2.00 1/
May 28	2.25	1.75
July 27	1.44 3/	2.20 2/
August 5	5.46	4.54
August 15	4.21 3/	2.49
August 25	3.24 3/	2.77

1/ Failed to obtain liver samples from 2 steers; figures for the first period are averages of six steers.
2/ One steer died as result of biopsy; figures in this group are averages of seven steers.
3/ Failed to obtain liver sample from one steer on these dates; figures for these dates are averages of seven steers.

is approximately ten times the daily vitamin A requirement for five hundred pound steers. It will be noted, however, that as soon as vitamin A supplementation stopped, the plasma-vitamin A levels rapidly decreased. By May 28, five days after the last supplemental vitamin A was given, the plasma-vitamin A value had almost returned to the levels present before supplemental vitamin A was administered.

The liver vitamin A levels, however, decreased by .06 to .25 micrograms of vitamin A per gram of liver during the ten days between the two liver biopsies of the first experimental period. No liver samples were collected on the fifth day when the last vitamin A capsule was administered; therefore, it was not possible to determine whether any liver storage had occurred at that time. However, the data indicates that, when steers have been on vitamin A deficient rations, supplementation at a level of 50,000 I. U. daily for a period of five days was not sufficient to stop further depletion of liver stores of vitamin A for any appreciable length of time.

The higher levels of supplementation and/or longer period of supplementation during the second experimental period produced more definite results. The administration, via capsule, of either 50,000 I. U. or 100,000 I. U. of vitamin A daily per steer for ten days produced substantial increases in plasma-vitamin A content, with the greater response from the higher level of supplementation. It is interesting to note that the peak plasma levels of vitamin A were reached from two to five days before supplementation stopped. The reason for this slight drop in plasma-vitamin A while the steers were still receiving supplemental vitamin A is not known. When vitamin A supplementation stopped, there was a comparatively rapid

decline in plasma-vitamin A levels. Typical responses from the two levels of vitamin A supplementation are presented graphically in Figures 3 and 4.

The increase in plasma-vitamin A during the supplemental periods was comparatively rapid with both levels of vitamin A supplementation. After supplementation stopped, the plasma-vitamin A levels declined sharply for the first ten days. Thereafter, the rate of decline in plasma-vitamin A levels tended to become slower as the plasma-vitamin A levels decreased.

When analyzed statistically (Ostle, 1954), the changes in plasma-vitamin A levels during the second experimental period were found to follow a quadratic type of regression (second degree equation). For each treatment, there was a highly significant ($P<.01$) correlation between the plasma-vitamin A levels and the date of the period.

During the second experimental period, the higher levels of vitamin A supplementation and/or longer period of supplementation also stimulated an increase in liver vitamin A content. It may be noted that the supplemental level of 50,000 I. U. of vitamin A per steer daily resulted in only a slight liver vitamin A storage during the ten day period (Table VIII and Figure 5). However, the administration of 100,000 I. U. of vitamin A per steer daily resulted in a substantial increase in liver-vitamin A content during the ten day period. After vitamin A supplementation was stopped, the amount of vitamin A in the liver decreased rapidly for the first ten days, after which the rate of depletion tended to level off. The steers which received yeast in the ration previous to the second experimental period had a slightly higher storage of vitamin A in the liver, with less subsequent depletion, than the steers which did not receive yeast in the ration.

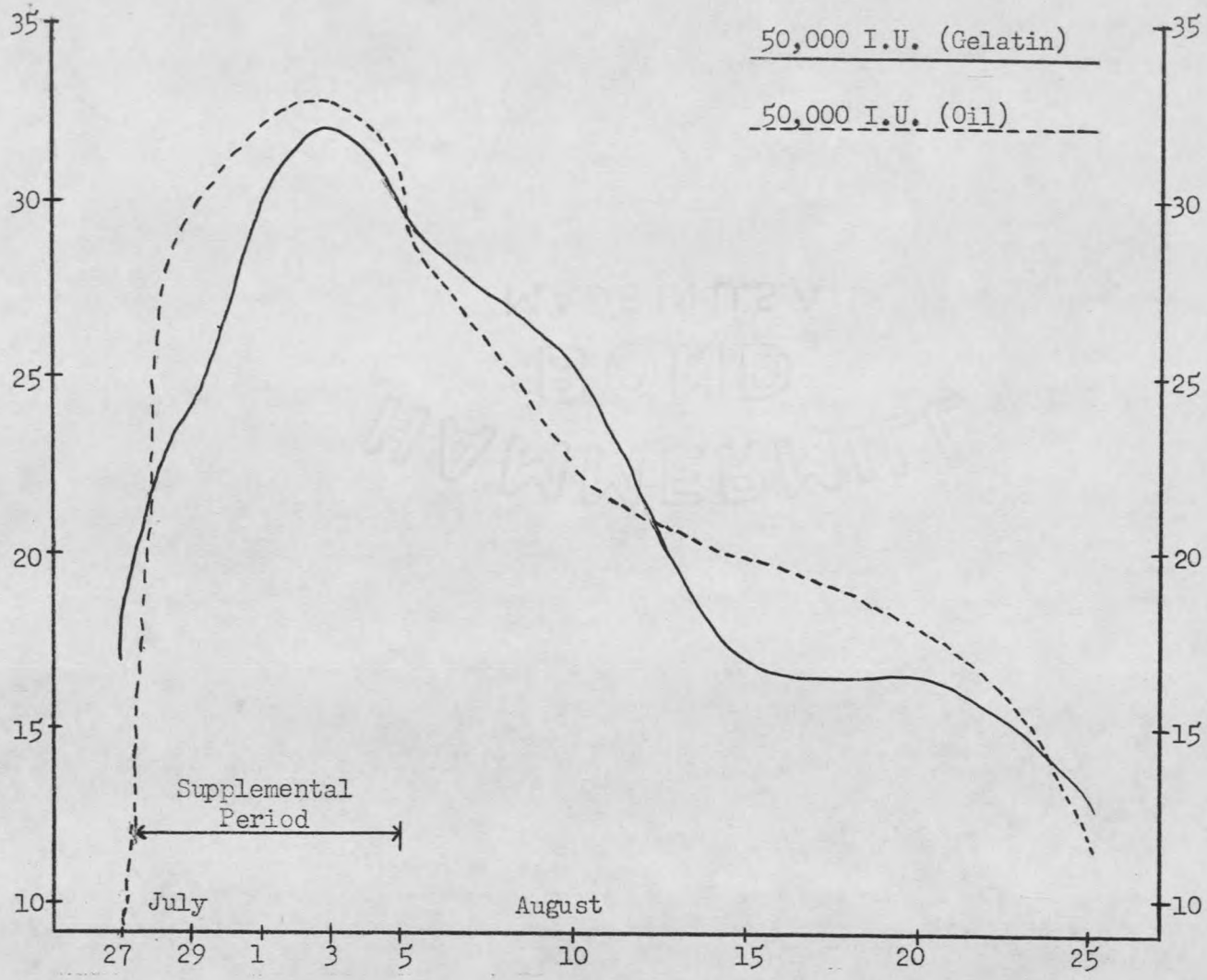

Figure 3. Plasma-Vitamin A Levels.

