

SOME EFFECTS OF DIETHYLSTILBESTROL AND RELATED HORMONES  
ON FEEDLOT PERFORMANCE, RATION DIGESTIBILITY AND  
CARCASS QUALITY OF LAMBS AND BEEF CALVES

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## INTRODUCTION

Feed costs account for approximately 75 percent of the cost of gain in lambs and cattle. Therefore, any saving that can be made in feed cost may act directly in lowering the total cost of fattening. Farmers and research workers are constantly looking for ways of decreasing the amount of feed, and therefore the cost, required per pound of gain.

Also, in most seasons and with most classes of market livestock, there is an economic desire to speed up gains in order to get the animals in desirable condition before the big "runs" of marketable livestock result in lower prices. The spring lamb producer for example, would like to market his lambs for slaughter by the first week in June, before the majority of the spring lambs arrive. The feeder who markets choice grade cattle after a winter feed would profit from faster gains allowing him to market before the seasonal price slump in March and April.

Another reason to speed gains is that it contributes to improved feed efficiency. In a fattening steer or lamb, about half the feed eaten each day is used for maintenance and about half for production of new tissue, or gain. If a steer or lamb gains faster, he will not have to be maintained as many days before he is ready for slaughter. Fewer days on feed also cuts down the risk of death loss and disease; it means less labor will be used and capital will not be invested for as long a time.

At the time the investigations reported in this thesis were begun, diethylstilbestrol (commonly called and hereafter referred to as stilbestrol) had been observed to greatly improve gains and feed efficiency

in fattening cattle, when fed at very low levels in the ration. There was much previous evidence that stilbestrol improved gains and feed efficiency of lambs and cattle when it was implanted in pelleted form under the skin of the animal. This procedure, however, was frequently accompanied by marked adverse effects on body metabolism and functions, as well as a significant lowering of carcass quality.

In the fall of 1953 there was no published information available on the value of stilbestrol in the ration of cattle under one year of age. The published reports on the use of stilbestrol and other hormones in lamb fattening rations were not conclusive.

Information was also needed on the effect the hormones would have on the value and desirability of the carcasses produced, ration digestibility, and physiological changes within the animal body. The investigations reported herein were designed to answer certain of these problems concerning the use of the compound.



## REVIEW OF LITERATURE

The effects of a number of natural and synthetic hormones on meat animal production have been observed and demonstrated for a number of years. As early as 1926, it was demonstrated that estrogenic substances in plants exerted certain physiological effects in rats and mice similar to those caused by estrogens of body origin.

There is a large amount of data concerning the role of hormones in reproduction. (Since the preparation of synthetic hormones such as diethylstilbestrol, reported by Dodds, et al. (1938), considerable study has been directed toward the possibility of influencing certain physiological processes in animals and poultry and thereby obtain more efficient meat production.)

Numerous observations in production of meat animals indicate definite hormonal effects. Gramlick and Thalman (1930), and Dinnuson et al. (1950) noted that spayed heifers did not gain as fast or as efficiently as intact heifers. Pregnant heifers produced fatter carcasses than similar non-pregnant heifers in a study reported by Snapp and Bull (1944). The carcasses of the bred heifers contained 20 percent more separable fat, 5 percent less lean and 10 percent less bone than similar non-pregnant heifers. Farmers often follow the practice of breeding cows or heifers that are being fattened. It is common knowledge that the animals will be more docile in the feedlot and will therefore fatten quicker and probably gain faster and more efficiently.

According to Bartlett et al. (1948) the increase in milk yield in



the spring when cows have access to young, succulent grass is greater than can be accounted for by the extra nutrients ingested by the animal. The galactopoietic effects of spring grass in lactating cows may be due to the presence of estrogenic materials in the green plants. Similarly, the excellent gains that lambs make on winter wheat pasture may possibly be enhanced by the estrogenic activity of the growing plant. Based on the nutrient content of wheat pasture and the pounds of dry matter normally required to put a pound of gain on a lamb in drylot, it can be calculated that a lamb would need to consume wheat pasture equivalent to about 25 percent of his body weight daily in order to make such rapid gains.

#### [ Effect of Stilbestrol on Metabolism in Farm Animals

From the available literature covering the effects of various hormones on ration digestibility and nutrient retention, only those articles that are particularly pertinent to the investigations reported herein have been reviewed.]

O'Mary et al. (1952), using the chromic oxide ratio technique, reported that implantation of stilbestrol had no effect on the digestibility of dry matter or protein in a lamb ration. Jordan and Bell (1952) found no effect on digestibility of rations when lambs were implanted with 12 mg. stilbestrol, and no difference in the amount of nitrogen retained. Details of the metabolism study were not reported. Whitehair et al. (1953) found no apparent effect on the digestibility of major nutrients in the lamb ration, except for a slight increase in the digestibility of crude fiber, when five of ten wether lambs were implanted with 24 mg. stilbestrol in the neck. The implanted lambs, however,

retained a larger percentage of the ingested nitrogen, calcium and phosphorus. Jordan (1953) reported a 30 percent increase in nitrogen retention among stilbestrol implanted lambs. The concentration of nitrogen in the urine was about the same in both groups, but there was a smaller quantity of urine excreted from the stilbestrol lambs.

The failure of the nitrogen retention work of Jordan and Bell (1952) to agree with that reported by Jordan (1953) or Whitehair et al. (1953) is not apparent, primarily because of the lack of information concerning the lambs, rations and procedure used in the first study.

Some investigators have been concerned that feeding stilbestrol might affect ration digestibility. Thompson et al. (1955) reported no significant effect on ration digestibility as the result of feeding 4 mg. stilbestrol per lamb daily, but stilbestrol in the feed did cause an increased retention of nitrogen, calcium and phosphorus. After the feeding of stilbestrol was discontinued there was a carry-over effect on calcium and phosphorus retention, but no carry-over effect on nitrogen retention.

Shroder and Hansard (1956) reported two to four mg. stilbestrol per day in the feed of wether lambs increased the digestibility of dietary calcium, but had no apparent effect on blood level or rate of blood disappearance of calcium<sup>45</sup> or phosphorus<sup>32</sup>.

Brooks et al. (1954) using lambs and an in vitro technique studied the effect of stilbestrol on nutrient digestibility. In the artificial rumen, stilbestrol increased digestion of cellulose and in lambs 10 or 20 mg. per day increased the digestion of both cellulose and crude protein.

More recently Struempler and Burroughs (1956) have reported nitrogen retention was increased in lambs receiving 1.2 or 2.4 mg. stilbestrol

per day if the ration contained 14 or 20 percent protein. Stilbestrol did not increase nitrogen retention consistently, however, on an 8 percent protein ration. In these trials dry matter and protein digestibility were not influenced by stilbestrol feeding.

In work reported by Campbell et al. (1956) 2 mg. stilbestrol per day increased nitrogen retention in lambs, but 10 days after stilbestrol was removed from the ration, the lambs which had received stilbestrol did not retain more nitrogen.

Using the lignin ratio technique with steers fed an all-pelleted ration, Erwin et al. (1955) found no effect of stilbestrol on digestibility of nutrients. Richardson et al. (1955) used eleven yearling steers in a digestion trial. Digestibility was determined on a standard ration with all of the steers, whereupon 10 mg. per day was included in the ration of each steer and digestibility was again measured. Each steer served as his own control. However, this procedure does not eliminate a possible environmental effect on digestibility. The results indicated a significant lowering of the digestibility of crude fiber, ether extract, crude fiber and nitrogen free extract when stilbestrol was fed.

Clegg (1952) stated that cattle implanted with 60 mg. stilbestrol retained twice as much nitrogen as the control cattle.

The above data is in agreement with the work of Schilling et al. (1950) and of Kochakian (1946) who demonstrated that administered estrogens increase the retention of nitrogen, calcium and phosphorus in humans.

Nutrient retention studies provide an estimate of the relative amounts of a nutrient retained in the body, but do not disclose where that nutrient is deposited. The increased nitrogen retention might mean

an increase in growth of any type of protein tissue in the body, such as lean muscle, connective tissue, epidermis, blood or wool. Clegg and Cole (1954) suggested the increased loin eye area among stilbestrol fed cattle would indicate that the increased nitrogen retained was being used to build muscle tissue. Extreme difficulty experienced in removing the pelts of stilbestrol implanted lambs would suggest the increased nitrogen retained may be used to build more connective tissue, primarily collagen, immediately under the lamb skin. This is supported by the slightly increased skin thickness among stilbestrol implanted lambs reported by Wilkinson et al. (1955). Also Clegg and Carroll (1956) reported that histological studies of the seminal vesicles of stilbestrol implanted and control cattle indicated stilbestrol stimulated the growth of epithelium as well as an increased development of fibrous tissue. There was no effect on smooth muscle tissue in the seminal vesicles.]

#### Growth and Carcass Composition Studies

A review of the recent literature disclosed many publications on the use of various hormones, particularly stilbestrol, in lamb and cattle feeding. An attempt was made to select from these publications research that was particularly outstanding or which summarized a particular phase of stilbestrol research in lamb and cattle feeding.

#### Lambs

Since the research on the effect of stilbestrol and testosterone on fattening lambs by Andrews et al. (1949) there have been numerous published reports on the use of various hormone implants, particularly stilbestrol, in lamb fattening. In a vast majority of the studies a significant increase in rate of gain and corresponding improvement in

feed efficiency have been noted. Numerous "side-effects" were apparent, however, which raised many questions as to the use of the material. Lambs were noticeably restless in the feedlot and there was considerable riding. Prolapse of the uterus and vagina among ewe lambs and prolapse of the rectum among wether lambs were common, and blockage of the urinary tract was occasionally observed. In addition, the dressing percent, carcass grade, and fat content were lowered in many cases and the carcasses carried more moisture, connective tissue and bone. Much of this work, conducted at several experiment stations, was reviewed by Soule (1955).

The seriousness of the side effects were definitely related to the quantity of stilbestrol implanted, but serious enough at even the lowest levels that stilbestrol implants were considered impractical.

Later, Hale et al. (1953, 1954) reported improved gains and feed efficiency in lambs as the result of feeding 2 mcg. stilbestrol per pound of ration, with no apparent effect on carcass quality. Further studies resulted in the recommendation of a level of 2 mg. per lamb per day in the ration. These studies were reported in detail by Hale et al. (1955). The inclusion of 0.6 mg. of stilbestrol per pound of ration resulted in approximately a 22 percent increase in rate of gain, with no consistent increase in feed consumption. Lambs receiving 1.2 mg. stilbestrol per pound of ration did not gain faster than the control lambs. There was no observed riding among the lambs in the feedlot, but the prepuce and bulbo-urethral gland were larger in the treated lambs, apparently in proportion to the quantity of stilbestrol in the ration.

There was constant dripping of urine among several lambs receiving



1.2 mg. stilbestrol per pound of ration. The prepuce in some of these lambs was ulcerated, and there was some mammary development and edema of the anal area in the wethers receiving the higher levels of stilbestrol. The lower level of stilbestrol apparently had a slight lowering effect on carcass grade; the higher level had a marked lowering effect.

Bell and Erhart (1955) fed 2 mg. stilbestrol per day to fattening lambs and reported a 23 percent improvement in rate of gain and a corresponding improvement in feed efficiency. Carcass data were not reported, but the fleece weight of the stilbestrol lambs was less even though the lambs were larger.

Jordan et al. (1955) fed 0.1, 0.5 or 1.5 mg stilbestrol daily to fattening lambs. Rate of gain and feed efficiency were slightly, but not significantly, improved at all three levels. There was no difference in daily feed consumption. Lambs receiving stilbestrol graded as high or higher than the control lambs, although the carcass yield was slightly lower in all cases.

In the summer of 1954, the Food and Drug Administration approved the use of a pellet including 10 mg. estradiol and 250 mg. progesterone for use in fattening lambs. Michigan workers (1954) using this combination in fattening lambs, reported a 27 percent improvement in rate of gain, accompanied by a 15 percent improvement in feed efficiency. The lambs had a lower dressing percent, however, and the carcasses shrank more in the cooler. In one of their two tests, the hormone treatment markedly lowered carcass grade.

Bell and Erhart (1955), using this combination material, obtained a 27 percent increase in rate of gain with a 19 percent saving in feed cost. Although carcass data were not available the fleece weights from

these heavier lambs were much less than those of the control lambs.

Bush (1955) in a preliminary study, observed a 16 percent improvement in gains and feed efficiency in lambs implanted with the same hormone combination.

Davy et al. (1956) fed three lots of lambs during a 91 day trial. The treatments were (1) control, (2) progesterone-estradiol combination implant and (3) 2.5 mg. stilbestrol per day during the first half of the experiment and 5 mg. per day during the last half. Carcasses from lambs which received either hormone treatment graded lower. In a second and similar trial the same effects on carcass grades were observed. Chemical analysis of meat from 9-rib racks showed a slightly higher moisture and protein, and lower fat percentage in the meat from treated lambs.

#### Mature Cattle

Many experiments on the use of stilbestrol and related hormones in fattening mature cattle, mainly yearlings, have been published. The earlier work with stilbestrol was concerned with using implants of 12 to 60 mg. per animal. Rate of gain and feed efficiency were consistently improved, but numerous detrimental side-effects were observed, and carcass quality was often lowered. Beginning in 1953, workers at several experiment stations have used small quantities of stilbestrol in the rations of fattening cattle. In nearly every case, an improvement in rate of gain and feed efficiency has resulted, without the side-effects and lowered carcass quality observed in many of the experiments with implants. This work has been extensively reviewed by Sykes et al. (1953), Andrews et al. (1955) and Burroughs et al. (1955).

[In 18 of 19 experiments summarized by Burroughs et al. (1955), cattle gains were improved when the ration contained 10 mg. or less stil-



bestrol per day. ] The average increase in rate of gain was 16 percent. Greater improvement was noted on high-grain rations than on high-roughage rations. Steers responded a little more than heifers on comparable rations and there was a tendency for more gain improvement with heavy weight cattle than with calves. The average reduction in feed costs per pound of gain in these experiments was 12 percent. Daily feed consumption was increased an average of 3 percent in the group of experiments, which included a number of experiments where there was no increase in feed consumption.

In the experiments where carcass studies were included no consistent effect on carcass yield or quality was observed.

In some cases where stilbestrol has been fed there have been noticeable side effects, such as longer teats on steers and a relaxation of the pelvic area. [ Andrews et al. (1955) cited several of these cases. ] These side effects, however, have not been considered serious in comparison with the effects that were observed earlier when implants were used.

Mitchell et al. (1955) fed 3 lots of 7 yearling steers averaging 850 pounds as follows: steers in Lots 2 and 3 received 10 mg. stilbestrol per day, and were fed until the steers averaged 1100 pounds in weight. The steers in Lots 1 and 3 were fed equal total concentrates in about the same length of time. In Table 1 is a summary of their results.

Steers in Lots 1 and 2 drank an average of 4.93 and 5.33 gallons of water per day for a 72 day period.

This is a practical illustration that stilbestrol fed cattle gain faster and more efficiently when there is no increase in daily feed consumption. It also demonstrated the response due to stilbestrol is great-

er, however, when the cattle are allowed to eat ad libitum.

Table 1. Summary of data reported by Mitchell et al. (1955)

Lot	<u>Control</u>	<u>Stilbestrol, 10 mg./day</u>	
	Full-fed	Full-fed	Total concen- trates and feeding time equal to Lot 1
	1	2	3
Length of feeding period, days	124	96	125
Final weight, lbs.	1106	1100	1141
Daily gain, lbs.	1.99	2.56	2.34
Concentrates per cwt. of gain, lbs.	846	665	719
Feed cost per cwt. gain, \$	26.39	21.29	22.63
Shrink, feedlot to slaughter, lbs.	27	27	20
Yield = $\frac{\text{cold carcass}}{\text{live wt.}} \times 100$	64.39	64.44	63.42

In a preliminary test, Marion (1955) fed 2 groups of 937 pound steers a limited grain ration of 2 pounds cottonseed meal, 4 pounds sorghum grain, 2 pounds alfalfa hay per day, and chopped hegari bundles, full-fed. The steers in one lot received 15 mg. stilbestrol per day. During the 60 day feeding period the stilbestrol-fed steers gained 39 lbs. (26 percent) more per head. After being trucked 230 miles to market, the difference was only 15 pounds per steer. The carcass yield for the stilbestrol fed steers was lower and carcass weights and grades for the steers from both lots were the same.

