AEC Carbon

ANNUAL PROGRESS REPORT FOR STATE SUPPORTED PROJECTS
OF THE
NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION
(Three copies to be submitted to Director's Office)

1. PROJECT: (Fund, number, and title): **AEC - Contract No. AT-(40-1)-1324, A Study of the Effect of Composition of the Diet on Lipide Metabolism Using C^{14}.**

2. DEPARTMENTS AND COOPERATING AGENCIES: **Depts. of Animal Industry, Statistics and Physics, N. C. State College, and the Atomic Energy Commission.**

3. PERSONNEL:
S. B. Tove, H. L. Lucas, G. H. Wise, F. H. Smith, and N. Underwood

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

5. USEFULNESS OF FINDINGS(Benefits to Agriculture and the general public and contributions to science):

6. WORK PLANNED FOR NEXT YEAR:
This project should be closed out. Work has been incorporated into P.H.S. (Tove)-Metabolism of Unsaturated Fatty Acids.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

8. Prepared by **S. B. Tove** Approved _____
Director

Date **February 20, 1959** Date _____

A.E.C. CONTRACT NO. AT-(40-1)-1324

Final Report

September 7, 1960

Prepared By: _____
Project Leader

Approved By: _____
Director, North Carolina
Agricultural Experiment Station

_____
Business Manager
North Carolina State College

Final Report to

U. S. ATOMIC ENERGY COMMISSION
Research and Development Division
Post Office Box E
Oak Ridge, Tennessee

A. E. C. Contract No. AT-(40-1)-1324

Contractor: North Carolina State College.

Title: A Study of the Effect of Composition of the Diet on Lipide Metabolism Using C^{14}.

This report covers the period from July 1,1951, to October 31, 1959.

Objectives:

When this project was initiated, it was hoped that lipide metabolism could be studied by investigating the effects of various nutritional factors (e.g., caloric intake, essential fatty acids, pantothenic acid, biotin) on the turnover of liver fatty acids. As the work progressed, the high degree of variability encountered in the data resulted in a shift of the objectives toward a more fundamental study of the nature of turnover curves.

Results:

In the initial phase of the project the effect of a deficiency of pantothenic acid, biotin, linoleic acid, and pyridoxine on the turnover of palmitic acid, stearic acid, and the unsaturated fatty acids was studied. In addition, the effects of caloric insufficiency and thyroxine feeding were also studied. In all of these experiments a single injection of carboxyl-labeled acetate was used as the tracer dose. In the first

experiments observations were taken at 12-hour intervals; and although the deficient groups were variable, the control groups clearly showed two turnover curves within 48 hours. The latter provided a basis for a paper entitled "Turnover of Palmitic, Stearic and Unsaturated Fatty Acids in Rat Liver", published in the Journal of Biological Chemistry (218: 275, 1956). In subsequent experiments the observations were taken at shorter time intervals.

The most characteristic feature of the data of all of these later experiments was the marked degree of variability between animals of the same experiment and between different experiments. This marked degree of variability was such that it was impossible to decide whether or not any of the nutritional variables under study had any effect on the metabolism of lipides. Thus the problem became a study of the variation; and as such, distinguishing animal variation of the usual random type from that which was not random but represented a phenomenon associated with metabolic turnover, became of paramount importance. It was considered that metabolic turnover might not be represented as a smooth curve but rather a curve consisting of a complex series of damped cycles. With such a situation highly variable data would be expected, for the variation would thus be composed of two components: (1) variability in the total amount of tracer that passes through a pool, and (2) variability in the rate of transfer from one pool to another, i.e., the degree with which metabolic cycles of different animals are in or out of phase. Clearly what is needed is a continuous record of the turnover of a single compound in one animal. Accordingly, the turnover of several compounds, as measured by expired carbon dioxide, was investigated.

In these studies, the specific activity time curves for expired carbon dioxide were constructed from observations taken at 15-minute intervals

over periods of from 20 to 50 hours. The tracer compounds injected were carboxyl-labeled acetate, 1-5 labeled citrate, and labeled bicarbonate. All of the curves obtained were similar, clearly showing the presence of damped cycles. Major cycles of about 20 hours duration were observed in good agreement with the previously observed binodal turnover curves of liver fatty acids. Superimposed on the major cycles were minor cycles showing some degree of regularity. During some of these experiments, observations on the activity of the animals were taken, but neither time of food ingestion nor animal activity could be correlated with the cycles.

Considerable time and effort were expended during the project on attempts to fit the data to multi-exponential equations. In all cases these attempts met with complete failure. The chief reason for this lay in the fact that statistical knowledge for the fitting of the data to non-linear equations was inadequate.

