THE EFFECT OF ALFALFA ASH AND CERTAIN OF ITS MINERAL CONSTITUENTS ON THE UTILIZATION OF COTTONSEED HULLS BY SHEEP

By

CHARLES WAYNE TEFFT

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Thesis Approved:

Allen D. Tillman
Thesis Adviser

Robert J. Sirny

Robert MacVicar
Dean of the Graduate School

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TABLE OF CONTENTS

INTRODUCTION

In recent years, a new approach to the problem of ruminant nutrition has been developing. This new approach is based on the fact that the first step in ruminant nutrition is microbial nutrition. When the microorganisms are properly fed, they are then able to more efficiently break down the cellulosic material in the roughages that normally make up a major part of a ruminant ration. This concept has developed largely as a result of a need to more efficiently use the potential feed energy in low quality roughages.

It appears that the relatively poor growth responses reported in previous years from feeding low quality roughages can now be attributed largely to a defiency or improper balance of nutrients required by rumen microorganisms. Furthur improvements in the utilization of low quality roughages would seem to involve determining the requirements of the ruminant microorganisms and developing supplements for deficient rations to fulfill these needs.

The problem of utilization is economic as well as nutritional. Higher feed prices and lower livestock market prices have made it desirable, and new machinery and methods of handling have made it possible and profitable, to use the bulky, low quality roughages such as corn cobs, wheat straw, and cottonseed hulls in the rations of ruminant animals.

Recent experiments on improving the efficiency of utilization of low quality roughages have shown that the addition of alfalfa hay or meal, or the ash from these materials will improve the utilization of some of these roughages. The investigation reported in this paper was undertaken to determine: (1) the effect of alfalfa ash on the utilization of cottonseed hulls, and (2) the mineral or minerals in alfalfa ash responsible for the effect.

REVIEW OF LITERATURE

Cottonseed Hull Feeding

An increasing amount of cottonseed hulls has been fed as part of ruminant rations during recent years. Moore (1951) reported that the strong demand for cottonseed hulls was keeping many mills busy trying to supply their customers, and that some mills were currently enjoying the best local demand they ever had. He also stated that other mills have been shipping hulls to distant markets that ordinarly would not pay the freight cost to ship such a bulky feed.

The earliest reports found in the literature on digestibility trials with cottonseed hulls are those of Emery and Kilgore (1891 a) Emery et al (1891 b), and Emery and Kilgore (1892). These workers ran an extensive series of experiments to determine the digestibility of cottonseed hulls alone, the effect of the addition of cottonseed meal upon the digestibility of cottonseed hulls, and the value of cottonseed hulls when supplemented with cottonseed meal for beef production. The effects of the addition of cottonseed meal upon the digestibility of ration components contained in cottonseed hulls were as follows:

The Effect of Cottonseed Meal on the Digestibility of Cottonseed Hulls (Emery and Kilgore, 1891 a)

Ration	Apparent Digestibility					
	Dry Matter %	Ash %	Crude Protein %	Ether Extract %	N-Free Extract %	Crude Fiber %
Cottonseed Hulls	35.9	27.1	24.6	80.6	40.3	27.1
Cottonseed Hulls and Cottonseed Meal	44.9	34.2	44.3	81.0	51.4	33.9

The results obtained in this digestibility trial clearly demonstrated the value of additional protein. These workers found that the animals receiving the cottonseed hulls and meal remained in thrifty condition for the duration of the test while some of the sheep receiving only cottonseed hulls did not.

Fraps (1914) conducted digestibility trials in which cottonseed hulls were fed with cottonseed meal and alfalfa hay. He found higher digestion coefficients for ether extract, crude fiber and nitrogen-free extract than the average of those reported in the literature. The digestibility of the protein was minus 21.0 percent, that is, feeding cottonseed hulls reduced the digestibility of the protein in the cottonseed meal and alfalfa hay fed with it.

Lush et al (1933) in studies comparing cottonseed hulls and grass hays found that cottonseed hulls, when supplemented with calcium, green feed and protein, were superior to hill land carpet and Bermuda grass hay for milk production. Cottonseed hulls were almost equal to high quality Bermuda and inferior to mixed clover hay. These workers concluded that when cottonseed hulls or non-legume hay is fed, adequate protein, green feed and minerals should be supplied.

Starkey and Godbey (1937) fed rations containing either cottonseed hulls or corn stover supplemented with cottonseed meal to steers and found that the cottonseed hulls-fed steers gained 0.85 pound per day more than the stover-fed steers.

Hostetler et al (1937) found that cottonseed hulls could be substituted for part of the lespedeza hay for steers being full-fed a ration of corn plus 2 pounds of cottonseed meal and lespedeza, free-choice. There was no significant difference in the gains, although the cottonseed hulls-fed steers were less efficient in their gains.

Forbes and Garrigus (1949) and Garrigus (1951) carried out studies to formulate a nutritionally adequate ration containing a considerable amount of cottonseed hulls. The rations fed contained: (1) corn and alfalfa hay, (2) corn, cottonseed hulls, molasses, cottonseed meal, and alfalfa meal, and (3) corn, cottonseed hulls, and cottonseed meal. They found that although the rations were as nearly equal in gross nutrients as they could be made, the lambs on cottonseed hulls digested 91 percent as much dry matter, 82 percent as much protein, 117 percent as much fat, 92 percent as much nitrogen-free extract, and 93 percent as much energy as those on the alfalfa and corn. These workers suggested that the higher lignin content of the cottonseed hull ration could be forming some sort of barrier to the action of the digestive juices. They concluded that cottonseed hulls can be used as roughage for fattening lambs or cattle provided minerals, protein, and carotene are adequately supplied by pasture forage, or if the hulls are supplemented with cottonseed meal, molasses, and alfalfa meal to supply the necessary nutrients.

Melton et al (1950) compared rations containing various levels of ground cotton stalks, ground gin trash and cottonseed hulls combined with alfalfa hay. Half of the steers on each ration also received 2 pounds of molasses. Animals receiving cottonseed hulls ate more and gained faster than those receiving stalks or gin trash. Although the appetite of the steers fed stalks and gin trash improved when molasses was added to their ration, their gains were not as rapid.

Hussain et al (1951) compared the chemical composition and digestibility of cottonseed hulls and wheat straw. They reported that the chemical composition was fairly similar although the cottonseed hulls were much lower in total ash (3.4 percent) than the wheat straw (9.4 percent). Digestibility was about equal except cottonseed hulls were slightly higher in digestible ether extract. Neither roughage had much digestible protein. These workers recommended that cottonseed hulls be supplemented with green fodder or a good legume hay for best results.

Effect of Alfalfa Hay or Meal on the Digestibility and Utilization of Various Rations

Combinations of feedstuffs apparently affect digestibility of rations by ruminant animals through the agency of rumen organisms which grow on the food ingested by the host animal and are then digested by the animal. This was pointed out by Forbes et al (1943) who found in experiments comparing alfalfa and timothy hay that the digestible nutrients and metabolizable energy of corn were one-fourth higher when fed with alfalfa hay than when fed with timothy hay.

Burroughs et al (1950 b) determined the digestibility of the dry matter of corn cobs with steers fed rations containing variable amounts of alfalfa hay. They found by feeding five rations containing from 0.5 pound to 4.0 pounds of alfalfa hay per steer that the digestibility of the corncob dry matter increased gradually from 39.7 percent to 54.9 percent as the amount of alfalfa hay in the ration increased. They believed that alfalfa hay contained one or more essential nutrients required by the rumen microorganisms for efficient cellulose digestion.

Beeson and Perry (1952) supplemented corn cob rations for steers with one and two pounds of alfalfa meal and found that daily gains on the two rations were increased 0.13 and 0.27 pound per day, respectively. In another experiment, also with steers, they found that supplementing a ration with alfalfa meal and soybean meal gave increased gains in comparison to rations supplemented with soybean meal alone, distiller's solubles plus urea, and brewer's yeast plus soybean meal. Addition of vitamin B_{12}, fish meal, live cell yeast, or brewer's yeast to the control ration did not result in a significant increase in gain; however, the replacement of 2 pounds of cobs with 2 pounds of alfalfa meal did give a significant increase. They believed these data substantiate a new concept in livestock feeding, this concept being that to properly feed ruminants on poor quality roughages, a supplement must be provided to nourish the billions of microorganisms in the rumen.