After conducting an experiment with yearling steers on native grass during the spring and summer, Baker et al. (1955) reported 10 mg. stilbestrol per day consistently increased rate of gain and improved feed efficiency of cattle that had received stilbestrol in the feed for 112 days, whether or not the cattle had previously received (stilbestrol). The use of stilbestrol in the ration while the animals were young did not limit the benefit from stilbestrol later in the animals' life.

(Klosterman et al. (1955) have reported that stilbestrol did not increase the rate of gain of steers in drylot on a fattening ration which was low in protein. When the protein content of the ration was adequate, however, average daily gains were increased .45 and .47 pounds per steer.)

#### Calves

At the time the investigations reported in this thesis were begun there were no published reports on the use of stilbestrol in the rations of cattle under one year of age. Since that time, however, the following work has been reported.

Andrews et al. (1955) increased the rate of gain about 20 percent, from 2.37 to 2.84 pounds per day on 450 pound steer and heifer calves, when they included 10 milligrams stilbestrol per day in a typical drylot fattening ration. The cost of gain was reduced about 15 percent during the 112 days the calves were on feed. The calves, 9 per treatment, were still on feed at the time the report was published.

Culbertson et al. (1955) reported a similar experiment where they included 5 or 10 mg. stilbestrol in a fattening ration for 440 pound steer calves for a 251 day feeding period with 9 calves per treatment. Feed consumption was increased slightly at the 5 mg. level, but not at

the 10 mg. level. Rate of gain was increased 14 percent, from 2.12 to 2.42 pounds per day on the lower level of stilbestrol, and was increased 8.5 percent on the higher level. Feed efficiency was also improved, suggesting a lower level of stilbestrol for these light-weight calves would be more nearly optimum than the level of 10 mg. per day which had been considered best for heavier, more mature cattle. There was about as much benefit from stilbestrol during the first part of the experiment, when the calves weighed about 500 pounds, as there was later when they were heavier and more mature.

~~Baker~~ Richardson et al. (1955) fed 10 mg. stilbestrol per day to 20 of 40 steers/calves weighing approximately 450 pounds in a wintering-type ration containing 4 pounds of milo and 1 pound of soybean oilmeal per day in addition to a full feed of sorghum silage. There was essentially no difference in feed consumption, rate of gain or feed efficiency during the 140 days the calves were on feed. Five 340 pound heifer calves receiving 10 mg. stilbestrol per day gained approximately 6 percent faster than their controls, all of which were on the same ration as the steers mentioned above.

Work reported by Hentges et al. (1955) indicated that average daily gains were increased from 2.7 to 3.1 pounds when 10 mg. stilbestrol were added to a fattening ration composed of corn and citrus pulp for steers averaging 536 pounds. Six steers per lot were fed for 109 days. Feed required per pound of gain was reduced slightly. No marked effect on carcass yield or quality was observed.

In a 112-day Nebraska test, feeding 10 mg. stilbestrol daily to calves averaging 535 pounds increased average daily gains 5 percent over gains of control calves. The stilbestrol-fed calves also ate an average

of 2 percent less feed per pound of gain.

Further evidence on the value of stilbestrol in improving gains among calves was presented by Smith et al. (1956). Calves fed 5 mg. stilbestrol per day from 475 to 600 pounds and 10 mg. per day from then until they weighed about 1000 pounds gained 13 percent faster and consumed 5 percent less feed per pound of gain. Very similar performance was made by a third lot of cattle receiving 40 mg. oxytetracycline per day to 600 pounds and 80 mg. per day after 600 pounds, in addition to the stilbestrol. All these cattle were on a full feed of corn silage and received 3 pounds of a 30 percent protein supplement per day.

Bredenstein et al. (1956) reported no significant differences in carcass grade, dressing percent, cooler shrink, specific gravity of the rib or other measured carcass traits when heifer calves were fed 5 mg. stilbestrol per day in comparison with control calves. Both groups received grass silage and a full feed of grain for 194 days.

Using 435 pound steer calves on a fattening ration, Stothers et al. (1956) obtained the following results. Ten mg. stilbestrol per day increased gains 5 percent and feed efficiency was improved by about 7 percent. A combination of stilbestrol and 10 mg. chlortetracycline per 100 pounds body weight per day improved gains and feed efficiency by 15 and 11 percent respectively. There were no appreciable differences in carcass grade.

More consistent benefit has been obtained from feeding 5 mg. stilbestrol per day to fattening calves than from feeding 10 mg. per day. Therefore, the level of 1 mg. stilbestrol per 100 pounds body weight per day may be more satisfactory. As is true with mature cattle, more improvement has been observed on high grain rations than on high roughage

rations. Very little carcass data from stilbestrol fed calves is available.

#### Cooking and Organoleptic Studies

Bell et al. (1955) at the Kansas station conducted cooking and palatability studies on legs of lambs that had been implanted with 15 mg. stilbestrol, or 12 mg. stilbestrol and 120 mg. progesterone, in comparison with legs from control lambs. The legs from the stilbestrol implanted lambs lost less weight during roasting and the lean had a much lower shear value, indicating more tenderness. This was not supported, however, by organoleptic scores of tenderness. There was very little difference among the scores for tenderness, juiciness and aroma.

In the report by Marion et al. (1955), the average cooking shrink of wholesale rib cuts from mature steers which had received 10 or 20 mg. stilbestrol per day in their ration was not materially different from the shrink of rib cuts from control cattle.

Simone et al. (1955) published a rather extensive carcass and organoleptic study on the effect of implanting barley-fattened cattle with stilbestrol. The animals were slaughtered when they graded choice and the carcasses were aged 11 days before the cuts were removed for cooking. Round steaks and rib roasts were scored by panel members for tenderness, juiciness and flavor. The complete study was conducted two different years. The first year there was no consistent difference in scores. The second year the cuts from the stilbestrol steers were scored slightly but consistently lower in all factors.

Cooperative research conducted by the Ralston Purina Company, Eli Lilly and Company and the Quartermaster Food and Container Institute for

the Armed Forces was reported by Secondino (1956). Twenty choice feeder heifers were used in the experiment, 10 receiving the recommended level of stilbestrol in the form of Stilbosol during the feeding period. Following slaughter of the fattened animals, loin steak, rib roast, hamburger from the neck and shoulder, and liver from each animal were used in taste preference and cooking studies. Panel members indicated preference for meat from the control animals in 560 of 1075 comparisons. Meat used in each comparison was from animals which graded the same. There was no marked difference in the moisture content of the raw steaks or moisture loss during cooking.

Taste panel tests of rib roasts conducted by the United States Department of Agriculture (Bartilson, 1956) indicated the meat from both control and stilbestrol fed steers "graded moderately tender and had taste scores averaging six out of a possible seven points." A panel was used to appraise the taste and a Warner-Bratzler shear was used to measure tenderness.



## INVESTIGATIONS

The investigations reported herein were conducted at the Oklahoma and Iowa Agricultural Experiment Stations during the years 1954 through 1957. The objectives were to determine the effect of feeding low levels of stilbestrol on: (1) nutrient digestibility and retention in lambs; (2) growth, feed efficiency, and carcass merit of fattening lambs; (3) growth, feed efficiency and carcass merit of creep-fed calves; (4) cooking shrink, shear values and organoleptic scores of cuts from fattened lambs, and the amount of connective tissue produced in the muscle tissue as well as below the epidermis of a fattening lamb.

### Part I. Digestibility and Retention Studies with Lambs

#### Experimental Procedure

Experiment 1 was conducted in the Animal Husbandry arena at Oklahoma Agricultural and Mechanical College in the fall of 1954. Twenty western feeder lambs, ranging from 50 to 60 pounds in weight were used in this digestion trial. The lambs were fed in stanchions, morning and evening, and bags were used for collection of feces.

The lambs were assigned to the stanchions randomly and were put on feed gradually. The hulls and previously mixed concentrate portion of the ration, listed in Table 2, were weighed separately and mixed by hand for each lamb. When every lamb was eating 2 pounds per day of the ration 2 mg. stilbestrol was added to the daily ration of a random half of the lambs.

Table 2. Basal ration used for Experiments 1 and 2.

<u>Ingredient</u>	<u>Pounds</u>
Cottonseed hulls	35
Yellow corn	49.5
Cottonseed meal	12.4
Vitamin A and D oil	0.2
Salt	0.5
Dicalcium phosphate	<u>2.4</u>
Total	100.0
<u>Calculated ration analysis</u>	<u>Percent</u>
Crude protein	10.97
Fiber	18.43
Total digestible nutrients	63.75
Calcium	.805
Phosphorus	.742
Magnesium	.107
Sodium	.212
Copper, ppm.	14.58
Iron, ppm.	41.70
Cobalt, ppm.	0.17
Potassium	.635

Fecal collections were begun 13 days later and continued for 10 days. The daily collection from each lamb was dried 24 hours in a 60° C oven and then put in a metal can for storage. At the end of the collection period, the total collection from each lamb was allowed to stand for several days to become uniformly air dry. The total collections were then weighed and sampled for chemical analysis by conventional A. O. A. C. (1950) methods.

Experiment 2 was conducted in the metabolism room under the bleachers of the Animal Husbandry arena in the summer of 1955. Twelve fine wool feeder lambs ranging from 58 to 82 pounds were used in this digestion and retention experiment, and were assigned to metabolism crates randomly. The ration and procedure were the same as for the first trial except that fecal and urine collections were begun 10 days after stilbestrol was added to the ration of half the lambs and the feces were collected in pans rather than bags. The urine was collected under acid in glass jars, made to a standard volume with tap water, and a 5 percent aliquot was saved under refrigeration for a composite sample. At the end of the 10 day collection period the composite sample from each lamb was stirred and sampled for chemical analysis.

An assay of the ration used in Trial 2, provided by Eli Lilly and Co., indicated the stilbestrol fed lambs received approximately 2.11 mg. stilbestrol activity per day.

### Results and Discussion

Average coefficients of digestibility obtained in Experiment 1 and 2 are contained in Tables 3 and 4 respectively. Nitrogen retention values obtained in Experiment 2 are also given in Table 4. The significance of the decrease in digestibility of ether extract among the

Table 3. Coefficients of digestibility, Experiment 1.

	No Stilbestrol	2 mg. Stilbestrol/day
No. lambs <sup>1/</sup>	9	9
Dry matter	55.98	52.59
Organic matter	56.90	56.00
Crude protein	48.80	47.37
Ether extract	87.41	84.95 <sup>2/</sup>
Crude fiber	15.99	13.29
Nitrogen free extract	68.47	68.14

<sup>1/</sup>Ten lambs per treatment were selected for this study. One lamb per treatment went off feed during the experiment. Data from these lambs are not included in the summary.

<sup>2/</sup>Significant at  $P = 0.05$  or less.

Table 4. Coefficients of digestibility and nitrogen retention values, Experiment 2.

	No Stilbestrol	2 mg. Stilbestrol/day
No. lambs <sup>1/</sup>	5	5
Dry matter	64.58	65.77
Organic matter	65.20	66.68
Crude protein	55.78	61.14 <sup>2/</sup>
Ether extract	80.71	80.28
Crude fiber	41.16	44.50
Nitrogen free extract	73.66	73.84
Nitrogen retained, % of that consumed	33.65	43.11 <sup>2/</sup>

<sup>1/</sup> Six lambs per treatment were selected for this study. A control lamb went off feed and was removed at the time collections were started. One of the stilbestrol-fed lambs voided an abnormally large weight of feces which were abnormally high in percent protein. Therefore, no digestion coefficients for this lamb were included in the summary of data.

<sup>2/</sup> Significant at P = 0.01 or less.

stilbestrol fed lambs in Experiment 1 is questionable. There is evidence that in some species the fat content of feces is rather constant and therefore does not give a true indication of the digestibility of ingested fat. The differences in digestion coefficients of the other nutrients were small and in every case there was considerable variation among the lambs on each treatment.

The digestibility of protein and retention of nitrogen among lambs receiving 2 mg. stilbestrol per day in Experiment 2 were significantly increased ( $P = 0.01$  or less). Nitrogen retained in relation to that consumed was increased 27 percent. There was also a noticeable but non-significant increase in the average digestibility of crude fiber. The digestion coefficients for the factors studied among lambs within each treatment were much more uniform than in the previous experiment. The digestion coefficients for all factors, except ether extract, were also higher in Experiment 2, perhaps indicating the lambs were in better condition, were more content, or had a better nutritional history than the lambs in Experiment 1. This may also explain the difference in the effect of stilbestrol in the two experiments.

#### Summary

Data obtained from 28 wether lambs used in metabolism experiments, where half the lambs received two mg. stilbestrol in their daily ration, may be summarized as follows:

1. No significant differences were noted among lambs in Experiment 1 where bags were used for fecal collections and where digestion coefficients for all nutrients were relatively low.
2. In Experiment 2, where the lambs were kept in crates, the nitrogen retained in relation to that consumed was 28 percent higher among the stilbestrol fed lambs. In this experiment, digestion coefficients for all nutrients were relatively high.

## Part II. Growth and Carcass Studies With Lambs

The four experiments reported in this section are presented complete in chronological order. Each is considerably different from the others in design as well as in procedure. At the end of this section an attempt is made to briefly consolidate and discuss the results of the four experiments.

### Experiment 1

In the fall of 1954 the Food and Drug Administration approved the use of a pellet containing progesterone and estradiol for use in implanting lambs being fattened for slaughter. This experiment was designed, therefore, not only to obtain information on the effects of low levels of stilbestrol in lamb fattening rations, but also to study the effects of the progesterone-estradiol combination pellet on fattening lambs.

### Experimental procedure

One-hundred-twenty western feeder lambs obtained from the range area in Texas and averaging 66 pounds were randomly allotted to three pens according to weight, sex and condition, and were gradually started on feed November 5, 1954, at the Ft. Reno Experiment Station. The lambs had been maintained on hay and dry pasture since early October and during that time were ear tagged, paint branded and drenched with phenothiazine containing arsenic. After gradually becoming accustomed to the ration, all lambs were self-fed a ration composed of 45 parts kafir, 5 parts molasses, and 50 parts good quality alfalfa hay, ground and mixed. Lot one was the control lot. One-half mg. crystalline stilbestrol per pound of feed was included in the ration for lambs in Lot 2. Each lamb in Lot 3 was implanted subcutaneously under the jaw with eight pellets containing a total of 250 mg. progesterone and 10 mg. estradiol the



day the lambs were allotted. Five lambs were removed from each of the lots after they had been on feed 61 days and were slaughtered at the College meats laboratory. Measurements and observations made on these lambs included (1) yield, (2) cooler shrink, (3) carcass grade, (4) weight of wool, (5) weight of sheared pelt and (6) thickness of skin. The remainder of the lambs were trucked to Oklahoma City and slaughtered after 92 days on trial. Measurements and observations made on these lambs included (1) yield, (2) carcass grade, (3) difficulty in removing pelts and (4) weight of woolled pelt.

#### Results and discussion

Carcass data for the lambs fed 61 days and slaughtered in the College meat laboratory are in Table 5. Feedlot and carcass data for the lambs fed 91 days and slaughtered in Oklahoma City are in Table 6.

