THE EFFECT OF SUPPLEMENTAL FAT AND METHIONINE HYDROXY ANALOGUE ON CELLULOSE DIGESTION IN WETHER LAMBS

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by
Donald Thomas Lyons
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Dr. W. H. Pfander, Thesis Supervisor

The undersigned, appointed by the Dean of the Graduate Faculty, have examined a thesis entitled

THE EFFECT OF SUPPLEMENTAL FAT AND
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CELLULOSE DIGESTION IN
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a candidate for the degree of Master of Science

and hereby certify that in their opinion it is worthy of acceptance.

W H Pfander
[illegible]
G. B. Garner

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CHAPTER I

INTRODUCTION

Ruminants are able to obtain much of their needed energy by consuming large quantities of roughage. Many of the roughages utilized by ruminants are of poor quality due to environmental conditions or the portion of the plant harvested. Supplements must be added to these roughages to provide the needed energy. Carbohydrates have supplied most of the additional energy, but fat, in recent years, has become an important energy source for non-ruminants and its value for ruminants should be investigated in detail. Synthetic detergents and vegetable oils have replaced animal fat for the manufacture of soap and for cooking purposes. Fat has also been found to be implicated with arteriosclerosis in humans. For this reason more fat is being trimmed from each retail and wholesale cut of meat. Therefore, animal fat has accumulated in large quantities and this surplus fat needs to find another market.

Fats, nutritionally, are primarily energy sources and must compete with the lowest priced non-fat energy supplements. Fat as an energy source was found to contain 2.25 times as much energy as number 2 corn. When compared to barley and oats, this value for fat was even higher (1). Studies carried out at Cornell University and the American

Meat Institute Foundation (2) indicated that up to 8 per cent fat was a good calorific replacement for carbohydrates in animal feed. These workers also believed that fat may have further nutritional advantages above this calorific value.

It is now known that ruminants are able to utilize inorganic sulfur in the synthesis of cystine and methionine. Also sheep make better utilization of non-protein nitrogen sources like urea when inorganic sulfur is available.

Recently it was reported that methionine added to a diet adequately supplied with sulfur would stimulate cellulose digestion. This work was demonstrated both *in vivo* and *in vitro*.

The purpose of this study was to determine the effects of different levels of two types of fat on cellulose digestion in lambs on a poor quality roughage. Methionine hydroxy analogue, the alpha keto acid of methionine, was studied to determine its effect on a low sulfur ration made up of natural feeds.

CHAPTER II

REVIEW OF LITERATURE

A. Effects of Supplemental Fat on Nutrient Digestibility

Fats, although making up only a small portion of the ruminant's diet, provide essential fatty acids, transport fat soluble vitamins and serve as an energy source for the animal. On the other hand, supplemental fat may decrease the digestibility of the ration.

In 1941 work with dairy cows, by Allen and Fitch (3), indicated that the feeding of corn oil, at a level of 1.5 lbs. daily over a 50-day period, greatly decreased the fat content of milk and the yield of milk fat. The depressing effect of corn oil was more pronounced with continued feeding.

Lucas and Loosli (4) conducted a series of digestion studies with dairy cows comparing the effects of different sources and levels of fat upon the apparent digestibility of nutrients. Different fat levels were obtained by adding varied amounts of concentrates, containing different levels of ether extract, to rations containing timothy hay and beet pulp. They reported no difference in the apparent digestibility of rations containing 1.6 and 2.6 per cent ether extract; however, nitrogen-free extract and crude fiber were less digestible in a ration containing 7 per cent ether extract than in one containing 1 per cent ether extract. The crude fiber digestibility was significantly lower for the rations containing ground soybeans and corn or soybean oil than for the rations containing solvent extracted soybean meal alone.

Swift et al. (5) found that the addition of 34 gms. (3.6 per cent) of corn oil to a basal ration containing 2.8 per cent ether extract increased the digestibility of all feed constituents. When they increased the corn oil to 68 gms. (7.0 per cent), all the digestion coefficients except that for ether extract were reduced. The digestion coefficients for crude fiber of the 2.8, 6.4, and 9.8 per cent ether extract rations were 49.0, 50.9, and 40.7. The digestion coefficients for dry matter of the preceding rations were 74.7, 76.2, and 72.8.

Swift and his co-workers (6) fed wether lambs six rations which contained different levels of ether extract. The levels used in this study were 3, 4, 5, 6, 7, and 8 per cent. As the fat content of the ration increased, the digestibility of nitrogen-free extract and dry matter decreased. There was no significant decrease in digestibility when the fat was increased 1 per cent; however, a 3 per cent increase in fat significantly decreased the dry matter and nitrogen-free extract digestibility. No constant effect was noted on the digestibility of crude fiber or crude protein.

Brooks *et al*. (7) studied the effect of added fat on cellulose digestion by sheep rumen microorganisms in the artificial rumen. The addition of 10-170 mg. corn oil per 25 ml. flask significantly reduced cellulose digestion 40 to 94 per cent. The addition of 32 or 64 gms. of corn oil to a cottonseed hull and casein basal ration significantly reduced cellulose digestion 70 per cent and protein digestion 36 per cent. Thirty-two or 64 gms. of lard added to the basal ration reduced cellulose digestion by 53 per cent and protein digestion by 33 per cent. The added corn oil caused a greater reduction in cellulose digestion than the lard.

Workers at the Kentucky Station followed up this work. Summers *et al*. (8) reported that 2 per cent corn oil added to a 65 per cent corn cob ration significantly reduced the digestibility of cellulose, organic matter, and crude protein. Rhodes *et al*. (9) found that the apparent digestibility of cellulose and protein was significantly depressed by replacing corn syrup with 1.8, 3.0, and 4.2 per cent corn oil in rations containing from 65 to 80 per cent cottonseed hulls. In 1957 Grainger *et al*. (10) showed that replacing corn syrup with 6 per cent corn oil significantly reduced the digestibility of cellulose, organic matter, and protein. These workers found that 5 per cent corn oil significantly decreased organic matter and protein digestibility. Baker *et al.* (11) showed in a study with crossbred wethers that 5 per cent corn oil progressively depressed cellulose digestion during a 40-day period. Cellulose digestion recovery was not complete until 17 days after a ration change was made omitting the added corn oil. These workers also found that mixing corn oil on the concentrate portion of the ration increased slightly the depressing action of the corn oil on cellulose digestion. They suggested that added fat decreased microbial metabolic activity and modified the microbial population of the rumen, thus depressing cellulose digestibility and decreasing the availability of roughage energy to the host.

Ward *et al.* (12) found that the addition of 2.6 or 10 per cent corn oil to rations for growing lambs decreased weight gains and increased the pounds of feed required for a pound of gain. They also reported that the addition of 3 per cent or more corn oil to rations for sheep containing high levels of cottonseed hulls or corn cobs significantly reduced the digestibilities of most ration components, especially that of crude fiber.

Later work by Brethour *et al.* (13) in growth and digestibility trials with sheep showed that the addition of 15 per cent animal fat to a cottonseed hull basal ration significantly decreased the digestibilities of organic matter and dry matter. Weight gains were also reduced. In another trial these workers reported that the addition of 10 per cent corn oil reduced weight gains regardless of whether it was absorbed on the roughage or concentrate portion of the ration.

Hale and King (14) added corn oil, prime tallow, and hydrogenated animal fat to a standard lamb fattening ration. These fats were added at the following levels to the basal ration: 0, 4, 8, and 12 per cent. Equal amounts of corn in the ration were replaced by the fat. The 4 per cent fat level appeared to have very little effect on dry matter digestibility. The two higher levels markedly reduced the digestibility of the dry matter.

In 1957 Embry _et al_. (15) demonstrated that 5 per cent added lard significantly increased digestibility of ether extract and crude protein but decreased digestibility of nitrogen-free extract and crude fiber. Total organic matter digestibility was not affected.

Very little information is available concerning the optimum level of fat in the rations of fattening beef cattle. Willey _et al_. (16) added 5 per cent cottonseed oil to rations of fattening steers and obtained a 12 per cent increase in the efficiency of feed utilization, but no improvement in rate of gain or carcass grade.

Matsushima and Dowe (17) fed 5 per cent beef tallow pellets to yearling steers over a 150-day feeding period. These steers consumed about 3/4 of a pound of beef tallow per day. They found that the added beef tallow increased the efficiency of feed utilization but had no effect on carcass quality. These investigators reported that the fat pellets were fed during the winter months. They stated that under warm weather conditions the feeding of high fat rations may not be desirable.

Hentges et al. (18) added 5 per cent raw ground waste beef tallow to steer fattening rations. In a trial with 18-month-old steers the 5 per cent added fat markedly increased the rate of gain. In another trial using 8-month-old steer calves there was little difference between the basal and the 5 per cent fat rations while 10 per cent added fat markedly reduced the rate of gain. In both trials less feed was required per unit of gain when the raw beef tallow was fed.

Work by Matsushima et al. (19) showed that as the beef tallow content of a steer fattening ration was increased, fiber digestibility was decreased and ether extract digestibility increased. These workers found that the efficiency of feed utilization increased as the level of added beef tallow and protein in the ration increased.

Bohman (1) added animal fat to fattening steer rations at levels of 0, 5, and 10 per cent of the entire ration. The cattle that were fed the 5 per cent fat level had an average daily gain of 2.57 lbs., while those on 10 per cent fat gained 2.27 lbs. compared to controls that gained 2.01 lbs. per day. These cattle were on feed for 120 days.

Bohman (20) in a two-year study found that 0.5 lbs. of inedible animal fat fed daily to wintering beef cattle had no effect on winter gains. The cattle that had received added fat during the winter gained faster the following summer.

Erwin *et al*. (21) found that 7 per cent edible beef tallow added to steer fattening rations significantly decreased the digestibility of crude fiber and dry matter. In 1956 Erwin *et al*. (22) showed that inedible animal fat increased the rate of gain of steers consuming alfalfa as the only roughage source but reduced the rate of gain of steers fed wheat straw. Another study by the same men (23) demonstrated that inedible animal fat, fed as 7 per cent of the total ration, significantly reduced the digestibility of crude fiber and dry matter but increased the digestibility of ether extract.

Bohman *et al*. (24) reported that 5 per cent animal fat significantly increased the rate of gain of fattening steers. Ten per cent added fat was not as effective as the lower level. Five per cent added fat reduced the amount of feed required by 23 per cent and 10 per cent added fat by 16 per cent.

Work by Dyer _et al_. (25) showed that edible beef tallow fed at 7 per cent of the total ration did not significantly increase rates of gain on a pelleted steer ration. They suggested that this was probably due to the accompanying reduction in digestibility of crude fiber and dry matter.

Hoflund _et al_. (26) demonstrated that polyunsaturated fatty acids can be hydrogenated in the rumen. Rumen samples were taken at regular intervals following the introduction of 33 per cent emulsified linseed oil into the rumen of a fistulated sheep. Conversion of linolenic acid to linoleic acid occurred, suggesting that the rumen microorganisms hydrogenated one double bond of the dietary polyunsaturated fatty acids in the rumen.

Reiser and Reddy (27) fed alfalfa meal containing 10 per cent linseed or cottonseed oil to goats. A comparison of the fatty acids of the rumen, stomach and caecum contents to those in the feed indicated that the original high levels of linolenic and linoleic were reduced to very low levels in the rumen. There was a comparable increase in the saturated acids found in the rumen. They reported that monoethenoid acids were increased in goats fed linseed oil and in one animal after cottonseed oil ingestion.