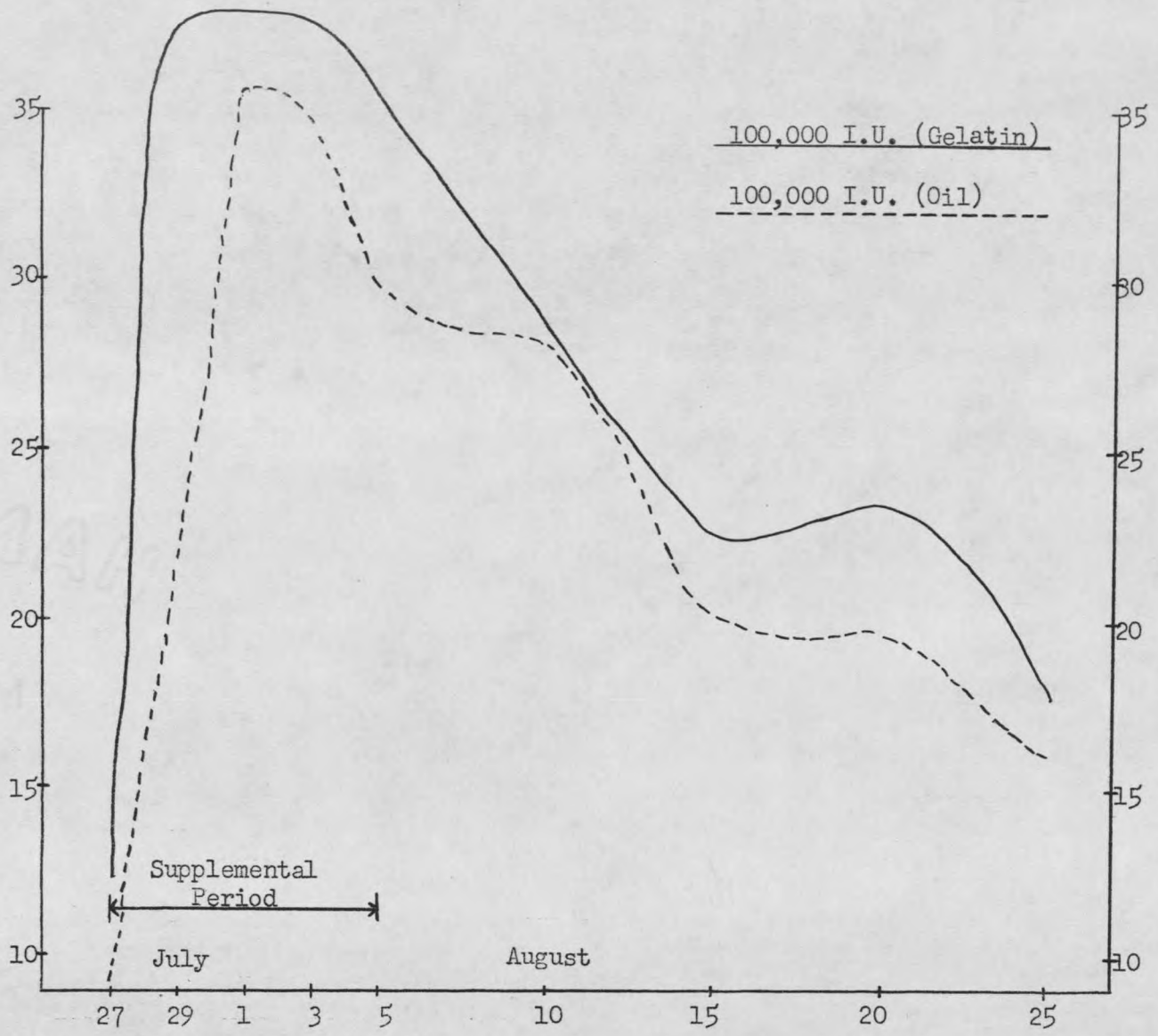

Figure 4. Plasma-Vitamin A Levels.

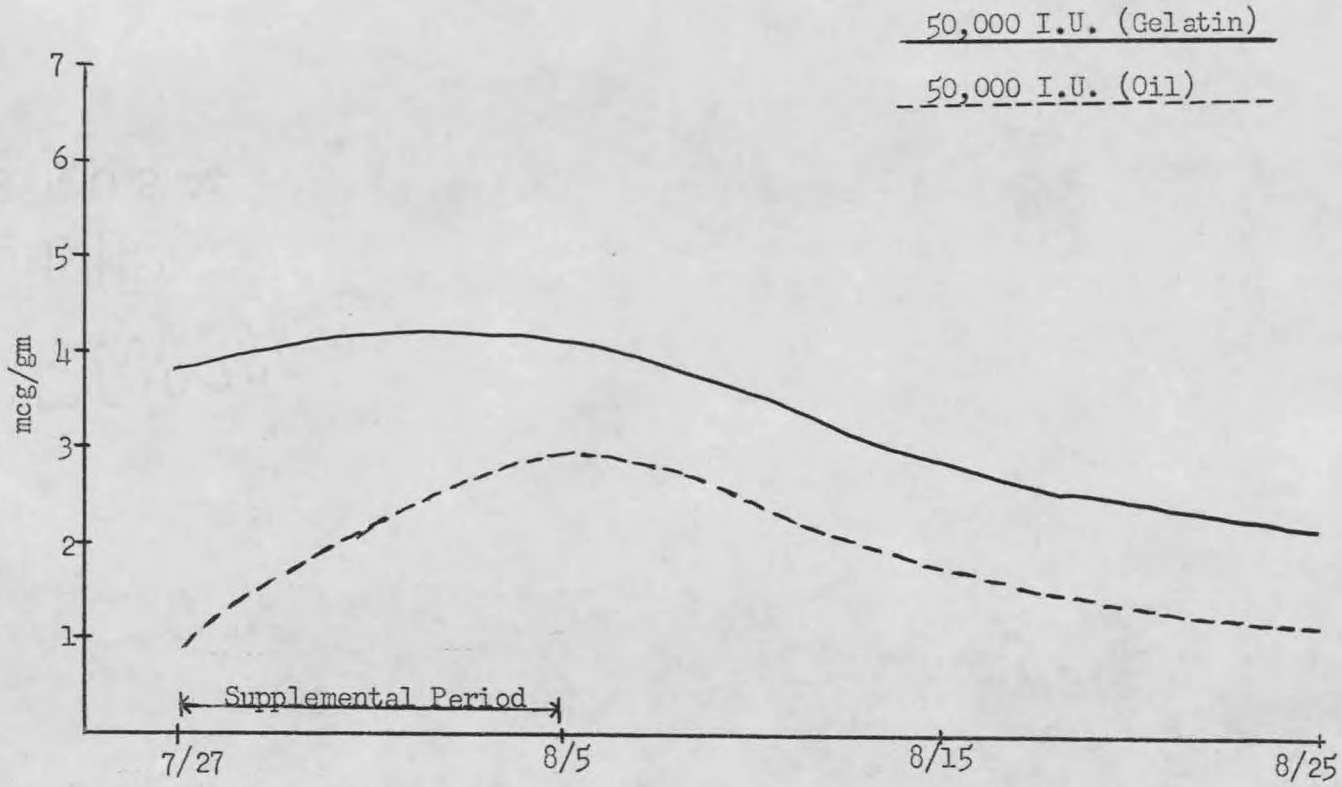

Figure 5. Liver–Vitamin A Levels for Steers Fed 50,000 I. U.

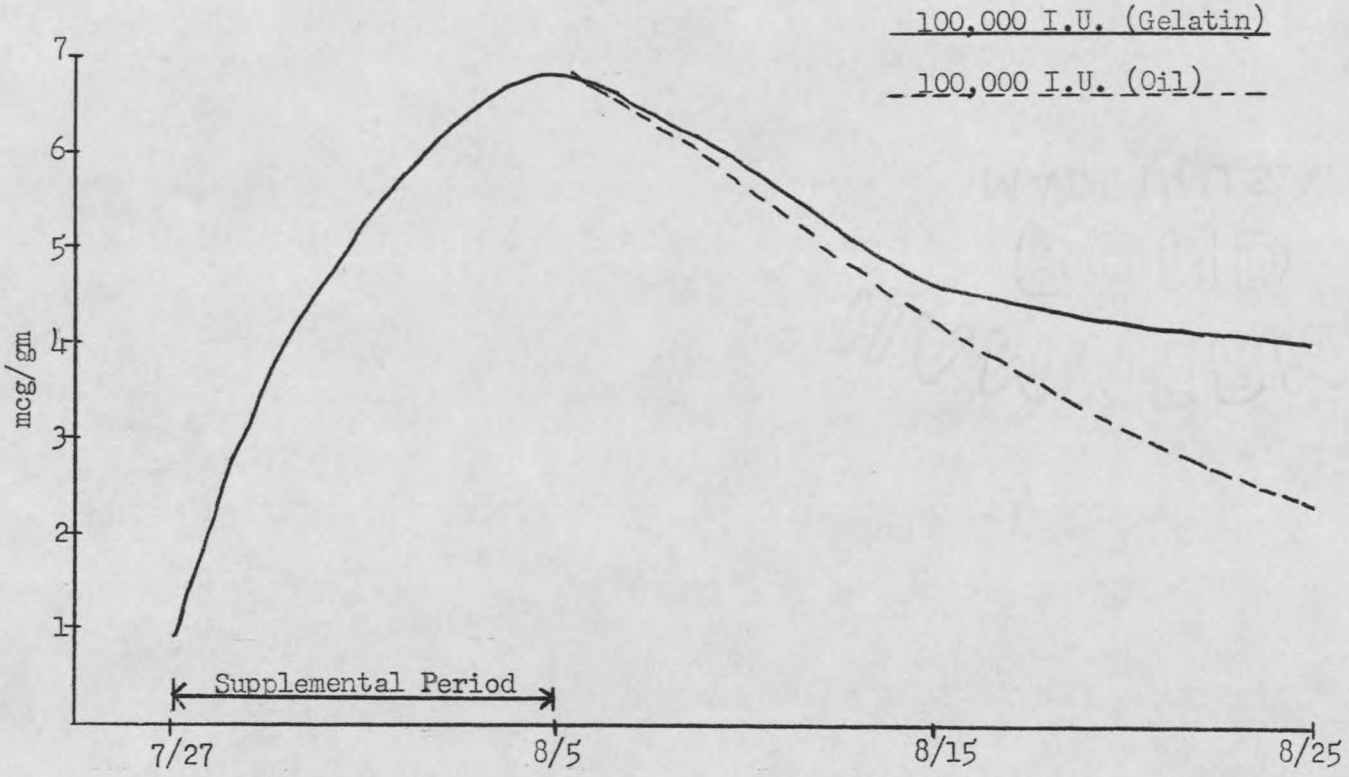

Figure 6. Liver-Vitamin A Levels for Steers Fed 100,000 I. U.

The data seemed to indicate that a relatively small proportion of the vitamin A administered orally subsequently appeared in the blood plasma and livers of the steers. Therefore, an estimation was made of the total increase of plasma and liver vitamin A per steer during the second period of vitamin A supplementation. The steers were not slaughtered at the end of the trial, so no data was collected on the average liver weights or total blood per steer, nor were accurate body weights available during the period of supplementation. Therefore, it was not possible to determine the total amount of vitamin A which appeared in the blood and livers with any degree of accuracy. It was believed, however, that even an approximate determination would be of some value.