The feeding of stilbestrol at the level used and under the conditions of this experiment resulted in approximately a 15 percent improvement in gain and feed efficiency. The market value of the lambs was less than that of the controls, however, because of (1) slightly lower yield and grade and (2) the fact that nine of the 34 lambs killed at the end of the feeding period were classed as yearlings on the rail. Packers estimate the value of a yearling carcass as approximately half that of a lamb carcass. In calculations used to determine market value and net return per lamb (Table 6), yearling carcasses were considered to be worth 75 percent of the value of lamb carcasses.

Implanted lambs which finished the 92-day trial gained 44 percent faster than the control lambs and on 29 percent less feed per pound of gain. Their market value was much lower than lambs in either Lots 1 or 2. Twenty of the 34 implanted lambs killed at Oklahoma City were classed

Table 5. Carcass data for lambs fed 61 days in Experiment 1.<sup>1/</sup>

Lot	1 Control	2 0.5 mg. stil- bestrol per lb. feed	3 10 mg. estradiol and 250 mg. pro- gesterone per lamb
No. lambs	5	2	5
Yield, % <sup>2/</sup>	49.29	49.63	47.27
Cooler shrink, 5 days, %	4.31	4.43	5.66
Carcass grade <sup>3/</sup>	3.8	4.0	2.4
Sheared pelt, % of live weight	5.46	--	6.44
Wool, % of live weight	3.57	--	2.92
Thickness of skin <sup>4/</sup> (mm.)	1.8	2.3	2.7

<sup>1/</sup> These data were not subjected to statistical analysis, since all lambs on each treatment were fed in a single pen there is no satisfactory error term for testing treatment effect.

<sup>2/</sup> Yield =  $\frac{\text{carcass wt. (chilled 2 days)}}{\text{live wt.}} \times 100$

<sup>3/</sup> A score of 3 = low good, 4 = ave. good, 5 = high good, 6 = low choice.

<sup>4/</sup> Average of 10 readings per lamb from a 2 cm. length of skin. Readings taken 24 hours after fixing in formal saline, on a slide and on cut surface in paraffin block. Skin was dried before blocking.

Table 6. Feedlot and carcass data for lambs fed 92 days in Experiment 1.<sup>1/</sup>

Lot	1 Control	2 0.5 mg. stil- bestrol per lb. feed	3 10 mg. estradiol and 250 mg. progesterone per lamb
No. lambs <sup>2/</sup>	35	34	34
Initial wt., lbs.	66	66	66
Final wt., lbs.	97	102	111
Ave. daily gain, lbs.	.34	.39	.49
Feed per 100 lbs. gain, lbs.	983	825	696
Yield, % <sup>3/</sup>	50.9	49.8	49.4
Carcass grade <sup>4/</sup>	4.86	4.71	4.50
% classed as yearlings	0	26	59
Ease of removing pelts <sup>5/</sup>	1.6	2.0	3.6
Pelt, % of live wt.	14.16	14.36	14.97
Value per cwt., \$ <sup>6/</sup>	19.81	18.17	15.37
Cost of feeder lamb, \$	10.96	10.96	10.96
Cost of gain per lamb, <sup>7/</sup> \$	6.45	6.33	7.22
Net return per lamb, \$	1.81	1.24	-1.12

<sup>1/</sup> These data were not subjected to statistical analysis. Since all lambs on each treatment were fed in a single pen there is no satisfactory error term for testing treatment effect.

<sup>2/</sup> A lamb in Lot 2 died of coccidiosis; a lamb in Lot 3 died of pneumonia.

<sup>3/</sup> Yield =  $\frac{\text{cold carcass wt.}}{\text{live full wt.}} \times 100.$

<sup>4/</sup> A score of 3 = low good, 4 = ave. good, 5 = high good, 6 = low choice.

<sup>5/</sup> A score of 1 = easy, 2 = normal, 3 = difficult, 4 = very difficult.

<sup>6/</sup> A calculated value based on yield, carcass value and pelt value.

<sup>7/</sup> Includes 4 ¢ worth of stilbestrol per lamb in Lot 2 and a 50 ¢ charge per lamb for implants used in Lot 3.

as yearlings on the rail. Those carcasses which were classed as yearlings were not classed as such because of weight; they were no heavier than other carcasses from their lots. The main reasons for down-grading the carcasses were shape, color and texture of meat, and appearance of bone. Perhaps blockier, shorter-legged lambs than those used in this test would not be so greatly affected by the hormone treatments and might have been classed as lambs. It is recognized, however, that most lambs fattened in the wheat pasture or corn belt areas are of the fine wool, or western type.

#### Side effects

Other effects of the hormones were also noted in the feedlot and at the time of slaughter. The economic consequence of these effects is difficult to measure. There was some riding and also dribbling urine among the lambs in both hormone lots throughout the feeding period; neither of these effects were noticed in the control lot.

Urogenital tract From examination at the time of slaughter it was apparent that the sexual organs which surround the urethra, through which urine passes, had swollen somewhat in the stilbestrol lambs and greatly in the implanted lambs, decreasing the size of the opening in the urethra. Urinary calculi or kidney stones, commonly a threat in feeding wether lambs, could be caught more easily and urine flow could be blocked completely when the size of the urethra is reduced. Three lambs had been removed temporarily during the feeding period from the implanted lot with apparent symptoms of urinary calculi. At the time of slaughter a calculi was found in the urinary tract of one control lamb and one or more calculi were found in four stilbestrol and four implanted lambs. Purulent cystitis (pusy inflammation of the bladder) was noted in two of the implanted lambs.

Pelting. Tremendous difficulty was experienced in removing the pelts from the implanted lambs. A numerical score was attached to the difficulty in pelting and the averages per treatment are given in Table 6. The difficulty in removing the pelts was apparently due to the presence of an abnormal abundance of connective tissue (primarily collagen fibers) immediately under the skin. In removing the pelts, some of the collagen remained on the carcass and some remained on the pelts, resulting in heavier pelts. After the carcasses chilled, the collagen remaining on the carcass looked like a layer of fat, which might result in higher carcass grades than merited.

Wool. Pelts from the five control and five implanted lambs killed earlier in the experiment were sheared and both the sheared pelts and wool were weighed. As can be noted in Table 5, the implanted lambs yielded less wool, expressed as a percent of live weight. Slower wool growth may have resulted from an impaired blood supply to the skin area because of the abnormal quantity of connective tissue present there. In support of this possibility, severe shedding was noted among the lambs in the implanted lot.

The thicker skins in both hormone lots reported in Table 5 and the slight difficulty encountered in pelting the stilbestrol lambs would imply a similar, but less serious, interference with wool growth may have resulted among the stilbestrol lambs.

## Experiment 2

### Experimental procedure

Seven uniform pairs of lambs primarily of Hampshire breeding were used in this experiment. They are further described in Table 7. Numbers 1 through 8 were from a relatively uniform ewe flock and were presumed

Table 7. Description of lambs used in Experiment 2.

Pair	Animal and lot number	Initial weight, lbs.	Description	Treatment <sup>1/</sup>
1	1	70.5	wether	S
	2	80	wether	C
2	3	63	wether	C
	4	66.5	wether	S
3	5	60	wether	S
	6	59.5	wether	C
4	7	59	wether	S
	8	58.5	wether	C
5	9	54.5	twin wether	C
	10	57	twin wether	S
6	11	81.5	twin ewe	C
	12	83	twin ewe	S
7	13	40	twin ewe	C
	14	44.5	twin ewe	S

<sup>1/</sup> S = stilbestrol; C = control.

to be all sired by the same ram. They were paired according to weight. Numbers 9 and 10 were twin wethers from that same flock. Numbers 11 through 14 comprised two pairs of ewe twins from a different flock.

The lambs were worked gradually up to a full feed of alfalfa hay and shelled corn. During this time they were drenched with phenothiazine, shorn and paint branded. They were then weighed and randomly allotted to individual feeding pens which were about 6 x 3½ feet in area. The lambs remained in these pens for the duration of the experiment except for a 2 to 3 hour period each afternoon when they were let out into a common exercise dry lot.

At the time of allotment the lambs were changed to the ration listed in Table 8. One-half mg. stilbestrol<sup>1</sup> per pound of ration was added for a random member of each pair of lambs. The lambs were worked up to full feed in 10 days, and for the remainder of the trial an excess was available at all times. The lambs were weighed weekly and feed consumption records were kept. A mixture of 1 part trace mineralized salt:1 part bonemeal was available at all times in each pen and the amount consumed by each lamb was recorded. Daily water consumption records were also kept.

Slaughter Data. Two pairs of lambs were killed after 64 days on feed. The remaining lambs were slaughtered after 73 days on feed. It was estimated that the lambs would grade low choice at the time of slaughter. The lambs were weighed full during the afternoon on the day before slaughter, then held without feed or water for 16 hours and

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<sup>1</sup> Sample of this ration was assayed for stilbestrol by Ely Lilly and Company. This assay indicated the ration contained .505 mg. stilbestrol activity per pound.



Table 8. Basal ration used in Experiment 2.

<u>Ingredient</u>	<u>Pounds</u>
Cottonseed hulls	25
Ground alfalfa hay	25
Kafir	40
Molasses	<u>10</u>
Total	100
<u>Calculated ration analysis</u>	<u>Percent</u>
Crude protein	9.59
Fiber	19.05
Fat	1.94
Total digestible nutrients	61.85
Calcium	0.49
Phosphorus	0.21

weighed again immediately before slaughter. During slaughter a numerical score was attached to the difficulty encountered in removing the pelts. Also, the livers and kidneys were weighed.

Carcass measurements. The carcasses were held in the cooler 2 days, then graded, weighed and cut into wholesale cuts. Each leg was removed from the carcass at the front edge of the pelvic bone. The shank was removed at the stifle joint, the tail bones were removed and a uniform amount of fat was trimmed from each leg around the tail area. The trimmed left leg of each lamb was wrapped in butcher's paper and held in the cooler for cooking tests which were conducted within three days. The right leg of each carcass was wrapped and frozen for cooking tests which

were conducted two weeks later.

Both nine-rib racks were separated by knife into edible and inedible portions which were weighed. Each rack included the fourth to twelfth rib, using the procedure described by Hankins (1947), who obtained correlations of 0.98 between the separable fat content of the rack and the separable fat content of the lamb carcass. The edible portion of the racks from each lamb was ground three times and a half-pound sample was taken and frozen in a glass jar for later chemical analysis. The chemical analyses included (1) moisture, (2) ether extract, (3) ash and (4) crude protein.

Cooking tests. The lamb legs were roasted in preheated ovens at 325° F. in uncovered aluminum roasters with one roast to a pan and one pan to each oven. Each leg was placed on a perforated grid in the roasting pan, with the outside of the leg up, and were cooked until the internal temperature of the roast at the thickest portion reached 180° F. Weights were taken 10 minutes after the legs were removed from the ovens to determine cooking losses.

Tenderness and organoleptic measurements. The fell and excess fat were removed from the roast. The semimembranosus and semitendinosus muscles (diagrammed in Figure 1) were removed and cut in ½ inch slices for palatability scoring. Panel members were served coded samples on trays and scored each sample on a score sheet similar to that in Figure 2. Aroma was appraised from the remaining uncut leg of lamb in the roasting pan. Seven persons served on the panel for the left legs; five served for the right legs.

The rectus femoris muscle was removed whole, wrapped in waxed paper and refrigerated three hours before cutting in ¾ inch slices. Four or

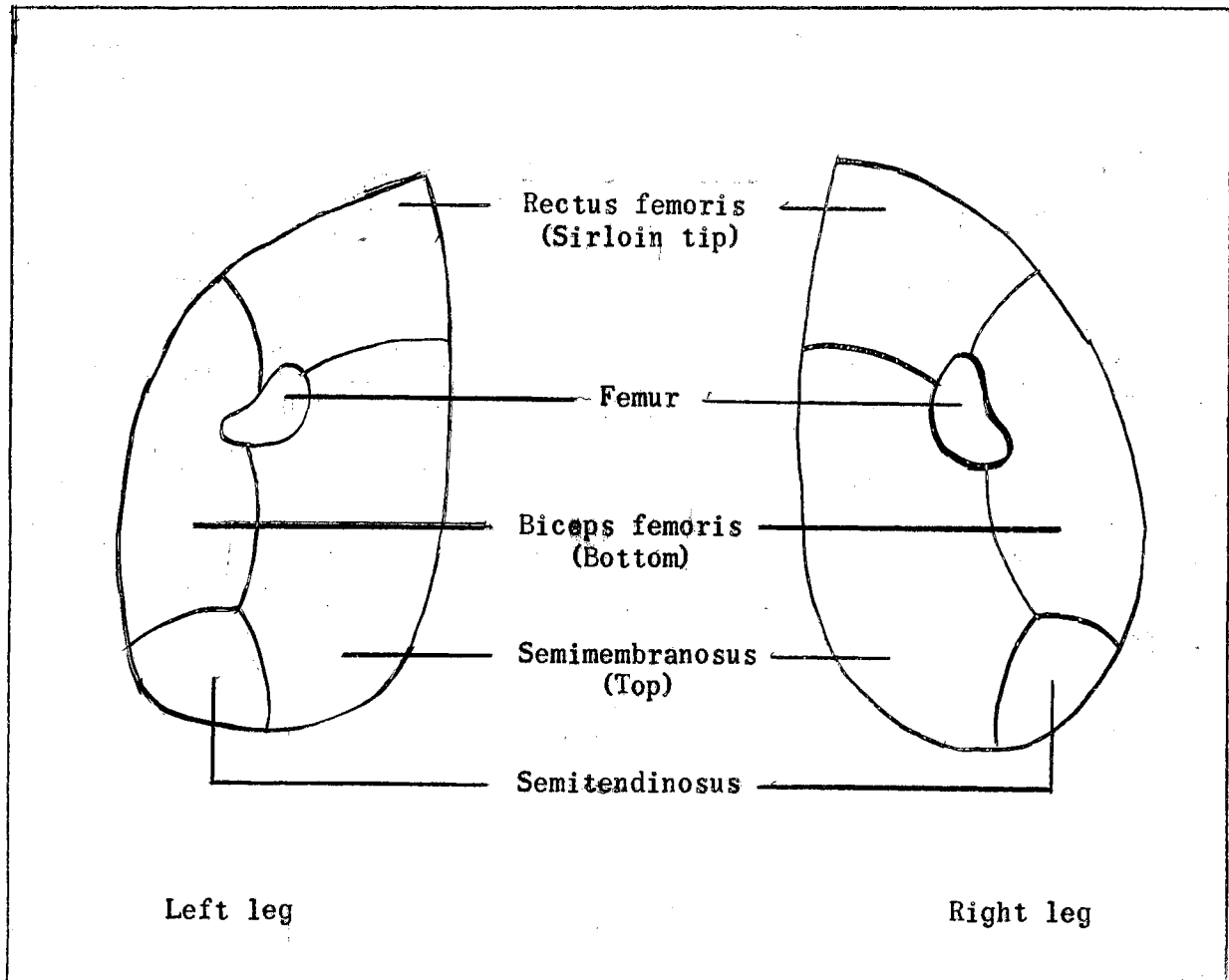


Fig. 1. Transverse cross-section of the right and left legs of lamb, indicating the muscles used in organoleptic and shear tests, and their approximate location (Sisson and Grossman, 1938).

SAMPLE	Date	Judge		Rank	Comments	
		Desirable or Standard	Undesirable			
			Too little	Too much		
1. Moistness or juiciness						
2. Tenderness						
3. Flavor						
4. Aroma						

SAMPLE	Date	Judge		Rank	Comments	
		Desirable or Standard	Undesirable			
			Too little	Too much		
1. Moistness or juiciness						
2. Tenderness						
3. Flavor						
4. Aroma						

Key:

Desirable = 3 (moist, tender, mild)  
 Too little = 2 or 1 (dry, mealy, tasteless)  
 Too much = 4 or 5 (greasy, tough, strong)

Fig. 2. Sample of the score sheet used for organoleptic appraisal of legs of lamb in Experiment 2.

