EFFECTS OF VARIOUS PROCESSING TEMPERATURES ON THE UTILIZATION OF SOLVENT-EXTRACTED SOYBEAN AND COTTONSEED MEALS BY SHEEP

By

LLOYD B. SHERROD

Bachelor of Science
South Dakota State College
Brookings, South Dakota
1958

Master of Science
University of Arkansas
Fayetteville, Arkansas
1960

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the Oklahoma State University
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Thesis Approved:

Allen D. Tillman
Thesis Adviser

L. J. Bush

W. S. Newcomer

Roger E. Koeppe

Robert D. Morrison

Dean of the Graduate School

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INTRODUCTION

In recent years, there has been considerable interest in the effects of processing temperature upon the utilization of oil meals by ruminant animals. Temperatures encountered during processing are known to reduce the protein solubility of these meals thereby altering their nutritive value as protein sources for mature ruminants. The degree of alteration in nutritive value seems to vary considerably depending on conditions of processing. The purpose of this series of studies was to determine the effects of some specific processing conditions upon the nutritive value of solvent-extracted soybean and cottonseed meals for sheep.

REVIEW OF LITERATURE

Protein Metabolism by Ruminant Animals

Protein ingested by ruminant animals is first subjected to the proteolytic activities of the rumen microorganisms. These organisms possess enzymatic capabilities for the hydrolysis of the food protein into peptides and amino acids, which can be deaminated with the release of free ammonia. Simultaneously, these breakdown products are utilized for synthesis of new microbial proteins which are later digested by the host animal. Pearson and Smith (1934) were first in clearly showing that both synthesis and breakdown of protein occurred in the rumen. McDonald (1948, 1952) found that ammonia constituted the main component of ruminal non-protein nitrogen when natural proteins were fed. The author concluded that ammonia was an important intermediate in the microbial degradation of dietary proteins and that it was subsequently used for resynthesis of microbial proteins. Excess ammonia was found to be absorbed directly through the ruminal wall into the venous blood, converted to urea by the liver, and excreted in the urine or returned to the rumen in the saliva. Only traces of ammonia were present in the peripheral venous blood. The amount of ammonia nitrogen absorbed through the ruminal wall was 4 to 5 gm. per day; about 0.5 gm. urea was secreted in the saliva and returned to the rumen. Nitrogenous materials leaving the rumen were composed largely of undigested food protein and the protein of the microorganisms with only trace amounts of ammonia passing from the rumen to the omasum.

Lewis et al. (1957) reported that changes in ammonia concentration in the rumen were closely correlated with similar changes in the portal blood which indicated rapid transfer of ammonia through the ruminal epithelium into the venous blood draining the rumen. Blood urea concentrations were relatively constant throughout the 12-hour sampling period. Lewis (1957) also found that the blood urea concentration in sheep was constant under a constant feeding regime, but changes in the ration led to different blood urea concentrations which were paralleled with the different ruminal ammonia concentrations resulting from the respective rations. The change in blood urea concentration followed increases or decreases in ruminal ammonia after a delay period of 4 to 8 hours. Peripheral blood ammonia concentrations remained quite low regardless of changes in ruminal ammonia level indicating that ammonia was efficiently converted to urea in the liver when the ration contained various natural proteins. He proposed that blood urea concentrations were not due to changes in total protein intake but rather to the rate of proteolysis and ammonia production in the rumen following ingestion of the various proteinacous materials.

Elliott and Topps (1963) examined the urinary nitrogen components in relation to efficiency of protein utilization by steers and found the most efficient protein utilization associated with low urea excretion.

The rate of ammonia production in the rumen and subsequent assimilation of microbial protein exert important influences on the nutritive value of protein supplements for ruminants. The major factors influencing the balance of these processes appear to be the solubility of the dietary protein and the amount and type of carbohydrate in the ration. Pearson and Smith (1943) first presented evidence of the importance of protein solubility in

ruminant protein utilization. They found greater net protein synthesis when blood meal was incubated with rumen contents than when either casein or gelatin was incubated, and attributed this to the lower protein solubility of blood meal. McDonald (1948, 1952), Annison et al. (1954), Chalmers et al. (1954), Chalmers and Synge (1954a, b), Annison (1956), Lewis (1957), Lewis et al. (1957), and Phillipson et al. (1959) have shown that the degree and rapidity of protein degradation in the rumen were directly related to protein solubility. Highly soluble proteins were rapidly dissimilated with the release of large amounts of ammonia within the first 4 hours after feeding. Ammonia was then absorbed through the ruminal epithelium into the venous blood, converted to urea, and excreted as urinary nitrogen. High levels of urinary nitrogen excretion reduced the net amount of nitrogen which could ultimately become available for use by the animal. Any treatment, such as heating, which reduced the protein solubility reduced ruminal ammonia production and urinary nitrogen excretion and increased nitrogen retention by the animal. These English investigators have also determined that addition of soluble carbohydrates to a ration containing highly soluble protein reduced the amount of ammonia absorbed directly from the rumen presumably by furnishing the carbon fragments necessary for synthesis of microbial protein.

Warner (1956) and Lewis and McDonald (1958) made extensive studies of the metabolic interactions of proteins and carbohydrates in the rumen by administering casein with various carbohydrate sources. The ammonia concentration was reduced when soluble carbohydrates such as levan, starch, or glucose were present. The authors concluded that the most efficient utilization of protein supplements was obtained when a carbohydrate was

present which could be fermented at a rate comparable with that of the protein.

El-Shazly (1952b) found that the amino acids of a casein hydrolysate were degraded to ammonia, carbon dioxide, and volatile fatty acids during anaerobic in vitro fermentation with whole rumen contents or washed suspensions of rumen microorganisms. The reaction products were in roughly equimolar proportions. The deaminating capacity of the in vitro fermentation medium was increased or decreased proportionally with the protein intake of the donor animal. Sirotnak et al. (1953) found that of 22 amino acids studied only aspartic acid, glutamic acid, serine, arginine, and cysteine were dissimilated by washed suspensions of rumen microorganisms. The major degradation products were ammonia, carbon dioxide, and volatile fatty acids. The authors postulated that dissimilation of these amino acids might function as the ammonia supply for bacterial protein synthesis. Similar studies by Lewis (1955) indicated that deamination of individual amino acids occurred at a slower rate than mixtures of amino acids or casein hydrolysate.

Several investigators have determined the extent of conversion of food nitrogen to microbial protein under normal feeding conditions. McDonald (1954) fed sheep a partially purified ration in which zein contributed 94 percent of the total nitrogen and found that approximately 40 percent of the zein was utilized by rumen microorganisms for synthesis of their own proteins. McDonald and Hall (1957) fed sheep fitted with ruminal and duodenal fistulae a basal ration of straw, rice starch, and molasses supplemented by daily feedings of 100 gm. of casein or casein digesta. They reported a maximum concentration of 47 percent casein in the abomasal

proteins 2 hours after feeding. When casein furnished 87 percent of the ration nitrogen at least 90 percent was degraded in the rumen and utilized for the synthesis of microbial proteins.

Moore and King (1958) reported that 65 to 78 percent of the nitrogenous components in the rumen ingesta 4 hours after feeding was found in feed residues and microbial proteins regardless of dietary treatment with only small amounts (less than 2 percent) being present in the form of dissolved protein. Maximum concentrations of organic non-protein nitrogen and ammonia were observed 1 hour after feeding; within 3 hours after feeding total nitrogen was not different from the prefeeding levels.

Phillipson et al. (1959) determined the effects of different rations on the net changes of the ammonia content in rumen liquor and the effect of starch on in vitro assimilation of ammonia nitrogen. Total ammonia increased during incubation of rumen fluid from sheep fed grass, hay, or hay with protein supplements and decreased when sheep were fed hay with either beet fodder or a mixture of flaked maize and maize gluten meal. The reduction in ammonia was accompanied by an increase in trichloracetic acid precipitate which indicated a net protein synthesis during incubation. The authors concluded that addition of soluble carbohydrates to high protein rations depressed ammonia nitrogen concentration because of increases in concentration of bacteria capable of assimilation of ammonia nitrogen.

Blackburn and Hobson (1960) studied the breakdown and redistribution of protein nitrogen in the rumen of sheep fed casein in a soluble form, a heat treated insoluble form, and a dissolved form to supplement a basal ration of chopped wheat straw and molasses. There was a rapid breakdown of the soluble casein almost immediately after feeding as indicated by an increase

1 hour after feeding in non-protein nitrogen from 10 to 50 mcg. atoms nitrogen per ml. rumen contents; this being the maximum concentration. Proteolytic activity and numbers of proteolytic bacteria in the sheep rumen were highest when the soluble casein was fed and lowest when the heat-treated casein was fed, the dissolved casein being intermediate. The authors concluded that rapid breakdown of foodstuff protein in the rumen immediately after feeding was due more to the relatively constant proteolytic activity in the rumen rather than to a sudden increase in enzyme activity immediately following ingestion of a highly soluble protein.

Effects of Processing upon the Nutritive Value of Proteins for Ruminants

Miller and Morrison (1944) compared the nutritive values of ground soybeans, solvent-extracted soybean oil meal, and solvent-extracted soybean oil meal heated for 70 minutes at 250°F in nitrogen-balance experiments with lambs. Average percent protein digestibility was 62.9, 69.0, and 70.6; percent ration nitrogen retained was 18.2, 23.7, and 26.3; and percent digestible nitrogen retained was 28.5, 34.1, and 37.2 for ground soybeans, solvent-extracted soybean meal, and heated solvent-extracted soybean meal, respectively. Responses were significantly less for raw soybeans by all criteria studied. The authors concluded that the lower response to the raw soybeans was due primarily to lower digestibility rather than differences in percent retention of digested nitrogen.

Cuthbertson and Chalmers (1950) found that pregnant ewes excreted 60 to 70 percent of the nitrogen furnished by a daily feeding of 50 gm. casein. Nitrogen retention was increased when the casein supplement was introduced through fistulae into the rumen or duodenum, with greatest retention resulting from duodenal administration. Ewes fed a low-energy roughage ration

utilized casein administered by all methods to a lesser extent than those fed high levels of concentrates. Chalmers et al. (1954) made similar observations from nitrogen-balance studies with sheep fed casein. They found that processing the casein by heating and browning greatly improved nitrogen retention even though digestibility was reduced. The processed casein resulted in lower ruminal ammonia concentrations but greater efficiency of conversion of dietary nitrogen into microbial protein. These results were interpreted as evidence that evaluation of proteins solely in terms of digestibility may not reflect their true nutritive value for ruminants. In further work, Chalmers and Synge (1954a) compared herring-meal supplements and casein in nitrogen-retention and growth trials with sheep fed a low-protein basal ration. Both nitrogen retention and growth rate were higher in the animals fed the herring-meal supplements. Ruminal ammonia production was less extensive than with the casein supplements.

Ellis et al. (1956) fed lambs a purified ration supplemented with either urea, gelatin, casein, soybean protein, or bovine blood fibrin as the protein source. There was no significant difference in digestibility of nitrogen from any of the sources with all values between 82.0 and 85.4 percent. Average daily nitrogen retention was 0.78, 1.66, 2.16, 2.78, and 2.86 gm. for urea, gelatin, casein, blood fibrin, and soybean protein, respectively. Nitrogen retention from urea was significantly less than from all other sources and that from casein was significantly less than from blood fibrin and soybean protein. Gelatin promoted less nitrogen retention than casein. Ruminal ammonia concentrations varied only slightly with the different nitrogen sources, with the exception of urea which gave significantly higher values 3 hours after feeding than the other supplements.