In 1957 Shorland *et al*. (28) found that oleic, linoleic or linolenic acid incubated with sheep rumen contents in a carbon dioxide atmosphere at 37^{o}, resulted in the production of stearic acid along with trans and positional isomers of the unsaturated acids.

B. The Effect of Added Sulfur on Ruminant Rations

Rimington and Bekkar (29) proposed that the intestinal flora of sheep may be able to convert inorganic sulfur to cystine which could then be utilized for synthesis of body tissues and keratins.

Van Koetsveld (30) found that cattle drinking water containing high levels of sulfate had diarrhea and/or showed symptoms of copper deficiency. The per cent sulfate in milk varied from 7.0 to 14.6 mg. in cows on a total sulfate intake of 75 to 150 grams per day. The total sulfate in milk ranged from 780 to 2774 mg. daily. He reported that sulfate in the blood of seven normal cows varied from 12.6 to 13.9 mg. per cent, while eight cows with grass tetany showed levels of 15.2 to 19.0 mg. per cent. Magnesium in the blood of the cows with grass tetany ranged from 0.3 to 0.5 mg. per cent. When the magnesium level was 0.8 to 1.6, the sulfate in the blood was 14.1 to

18.6 mg. per cent. Van Koetsveld found no value for sulfate higher than 21 mg. and he suggested that sulfate was a threshold substance.

Early work by Loosli and Harris (31) in a balance study with growing lambs showed that the addition of methionine to a urea containing ration greatly enhanced protein synthesis from urea in the rumen of lambs.

Garrigus *et al*. (32) fed growing fattening lambs (1) a methionine-deficient basal ration, (2) the basal ration plus supplemental elemental sulfur, and (3) the basal ration plus supplemental methionine. The basal ration was significantly improved by the addition of methionine. The improvement from elemental sulfur approached significance.

Thomas *et al*. (33) studied the sulfur and nitrogen metabolism of lambs fed purified diets with and without sulfates. The lambs on the sulfur-deficient diet were in negative nitrogen and sulfur balance. There was a marked decrease in rate of wool growth on the sulfur-deficient animals when compared to the lambs on the sulfur-supplemented ration. These workers found that the amino acid composition of the wool produced by the sulfur-deficient lambs appeared normal.

In 1952 Gallup *et al*. (34) demonstrated that supplemental methionine added at the rate of 3 or 6 grams per day failed to improve a low protein ration adequately supplied with sulfur. When methionine was added to a ration containing urea at the rate of 1.6, 2, and 3 grams per day, it increased the digestibility of nutrients and nitrogen utilization.

Starks _et al_. (35) studied the influence of elemental sulfur on nitrogen retention by lambs fed a low sulfur, urea containing purified ration. The sulfur-deficient lambs showed the following symptoms: poor appetite, loss of wool, excessive lacrimation, profuse salivation, weakness, dullness, cloudy eyes, emaciation, and finally death. The lambs receiving elemental sulfur increased their wool growth, retained more nitrogen and sulfur, and came closer to retaining their weight than those on the sulfur-deficient ration. These investigators concluded that the addition of elemental sulfur definitely improved the utilization of feed nitrogen.

Lofgreen _et al_. (36) studied the addition of sodium sulfate to a ration composed of natural feedstuffs to see if it would produce comparable results to the classical purified diet work. The addition of 0.2 per cent sodium sulfate to a basal ration made up of 87 per cent natural feeds with 40 per cent of the total nitrogen furnished by urea, had no effect on weight gains, feed utilization, nitrogen utilization, wool growth, or serum sulfate levels. These workers believed that the sulfur present in natural feedstuffs was readily available and was present in large enough quantities to meet the sulfur requirement for growing lambs. The addition of inorganic sulfur was not beneficial under these conditions.

Starks *et al*. (37) conducted investigations to determine if organic sulfur was converted to protein sulfur at a rate to permit optimum growth in lambs. The basal ration, containing 0.054 per cent sulfur with 92 per cent of the nitrogen supplied by urea, was supplemented by three levels of: elemental sulfur (0.2, 0.4, and 0.6 per cent); sodium sulfate (0.89, 1.33, and 1.78 per cent); and DL-methionine (0.2, 0.5, and 0.7 per cent). Weight gains and wool growth were significantly increased by the addition of the sulfur supplements. There was no significant difference between the three forms of sulfur or the levels of each.

Noble *et al*. (38) studied the value of added methionine to lamb fattening rations. Urea and soybean meal, alone and with methionine, were compared in three feed lot trials as nitrogen supplements to a low-protein basal ration for fattening lambs. Methionine and urea failed to improve rate of gain when added separately to the basal ration. When methionine and urea were added in combination they increased slightly the daily gain in all trials. Feed efficiency and rate of gain were consistently improved when soybean meal was added as a supplement to the basal ration.

Other investigators used radioactive forms of sulfur in studying sulfur metabolism in ruminants. Block and Stekol (39) found that methionine and cystine in the milk proteins of a Holstein cow showed considerable radioactivity several days after radioactive sodium sulfate was fed. They believed that sulfate sulfur when fed to a ruminant was converted in appreciable quantities to cystine and methionine.

Block *et al*. (40) studied the conversion of sulfate sulfur to methionine and cystine by the goat and by the microorganisms in the rumen of the ewe. Radioactive sodium sulfate showed its peak activity in the milk protein 24 hours after the initial feeding to a goat. Eighty per cent of the radioactive sulfur was found in the protein fraction of the milk and 20 per cent in the filtrate. Radioactive sodium sulfate was fed to a ewe in three equal doses. The greatest activity was observed in the protein fraction of the rumen contents. After hydrolysis of the rumen contents with trichloroacetic acid, the radioactivity of the cystine present was equal to that of the methionine.

Keener *et al*. (41) investigated the metabolic fate of sulfur from sulfur dioxide used as a silage preservative. This silage was put in large drums and preserved with S^{35}-sulfur dioxide. They fed silage made from ladino clover, alsike clover, and a small amount of timothy to lactating cows. Two-thirds of the radioactive sulfur fed was absorbed and then most of it was eliminated by the kidneys. Fifty-four hours after the last silage had been eaten there was a marked decrease in urinary and mammary excretion of radioactive sulfur. Some activity was noted in the feces two to three days later. They believed that the sulfur from the S^{35}-sulfur dioxide could possibly be of some nutritional value because it was synthesized into the proteins of the blood and the milk.

Hale and Garrigus (42) used radioactive isotopes to see if elemental sulfur and sulfate sulfur could be utilized for wool growth. They fed one yearling wether lamb radioactive neutral sulfur and another radioactive sodium sulfate. Within two weeks these workers found radioactivity in the cystine of the wool protein of both sheep, the higher amount being in the sulfate-fed sheep. This study demonstrated that inorganic sulfur was synthesized into protein sulfur by sheep.

Kulwich *et al*. (43) studied the uptake of radiosulfur in lambs using labeled sodium sulfate. Two three-month-old lambs were fed pelleted rations *ad libitum*. Lamb A was fed a ration with soybean protein as the chief source of protein while lamb B received a similar ration in which 1.2

per cent urea replaced part of the protein. Eleven doses of radioactive sodium sulfate were administered to each lamb over a 24-day period. The excreta was analyzed for radiosulfur content. Lamb A's urine contained radiosulfur equivalent to 72 per cent of a single dose while the feces contained radiosulfur equivalent to 39 per cent of a single dose. The values for lamb B were 55 and 59 per cent, respectively. The concentration of radiosulfur was higher in the wool than in any of the tissues analyzed. Lamb B had a higher tissue concentration of radiosulfur than Lamb A.

Dick (44) investigated the effect of inorganic sulfate on the excretion of molybdenum by sheep on a daily intake of 10 mg. molybdenum from ammonium molybdate. When the sulfate intake was low very little molybdenum was excreted in the urine and the blood molybdenum values were high. When the sulfate intake was increased large quantities of molybdenum were excreted in the urine and the molybdenum level in the blood fell sharply. Since more molybdenum was excreted than could be accounted for in the extracellular fluid and blood, Dick believed that the animals' molybdenum stores were depleted by the sulfate. He reported that due to the similar size of the sulfate ion and molybdenum, they competed for reabsorption in the kidney tubules after filtration through the glomeruli. A follow-up (45) demonstrated that molybdenum limited copper storage in the liver of sheep only when adequate inorganic sulfate was present in the diet. When the total sulfate intake of sheep was around 2 grams daily, as little as 0.5 mg. of molybdenum would significantly limit copper storage.

Whiting *et al*. (46) reported that the sulfur requirement of the mature range ewe did not exceed 0.1 per cent of the total ration since this level was found adequate for wool and lamb production.

In 1956 Albert *et al*. (47) determined the requirements for methionine, sodium sulfate, and elemental sulfur of growing fattening lambs. The three sulfur sources were added at varying levels to a low sulfur basal ration containing 4 per cent urea. Average daily gains were used to measure the optimum level. On the basis of total sulfur about 70 per cent less sulfur was needed as methionine than as elemental sulfur in the diet, and about 50 per cent less as sulfate sulfur. In this study methionine appeared to be the most efficient source of sulfur for growing-fattening lambs.

Hunt *et al*. (48) used rumen microorganisms to determine the effect of sulfur and starch on B-complex vitamin synthesis, cellulose digestion, and urea utilization. Starch increased B-complex vitamin synthesis and urea utilization but decreased cellulose digestion.

Inorganic sulfur as sodium sulfate and methionine stimulated urea utilization, cellulose digestion, and B-vitamin synthesis, the only exception being panthothenic acid. It was postulated that the microorganisms responsible for panthothenic acid synthesis have a lower sulfur requirement than the other strains of rumen organisms.

Emery _et_ _al_. (49) used radioactive sulfur to determine if sulfur is utilized by rumen microorganisms. Rumen fluid from four fistulated cows fed either hay alone or 75 per cent grain rations were incubated anaerobically with the substrate on which they had developed. Sulfate incorporation was greater by the grain microorganisms than by the hay microorganisms (there was more sulfur present in the grain). Most of the sulfate incorporation was in the cystine (cysteine) and methionine with glutathione showing less activity. These workers believed that the incorporation rate was less than 3 per cent per minute of the sulfate available at any given time. The rate with grain appeared to be a little higher than with hay.

Lewis (50) in 1953 studied the reduction of sulfate by rumen microorganisms in _in_ _vivo_ and _in_ _vitro_ experiments with mature wethers. Sulfate was reduced to sulfide in the rumen. This was also demonstrated under _in_ _vitro_ conditions using washed cells. The optimum pH for the reduction was 6.5. Thiosulfite and sulfite were also reduced to sulfide. The mature wethers tolerated 150 grams $Na_2SO_4 \cdot 10H_2O$, and showed no toxic effects even when the concentration of $S^=$ reached 1.47 m. moles/100 ml. rumen liquor.

