IN VITRO AND *IN VIVO* STUDIES TO EVALUATE VARIOUS NITROGEN SOURCES FOR RUMINANTS

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INTRODUCTION

Feedstuffs used as protein supplements are usually processed in some manner prior to use in order to render the protein more available to the animal or in order to isolate other products. These various methods of processing may have an effect on the nutritive value of the protein supplements.

Although considerable work has been conducted with simple-stomached animals to study the value of protein supplements processed by different methods, little work has been conducted with ruminants to study this problem. Since different methods of processing involve various types of oil extraction and temperature during processing, questions have arisen as to the best method of oil extraction and the optimum operating temperature for processing protein supplements.

In several studies cottonseed meal has been shown to be of less value for ruminants than certain other protein supplements. The exact cause or nature of this is not known although it is thought to be due to the presence of gossypol or having protein of lower quality. The problem of cottonseed meal being of less value has been of considerable concern to the cotton oil industry.

Since little experimental work has been conducted to study the effect of processing upon the nutritive value of protein supplements for ruminants and little experimental evidence is present as to why cottonseed meal is of less value for ruminants, three studies were undertaken to study these problems. The studies conducted were a digestibility and nitrogen balance trial, a growth trial, and an artificial rumen study. The digestibility and nitrogen balance trial and the growth trial were used to study the effect of different methods of processing and quality of protein in the various supplements. The artificial rumen study was conducted to compare different protein supplements as sources of nitrogen for cellulose digesting microorganisms and the effect of gossypol on these microorganisms.

REVIEW OF LITERATURE

It is a well established fact that protein quality is of much greater consequence in the feeding of simple-stomached animals than in ruminants. Maynard (1951) describes a high quality protein feed as one "which supplies all of the amino acids needed in proportions most nearly like those in which they exist in the protein to be formed." Cottonseed meal has been shown to be of little value for the simple-stomached animals when fed as the major supplement in combinations or alone, and of less value for ruminants than certain other protein supplements.

Altschul (1958) states that gossypol has two definitions, bound and free. Free gossypol has the practical significance of being physiologically active; that is, toxic when fed in sufficient quantities to simple-stomached animals and responsible for discoloration of eggs when hens are fed cottonseed meal. Bound gossypol does not exhibit these physiological effects. Processing affects the use of cottonseed meal more for simple-stomached animals than for ruminants. Various types of extraction of oil in processing cottonseed meal are: hydraulic press; screw press; prepress solvent-extracted; and solvent extraction, direct and direct chemically treated. The amount of free gossypol found in cottonseed meal after the various extraction processes is: 0.04-0.22%; 0.03-0.08%; 0.02-0.06%; 0.05-0.60%; and 0.02-0.04%, respectively. However, as will be shown later the effect of bound gossypol upon the proportion of essential amino acids must be considered also.

Cottonseed Meal For Beef Cattle

Numerous workers have compared various protein supplements for beef cattle when fed separately or in combinations with other supplements or with different grains under varying conditions or situations. Pope et al. (1951) compared the relative value of cottonseed meal, soybean meal, and a sesame meal-soybean meal mixture as protein supplements for two-year-old steers being wintered on native grass. The supplements were fed with and without ground limestone or bone meal so that each supplement supplied the same amount of calcium and phosphorus. Steers fed cottonseed meal lost an average of 13 lbs. per steer while those on soybean meal lost an average of 1 lb. per steer. When ground limestone was added to cottonseed meal, steers gained an average of 27 lbs. Those fed soybean meal plus bone meal lost 4 lbs. on the average. A combination of two parts soybean meal and one part sesame meal plus ground limestone resulted in an average loss of 12 lbs. Two steers which were unthrifty accounted for almost the entire weight loss in this group.

Linseed meal, cottonseed meal, and corn gluten meal were compared by Anderson et al. (1929) as supplements in cattle fattening rations. The supplements were fed separately, in combinations of two, and as a combination of all three. When fed separately, average daily gains were 2.08, 2.22, and 2.11 lbs., respectively for cottonseed meal, linseed meal, and corn gluten meal. Feed (concentrate plus roughage) required per 100 lbs. gain was 993, 936, and 947 lbs. in the same order as above. A combination of linseed meal and corn gluten meal in equal parts resulted in better gains and feed efficiency than any other combinations.

Stanley and Walker (1940) studied the value of cottonseed meal, fish meal, and meat meal for fattening steers when each meal furnished the same amount of protein. Steers fed cottonseed meal made slightly more rapid gains, 2.20 lbs., than those fed fish meal, 2.08, or meat meal, 2.10. Slightly less feed was required per 100 lbs. of gain by steers fed cottonseed meal. It was stated that when these supplements are fed on an equivalent protein basis, cottonseed meal is worth more pound for pound.

An experiment was conducted by Jacob and Duncan (1938) for three consecutive years to compare cottonseed meal, cottonseed meal-tankage combination, peanut meal, and soybean meal when fed with silage for fattening steers. Cottonseed meal and tankage were combined in a 3:1 ratio. All supplements were fed at the same level daily. Steers fed either cottonseed meal, cottonseed meal tankage, or soybean meal made higher daily gains on less feed than those fed peanut meal.

Briggs <u>et</u> <u>al</u>. (1946a) conducted a digestibility trial with yearling steers to determine the nutritive value of cottonseed meal, soybean meal, peanut meal, and a combination of all three meals. Prairie hay was the basal diet. One lb. of the supplement was fed daily. There were only small differences in the digestibility of the nutrients of rations containing the different supplements with the nutrients of the ration containing the combination of supplements being slightly more digestible. The biological values of the protein in the various rations were 65 for the basal, 73 for both cottonseed meal and the combination, 71 for soybean meal, and 68 for peanut meal.

Jones <u>et</u> <u>al</u>. (1946) compared cottonseed meal and peanut meal in six trials with yearling steers at three different Texas experiment stations. The average results showed there to be no differences between the supplements as measured by daily rate of gain. Massey (1941) found similar results when he averaged data from four consecutive years.

Two-year-old steers on fattening rations were used by Skinner and King (1924) to compare soybean meal, cottonseed meal, and whole soybeans. Steers fed whole soybeans made the most rapid gains, 2.25 lbs. daily, but this was not significantly higher than those fed soybean meal or cottonseed meal, 2.17 lbs. and 2.16 lbs. Feed efficiency was similar in all lots.

Rusk and Snapp (1924) compared soybean meal to cottonseed meal for fattening steers and found that these two protein supplements had practically the same feeding value. Steers fed soybean meal made faster and more economical gains than those fed cottonseed meal but the differences were not significant.

Pope et al. (1952) using yearling steers being wintered on dry native grass compared cottonseed meal to soybean meal with and without the calcium and phosphorus levels of the supplements being equalized. Cottonseed meal was compared to corn gluten meal without equalizing the calcium and phosphorus level. Solvent extracted cottonseed meal was also compared to hydraulic processed meal. Winter gains were larger when the calcium and phosphorus levels were not equalized with soybean meal fed steers gaining the most. Corn gluten meal was found to produce smaller gains than the other supplements. No difference was found between soybean meal and cottonseed meal with respect to winter gain when calcium and phosphorus levels were equal in the two supplements. Solvent extracted cottonseed meal produced slightly greater gains than hydraulic processed meal.

A similar study was made by Pope et al. (1953b) except that two-year-old steers were used and a combination supplement of linseed, cottonseed, and soybean meals was used in the place of corn gluten meal. The feeding of soybean meal produced winter gains of 17 lbs. while cottonseed meal feeding resulted in an average loss of 21 lbs. Upon the addition of ground limestone to cottonseed meal gains were increased to 12 lbs. Bonemeal supplementation to soybean meal increased average gains to 26 lbs. Equalization of the calcium and phosphorus content of the combination supplement resulted in an average loss of 3 lbs. per steer. When solvent extracted cottonseed meal was compared to hydraulic processed cottonseed meal, average weight losses were 25 and 21 lbs., respectively.

A metabolism study was conducted by Briggs et al. (1948) to determine the nutritive value of solvent-extracted soybean meal and cottonseed meal, hydraulic-processed cottonseed meal, and expeller-processed soybean meal. Each meal supplied the same amount of protein daily. Higher apparent digestibility of protein was obtained with both soybean meal samples with expeller-processed meal having the highest. Nitrogen retention was very similar when either pressure-treated or solvent-extracted meals were fed. A similar study was conducted by the same investigators in 1949. Apparent digestibility of protein was again higher for soybean meal. Solvent extracted meals resulted in slightly higher nitrogen retention than did pressure-treated meals.