El-Shazly (1958) fed adult sheep isonitrogenous levels of cottonseed cake, linseed oil meal, beans, meat meal, fish meal, casein, and barseem as supplements for wheat straw and found that protein digestibility ranged from 71.4 percent for casein to 46.0 percent for meat meal. The only supplements which promoted positive nitrogen balance were barseem, cottonseed cake, and linseed meal. Nitrogen retention from beans was significantly improved when 70 gm. starch was added to the daily ration. From these results the author concluded that: (1) ruminal ammonia production is directly related to the nitrogen solubility of the protein supplement, (2) solubility should not be used as a sole criteria to assess the value of a protein because highly insoluble proteins may have a low nutritive value for ruminants even though ruminal ammonia concentrations are low, and (3) protein digestibility is a relatively inaccurate single criteria for evaluating a protein supplement for ruminants because highly digestible supplements may promote rapid ammonia production in the rumen. In similar studies Whitelaw et al. (1961) fed eleven week old calves commercial groundnut meal, heat-treated groundnut meal, and Peruvian fishmeal in isonitrogenous rations containing 16.5 percent crude protein. The respective solubilities of the total nitrogen soluble in M NaCl were 47.6, 39.6, and 17.6 percent. Nitrogen retention was 17.2, 18.9, and 21.6 gm. per day and average daily gains were 363, 442, and 636 gm. per day, respectively, for the three supplements in the order listed above. Blood urea concentrations were directly related to the solubility of the protein in the ration.

Drori and Loosli (1961) found that the magnitude and direction of postprandial changes in blood urea were indicative of changes in levels of ruminal ammonia in sheep fed a basal ration of timothy hay and cane molasses

supplemented with either soybean meal, urea plus glucose, or urea plus starch. These investigators reported that blood urea decreased when soybean meal was fed and increased when urea was the nitrogen source. The excretion of urinary urea accounted for the differences in the retention of absorbed nitrogen.

Tagari et al. (1962) studied the nutritive value for sheep of three soybean oil meals from solvent-extracted soybeans which had been subjected to a temperature of 70-80° F for 20 minutes during extraction. Subsequent heat treatments were: (raw) no further heating, (untoasted) heated for 10 minutes at 80° F, and (toasted) steamed for 15 minutes at 120° F. The protein solubility was 61.2, 40.7, and 13.1 percent for the raw, untoasted, and toasted meals, respectively. Protein digestibility was 55.3, 58.2, and 61.6 percent, respectively, for the raw, untoasted, and toasted meals. Nitrogen-retention was 0.62, 1.65, and 2.32 gm. per day for the meals in the same order. Ruminal ammonia production and blood urea concentration were highest for the raw meal and lowest for the toasted meal with the untoasted meal being intermediate when these meals were fed at maintenance and at twice maintenance levels of protein. According to the authors the major factor contributing to the different efficiencies of utilization was the different protein solubilities of the meals. The increasing increments of heating rendered the meals less soluble and more resistant to rapid deamination in the rumen. Elliott and Topps (1963) also found that the most efficient protein utilization by steers was associated with low urinary urea excretion.

Woods et al. (1962) reported that protein digestibility by sheep was related to protein solubility in studies comparing a cottonseed meal of low

solubility (CSM-13), a cottonseed meal of high solubility (CSM-45), and a soybean meal of high solubility (SBM). The respective protein solubilities of the meals in percent were 30.1, 65.9, and 81.0. Protein digestibility was 41.8, 47.2, and 53.7 percent for the CSM-13, CSM-45, and SBM rations, respectively. Nitrogen retention was highest in lambs fed the CSM-45 with 1.51 gm. nitrogen retained per day. Nitrogen retention was 1.40 and 0.89 gm. per day for CSM-13 and SBM, respectively. The lambs fed CSM-13 made the most efficient utilization of the nitrogen that was absorbed (41.9 percent) and the least efficient utilization of absorbed nitrogen was from the soybean meal with 20.8 percent of absorbed nitrogen retained. The meals of higher nitrogen solubility promoted higher daily gains with greater feed efficiency than the less soluble meals when fed to lambs in high concentrate rations. However, when high roughage rations were fed, the meal of higher nitrogen solubility gave the poorest results in terms of daily gain and feed efficiency.

Little et al. (1963) found no consistent relationships between nitrogen solubility and in vitro ammonia production using rumen fluid as the incubation medium. Correlations between ammonia production from various protein supplements and nitrogen solubility in rumen fluid, dilute sodium hydroxide, and distilled water were 0.93, 0.52, and 0.38, respectively. There was little difference in protein digestibility and nitrogen retention between groups of lambs fed corn gluten meal with low nitrogen solubility. Lambs fed corn gluten meal gained significantly less than those fed commercially processed soybean or linseed oil meals and heated (110° C dry heat for 24 hours) soybean oil meal. However, addition of three percentage units of protein equivalent from urea to the corn gluten meal rations resulted in

lamb performance which was equal to the other rations. The authors suggested that some readily available nitrogen is necessary for normal microbial protein metabolism in the rumen when relatively insoluble protein supplements such as corn gluten meal represent the only other source of nitrogen in the ration.

Effect of Gossypol upon the Nutritive Value of Proteins for Ruminants

Lyman et al. (1959) reported that the level of free gossypol in cottonseed meal was reduced during normal processing by the reaction of free gossypol with the free epsilon amino groups of lysine in cottonseed protein. This reaction causes a subsequent increase in the bound gossypol level. Baliga and Lyman (1957) and Lyman et al. (1959) found that in vitro digestibility of the protein-gossypol complex by pepsin and trypsin was quite low which might be partial explanation for the negative correlations between level of bound gossypol and nutritive value of cottonseed meal for non-ruminants. These investigators suggested that bound gossypol might reduce the utilization of cottonseed proteins by non-ruminants by blocking the epsilon amino groups of lysine that are necessary for the action of these proteolytic enzymes.

Woods et al. (1957, 1958, 1962) reported that reduced digestibility and utilization of cottonseed proteins by ruminants was related to high levels of bound gossypol. Tillman and Kruse (1962) found no significant effect on protein digestibility and nitrogen retention by sheep with the addition of 1 percent gossypol acetic acid to soybean meal. However, the combination of gossypol plus heat significantly reduced both protein digestibility and nitrogen retention.

EXPERIMENT I

Previous nitrogen utilization studies from this laboratory (Woods et al. 1962) have shown that oil meals produced under commercial conditions gave inconsistent results when fed to ruminants. The temperature and length of time the meals are heated are only approximations and may vary in commercially produced oil meals. The experiment described herein was conducted for the purpose of determining the effects of some specific processing conditions upon the nutritive value of three different protein supplements for sheep.

Experimental Procedure

Digestibility and Nitrogen Retention Studies

One cottonseed meal was extracted with an azeotrope (acetone, hexane, and water) solvent and contained low levels of free and bound gossypol. The other cottonseed meal and the soybean meal were specially prepared by cold-hexane extraction with no heat being applied at any time prior, during, or after extraction. Portions of each meal were then subjected to the following heat treatments: (1) no heat, (2) autoclaved under 15 lb. per square in. steam pressure at 250° F for 45 minutes, and (3) autoclaved under the same conditions for 90 minutes. For autoclaving, the meals were placed in metal pans which were previously lined with heavy paper and leveled to a uniform depth of 1/2 inch. After autoclaving, the meals were dried, reground to their original particle size, and compared in isonitrogenous rations,

compositions of which are shown in Tables I and II.

Eighteen crossbred wethers averaging 100 lb. and about 7 months of age were used in a 3 x 3 factorial arrangement of treatments during three separate trials; two animals per treatment were used during each trial. The animals were randomly assigned to the treatments in all trials. A 7-day adjustment period preceded successive 10-day preliminary and 10-day collection periods. The animals were removed from the metabolism stalls (Briggs and Gallup, 1949) for a 10-day rest period following the completion of trials 1 and 2.

Each lamb was fed 500 gm. of his assigned diet twice daily during all trials; water was available at all times. The ration supplied nutrient levels which were slightly above the maintenance requirements established by the National Research Council (N.R.C., 1957). Feces and urine were collected and prepared for analyses as described by Tillman and Swift (1953).

Blood samples were obtained by jugular puncture from all animals 3 hours after the morning feeding on the last day of each trial. Heparin was the anticoagulant and the plasma was separated by centrifugation and then stored at -10° F until analyzed. After completion of the final metabolism trial, the animals were maintained on their respective diets and samples of rumen fluid were taken by stomach pump from two animals on each treatment at 0, 1, 2, 3, 4, 5, 6, 8, 10, and 12 hours after the morning feeding for the determination of pH and ammonia-nitrogen (NH_3-N) level.

Proximate analyses of feed, feces, urine, and total nitrogen in the plasma were determined by the methods of A.O.A.C. (1960). Non-protein nitrogen (NPN) was determined by the method of Folin and Wu (1919). Protein nitrogen was calculated by subtracting the NPN from total nitrogen. Ammonia

TABLE I

CHEMICAL ANALYSES OF OIL MEALS USED IN ALL STUDIES

Meal designation[a]	Special SBM[c]			Azeotrope CSM[c]			Special CSM			Oklahoma CSM	
Heat treatment[b]	0	45	90	0	45	90	0	45	90	0	45
Particular components											
Total gossypol, %	0	0	0	0.34	0.23	0.12	2.30	1.67	1.03	0.78	0.53
Free gossypol, %	0	0	0	0.03	0.02	0.01	2.08	0.84	0.38	0.61	0.12
Bound gossypol, %	0	0	0	0.31	0.21	0.11	0.22	0.83	0.65	0.17	0.41
Nitrogen soluble in 0.02 N NaOH, % of total	87.3	60.1	36.8	73.70	47.50	26.40	76.40	38.20	31.10	82.60	59.10
Proximate composition, % of dry matter											
Organic matter	92.6	93.0	93.0	91.8	91.4	91.4	92.1	92.3	92.2	92.3	92.2
Ash	7.4	7.0	7.0	8.2	8.5	8.6	7.9	7.7	7.8	7.7	7.8
Protein (N X 6.25)	47.6	48.0	48.1	56.7	58.5	58.4	48.6	48.9	50.5	57.3	57.2
Ether extract	7.2	7.8	7.9	1.1	0.6	0.8	7.6	6.8	6.9	1.7	1.6
Crude fiber	5.6	7.1	11.1	6.2	7.4	9.2	5.5	7.3	6.5	4.4	4.3
NFE	32.2	30.1	25.9	27.8	25.0	23.0	30.4	29.3	28.3	28.9	29.1

[a]See text for description of meals.
[b]Minutes autoclaved (see text for details of processing).
[c]SBM - soybean meal; CSM - cottonseed meal.

TABLE II

COMPOSITION OF RATIONS USED IN SHEEP DIGESTIBILITY AND NITROGEN RETENTION STUDIES

Ration designation[a]	Special SBM			Azeotrope CSM			Special CSM		
Heat treatment[b]	0	45	90	0	45	90	0	45	90
Ration number	1	2	3	4	5	6	7	8	9
	%	%	%	%	%	%	%	%	%
Ingredient									
Cottonseed meal	-	-	-	18.9	18.5	18.6	22.3	22.4	21.4
Soybean meal	22.8	22.7	22.8	-	-	-	-	-	-
Corn dextrose	16.9	16.9	16.9	16.9	16.9	16.9	16.9	16.9	16.9
Corn starch	16.9	16.9	16.9	16.9	16.9	16.9	16.9	16.9	16.9
Corn oil	3.5	3.5	3.5	4.8	4.8	4.8	3.5	3.5	3.5
Cottonseed hulls	10.0	10.0	10.0	8.9	8.9	8.9	8.9	8.9	8.9
Mineral mix[c]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Purified cellulose[d]	24.8	24.9	24.8	28.5	28.9	28.8	26.4	26.3	27.3
Vitamins A and D[e]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Proximate composition, dry matter basis, %									
Organic matter	93.3	93.6	93.3	93.3	93.0	93.5	92.6	92.4	93.1
Ash	6.7	6.4	6.7	6.7	7.0	6.5	7.4	7.6	6.9
Crude protein	11.3	11.5	11.4	11.2	11.5	11.4	11.2	11.2	11.6
Ether extract	7.3	7.0	6.6	6.6	7.7	7.5	6.7	6.4	6.3
Crude fiber	24.2	24.0	24.0	26.3	25.4	27.3	26.8	25.6	24.7
NFE	50.5	51.1	51.3	49.2	48.4	47.3	47.9	49.2	50.5

[a]See text for description of meals.
[b]Minutes autoclaved (see text for details of processing).
[c]Oltjen et al. (1959).
[d]"Solka Floc," Brown & Company, New Hampshire.
[e]Contained 20,000 I.U. and 2500 U.S.P. units per gram of vitamins A and D, respectively.

nitrogen (NH_3-N) in blood and rumen fluid was determined by the micro-diffusion method described by Conway (1957). Nitrogen solubility of the meals was determined by the method described by Lyman et al. (1953).

