EFFECT OF A PURIFIED DIET UPON REPRODUCTION IN EWES

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INTRODUCTION

Purified diets have become very useful tools in ruminant nutrition research. Most research institutions involved in this area of research use some form of a purified diet. However, these diets vary greatly in composition from the semi-purified diets in which casein, alfalfa meal, isolated soy-protein, or some other form of plant or animal protein is added, to the completely purified diet in which urea or other non-protein nitrogenous compounds serve as the sole source of nitrogen. As purified diets are expensive, they have no practical value for production; however, in the area of experimental feeding they are important. All nutrients are supplied individually and in as pure a form as possible, thus addition or removal of any given amount of a nutrient is performed with minimum disturbance of other nutrients. These diets are used in studies for finding optimum required levels of known nutrients and have application in studying feed additives.

Many investigators have found their purified diets will produce satisfactory gains and feed efficiencies. However, only few reports have been published concerning the ability of ruminants to reproduce on such diets. No synthetic or purified diet can be considered truly adequate until it has been shown to support growth as well as reproduction and

lactation over prolonged periods of time. The objective of the study reported herein was to determine the adequacy of a synthetic diet developed at this station for reproduction in ewes.

REVIEW OF LITERATURE

Probably the earliest work in the area of purified diets for ruminants was that of Woodward and McCay (1932), who maintained a goat for 152 days on a semi-purified diet consisting of 30% corn starch, 15% casein, 10% sucrose, 5% yeast, 7% salt mixture, and 3% lard. The goat was maintained in nitrogen balance and was quite fat when it died of a kidney infection. Along with the above study, twenty young rabbits were fed a synthetic diet of similar composition. The rabbits ceased to grow and started losing weight in about one month, and by the end of six weeks on the diet had paralysis in the rear legs. Histological examination revealed degeneration of muscle tissue in the paralyzed legs.

Johnson et al. (1940) fed two to ten day old calves a semi-purified diet consisting of casein, lactalbumin, sugar, butter or lard, minerals, and water as a substitute for milk. When the calves were about three months of age, they were gradually transferred to dry feed composed of casein, starch, sugar, cottonseed oil, cellophane, and minerals. Growth rates of these calves were below normal, which they thought were caused by poor food consumption associated with periodic digestive upsets. They found it necessary to supply a daily allowance of about 25 mg. magnesium per kg. of body weight to prevent convulsions, paralysis, and death.

In later studies, Woods and Tillman (1956) tested the effect of soybean meal ash upon gains of sheep which were consuming a purified diet and found that the ash improved gains. Byers et al. (1956), in their studies to develop a purified diet for dairy cattle, found that when a soybean product replaced urea as the nitrogen source on a protein equivalent basis gains were greatly improved. They also found that steers, fed a purified diet with urea as the sole source of nitrogen, developed enlarged pasterns, swollen knees and hocks, and posture difficulties after four weeks on the purified diet.

Matrone et al. (1957) attempted to improve their purified diet by adding a total of 39.1% salts of acetic, propionic, and butyric acids. It was found that this diet gave a superior response over diets with or without cellulose, and that lambs on the VFA supplemented diet did not eat their own wool, a characteristic which is typical of sheep fed a purified diet. Later, Matrone et al. (1959) found supplementing their purified diet with 4.4% $KHCO_3$ plus 7.3% $NaHCO_3$ gave a response equal to that of the VFA supplemented diet suggesting that the response was in fact due to an increased rumen buffering capacity associated with Na and K cations.

Oltjen et al. (1962c) compared purified diets containing urea, casein, isolated soy-protein, and soybean meal when they were fed in isonitrogenous diets. Sheep receiving a diet in which urea supplied 98.7% of the dietary nitrogen

performed quite satisfactorily; their gains were 80 and 86% as fast as those made by sheep on the isolated soy-protein and the soybean meal rations, respectively. Such gains indicate considerable improvement over those obtained in earlier reports. These workers found that the addition of alanine, methionine, or lysine to the urea-containing purified diet did not influence gains.

Clifford and Tillman (1967) studied purified diets in which urea was replaced by soybean protein at the rates of 0%, 25%, 50%, and 100%. Differences in nitrogen retention between those diets in which nitrogen was supplied by all urea or by all soybean protein were not significant.

In further studies, Oltjen et al. (1962b) used a 3 X 3 factorial arrangement of treatments to test the response of growing lambs to three levels of minerals (3.5, 5.0, and 6.5% of the ration) and three levels of cellulose (30, 40, and 50% of the ration). Sheep consuming the 30% cellulose rations produced faster and more efficient gains than sheep consuming the 50% rations. Mineral levels did not significantly affect gains or feed efficiencies, and there was no significant interaction between mineral and cellulose levels. The combination of 30% cellulose and 6.5% alkaline mineral mixture supported the fastest and most efficient gains. In a similar study, Smith et al. (1966) determined the effect of increasing levels of cellulose in purified diets on growth, digestibility, and rumen function of eight Holstein calves. They used 0 and 25% cellulose in pelleted purified

diets and 50% cellulose in a purified diet fed unpelleted. The purified diet containing 25% Solka floc supported significantly faster growth than other purified diets. Calves on the purified diet containing 25% Solka floc did not show signs of regurgitation; however, they did not chew wood as did the calves on the other two purified diets. Dry matter digestibility appeared to decrease with increasing levels of cellulose. The purified diet containing no cellulose had significantly higher digestibilities of dry matter and energy than other diets, while the diet containing 25% Solka floc had significantly greater digestible energy and dry matter than did the diet containing 50% Solka floc.

Oltjen et al. (1962a) studied the effects of an acid (pH 5.4) and a basic (pH 10.1) mineral mixture upon rumen function of a pair of identical-twin Angus steers fed the purified diet. The rumen content of the steer receiving the alkaline mineral mixture had a greater concentration of riboflavin and thiamine and contained significantly greater quantities of acetate, propionate, and butyrate plus higher molecular weight VFA than the rumen content of the steer receiving the acid mineral mixture. The inoculum from the steer fed the basic mineral mix digested significantly more cellulose than inoculum from the acid fed steer. They found that, at the end of the 30 day period, the steer receiving the acid mineral mixture consumed his diet very slowly, had a dull haircoat and had lost weight, while his identical twin appeared to be normal and had gained weight.

Oltjen et al. (1965) pelleted a purified diet and compared it to the loose purified diet. Crude fiber digestibility was 11% lower as a result of pelleting the diet. Pelleting did not, however, significantly alter protein utilization.