Three protein supplements, cottonseed meal, cottonseed feed, and linseed meal, were compared in a winter feeding test conducted by Tomhave and Bentley (1923). Cottonseed feed was found to be an uneconomical protein supplement by these investigators. Linseed meal and cottonseed meal were found to be of approximately equal value when fed with silage. The feeding of cottonseed meal and linseed meal with and without shelled corn was also studied. Corn was fed for the last 56 days in one trial and the last 84 days in another trial. When fed for 56 days, average daily gains were 2.0 lbs. for steers on cottonseed meal and 2.27 lbs. for linseed meal fed steers. The control animals or those fed no corn gained 1.93 and 1.97 lbs. daily on cottonseed meal and linseed meal, respectively. Steers fed corn for 84 days gained 2.38 and 2.62 lbs., respectively, and the controls gained 2.53 lbs. on cottonseed meal and 2.33 lbs. on linseed meal.

The nutritive value of the protein of linseed meal and cottonseed meal for beef calves fed fattening rations was compared by Bethke _et al._ (1928). The meals were fed so as to supply the same amount of protein daily. Daily gains were very similar on both rations with feed efficiency being in favor of cottonseed meal.

The use of cottonseed meal as a replacement for corn was the basis of a study conducted by McCampbell _et al._ (1926). One lot of yearling steers received cottonseed meal as the only concentrate and another lot received one pound of cottonseed meal and enough corn to equal the amount of concentrate fed to animals in the other lot. Steers receiving 11 lbs. of cottonseed meal made average daily gains of of 2.39 lbs. as compared to 2.43 lbs. for those on corn. Feed efficiency was in favor of corn-fed steers but this advantage was small.

Gayle (1917) made a study of cottonseed meal alone, of a combination of cottonseed meal and shelled corn, and of shelled corn alone for fattening calves. There was little difference in daily rate of gain between the three rations. Pounds of concentrate to produce 100 lbs. gain was very much in favor of cottonseed meal alone, 213 lbs. cottonseed meal against 489 lbs. of corn. Corn silage was just the reverse, 1318 lbs. required for 100 lbs. gain by steers on cottonseed meal to 739 lbs. for steers on corn. It was stated that steers fed cottonseed meal tended to grow more and fatten less.

Another experiment to study the value of cottonseed meal as a replacement for corn in a fattening ration was conducted by Edwards and Massey (1934). Different proportions of cottonseed meal to corn were used. The proportions were: 1 part cottonseed meal to 6 parts corn, 1 to 3, and 2 to 3. There was no difference in daily rate of gain with all lots averaging 2.0 lbs. Differences in pounds of concentrates per 100 lbs. gain were small with the greatest difference being between the 1:6 and the 1:3 in favor of the 1:6 proportion.

Knox and Neale (1939) used yearling steers in a three year test to determine the value of cottonseed meal in combination with ground kafir in fattening rations. Rations fed were Lot 1, ground kafir as the chief concentrate and enough cottonseed meal to supply ample protein; Lot 2, equal amounts of kafir and meal; Lot 3, cottonseed meal alone; and Lot 4, cottonseed meal alone but deferred until the second half of the experiment. Steers in Lots 2 and 3 had higher daily gains and better feed efficiency but the differences between these two lots were small in all years. Finish was also higher for animals in these lots. The behavior of the steers in Lot 3 followed a definite pattern in all three years. Early in the test they had the keenest appetite, but when the amount of meal reached 10 lbs. daily, there was an increased laxative nature of the ration. A distaste for the meal was shown toward the end of the test when levels of meal reached as high as 14 lbs. daily.

The influence of solvent-extracted and hydraulic-processed cottonseed meals upon the performance and the level of plasma carotene, vitamin A, and fat in the blood of wintering beef cows was studied by Parham et al. (1950). Cows on hydraulic-processed meal gained 11 pounds more than cows on solvent-extracted meal but this difference was not significant. Blood samples taken at the beginning of the experiment showed that blood fat levels were similar. At the end of the trial, cows fed hydraulic-processed meal had blood fat values of 187.5 mg. per 100 ml. (an increase of 11.2 mg.) while those cows on solvent-extracted meal had values of 176.6 mg. per ml. of blood (a decrease of 2.0 mg.). Blood carotene decreased 20 mcg. per 100 ml. for cows on solvent extracted meal and 4 mcg. per 100 ml. when hydraulic-processed meal was fed. Solvent extracted meal produced blood vitamin A levels 20 mcg. higher per 100 ml. than did hydraulic processed meal.

Using a fistulated steer, Stallcup and Looper (1958) studied the fate of the nitrogen of soybean meal, cottonseed meal, Morea[1], and cottonseed hulls. Relatively high levels of nitrogen were present in the rumen when soybean meal was fed. Total nitrogen present when cottonseed meal was fed paralleled that of soybean meal but at a lower level. Concentrations of ammonia released in the rumen reached a peak three hours following the feeding of soybean meal and remained rather constant 4 to 11 hours after feeding. A peak concentration of ammonia when cottonseed meal was fed was reached two hours after feeding and declined rapidly. Six to 12 hours after feeding cottonseed meal the release was only slightly higher than that released by a cottonseed hull

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[1]Morea is a commercial supplement consisting of urea, molasses, alcohol, and minerals which was furnished by Feed Service Corporation, Crete, Nebraska.

ration. Protein nitrogen levels were higher at all times when soybean meal was fed. The authors suggested that this may be due to the fact that most protein is in solution and suspension.

Pope *et al.* (1953a) studied the use of solvent-extracted and hydraulic-processed cottonseed meal, the use of ammoniated furfural residue to replace one-half cottonseed meal, and the use of alfalfa hay or dehydrated alfalfa meal pellets to replace cottonseed meal in rations for fattening steer calves. The feeding of solvent-extracted meal did not affect daily gain but did decrease feed efficiency. Ammoniated furfural residue decreased rate of gain 0.22 lb. Dehydrated alfalfa meal pellets increased gains the same amount as did alfalfa hay. Feed efficiency was increased by the use of either alfalfa hay or the pellets.

Cottonseed cake, soybean cake, and urea pellets were compared by Darlow *et al.* (1946) for wintering two-year-old steers on grass. Soybean cake proved to be the best supplement in this trial with gains of 0.45 lb. daily as compared to 0.26 lb. for steers receiving cottonseed cake and 0.05 lb. for steers being fed urea pellets.

Halverson and Sherwood (1930) reported that under the conditions of their investigation, deleterious effects in beef cattle thought to be due to cottonseed meal poisoning were due to nutritive deficiencies of cottonseed meal. It was found by the authors that cottonseed meal did not contain sufficient calcium or vitamins A, D, or B complex to fully meet the needs of cattle.

Cottonseed Meal For Sheep

Woods *et al.* (1957) compared the nutritive value of a high and low nitrogen soluble cottonseed meal with the value of a 1:2 mixture of sesame meal-soybean meal and sesame meal alone. The supplements were compared in growth trials and digestibility-nitrogen balance trials. Daily gains, digestibility, and nitrogen retention were lower for lambs fed cottonseed meal. Sesame meal provided for better protein digestion and higher nitrogen balance. The sesame-soybean meal mixture promoted better growth although this difference was small. The same investigators compared soybean meal, sesame meal, and cottonseed meal at three protein levels (4, 6, and 8%) in digestion and nitrogen balance trials. Digestibility of protein was significantly lower for lambs fed cottonseed meal. Nitrogen retention was lowest for lambs fed cottonseed meal and highest for those fed sesame meal at the three protein levels.

Briggs et al. (1946b) used lambs in nitrogen balance studies to determine the nutritive value of cottonseed meal, soybean meal, and peanut meal when fed separately and together as a supplement to prairie hay. The supplements were fed at an equal amount daily. Apparent digestibility of protein was lowest for lambs fed cottonseed meal and highest for those fed soybean meal. Peanut meal was the least efficient for nitrogen storage and soybean meal was significantly better than cottonseed meal or the mixture.

