INFLUENCE OF DIETHYLSTILBESTROL UPON PROTEIN UTILIZATION IN RUMINANTS

by

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INTRODUCTION

The knowledge of nutrition has expanded rapidly during the first half of the Twentieth century. During this period important contributions have been made relating to the discovery, function, and essential nature of many nutrients. Some of the amino acids, many of the minerals, and all of the recognized vitamins have been discovered and their functions evaluated. With the development of biochemistry and the related biological sciences, it would seem unwise to predict that in the future no new vitamin, amino acids, enzyme or growth factor would not be discovered. Even basic concepts, such as the calorie-protein relationship are being constantly changed. The field of nutrition is not in an equilibrium state;it is constantly being altered as new discoveries are being introduced.

During this same period the importance of hormones has been realized and a new field of nutritional application is being developed in this area. As a result, unknown relationships between known fundamental nutritional facts and hormone action must be investigated. As these unknown problems are solved, as is true to science, others arise.

Diethylstilbestrol (stilbestrol), a synthetic hormone, has made its application in animal nutrition. Feedlot acceptance of the discovery of stilbestrol has been exceedingly prompt and the results outstanding. Its use in cattle and lamb

feeding manifests greater feed efficiency and rate of gain. The explanation for increased feed efficiency is not apparent, however, increased growth and feed efficiency suggests that protein metabolism is being altered.

Since nitrogen retention is associated with growth, it would seem suggestive that nitrogen balance studies would be an ideal tool with which to study the mechanism of stilbestrol on growth. For this reason, one objective of this study was to determine the influence of stilbestrol upon protein utilization in wether lambs receiving different levels of stilbestrol, protein, energy, and kind of protein.

Since growth is dependent upon growth (somatotrophic) hormone, a further purpose of this study was to assay the pituitary glands of stilbestrol treated steers in an attempt to correlate any increase in growth of steers from stilbestrol treatment with the size and growth hormone content of the anterior pituitary gland.

A final objective of this investigation was to study the relationship between growth hormone and stilbestrol administration on protein utilization in lambs receiving two different levels of protein and energy intake.

REVIEW OF LITERATURE

It is beyond the scope of this thesis to review completely the basic concepts of nutrition that affect nitrogen balance in animals. It is, however, seemingly important to review the basic calorie-protein relationship and its application to nitrogen balance and growth.

It has been known for some time that a calorie-protein relationship exists, and only recently has this aspect been reinvestigated and applied to poultry management, and to a limited degree, in swine feeding. No new data on calorie-protein ratio has been obtained recently in ruminant nutrition, but if there exists an optimum calorie-protein relationship in laboratory animals and in poultry, where nutritional requirements are more sensitive, then it is reasonable to expect an optimum caloric-protein relationship for ruminants as well.

Rosenthal and Allison (54) studied the response of rats subjected to caloric restriction but fed a constant nitrogen intake of 250 mg. of casein daily. On an energy intake of 43 calories per day, the rats maintained body weight and retained 50 mg. of nitrogen daily. When the energy intake was increased to 74 calories daily, the rats increased 24 per cent in weight and retained 100 mg. of nitrogen daily. If, however, on the same nitrogen intake energy was restricted to 18 calories per day per rat, a 33 per cent loss of weight oc-

curred and the nitrogen balance was near equilibrium, a condition, where the workers point out, the overall catabolic activity of the labile protein stores were probably reduced to conserve nitrogen. This work demonstrates that animals may manifest a positive nitrogen balance when subjected to a dietary caloric deficiency, although the reduction in nitrogen balance that follows lowered energy intake is characterized with a loss of labile stores. Therefore, an animal on a restricted caloric intake could remain on a positive nitrogen balance until body energy pools are depleted.

To demonstrate the relationships between nitrogen intake and its utilization, Callaway and Spector (19) restricted rats to one-half of their caloric requirements and fed egg albumin nitrogen from zero to 604 mg. daily per animal. Positive nitrogen balance was never attained and the degree of negative retention was inversely proportional to nitrogen intake.

Similar work along this line was reported by Muno and Naismith (46) who, using the nitrogen balance technique, studied the effects of different ranges of energy intake on nitrogen metabolism in rats. On diets providing low amounts of protein, these workers found that increments of energy intake resulted in a linear improvement in nitrogen retention. When the diet contained no protein, additions of energy failed to improve materially nitrogen balance.

Bosshardt, et al. (13) reported that in rats there was a marked reduction in protein utilization when caloric intake

was restricted to below 80 per cent of the maximum obtained in ad libitum feeding. Under similar conditions in mice, it was demonstrated that a sharp reduction in protein utilization occurred when the caloric intake was reduced to 95 per cent of the maximum intake obtained under ad libitum feeding conditions. In dogs, Allison, et al. (2) reported that utilization of dietary protein, as measured by nitrogen balance, was not affected until the caloric intake was reduced to 50 per cent or below the normal allowance.

Lofgreen, et al. (41) investigated the calorie-protein relationship by conducting nitrogen balance studies on dairy calves when they reached 150, 200, 250, and 300 pounds. The calves were fed the following dietary regimes: low energy-low protein, high energy-low protein, low energy-high protein, and high energy-high protein. Their results indicated that on a low protein intake a 20 per cent increase in non-nitrogen TDN (total digestible nutrients) increased nitrogen retention significantly, whereas increasing the energy in calves fed the high protein intake did not result in better protein utilization. It was of interest to note that there was no significant difference in liveweight gains between groups of calves fed the same protein intake.

In using the nitrogen balance technique on wether lambs, Woods, et al. (63) studied protein utilization by feeding a low quality timothy hay supplemented with soybean meal to increase the protein content of the ration to approximately

7.0, 11.0, and 13.6 per cent and added cerelose in increasing increments of 0.0, 3.3, and 6.2 per cent of the rations. Lambs fed the 7.0 per cent protein ration exhibited negative nitrogen balance, even on the high cerelose intake. Lambs fed the 11.0 per cent protein ration exhibited a negative nitrogen balance without cerelose supplementation; with cerelose lambs manifested a positive balance. Further increasing of the protein content to 13.6 per cent did not result in any significant increase in nitrogen retention over the 11.0 per cent protein ration. The nutrient intake for all treatments may have been low because in no treatment did any lamb retain over 0.90 gm. of nitrogen daily.

Fontenot, et al. (28) also made studies on the effect of energy intake on nitrogen utilization using steers fed a wintering ration consisting of prairie hay and cottonseed meal and supplemented with varying amounts of cerelose. Daily additions of 700 and 1050 gm. of cerelose resulted in a significant increase in nitrogen retention when the steers were fed the 12 per cent protein ration. If, however, cerelose was added to steers fed an 8 per cent protein ration, a depression of nitrogen retention resulted. The authors indicated that addition of cerelose was accompanied by a decrease in apparent protein and fiber digestibility. This may account for the decrease in nitrogen retention on the low level of protein intake.

Further importance of energy in sparing protein was

demonstrated by Robinson, et al. (53) who fed Hereford steers a 9.5 per cent protein ration consisting of 3000 gm. of prairie hay and one pound of cottonseed meal. Supplementation of this ration with 200 gm. of corn oil increased nitrogen retention from 21.1 per cent of the dietary intake to 24.7 per cent. The fecal nitrogen was not affected.

If energy considerations are deemed important in the interpretation of nitrogen balance data, then the age old question of ad libitum versus paired-feeding technique becomes more important. The paired-feeding method has been used by most workers in large animal nitrogen balance studies and many advances have been made by its use. However, this technique is not adapted to all nutritional studies.

Barnes, et al (10) studied the growth-promoting quality of proteins when fed to rats and concluded that the paired-feeding method gave an entirely artificial value for the better protein. These workers preferred the ad libitum feeding method because if food restriction was severe some of the higher quality protein was wasted for energy for which sucrose or fats could have provided as well. About twice as high protein efficiency (gain in body weight per gm. of protein consumed) was obtained with ad libitum as compared to the controlled feeding of rats and mice.

Boutwell, et al. (14) believe that the ad libitum method of feeding does not penalize the most efficient ration and serves to emphasize any nutritional differences. These workers

state:

> We think that we are correct when we say that no new dietary factor has ever been disclosed by the use of the paired-feeding method. A control animal limited in its intake to the amount of feed consumed by an animal suffering from a deficiency must sooner or later have its consumption reduced to zero. Ultimately that is exactly the consumption level reached by an animal fed a deficient ration. Only short-time records will obliterate such a condition unless internal synthesis wholly or partially intervenes.

The question whether an animal which is not allowed to satisfy its appetite is comparable with one that eats as much as it wishes is especially important in quantitative nitrogen balance studies. Brody (15) pointed out that there is no sharp dividing line between ad libitum and paired-feeding methods and that there are occasions for using both, depending on circumstances.

The remarkable gains and feed efficiency which result in feeding or implanting stilbestrol in cattle and sheep suggests that increased digestibility of nutrients might occr. Some of the earliest workers reporting on digestion and nitrogen balance studies with stilbestrol were O'Mary, et al. (48) who indicated that the greater "efficiency" of stilbestrol in lambs did not appear to be in an increase in apparent dry matter or protein digestibility. About the same time Clegg (21) used two steers per treatment and found that the total nitrogen retained per day by the stilbestrol implanted steers was twice in excess of the controls. A somewhat different picture was presented by Jordan and Bell (38). These workers

did not find any significant difference in the amount of nitrogen retained between the two groups of lambs. Neither was there any difference in digestibility of nutrients. In the following year, Jordan (37) reported that stilbestrol treatment increased nitrogen retention 30 per cent when lambs were fed a ration of shelled corn and good quality alfalfa hay. No differences were noted in nutrient digestibility. Less urine was voided by the treated animals which resulted in approximately equal concentrations per ml. of urine. The worker suggested that stilbestrol increased protein anabolism and any differences in nitrogen retention between the two groups of lambs were not due to lower concentrations of nitrogen in the urine but rather to a smaller amount of urine voided.

Clegg and Cole (22) made a rather extensive study on the action of stilbestrol on the growth response in ruminants. A phase of their study included a balance trial on steers and their results indicated that implanted stilbestrol did not affect fecal nitrogen, however, urinary nitrogen decreased. As a result, nitrogen retention was almost doubled.

Richardson, et al. (51) conducted digestion trials on 11 steers serving as their own controls and reported that stilbestrol significantly lowered the digestibility of crude protein, crude fiber, ether extract, nitrogen free-extract and also the per cent of TDN[a]. The rations consisted of three

[a]Total digestible nutrients.

parts of cracked milo and one part of alfalfa hay.

An *in vitro* study made by Brooks, *et al.* (16) indicated that stilbestrol increased cellulose digestion significantly. These workers also reported that stilbestrol treatment in wethers increased cellulose digestion from 42 to 48 per cent and likewise increased protein digestion from 38 to 44 per cent. However, this writer (59) using the artificial rumen, could not obtain any increase in cellulose digestion from stilbestrol.

Thompson, *et al.* (60) did not find any differences in the digestibility of ration constituents in stilbestrol treated wethers fed a ration of 650 gm. of timothy hay and 143 gm. of soybean meal daily. The nitrogen balance results showed that the four control lambs retained 0.74 gm. of nitrogen daily. The addition of 2.5mg. stilbestrol daily increased nitrogen retention to 0.93 gm. Stilbestrol intake of 5 gm. daily increased nitrogen retention further to 1.35 gm. daily per lamb. These lambs appeared to be on a low level of feed intake which may account for the low nitrogen retention values.