Clifford et al. (1966) supplemented the purified diet with amylase, chlortetracyline, diethylstilbestrol, and all combinations of these additives. Treatment effects for gain and feed intake were not significant except for an interaction between amylase and chlortetracycline. Diethylstilbestrol significantly increased the efficiency of gains.

Composition of ration has an effect upon both the types and number of rumen micro-organisms. Gall et al. (1951) fed sheep a purified diet consisting of 42% corn starch, 25% corn sugar, 20% cellophane, 5% minerals, and 4% urea and found that casein or urea plus sulphur in the rations supported a bacterial population of about double that found in the rumen contents of animals fed a ration containing urea but no sulphur. Animals fed urea plus sulphur had Gram positive slender, curved rods or Gram positive large, fat rods as the predominating organism, while the animals on the urea ration had either Gram negative, medium rods or Gram positive, fat rods. Animals consuming the casein ration had Gram positive cocci in masses as the predominating organisms. Bacteria isolated from the sheep fed rations containing urea plus sulphur and casein were almost all obligate anaerobes while most of the bacteria in the rumen of sheep

fed the urea ration were faculative anaerobes. Oltjen et al. (1966), in a comparison of a purified and natural diet, found that steers consuming a purified diet had an increase in total bacterial numbers while the total protozoal numbers decreased in comparison to those animals receiving the natural diet.

Various reports indicate that the feeding of a purified diet results in a lowered rumen pH. Gall et al. (1951) found that the bacteria isolated from sheep fed purified diets containing urea produced a low pH in vitro, while the sheep fed casein tended to support flora which did not lower the pH quite as much. Matrone et al. (1957) found a similar reduction in rumen pH on sheep fed a purified diet and suggested that since the starch and glucose content was higher (56%) than found in natural rations, the lower pH could be caused by lactate remaining in the rumen in considerably higher than normal concentrations. Oltjen et al. (1965) reported a significant decrease in rumen pH when steers were fed a purified diet in comparison with a conventional diet consisting of timothy hay, soybean meal, and molasses. In a similar study, Oltjen (1966) observed the same depression in rumen pH on the steers consuming the purified diet. Smith et al. (1966) also found rumen pH to be lower for calves on purified diets than for those on a control diet, and suggested that the lower pH was either the result of increased VFA production or decreased buffering capacity of the rumen when the purified diet was fed.

Oltjen et al. (1965) reported that steers fed a purified diet had a higher total VFA production and a higher molar percent of propionate than steers fed a conventional diet. These findings were in accord with the earlier findings of Matrone et al. (1964) who reported that the addition of 5% alfalfa to a urea containing purified diet lowered the propionate and raised the buturate concentration. In 1966, Oltjen et al. again reported increased VFA concentration on steers consuming a purified diet; however, in disagreement with the findings of Matrone, Oltjen reported the molar percent of butyric acid to be higher when steers were fed the purified diet. In this same study, Oltjen found the molar percent of acetic acid to be significantly lower in ruminal fluid of steers fed the purified diet, and these results are in agreement with those of Fontenot et al. (1962) and Virtanen (1966). Smith et al. (1966), however, in his comparison of the corn plus Purdue Supplement A ration to various purified diets reported no significant treatment differences in total concentration of VFA, molar percent of VFA, or acetate to propionate ratio.

Composition of blood from ruminants fed purified diets does not seem to differ from that of animals fed natural diets; however, there are exceptions to this general idea. Matrone et al. (1957) reported that calcium, magnesium, phosphorus, iron, and copper levels of serum, hemoglobin, and glucose levels of the blood were within the normal range when lambs were fed a semi-purified diet. Later,

Matrone et al. (1964) found that lambs fed a purified diet with urea as the sole source of nitrogen had slightly lower serum phosphorus and higher serum calcium than lambs fed various semi-purified diets; however, marked differences among diets for any serum constituent were not obtained.

Oltjen and Putman (1966) reported that the total concentration of plasma amino acids was similar for steers fed purified diets in which isolated soy protein or urea served as the nitrogen source. However, in steers consuming the urea diets, serine and glycine were found in greater concentrations, while valine, isoleucine, leucine, and phenylalanine were found in lower concentrations than in steers consuming isolated soy protein diets. In disagreement with these findings, Clifford and Tillman (1967) found the plasma concentration of serine to be lower in lambs fed all urea versus soybean protein purified diets. However, concentrations of threonine, asparagine, proline, alanine, isoleucine, tyrosine, and phenylalanine were 50-75% higher in lambs fed soybean protein in the purified diet. Virtanen (1966), working with dairy cows, found that concentrations of most of the free amino acids, and particularly the essential amino acids, in the plasma of lactating cows fed a purified diet were slightly lower than that of control cows fed diets containing natural feeds. The relative decrease in the concentration of free histidine in plasma was greater, in cows fed the purified diet, than the decrease for any other amino acid. Since the histidine content of hemoglobin is high, he

suggested that the low blood histidine levels could explain the reduced hemoglobin level in cows fed the purified diet. Virtanen also found the plasma concentration of total volatile fatty acids to be about the same for both groups of cows, while total plasma lipids were much higher for cows fed the natural ration than for those on the purified rations.

The purified diet has found application in a variety of research studies. Virtanen (1966) has used it to investigate the origin of the flavor substances of milk. Martin et al. (1964) used a purified diet to study the effect of magnesium and sulfur upon cellulose digestion and of magnesium upon voluntary feed intake. Ott et al. (1965) used a purified diet to quantitate the zinc requirement of growing lambs, and, based on growth data, he suggested that the requirement lies between 18 and 33 mg. of zinc per kg. of diet. Wright and Bell (1964) as well as Hopkins et al. (1964) have used purified and semi-purified diets to study the relationship of dietary α-tocopherol and selenium to nutritional muscular dystrophy in sheep.

In the studies of Wright and Bell (1964), forty-eight ewes were placed on a purified ration at the time of mating and divided into four groups in a factorial arrangement of the following variables: zero or 0.5 ppm selenium as sodium selenite in the ration, and zero or 100 IU of vitamin E per day as the acetate in a drench. All rations were consumed readily and body weight gains by the ewes were large enough

to allow normal development of fetal lambs. Ewes fed the basal ration gained less than those receiving supplemented α-tocopherol or selenium, or both. No congential abnormalities were observed in any of the lambs, which were removed from the ewe at approximately the 135th day of gestation.