The use of cottonseed meal, linseed meal, and corn gluten meal fed separately, in combinations of two, and all together was studied by Jordan and Peters (1934). During the first trial, the trio mixture produced the highest rate of gain, 0.34 lb. A combination of linseed meal and corn gluten meal produced the best rate of gain 0.32 lb., when two meals were combined. When the meals were fed separately, linseed meal fed lambs gained the most, 0.33 lb. Control lambs fed corn and alfalfa hay gained 0.24 lb. daily. In a second trial daily gains were 0.50 lb. for lambs fed the trio mixture of supplements. The meals were not studied in combinations of two in this trial. Linseed meal produced the best gains, 0.48 lb., for supplements fed alone. Paterson (1919) compared the same meals and found similar results. Differences in gain by lambs on different supplements were small in all cases.

Skinner and Starr (1918) found no difference in daily rate of gain between lambs fed either cottonseed meal, linseed meal, or ground soybeans. Skinner and Vestal (1921) reported that lambs fed cottonseed meal made more rapid gains, required less feed per pound of gain, and were more profitable than lambs fed linseed meal. These differences were small, however.

Three digestion trials were conducted by Briggs and Heller (1942) to determine the effects of adding large amounts of cottonseed meal to a lamb fattening ration. One ration contained 46 grams of meal and the other contained 227 grams of meal. Apparent digestibility of protein and fat was higher for the ration containing the high level of meal. Nitrogen retention was extremely higher for lambs fed the high-meal ration as compared to that of lambs on the low-meal, 73.3 grams to 38.8 grams.

Briggs (1943) studied the effects of levels of cottonseed meal in excess of that amount needed to balance the ration of pregnant ewes. No abortions resulted from feeding as excessive amount.

Cottonseed Meal For Simple-Stomached Animals

Altschul _et al_. (1957) reported the results of a collaborative study on the use of cottonseed meal processed by different methods. The processing methods and a number assigned to each meal were as follows: prepress, solvent-extracted CM-6, CM-10, and CM-45; high speed screw press CM-13; hydraulic press CM-16; solvent-extracted, degossypolized CM-49. Each meal was tested while contributing three levels of supplemental protein. The rations used were: (1) cottonseed meal supplying 100% of supplemental protein; (2) 75% cottonseed meal and 25% soybean meal on a nitrogen basis; and (3) 50% cottonseed meal and 50% soybean meal also on a nitrogen basis. The soybean meal used was a 52% protein meal which was also used as the supplemental protein for the control ration. Approximately 12,000 chicks were used in this study. Growth response was found to be negatively correlated with total gossypol. A poor correlation was found between free gossypol and growth response. Protein solubility in 0.02N NaOH was found to be correlated with growth response.

Aines (1957) compared seven cottonseed meals processed by different methods, CM-10, 13, 19, 21, 36, 45, and 49, to soybean meal when cottonseed meal replaced 50%, 75%, and 100% of the soybean meal in chick rations. CM-49 was superior to all other cottonseed meals compared. CM-49, 21, and 45 were found to be similar in feeding value when used to replace 75% of the soybean meal. Other meals processed by lowspeed screw-press and prepress solvent-extracted were appreciably lower in value than CM-21 or 45. High speed screw-processed meal was inferior to all other meals at both the 75% and 100% levels. Chicks fed rations containing the lowest level of cottonseed meal, 50%, had similar performance on each meal except for solvent-extracted meal which was poor even at this level. It was also found by this investigator that the addition of lysine restored optimum nutritive value of cottonseed meal for chich growth.

The addition of dl-lysine to rations of chicks containing varying amounts of cottonseed meal was studied by Bucek (1957). Soybean meal was used in the control ration. Levels of cottonseed meal used were 100%, 75%, and 50%. Varying amounts of dl-lysine were also used. The amounts of lysine used were: none, 2.0, 3.0, 4.0, and 5.0 grams; none, 1.0, 2.0, 3.0, and 4.0 grams; and none, 0.70, 0.85, and 1.0 gram per lb. of ration for the 100%, 75% and 50% cottonseed meal rations, respectively. No lysine was added to the basal ration. Cottonseed meals used were CM-10, 36, and 49. In rations which cottonseed meal supplied 100% of the supplemental protein, 4 grams of lysine resulted in the best feed efficiency, 2 grams in the 75% rations, and 0.85 gram in the 50% rations. Before supplementation, CM-10, either as 75% or 100%, resulted in feed efficiency of approximately four lbs. of feed per pound of gain while CM-36 and 49 rations only required 2.5 and 2.7 lbs. respectively. After supplementation, CM-10 was improved to 2.5 lbs. of feed per lb. of gain, CM-36 to 2.2 lbs., and CM-49 to 2.5 lbs. All of these values equaled or were superior to those of the control ration.

Hunter <u>et al</u>. (1957) compared CM-6, 10, 13, 19, 21, 36, and 49 to soybean meal in chick rations. All chicks were raised to two weeks of age on the same ration and then allotted on an equal weight basis. Three levels of cottonseed meal were fed. Rations containing CM-21 or 49 at the lowest level gave the best results as measured by per cent gain per day, 6.7 and 6.6%, respectively. Percent gain per day was calculated by the formula $\left(\frac{\text{average gain X 100}}{\text{average weight X number of days}}\right)$. Soybean meal resulted in 6.5% gain per day. Of rations supplying the greatest proportion of protein as cottonseed meal, CM-49 produced the best growth, 6.1% gain.

The value of cottonseed meal for chickens after various methods of processing and subjection to various temperatures was studied by Graw and Zweigart (1954). The types of processing and the temperatures were: screw press from 185°F to 240°F; four commercial screw press meals at 200, 240, and 250°F; four prepress solvent-extracted meals at 190, 200, 220°F; a hydraulic-pressed meal at 225°F; a hydraulic-pressed, solvent-extracted meal at 230°F; and solvent-extracted meals at 190-200°F for 60 minutes, 200-226°F for 60 minutes, 203-224°F for 60 minutes, and 190-210°F for 36 minutes. High quality fish meal supplied the protein for the control diet. The gossypol level in the diet was below 0.02% at all times so that growth limiting effects of gossypol did not have to be considered. The value of the meals was determined by per cent gain per day. When cottonseed meal was used in the diets, best growth was obtained from meals processed at temperatures less than 200°F. Commercial meals used resulted in good growth.

Morgan and Willimon (1953) used cottonseed meal alone and in combination with soybean meal in broiler rations. Cottonseed meal used was processed either by solvent extraction, screw press, or hydraulic press. All of the rations in which cottonseed meal and soybean meal were combined produced gains equal to or greater than those produced by soybean meal alone in the control diet. Chicks fed a ration supplemented with solvent-extracted cottonseed meal gained less than those on soybean meal but this difference was not significant. Rations supplemented with cottonseed meal produced by the other two processing methods produced significantly less gains than soybean meal. Feed efficiency was higher when chicks were fed a combination of soybean meal and cottonseed meal than when cottonseed meal was fed alone.

Dowell and Menaul (1923) studied the effect of autoclaving cottonseed meal to reduce gossypol toxicity in swine. Commercial cottonseed meal was used. One-half of the pigs received meal which had been autoclaved for 20 minutes at 15 lbs. pressure and the others received regular meal. No difference was noticed up to three weeks of age; but after that time, pigs fed regular meal were noticeably inferior. At the end of 73 days, total gains were 33 lbs. for pigs fed autoclaved meal and 23.5 for the other group. Cottonseed meal was removed but five days later one pig receiving the regular meal died and after another five days another died. These deaths were found to be due to effects of cottonseed meal. No pigs fed autoclaved meal showed ill effects.

Autoclaving and steaming cottonseed meal for pigs were studied by Gallup (1926). The autoclaving was for one hour at 20 lbs. of pressure. Pigs on either autoclaved meal or steamed meal were in the best condition during the entire experiment and had the keenest appetite. Gains for the entire experiment were 13 lbs. for untreated meal, 22 lbs. for autoclaved meal, and 26 lbs for steamed cottonseed meal. Cottonseed meal was found to be safe for swine after steaming or autoclaving.