In making the approximation, the livers were considered to weigh 1.04 percent of the liveweight of the steers (Edelmann et al, 1943), and the weight of the blood was considered to be 7.7 percent of the liveweight (Dukes, 1947). On the basis of this information, the average weight of blood and liver per steer were calculated from the average weights per steer recorded on June 14. (These were the last weights taken during the trial). The total weight of blood per steer was then converted to milliliters by utilizing the specific gravity of steer blood and plasma was considered to be 60 percent of the blood (Dukes, 1947). Once the total volume of blood plasma, and weight of liver, per steer was known, the results of the chemical analyses of the blood plasma and liver samples were used to determine the total number of micrograms of vitamin A per steer appearing in the blood plasma and livers. To convert the micrograms of vitamin A to international units, 0.23 micrograms of vitamin A was considered to be equal to

one international unit (Everett, 1946).

When these calculations were made, it was found that the increase in plasma and liver-vitamin A accounted for approximately 5 percent of the vitamin A fed at a level of 50,000 I. U. per steer daily over the ten day period. When a level of 100,000 I. U. was fed daily for ten days, approximately 8 percent of the vitamin A could be accounted for in the blood and livers of the steers. Since the steers were definitely deficient in vitamin A when supplementation started, it is probable that substantial amounts of vitamin A were removed from the blood stream by the body tissues when the plasma-vitamin A levels began to rise. In spite of the fact that this would account for some of the vitamin A fed, it seems apparent that the major part of the vitamin A that was fed to the steers was either destroyed or failed to be absorbed by the intestinal tract.

No definite conclusions can be made about the effects of the various treatments used in the trial because of the small number of animals available for each treatment. The failure to obtain liver samples from some of the steers, and the death of one steer, further decreased the amount of data obtained. However, certain trends were noted which would bear further investigation. Adding yeast to the rations had very little effect on plasma-vitamin A levels, but there was an increase in liver-vitamin A storage and a slight decrease in the rate of depletion of liver stores. However, no liver samples was obtained from steer number 77 on July 27, and this steer had substantially higher than average liver-vitamin A levels during the rest of the period. Conversely, steer number 63, from which no liver samples were obtained on August 15 and 20, had slightly lower than average liver-

vitamin A levels during the first half of the period. Therefore, it is possible that the apparent differences noted may have been due to experimental error.

The two types of vitamin A concentrate used in the trial produced only minor variations in the increase in plasma-vitamin A. Liver storage from the two types of vitamin A was practically identical. However, there was an indication that when vitamin A palmitate in gelatin was fed, the resultant liver stores were maintained for a longer period of time after vitamin A supplementation stopped. While steers that had received both types of vitamin A concentrate had a depletion in liver stores of vitamin A after supplementation stopped, the rate of decline was more rapid in the steers which had received the corn-oil suspension of vitamin A.

SUMMARY

Sixteen Hereford steer calves were used to study the effects of two types of concentrated vitamin A supplements, and the effects of active dry yeast in the ration, upon the steers' blood plasma and liver vitamin A content. The two types of concentrated vitamin A used were: a corn-oil suspension of vitamin A with a potency of 1,600,000 I. U. per gram, and vitamin A palmitate in gelatin with a potency of 250,000 I. U. per gram.

The trial consisted of two periods of vitamin A supplementation, each of which was preceded by a period of depletion of vitamin A stores. The depletion rations consisted of approximately seven pounds of wheat straw per steer daily plus three pounds of a protein supplement (30 percent crude protein). Half the steers received 0.1 pound of active dry yeast per day in the supplement. Good results were achieved with the depletion rations used and no difficulty was encountered in reducing the plasma-vitamin A levels to the desired value of approximately 12 micrograms per hundred milliliters.

During the two periods of vitamin A supplementation, each type of concentrated vitamin A was fed to half the steers on each ration. During the first period, the steers were fed 50,000 I. U. of vitamin A per steer daily. During the second period, the length of the supplemental period was increased to ten days, and the steers were fed either 50,000 I. U. or 100,000 I. U. daily of the applicable vitamin A concentrate. Blood samples were taken from the jugular vein throughout the trial and were analyzed for plasma-vitamin A and carotene content. Liver samples were collected during the periods of supplementation and were analyzed for vitamin A content.

The results indicated that, while the feeding of 50,000 I. U. of vitamin A daily for five days caused a substantial rise in plasma-vitamin A content, the five-day period of supplementation was not sufficient to provide for storage of vitamin A in the liver. However, when the period of vitamin A supplementation was increased to ten days, a level of 50,000 I. U. daily was sufficient to initiate liver storage (0.2 to 2.0 micrograms per gram of liver). A level of 100,000 I. U. of vitamin A daily per steer for ten days was found to provide a substantial liver storage (approximately 6 micrograms per gram of liver). Both levels of supplementation increased plasma-vitamin A levels markedly, with greater increases from the higher level of supplementation. It was found that, after vitamin A supplementation stopped, the decrease in plasma and liver-vitamin A was most rapid during the early stages of depletion, and tended to level off as the plasma and liver-vitamin A values decreased.

Statistically, the plasma-vitamin A values during the depletion period followed a pattern of simple linear regression with highly significant ($P<.01$) correlation between the number of days the steers were on depletion rations and the plasma-vitamin A content. During the experimental periods of vitamin A supplementation, both the plasma and liver-vitamin A values followed a quadratic regression with a highly significant ($P<.01$) correlation between the date of the period and the plasma or liver-vitamin A content.

The addition of yeast to the ration had no apparent effect upon plasma-vitamin A values, but there was an indication that the feeding of yeast induced higher liver stores of vitamin A with slower subsequent depletion of

liver stores. There were no consistent differences between the effects of the two types of vitamin A supplements used on either the increase of plasma-vitamin A content, or the amount of vitamin A storage in the liver. However, the depletion of vitamin A stores from the livers was faster when the steers had received the corn-oil suspension of vitamin A.

The rise in blood plasma and liver-vitamin A content during the supplemental periods accounted for only a small portion of the total vitamin A fed; approximately five percent when a level of 50,000 I. U. was fed and approximately eight percent when a level of 100,000 I. U. was fed.

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II. THE EFFECT OF FEEDING STILBESTROL AND ANTIBIOTICS TO CATTLE ON A HIGH BARLEY FATTENING RATION

INTRODUCTION

Within the past fifty years, the feeding of livestock has rapidly become a scientific enterprise. At the turn of the century, the information on livestock feeding and animal nutrition was extremely fragmentary; specific knowledge and accepted practices were more often based on the experience of successful feeders than upon the results of actual experiments. Only carbohydrates, fats, proteins and a few minerals were recognized as essential dietary constituents. Since then, and especially during the last twenty to thirty years, rapid progress in animal feeding has been made through well controlled research projects. Some forty different nutrients are now known to be needed by the growing, fattening and/or reproducing animal. The discoveries of the vitamins, of the role of the amino acids, and of several more essential mineral elements have been among the comparatively recent important contributions of research which have enabled the scientific feeder of today to continually increase the production of his animals, and at the same time decrease the feed required per unit of production.

In addition to nutrient requirements, other discoveries have been made which have raised the level of production, decreased the cost of production, or both. The discovery that ruminants can utilize nonprotein-nitrogen compounds, such as urea, in their rations has provided a cheap source of protein. Antibiotics are now used extensively in the swine and poultry industries. Also falling into this category is the use of synthetic products,

such as diethylstilbestrol.

Diethylstilbestrol, commonly known as stilbestrol, is a synthetic chemical compound with activity similar to the female hormone "Estradiol". Stilbestrol is not a new product, for it has been manufactured commercially for about twenty years. It has been used for about fifteen years in human and veterinary medicine as a therapeutic agent in treating various disorders of the reproductive tract. It has also been used commercially in the poultry industry for several years, primarily for the fattening of cockerels. In poultry, stilbestrol is placed under the skin of the bird, usually in the neck, in the form of a pellet implant.

The pellet implant has also been tried experimentally on most species of farm animals, including cattle. In the case of fattening cattle, increases in rate of gain and feed efficiency have been achieved by using stilbestrol implants; however, these advantages have been accompanied by undesirable side effects such as restlessness of the cattle, riding, high tailheads, sagging backs, mammary development, lowered carcass quality and, in some cases, residual pellets remaining in the meat. For this reason, implants in cattle have not been recommended for on-farm use, although recent experiments have shown more favorable results when lower levels of stilbestrol implants were used.

The idea of incorporating stilbestrol in the feed rather than administering it as pellet implants was developed at the Iowa Agricultural Experiment Station about five years ago. In 1951, nutritionists at Iowa State College noticed that lambs on a certain fattening ration made faster gains than could reasonably be expected from the type of ration they were fed.

Samples of the ration were taken to the laboratory where careful analyses revealed traces of hormone activity in parts of the ration. It was also found that several other feeds showed variable traces of estrogenic activity. It was this clue which led Dr. Wise Burroughs and his associates to begin a series of experiments designed to explore the possibilities of adding hormone-like substances to fattening rations for cattle. Stilbestrol was used in the experiments because it was the cheapest and most readily available synthetic female sex hormone. As a result of these investigations, The Federal Food and Drug Administration released stilbestrol for on-farm use in rations for beef cattle weighing over 600 pounds being fattened for slaughter.