Bell, *et al.* (11) made calcium and phosphorus, as well as nitrogen, balance studies on lambs fed stilbestrol. Four mg. of stilbestrol daily did not affect the digestibility of ration components, however, the treated lambs retained 4.9 gm. of nitrogen daily per lamb as compared to 3.9 for the controls. This value was significant. Based on nitrogen balance studies,

there was no carry-over effect of stilbestrol once it was discontinued in the ration. Stilbestrol also increased calcium and phosphorus retention significantly, even after stilbestrol was stopped.

Feeding lambs a semi-purified ration with wheat straw as the source of fiber and containing 1.6 per cent nitrogen of which two-thirds of the nitrogen was provided by urea, biuret or a combination of these materials, Campbell, et al. (20) obtained a significant increase in nitrogen utilization when each lamb received 2 mg. of stilbestrol daily. Dry matter and protein digestibility were not influenced by stilbestrol treatment. Based on nitrogen balance results, it was interesting to note that any possible carry-over effect of stilbestrol in lambs was lost in ten days.

Erwin, et al. (27) using the lignin ratio technique did not find any significant differences in the digestibility of ether extract, crude protein, crude fiber or dry matter in stilbestrol treated steers as compared to controls. Acker (1) did not find any difference in protein digestibility coefficients upon feeding lambs 2 mg. of stilbestrol daily. However, Story, et al. (58) reported that nitrogen retention, likewise protein and dry matter digestibility coefficients, were enhanced in lambs fed increasing levels of stilbestrol. A somewhat different result is presented by Grainger (31) who reported that feeding 0.5 mg. of stilbestrol per pound of ration to wether lambs produced a significant decrease in the

digestibility of the protein and fiber in the ration.

Lee and Schaffer (40) were among the earliest workers to investigate the effects of growth hormone administration upon the gain in weight in rats and upon the composition of that gain, with the factor of food consumption being carefully controlled. These investigators selected rats in quadruple, all of the same litter, sex, and weight. One animal was chosen for a control, a pair-fed mate was chosen to be treated with the growth hormone. The third rat was designed as a "check" to be killed at the beginning of the experimental period and its analyzed carcass composition was assumed to be the composition of its two litter mates at that time. The fourth rat was treated with growth hormone and fed ad libitum. In nitrogen balance studies, the pair-fed, growth hormone treated rats retained more nitrogen than their controls and carcass studies revealed a higher content of water and a lower content of fat than the controls. These pair-fed treated animals retained almost all of their initial composition in all constituents and in the energy value of their tissues.

Gordon, et al. (30) made several studies on growth hormone treatment in rats fed a diet that varied in the quality and quantity of protein. It was demonstrated that growth hormone treated rats fed a 6 per cent casein diet produced nitrogen retention without gain in body weight. With the addition of DL-methionine to this diet, growth hormone produced nitrogen retention and concomitant weight gains. When the

dietary casein content was increased to 12, 18, 24, and 48 per cent without amino acid supplementation, the average daily nitrogen retention produced upon growth hormone administration was 67, 92, 119, and 121 mg. per rat per day, respectively. Likewise, the amount of body gain increased with increasing dietary protein until the 24 per cent casein level was reached. These data also indicated that there was a correlation between the amount of nitrogen retained and the body weight gain during the five day injection period.

Hall and Bieri (33) reported that growth hormone administration stimulated body growth in rats fed an adequate diet, but was ineffective in prevention weight loss when the diet was deficient in choline.

In a study dealing with the endocrine aspects of growth in swine, Baird, et al. (8) assayed the anterior pituitary glands for growth hormone. These pigs were slaughtered at various ages and belonged to two genetically different lines which were selected for rapid and slow rates of growth. When expressed in relation to body weight, the thyroid and the pituitary glands from the rapid-line pigs contained significantly greater amounts of growth hormone per unit of tissue than did the same amount of tissue from the slow-line. It is interesting that within each line the hormone potency per unit of anterior pituitary gland remained constant with age. These authors indicated that the thyroid and pituitary gland weights in swine are a function of body weight, i.e., pitui-

tary gland weight is dependent upon total body weight and a given amount of pituitary tissue produces the same amount of growth hormone, irrespective of the age or the rate the animals are growing. Furthermore, the ratio of anterior pituitary gland weight generally decreases with age.

Armstrong and Hansel (4) made biological assays on the anterior pituitary glands of Holstein heifers raised on low, medium, and high levels of nutrition, and which were slaughtered at different ages in an attempt to relate any differences in growth rate to growth hormone and thyrotropin production. Based on growth hormone content per gram of anterior pituitary tissue and also on growth hormone content per 100 pounds of body weight, the workers found that a greater concentration of growth hormone was contained in the pituitary glands of the younger animals that were making the fastest gains; this growth hormone content gradually decreased with age of the animals. No differences in any of the above measurements could be detected due to the plane of nutrition. The authors believe that quantities of growth hormone detected in pituitary gland bioassays are fairly close approximations of the actual secretion rate of the growth hormone. Furthermore, these workers believe that differences in rate of growth of heifers of different ages are at least partly due to differences in the amount of growth hormone and thyrotropin being produced, but the differences in growth rates of heifers on different feed intakes are due to the dif-

ferences in the amount of nutrients available for growth, rather than differences in growth hormone production.

Baker, et al. (9) assayed the anterior pituitary glands of swine to compare growth hormone potency with growth rate throughout most of the life span which ranged from fetal to maturity and pregnancy. Their results indicated that the anterior pituitary gland weight increased rapidly until the age of 225 days, after which the rate of increase slowed but continued. The ratio of anterior pituitary weight to body weight decreased until the age of 225-300 days when the ratio became constant. Neither breed nor reproductive states had any effect on pituitary growth potency. The authors believe that reduction in growth rate in the "mature" animal is due to the "dilution" of the available circulating growth hormone to the point where the body can no longer respond to growth to the lowered concentration.

To study the effect of stilbestrol on protein utilization in ruminants, Clegg and Cole (22) conducted the aforementioned nitrogen balance studies and made detailed studies of endocrine glands of stilbestrol tested steers. They reported that the pituitary and adrenal glands of the treated steers and heifers were larger than the controls, whereas the thyroid glands were only slightly larger. The pituitary assay of the treated heifers indicated that the growth hormone content per gm. of the anterior tissue was approximately twice that of the controls. In contrast, the stilbestrol treated steers had less

than the untreated steers, however, since the treated steers had larger pituitaries the total units of growth hormone potency was similar between the two groups. Using the regression of the thymus weight in four-day-old rats as the assay method, the ACTH (adrenocorticotropic hormone) content of the treated steer pituitary glands were not different from the controls. It is of interest to note that the treated steers gained markedly faster than did the controls, whereas the treated heifers manifested only a small increase in gain over the controls. The authors believe that stilbestrol administration in ruminants results in anterior pituitary hypertrophy. This results in increased ACTH secretion which in turn caused adrenal hypertrophy and an accompanying increase in androgen production. They theorize that the androgens in turn accelerate protein anabolism.

EXPERIMENTAL PROCEDURE

This thesis is concerned with the influence of stilbestrol upon growth and protein utilization in ruminants. Nitrogen retention is generally regarded as a sensitive index for growth. To investigate the effects of stilbestrol upon growth and protein utilization, a series of six nitrogen balance and digestibility studies were conducted using wether lambs as the experimental animal. For the first five balance studies, the lambs were fed a basal ration consisting of approximately 8, 14, and 20 per cent protein, which according to the National Research Council[1] standards are considered, respectively, low, adequate, and high in protein needs for lambs. These rations varied in amino acid composition and were fed at different energy intakes. The sixth and final balance study was made to investigate the relationship between growth hormone and stilbestrol on nitrogen retention in lambs. The lambs received a ration which was either low or adequate in protein and was fed at either a low or high level of energy intake.

Since the anterior pituitary gland is the site of growth hormone secretion, the final phase of this study included a growth hormone bioassay of the anterior pituitary glands of

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[1]National Research Council. Recommended nutrient allowance for sheep. 2101 Constitution Avenue, N. W., Washington 25, D. C. National Research Council. 1949.

stilbestrol treated steers in an attempt to relate any increase in growth stimulation from stilbestrol with the size and growth hormone content of the anterior pituitary gland.

General Lamb Experimental Procedure

The experimental procedure as described under this heading applies to all of the lamb experimental studies.

Wether lambs of approximately uniform breeding and of western origin were used for the nitrogen balance experiments. After their arrival at Iowa State College, the lambs were fed and housed on the campus for approximately a two to three week adjustment period. During this time the lambs were vaccinated simultaneously for sore mouth (contagious exanthema) and overeating disease (enterotoxemia). Also, within this period the animals were gradually accustomed to a grain-type ration. Salt and water were available before the lambs at all times and wood shavings served as bedding.

For the collection studies, the animals were randomly allotted within weight outcome groups and assigned to the stilbestrol treatments. Some lambs were used for two experiments. When this procedure occurred, the lambs were group fed a common ration for at least 18 days, relotted at random, irrespective of previous treatments, using outcome groups to the stilbestrol treatments. At least a 28-day period elapsed before any lamb was again placed in the metabolism cage.

During the preliminary feeding period, each lamb in Experiments 1, 2, and 3 was fed his ration from an individual self-feeder twice daily. For the remainder of the experiments the lambs during the preliminary period were confined to a small individual pen in the metabolism barn and were allowed access to their feed at all hours of the day. Preliminary feeding periods were of at least 10 days duration and the collections were made for six days. After the lambs were placed in the metabolism cages, a one-day period lapsed before collections began. Feces were collected in canvas bags and transferred at frequent intervals to metal containers over a three day interval. The feces were then placed in a forced air drying oven until a constant weight was reached. At the completion of the collection period, the dried feces from each lamb were pooled, thoroughly mixed and a sample ground in a Wiley mill for nitrogen analysis. Urine was collected in gallon glass jugs into which 10 ml. of toluene had been added as a preservative. Testing the urine twice daily with litmus paper, just enough 6 N sulfuric acid was added to keep the urine in an acid condition. At the end of the 6-day collection period, the urine was pooled, measured, and a sample taken for analysis. During the warmer summer season, each day's collection of feces was refrigerated until placed in the oven for drying. The urine was likewise refrigerated daily. The ration and excreta samples were analyzed in duplicate for total nitrogen by the Kjeldahl pro-

cedure.

One week prior to the collection period, feed intake for all lambs, except the lambs in Experiment 1, were equalized on a weight basis using the following equation (42):

$$\text{Lb. feed for maintenance} = \frac{.0436\ W^{.73}}{\%\ \text{TDN}}$$

TDN (Total digestible nutrients) values were estimated using Morrison's tables (45). Likewise, the energy value assigned to the rations were based on Morrison's TDN values. Cobalt was added to all the rations to supply 0.5 parts per million. The lambs were fed twice daily, receiving one-half of their ration at each feeding. The temperature of the metabolism barn during the winter months was maintained at approximately 45° F. During the summer months the temperature could not be controlled and, therefore, varied with the environmental temperature.