Klosterman _et al_ (1953) studied the effect of substituting dehydrated alfalfa meal in place of part or all of the soybean meal in a corncob ration. The substitutions were made in such way that equal amounts of total crude protein were fed in all rations. Gains of the cattle increased as the amount of alfalfa meal increased and when either one half or all the soybean meal was replaced with alfalfa meal the increase in gains was highly significant.

Richardson _et al_ (1953) found that supplementation of a ration composed of wheat straw, soybean meal and milo with 1 pound of dehydrated alfalfa pellets increased rate and efficiency of gain with dairy calves.

Effect of Various Minerals on Cellulose Digestion

Riddell _et al_ (1934) reported that phosphorus deficiency did not decrease the digestive functions of dairy cattle although there was a failure of appetite. Klieber *et al* (1936), however, working with beef heifers, reported that phosphorus deficiency decreased partial efficiency of energy utilization, the efficiency of food protein for sparing body protein, and appetite of animals. In both cases, addition of phosphorus improved the abnormal conditions.

Weber *et al* (1940) reported that adding ground limestone to a calcium-deficient ration resulted in an increase in gain in weight, more efficient utilization of feed and slightly higher slaughter grades.

Becker and Smith (1949) found that when lambs fed a ration containing grass hay which was deficient in cobalt were supplemented with cobalt, they digested the ether soluble and nitrogen-free extract fractions of the ration more efficiently. Crude fiber digestion, however, was not affected significantly.

By the use of digestion studies with cattle and by the use of an artificial rumen technique, Cardon (1953) showed that an increased salt content of the rumen caused by feeding a high level of salt did not decrease rumen microbial activity. Both types of experiments showed that the digestion of cellulose as well as the digestion of gross energy was not altered by the increased consumption of salt.

Effect of Complex Mineral Mixtures, Extracts and Ashs of Alfalfa, and Other Materials on Cellulose Digestion

Burroughs *et al* (1949) and Burroughs *et al* (1950 c) reported the results of *in vitro* studies in which the digestion of cellulose of good and poor quality roughages was measured. These workers found that good quality roughages (alfalfa hay, clover hay, and immature rye hay) were digested efficiently by rumen microorganisms when no supplement was added. Poor quality roughages (corn stover, wheat straw, corncobs, and mature timothy-blue grass hay), however, were not efficiently digested until supplemented with a complete mineral mix or an autoclaved extract of cow manure. They believed this indicated that the efficiency with which a roughage is digested depends on the presence or absence of essential nutrients in the ration which are required for maximum function of rumen microorganisms.

Burroughs et al (1950 b) working with cattle found that as the ratio of alfalfa to corn cobs in a ration increased, the digestibility of the corn cob dry matter increased. They also found that daily supplementation with a water extract from 4 pounds of alfalfa meal increased the digestibility of the corn cob dry matter from 34.4 to 48.9 percent and that the ash from 4 pounds of meal increased it from 38.5 to 52.0 percent. They postulated that the alfalfa extract or ash supplied some nutrient(s) required by the rumen microorganisms so that when fed with a poor quality roughage the digestion of the poor quality of a roughage may depend to a large extent on its mineral makeup.

In preliminary in vitro studies on factors affecting cellulose digestion by rumen microorganisms, Burroughs et al (1950 a) found that the ability of rumen organisms to digest cellulose depended on the addition or withdrawl of certain substances from the nutrient medium. The substances found to increase digestion in this trial were alfalfa ash, a complex mineral mixture resembling the minerals in sheep saliva, autoclaved rumen liquid and autoclaved cow manure extract. In furthur experiments in which the same procedure was used, Burroughs et al (1951) found that a water extract from dehydrated clover meal and molasses ash gave similar increases in cellulose digestion. The amount of molasses ash necessary for efficient cellulose digestion varied with the amount of available energy. They also found that added iron and phosphorus aided digestion; iron alone increased digestion significantly, but phosphorus did not unless added in the presence of iron. These workers said the results obtained from adding molasses were not specific with the molasses, but with some of its constituents.

Meites et al (1951) found that the addition of alfalfa ash or ash of rumen liquor to dialyzed rumen liquor increased in vitro digestion of cellulose. They also found that the addition of small quantities of ferrous sulfate or cobaltous nitrate failed to stimulate digestion.

Swift et al (1951) reasoned that the increased cellulose digestion resulting when alfalfa ash was added would be of no value to the animal if methane production was greatly increased. They found that alfalfa ash increased crude fiber digestion from 43.0 to 53.8 percent. Although there was an appreciable increase in methane production, there was a large increase in metabolizable energy, the net effect being to make more feed energy available to the animal.

Bentley and Moxon (1952) conducted an experiment in which they supplemented a semi-synthetic ration composed of urea, cerelose, iodized salt, $CaCO_3$, $Ca_3(PO_4)_2$, vitamin A and D oil, corn and cob meal, and a poor quality timothy hay with either alfalfa ash, a trace mineral mixture or reduced iron. Alfalfa ash and the trace mineral mixture improved the average daily gains by 43 percent. Iron supplement alone had no effect.

Chappel (1952) in the first of a series of experiments on factors affecting the digestibility of low quality roughages found that lambs supplemented with alfalfa ash digested the organic matter of corn cobs 20 percent more efficiently than lambs receiving five other rations containing various mineral mixtures. A "complete" mineral mixture had no effect, and he furthur observed that the effect of alfalfa ash was not due to sodium, potassium, calcium, phosphorus, cobalt or iodine in this experiment. In another experiment of the same series, he found that alfalfa ash increased the digestion of all fractions of the ration, and especially crude fiber. A "synthetic" alfalfa ash containing all the minerals found in alfalfa ash and in the same proportions gave similar increases, but to a slightly lower degree. The complete mineral mix again had much less effect.

In an experiment reported by Chappel (1952) and extended by Tillman et al (1954 a), prairie hay rations supplemented, respectively, with a complete mineral mixture and alfalfa ash were compared to a basal ration. No significant effect on digestibility of organic matter, crude protein or crude fiber was observed with either of the supplements.

Bentley and Klosterman (1953) and Klosterman et al (1953) reported that supplementation of a corn cob ration with alfalfa ash, molasses ash, and a trace mineral mix containing copper, zinc, manganese, iron and cobalt significantly increased gains with steers. Then in a furthur experiment reported by Bentley and Klosterman (1953) it was found that the trace mineral mixture or cobalt alone produced striking improvement in gain with the corn cob ration. They believed that borderline trace mineral deficiencies are more likely to occur on poor quality roughages such as corn cobs, stover, etc., and that the kind and quality of roughage available may be the deciding factor in whether or not to feed trace minerals.

Using in vitro experiments, Bentley et al (1953) obtained a two- or three-fold increase in cellulose digestion when the medium of cellulose, urea, minerals, and glucose was supplemented with either autoclaved rumen juice, a hot water extract of alfalfa leaf meal or hay. When nine B vitamins were added, there was no significant effect on cellulose digestion, but when alfalfa ash was also added, cellulose digestion was doubled.

Tillman et al (1954 b) found that addition of alfalfa ash to a cottonseed hull basal ration increased the digestibility of all ration components significantly. Crude fiber digestion was increased from 35.6 to 46.5 percent. In a depletion-repletion type experiment used by these workers, it was found that sheep which had lost weight and developed abnormal appetites and behavior after ten days on a basal diet started gaining weight immediately and displayed more normal behavior when alfalfa ash was added to their ration. Response to the addition of a synthetic alfalfa ash was as good as with natural alfalfa ash, while the addition of cobalt to the ration had no effect. They believed these results supported the theory that alfalfa ash supplies one or more nutrient(s) needed by rumen microorganisms and postulated that other factors such as total ash, anion-cation balance, buffering effect, and balance of inorganic elements might be contributing to the observed effects of alfalfa ash.

EXPERIMENTAL OBJECTIVES

The purposes of the trials reported here are as follows:

Trial I To study the mineral(s) responsible for the effect of alfalfa ash on the utilization of a semi-purified type ration containing cottonseed hulls as the roughage.

