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Effects of diethylstilbestrol feeding on the bovine reproductive tract

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EFFECTS OF DIETHYLSTILBESTROL FEEDING ON THE BOVINE REPRODUCTIVE TRACT

by

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INTRODUCTION

Since its synthesis almost 20 years ago, diethylstilbestrol (hereinafter called stilbestrol) has been widely used for estrogen therapy in veterinary medicine. Stilbestrol is preferred over natural estrogens because it is cheap and effective orally as well as parenterally. Current interest in the body intake of estrogenic substances is due to several events. Over 10 years ago stilbestrol implants were found to increase the efficiency of meat production in fattening cockerels. Later, the marked effects of Australian clover on grazing sheep startled animal nutritionists. There followed a great many investigations on the effects of prolonged low levels of estrogen within the animal body, climaxed in 1954 when the Federal Food and Drug Administration authorized the inclusion of stilbestrol in cattle feeds.

Following the favorable reports in scientific literature and farm journals on the feeding of stilbestrol to fattening cattle, livestock feeders developed a growing interest in the use of this drug. About 40 per cent of the cattle on feed in the United States were receiving stilbestrol in 1955, and usage has increased to an estimated 70 per cent in 1957. Prior to the feeding of stilbestrol, implants in ruminant animals had been tried with some beneficial and some undesirable results. Oral administration has lessened the

objectionable results such as excessive mammary development, vaginal prolapse, and lowered carcass quality (Clegg and Cole 1954, Maynard and Loosli 1956, Andrews et al. 1957). While there are conflicting reports on the amount of growth stimulation, increased feed efficiency or increased appetite that can be attributed to stilbestrol, the fact that stilbestrol feeding results in larger profits to the beef producer is accepted with little dispute. This reality has been responsible for the drug's widespread use in cattle feeds. Stilbestrol feeding is still in the experimental stage in many respects. For example, little is known concerning the comparative economics of feeding the hormone to animals of either sex, to animals at different ages, or to animals to be maintained as breeding stock. The solutions to these problems lie in learning precisely how the hormone acts in the body, and in what manner it is metabolized and/or eliminated.

This investigation was undertaken with the hope of finding out more about the effects of the oral administration of stilbestrol on the reproductive organs.

REVIEW OF LITERATURE

Stilbestrol Feeding to Heifers

Burroughs et al. (1954), Breidenstein et al. (1956), Reynolds et al. (1956) and Andrews et al. (1957) observed that the feeding of stilbestrol to fattening heifers created no serious management problems. The favorable effects were similar, though less marked, to those observed in steers. This difference in response between heifers and steers is not surprising when one considers that heifers on feed gain more slowly, have a lower feed efficiency and usually sell for less than steers when finished. The natural female hormone, estradiol, circulating throughout the female body may be responsible for the lower response observed with heifers. While stilbestrol, a synthetic product, has many of the physiological effects of estradiol, the two compounds are neither identical chemically (Zondek et al. 1943) nor are they similar in all of their properties.

Stilbestrol in large doses can cause nymphomania, follicular cysts, high tailheads and even abortion. Fearing similar results from small amounts of the hormone in the feed, ruminant nutritionists have been cautious in advocating its use in the diet of female animals. This caution is also based on the problems presented in Australian ewes

where plant estrogens brought about extensive genital development and adversely affected reproduction (Bennetts, Underwood and Shier 1946). The rams were not affected. The estrogens in Australian clover were sufficiently potent to prevent atrophy of the uterus or to restore the atrophic uterus to its normal state, in spayed ewes fed on the green forage (Bennetts and Underwood 1951). Alfalfa, clover, wheat, oats, soybean oil meal, corn silage and legume silage are some of the common animal feeds in which estrogenic activity has been detected (Story 1954, Pieterse and Andrews 1956a, Pieterse and Andrews 1956b). Assays have shown that the hormonal content of farm crops varies with the season, stage of growth and environment. American pastures have not been found sufficiently high in estrogenic material to cause any harmful symptoms in farm animals. However, it is conceivable that milk production and reproductive efficiency may vary directly with the estrogens in the forage and grain.

Folley and Malpress (1944) fed synthetic estrogens to dairy heifers to induce udder development and lactation. From 50 to 200 milligram daily doses for 23 weeks were necessary before milk production began, and daily doses of less than 50 milligrams did not initiate lactation.

Fecal Elimination of Stilbestrol

The fate of stilbestrol in the body is largely unknown and appears to vary in different species. The elimination of this substance is of interest both from an academic and a practical point of view where swine depend on the cattle droppings for part of their diet. The estrogenic content of moldy grain consumed by swine has caused enlargement of the vulva and vaginal prolapse in gilts, and, initiated preputial swelling, mammary development and inflamed teats in barrows. At times these symptoms have been severe enough to cause death (Koen and Smith 1945, McErlean 1952). Apparently the estrogenic content of droppings from cattle on stilbestrol feed is not sufficiently high to cause the above condition in swine (Culbertson et al. 1955, Stob 1956) although there have been unauthentic reports of mild forms of such genital disorders (Turner 1955).

Stob (1956) found that cattle receiving stilbestrol did eliminate more estrogen in the feces than control cattle receiving no stilbestrol, but he did not assay the amount quantitatively. Ordinarily, feeder cattle do not give off estrogens in detectable amounts because natural estrogens are rapidly destroyed by the liver and eliminated with the bile. Stob (1956) postulated that stilbestrol given parenterally does not necessarily pass through the liver,

whereas all of the hormone given orally does. Consequently, less active stilbestrol is found in the feces after oral dosage than after a similar parenteral intake. An excellent literature review on the excretion of synthetic estrogens has been presented by Story et al. (1957) who also conducted work in which lambs receiving one milligram of stilbestrol daily excreted 51 per cent of it in the feces and 25 per cent in the urine. These workers postulated that the remaining 24 per cent was not stored in the body, but was degraded by rumen microorganisms and/or by metabolic processes after absorption.

Vaginal Smears and the Sexual Cycle

The female reproductive tract undergoes cyclic changes under the influence of ovarian hormones. Thus alterations in the vaginal epithelium should be a criterion of ovarian function and of sex hormones administered for therapeutic purposes. Originally Stockard and Papanicolaou (1917) analyzed the sexual cycle in the guinea pig by making smears of the vaginal contents. Allen (1922) reported that the vaginal epithelium of a mouse in estrus underwent proliferation and cornification; later the degenerating cells were removed by phagocytosis. The detection of these changes was more reliable than external signs in discovering the presence of

"heat" or estrus. Recently, spayed mice used in estrogen assays have been checked for completness of ovarectomy by taking vaginal smears (Pieterse and Andrews 1956).

The informative work on rodent smears led to research in comparative vaginal cytology. Studies indicated that facts established about the number, type and form of free cells in the vagina in one species cannot always be applied in other species. The vaginal smear provides no accurate indication of the stage of the estrous cycle in either the sow or mare. Caprine and ovine vaginal smears are less diagnostic than those of rodents although some cyclic phenomena have been detected (Hamilton and Harrison 1951) (Roberts 1956). The stages of the sexual cycle have been quite accurately depicted in the dog by means of vaginal cytology (Newberry and Gier 1952).

The marked cyclic changes seen in the vaginal epithelium of rodents and carnivora have not been found in the human female (Papanicolaou 1946). However the careful study of vaginal smears, made daily, has been a guide in estimating ovulation time, in calculating therapeutic doses of sex hormones, and in early detection of cancer (Goldhar et al. 1952, Allan 1955). According to present concepts, the vaginal mucous membrane increases in thickness under the influence of estrogen. The superficial cells on being removed farther and farther away from their blood supply undergo

degeneration and exfoliation. Thus the amount of estrogen production may be established in the human female by the degree of cornification seen in vaginal smears (Talbot et al. 1952, p. 313).

Cyclic cellular changes in the bovine vagina have been described in detail, and have been correlated with the reproductive cycle in the cow (Zupp 1926, Wolfsteller 1952). However these findings have not been generally accepted because other research workers discovered that many bovine vaginal smears deviate for no apparent reason from a predictable pattern (Cole 1930, Hansel et al. 1949, Roberts 1956). The irregular cellular changes of the cow's vagina have been attributed to low hormone levels, relatively short estrual periods, and a vaginal structure histologically different from that of other animals (Trautmann 1952, p. 300). Perhaps the difficulty of procuring a smear from a similar area in the vagina using exactly the same technique each time has also been a cause of unexplained variations in the bovine smear (Casas 1955).

Stilbestrol Feeding to Bulls

Since bulls are not of major importance as a source of meat, little experimental work has been done with the addition of stilbestrol to their rations. While bulls make

more rapid and economical gains when fattened in a dry lot, than either steers or heifers, they sell for a lower price per cwt. when marketed (Morrison 1956). The male hormones produced by bulls seem to stimulate body growth and weight gain. As the chemistry of both estrogens and androgens is similar, the manner by which rate of gains are stimulated may also be similar for the two hormones. Several experiments on the implantation of young bulls with stilbestrol have been carried out at the Ohio Agricultural Experiment Station (Klosterman et al. 1955a, Klosterman et al. 1955b). Stilbestrol administration boosted weight gains but did not noticeably affect feedlot behavior. The implantations retarded sexual development and raised carcass grades. The treated bulls did not develop as heavy a crest, as masculine a head, and as large testes. Elevated tailheads and teat development were present to a slight degree.