Analyses for total, free, and bound gossypol were conducted by the methods of Pons et al. (1958) and Pons and Hoffpauir (1957). The data were analyzed statistically by analysis of variance. Orthogonal comparisons were made between combined groups using the unheated meals as controls.

Sheep Growth Studies

The unheated meals, which have been described, were autoclaved for 45 minutes and included in isonitrogenous sheep diets fed during a 30-day growth trial. Compositions of the rations are shown in Table III. Sixteen cross-bred lambs weighing an average of 46 lb. were randomly divided by sex into four groups with four animals in each group. The animals were individually-fed twice daily more of their assigned diets than they would consume; uneaten feed was removed and weighed every third day. Response criteria were weight gains and feed efficiency. A 16-hour shrink period during which time feed and water were removed preceded the initial and final weights.

Rat Growth Studies

The treatments and composition of rations are shown in Table IV. The special soybean meal, azeotrope-extracted cottonseed meal, and special cottonseed meal have been described. The other cottonseed meal (Oklahoma cottonseed meal) was prepared in the same manner as were the special meals using cottonseed from a low-gossypol strain of cotton. As shown in Table IV, each of the protein supplements were fed either unheated or were auto-claved for 45 minutes and the supplements under test supplied all the dietary

TABLE III

COMPOSITION OF RATIONS USED IN SHEEP GROWTH STUDIES

Ration designation[a]	Special soybean meal		Special cottonseed meal	
Heat treatment[b]	0	45	0	45
Ration number	1	2	3	4
Ingredient				
Cottonseed meal	- -	- -	24.6	24.6
Soybean meal	25.0	25.0	- -	- -
Corn dextrose	19.2	19.2	20.4	20.4
Corn starch	19.2	19.2	20.4	20.4
Corn oil	1.5	1.5	1.5	1.5
Cottonseed hulls	30.0	30.0	28.0	28.0
Mineral mix[c]	5.0	5.0	5.0	5.0
Vitamins A and D[d]	0.1	0.1	0.1	0.1
Total[e]	100.0	100.0	100.0	100.0

[a]See text for description of meals.
[b]Minutes autoclaved (see text for details of processing).
[c]Oltjen et al. (1959).
[d]Contained 20,000 I.U. and 2,500 U.S.P. units per gram of Vitamins A and D, respectively.
[e]Using the chemical analyses of individual components of each diet, the proximate composition of all rations were, as a percent of dry matter, as follows: ash, 8.1; crude protein, 13.0; ether extract, 3.3; nitrogen-free extract, 59.5; crude fiber, 16.1; and organic matter, 91.9.

TABLE IV

COMPOSITION OF RATIONS USED IN RAT GROWTH STUDIES

Ration designation[a]	Casein		Special SBM[c]		Azeotrope CSM[c]		Oklahoma CSM		Special CSM	
Heat treatment[b]	0	45	0	45	0	45	0	45	0	45
Ration number	1	2	3	4	5	6	7	8	9	10
	%	%	%	%	%	%	%	%	%	%
Ingredient										
Casein	12.20	12.20	-	-	-	-	-	-	-	-
Special SBM	-	-	22.80	22.70	-	-	-	-	-	-
Azeotrope CSM	-	-	-	-	19.00	18.50	-	-	-	-
Oklahoma CSM	-	-	-	-	-	-	19.70	19.70	-	-
Special CSM	-	-	-	-	-	-	-	-	22.30	22.40
Corn dextrose	74.65	74.65	66.75	66.85	69.15	69.65	68.15	68.15	67.35	67.25
Corn oil	5.00	5.00	3.50	3.50	4.80	4.80	4.80	4.80	3.40	3.40
Mineral mix[d]	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50
Vitamin mix[e]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Choline chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Purified cellulose[f]	3.20	3.20	2.00	2.00	2.10	2.10	2.40	2.40	2.00	2.00
Total[g]	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[a] See text for description of meals.
[b] Minutes autoclaved (see text for details of processing).
[c] SBM - soybean meal; CSM-cottonseed meal.
[d] Salt mixture U.S.P. XIV, Nutritional Biochemicals Corporation, Cleveland 28, Ohio.
[e] Mameesh et al. (1959).
[f] "Solka floc," Brown and Company, Berlin, Hampshire.
[g] Using the chemical analyses of individual components of each diet, the proximate composition of all diets were, as a percent of dry matter, as follows: ash, 6.4; crude protein, 10.9; ether extract 5.4; nitrogen-free extract, 73.8; and organic matter, 93.6.

protein in the isonitrogenous diets.

Weanling female rats weighing initially an average of 48 gm. were randomly allotted to 10 groups of 10 each for the 28-day trial. The animals were individually-fed more of their assigned diets than they would consume once daily; uneaten and wasted feed were removed and weighed daily. Water was available at all times and the animals were weighed twice weekly. Response criteria were weight gains, feed efficiency, and mortality.

Results and Discussion

Digestibility and nitrogen retention results are shown in Tables V and VI. When all heat treatments were combined and the meals compared, it was found that the digestibility of organic matter in soybean meal was higher ($P < .01$) than that of the cottonseed meals. Likewise the digestibility of organic matter in the azeotrope-extracted meal was higher ($P < .01$) than that of the special cottonseed meal, which contained high levels of free and bound gossypol. Autoclaving of all meals for 45 minutes (rations 2, 5, 8) improved ($P < .05$) organic matter digestibility when these were compared to all unheated rations (rations 1, 4, 7). When the meals were autoclaved for 90 minutes, the favorable effect of heat upon organic matter digestibility noted when they were autoclaved for only 45 minutes was not obtained; differences between organic matter digestibilities when the two treatments were compared were in favor of the shorter time ($P < .10$).

When all heat treatments were combined, nitrogen-free extract digestibilities were higher ($P < .01$) in the rations containing the special soybean meal than in those containing either of the cottonseed meals; and higher ($P < .10$) in the rations containing the azeotrope-extracted cottonseed meal than those with the special cottonseed meal. Differences between rations

TABLE V

EFFECTS OF HEATING PROTEIN SUPPLEMENTS ON SHEEP DIGESTIBILITY OF RATION COMPONENTS

Protein supplement[a]	Special SBM			Azeotrope CSM			Special CSM		
Heat treatment[b]	0	45	90	0	45	90	0	45	90
Ration number	1	2	3	4	5	6	7	8	9
Number of animals	6	6	6	6	6	6	6	6	6
Digestibility, %									
Organic matter	71.3 (1.8)[c]	72.4 (1.8)	70.4 (1.9)	67.7 (3.1)	69.8 (2.3)	66.3 (2.2)	60.1 (1.5)	66.3 (1.5)	63.6 (1.9)
Protein	66.6 (2.8)	62.8 (1.8)	59.7 (2.1)	60.0 (2.3)	61.4 (1.2)	51.3 (1.5)	63.9 (2.8)	55.1 (1.7)	51.6 (2.1)
Ether Extract	81.9 (6.5)	79.2 (4.1)	79.4 (4.8)	82.6 (4.2)	86.4 (3.9)	84.0 (4.4)	72.7 (6.5)	84.4 (3.6)	70.7 (6.5)
Crude Fiber	49.9 (3.6)	54.7 (4.9)	47.4 (4.1)	50.7 (5.7)	50.5 (6.4)	47.8 (5.8)	28.9 (3.0)	44.4 (3.8)	42.6 (4.8)
N-free Extract	83.3 (1.3)	84.4 (1.2)	84.5 (1.1)	79.7 (2.4)	82.0 (1.6)	80.3 (1.7)	76.9 (1.8)	80.9 (1.6)	78.1 (2.0)

	Significant Differences[d]
Organic matter	1,2,3 > 4,5,6,7,8,9**; 4,5,6 > 7,8,9**; 2,5,8 > 1,4,7*; 2,5,8 > 3,6,9[e]
Protein	1,2,3 > 4,5,6,7,8,9**; 2,5,8 > 3,6,9**; 1,4,7 > 2,3,5,6,8,9**
Crude Fiber	1,2,3 > 4,5,6,7,8,9[e]; 4,5,6 > 7,8,9**
N-free Extract	1,2,3 > 4,5,6,7,8,9**; 4,5,6 > 7,8,9[e]

[a]See text for description of meals.

[b]Minutes autoclaved (see text for details of processing).

[c]Standard error.

[d]Orthogonal Comparisons. Ration numbers separated only by commas represent the average for that group.

[e]P < .10; *P < .05; **P < .01.

TABLE VI

EFFECTS OF HEATING PROTEIN SUPPLEMENTS ON SHEEP RETENTION OF DIETARY NITROGEN AND BLOOD PLASMA COMPONENTS

Protein supplement[a]	Special SBM			Azeotrope CSM			Special CSM		
Heat treatment[b]	0	45	90	0	45	90	0	45	90
Ration number	1	2	3	4	5	6	7	8	9
Number of animals	6	6	6	6	6	6	6	6	6
Nitrogen balance, gm.									
Nitrogen intake	16.40	16.60	16.50	16.20	16.60	16.50	16.00	15.90	16.90
Nitrogen in feces	5.48	6.17	6.64	6.48	6.40	7.99	5.78	7.14	8.18
Nitrogen in urine	7.32	5.57	4.91	5.84	5.44	4.19	6.31	4.46	4.24
Nitrogen retention	3.60	4.86	4.95	3.88	4.76	4.32	3.91	4.30	4.48
	(0.52)[c]	(0.37)	(0.50)	(0.36)	(0.57)	(0.45)	(0.29)	(0.29)	(0.32)
N balance, % intake	21.9	29.3	30.0	23.9	28.6	26.2	24.4	27.0	26.5
	(3.1)	(2.3)	(3.1)	(2.2)	(3.4)	(2.7)	(1.8)	(2.0)	(1.9)
N balance, % absorbed	32.9	46.5	50.1	39.9	46.7	50.8	38.2	49.2	51.4
	(4.3)	(3.1)	(4.6)	(3.7)	(5.5)	(4.7)	(2.2)	(3.5)	(3.5)
Plasma									
Total N gm./100 ml.	0.95	0.94	0.97	1.00	1.00	0.96	0.93	0.94	0.94
	(0.03)	(0.02)	(0.04)	(0.03)	(0.03)	(0.03)	(0.03)	(0.03)	(0.06)
Prot. N gm./100 ml.	0.83	0.88	0.91	0.92	0.92	0.90	0.82	0.89	0.88
	(0.03)	(0.03)	(0.04)	(0.04)	(0.03)	(0.03)	(0.02)	(0.03)	(0.06)
NPN gm./100 ml.	0.12	0.06	0.06	0.08	0.08	0.06	0.11	0.05	0.06
	(0.01)	(0.01)	(0.01)	(0.02)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
NH_3 N mg./L.	1.05	1.05	1.16	1.11	1.04	1.10	1.15	1.10	1.14
	(0.04)	(0.04)	(0.08)	(0.05)	(0.07)	(0.05)	(0.06)	(0.04)	(0.05)

	Significant Differences[d]
Nitrogen retention	2,3,5,6,8,9 > 1,4,7**
N balance, % absorbed	2,3,5,6,8,9 > 1,4,7**

[a] See text for description of meals.