Robinson (1934) studied the use of cottonseed meal alone and in combination with tankage, cottonseed meal autoclaved for 30 minutes and one hour, cottonseed meal as 20% of ration and 8% of ration, and tankage plus linseed meal for pigs. Cottonseed meal alone and not autoclaved produced gains of 0.64 lb. on approximately 526 lbs. of feed per 100 lbs. gain. No deaths occurred in this group. Meal autoclaved for 30 minutes produced 0.73 lb. gain daily. Feed efficiency was similar to that of regular cottonseed meal. Two deaths occurred on this ration and these were due to gossypol toxicity. Autoclaving for one hour increased gain and feed efficiency and produced no deaths. Linseed meal plus tankage produced gains of 1.11 lbs. and feed efficiency of 415 lbs. No deaths occurred from this ration. When cottonseed meal was fed as 20% and 8% of the ration, results were similar to those produced by linseed meal and tankage.

Hall and Lyman (1957) fed pigs 80 to 100 days old various levels of gossypol with the protein at two levels. Protein levels were 15% and 30%. All rations except the control ration contained cottonseed meal and soybean meal. The gossypol was present in two different cottonseed meals having different levels of free gossypol. When pigs were fed a 15% protein ration, control pigs fed soybean meal gained an average of 1.92 lbs. daily. This gain was equaled by pigs receiving 0.0013% free gossypol. No deaths occurred at this protein level from gossypol poisoning until the level of free gossypol reached 0.019% although two pigs had gossypol poisoning symptoms when the free gossypol was 0.015%. When pigs were fed rations to compare 15% protein to 30% protein, free gossypol levels went as high as 0.03%. No deaths occurred in pigs at the 30% protein level, but six pigs died of gossypol poisoning when fed the 15% protein feed.

Cottonseed meal was compared to soybean meal as a source of supplemental protein for pigs by Hillier et al. (1955). The value of adding lysine was also studied. Daily gains decreased from 1.92 lbs. for pigs fed soybean meal as the only supplemental protein source to 1.58 lbs. for pigs fed cottonseed meal. Feed efficiency decreased in the same way. Adding lysine increased gains slightly but did not increase feed efficiency. The cottonseed meal used was a low gossypol, prepressed, solvent-extracted meal.

Hillier et al. (1956) used cottonseed meal in combination with soybean meal for pigs in one trial and in a second trial used it alone, with tankage, with soybean meal, and with blood meal. When cottonseed meal and soybean meal were combined (1:3), gain and feed efficiency were equal to that of soybean meal alone. One-half cottonseed meal and one-half soybean decreased gains by 0.18 lb. and feed efficiency by 35 lbs. In the second trial, all sources of protein produced gains less than those of soybean meal alone. Feed efficiencies were also reduced below that of soybean meal. Cottonseed meal alone produced gains of only 0.66 lb. and resulted in the death of nine pigs from gossypol poisoning.

Hillier et al. (1957) compared soybean meal alone with cottonseed meal alone and combinations of the two meals. The combinations studied were: 2 parts soybean meal: 1 part cottonseed meal; 1:1; and 1:2. Daily gain and feed efficiency were the same for soybean meal alone and the 2:1 combination. Equal parts of the two meals produced gains equal to soybean meal but decreased feed efficiency. Soybean meal and cottonseed meal in a 1:2 combination decreased daily gain and feed efficiency as did cottonseed meal alone. Low gossypol, prepressed, solvent-extracted cottonseed meal was used.

Gallup (1926) fed cottonseed which was either untreated, autoclaved for one hour, or autoclaved for two hours to albino rats. The untreated cottonseeds produced animals in poor condition and which died shortly after the experiment terminated. Autoclaving was found to be beneficial and allowed large quantities to be used. Autoclaving for two hours did not increase the advantage.

The apparent digestibility of cottonseed meal and cottonseeds was determined by Gallup (1927) using albino rats. The effect upon digestibility of adding gossypol and autoclaving for one hour at 20 lbs. of pressure were studied. Cottonseed meal which had not been treated in any manner had protein digestibility of 68.5%. When the meal was autoclaved, protein digestibility was reduce to 51.7%. Cottonseeds had values of 76.9 and 66.7%, respectively. Extracting cottonseed with ether to remove gossypol did not affect protein digestibility. Addition of small amounts of free gossypol to extracted cottonseeds had little effect upon digestibility. Fatal results were obtained when gossypol levels reached amounts equivalent to 1% of the protein. When meal was used and the gossypol was in a less soluble form, amounts of gossypol equivalent to 2% of the protein were fed for several months before toxic symptoms occurred.

Olcott and Fontaine (1941) studied the effect of autoclaving on the nutritive value of cottonseed meal. Rats were fed rations containing 12 and 24% protein. The meal was autoclaved at 17 lbs. pressure for 30 minutes, 1 hour, and 2 hours. Rats fed a 12% protein ration gained 1.99 grams per gram of protein when the meal had not been autoclaved. Gains were 1.58, 1.08, and 0.28 grams when the meal had been autoclaved for 30 minutes, 1 hour, and 2 hours, respectively. When the 24% protein ration was fed, the gains per gram of protein were 1.84, 1.84, 1.31, and 0.52 grams, respectively. Cottonseed meal used had been ether-extracted to make it low in gossypol.

OBJECTIVES

The objectives of the work presented in this thesis were:

(1) To determine the effect of method of processing of protein supplements upon their nutritive value for lambs as measured by digestibility, nitrogen balance, growth, and feed utilization;

(2) To compare the quality of protein of soybean oil meal and four samples of cottonseed meal as measured by digestibility, nitrogen balance, growth, and feed utilization;

(3) To compare different nitrogen sources for rumen microorganisms digesting cellulose *in vitro*; and

(4) To study the effect of gossypol on rumen microorganisms digesting cellulose *in vitro*.

PART I DIGESTIBILITY AND NITROGEN BALANCE TRIALS

EXPERIMENTAL

Three digestion and nitrogen balance trials were conducted with cross-bred western wether lambs to compare cottonseed meal 13, cottonseed meal 45, and soybean oil meal. Cottonseed meal 13 was a high speed screw-press processed meal and cottonseed meal 45 was a prepress solvent-extracted meal. The number of lambs was 10, 8, and 12 in trials 1, 2, and 3, respectively. The numbers varied because of not being able to keep all lambs eating a constant quantity of the ration. Each trial was conducted as a new experiment and lambs were randomly allotted to stalls and treatments. Some animals were used for two trials and if they received the same supplement both times this was due entirely to chance. All lambs were fed the same ration for 10 days preceding each trial and then placed in metabolism stalls as described by Briggs and Gallup (1949). They were then fed the various rations for a 10-day preliminary period and a 10-day collection period.

The lambs were fed 300 gms. twice daily of the rations in Table 1. Water was available at all times. Feces were collected once daily and the total daily collection was dried in an oven at approximately 70°C for 24 hours. After drying, the feces were stored in open cans until the collection period was completed at which time the total collection was mixed and sampled for analysis. The urine was collected in glass jars containing hydrochloric acid. Daily aliquots (5%) were taken and stored

TABLE I COMPOSITION OF RATIONS FED TO LAMBS ON DIGESTIBILITY AND NITROGEN BALANCE TRIALS

Ingredient (gms. per day)	Rations CSM-13	CSM-45	SBOM
Concentrate mixture			
Cottonseed meal 13	122.0	---	---
Cottonseed meal 45	---	117.0	---
Soybean oil meal	---	---	96.0
Cerelose	88.5	91.0	101.5
Starch	88.5	91.0	101.5
Vitamin A & D supplement[1]	1.0	1.0	1.0
Mineral mixture[2]	30.0	30.0	30.0
Cellulose	180.0	180.0	180.0
Cottonseed hulls	60.0	60.0	60.0
Corn oil	30.0	30.0	30.0

[1] Vitamin A and D supplement was Quadrex which contains 10,000 vitamin A units and 1250 vitamin D units per gram.

[2] Composition of mineral mixture (gms.); NaCl, 378.0; KH_2PO_4, 668.0; $CaHPO_4 \cdot 2H_2O$, 746.0; $MgSO_4$, 207.0; $CaSO_4 \cdot 2H_2O$, 875.0; $CaCO_3$, 63.3; $FeSO_4$, 16.2; KI, 1.7; $ZnSO_4$, 0.6; $CuSO_4 \cdot 5H_2O$, 0.7; $CoSO_4 \cdot H_2O$, 0.4; CaF_2, 0.5; $MnSO_4 \cdot H_2O$, 3.0.

under refrigeration until the collection period was completed and then were mixed and sampled for analysis.