Trial II To study the effect of adding alfalfa ash on the utilization of a ration composed of natural feedstuffs containing cottonseed hulls as the roughage.

Trial III To furthur study the mineral(s) responsible for the effect of alfalfa ash on the utilization of a semi-purified type ration containing cottonseed hulls as the roughage.

EXPERIMENTAL PROCEDURE

Trial I

The basal ration in this trial was a semi-purified type consisting of (in percent): cottonseed hulls, 60.00; corn oil, 2.40; cerelose, 30.80; urea, 2.00; di-calcium phosphate, 2.40; sodium sulfate, 0.32; gelatin, 1.60; vitamin A and D oil, 0.08; and sodium chloride, 0.40. The mineral supplements fed were based on a "synthetic" alfalfa ash (Tillman et al, 1954 b) compounded from mineral salts to duplicate, as nearly as possible, the mineral composition of natural alfalfa ash. It consisted of:

Material	grams	Material	grams
$KHCO_3$	960.00	$Na_2B_4O_7 \cdot 10\ H_2O$	5.70
K_2HPO_4	348.00	$MnSO_4 \cdot H_2O$	3.00
$CaCl_2$	277.00	$CuSO_4 \cdot 5\ H_2O$	5.00
$Ca(OH)_2$	348.00	ZnO	1.06
$MgSO_4 \cdot 7\ H_2O$	592.00	$CoCl_2 \cdot 6\ H_2O$	0.007
$FeSO_4 \cdot 7\ H_2O$	500.00	MoO_3	0.003
$NaHCO_3$	319.00		

Since Tillman and associates obtained equally good results in the utilization of cottonseed hulls with synthetic alfalfa ash as with natural alfalfa ash, it was decided to use the basal ration supplemented with synthetic ash as the control ration. The other lots received the basal ration supplemented with synthetic ash minus certain of its mineral constituents in order to determine the mineral(s) contained in alfalfa ash responsible for the increase in utilization of cottonseed hulls. In this procedure, the animals received all the minerals present in the synthetic alfalfa ash except for the one being studied. The rations fed in this trial were as follows:

1. Basal plus synthetic ash (Control).
2. Basal plus synthetic ash minus magnesium.
3. Basal plus synthetic ash minus iron and copper.
4. Basal plus synthetic ash minus molybdenum.
5. Basal plus synthetic ash minus manganese.

The synthetic alfalfa ash supplements were fed at the level supplying the mineral elements contained in three-fourths pound of alfalfa hay.

Twenty-five western wether lambs were the experimental animals in this trial. They were paint branded, for easier identification, on the day after their arrival from the Oklahoma City stockyards. They were also drenched with a phenothiazine-water solution to minimize possible intestinal parasite infestation. Previous to the start of the experiment, they were fed prairie hay, free-choice, and one-half pound of soybean meal per head daily for seven days in order to accustom them to the surroundings and attendant. The animals were kept in large box stalls which were equipped with individual stanchions, into which they were locked at feeding time. When not eating, they were allowed the freedom of the stall and free access to water. At the end of the seven days, all the refused hay and concentrate were cleaned out of the feed bunks and the loose hay and bedding cleaned out of the pen before feeding of the experimental ration began.

A depletion-repletion type experiment as described by Tillman et al (1954 b) was used for this trial. The lambs were weighed at the beginning of the depletion phase, and were group-fed twice daily the semi-purified basal diet for 33 days. At the end of the depletion period, the lambs were again weighed and allotted in a randomized manner into five approximately equal groups on the basis of their weight loss during this phase. In the repletion phase that followed immediately, the effect of removal of the various minerals was studied. During this phase, which lasted 21 days, the lambs were fed in individual stanchions. Weekly weights were taken, and individual feed records kept. Whenever the previous feeding was not cleaned up, the roughage and concentrate part of the ration was decreased although the full amount of the mineral supplements were fed each time. Feed refusals were also weighed back at weekly intervals. Blood samples were taken from the sheep in lots 1 and 3 for red blood cell count and hemoglobin determination at the end of the repletion period. The data were analyzed by methods of analysis of variance, and least significant difference as described by Snedecor (1946).

Trial II

Forbes and Garrigus (1949) and Garrigus (1951) found that the utilization of a cottonseed hull, cottonseed meal, and corn ration by sheep could be improved by supplementation with alfalfa meal and molasses. The trial reported here was undertaken to determine if natural alfalfa ash, alone would improve gains of sheep fed a similar ration. Two rations were fed, with ten sheep per ration. The basal ration, which was balanced according to Morrison's (1948) standards, was composed of 50 percent cottonseed hulls and 50 percent concentrate mix consisting of (in percent): corn, 79.90; cottonseed meal, 19.90; vitamin A and D oil, 0.10; and di-calcium phosphate, 0.10. The second ration consisted of the basal ration plus the ash from three-fourths pound of alfalfa hay per day.

Twenty of the sheep from the previous experiment were used in this trial. They were allotted in a randomized manner into four groups of five animals with one animal in each group from each of the lots in the previous trial. The animals were fed twice daily in individual stanchions for 35 days. Weights were recorded twice during the experiment, individual feed records were kept on each sheep, and feed refusals were weighed back at weekly intervals. The data were analyzed by the method of analysis of variance as described by Snedecor (1946).

Trial III

The procedure followed in this trial, except for the differences listed here, was the same as for Trial I.

The following nine rations were fed in Trial III.

1. Basal.
2. Basal plus synthetic ash.
3. Basal plus synthetic ash minus copper.
4. Basal plus synthetic ash minus iron.
5. Basal plus synthetic ash minus zinc.
6. Basal plus synthetic ash minus molybdenum.
7. Basal plus synthetic ash minus cobalt.
8. Basal plus the major minerals plus iron.[1]
9. Basal plus natural alfalfa ash (Control).

[1]All the trace minerals except iron were omitted from the synthetic ash mix for this mineral supplement. It consisted of the first seven mineral salts listed in the mineral composition of synthetic ash as described in Trial one.

Thirty-six western wether lambs were used in this experiment. One week prior to the start of the depletion phase, all the sheep were sheared. Following the depletion period of 28 days, the lambs were allotted at random into nine lots of 4 lambs per lot. The regular repletion period lasted for 25 days. At the end of this period, blood samples were taken from lots 1, 2, 3, and 4 for red blood cell count and hemoglobin determination. Three sheep were selected at random from the basal lot, and the lots supplemented, respectively, with synthetic ash, synthetic ash minus copper, and synthetic ash minus iron. These sheep were then supplemented with complete synthetic ash for 17 days in order to determine if this treatment would improve the performance of the sheep; the three sheep from the synthetic ash lot being kept as controls. Because of rather unusual results obtained during Trials I and III, three sheep from the synthetic ash minus molybdenum lot were also retained, but were continued on their usual ration. It was planned to keep these sheep on their ration indefinitely in order to determine the long-time effect of supplementation of the cottonseed hull basal ration with synthetic alfalfa ash minus molybdenum. The three animals used as controls during the 17 day recovery phase were retained, and continued on their usual ration as controls for this group. After five and one-half months, one sheep from the synthetic ash minus molybdenum lot, and one from the synthetic ash lot were sacrificed and the xanthine oxidase activity of the liver was determined by an adaptation of the method of Dietrich et al (1954). The liver xanthine oxidase activity was also determined for one sheep that had been on wheat pasture and later fed prairie hay, free-choice, plus a concentrate mixture of natural feedstuffs. In the actual determination of liver xanthine oxidase activity, it was observed that xanthine added to the liver homogenates, as prescribed in the procedure outlined by these workers for mouse livers, appeared to depress the formation of allantoin. In this procedure allantoin production is the index of the enzyme's activity. In studies using mouse livers, these workers reported a similar depression when xanthine was added in amounts larger than recommended in their procedure. This seemed to indicate that the endogenous xanthine of sheep liver was sufficient for optimum enzyme activity; for this reason, only the endogenous activity of the enzyme system is shown in the results.