[b] Minutes autoclaved (see text for details of processing).

[c] Standard error. ** $P < .01$.

[d] Orthogonal Comparisons. Ration numbers separated only by commas represent the average for that group.

were not significant when ether extract digestibility was considered.

Differences between the soybean meal and the azeotrope-extracted cottonseed meal were not significant when digestibility of crude fiber was considered over all treatments; however, both of these meals were superior ($P < .01$) in this response criterion to the special cottonseed meal. Autoclaving had no effect upon the digestibility of crude fiber, when the soybean meal or azeotrope-extracted meal were considered; however, autoclaving for either 45 or 90 minutes improved crude fiber digestibility in the special cottonseed meal.

The digestibility of protein in the special soybean meal, when all heat treatments were combined, was higher ($P < .01$) than the protein of the cottonseed meals. Autoclaving for either 45 or 90 minutes reduced ($P < .01$) the digestibility of protein in all of the meals. Protein digestibilities of meals autoclaved for 90 minutes were lower ($P < .01$) than those of the meals autoclaved for 45 minutes.

The effect of autoclaving time upon fecal nitrogen excretion, urinary nitrogen excretion, and nitrogen retention did not differ from linearity ($P > .01$; $P > .01$; $P > .05$, respectively) when the meals were combined in regard to the different heat treatments. As there were some differences between meals, regression equations were calculated for each of the three meals. The regression equations for fecal nitrogen were as follows:

$Y = 5.488 + 0.0133X$ (special soybean meal)
$Y = 6.205 + 0.0167X$ (azeotrope cottonseed meal)
$Y = 5.840 + 0.0267X$ (special cottonseed meal)

where Y is gm. of fecal nitrogen and X is time autoclaved in minutes.

The regression equations for urinary nitrogen were as follows:

$Y = 7.140 - 0.0268X$ (special soybean meal)
$Y = 5.986 - 0.0183X$ (azeotrope cottonseed meal)
$Y = 6.061 - 0.0232X$ (special cottonseed meal)

where Y is gm. of urinary nitrogen and X is time autoclaved in minutes.

The regression equations for nitrogen retention were as follows:

$Y = 3.790 + 0.0151X$ (special soybean meal)
$Y = 4.098 + 0.0049X$ (azeotrope cottonseed meal)
$Y = 3.493 + 0.0063X$ (special cottonseed meal)

where Y is gm. of nitrogen retained and X is the time autoclaved in minutes.

When nitrogen retained was expressed as a percentage of the absorbed nitrogen, it was found that autoclaving improved ($P < .01$) all meals; differences between the three meals were not significant ($P > .05$).

Increasing the time of autoclaving in the present experiment decreased urinary nitrogen loss at a faster rate than it increased fecal nitrogen loss; thus, nitrogen retention was improved by each increase in time when the special meals were considered. This could indicate that further heating could result in further improvement in nitrogen retention as long as the decreases in urinary nitrogen loss would compensate for increases in fecal nitrogen loss. Miller and Morrison (1944) heated solvent-extracted soybean meal at 250° F for 70 minutes and found an improvement in nitrogen retention of sheep in comparison to the same product heated for a shorter period of time.

Nitrogen components in peripheral blood plasma are shown in Table VI. No differences between treatments were obtained when total nitrogen, protein nitrogen, and ammonia nitrogen were the response criteria; however, sheep fed the unheated special soybean meal or unheated special cottonseed meal had higher levels of plasma non-protein nitrogen than those fed the unheated azeotrope-extracted meal or any of the heated meals. Heating for 45 minutes was just as effective in reducing the plasma non-protein nitrogen level as heating for 90 minutes.

The effect of various treatments upon ruminal ammonia levels are shown in Table VII. Sheep consuming the unheated meals had higher ($P < .05$) ruminal ammonia values during the second, third, and fourth hours after feeding than those receiving the meals which were heated for either 45 or 90 minutes; differences between autoclaving for 45 or 90 minutes were not significant.

The results of the sheep growth experiment are shown in Table VIII. Autoclaving either the soybean or cottonseed meals improved ($P < .05$) daily gains and efficiency of feed utilization by sheep.

The basic principles concerning the utilization of dietary proteins by ruminants were established by McDonald (1948, 1952), Annison et al. (1954), Chalmers et al. (1954), Chalmers and Synge (1954a, b), and Lewis (1957), who reported that soluble proteins are rapidly hydrolyzed by the rumen microflora causing high ruminal ammonia levels, high plasma non-protein nitrogen levels, high digestibility of dietary nitrogen, high urinary nitrogen excretion, and low retention of dietary nitrogen. They found that any treatment which reduces the solubility of the protein or provides carbon fragments and other factors which are needed for microbial synthesis of protein will reduce ruminal ammonia, reduce the digestibility of dietary nitrogen, lower the plasma non-protein nitrogen level, decrease urinary nitrogen losses, and increase the retention of dietary nitrogen. Inspection of Tables I, V, VI, VII, and VIII reveals that the results obtained in the present experiment, when the special cottonseed and soybean meals were fed to sheep, conform to these basic principles. The azeotrope-extracted cottonseed meal, in general, followed the same trends with the exception that the unheated product appeared to be less

TABLE VII

EFFECTS OF HEATING PROTEIN SUPPLEMENTS ON LEVEL OF RUMINAL AMMONIA

Meal designation[a]	Special SBM			Azeotrope CSM			Special CSM		
Heat treatment[b]	0	45	90	0	45	90	0	45	90
Ration number	1	2	3	4	5	6	7	8	9
Hours after feeding	millimoles NH_3/liter rumen fluid								
0	6.71	8.63	10.04	8.78	6.89	7.31	6.84	9.87	8.66
	(1.49)[c]	(0.82)	(3.63)	(1.03)	(1.53)	(1.60)	(1.12)	(1.83)	(2.50)
1	15.16	9.81	9.87	10.41	7.73	9.48	12.67	11.43	9.89
	(3.10)	(0.88)	(1.26)	(3.29)	(1.41)	(2.08)	(1.94)	(2.34)	(2.20)
2	22.91	11.44	13.26	12.97	10.69	9.54	14.24	13.97	10.09
	(4.79)	(2.38)	(2.95)	(1.55)	(2.21)	(2.03)	(2.63)	(1.64)	(2.93)
3	20.03	17.59	15.74	17.13	11.81	9.93	23.92	11.84	13.42
	(2.23)	(2.81)	(2.66)	(1.77)	(2.34)	(2.39)	(2.11)	(0.93)	(2.41)
4	17.45	17.13	15.09	15.71	11.62	11.43	19.97	11.61	11.78
	(2.76)	(1.31)	(2.47)	(1.20)	(2.55)	(1.33)	(1.72)	(1.79)	(1.99)
5	14.76	16.95	14.81	12.37	10.97	11.14	18.76	10.79	9.84
	(1.58)	(3.54)	(1.89)	(1.89)	(0.99)	(2.58)	(2.12)	(2.42)	(1.08)
6	11.40	17.29	14.77	12.11	11.09	10.73	17.40	10.48	8.86
	(1.80)	(2.38)	(2.19)	(2.62)	(2.14)	(1.08)	(2.11)	(2.15)	(1.83)
8	11.30	15.96	12.98	11.70	9.41	11.06	14.54	9.89	9.03
	(1.99)	(2.69)	(2.49)	(1.21)	(2.21)	(2.11)	(1.67)	(2.51)	(1.50)
10	8.52	10.71	13.28	9.45	7.39	9.44	9.37	9.93	8.71
	(2.56)	(3.41)	(2.33)	(2.58)	(1.31)	(1.85)	(1.52)	(1.48)	(1.05)
12	7.63	8.50	10.61	8.52	6.93	7.26	7.39	9.88	8.43
	(2.17)	(1.72)	(1.29)	(2.20)	(1.23)	(2.21)	(1.70)	(1.43)	(1.76)
Total	135.87	134.01	130.45	119.15	94.53	97.32	145.10	109.69	98.71

	Significant Differences[e]
1	1,4,7>2,3,5,6,8,9[d]
2	1,4,7>2,3,5,6,8,9*
3	1,4,7>2,3,5,6,8,9**
4	1,4,7>2,3,5,6,8,9**

[a]See text for description of meals.
[b]Minutes autoclaved (see text for details of processing).
[c]Standard error. [d]$P < .10$; *$P < .05$; **$P < .01$.
[e]Orthogonal Comparisons. Ration numbers separated only by commas represent the average for that group.

TABLE VIII

DAILY GAIN AND FEED CONVERSION OF LAMBS FED VARIOUS PROTEIN SUPPLEMENTS

Protein supplement[a]	Special SBM		Special CSM		
Heat treatment[b]	0	45	0	45	Significant
Ration number	1	2	3	4	Differences[d]
Number of animals	4	4	4	4	
Av. daily gain, lb.	0.07 (0.02)[c]	0.20 (0.04)	0.10 (0.06)	0.16 (0.04)	2,4>1,3*
Av. daily feed consumption, lb.	1.70 (0.06)	1.70 (0.06)	1.70 (0.09)	1.60 (0.09)	- - - -
Feed efficiency, feed/gain, lb.	30.7 (8.0)	10.2 (2.9)	22.9 (7.0)	12.4 (4.4)	1,3>2,4*

[a]See text for description of meals.
[b]Minutes autoclaved (see text for details of processing).
[c]Standard error.
[d]Orthogonal Comparisons. Ration numbers separated only by commas represent the average for that group.
*P<.05.

soluble than the special meals and autoclaving for 45 minutes did not affect fecal nitrogen loss, ruminal ammonia levels, or plasma non-protein nitrogen levels as greatly as when the special meals were heated for 45 minutes. Some denaturation of the protein of the azeotrope-extracted meal may have occurred during extraction.

The results of the rat growth trial are shown in Table IX. Rats consuming rations containing the special cottonseed meal, heated or unheated, were all dead within 11.4 days; thus, the performance data of these animals were not included in the statistical analyses. Daily gains were significantly ($P<.01$) lower in the rats fed the unheated soybean, autoclaved azeotrope cottonseed, and unheated Oklahoma cottonseed meals than in those fed all other rations; however, the azeotrope meal autoclaved 45 minutes promoted a significantly ($P<.01$) faster daily gain than the unheated special soybean meal or Oklahoma cottonseed meal. Daily feed consumption of the ration containing the autoclaved azeotrope cottonseed meal was significantly ($P<.05$) lower than when this meal was unheated. Feed efficiency was significantly ($P<.01$) lower in the unheated soybean meal and unheated Oklahoma cottonseed meal than when these meals were heated. The unheated soybean meal and unheated Oklahoma cottonseed meal were less ($P<.01$) efficiently utilized than the autoclaved azeotrope meal, while the unheated soybean meal was less ($P<.01$) efficiently utilized than the unheated Oklahoma cottonseed meal.