Trenkle et al. (51) studied the effects of different amino acids on cellulose digestion by rumen microorganisms. They found that DL - proline, DL - alanine, and DL - methionine consistently increased cellulose digestion by rumen microorganisms. These authors used washed suspensions of rumen microorganisms in their work. Methionine was found to be more effective, when added to a basal medium containing adequate sulfur, in stimulating cellulose digestion than either proline or alanine. Combinations of methionine and proline or methionine and alanine gave responses equal to those of methionine alone. These in vitro results were confirmed in a lamb feeding experiment using a purified diet. The lambs supplemented with methionine showed increased live weight gains which approximated the increased cellulose digestion noted in the in vitro studies.

CHAPTER III

MATERIALS AND EXPERIMENTAL PROCEDURES

A. Artificial Rumen Procedure

1. Equipment

Artificial Rumen--A 50 ml. centrifuge tube fitted with a two-hole rubber stopper was used. Through the stopper holes were two pieces of glass tubing, one long piece extending to within one-fourth inch of the bottom of the tube and the other piece extending one inch below the stopper. A one-inch portion of both pieces of tubing projected out of the top of the stopper.

Glass and Metal "T" tube series--constructed so that a piece of rubber tubing was connected to an artificial rumen from the bottom of the "T," while the tops of the "T's" were connected into a series with rubber tubing. The tubes were placed in series of either two or three. Figure 1, Appendix, shows a trial in progress.

CO_2 cylinder--equipped with a regulating valve.

Pipettes--one 10 ml. and one 5 ml.

International centrifuge--size 1, Model SBV.

Magnetic stirrer--Fisher Scientific Co., 115 volts.

Water Bath--Precision Scientific Co., No. J-10. Maintained at 39° C. for the incubation period.

Stainless steel water bath--for cellulose digestion procedure, 12" wide by 15" long by 5" deep.

Wire test tube rack--capacity ten 50 ml. centrifuge tubes.

Aspirator.

Selas previously ignited crucibles, coarse, 40 ml.

Desiccator--vacuum type, 10" in diameter.

Oven--Blue Line, Model OV490.

Muffle furnace--Ward Leonard Electric Co.

Analytical balance--Christian Becker, Chainomatic.

2. Materials and Methods of Preparation

Mineral Media for Artificial Rumen:

Solution (1)

$NaHCO_3$	2.00 gm.	Dissolved in 100 ml. distilled water.
$KHCO_3$	0.40 gm.	
$Na_2HPO_4 \cdot 12H_2O$	3.34 gm.	
$NaH_2PO_4 \cdot H_2O$	1.25 gm.	
KCl	1.89 gm.	

The disodium phosphate was placed in a beaker first, followed by KCl, then the bicarbonates.

Solution (2)

$CaCl_2.2H_2O$	0.366 gm.	Dissolved in 100 ml. distilled water.

Solution (3)

$MgSO_4.7H_2O$	0.617 gm.	Dissolved in 100 ml. distilled water.

Solution (4)

$MnSO_4.H_2O$	3.085 gm.	Dissolved in 500 ml. distilled water.
$FeSO_4.7H_2O$	1.220 gm.	
$CuSO_4.5H_2O$	0.03935 gm.	
$CoSO_4.7H_2O$	0.02375 gm.	
$ZnSO_4.7H_2O$	0.01095 gm.	

Twenty-five ml. of solutions 1, 2, and 3 and 2.5 ml. of solution 4 were put in a 250 ml. volumetric flask, made to volume with 39^{o} C. CO_2-saturated distilled water and used as mineral media. For larger or smaller volumes, 10 volumes of solutions 1, 2, 3, and 1 volume of solution 4 were used in a total of 100 volumes.

Solka-Floc--purified wood pulp, product of the

Brown Company.[1]

Filter Paper Cellulose--pure finely ground cellulose powder.[2]

Rumen inoculum--was obtained from a mature wether on a good quality alfalfa hay diet. The sample was taken to the laboratory, shaken for two or three minutes and then centrifuged at 2000 rpm. for 20 minutes to spin down the coarse particles and the protozoa. The supernatant was used to inoculate the artificial rumen. The rumen fluid was transferred to a large beaker, placed on the magnetic stirrer and CO_2 was bubbled into the contents of the beaker. Then 5 ml. of rumen fluid were added to each of the tubes in the water bath. Each tube was shaken as the inoculum was added to insure complete mixing.

Ethyl alcohol--95 per cent.

Benzene (Benzol)

Glacial Acetic Acid.

Concentrated Nitric Acid.

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[1]Brown Company Mills, Berlin, New Hampshire.

[2]Whatman Cellulose Powder, W. & R. Balston, Ltd., London, England.

3. Procedure

a. Preparation of artificial rumen. Each test tube contained 100 to 3200 mg. of the cellulose substrate, corncobs, Solka-Floc, or Filter Paper Cellulose, 5 ml. of the mineral media, 5 ml. of the mineral media plus different levels of corn starch and 5 ml. of the mineral media plus 0.4 per cent urea (0.1 per cent of total). Different levels of fat, either corn oil or lard, were added to the cellulose in some of the trials. Methionine hydroxy analogue was also added to the mineral media in later trials. When the tubes were charged, the stoppers were equilibrated with CO_2 one hour before the rumen fluid was added in the 39° C. water bath. A 24-hour incubation period with CO_2 bubbling through was used in most trials.

b. Preparation for cellulose determination. After 24 hours, the artificial rumens were removed from the water bath. The tubes were centrifuged at 2500 rpm. for 25 minutes and the supernate from each tube was removed. Care was taken to prevent cellulose from being lost.

c. Determination of cellulose. The method was modified from Crampton and Maynard (52). Twelve ml. of glacial acetic acid and 1.5 ml. of concentrated nitric acid was added to each of the tubes. The tubes were swirled to insure complete mixing of the acid, and placed into a boiling water bath, located under the hood, for 20 minutes. The tubes were removed and cooled immediately by placing them in an ice bath.

After the samples were completely cool, they were transferred with 70° C. ethyl alcohol to previously ignited Selas filter crucibles set in an aspirator. The samples were then washed successively with 70° C. benzene and 70° C. ethyl alcohol, and allowed to dry thoroughly at room temperature for two or three hours, then placed in a 100° C. drying oven overnight. The crucibles were removed from the oven and placed in a desiccator until cool. They were then weighed on an analytical balance. The crucibles plus the samples were ignited overnight in the muffle furnace, starting with a cold furnace and going up to 600° C. They were placed back in the desiccator to cool and the second weighing was recorded. The difference between the two weighings was reported as per cent cellulose. Control tubes were used to determine the cellulose content of the substrate used. The average value of the control tubes was used as a correction factor in the determinations.

B. Digestion Trial

1. Design of the experiment

A replicated 2 x 2 x 4 factorial design was used in which the variables studied were: effects of lard versus emulsified lard,[3] methionine hydroxy analogue[4] versus no methionine hydroxy analogue and the following four added fat levels (0, 1, 2½, and 5½ per cent of the ration). Cellulose digestion, feed utilization, and growth rate of wether lambs were used as criteria for the comparisons of results. The lambs were randomly selected for the different treatment levels. The experiment was conducted in the winter of 1958 at the Animal Nutrition Farm at the University of Missouri. The experiment consisted of two separate trials following the same factorial design.

2. Animals

Sixteen 6-month-old Texas feeder lambs, all wethers, were used in this experiment. These wethers were part of a 100-lamb truckload purchased from Swift and Company, South St. Joseph, Missouri. They weighed an average of 63 pounds per lamb when they arrived in Columbia December 10, 1957.

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[3]Emulsified lard was obtained from Armour and Company, Chicago, Illinois.

[4]Methionine hydroxy analogue was obtained from Monsanto Chemical Company, St. Louis, Missouri.

The lambs were drenched with phenothiazine and vaccinated for sore mouth[5] before being put on trial. They received good quality alfalfa hay *ad libitum* until December 22, when they were placed on the basal ration.

3. Equipment

Feeders and loafing area--The lambs were fed in individual feeding stalls[6] located on a concrete floor in an enclosed building. They were kept in a closed shed when not in the feeders. The lambs also had access to a 10 by 55-foot dirt lot for exercise. Water was available to the lambs except when they were in the feeding stalls.

Fecal collection bags--waterproof lightweight canvas bags individually fitted to each lamb. Details of this bag are shown in Figure 2, Appendix.

Pyrex dishes--12 by 7½ by 2 inches.

Electric drying oven.

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[5]Ovine Ecthyma Vaccine, Dr. James English, Veterinary Clinic, University of Missouri, Columbia, Missouri.

[6]C. C. Brooks, "Factors Which Affect the Utilization of Rations High in Cellulose" (Ph. D. Thesis, The University of Missouri, Columbia, 1954), Figure 3, Appendix.

4. Rations

a. Composition of rations. The basal ration shown in Table I was made up of natural feedstuffs for the most part and had a crude protein and total digestible nutrient level that was adequate to meet the requirements of growing wether lambs. The calculated composition of the basal ration was as follows: crude protein, 11.60 per cent; total digestible nutrients, 57.76 per cent; fat, .56 per cent; and crude fiber, 21 per cent. The basal ration was very low in dietary fat content; this was necessary in order to study the different fat levels in the experiment. Treatment levels added to the basal ration are shown in Table II. The controls, lambs on rations A and H, received 1000 grams of the basal ration daily. Lambs on the other treatments received the basal ration plus fat equivalent to the amount assigned in the treatments outlined in Table II; thus all animals maintained a constant intake of cellulose and all other ingredients except for fat. During the second trial the lambs received the first trial ration plus 420 grams of ground milo. Lamb 75, a control, went off feed during the preliminary period between the first and second trials and was replaced by lamb 67 (ration C). Therefore ration C was not used during the second trial.

TABLE I

BASAL RATION

Ingredients	Per Cent in Ration
Ground Corn Cobs (¼" screen)	61.5
Soybean Oil Meal, 44% (solvent)	20.4
Corn Grits	10.2
Corn Syrup	6.1
Dicalcium Phosphate (18½%P)	0.41
Ground Limestone	0.31
Salt	0.93
Vitamin A & D Supplement[1]	0.15
Cobalt Chloride[2]	+
	100.0

[1]Vitamin A and D supplement contained 2250 I.U. of vitamin A and 400 I.U. of vitamin D per gram.

[2]Each 100 pounds of the basal ration contained 21.2 mg. of $CoCl_2.6H_2O$.

TABLE II

TREATMENTS

Sheep Number	Ration Letter	Type of Added Fat	Fat Added %	Plus or Minus 2 gms. M.H.A.[1]
66	A	None	0	+
75	A	None	0	+
79	B	Lard	1.0	+
67	C	Emulsified Lard	1.0	+
77	D	Lard	2.5	+
72	E	Emulsified Lard	2.5	+
70	F	Lard	5.5	+
73	G	Emulsified Lard	5.5	+
69	H	None	0	-
78	H	None	0	-
74	I	Lard	1.0	-
80	J	Emulsified Lard	1.0	-
68	K	Lard	2.5	-
76	L	Emulsified Lard	2.5	-
81	M	Lard	5.5	-
71	N	Emulsified Lard	5.5	-

[1]Methionine hydroxy analogue

b. Preparation of rations. The corn cobs were ground with a portable mill[7] using a one-quarter inch screen. The balance of the ration ingredients, except for the syrup, fat, and methionine hydroxy analogue, were added to the ground corn cobs and mixed in the portable mixer.