Matrone et al. (1965) reported that a purified diet containing 570 mg. of α-tocopherol acetate per 100 lbs. ration did not sustain normal reproduction in yearling ewes. He found that many of the ewes did not conceive, and the ewes that became pregnant carried their fetus almost to term, but the fetuses died in utero. As two-year-olds, the ewes, which had been fed the purified diet from the time they were 2.5 to 3 months of age, were given the purified diet containing 5% alfalfa leaf meal and bred to a ram fed a normal hay plus grain diet. Nine ewes gave birth to a total of 15 lambs; two lambs were born dead and two died several hours after birth. Birth weights of the lambs ranged from 2.5 to 5.5 kg. with a mean of 3.95 kg. Since gains of these lambs were comparable to those obtained in practice, Matrone presumed that lactation was adequate during the six-week period. He concluded that alfalfa meal supplied some factor or factors to the purified diet and that these were necessary for reproduction.

Virtanen (1966) has found little reproductive trouble with dairy cows fed the purified diet. Estrus periods of the cows were regular and easy to detect. However, during the first year of his study, the cows were not given supple-

mental vitamin E, and many cows required several services before conceiving. All cows were then fed 330 to 500 mg. of ∝-tocopherol per head per day in his later studies. Virtanen has suggested that the vitamin E content of plant oils commonly used in the purified diet is not sufficient to satisfy the need of the cow for this vitamin, especially since the polyunsaturated fatty acids found in such oils increase the requirement for vitamin E.

Hertin and Drury (1963) determined the total and ∝-tocopherol content of various vegetable oils and found that corn oil, a very common constituent of the purified diet, contains from 147 to 236μg ∝-tocopherol per gram.

The manifestations of vitamin E deficiency in various animal species are quite variable. Muscular dystrophy, hepatic necrosis, infertility and abortion, encephalomalacia and anemia have been reported for various species. Edwin et al. (1961) has suggested that the specific syndrome of vitamin E deficiency in various animals may be related to a local tissue deficiency of this vitamin. In a study of the distribution of vitamin E in 14 tissues in rats, he found highest concentrations to be present in the adrenal gland, heart, uterus, and nerve. Likewise, it appears that tissues lose their vitamin E at different rates during depletion; tissues such as adrenal gland and nerve retained high concentrations of tocopherol, whereas the uterus and liver readily lost their tocopherol. He concluded that the susceptibility of the uterus to tocopherol depletion and admin-

istration was related to the gestation-resorption syndrome in the rat. Green et al. (1961) determined the distribution of vitamin E in tissues of rabbits and, in support of Edwin's hypothesis, found that skeletal muscles in rabbits contained little α-tocopherol and that it readily became depleted to exceptionally low levels thereby offering a possible explanation for muscular dystrophy, which readily occurs in that species. In contrast to the rat uterus, the uterus of the rabbit is affected very little by tocopherol levels.

Hopkins et al. (1964) fed growing lambs a semi-purified diet containing torula yeast and stripped lard and found that of 19 lambs receiving neither vitamin E nor selenium, eight developed muscle stiffness and subsequent immobility and eight died of apparent heart failure within a period of 23 to 28 days after the initiation of the experiment. During the first nine weeks of the experiment, those lambs receiving selenium or selenium plus vitamin E grew significantly faster than the lambs given vitamin E alone. After nine weeks of age, however, growth of lambs receiving either selenium or vitamin E was nearly the same, while those lambs receiving both selenium and vitamin E continued to grow rapidly. Hopkins found that a rise in levels of the enzyme serum glutamic oxaloacetic transaminase (SGOT) occured early in the experiment among the non-supplemented animals, was delayed but not prevented by selenium supplementation, and was completely prevented by supplementation with 250-500 mg. of α-tocopherol per lamb weekly. These findings are in

agreement with those of Wright and Bell (1964) who found that SGOT levels were elevated in ewe lambs during the ninth week for those receiving no supplemental vitamin E or selenium, during the twelfth week for those receiving selenium supplementation only, and remained relatively constant in the case of ewes receiving 100 IU of vitamin E per day. They also reported that four and five year old ewes receiving rations not supplemented with vitamin E maintained normal transaminase levels throughout the 18 week experiment, indicating larger body reserves of α-tocopherol in the aged ewes. Green et al. (1961) reported that rabbits kept on a vitamin E deficient diet for 15 weeks still contained large reserves of vitamin E in adipose tissue.

It appears that SGOT assay is a more sensitive test than histological examination for determining nutritional muscular dystrophy. Hopkins et al. (1964) found that there is a correlation between SGOT values and the degree of degeneration observed histologically. They found that of eight lambs fed a low vitamin E diet only two cases were diagnosed as muscular dystrophy, whereas seven of the eight lambs had SGOT values that were much higher than the controls.

Ehlig et al. (1967) compared a selenium-methionine mixture to Na_2SeO_3, each fed with and without vitamin E as sources of selenium for lambs. They found that lambs on the selenium-methionine supplement treatment retained significantly more selenium, had significantly higher biological

values for selenium, had slightly higher selenium blood values, and had significantly greater amounts of selenium in their tissues than lambs supplemented with Na_2SeO_3. SGOT values remained low for all lambs, and absorption and retention of selenium were not significantly affected by oral doses of 110 IU of vitamin E daily.

The general hypothesis concerning the action of vitamin E has been that this vitamin acts as a cellular antioxidant protecting a variety of membrane lipids from oxidation. In view of the increase in the activity of certain catabolic enzymes with change in vitamin E deficiency, Olson and Carpenter (1967) have offered the hypothesis that an unchecked biosynthesis of these enzymes occurs in the absence of α-tocopherol. By this line of reasoning, vitamin E may be thought of as a repressor of specific protein synthesis. The multitude of unanswered questions concerning vitamin E directly indicates the need for a more thorough probing into this area of research.

Since purified diets have become increasingly important in ruminant research, it is evident that further studies involving reproduction are necessary to establish these diets as nutritionally adequate. In view of the apparent lack of published information pertaining to this subject, the experiment reported herein was undertaken to determine the effects of a synthetic diet on the reproductive performance of ewes.

EXPERIMENT

Responses obtained during recent years with an improved purified diet developed at this station indicate that the diet will promote satisfactory growth and development in lambs. As it was not known whether this diet would support normal reproduction and lactation in ewes, this experiment was designed to supply information on this question.