The advisability of using stilbestrol feeds for fitting bulls to show or to sell as sires has not been investigated.

Vasectomy in Bulls

One of the most difficult problems with artificial insemination is the detection of heat in the cows. The use of vasectomized bulls has been advocated for this purpose.

In parts of Africa, where artificial insemination is practised to enable bulls susceptible to East Coast Fever to sire calves without contacting the disease, and where estrus symptoms in Zebu cattle are not very noticeable, the use of vasectomized bulls and marking ointment has been a boon to livestock breeding (Rollinson 1956). Bovine vasectomy rather than castration is being advocated in parts of Italy because calves retain the excellent growing qualities of bulls, but cannot reproduce (Borrelli 1956). Detailed descriptions of how to perform the operation have been recorded (Hammond 1927, Tharp 1955, Borrelli 1956). At one time, vasectomy was thought to halt spermatogenesis due to pressure atrophy in the testicle. Later research workers have found live sperm in the vas deferens below the site of vasectomy or vasoligation indicating that sperm produced in the testicle of such sterilized males undergo resorption within the epididymis and the vas deferens (Rice and Andrews 1951, p. 120, Knaus 1937). Research work has been done on the male guinea pig showing that spermatozoa are continuously produced, and, if ejaculation does not occur, they disintegrate and are absorbed (Simeone and Young 1931). Literature references are rather confusing as to whether or not this operation results in more libido and testosterone production due to increased interstitial (Leydig) cell proliferation; Moore (1939) and Hammond (1927, p. 4) report that hyperplasia of the Leydig

cells does not occur after obstruction of the vas deferens, whereas other scientists report the opposite (Wolstenholme 1953, p. 27). One fact appears to have been well established; vasectomy is a simple permanent device for excluding the sperm from union with the egg without interfering with copulation or any other masculine trait.

EXPERIMENTAL

Studies with Heifers

Two experiments were conducted to study the effects of the oral administration of stilbestrol to heifers before and after sexual maturity. One experiment was carried out with yearling heifers about 15 months old; the other with heifer calves about seven months old. All the animals were healthy and normal as determined by examination and available history.

The yearling heifers were group fed. Studies with them involved the efficient production of wholesome beef, the fecal elimination of estrogens, and the functioning of the reproductive organs.

The heifer calves were individually fed. Studies with them involved economical gains in body weight and growth, and the development of the reproductive organs.

Experiment with yearling heifers

Methods and materials. Eighteen Hereford heifers of similar type and size were procured from a South Dakota range. They were not pregnant and had no genital abnormalities detectable by rectal palpation. The animals were

randomly divided into three lots of six animals each. They were kept under uniform conditions, the only treatment between lots being different amounts of stilbestrol as shown in Table 1. At the end of a 148-day feeding period (December 19, 1956) the three heaviest heifers in each lot were slaughtered and the three lightest ones from each lot were turned with a fertile bull to determine their ability to conceive. The nine heifers turned with the bull were full-fed a fattening ration without stilbestrol for 91 days (until March 20, 1957) when they were slaughtered. Pertinent data concerning the rate of gain, feed efficiency and carcass evaluations are recorded in Tables 1, 2, 3, and 4.

The ration fed to the heifers was a nutritionally adequate fattening diet containing approximately 10.9 per cent protein. Stilbestrol was added to the supplement in the amounts shown in Table 1 in the form of "Stilbosol". This product, made by Eli Lilly and Co., contains one gram of diethylstilbestrol per pound of carrier made up of soybean meal and vegetable oil. The ground corn cobs and cane molasses, mixed in a four to one ratio, were hand-full-fed twice daily along with the ground shelled corn. The 60 per cent protein supplement, hand-fed twice daily, contained 80 parts soybean meal, 9 parts urea, and 11 parts mineral and vitamin compounds. Block salt was self-fed.

Table 1. Data from the experiment with yearling heifers from July 24, 1956 to December 19, 1956 (148 days)

	Lot I	Lot II	Lot III
Number of heifers per lot	6	6	6
Mg. stilbestrol fed/heifer/day	0	10	20
Average initial weight in lb.	635.2	637.3	624.2
Average final weight in lb.	930.8	963.7	967.6
Average gain/heifer/day in lb.	2.0	2.2	2.3
Average feed consumed/heifer/day:			
Corn cobs[a] & molasses in lb.	10.9	10.9	10.5
Ground shelled corn in lb.	11.7	11.6	11.4
Supplement in lb.	2.5	2.5	2.4
Lb. feed consumed/1000 lb. live weight/day	32.1	31.3	32.0
Lb. feed consumed/100 lb. gain	1259	1137	1048
Feed cost per 100 lb. gain	$30.9	$27.5	$25.7
Necessary selling price per cwt. to pay for heifers and feed	$22.1	$21.4	$20.9

[a]All feed was calculated on an airdry basis except the ground shelled corn which was figured on a 14 per cent moisture basis.

Table 2. Data from July 24, 1956 to March 20, 1957 (239 days) on the nine yearling heifers slaughtered in March, 1957

	Lot I	Lot II	Lot III
Numbers of heifers per lot	3	3	3
Stilbestrol fed/heifer/day up to 148 days	0 mg.	10 mg.	20 mg.
Stilbestrol fed/heifer/day after 148 days	0 mg.	0 mg.	0 mg.
Avg. initial weight[a]	590.8	590.8	580.2
Avg. 148-day weight	867.2	886.9	880.5
Avg. final weight	1042.8	1035.1	1005.1
Avg. gain/heifer/day from 0 to 148 days	1.93	2.00	2.03
Avg. gain/heifer/day from 148 to 239 days	1.83	1.63	1.37
Avg. gain/heifer/day from 0 to 239 days	1.89	1.86	1.78
Gain per heifer from 0 to 148 days	285.4	296.1	300.3
Gain per heifer from 0 to 239 days	452.0	444.3	424.9

[a]All weights and gains are given in pounds.

Table 3. Data from July 24, 1956 to December 19, 1956 (148 days) on the nine yearling heifers slaughtered in December, 1956

	Lot I	Lot II	Lot III
Number of heifers per lot	3	3	3
Stilbestrol fed/heifer/day	0 mg.	10 mg.	20 mg.
Avg. initial weight[a]	679.6	683.5	668.2
Avg. final weight	985.3	1040.5	1054.7
Avg. gain/heifer/day	2.07	2.41	2.61
Gain per heifer	305.7	357.0	386.5

[a]All weights and gains are given in pounds.

Table 4. Yearling heifer carcass evaluations[a]

	Heifers slaughtered December, 1956			Heifers slaughtered March, 1957		
	Lot I	Lot II	Lot III	Lot I	Lot II	Lot III
Dressing per cent	60.73	59.49	59.04	63.25	60.97	63.31
Sq. in. loin eye area	11.18	11.34	11.81	10.65	10.47	10.90
Fat on loin eye area[b]	25.55	20.88	24.55	29.22	23.11	26.83
Marbling[c]				6.3	5.7	6.7
Carcass grade[d]	Choice+	Choice	Choice	Choice+	Choice	Choice

[a]All values are an average of three animals.

[b]Thickness of fat over the loin eye muscle is given in centimeters calculated by the method of Clifton and Shepherd (1953).

[c]Marbling was scored from one to nine with the higher digits denoting more abundant marbling.

[d]Choice+ in this table indicates that one carcass out of the three averaged, had a higher grade than the carcasses whose average is given as choice. For example, if two carcasses were graded choice and one choice+, they were averaged as choice+ to show their superiority over the carcasses that averaged choice.

The technique used to measure fecal elimination of estrogen from the yearling heifers was similar to the method of Preston et al. (1956) for determining the estrogenic residues in the tissues of beef cattle. Sixty days after the beginning of the experiment with yearling heifers some feces were taken manually from the rectum of each heifer, placed in individual shallow pans, and dried at 130 F. for 30 hours. Eight grams of dried feces from each heifer were then finely ground in a Wiley mill and pooled with feces from the other heifers within that lot. The resulting composite sample from each lot was refrigerated until used. To assay for estrogenic activity measured quantities of the ground feces were incorporated into a basal mouse diet made of 72 parts ground yellow corn, 20 parts dried skim milk, 6 parts corn oil, and 2 parts mineral mixture. This mouse diet was then fed to immature female white mice weighing about 10 grams each. Groups of 10 mice, in two replicates of five mice each, were used for every dietary treatment. Feed consumption averaged two grams of feed per mouse per day during the six-day feeding period after which the mice were killed. Their uteri were removed, fixed in Bouin's fluid, trimmed, and weighed after the excess fluid was removed with filter paper. The criterion of this method of assay was the increase in uterine weights if the mice had

consumed any feces containing stilbestrol, or any other estrogenic compound in the diet.