The complete rations were prepared before the start of the first trial. The following procedure was used. Enough of the basal ration minus the corn syrup was weighed out for 90 days for each lamb. The fat and corn syrup were heated on a 10-inch hot plate and added in small portions into the mixer[8] with the basal ration while the machine was running. The methionine hydroxy analogue was added to 10 pounds of the basal ration in order to insure complete mixing. This mixture was added to the mill along with the fat and the corn syrup and mixed for 30 minutes. The complete rations were then sacked and stored in an upright position.

Fifty-pound lard pails were filled with the different rations and labeled with their respective letters. The daily allowance for each lamb was weighed out and put into

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[7]The Daffin Manufacturing Company, Lancaster, Pennsylvania, operated by the Farm Bureau, Columbia, Missouri.

[8]Three-fourths ton Kelly Duplex vertical feed mixer.

paper sacks. The lambs were individually fed twice a day, at 6 A.M. and 4 P.M.

There was some difficulty in getting the lambs to eat the basal ration and the basal ration plus the lowest fat level. This was probably due to the extreme dustiness of the basal ration. Water was sprayed on these rations with a small hand sprayer. This helped to eliminate the excessive dustiness of the rations. When the lambs would go off feed, a small amount of supplemental B_{12} helped to stimulate their appetites.

Orts were removed from the feeding stalls, weighed back and recorded at the end of each week during the preliminary period.

5. Collecting Technique

Each collection period was preceded by a preliminary feeding phase. The preliminary phase for the first trial was 39 days and the preliminary period for the second trial was 29 days. These periods allowed the lambs ample time to adjust to the new ration.

At the end of the preliminary feeding period, the individual fecal collection bags (shown in Figure 2, Appendix) were put on each lamb. These were adjusted so that there would be no feces lost from the bags. The bags were on the lambs for a 24-hour adjustment period and then the feces from each lamb was thrown away. The lambs were afraid of the bags at first and were left in the feeding stalls overnight to help them overcome this.

Fecal collections were made between 8:00 and 9:30 A.M. each day for six days during the first trial. The collections during the second trial were made between 2:00 and 4:00 P.M. The fecal collection period for the second trial consisted of a four-day period.

After each day's collection, the total weight of the feces from each lamb was recorded. The feces were then mixed in the weighing pan and one-fifth of the total weight was saved. This sample was placed in a pyrex dish and dried in an oven for twenty-four hours at 65° C. When the feces were dry, they were removed from the dish and placed in a properly labeled paper sack.

After the final collection was added to the sack, the sample was left to dry in the collection room for a week, then reweighed and the air dry weight was recorded.

A daily feed sample was also taken for each lamb during the collection period. A representative feed sample was taken with a sampling probe for each lamb on trial. This feed sample was stored in a paper sack until it could be ground. Feed refusals were collected, weighed, and then saved for analysis.

The fecal samples, feed samples, and the orts were all ground through a 40-mesh screen in a Wiley Mill. After grinding, each sample was mixed thoroughly and placed in a separate screw cap glass jar and labeled with the proper Animal Husbandry and Experiment Station numbers.

6. Chemical Analysis

The following analyses[9] were made on the different rations and feces collected: moisture, ash, nitrogen, fat, and cellulose. Cellulose was determined by the Crampton-Maynard method. Moisture, ash, nitrogen, and fat were determined by the methods described in the 7th edition of the "Official Methods of the Association of Official Agricultural Chemists."

7. Statistical Analysis of Data

The method of analyzing was to compute the means and where differences were apparent, the "t" test for paired data was used to determine the degree of significance.

[9] The analyses were done by the Station Analytical Laboratories.

CHAPTER IV

RESULTS AND DISCUSSION

A. Artificial Rumen Results

1. Introduction

The artificial rumen was used to determine the effects of fat and methionine hydroxy analogue on cellulose digestion. Preliminary tests were run to establish cellulose source, starch and cellulose levels. Each artificial rumen trial represents at least eight individual artificial rumina. Cellulose digestion is shown as milligrams of cellulose disappearing from the artificial rumen in 24 hours.

2. Forms of Cellulose

Corn cobs, ground through a 40-mesh screen in a Wiley mill, and Solka-Floc cellulose were compared in the artificial rumen in determine the best substrate for use in the studies. Table III summarizes the results obtained from comparing different levels of these two cellulose sources. In Trial 1 Solka-Floc cellulose digestion was 39 per cent while corn cob cellulose was apparently not digested. Failure to get digestion of corn cobs was attributed to the poor recovery of the corn cob cellulose in the trial. In Trial 2 three levels of corn cob cellulose were compared to Solka-Floc cellulose. At the three levels studied Solka-Floc cellulose increased cellulose digestion 29 per cent on the average over that of the corn cob cellulose. The 20 and 200 mg. levels of both cellulose sources showed the best digestion. Four times as much Solka-Floc cellulose was digested as corn cob cellulose at these two levels.

Although the 20 mg. Solka-Floc level showed the best digestion expressed percentagewise, the 200 mg. level showed the highest recovery of cellulose actually digested. Further trials were run to determine the best level of cellulose to use in the artificial rumen.

3. Levels of Cellulose

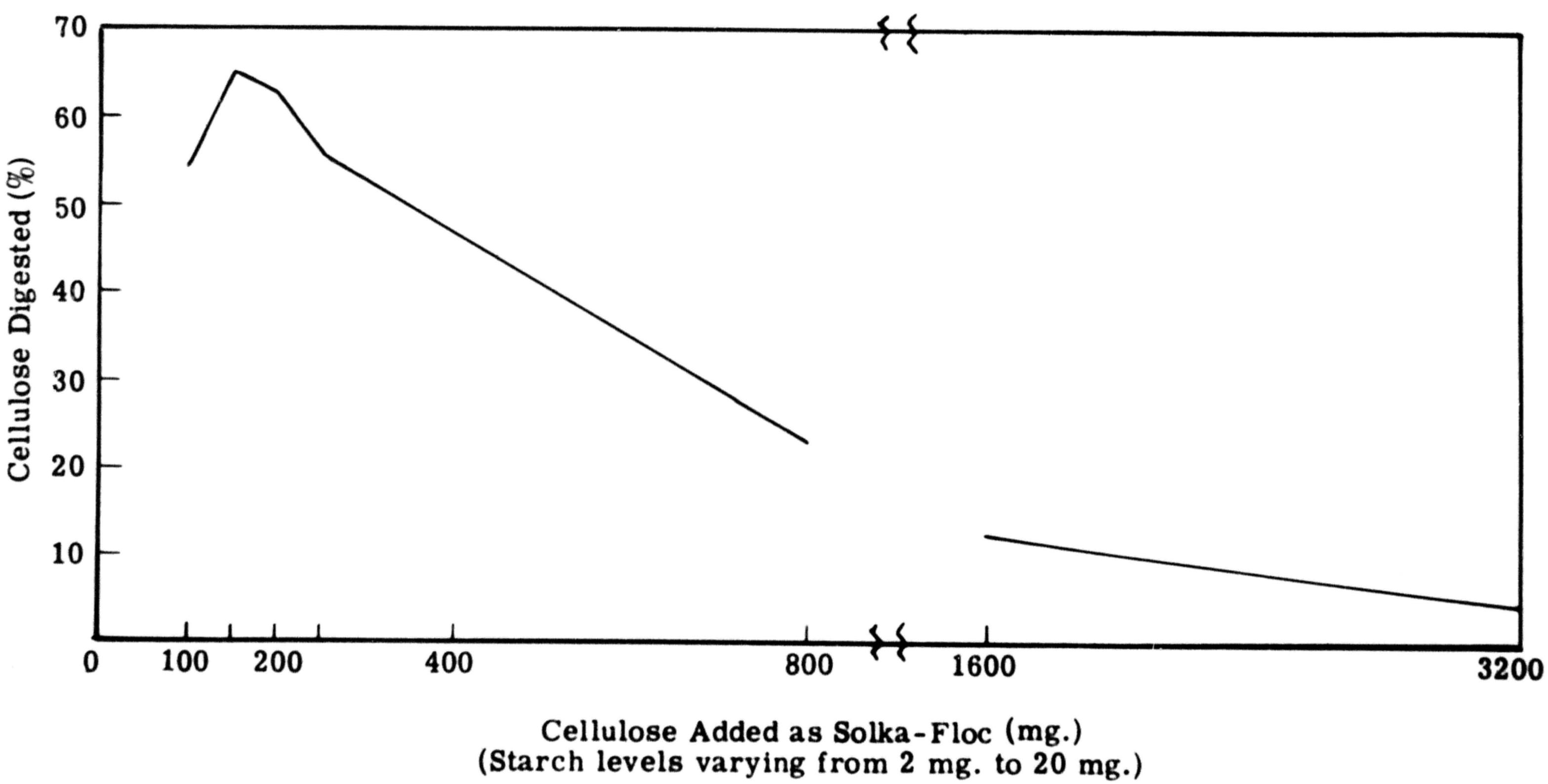
The effect of Solka-Floc cellulose on cellulose digestion was studied in Trials 3 through 5 at levels ranging from 100 to 3200 mg. per rumen. Figures 1 and 2 summarize these results. Figure 1 shows that the highest percentage cellulose digestion occurred at the 150 and 200 mg. levels. This corresponds to the concentration used in artificial rumen work by Garner (53), who used 175 mg. of cellulose substrate per artificial rumen tube. Figure 2 shows cellulose digestion plotted against added cellulose and demonstrates a typical dose response curve. The greatest increase in milligrams of cellulose digested was

TABLE III

A COMPARISON OF THE DIGESTIBILITIES OF SOLKA-FLOC AND CORN COB CELLULOSE IN THE ARTIFICIAL RUMEN

Cellulose Level (mg.)		Type of		No.	Cellulose Digestion	
Inoculated	Recovered	Cellulose	Trial	Rumina	%	mg.
200	146	Corn Cob	1	3	0	0
200	186	Solka-Floc	1	3	39.4	73.3
20	15.5	Corn Cob	2	3	23.4	3.6
200	108.4	Corn Cob	2	3	22.3	24.1
2000	1,024.0	Corn Cob	2	3	4.2	43.2
20	16.2	Solka-Floc	2	3	79.0	12.8
200	156.9	Solka-Floc	2	3	60.8	95.4
2000	1,673.7	Solka-Floc	2	3	6.5	107.9

Figure 1. Effect of cellulose level on per cent cellulose digestion in the artificial rumen.