RESULTS AND DISCUSSION

Trial I

Results during the depletion phase of this trial indicate much the same reaction to the basal ration as reported by Tillman et al (1954 b). After only seven days, indications of depraved appetites in the experimental animals were evidenced by their chewing on the clothes of the attendant. About two weeks later, they were chewing on the wooden feed bunks and door frames. Shortly before the start of the repletion period, some of the sheep started pulling and eating wool from the other sheep. It was also noticed that the feces produced during the depletion period contained a considerable amount of cotton linters. The lambs weighed an average of 71 pounds at the start of the depletion phase, and lost an average of 8 pounds or 0.26 pound per day for the 33 days. It was found by a linear regression study that there was no relation between initial weight and loss of weight during the depletion phase.

The average daily gains during the depletion and repletion periods of this trial are shown as lot averages in Table 1. The results in detail are given in Table I of the appendix. In Figure 1, the average weekly weights for each lot are plotted as percentages of average weights at the start of the repletion phase.

Only the control and synthetic ash minus manganese lots gained weight during the repletion phase. Although the gains with synthetic ash were not as great as those reported by Tillman et al (1954 b),

Table 1

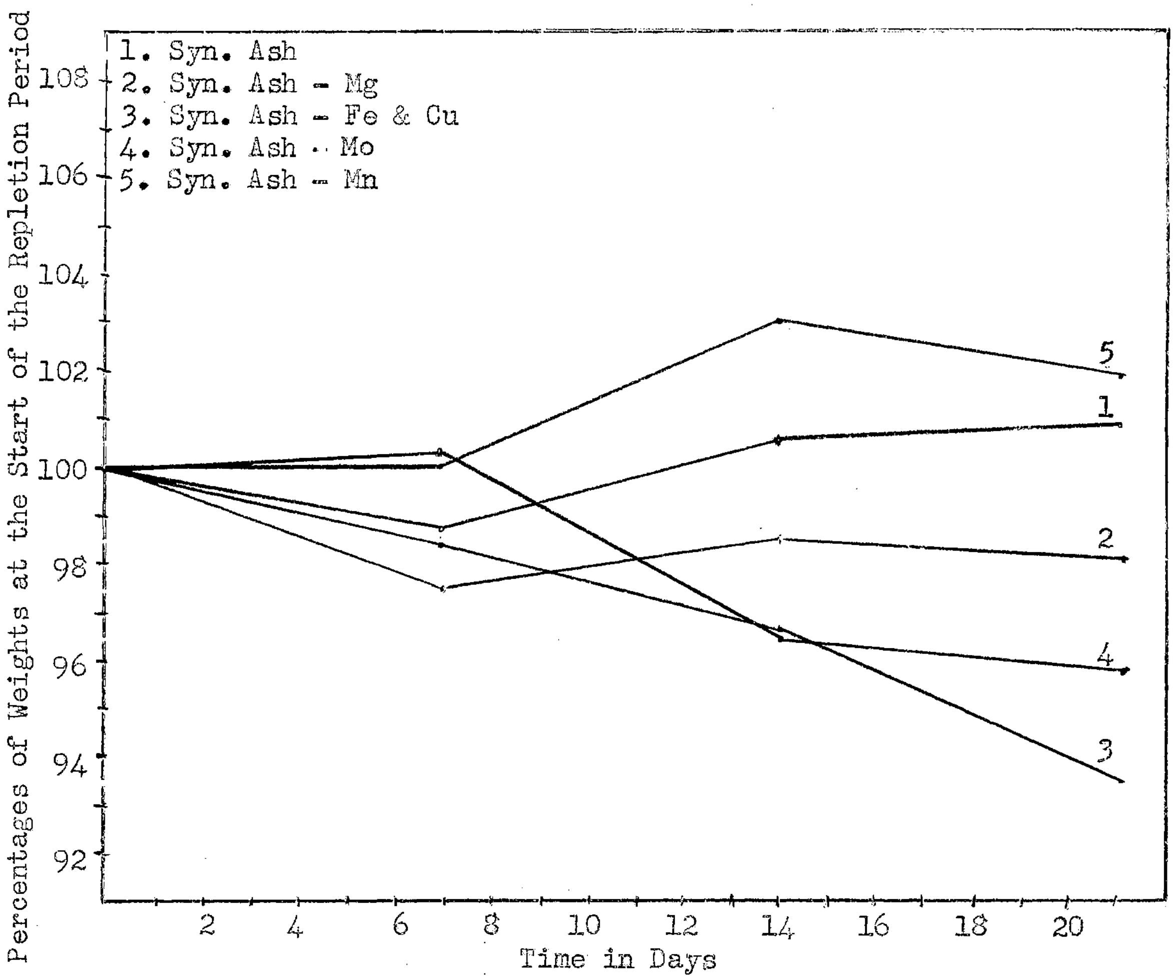
Average Daily Gains During the Depletion and Repletion Periods of Trial I

	Average Daily Gains	
	Depletion	Repletion
1. Syn. Ash	-0.25	0.03
2. Syn. Ash - Mg	-0.23	-0.06
3. Syn. Ash - Fe & Cu	-0.27	-0.19*
4. Syn. Ash - Mo	-0.30	-0.12[1]
5. Syn. Ash - Mn	-0.27	0.06[1]
Average	-0.26	-0.06

*Significant at the 5 percent level.
[1]One lamb had to be removed from these lots during the trial.

Figure 1

Average Weekly Weight Changes During the Repletion Period of Trial I (Percentage of Weights at the Start of the Repletion Period)



they were significantly greater than the average daily gains (all negative) during the depletion phase of the trial. It was found that only the lot supplemented with synthetic ash minus iron and copper had a rate of gain significantly different from that of the control lot during the repletion phase. As can be seen from the growth curve in Figure 1, this lot continued to lose weight during the repletion phase, and at a slightly increasing rate as the phase progressed. Although the average daily weight loss during this period was not as great as that during the depletion period, the two were not significantly different. Because both iron and copper had been left out of the synthetic ash supplement in this lot, it was impossible to tell which mineral was responsible for the observed results. The lot supplemented with synthetic ash had a red blood cell count of 7,650,000 per Cmm as compared to 6,830,000 for the lot supplemented with synthetic ash minus iron and copper, and a hemoglobin value of 11.9 percent as compared to 11.3 percent. Neither of these differences was found to be significant.

The average daily gains for the sheep supplemented with synthetic ash minus molybdenum were not significantly different from those of the control lot. However, three of the sheep in this lot, numbers 2, 24, and 25 became quite thin during the repletion period and developed a slightly stiff, awkward gait, hunching their backs as they moved. Number 25 sheep developed soreness and lameness in his hind legs, became unable to rise without assistance, and was barely able to stand when set on his feet. He was finally removed from the test and given a ration of corn, cottonseed meal, cottonseed hulls and alfalfa hay. He seemed to improve almost immediately, and on the fourth day on this ration was able to rise unaided. Although molybdenum has been considered only as a toxic mineral in the past, recent work has shown it to be needed by rats. Richert and Westerfeld (1953) and De Renzo <u>et al</u> (1953 a,b) found that molybdenum salts were necessary for the deposition and maintenance of normal levels of intestinal xanthine oxidase. In a textbook by Sumner and Somers (1953), xanthine oxidase is described as one of the "yellow enzymes", having as one of its functions the dehydrogenation of Co-enzyme I, which is necessary in the hydrogen transfer mechanism in Carbohydrate metabolism. These observations indicate that molybdenum is an essential mineral. Since the findings in this trial indicated a possible need for molybdenum by sheep, it was decided to include a synthetic ash minus molybdenum-mineral supplement in another trial to see if the observed results could be repeated.

One sheep had to be removed from the synthetic ash minus manganese lot because of refusal to eat the ration. This was not believed due to the mineral supplement, however, because the other sheep in the lot ate well and gained slightly more than the average for the control lot. The gains of the lots having manganese and magnesium, respectively, removed from the synthetic ash supplements were not significantly different from those of the control lot. This indicated that these two minerals were being supplied in sufficient amounts by the cottonseed hulls.

<u>Trial II</u>

A summary of the results in this trial is given in Table 2, and the results in detail are given in Table II of the appendix.