The fecal assay procedure in this investigation can be divided into two distinct parts. The first part gave an estimate of how much, if any, estrogenic activity was present in the feces. Such activity was determined by comparing the uterine weights of mice fed a diet containing feces from cattle receiving no stilbestrol, with the uterine weights of mice fed feces from cattle receiving stilbestrol. The amount of estrogenic residue present in the feces was then calculated by comparing the mean uterine weights from mice showing uterine weight increases with the mean uterine weight response to known amounts of stilbestrol fed to the respective groups of mice. The known amounts of stilbestrol added per gram of basal mouse diet were 0.000, 0.005, 0.010, and 0.020 micrograms respectively. The values thus obtained gave a standard uterine weight response curve. The results of one such assay trial are summarized in Table 5.

In the second part of the assay procedure a method of analysis was employed which could be more precisely analyzed statistically than the method of the first part. Table 6 shows the design of, and the data obtained by, this method with feces collected from the yearling heifers 85 days after these animals had been on the experimental diets. The mean uterine weights resulting from the 12 dietary mouse treatments

Table 5. Uterine weight responses obtained by a trial addition of stilbestrol or feces to the basal mouse diet in the first part of the fecal assay

Addition to the basal mouse diet	Mean uterine weight[a]
0.000 Mcg. stilbestrol/gm. of mouse diet	6.1 mg.
0.005 Mcg. stilbestrol/gm. of mouse diet	14.6 mg.
0.010 Mcg. stilbestrol/gm. of mouse diet	27.9 mg.
0.020 Mcg. stilbestrol/gm. of mouse diet	60.8 mg.
1.25% Feces from Lot I heifers in mouse diet	7.2 mg.
2.50% Feces from Lot I heifers in mouse diet	5.9 mg.
1.25% Feces from Lot II heifers in mouse diet	36.1 mg.
2.50% Feces from Lot II heifers in mouse diet	64.1 mg.
1.25% Feces from Lot III heifers in mouse diet	62.6 mg.
2.50% Feces from Lot III heifers in mouse diet	72.9 mg.

[a]To make an estimate of the fecal estrogenic content the amounts of feces added to the basal diet were varied until all of the mean uterine weights came within the range of values of the standard curve established by adding stilbestrol to the basal diet. The assay was repeated several times before this goal was attained. The uterine weights in this table do not come within the standard curve range. They indicate that a lower level of feces should be used in subsequent trials.

Table 6. Unprocessed data from the second part of the fecal assay for estrogenic activity

Source of .4% feces in mouse diet	Mcg. stilbestrol per gm. mouse diet											
	0.000			0.005			0.010			0.020		
	P[a]	N[b]	M[c]	P	N	M	P	N	M	P	N	M
Lot I heifers	21	4		41	5		75	5		161	5	
	22	5	4.8	46	5	9.7	86	5	16.1	156	4	35.2
Lot II heifers	34	5		71	5		137	5		224	5	
	32	5	6.6	38	3	13.6	112	5	24.9	260	5	48.4
Lot III heifers	48	5		82	5		181	5		201	4	
	45	5	9.3	123	5	20.5	166	5	34.7	348	5	61.0

[a]P = total weight of all the mouse uteri per pen or replicate in milligrams. There were two replicates per dietary treatment.

[b]N = number of live mice per pen at the end of the six-day mouse feeding period.

[c]M = mean uterine weight per treatment expressed in milligrams.

come within the range of the standard curve response obtained by the first part of the assay. The statistical procedure employed for processing this data was that of Homeyer and Pauls (1955); the estrogenic content of the feces from the cattle in Lots II and III was estimated by plotting the estrogenic response curve to a curve established by the response with feces from control cattle.

The estrous cycles of the heifers were followed by vaginal smears, rectal palpation of the genitalia, and by the use of vasectomized bulls. The study of bovine vaginal smears was undertaken to describe the normal bovine vaginal cycle by using such smears, and to use the basal pattern thus established as a standard with which to compare abnormal cases. Vaginal smears were taken daily from mature dairy cows with normal estrous cycles, and weekly from the experimental heifers. A technique similar to that described by Zupp (1926) was employed during which the cow's vulva was cleansed with cotton, the labia held open, and a clean plastic inseminating pipette introduced about five inches, gently rolled and withdrawn. The end of the pipette was then touched to a drop of saline on a glass slide. The slide was dried over a gentle heat and immediately immersed in a fixing solution. The following staining methods were tried: hematoxylin and eosin, Schorr's single stain, Schorr's trichromatic stain (de Allende and Orias 1950,

p. 18-24), Papanicolaou's stain (Papanicolaou 1954, p. 3-6), and a modification of Papanicolaou's stain. On the basis of these preliminary studies, the modification of Papanicolaou's stain was routinely used. This stain was relatively easy to use and resulted in slides with marked differences in staining intensity and color, an asset in the study of cellular definition. The procedure for this staining method is given in Table 7. All stains used were National Aniline and Chemical Company certified. Each slide was studied under both the low and high power of a binocular microscope.

Results and discussion. According to the data in Table 1 for the initial 148 days of this experiment, the heifers in Lot III made faster and cheaper gains than the ones in Lot II. Likewise the heifers in Lot II showed superior performance to the control heifers in Lot I. Ten milligrams of stilbestrol per day increased gains by 10 per cent and feed efficiency by 10 per cent, whereas 20 milligrams per day increased gains by 15 per cent and feed efficiency by 16 per cent. In both the Lot II and Lot III heifers the savings in feed costs approximately paralleled the gains in feed efficiency. The increased rates of gain in this experiment are similar to those obtained in other such experiments with heifers (from 0.1 to 0.4 pounds per day). However the number of experiments conducted with stilbestrol

Table 7. Modification of Papanicolaou's method for staining smears

Sequence	Solutions used	Time
1	95% alcohol (fixative)	at least 30 min.
2	Mayer's hematoxylin	1 1/2 min.
3	tap water	6 min.
4	70% and 95% alcohols	20 sec. each
5	OG 6[a]	1 1/2 min.
6	95% alcohol	10 sec.
7	EA 36[b]	1 1/2 min.
8	95%, 95% and 100% alcohols	3 sec. each
9	xylene (clarifier)	2 min.
10	Harleco synthetic resin (mountant)	

[a]OG 6: Phosphotungstic acid 0.015 gm.
Orange G--0.5% soln. in 95% alcohol 100.0 cc.

[b]EA 36: Phosphotungstic acid 0.2 gm.
Bismark Brown--0.5% soln. in 95% alcohol 10.0 cc.
Lithium carbonate, saturated aqueous soln. 1 drop
Light Green SF yellowish--0.1% soln. in 95% alcohol 45.0 cc.
Eosin yellowish (water & alcohol soluble)--0.5% soln. in 95% alcohol 45.0 cc.

in heifers is inadequate to draw any valid conclusions as to when and how to feed the hormone for maximum stimulation. The variable benefits from stilbestrol in steers receiving different treatments such as high and low dietary levels of protein and energy, are ample evidence of the necessity of a large number of experiments. In this investigation with yearling heifers the feeding of 20 milligrams of stilbestrol per heifer per day, rather than the conventional 10 milligrams recommended for feed lot cattle, gave the best results. The higher dosage effected a gain per day 0.1 pounds greater than the lower dosage and 0.3 pounds greater than the untreated heifers.

In contrast to the heifer gains during the first 148 days, the gains for the last 91 days (recorded in Table 2) are greatest for the control heifers, and least for the Lot III heifers. Also the gains for the entire 239 days are highest for the control cattle, intermediate for the Lot II cattle, and least for the Lot III cattle. According to these results the feeding of stilbestrol to yearling heifers should be continued until they are marketed or slaughtered. If stilbestrol is added to the ration for part of the feeding period and then discontinued, the advantages in gain and feed efficiency may be lost making the use of the hormone of questionable value. Apparently the favorable

stimulation from stilbestrol is followed by a depression of gains when stilbestrol feeding is discontinued.

The carcasses of all the heifers were fairly uniform in quality and grade. Some carcass evaluations are given in Table 4. The heifers receiving no stilbestrol were equal to or slightly higher than the stilbestrol fed heifers in dressing percent and grade, but, were equal to or slightly lower in muscle volume, as measured by the loin eye area, than the stilbestrol fed heifers. In measuring loin eye areas, carcasses were divided between the 12th and 13th ribs, tracings were made on acetate paper of the exposed cross-sections of the longissimus dorsi muscles (loin eyes), and, the areas on the tracings were then measured with a planimeter. No appreciable differences in carcasses due to stilbestrol treatment were detected.