Discussion:

It is clear from these studies conducted under this contract that metabolic turnover curves are cyclic in nature. In speculating on mechanisms that would account for cyclic turnover curves, four possibilities have been considered:

1. There could be more than one metabolic pool for a given compound. These different pools might reside in different tissues within an animal, different cells within a given tissue, different parts of a single cell, or even in different parts of a large molecule. Thus, even though a pure compound was isolated, it would represent the mean of several parts.

Should the individual pools have metabolic rates sufficiently different, the mean specific activity time curves would contain cycles.

2. Similarly, cycles would occur in the specific activity time curve of a metabolite that arose from a common precursor over two or more alternate pathways with different metabolic rates.

3. A time delay in the recycling of a metabolite might occur. For example, Stetten and Stetten (J. Biol. Chem., 213: 723, 1955) found that shortly after the injection of labeled glucose, the peripheral tiers of liver glycogen were more highly labeled than the center tiers. As time went on, the radioactivity of the center tiers rose, while that of the peripheral tiers fell, until finally the center tiers of the glycogen had a higher specific activity than the outer tiers. Thus the glucose move in a loop from the periphery of the glycogen molecule to the center and then out. The specific activity of carbon dioxide from such animals would be high initially, decrease as the glucose was in the center of the glycogen molecule, and then increase as the glucose which occupied the center tiers was oxidized. Tolbert et al. (U.C.R.L., 2941, 1955) did obtain a cycle in the specific activity time curve of the expired carbon dioxide following the injection of radioglucose. It is not unreasonable that similar events would occur with any large molecule not in true solution in a cell.

4. That a specific activity time curve should be uniform is based on the assumption that metabolism flows continuously smoothly and evenly. It would seem logical that such an assumption is an oversimplification of the true picture of metabolism particularly in an intact animal. It is more likely metabolism is basically a more or less discontinuous process. As such, a given metabolic pathway may function at an accelerated rate for a given period and at a reduced rate for the next period. At the same time, a second system could follow a reverse time pattern. The specific activity time curves resulting from these systems would clearly be cyclic.

It was a great disappointment that no progress could be made in fitting the specific activity time data to exponential expressions of rate. It is hoped that with improvements in the estimation of non-linear equations, this goal will be achieved in the future.

Finally, despite the vast amount of work on the estimation of turnover rates, one can question what is measured in these investigations. In almost every instance (where sufficient observations were taken) these curves show evidence of cycles that were assumed to be random variation and hence were ignored. This does not mean to imply that genuine turnovers were not measured in these cases, but the difficulty arises in deciding which metabolic compartment is represented by the turnover values obtained.

Incidents:

No incidents or accidents occurred other than those of a minor nature during the contract period.

Termination:

With this report, work on A.E.C. contract No. AT-(40-1)-1324 will be terminated.

Expenditure of Funds:

The financial statement for the contract has been made previously, and no further expenditure of funds has been made since the last report.

ANNUAL PROGRESS REPORT

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION STATE PROJECTS

1. PROJECT:(Fund, number, and title): Miscellaneous Gifts - A.E.C. Contract No. AT-(40-1)-1324. A Study of the Effect of Composition of the Diet on Lipide Metabolism Using C^{14}.
2. DEPARTMENTS AND COOPERATING AGENCIES: Departments of Animal Industry, Statistics and Physics, N. C. State College, and the Atomic Energy Commission.

PERSONNEL:
S. B. Tove, R. L. Lucas, G. H. Wise, F. H. Smith, and N. Underwood

RESEARCH ACCOMPLISHMENTS OF THE YEAR(Confidential information should be so marked):

Previous studies have shown that the turnover curves of various metabolites follow a rapid cyclic course.

This year the pattern of the specific activity of the expired CO_2 arising from the metabolism of carboxyl-labled acetate by resting yeast cells was studied. The results indicate that when an exogenous source of carbohydrate is present no cycles appear. However, when the exogenous carbohydrate supply is exhausted, cycles similar to those seen with rats are observed.

5. USEFULNESS OF FINDINGS(Benefits to agriculture and the general public and contributions to science.):
These studies represent an attempt to quantitatively measure intermediary metabolism in intact animals. Such measurements would be of great value in interpreting the effects of various environmental, physiological, and nutritional factors on animal performance.

6. WORKED PLANNED FOR NEXT YEAR:
Project to be terminated.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

8. Prepared by S. B. Tove Approved _____ (Director)

Date March 5, 1958

SES—Form 8
(June 12, 1957)

North Carolina AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, FEDERAL-GRANT PROJECTS, 1958

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): Hatch H-18, RIBOFLAVIN CONTENT OF SOYBEANS AND COWPEAS.--A. GROWTH STIMULANT(S) ASSOCIATED WITH RIBOFLAVIN IN SOYBEANS.