5 slices, depending on the size of the muscle, were cut and  $\frac{1}{2}$  inch cores were cut from 2 positions on each slice. Shear values were determined with the Warner-Bratzler shear machine, therefore, on 8 to 10 samples per leg.

#### Results and discussion.

Growth data. These data are given in Table 9. The addition of  $\frac{1}{2}$  mg. stilbestrol per pound of ration for a member of each pair of lambs in this experiment caused a slight but non-significant improvement in rate of gain and feed efficiency. Feed consumption was increased slightly among the stilbestrol lambs, but the difference did not approach statistical significance. The stilbestrol lambs did consume significantly ( $P = .05$  or less) more water per day throughout the feeding period. The average difference in mineral consumption is not considered of any consequence, since there was considerable waste in some pens.

The reason for the small improvement in gains and feed efficiency as the result of feeding stilbestrol is not apparent. The assay of a sample of the experimental ration, mentioned previously, indicated that the stilbestrol was thoroughly mixed in the experimental ration and that it had maintained its potency through several months of storage. This experiment was conducted during the summer months of 1955 when the outside temperature was consistently over  $100^{\circ}$  F. Perhaps the intense heat (1) counteracts a potentially beneficial metabolic effect of stilbestrol, or (2) discourages the lambs from increasing their daily feed consumption enough to gain some of the benefit from stilbestrol. It is also possible that the length of day had an influence on the response to stilbestrol. Few reports have been published indicating an improvement in rate of gain or feed efficiency as the result of feeding stilbestrol to lambs during

Table 9. Rate of gain, feed consumption and water consumption data for Experiment 2.

	Control	Stilbestrol ( $\frac{1}{2}$ mg./lb. ration)
No. lambs	7	7
Ave. initial wt., lbs.	64.4	64.1
Ave. final wt., lbs. <sup>1/</sup>	90.5	94.0
Ave. daily gain, lbs.	.400	.426
Feed per day, lbs.	3.46	3.65
Feed per lb. gain, lbs.	8.74	8.63
Water per day, ml.	3933	4351. <sup>2/</sup>
Total mineral consumed, lbs.	3.78	3.24

<sup>1/</sup> The lambs had full access to feed and water up to the time these weights were taken.

<sup>2/</sup> Significant at  $P = .05$  or less.

the summer months.

Carcass data. Based on weights recorded in Table 10 the stilbestrol lambs had a lower yield, expressed as a percent of the live full weight, and there was essentially no difference in the weight of carcasses produced from lambs that had averaged the same weight at the time they went on feed. Further evidence that there was sufficient stilbestrol activity in the experimental ration was the fact that the pelts were more difficult to remove from the stilbestrol lambs than from the control lambs. The livers of the stilbestrol lambs were significantly ( $P = .06$  or less) larger than those of the control lambs. Other differences recorded were small.

Chemical analysis. The average composition of edible tissue from the nine-rib racks is reported in Table 11. The slightly lower moisture and protein content of the edible tissue from the stilbestrol lambs and the higher percentage of ether extract are in accord with the slightly higher average grade of the stilbestrol lambs reported in Table 10. It is known that as the percentage of fat increases in a carcass the percentage of moisture and protein decline, and finish is the main factor considered in grading lamb carcasses. The protein analysis figures may be further evidence that the additional nitrogen retained during stilbestrol feeding may be used for the building of tissue other than muscle tissue.

Ash content of fore shank bones is also recorded in Table 11. Shank bones from the stilbestrol lambs had a significantly higher percent ash ( $P = .01$  or less) than those from control lambs. This is in agreement with retention work previously reported, indicating that stilbestrol increases the percent retention of calcium and phosphorus.



Table 10. Slaughter data for Experiment 2.

	Control	Stilbestrol (½ mg./lb. ration)
Shrunk live wt., lbs. <sup>1/</sup>	82.4	84.2
Cold carcass wt., lbs.	44.8	44.6
Yield, % <sup>2/</sup>	49.45	47.88
Cooler shrink, %	2.62	2.86
Liver wt., gms.	540	631 <sup>6/</sup>
Kidney wt., gms.	94.3	88.9
Ease of removing pelts <sup>3/</sup>	1.86	2.50 <sup>6/</sup>
Carcass grade <sup>4/</sup>	5.43	5.71
Wt. of 9-rib racks, lbs. <sup>5/</sup>	5.00	5.08
Edible portion, %	79.17	80.35
Inedible portion, %	20.83	19.65

<sup>1/</sup> The lambs were held off feed and water 16 hours before slaughter.

<sup>2/</sup> Yield =  $\frac{\text{chilled carcass wt.}}{\text{live full wt.}} \times 100.$

<sup>3/</sup> A score of 1 = easy, 2 = normal, 3 = difficult, 4 = very difficult.

<sup>4/</sup> A score of 7 = ave. choice, 6 = low choice, 5 = high good, 4 = ave. good, 3 = low good.

<sup>5/</sup> The edible and inedible portions were separated by knife and weighed immediately. This cut was used because of the correlations reported by Hankins (1947).

<sup>6/</sup> Significant at P = 0.06 or less.

Table 11. Average chemical composition of edible tissue removed from 9-rib racks and of fore shank bones, Experiment 2.

	Control	Stilbestrol (½ mg./lb. ration)
<u>Edible tissue</u>		
Moisture, %	41.27	40.80
Ether extract, %	46.28	47.16
Ash, %	0.65	0.64
Protein, %	12.01	12.12
<u>Fore shank bone</u>		
Ash, %	57.93	60.31 <sup>1/</sup>

<sup>1/</sup>Difference significant at P = 0.01.

Cooking, organoleptic and tenderness data. Data from these studies are recorded in Table 12. Weight loss during roasting, both by evaporation and loss of liquid, of the legs of the stilbestrol lambs was not markedly different from the loss encountered during roasting of the legs from the control lambs. The average total shrink was nearly the same, although the legs from the stilbestrol lambs lost slightly more by evaporation and less in the form of liquid.

Shear values, as a measure of tenderness, showed no consistent differences. Average scores of panel members for the organoleptic study indicated the samples from the stilbestrol lambs were slightly less tender and had significantly less flavor. See Figure 2 for the scoring sheet and numerical scores used to appraise these various factors.

It must be pointed out that the panel members who participated in the organoleptic appraisal of the lamb samples were not trained for this

work, nor were they tested for repeatability or ability to discriminate among different degrees of tenderness, juiciness, flavor or aroma. It was also impossible to estimate the repeatability of the panel members by comparing their scores on the right and left legs of the same lamb, since the one leg was cooked relatively soon after the lamb was slaughtered and the other leg was held in the freezer for a two week period. Therefore, the value and validity of this portion of the experiment is in question.

Table 12. Cooking shrink, shear values and organoleptic scores of legs of lambs in Experiment 2.

	Control	Stilbestrol (½ mg./lb. ration)
No. legs	14	14
Total cooking shrink, %	25.9	26.0
Evaporation loss during cooking, %	16.4	17.0
Liquid loss during cooking, %	9.5	9.0
Shear value, lbs.	4.24	4.22
Organoleptic scores:		
Juiciness	2.51	2.54
Tenderness	2.99	3.14
Flavor	2.77	2.56 <sup>1/</sup>
Aroma	2.81	2.82

<sup>1/</sup>Significant at P = 0.05 or less.

### Experiment 3

Two hundred forty western wether lambs were purchased for this study, which was conducted in the fall of 1955 at the Iowa Agricultural Experiment Station, Ames, Iowa.

#### Experimental procedure

After arrival at Ames, the lambs were fed  $\frac{1}{4}$  pound soybean meal per day and alfalfa - bromegrass hay, free-choice. The lambs were vaccinated for enterotoxemia and after several days shelled corn was introduced into the ration. The lambs were started on experiment two weeks after arrival and at that time were eating an average of  $\frac{1}{4}$  pound shelled corn and two pounds hay. The proportion of concentrate was increased and the hay decreased during the feeding period until the lambs in all lots were eating approximately 60 percent concentrate, hand-fed twice daily.

Six ration treatments were assigned to 12 lots as indicated in the experimental design (Fig. 3). Two mg. stilbestrol was incorporated in the daily ration for the lambs receiving rations 3 and 5. The average composition of rations 2 and 4, which served as controls for the stilbestrol treatment, are listed in Table 13. Only data from the stilbestrol lots and the corresponding control lots are reported in this thesis.

The lambs, within each replicate, were placed in uniform outcome groups of 6 lambs according to weight and condition. These lambs were then allotted randomly to the pens within the replicate. The lambs in the heavy replicate (Replicate 2) were on feed 73 days; the lambs in the light replicate were fed 90 days. The lambs were weighed monthly and feed consumption records were kept.

At the end of the feeding period the lambs were weighed individually and trucked to Des Moines for slaughter the following morning.

Ration Number	Treatment	Replicate		Total lambs per treatment
		1 (light)	2 (heavy)	
1	Shelled corn + soybean meal	20	20	40
2	Mixed concentrate including cob	20	20	40
3	Ration 2 + 2 mg. stilbestrol per lamb per day	20	20	40
4	Mixed concentrate including cob and fat	20	20	40
5	Ration 4 + 2 mg. stilbestrol per lamb per day	20	20	40
6	Ration 2 + 6 mg. squalene and 10 mg. cholesterol per lamb per day	20	20	40

Fig. 3. Design for Experiment 3, indicating the number of lambs started per lot and per treatment.

Table 13. Average composition of basal rations for lambs in Experiment 3.

<u>Ingredients (lbs.)</u>	<u>Ration 2</u>	<u>Ration 4</u>
Alfalfa-bromegrass hay	20.30	20.95
Ground corn cob	16.00	15.80
Cracked corn	46.66	43.71
Molasses	12.00	12.00
Soybean meal	4.00	4.00
Dehydrogenated animal fat	---	2.40
Urea	0.80	0.80
Bonemeal with cobalt <sup>1/</sup>	0.20	0.20
Vitamin A concentrate <sup>2/</sup>	<u>0.04</u>	<u>0.04</u>
Total	100.00	100.00

<sup>1/</sup> Ten ounces cobalt sulfate per 100 lbs. bonemeal.

<sup>2/</sup> 2,500,000 I.U. vitamin A per pound.

Carcass grades and chilled carcass weights were obtained one day later.

#### Results and discussion

Feedlot and carcass data are contained in Table 14. The addition of 2 mg. stilbestrol to the daily ration of the wether lambs caused a 22 percent increase in rate of gain under the conditions of this experiment, and a 17 percent improvement in feed efficiency. The differences in rate of gain and feed efficiency were highly significant ( $P = 0.01$  or less). The addition of stilbestrol to the ration did not cause any noticeable change in daily feed consumption.

As indicated in the experimental design, stilbestrol was added to two different basal rations, one which contained a large percentage of ground corn cob and one which contained added fat. The improvement in rate of gain and feed efficiency was nearly the same with the two types of rations. Data from lambs on the two basal rations and lambs on the two stilbestrol rations are pooled in Table 14, because there was little difference in response on the two rations, and also because the analysis of variance used required pooling of the data.

Stilbestrol apparently caused a decrease in yield. The average decrease in carcass grade was only about two percent of a grade, so would not be considered of any consequence.

A numerical score was attached to the difficulty encountered in removing the pelts from the lambs in Replicate 2. The average scores for pelting difficulty indicate that the stilbestrol lambs were definitely more difficult to pelt. Often two men were required to pull the pelt from a stilbestrol-fed lamb. The only reliable method of statistically analyzing this data, since only lambs in Replicate 2 were scored, was to consider the two levels of fat in the ration as two replicates, and the



Table 14. Growth and carcass data for Experiment 3.

	Control	Stilbestrol (2 mg./lamb/day)
No. of lambs	79	80
Initial wt., lbs.	76.6	76.6
Final wt., lbs.	106.2	112.2
Daily gain, lbs.	.364	.444 <sup>1/</sup>
Feed per day, lbs.	3.52	3.55
Feed per 100 lbs. gain, lbs.	968	805 <sup>1/</sup>
Yield, % <sup>2/</sup>	49.68	48.85 <sup>3/</sup>
Carcass grade <sup>4/</sup>	5.48	5.30
Ease of removing pelts <sup>5/</sup>	2.38	2.88 <sup>6/</sup>
Cooler shrink, %	2.40	2.55

<sup>1/</sup> Significant at P = 0.01 or less.

<sup>2/</sup>  $\frac{\text{Cold carcass wt.}}{\text{Ames wt.}} \times 100.$

<sup>3/</sup> Significant at P = 0.06 or less.

<sup>4/</sup> A score of 3 = low good, 4 = ave. good, 5 = high good, 6 = low choice.

<sup>5/</sup> A score of 1 = easy, 2 = normal, 3 = difficult, 4 = very difficult. This estimate was made only on the light replicate of lambs.

<sup>6/</sup> Significant at P = 0.10 or less.

presence or absence of stilbestrol in the ration as treatment. This analysis gave an F value for stilbestrol effect of 57.14 which was significant at  $P = 0.10$  or less. Since the error term had only one degree of freedom (fat x stilbestrol) the required F value for significance at  $P = 0.05$  was 161. Evidence during pelting on the kill floor and evidence previously presented would indicate, however, there was a true difference in difficulty of pelting.

The difference in percent cooler shrink was small, and because of considerable variability among lambs on the same treatment the difference did not approach significance.

#### Experiment 4

This trial was conducted in the winter and spring of 1956 at the Iowa Agricultural Experiment Station and involved 160 western ewe lambs.

#### Experimental procedure

The lambs used for this experiment were handled in the same manner as described for lambs in Experiment 3. All lambs were fed  $\frac{1}{4}$  pound soybean meal per head daily, shelled corn, and alfalfa hay. During the early part of the experiment the corn was increased and hay decreased gradually until all lambs were consuming approximately 60 percent total concentrate. Iodized salt was also offered free-choice.

The experimental design is diagrammed in Figure 4. As in the previous experiment only data from the control and stilbestrol lots are included in this thesis, though all treatments were included in the analysis of variance to provide a more reliable estimate of the experimental error. The lambs in Replicate 1 were on feed 82 days; those in Replicate 2 were on feed 68 days.

Ration Number	Treatment	Replicate		Total lambs per treatment
		1 (light)	2 (heavy)	
1	Control	20	20	40
2	2 mg. stilbestrol per lamb per day	20	20	40
3	3.5 mg. stilbestrol per lamb per day	20	20	40
4	3 mg. squalene and 2 mg. cholesterol per lamb per day	20	20	40

Fig. 4. Design for Experiment 4, indicating the number of lambs started per lot and per treatment.

#### Results and discussion

Feedlot and carcass data are presented in Table 15. Under conditions of this experiment the addition of 2 mg. stilbestrol to the daily ration of fattening ewe lambs caused only a slight improvement in rate of gain and feed efficiency. The higher level of stilbestrol, 3.5 mg. per day, caused approximately a 20 percent increase in rate of gain and a 16 percent improvement in feed efficiency, both of which were significantly different from the controls at  $P = 0.10$  or less.

The slight increase in daily feed consumption was no more than would be attributed to the faster growth of the stilbestrol fed lambs and the resulting fact that they were heavier lambs than the controls during the latter portion of the experimental period. Yield and carcass grade, though consistently lower in the stilbestrol fed lambs, did not differ significantly among the treatments. Over 90 percent of the carcasses of the stilbestrol lambs were classed, on the rail, as yearlings by the

Table 15. Growth and carcass data for Experiment 4.