EXPERIMENTAL PROCEDURE

Ten Dorset X western crossbred ewes averaging 30.6 kg. and four months in age were started on the purified diet February 7, 1966. The ewes were housed as a group on a slatted floor 10 X 2 meter pen, and an environmental temperature from 10° to 26.5°C was maintained. Fresh water and the diet shown in Table I were provided ad libitum. The animals were weighed at 0, 30, 100, and 200 days after the experiment was initiated. Hooves were trimmed twice a year, and the ewes were shorn annually.

The ewes were fed for 215 days, and then were exposed to a yearling Dorset ram over a 100 day breeding period. The ram was individually fed once daily a ration of hay plus a commercial sheep pellet and had free access to the purified diet when with the ewes. As the ewes pulled wool from the ram, he was allowed to be with them only during periods

TABLE I

PERCENTAGE COMPOSITION OF DIET

Ingredients	%	Ingredients	%
Corn starch	34.37	$CaHPO_4$	1.32
Glucose[a]	24.37	$MgSO_4$	0.12
Cellulose[b]	30.00	$MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$	0.28
Corn oil[c]	1.00	Na_2SO_4	0.25
Polyethylene resin[d]	1.00	NaCl	0.62
Choline chloride	0.10	Trace minerals[f]	0.10
Vitamins A and D[e]	0.02	Urea[g]	4.20
K_2CO_3	2.25		
		Total	100.00

[a]Cerelose. Corn Products Co., Argo, Illinois.

[b]Solka-Floc. B-W20, Brown Co., Berlin, New Hampshire.

[c]Mazola. Santoquin added to give 0.0125% in total ration.

[d]Alathon. E. I. du Pont de Nemours, Inc., Wilmington, Deleware.

[e]Containing 20,000 I.U. and 2,500 Units/gm. of vitamins A and D, respectively. Courtesy NOPCO Chemical Co., Harrison, New Jersey.

[f]Composition of the trace mineral mixture; (mg/100 gm. diet) $FeSO_4$, 42.51; $MnSO_4 \cdot H_2O$, 15.37; $Na_2B_4O_7$, 12.56; $ZnSO_4 \cdot 7H_2O$, 26.35; $CuCO_3 \cdot Cu(OH)_2$, 1.97; KI, 0.07; $CoCl_2 \cdot 6H_2O$, 0.05; CaF_2, 0.20; $Na_2MoO_4 \cdot 2H_2O$, 0.50; $Cr_2(SO_4)_3$, 0.04; Na_2SeO_4, 0.02.

[g]Crystalline urea. Courtesy Nipak Chemical Co., Pryor, Oklahoma.

of heat using the following procedure: Each evening the ram was placed with the ewes for approximately 15 minutes. If a ewe showed signs of heat, the ram was left with the flock for 24 hours, otherwise he was returned to his separate pen. A marking harness was used on the ram, and all estrus periods and breeding dates were recorded for each ewe.

When signs of lambing were noted, the ewe was separated from the remainder of the flock. Birth weights and dates were recorded. An autopsy was performed by a licensed veterinary pathologist on ewes and lambs which died during the course of the experiment.

On the 396th day of the experiment, five of the six remaining ewes were selected at random and fasted for 12 hours. Three hours after feeding, blood samples were collected in heparinized tubes by jugular puncture. In a like manner, blood samples were taken from five ewes of similar breeding fed a natural diet. All samples were kept under refrigeration until analyzed.

Milk samples were collected from four ewes producing a large enough volume for such a sample to be obtained and frozen until analyzed. Biweekly weights of a single surviving lamb were recorded over a 16 week period as an indication of milk production of the dam.

On the 437th day of the experiment the two remaining ewes were injected intramuscularly with 1,000,000 IU of vitamin A plus 500 IU vitamin E. Three weeks later vitamin E injections were repeated. Likewise, the lamb mentioned

above was injected with 500,000 IU vitamin A plus 250 IU vitamin E at birth followed by a 250 IU vitamin E injection at one week of age.

Analytical procedures were as follows:

(a) Blood samples. As soon as possible after bleeding a ten ml. aliquot of whole blood was set aside for determination of hematocrit by the method of Strumia *et al.* (1954), hemoglobin by the method of Cannan (1958), and red blood cell count by the method of Berkson *et al.* (1940). The remainder of the sample was centrifuged and the plasma collected. From the hematocrit, hemoglobin, and RBC count, the values presented below were obtained using the formulas of Wintrobe (1961) as follows:

$$\text{Mean corpuscular hemoglobin, } \mu\mu g. = \frac{\text{hemoglobin, g/1000 ml.}}{\text{RBC, millions/ccm.}}$$

$$\text{Mean corpuscular volume, } c\mu, = \frac{\text{hematocrit, ml/1000 ml.}}{\text{RBC, millions/ccm.}}$$

$$\text{Mean corpuscular hemoglobin concentration, \%, } = \frac{\text{hemoglobin, g/100 ml.}}{\text{hematocrit, ml/100 ml.}} \times 100$$

From the plasma, aliquots were taken for phosphorus determination by the method of Fiske and Suba Row (1925) and chloride by the method of Schales and Schales (1941). Calcium, potassium, magnesium, sodium, zinc, iron, and copper were determined by atomic absorption spectrophotometry (Perkin-Elmer, 1964). Plasma crude protein was determined by Kjeldahal (AOAC 1960) and blood urea nitrogen by the Hycel procedure (1960). Serum glutamic oxaloacetic transaminase

(SGOT), an enzyme indicating vitamin E status, was measured by the method of Freedland et al. (1965).

(b) Milk samples: These samples were thawed, mixed thoroughly, and appropriate aliquots were taken for the determination of total solids (AOAC 1960), fat (AOAC 1960), and crude protein by Kjeldahal (AOAC 1960). Ash was determined from five g. aliquots by heating in a muffle furnace at 550°C. for 12 hours.

Data were subjected to analysis of variance (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Table II shows the effects of the purified diet upon gains, which were lower than those reported by Oltjen *et al.* (1962a,b,c), Wright and Bell (1964), and Matrone *et al.* (1965); however, they are similar to gains of lambs fed urea-rich purified diets observed by Gall *et al.* (1951) and Matrone *et al.* (1964). The ewes of the present experiment were approximately four months of age at the start of the trial and almost one year of age when the last weights were recorded, while the authors in the above mentioned reports used young lambs. Differences in age could be responsible for the slower gains obtained here, particularly in the latter part of the growth period.