2. DEPARTMENTS AND COOPERATING AGENCIES:
   Animal Industry and Chemistry
3. PERSONNEL:
   F. W. Sherwood, Virginia W. Smart, G. H. Wise and W. P. Ingram, Jr.
4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

   Assays of solvent-extracted soybean-oil meal for riboflavin, using fluorimetric and microbiological methods, indicated that incubation with pepsin at pH 1.5 to 2.5 was more efficient than autoclaving in dilute acid, but liberated only about half of the riboflavin-active material indicated by rat-growth assays. Incubation in three stages with clarase at pH 4.5, pepsin at pH 1.5-2.5, and trypsin at pH 8.0 gave estimates no higher than those obtained with trypsin alone, which yielded values equivalent to those obtained by rat-growth assays.

   Results from a rat-growth assay of extract and of residue following autoclaving in 0.045N sulfuric acid, incubation with trypsin at pH 8.0 and autoclaving at pH 1.7 were similar to those previously obtained by rat-growth assays of fat-free meal. The bulk of the activity was associated with the extract, the estimate of which was in agreement by all three assay methods.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science):

   The findings indicate that the substitution of tryptic-incubation at pH 8.0 for clarase-incubation at pH 4.5 will make possible more nearly accurate estimates of the riboflavin content of high-protein materials by fluorimetric and microbiological methods.

6. WORK PLANNED FOR NEXT YEAR:

   Prepare data for publication
   Terminate the project

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

   Ph.D. Thesis in preparation

8. Prepared by W. P. Ingram, Jr. Approved _____ (Director).

   Date March 5, 1958 Date _____

SES—Form 8
(June 12, 1957)

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, FEDERAL-GRANT PROJECTS, 1959

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): HATCH H-23, NUTRITIONAL REQUIREMENTS OF THE SUCKLING PIG.

2. DEPARTMENTS AND COOPERATING AGENCIES: Animal Industry (Husbandry and Nutrition)

3. PERSONNEL: Gennard Matrone, A. J. Clawson, M. B. Wise, E. R. Barrick, G. H. Wise, S. B. Tove and Clara R. Bunn.

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

Continuation of the study to characterize the Mn-Fe antagonism found to occur in animal nutrition was centered on defining the minimum level for dietary Mn that interferes with hemoglobin formation in baby pigs. The results of two experiments (one presented in progress report of 1957) involving dietary levels of 0, 50, 125, 250, 500, 1250 and 2000 ppm of supplemental Mn showed that the minimal level of Mn, which interfered with hemoglobin formation, was between 50 and 125 ppm. It was found, moreover, that dietary levels of either 1250 or 2000 ppm of Mn also depressed growth of baby pigs. The growth depression was probably the result of the anemia rather than the direct effect of Mn on rate of gain.

Preliminary results indicate that the iron requirement of the baby pig is approximately 50 ppm.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science):

Better understanding of mineral nutrition and estimation of Fe requirements of baby pigs.

6. WORK PLANNED FOR NEXT YEAR:

Investigation of the nutrition of the neo-natal porcine with particular emphasis on protein. Project will be revised.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

Gennard Matrone, R. H. Hartman and A. J. Clawson. Studies of a Manganese-Iron Antagonism in the Nutrition of Rabbits and Baby Pigs. In press, J. Nutrition.

8. Prepared by G. Matrone Approved _____ (Director).

Date February 20, 1959 Date _____

SES—Form 8
(June 12, 1957)

North Carolina AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, FEDERAL-GRANT PROJECTS, 19 58

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): Hatch H-23, NUTRITIONAL REQUIREMENTS OF THE SUCKLING PIG.

2. DEPARTMENTS AND COOPERATING AGENCIES: Animal Industry (Husbandry and Nutrition)

3. PERSONNEL: Gennard Matrone, A. J. Clawson, M. B. Wise, E. R. Barrick, G. H. Wise, S. B. Tove and Clara R. Bunn

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

The problem investigated was to determine the minimum amount of dietary Mn that would interfere with the utilization of the normal level of iron in the diets of baby pigs. Preliminary results obtained indicate a progressive increase in the interfering effect of manganese on Hb regeneration with increasing increments of manganese. It appears that the lowest level of Mn, 125 ppm, tested was sufficient in amount to exert a detrimental effect.

In addition, and in conjunction with the above problem, an investigation of the Fe requirement of baby pigs was initiated.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science):

Better understanding of mineral nutrition and estimation of Fe requirements of baby pigs.