Statistical treatment of the data were analyzed according to Snedecor (56). The use of the term significant designated statistical significance at P = .05 or less; highly significant indicated P = .01 or less. The deleted values in the tables shown in the Results section represented, in most cases, feed refusals. The lambs' weights in these tables are expressed as the average beginning and final weight during the collection period. For the sake of brevity, certain words will at times be abbreviated, namely, as follows:

TDN - Total digestible nutrients

DES - Diethylstilbestrol

NRC - National Research Council

AP - Anterior pituitary gland

LE - Low energy

HE - High energy

Lamb Experiment 1. (Fall and winter, 1955-1956)

The purpose of this experiment was to determine the effect of stilbestrol on nitrogen retention in lambs which were fed a ration that was low (8 per cent) or adequate (14 per cent) in protein according to the National Research Council standards (47).

The lambs were placed on a nitrogen balance and digestibility study and each lamb was fed one pound daily of the following experimental ration:

Ingredients	Protein per cent	
	8	14
Molasses	10.0	10.0
Ground cobs	25.0	25.0
Ground alfalfa hay	15.0	15.0
Cracked corn	49.5	34.8
Soybean meal	---	15.0
Dicalcium phosphate	0.3	0.2
Sodium diphosphate	0.2	---
	100.0	100.0

The rations contained approximately 8 or 14 per cent protein. Since a constant feed source was not available for the duration of the trial, the protein percentage varied slightly from time to time between collection periods. Stilbestrol was added in

both the 8 and 14 per cent protein rations at the rate of 0, 300, 600, and 1200 mcg. to each pound of mixed ration. Stilbestrol was incorporated in the feed mixtures containing this material by dissolving a known quantity of stilbestrol in 95 per cent ethanol and mixing this solution in finely ground corn. This pre-mixture was then incorporated into the remainder of the mixed ration by the use of a mechanical mixer. The energy content of the rations was then assumed to be equal with an estimated 64 per cent TDN. Block salt and water were provided ad libitum in the metabolism cages.

A second collection was made on some of the lambs with either a two or three week interval spaced between collection periods. Using this procedure, six collections were made from four lambs receiving the 8 per cent protein ration and ten collections were made from six lambs fed the 14 per cent protein ration.

Lamb Experiment 2. (April and May, 1956)

The purpose of this experiment was to determine the effect of stilbestrol on nitrogen retention and digestibility of nutrients in lambs fed either an adequate (14 per cent) or an excess (20 per cent) of protein in the ration.

Sixteen lighter weight lambs of Experiment 1 and weighing approximately 90 pounds were placed on a nitrogen balance experiment and fed the following rations:

Ingredients	Protein per cent 14	20
Cracked corn	34.8	18.8
Soybean meal	15.0	31.1
Ground cobs	25.0	25.0
Ground alfalfa hay	15.0	15.0
Molasses	10.0	10.0
Dicalcium phosphate	0.2	0.1
	100.0	100.0

The ingredient composition in this 14 per cent protein ration was the same as the 14 per cent protein ration of the previous trial. The only essential difference between the two ration ingredients was a substitution of soybean meal for corn to alter the protein per cent. The energy content between the two rations was assumed to be equal with an estimated TDN value of 64 per cent. Stilbestrol was added to the mixed rations to those animals receiving this treatment to supply 2.3 mg. of stilbestrol daily per lamb. Feed intake was adjusted to an estimated 150 per cent of body maintenance requirements. Duplicated collections were made with each lamb with a week's interval spaced between collection periods.

Lamb Experiment 3. (June through August, 1956)

The purpose of this experiment was to determine the effect of stilbestrol upon nitrogen retention in lambs fed an 8 or 14 per cent protein ration in which a large part of the protein was supplied from either alfalfa or soybean meal.

A new group of lambs was obtained for this nitrogen balance and digestibility study. The basal rations fed to the

experimental animals are shown in Table 1. On each level of protein intake either alfalfa or soybean meal protein dominated a large part of the protein in the ration. Eight hundred mcg. of stilbestrol were added to each pound of mixed

Table 1. Composition of rations fed to lambs in Experiment 3

Protein per cent	8				14			
Designation	C[a]	DES[b]	C	DES	C	DES	C	DES
Treatment number	1	2	3	4	5	6	7	8
Gr. alfalfa hay	40.0		14.7		40.3		15.0	
Soybean meal	4.0		11.0		17.0		24.0	
Starch	45.0		38.0		32.0		25.0	
Ground cobs	0.0		25.0		0.0		25.0	
Molasses	10.0		10.0		10.0		10.0	
Dicalcium phosphate	0.0		1.0		0.0		1.0	
Sodium phosphate	1.0		0.3		0.7		0.0	
	100.0		100.0		100.0		100.0	

[a]Control treatment.

[b]Stilbestrol treatment.

feed to those animals receiving this treatment. Also, to each pound of mixed ration was added 5000 USP units of vitamin A.

Two collections were made on the same lamb with a week's

interval spaced between collection periods. Feed intake was adjusted to an estimated 150 per cent of body maintenance requirements, based on the following percentages of TDN values: Treatments 1 and 2, 69.5; 3 and 4, 67.5; 5 and 6, 68.0, and 7 and 8, 66.0. The detailed experimental procedure was essentially the same as that described in Experiment 1 and in the General Experimental Procedure section, except that each lamb was drenched with 0.7 ounces of phenothiazine five days post-vaccination for the treatment of internal parasites. In addition, Stilbosol[a] was now used to supply the stilbestrol added in the ration and the feces bags were discontinued. In substitution, the feces were collected on an 8 x 8 hardware cloth placed over the urine pan.

Lamb Experiment 4. (September and October, 1956)

The purpose of this experiment was to determine the effect of stilbestrol on nitrogen retention in lambs fed a 14 per cent protein ration in which the protein was supplied essentially from alfalfa. In addition, treatments 5 and 6 of the previous trial were repeated which consisted of stilbestrol treated lambs fed a 14 per cent protein ration in which only a part of the protein was supplied from alfalfa.

Twenty-four wethers weighing approximately 65 pounds were placed on a nitrogen balance and digestibility study and fed

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[a]Diethylstilbestrol premix, Eli Lilly & Co., Indianapolis, Indiana.

the following rations:

Ingredients	Protein per cent 14	14
Ground alfalfa hay	85.5	39.3
Soybean meal	2.5	17.0
Starch	0.0	32.0
Molasses	10.0	10.0
Salt	1.0	1.0
Sodium phosphate	1.0	0.7
	100.0	100.0

It is noted that both rations contained 14 per cent protein. As in the previous experiment, 800 mcg. of stilbestrol were added to each pound of mixed ration to those animals receiving this treatment. Feed intake was adjusted to an estimated 150 per cent of body maintenance requirements based upon a TDN value of 50 per cent for the high-alfalfa ration and 68 per cent for the low-alfalfa ration. One per cent salt was added to the mixed ration rather than providing salt ad libitum. The remainder of the experimental continuity was essentially a repetition to that described in Experiment 3.

Lamb Experiment 5. (November and December, 1956)

The objective of this experiment was to determine the effect of different levels of stilbestrol and energy intake on nitrogen balance in lambs fed a 14 per cent protein ration, which in the two previous nitrogen balance studies failed to enhance nitrogen retention in stilbestrol treated lambs when fed at a low level of energy intake.

Thirty-six wether lambs weighing approximately 75 pounds

were placed on a nitrogen balance experiment and fed the following ration:

Ground alfalfa hay	39.3
Soybean meal	17.0
Starch	32.0
Molasses	10.0
Salt	1.0
Sodium phosphate	0.7
	100.0

It is noted that this 14 per cent protein ration was formulated similar to the one fed in treatment 5 and 6 of Experiment 3, and similar to the low-alfalfa ration fed in Experiment 4. Stilbestrol was added at the rate of 0.8 and 1.2 mg. per pound of mixed ration to those lambs receiving this treatment. In one comparison, feed intake was purposely controlled at an estimated 210 per cent of body maintenance requirements for each level of stilbestrol addition. In the other comparison, feed intake was controlled to 150 per cent of maintenance requirements. The ration was estimated to contain 68 per cent TDN. The manner of handling the lambs, making the collections, sampling, and analyzing the excreta were similar to those described for Experiment 4.

Lamb Experiment 6. (January through March, 1957)

The purpose of this experiment was to determine the effect of growth hormone and stilbestrol, alone or in combination, upon nitrogen retention and digestibility of nutrients in lambs receiving either a low (8.5 per cent) or medium (13.0 per cent) protein ration which was fed at either a low or high

level of energy intake.

Lambs were placed on a nitrogen balance experiment for 36 days and fed an 8.5 or a 13.0 per cent protein ration with and without stilbestrol additions. Treatments were further divided into low energy and high energy intakes for the experimental lambs. Two different groups of 12 lambs were used for two collection series of this experiment. The first collection series of 36 days consisted of the low energy treatment. Feed intake was estimated to be 110 per cent of body maintenance requirements. This collection was conducted on 12 of the lighter weight lambs of the previous experiment which weighed an average of 81 pounds at the beginning of this collection period. The second collection series of 36 days consisted of the high energy treatment. Feed intake was estimated to be 170 per cent of body maintenance requirements. This collection was conducted on a new group of 12 lambs which weighed an average of 72 pounds at the start of the 36 day collection period.

The first 12 days of each 36 day collection series was designated as a pre-growth hormone collection period. This was immediately followed by a 12 day growth hormone injection and collection period. During this time each lamb received 12.5 mg. of purified growth hormone preparation[a] which was

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[a]Bovine Growth Hormone. Lot No. R-50109. SOMAR-A, The Armour Laboratory, Kankakee, Illinois. Supplied as a gift from the National Institutes of Health.

dissolved in one-half ml. of sterile saline and injected daily in single intramuscular injections. Immediately following the growth hormone injection period was the final 12-day collection period designated as a post-growth hormone collection period.

The excreta were collected and analyzed over a 4-day period. Thus, collections 1, 2, and 3 represented the 12-day pre-growth hormone collection period. Likewise, collections 4, 5, and 6 represented the 12-day growth hormone collection period. Finally, collections 7, 8, and 9 constituted the 12-day post-growth hormone collection period. To study the effects of disturbances resulting from injections, each lamb during collections 2 and 8 on the low energy treatment received daily, one-half ml. of sterile saline injected intramuscularly.

The two rations fed are shown in Table 2. The only difference in ration ingredients between the 8.5 and 13.0 per cent protein rations was a substitution of 11 per cent of corn in place of a similar amount of soybean meal. Stilbosol was added to supply stilbestrol at the rate of 1.2 mg. per pound of mixed ration to those lambs receiving the treatment. The energy intake of lambs was altered by increasing or decreasing the amount of ration fed. The energy content of both rations was assumed to be equal with an estimated 64 per cent TDN.

Table 2. Rations fed to lambs in Experiment 6

Ingredients	Protein per cent	
	8.5	13.0
Soybean meal	0.0	11.0
Cracked corn	44.0	33.0
Ground alfalfa hay	25.0	25.0
Ground cobs	15.0	15.0
Molasses	15.0	15.0
Salt	1.0	1.0
	100.0	100.0

Growth Hormone Bioassay Studies

The purpose of this aspect of study was to assay the anterior pituitary glands of stilbestrol treated steers for growth hormone content. This investigation was conducted in an attempt to relate any increase in growth of steers from stilbestrol treatment with the size and growth hormone content of the anterior pituitary gland.