TABLE II

EFFECTS OF THE PURIFIED DIET UPON WEIGHT GAIN

Feeding Period	Initial Weight (kg.)	Final Weight (kg.)	Average Daily Gain (gm.)
0 - 30 days	30.6	32.7	70
30 - 100 days	32.7	39.0	89
100 - 200 days	39.0	42.1	31

Reproduction data are shown in Table III. With two exceptions, all ewes settled during the first or second estrus period, all of which were normal in length and easy to observe. Virtanen (1966) found that cows fed purified diets, not supplemented with vitamin E, required several services before conceiving. Since no attempt was made to measure vitamin E status of these ewes at the time of breeding, it is possible that their requirement for this vitamin was being fulfilled from vitamin E stores. However, the initial vitamin E stores of the ewes were not determined. Green et al. (1961) found rabbits kept on a vitamin E deficient diet for 15 weeks still contained large reserves of vitamin E in their adipose tissue. In addition, naturally occurring α-tocopherol in the corn oil supplied some dietary vitamin E. Based on α-tocopherol levels of corn oil, Hertin and Drury (1963), this would amount to 1/15 to 1/25 of the daily vitamin E requirement.

Average gestation length for the six ewes that lambed was approximately one week shorter than the 147 day period suggested by Kammlade and Kammlade (1955) for ewes of similar breeding and could explain the light birth weights obtained in the present experiment. Matrone et al. (1965) reported an average birth weight of 3,950 gm. for lambs obtained from ewes fed a semi-purified diet containing five percent alfalfa leaf meal. Their lambs were vigorous and healthy while most of the lambs obtained in this experiment were very weak and sickly in appearance at birth. Only one

TABLE III

EFFECTS OF THE PURIFIED DIET UPON REPRODUCTIVE PERFORMANCE

EWE NO.[a]	WEIGHT AT BREEDING (Kg.)	HEAT PERIODS PER CONCEPTION	LENGTH OF GESTATION (days)	BIRTH WEIGHT OF LAMB (gm.)	NO. DAYS LAMB LIVED	NO. DAYS EWE LIVED AFTER LAMBING
1	49.0	1	142	2,355	2	3
2	45.4	1	141	2,120	2	40
3	36.3	4+[b]	---	-----	-	----
4	40.4	2	---	-----	-	-15[c]
5	39.5	3	---	-----	-	-15[c]
6	44.0	1	148	3,160	Living	140[d]
7	43.1	1	145	2,965	5	36
8	41.8	2	126	2,100	0	215[d]
9	39.0	1	142	2,270	0	0

[a]Ewe 10 died before the breeding period was initiated.

[b]Indicates the ewe never conceived.

[c]The minus (-) sign indicates the ewe died before lambing. Approximate number of days are shown.

[d]These ewes died after the experiment had ended.

lamb survived, and he was injected with 500,000 IU vitamin A and 250 IU vitamin E at birth followed by a 250 IU vitamin E injection at one week of age. This lamb was much stronger and more active at birth than were the other lambs; its dam had been injected with vitamins A and E three weeks prior to parturition. With the exception of this ewe and a ewe receiving a similar injection, the remaining ewes died before or shortly after lambing. General nervous disorders and muscle paralysis appeared to be responsible for their death. These symptoms are discussed in more detail later.

Hematology data and SGOT levels are shown in Table IV.

TABLE IV

EFFECTS OF THE PURIFIED DIET UPON BLOOD VALUES

	Diets		
	Natural	Purified	SE[a]
Number of animals	5	5	----
Hematocrit, %	35.5	38.1	1.45
Hemoglobin, gm./100 ml.	12.6	14.3	0.64
RBC X $10^6/mm^3$	7.58	9.14	0.50
Mean corp. Hb., μμg.	16.77	15.68	0.78
Mean corp. vol., μ^3	47.07	41.98	1.77
Mean corp. Hb., conc., %	35.52	37.40	0.64
SGOT, μmoles/100 ml.	1.7[b]	22.3[c]	4.89

[a]Standard error.

[b,c]Horizontal values with different superscripts differ ($P<.025$).

Hematology measurements on animals fed the purified diet did not differ ($P<.05$) from those animals fed a natural diet. The purified diet promoted higher ($P<.025$) SGOT levels than did the natural diet, indicating a deficiency of vitamin E. Elevated transaminase levels have likewise been reported by Wright and Bell (1964) and Hopkins et al. (1964) for lambs fed diets deficient in vitamin E.

Plasma magnesium levels were lower ($P<.05$) for ewes fed the purified diet (Table V), and no logical explanation for this can be offered, but it should be pointed out that both values are considered to be normal. Differences in plasma iron levels approached significance. Higher concentrations of plasma iron in ewes receiving the purified diet were thought to be a result of greater hemolysis in this group; this condition is a characteristic symptom of vitamin E deficiency. Hopkins et al. (1964) found that red blood cells of lambs fed a vitamin E free semi-purified diet for 13 weeks were so fragile that they ruptured in saline buffer solution. In the present experiment, samples from ewes fed the purified diet showed much hemolysis even though they were centrifuged within one hour after bleeding. On the contrary, blood samples from ewes fed a natural diet showed very little hemolysis upon centrifugation after storage for as long as 24 hours post bleeding. No other differences existed ($P<.05$) between plasma mineral levels of ewes fed the natural or purified diet.

TABLE V

EFFECTS OF THE PURIFIED DIET UPON THE CONCENTRATION OF BLOOD PLASMA CONSTITUENTS

	Diets		
	Natural	Purified	SE[a]
Number of animals	5	5	----
Protein, %	6.30	6.13	0.16
Urea N, mg./100 ml.	6.12	7.31	0.40
Plasma Minerals			
Calcium, mg./100 ml.	10.76	10.38	0.26
Phosphorus, mg./100 ml.	4.76	5.52	0.24
Chlorine, mequiv./ml.	3.86	3.71	0.13
Potassium, mg./100 ml.	23.03	24.16	1.28
Magnesium, mg./100 ml.	2.74[b]	2.29[c]	0.12
Sodium, mg./100 ml.	374.24	369.14	10.95
Zinc, ppm.	0.43	0.62	0.06
Iron, ppm.	2.61	7.72	1.75
Copper, ppm.	1.06	1.28	0.09

[a]Standard error.

[b,c]Horizontal values with different superscripts differ ($P<.05$).

Ewes receiving the purified diet tended to have higher blood urea nitrogen levels; however, these differences were not significant. Since this group received urea as the sole nitrogen source, this trend was expected.