Woods et al. (1957, 1958, 1962) and Tillman and Kruse (1962) reported that gossypol and heat applied during processing were the major factors affecting the nutritive value of cottonseed meal for ruminants. In those trials, it was found that nitrogen digestibility of cottonseed meals was

TABLE IX

DAILY GAIN AND FEED CONVERSION OF RATS FED VARIOUS PROTEIN SUPPLEMENTS

Protein supplement[a]	Casein		Special SBM[b]		Azeotrope CSM[b]		Oklahoma CSM		Special CSM	
Heat treatment[c]	0	45	0	45	0	45	0	45	0	45
Ration number	1	2	3	4	5	6	7	8	9	10
Number of animals	10	10	10	10	10	10	10	10	10	10
Av. daily gain, gm.	2.7 (0.2)[e]	2.7 (0.2)	0.6 (0.2)	2.8 (0.3)	3.1 (0.2)	1.8 (0.1)	0.8[d] (0.3)	2.8 (0.1)	-	-
Av. daily feed consumption, gm.	11.9 (0.4)	11.8 (0.4)	8.0 (0.5)	12.9 (0.7)	13.3 (0.5)	11.2 (0.4)	6.9 (0.4)	12.8 (0.2)	5.5	4.4
Feed efficiency feed/gain, gm.	4.3 (0.1)	4.6 (0.2)	391.6 (150.6)	5.0 (0.5)	4.4 (0.2)	6.3 (0.3)	11.0 (4.1)	4.6 (0.2)	-	-
% mortality.	0	0	0	0	0	0	40	0	100	100
Av. survival time, days.	-	-	-	-	-	-	9.0	-	4.0	11.4

	Significant Differences[f]
Av. daily gain	1,2,4,5,8>3,6,7**; 6>3,7**
Av. daily feed consumption	1,2,4,5,6,8>3,7**; 5>6**
Feed efficiency	3,6,7>1,2,4,5,8**; 3,7>6**; 3>7**

[a]See text for description of meals.
[b]SBM-soybean meal; CSM-cottonseed meal.
[c]Minutes autoclaved (see text for details of processing).
[d]Calculated on the 6 surviving animals.
[e]Standard error.
[f]Individual mean comparisons.
**$P < .01$.

related to nitrogen solubility and bound gossypol levels. The results of the present studies (Tables V and VI) reveal that the azeotrope-extracted cottonseed meal, which contained very low levels of total and bound gossypol, was not superior to the special cottonseed meal containing much higher levels of gossypol, indicating that gossypol is considerably less important than the effects of heating upon ruminant utilization of cottonseed protein. Chick (Mann et al. 1962) and rat (Table IX) tests offer evidence that the low gossypol azeotrope-extracted cottonseed meal is a satisfactory source of protein for non-ruminants. However, nothing is known regarding treatment of the azeotrope meal prior to the time it was extracted with the azeotrope solvent.

Summary

Digestibility, nitrogen-retention, and growth trials were conducted to determine the effects of three heat treatments upon the utilization of cottonseed and soybean meals. One of the cottonseed meals was extracted with a mixture of acetone, hexane, and water (azeotrope) and contained a low level of total and bound gossypol while the soybean meal and the other cottonseed meal were produced by extracting with cold hexane with no heat being applied during extraction. Portions of each of the above products were then subjected to the following heat treatments: (1) no heat, (2) autoclaved under 15 lb. steam pressure per square inch at 250° F for 45 minutes, and (3) autoclaved for 90 minutes. The digestibility and nitrogen-retention trials were conducted using crossbred wethers in a 3 x 3 factorial experiment.

Organic matter digestibility was higher ($P < .01$) in the rations containing the soybean meal than those containing both cottonseed meals when

the meals were compared with all heat treatments combined. The azeotrope-extracted cottonseed meals had higher ($P < .01$) digestibility of organic matter than the special cottonseed meals. Autoclaving for 45 minutes improved ($P < .05$) organic matter digestibility when compared to all unheated meals with no further improvement from autoclaving for 90 minutes. When digestibility of crude fiber was considered over all treatments the soybean and azeotrope-extracted cottonseed meals were superior ($P < .01$) to the special cottonseed meal. Autoclaving improved crude fiber digestibility in the special cottonseed meal, but had no effect on the crude fiber digestibility of the rations containing the other meals. Autoclaving had no effect upon nitrogen-free and ether extract digestibilities when all meals were considered. Protein digestibility was reduced ($P < .01$) in all meals after autoclaving for either 45 or 90 minutes. The effect of increased autoclaving times did not differ from linearity ($P > .05$) when fecal nitrogen loss (increased), urinary nitrogen loss (decreased), and nitrogen retention (increased) were considered for all meals. Autoclaving reduced ruminal ammonia and plasma non-protein nitrogen levels.

The special soybean and cottonseed meals autoclaved for 0 and 45 minutes were fed to growing lambs in a completely random experiment. Average daily gain and feed efficiency were higher ($P < .05$) when the animals consumed the autoclaved meals.

EXPERIMENT II

In the previous experiment, nitrogen retention in sheep was improved by autoclaving in increments that did not differ from linearity when the cold hexane-extracted cottonseed meal was autoclaved under 15 lb. steam pressure for 0, 45, and 90 minutes. Urinary nitrogen loss was inversely and fecal nitrogen loss directly related to autoclaving time; and urinary nitrogen loss decreased at a faster rate than the fecal nitrogen loss increased. These results were interpreted as evidence that longer autoclaving times could result in further increases in nitrogen retention as long as the decreased urinary nitrogen loss compensated for the increased fecal nitrogen loss. The studies in this experiment were designed to study the effects of various autoclaving times up to 240 minutes upon the utilization of cottonseed meal by ruminants.

Experimental Procedure

Cottonseed as in Experiment I were extracted with cold hexane with no heat being applied at any time prior, during, or after extraction. Portions of this meal were then subjected to six different heat treatments: (1) no heat, (2) autoclaved under 15 lb. per square in. steam pressure at 250° F for 20 minutes, (3) autoclaved under the same conditions for 60 minutes, (4) autoclaved for 120 minutes, (5) autoclaved for 180 minutes, and (6) autoclaved for 240 minutes. For autoclaving, the meal was leveled at a uniform depth of 1/2 inch in metal pans lined with heavy paper and after autoclaving all meals were dried and reground to their original

particle size. Composition of the meals is shown in Table X.

Digestibility and Nitrogen Retention Studies

Fifteen crossbred wethers averaging 99 lb. and about 8 months of age were used in a completely random experiment with an orthogonal set of treatments (excluding the ration containing the meal autoclaved for 20 minutes) in two trials using three animals per treatment in each trial. The animals were randomly assigned to the treatments in each trial. Following completion of the second trial six animals were randomly selected for a third trial and fed the ration containing the cottonseed meal autoclaved for 20 minutes. All rations were isonitrogenous and were essentially isocaloric and their compositions are shown in Table XI. The animals were placed in metabolism stalls (Briggs and Gallup, 1949) for a 7-day adjustment period preceding successive 10-day preliminary and 10-day collection periods. The animals were removed from the metabolism stalls for a 10-day rest period between successive trials.

Each lamb was fed 550 gm. of his respective ration twice daily during all trials; water was available at all times. This level of intake furnished nutrient levels which were above the maintenance requirements established by the National Research Council (N.R.C., 1957). Feces and urine were collected and prepared for analyses as described by Tillman and Swift (1953).

Proximate analyses of feed, feces, and urine were determined by the methods of A.O.A.C. (1960). Nitrogen solubility of the cottonseed meals was determined by the method described by Lyman et al. (1953). Analyses for total, free, and bound gossypol in the cottonseed meals were conducted by the methods of Pons and Hoffpauir (1957) and Pons et al. (1958).

TABLE X

CHEMICAL ANALYSES OF COTTONSEED MEALS USED IN ALL STUDIES

Heat Treatment[a]	0	20	60	120	180	240
Particular Components						
Total gossypol, %	1.41	1.22	0.90	0.58	0.38	0.25
Free gossypol, %	0.99	0.50	0.25	0.11	0.06	0.05
Bound gossypol, %	0.42	0.72	0.65	0.47	0.32	0.20
Nitrogen soluble in 0.02 N NaOH, % of total	78.2	35.8	30.1	21.5	23.3	25.5
Proximate composition, % of dry matter						
Organic matter	92.2	92.2	92.2	92.1	92.0	91.9
Ash	7.8	7.8	7.8	7.9	8.0	8.1
Protein (N x 6.25)	49.4	49.7	49.0	50.3	49.6	50.7
Ether Extract	5.1	6.5	4.4	4.4	4.8	5.3
Crude fiber	8.7	6.7	6.4	7.5	7.7	7.2
NFE	29.0	29.3	32.4	29.9	29.9	28.7

[a]Minutes autoclaved (see text for details of processing).

TABLE XI

COMPOSITION OF COTTONSEED MEAL RATIONS USED IN SHEEP DIGESTIBILITY AND NITROGEN RETENTION STUDIES

Heat Treatment of CSM[a]	0	20	60	120	180	240
Ingredient	%	%	%	%	%	%
Cottonseed meal	22.7	22.5	22.1	21.5	22.1	21.7
Corn dextrose	17.8	17.8	17.8	17.8	17.8	17.8
Corn starch	17.8	17.8	17.8	17.8	17.8	17.8
Corn oil	2.0	2.0	2.0	2.0	2.0	2.0
Cottonseed hulls	8.9	8.9	8.9	8.9	8.9	8.9
Mineral mix[b]	5.0	5.0	5.0	5.0	5.0	5.0
Purified cellulose[c]	25.7	25.9	26.3	26.9	26.3	26.7
Vitamins A and D[d]	0.1	0.1	0.1	0.1	0.1	0.1
Total	100.0	100.0	100.0	100.0	100.0	100.0
Proximate composition, dry matter basis, %						
Organic Matter	93.5	93.5	93.3	93.4	93.3	93.2
Ash	6.5	6.5	6.7	6.6	6.7	6.8
Crude Protein	11.6	11.4	11.9	12.0	11.8	12.1
Ether Extract	8.3	6.6	7.6	7.0	8.0	7.5
Crude Fiber	28.7	27.3	29.1	28.8	28.2	28.8
NFE	44.9	48.2	44.7	45.6	45.3	44.8

[a]Minutes autoclaved (see text for details of processing).
[b]Oltjen *et al.* (1959).
[c]"Solka floc," Brown & Company, Berlin, New Hampshire.
[d]Contained 20,000 I.U. and 2,500 U.S.P. units per gram of vitamins A and D, respectively.

The data were analyzed statistically by analysis of variance with orthogonal comparisons being made between combined groups using the meal autoclaved for 60 minutes as the control. Data on the ration containing the cottonseed meal autoclaved for 20 minutes were not included in the orthogonal comparisons; this treatment was compared to the others using the multiple range test described by Duncan (1955).

Rumen Fluid and Blood Studies

Two crossbred sheep averaging 75 lb. and fitted with permanent ruminal fistulae were used for in vivo rumen fluid and blood studies. The animals were fed each of the rations (Table XI) containing the special cottonseed meal autoclaved for 0, 60, 120, 180, and 240 minutes for a preliminary period of 10 days prior to collection of samples of rumen fluid and peripheral venous blood. The sequential order in which the rations were fed was determined by random selection before initiation of the first preliminary feeding period; this sequential order was the rations containing the cottonseed meal autoclaved for 240, 120, 60, 180, and 0 minutes, respectively. The animals were maintained on a standardization ration for a period of at least 7 days between each 10-day preliminary feeding period.

The animals were individually-fed 425 gm. twice daily of the respective experimental ration. This level was held constant throughout the entire experiment to insure that all feed allotted would be consumed within a short time after feeding. Water was available at all times during the 10-day preliminary feeding period. On the day before sampling the water was removed after the animals had been allowed to drink following the night feeding. The animals were allowed to drink approximately 1/2 gallon

of water at the morning feeding on the day of sampling. This was done to partially equalize the volume of rumen contents between the two sheep. Response criteria were levels of ruminal ammonia, ruminal volatile fatty acids, plasma urea, plasma ammonia, and molar percentages of ruminal volatile fatty acids.