The first part of the fecal assay for estrogenic activity was positive for estrogens in the feces from the heifers receiving stilbestrol with the amount of estrogen eliminated varying directly as the quantity of stilbestrol consumed. Also these initial determinations indicated that the inclusion of less than 0.5 per cent feces in the basal mouse diet during the second part of the assay should yield accurate estimates. The second part of the fecal assay was carried out with 0.2 per cent, and 0.4 per cent feces in the mouse diet. As the 0.4 per cent fecal diet gave a more marked

uterine weight response, the data from this trial given in Table 6, were used in making the estrogenic estimates. The quantity of estrogen in addition to the known amounts added during the assay was 0.004233 micrograms per gram of mouse diet for the feces from the Lot II heifers. Since the mouse diet was 0.4 per cent feces the calculated estrogenic substance per gram of heifer feces was 1.056 micrograms. The daily feed consumption per heifer when the fecal samples were taken from the heifers was 24 pounds. As the feed was calculated as being 61.92 per cent digestible (Morrison 1956), 9.14 pounds of feces were excreted from each heifer daily. Assuming that the excreted fecal estrogenic activity was due to stilbestrol, the Lot II heifers excreted 43.8 per cent of the stilbestrol consumed. Likewise the heifers in Lot III eliminated an estimated 42.5 per cent of their stilbestrol intake in the droppings. If the entire fecal estrogenic substance found was stilbestrol, and if about 25 per cent of this drug is eliminated in the urine (Story et al. 1957) then the fate of almost one-third of stilbestrol administered orally is unexplained. Apparently about 33 per cent of the hormone given in the feed is inactivated by rumen fermentation and body metabolism.

While the contents of the vaginal smears studied in this experiment followed certain trends, some apparently normal cycles yielded atypical smear patterns disgressing

from the regular predictable type. During proestrus and estrus more large epithelial cells with folding edges were present than at any other time. True cornification or keratinization was not observed; all of the epithelial cells contained nuclei. Leucocytes and small round deep-staining epithelial cells were most prevalent during postestrus and early diestrus. Whenever an abundance of leucocytes occurred a few erythrocytes were seen also. Clumping of epithelial cells was most evident around the time of estrus coinciding with the period of maximum cell proliferation. All the epithelial cells were eosinophilic except in late diestrus, in proestrus and estrus when the majority of the large epithelial cells were basophilic. The inclination toward basophilia seemed to increase with the age of the cell. In atypical smears epithelial and blood cells showed variations in number and character having little relation to the stage of the cycle. Irregular smears were seen on one or two, or on many slides of a cycle and the cause of such smears could not be determined.

The vaginal leucocytosis seen during postestrus is somewhat like the defense mechanism of an inflammation. This seems to be nature's way of resisting any infection or foreign material introduced into the vagina along with the semen at copulation. Since erythrocytes pass through vaginal wall by diapedesis during leucocytosis, erythrocytes

appear in vaginal smears whenever there is an abundance of leucocytes and not only after ovulation as formerly believed.

Unpredictable variations occuring in bovine vaginal smears can possibly be attributed to:

1. The stages of the sexual cycle (proestrus, estrus, postestrus and diestrus) may differ in their respective lengths without greatly altering the total cycle length. Therefore, smear patterns in cycles of the same length will not be similar unless the different phases of the cycles are also of the same duration. Such a hypothesis is difficult to evaluate because estrus is the only stage whose length can precisely be measured.
2. Exfoliated cells separated from their site of origin and freed from the pressure of surrounding cells may assume forms differing from similar cells as they appear in tissue sections. Dehydration or changes in cell turgidity may also be a factor.
3. Common pathological conditions like cervicitis, endometritis, and vaginitis may be present altering the normal cell picture, yet, may be difficult to detect clinically.
4. Most of the cells in a vaginal smear come from the vagina, yet cells may also be present from the

vestibule, cervix, uterus, and uterine tubes. Cells from the various parts of the genitalia are difficult to defferentiate after they have separated from their site of origin.

Because of the frequency with which atypical vaginal smears occur, a single smear or an occasional smear is of little value in accurately estimating the stage of the bovine sexual cycle. As diagnosis depends on a succession of multiple changes, a series or several series of smears may be indicated. One can postulate, but not state with certainty, why some apparently normal sexual cycles yield atypical vaginal smears. The bovine reproductive organs cannot be considered normal or abnormal on the basis of vaginal smears alone.

Examination and rectal palpation of the genital organs of the heifers revealed no abnormalities such as cystic ovaries or hypertrophied genitalia. Excessive riding, vaginal prolapse, and swollen mammae were not noticeable. The heifers were checked for estrus every eight hours by being turned with vasectomized bulls. Checking the heifers every 12 hours was tried with unsatisfactory results; apparently many heat periods in heifers are of shorter duration than 12 hours. Even by checking the heifers every eight hours some of the sexual periods could not be detected because of very short time intervals during which service by the male

was accepted, or because of temporarily inactive ovaries. Anestrus caused by excessive fat deposition around the ovaries (Maynard and Loosli 1956, p. 387) or a subclinical genital infection has been recorded. The vaginal smears taken during anestrus had an abundance of leucocytes and cellular detritis indicative of primary or secondary infection. The incidence of mating was 40 per cent higher in the morning and late evening than in the afternoon. This behavior points to the early forenoon and late evening as the most probable time for the detection of estrus in Iowa cattle. There was no difference between lots in the frequency of estrus or in its duration.

The three heifers from each lot turned with a bull in December, 1956 were slaughtered as soon as a tentative diagnosis of pregnancy could be made by rectal palpation. Carcass examinations at the end of the experiment (March, 1957) confirmed pregnancy in all nine heifers ranging in duration from 23 to 60 days. Each uterus contained a grossly normal embryo.

Stilbestrol feeding for five months to the heifers in this experiment did not interfere with either the sexual cycles or the fertility.

Summary. Feedlot performance, fecal estrogenic activity and function of the reproductive organs were studied in yearling heifers receiving stilbestrol.

Ten and 20 milligrams of stilbestrol in the feed improved rate of gain and feed efficiency without appreciably altering carcass quality. Twenty milligrams gave somewhat more favorable results than did 10 milligrams.

The fecal elimination of estrogens varied directly as the amount of stilbestrol fed in the heifer diets. Over 40 per cent of the stilbestrol fed, was recovered in the feces, if the estrogenic activity in the feces was stilbestrol. Possibly, the remaining 60 per cent was either eliminated in the urine or inactivated during the processes of intermediary metabolism within the body.

Vaginal smears, highly diagnostic of the stage of the sexual cycle in rodents and carnivorous animals, do not always follow a regular pattern in cattle. Due to unknown reasons some apparently normal cycles yield atypical smears making a diagnosis on the basis of vaginal smears alone, impossible.

The stilbestrol, fed to the heifers about 148 days, did not interfere with the regular manifestation of normal estrus, or the ability to conceive. No adverse effects from the use of the drug were noted. Vaginal smears were studied as a means of more closely following the stages of the bovine sexual cycle.

Experiment with heifer calves

Methods and materials. Twenty-four native Iowa heifer calves were randomly allotted into three groups of eight animals each. All of these heifers were beeftype, nonpregnant and without any detectable genital abnormality. The three groups of animals were run together in a drylot except during the feeding period of about an hour and a half twice a day when they were tied to individual feed mangers. Each manger was constructed with a hopper above it enabling a three day's supply of feed to be placed into the hopper at one time. Since the mangers were covered except at the feeding periods, exact records of individual feed and stilbestrol intake were made possible. No actual records were kept of daily feed consumption, but the quantity eaten by a heifer was calculated from the feed put into her hopper minus that remaining at the end of a feeding period, and that which was discarded at intervals because it was unpalatable. The only treatment between groups was the different amounts of stilbestrol fed as shown in Table 8.

Beginning February 17, 1957, 66 days after the calves were put on feed, the frequency and length of estrus was determined by turning a vasectomized bull with the heifers at 12-hour intervals.

Table 8. Composite data from the experiment with heifer calves from December 13, 1956 to May 25, 1957 (163 days)

	Group I	Group II	Group III
Number of heifers per group	8	8	8
Mg. stilbestrol fed/heifer/day	0	5	10
Average initial weight in lb.	412	434	439
Average final weight in lb.	679	696	733
Average gain/heifer/day in lb.	1.64	1.61	1.79
Ratio of feed ingredients:			
Shelled corn	700	700	700
Whole oats	600	600	600
Chopped alfalfa hay	600	600	600
Linseed oil meal	100	99.33	98.67
Stilbosol	0.00	0.67	1.33
Mineral mixture	free choice	free choice	free choice
Lb. feed[a] consumed/heifer/day	13.50	12.99	14.23
Lb. feed consumed/1000 lb. live weight/day	24.76	23.50	24.29
Lb. feed consumed/100 lb. gain	825	808	971

[a]All feed was calculated on an airdry basis.

The heifers were full-fed a fattening ration containing 12.8 per cent protein. Prior to being fed, the ingredients were mixed in the proportions shown in Table 8. The Stilbosol, previously described, and linseed oil meal were pelleted. The shelled corn was cracked (very coarsely ground). A mineral mixture of steamed bone meal 132 parts, calcium carbonate 90 parts, salt 60 parts and trace minerals 18 parts, was fed free choice.