Table 2

Average Daily Feed Intake, Daily Gains, and Feed Efficiency (Trial II)

	Average daily feed intake (gms.)	Average daily gains (lbs.)	Feed efficiency (lbs. feed/100# gain)
Basal	985	0.26	1037
Basal plus Alfalfa Ash	955	0.32	703
Average	970	0.29	870

The average daily feed intake for the sheep on the two rations was nearly the same, although the unsupplemented sheep ate slightly more. This would seem to indicate that the palatibility of the ration was not affected by supplementation with alfalfa ash. Alfalfa ash supplementation increased the average daily gain 23 percent in this trial from 0.26 to 0.32 pound per day. This difference, although considerable, was not statistically significant.

The results of this trial are likely to interest a commercial feeder in the fact that the sheep on the unsupplemented basal ration required 47 percent more feed per 100 pounds gain than those supplemented with alfalfa ash. This difference approachs significance at the 5 percent level. It is interesting to note the similarity of these results to those reported by Garrigus (1951). He found that the supplementation of a ration containing cottonseed hulls, cottonseed meal, and corn with alfalfa leaf meal and molasses reduced the feed required per 100 pounds gain by lambs from 1133 to 754 pounds. The unsupplemented lambs in his trial required 50 percent more feed per 100 pounds of gain than the supplemented ones. From this comparison it appears that the mineral part of the alfalfa meal and molasses were largely responsible for the increased feed efficiency. Furthur support for this view can be found in the results of *in vitro* studies by Burroughs *et al* (1950 a) and (1951) in which both alfalfa ash and molasses ash were found to increase cellulose digestion. These workers concluded that the results obtained from adding molasses weren't specific with the molasses, but with some of its constituents. These data indicate that a ration of cottonseed hulls, cottonseed meal, and corn, should be supplemented either with the proper minerals or with a small amount of alfalfa hay or meal to provide for efficient utilization of the ration by ruminants.

Trial III

During the 28 day depletion period, the abnormal behavior characteristic of sheep receiving the basal ration again appeared. Since the sheep had been sheared a week previous to the start of the experiment, there was no evidence of wool pulling in this experiment. The sheep had an average weight of 63.6 pounds at the start of the depletion phase, and lost an average of 4 pounds or 0.15 pound per day for the 28 days.

Table 3 shows the average daily gains during the depletion and repletion periods and the recovery period following the repletion period for four of the lots. The results of this trial are given in detail in Table III of the appendix. Figure 2 shows the average weekly weights for the various lots during the repletion and recovery periods plotted as percentages of their average weights at the beginning of the repletion period.

The lots having gains significantly different from those of the lot receiving natural alfalfa ash were the basal lot, and the lot

Table 3

Average Daily Gains for the Depletion, Repletion, and Recovery Periods of Trial III

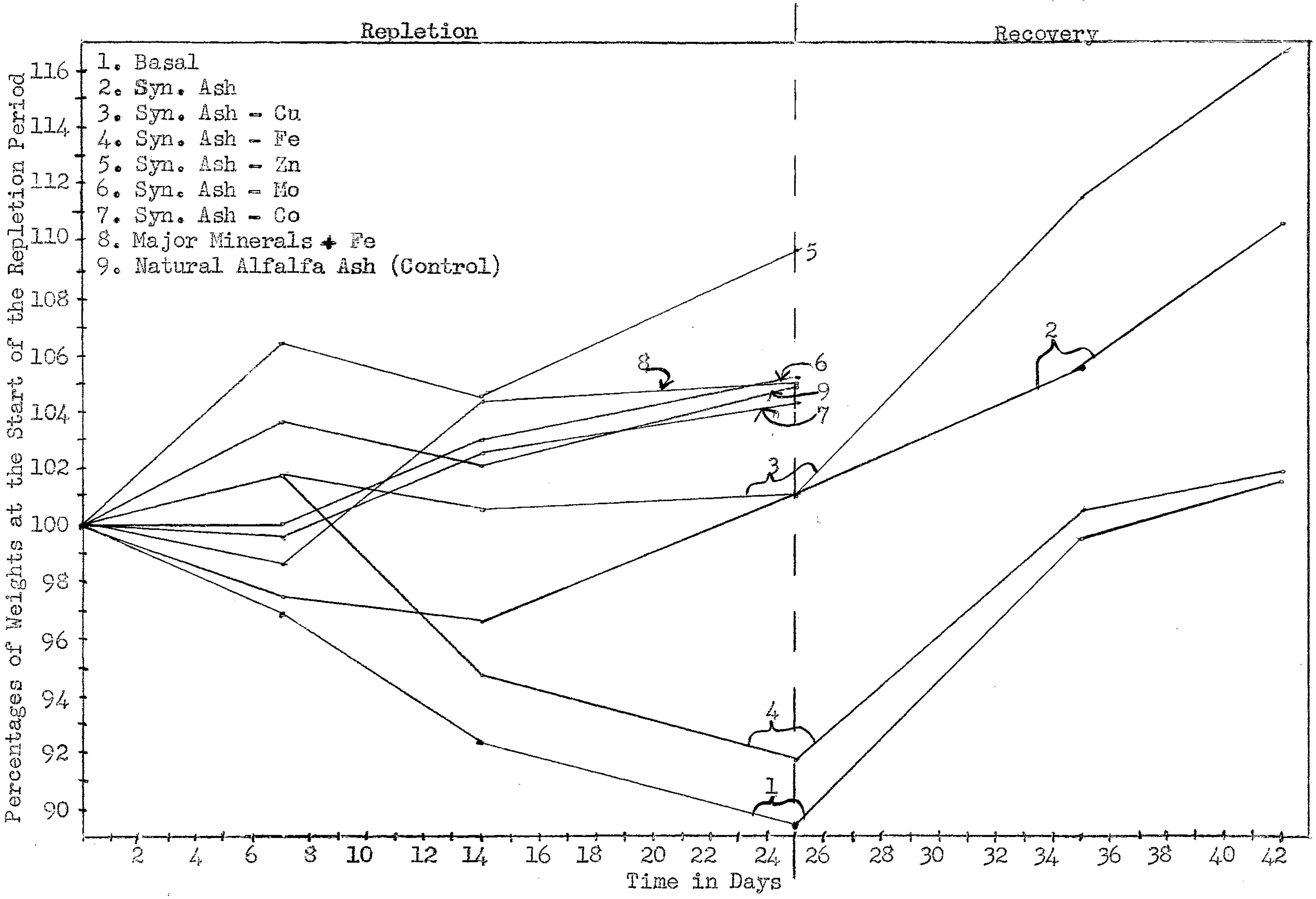
	Average Daily Gains		
	Depletion	Repletion	Recovery
1. Basal	-0.18	-0.25*	0.39
2. Syn. Ash	-0.15	0.02	0.29
3. Syn. Ash - Cu	-0.15	0.02	0.49
4. Syn. Ash - Fe	-0.16	-0.19*	0.39
5. Syn. Ash - Zn	-0.16	0.24	
6. Syn. Ash - Mo	-0.14	0.14[1]	
7. Syn. Ash - Co	-0.14	0.10	
8. Major Minerals + Fe	-0.13	0.12	
9. Natural Alfalfa Ash (Control)	-0.13	0.12	
Average	-0.15	0.04	0.39

*Significant at the 5 percent level.
[1]One sheep in this lot died during the repletion period.

supplemented with synthetic ash minus iron. This difference is demonstrated in Figure 2. Figure 2 appears somewhat difficult to interpret due to the grouping of the growth curves of several treatments around that of the control lot. This grouping, however, helps to point out the extent of the differences between the two lots whose gains were significantly different from those of the control lot and the majority of the lots whose gains were not significantly different. Although the synthetic ash minus copper lot gained less than the control lot, the difference was not significant. There was no significant difference between these lots and the lot supplemented with synthetic ash minus all the trace minerals except iron. Red blood cell counts and hemoglobin values for the sheep from lots 1, 2, 3, and 4 are shown in Table 4. The sheep receiving synthetic ash again had higher red blood cell counts and hemoglobin values than the sheep receiving no mineral supplement or synthetic minus iron or copper although the differences

Figure 2

Average Weekly Weight Changes During the Repletion and Recovery Periods, Trial III
(Percentages of Weights at the Start of the Repletion Period)



were not significant.