The feeding of stilbestrol to fattening cattle probably has been accepted faster than any other comparable discovery in feeding history. Within the first six months after stilbestrol became available, it was being fed to more than 50 percent of the cattle on fattening rations in this country. Montana cattle producers, in common with feeders elsewhere, have also exhibited a great deal of interest in the feeding of stilbestrol. Accordingly, the Montana Agricultural Experiment Station recently conducted the experiment reported herein. This experiment was designed to study the effects of adding stilbestrol, aureomycin, and aureomycin plus stilbestrol to a ration containing barley as the principle component, because barley is the most abundant and economical fattening feed for this area.

LITERATURE REVIEW

In poultry, the administration of stilbestrol by subcutaneous pellet implants has resulted in increased growth rates and fat deposition (Lorenz, 1943 and 1945; Andrews and Bohren, 1947). Increased rate of gain and improved feed efficiency through the use of stilbestrol implants have also been demonstrated in lambs (Andrews et al, 1949; Jordan, 1950; Perry et al, 1951), and in cattle (Dinusson et al, 1950; Andrews et al, 1949 and 1950). However, these advantages were often accompanied by such undesirable side effects as lowered carcass yield, elevated tailheads, lowered loins, restlessness, riding, etc.

After preliminary exploratory experiments produced encouraging results from the use of stilbestrol in cattle fattening rations, the Iowa Agricultural Experiment Station conducted a series of experiments designed to study the effects of feeding stilbestrol with various rations to cattle weighing over 600 pounds. Burroughs et al (1954) published several reports presenting the results of the various experiments conducted. In the first experiment, four lots, of eight steers each, were full-fed a 60-40 corn and cob meal mixture plus three pounds per steer daily of a supplement containing stilbestrol. For the first 112 days, the levels of stilbestrol fed were 0, 0.06, 0.24, and 0.60 milligrams per steer daily for the four lots, respectively. At the end of 112 days, when it became evident that the levels being fed were too low, the amounts of stilbestrol fed to lots 3 and 4, respectively, were increased to 5 and 10 milligrams per steer daily. While feeding levels of stilbestrol below 0.60 milligrams produced little or no stimulation in gains, the feeding of 5 or 10 milligrams daily per steer

resulted in increased gains with less feed required per pound of gain. There were no apparent differences in carcass quality between the steers on different treatments. In addition to these advantages, no undesirable side effects such as restlessness, riding, high tailheads or lowered loins could be noted at any time during the experiment. In the second experiment, stilbestrol was fed at the levels of 0, 2.75, 5 and 11 milligrams per steer daily with a conventional corn-hay type fattening ration. The results showed progressively faster rates of gain as the amount of stilbestrol fed was increased. The fastest gaining lot of animals (receiving 11 milligrams of stilbestrol daily) gained 37 percent faster than the control animals during the 112-day feeding period. The appetites of the steers receiving stilbestrol were increased and the feed required per pound of gain was decreased. In a 113-day feeding period, Angus-Hereford crossbred, yearling heifers were fed a 60-40 corn and cob meal mixture plus a protein supplement. The addition of 6 or 12 milligrams of stilbestrol to the daily ration of each heifer substantially stimulated liveweight gains with the greatest stimulation obtained from the higher level. While feed consumption was not materially altered, feed costs per pound of gain were reduced by 10 percent. No adverse effects were noted at any time. Steers fed 5.5 milligrams of stilbestrol daily with a 65-35 corn and cob meal mixture made faster gains than animals not receiving stilbestrol with either a 65-35 corn and cob meal mixture or a conventional corn-hay type ration. In another trial, steers were fed low-grade roughage (cornstalk silage) plus supplements the first two-thirds of a feeding period and a ground corn finishing ration plus the same supplements the last one-third of the period. The administration of

10 milligrams of stilbestrol per steer daily in the supplements again increased the gains and decreased the cost of gain. In one experiment when levels of 20 milligrams of stilbestrol were fed, gains were further increased over the 10 milligram level. However, various disease conditions encountered during the experiment made it difficult to evaluate the final results.

In summarizing the results of these six experiments, Culbertson et al (1954) concluded that 10 milligrams of stilbestrol daily per animal was the most desirable feeding level. It was found that, on the average, gains were stimulated by 19 percent, appetite by 5 percent, and feed efficiency improved by 11 percent. Carcass evaluations were fully as good when stilbestrol had been included in the ration as they were when carcasses were produced with no hormone addition. No difficulties such as riding, restlessness, high tailheads or lowered loins were noted at any time.

Since this preliminary work was completed, a number of stilbestrol feeding experiments have been conducted by several experiment stations under a wide variety of feeding and environmental conditions.

Deans et al (1955) found that liveweight gains were significantly increased when a level of 10 milligrams of stilbestrol daily was fed the last 84 days of a 140-day feeding trial. Carcass grade, dressing percent and rib-eye lean-moisture content were not affected. An increase in moisture content of the external fat approached significance. Murphree et al (1955) reported on two trials in which yearling steers were fed stilbestrol. Feeding 10 milligrams of stilbestrol daily increased gains 20 percent over gains of control animals, while steers fed 5 milligrams gained faster in one

trial but made no increase in gain over the controls in the second trial. The data also indicated that the major response from stilbestrol was during the first 100 days of the feeding period. Baker (1955) reported that stilbestrol fed at levels of 10 and 20 milligrams per head daily resulted in 10 and 16 percent greater gains, respectively, and approximately 6 to 7 percent greater feed consumption when compared to control animals not receiving stilbestrol. The rations used consisted of ground-snapped corn, citrus molasses, cottonseed meal and hay. Bell et al (1955) found that the addition of stilbestrol to a high urea ration (urea provided 32 percent of the nitrogen fed) improved rate of gain, feed efficiency and financial returns per animal.

Aunan et al (1955) reported an increase of 15 percent in daily gains with 8.4 percent less feed when steers were fed 10 milligrams of stilbestrol daily in dry lot. When compared to control steers fed the same length of time, no differences were apparent in carcass characteristics except a larger area of rib-eye muscle in the supplemental group. Mitchell et al (1955) found that when stilbestrol (10 milligram level) and nonstilbestrol fed steers were fed to equal slaughter weights (1100 pounds), the stilbestrol-fed steers made 28.6 percent more rapid gains on 20.4 percent less concentrates. When the stilbestrol-fed steers were fed until they consumed the same amount of concentrates as the control steers, the stilbestrol-fed steers gained 17.6 percent more rapidly on 18.8 percent less concentrates per unit of gain.

Beeson et al (1955) reported that feeding 10 milligrams of stilbestrol per head daily increased gains of yearling steers by 22 percent but feeding

175 milligrams of testosterone increased gains only slightly. A combination of stilbestrol and testosterone produced higher gains than testosterone alone, but not as high as gains resulting from stilbestrol alone.

Perry et al (1955) reported a highly significant increase in gains when steers were fed 10 milligrams of stilbestrol per head daily with 9 to 12 percent less feed required per unit of gain. In this experiment, increase in teat length, some elevation of the tailhead and some relaxation of the lumbar vertabrae could be recognized, though the effects were not as marked as when following pellet administration. Also, the carcass grades of the stilbestrol-fed animals were slightly inferior to the control steers.

Klosterman et al (1955) found that, when an essential nutrient, protein, is definitely limited, stilbestrol is ineffective in stimulating gains. However, when 0.75 pound of 1.5 pounds of soybean oil meal per head daily was fed, the response from stilbestrol at the lower level of protein supplementation was fully equal to that obtained with the higher level of protein supplementation.

Burroughs et al (1955) presented data which indicated that liveweight gains of steers, following the removal of stilbestrol from wintering rations, were not lessened during a subsequent pasture feeding season (when compared to gains made by cattle on pasture which had not previously received stilbestrol). In fact, in the 160 and 180-day pasture feeding periods reported, there was slight beneficial carry-over effect from the previous stilbestrol feeding. Baker et al (1955) found that, when yearling steers on pasture received supplementary feed, the addition of 10 milligrams of stilbestrol per steer daily to the supplement increased gains by 20 percent and decreas-

ed the amount of feed and number of pasture days required per hundred pounds gained. Albert et al (1955) reported that steers being full-fed ground ear corn and a protein supplement while on pasture gained 12.7 percent faster with a 9.5 percent saving in feed cost when 7.5 milligrams of stilbestrol daily was added to the ration.

Results reported by Culbertson et al (1955) showed that, with steer calves weighing 440 pounds, liveweight gains were stimulated by adding stilbestrol to fattening rations. However, the stimulus to rate of gain was slightly less than that obtained with heavier cattle. Greater stimulus was obtained with 5 milligrams of stilbestrol daily than with 10 milligrams, indicating that lower levels of stilbestrol are required for maximum effects with lighter animals. The feed saving of 8 percent was also somewhat lower than that obtained with heavier cattle. No undesirable side effects were noted. Andrews et al (1955) reported that both steer and heifer calves (450 pounds) receiving 10 milligrams of stilbestrol daily gained 20 percent faster than control animals and required 16 percent less feed per unit of gain. All animals fed hormone-like materials exhibited mammary development and heifers exhibited enlargement of the vulva. Richardson et al (1955) found that when 10 milligrams of stilbestrol daily was added to wintering rations for beef steer and heifer calves (335 to 450 pounds), differences in gain were very slight. A digestion trial with yearling steers showed a significant lowering of digestibility when stilbestrol was added to the ration. Baker and Jackson (1955) reported similar results with 535 pound calves on wintering rations. The addition of 10 milligrams of stilbestrol daily per head resulted in rates of gain only slightly faster than the

control animals. The feed efficiency was unchanged.