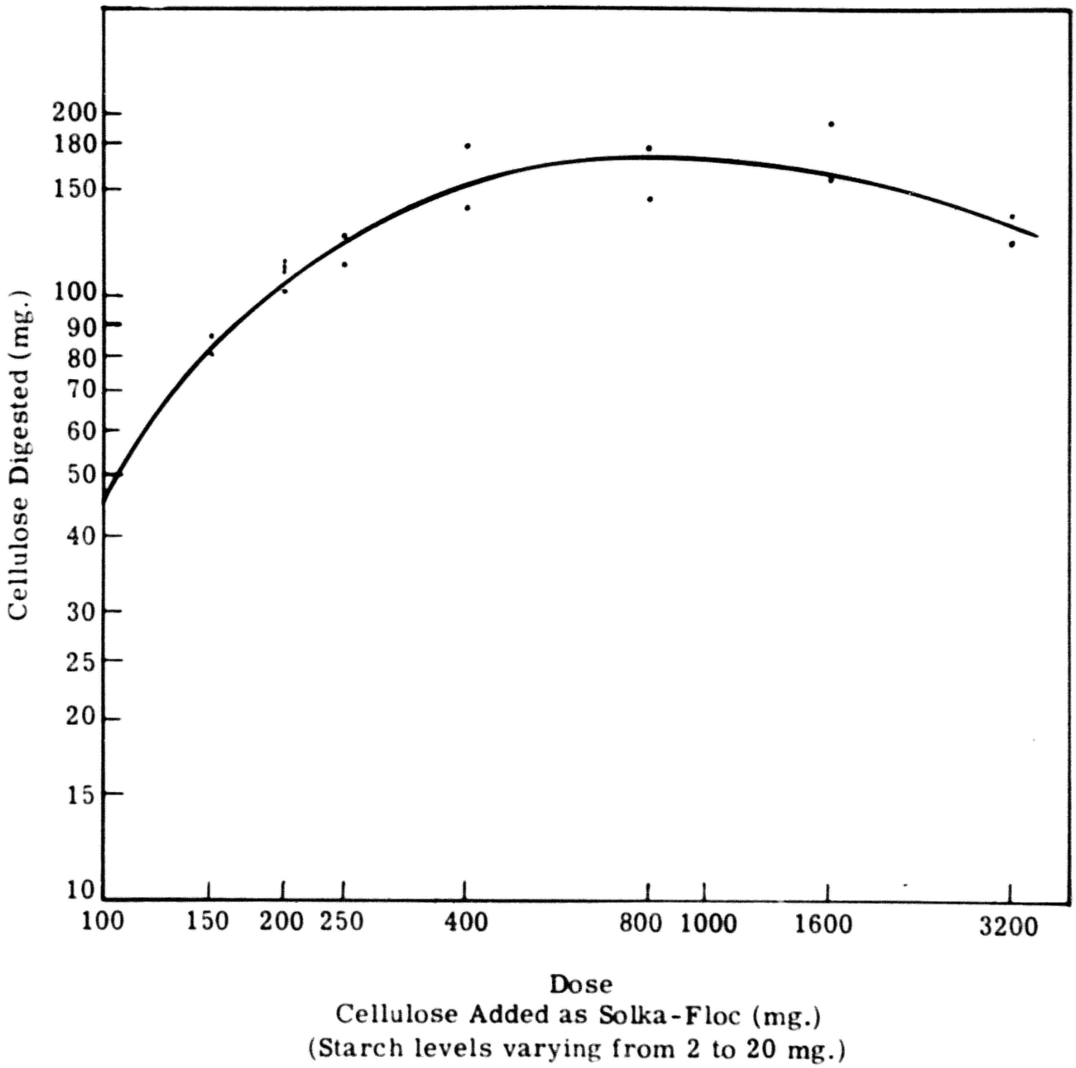


Figure 2. Effect of cellulose level on milligrams cellulose digested in the artificial rumen.

between the 100 and 200 mg. levels of added Solka-Floc cellulose. A gradual increase was noted from the 200 mg. through the 800 mg. levels. The curve indicates a slight increase between the 800 and 1600 mg. levels of added Solka-Floc cellulose. At levels above 1600 mg. a gradual decline was noted in mg. cellulose digested.

The maximum amount of cellulose actually digested was 177 mg. (at the 1600 mg. level) and the minimum amount digested was 47 mg. (at the 100 mg. level). The highest per cent cellulose digestion occurred at the 150 mg. level (65 per cent); the 200 mg. level being only slightly lower (63 per cent). These results indicated that the most useful concentrations to use in further trials were the 150 and 200 mg. levels of Solka-Floc cellulose.

4. Corn Starch Levels

The effect of added corn starch was tested in Trials 3 through 5 at levels ranging from 2 to 200 mg. per rumen. Results are summarized in Figures 3 and 4. Figure 3 demonstrates the effect of three different concentrations of corn starch on mg. Solka-Floc cellulose digested. Two hundred mgs. of corn starch depressed cellulose digestion at all levels of added cellulose when compared to the 2 mg. and the 20 mg. of corn starch. Corn starch additions of 2 and 20 mgs. resulted in almost equal cellulose

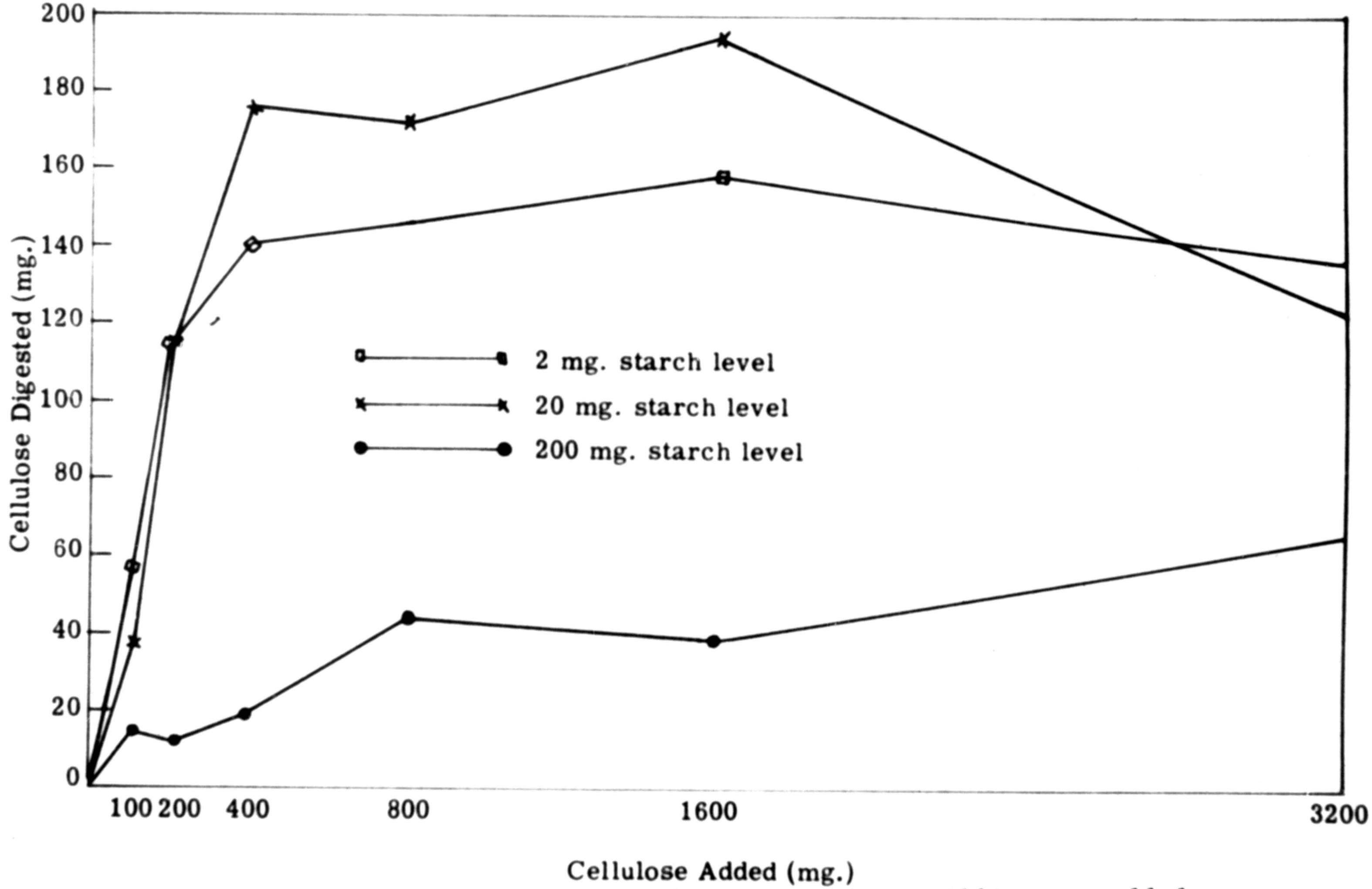


Figure 3. Effect of varying levels of corn starch on milligrams cellulose digested in the artificial rumen.

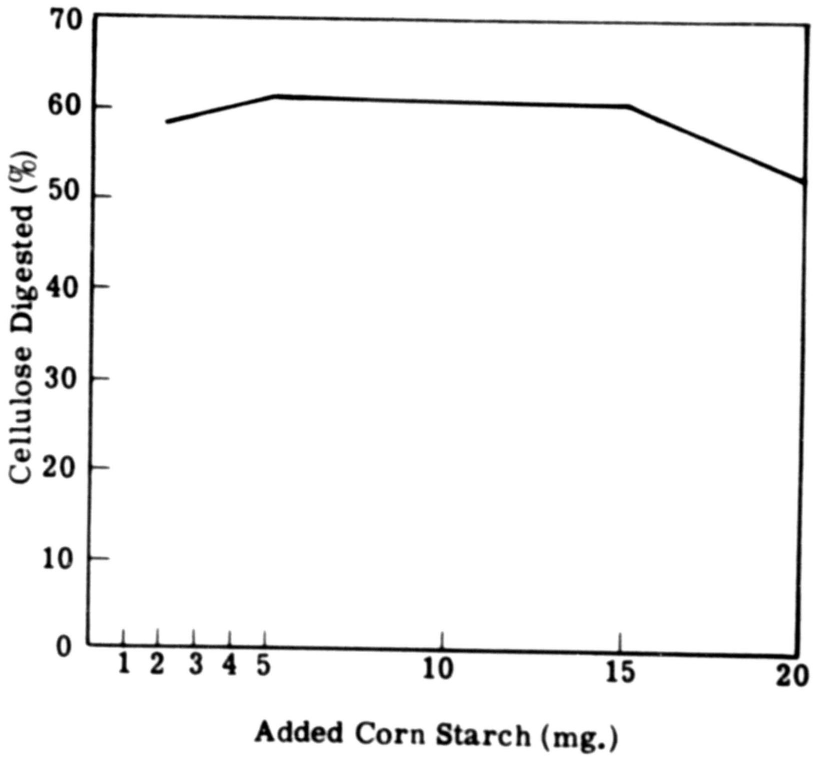


Figure 4. Effect of varying levels of corn starch on per cent cellulose digestion in the artificial rumen.

digestion at the 200 mg. Solka-Floc level, but at 400 through 1600 mg. Solka-Floc levels the average for the 20 mg. corn starch addition was 182 mg. of cellulose digested, as compared to 149 mg. digested at 2 mg. of added starch. An average of 33 mg. more cellulose was digested at the 20 mg. starch level.

Starch additions varying from 2 to 20 mg. were compared in the presence of 100 to 800 mg. cellulose. Results are presented in Figure 4. Little or no difference was observed between the 5, 10, and 15 mg. doses of corn starch on per cent cellulose digested. Therefore, later artificial rumen studies were run with 10 or 15 mg. corn starch per tube.

5. Emulsified Lard and Corn Oil

The effect of emulsified lard and corn oil was tested in Trials 6 through 9 at levels ranging from 3 to 30 mg. per tube. When 15 or 30 mg. of emulsified lard were added in Trial 6, cellulose digestion increased slightly in both cases. Additions of 30 mg. and 15 mg. increased cellulose digestion 9 per cent and 3 per cent, respectively, when compared to the control tubes. The emulsified lard cooled off rapidly and formed large lumps in the cellulose material, making it difficult to obtain a representative sample. Due to these difficulties, corn oil was used in later fat trials. The effect of fat additions on cellulose digestion is summarized in Table IV.