Table 4

Red Blood Cell Counts and Hemoglobin Values
for Lots 1, 2, 3, and 4 (Trial III)

Lot Number	1	2	3	4
Red Blood Cells (Million per Cmm)	6.93	7.99	6.93	6.90
Hemoglobin volume percent	10.80	11.20	10.00	9.90

Experiments reported in the literature on the effect of iron supplements on cellulose digestion show conflicting results. Burroughs *et al* (1951) carried out *in vitro* cellulose digestion studies using rumen microorganisms and found that iron and phosphorus aided digestion. Iron alone, as ferrous sulfate, increased digestion significantly, while phosphorus as sodium diphosphate failed to increase digestion unless added in the presence of iron. Meites *et al* (1951), however, also using the *in vitro* technique, failed to get a stimulation of cellulose digestion when ferrous sulfate was added. Bentley and Moxon (1952) feeding a semi-synthetic ration containing timothy hay to cattle reported that supplementation with reduced iron had no effect on feed efficiency or gain. It should be remembered that none of these experiments were carried out with cottonseed hulls as the source of cellulose. Workers at the Buckeye Cotton Oil Company (1949) found in a chemical analysis of various cottonseed feed products that the ash of cottonseed hulls was very low in iron. Cottonseed hulls contained only 22 parts per million of iron, while cottonseed meal contained 175 parts per million.

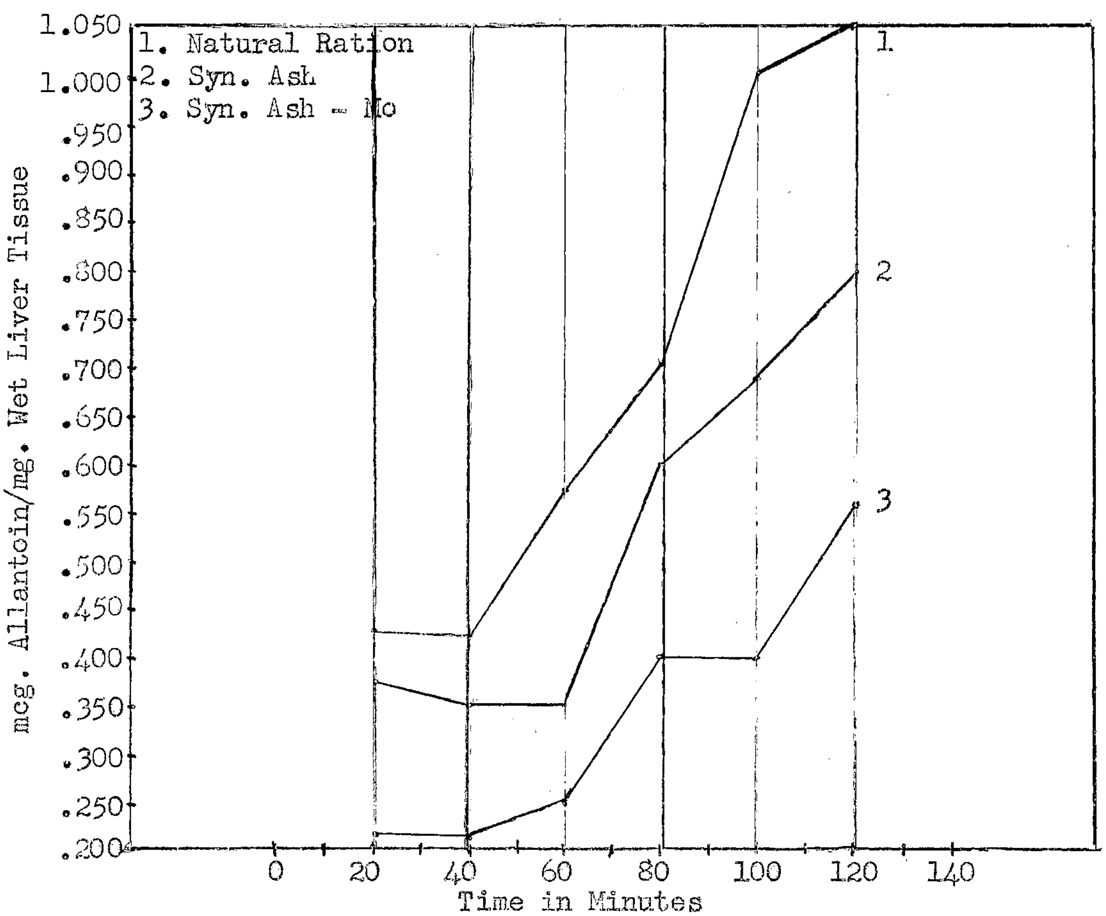
One of the surprising results to be noticed in Table 3 is the extent of the difference between the average gains of the sheep in the synthetic ash lot during the repletion period and during the recovery period. Figure 2 shows that they lost weight during the first two weeks of the repletion phase and then gained at a fast rate during the last eleven days. They then continued at essentially the same rate of gain until the end of the recovery phase. The average daily gains for the synthetic ash lot during the repletion period were not statistically different from those of the natural ash lot, however, when compared by the least significant difference method. The reason for the somewhat poorer gains of the synthetic ash lot during the repletion period is not clear, especially since four of the lots that received synthetic ash minus one or more of its mineral constituents gained nearly as well or better than the control lot.

Figure 2 and Table 3 also show the performance of the sheep during the recovery phase in which they received synthetic alfalfa ash. Lot 3, the group which had received synthetic ash minus copper during the repletion phase gained an average of 0.49 pound per day. Lot 4, the group which had received synthetic ash minus iron, and lot 1, the group on the unsupplemented basal ration during the repletion phase, each gained 0.39 pound per day. Lot 2, the group which had received complete synthetic ash during both periods, gained 0.29 pound per day during the recovery period. The differences in gains between the four lots during the recovery period were not significant. There was, however, a highly significant difference in gains of the sheep in lots 1, 3, and 4 during the recovery period as compared to their respective gains during the repletion period ($P < .01$). The average daily gains of the sheep in lot 2, which had received complete synthetic ash during both periods, were essentially the same during the recovery period as during the last eleven days of the repletion period. If compared to their average daily gains during the entire repletion period, however, the difference would be statistically significant ($P < .05$). From the blood tests made in Trials I and III of this study and the blood test reported by Tillman et al (1954 b), it can be seen that in every case where sheep received the unsupplemented basal ration or the basal ration supplemented with synthetic ash minus iron and/or copper, the red blood cell counts and hemoglobin values were slightly lower than when the sheep received the basal ration supplemented with natural or synthetic alfalfa ash. These trials were of relatively short duration and it is not too surprising that none of the observed differences were large enough to be statistically significant or indicative of anemia. They do indicate a trend toward lower red blood cell counts and hemoglobin values when sheep on rations containing cottonseed hulls as the roughage receive no supplementation with iron and/or copper. The data presented in this trial and in Trial I strongly indicate that iron is the mineral principally responsible for the effect of alfalfa ash on the utilization of cottonseed hulls by sheep. The fact that there was a highly significant increase in gains of the sheep in the lot receiving synthetic ash minus copper when they were supplemented with complete synthetic ash, together with the trend toward lower red blood cell counts and hemoglobin values in lots receiving no supplementation with copper indicate that copper is also partially responsible for the effect.