Chemical analyses were made according to the Methods of Analysis of the Association of Official Agricultural Chemists (1955). Statistical analysis of the data was made according to the methods of Snedecor (1956).

RESULTS AND DISCUSSION

Digestibility of the various nutrients and nitrogen balance data are given in Table 2. In general the nutrients of cottonseed meal 13 were slightly less digestible than those of cottonseed meal 45 or soybean oil meal Only protein digestibilities, 41.82%, 47.18%, and 53.68% for cottonseed meal 13, cottonseed meal 45, and soybean oil meal, respectively, were

TABLE II DIGESTIBILITY AND NITROGEN BALANCE DATA OBTAINED BY FEEDING DIFFERENT PROTEIN SUPPLEMENTS

	Rations		
	CSM-13	CSM-45	SBOM
Digestibility (%)			
Organic matter	71.98	73.54	76.19
Protein	41.82	47.18	53.68
Ether extract	90.15	90.94	87.15
Crude fiber	67.70	69.57	73.84
Nitrogen free extract	78.67	79.28	80.97
Nitrogen Balance			
Nitrogen intake, gms.	7.97	8.07	7.97
Nitrogen in feces, gms.	4.63	4.27	3.69
Nitrogen in urine, gms.	1.94	2.29	3.39
Nitrogen retained, gms.	1.40	1.51	0.88
As percent of intake	17.56	18.71	11.71
As percent of digested nitrogen	33.48	32.01	16.39

significantly different and these differences were highly significant ($P < 0.01$). The difference in protein digestibility of cottonseed meal 13 and cottonseed meal 45 was significant at the 5% level.

The differences in crude fiber content, 4.55% in cottonseed meal 13, 4.84% in cottonseed meal 45, and 1.67% in soybean oil meal, may have contributed to the significant difference in protein digestibility. Other dietary factors may also have contributed to this difference. Therefore, it should not be stated that there is a difference in quality of protein in these protein supplements.

Grams of nitrogen retained by lambs when fed the various rations were 1.40, 1.50, and 0.88 for cottonseed meal 13, cottonseed meal 45, and soybean oil meal rations, respectively. These differences were significant ($P < 0.05$). Nitrogen retention was significantly ($P < 0.01$) less when soybean oil meal was fed. Little difference was found in nitrogen retention of lambs fed cottonseed meal 13 or cottonseed meal 45.

The differences in nitrogen retention was due largely to high urinary excretion of nitrogen by lambs on soybean oil meal. Expressed as percent of nitrogen intake, nitrogen retention was 17.56, 18.71, and 11.17 for cottonseed meal 13, cottonseed meal 45, and soybean oil meal, respectively. When expressed as percent of digested nitrogen, nitrogen retention was 33.48, 32.01, and 16.39 for cottonseed meal 13, cottonseed meal 45, and soybean oil meal, respectively. Low nitrogen retention by lambs fed soybean oil meal is not in agreement with Woods _et al._ (1958) or Briggs _et al._ (1946b).

Differences in digestibility of nutrients and nitrogen retention between the different trials were not significant. This was also true for treatment-trial interaction.

PART II GROWTH TRIALS

EXPERIMENTAL

Two growth trials were conducted with crossbred western lambs to compare soybean oil meal with a low and a high nitrogen cottonseed meal which were processed similar to cottonseed meal 13 and cottonseed meal 45 used in the digestibility and nitrogen balance trials. Nitrogen analyses of the protein supplements used were 7.32%, 6.40% and 7.40% for soybean oil meal, low nitrogen cottonseed meal, and high nitrogen cottonseed meal, respectively. In one trial the lambs were fed rations which contained insufficient energy. The rations (Table 3) which provided sufficient energy contained 8% protein. The ration which provided insufficient energy was cottonseed hulls ad libitum, a mineral mixture, and the different protein supplements fed in amounts which furnished equal protein. Daily amounts of the supplements fed were 0.58 lb. of low nitrogen cottonseed meal and 0.50 lb. of high nitrogen cottonseed meal or soybean oil meal.

Thirty lambs were used in the trial in which sufficient energy was fed and 15 were used in the trial in which insufficient energy was fed. Before being placed on experiment, the lambs were drenched with phenothiazine and fed a standardization ration. Lambs were allotted to the various rations within each trial according to weight and sex and placed in individual pens. Lambs fed sufficient energy were fed all they would consume. The trial was conducted for a period of 68 days. The second

TABLE III COMPOSITION OF RATIONS FED TO LAMBS ON GROWTH TRIAL (SUFFICIENT ENERGY)

Ingredient (%)	Rations		
	CSM-High N	CSM-Low N	SBOM
Cottonseed meal high nitrogen	11.80	---	---
Cottonseed meal low nitrogen	---	13.60	---
Soybean oil meal	---	---	11.90
Cerelose	7.10	8.00	8.00
Starch	7.10	8.00	8.00
Cane molasses	20.00	20.00	20.00
Corn oil	1.00	1.00	1.00
Cottonseed hulls	50.00	50.00	50.00
NaCl	.50	.50	.50
$CaHPO_4$	---	---	.50
$CaCO_3$	.60	.60	---
Vitamin A & D	.10	.10	.10

trial lasted 49 days. Initial and final weights were taken after 16-hour shrink periods. Intermediate weights were taken every two weeks.

RESULTS AND DISCUSSION

Results of the sufficient energy growth study are presented in Table 4 and are an average of 10 lambs per treatment. Soybean oil meal rations produced slightly greater gains, 0.43 lb. for soybean oil meal, 0.39 lb. for low nitrogen cottonseed meal, and 0.41 lb. for high nitrogen cottonseed meal. Daily gains were not significantly different. Feed efficiency and protein efficiency for soybean oil meal, 8.63 lbs. and 0.69 lb., were better than for low nitrogen cottonseed meal, 9.75 lbs. and 0.78 lbs., or high nitrogen cottonseed meal, 9.47 lbs. and 0.75 lb. These differences approached significance at the 10% level of probability. Feed consumption was approximately the same for all three rations.

The differences in gain by lambs on rations which contained sufficient energy were small and not significant but the trend was in agreement with

TABLE IV AVERAGE GAIN, FEED CONSUMPTION, FEED EFFICIENCY, AND PROTEIN EFFICIENCY DATA OBTAINED FROM LAMBS ON GROWTH TRIAL (SUFFICIENT ENERGY)

	Rations		
	CSM-Low N	CSM-High N	SBOM
Average daily gain	0.39	0.41	0.43
Average feed consumption (total)	264.0	272.0	259.0
Feed efficiency	9.75	9.47	8.63
Protein efficiency	0.78	0.75	0.69

work conducted at Indiana (1942, 1943). There was little difference in daily gain by lambs on the two cottonseed meals and no definite statement can be made as to which one of these cottonseed meals is superior for daily gain. There was a definite trend in favor of soybean oil meal with respect to feed and protein efficiency. The high nitrogen cottonseed meal appeared to be superior to the low nitrogen cottonseed meal for both feed and protein efficiency.

Gain, feed consumption, feed efficiency, and protein efficiency data for the lambs fed insufficient energy are given in Table 5 and are an average of 5 lambs per treatment. Because of the nature of this study in that the lambs were limited in the amount that they could gain, the data are not applicable to the normal population of lambs fed fattening type rations. Feed consumption, which includes cottonseed hulls and protein supplement, was similar for all three rations. Because of the variation in response, which was probably due to the limitations placed on the lambs, no attempt was made to analyze these data for significance.

Results obtained from the trial with rations containing insufficient

TABLE V GAIN, FEED CONSUMPTION, FEED EFFICIENCY AND PROTEIN EFFICIENCY (INSUFFICIENT ENERGY)[1]

	Rations		
	CSM-Low N	CSM-High N	SBM
Gain	2.7	1.6	3.2
Feed consumption	820	825	810
Feed efficiency	63.1	103.1	50.6
Protein efficiency	5.05	8.25	4.05

[1] Due to the nature of this trial, some lambs did not gain and one lamb lost weight. For this reason data are averages of all animals on a treatment.

energy tended to contradict the results obtained with the cottonseed meals in the first trial. Average gains and feed and protein efficiency were less for high nitrogen cottonseed meal than for low nitrogen cottonseed meal although most of these differences were due to one lamb losing weight.