Peripheral blood and rumen fluid were collected so the approximate midpoint of the sampling period would correspond with the times of maximum concentrations of ruminal ammonia and blood urea after feeding. These were based on the results of Lewis et al. (1957), Stallcup and Looper (1958), and Tagari et al. (1962) who reported that maximum ruminal ammonia levels were reached between 1 and 3 hours after feeding when oil meals constituted the protein source in the ration. Lewis (1957) and Tagari et al. (1962) also found that a time interval of approximately 4 hours elapsed between maximum ruminal ammonia levels and subsequent maximum urea concentrations in the peripheral blood which corresponded to a period of between 5 and 7 hours after feeding. These investigators also reported that both ruminal ammonia and blood urea reached only one maximum concentration then returned to prefeeding levels during the next several hours.

Samples of rumen fluid were taken immediately before feeding and at hourly intervals for 4 hours afterwards. Rumen fluid was removed through the rumen fistulae at each sampling interval using a suction strainer technique (Raun and Burroughs, 1962). The pH was determined immediately after collection with a portable Beckman pH meter. Solid food particles were removed by centrifugation and the supernatant was stored at -10° F until analyzed. Blood samples were taken by jugular puncture immediately before and at hourly intervals for 8 hours following feeding. Heparin was used as the anti-coagulant and the plasma was separated by

centrifugation and stored at -10° F until analyzed. The samples taken from 0 to 4 hours were analyzed for ammonia and those taken from 4 to 8 hours for urea.

Artificial Rumen Studies

In vitro fermentations were conducted in duplicate by the method described by Kuhlman (1963) using rumen fluid from the animals after they had received the standardization ration for several days. Urea was omitted from the mineral solution and the rations (Table XI) containing the cottonseed meal autoclaved for 0, 60, 120, 180, and 240 minutes were added to supply the equivalent amount of nitrogen. This required 3.2 gm. of the rations per flask. The sampling intervals and preparation of the samples for storage were the same as that used for the in vivo rumen samples. Response criteria were volatile fatty acid production and molar percentages of the volatile fatty acids.

Ruminal ammonia, plasma ammonia, and urea analyses were determined by the methods described by Conway (1957). Samples were prepared for volatile fatty acid analyses by the method described by Erwin et al. (1961). Volatile fatty acids were separated using an Aerograph Model A-600-B "Hy-Fi" gas chromatograph equipped with a hydrogen flame ionization detector and recorder. The data were analyzed statistically by analysis of variance and orthogonal comparisons were made between combined groups.

Sheep Growth Studies

The special cottonseed meal was autoclaved for 0, 60, 120, and 240 minutes and the four meals were compared in a 30-day lamb growth trial by feeding isonitrogenous rations containing two (2:1 and 1:2) concentrate: roughage (C:R) ratios. Compositions of the rations are shown in Table XII.

TABLE XII

COMPOSITION OF COTTONSEED MEAL RATIONS USED IN SHEEP GROWTH STUDIES

Concentrate:Roughage Ratio	1:2				2:1			
Heat treatment of CSM[a]	0	60	120	240	0	60	120	240
Ingredient	%	%	%	%	%	%	%	%
Cottonseed meal	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
Corn dextrose	3.6	3.6	3.6	3.6	20.2	20.2	20.2	20.2
Corn starch	3.7	3.7	3.7	3.7	20.3	20.3	20.3	20.3
Corn oil	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Cottonseed hulls	33.3	33.3	33.3	33.3	16.7	16.7	16.7	16.7
Purified cellulose[b]	33.3	33.3	33.3	33.3	16.7	16.7	16.7	16.7
Mineral mix[c]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Dicalcium phosphate	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Limestone ($CaCO_3$)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamins A and D[d]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Proximate composition dry matter basis, %								
Organic matter	95.1	94.9	95.0	95.0	95.3	95.1	94.9	95.3
Ash	4.9	5.1	5.0	5.0	4.7	4.9	5.1	4.7
Crude protein[e]	11.2	11.1	11.3	11.2	11.3	11.2	11.4	11.5
Ether extract	6.2	5.9	4.3	4.5	6.7	5.9	6.0	5.0
Crude fiber	44.7	45.0	42.6	46.5	23.8	24.3	24.1	25.4
NFE	33.0	32.9	36.8	32.8	53.5	53.7	53.4	53.4

[a]Minutes autoclaved (see text for details of processing).
[b]"Solka Floc," Brown and Company, Berlin, New Hampshire.
[c]Oltjen et al. (1959).
[d]Contained 20,000 I.U. and 2,500 USP units per gram A and D, respectively.
[e]Includes only cottonseed meal protein.

Forty-eight crossbred lambs averaging 62 lb. were randomly allotted within sex to a 2 x 4 factorial arrangement of treatments with six animals per treatment. The animals were fed individually twice daily more of their rations than they would consume; uneaten feed was removed, weighed, and discarded every third day. The sheep were weighed every two weeks. Feed and water were removed for a 16 hour shrink preceding the initial and final weighings. Response criteria were weight gains, feed consumption, and feed efficiency. Differences between treatments were tested for significance by analysis of variance and orthogonal comparisons.

Results and Discussion

Digestibility and Nitrogen-Retention Studies

Digestion coefficients and nitrogen balance data are presented in Table XIII. All autoclaved meals had higher organic matter digestibilities than the unheated meal and autoclaving for 60 minutes resulted in improved ($P<.10$) organic matter digestibility when compared to other heated meals. These results are in agreement with those of Experiment I in which it was found that organic matter digestibility was improved by autoclaving the cottonseed meal for 45 minutes and that no further improvement was obtained by autoclaving it for 90 minutes.

Ether extract digestibility was higher ($P<.05$) in the rations containing autoclaved meals than in that with the unheated meal. Nitrogen-free extract digestibility was similar for all rations and orthogonal comparisons did not reveal any significant differences between the rations.

The digestibility of crude fiber was higher ($P<.10$) by the animals consuming the meal autoclaved for 60 minutes than in other heated meals and,

TABLE XIII

EFFECTS OF HEATING COTTONSEED MEAL ON SHEEP DIGESTIBILITY OF RATION COMPONENTS AND RETENTION OF DIETARY NITROGEN

Heat treatment of CSM[a]	0	60	120	180	240	
Ration Number	1	2	3	4	5	
Number of animals	6	6	6	6	6	Significant Differences[d]
Digestibility, %						
Organic matter	61.7 (0.8)[b]	67.1 (1.1)	65.6 (2.3)	63.0 (1.5)	64.4 (2.0)	2>1,3,4,5[c]
Protein	53.6 (1.7)	48.9 (1.6)	42.8 (1.5)	36.8 (0.6)	33.3 (1.1)	2>1,3,4,5**; 1>3,4,5**; 3>4,5**; 4>5[c]
Ether extract	81.8 (1.4)	86.7 (1.1)	82.1 (1.7)	82.1 (1.7)	85.4 (1.1)	2>1,3,4,5*
Crude fiber	36.6 (1.7)	54.1 (3.3)	50.6 (5.4)	47.4 (3.9)	50.3 (4.1)	2>1,3,4,5[c]; 1<3,4,5*
N-free extract	76.1 (1.4)	77.1 (1.2)	78.6 (1.5)	76.8 (0.9)	78.4 (1.6)	- - - - - - - - - - - -
Nitrogen balance, gm.						
Nitrogen intake	18.04	18.42	18.95	18.47	18.84	- - - - - - - - - - - -
Nitrogen in feces	8.38	9.41	10.83	11.67	12.57	- - - - - - - - - - - -
Nitrogen in urine	5.97	4.68	4.38	4.13	3.68	- - - - - - - - - - - -
Nitrogen retention	3.69 (0.14)	4.34 (0.47)	3.74 (0.33)	2.67 (0.36)	2.60 (0.30)	2>1,3,4,5**; 1>3,4,5[c]; 3>4,5*
N balance, % intake	20.4 (0.8)	23.6 (2.5)	19.7 (1.8)	14.5 (1.9)	13.8 (1.6)	2>1,3,4,5**; 1>3,4,5**; 3>4,5**
N balance, % absorbed	38.5 (2.4)	47.8 (4.5)	45.9 (3.5)	39.0 (5.0)	41.1 (4.3)	- - - - - - - - - - - -

[a]Minutes autoclaved (see text for details of processing). [b]Standard error. [c]P<.10; *P<.05; **P<.01. [d]Orthogonal Comparisons. Ration numbers separated only by commas represent the average for that group

as in Experiment I, all of the autoclaved meals caused higher ($P<.05$) crude fiber digestibility than the unheated meal. These results are also in agreement with those of Annison et al. (1954), Chalmers et al. (1954), and Lewis and McDonald (1958), in which it was reported that cellulose digestibility and utilization were reduced when ruminants were fed highly soluble proteins.

Protein digestibility was successively reduced with increased autoclaving times. The unheated meal and that autoclaved for 60 minutes had higher ($P<.01$) protein digestibility than those autoclaved for 120, 180, or 240 minutes. Similarly, the cottonseed meal autoclaved for 120 minutes had a higher ($P<.01$) protein digestibility than when autoclaved for 180 or 240 minutes and that autoclaved for 180 minutes higher than the 240 minutes autoclaving time ($P<.10$).

Nitrogen retention was higher ($P<.01$) in sheep fed the meal autoclaved for 60 minutes than for those fed the other meals. The unheated meal and the one autoclaved for 120 minutes promoted greater ($P<.05$) nitrogen retention than the meals autoclaved for 180 and 240 minutes.

The nitrogen balance data in this experiment are shown graphically in Figure 1 and it can be seen that fecal nitrogen losses increased and urinary nitrogen losses decreased and that neither differed from linearity ($P<.01$) when plotted against autoclaving times. Regression equations were $Y = 8.420 + .0181X$ and $Y = 5.600 - .0086X$ for fecal and urinary nitrogen, respectively, where X is autoclaving time in minutes and Y is gm. nitrogen excreted. Both the linear and cubic component of the curve were significant ($P<.01$ and $P<.05$, respectively) for nitrogen retention, indicating that a significant deviation from linearity existed between autoclaving time and grams of nitrogen retained. Autoclaving for