It will be recalled that in Trial I, three sheep in the lot receiving synthetic ash minus molybdenum became thin and weak, with one animal becoming so weakened and lame as to necessitate removal from the experiment. During Trial III, one sheep in the synthetic ash minus molybdenum lot died after 21 days of the repletion phase, and on autopsy was found to be very badly constipated. He had been eating very little for several days prior to his death, but did not appear unusually weak or lame at any time. No abnormal external symptoms appeared in the other sheep in this lot, either during the repletion period or during the time they were retained after the repletion period. The carcass of the sheep from this lot that was sacrificed for the purpose of making a liver xanthine oxidase activity determination, as well as the carcass of the sheep from the lot receiving synthetic ash, was surprisingly well finished considering the type of ration they had been receiving. Results of the liver xanthine oxidase activity determinations based on the production of allantoin are shown in Figure 3. Since xanthine added to the liver homogenates as called for in the procedure outlined by Dietrich et al (1954) appeared to cause depression of xanthine oxidase activity, only the endogenous activity of the enzyme system is shown. In this preliminary study using only one sheep from each treatment, the liver tissue of the sheep receiving synthetic ash minus molybdenum had considerably less activity than the tissue of the sheep receiving complete synthetic ash. The liver tissue of the sheep that had been receiving a ration composed of natural feedstuffs showed considerably more xanthine oxidase activity than did tissue from the sheep receiving synthetic ash, and approximately twice that of the tissue from the sheep receiving synthetic ash minus molybdenum. It can not be said that these differences are significant since the determinations were conducted using only one sheep per treatment. Also, since no information is available on the normal range of xanthine oxidase activity in sheep liver tissue, these results are not definite proof as yet that either the activity of this enzyme in the sheep receiving synthetic ash minus molybdenum was abnormally low, or that molybdenum is a dietary essential for sheep. However, these results, together with the weakness and lameness which developed in three sheep in Trial I and the death of one sheep in Trial III, all suggest that molybdenum is another of the factors in alfalfa ash which favorably affect utilization of cottonseed hulls. Furthur closely controlled feeding trials as well as furthur study of the normal and abnormal levels of xanthine oxidase activity in sheep seem clearly justified.

Figure 3

Endogenous Liver Xanthine Oxidase Activity
(mcg. Allantoin/mg. Wet Liver Tissue)



SUMMARY

Three experiments were conducted to determine the effect of alfalfa ash on the utilization of cottonseed hulls by sheep, and the mineral(s) responsible for the effect. The effect of alfalfa ash was studied by feeding it as a supplement to a ration similar to those used by commercial feeders for many years. To study the mineral(s) responsible for the effect, a semi-purified cottonseed hull basal ration supplemented with a synthetic alfalfa ash minus certain of its mineral components was fed.

In Trial I, which was conducted to determine the mineral(s) contained in alfalfa ash responsible for the increased utilization of cottonseed hulls, five sheep per treatment were used in a depletion-repletion regimen. During the depletion phase, a marked weight loss occurred in all animals. During the repletion phase, the animals receiving synthetic alfalfa ash minus magnesium and synthetic ash minus manganese made gains comparable to the control group which received complete synthetic ash. The animals receiving synthetic alfalfa ash minus iron and copper made gains significantly lower than the controls. This indicated that cottonseed hulls were low in one or both of these minerals. The group receiving synthetic alfalfa ash minus molybdenum continued to lose weight during the repletion period, but their average daily gains (all negative) were not significantly different from those of the control lot. Three sheep in this lot, however, became thin and weak, with one sheep becoming lame enough, particularly in his

hind legs, to necessitate removal from the experiment.

Ten sheep per treatment were used in Trial II to determine the effect of alfalfa ash on the utilization of a ration composed of natural feedstuffs with cottonseed hulls as the roughage. Supplementation with alfalfa ash gave a 23 percent increase in average daily gains. The unsupplemented lot required 47 percent more feed per 100 pounds of gain. These data indicate the value of supplementing a cottonseed hull ration with the proper minerals or with a small amount of alfalfa hay or meal.

In Trial III, four sheep per treatment were used to furthur study the mineral(s) responsible for the effect of alfalfa ash on the utilization of cottonseed hulls by sheep. In this trial, the depletion-repletion regimen was again followed. During the depletion period, a marked loss of weight was again observed. During the repletion period, it was found that the sheep remaining on the basal diet continued to lose weight. This was also true of the group receiving synthetic ash minus iron. The gains for these two lots were significantly different from the gains of the control lot and the gains of a lot supplemented with synthetic ash minus all the trace minerals except iron. During this phase, there was no significant difference between the gains of the control lot and the gains of the lots receiving complete synthetic ash, synthetic ash minus copper, synthetic ash minus all the trace minerals except iron, synthetic ash minus zinc, synthetic ash minus molybdenum, and synthetic ash minus cobalt respectively. From blood tests made in Trials I and III, (and one test reported in the literature made with sheep receiving the same basal ration), it was found that there was a trend toward lower red blood cell and hemoglobin values when the sheep received the cottonseed hulls basal ration with no iron and/or copper supplementation. In a recovery period following the regular experiment, average daily gains for the sheep in the basal, synthetic ash minus copper, and synthetic ash minus iron were greatly improved when they were supplemented with complete synthetic ash. The differences in gains between these two phases were highly significant for all three lots ($P < .01$). The results from these experiments indicate that iron and copper are the minerals principally responsible for the effect of alfalfa ash on the utilization of cottonseed hulls by sheep, with iron being the more effective of the two. Furthur study to determine whether the effect is a direct one on the host, or an indirect one through the microorganisms in the rumen, or a combination of both conditions seems to be indicated. Preliminary determinations involving one sheep per treatment showed a considerable lower xanthine oxidase activity in liver tissue from a sheep receiving synthetic ash minus molybdenum as compared to that in tissue from a sheep receiving complete synthetic ash and another sheep receiving a ration composed of natural feedstuffs. It is realized that this is insufficient evidence to prove conclusively that molybdenum is a dietary essential for sheep. However, these results, together with the weakness and lameness which developed in three of the sheep in Trial I and the death of one sheep in Trial III, all suggest that molybdenum is another of the factors in alfalfa ash which favorably affect utilization of cottonseed hulls. It is felt, since no evidence to the contrary has been obtained in this work, that furthur more extensive studies of role of molybdenum in sheep are clearly justified.

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APPENDIX

Table I

Gains and Feed Intake of
Lambs in Trial I

	Depletion			Repletion					
	Weight		Daily	Daily	Weight				Daily
Sheep Number	In.	Final	Gain	Feed Intake	In.	1st Wk.	2nd Wk.	Final	Gain
	lbs.	lbs.	lbs.	gms.	lbs.	lbs.	lbs.	lbs.	lbs.
				Ration 1, Synthetic Ash					
1	72	62	-0.30	345	62	60	58	58	-0.19
9	70	65	-0.15	703	65	64	66	67	0.09
10	71	58	-0.39	593	58	58	60	60	0.09
17	71	67	-0.12	660	67	66	68	68	0.05
21	73	64	-0.27	653	64	64	66	66	0.09
Ave.	71.3	63.2	-0.25	591	63.2	62.4	63.6	63.8	0.03
				Ration 2, Synthetic Ash minus Magnesium					
8	76	64	-0.36	534	64	63	63	62	-0.09
11	72	64	-0.24	653	64	64	65	65	0.05
12	81	72	-0.18	659	72	68	70	71	-0.05
14	65	59	-0.33	606	59	57	57	55	-0.19
22	67	65	-0.06	556	65	64	64	65	0.00
Ave.	72.2	64.8	-0.23	602	64.8	63.2	63.8	63.6	-0.06
				Ration 3, Synthetic Ash minus Iron and Copper					
3	83	74	-0.27	645	74	75	74	70	-0.19
4	66	60	-0.18	572	60	59	60	59	-0.05
15	57	48	-0.27	400	48	47	49	47	-0.05
16	72	64	-0.24	577	64	62	59	58	-0.28
18	74	62	-0.36	287	62	60	56	54	-0.38
Ave.	70.4	61.6	-0.27	496	61.6	60.6	59.6	57.6	-0.19
				Ration 4, Synthetic Ash minus Molybdenum					
2	66	55	-0.33	268	55	56	53	50	-0.24
5	75	66	-0.27	595	66	66	64	64	-0.09
13	71	62	-0.27	632	62	61	59	59	-0.14
24	65	53	-0.36	372	53	54	52	53	0.00
25	76	67	-0.27	—	67	66	62	—	—[1]
Ave.	70.6	60.6	-0.30	467	59.0	59.3	57.0	56.5	-0.12
				Ration 5, Synthetic Ash minus Manganese					
6	81	67	-0.42	591	67	71	72	73	0.28
7	68	59	-0.27	652	59	57	60	58	-0.05
19	59	51	-0.24	—	51	48	46	—	—[1]
20	75	69	-0.18	666	69	66	68	67	-0.09
23	78	70	-0.24	537	70	71	73	72	0.09
Ave.	72.2	63.2	-0.27	611	66.3	66.3	68.3	67.5	0.06

[1]This sheep was removed; data not included in the repletion period average.