Since Brooks (54) found that the addition of 10 mg. of corn oil decreased *in vitro* cellulose digestion 40 per cent, corn oil levels varying from 3 to 12 mgs. were studied in Trials 7 through 9 to determine the minimum amount of corn oil needed to depress cellulose digestion.

In Trial 7, the addition of 6 or 12 mg. of corn oil resulted in only a 2 per cent decrease in cellulose digestion.

The addition of 3 mg. of corn oil in Trial 8 resulted in a 4 per cent increase in cellulose digestion, while 6 mg. of corn oil resulted in a 14 per cent decrease. Twelve mg. of added corn oil depressed cellulose digestion 18 per cent when compared to the control value. The differences in cellulose digestion were significant: 3 mg. vs. 6 mg. $P < .05$; 3 mg. vs. 12 mg. $P < .02$.

In Trial 9 no depression due to added corn oil was demonstrated, and increased cellulose digestion was noted at all three levels. There was a 16 per cent increase in cellulose digestion at the 3 mg. corn oil level. Six and 12 mg. of corn oil in this trial apparently increased cellulose digestion 11 and 9 per cent, respectively.

Apparently 3 mg. corn oil may have a stimulating

TABLE IV

THE EFFECT OF VARYING LEVELS OF EMULSIFIED LARD, CORN OIL AND METHIONINE HYDROXY ANALOGUE ON CELLULOSE DIGESTION IN THE ARTIFICIAL RUMEN

Trial	Variable Studied	Level per Rumina	Cellulose Digested Control	Cellulose Digested Treated
6	Fat-Emulsified Lard	15 mg.	45.5(4)[1]	48.9(3)
		30 mg		54.7(4)
7	Fat-Corn Oil	6 mg.	87.2(4)	85.3(4)
		12 mg.		85.6(4)
8	Fat-Corn Oil	3 mg.	66.3(2)	70.8(2)
		6 mg.		*52.6(2)
		12 mg.		**48.2(2)
	M.H.A.	.002%	57.2(4)	61.7(4)
9	Fat-Corn Oil[2]	3 mg.	35.4(3)	51.4(3)
		6 mg.		46.3(3)
		12 mg.		44.5(3)
	M.H.A.	.002%	44.0(4)	38.0(4)
		.004%		51.0(4)

[1]Number of rumina at each level.

[2]Filter paper cellulose was used in this trial.

** Difference in cellulose digestion (3 mg. level vs. 12 mg. level) significant at .02.

* Difference in cellulose digestion (3 mg. level vs. 6 mg. level) significant at .05.

effect on cellulose digestion under the conditions studied. Solka-Floc cellulose recovery in Trial 8 was only 65 per cent while the recovery reported in Trial 9 with filter paper cellulose was 96 per cent. Due to the contrasting results reported in Trials 8 and 9 and the small number of rumina involved in these trials, the minimum level of corn oil needed to show depression in cellulose digestion was not determined. However, it is amply demonstrated that the depression can occur at extremely low levels.

6. Methionine Hydroxy Analogue

The effect of adding methionine hydroxy analogue on cellulose digestion is summarized in Table IV. In Trial 8 the .002 per cent of methionine hydroxy analogue stimulated cellulose digestion 4 per cent, while in Trial 9, it depressed cellulose digestion 6 per cent. Methionine hydroxy analogue added at .004 per cent in Trial 9 stimulated cellulose digestion 7 per cent. Since these low levels failed to give a positive response, higher levels were used in the digestion trials.

These data indicate that higher levels of methionine hydroxy analogue may have a beneficial effect on *in vitro* cellulose digestion.

B. Digestion Trial Results

1. Introduction

The weight gains, feed efficiencies, and the coefficients of digestibility of cellulose, nitrogen, fat, organic matter, and nitrogen-free extract for the individual lambs are reported in Tables IX through XII, Appendix. The calculated total digestible nutrients of the lambs on the basal ration (Trial I) are shown in Table XIII of the Appendix.

Some lambs, especially those on the control and the high fat diets, did not readily consume a constant level of feed. Spraying water on the dusty control ration and giving small oral doses of Vitamin B_{12} to all lambs not eating, helped stimulate appetite.

2. Trial 1

The trial was conducted from January 6 to February 20, 1958. During the last seven days the lambs wore collection harnesses. The mean temperature during this period was 27° F.

The lambs used in this study had been on good quality alfalfa hay and were in thrifty condition at the beginning of the first preliminary period. The lambs were changed to the first trial rations when the mean temperature was 30° F., and they lost an average of 2.3 pounds per animal during the first 7 days of the preliminary period. During the following 7 days the lambs gained an average of 5.2 pounds per animal. This weight fluctuation was probably due to erratic feed intake during this period. After this initial two-week period the gains were relatively constant at 1/4 pound per day. The gains made by the lambs in this trial were good considering that the ration was primarily for maintenance and growth. The summary of the results of Trial 1 is given in Tables V and VI.

The addition of methionine hydroxy analogue or the substitution of emulsified lard for lard did not significantly affect gain or feed efficiency.

One per cent added fat significantly increased ($P < 0.05$) both average daily gain and feed efficiency in this trial. The higher fat levels increased the feed efficiency and daily gain over that of the control (no added fat) level but not to the extent of the 1 per cent level. These results indicate that the addition of 1 per cent fat to a low fat basal ration increased feed efficiency and daily gains in wether lambs under cold weather conditions. Added fat and fat present in the average ration may produce different effects.

The summary of the apparent digestibilities of the ration constituents in Trial 1 is given in Table VI.

TABLE V

MEAN VALUES OF THE DAILY GAINS AND FEED EFFICIENCIES OF WETHER LAMBS IN TRIAL 1 (BASAL RATION)

No. of Animals	Rations Involved	Variable Studied	Average Mean Daily Gain	Ave. Mean of Feed Eff. Lbs. Feed/Lb.Gain
8	A, B, C, D, E, F, G	+M.H.A.[1]	.22	10.3 lbs.
8	H, I, J, K, L. M, N	-M.H.A.	.23	9.7 lbs.
6	C, E, G. J. L. N	Emulsified Lard	.25	9.2 lbs.
6	B, D, F, I, K, M	Lard	.24	9.5 lbs.
4	A, H	No Added Fat	.18	12.0 lbs.
4	B, C, I, J.	1% Added Fat	.28*	8.0 lbs.*
4	D, E, K, L	2.5% Added Fat	.25	9.0 lbs.
4	F, G, M, N	5.5% Added Fat	.21	11.0 lbs.

*Difference in average daily gain and feed efficiency (no added fat vs. 1% added fat) significant at .05.

[1]Methionine hydroxy analogue, 2 gms. per animal per day.

TABLE VI

AVERAGE MEAN VALUES OF THE COEFFICIENTS OF DIGESTIBILITY OF THE RATION CONSTITUENTS IN THE BASAL RATION (TRIAL 1)

No. of Animals	Rations Involved	Variable Studied	Average Mean of C.O.D.				
			Cellulose	N	Fat	O.M.	N.F.E.
8	A, B, C, D, E, F, G	+M.H.A.[1]	61.2	71.2	80.5	66.5	66.8
8	H, I, J, K, L, M, N	-M.H.A.	58.8	75.4	81.1	67.2	67.7
6	C, E, G, J, L. N	Emulsified Lard	59.4	73.8	89.4	67.0	66.7
6	B, D, F, I, K, M	Lard	57.6	74.0	86.2	66.2	66.6
4	A, H	No Added Fat	64.5	73.9	59.8	68.1	68.8
4	B, C, I, J	1% Added Fat	64.2	70.2	81.0*	67.8	68.6
4	D, E, K, L	2.5% Added Fat	64.2	74.2	89.2**	68.7	68.2
4	F, G, M, N	5.5% Added Fat	47.2*	77.2	93.3**	63.3	63.2

*Difference in C.O.D. (no added fat vs. added fat) Significant at .05.

**Difference in C.O.D. (no added fat vs. added fat) Significant at .01.

[1]Methionine hydroxy analogue, 2 gms. per lamb per day.

The substitution of emulsified lard for lard or the addition of methionine hydroxy analogue did not significantly affect cellulose, nitrogen, fat, organic matter and nitrogen-free extract digestibility in this trial.

The addition of 5.5 per cent fat significantly depressed ($P < 0.05$) cellulose digestion. No difference was noted between the rations with 1 or 2.5 per cent added fat and the control ration.

The effect of fat additions on the digestibility of fat was very striking. With each increase in fat level there occurred a corresponding increase in the coefficient of digestibility of fat in the ration. The 5.5 and 2.5 per cent added fat levels very significantly ($P < 0.01$) increased the digestibility of fat over that of the basal ration. One per cent added fat significantly ($P < 0.05$) increased fat digestibility in this trial. The lambs on the two highest fat levels were more alert and had firmer, larger loins than the lambs on the control and 1 per cent added fat rations.

Organic matter and nitrogen-free extract digestibility were decreased at the 5.5 per cent added fat level. When Trials 1 and 2 were combined, organic matter digestibility was very significantly ($P < 0.01$) decreased at the 5.5 per cent fat level.

The summary of the calculated total digestible nutrients of the rations used in Trial 1 is shown in Appendix Table XIII. The results obtained did not seem to follow any set pattern because there were both high and low values at each treatment level.

3. Trial 2

The second trial was conducted from March 2 to April 5, 1958. The collection harnesses were on the lambs the last five days of this period. The mean temperature during this trial was 47° F. The purpose of this trial was to study the effect of the test substances in Trial 1 when added to a higher energy ration calculated to be adequate for fattening. The energy level of the basal ration was raised by supplementing it with finely ground milo. A slight palatability problem was encountered with some of the lambs when the milo was first added to their rations. The daily milo allowances were increased until all lambs were receiving 420 grams in addition to the basal ration fed in Trial 1.

The summary of the results of Trial 2 is given in Tables VII and VIII.

The addition of methionine hydroxy analogue or the substitution of emulsified lard for lard in Trial 2 did not significantly affect feed efficiency or daily gain.

The addition of 1 per cent fat to the basal ration

TABLE VII

MEAN VALUES OF THE DAILY GAINS AND FEED EFFICIENCIES OF WETHER LAMBS IN TRIAL 2 (BASAL RATION PLUS MILO)

No. of Animals	Rations Involved	Variable Studied	Average Mean Daily Gain	Ave. Mean of Feed Eff. Lbs.Feed/Lb.Gain
7	A, B, D, E, F, G	+M.H.A.[1]	.35	9.1 lbs.
8	H, I, J, K, L, M, N	-M.H.A.	.38	8.4 lbs.
5	E, G, J, L. N	Emulsified Lard	.35	8.6 lbs.
6	B, D, F, I, K, M	Lard	.34	9.5 lbs.
4	A, H	No Added Fat	.40	7.7 lbs.
3	B, I, J	1% Added Fat	.36	7.9 lbs.
4	D, E, K, L	2.5% Added Fat	.37	9.1 lbs.
4	F, G, M, N	5.5% Added Fat	.31	10.1 lbs.