	Control	2 mg. Stilbestrol per day	3.5 mg. Stilbestrol per day
No. lambs	40	40	40
Ave. initial wt., Lbs.	80.4	79.4	79.1
Ave. final wt., lbs.	107.8	109.0	112.0
Ave. daily gain, lbs.	.366	.392	.438 <sup>4/</sup>
Ave. daily feed consumption, lbs.	2.906	2.964	2.931
Feed consumed/lb. gain, lbs.	7.94	7.55	6.70 <sup>4/</sup>
Yield, % <sup>1/</sup>	50.13	49.74	49.52
Cooler shrink, %	2.63	2.59	2.75
Carcass grade <sup>5/</sup>	5.00	4.75	4.82
Percent yearlings	23.15	85.00 <sup>6/</sup>	97.50 <sup>6/</sup>
Uterus wt., gms.	27.65	28.75	30.25
Ovary wt., gms.	1.33	1.01 <sup>2/</sup>	.90 <sup>3/</sup>
Developing follicles per lamb, over 2 mm.	1.250	.075 <sup>2/</sup>	0 <sup>2/</sup>

<sup>1/</sup> Yield =  $\frac{\text{warm carcass wt.}}{\text{live wt.}} \times 100.$

<sup>2/</sup> Significantly different from control at P = 0.05 or less.

<sup>3/</sup> Significantly different from control at P = 0.01 or less.

<sup>4/</sup> Significantly different from control at P = 0.10 or less.

<sup>5/</sup> A score of 4 = good, and a score of 7 = choice. The carcasses were not graded to the nearest third of a grade.

<sup>6/</sup> Significantly different from control at P = 0.005 or less.

government grader. As was true in Experiment 1 they were not classed as yearlings because they were heavier carcasses. In fact, those carcasses which were classed as yearlings averaged lighter in weight than those which were classed as lambs.

The uteri removed from the stilbestrol lambs were noticeably more turgid and brighter pink in color, though only slightly heavier in weight. Estrogens are known to cause a generalized hyperemia and edema of the uterine tract according to Turner (1949). There was an apparent inhibition of ovarian activity among the stilbestrol fed lambs. Not only were the ovaries significantly smaller in weight but there were only 2 lambs among the 80 fed stilbestrol with developing follicles larger than 2 mm. in diameter. All but seven of the 40 control lambs had from one to four developing follicles of that size. Prolonged use of low levels of stilbestrol may depress the pituitary, which normally produces adequate gonadotropin for functioning of the ovaries and development of follicles.

#### Summary

Observations made on the growth, feed utilization and carcass merit of 412 ewe and wether lambs fattened in drylot in the previous four experiments are summarized below.

1. The addition of stilbestrol to the lamb fattening rations at the rate of .5 mg. per pound of feed or 2 or 3.5 mg. per day caused an increase in rate of gain of 6.5 percent (Experiment 2) to 22 percent. Experiment 2 was conducted during the summer in 1955 when the temperature was over 100° F most days. The other experiments were carried on during the fall, winter or spring. Slightly greater improvement was noted among

wethers than among ewe lambs when fed comparable levels of stilbestrol.

2. Lambs fed stilbestrol gained more efficiently. In Experiments 1, 3 and 4 the saving in feed consumed per pound of gain ranged from 5 to 17 percent. There was essentially no improvement in feed efficiency among lambs fed stilbestrol in Experiment 2.
3. The pelts were more difficult to remove from the stilbestrol fed lambs. With a score of 1 = easy, 2 = normal, 3 = difficult and 4 = very difficult, the lambs which had received stilbestrol were scored approximately 0.5 points higher than the control lambs.
4. The pelts, weighed in Experiment 1 and expressed as a percent of the live weight, were heavier among the stilbestrol lambs. The surface area of an animal, as a function of body weight, decreases as an animal grows and fattens. Therefore, the average surface area per unit of body weight should have been less among the stilbestrol fed lambs than among the controls.

The skin from a sample of the lambs in Experiment 1 indicated the stilbestrol fed lambs had thicker skins which would contribute toward the heavier pelt weights. Histological stains of a cross section of these skins indicated an abundance of collagen fibers in the skin of the stilbestrol fed lambs. Further research on this topic is included in Part IV.

5. Dressing percent or yield was slightly, but consistently, lower among lambs fed stilbestrol. In only one case, Experiment 3, did the difference approach statistical significance.
6. There was no consistent effect on the grade of the chilled car-

cass. Any differences in average grades were small, between one-tenth and one-twentieth of a grade.

7. A larger percentage of the stilbestrol fed animals were classed as yearlings by the government grader. The extent of this effect was greater with older lambs and was somewhat proportional to the level of stilbestrol fed.
8. In all but one case the carcasses from the stilbestrol lambs shrank more in the cooler. The differences were small, however, and did not approach significance. This increased cooler shrink, if a true difference, would imply a larger percentage of moisture in the hot carcass. Such moisture might be present within any of the tissues, or may adhere to the outside of the carcass because of increased amounts of collagen present there.
9. The uteri among stilbestrol fed ewe lambs were slightly, but consistently, larger in Experiment 4 which included 120 lambs. The ovaries of the stilbestrol fed lambs were much smaller and most were devoid of developing follicles. The same effects are indicated among heifer calves discussed in Part III.
10. Detailed chemical analyses, cooking and organoleptic tests of cuts from the carcasses of lambs in Experiment 2 did not reveal any striking differences. The fore shank bones of the stilbestrol-fed lambs had a higher percent ash than those of the control lambs.

### Part III. Growth and Carcass Studies With Creep-Fed Calves

At the time this series of experiments was initiated (spring of 1954), there was no reported information on the value of low levels of



stilbestrol in rations of cattle under 500 pounds. In the fall of 1953, a project was started at the Ft. Reno Experiment Station to study different management methods in the production of fall-dropped calves to be sold for slaughter the following spring. In the early spring of 1954, 1955 and 1956 certain calves from this project were selected and reallocated, with their dams, to study the value of stilbestrol in the creep rations for approximately a two month period. Results of the three trials are grouped together for simplicity.

#### Experimental Procedure

Four steer and four heifer calves in each of the three lots of the management project were selected in May, 1954. The dams of these calves had been on different feeding regimes during the winter of 1953-1954, but all were being maintained on native grass pasture during the spring and summer of 1954. The calves, within each sex group and lot were divided into two groups as equally as possible, based on age, weight and condition. The calves of one of the groups, with their dams, were removed from their respective lots and placed together in a fourth pasture. The calves of the other group, serving as controls, remained in their original lots.

Stilbestrol was included in the creep ration of the removed calves at the rate of approximately 10 mg. stilbestrol per calf per day for 56 days. Since the creep feed was available ~~ad libitum~~ and the calves varied in their consumption from day to day, it was impossible to get exactly 10 mg. stilbestrol into every calf every day. The basal creep ration used in 1954, as well as those used in 1955 and 1956 are listed in Table 16.

At the end of 56 days the control calves and those that had received



stilbestrol were marketed and slaughtered in Oklahoma City. The measurements and observations that were obtained during and after slaughter included (1) yield, (2) grade, and (3) liver weights on all calves, as well as (4) uterus weight, (5) ovary weights, and (6) number of developing follicles for the heifer calves.

Table 16. Basal creep rations used for calves.

Ingredient	1954	1955	1956
Cracked corn, lbs.	60	55	
Milo, lbs.			55
Oats, lbs.	30	30	30
Cottonseed meal, lbs.	10	10	10
Dried molasses, lbs.	—	<u>5</u>	<u>5</u>
Total lbs.	100	100	100

In the spring of 1955, twelve calves from each of the three winter treatment lots were divided, with their dams, as equally as possible into two groups according to previous treatment, age, sex, weight and condition. One group of calves, with their dams, served as a control group. The second group of calves received 5 mg. stilbestrol per calf per day in the creep ration. The remainder of the calves from the original project, with their dams, were grouped in a third lot.

The calves were weighed for allotment on April 28, 1955, but were not put into the experimental groups until May 2, when the stilbestrol-containing creep rations were made available to the experimental calves. The three groups, with their respective creep feeders, were rotated

among the three pastures every two weeks. The stilbestrol creep feed was mixed daily or every other day in an attempt to maintain the 5 mg. daily intake of stilbestrol. The crystalline stilbestrol was premixed in cottonseed meal before being incorporated into the complete creep ration.

The calves were weighed every two weeks during the experimental period and were weighed before and after trucking to Oklahoma City for slaughter at the end of the experiment on June 14. Average daily gains were calculated for the 47 day period from April 28, but the amount of feed consumed from May 2 to June 14 was used to calculate daily feed intake and feed consumed per 100 pounds gain. Measurements obtained at slaughter were similar to those made the previous year, and in addition hide weight and eye muscle area were recorded.

On April 19, 1956, 15 calves from each lot were divided with their dams, into three groups as in the previous year. The ration and procedure were similar to that used in 1955, except that the calves in Lot 3 received 5 mg. stilbestrol and 40 mg. terramycin per day. Terramycin was included to see if it would improve gains above and beyond the improvement caused by stilbestrol. Additional observations made at the end of the experiment were liver weight and marbling score of the loin eye muscle.

### Results and Discussion

Growth and slaughter data for the 1954 study are contained in Table 17. The addition of 10 mg. stilbestrol per day to the creep ration of suckling calves grazing native grass pasture did not cause a substantial change in rate of gain, yield or carcass grade. The average values for the stilbestrol calves were slightly larger in each of these factors

Table 17. Gain and slaughter data for creep fed calves, 1954.

	Control	Stilbestrol 10 mg./day
No. calves	12	12
Days on experiment	56	56
Ave. initial wt., lbs.	458	470
Ave. final wt., lbs.	571	585
Ave. daily gain, lbs.	2.01	2.06
Yield, % <sup>1/</sup>	55.17	55.42
Carcass grade <sup>2/</sup>	4.17	4.50
Liver wt., lbs.	7.34	7.35
Uterus wt., gms. <sup>3/</sup>	50.12	67.08
Ovary wt., gms. <sup>3/</sup>	5.61	7.71
Developing follicles per calf <sup>3/</sup>	8.33	2.20

<sup>1/</sup> Yield =  $\frac{\text{Hot carcass wt.}}{\text{Live full wt.}} \times 100$

<sup>2/</sup> A score of 3 = low good, 4 = ave. good, 5 = high good, 6 = low choice.

<sup>3/</sup> Six heifer calves per treatment.

but the differences were not significant. Since there was no replication of treatment on these experiments the error term used to test treatment effect was the discrepancy of animals of the same sex and treatment. This is not legitimate and probably gives an underestimate of experimental error.

The average weights of the uteri and ovaries from the stilbestrol fed heifers were substantially larger, but because of the large variability within treatment, the differences did not approach statistical significance. The uteri of the stilbestrol heifers were noticeably more turgid and were brighter pink in color. Although the ovaries from the stilbestrol heifers were larger, on the average, they carried fewer developing follicles.

The size, turgidity and color of the uteri of the stilbestrol heifers clearly indicated that stilbestrol or a converted product of stilbestrol was causing physiological effects. The failure of the calves to respond, in terms of growth, to stilbestrol may have been due to too high level of stilbestrol in the ration, or failure of the calves to consume enough feed so that nutrients would be available for any potential growth promoting effect of stilbestrol. Since the control calves were maintained in three separate lots which also contained other calves, feed consumption records could not be compared. Further, the possibility remains that the grass contained estrogens in ample quantities to lessen the effect of stilbestrol on the calves.

At the time this work was initiated, 10 mg. stilbestrol per day was being recommended for cattle, based on research conducted with yearling or older cattle. However, it had been earlier demonstrated that 20 mg. stilbestrol per day did not increase gain as much in yearling cattle as

10 mg. per day. Perhaps the requirement or optimum level is a function of body weight. Since this work was initiated, <sup>Burroughs</sup> Culberson et al. (1955) and Richardson et al. (1955) have published reports which would indicate 5 mg. per day is a more beneficial level than 10 mg. for increasing daily feed consumption and rate of gain in calves.

Growth, feed and carcass data for the 1955 study are contained in Table 18. The addition of approximately 5 mg. stilbestrol per day to the creep ration of suckling calves grazing native grass pasture resulted in a substantial, but statistically nonsignificant, increase in rate of gain over a 47 day period. Stilbestrol apparently stimulated creep feed consumption, perhaps causing the calves to get a larger proportion of their nutrients from the creep ration and a smaller proportion from grass. The slightly higher yield and carcass grade among the stilbestrol calves would support this possibility, although it must be recognized that the differences are small. Since each whole number used in the grade coding represents only one third of a grade, the actual difference in grade is only approximately .06 percent of a grade.

The weight of the hide, expressed as a percent of body weight, was slightly less in the stilbestrol calves. This would be expected, however, since the stilbestrol calves were larger at the time of slaughter and as the weight of an animal increases the relative amount of surface area decreases.

Eye muscle area, measured by a planimeter from a tracing, was slightly larger in the stilbestrol calves. Much of this difference, however, is probably due to larger weight of those carcasses. The uteri of the stilbestrol fed heifers in this trial were significantly larger ( $P = .05$ ) and were also more turgid and a brighter pink in color. The ovaries

Table 10. Weight gain, feed consumption and slaughter data for creep fed calves, 1955.

	Control	Stilbestrol 5 mg./day
No. calves	18	18
Days on experiment	47	47
Ave. initial wt., lbs.	417	418
Ave. final wt., lbs.	516	529
Ave. daily gain, lbs.	2.11	2.36
Daily feed consumption, lbs.	3.38	4.13
Creep feed per 100 lbs. gain, lbs.	159	174.7
Transit shrink, %	3.39	3.15
Yield, % <sup>1/</sup>	56.51	56.79
Carcass grade <sup>2/</sup>	5.11	5.28
Hide wt., percent of live wt.	10.35	10.12
Eye muscle area, sq. in. <sup>3/</sup>	7.45	7.59
Uterus wt., gms.	44.94	65.46 <sup>4/</sup>
Ovary wt., gms.	6.42	5.39
Developing follicles per calf	27.3	20.9

<sup>1/</sup> Yield =  $\frac{\text{hot carcass wt.}}{\text{live full wt.}} \times 100.$

<sup>2/</sup> A score of 3 = low good, 4 = average good, 5 = high good, 6 = low choice.

<sup>3/</sup> Measurement taken with a planimeter from a tracing of the loin eye.

<sup>4/</sup> Significant at P = 0.05 or less.

were slightly smaller, in contrast to the observations in the previous trial. As was true in the 1954 trial, the number of developing follicles was less in the stilbestrol heifers.

Growth, feed and carcass data for the 1956 experiment are summarized in Table 19. As in the previous study, the addition of 5 mg. stilbestrol to the daily creep ration caused only a slight increase in rate of gain. The combination of 5 mg. stilbestrol and 40 mg. terramycin per day caused a highly significant increase in rate of gain, however. Cattle in the two lots receiving stilbestrol were slightly more variable in gains than the control calves.

The calves fed stilbestrol were similar to the control calves in yield and carcass grade, but had slightly more marbling in the rib eye muscle. The fact that the hides of the stilbestrol fed calves were heavier, expressed as a percent of the live weight might be worth noting, particularly since the stilbestrol fed calves were heavier and should have less surface area per unit weight. This same effect was noted in lambs, reported in Part II, Experiment 1. Calves fed the combination of stilbestrol and terramycin were similar to the controls in carcass observations, except for a slightly lower yield.

Stilbestrol caused an increase in uterine weights, as was true in both previous trials. There was also a slight decrease in ovary weights and number of developing follicles per calf in the heifers which received only stilbestrol. This effect was not noted among heifers in Lot 3, however, which received both stilbestrol and terramycin. This may have been due to failure of these calves to consume the intended amount of creep feed which would have supplied 5 mg. stilbestrol per day. As was pointed out before, the effect of estrogens on uterine activity may

Table 19. Gain, feed and slaughter data for creep fed calves, 1956.