Pituitary glands were collected from the steers fed as described in Iowa State College Leaflet 211 (35). These steers prior to any stilbestrol treatment were fed a standard fat-

tening ration for an 84-day period for the purpose of allotting the animals into slow and fast gaining groups. At this point the slow and fast gaining groups were allotted and fed a medium protein (10.5 per cent) and a high protein (13.5 per cent) ration. These animals were further divided into stilbestrol treatments of 0, 5, and 10 mg. daily per steer. Twelve lots of six animals each were group fed fattening rations for 203 days. The cattle experimental design was as follows:

DES Treatment	Medium protein ration		High protein ration	
	Low-gainers	High-gainers	Low-gainers	High-gainers
No DES	Lot 1	Lot 2	Lot 3	Lot 4
5 mg. DES	Lot 5	Lot 6	Lot 7	Lot 8
10 mg. DES	Lot 9	Lot 10	Lot 11	Lot 12

The pituitary glands were collected at the time of slaughter, frozen immediately with dry ice and kept in a frozen condition until prepared for assay. Preparation for assay consisted of thawing the pituitary glands of each lot of steers and carefully dissecting the anterior lobe from the surrounding tissue. The anterior pituitary gland was then weighed and further prepared by adding 3 ml. of 0.85 per cent of physiological saline to each gm. of tissue and homogenized in a Potter-Elvehjem type of tissue homogenizer. The resulting solution was centrifuged for 15 minutes at 500 RPM, at 830 x gravity and the decanted extract further diluted with physio-

logical saline to a final volume of 1 gm. of anterior pituitary tissue to 16 ml. of saline. This extract was refrigerated at 1° C. until assayed.

Hypophysectomized female rats of approximately four weeks of age were used as the assay animal. Following a 13-day post-operative period, three rats were randomly allotted to each lot of pituitary material to be assayed. The extracts were administered by intraperitoneal injections of one-half ml. of the prepared extract daily for four days. Twenty-four hours after the last injection the rats were sacrificed, the right tibia dissected, split with a sharp razor blade at the proximal end in the mid-sagittal plane, and stained according to the method of Russell (55). The growth hormone content of the anterior pituitary extracts was estimated by measuring the average width of the uncalcified epiphyseal cartilage of the tibia. The desired response was to obtain a working range between 190 and 340 micra (32). Several preliminary bioassay trials were conducted to determine the extraction procedure of the pituitary glands and the proper dilution of the pituitary extract necessary in meeting this criteria. Since the primary objective of the experiment was to obtain relative differences rather than quantitative determinations of growth hormone potency between the growth hormone content of the pituitaries of stilbestrol treated steers as compared to their controls, no standard curve was established. Instead, any differences in growth hormone potency between pituitary glands were based

on differences expressed in micra, in the width of the uncalcified epiphyseal cartilages of the rat tibiae.

RESULTS

Lamb Experiments

Lamb Experiment 1. (Fall and winter, 1955-1956)

The addition of 0.6 and 1.2 mg. of stilbestrol per pound of ration increased nitrogen retention significantly in lambs fed the 14 per cent protein ration. The control lambs and those receiving 0.6, 1.2, and 2.4 mg. of stilbestrol daily and fed the 14 per cent protein ration retained, respectively, 4.24, 4.22, 5.40, and 4.87 gm. of nitrogen daily per lamb. On the other hand, stilbestrol, regardless of the amount given, failed to enhance nitrogen retention in lambs fed the 8 per cent protein ration (Table 3). Neither apparent dry matter nor protein digestibility appeared to be affected by any level of stilbestrol treatment on either level of protein intake.

Regardless of level of stilbestrol intake, stilbestrol did not alter water consumption to any extent in lambs fed the 14 per cent protein ration. However, the lambs receiving the higher levels of stilbestrol and fed the 8 per cent protein ration consumed approximately the same volume of water as did the lambs fed the higher protein ration and likewise voided approximately the same quantities of urine. It is of interest to note that the lambs on the low protein-high stilbestrol treatments consumed approximately 25 per cent more water and voided a corresponding amount of urine as compared to those

Table 3. Experiment 1. Effect of stilbestrol upon nitrogen retention in lambs fed either an 8 or 14 per cent protein ration (daily basis)

	8 per cent protein ration DES intake/day, mg.				14 per cent protein ration DES intake/day, mg.			
	0.0	0.6	1.2	2.4	0.0	0.6	1.2	2.4
Number of lambs	6	4	6	4	10	10	10	10
N. intake per kg. of body weight, gm.	0.34	0.36	0.36	0.35	0.57	0.56	0.55	0.54
Nitrogen in feed, gm.	12.09	12.18	12.10	12.08	19.44	19.44	19.44	19.44
Nitrogen in feces, gm.	5.68	5.99	5.83	5.91	5.37	5.63	5.35	5.72
Nitrogen digested, %	53.0 ±1.11[a]	50.8 ±2.47	51.8 ±1.62	51.1 ±2.45	72.4 ±0.89	71.0 ±1.08	72.5 ±0.75	70.6 ±0.75
Water intake, ml.	1402	1486	1801	1896	1899	2164	2153	1945
Urine volume, ml.	358	358	706	721	924	938	992	748
Creatinine[b], gm.	---	---	---	---	1.57	1.36	1.36	1.82
Nitrogen in urine, gm.	3.28	2.65	2.98	3.07	9.83	9.59	8.69	8.85
Nitrogen retained, gm.	3.14 ±0.15	3.54 ±0.30	3.29 ±0.29	3.10 ±0.50	4.24 ±0.30	4.22 ±0.26	5.40[c] ±0.53	4.87[c] ±0.58

[a]Standard error of the mean.

[b]Creatinine values average of four lambs out of 10 lambs used per treatment.

[c]Treatment effect significant at the 5% level of probability.

lambs on the low protein-low stilbestrol treatment. Within all treatments, urine elimination was correlated with water intake.

Using four lambs for each level of stilbestrol treatment, creatinine values were determined in lambs fed the 14 per cent protein ration in an attempt to relate any correlation between nitrogen retention and creatinine excretion. No consistant creatinine excretion pattern was detected.

Feed intake in this experiment was regulated to 2 pounds daily per lamb, regardless of body weight. This gave an energy intake value of approximately 125 per cent of that calculated to cover body maintenance requirements (Table 4). Even on this low level of feed intake, difficulty was experienced in getting some of the lambs to consume the low protein ration. Lambs fed the 8 per cent protein ration exhibited lower apparent dry matter and protein digestibility coefficients than did those lambs fed the higher protein ration.

Lamb Experiment 2. (April and May, 1956)

Stilbestrol administration in this experiment increased nitrogen retention significantly and by about the same extent, 6.50 gm. of nitrogen daily per lamb, when fed both the 14 and 20 per cent protein rations. Without stilbestrol, the lambs fed the 20 per cent protein ration failed to retain any more nitrogen than those fed the 14 per cent protein ration. Both control groups of lambs retained approximately 4.85 gm. of

Table 4. Experiment 1. Nutrient intake of lambs and effect of stilbestrol upon apparent dry matter digestibility in lambs fed either an 8 or 14 per cent protein ration (daily basis)

	8 per cent protein ration DES intake/day, mg.				14 per cent protein DES intake/day			
	0.0	0.6	1.2	2.4	0.0	0.6		4
Number of lambs	6	4	6	4	10	10		
Weight of lamb, lb.	76	74	76	76	76	77	79	79
DES intake/100 lb. body weight, mg.	0.00	0.81	1.58	3.16	0.00	0.78	1.52	3.04
Energy intake: Level of maintenance based on 64% TDN of ration	1.25	1.28	1.26	1.25	1.25	1.24	1.22	1.22
Dry feed intake, gm.	801	802	802	801	800	800	800	800
Dry feces, gm.	260	275	262	275	236	249	244	235
Dry matter dig. %	67.5 ±1.08[a]	65.8 ±2.41	67.3 ±0.93	65.7 ±3.31	70.5 ±1.00	68.9 ±0.96	69.5 ±1.04	70.6 ±0.72

[a]Standard error of the mean.

nitrogen daily per lamb (Table 5). Again, as in Experiment 1, neither apparent protein or dry matter digestibility was influenced by stilbestrol treatment. Apparent protein digestibility, however, was greater on the lambs fed the higher protein ration. Lambs consumed more water when fed the higher protein ration. This may be expected because less water is formed from protein than from carbohydrate catabolism. Likewise, more water is needed as a solvent to excrete the catabolic products such as urea. Also, stilbestrol treated lambs consumed more water than did their controls. Urine elimination was related with water intake.

On the basis of 100 pounds of body weight, each lamb received approximately 2.5 mg. of stilbestrol daily (Table 6). This level appeared to give a maximum nitrogen retention in lambs fed the 14 per cent protein ration in the previous trial. The level of protein intake did not appear to influence dry matter digestibility coefficients. All lambs consumed their ration readily on the basis of feed intake adjusted to 150 per cent of maintenance requirements. An indication of uniform feed consumption was indicated by the small error of the mean of the dry matter and protein digestibility coefficients. The missing values in the tables resulted from a prolapsed rectum difficulty in one lamb during his first collection period.

Table 5. Experiment 2. Effect of stilbestrol upon nitrogen retention in lambs fed either a 14 or 20 per cent protein ration (daily basis)

	14 per cent protein ration DES intake/day, mg.		20 per cent protein ration DES intake/day, mg.	
	0.00	2.30	0.00	2.31
Number of lambs	8	6	8	8
N. intake per kg. of body weight, gm.	0.65	0.66	0.96	0.91
Nitrogen in feed, gm.	27.28	26.92	38.54	38.54
Nitrogen in feces, gm.	6.81	6.82	6.97	6.84
Nitrogen digested, %	75.0 ±0.40[a]	74.7 ±0.75	81.9 ±0.40	82.2 ±0.23
Water intake, ml.	2028	2318	2545	2821
Urine volume, ml.	736	815	1244	1528
Nitrogen in urine, gm.	15.58	13.67	26.75	25.10
Nitrogen retained, gm.	4.89 ±0.35	6.43[b] ±0.47	4.82 ±0.33	6.60[b] ±0.63

[a]Standard error of the mean.

[b]Treatment effect significant at the 5% level of probability.

Table 6. Experiment 2. Nutrient intake of lambs and effect of stilbestrol upon apparent dry matter digestibility in lambs fed either a 14 or 20 per cent protein ration (daily basis)

	14 per cent protein ration DES intake/day, mg.		20 per cent protein ration DES intake/day, mg.	
	0.00	2.30	0.00	2.31
Number of lambs	8	6	8	8
Weight of lamb, lb.	90	89	88	93
DES intake/100 lb. body weight, gm.	0.00	2.58	0.00	2.48
Energy intake: Level of maintenance based on 74% TDN of ration	1.43	1.43	1.45	1.39
Dry feed intake, gm.	1039	1026	1039	1039
Dry feces, gm.	300	297	299	283
Dry matter dig.%	71.1 ±0.64[a]	71.1 ±0.88	71.2 ±0.61	72.8 ±0.50

[a]Standard error of the mean.

Lamb Experiment 3. (June through August, 1956)

Nitrogen retention in this experiment was increased from 2.34 to 3.90 gm. daily per lamb when 0.8 mg. of stilbestrol was added to the 14 per cent protein ration that consisted of protein supplied largely from soybean meal. On the other hand, the addition of 0.8 mg. of stilbestrol per pound of ration failed to increase nitrogen retention in lambs fed the 14 per cent protein ration that consisted of protein supplied in a large part from alfalfa. Both the control and stilbestrol treated group fed this ration retained approximately 4.00 gm. of nitrogen daily per lamb. Again, as in Experiment 1, the stilbestrol treated lambs fed either of the two 8 per cent protein rations failed to retain more nitrogen than their controls (Table 7). This suggests that stilbestrol will not increase nitrogen retention in lambs fed a ration low in proteins. Neither was apparent protein nor dry matter digestibility of any of the rations fed influenced by stilbestrol treatment. Once again, as in Experiment 1, the stilbestrol treated lambs fed the 8 per cent protein rations consumed more water and voided more urine than did their controls. Stilbestrol treatment, however, did not influence either water intake or urine voided by lambs fed either of the two 14 per cent protein rations in this experiment.