PART III ARTIFICIAL RUMEN STUDIES

EXPERIMENTAL

Artificial rumen studies were conducted to compare various nitrogen sources for rumen microorganisms digesting cellulose. Nitrogen sources used were cottonseed meal, soybean oil meal, torula yeast, and urea. Nitrogen analyses of the samples used are shown in Table 6. Also studied was the effect of adding gossypol to all of the nitrogen sources except cottonseed meal.

The apparatus and procedure used for these studies were similar to that described by Cheng et al. (1955). The amount of nitrogen source used in all cases supplied the same amount of nitrogen as 20 mg. of urea. The amount of cellulose (Solka-Floc) used in each tube was 120 mg. All weighings were made to the nearest 0.1 mg. on a Mettler electronic balance.

Rumen fluid was taken from a 900 lb. Hereford steer approximately five hours after feeding. The ration for this steer consisted of 6 lbs. cottonseed hulls, 3 lbs. ground milo, 1 lb. cottonseed meal, prairie hay ad libitum, vitamins A and D, and a 2:1 mixture of salt and bonemeal. The rumen samples were taken through a permanent rumen fistula, strained through 2 layers and then 4 layers of cheesecloth into a thermos bottle which had previously been warmed to 40°C.

The sample was immediately taken to the laboratory where a pH reading was made and then centrifuged for one minute at 3000 r.p.m.

TABLE VI NITROGEN ANALYSIS OF NITROGEN SOURCES USED IN ARTIFICIAL RUMEN STUDIES

Nitrogen Source	Percent Nitrogen
Cottonseed meal	6.51
Soybean oil meal	7.41
Torula yeast	6.89
Urea	46.62

After being centrifuged, the supernatant material was strained through 4 layers of cheesecloth and 5 ml. of this material added to each tube. Also added to each tube was 15 ml. of a salt solution containing the same compounds but in different concentrations as described by Cheng et al. (1955). The salt solution used is shown in Table 7. Before adding this salt solution, saturated Na_2CO_3 was added and CO_2 was bubbled through it to adjust the pH to 6.8 to 7.0.

After the rumen fluid and the salt solution had been added, the tubes were placed in a water bath set at 39°C and CO_2 bubbled through the tubes for 24 hours. The purpose of the CO_2 was to maintain anaerobic conditions and also for agitation of the cellulose and nitrogen source. After the 24 hours the tubes were centrifuged at 3000 r.p.m. for 5 minutes and the supernatant material was then poured off. Twelve ml. of glacial acetic acid and 1.5 ml. of concentrated nitric acid were added to each tube. The tubes were then placed in a boiling water bath for 20 minutes after which time they were removed, allowed to cool and then emptied into Gooch crucibles having asbestos in the bottom. Each tube was washed with 95% ethyl alcohol to remove all cellulose. The crucibles were dried at

TABLE VII COMPOSITION OF SALT SOLUTION USED IN ARTIFICIAL RUMEN STUDIES

Salt	Gm. per 20 liters of water
KH_2PO_4	6.00
$Na_2HPO_4\ 7H_2O$	12.00
$NaHCO_3$	35.00
KCl	40.00
NaCl	40.00
$MgSO_4$	1.50
$CuSO_4\ 5H_2O$	0.02
$MnSO_4\ H_2O$	0.0028
$ZnSO_4\ 7H_2O$	0.0008
$FeSO_4\ 7H_2O$	0.75
$CoCl_2\ 6H_2O$	0.02
$CaCl_2$	5.50

100°C for at least 4 hours, then cooled in a dessicator and weighed. After being weighed they were ashed at 600°C for 2½ hours, cooled and reweighed.

Tubes used as "blanks" to determine cellulose digestion had only cellulose added to them and 5 ml. of the supernatant material and 15 ml. of the salt solution. They were then centrifuged for 5 minutes, the supernatant material poured off and 12 ml. of acetic acid added. They were covered and left in the open for 24 hours and then handled the same manner as the other tubes.

The average amount of cellulose which disappeared from the "blanks" was added to the amount of fiber in the nitrogen sources. The amount of cellulose which disappeared from the experimental tubes was subtracted from the value determines for the "blanks" and the remainder was divided by the value for the "blanks" to give percent cellulose digested.

The tubes were arranged in a 4X4 Latin Square design whenever possible so that all treatments would be supplied by the same CO_2 line. When a Latin Square design could not be used because of too many tubes, a completely randomized design was used. Each days trial was repeated on at least one other day. A total of at least 8 tubes were used per nitrogen source per day.

Gossypol levels studied were 0.04%, 0.08%, 0.12%, and 1.0% of the total fluid volume of the tubes. The first three levels were studied only in solution but the 1.0% level was studied both in solution and not in solution. Nitrogen sources used were soybean oil meal, urea, and torula yeast. The gossypol was put into solution by mixing one gram of gossypol in one ml. of 95% ethyl alcohol. Later a solvent of corn oil (2.5 ml.), water (7.46 ml.), and tri-con X-100 (0.04 ml.) was used. Ethyl alcohol was again used as the solvent but was evaporated before the microorganisms were introduced into the tubes. In all instances where a solvent was used, the same amount of solvent to which gossypol had not been added was added to tubes to determine if the solvent was affecting cellulose digestion.

Statistical analysis for all data was made according to the methods of Snedecor (1956) or Federer (1955).

RESULTS AND DISCUSSION

TRIAL A

The results of the artificial rumen studies are presented in Table 8.

TABLE VIII AVERAGE CELLULOSE DIGESTION OBTAINED FROM ARTIFICIAL RUMEN STUDIES

Treatment	Cellulose Digestion %	No. of tubes per treatment	No. days
Trial A			
Soybean oil meal	66.6	32	2
Cottonseed meal	53.9	32	2
Soybean oil meal	62.8	16	2
Cottonseed meal	49.8	16	2
Torula yeast	73.7	16	2
Controls	19.8	16	2
Trial B			
Soybean oil meal	17.8	16	2
SBOM + 0.12% alcohol	8.1	16	2
SBOM + 0.04% gossypol	7.5	16	2
SBOM + 0.08% gossypol	5.6	16	2
SBOM + 0.12% gossypol	6.8	16	2
Trial C			
Torula yeast	36.1	16	2
TY + 0.12% detergent	19.2	16	2
TY + 0.04% gossypol	28.2	16	2
TY + 0.08% gossypol	25.5	16	2
TY + 0.12% gossypol	16.9	16	2
Soybean oil meal	33.4	16	2
SBOM + 0.12% detergent	14.0	16	2
SBOM + 0.04% gossypol	7.0	16	2
SBOM + 0.08% gossypol	7.3	16	2
SBOM + 0.12% gossypol	6.4	16	2
Urea	34.4	16	2
Urea + 0.12% detergent	16.6	16	2
Urea + 0.04% gossypol	26.4	16	2
Urea + 0.08% gossypol	19.2	16	2
Urea + 0.15% gossypol	9.9	16	2
Trial D			
Urea	28.4	24	3
Urea + 0.12% alcohol	27.5	24	3
Urea + 0.12% gossypol	19.4	24	3
Trial E			
Urea	28.6	32	4
Urea + 1.0% alcohol	27.5	32	4
Urea + 1.0% gossypol solution	4.0	32	4
Urea + 1.0 mg. gossypol	23.9	32	4

A highly significant ($P < 0.01$) difference in cellulose digestion was found between cottonseed meal and soybean oil meal, 53.3% and 66.6%, when compared on two different days as nitrogen sources for rumen microorganisms. Sixteen tubes were used for each source on each day.

A significant ($P < 0.01$) increase in cellulose digestion was found when torula yeast, soybean oil meal, and cottonseed meal were used as nitrogen sources and compared to digestion of cellulose in tubes to which no nitrogen had been added. Average digestion for two days was 62.8%, 49.8%, 73.7%, and 19.8% for soybean oil meal, cottonseed meal, torula yeast, and the controls or nitrogen tubes. Orthogonal comparisons indicated that there was a highly significant ($P < 0.01$) difference in cellulose digestion between torula yeast and soybean oil meal, between soybean oil meal and cottonseed meal, and between the controls and the nitrogen source tubes.