	Control	5 mg. Stilbestrol per day	5 mg. Stilbestrol and 40 mg. Terramycin per day
No. calves	15	15	15
Days on experiment	47	47	47
Ave. initial wt., lbs.	470	473	470
Ave. final wt., lbs.	563	472	578
Ave. daily gain, lbs.	1.97	2.09	2.30 <sup>4/</sup>
Daily feed consumption, lbs.	5.40	4.41	4.87
Creep feed per 100 lbs. gain, lbs.	274	211	212
Yield, % <sup>1/</sup>	59.37	59.17	58.54
Carcass grade <sup>2/</sup>	5.53	5.73	5.73
Eye muscle area, sq. in.	7.59	7.41	7.48
Marbling score <sup>3/</sup>	3.37	2.97	3.32
Hide wt., % of live wt.	8.46	8.90 <sup>5/</sup>	8.42
Liver wt., lbs.	7.92	8.19	8.29
Uterus wt., gms.	69.83	78.23	95.83
Ovary wt., gms.	6.20	5.72	6.87
Developing follicles per calf <sup>6/</sup>	8.5	7.1	12.8

<sup>1/</sup> Yield =  $\frac{\text{hot carcass wt.}}{\text{live wt.}} \times 100.$

<sup>2/</sup> A score of 3 = low good, 4 = average good, 5 = high good, 6 = low choice.

<sup>3/</sup> A score of 1 = abundant, 2 = very good, 3 = good, 4 = slight, 5 = very scanty.

<sup>4/</sup> Significantly different from the controls at P = 0.01 or less.

<sup>5/</sup> Significantly different from the controls at P = 0.10 or less.

<sup>6/</sup> Six heifer calves per treatment.



differ tremendously, depending on the amount of estrogen administered.

### Summary

Three years' trials involving 105 fall-dropped creep-fed calves which were sold for slaughter the following spring at 500 to 600 pounds in weight are reported in Part III. Forty-five of these calves served as controls, 12 received 10 mg. stilbestrol in their creep ration per day for approximately a two month period before slaughter, 33 received 5 mg. stilbestrol per day, and 15 received 15 mg. stilbestrol and 40 mg. terramycin per day. The three years' experiments are summarized below.

1. Ten mg. stilbestrol per day caused little change in gains or carcass merit but its physiological effect was reflected in inhibition of the developing follicles on the ovaries of these immature heifers.
2. Five mg. stilbestrol per day increased gains 6 to 12 percent. These increases, however, were not statistically significant.
3. The 5 mg. level of stilbestrol did stimulate creep feed consumption during the 1955 trial, causing the calves to obtain a larger proportion of their nutrients from the creep ration and resulted in carcasses that were fatter and graded slightly higher.
4. Stilbestrol caused a slight increase in liver weights, probably due to increased feed consumption.
5. Heifer calves receiving stilbestrol had heavier uteri in every trial, and had lighter ovaries with fewer developing follicles in all cases except one. This effect was not observed in one group receiving both stilbestrol and terramycin in the 1956 trial, perhaps because of low feed consumption resulting in a very

low stilbestrol intake.

6. Calves receiving 5 mg. stilbestrol and 40 mg. terramycin per day gained 17 percent faster than control calves. This difference was significant at  $P = 0.01$  or less.

#### Part IV. Connective Tissue and Cooking Studies With Lamb.

Evidence presented in Part II indicated the need for more extensive research which would conclusively determine if stilbestrol in the ration of fattening lambs causes an increase in growth of connective tissue and, if so, the effects of this connective tissue on the desirability of the meat for human consumption and on the amount of wool produced. The research reported herein, done at the Iowa Experiment Station, was designed to approach these problems by means of the following observations: (1) pelting score, (2) weight of shorn skin, (3) weight of fleece, (4) thickness of dermis and microscopic appraisal of dermis cross-section, (5) chemical determination of connective tissue components in the loin eye muscle, or longissimus dorsi, (6) weight loss during roasting of lamb legs, (7) organoleptic scores, including tenderness, flavor and juiciness of slices from roasted lamb legs, and (8) tenderness of muscles of the cooked product as measured by the Warner-Bratzler shear.

#### Experimental Procedure

Seventy-two California white-faced wether lambs, approximately five months old and averaging 80 pounds, were divided into two groups of 36 lambs according to amount of finish. Each finish group was then divided into two groups of 18 lambs according to weight and randomly allotted to the experimental design in Figure 5. Each lamb was individually fed morning and evening in a crate. The remainder of the time they were

Energy	Percent Protein	Mg. Stilbestrol per pound of ration
Low	9	0
		0,3
		0,6
	13	0
		0,3
		0,6
	17	0
		0,3
		0,6
High	9	0
		0,3
		0,6
	13	0
		0,3
		0,6
	17	0
		0,3
		0,6

Fig. 5. Experimental design for each of the four replicates in Part IV.

together in small groups in drylot. Composition of the rations fed are listed in Table 20.

Table 20. Basal rations for lambs used in Part IV. <sup>1/</sup>

Ingredient, lbs.	Low energy	High energy
Ground corn cob	40	10
Ground alfalfa	20	20
Molasses	10	10
Cracked corn	23.8	59.5
Soybean meal	5.5	0
Dicalcium phosphate	0.2	0.2
NaH <sub>2</sub> PO <sub>4</sub> · H <sub>2</sub> O	<u>0.5</u>	<u>0.3</u>
Total	100.00	100.00

<sup>1/</sup> Rations contained 9 percent crude protein. The percent protein was varied by adjusting the proportions of corn and soybean meal.

It was planned to remove each lamb individually from the experiment so that all lambs within each replicate would gain an equal amount, as nearly as possible. It was recognized that it would also be desirable to have the slaughter weights of the lambs as nearly alike as possible, thus it was planned that the lambs comprising each of the four replicates average the same weight at the time of slaughter. Therefore, lambs in the light replicates at the beginning of the experiment were allowed to put on more gain than those in the heavier replicates, and hence were fed longer.

At the time of slaughter a numerical score was used to express the

difficulty encountered in pelting each lamb. Before the pelts were removed, however, and with the bled lamb hanging from a rail, a half-inch core of skin was removed with a stainless steel biopsy punch from the right loin of the lamb, approximately two inches behind the last rib and two inches to the right of the backbone. The core was removed, then, from directly above the right loin eye muscle and was deep enough so that the fell was included in the sample. Wool on the sample was trimmed with a scissors and the fat present below the fell was also trimmed away.

Each skin sample was then placed in a small sample bottle and fixed as quickly as possible in Bouin's solution (75 ml. saturated aqueous solution of picric acid, 25 ml. 40 percent formaldehyde and 5 ml. glacial acetic acid.) For fixing, the sample was laid on a small paper tag with the wool side up. The samples adhered fairly well to the tags and therefore curled very little while in the fixative. Approximately 24 hours later, the Bouin's solution was replaced with 70 percent alcohol in the sample bottles. This alcohol was replaced daily until no yellow color of picric acid in the bottles remained. They were then held in alcohol for approximately three months.

The pelts were allowed to hang on a rack for 24 hours after slaughter, or until the wool was air-dry. Each pelt was laid on a table and all the wool shorn and weighed. The shorn pelt, or skin, which remained was also weighed.

Carcasses from the lambs were held in a 35° F cooler for two days before they were cut into wholesale cuts. Each leg was removed from the carcass at the front edge of the pelvic bone. The shank was removed at the stifle joint, the tail bones were removed and a uniform amount of fat was trimmed from each leg around the tail area and on

the inside of the pelvis. The legs were then individually wrapped in aluminum foil and butcher paper, sharp frozen at  $-20^{\circ}$  F and held at  $0^{\circ}$  F for cooking tests conducted approximately three months later.

Both longissimus dorsi muscles from the nine-rib racks were removed and chopped in a silent cutter. A representative sample was wrapped and frozen for later chemical determination of connective tissue.

Both metacarpal, or lower shank bones, from each lamb were removed and cooked in lye water to remove the various soft tissues covering the bone. A Carver press was then used to determine pounds of pressure required to break the bones.

Skin studies. Each skin section was cut in half vertically and then put through a three-step dioxan series. The tissue was then placed in melted paraffin and left for three and one half hours and then it was imbedded in paraffin. Later, fifteen-micron slices were cut from a cross section of the imbedded skin sample, placed on a microscope slide and stained with haematoxylin and eosin. Thickness of the skin sample was then measured by use of a Bausch and Lomb microscopic projector, with the projection surface calibrated in units of 10 microns.

Chemical determination of connective tissue. Longissimus dorsi samples from the rack of the 36 high energy lambs were used in this phase of the investigations because (1) the most consistent improvement in gains from the feeding of stilbestrol was noted in the high energy lambs, (2) chemical determination of connective tissue components is very costly and (3) only legs from the high energy lambs were used in the cooking tests.

Rather than determine only the total amount of connective tissue in the samples, the amounts of the individual components, elastin, col-

lagen and mucoprotein were measured. Mucoprotein hexosamine was determined according to a method worked out by Miller and McIntosh (1957). Collagen and elastin were measured according to the methods of Miller and Kastelic (1956).

Cooking tests. Both legs from each of two randomly selected lambs within each treatment of the high energy group were cooked.

Approximately 24 hours before each leg was cooked, it was removed from the freezer and allowed to thaw at room temperature until it reached an internal temperature of 27° F. It was then stored in a refrigerator overnight. The legs of lamb were roasted in preheated ovens at 325° F. in uncovered pans with one roast to a pan. Each leg was placed on a perforated grid in the pan, with the outside of the leg up, and were cooked until the internal temperature at the thickest portion reached 167° F.

Tenderness and organoleptic measurements. The fell and excess fat were removed from the cooked roast. Slices, including a cross section of the semitendinosus and parts of the semimembranosus and biceps femoris muscles were cut into slices approximately 3/8 inch thick for palatability scoring. Six panel members were served coded samples on hot plates and scored each sample on a score sheet similar to that shown in Figure 6. Panel members, during the tasting and scoring procedure, were in individual booths equipped with red fluorescent lights to block out the color of the samples.

The remainder of the semimembranosus and biceps femoris muscles were removed after the leg was refrigerated 15 to 18 hours. One inch cores were removed from the muscles and wrapped in polyethylene. When the cores had reached room temperature shear values were determined

SCORE CARD

Roast Lamb Palatability Test

Judge \_\_\_\_\_ Date \_\_\_\_\_ Time \_\_\_\_\_  
Slice \_\_\_\_\_

Score on basis of 10 to 0

SAMPLE NUMBER						
	Check	Score	Check	Score	Check	Score
Flavor of Meat <u>Lacking in flavor</u> Pronounced or "muttony"						
Flavor of Fat <u>Lacking in flavor</u> Pronounced or "muttony"						
Moistness <u>Dry</u> Greasy						
Tenderness						
Abnormal Flavor* 1. Lean <u>Faint</u> <u>Noticeable</u> Pronounced						
2. Fat <u>Faint</u> <u>Noticeable</u> Pronounced						

\* Score on basis of 0 to 10

Sample No.	COMMENTS

Fig. 6. Sample of the score sheet used for organoleptic appraisal of legs of lamb in Part IV.



with the Warner-Bratzler shear. Two cores were removed from the semi-membranosus muscle and one, two or three readings per core were taken, depending on the length of the core. One core from the biceps femoris muscle was used and two or three readings per core were taken. Each value reported in Table 25, therefore, represents the average of 8 to 24 readings.

### Results and Discussion

Data on wool and skin weights, as well as pelting score and average breaking strength of shank bones, are contained in Table 21. Although the addition of stilbestrol resulted in a slight decrease in yield of clipped wool, the difference was small and inconsistent among the various protein and energy treatments. Additional protein in the ration, however, resulted in consistently greater wool weight. There was a 10.6 percent difference in the average weight of wool from the lambs on low and high levels of protein. Level of energy in the ration had no consistent effect on wool weights.

The apparent effects of protein and energy on wool growth in this experiment are in agreement with the work of Whiting and Slen (1956), where increased protein resulted in more wool, but differences in energy content of the ration had no apparent effect.

Average scores in Table 21 indicate a difference among treatments in difficulty of removing the pelts. In comparing the stilbestrol treatments, the highest pelting scores, on the average, were given to those lambs on the high level of stilbestrol. It should be noted this was consistently true within each level of protein. However, scores obtained on the difficulty of removing the pelts from the lambs in this experiment are considered of little significance. Inexperienced students

Table 21. Wool, skin, pelting score and bone breaking data for lambs in Part IV (8 lambs per treatment).

Percent protein	Mg. stilbestrol per lb. ration			Ave.
	0	0.3	0.6	
<u>Wool, lbs.</u>				
9	3.05	2.55	2.92	2.84
13	2.83	3.13	2.78	2.91
17	3.38	2.94	3.08	3.14
Ave.	3.08	2.87	2.93	2.96
<u>Skin, lbs.</u>				
9	6.80	7.32	7.02	7.04
13	7.69	7.62	7.12	7.47
17	7.94	7.24	8.14	7.77 <sup>1/</sup>
Ave.	7.48	7.39	7.43	7.43
<u>Pelting score</u>				
9	2.12	2.00	2.25	2.12
13	2.12	2.50	2.75	2.46
17	2.75	2.25	3.00	2.67 <sup>2/</sup>
Ave.	2.33	2.25	2.67	2.41
<u>Bone breaking strength, lbs. pressure</u>				
9	684.3	625.9	640.4	650.2
13	637.8	639.3	637.3	638.1
17	588.0	541.3	640.5	589.9
Ave.	636.7	602.2	639.4	626.1

<sup>1/</sup> Linear effect of protein significant at P = 0.01 or less.

<sup>2/</sup> Linear effect of protein significant at P = 0.05 or less.

did most of the pelting and were neither skilled nor consistent in their method of removing the pelts. Therefore, nearly every lamb was difficult to pelt, regardless of the ration treatment. There was a positive correlation of .21, however, between skin weight and pelting score.

Data on the force required to break the fore shank bones of the lambs showed few consistent differences among the various treatments. On the average, shanks from the lambs fed a high protein ration required less pressure for breaking, but this was not consistently true within each stilbestrol level. Many of the bones split longitudinally, giving an erroneous value for breaking strength.

Measures of skin thickness, made from cross sections projected from a microscope slide and reported in Table 22, indicate a significant increase in thickness as the protein content of the ration was increased. This is in agreement with skin weights, reported in the previous table.

Table 22. Thickness of skin from lambs in Part IV, measured in millimeters.

Percent protein	Mg. stilbestrol per lb. ration			Ave.
	0	0.3	0.6	
9	3.00	2.80	2.84	2.88
13	3.06	2.49	3.47	3.01
17	2.93	2.94	3.58	3.15 <sup>1/</sup>
Ave.	2.99	2.76	3.28 <sup>2/</sup>	3.02

<sup>1/</sup> Linear effect of protein significant at P = 0.10 or less.

<sup>2/</sup> Average effect of stilbestrol significant at P = 0.05 or less.

In comparing levels of stilbestrol the thickest skins, on the average, were from those lambs on the highest level of stilbestrol. This increased thickness was apparently caused by an increase in collagen.

Organoleptic scores. Subjective scores for the amount of flavor of lean and fat, moistness and tenderness of roasted legs of lamb by 6 panel members showed few marked differences. Average values per ration treatment are contained in Table 23.

According to the data, legs from lambs receiving stilbestrol tended to have more flavor of both lean and fat, on the average, and were more moist. These trends however, were not marked and were not consistent within protein levels. Each value recorded in Table 23 represents an average of 24 scores.

The level of protein in the ration had no apparent effect on organoleptic scores except that those legs from lambs on the higher levels of protein were slightly less tender.

In all cases the differences among the average organoleptic scores were small and there was much variation among panelists and between lambs on the same treatment. For this reason and because the cooking schedule prevented statistically removing the variation in scoring due to day of cooking and scoring, no statistical analysis of this data was attempted.