It should be noted that this trial was conducted under extremely warm weather, and as a result, feed intake was somewhat erratic. Daily stilbestrol intake, on the basis of 100

Table 7. Experiment 3. Effect of stilbestrol upon nitrogen retention in lambs fed largely an alfalfa or soybean meal type protein in either an 8 or 14 per cent protein ration (daily basis)

	8 per cent protein ration Treatment number				14 per cent protein ration Treatment number			
	1	2	3	4	5	6	7	8
DES intake per 100 lb. body wt., gm.	0.00	2.36	0.00	2.32	0.00	2.28	0.00	2.26
Number of lambs	7	8	8	7	8	6	8	7
N. intake per kg. of body weight, gm.	0.39	0.39	0.38	0.38	0.65	0.65	0.64	0.64
Nitrogen in feed, gm.	14.18	14.61	14.16	13.87	25.37	24.99	25.26	25.54
Nitrogen in feces, gm.	7.34	7.51	7.31	7.02	7.36	7.86	7.29	6.97
Nitrogen digested, %	48.2 ±1.95[a]	48.6 ±1.18	48.3 ±2.06	49.4 ±1.59	71.0 ±1.00	68.5 ±1.00	71.2 ±1.32	72.7 ±0.83
Water intake, ml.	2687	3136	3157	3681	4140	4065	4741	4886
Urine volume, ml.	578	1117	1015	1425	1832	1838	2555	2757
Nitrogen in urine, gm.	4.28	4.49	4.94	4.83	13.90	13.21	15.63	14.67
Nitrogen retained, gm.	2.56 ±0.56[a]	2.61 ±0.27	1.91 ±0.36	2.02 ±0.43	4.11 ±0.58	3.92 ±0.69	2.34 ±0.71	3.90 ±0.70

[a]Standard error of the mean.

pounds of body weight (Table 8), was approximately 2.3 mg. for all the lambs receiving this treatment. Stilbestrol treated lambs manifested a slight but probably an insignificant increase in dry matter digestibility in treatment 8 over its control, treatment 7. When cobs constituted a part of the ration composition (treatments 3 and 4, 7 and 8), a slight lowering of dry matter digestibility occurred as compared to each respective protein ration that contained no cobs. It is interesting to note that lambs consumed more water when fed either the 8 or 14 per cent protein rations when cobs constituted a part of the ration ingredients.

Lamb Experiment 4. (September and October, 1956)

A highly significant increase in nitrogen retention resulted in this experiment when lambs were fed 0.8 mg. of stilbestrol per pound of the 14 per cent protein ration containing 85.5 per cent alfalfa. In contrast to this result and in conformity with the nitrogen balance results of the previous trial, the same addition of stilbestrol failed to increase nitrogen retention in lambs fed the 14 per cent protein ration that contained approximately 40 per cent alfalfa (Table 9). In both rations a slight but insignificant upward trend in protein digestibility was noted in stilbestrol treated lambs when compared to their controls.

The lambs fed the high alfalfa, 14 per cent protein ration received 0.90 gm. of nitrogen daily per 100 pounds of

Table 8. Experiment 3. Nutrient intake of lambs and effect of stilbestrol upon apparent dry matter digestibility in lambs fed largely an alfalfa or soybean meal type protein in either an 8 or 14 per cent protein ration (daily basis)

	8 per cent protein ration Treatment number				14 per cent protein ration Treatment number			
	1	2	3	4	5	6	7	8
Number of lambs	7	8	8	7	8	6	8	7
Weight of lamb, lb.	82	83	82	80	86	85	87	88
DES intake/100 lb. body weight, mg.	0.00	2.36	0.00	2.32	0.00	2.28	0.00	2.26
Energy intake: Level of maintenance based on TDN of ration	1.54	1.55	1.47	1.47	1.48	1.47	1.44	1.44
Dry feed intake, gm.	950	966	950	931	970	956	978	989
Dry feces, gm.	277	286	319	312	268	271	313	289
Dry matter dig. %	70.9 ±0.83[a]	70.4 ±0.79	66.4 ±0.69	66.5 ±1.18	72.4 ±0.88	71.7 ±0.95	68.0 ±1.95	70.8 ±1.07

[a]Standard error of the mean.

Table 9. Experiment 4. Effect of stilbestrol upon nitrogen retention in lambs fed a 14 per cent protein ration that consisted of 85.5 or 39.3 per cent alfalfa (daily basis)

	85.5% alfalfa DES intake/day, mg.		39.3% alfalfa DES intake/day, mg.	
	0.00	2.19	0.00	1.54
Number of lambs	6	6	6	6
N. intake per kg. of body weight, gm.	0.90	0.93	0.71	0.70
Nitrogen in feed, gm.	27.39	27.84	20.17	20.17
Nitrogen in feces, gm.	8.81	8.88	6.25	5.95
Nitrogen digested, %	67.8 ±0.95[a]	68.1 ±0.68	69.0 ±1.00	70.5 ±1.11
Water intake, ml.	2765	3346	2419	2556
Urine volume, ml.	1349	1491	1597	1445
Nitrogen in urine, gm.	15.62	14.85	11.87	12.31
Nitrogen retained, gm.	2.96 ±0.35[a]	4.11[b] ±0.28	2.05 ±0.47	1.91 ±0.41

[a]Standard error of the mean.

[b]Stilbestrol treatment significant at the 1 per cent level of probability.

body weight. This amount of protein is equal to the nitrogen intake of the lambs fed the 20 per cent protein ration in Experiment 2. The stilbestrol treated lambs fed the high alfalfa ration consumed more water than did their control, however, only a very little water consumption was noted in the stilbestrol treated lambs fed the low alfalfa ration. Urine elimination was correlated with water intake, however, in relation to water intake, urine elimination was less on the high alfalfa ration than its comparative low alfalfa ration.

As was true in the case of protein digestibility coefficients in this experiment, the stilbestrol treated lambs manifested a slight upward trend in dry matter digestibility (Table 10). Based on 100 pounds of body weight, the lambs fed the high alfalfa ration received 3.32 mg. of stilbestrol daily as compared to 2.40 mg. to those fed the low protein ration. This occurred as the result of feed intake adjusted to body maintenance requirements, differences in TDN values between the two rations, and equal addition of stilbestrol per pound of both rations. Consequently, the lambs fed the high roughage, less digestible ration (85.5 per cent alfalfa) received a greater dry matter intake of feed as compared to those lambs fed the more digestible ration (39.3 per cent alfalfa), resulting in a greater intake of stilbestrol per lamb. With feed intake adjusted to 150 per cent of maintenance, the high alfalfa ration provided considerable bulk. Under these con-

Table 10. Experiment 4. Nutrient intake and effect of stilbestrol upon apparent dry matter digestibility in lambs fed a 14 per cent protein ration that consisted of 85.5 or 39.3 per cent alfalfa (daily basis)

	85.5% alfalfa DES intake/day, mg.		39.3% alfalfa DES intake/day, mg.	
	0.00	2.19	0.00	1.54
Number of lambs	6	6	6	6
Weight of lamb, lb.	67	66	63	64
DES intake /100 lb. body weight, mg.	0.00	3.32	0.00	2.40
Energy intake: Level of maintenance based on TDN of ration	1.43	1.47	1.50	1.47
Dry feed intake. gm.	1036	1086	771	771
Dry feces, gm.	444	453	211	198
Dry matter digested, %	57.1 ±0.58[a]	58.3 ±1.51	72.6 ±0.48	74.3 ±0.93

[a]Standard error of the mean.

ditions, in spite of its palatability, this amount of feed intake appeared to be about all that most lambs could be safely relied upon to consume. Again, as manifested in the previous experiment, the lambs fed the higher fibrous ration exhibited a lower dry matter digestibility coefficient. This observation confirms the well known fact that a ration high in fiber will be digested less completely than a ration lower in fiber.

Lamb Experiment 5. (November and December, 1956)

The lambs in this experiment receiving 0.0, 0.8, and 1.2 mg. of stilbestrol per pounds of 14 per cent protein ration with feed intake adjusted to an estimated 210 per cent of body maintenance requirements retained, respectively, 5.08, 8.41, and 8.27 gm. of nitrogen daily per lamb. When, however, feed intake was adjusted to 150 per cent of maintenance, the control lambs and those fed 0.8 and 1.2 mg. of stilbestrol per pound of the same 14 per cent protein ration retained, respectively, 3.08, 3.51, and 4.09 gm. of nitrogen daily per lamb (Table 11). In contrast, to these results, the nitrogen balance data in the two previous experiments indicated that stilbestrol, when added at the rate of 0.8 mg. per pound of the same 14 per cent protein ration and fed to lambs with feed intake adjusted to 150 per cent of maintenance, failed to increase nitrogen over the control group of lambs.

Within energy treatments, no difference was noted in

Table 11. Experiment 5. Effect of stilbestrol and energy intake upon nitrogen retention in lambs fed a 14 per cent protein ration containing 39.3 per cent alfalfa (daily basis)

	2.1 times maintenance DES intake/lb. of ration, mg.			1.5 times maintenance DES intake/lb. of ration, mg.		
	0.0	0.8	1.2	0.0	0.8	1.2
Number of lambs	3	4	5	5	6	6
N. intake per kg. of body weight, gm.	0.99	0.92	0.91	0.68	0.66	0.69
Nitrogen in feed, gm.	34.81	34.06	35.55	23.72	23.75	23.56
Nitrogen in feces, gm.	10.50	11.00	11.12	6.72	6.96	6.82
Nitrogen digested, %	69.8 ±0.47[a]	67.7 ±0.76	68.7 ±1.81	71.7 ±1.62	70.7 ±0.73	71.0 ±2.05
Water intake, ml.	3503	3133	3656	2558	2029	2643
Urine volume, ml.	1350	1201	1279	1648	1210	1344
Nitrogen in urine, gm.	19.23	14.65	16.16	13.92	13.28	12.65
Nitrogen retained, gm.	5.08 ±1.05[a]	8.41 ±0.61	8.27 ±1.23	3.08 ±0.61	3.51 ±0.33	4.09 ±0.36

[a]Standard error of the mean.

either protein or dry matter digestibility as a result of any level of stilbestrol additions. However, the lambs receiving the lower nutrient intake manifested a slight increase in both protein and dry matter digestibility coefficients. This demonstrated the fact that animals usually digest a ration more completely when fed sparingly as compared to one fed liberally. No correlation was exhibited between stilbestrol treatment and water consumption. However, those lambs receiving the higher nutrient intake consumed more water than those fed at the lower level.

The unpalatibility of the starch in this ration aided in the difficulty of maintaining a constant feed intake of lambs receiving the high feed intake. As indicated in Table 12, some of the heavier lambs on the high energy intake consumed almost 4 pounds of air dry feed daily. Because no values are included where a weigh-back of feed occurred, only three, four, and five observations are shown per stilbestrol treatment in lambs fed the high level of feed intake. The lambs on the high level of feed intake received, per 100 pounds of body weight, 4.87 mg. of stilbestrol daily. This rate far exceeded stilbestrol intake of any previous trial. No prolapsed rectum occurred as a result of this high level of stilbestrol intake.