[1]Methionine hydroxy analogue, 2 gms. per animal per day.

TABLE VIII

AVERAGE MEAN VALUES OF THE COEFFICIENTS OF DIGESTIBILITY OF THE RATION CONSTITUENTS IN THE BASAL RATION PLUS MILO (TRIAL 2)

No. of Animals	Rations Involved	Variable Studied	Average Mean of C.O.D.				
			Cellulose	N	Fat	O.M.	N.F.E.
7	A, B, D, E, F, G	+M.H.A.[1]	51.4	71.0	79.6	69.3	74.4
8	H, I, J, K, L, M, N	-M.H.A.	46.0	74.0	81.4	69.6	74.2
5	E, G, J, L, N	Emulsified Lard	43.8	74.6	84.4	70.3	75.0
6	B, D, F, I, K, M	Lard	48.0	71.4	85.2	67.8	72.7
4	A, H	No Added Fat	55.1	72.0	68.7	71.3	75.8
3	B, I, J	1% Added Fat	47.9	74.1	80.7	70.1	74.9
4	D, E, K, L	2.5% Added Fat	52.7	72.6	84.4*	71.8	77.0
4	F, G, M, N	5.5% Added Fat	38.2	72.3	88.4**	65.2	69.6

*Difference in C.O.D. (no added fat vs. added fat) significant at .05.

**Difference in C.O.D. (no added fat vs. added fat) significant at .01.

[1]Methionine hydroxy analogue, 2 gms. per lamb per day.

plus milo, markedly decreased average daily gain, but feed efficiency remained essentially unchanged. The 2.5 per cent added fat level produced daily gains equivalent to those noted at the 1 per cent level. However, feed efficiency was markedly reduced at the 2.5 per cent fat level compared to 1 per cent added fat. The addition of 5.5 per cent fat to the basal ration plus milo produced the lowest average daily gains noted in this trial. Also, more feed was needed to produce a pound of gain at this fat level. The addition of supplemental fat to the higher energy rations in Trial 2 did not improve feed efficiency or daily gains in wether lambs. This failure to respond to supplemental fat was apparently due to the fact that the milo contained 2.2 per cent fat and was able to meet the minimal fat requirements of the lambs. The daily gains reported in Trial 2 were excellent, indicating that rations of this type could be used in future lamb fattening trials.

Coefficients of digestibility of the ration constituents in Trial 2 are summarized in Table VIII. The individual coefficients of digestibility for each lamb are given in Table XII of the Appendix.

Substitution of emulsified lard for lard or the addition of methionine hydroxy analogue did not significantly affect the digestibilities of the ration constituents of the milo-supplemented ration in Trial 2.

The addition of 5.5 per cent fat depressed cellulose digestion 17 per cent when compared to the control (no added fat) level. A 7 per cent decrease in cellulose digestion was noted at the 1 per cent fat level while at the 2.5 per cent level cellulose depression was only 2 per cent. The coefficients of digestibility of cellulose in Trial 2 were lower than those observed in Trial 1. This response was similar to the cellulose depressing effect noted when high levels of corn starch were added to the artificial rumen tubes.

The result of adding fat on fat digestibility in Trial 2 was the same as that reported in Trial 1. The more total fat consumed the higher the resulting coefficient of digestibility. Five and one half per cent added fat very significantly ($P < 0.01$) increased the digestibility of fat when compared to the control (no added fat) level. Fat digestibility was significantly ($P < 0.05$) increased at the 2.5 per cent fat level. There was a 12 per cent increase in fat digestibility observed between 1 per cent supplemental fat and the control level.

The highest fat level in Trials 1 and 2 decreased the digestibilities of organic matter and nitrogen-free extract.

The basal ration was calculated to contain 0.10 per cent sulfur; however, by analysis the sulfur content of ration H (basal ration) was .145 per cent. The addition of methionine hydroxy analogue raised the sulfur content to .150 per cent. The milo contained .102 per cent sulfur. The addition of milo in Trial 2 would lower the per cent sulfur content of the ration. The requirement for sulfur for the feeder lambs was apparently less than .14 per cent of the ration.

CHAPTER V

SUMMARY AND CONCLUSIONS

A. Summary

This thesis reports the results of:

1. Five trials to determine levels of starch, cellulose, and type of cellulose for the artificial rumen studies.

2. Four trials to determine fat levels and two trials to test the effect of methionine hydroxy analogue on cellulose digestion in the artificial rumen.

3. Two feeding and digestion trials with sixteen lambs to determine effects of supplemental methionine hydroxy analogue, three levels of fat, and the value of emulsifying lard on daily gains, feed efficiency, and the coefficients of digestibility of cellulose, fat, nitrogen, organic matter, and nitrogen-free extract. These effects were studied on both high and low level energy rations.

The data presented show:

Six mg. of added fat as corn oil significantly ($P < 0.05$) depressed cellulose digestion. Twelve mg. of corn oil very significantly ($P < 0.02$) decreased cellulose digestion in the artificial rumen. The addition of 3 mg. of corn oil stimulated cellulose digestion under the conditions studied.

Fifteen and 30 mg. of emulsified lard did not significantly affect cellulose digestion in the artificial rumen.

Methionine hydroxy analogue, when added at very low levels, demonstrated a slight stimulatory effect on cellulose digestion in the presence of supplemental fat.

Solka-Floc cellulose proved to be the most readily available cellulose substrate studied. Either the 150 or 200 mg. level of cellulose was shown to give the optimal response in per cent cellulose digested.

Additions of corn starch ranging from 5 to 20 mg. per tube showed very little difference between these levels on per cent cellulose digested. Two hundred mg. of corn starch depressed cellulose digestion at Solka-Floc cellulose levels ranging from 100 to 3200 mg. in the artificial rumen.

In Trial 1 the addition of 5.5 per cent fat significantly ($P < 0.05$) depressed cellulose digestion in wether lambs. This depression was also noted in Trial 2 on a milo-supplemented basal ration, although it was not significant. No significant depression was observed at the 2.5 and 1 per cent fat levels. Fat digestibility was significantly increased with each addition of fat in Trial 1. Trial 2 demonstrated a similar response except that the increase at the 1 per cent level was not significant.

When the results from both trials are considered, organic matter digestibility was very significantly ($P < 0.01$) decreased at the 5.5 per cent fat level.

One per cent added fat significantly increased ($P < 0.05$) both daily gain and feed efficiency in Trial 1 (low fat basal ration).

The substitution of emulsified lard for lard did not significantly affect cellulose digestibility in this trial. Average daily gain and feed efficiency were not affected by this substitution.

Methionine hydroxy analogue, 2 grams per lamb per day, did not significantly affect cellulose digestibility in either trial studied. Feed efficiency and daily gain were not improved by the addition of methionine hydroxy analogue to a basal diet containing 0.13 or 0.15 per cent sulfur.

B. Conclusions

From these tests it may be concluded that:

1. One hundred fifty to 200 mg. of cellulose and 5 to 15 mg. of corn starch are optimal for studying cellulose digestion in the 20 ml. artificial rumen.

2. Six and 12 mg. of corn oil depress cellulose digestion in the artificial rumen. A minimal amount of fat is needed to produce a maximal effect on cellulose digestion in the artificial rumen. Higher levels of

animal fat (i.e., emulsified lard) are tolerated without this depressing effect.

3. A percentage of 0.004 methionine hydroxy analogue stimulates cellulose digestion in the presence of supplemental fat.

4. Five and one-half per cent added animal fat significantly depresses cellulose digestion and organic matter digestibility in wether lambs. This depression is not observed at the 1 per cent and 2.5 per cent fat levels.

5. Two and one-half per cent animal fat added to a low fat-high roughage ration, under cold weather conditions, is an efficient energy source for growing lambs. Emulsified lard as compared to lard does not affect daily gain, feed efficiency or digestibility of the ration constituents.

6. Methionine hydroxy analogue when added to a ration containing .145 per cent sulfur does not significantly affect cellulose digestion in wether lambs on a fat-supplemented, poor quality roughage ration. Daily gain and feed efficiency are not significantly affected in these trials.

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APPENDIX

TABLE IX

AVERAGE DAILY GAIN, DAILY FEED INTAKE AND POUNDS OF FEED/POUNDS OF GAIN ON WETHER LAMBS IN TRIAL 1 (BASAL RATION)[4]

Sheep No.	Ration Letter	Variable: Added Fat Level(%) and Type	Variable: + or - M.H.A.[3]	Av. Daily Gain	Daily Feed Intake (lbs.)	Lbs.Feed/ Lbs. Gain (lbs.)
66	A	None	+	.14	2.1	15.0
75	A	None	+	.20	2.1	10.6
79	B	1-L[1]	+	.29	2.1	7.3
67	C	1-E.L.[2]	+	.23	2.3	9.8
77	D	2.5-L.	+	.24	2.2	9.2
72	E	2.5-E.L.	+	.29	2.3	7.9
70	F	5.5-L.	+	.22	2.2	9.8
73	G	5.5-E.L.	+	.18	2.2	12.6
69	H	None	-	.18	2.2	12.5
78	H	None	-	.21	2.1	9.9
74	I	1-L.	-	.31	2.2	7.0
80	J	1-E.L.	-	.27	2.1	8.0
68	K	2.5-L.	-	.20	2.1	0.5
76	L	2.5-E.L.	-	.27	2.3	8.4
81	M	5.5-L.	-	.18	2.3	12.9
71	N	5.5-E.L.	-	.25	2.1	8.6

[1] Lard
[2] Emulsified Lard
[3] Methionine hydroxy analogue, 2 gms. per lamb per day.
[4] Lambs consumed 1 oz. per lamb per day of the following mineral mix: NaCl 100 lbs.; $FeSO_4$ 1 lb.; $CoCl_2.6H_2O$ 22 gm.; KI (stabilized) 5 gm.; and $CuSO_4$ (crystal) 90 gm.

TABLE X

AVERAGE DAILY GAIN, DAILY FEED INTAKE AND POUNDS OF FEED/POUND OF GAIN ON WETHER LAMBS IN TRIAL 2 (BASAL RATION PLUS MILO)[4]

Sheep No.	Ration Letter	Variable: Added Fat Level (%) and Type	Variable: + or - M.H.A.[3]	Av. Daily Gain	Daily Feed Intake (lbs.)	Lbs. Feed/ Lb. Gain (lbs.)
66	A	None	+	.43	2.9	6.7
67	A	None	+	.38	3.1	8.2
79	B	1-L.	+	.38	2.8	7.3
77	D	2.5-L[1]	+	.24	3.0	12.6
72	E	2.5-E.L.[2]	+	.43	3.2	7.4
70	F	5.5-L.	+	.29	3.2	11.3
73	G	5.5-E.L.	+	.29	2.9	10.0
69	H	None	-	.33	3.1	9.2
78	H	None	-	.48	3.1	6.6
74	I	1-L.	-	.33	2.9	8.8
80	J	1-E.L.	-	.38	2.9	7.5
68	K	2.5-L.	-	.52	3.0	5.7
76	L	2.5-E.L.	-	.29	3.0	10.6
81	M	5.5-L.	-	.29	3.3	11.4
71	N	5.5-E.L.	-	.38	2.8	7.4

[1]Lard

[2]Emulsified lard

[3]Methionine hydroxy analogue, 2 gm. per lamb per day.