The individual weights and gains for the heifer calves are given in Table 9, and the composite experimental results by group treatment are given in Table 8. The first eight animals recorded in Table 9 received no stilbestrol, the second eight received five milligrams, and the last eight received 10 milligrams of stilbestrol. Carcass studies on the experimental heifers were not possible because some of the heifers were to be used as dams in a progeny testing project.

Results and discussion. The beef producer is highly interested in keeping stock that will gain both rapidly and efficiently. Individual feeding makes possible an accurate record of feed consumption which can be than correlated with rate of gain. Individual feeding also assures the timid animals in a group an opportunity to consume as much feed as the more aggressive animals. In Table 9, large

Table 9. Individual data[a] from the experiment with heifer calves from December 13, 1956 to May 25, 1957 (163 days)

Animal number	Initial weight	Final weight	Average gain/day	Average feed/day	Feed/1000 lb. live weight/day	Feed/100 lb. gain
35	462	694	1.42	13.67	23.65	961
82	380	576	1.20	12.10	25.31	1006
265	342	596	1.56	12.20	26.01	783
409	434	745	1.91	15.84	26.87	830
411	396	677	1.72	13.11	24.43	761
412	366	664	1.83	13.43	26.08	734
415	484	746	1.61	13.93	22.65	866
522	430	730	1.84	13.58	23.41	738
36	424	684	1.60	12.82	23.14	803
81	412	632	1.35	12.35	23.66	915
222	412	616	1.25	11.10	21.60	887
263	404	698	1.80	13.91	25.25	771
400	532	890	2.20	15.48	21.77	705
405	436	700	1.62	13.87	24.42	856
407	430	678	1.52	10.63	19.19	698
410	418	666	1.52	13.72	25.31	902
34	400	648	1.52	13.40	25.57	881
80	562	840	1.71	16.66	23.77	977
224	366	542	1.08	10.02	22.07	935
264	372	666	1.80	12.86	24.79	713
401	534	828	1.80	16.87	24.77	936
403	438	784	2.12	15.66	25.63	738
404	460	812	2.16	14.29	22.47	662
414	382	740	2.20	14.01	24.97	638

[a]All values are expressed in pounds.

variations are shown between animals receiving the same treatment. For example animal 414 had the highest daily gain in the group (2.20 pounds) as well as the highest feed efficiency, requiring 638 pounds of feed for 100 pounds of gain. In contrast, animal 224 gained only 1.08 pounds per day while requiring 935 pounds of feed to put on 100 pounds of gain. Animal 414 gained 358 pounds during the 163 day experimental period, whereas animal 224 gained 176 pounds in the same length of time. These two animals are examples of the differences in feedlot performance that exist in cattle. Individual feeding is the only method of studying such differences. The sampling nature of gene inheritance plus the influence of environment make several such progeny tests advisable before passing judgement on the merits of the progeny of any mating.

The expected daily gains for growing heifer calves like the calves in this experiment, are 1.4 pounds, and for such calves on a fattening ration the expected gains are 2.7 pounds (National Research Council 1950). Recommended feed intake is 16 pounds daily. Thus both gains and feed consumption for experimental heifers full-fed a fattening diet, were low as shown by the data presented in Table 8. In fact, the gains were little better than could be expected for growing beef heifers on a non-fattening diet. Five milligrams of stilbestrol per day did not stimulate daily

gains but did somewhat improve feed efficiency. Ten milligrams of stilbestrol boosted both daily gains and appetite slightly. Stilbestrol feeding may have little effect on heifer calves at this age. The low gains made by all of the experimental heifers and the unusual response to stilbestrol may also have been due to the manner in which this experiment was conducted. The alfalfa hay fed to the heifers was mostly of mediocre quality and finely chopped. This resulted in lower feed intake and some bloating in the heifers. Whether or not individual feeding lowers feed consumption in cattle by removing competition at the trough is debatable. Kidwell et al. (1954) have given a literature review on this subject and did work in which they found that animals on nutritious diets eat more feed with less waste when group fed. The length of time the individually-fed animals have access to feed and the frequency with which rejected feed is removed may greatly influence appetite and rate of gain. Gerlaugh et al. (1945), after comparing the performance of individually-fed steers with group-fed steers, suggest that individual feeding be conducted on a three or four times-a-day feeding schedule rather than twice-a-day schedule. The proper management of individually-fed cattle should yield results that can have practical application. Originally, the amount of stilbestrol added to the ration fed to the heifers, was calculated to supply five and 10 milligrams

per day to the heifers in Group II and III respectively, if 15 pounds of feed were consumed daily. Since the heifers consumed less than the reckoned 15 pounds, the stilbestrol intake was also lower than five or 10 milligrams per heifer per day.

Sixteen of the heifers in this experiment were receiving stilbestrol before puberty, the age at which the sexual organs begin to function. Puberty usually occurs between the ages of 10 to 12 months in beef breeds. Since the heifers were seven months old at the beginning of the experimental period, this research provided an opportunity to observe the effects of stilbestrol on the sexual development of heifers. On February 17, 1957 after some of the heifers began to show signs of estrus, a vasectomized bull was used twice daily to study the heat periods. While fewer heat periods would have missed detection if the bull had been turned with the heifers every eight hours, that was not possible with the personnel and facilities available for this research. Data on sexual development is recorded in Table 10. Eight heat periods were detected in the control Group, 21 in Group II, and 22 in Group III, indicating that stilbestrol hastened the onset of puberty. Also the stilbestrol-treated heifers seemed to have longer and more pronounced heat periods, although the differences in length and intensity of estrus was not great. The enlargement of

Table 10. Data on the estrus periods and external genitalia in the heifer calves

	Group I	Group II	Group III
Length of heat periods[a]:			
Less than one day	7	18	18
More than one day	1	3	4
External genitalia[b]	7	7	4
Teats and udder[c]	8	10	7
Tailhead[d]	variable	variable	variable

[a]Heifers were checked for estrus twice daily. Those in heat on any part of two consecutive days were designated as being in heat more than one day.

[b]Vulvar size and vestibular mucous membrane visible were scored as zero, one or two, with two indicating the most genital hypertrophy. This work was carried out by two dairy geneticists from the Iowa State College staff.

[c]Teats and udder were scored in a method similar to that used for the external genitalia.

[d]Height of the tailhead (lumbo-sacral angle) varied at intervals within the same animal making an evaluation of this observation difficult.

the vulva, perineum and mammary glands was not very prominent in any one group, but there were marked differences in individual animals within the groups. Such differences do not lend themselves to accurate measurement in live animals. From the limited number of heifers studied in this work, stilbestrol tends to hasten the onset of puberty, making the heat periods more intense. The small differences between the treated and control heifers were not objectionable and might easily go unnoticed. During the five months that the heifers were receiving stilbestrol, vaginal prolapses, excessive sexual activity or unusual feedlot behavior did not occur.

Summary. Sexual development was studied in three groups of heifer calves individually-fed zero, five and 10 milligrams of stilbestrol per day in a growing-fattening basal diet containing 12.8 per cent protein.

The daily gains in all three groups of heifers were lower than expected probably due to the way the animals were fed and managed. Low feed intake, individual feeding and the frequent incidence of bloat are possible reasons for the low rate of gain. The heifers in this experiment received little benefit from stilbestrol in the diet.

The onset of puberty in the heifers was observed with the aid of a vasectomized bull, and by clinical observations.

There were some indications that stilbestrol hastened and accentuated the development of the reproductive organs in the heifer calves. Objectionable results that have been attributed to stilbestrol (vaginal prolapse, excessive mammary growth, persistent riding, low backs and high tailheads) were not noticed even after the heifers had received stilbestrol 163 days.

Studies with Bulls

Experiment with bull calves

Methods and materials. Twenty-one native Iowa bull calves were randomly allotted into three groups of seven animals each. All of the animals used were normal beeftype calves. These animals were fed and managed in the same way as were the heifer calves previously described. Treatments, weights and gains for the bulls are recorded in Tables 11 and 12. The treatments between groups for the bull calves like those for the heifers, were zero, five and 10 milligrams of stilbestrol for Groups I, II and III respectively. The first seven animals recorded in Table 12 received no stilbestrol, the next seven received five milligrams and the last seven received 10 milligrams.

Table 11. Composite data from the experiment with bull calves from December 6, 1956 to May 23, 1957 (168 days)

	Group I	Group II	Group III
Number of bulls per group	7	7	7
Mg. stilbestrol fed/bull/day	0	5	10
Average initial weight in lb.	505	513	513
Average final weight in lb.	873	903	865
Average gain/bull/day in lb.	2.19	2.31	2.09
Ratio of feed ingredients:			
Shelled corn	700	700	700
Whole oats	600	600	600
Chopped alfalfa hay	600	600	600
Linseed oil meal	100	99.33	98.67
Stilbosol	0.00	0.67	1.33
Mineral mixture	free choice	free choice	free choice
Lb. feed[a] consumed/bull/day	16.42	17.56	17.21
Lb. feed consumed/1000 lb. live weight/day	23.95	24.83	24.91
Lb. feed consumed/100 lb. gain	750	755	823

[a]All feed was calculated on an airdry basis.