Belasco (1954) also found that cellulose digestion was considerably reduced when no nitrogen was added to tubes in *in vitro* studies. The differences in cellulose digestion when different nitrogen sources were used might be explained as being due to the amount of available nitrogen present in the tube. Thus, the increase in cellulose digestion in tubes to which soybean oil meal had been added above that obtained in cottonseed meal tubes could be supported by the work of Stallcup and Looper (1958) who found higher levels of nitrogen present by feeding soybean oil meal than when cottonseed meal was fed.

TRIAL B

The addition of gossypol in an alcohol solution to tubes containing soybean oil meal was found to decrease ($P < 0.01$) cellulose digestion.

A decrease in cellulose digestion was also noted when alcohol alone was added to tubes. There were no significant differences between adding 0.04% or 0.08% gossypol nor between adding 0.12% gossypol or 0.12% ethyl alochol. Cellulose digestion was 17.8%, 8.1%, 7.5%, 5.6%, and 6.8%, for controls, 0.12% alcohol, 0.04% gossypol, 0.08% gossypol, and 0.12% gossypol, respectively.

TRIAL C

Gossypol in a solvent consisting of corn oil, a fat detergent (tri-con X-100), and water significantly ($P < 0.01$) decreased cellulose digestion by rumen microorganisms in tubes to which torula yeast had been added. No significant differences were found between tubes containing 0.04% and 0.08% gossypol (28.2% and 25.5%) or between the 0.12% level of detergent and the 0.12% level of gossypol (19.2% and 16.9%). Cellulose digestion for the controls was 36.1%.

When soybean oil meal was used as a nitrogen source, cellulose digestion was reduced ($P < 0.01$) from 33.4% for the controls by the addition of gossypol or the solvent. A significant ($P < 0.01$) difference in cellulose digestion was obtained between tubes to which 0.12% gossypol and 0.12% of the solvent had been added (6.4% and 14.0%). The difference in cellulose digestion between the addition of 0.04% and 0.08% gossypol was small (7.0% and 7.3%)

Cellulose digestion in tubes having urea as the nitrogen source was highly significantly ($P < 0.01$) reduced by the addition of gossypol or the solvent. Cellulose digestion for the controls was 34.4%. The difference in cellulose digestion between tubes to which 0.12% gossypol or 0.12% or the solvent had been added was statistically significant

($P < 0.01$). There was a difference of 6.7% digestion in favor of the solvent. There was also a difference ($P < 0.01$) between tubes to which 0.04% or 0.08% gossypol had been added (26.4% and 19.2%).

The addition of gossypol to tubes containing various nitrogen sources decreased cellulose digestion by rumen microorganisms. The solvents also caused a decrease in cellulose digestion. The cause of the decrease by the solvent was thought to be due to the solvent in some manner destroying or reducing the number of microorganisms in the tubes.

TRIAL D

In a study conducted for three days using urea as the nitrogen source and alcohol as the solvent and evaporating the alcohol before introducing the rumen microorganisms, cellulose digestion was greater ($P < 0.01$) in the control tubes, 28.4%, than in tubes to which 0.12% alcohol, 27.5%, and 0.12% gossypol, 19.4%, had been added. The greater cellulose digestion in the alcohol tubes was highly significantly ($P < 0.01$) different from the gossypol tubes.

When alcohol was used as the solvent and evaporated before the microorganisms were introduced, differences in cellulose digestion were small between control tubes and tubes to which alcohol but not gossypol had been added. This indicated that the alcohol was affecting the microorganisms in some way but evaporating the alcohol would not appreciably decrease cellulose digestion.

TRIAL E

The average of studies made on four days to determine the effect of adding gossypol, free or in an alcohol solution, upon cellulose digestion using urea as a nitrogen source showed that the addition of gossypol significantly ($P < 0.01$) decreased cellulose digestion. Alcohol, used as the solvent, was evaporated. Cellulose digestion was greater ($P < 0.01$) in tubes containing the free gossypol, 23.9%, than in tubes containing the gossypol solution, 4.0%. Cellulose digestion in the control tubes was 28.6% and this was greater than in tubes to which alcohol had been added, 27.5%. This difference approached significance at the 2.5% level.

The results of these studies suggest that gossypol must be in solution or broken down before it will appreciably decrease cellulose digestion. There was approximately a 20% difference in cellulose digestion between tubes to which gossypol not in solution and in solution had been added.

SUMMARY

In a digestibility and nitrogen balance study using crossbred wether lambs, the protein of the soybean oil meal ration was more digestible than that of cottonseed meal 13 or cottonseed meal 45 rations. The digestibility of protein for the three rations was 53.68%, 41.82%, and 47.18%, respectively. The difference between soybean oil meal and cottonseed meal rations was highly significant ($P < 0.01$). Crude fiber content of the different supplements may have been one of the dietary factors that contributed to this significant difference.

Lambs fed the soybean oil meal ration retained less nitrogen than lambs fed cottonseed meal 13 or cottonseed meal 45 rations. The low retention by lambs fed the soybean oil meal ration was due largely to high urinary excretion of nitrogen. When expressed as percent of nitrogen intake, nitrogen retention was 17.56, 18.71 and 11.71 for cottonseed meal 13, cottonseed meal 45, and soybean oil meal, respectively.

When lambs were fed rations containing sufficient energy, there were no significant differences in rate of gain, feed consumption, feed efficiency, or protein efficiency when fed soybean oil meal, low nitrogen cottonseed meal, or high nitrogen cottonseed meal. Lambs fed soybean oil meal gained more and had better feed and protein efficiency than lambs fed either of the cottonseed meals.

When insufficient energy rations were fed, lambs fed the soybean oil meal ration gained more than lambs fed the cottonseed meal rations.

The feed and protein efficiency was also better for lambs fed soybean oil meal.

Torula yeast, when used as a nitrogen source for rumen microorganisms digesting cellulose, promoted greater cellulose digestion than soybean oil meal or cottonseed meal.

The addition of gossypol decreased cellulose digestion when soybean oil meal was used as a nitrogen source for rumen microorganisms. Alcohol, when used as a solvent, also decreased cellulose digestion.

Gossypol also decreased cellulose digestion when added to tubes containing torula yeast, soybean oil meal, or urea as nitrogen sources. A solvent of corn oil, water, and a fat detergent (tri-con X-100) also decreased cellulose digestion. Cellulose digestion, in general, decreased as the level of gossypol increased. When gossypol was added in an alcohol solution and the alcohol later evaporated, cellulose digestion was not appreciably decreased by the alcohol. It was reduced 9.0% by the addition in solution. Cellulose digestion was not reduced appreciably when gossypol not in solution was added.

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A P P E N D I X

TABLE IX NITROGEN BALANCE DATA FOR LAMBS FED VARIOUS PROTEIN SUPPLEMENTS TRIAL I

Ration[1]	Lamb No.	Intake		Excretion		Nitrogen retained gms.
		Dry Matter	Nitrogen	Fecal	Urinary	
CSM-13	12	561.88	7.97	5.05	1.96	0.96
CSM-13	52	561.88	7.97	5.06	2.14	0.77
CSM-13	74	561.88	7.97	4.75	1.58	1.64
CSM-45	68	560.86	8.07	4.02	2.52	1.53
CSM-45	38	560.86	8.07	4.21	2.02	1.84
CSM-45	64	560.86	8.07	5.12	3.04	-0.09
SBOM	56	560.03	7.97	3.45	4.20	0.32
SBOM	53	560.03	7.97	3.69	3.06	1.22
SBOM	73	560.03	7.97	3.54	3.14	1.29
SBOM	2	560.03	7.97	4.83	2.84	0.30

[1] CSM-13 is cottonseed meal 13
CSM-45 is cottonseed meal 45
SBOM is soybean oil meal

TABLE X APPARENT DIGESTION COEFFICIENTS FOR TRIAL I

Ration[1]	Lamb No.	Dry Matter Intake gms.	Apparent Percent Digestibility					
			Dry Matter	Organic Matter	Crude Protein	Ether Extract	Crude Fiber	Nitrogen-free Extract
CSM-13	12	561.88	63.33	65.34	36.56	76.76	55.85	76.03
CSM-13	52	561.88	68.98	70.19	36.42	93.11	66.77	75.94
CSM-13	74	561.88	70.37	72.50	40.36	86.96	70.02	78.44
CSM-45	68	560.86	71.90	73.17	50.29	92.21	71.31	76.55
CSM-45	38	560.86	70.45	72.20	47.97	92.03	63.24	80.76
CSM-45	64	560.86	64.50	66.20	36.63	84.89	63.36	71.66
SBOM	56	560.03	75.87	79.62	56.72	81.23	78.13	84.59
SBOM	53	560.03	75.55	77.79	53.71	83.07	78.36	81.21
SBOM	73	560.03	76.62	78.57	55.64	91.66	73.13	84.69
SBOM	2	560.03	66.98	67.26	39.49	71.13	68.54	75.63