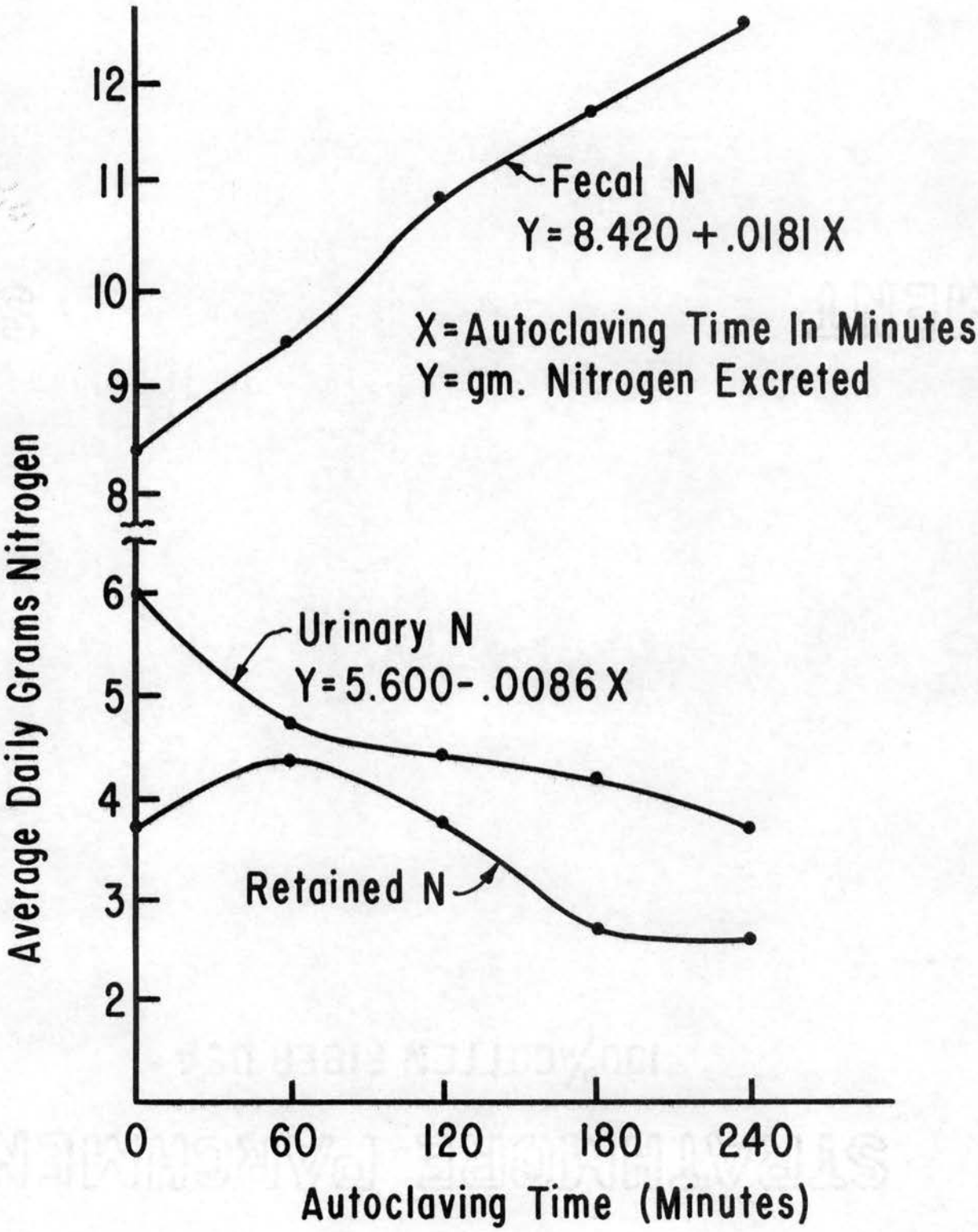

Figure 1. Effects of Heating Cottonseed Meal on the Nitrogen Excretion and Retention Patterns of Sheep.

60 minutes resulted in increased nitrogen retention but successive increases in time caused decreased nitrogen retention at a reasonably constant rate up to the 180 minute time; the rate of decrease in nitrogen retention was slower when time was extended from 180 to 240 minutes. This probably accounts for the significant cubic response.

There was a significant ($P<.01$) quadratic response for nitrogen retention when autoclaving times between 0 and 180 minutes were considered. In Experiment I, a linear increase in nitrogen retention was found when autoclaving time was extended up to 90 minutes; thus it was proposed that there must be a point at which increased fecal nitrogen loss would not be compensated by decreased urinary nitrogen loss. If such a point were reached further heating would result in decreased nitrogen retention. Apparently, heating for 120 minutes was sufficient to obtain this effect.

Fundamental relationships between proteolysis in the rumen and protein solubility were reported by McDonald (1948, 1952), Annison et al. (1954), Chalmers et al. (1954), Chalmers and Synge (1954a, b), and Lewis (1957), who established that the rate of proteolysis and level of free ammonia in the rumen were proportional to the solubility of the protein. It was found that free ruminal ammonia could be absorbed directly into the venous blood and subsequently excreted as urinary nitrogen.

When raw cottonseed meal was autoclaved for 20 minutes, higher ($P<.05$) organic matter and nitrogen-free extract digestibilities were obtained than with all treatments. Protein digestibility and urinary nitrogen loss were not significantly ($P>.05$) different from that obtained with the raw meal so this level of heating did not improve nitrogen retention.

Ruminal ammonia and plasma nitrogen component levels in the two experiments, when the raw meal was fed, were in such close agreement that the data of this experiment are not exhibited in tabular form. In both experiments, ruminal ammonia levels increased rapidly and reached a peak level 3 hours after feeding the raw meal. Afterwards, the ruminal ammonia level steadily decreased until there was a lower level of ruminal ammonia in the animals fed the raw meal. In comparison, ruminal ammonia levels of sheep fed the autoclaved meals neither increased as fast nor reached as high levels. Also, as in Experiment I, differences between the autoclaved meals were not significant ($P > .05$). Significant ($P < .05$) and positive correlation coefficients existed between protein solubility and ruminal ammonia levels found at 3 hours after feeding ($r = .94$) and between protein solubility and plasma urea levels at 6 hours after feeding ($r = .99$). These values are in agreement with those of Lewis (1957) and lend further support to the idea that previously-described basic principles pertaining to the effect of protein solubility upon its utilization by ruminants can be used to interpret the results of these experiments.

Levels of total volatile fatty acids produced in vivo are given in Table XIV. The unheated cottonseed meal resulted in higher ($P < .05$) total volatile fatty acid concentration at all sampling times after feeding than the autoclaved meals. Total ruminal volatile fatty acid levels in sheep fed the meal autoclaved for 120 minutes was lower ($P < .05$) than in those receiving meals autoclaved for 180 or 240 minutes; the 180 minutes meal gave a lower ($P < .05$) value than that one autoclaved for 240 minutes. In comparison to the heated meals, total volatile fatty acid concentration increased at a faster rate when the raw meal was fed. Similar patterns

TABLE XIV

EFFECTS OF HEATING COTTONSEED MEAL ON TOTAL VOLATILE FATTY ACID CONCENTRATIONS IN RUMEN FLUID

Heat treatment[a]	0	60	120	180	240	Significant
Ration number	1	2	3	4	5	Differences[c]
Hours after feeding	Total millimoles/liter rumen fluid					
0	35.9 (7.8)[b]	38.2 (5.1)	18.1 (1.0)	46.7 (13.5)	86.7 (29.8)	
1	65.6 (24.1)	41.5 (11.1)	29.7 (1.2)	53.5 (8.5)	87.6 (34.4)	1>2,3,4,5*; 3<4,5*; 4<5*
2	96.8 (37.7)	51.1 (9.8)	42.4 (5.7)	54.5 (6.9)	89.9 (36.6)	1>2,3,4,5*; 3<4,5*; 4<5*
3	115.6 (52.7)	60.5 (7.0)	49.4 (2.4)	48.8 (0.8)	102.1 (45.2)	1>2,3,4,5*; 3<4,5*; 4<5*
4	140.7 (55.9)	64.0 (8.5)	58.9 (0.4)	47.8 (2.6)	103.9 (34.3)	1>2,3,4,5*; 3<4,5*; 4<5*
	Average percentage of total volatile fatty acids					
acetic	52.8	57.7	63.1	72.5	66.0	
propionic	29.5	24.1	21.2	17.4	22.0	
butyric and iso-butyric	11.9	13.2	11.9	8.0	10.7	
valeric and iso-valeric	5.8	5.0	3.7	2.1	1.3	
acetic:propionic ratio	1.8	2.4	3.0	4.2	3.0	

[a]Minutes autoclaved (see text for details of processing).
[b]Standard error.
[c]Orthogonal Comparisons. Ration numbers separated only by commas represent the average for that group.
*$P < .05$.

of volatile fatty acid production were obtained from the in vitro fermentations (Table XV). The raw cottonseed meal produced higher ($P<.05$) concentrations of volatile fatty acids and at a faster rate similar to that in the in vivo trials, thereby adding further evidence to the idea that autoclaving decreased the rate of microbial protein degradation. This idea is also supported by the results of El-Shazly (1952a), Sirotnak et al. (1953), and Annison (1954, 1956) who observed increased concentrations of total volatile fatty acids with decreases in the relative proportions of acetic acid when highly soluble proteins were fed.

The general pattern of change in ratio of acetic and propionic acids in these studies was an increase in percent acetic and a decrease in percent propionic as the autoclaving time of the cottonseed meal increased. The molar percentages of the various acids for each heat treatment were relatively constant at all sampling intervals. It is interesting to note that the increases in percent acetic acid were associated with the higher crude fiber digestibilities (Table XIII) and decreases in protein solubility of the cottonseed meals (Table X), indicating that the highly soluble unheated cottonseed meal provided optimum nitrogen to the rumen microorganisms only long enough for the soluble carbohydrate fraction to be converted to propionic acid, but when the cellulolytic microorganisms became active breaking down cellulose a limited nitrogen supply reduced their activities.

The percentages of butyric and valeric acids in these studies were directly related to protein solubility and ruminal ammonia levels. Increases in the proportions of the longer chain volatile fatty acids were closely related to increased ruminal ammonia production when the more soluble protein supplements constituted the dietary protein. El-Shazly

TABLE XV

EFFECTS OF HEATING COTTONSEED MEAL ON TOTAL VOLATILE FATTY ACID CONCENTRATIONS IN VITRO

Heat treatment[a]	0	60	120	180	240	Significant
Ration number	1	2	3	4	5	Differences[c]
Hours incubation	Total millimoles/liter					
0	98.4	98.4	98.4	98.4	98.4	
1	116.9 (2.0)[b]	102.5 (2.2)	106.4 (4.9)	112.0 (1.9)	109.4 (1.3)	1>2,3,4,5*
2	130.0 (2.6)	130.6 (3.2)	115.2 (2.7)	120.3 (1.6)	123.8 (3.5)	1>2,3,4,5*
3	154.2 (4.1)	134.2 (4.1)	132.1 (1.8)	123.6 (3.5)	127.5 (2.7)	1>2,3,4,5*
4	173.4 (2.2)	149.6 (3.8)	147.8 (6.2)	142.0 (1.9)	136.2 (1.7)	1>2,3,4,5*
Average percentage of total volatile fatty acids						
acetic	58.7	63.6	67.4	70.1	70.7	
propionic	24.0	21.4	19.0	17.8	17.3	
butyric and iso-butyric	11.2	10.6	10.2	9.5	9.1	
valeric and iso-valeric	6.1	4.4	3.4	2.6	2.9	
acetic:propionic ratio	2.4	3.0	3.6	3.9	4.1	

[a]Minutes autoclaved (see text for details of processing).
[b]Standard error.
[c]Orthogonal Comparisons. Ration numbers separated only by commas represent the average for that group.
*$P < .05$.

(1952a) observed a similar relationship between the ruminal concentrations of ammonia and of the longer chain volatile fatty acids. Annison (1954, 1956) also found that the mixture of volatile fatty acids resulting from protein degradation in the rumen contained considerably higher proportions of butyric and valeric acids than is normally found in rumen contents.

Sheep Growth Studies

The results of the growth trial are presented in Table XVI. Average daily feed consumption was higher ($P<.01$) in the animals fed the 1:2 concentrate roughage (C:R) ratio than by those fed the 2:1 C:R ratio. Differences in average daily gain and feed efficiency were not significant between the ratios. Average daily gain and feed efficiency were higher ($P<.05$) when the animals consumed the cottonseed meal autoclaved for 60 minutes than all other heat treatments. The ratio X time (1:2 vs 2:1 X 0 vs 120, 240) interaction was significant ($P<.05$) for average daily gain and feed efficiency, indicating that response to each C:R ratio was dependent upon autoclaving time. Also responses to rations containing different C:R ratios with meals autoclaved for 0 and 240 minutes were different; average daily gain and feed efficiency were higher when the unheated meal was fed in the 1:2 C:R ratio than in the 2:1 C:R ratio, however, the reverse was true when the meal was autoclaved for 240 minutes. Differences in average daily gain and feed efficiency between the two C:R ratios were of greater magnitude when unheated cottonseed meal was the protein source than when the meal was autoclaved for 240 minutes and, perhaps, indicates that response to the rations containing the unheated meal contributed the larger portion of the variance component associated with the interaction. Variance components of the other interactions were small and did not

TABLE XVI

DAILY GAIN AND FEED CONVERSION OF LAMBS FED THE COTTONSEED MEALS IN RATIONS CONTAINING DIFFERENT CONCENTRATE:ROUGHAGE RATIOS

Concentrate:Roughage Ratio	1:2				2:1			
Autoclaving Time, Minutes[a]	0	60	120	240	0	60	120	240
Number of Animals	6	6	6	6	6	6	6	6
Av. daily gain, lb.	0.45 (0.06)[b]	0.50 (0.07)	0.39 (0.05)	0.32 (0.06)	0.25 (0.06)	0.48 (0.06)	0.39 (0.06)	0.36 (0.06)
Av. daily feed consump., lb.	2.83 (0.25)	2.65 (0.27)	2.75 (0.17)	2.78 (0.16)	1.94 (0.23)	2.22 (0.18)	2.43 (0.19)	2.39 (0.12)
Feed efficiency, fed/gain, lb.	6.6 (0.4)	5.6 (0.6)	7.6 (1.0)	10.5 (2.0)	11.6 (3.3)	4.8 (0.4)	6.8 (0.9)	8.0 (1.6)

	Significant Differences[c]		
Between,	Av. Daily Gain	Av. Daily Feed Consump.	Feed efficiency, feed/gain
Concentrate:Roughage ratios	- - - - -	1:2>2:1**	- - - - -
Autoclaving Times	60>0,120,240*	- - - - -	60>0,120,240*

[a]Minutes autoclaved (see text for details of processing).
[b]Standard error.
[c]Orthogonal Comparisons. Ration numbers separated only by commas represent the average for that group. *P<.05; **P<.01.

contain a significant amount of the total interaction variance.