Table II

Feed Intake, Feed Efficiency, and Gains of Lambs in Trial II

Sheep Number	Feed Intake Daily gms.	Total lbs.	Per 100 lbs. Gain lbs.	Weight In. lbs.	16 day lbs.	Final lbs.	Gain Total lbs.	Daily lbs.
			Ration 1, Basal					
2	729	56.1	431	50	61	63	13	0.37
3	1093	84.2	598	70	81	84	14	0.40
4	864	66.6	2216	59	61	63	3	0.08
6	893	68.8	1719	73	79	77	4	0.11
8	964	74.3	1062	62	66	69	7	0.20
10	1043	80.3	723	60	66	71	11	0.31
11	1050	80.9	897	65	73	74	9	0.26
13	1007	77.6	597	59	65	72	13	0.37
17	1071	82.5	1031	68	72	76	8	0.23
20	1136	87.5	1093	67	73	75	8	0.23
Ave.	985	75.8	1037	63.3	70.0	72.3	9	0.26
			Ration 2, Basal plus Alfalfa Ash					
5	1028	72.2	556	64	75	77	13	0.37
7	1100	84.7	652	58	67	71	13	0.37
9	1036	79.8	885	67	72	76	9	0.26
12	1142	88.0	519	71	84	88	17	0.49
16	657	50.6	840	58	64	64	6	0.17
18	743	57.2	1144	54	61	59	5	0.14
21	1086	83.6	644	66	76	79	13	0.37
22	850	65.5	655	65	68	75	10	0.28
23	1014	78.1	703	72	80	83	11	0.31
24	893	68.7	433	53	64	69	16	0.46
Ave.	955	73.5	703	62.8	71.1	74.0	11	0.32

Table III

Gains and Feed Intake of
Lambs in Trial III

Sheep Number	Depletion Weight In.	Depletion Weight Final	Depletion Daily Gain	Repletion Daily Feed Intake	Repletion Weight In.	Repletion Weight 1st Wk.	Repletion Weight 2nd Wk.	Repletion Weight Final	Repletion Daily Gain	Recovery Weight In.	Recovery Weight 10 day	Recovery Weight Final	Recovery Daily Gain
	lbs.	lbs.	lbs.	gms.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
Ration 1, Basal													
2	55	50	-0.18	522	50	50	47	43	-0.28	43	49	50	0.41
20	68	60	-0.29	332	60	61	57	56	-0.16	56	63	64	0.47
12	82	74	-0.29	428	74	72	70	68	-0.24	68	71	73	0.29
36	52	53	0.04	310	53	47	45	45	-0.32				
Ave.	64.3	59.3	-0.18	398	59.3	57.5	54.8	53.0	-0.25	53.0	61.0	62.3	0.39
Ration 2, Synthetic Ash													
1	67	60	-0.25	428	60	58	57	59	-0.04	59	61	64	0.29
4	56	55	-0.04	483	55	54	51	53	-0.08				
21	71	64	-0.25	534	64	64	65	68	0.16	68	71	73	0.29
28	57	55	-0.07	475	55	52	53	56	0.04	56	57	61	0.29
Ave.	62.8	58.5	-0.15	480	58.5	57.0	56.5	59.0	0.02	59.0	63.0	66.0	0.29
Ration 3, Synthetic Ash minus Copper													
6	59	54	-0.18	521	54	55	53	55	0.04	55	61	64	0.53
23	76	74	-0.07	606	74	73	75	76	0.08	76	79	84	0.47
27	52	47	-0.18	491	47	50	49	48	0.04	48	55	56	0.47
41	51	46	-0.18	382	46	47	45	44	-0.08				
Ave.	59.5	55.3	-0.15	500	55.3	56.3	55.5	55.8	0.02	55.8	56.3	57.0	0.49

Table III (Continued)

Sheep Number	Depletion Weight In. lbs.	Depletion Weight Final lbs.	Depletion Daily Gain lbs.	Repletion Daily Feed Intake gms.	Repletion Weight In. lbs.	Repletion Weight 1st Wk. lbs.	Repletion Weight 2nd Wk. lbs.	Repletion Weight Final lbs.	Repletion Daily Gain lbs.	Recovery Weight In. lbs.	Recovery Weight 10 day lbs.	Recovery Weight Final lbs.	Recovery Daily Gain lbs.
Ration 4, Synthetic Ash minus Iron													
7	64	59	-0.18	483	59	62	60	53	-0.24	53	60	62	0.53
11	66	60	-0.21	532	60	64	60	58	-0.08				
19	61	59	-0.07	426	59	54	49	51	-0.32	51	55	55	0.24
30	55	50	-0.18	476	50	52	47	47	-0.12	47	54	54	0.41
Ave.	61.5	57.0	-0.16	479	57.0	58.0	54.0	52.3	-0.19	52.3	56.3	57.0	0.39
Ration 5, Synthetic Ash minus Zinc													
16	54	52	-0.07	443	52	54	51	55	0.12				
17	69	67	-0.07	784	67	73	71	74	0.25				
33	59	52	-0.25	694	52	58	57	60	0.32				
39	83	76	-0.25	785	76	78	79	82	0.24				
Ave.	66.5	61.8	-0.16	677	61.8	65.8	64.5	67.8	0.24				
Ration 6, Synthetic Ash minus Molybdenum													
3	74	73	-0.04	746	73	72	75	79	0.24				
31	53	52	-0.04	----	52	49	44	--	----[1]				
38	69	62	-0.25	767	62	62	64	66	0.16				
42	78	71	-0.25	716	71	70	73	72	0.04				
Ave.	68.5	64.5	-0.14	743	68.7	68.7	70.7	72.3	0.14				

[1] This sheep died: data not included in the repletion period averages.

Table III (Continued)

Sheep Number	Depletion Weight In.	Depletion Weight Final	Depletion Daily Gain	Repletion Daily Feed Intake	Repletion Weight In.	Repletion Weight 1st Wk.	Repletion Weight 2nd Wk.	Repletion Weight Final	Repletion Daily Gain	Recovery Weight In.	Recovery Weight 10 day	Recovery Weight Final	Recovery Daily Gain
	lbs.	lbs.	lbs.	gms.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
Ration 7, Synthetic Ash minus Cobalt													
8	64	59	-0.18	641	59	57	60	59	0.00				
22	59	58	-0.04	544	58	56	57	59	0.04				
26	57	56	-0.04	732	56	57	61	62	0.24				
34	74	65	-0.30	650	65	67	66	68	0.12				
Ave.	63.5	59.5	-0.14	642	59.5	59.3	61.0	62.0	0.10				
Ration 8, Major Minerals plus Iron													
9	69	65	-0.14	770	65	67	70	68	0.12				
10	62	57	-0.18	719	57	55	62	62	0.20				
14	66	66	0.00	692	66	64	66	68	0.08				
32	51	46	-0.18	473	46	45	46	48	0.08				
Ave.	62.0	58.5	-0.13	664	58.5	57.8	61.0	61.5	0.12				
Ration 9, Natural Alfalfa Ash													
5	69	62	-0.25	722	62	66	66	68	0.24				
13	67	66	-0.04	796	66	72	68	70	0.16				
35	74	68	-0.21	749	68	68	67	68	0.00				
37	49	49	0.00	588	49	48	49	51	0.08				
Ave.	64.8	61.3	-0.13	714	61.3	63.5	62.5	64.3	0.12				

VITA

Charles Wayne Tefft
candidate for the degree of
Master of Science

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Major: Animal Nutrition

Biographical and Other Items:

Born: November 1, 1931 at Enid, Oklahoma

Undergraduate Study: O. A. M. C., 1949-53

Graduate Study: O. A. M. C., 1953-54

Member of Alpha Zeta, Block and Bridle, and Phi Kappa Phi.

Date of Final Examination: August, 1954

THESIS TITLE: THE EFFECT OF ALFALFA ASH AND CERTAIN OF ITS MINERAL CONSTITUENTS ON THE UTILIZATION OF COTTONSEED HULLS BY SHEEP.

AUTHOR: Charles Wayne Tefft

THESIS ADVISER: Dr. A. D. Tillman

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