Table VI shows the proximate composition of milk from

ewes fed the purified diet. Since most of the lambs died shortly after birth, milk samples were difficult to obtain, and the resulting values cannot be considered representative of the lactation period. Ewes 6 and 7 gave birth to lambs which survived for five days or more, and with the exception of the percentage of fat, produced milk the composition of which did not differ greatly from normal values presented by Petersen (1950).

TABLE VI

COMPOSITION OF MILK FROM EWES FED THE PURIFIED DIET

Ewe No.	Sample Type[a]	% Total Solids	% Crude Protein[b]	% Fat	% Ash
2	Colostrum	45.24	14.33	27.5	1.10
8	Colostrum	37.24	7.56	28.0	0.84
6	Whole Milk	11.65	3.96	3.5	0.99
7	Whole Milk	12.01	5.11	2.0	1.05
Normal[c]	Whole Milk	17.0	5.2	6.2	0.90

[a]Samples collected 3 days after lambing are designated as colostrum. Samples collected 1 week or more after lambing are designated as whole milk.

[b]Computed as % N X 6.38.

[c]Approximate normals for sheep taken from Dairy Science by William E. Petersen (1950).

Visual observations of the ewes is a valuable part of this experiment, thus a discussion of these seems necessary. The following describes the physical condition of each ewe

prior to death and various clinical findings upon postmortem examination:

The case histories of Ewes 2 and 7 are similar. Ewe 2 developed a muscular paralysis of the legs two weeks after parturition. Prior to this time she was active and had no apparent difficulties during parturition. Once in the paralyzed condition, this ewe consumed little feed, slowly lost weight, and in general, degenerated in condition. No response was obtained by drenching with 500 IU ∝-tocopherol. Ewe 7 also became paralyzed one week after lambing. She gradually degenerated in condition, and again, no response was obtained from a 500 IU drench of ∝-tocopherol. In an attempt to stimulate use of the leg muscles, Ewe 7 was placed in a leather sling hung from the rafters of the housing unit whereby her feet touched the floor of the pen. Instead of holding her legs straight as in an attempt to stand, the ewe bent her hooves back allowing her weight to rest on the pasterns. This exercise was repeated for several days but was unsuccessful in improving the condition of the ewe. Unfortunately, both Ewe 2 and Ewe 7 were too decomposed for the postmortem diagnosis to be meaningful.

The lamb from Ewe 7 survived for five days, during which time it was weak, but it was quite active and nursed regularly. Postmortem examination revealed that its liver was greatly congested and friable, also, a yellow mucus was present in the alveolar spaces of the lung. The heart contained swollen muscle fibers and cellular hyperplasia.

Cause of death was diagnosed as white muscle disease and fat necrosis.

Ewes 3 and 4 were found dead, having given no previous indications of ailment. Ewe 3 was not pregnant at the time of death. Apparently this ewe never conceived; she had returned to heat each month during the four month breeding period. On autopsy, Ewe 4 was found to be carrying a fully developed fetus; however, she had shown no indications of labor previous to death. The liver was bile stained in areas and had a yellow-brown cast to the surface; the gall bladder contained a thick, yellow-green material. The larynx contained free blood, while in the lower portion of the trachea and major bronchi a thick yellow material was noted. Surrounding the heart were yellowish-white adipose deposits, and the pericardial sac contained a blood-red fluid. The ewe was too decomposed for an adequate diagnosis to be made. Postmortem decomposition seemed to occur much more rapidly in these animals than that noticed in animals fed natural diets.

Ewe 9 appeared quite normal until parturition, during which time she had great difficulty: Uterine contractions were slight and widely spaced in time. Manual manipulation of the vagina increased uterine contractions; however, these attempts were unsuccessful in delivering the lamb which was later removed by Caesarean section. The ewe did not survive the operation. Internal structures of the fetus appeared normal in size, location, and texture; however, it was de-

composed upon delivery. Postmortem examination of the ewe revealed that her liver was soft, friable, and yellow in color. Her lungs contained much blood, and numerous balls of wool were found in the rumen. The muscle mass was light in color indicating a lack of vitamin E. Cause of death was diagnosed as pulmonary edema.

Ewe 1, the largest ewe of the group, remained in good health until parturition. She had no difficulty lambing but retained most of the placental membranes, which were pulled from her as completely as possible; however, she died three days after parturition as a result of pulmonary edema. Autopsy revealed extensive hemorrhaging in the subcutaneous tissue of the cervical area, the wall of the trachea, and lungs. The lamb had nursed frequently, but was extremely weak at birth, had much difficulty standing on the slatted floor pen, and died two days later. Postmortem examination disclosed congestion of the kidneys, liver, spleen, lungs, and gastro intestinal tract which in addition had hemorrhages scattered throughout and contained a greenish yellow ingesta. Cultures were taken from the spleen as well as the contents of the gastro intestinal tract, both of which were found to contain E. coli; cause of death was diagnosed as colibacillosis.

Ewe 5 died prior to lambing following one week of extreme nervousness. These symptoms included glassy eyes, ear and head twitching, and a staggering gait when walking. The carcass was too badly decomposed for a complete diagnosis to

be made; however, the ewe was carrying a fully developed fetus at the time of death.

Ewe 10 died two months prior to the breeding period having been fed the purified diet for only five months, but no pathological information is available as to cause of death.

Ewe 8 was one of two ewes surviving the experiment. This ewe aborted three weeks before she was due to lamb. Postmortem examination revealed that all internal structures of the fetus were normal in size, shape, and position, thus cause of abortion could not be determined. Contrary to the performance of the previous mentioned ewes, Ewe 8 continued to survive on the purified diet for 67 days after lambing at which time she was injected intramuscularly with 1,000,000 IU vitamin A and 500 IU vitamin E. This ewe was fed the purified diet for 19 months and was fleshy and in good condition at the end of the experiment (Figure I).