Feed consumption and sheep gains were somewhat lower when raw cottonseed meal was fed in the 2:1 than in the 1:2 ratio. These results are not in agreement with those reported by Phillipson et al. (1959), Chalmers and Synge (1954a), and Lewis and McDonald (1958) who found that highly soluble proteins were more efficiently utilized when soluble carbohydrates such as starches or sugars were added to the ration. A possible explanation of the apparent deviation of the results in this experiment could concern the effect of rate of food passage through the reticulo-rumen and ruminal pH upon the detoxification of gossypol in the rumen. Food passage would be faster in animals fed the 2:1 C:R ratio (Balch, 1957) so time for ruminal gossypol detoxification reactions would be reduced. Dietary proteins are less soluble in acid mediums (Preston, 1963) and gossypol detoxification in the rumen is dependent upon soluble proteins (Reiser and Fu, 1962). In this experiment, rumen fluid from animals fed the 2:1 C:R ratio had lower rumen pH (2.5×10^{-1} greater average concentration of hydrogen ion per liter) than those fed the 1:2 C:R ratio. The rumen pH readings were 5.9 and 5.3 at 3 hours after feeding for the animals receiving the 1:2 and 2:1 C:R ratios, respectively. These conditions may have allowed some of the free gossypol to escape detoxification in the rumen and pass into the lower digestive tract where it could have been absorbed. Eagle and Harrell (1950) found that free gossypol can act as an appetite depressant in rats, thereby offering a possible explanation for the decreased intake noted when the raw meal was fed in the 2:1 ratio.

Ruminal ammonia values were also lower for the first 6 hours after feeding in the sheep receiving the 2:1 C:R ratio indicating that protein

breakdown was less than from the 1:2 C:R ratio.

The animals in the growth trial were allotted so that the animals of one sex were randomly assigned to each concentrate:roughage ratio in each replication which caused the animals of each sex to consume the rations containing the same concentrate:roughage ratio only in alternate replications. This method of allotment caused confounding of treatment with replication which could allow the treatment differences to be affected by differences between replications. However, in these data, the replication mean squares were approximately equal to the error mean squares and it is possible that the relative consistency between replications indicates that the variance component due to replications did not appreciably affect treatment differences or the estimate of error in spite of this apparent error in allotment.

The results of the growth trial closely agree with the data of the digestibility and nitrogen balance studies. They also agree with the growth trial data of Experiment I in which it was found that significant improvements in lamb gain and efficiency were obtained by autoclaving a similar raw cottonseed meal for 45 minutes. The results of these experiments indicate that autoclaving cold-hexane extracted cottonseed meal for 60 minutes under 15 lb. per square inch steam pressure improved cottonseed meal for sheep over that found in any other heat treatment.

Summary

Digestibility, nitrogen-retention, and growth trials were designed to study the effects of autoclaving a raw cottonseed meal for various times up to 240 minutes upon its utilization by ruminants. The raw cottonseed meal was produced by cold-hexane extraction with no heat being applied during

extraction. Portions of this meal were then subjected to the following heat treatments: (1) no heat, (2) autoclaved under 15 lb. steam pressure per square inch at 250° F for 20 minutes, (3) autoclaved for 60 minutes, (4) autoclaved for 120 minutes, (5) autoclaved for 180 minutes, and (6) autoclaved for 240 minutes. Digestibility and nitrogen-retention trials were conducted using crossbred wethers in a completely random experiment with an orthogonal set of treatments (excluding the 20 minute ration); the meal autoclaved for 20 minutes was compared to all other treatments using the multiple range test.

In orthogonal comparisons, all autoclaved meals had higher organic matter and crude fiber digestibilities than the unheated meal. Cottonseed meal autoclaved for 60 minutes resulted in higher ($P<.10$) organic matter and crude fiber digestibilities than all other treatments. Nitrogen-free extract digestibility was similar for all rations. Protein digestibility was reduced ($P<.01$) with increased autoclaving times. Fecal nitrogen (increased) and urinary nitrogen (decreased) losses did not differ from linearity ($P>.01$) when plotted against autoclaving times. The nitrogen retained curve had significant linear and cubic components. The cubic component was expressed by greatest nitrogen retention when the cottonseed meal was autoclaved for 60 minutes and decreased retention at a relatively constant rate when the autoclaving time was increased from 60 to 180 minutes; a reduction in rate of decrease was obtained when the autoclaving time was extended from 180 to 240 minutes.

Cottonseed meal autoclaved for 20 minutes resulted in higher ($P<.05$) organic matter and nitrogen-free extract digestibilities than all other treatments. Protein digestibility and urinary nitrogen loss

were not significantly different from that obtained with the raw meal so this level of heating did not improve nitrogen retention.

In an *in vivo* study involving fistulated sheep, the maximum ruminal ammonia and plasma urea levels were reached 3 and 6 hours, respectively, after feeding. The maximum concentrations of these components resulting from the unheated meal were higher ($P<.01$) than from all autoclaved meals. Differences between the autoclaved meals were not significant. Peripheral plasma ammonia was low and quite constant for all meals. The unheated cottonseed meal resulted in higher ($P<.05$) total volatile fatty acid concentrations which were produced at a faster rate than the autoclaved meals. Similar total volatile fatty acid values were obtained with *in vitro* fermentations.

Cottonseed meals autoclaved for 0, 60, 120, and 240 minutes were fed in two (1:2 and 2:1) concentrate:roughage ratios to growing lambs allotted in a 2 x 4 factorial experiment. Average daily feed consumption was higher ($P<.01$) by lambs fed the 1:2 concentrate:roughage (C:R) ratio than those fed the 2:1 C:R ratio. Differences in average daily gain and feed efficiency were not significant between the two C:R ratios. Average daily gain and feed efficiency were higher ($P<.05$) when the animals consumed the cottonseed meal autoclaved for 60 minutes than all other heat treatments. The ratio X time interaction was significant ($P<.05$) for average daily gain and feed efficiency indicating that responses on each C:R ratio were dependent upon the autoclaving time of the cottonseed meal.

GENERAL SUMMARY

Digestibility, nitrogen-retention, and growth studies were conducted to determine the effects of various autoclaving times upon the utilization of raw soybean and cottonseed meals by sheep. One of the cottonseed meals was extracted with an azeotrope (acetone, hexane, and water) solvent while the special soybean meal and the special cottonseed meal were produced by cold-hexane extraction with no heat being applied during extraction. In Experiment I, portions of each meal were subjected to the following heat treatments: (1) no heat, (2) autoclaved under 15 lb. steam pressure per square in. at 250°F for 45 minutes, and (3) autoclaved for 90 minutes. In Experiment II, portions of the special cottonseed meal were subjected to the following heat treatments: (1) no heat, (2) autoclaved for 20 minutes, (3) autoclaved for 60 minutes, (4) autoclaved for 120 minutes, (5) autoclaved for 180 minutes, and (6) autoclaved for 240 minutes.

Organic matter digestibility (Experiment I) was higher ($P<.01$) in rations containing soybean meal than both cottonseed meals and higher ($P<.01$) in those containing the azeotrope-extracted cottonseed meal than the special cottonseed meal when all heat treatments were combined. Autoclaving all meals for 45 minutes improved ($P<.05$) organic matter digestibility when compared to the unheated meals with no further improvement from autoclaving for 90 minutes. When the raw cottonseed meal of Experiment II was autoclaved for times up to 240 minutes, organic matter digestibility was higher ($P<.01$) in the ration containing the meal autoclaved

for 60 minutes than all other treatments. Crude fiber digestibility (Experiment I) was higher ($P < .01$) in rations containing soybean and azeotrope-extracted cottonseed meals than in those containing the special cottonseed meal; autoclaving improved crude fiber digestibility in the special cottonseed meal but had no effect on the crude fiber digestibility of the rations containing the other meals. The special cottonseed meal autoclaved for 60 minutes resulted in higher ($P < .01$) crude fiber digestibility than all other autoclaving times in Experiment II. In both experiments, ether extract and nitrogen-free extract digestibilities were slightly improved in all rations containing the meals autoclaved for the shorter times with no further improvement from longer autoclaving times.

In Experiment I, protein digestibility was reduced ($P < .01$) in all meals after autoclaving for either 45 or 90 minutes. Fecal nitrogen loss (increased), urinary nitrogen loss (decreased), and nitrogen retention (increased) from all meals did not differ from linearity ($P > .01$) when plotted against autoclaving times. Protein digestibility was also reduced ($P < .01$) with increased autoclaving times in Experiment II. Fecal nitrogen (increased) and urinary nitrogen (decreased) losses did not differ from linearity ($P > .01$) when plotted against autoclaving times. The nitrogen retained curve had significant linear and cubic components; the latter being expressed by greatest nitrogen retention when the meal was autoclaved for 60 minutes and decreased retention with the longer times. Autoclaving reduced ruminal ammonia, ruminal total volatile fatty acids, and plasma non-protein nitrogen levels.

In lamb growth studies, the special soybean and cottonseed meals autoclaved for 45 minutes promoted higher ($P < .05$) average daily gains and feed

efficiencies than the unheated meals. Further growth studies revealed that lambs fed the special cottonseed meal autoclaved for 60 minutes had higher ($P<.05$) average daily gain and feed efficiency than those fed this meal autoclaved for 0, 120, or 240 minutes.

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VITA

Lloyd B. Sherrod

Candidate for the Degree of

Doctor of Philosophy

Thesis: EFFECTS OF VARIOUS PROCESSING TEMPERATURES ON THE UTILIZATION OF SOLVENT-EXTRACTED SOYBEAN AND COTTONSEED MEALS BY SHEEP

Major Field: Animal Nutrition

Biographical:

Personal Data: Born at Goodland, Kansas, March 5, 1931, the son of Charles and Helen Sherrod.

Education: Received the Bachelor of Science degree from South Dakota State College, with a major in Animal Husbandry in June, 1958; received the Master of Science degree from the University of Arkansas, with a major in Animal Nutrition in June, 1960.

Experience: Served in the United States Army from October, 1951 to October, 1953; Graduate Assistant at the University of Arkansas, 1958-60; Graduate Assistant and Research Associate at Oklahoma State University, 1960-63.

Professional Organizations: Phi Kappa Phi, Gamma Sigma Delta, Sigma Xi, American Society of Animal Science, and American Association for the Advancement of Science.

Date of Final Examination: November, 1963.