Burroughs et al (1955) found that, in a preliminary study with limited numbers, the feeding of 10 milligrams of stilbestrol daily per cow stimulated gains and reduced feed costs when fed to non-pregnant, beef breeding cows. However, with pregnant cows, gains were slightly reduced, feed costs slightly increased, and the birth weight of calves was slightly lower when 10 milligrams of stilbestrol daily per cow was fed during gestation.

Because it is often a standard practice for feeders to have hogs following cattle in the feedlot, speculation has arisen as to whether or not breeding gilts or sows may be adversely affected when following cattle being fed stilbestrol. To date, very little information is available to answer this question. Culbertson et al (1955) reported that twelve gilts following steers receiving 10 milligrams of stilbestrol daily apparently suffered no ill effects when compared to control gilts. The gilts were bred while in the lots with the cattle, and farrowed normal litters. There was no indication that any stilbestrol which may have been picked up from the cattle droppings had a detrimental effect on breeding performance. Andrews et al (1955) reported that open gilts following stilbestrol-fed calves exhibited teat development and enlarged vulva.

Kastelic et al (1955) reported the results of carcass evaluation studies with 92 cattle from four Iowa experiments. There were no consistent differences in shrinkage, dressing percentages or live animal or carcass grades. The stilbestrol-fed animals had a slightly higher percentage of lean meat in the rib cuts and larger area of rib-eye muscle. It was noted that individual variation was greater than variation between groups of

animals from different treatments. The stilbestrol-fed animals had a slightly lower moisture content in the rib-eye muscle.

Preston et al (1956) examined cattle tissues by mouse uterine-weight technique sensitive enough to detect 1.4 to 3 micrograms of stilbestrol per kilogram of tissue (depending on whether the tissue was muscle or fat in composition). Tissues of cattle from four experiments receiving up to 12 milligrams of stilbestrol daily were tested. Tissues assayed included lean muscle, fat, liver, kidney, heart, pooled offal including tripe, intestinal tract, lungs and spleen. No estrogenic residues were found in any of the tissues studied. Turner (1956), using the same technique, found no detectable residues in edible red meat, rib-eye, neck trimmings, tongue, liver, heart, spleen, brain, depot fat or digestive tracts from animals which received 10 milligrams of stilbestrol per day in the ration. However, evidences of estrogenic activity were found in the kidneys (4 parts per billion) and lungs (10 to 12 parts per billion). It was also found that when dairy cows were fed the 10 milligram level of stilbestrol, there was as much estrogenic activity in the dried feces as was present in the feed. Perry et al (1955) reported no residual hormone in the meat of beef animals whether hormone feeding was stopped either one or seven days before slaughter.

While there has not been, to date, any reports concerning the mechanism by which the increased growth rate is brought about through feeding stilbestrol, there have been studies conducted with cattle implanted with stilbestrol. Clegg and Cole (1954) reported nitrogen retention to be almost doubled in beef steers receiving pellet implants of 60 milligrams of stil-

bestrol in the ear. Fecal nitrogen remained unchanged, the differences being in excretion of urinary nitrogen, indicating a true increase in nitrogen storage. Blood plasma total lipids remained unchanged, indicating that increased gains are not due to increased fat deposition. The weights of pituitary and adrenal glands were significantly larger in treated animals. Thyroids were larger in steers but smaller in heifers. Ovarian weight was not altered, but corpora lutea formation was depressed. Growth hormone and adrenocorticotrophic hormone content of treated steer pituitaries was not significantly different from controls, but the pituitaries of treated heifers contained twice as much growth hormone as the untreated. Clegg and Carrol (1956) reported further studies on carcass composition and blood constituents. Carcass evaluations indicated decreased fat deposition and increased protein anabolism. Cross-sectional area of the eye muscle was significantly increased. Steers had enlarged kidneys and stimulated seminal vessicle epithelium. No difference was found in bone and moisture content. There were no differences in plasma-glucose, non-protein-nitrogen, serum protein-bound iodine (indicating no thyroid effect), or potassium or sodium ions.

Several workers have reported increased rate of gain and/or improved feed efficiency when aureomycin is included in high-roughage type wintering rations for beef cattle (Burroughs et al, 1954; Duitsman and Kessler, 1953, 1954, and 1955; Perry et al, 1954; Beeson and Perry, 1954; and Beeson et al, 1955). However, Totusek and Lynd (1954), and Totusek et al (1955), failed to obtain increased rate of gain or feed efficiency by adding aureomycin to wintering rations for steer calves.

When incorporated into high-concentrate rations, the feeding of aureomycin has given inconsistent results. Duitsman and Kessler (1955) reported less feed required per unit of gain when aureomycin was fed to beef steers on a fattening ration. Perry et al (1954) reported both increased rate of gain and decreased feed required per unit of gain when aureomycin was fed in a high-concentrate ration. However, in a second experiment, supplementation of a high-concentrate ration with aureomycin did not affect either rate of gain or feed efficiency. Beeson and Perry (1954) obtained significantly faster gains with 10 percent less feed per pound of gain when aureomycin was added to fattening rations of steers and heifers.

Baker and Jackson (1955) reported decreased gains and increased feed required per unit of gain when aureomycin was added to fattening rations for two-year-old steers. A mixture of aureomycin and stilbestrol increased gains over control animals, but did not equal gains made by animals fed stilbestrol only. Burroughs et al (1955) found that the addition of either aureomycin or stilbestrol to high-roughage rations decreased costs per pound of gains. Adding a combination of aureomycin and stilbestrol to the ration resulted in increased feed costs per pound of gain when compared to the addition of either ingredient alone.

Hentges et al (1955) reported an 11 percent increase in gain when steers were fed 10 milligrams of stilbestrol daily. Aureomycin-fed steers gained faster than the controls the first 28 days of the 109-day feeding period but thereafter exhibited lower weight gains, less appetite, and were difficult to keep on feed. Steers receiving a combination of aureomycin and stilbestrol made the fastest gains the first 35 days of the experiment,

but finished the trial only slightly ahead of the control animals. Baker (1956) reported that the addition of either terramycin or aureomycin to steer fattening rations containing stilbestrol slightly decreased gains with very little difference in feed efficiency.

PROCEDURE

Forty yearling Hereford steers, previously used for a winter feeding experiment, were used in the trial reported herein. After the wintering trial, all the animals were handled and fed together for three weeks to minimize the effects of the previous treatments before the fattening experiment started. Five days before the beginning of the experiment, the steers were randomly divided into four lots of ten head each. Animals from each of the previous winter-feeding treatments were distributed equally among the four lots. The dry-lot pens used for the experiment consisted of a sheltered area inside a barn and an outside exercise area. In August, the inside area of each pen was sprayed with Lindane and the steers were sprayed with D. D. T. to control flies.

The steers were weighed for three consecutive days at the beginning of the experiment. The three-day average was taken for the starting weight and the third day (June 15) was considered the initial day of the experiment. A basal ration consisting of two-thirds barley and one-third beet pulp was group-fed to each lot. Alfalfa hay was group-fed in outside feed racks. Supplemental pellets with and without stilbestrol and aureomycin were group-fed at the rate of one pound per head per day. The design of the experiment and the composition of the pelleted supplements is shown in Table I.

There was considerable precipitation during the first part of June, and the feeding pens became very muddy. By mid-June steers in all lots were exhibiting symptoms of coccidiosis and went off feed. Steers with the most severe symptoms were in lot 1, and two steers in that lot received

Table I: Experiment Design and Supplemental Ration Formulation

Lot No:	1	2	3	4
Ration No:	4	2	1	3
Treatment:	Ayreomycin & Stilbestrol	Aureomycin	Control	Stilbestrol
Ingredients (Lbs. per ton)				
Wheat mixed feed	600	600	600	600
Ground barley	724	692	734	714
Dehydrated alfalfa meal	300	300	300	300
Soybean meal	100	100	100	100
Urea (262)	50	50	50	50
Cane molasses	150	150	150	150
Salt	20	20	20	20
Dicalcium phosphate	40	40	40	40
"CCC" Trace mineral	4	4	4	4
Vitamin D-3 supplement 1/	2	2	2	2
Lederle Aurofac 2-A 2/	---	42	---	---
"Stilbosol" (Lilly) 3/	---	---	---	20
Lederle Aureomycin-stilbestrol supplement 4/	10	---	---	---
	2000 Lbs.	2000 Lbs.	2000 Lbs.	2000 Lbs.

1/ 3,000 units vitamin D-3 per gram.
2/ Provides 75 mg. aureomycin per pound of supplement.
3/ Provides 10 mg. stilbestrol per pound of supplement.
4/ Provides 10 mg. stilbestrol and 75 mg. aureomycin per pound of supplement.

treatment with "Sulmet" (90 grams over a three-day period for each steer). After June 15, the weather became warm and dry, and the coccidiosis symptoms gradually disappeared.

During the three-week period before the start of the trial, the feed given the steers had been gradually increased to about five and one-half pounds of grain per steer per day; however, as a result of the coccidiosis, it was necessary to decrease the feed level to two pounds of the basal ration per steer daily. This amount was gradually increased until the steers were consuming seven and one-half pounds per head daily at the end

of three weeks. Each lot of steers received an equal amount of grain daily during this starting period. Throughout the rest of the trial, each lot was fed the basal ration on the basis of appetite, so that the feed given in a twenty four hour period was just cleaned up. The animals were fed the basal ration once a day, in the morning, until a consumption of ten pounds per head daily was reached. Thereafter, the basal ration was fed twice daily, the first feeding at 8:00 A.M. and the second at 4:00 P.M.