Table 12. Individual data[a] from the experiment with bull calves from December 6, 1956 to May 23, 1957 (168 days)

Animal number	Initial weight	Final weight	Average gain/day	Average feed/day	Feed/1000 lb. live/weight/day	Feed/100 lb. gain
39	480	790	1.85	14.07	22.16	763
498	514	880	2.18	17.93	25.72	823
499	514	942	2.55	17.55	24.11	688
586	514	938	2.52	18.92	26.06	752
587	460	816	2.12	14.86	23.29	701
588	488	832	2.05	15.04	22.79	734
594	564	912	2.07	17.77	24.08	858
42	630	1076	2.65	20.37	23.96	767
486	410	824	2.46	14.96	24.25	607
494	564	928	2.17	18.48	24.77	853
495	512	870	2.13	17.01	24.62	798
584	440	800	2.14	16.04	25.87	748
585	470	862	2.33	16.58	24.89	711
593	562	964	2.38	19.49	25.54	819
40	540	944	2.40	21.10	28.44	877
237	460	836	2.24	17.23	26.59	770
238	570	950	2.26	19.59	25.78	866
492	440	784	2.05	11.97	19.56	586
591	518	882	2.17	16.05	22.93	741
597	490	846	2.12	17.13	25.64	808
599	574	810	1.40	17.44	25.20	1241

[a]All values are expressed in pounds.

The sexual development of the bulls was determined by taking semen samples on the 132nd and 162nd day of the experiment and by measuring testicular size on the 168th day. Evaluations of the semen samples are tabulated in Table 13 and testicular measurements are given in Table 14. As some of the bulls were to be progeny tested as sires, carcasses from the animals were not available for study.

Results and discussion. Young bulls should gain from 2.75 to 3.00 pounds and consume about 19 pounds of feed daily when full-fed a fattening diet (Klosterman et al. 1955a, Klosterman et al. 1955b, National Research Council 1950). As recorded in Table 11, the feed consumption and the daily gains ranging from 1.40 to 2.65 pounds, were small. These small gains, like those in the preceding experiment, can be attributed to individual feeding, low feed intake, the frequent occurrence of bloat, and the possibility that stilbestrol has no beneficial effect on animals of this age. The animals affected with bloat rarely needed any therapy to relieve the symptoms, but the affliction lowered feed intake. The bull calves receiving five milligrams of stilbestrol per day showed a slight stimulation in weight gains and the calves receiving 10 milligrams per day gained less than the control animals. Thus stilbestrol feeding had no effect on weight gains and feed efficiency in these individually-fed bull calves.

Table 13. Evaluation of semen samples[a] collected from the bull calves at five weeks and at one week before the end of the experiment, May 23, 1957

Animal		Semen					
no.	wt.	vol.	motil.	concentr.	color & density	pH	morphology & miscellaneous material
39	745	2.5	60%, 3	122,000	watery & opaque	7.0	some abnormal sperm, blood, epith. cells
		1.5	90%, 1	6,000	creamy & opaque	7.5	normal sperm, no foreign matter
498	842	3.5	80%, 2	244,000	creamy & opaque	7.0	protoplasmic droplets, epith. cells
		6.0	90%, 1	290,000	creamy & opaque	7.0	normal morphology, no foreign matter
499	888	2.5	85%, 1	160,000	creamy & opaque	7.5	epith. cells & cellular detritis
		4.0	85%, 2	250,000	creamy & opaque	7.5	normal sperm, epith. cells
586	886	5.5	60%, 2	287,000	creamy & opaque	7.5	protoplasmic droplets, blood cells
		4.5	90%, 1	300,000	creamy & opaque	7.0	normal sperm, some cellular detritis
587	778	2.0	50%, 3	130,000	creamy & opaque	7.5	droplets, epith. cells & detritis
		1.5	95%, 1	2,000,000	creamy & opaque	7.5	normal sperm, no foreign matter
588	780	3.5	none	0	watery & milky	7.0	no sperm, some epith. cells
		1.5	80%, 2	50,000	creamy & opaque	7.5	normal sperm, some epith. cells
594	888	3.5	80%, 2	1,000	watery & milky	7.5	normal sperm, some epith. cells
		6.5	95%, 1	170,000	creamy & opaque	7.0	normal sperm, no foreign matter
Avg.	830	3.4	75%	286,000			
42	1003	4.0	65%, 2	800,000	creamy & opaque	7.5	normal sperm, few epith. cells
		3.0	80%, 2	1,000	creamy & opaque	7.5	mostly seminal fluid, many epith. cells
486	782	2.5	60%, 2	100,000	creamy & opaque	7.5	protoplasmic droplets, foreign matter
		1.0	75%, 2	400,000	creamy & opaque	7.5	normal morphology, no foreign matter
494	891	4.0	50%, 1	516,000	bloody & milky	7.0	droplets, blood & epith. cells
		3.5	90%, 3	400,000	creamy & opaque	7.0	normal sperm, few epith. cells
495	826	3.5	20%, 1	110,000	creamy & opaque	7.5	droplets, epith. cells, detritis
		1.5	85%, 3	3,000	creamy & opaque	7.5	normal sperm, few epith. cells
584	770	4.0	65%, 2	470,000	creamy & opaque	7.0	protoplasmic droplets, epith. cells

494	891	4.0	50%, 1	516,000	bloody & milky	7.0	droplets, blood & epith. cells
		3.5	90%, 3	400,000	creamy & opaque	7.0	normal sperm, few epith. cells
495	826	3.5	20%, 1	110,000	creamy & opaque	7.5	droplets, epith. cells, detritis
		1.5	85%, 3	3,000	creamy & opaque	7.5	normal sperm, few epith. cells
584	770	4.0	65%, 2	470,000	creamy & opaque	7.0	protoplasmic droplets, epith. cells
		3.5	90%, 3	350,000	creamy & opaque	7.5	normal sperm, no foreign matter
585	816	3.0	65%, 2	100,000	creamy & opaque	7.0	droplets, epith. cells & detritis
		5.0	65%, 2	6,800	creamy & opaque	7.5	fairly normal sperm, cellular detritis
593	938	3.0	90%, 3	150,000	creamy & opaque	7.5	protoplasmic droplets, few epith. cells
		4.5	85%, 3	750,000	creamy & opaque	7.5	normal sperm, few epith. cells
Avg.	861	3.2	69%	296,000			
40	902	3.5	75%, 2	270,000	yellow & watery	6.5	curled tails, dirt, urine, epith. cells
		4.0	75%, 2	250,000	creamy & opaque	7.5	normal sperm, cellular detritis
237	793	3.2	60%, 1	80,000	creamy & opaque	7.5	droplets, epith. cells & dirt
		2.5	90%, 3	6,000	creamy & opaque	7.5	normal sperm, few epith. cells
238	800	5.0	70%, 2	330,000	creamy & opaque	7.5	protoplasmic droplets, few epith. cells
		1.5	90%, 3	500,000	creamy & opaque	7.5	normal sperm, some epith. cells
492	762	2.5	75%, 3	90,000	milky & dirty	7.0	droplets, epith. cells, foreign matter
		2.0	none	0	clear & watery	7.0	transparent accessory fluid
591	850	4.8	none	4,000	milky & watery	7.5	protoplasmic droplets, epith. cells
		3.0	90%, 3	300,000	creamy & opaque	7.0	normal sperm, no foreign matter
597	808	3.5	80%, 1	10,000	milky & opaque	7.5	normal sperm, few epith. cells
		2.5	90%, 3	1,300,000	creamy & opaque	7.5	normal sperm, some epith. cells
599	802	2.5	85%, 3	700,000	creamy & opaque	7.0	some protoplasmic droplets, epith. cells
		2.0	90%, 3	400,000	creamy & opaque	7.5	normal sperm, no foreign matter
Avg.	817	2.9	65%	298,000			

[a]Animal weight is the approximate weight in pounds when the semen samples were collected. Motility was classified as one, two or three with one indicating sluggish circling, two denoting convulsive motion, and three denoting progressive movement. Concentration is expressed as the sperm count per cubic millimeter. Since color, opacity and consistency are closely related, they were grouped under color and density.