[1] CSM-13 is cottonseed meal 13
CSM-45 is cottonseed meal 45
SBOM is soybean oil meal

TABLE XI NITROGEN BALANCE DATA FOR LAMBS FED VARIOUS PROTEIN SUPPLEMENTS TRIAL II

Ration[1]	Lamb No.	Intake		Excretion		Nitrogen retained gms.
		Dry Matter	Nitrogen	Fecal	Urinary	
CSM-13	105	561.88	7.97	4.95	2.30	0.72
CSM-13	8	561.88	7.97	4.75	1.91	1.31
CSM-13	63	561.88	7.97	4.42	2.04	1.51
CSM-13	30	561.88	7.97	4.60	2.09	1.28
CSM-45	55	560.86	8.07	3.76	2.78	1.53
CSM-45	98	560.86	8.07	4.64	1.90	1.53
SBOM	23	560.03	7.97	4.08	3.34	0.55
SBOM	4	560.03	7.97	3.62	3.10	1.25

[1] CSM-13 is cottonseed meal 13
CSM-45 is cottonseed meal 45
SBOM is soybean oil meal

TABLE XII APPARENT DIGESTION COEFFICIENTS TRIAL II

Ration[1]	Lamb No.	Dry Matter Intake	Apparent Percent Digestibility					
			Dry Matter	Organic Matter	Crude Protein	Ether Extract	Crude Fiber	Nitrogen-free Extract
		gms.						
CSM-13	105	561.88	68.10	70.00	37.79	88.78	65.90	76.51
CSM-13	8	561.88	70.60	73.09	40.34	85.03	72.24	78.34
CSM-13	63	561.88	73.71	75.74	44.50	91.73	72.69	81.70
CSM-13	30	561.88	72.66	73.93	42.25	93.30	72.25	78.52
CSM-45	55	560.86	73.03	74.99	53.45	92.25	68.76	81.32
CSM-45	98	560.86	72.14	74.33	42.61	92.12	71.12	80.53
SBOM	23	560.03	61.14	62.79	48.86	81.89	57.87	66.18
SBOM	4	560.03	74.17	76.36	54.65	89.99	69.70	82.95

[1] CSM-13 is cottonseed meal 13
CSM-45 is cottonseed meal 45
SBOM is soybean oil meal

TABLE XIII NITROGEN BALANCE DATA FOR LAMBS FED VARIOUS PROTEIN SUPPLEMENTS TRIAL III

Ration[1]	Lamb No.	Intake		Excretion		Nitrogen retained gms.
		Dry Matter	Nitrogen	Fecal	Urinary	
CSM-13	3	561.88	7.97	4.10	2.08	1.79
CSM-13	39	561.88	7.97	4.56	1.46	1.95
CSM-13	52	561.88	7.97	4.40	2.00	1.57
CSM-13	63	561.88	7.97	4.32	1.78	1.87
CSM-45	49	560.86	8.07	3.80	2.06	2.21
CSM-45	44	560.86	8.07	3.82	2.01	2.24
CSM-45	8	560.86	8.07	4.08	2.30	1.69
CSM-45	116	560.86	8.07	4.97	1.98	1.12
SBOM	58	560.03	7.97	3.51	3.52	0.94
SBOM	30	560.03	7.97	3.35	3.64	0.98
SBOM	5	560.03	7.97	3.10	4.14	0.73
SBOM	2	560.03	7.97	3.78	2.92	1.27

[1] CSM-13 is cottonseed meal 13
CSM-45 is cottonseed meal 45
SBOM is soybean oil meal

TABLE XIV APPARENT DIGESTION COEFFICIENTS FOR TRIAL III

			Apparent Percent Digestibility					
Ration[1]	Lamb No.	Dry Matter Intake	Dry Matter	Organic Matter	Crude Protein	Ether Extract	Crude Fiber	Nitrogen-free Extract
		gms.						
CSM-13	3	561.88	64.95	65.85	48.51	94.90	48.06	77.77
CSM-13	39	561.88	72.30	73.63	42.75	93.72	71.44	78.33
CSM-13	52	561.88	75.01	76.39	44.80	93.52	74.35	81.53
CSM-13	63	561.88	73.86	75.10	45.74	93.82	69.28	82.27
CSM-45	49	560.86	77.03	78.42	52.94	94.35	75.26	83.60
CSM-45	44	560.86	77.20	79.23	52.80	94.69	75.84	84.81
CSM-45	8	560.86	71.49	73.31	49.50	90.04	68.00	79.60
CSM-45	116	560.86	68.02	69.97	38.47	85.22	69.28	74.67
SBOM	58	560.03	75.15	76.99	55.96	90.21	71.60	85.42
SBOM	30	560.03	79.24	80.77	57.95	91.23	76.25	86.59
SBOM	5	560.03	78.62	80.55	61.18	91.14	75.29	86.18
SBOM	2	560.03	79.31	81.20	52.65	89.99	82.44	84.59

[1] CSM-13 is cottonseed meal 13
CSM-45 is cottonseed meal 45
SBOM is soybean oil meal

TABLE XV INDIVIDUAL FEED CONSUMPTION AND GAIN OF LAMBS FED INSUFFICIENT ENERGY RATIONS

Ration[1]	Lamb No.	Feed Consumption lbs.	Gain lbs.
1	64	160	4
1	100	156	4
1	91	168	0
1	60	167	3
1	98	170	2
	Average	164	2.6
2	93	166	0
2	54	163	2
2	63	166	-2
2	89	164	2
2	97	164	6
	Average	169	1.6
3	92	167	1
3	74	166	9
3	14	167	1
3	99	167	3
3	90	143	2
	Average	162	3.2

[1] Low nitrogen cottonseed meal was fed in ration 1, high nitrogen cottonseed meal was fed in ration 2, and soybean oil meal was fed in ration 3.

TABLE XVI INDIVIDUAL FEED CONSUMPTION, GAIN, FEED EFFICIENCY AND PROTEIN EFFICIENCY OF LAMBS FED SUFFICIENT ENERGY RATIONS

Ration[1]	Lamb No.	Feed Consumption lbs.	Gain lbs.	Feed Efficiency[2]	Protein Efficiency[3]
1	1	250	32	7.80	0.62
1	48	279	31	8.99	0.72
1	25	218	22	9.93	0.79
1	11	249	34	7.31	0.58
1	37	286	31	9.23	0.74
1	2	273	33	8.26	0.66
1	6	239	30	7.97	0.64
1	5	236	30	7.88	0.63
1	8	289	31	9.32	0.75
1	10	272	26	10.47	0.84
	Average	259	30	8.72	0.70
2	3	209	23	9.10	0.73
2	28	253	25	10.11	0.81
2	9	288	36	8.00	0.64
2	36	274	27	10.16	0.81
2	16	264	30	8.79	0.70
2	18	271	19	14.26	1.14
2	15	286	26	11.00	0.88
2	26	244	24	10.16	0.81
2	34	281	31	9.05	0.72
2	49	275	30	9.16	0.73
	Average	265	27	9.98	0.80
3	14	284	31	9.17	0.73
3	13	236	31	7.60	0.61
3	39	298	35	8.50	0.68
3	44	282	30	9.41	0.75
3	12	258	23	11.20	0.90
3	4	239	21	11.39	0.91
3	31	273	31	8.80	0.70
3	33	278	29	9.59	0.77
3	41	288	30	9.59	0.77
3	35	284	26	10.92	0.87
	Average	272	29	9.62	0.77

[1] Soybean oil meal was fed in ration 1, low nitrogen cottonseed meal was fed in ration 2, and high nitrogen cottonseed meal was fed in ration 3.

[2] Pounds of feed to produce 1 lb. of gain.

[3] Pounds of protein to produce 1 lb. of gain.

VITA

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