The pelleted supplements were fed at a rate of one pound per steer daily throughout the trial and were mixed with the basal ration at the time of the morning feeding. The pellets were one-fourth inch in diameter and were fed intact during the first part of the experiment. As the daily feed consumption reached a level of ten pounds of concentrate per head, the steers in lots 1 and 2 (which received aureomycin incorporated into the pellets) began sorting their grain and leaving the pellets in the feed bunks. However, when the pellets were reduced to crumble form by passing them through a rolling mill, the sorting stopped and the steers again completely cleaned up their feed. After July 23, all lots received the supplements in crumbled form to effect uniform treatment. No other sorting of concentrates was encountered throughout the rest of the experiment. Samples of each of the pelleted supplements were collected during the trial and sent to Eli Lilly and Company for assay to check the stilbestrol content.

At the beginning of the trial, the hay was fed at a level of seven pounds per steer daily. After the steers reached a concentrate consumption of approximately ten pounds daily, it was no longer necessary to limit the hay. Therefore, during the remainder of the experiment, the hay was fed

according to appetite in the same manner as the concentrates. The hay was weighed into open feed racks each morning, immediately after the concentrates were placed in the feed bunks. Refused stems were weighed back and recorded once or twice each week.

In addition to the basic treatments, half the steers in each lot were randomly selected for treatment with approximately 40 grams of phenothiazine (administered by capsule) on July 1. At this time, fecal samples were taken from each steer for worm egg counts to determine the degree of parasite infestation.

The steers were individually weighed at 28-day intervals. Daily records were kept of feed consumed. At the close of the experiment, weights were again recorded for three successive days (December 8, 9 and 10), the average being taken as the final weight. December 10 was considered as the final day of the feeding phase of the experiment. The steers were given a normal feed Sunday morning (December 11) and at 4:00 P. M. all remaining grain was weighed back and the steers were fed only hay that night. On December 12, the steers were trucked to Great Falls, Montana, where they were slaughtered on December 13. Individual weights of the steers were recorded on December 11 when the grain was removed, and on December 12 immediately prior to being loaded onto the trucks to obtain shrinkage data. Unfortunately, it was not possible to obtain individual weights at Great Falls when the steers were unloaded from the trucks. A total group weight was obtained, however, and this allowed the average shrinkage for the transit period to be determined, even though it was not possible to determine lot differences.

Each steer's individual identity (eartag) was transferred to the carcass on the killing floor. Carcass weights were recorded immediately after the steers were dressed and hung on the rail and again after a twenty four hour shrink in the cooler. The chilled carcasses were graded by a federal grader and the grades recorded. The penes from the control and stilbestrol fed steers were also collected, and urethral diameters were measured to determine the effect of feeding stilbestrol.

RESULTS AND DISCUSSION

The design of the experiment provided that the pelleted supplements used should be identical for each lot of steers, except for the level of aureomycin and/or stilbestrol included in the pellets. The four supplements were formulated to provide: (1) 10 milligrams of stilbestrol per steer daily, (2) 75 milligrams of aureomycin per steer daily, (3) 10 milligrams of stilbestrol plus 75 milligrams of aureomycin per steer daily, or (4) control containing neither stilbestrol nor aureomycin (see Table I). When the samples of the four supplements were analyzed for stilbestrol content, it was found the two supplements which contained stilbestrol actually provided 9.7 and 9.5 milligrams of stilbestrol to the steers fed stilbestrol only and a combination of stilbestrol and aureomycin, respectively. The supplement which was formulated to contain aureomycin only was free of stilbestrol. However, the control pellet was found to contain sufficient stilbestrol to provide a level of 1.8 milligrams of stilbestrol per steer daily. The reason for this presence of stilbestrol in the control supplement is not known, but it cannot be ignored for it is quite possible that a level of 1.8 milligrams of stilbestrol per steer daily could cause some stimulation in gains. Therefore, although a control lot of steers is designated for clarity of presentation, it must be remembered that this is technically inaccurate because of the stilbestrol content of the control supplement.

The results of the treatments on gains, feed consumption and efficiency, and financial returns per steer are summarized in Table II. The increase in average gain made by lot 4 (stilbestrol fed) over lot 3 (con-

Table II. Weight Gains, Feed Consumption and Financial Returns.

Lot No: Treatment	1 Aureomycin & Stilbestrol	2 Aureomycin	3 Control	4 Stilbestrol
Length of period (Days)	178	178	178	178
Steers per lot	10	10	10	10
Average weight per steer (lbs.)				
Initial	560	550	553	554
Final	971	979	995	1037
Gain	411	429	442	483
Daily gain	2.31	2.41	2.49	2.71
Average daily feed consumption per steer (lbs.)				
Supplement	1	1	1	1
Hay	5.2	4.9	4.5	4.5
Grain mix	14.5	14.6	15.8	16.7
Total	20.7	20.5	21.3	22.2
Feed per cwt. gain (lbs.)				
Supplement	43.7	41.7	40.5	37.1
Hay	231.1	203.2	180.5	164.5
Grain mix	639.2	604.9	636.7	616.7
Total	914.0	849.8	857.7	818.3
Feed cost per cwt. gain 1/	$18.99	$17.73	$17.82	$17.28
Investment per steer				
Value June 15 1/	$126.05	$123.68	$124.42	$124.72
Feed cost of gain	$76.49	$76.04	$78.82	$83.38
Total	$202.54	$199.72	$204.24	$208.10
Return per steer 2/	$185.33	$185.05	$187.76	$195.92
Loss per steer 4/	$17.20	$14.67	$15.48	$12.18

1/ Prices used for figuring feed cost: Barley $45.00/ ton; beet pulp $49.10/ton; hay $20.00/ton. Protein supplements: Ingredients $50.00/ ton plus pelleting cost $12.00/ton plus cost of premix where applicable. Premixes added to one ton of pellets: Stilbosol $10.00; Aurofac $18.48; stilbestrol plus aureomycin $23.50.

2/ The value on June 15 was based on a visual appraisal at $22.50/cwt. This appraisal was made by a committee consisting of: E. P. Orcutt and N. A. Jacobsen, Extension Livestock Specialists, and R. H. Ellerd, Bozeman Livestock Auction Company.

3/ The steers were sold to Great Falls Meat Co. on the basis of carcass grade and weight. The price received was $32.00 per cwt. of carcass.

4/ Labor costs not included.

trol) represents an increase of 9.3 percent. The decreases in average gain made by lots 2 (aureomycin) and 1 (aureomycin plus stilbestrol) in comparison to the control animals were 2.9 percent and 7.0 percent, respectively.

Statistical analysis--analysis of variance--(Ostle, 1954) showed the differences in gains among the four lots of steers to be statistically significant ($P<.05$). However, at the 5 percent level of probability, there were no significant differences due to individual treatments when compared to the control steers. Because the validity of the control is questionable, separate analyses of variance were made of three other treatment comparisons (control versus stilbestrol, stilbestrol versus stilbestrol plus aureomycin, and aureomycin versus stilbestrol plus aureomycin). In each of these three analyses of variance, only the data from the two groups of steers in question was included in the analysis. The results revealed a significant ($P<.05$) difference in gain between the stilbestrol-fed steers and the steers fed stilbestrol plus aureomycin, suggesting a possible interaction. There were no significant differences in gain (at the 5 percent level of probability) between the aureomycin-fed steers and the steers fed stilbestrol plus aureomycin or the stilbestrol-fed steers and the controls. The statistical analyses were made with the assumption that there were no lot effects. (Analysis of variance tables are shown in the appendix).

The cumulated average daily gains are presented in graphic form in Figure 2. The greatest increase in average daily gain demonstrated by lot 4 (stilbestrol), compared to the controls, was at the end of the third and fourth periods when there was an increase of 12.9 percent and 11.4 percent,

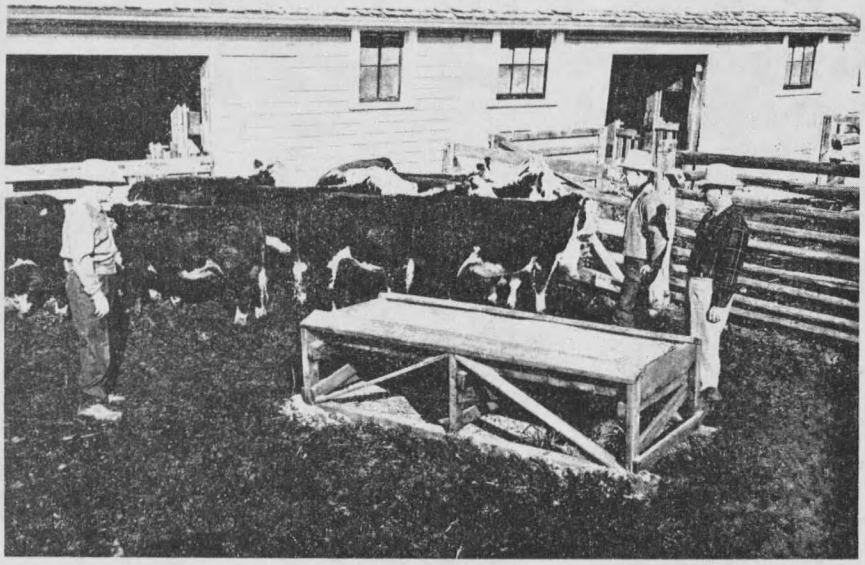

Figure 1. The stilbestrol-fed steers (lot 4) approximately six weeks before the end of the experiment.