Cooking shrink. Average values for percent weight lost during roasting are shown in Table 24. Each figure in the body of the table represents an average of four legs, so the average for each level of stilbestrol or protein represents observations on 12 legs.

Legs from lambs fed higher protein rations shrank significantly less ( $P = 0.01$  or less) during cooking. This difference was mainly a

Table 23. Organoleptic scores of legs from lambs in Part IV.

Percent Protein	Mg. stilbestrol per lb. ration			Ave.
	0	0.3	0.6	
<u>Amount of flavor of lean</u>				
9	9.88	9.54	10.10	9.84
13	9.75	9.79	10.19	9.91
17	9.69	10.21	9.75	9.88
Ave.	9.77	9.85	10.02	9.88
<u>Amount of flavor of fat</u>				
9	10.40	10.30	11.16	10.62
13	10.10	10.93	10.93	10.47
17	10.46	11.10	10.58	10.72
Ave.	10.31	10.59	10.89	10.60
<u>Moistness</u>				
9	8.31	8.44	8.65	8.47
13	8.52	8.65	8.57	8.58
17	8.02	8.54	8.44	8.33
Ave.	8.28	8.54	8.55	8.46
<u>Tenderness</u>				
9	8.06	8.02	7.50	7.86
13	7.83	7.78	7.74	7.78
17	7.48	7.58	7.25	7.44
Ave.	7.79	7.79	7.50	7.67

Table 24. Cooking shrink of legs from lambs in Part IV.

Percent protein	Mg. stilbestrol per lb. ration			Ave.
	0	0.3	0.6	
<u>Total weight loss during cooking, %</u>				
9	25.67	25.42	24.92	25.33
13	23.61	23.58	24.13	23.77
17	24.89	23.00	22.11	23.33 <sup>1/</sup>
Ave.	24.72	24.00	23.72 <sup>2/</sup>	24.15
<u>Volatile weight loss during cooking, %</u>				
9	16.37	16.69	14.82	15.96
13	16.63	15.56	15.99	16.06
17	16.20	15.59	15.22	15.67
Ave.	16.40	15.95	15.35 <sup>2/</sup>	15.90
<u>Liquid weight loss during cooking, %</u>				
9	9.30	8.73	10.08	9.37
13	6.98	8.01	8.14	7.71
17	8.69	7.40	6.89	7.66 <sup>1/</sup>
Ave.	8.32	8.05	8.37	7.50

<sup>1/</sup> Linear effect of protein significant at P = 0.01 or less.

<sup>2/</sup> Linear effect of stilbestrol significant at P = 0.10 or less.

difference in loss of liquid ("drip") rather than volatile loss. Difference in size, and therefore relative amount of surface area, would not have accounted for the greater percentage shrink of legs from lambs fed the 9 percent protein ration. There was very little difference in the average weights of the legs. Since a large proportion of the liquid loss is fat, the difference in shrink may have been due to the fact that the legs from the lower protein lambs were slightly fatter.

Stilbestrol may have caused a decrease in volatile shrink from the roasted legs. Although the difference was not highly significant ( $P = 0.10$  or less) the decrease in percentage of total and of volatile shrink was almost directly proportional to the amount of stilbestrol in the ration.

Shear values. According to data presented in Table 25 the shear value of the semimembranosus muscle was directly proportional to the amount of protein in the ration (linear effect significant at  $P = 0.01$  or less). Average values indicate a greater force was required to cut the cores from legs of lambs fed higher levels of protein. This is in agreement with organoleptic tenderness scores for tenderness presented in Table 23. Stilbestrol had no apparent effect. The correlation between the shear value of the semitendinosus and organoleptic tenderness score was  $-.17$ .

Shear values for the biceps femoris muscles were less consistent and there was considerably more variation within treatments. This may have been due to the small size of the muscle and difficulty in obtaining representative samples. Of interest was the fact that the biceps femoris from the right leg was less tender than the same muscle from the left leg, as measured by the shear, in 17 of the 18 lambs. The

Table 25. Shear values of leg muscles from lambs in Part IV.

Percent protein.	Mg. stilbestrol per lb. ration			Ave.
	0	0.3	0.6	
<u>Semimembranosus muscle, lbs. pressure</u>				
9	20.1	17.7	18.8	18.8
13	22.7	26.5	20.2	23.1
17	23.6	28.5	22.0	24.7 <sup>1/</sup>
Ave.	22.1	24.2	20.4 <sup>2/</sup>	22.2
<u>Biceps femoris muscle, lbs. pressure</u>				
9	18.0	18.0	15.8	17.3
13	18.1	19.0	18.8	18.6
17	16.4	15.8	18.7	17.0
Ave.	17.5	17.6	17.8	17.6

<sup>1/</sup> Linear effect significant at  $P = 0.01$  or less.

<sup>2/</sup> Quadratic effect significant at  $P = 0.05$  or less.



right semitendinosus was less tender than the left in 12 of the lambs and the reverse was true with the other six.

Connective tissue content of muscle. The amounts of different components of connective tissue contained in the longissimus dorsi muscles from the lamb racks are summarized in Tables 26 and 27. In Table 26, the average values for collagen nitrogen, elastin nitrogen and hexosamine are expressed in proportion to the total nitrogen contained in the muscles. In Table 27, the average values for these components are expressed as a percent of the moisture-free, fat-free tissue. At the present time there is no known method for combining these three components mathematically to give an over-all estimate of the connective tissue content of the muscle. Since hydroxyproline is used as the basis for the collagen and elastin determinations, it is possible to combine these two components with considerable confidence. Hexosamine is used, however, as an index of the amount of mucoprotein in meat and the exact amount of hexosamine in skeletal mucoprotein is not known. A common method of grossly appraising connective tissue content of meat has been to measure the relative amount of tissue insoluble in certain concentrations of KCl. These procedures give figures considerably higher than would be obtained by adding together collagen, elastin and mucoprotein.

It is apparent from Table 27 that increased levels of stilbestrol in the ration of lambs caused a consistent and highly significant increase in elastin and hexosamine content of lamb muscle expressed as a percent of the moisture-free, fat-free tissue. This trend is also apparent with elastin expressed as a percent of the total nitrogen (Table 26). When the hexosamine was expressed in proportion to the total

Table 26. Collagen nitrogen, elastin nitrogen and hexosamine content of fresh lamb tissue, Part IV.

Percent protein	Mg. stilbestrol per lb. ration			Ave.
	0	0.3	0.6	
<u>Collagen nitrogen, % of total nitrogen</u>				
9	1.09	1.18	1.25	1.19
13	1.27	1.20	1.21	1.26
17	1.15	1.29	1.14	1.22
Ave.	1.20	1.25	1.23	1.23
<u>Elastin nitrogen, % of total nitrogen</u>				
9	.57	.50	.70	.58
13	.37	.54	.65	.52
17	.38	.54	.58	.51
Ave.	.43	.53	.64 <sup>1/</sup>	.54
<u>Ratio of hexosamine : total N (multiplied by 100)</u>				
9	.170	.155	.246	.190
13	.185	.148	.208	.180
17	.155	.180	.207	.181
Ave.	.170	.161	.220 <sup>2/</sup>	.184

<sup>1/</sup> Linear effect significant at  $P = 0.005$  or less.

<sup>2/</sup> Linear effect significant at  $P = 0.005$  or less and quadratic effect significant at  $P = 0.05$  or less.

Table 27. Collagen nitrogen, elastin nitrogen and hexosamine as a percent of moisture-free fat-free tissue, Part IV.

Percent protein	Mg. stilbestrol per lb. ration			Ave.
	0	0.3	0.6	
<u>Collagen nitrogen</u>				
9	.156	.172	.181	.170
13	.191	.179	.178	.180
17	.195	.189	.170	.183
Ave.	.179	.179	.176	.178
<u>Elastin nitrogen</u>				
9	.083	.076	.102	.086
13	.055	.083	.096	.078
17	.057	.058	.086	.069
Ave.	.064	.073	.094 <sup>1/</sup>	.078
<u>Hexosamine</u>				
9	.0251	.0226	.0359	.0273
13	.0220	.0220	.0307	.0249
17	.0226	.0264	.0306	.0269
Ave.	.0231	.0234	.0321 <sup>2/</sup>	.0263

<sup>1/</sup> Linear effect significant at  $P = 0.025$  or less.

<sup>2/</sup> Linear effect significant at  $P = 0.005$  or less, and quadratic effect significant at  $P = 0.025$  or less

nitrogen, however, 0.3 mg. stilbestrol per pound of ration did not cause an increase but there was a marked effect at the 0.6 mg. level. Statistical analysis did indicate, however, a significant linear effect of stilbestrol on hexosamine. Stilbestrol apparently caused no consistent effect on the collagen content of this muscle, although recent research by Miller et al. (1957) has indicated stilbestrol causes an increase in the collagen content of the semitendinosus muscle.

It is apparent from these data that stilbestrol in the ration, at the 0.3 and 0.6 mg. levels used in these tests, did cause an increase in certain of the connective tissue components of the loin eye muscle of lambs. Kastelic (1955), using KCl insoluble nitrogen as an appraisal of connective tissue in beef muscle, found that 5 and 10 mg. stilbestrol in the daily ration of yearling steers caused a consistent increase in connective content of muscle on a fat-free basis.

The only consistent effect of level of protein in the ration on any of the connective tissue components was the decrease in elastin as the protein content of the ration was increased.

There was a negative correlation (-.28) between the collagen and elastin content of the longissimus dorsi muscle, when both were expressed as a percent of the total nitrogen. There was a positive correlation of .31 between elastin and hexosamine, expressed on a similar basis. This would give confidence to the data in Table 26 where both elastin and hexosamine were consistently increased by an increase in the stilbestrol content of the ration, while there was no consistent change in collagen content.

#### Summary

Seventy-two wether lambs were individually fed in a 2 by 3 by 3

factorial experiment with 4 replicates. Treatments included 0, 0.3 and 0.6 mg. stilbestrol per pound of ration; 9, 13 and 17 percent crude protein; and 2 energy levels. Observations were made to determine if stilbestrol or protein level caused an increase in growth of connective tissue or on the desirability of lamb for human consumption. Lambs fed low energy rations were not used for cooking or chemical studies. Results of the research are summarized below.

1. Lambs fed stilbestrol yielded slightly less wool. The difference was very small and did not approach statistical significance.
2. It was more difficult to remove the pelts from the lambs fed the high level of stilbestrol. These lambs also had significantly thicker skins ( $P=0.05$  or less).
3. Organoleptic scores indicated lambs receiving the high level of stilbestrol were slightly less tender. The difference was small but was apparent within each level of protein.
4. There was a linear decrease in volatile weight loss during roasting of legs of lambs fed increased levels of stilbestrol. The reason for this trend is not apparent.
5. Stilbestrol caused a highly significant linear increase ( $P=0.025$  or less) in elastin content of the longissimus dorsi. It also caused a similar increase in hexosamine. In the latter case, the linear component was significant at  $P=0.005$  or less and the quadratic component was significant at  $P=0.05$  or less. There was no apparent effect of stilbestrol on collagen content of this muscle.
6. As the percent crude protein in the ration increased there

were significant linear increases in skin thickness, skin weight and difficulty of pelting, and a slight increase in wool produced.

7. The only apparent effect of protein level on organoleptic scores was a slight decrease in tenderness as percent protein in the ration increased. This is supported by significantly higher shear values of the semimembranosus at the higher protein levels.
8. Legs from lambs fed higher levels of protein lost less liquid during roasting. The linear effect was significant at  $P=0.01$  or less.
9. The only consistent effect of level of protein on any of the connective tissue components was a decrease in elastin as the percent protein in the ration was increased.

## GENERAL SUMMARY

(Studies reported in this thesis involved ~~512 feeder lambs and 105~~ creep-fed calves. Experiments were conducted to determine the effects of feeding low levels of stilbestrol in the rations of ~~feeder lambs~~ ranging in age from ~~4 to 10 months~~. ~~Additional studies included feeding low levels of stilbestrol to creep-fed suckling beef calves~~) and ~~im-~~planting feeder lambs with a pellet containing progesterone and ~~estra-~~diol.

Metabolism studies with 18 lambs indicated retention of nitrogen, in relation to that consumed, was 28 percent higher among lambs receiving 2 mg. stilbestrol per day. Digestion and retention of other ration components was not consistently affected.

Levels of 0.5 to 3.5 mg. stilbestrol per day caused a 6 to 22 percent increase in rate of gain of feeder lambs. Savings in feed consumed per pound of gain ranged from 0 to 17 percent. The least improvement in gains and feed efficiency was observed in the one experiment conducted during the hot summer. It was consistently more difficult to remove pelts from lambs fed stilbestrol, apparently because of an increase in growth of connective tissue immediately below the skin. Stilbestrol-fed lambs yielded slightly less and the carcasses shrank slightly more while in the cooler. Stilbestrol had no large or consistent effect on grade of chilled carcass, weight loss during cooking, palatability scores or tenderness as measured by the Warner-Bratzler shear. Among the ewe lambs, all levels of stilbestrol caused an increase in weight of the

uterus, and a decrease in weight of ovary and the number of developing follicles per ovary.

Levels of 0.3 and 0.6 mg. stilbestrol per pound of ration resulted in a highly significant increase in two connective tissue components, elastin and mucoprotein, in the longissimus dorsi. In this experiment stilbestrol had no apparent effect on collagen content of this muscle.

Implants of 250 mg. progesterone and 10 mg. estradiol caused a 44 percent increase in rate of gain and a 29 percent saving in feed per pound of gain. Market value of these lambs was low, however, partly because 59 percent of the carcasses were classed as yearlings. These lambs yielded lower and the carcasses graded lower. The pelts were much more difficult to remove and were heavier as a percent of the live weight. These effects were greater among these lambs than among lambs fed stilbestrol.

( Five mg. stilbestrol per day increased gains of suckling calves from 6 to 12 percent and apparently caused them to eat more creep feed, resulting in slightly fatter carcasses at slaughter. Ten mg. stilbestrol per day increased gains very little. Five mg. stilbestrol and 40 mg. terramycin per day caused a 17 percent increase in gains. Stilbestrol reduced the number of developing follicles on the ovaries of the heifers in all experiments, and in most cases the weights of the ovaries were reduced and the weights of the uteri were increased. )



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## A P P E N D I X

Table A. Analyses of variance for Part I, Experiment 1.

Sources of variation	Degrees of freedom	Mean squares					
		Dry matter	Organic matter	Crude protein	Ether extract	Crude fiber	Nitrogen free extract
Stilbestrol	1	51.97	3.74	9.0	27.09 <sup>1/</sup>	32.94	0.49
Experimental error	16	20.92	12.78	12.2	4.18	50.26	13.15
Total	17						

<sup>1/</sup> Significant at P = 0.05 or less.

Table B. Analyses of variance for Part I, Experiment 2.

Sources of variation	Degrees of freedom	Mean squares						
		Dry matter	Organic matter	Crude protein	Ether extract	Crude fiber	Nitrogen free extract	Nitrogen retention
Stilbestrol	1	3.55	5.52	71.88 <sup>1/</sup>	.45	27.82	0.08	223.35 <sup>1/</sup>
Experimental error	9	4.46	4.93	5.17	3.73	16.89	51.99	13.08
Total	10							

<sup>1/</sup> Significant at P = 0.01 or less.



Table C. Analyses of variance for Part II, Experiment 2, growth, feed and water data.

Sources of variation	Degrees of freedom	Mean squares			
		Daily gain	Daily feed consumption	Feed per lb. gain	Daily water consumption
Stilbestrol	1	0.002419	0.1282	0.0424	613,207 <sup>1/</sup>
Pairs	6	0.005762	0.4117	1.6123	678,324
Experimental error	6	0.002599	0.0418	0.7624	62,934
Total	13				

<sup>1/</sup> Significant at P = 0.05 or less

Table D. Analyses of variance for Part II, Experiment 2, carcass data.