[4]Lambs consumed 1 oz. per lamb per day of the following mineral mix: NaCl 100 lbs.; $FeSO_4$ 1 lb.; $CoCl_2.6H_2O$ 22 gm.; KI (stabilized) 5 gm. and $CuSO_4$ (crystal) 90 gm.

TABLE XI

THE COEFFICIENTS OF DIGESTIBILITY OF THE RATION CONSTITUENTS (BASAL RATION) IN TRIAL 1 WITH WETHER LAMBS

Sheep No.	Ration Letter	Variable: Added Fat Level (%) and Type	Variable: + or - M.H.A.[3]	Coefficients of Digestibility: Cellulose	N	Fat	O.M.	N.F.E.
66	A	None	+	63.6	74.2	54.6	67.0	66.9
75	A	None	+	75.4	76.3	62.9	74.0	72.9
79	B	1-L[1]	+	69.2	72.5	83.7	70.7	70.6
67	C	1-E.L.[2]	+	57.6	62.1	83.4	62.3	64.2
77	D	2.5-L.	+	57.5	67.6	87.6	63.7	64.6
72	E	2.5-E.L.	+	65.5	75.3	89.5	69.2	67.6
70	F	5.5-L.	+	48.8	75.1	89.4	61.8	62.3
73	G	5.5-E.L.	+	51.8	75.9	93.1	65.3	64.9
69	H	None	-	52.1	70.8	54.0	61.5	63.6
78	H	None	-	67.0	74.2	67.5	69.9	72.0
74	I	1-L.	-	59.8	70.7	69.5	65.4	66.6
80	J	1-E.L.	-	70.2	75.7	87.2	72.9	73.1
68	K	2.5-L.	-	66.0	78.3	91.2	73.0	73.4
76	L	2.5-E.L.	-	67.6	75.6	88.4	69.0	67.1
81	M	5.5-L.	-	44.6	79.6	95.6	62.5	62.2
71	N	5.5-E.L.	-	43.6	78.1	95.1	63.6	63.3

[1] Lard
[2] Emulsified lard
[3] Methionine hydroxy analogue, 2 gm. per lamb per day

TABLE XII

THE COEFFICIENTS OF DIGESTIBILITY OF THE RATION CONSTITUENTS (BASAL RATION PLUS MILO) IN TRIAL 2 WITH WETHER LAMBS

Sheep No.	Ration Letter	Variable: Added Fat Level (%) and Type	Variable: + or - M.H.A.[3]	Coefficients of Digestibility: Cellulose	N	Fat	O.M.	N.F.E.
66	A	None	+	55.9	71.2	69.3	70.4	74.9
67	A	None	+	61.1	72.7	72.1	73.8	78.3
79	B	1-L.	+	49.0	70.2	80.6	68.4	73.3
77	D	2.5-L.[1]	+	51.5	68.2	86.0	69.6	74.6
72	E	2.5-E.L.[2]	+	61.1	75.6	74.9	74.1	78.2
70	F	5.5-L.	+	47.9	64.5	83.7	62.7	70.7
73	G	5.5-E.L.	+	33.4	74.8	90.8	66.4	70.5
69	H	None	-	46.1	72.3	64.8	68.0	73.8
78	H	None	-	57.2	71.6	68.6	71.9	76.3
74	I	1-L.	-	45.2	75.2	80.2	69.1	74.0
80	J	1-E.L.	-	49.5	76.8	81.2	72.8	77.3
68	K	2.5-L.	-	45.7	76.6	89.8	71.1	76.3
76	L	2.5-E.L.	-	52.5	69.8	87.1	72.4	78.9
81	M	5.5-L.	-	49.0	73.9	91.1	65.9	67.2
71	N	5.5-E.L.	-	22.7	75.9	88.0	65.6	70.2

[1]Lard
[2]Emulsified lard
[3]Methionine hydroxy analogue, 2 gm. per lamb per day

TABLE XIII

TOTAL DIGESTIBLE NUTRIENTS OF RATIONS USED IN TRIAL 1 (BASAL RATION) WITH WETHER LAMBS

Sheep No.	Ration Letter	Variable Studied: Added Fat Level (%) and Type	Variable Studied: + or - M.H.A.	Calculated Per Cent T.D.N.
66	A	None	+	61.1
75	A	None	+	67.6
79	B	1-L.	+	65.9
67	C	1-E.L.	+	58.6
77	D	2.5-L.	+	61.1
72	E	2.5-E.L.	+	66.2
70	F	5.5-L.	+	63.0
73	G	5.5-E.L.	+	67.5
69	H	None	-	55.8
78	H	None	-	63.4
74	I	1-L.	-	61.3
80	J	1-E.L.	-	68.4
68	K	2.5-L.	-	69.9
76	L	2.5-E.L.	-	65.5
81	M	5.5-L.	-	65.5
71	N	5.5-E.L.	-	64.8

TABLE XIV

COMPOSITIONS OF RATIONS (BASAL RATION AND MILO)
AVERAGE PER CENT IN RATION

Ration	Water	Fat	Ash	N	Cellu-lose	N.F.E.[1]
A	5.78	0.70	3.56	1.98	25.90	51.68
B	5.60	1.75	3.72	1.86	24.53	52.77
C	5.98	1.88	3.39	1.51	25.15	54.16
D	5.57	2.80	3.22	1.72	25.47	52.19
E	5.50	3.17	3.33	1.78	25.65	51.22
F	5.65	5.93	3.07	1.65	24.95	50.09
G	4.93	6.73	3.67	2.01	22.32	49.79
H	5.82	0.73	4.14	1.93	24.33	52.92
I	4.93	1.78	3.64	1.93	24.54	53.00
J	5.70	2.08	3.83	2.13	23.38	51.70
K	5.68	3.45	3.68	1.99	23.85	50.90
L	5.90	2.68	3.39	1.87	26.74	49.60
M	4.40	6.68	3.35	1.92	23.00	50.57
N	5.80	6.63	3.86	2.12	22.02	48.44
Milo	10.65	2.20	1.79	1.76	4.69	69.67

Ration	Sulfur Analysis*
A	.150
H	.145
Milo	.102

*Spectrographic analysis done by the Station Laboratories

[1]N.F.E. was determined as 100- ether extract + cellulose + water + ash + (N x 6.25).

APPENDIX FIGURES

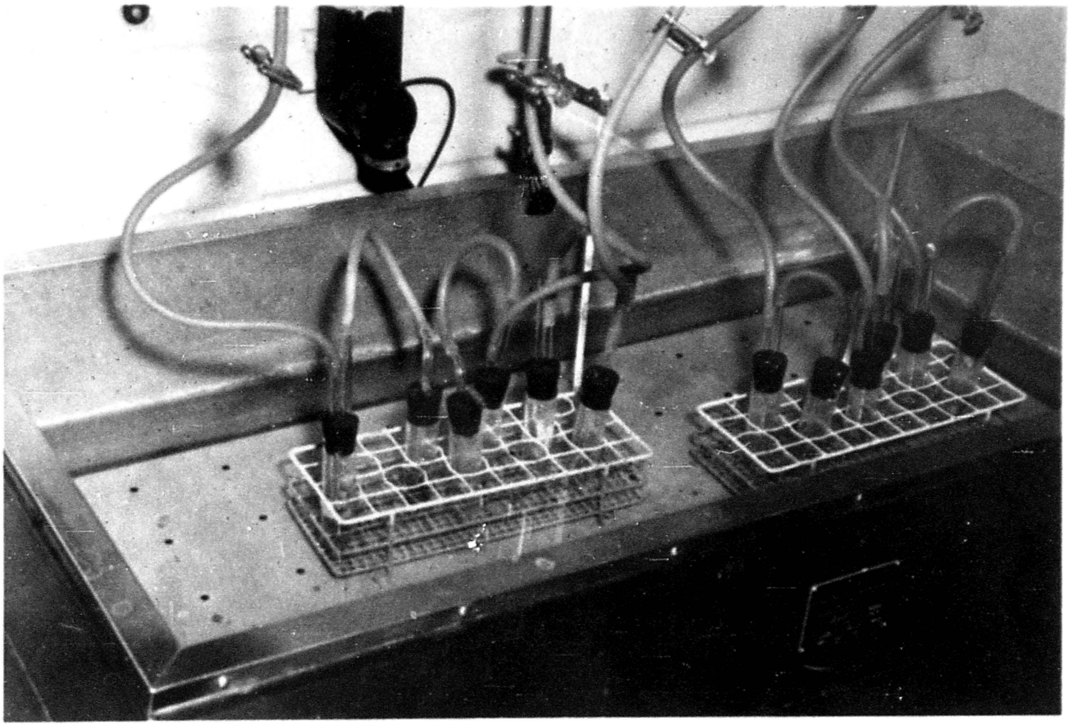

Figure 1. Artificial rumen in waterbath.

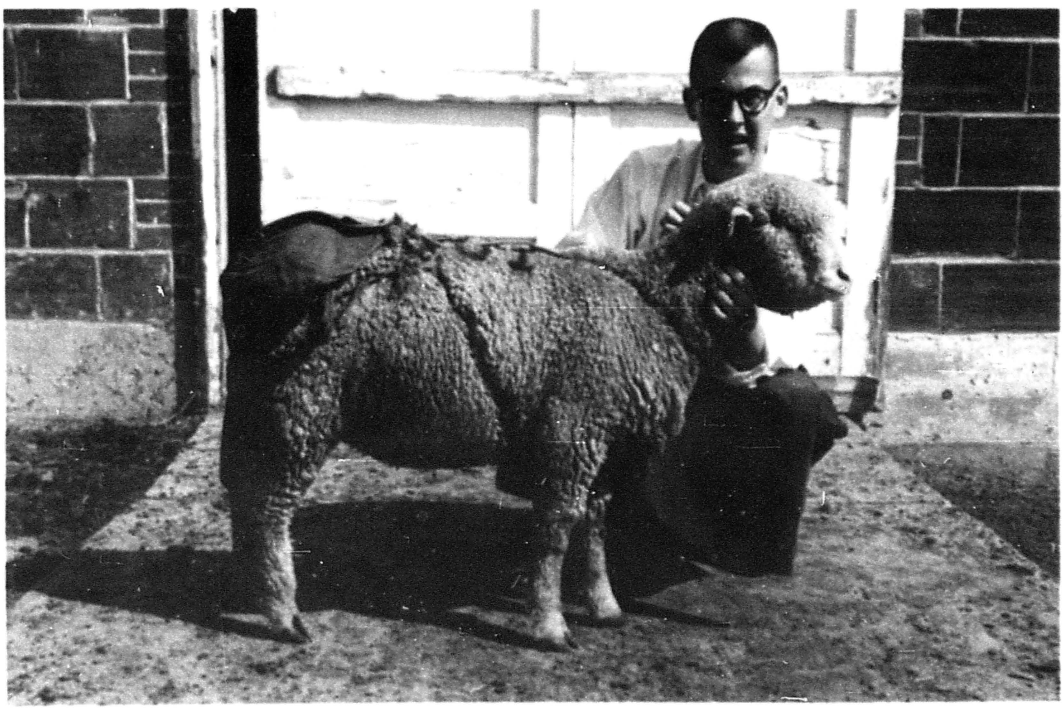

Figure 2. Lamb wearing canvas fecal collection bag.