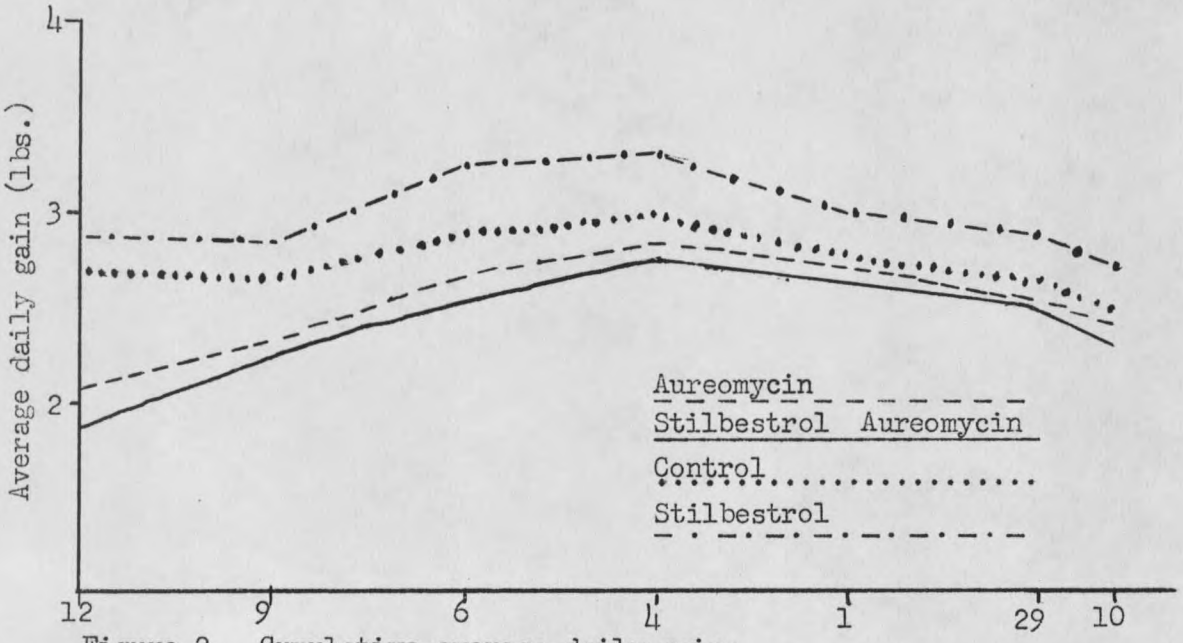

Figure 2. Cumulative average daily gains.

respectively. After the end of the fourth period on October 4, the difference decreased slowly until the final date of the experiment. However, the differences for lots 1 and 2 follow a quite different pattern, with the greatest decrease being at the beginning of the experiment (30 percent and 22 percent respectively). These differences gradually decreased until the end of the fifth period (November 1) when there was only 4 percent and 1.4 percent decrease in average daily gain for lots 1 and 2, respectively. Thereafter, the differences increased slightly until the end of the experiment.

The figures for average daily feed consumption and feed efficiency presented in Table II are the overall averages for the experimental period. The addition of 10 milligrams of stilbestrol to the daily ration per steer increased the average daily feed consumption by 0.9 pounds (4.2 percent). The addition of 75 milligrams of aureomycin or a combination of 75 milligrams of aureomycin and 10 milligrams of stilbestrol to the daily ration decreased the daily feed consumption by 0.8 pounds (3.75 percent) and 0.6 pounds (2.8 percent), respectively. It may be noted that, with the exception of lot 1, the differences in feed consumption were due mainly to concentrate intake. The feed required per hundredweight gain was decreased by 4.6 percent and one percent by the addition of stilbestrol and aureomycin respectively. The addition of both aureomycin and stilbestrol increased the feed required per hundredweight gain by 6.6 percent. These differences in feed efficiency resulted in a saving of feed costs per unit of gain of 3 percent and 0.5 percent for lots 4 (stilbestrol) and 2 (aureomycin), respectively, when compared to the control lot. Lot 1 (aureomycin

plus stilbestrol) had an increase of feed costs per unit of gain of 6.6 percent when compared to the control lot.

As previously mentioned, all four lots of steers were given the same amount of feed only while they were being started on concentrates. When the concentrate intake reached a level of seven and one-half pounds per head daily, appetite differences became apparent. After this time, each lot was full-fed according to appetite. The data on average feed consumption per period presented in Table III shows that lots 3 and 4 exhibited increased appetite very early in the experiment. The increase in feed consumption by the stilbestrol-fed steers over the controls did not become apparent until the third period. From the end of the second period (August 9) until the end of the experiment, however, the steers receiving stilbestrol in the ration consistently consumed substantially more feed per day than any of the other three lots of steers. The two lots of steers receiving aureomycin consistently had a lower feed consumption than the control steers.

The feed required per hundredweight gain by periods is presented in Table IV. The differences in feed efficiency from period to period are not consistent. However, the figures do indicate certain trends. For example, the data indicates that the greatest improvement in feed efficiency exhibited by the stilbestrol steers came during the first 84 days of the trial (this was also the period of greatest increase in rate of gain). Also, the increased hay consumption by lots 1 (aureomycin plus stilbestrol) and 2 (aureomycin) accounted for the major portion of the differences in feed efficiency for these lots.

Table III. Daily Feed Consumption Per Steer by Periods.

Lot No. Treatment	1 Aureomycin & Stilbestrol		2 Aureomycin		3 Control		4 Stilbestrol	
	Feed Consumed (Pounds) 1/							
Period:	Grain	Hay	Grain	Hay	Grain	Hay	Grain	Hay
June 15 to July 12	5.5	6.6	5.5	6.6	5.8	6.5	5.8	6.5
July 13 to Aug. 9	11.9	7.0	12.1	6.8	14.2	5.2	14.2	5.4
Aug. 9 to Sept. 6	15.8	5.5	16.7	4.4	17.6	3.0	18.6	3.9
Sept. 7 to Oct. 4	17.8	4.3	18.1	3.7	17.7	4.8	20.6	3.8
Oct. 5 to Nov. 1	18.6	4.2	17.7	4.1	20.4	3.8	21.5	3.7
Nov. 2 to Nov. 29	17.4	4.2	16.1	4.1	18.7	3.8	19.1	3.8
Nov. 30 to Dec. 10	14.3	4.3	15.1	4.2	17.6	3.7	18.1	3.7
Average for all periods	14.5	5.2	14.6	4.9	15.8	4.5	16.7	4.5

1/ Figures do not include the protein supplement which was fed at the rate of one pound per head daily.

Table IV. Feed Required Per Hundredweight Gain Per Steer by Periods 1/

Lot No. Treatment	1 Aureomycin & Stilbestrol		2 Aureomycin		3 Control		4 Stilbestrol	
	Feed Per Hundredweight Gain (Pounds) 2/							
Period:	Grain	Hay	Grain	Hay	Grain	Hay	Grain	Hay
June 15 to July 12	305.3	365.6	272.0	325.7	222.2	250.1	207.4	234.3
July 13 to Aug. 9	447.4	265.9	470.8	262.8	546.8	202.5	509.6	192.2
Aug. 10 to Sept. 6	512.4	178.0	511.4	130.2	530.3	91.3	461.2	95.5
Sept. 7 to Oct. 4	520.2	125.2	547.6	113.3	536.3	145.4	582.8	107.5
Oct. 5 to Nov. 1	840.3	188.1	785.1	181.9	1079.1	200.0	1296.8	220.7
Nov. 2 to Nov. 29	962.0	230.7	1022.6	248.9	900.7	184.5	802.6	160.5
Average for all periods	639.2	231.1	604.9	203.2	636.7	180.5	616.7	164.5

1/ The final 10-day period is not included, since the steers made practically no gains or actually lost weight and feed efficiency figures for this single period would be meaningless.

2/ Figures do not include the amount of protein supplement fed.

The general trends indicated by the pertinent data are fairly consistent with what might be expected from this type of a trial. As the feed intake increased, the gains of the steers increased. As the concentrate consumption increased, the relative hay consumption decreased, effecting wider concentrate-roughage ratios at the higher levels of feed consumption. Considering the entire experimental period, as feed consumption and gains increased, the feed required per hundred pounds of gain became lower and the feed cost per hundred pounds of gain decreased. (Lot 2 is a minor exception).

In addition to the coccidiosis outbreak at the very beginning of the experiment, two severe cases of foot-rot were encountered during the experiment. Whether these disease conditions influenced the results of the trial to any great extent is a matter for speculation. The most severe symptoms of coccidiosis were present in the steers of lots 1 and 2. These two lots also made the slowest comeback when the symptoms of coccidiosis cleared so it is possible that the final results might have been somewhat altered by this early outbreak of coccidiosis.

The first case of foot-rot occurred in October when one steer in the stilbestrol-fed lot became afflicted. The steer was treated with "Sulmet" and penicillin and recovered in about three weeks time. The steer did not go off-feed during the illness but failed to make any gain during October, which probably accounts for the poor feed efficiency of the stilbestrol fed steers during that month. However, the individual records showed that the steer made a very good comeback during the last six weeks of the experiment so it is doubtful whether the final results were materially affected.