Ewe 6 likewise survived the 19 month feeding period. After receiving injections of 1,000,000 IU vitamin A plus 500 IU vitamin E 23 days prior to parturition, this ewe gave birth to the only lamb surviving the experiment. Apparently she had either aborted or reabsorbed an earlier embryo, for after being bred in September she did not return to estrus during October or November and was therefore assumed pregnant. However, she returned to estrus in December, and it was from this mating that the above mentioned lamb was born. On the day following lambing, she received an additional

PLATE I

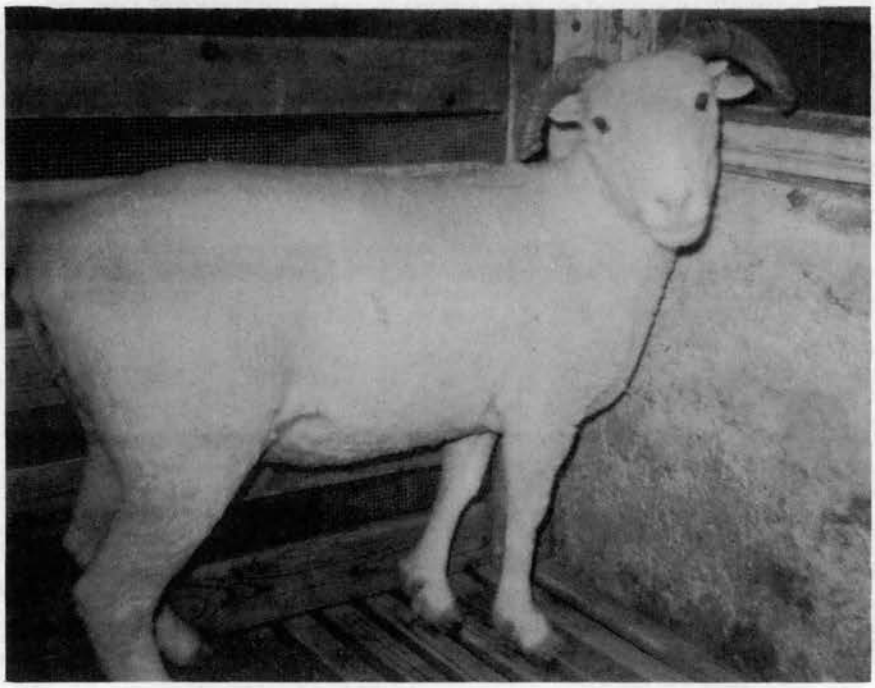

Figure 1. Ewe 8 After Being Fed the Purified Diet for 18 Months.

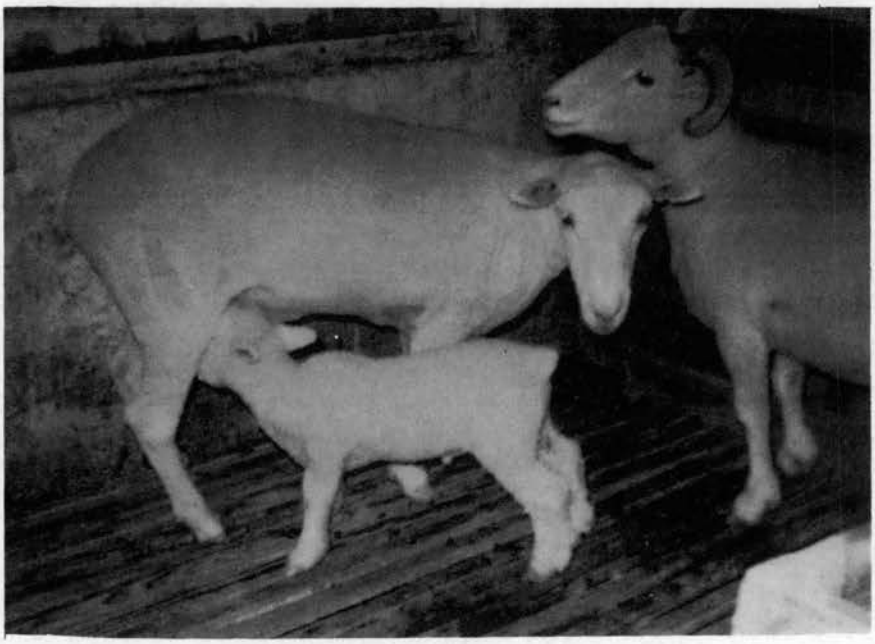

Figure 2. Ewe 6 (Nursing Lamb) 1 Month After the Lamb was Born.

500 IU vitamin E and was in good health at this stage (Figure 2); however, her condition continuously worsened as the lactation period progressed (Figures 3 and 4).

The lamb of Ewe 6 was a much stronger lamb at birth than previously born lambs. At birth this lamb was injected with 500,000 IU vitamin A and 250 IU vitamin E followed by an additional injection of the same quantity of vitamin E at one week of age. Unlike the other lambs, this lamb adjusted quite readily to the slatted floors, and was much more active and nursed more frequently than previously mentioned lambs. No physical abnormalities were noted in the young lamb (Figure 5), and it developed quite well during the post weaning period (Figure 6).

Gains made by the lamb (Table VII) were well above those of the ewe flock during the previous year. Gains during the first ten weeks indicate that milk production of the dam was normal. Since the lamb was never separated from its dam, reduced gains from the eleventh through the fourteenth week are a result of the natural weaning period during which time the lamb slowly adjusted to the purified diet. Post-weaning gains were equal to those found by Oltjen et al. (1962a,b,c) and Matrone et al. (1965), and were superior to gains found by Woods and Tillman (1956), Matrone et al. (1964), and Clifford (1967) for young lambs fed urea-rich purified diets.

PIATE II

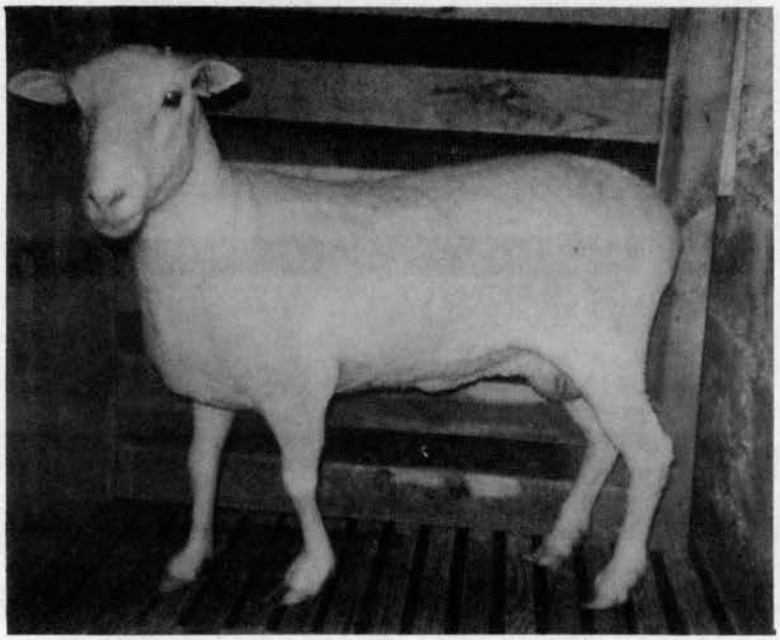

Figure 3. Ewe 6 Two Months After the Lamb was Born.