Sources of variation	Degrees of freedom	Mean squares						Percent edible portion in racks
		Yield	Cooler shrink	Liver weight	Kidney weight	Removing pelts	Carcass grade	
Stilbestrol	1	8.6586	0.1968	24,120 <sup>1/</sup>	103.0	1.4464 <sup>1/</sup>	0.29	5.91
Pairs	6	16.7846	3.3030	8,828	206.1	0.0714	1.83	8.24
Experimental error	6	8.9923	2.2252	3,914	37.5	0.2475	1.45	5.65
Total	13							

<sup>1/</sup> Significant at P = 0.06 or less.

Table E. Analyses of variance for Part II, Experiment 2, chemical analysis of tissue.

Sources of variation	Degrees of freedom	Mean squares				Bone ash
		Moisture	Edible tissue Ether extract	Ash <sup>1/</sup>	Protein <sup>2/</sup>	
Stilbestrol	1	0.1738	0.0008	0.0002	0.0397	29.7779 <sup>3/</sup>
Pairs	6	23.7491	38.6452	0.0058	4.1863	6.4178
Experimental error	6	14.7500	23.1710	0.0034	1.5007	1.8203
Total	13					

<sup>1/</sup> Data for one lamb missing, so that pair was eliminated in the analysis of variance and the degrees of freedom were reduced accordingly.

<sup>2/</sup> Data for one lamb in each of two pairs was missing, so those pairs were eliminated in the analysis of variance and the degrees of freedom were reduced accordingly.

<sup>3/</sup> Significant at  $P = 0.01$  or less.

Table F. Analyses of variance for Part II, Experiment 2, cooking, shear, and organoleptic data.

Sources of variation	Degrees of freedom	Total cooking shrink	Evaporation loss	Shear values	Mean squares			
					Juciness	Tender-ness	Flavor	Aroma
Stilbestrol	1	0.130	3.09	0.2050	0.0089	0.1575	0.3004 <sup>1/</sup>	0.0014
Pair	6	8.287	7.17	0.6901	0.1733	0.1123	0.0923	0.1074
Experimental error A	6	3.817	1.99	0.4701	0.1064	0.2875	0.0470	0.0061
Sub-total	13							
Leg (left vs. right)	1	18.580	16.51	0.3796	0.0804	0.1032	0.0004	0.1157
Leg x stilbestrol	1	0.020	0.22	0.0417	0.3432	0.0090	0.0432	0.1429
Experimental error B	12	8.400	6.71	0.1346	0.0668	0.0861	0.1718	0.2268
Total	27							

<sup>1/</sup> Significant at P = 0.05 or less.

Table G. Analyses of variance for Part II, Experiment 3.

Sources of variation	Degrees of freedom	Mean squares					
		Gains	Feed consumption	Feed efficiency	Yield	Carcass grade	Cooler shrink
Treatment	5 <sup>1/</sup>	.005074 <sup>2/</sup>	.02670	20,641 <sup>2/</sup>	1.08593	.04917	0.04927
Stilbestrol	1	.012720 <sup>2/</sup>	.00180	53,301 <sup>2/</sup>	1.38611 <sup>3/</sup>	.06661	0.04500
Other	4	.003162	.13170	49,904	1.01088	.04481	0.05034
Replicate	1	.000004	.04810	4,409	0.81641	.23240	6.42097
Experimental error	5	.000123	.00766	540	0.21977	.04831	0.07151
Total	11						

<sup>1/</sup> All lots were included in the analyses, even though only 4 of the lots were concerned with stilbestrol treatment, in order to obtain a more accurate estimate of experimental error.

<sup>2/</sup> Significant at P = 0.01 or less.

<sup>3/</sup> Significant at P = 0.06 or less.

Table H. Analysis of variance for Part II, Experiment 3,  
pelting data.

Sources of variation	Degrees of freedom	Mean squares
Stilbestrol	1	.2400 <sup>1/</sup>
Fat	1	.0036
Experimental error	1	.0042
Total	3	

<sup>1/</sup> Significant at P = 0.10 or less.

Table I. Analyses of variance for Part II, Experiment 4.

Sources of variation	Degrees of freedom	Mean squares								
		Rate of gain	Daily feed consumption	Feed per lb. gain	Yield	Cooler shrink	Carcass grade	Uteri weights	Ovary weights	Developing follicles
Treatment	3	.001956	.001399	0.5965	0.158	.0175	0.0333	2.30	.1416 <sup>2/</sup>	0.6403 <sup>2/</sup>
Control vs. 2 mg. stilbestrol	1	.000702	.002304	0.1482	0.160	.0020	0.0600	1.21	.1024 <sup>2/</sup>	1.3806 <sup>2/</sup>
Control vs. 3.5 mg. stilbestrol	1	.005112 <sup>1/</sup>	.000650	1.5129 <sup>1/</sup>	0.384	.0144	0.0289	6.76	.1849 <sup>3/</sup>	1.5625 <sup>2/</sup>
Replicate	1	.000008	.078606	0.4141	1.411	.0435	1.2483	16.53	.0145	0.0378
Treatment x replicate	3	.000658	.006935	0.2111	0.617	.0310	0.0773	2.51	.0048	0.0513
Total	7									

<sup>1/</sup> Significantly different from control at P = 0.10.

<sup>2/</sup> Significantly different from control at P = 0.05.

<sup>3/</sup> Significantly different from control at P = 0.01.

Table J. Analyses of variance for Part III, 1954 data.

Sources of variation	Degrees of freedom	Mean squares		
		Gains	Yield	Carcass grade
Stilbestrol	1	51	0.40	1.00
Sex	1	1276 <sup>1/</sup>	1.87	2.00
Sex x stilbestrol	1	651	0.46	0.00
Experimental error	20	301	1.55	1.05
Total	23			

<sup>1/</sup> Significant at P= 0.06 or less.

Sources of variation	Degrees of freedom	Mean squares	
		Uterus weight	Ovary weight
Stilbestrol	1	862.75	15.939
Experimental error	10	695.82	10.180
Total	11		



Table K. Analyses of variance for Part III, 1955 data.

Sources of variation	Degrees of freedom	Mean squares			
		Gains	Yield	Carcass grade	Eye muscle area
Stilbestrol	1	1,225	0.689	0.25	0.165
Sex	1	0	3,542	0.32	1.912
Sex x stil- bestrol	1	150	4,064	1.94	0.875
Experimental error	32	413	2,138	2.72	0,560
Total	35				

Sources of variation	Degrees of freedom	Mean squares	
		Uterus weight	Ovary weight
Stilbestrol	1	1993.60 <sup>1/</sup>	5.047
Experimental error	17	389.97	2.652
Total	18		

<sup>1/</sup> Significant at P = 0.05 or less.

Table L. Analyses of variance for Part III, 1956 growth and carcass data.

Sources of variation	Degrees of freedom	Mean squares						
		Gains	Yield	Carcass grade	Eye muscle area	Marbling score	Percent hide	Liver weight <sup>4/</sup>
Treatment	2	901 <sup>1/</sup>	2.75	0.200	0.1176	0.9875	1.060	0.4887
Control vs. stilbestrol	1	241	0.31	0.300	0.2323	1.8750	1.426 <sup>3/</sup>	0.5237
Control vs. stilbestrol and terramycin	1	1763 <sup>2/</sup>	5.15	0.300	0.0822	0.1688	0.015	0.8936
Sex	1	507	0.26	0.830	8.3636 <sup>2/</sup>	1.6725	0.102	0.8335
Treatment x sex	2	127	2.30	0.135	0.0351	0.6598	0.378	0.1171
Experimental error	39	215	2.17	2.167	0.3322	1.5978	0.485	0.6339
Total	44							

<sup>1/</sup> Significant at P = 0.05 or less.

<sup>2/</sup> Significant at P = 0.01 or less.

<sup>3/</sup> Significant at P = 0.10 or less.

<sup>4/</sup> Degrees of freedom were 33 for experimental error and 44 for total. One observation was missing from each sex within each treatment.

Table M. Analyses of variance for Part III, 1956 reproductive organ data.

Sources of variation	Degrees of freedom	Mean squares	
		Uterus weight	Ovary weight
Treatment	2	1054	200
Experimental error	15	417	423
Total	17		

Table N. Analyses of variance for wool and skin weights and pelting scores for lambs in Part IV.

Sources of variation	Degrees of freedom	Mean squares		
		Wool	Skin	Pelting score
Treatment	17	0.4613	2.2691	0.5882
Stilbestrol	2	0.2889	0.0457	0.9167
Energy	1	0.2990	7.7947 <sup>1/</sup>	0.0555
Protein	2	0.5831	3.2307 <sup>2/</sup>	1.7916
Linear	1	1.0680	6.3948 <sup>1/</sup>	3.5208 <sup>2/</sup>
Stilbestrol x protein	4	0.4744	1.5497	1.0417
Stilbestrol x energy	2	0.1861	0.1622	0.3890
Protein x energy	2	1.3715 <sup>3/</sup>	8.2077 <sup>1/</sup>	0.0972
Stilbestrol x protein x energy	4	0.1947	0.3219	0.7430
Replication	3	0.4066	3.0177	0.2037
Experimental error	51	0.4482	0.8935	0.7625
Total	71			

<sup>1/</sup> Significant at P = 0.01 or less.

<sup>2/</sup> Significant at P = 0.05 or less.

<sup>3/</sup> Significant at P = 0.06 or less.

Table O. Analyses of variance for skin thickness of lambs in Part IV.

Sources of variation	Degrees of freedom	Mean squares
Protein	2	1.3021
Linear	1	2.6000 <sup>1/</sup>
Quadratic	1	0.0042
Stilbestrol	2	4.4926 <sup>2/</sup>
Linear	1	2.8728
Quadratic	1	6.1124 <sup>2/</sup>
Protein x stilbestrol	4	4.782 <sup>3/</sup>
Experimental error	23	.8882
Total	31	

<sup>1/</sup> Significant at P = 0.10 or less.

<sup>2/</sup> Significant at P = 0.05 or less.

<sup>3/</sup> Significant at P = 0.01 or less.

Table P. Analyses of variance for cooking shrink of legs from lambs in Part IV.

Sources of variation	Degrees of freedom	Mean squares		
		Total	Volatile	Liquid
Protein	2	13.25814 <sup>1/</sup>	0.48014	11.3644 <sup>1/</sup>
Linear	1	24.00000 <sup>2/</sup>	0.49307	17.5446 <sup>2/</sup>
Stilbestrol	2	3.23408	3.36562	0.3675
Linear	1	6.06015 <sup>2/</sup>	6.69398 <sup>3/</sup>	0.0150
Stilbestrol x protein	4	2.90791	1.37313	3.2661
Experimental error A	9	1.66958	1.70167	1.4219
Leg	1	3.25201	9.78647	1.7600
Leg x protein	2	0.19168	0.51814	0.3588
Leg x stilbestrol	2	0.01501	0.44094	0.2941
Leg x protein x stilbestrol	4	4.17861	2.84693	0.3527
Experimental error B	9	3.73386	3.12323	0.6596
Total	35			

<sup>1/</sup> Significant at P = 0.05 or less.

<sup>2/</sup> Significant at P = 0.01 or less .

<sup>3/</sup> Significant at P = 0.10 or less.

Table Q. Analyses of variance for shearing force of leg muscles from lambs in Part IV.

Sources of variation	Degrees of freedom	Mean squares	
		Semimembranosus	Biceps femoris
Protein	2	109.96 <sup>1/</sup>	9.3136
Linear	1	205.34 <sup>1/</sup>	0.4266
Stilbestrol	2	45.18 <sup>2/</sup>	0.2519
Linear	1	18.37	0.4538
Quadratic	1	72.00 <sup>2/</sup>	0.0501
Stilbestrol x protein	4	23.02	7.9444
Experimental error A	9	10.11	17.3633
Leg	1	13.93	73.3878 <sup>1/</sup>
Leg x protein	2	15.82	1.0770
Leg x stilbestrol	2	4.66	0.1003
Leg x stilbestrol x protein	4	4.83	1.1519
Experimental error B	9	14.02	3.3468
Total	35		

<sup>1/</sup> Significant at P = 0.01 or less.

<sup>2/</sup> Significant at P = 0.05 or less.

Table R. Analyses of variance for connective tissue components of longissimus dorsi from lambs in Part IV.

Sources of variation	Degrees of freedom	Mean squares					
		Percent of total nitrogen			Percent of moisture-free, fat-free tissue		
		Collagen	Elastin	Hexosamine	Collagen	Elastin	Hexosamine
Stilbestrol	2	.0133	.1107 <sup>2/</sup>	.011363 <sup>3/</sup>	.000021	.002597 <sup>5/</sup>	.032273 <sup>3/</sup>
Linear 1		.0121	.2204 <sup>3/</sup>	.012973 <sup>3/</sup>	.000024	.004902 <sup>4/</sup>	.051152 <sup>3/</sup>
Quadratic 1		.0145	.0010	.009754 <sup>5/</sup>	.000018	.000292	.013394 <sup>4/</sup>
Protein	2	.0138	.0164	.000286	.000394	.000778	.002540
Linear 1		.0045	.0294	.000337	.000704	.001536	.000925
Quadratic 1		.0232	.0035	.000235	.000085	.000020	.004156
Stilbestrol x protein	4	.0461	.0146	.001512	.000501	.000301	.002166
Replicate	3	.0180	.3564 <sup>3/</sup>	.000960	.001893	.007661 <sup>3/</sup>	.000105
Replicate x stilbestrol	6	.0057	.0811 <sup>4/</sup>	.002051	.000417	.001114	.003927
Replicate x protein	6	.0064	.0301	.000693	.000074	.001156	.001733
Experimental error	8 <sup>1/</sup>	.0322	.0112	.001576	.001180	.000475	.001757
Total	31						

<sup>1/</sup> Four missing values were computed for analyses, so degrees of freedom for error and total were reduced by four.

<sup>2/</sup> Significant at P = 0.01 or less.

<sup>3/</sup> Significant at P = 0.005 or less.

<sup>4/</sup> Significant at P = 0.025 or less.

<sup>5/</sup> Significant at P = 0.05 or less.



Table S. Correlation coefficients for data from lambs in Part IV.

	Correlation coefficient
<u>Coefficients discussed in Part IV.</u>	
1. Skin wt. x pelting score	.21
2. Shear value of semitendinosus x organoleptic tenderness score	-.17
3. Collagen N as percent of total N x elastin N as percent of total N	-.28
4. Collagen N as percent of total N x ratio of hexosamine:total N (mult. by 100)	.06
5. Elastin N as percent of total N x ratio of hexosamine:total N (mult. by 100)	.31
<u>Connective tissue components of longissimus dorsi x shear values or tenderness scores of leg muscles.</u>	
6. Collagen N as percent of total N x organoleptic tenderness score	-.06
7. Elastin N as percent of total N x organoleptic tenderness score	-.13
8. Ratio of hexosamine:total N (mult. by 100) x organoleptic tenderness score	-.23
9. Collagen N as percent of total N x shear value of semitendinosus	.33
10. Elastin N as percent of total N x shear value of semitendinosus	-.24
11. Ratio of hexosamine:total N (mult. by 100) x shear value of semitendinosus	-.17

VITA

Duane Calvin Acker  
Candidate for the Degree of  
Doctor of Philosophy

Thesis: SOME EFFECTS OF DIETHYLSTILBESTROL AND RELATED HORMONES ON FEED-  
LOT PERFORMANCE, RATION DIGESTIBILITY AND CARCASS QUALITY OF  
LAMBS AND BEEF CALVES

Major Field: Animal Nutrition

Biographical:

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