The second case of foot-rot occurred in lot 1 (aureomycin plus stilbestrol) on December 4. This steer received the same treatment as the first animal having foot-rot, and was beginning to recover when the experiment ended. Since this steer had no chance to regain lost weight, it is possible that this case of foot-rot may have slightly affected the final results for the lot receiving aureomycin plus stilbestrol. However, the average daily gain for the remaining nine steers was 2.29 pounds per steer daily. This is only a very slight increase over the 2.26 pounds per steer daily when the affected steer was included in the calculation and it is unlikely that the feed efficiency was materially affected.

None of the steers exhibited any tendency to go off feed throughout the trial (except at the very beginning when coccidiosis caused all lots to go off feed). However, it seemed that there may have been a decreased palatability when aureomycin was incorporated into the pelleted supplements, which might account for the lower concentrate consumption by lots 1 and 2. It was necessary to feed the pellets in crumbled form to stop the steers in lots 1 and 2 from sorting the grain and refusing the pellets. Also, a marked difference in feeding behavior was noticed. The morning feeding of concentrates was placed in the feed bunks first, with the hay weighed into the hay racks immediately afterward. It was observed that the steers in lots 1 and 2 (receiving aureomycin) would consistently leave the grain as soon as hay was available, and move to the hay racks. The steers in lots 3 and 4 (controls and stilbestrol-fed, respectively) would remain at the grain bunks for a considerable length of time before moving to the hay racks.

The financial returns presented in Table II reveal a financial loss for all four lots of steers. This loss was mainly due to the unfavorable negative margin encountered in the experiment. At the time the experiment began, the grass conditions were very favorable, causing a high demand for feeder stock. The result was an appraisal of $22.50 per hundredweight as the liveweight value of the steers at the beginning of the experiment. When the steers went to market, there was very little demand for fat cattle, resulting in a low market value. The $32.00 per hundredweight received for the carcasses converted to liveweight selling prices of: $19.14, $18.95, $18.79 and $18.85 per hundredweight for lots 1 through 4, respectively. If these figures are compared to the feed cost per hundredweight gained, presented in Table II, it will be seen that the feed cost per hundredweight gain was less than the selling price in each case. Therefore, had the margin been normal, the cattle would have made a positive financial return. As it was, however, the steers suffered financial losses of: $17.20, $14.67, $15.48 and $12.18 per steer for lots 1 through 4, respectively. When compared to the control animals, these figures represent a saving, after feed costs, of 21.3 percent and 5.2 percent for the stilbestrol-fed and aureomycin-fed steers, respectively. The steers fed a combination of aureomycin and stilbestrol returned 11.1 percent less than the control animals.

The pertinent carcass data is presented in Table V. It will be noted that neither the carcass grades nor the trucking shrink are included. The carcasses of all forty steers graded choice, so that there were no lot differences. Because it was impossible to calculate trucking shrinkage

for each individual steer, only the group shrinkage was determined. The average trucking shrinkage for the group was 3.64 percent. The dressing percentages were calculated by dividing the chilled carcass weights by the liveweights at Bozeman (minus the 3.64 percent transit shrink). This calculation was the only method available since individual liveweights were not obtained at Great Falls. Lot 1 had about a one percent higher dressing percentage, but there were only minor differences in dressing percentages among the other three lots. There were no appreciable differences in cooler shrink among the lots. Also, there were no condemned livers or carcasses from any of the steers in any of the lots.

Table V. Carcass Data.

Lot No. Treatment	1 Aureomycin & Stilbestrol	2 Aureomycin	3 Control	4 Stilbestrol
Avg. hot carcass wts. (lbs.)	590.6	587.8	598.2	626.2
Avg. chilled carcass wt. (lbs.)	575.8	573.1	583.3	610.5
Avg. carcass shrink (lbs.)	14.8	14.7	14.9	15.7
Chilling shrink (%)	2.5	2.5	2.49	2.51
Avg. dressing percent 1/	61.73	60.91	60.60	60.95

1/ Dressing percentages were figured by dividing the chilled carcass weights by liveweights at Bozeman (minus the 3.64% trucking shrink).

The administration of phenothiazine had very little effect upon the weight gains of the steers. The very slight differences in rate of gain were not consistent, but varied from lot to lot in favor of the treated or non-treated steers. Dressing percentages were consistently lower for the treated animals (0.2 to 0.9 percent). In three of the four lots returns per steer were slightly in favor of the non-treated animals. In all cases, the differences were very slight. Since all lots of steers

were group fed, and half the steers in each lot were treated with phenothiazine, it was not possible to determine differential feed consumption between the treated and untreated animals. None of the steers exhibited any external signs of parasite infestation, and fecal-egg counts before treatment with phenothiazine showed only normal parasite infestation. These results indicate that a single administration of phenothiazine is not beneficial for cattle which do not have an abnormal parasite infestation, on fattening rations.

The penes from the control and stilbestrol-fed steers were obtained at the time of slaughter for urethral measurement. The urethra were distended with liquid latex, clamped off, and placed in 10 percent formalin, 1.5 percent acetic acid solution for 36 hours. The latex casts were then removed, by cutting away the urethras, and measured every two centimeters. The results showed only slight differences in average urethral diameters (5.4 millimeters for the control steers compared to 5.2 millimeters for the stilbestrol-fed steers) or smallest urethral diameters (4.1 millimeters for the control steers compared to 4.0 millimeters for the stilbestrol-fed steers). These results indicate that stilbestrol has no effect on urethral diameter once the penis has reached mature growth.

SUMMARY

Forty yearling Hereford steers were randomly divided into four lots of ten head each for a fattening experiment designed to study the effects of adding stilbestrol and/or aureomycin to a high-barley fattening ration. The treatments used were: lot 1, fed a combination of 10 milligrams stilbestrol and 75 milligrams aureomycin; lot 2, fed 75 milligrams of aureomycin; lot 3, control; and lot 4, fed 10 milligrams of stilbestrol. The stilbestrol and aureomycin were incorporated into pelleted supplements which were group fed to each lot of steers at a rate of one pound per steer daily. The control supplement was intended to be free of both stilbestrol and aureomycin; however, assay results showed a level of 0.00039 percent stilbestrol was actually present in the control supplement. Since this would provide approximately 1.8 milligrams of stilbestrol per steer daily, it is possible that the gains of the control steers were affected. In addition to the pelleted supplements, each lot of steers was group fed a basal ration of two-thirds barley and one-third beet pulp according to appetite. Alfalfa hay was also fed according to appetite.

The average daily gains for the 178-day feeding period were 2.26, 2.41, 2.49 and 2.71 pounds per steer daily for lots 1 through 4, respectively. The animals receiving stilbestrol made 9.3 percent faster gains than the controls while the animals fed aureomycin or aureomycin plus stilbestrol gained 2.9 percent and 7 percent slower than the controls, respectively. The differences in rate of gain between the four lots of steers were statistically significant ($P<.05$), but there were no significant differences (at the 5 percent level of probability) due to individual

treatments when compared to the control steers. The difference in gain between lot 1 and lot 4 (stilbestrol plus aureomycin versus stilbestrol only) were significant (P .05), suggesting an interaction between stilbestrol and aureomycin.

The stilbestrol and aureomycin groups required 39 pounds and 8 pounds less feed per hundredweight gain, respectively, than the controls while the aureomycin-stilbestrol group required 57 pounds more. The aureomycin-stilbestrol lot had a slightly higher average dressing percent than the other lots. Otherwise, there were no appreciable differences in carcass data.

There was a financial loss for all lots due to an unfavorable margin. The average loss per steer for the four lots was as follows: stilbestrol $12.18; aureomycin $14.67; aureomycin plus stilbestrol $17.20; and control $15.48.

One-half the steers of each lot were treated with 40 grams of phenothiazine at the beginning of the trial. This treatment had no appreciable effect upon the gains, carcass data or financial returns.

The feeding of stilbestrol to yearling steers had no apparent effect, either on average urethral diameters or smallest urethral diameters. No undesirable side effects due to the feeding of stilbestrol were noted at any time during the experiment.

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APPENDIX

Table I. Analysis of Variance--Fattening Trial.

Source of Variation	d. f.	Sum of Squares	Mean Square
Among treatments	3	27,726.9	9,242.3*
Cont. vs. Aureo.-Stil.	1	4,898.45	4,898.45
Cont. vs. Aureo.	1	911.25	911.25
Cont. vs. Stil.	1	8,082.2	8,082.2
Within Treatments	36	109,520.2	3,042.2278

*Statistically significant ($P<.05$)

Table II. Analysis of Variance--Control vs. Stilbestrol.

Source of Variation	d. f.	Sum of Squares	Mean Square
Control vs. stilbestrol	1	8,080.2	8,080.2
Within treatments	18	43,880.6	2,437.8111

Table III. Analysis of Variance--Stilbestrol vs. Stilbestrol Plus Aureomycin.

Source of Variation	d. f.	Sum of Squares	Mean Square
Stilbestrol vs. Stil. plus Aureo.	1	25,561.25	25,561.25*
Within treatments	18	74,986.5	4,165.9167

*Statistically significant ($P<.05$)

Table IV. Analysis of Variance--Aureomycin vs. Stilbestrol Plus Aureomycin.

Source of Variation	d. f.	Sum of Squares	Mean Square
Aureo. vs. Stil. plus Auero.	1	1,584.2	1,584.2
Within Treatments	18	65,639.6	3,646.6444