Lamb Experiment 6. (January through March, 1957)

Regardless of the level of protein in the ration, either

Table 12. Experiment 5. Nutrient intake and effect of stilbestrol upon apparent dry matter digestibility in lambs fed a 14 per cent protein ration containing 39.3 per cent alfalfa (daily basis)

	2.1 times maintenance DES intake/lb. of ration, mg.			1.5 times maintenance DES intake/lb. of ration, mg.		
	0.0	0.8	1.2	0.0	0.8	1.2
Number of lambs	3	4	5	5	6	6
Weight of lamb, lb.	78	81	86	77	80	75
DES intake/100 lb. body weight, mg.	0.00	3.27	4.84	0.00	2.44	3.68
Energy intake: Level of maintenance based on 68% TDN of ration	2.20	2.09	2.10	1.51	1.48	1.53
Dry feed intake, gm.	1330	1301	1358	906	907	900
Dry feces, gm.	356	357	368	219	230	227
Dry matter digested, %	73.2 ±0.41[a]	72.6 ±0.25	72.9 ±0.87	75.8 ±0.74	74.6 ±0.41	74.8 ±1.33

[a]Standard error of the mean.

growth hormone or stilbestrol when administered alone or in combination, resulted in increased nitrogen retention. When growth hormone was given to lambs already receiving stilbestrol, very little additional increase in nitrogen retention occurred over that resulting from stilbestrol alone. Lambs receiving the higher energy intake, under all conditions, retained more nitrogen than those receiving the lower level of energy intake (Table 13). It should be noted that the lambs fed the 8.5 per cent protein ration received only 0.31 gm. of nitrogen per kg. of body weight yet exhibited an increase in nitrogen retention from stilbestrol treatment.

Neither stilbestrol nor growth hormone, alone or in combination, appeared to influence apparent protein digestibility. The lambs fed the low energy intake exhibited larger protein digestibility coefficients (Table 14). A slight progressive increase in protein digestibility occurred with increasing length of the collection period. This observation probably occurred as the result of the lamb gaining weight, although continuing to be fed a constant feed intake. As a result, as indicated in the previous experiment, an animal will digest its feed more completely. The large standard error of the mean of protein digestibility exhibited in the low protein-control-low energy treatment (Table 14) was largely the result due to one lamb. This lamb exhibited a consistent decrease in protein digestibility of approximately 10 per cent compared with the other two lambs on this same treatment.

Table 13. Experiment 6. The influence of growth hormone without and with stilbestrol on nitrogen retention in lambs receiving either a low or a medium protein ration which was fed at either a low or high level of energy intake (gm. nitrogen retained daily per lamb)

Ration treatment			Growth hormone treatment		
Protein	DES	Energy	None[a]	G.H. Adm.[b]	None[c]
8.5%	Control lambs	LE[d]	0.86	1.89	0.68
		HE[d]	2.68	4.42	2.23
	DES lambs	LE[d]	1.72	1.87	0.55
		HE[e]	4.52	5.00	3.92
13.0%	Control lambs	LE[d]	1.97	2.76	1.29
		HE[e]	3.94	5.82	1.99
	DES lambs	LE[d]	3.38	3.96	2.99
		HE[e]	6.00	6.76	4.64

[a]12-day pre-growth hormone collection period.

[b]12-day growth hormone injection and collection period.

[c]12-day post-growth hormone collection period.

[d]Three lambs per treatment.

[e]Two lambs per treatment.

Table 14. The effect of growth hormone administration with and without stilbestrol on protein digestibility in lambs receiving either a low or a high protein ration which was fed at either a low or a high level of energy intake

Ration treatment			Growth hormone treatment		
Protein	DES	Energy	None[a]	G.H. Adm.[b]	None[c]
8.5%	Control lambs	LE[d]	60.1[e]±3.88[f]	60.2±4.19	63.9±3.31
		HE[d]	59.5 ±1.32	59.4±0.98	60.5±0.93
	DES lambs	LE[d]	59.8 ±1.28	63.4±0.63	62.8±1.38
		HE[g]	57.1 ±2.01	59.7±0.16	61.9±1.10
13.0%	Control lambs	LE[d]	74.7 ±0.01	75.3±0.22	75.4±0.43
		HE[g]	69.3 ±2.30	73.2±0.35	72.0±1.40
	DES lambs	LE[d]	74.4 ±0.60	75.0±0.96	75.1±1.22
		HE[g]	66.6 ±1.65	69.2±0.30	71.2±0.85

[a]12-day pre-growth hormone collection period.

[b]12-day growth hormone injection and collection period.

[c]12-day post-growth hormone collection period.

[d]Three lambs per treatment.

[e]Apparent protein digestibility coefficients.

[f]Standard error of the mean.

[g]Two lambs per treatment.

Apparent dry matter digestibility coefficients, as shown in Table 15, manifested the same general continuity as the protein digestibility coefficients.

Some degree of correlation appeared to exist between nitrogen retention, water intake, and gain in body weight (Table 16). The lambs that retained the most nitrogen gained the most weight and likewise consumed the most water. This suggests that nitrogen retention is related to body weight gain. The lambs gained an average of 9 to 23 pounds per treatment during tne 36-day collection period.

With a few exceptions, most lambs exhibited a constant nitrogen retention value for each four day collection period within each 12-day per-growth, growth hormone, and post-growth hormone collection period. The largest variations occurred in those lambs receiving the high energy treatment, notably lambs 9 and 10, during the pre-growth hormone collection period (Table 21 in the Appendix). In all cases, nitrogen retention values were higher in the pre-growth hormone collection than in the post-growth hormone collection period. This was because the lambs were fed a constant feed intake and a greater demand was made on dietary nutrients for energy purposes as the lambs were gaining weight.

Three lambs were placed on each low and high energy treatment at the beginning of the collection series. All lambs receiving the low energy treatments satisfactorily completed the 36-day collection period. However, two lambs on

Table 15. The effect of growth hormone administration with and without stilbestrol on dry matter digestibility in lambs receiving either a low or high protein ration which was fed at either a low or high level of energy intake

Ration treatment			Growth hormone treatment		
Protein	DES	Energy	None[a]	G.H. Adm.[b]	None[c]
8.5%	Control lambs	LE[d]	74.3[e]±1.87[f]	74.5±2.24	76.4±1.68
		HE[d]	71.6 ±1.64	70.5±0.70	71.4±1.08
	DES lambs	LE[d]	75.3 ±0.91	76.2±0.41	76.7±0.28
		HE[g]	71.8 ±0.16	73.8±0.16	74.8±0.10
13.0%	Control lambs	LE[d]	76.7 ±0.21	77.3±0.30	77.6±0.50
		HE[g]	70.6 ±1.00	74.2±1.44	72.4±2.80
	DES lambs	LE[d]	76.4 ±1.00	77.2±0.44	77.6±0.34
		HE[g]	71.8 ±0.16	74.2±0.16	74.8±0.76

[a]12-day pre-growth hormone collection period.

[b]12-day growth hormone injection and collection period.

[c]12-day post-growth hormone collection period.

[d]Three lambs per treatment.

[e]Apparent dry matter digestibility coefficients.

[f]Standard error of the mean.

[g]Two lambs per treatment.

Table 16. The effect of growth hormone administration with and without stilbestrol on the daily water intake, total nitrogen retention and total gain in weight of lambs receiving either a low or high protein ration and fed at either a low or high level of energy intake (average per lamb based on a 36-day collection period)[a]

Ration treatment			Water intake (ml.)	Nitrogen retained (gm.)	Total gain in 36 days (lb.)
Protein	DES	Energy			
8.5%	Control lambs	LE[b]	1578	1.14	9
		HE[b]	2164	3.11	14
	DES lambs	LE[b]	1785	1.38	12
		HE[c]	2534	4.48	21
13.0%	Control lambs	LE[b]	2087	2.22	11
		HE[c]	2695	3.92	23
	DES lambs	LE[b]	2314	3.44	14
		HE[c]	2753	5.80	18

[a]For further explanation of experimental design see footnote of Table 13.

[b]Three lambs per treatment.

[c]Two lambs per treatment.

the high energy treatment refused feed during some part of the collection period. Another lamb was injured in the collection cage. Therefore, these abnormalities account for the deleted values in the tables.

Growth Hormone Bioassay Studies

Only whole, intact anterior pituitary glands were assayed in this study. Those damaged during removal at the time the steers were slaughtered were discarded. The average weights of the anterior pituitary gland from the control steers and from the steers that were fed 5 and 10 mg. of stilbestrol daily were 1.16, 1.41, and 1.48 gm., respectively. The control steers, and those that were fed 5 and 10 mg. of stilbestrol daily, weighed an average of 964, 1033, and 1057 pounds, respectively (Table 17). The anterior pituitary weight per unit of body weight increased with stilbestrol administration as follows: 12.0, 13.6, and 14.0 x 10^4.[a] Those steers fed 5 mg. of stilbestrol daily manifested a significantly ($P = .01$) larger anterior pituitary gland as compared to those steers fed no stilbestrol (Table 22 in the Appendix). Because of the previously mentioned damaged pituitary glands, no satisfactory statistical analysis could be made comparing the 10 mg. group

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[a]Anterior pituitary weight (gm.)/body weight (lb.) x 10^4.

Table 17. Summaries of the average weight of steers and of their anterior pituitary glands and width of tibiae of hypophysectomized rats injected with extract of the anterior pituitary glands of steers

		10.5%, medium protein		13.5%, high protein		
		Low-gainers	High-gainers	Low-gainers	High-gainers	Ave.
Control	Cattle lot no.	1	2	3	4	
	No. of intact A.P.[a]	4	4	4	5	
	No. of surviving rats	3	3	3	3	
	Wt. of A.P., gm.	1.40±.08[b]	1.05±.06	1.30±.06	0.96±.09	1.16
	Epiphysis width, u	255±21[b]	275±10	277±22	268±19	
	Wt. of steer, lb.	909	979	1008	991	964
5 mg. DES	Cattle lot no.	5	6	7	8	
	No. of intact A.P.	4	4	4	1	
	No. of surviving rats	3	3	3	3	
	Wt. of A.P., gm.	1.16±.06	1.61±.05	1.45±.12	1.51 ---	1.41
	Epiphysis width, u	208±14	273±50	278±29	256±22	
	Wt. of steer, lb.	960	1094	1047	1030	1033
10 mg. DES	Cattle lot no.	9	10	11	12	
	No. of intact A.P.	2	5	5	0	
	No. of surviving rats	1	1	2	did	
	Wt. of A.P., gm.	1.57±.11	1.55±.05	1.39±.04	not	1.48
	Epiphysis, width, u	314	240	251	assay	
	Wt. of steer, lb.	1043	1097	1024		1057

[a]Anterior pituitary glands.

[b]Standard error of the mean.

against the control group.

The average width of the uncalcified cartilage of the tibiae of rats in lots one through eight is rather constant. One exception, however, is lot five which exhibited a smaller response than the other lots. In most cases the standard error of the mean was large, therefore data of this type from one rat, as shown in lots nine and ten, might be misleading. When these data from lots one through eight were treated statistically (Table 23 in the Appendix) no difference in cartilage width, when expressed in micra, approached significance. A control group of three rats manifested an average response of 134 micra.