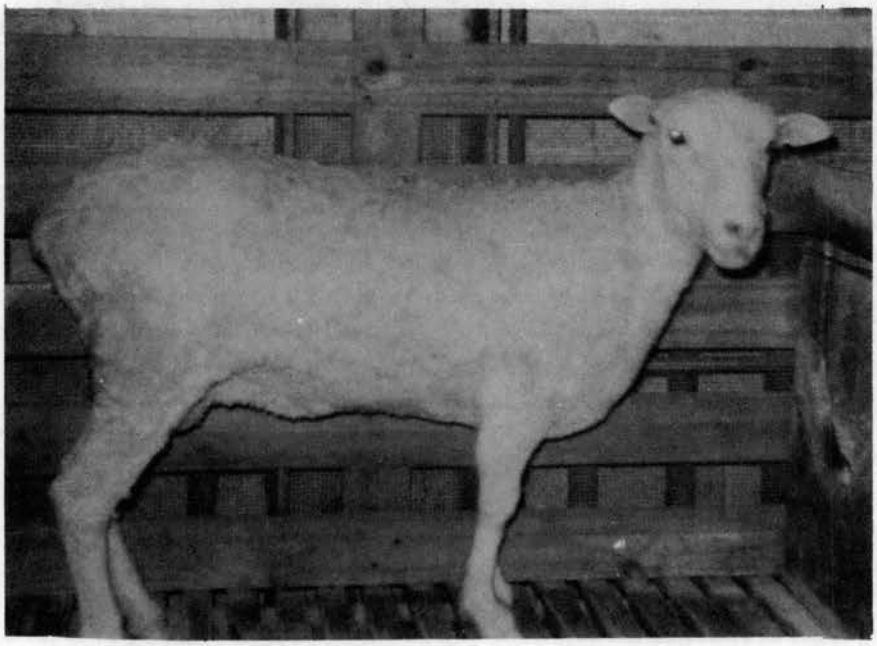

Figure 4. Ewe 6 Three Months After the Lamb was Born.

PLATE III

Figure 5. Lamb of the Sixth Ewe at 1 Month of Age.

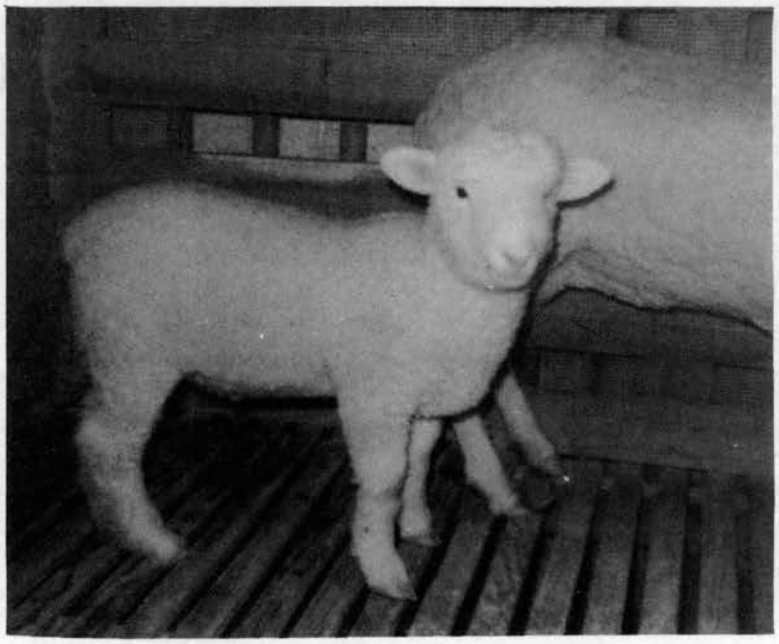

Figure 6. Lamb of the Sixth Ewe at 3 Months of Age.

TABLE VII

EFFECTS OF THE PURIFIED DIET UPON PRE- AND POST-WEANING GAINS OF THE ONE SURVIVING LAMB

Age	Weight (Kg.)	Average Daily Gain (gm.)
Birth	3.2	-----
1 week	4.6	201.4
2 weeks	5.6	152.0
4 weeks	8.0	168.0
6 weeks	10.2	158.5
8 weeks	12.0	129.7
10 weeks	13.5	105.4
12 weeks	13.4	0.0
14 weeks	14.5	81.1
16 weeks	17.0	178.4

The only difference between the two ewes and lamb surviving the experiment and the remainder of the flock was the injections of vitamins A and E received by these three animals. Three weeks after the experiment was completed, one of these ewes died, and the condition of the other became critical; she died two weeks later. Since this diet was adequate in vitamin A but supplied only 1/15 to 1/25 of the daily vitamin E requirement, these results indicate that vitamin E may have been the limiting nutritional factor responsible for poor reproduction. Other factors such as

serum glutamic oxaloacetic transaminase levels, rate of hemolysis, and various autopsy findings likewise indicate a deficiency of this vitamin; however, these results are based on too few animals for any positive conclusions to be made.

The purified diet used in this experiment did not support normal reproduction in the ovine.

SUMMARY

Ten ewes, which were four months old at the initiation of the experiment, were fed a purified diet containing urea as the nitrogen source during growth, gestation, and lactation for the purpose of ascertaining if the diet would support reproduction and lactation in sheep.

Eight of the ewes became pregnant; however, gestation periods were shorter and birth weights considerably lighter than normal. Five lambs were born; one was born dead, and three died within one week after birth. Most of the ewes died shortly before or after lambing. Two ewes and one lamb were alive at the end of the experiment, and these had received vitamins A and E injections. Postmortem examinations revealed congestion of the liver, spleen, respiratory tract, and gastro-intestinal tract.

When compared to ewes receiving a natural diet, there were no significant differences in any of the hematology measurements; however, ewes fed the purified diet had significantly higher serum glutamic oxaloacetic transaminase levels than those fed a natural diet. Animals receiving the purified diet had lower plasma magnesium levels, but diet had no effect upon other minerals. Blood samples from ewes fed the purified diet hemolyzed to a greater extent than those from ewes fed the natural diet.

Composition of milk from the experimental ewes did not differ greatly from normal values. The only ewe with a surviving lamb produced sufficient milk as was evident by average daily gain for this lamb as large as 200 gm. during the pre-weaning period.

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