Table 14. Testicular measurements[a] on bull calves taken May 23, 1957

0 Mg. stilbestrol[b]					5 Mg. stilbestrol					10 Mg. stilbestrol				
Animal number	Body wt.[c]	Testis length	Testis width	Testis volume	Animal number	Body wt.	Testis length	Testis width	Testis volume	Animal number	Body wt.	Testis length	Testis width	Testis volume
39	790	13.0	6.7	306.2	42	1076	14.0	7.9	458.6	40	944	14.0	5.8	247.3
		14.0	6.5	310.4			13.5	7.3	377.6			14.0	6.0	264.6
498	880	13.0	6.8	315.4	486	824	11.5	5.5	182.6	237	836	11.0	6.8	267.0
		12.5	6.4	268.8			11.0	5.7	187.7			11.5	6.7	271.0
499	942	10.7	6.0	202.2	494	928	12.0	6.7	282.7	238	950	12.0	6.1	308.7
		9.2	5.7	156.9			12.2	6.4	262.3			12.5	6.9	312.4
586	938	13.5	6.2	272.3	495	870	12.5	6.7	294.5	492	784	12.0	7.0	282.7
		13.0	6.2	262.4			13.5	6.9	337.4			11.5	6.5	255.0
587	816	13.0	6.8	315.6	584	800	11.5	7.2	312.9	591	882	13.0	6.7	306.3
		13.5	6.5	299.4			11.5	6.4	247.3			12.5	6.9	312.4
588	832	12.5	6.2	272.5	585	862	13.0	6.5	288.3	597	846	12.0	6.5	266.1
		13.0	6.5	290.8			13.0	6.6	297.3			11.5	6.7	271.0
594	912	13.0	7.0	334.4	593	964	14.0	7.0	360.2	599	810	13.5	5.9	246.7
		11.5	6.3	239.6			13.5	7.5	398.6			12.0	6.6	274.4
Avg.	873	12.5	6.4	274.8		903	12.6	6.7	306.3		865	12.4	6.5	277.5

[a]Measurements given in centimeters, were made on the testicles in situ with the scrotal skin drawn over them. Dimensions for the left testicle are recorded first followed by those for the right one. Volume was calculated with the formula for the volume of a prolate spheroid, volume = $4/3$ pi ab^2 where a and b are the major and minor semiaxes.

[b]Animals are arranged in groups according to the treatments.

[c]Body weights are given in pounds.

The onset of sexual activity in the reproductive organs in bull calves is usually accompanied by aggressiveness, indications of the desire to mate when in the presence of other animals, and the development of a masculine body conformation. This onset of puberty was difficult to detect in the experimental bull calves because tying them up twice daily made them docile and relatively quiet for bulls confined to a feedlot. Probably more sex drive would have been shown if they had been given access to cows or heifers in heat, a factor not included in the design of this experiment. Differences in body conformation were not detectable in the bull calves on the different treatments of stilbestrol. Had the experimental animals been slaughtered at the conclusion of this investigation masculinity differences, if present, would have been reflected in the statistics on dressing percentage and carcass grade.

A record of the findings in the semen samples is recorded in Table 13. These samples were collected by electroejaculation because the young, untrained bulls would not ejaculate into an artificial vagina. During electroejaculation a bull was confined in stocks with a multipolar electric probe conveying the electric stimulus into the rectum. The semen was collected in an artificial vagina prepared in the conventional manner. Single finger electrodes were used on any bulls that objected to the electric

probe when an electric stimulus was given. Some of the bulls displayed dislike for the probe by extreme restlessness and bellowing, or by lying down and refusing to get up. The single finger electrodes were fastened to two non-adjacent fingers of a gloved hand after which the hand was inserted into the bull's rectum to a depth of about eight inches.

As shown by the data in Table 13, the semen obtained by electroejaculation from these young bulls was less concentrated and contained more protoplasmic droplets and epithelial cells than normal bovine semen. These rather unsatisfactory semen samples may be attributed to (1) the immaturity of the bull calves that had not previously been used for service, and (2) the induction of ejaculation by electric stimuli rather than by the psychic stimulation of a cow in heat. In using the electric ejaculator small frequent stimuli were applied until the penis was protruded about six inches. The electric current applied was then gradually increased in intensity until some urine and seminal fluid were expelled. Thereupon the artificial vagina was put over the protruded penis for the collection of the semen. In working with the untrained young bulls the exact time when semen would be ejaculated was difficult to estimate. An error in this estimation resulted in an ejaculate with no spermatozoa in it, or in an ejaculate with much

seminal fluid along with the spermatozoa. Apparently the expulsion of semen was both preceded and followed by seminal fluid. Excellent samples such as the second sample from animal 587 and the first one from 597, were obtained when collection (1) began after discarding the initial seminal fluid, and (2) ceased before the semen was diluted with postejaculatory fluid. The wide range in volume and density of the semen samples can be attributed to unavoidable errors in technique. Semen collections with an electroejaculator from conditioned, mature bulls have been routinely found inferior to collections made with a cow or dummy and an artificial vagina (Rollinson 1956). The main uses of the electroejaculator seem to be for protrusion of the penis in examination and, for determination of the presence or absence of spermatozoa rather than their concentration, etc. Hydrogen ion concentration of minor importance in semen evaluation, was measured with pH indicator paper accurate for each half pH unit. The tendency toward alkalinity, pH of seven or above rather than below seven, denoted semen with much accessory gland fluid, a trait of collections by electroejaculation (Roberts 1956, p. 490, Rollinson 1956). All of the bulls in this experiment ejaculated semen containing spermatozoa, and showed no behavior indicative of infertility.

Since semen quality and testicular normality are closely related, the testes of the bull calves were examined at the termination of this experiment. The examination included size, form, consistency and sensitivity. None of the bulls showed any marked abnormality in the form, consistency and sensitivity of the testes. All of the testes were of the conventional ovoid form and of a turgid, elastic consistency. Sensitivity appeared to vary with the tractability of the animal and the method of approach to the scrotal area; animals that showed signs of pain when the scrotum was first palpated, did not object to the procedure when it was continued for several minutes. Since normal male animals show much variation in size and asymmetry of testes, the variations recorded in Table 14 can be considered normal. A statistical analysis of the estimated testicular volumes signified that the stilbestrol treatment had no large effect on testicular size (treatment effect was not significant), either before or after adjustment was made for body weight. Adjusting for the variations in body weight increased the F value from 1.45 to 1.95, making the effects of stilbestrol feeding on testicle size somewhat more obvious. A summary of the analysis of covariance calculated according to the method of Ostle (1954, p. 391) is recorded in Table 15. If there is a real difference in testicular size due to stilbestrol feeding such a difference must be small.

Table 15. Analysis of covariance of testicular volume with body weight[a]

Source of variation	Degrees of freedom[b]	Sums of squares and products			Deviations about regression		
		Σx^2	Σxy	Σy^2	$\Sigma y^2 - \frac{(\Sigma xy)^2}{\Sigma x^2}$	Degrees of freedom	Mean square
Among treatments	2	11,728	2,284.7	482.48			
Among calves treated alike	18	201,621	13,259.4	5,609.57	4,737.58	17	278.68
Between measurements within calves	21			867.23			
Total	41	213.349	15,544.1	6,959.28	5,826.77	19	
Difference for testing among adjusted treatment means					1,089.19	2	544.59

[a]x = body weight, and y represents testicular volume. Calculated $F = \frac{544.6}{278.7} = 1.95$. Tabular value of $F_{.05(2,17)} = 3.59$ (Ostle 1954, p. 456).

[b]Degrees of freedom listed refer to testicular volume only. Since there was only one measurement of body weight for each calf, the total degrees of freedom for x is 20 as obtained from the first two lines of the analysis of variance table.

Summary. The effects of stilbestrol on sexual development was studied in bull calves beginning when the calves were seven months old and ending when they were 12 months old. Although the calves were fed a growing-fattening diet, weight gains were small resulting in a rather narrow range of weights in the calves at the termination of the experiment. Five and 10 milligrams of stilbestrol had no large effect on weight gains or feed efficiency.

As far as could be ascertained from semen samples and the measurement of testicular volume, stilbestrol affected neither the size of the testicle nor the ability of the testicle to produce spermatozoa. No objectionable side effects from the use of this hormone were noted in the bulls.

Experiment with vasectomized bulls

Methods and materials. Three burebred bulls born and raised at the Iowa State College Dairy Farm were vasectomized and used in the detection of estrus in heifers. The Holstein bull, 1084, was vasectomized at 28 months of age after being in service for one year. The Holstein bull, 782, was vasectomized at nine months of age. The Brown Swiss bull, 876, was vasectomized at eight months of age. Bulls 782 and 876 had not served cows prior to the operation.

Vasectomy was carried out under local anesthesia with the bull recumbent on a large animal operating table similar to the method described by Borrelli (1956). After ligating the vas deferens 2½ inches of the duct were removed and identified by histological sectioning. The skin of the surgical incision was sewn up with plain catgut which disintegrated in one week to 10 days. The only post-operative treatment required was the application of screw worm spray to the surgical sites. The bulls were allowed to serve heifers in heat 28 days after surgery. This procedure was managed by turning one bull at a time with heifers twice daily for four minutes.

Results and discussion. Within 10 days after beginning the post-operative period of service, the bulls had lost much of their libido. Although their sexual interest did not entirely vanish the time required for complete copulation took several minutes; often a heifer in estrus was mounted without completing service. During this time of waning sexual drive the bulls mated an average of twice daily.