With the exception of the anterior pituitary glands of lots 2, 4, and 5, the average weight of the anterior pituitary glands among all lots appeared reasonably uniform. No apparent pattern was evident for the low pituitary weights of lots 2, 4, and 5.

Three rats were injected with the pooled anterior pituitary extract from each lot of steers. However, what appeared to be more than a chance effect, five out of nine rats succumbed in the four day injection period upon the treatment of the pituitary extracts from the steers fed 10 mg. of stilbestrol daily and none died from any of the other treatments. It should be noted that, in general, one day following the initial injection period most rats in all lots gained about 7 gm. In the succeeding day, the rats gained about three ad-

ditional gm. Some rats following the third day of injection would begin to lose weight and the day following the fourth and last injection period, or at the time of sacrifice, weighed less than their initial weight. Once rats started to lose weight, death was usually encountered within two to three days.

DISCUSSION

One of the objectives of livestock production is to increase the rate of gain in the growing animal. Since feed costs represent the most expensive phase of livestock production, the producer is always striving for improved feed efficiency. As indicated by Burroughs, et al. (17), stilbestrol has improved both the rate of gain and feed efficiency in ruminants.

If the excess growth caused by stilbestrol is not solely dependent upon the character of the ration or to increased feed intake, then it is reasonable to look for specific factors that influence the metabolic processes for this acceleration of body growth. Using the criterion of nitrogen retention as a sensitive index of growth, the data in this thesis suggest that stilbestrol will stimulate growth in wether lambs if adequate nutrients are available. It was demonstrated that an adequate level of dietary protein facilitated the action of stilbestrol in increasing nitrogen retention. Usually, the stilbestrol treated lambs fed a ration containing approximately an 8 per cent protein failed to retain more nitrogen than their controls. However, the levels of energy and stilbestrol appeared to influence nitrogen retention. When protein intake was increased to approximately 14 per cent, the stilbestrol treated lambs fed four different rations usually retained more nitrogen than those not receiving

stilbestrol. Energy and stilbestrol intake again affected nitrogen retention in lambs. These results seem to confirm, at least in part, the feed lot results of cattle (26, 39, 49). However, it appears that once the dietary protein needs are met, stilbestrol will not increase nitrogen retention upon feeding an excess of protein (Experiment 2). This is in agreement with Deans, et al. (24) who reported that stilbestrol treated steers under feed lot conditions and fed three pounds of soybean meal daily failed to gain faster than those fed two pounds daily. Also, stilbestrol treated lambs fed four different 14 per cent protein rations, retained more nitrogen than controls, suggesting that stilbestrol will enhance nitrogen retention in lambs fed an adequate dietary protein, regardless of its quality.

It is a well known fact that the energy needs of the body must first be met before growth can occur. McHenry stated (43) "...body weight can increase only if there is a surplus of food after the current expenditure of the animal has been paid". The importance of an adequate energy intake for maximum growth is demonstrated by the nitrogen balance results of Experiments 5 and 6. Lambs fed the higher energy intake retained more nitrogen than those receiving lower energy intake. However, those lambs receiving the higher energy intake with stilbestrol retained more nitrogen than those not fed stilbestrol. The net result is that the greater the energy and protein intake, the greater the nitrogen retention

from stilbestrol.

However, it appears that under certain conditions stilbestrol is without effect in lambs fed an adequate protein intake (14 per cent). This observation is indicated in stilbestrol treatment 6 over its control treatment 5, which failed to enhance nitrogen retention in lambs fed a 14 per cent protein ration in Experiment 3 and also in Experiment 4, yet increased nitrogen retention under a similar dietary condition in Experiment 5. The 14 per cent protein ration fed contained protein in which a large part of the protein was supplied from alfalfa. Experiments 3, 4, and 5 were conducted during the summer, early and late autumn seasons, respectively. It is possible that the physiological role of stilbestrol in wether lambs may be different during the seasons of the year. Story (57) and Acker (1) failed to obtain any increase in rate of gain from stilbestrol treated lambs during the summer months. However, the concurrent 14 per cent protein-stilbestrol study conducted in Experiment 3 would tend to disprove this speculation for it was shown that stilbestrol enhanced nitrogen retention in those lambs that were fed a 14 per cent protein ration that contained protein supplied largely by soybean meal. Likewise, the concurrent 14 per cent protein-stilbestrol study conducted in Experiment 4 indicated that stilbestrol increased nitrogen retention significantly ($P = .01$) in lambs fed a 14 per cent protein ration in which all the protein was supplied by alfalfa. On

the basis of these results, it is difficult to explain in that stilbestrol increased nitrogen retention in lambs fed a 14 per cent protein ration in which the protein was supplied in a large part from alfalfa in only one out of three trials. Based on these findings, the speculation that the physiological role of stilbestrol in lambs is different during the summer season as compared to the other seasons of the year may be queried. It is conceivable, however, that lambs, although fed a ration adequate in protein, may need a greater energy and/or stilbestrol intake during the summer season if maximum growth is to be obtained from stilbestrol treatment.

As indicated previously, the observation that stilbestrol treated lambs fed four different 14 per cent protein rations retained more nitrogen than did their controls would tend to rule out the speculation that certain rations, such as silages, although adequate in protein, may be deficient in amino acids and therefore render stilbestrol ineffective in stimulating gains in ruminants. Some studies have indicated that cattle fed silages failed to respond to stilbestrol treatment (36, 52). Another study indicated that cattle fed green chopped alfalfa showed less response to stilbestrol feeding than did those fed the cured and bailed alfalfa (23). Silages are lower in energy than most feed lot fattening rations. Based on the previously mentioned calorie-protein relationships, it is conceivable that insufficient energy intake may at least account for a part of the lack of response

from stilbestrol when cattle are fed silages.

These results are in general agreement with most workers (1, 11, 20, 22, 27, 37, 48, 60) that stilbestrol, administered either orally or by implantation, will not increase dry matter or protein digestibility. Neither did growth hormone administration increase dry matter or protein digestibility. It is known that animals usually digest a larger portion of their ration when fed a scanty ration than when they receive a liberal allowance. This fact is demonstrated by the dry matter digestibility coefficients in Experiments 5 and 6. The apparent dry matter digestibility coefficients were lowest on the lambs receiving the low level of nutrient intake. It was of interest to note that the lambs fed the 20 per cent protein ration did not manifest any increase in dry matter digestibility over those lambs fed the 14 per cent protein ration (Experiment 2). Increasing the per cent of protein in the ration increased apparent protein digestibility, even though actual protein digestibility was probably the same on all protein intakes. This is because the metabolic nitrogen of the higher protein rations made up a smaller proportion of the total nitrogen of the excreta.

Although Mitchell, et al. (44) reported that stilbestrol treated, paired-fed steers consumed more water than did their controls, the speculation that stilbestrol increases water consumption was not indicated in the results of these nitrogen balance studies. Water consumption was not increased in the

stilbestrol treated lambs fed the 14 per cent protein rations in Experiments 1, 3, and 5, even though nitrogen retention was enhanced, although the stilbestrol treated lambs in Experiments, 2, 4, and 6 did consume more water than their controls. It should be noted that stilbestrol appeared to increase water consumption of lambs fed the 8 per cent protein rations, even though nitrogen retention was not influenced. This may indicate that more water is needed to metabolize and excrete stilbestrol on a low protein ration. These studies, however, are in agreement with Baintner and Biro (7) who reported greater water consumption in cattle fed the higher protein rations.

The nitrogen retained from stilbestrol treatment was the result of decreased concentration of nitrogen in the urine. This is not in agreement with Jordan (37) who reported that the greater nitrogen retention exhibited from implanted stilbestrol was the result of less urine voided with a net result of less nitrogen per ml. of urine. This worker, however, used only four stilbestrol treated lambs and reported considerable variation in the amount of urine voided between lambs with a group.

In the interpretation of nitrogen balance data, considerations should be given to factors that will contribute to experimental error and affect nitrogen retention. Most large animal nitrogen balance experiments are conducted on restricted feed intake and under these conditions, as was demonstrated in

these studies, nitrogen retention will be affected. That is, gm. of nitrogen retained daily by a lamb receiving 800 gm. of a 12 per cent protein feed might be different than if the same lamb was receiving 1000 gm. of the same feed. If a nitrogen balance is to be meaningful, it should be designed to represent the conditions for which it was intended.

The number of animals per observation is very important. Forbes, et al. (29) stated that five lambs per treatment are sufficient in digestibility studies if the experimental technique is efficient and if the sheep have been successfully treated for parasites. However, more factors affect nitrogen balance values than digestibility coefficients, therefore emphasis must be stressed on numbers to reduce the variability in nitrogen balance studies. This fact was reflected in the nitrogen balance data in Experiment 6, which indicated large differences in nitrogen retention values between some lambs receiving the same treatment, especially lambs fed a high level of feed intake. These lambs were treated for parasites and certainly the experimental technique was considered improved over the beginning experiments.

The protein digestibility coefficients obtained in treatments 5 and 6, Experiment 3, as compared to the duplicate treatments made at a later date, as indicated in Experiment 4, would suggest that differences in only 2 per cent of dry matter and protein digestibility coefficients are of no significance in predicting digestibility trends. Stilbestrol in

Experiment 3 increased protein digestibility, whereas the latter trial indicated the opposite. Likewise, reducing largely the variation in nitrogen balance and digestibility data by repeating a collection on the same lamb may be queried. Most lambs in Experiment 6 exhibited similar nitrogen balance results and digestibility coefficients when consecutive four day collections were analyzed. Time of feeding (12), nitrogen fixation of feces (50), and nervous animals (22) are additional factors that affect the net nitrogen balance results.

Campbell, et al. (20) reported that all possible carry-over effect of stilbestrol was lost in 10 days in lambs. Bell, et al. (11) likewise indicated no carry-over effect of stilbestrol. All lambs in these studies had at least a 28 day interval between collections if they were used for two different studies. On this basis it seemed safe to use the same animal for two different experiments.

Stilbestrol, administered either orally or by implant, appears to manifest its protein anabolic response through a decrease in urinary nitrogen. Likewise, growth hormone treatment in lambs exhibited a similar action (Experiment 6). The pituitary gland is known to secrete various hormones, namely, thyrotrophic, gonadotrophic, adreocorticotrophic (ACTH), and the growth hormone. These hormones are known to influence animal growth.

These studies indicated that in relation to body weight,

stilbestrol treated steers had heavier anterior pituitary glands than the non-treated steers. This indicated that the size of the anterior pituitary gland was increased as the result of feeding stilbestrol and not as the result of an increase in body weight, due to the stilbestrol treatment. Other workers reported that stilbestrol treated cattle had heavier pituitary glands than did their controls (22, 18). However, Hentges, et al. (34) could not confirm this observation. The bioassay data did not reveal any difference in growth hormone potency when based on each gm. of pituitary tissue between the treated and control groups. It is conceivable that a heavier pituitary, if as the result of stilbestrol treatment, would secrete a greater quantity of growth hormone. Baird, et al. (8) believe that each unit of swine pituitary tissue, regardless of age, weight, or sex of swine, secrete a constant quantity of growth hormone. Armstrong and Hansel (4) did not confirm this observation from their results of growth hormone bioassay studies in heifers. (Such a measurement would be the most ideal method of measuring growth hormone secretion.) However, both workers believe that growth occurs only so long as the anterior pituitary is able to maintain an adequate concentration of growth hormone in the body as a whole. They believe that once maturity is reached the amount of circulating growth hormone falls until it is below the level necessary for growth. Based on this reasoning, one manner in which growth hormone production could be increased

is to increase the size of the pituitary gland. Another method is to increase the secretory activity of the cells of the pituitary gland.