About 45 days after the vasectomy operation the bulls were put on pasture for 28 days with a herd of pregnant cows. This period of sexual rest renewed sexual vigor. Thereafter the bulls quickly and accurately identified

heifers in heat, serving them repeatedly without becoming exhausted. About eight months after surgery, the vasectomized bulls were marketed making the surgical areas available for pathological study. These regions had a minimum of scar tissue without any evidence of abscess formation. About 50 per cent of the spermatozoa found in the vas deferens, were alive and about 80 per cent revealed protoplasmic droplets. A diverticulum one-fourth of an inch in diameter was located in the ligated vas deferens ventral to the site of ligation. This diverticulum, not described in any of the literature reviewed, may have been the result of the surgical procedure followed; the incisions in this experiment were closed with one row of skin sutures rather than with rows of superficial and deep sutures. The diverticulum may also have been the result of pressure caused by the continuous production of testicular secretion. The spermatic motility was greatest in the contents taken from this diverticulum. Since histological sections of the testicles were negative for any pathology the eventual increase in libido is difficult to explain with certainty.

Summary. In the three young bulls vasectomized in this study, vasectomy was a simple operation to perform. The only visible postoperative complication was a mild swelling of the scrotal neck which disappeared without

treatment. As the bulls did not settle heifers, when used 28 days after surgery, live sperm could not have been stored in the genital tract for this length of time.

The experimental bulls required over two months after surgery to regain their libido. Thereafter the animals displayed a greater-than-average sex drive and the usual amount of aggressiveness found in the bovine male species. The effects of the operation appeared to be the same when performed either before or after puberty.

The surgical sites and testicles studied after slaughter of the bulls, showed no unusual pathological changes and offered no basis for an explanation of the increased sexual drive eventually seen in the vasectomized bulls. The most prominent pathological lesion observed was a diverticulum of the vas deferens below the site of ligation. This diverticulum containing spermatozoa may have been the result of pressure produced in the vas deferens by continuous sperm production in the testicles.

GENERAL DISCUSSION

The overall effects of stilbestrol are worthy of consideration because of this hormone's widespread use in animal nutrition. The mass of experimental information available on gains and feed efficiency indicate that stilbestrol usage in cattle feeds will continue. If the addition of this hormone remains a feeding practice, knowledge on how to use the hormone for maximum benefits will come only after its precise metabolic pathways and effects on reproduction have been investigated. The results presented in this study reveal that feedlot benefits and reproductive consequences from stilbestrol feeds vary more widely than has been usually realized. None of the theories advanced on "how stilbestrol acts" can be used to explain all of the results obtained. Some of the common theories on mode of action are that the growth hormone output from the anterior pituitary is increased, that the anterior pituitary effect on the adrenal cortex (adrenocorticotrophic hormone output) is stimulated, or that calcium, phosphorus and nitrogen retention in the kidney is increased (Bell et al. 1955, Taylor 1955). It may be that the output of all the anterior pituitary hormones, including gonadotrophins, are increased. Both male and female sex hormones are closely related to meat production since both have stimulated livestock gains

(Burris et al. 1954, Acker et al. 1955). Thus the stilbestrol (estrogen) response may actually be an androgen response. As the results with hormone administration, especially with androgen, have been inconsistent, responses appear to be influenced by the genetic strain of the feeder animals, the ration and the environment. When certain changes occur in the digestive tract the body hormone level may increase, just as ensiling can increase the estrogenic activity of forage (Pieterse and Andrews 1956a).

The bovine feces assayed for estrogenic activity in this investigation revealed that over 40 per cent of the stilbestrol added to the feed was excreted in the feces. These results differ somewhat from those of other workers (Story et al. 1957). A wide range of values for excreted stilbestrol have been reported; in fact, no two workers have given the same values in their results. Whether the assay methods conducted by various workers have been faulty or whether the animals actually have given off different percentages of their stilbestrol intake is not known. Estrogens may come from natural sources in the body, from plants eaten by animals in the feed, and from administered estrogenic substances. Since assays of body excretions reveal only the estrogenic activity and not the source of such estrogenic activity, the various sources possible of estrogens excreted may confound the interpretation of assay

results. The abortions, excessive riding, etc. in gilts and sows following stilbestrol-fed cattle could have been due to chance (sampling error) rather than to stilbestrol treatment since such conditions have been reported by commercial feeders and have not been duplicated in scientifically-controlled experiments.

The results of this study suggest that the reproductive organs of young heifers receiving stilbestrol before the age of puberty show greater stimulation than those of yearling heifers receiving stilbestrol; heat periods occurred earlier in the younger animals whereas no sexual effects were noted in the older animals although the older ones received twice as much stilbestrol. There may be circumstances under which sexual stimulation in farm animals would be desirable. Seasonal anestrus in ewes, anestrus in cows during the winter and early spring, and, some other types of bovine infertility (Reynolds et al. 1956) are examples of such circumstances. Also estrogenic stimulation (suppression of masculinity) may be desirable in young bull calves fed for slaughter. In these animals stilbestrol administration has depressed masculine body characteristics such as a large neck and head, but has not altered the fast growing qualities peculiar to young bulls (Klosterman et al. 1955a). The resulting bull carcasses graded higher because of a greater percentage of high-priced cuts of meat. The

advisability and benefits of estrogen administration to growing and fattening stock appears to depend on the purpose for which animals are being kept.

Although stilbestrol did not hinder the development and function of the reproductive organs in the bull calves employed in this investigation, the use of the hormone for bulls being fitted to show or sell cannot be recommended at present. That this problem warrants further study is evidenced by the work in which stilbestrol depressed masculine body characteristics and raised carcass grade in bulls. The work of Stob (1956) is also relevant. By limiting the feed of young boars following stilbestrol-treated steers, he encouraged fecal consumption. At the end of four months of such treatment gross and histological examination of the testes revealed a lack of normal development.

The low gains of the individually-fed animals show that for most purposes in animal nutritional research, feeding small groups of animals will yield results that can be more readily duplicated in commercial feedlots than can results from individually-fed experimental cattle. Where production testing is desired, individual feeding is a necessity, and maintenance or "low rate of gain" feed requirements may be as important as the requirements when on full-feed. Fattening cattle tend to consume less feed when individually-fed than when group fed.

Vasectomized bulls were found satisfactory for allowing mating without impregnation in the experimental heifers. The use of such bulls may not become prevalent, even where artificial insemination is being widely practiced, because of disease transmission, and because such bulls retain the unreliable disposition of intact herd sires. Young vasectomized dairy bulls can be useful for experimental purposes and for the detection of cows in heat in certain large herds where artificial insemination is done. The presence of a "mean" bull can be avoided by procuring a young vasectomized bull for replacement every four to five months, or whenever indicated. Vasoligation, a reversible surgical operation performed in man, was not studied because such an operation has no use in cattle other than for experimental purposes.

SUMMARY

Stilbestrol was fed to yearling heifers, heifer calves, and bull calves, primarily to study its effects on the reproductive tract.

Three groups of yearling heifers were fed zero, 10 and 20 milligrams of stilbestrol per animal per day respectively in a fattening ration. Heat periods were recorded by turning vasectomized bulls with the heifers every eight hours. After 148 days one-half of the heifers from each group were slaughtered, and the remainder were turned with a virile bull to determine fertility. When 90 days later these heifers were slaughtered, they all carried normal embryos. Tissue sections were taken from the pituitary glands, ovaries, and uteri of the heifers, for later histological study. The stilbestrol had no observable effects on carcass quality, estrous cycles or conception in the experimental heifers. Ten milligrams of stilbestrol increased both gains and feed efficiency by 10 per cent; 20 milligrams increased gains by 15 per cent and feed efficiency by 16 per cent. The savings in feed costs paralleled the gains in feed efficiency.

Additional work on the yearling heifers included vaginal cytology and fecal assays for estrogens. Over 40 per cent of the estrogen equivalent of the stilbestrol added to the diet, was eliminated in the feces. The quantity of fecal estrogen

varied directly with the dietary estrogen intake. Since estrus is the only stage of the sexual cycle whose length can be accurately measured, an attempt was made to correlate vaginal cytology with the stages of the estrous cycle. Vaginal smears were an aid rather than an independent means of following ovarian changes and of determining genital abnormality. The smears were unreliable because some of them showed unpredictable variations for no apparent reason.

Three groups of heifer calves were fed zero, five and 10 milligrams of stilbestrol respectively per animal per day in a growing-fattening ration. Low feed intakes, due in part to individual twice-a-day feeding, resulted in small gains in the 163-day feeding period. Heat periods were plotted with the aid of a vasectomized bull. The stilbestrol treatment appeared to hasten and accentuate the onset of puberty; no other side effects were noted.

Three groups of bull calves were fed and managed similar to the heifer calves. The results with individual feeding indicate that unless carried out under excellent management, this method of feeding will result in low feed consumption and poor gains. No effects attributable to stilbestrol treatment were noted in feedlot behavior, testicular size, or in fertility as measured by semen quality.

The use of vasectomized bulls to detect estrous periods in the experimental heifers afforded an opportunity to study

various aspects of bovine vasectomy. It was a simple, effective way of preventing conception, while permitting copulation. The surgery was followed by a period of waning libido after which sexual drive was very strong. The eventual increased libido may have been due to pressure irritation from continuous testicular secretion.

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