It seems plausible that stilbestrol in ruminants may act as a precursor or coenzyme to increase the secretory activity of the growth hormone cells of the pituitary gland. Therefore, as a result of cell hyperactivity, the pituitary gland is enlarged. Turner (61) indicated that the presence of only three cytologically different kinds of cells in the anterior lobe constitutes an enigma which is difficult to harmonize with the large number of hormones which seem to be secreted from it. Very little is known about the secretion of hormones. Many of these nitrogen balance studies were initiated 10 days after stilbestrol was administered and nitrogen retention was obtained under these conditions. Similarly, Acker (1) and Story, et al. (58) obtained nitrogen retention shortly after stilbestrol administration. Conversely, Campbell, et al. (20) reported that, based on nitrogen balance studies, any possible carry-over effect of stilbestrol was lost in 10 days. Story, et al. (58) likewise confirmed similar results. This suggests that stilbestrol treatment per se, and not an enlarged pituitary gland, which may have resulted from stilbestrol administration is instrumental in the growth response manifested in ruminants from this treatment.

The evidence of side effects (femininity) from stilbestrol would suggest that a part of the growth response ob-

tained from this treatment is due to increased stimulation of growth of the accessory sex organs. However, when stilbestrol and growth hormones were administered concurrently (Experiment 6) very little increase in nitrogen retention was obtained over that when either was administered alone. This would strongly suggest that the type of growth response obtained from stilbestrol is in most part similar to the type of growth obtained from growth hormone alone. It would appear that if stilbestrol elicits most of its growth response other than an increase in growth hormone secretion, the nitrogen retention values obtained from concurrent administration of stilbestrol and growth hormone would have been more accumulative.

Further speculation that stilbestrol manifests most of its growth response by increasing growth hormone secretion is strengthened by the similarity of selectivity of tissue formation by both growth hormone and stilbestrol. Growth is characterized by retention of nitrogen, calcium, and phosphorus and synthesis of protein. Growth hormone is known to accelerate osteogenesis, fibrous and connective tissue, and increase protein synthesis (5). Likewise, stilbestrol appears to slightly accelerate connective and fibrous tissue formation (1, 62), retention of calcium (11), and appears to hasten maturity in lambs when break joints are used as a criterion of age.

Feed lot acceptance of stilbestrol was rapid. Because of the complexities and variability of biological systems, more

investigations are needed before the mode of action of stilbestrol as a growth stimulant in ruminants can be completely determined. The nutritional application of hormones represents a new field of study in animal production. Already large advances have been attained in the use of hormones in cattle and sheep feeding under practical farm conditions.

SUMMARY

A series of nitrogen balance experiments was made to study the effect of stilbestrol on nitrogen retention in wether lambs. In addition, a study was made on the effect of growth hormone and stilbestrol, both single and in combination, on protein metabolism in lambs fed two different levels of protein and energy intake. A study was also made on the effect of stilbestrol on growth hormone potency of the pituitary gland of steers.

An adequate level of dietary protein facilitated the action of stilbestrol in increasing nitrogen retention in wethers. Usually, the stilbestrol treated lambs fed rations containing approximately 8 per cent protein failed to retain more nitrogen than controls. However, the levels of energy and stilbestrol intake appeared to influence nitrogen retention. On the other hand, the stilbestrol treated lambs fed four different rations, each containing approximately 14 per cent protein, usually retained more nitrogen than those lambs not receiving stilbestrol. Energy and stilbestrol intake also affected nitrogen retention. Stilbestrol treated lambs fed an excess of dietary protein (20 per cent) failed to retain more nitrogen than lambs when fed a lower level of protein.

Lambs fed 8.5, 13.0, and 14.0 per cent protein rations, and receiving higher levels of energy intake, retained more nitrogen than those receiving lower energy intake. Lambs fed

higher protein and energy levels with stilbestrol retained more nitrogen than those that received the lower protein and energy level.

Lambs not receiving stilbestrol responded to growth hormone administration in which nitrogen retention was increased. If, however, growth hormone was administered to lambs receiving stilbestrol, very little increase in nitrogen retention occurred over that resulting from stilbestrol alone.

Neither stilbestrol nor growth hormone influenced apparent digestibility of protein or dry matter. Body weight increase in lambs appeared to be related to the amount of nitrogen retained.

The stilbestrol fed steers, in relation to body weight, had heavier anterior pituitary glands than controls. Growth hormone potency per gm. of anterior pituitary tissue was not influenced by stilbestrol treatment. It is suggested that stilbestrol in ruminants mediated its protein anabolic action primarily through an increased release of growth hormone secretion from the anterior pituitary gland.

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APPENDIX

Table 18. The effect of growth hormone administration without and with stilbestrol treatment on nitrogen retention in lambs receiving an 8.5 per cent protein ration fed at a low level of energy intake (gm. nitrogen retained daily per lamb)

Lamb no.	Pre-growth hormone collection period (12 days)				Growth hormone injection and collection period (12 days)				Post-growth hormone collection period (12 days)			
	Collection number[a]				Collection number				Collection number			
	1	2[b]	3	Ave.	4	5	6	Ave.	7	8[b]	9	Ave.
					Without stilbestrol							
1	0.53	1.32	1.81	1.22	1.58	2.37	2.48	2.14	0.63	0.05	-0.27	0.14
2	-0.35	1.06	0.35	0.35	2.15	2.68	1.91	2.25	1.76	1.00	0.64	1.13
3	1.26	2.51	-0.75	1.01	0.23	1.13	2.46	1.27	0.95	0.45	0.90	0.77
				0.86				1.89				0.68
					With stilbestrol							
4	1.56	2.90	1.79	2.08	2.63	1.88	1.44	1.98	0.94	0.63	0.41	0.66
5	2.67	2.54	1.30	2.17	2.50	2.46	2.49	2.48	1.46	0.45	1.22	1.04
6	1.22	1.63	-0.13	0.91	0.63	1.61	1.24	1.16	-0.04	-1.24	1.17	-0.04
				1.72				1.87				0.55

[a]Each collection number represented a four day collection period.

[b]Injected with saline.

Table 19. The effect of growth hormone administration without and with stilbestrol treatment on nitrogen retention in lambs receiving a 13.0 per cent protein ration fed at a low level of energy intake (gm. nitrogen retained daily per lamb)

Lamb no.	Pre-growth hormone collection period (12 days)				Growth hormone injection and collection period (12 days)				Post-growth hormone collection period (12 days)			
	Collection number[a]				Collection number				Collection number			
	1	2[b]	3	Ave.	4	5	6	Ave.	7	8[b]	9	Ave.
					Without stilbestrol							
7	2.47	2.88	2.31	2.55	2.21	2.25	2.28	2.25	1.29	1.26	1.50	1.35
8	1.77	1.86	1.77	1.80	3.15	3.25	3.37	3.26	1.42	0.40	1.10	0.97
9	1.95	1.32	1.45	1.57	2.70	2.82	2.76	2.76	1.02	1.93	1.67	1.54
				1.97				2.76				1.29
					With stilbestrol							
10	1.83	2.14	1.80	1.92	1.52	3.30	3.26	2.69	2.15	2.35	0.92	1.81
11	2.85	3.73	2.81	3.13	2.75	3.89	4.06	3.57	3.23	2.80	3.01	3.01
12	4.78	5.78	4.67	5.08	5.85	5.42	5.62	5.63	4.91	4.35	3.22	4.16
				3.38				3.96				2.99

[a]Each collection number represented a four day collection period.

[b]Injected with saline.

Table 20. The effect of growth hormone administration without and with stilbestrol treatment on nitrogen retention in lambs receiving an 8.5 per cent protein ration fed at a high level of energy intake (gm. nitrogen retained daily per lamb)

Lamb no.	Pre-growth hormone collection period (12 days)				Growth hormone injection and collection period (12 days)				Post-growth hormone collection period (12 days)			
	Collection number[a]				Collection number				Collection number			
	1	2	3	Ave.	4	5	6	Ave.	7	8	9	Ave.
					Without stilbestrol							
1	3.88	3.33	3.26	3.49	4.22	5.96	4.72	4.97	3.84	3.58	3.78	3.73
2	3.67	2.01	2.33	2.67	3.98	2.79	3.60	3.46	2.70	-1.15	-0.01	0.51
3	1.87	2.33	1.45	1.88	4.76	5.22	4.52	4.83	3.32	2.90	1.11	2.44
				2.68				4.42				2.23
					With stilbestrol							
4[b]												
5	5.22	3.96	4.09	4.42	4.74	5.56	5.35	5.21	4.93	4.14	3.37	4.15
6	4.61	4.30	4.99	4.63	5.12	4.97	4.28	4.79	3.90	3.89	3.28	3.69
				4.52				5.00				3.92

[a]Each collection number represented a four day collection period.

[b]Lamb numbered four failed to complete the experiment.

Table 21. The effect of growth hormone administration without and with stilbestrol treatment on nitrogen retention in lambs receiving a 13 per cent protein ration fed at a high level of energy intake (gm. nitrogen retained daily per lamb)

Lamb no.	Pre-growth hormone collection period (12 days)				Growth hormone injection and collection period (12 days)				Post-growth hormone collection period (12 days)			
	Collection number[a]				Collection number				Collection number			
	1	2	3	Ave.	4	5	6	Ave.	7	8	9	Ave.
					Without stilbestrol							
7	3.06	2.03	3.11	2.73	6.79	6.97	4.59	6.12	1.02	0.74	1.81	1.19
8[b]												
9	6.96	4.74	3.73	5.14	7.04	6.30	3.22	5.52	3.31	2.28	2.78	2.79
				3.94				5.82				1.99
					With stilbestrol							
10	8.53	6.03	3.62	6.06	5.54	6.28	5.83	5.88	5.84	4.68	3.64	4.72
11[b]												
12	5.93	5.88	6.02	5.94	8.70	8.19	6.07	7.65	3.61	4.03	6.02	4.55
				6.00				6.76				4.64

[a]Each collection number represented a four day collection period.

[b]Lambs numbered eight and 11 failed to complete experiment.

Table 22. Analysis of variance of anterior pituitary gland weight (gm.) of steers in lots 1-8

Source	Degrees of freedom	Mean square	F-value
Among lots	7	0.20365	
Protein levels	1	0.03314	
High vs. low gainers	1	0.10007	2.44
Protein x gain	1	1.94574	47.52**
Stilbestrol	1	0.46545	11.37**
Protein x stilbestrol	1	0.04268	1.04
Gain x stilbestrol	1	0.63786	15.58**
Protein x gain x stilbestrol	1	0.13340	3.26
Within lots	22	0.04094	
Total	29		

**Treatment effect significant at the 1 per cent level of probability.

Table 23. Analysis of variance of tibiae epiphyseal cartilage width (micra) upon treatment of anterior pituitary extracts of steers from lots 1-8[a]

Source	Degrees of freedom	Mean square
Lots	7	1631.8
Protein	1	1666.7
High vs. low gainers	1	1066.7
Protein x gainers	1	5104.2
Stilbestrol	1	1320.2
Protein x stilbestrol	1	560.7
Gainers x stilbestrol	1	384.0
Protein x gainers x stilbestrol	1	1320.2
Within lots	16	2663.8
Total	23	

[a]Treatment effect not significant at P = .05 or less.