6. WORK PLANNED FOR NEXT YEAR:

Continuation of investigation of Mn x Fe antagonism and of Fe requirement of baby pigs.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

None

8. Prepared by G. Matrone Approved _____ (Director).

Date March 5, 1958 Date _____

SES—Form 8
(June 12, 1957)

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, FEDERAL-GRANT PROJECTS, 19 60

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): HATCH, H-29, A STUDY OF THE ESSENTIALS OF THE RUMINANT DIET

2. DEPARTMENTS AND COOPERATING AGENCIES: Animal Industry

3. PERSONNEL: G. Matrone, H. A. Ramsey, J. J. McNeill, and G. H. Wise

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

Radioisotopes studies revealed that sodium (Na) and potassium (K) bicarbonates in roughage-free purified diets increase CO_2 fixation in propionate and the formation of butyrate from acetate. They also indicate that these cations shift the ruminal fermentation to the propionate side.

Lamb growth studies indicate that 10% propionate in a roughage-free purified diet already containing K and Na bicarbonates depresses growth, whereas glycerol was equal to but not superior to glucose. These results along with ruminal volatile acid determination suggest that an optimum acetate-propionate ratio is necessary for optimum growth.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science):

Better understanding of ruminant nutrition.

6. WORK PLANNED FOR NEXT YEAR:

Continue attempts to improve roughage-free purified diet; obtain insight into function of K and Na cations in rumen and optimum acetate-propionate ratio.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

Gennard Matrone, H. A. Ramsey and J. J. McNeill. Sodium and Potassium Bicarbonates and Glycerol in Purified Diets for Ruminants. Abs. No. 2111, Fed. Proc., 18(1): 536. 1959.

Gennard Matrone, H. A. Ramsey and G. H. Wise. Role of Sodium and Potassium Cations in Volatile Fatty Acid Metabolism of Ruminants. Cornell Nutr. Conf. 86-41. November, 1959.

8. Prepared by G. Matrone Approved (Director).

Date March 15, 1960 Date

SES—Form 8
(June 12, 1957)

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, FEDERAL-GRANT PROJECTS, 19 61

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): HATCH, H-29, A STUDY OF THE ESSENTIALS OF THE RUMINANT DIET.

2. DEPARTMENTS AND COOPERATING AGENCIES: Department of Animal Industry

3. PERSONNEL: G. Matrone, J. J. McNeill, C. R. Bunn, R. E. Cathey, G. H. Wise, P. F. Heinstein

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked): Roughage-free purified diets adequately supplemented with sodium and potassium in the form of bicarbonates are suitable for ruminants. Between 1 and 2% sodium and 1 and 2% potassium (on elemental basis) appeared optimum. Starch was not found to be superior to a combination of glucose and starch. In fact, animals adjusted more slowly to the starch diet. In vitro studies with rumen microorganisms indicate that potassium activates the acetate → butyrate reaction. Radiosodium studies suggest sodium enters the rumen from the blood more slowly in purified-diet-fed animals than in hay-fed animals.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science): Better understanding of ruminant nutrition.

6. WORK PLANNED FOR NEXT YEAR: Continue to improve roughage-free diet; obtain insight into role of potassium and sodium.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

Darrell R. Van Campen and G. Matrone. Investigation of Precursors of Ruminal Fatty Acids of Sheep Fed Purified Diets. J. Nutrition, 72: 277-282, 1960.

8. Prepared by G. Matrone Approved _____ (Director).

Gennard Matrone

Date March 28, 1961 Date _____

1. STATE	2. PROJ. NO.	3. DEPT.	4. ABBREV. TITLE
N C	H-29	Anim Indus	Study of Essentials of Ruminant Diet

7. TITLE, PROJECT OBJECTIVES AND DESCRIPTION OF WORK PROPOSED

5. REF.
6. X-REF.

A STUDY OF THE ESSENTIALS OF THE RUMINANT DIET -- Investigate, by means of the purified-diet method: the basic nutrient requirements of the ruminant; and nutritional and physical interrelationships of the diet.

DESCRIPTION OF WORK

A study of the dietary factors which regulate and control the energy compounds produced in the rumen and utilized by the ruminant. Role in ruminant metabolism of sodium and potassium.

8. INDICATE TYPE OF PROJECT	HATCH	RRF	AMA	NON-FED.	NEW	REV.	MAR-KETING	9. DURATION	10. COOPERATION	11. APPROVAL DATES	12. X-REF
	H							60		6-10-55	

13. STATE	14. PROJ. NO.	15. DEPT.	16. ABBREV. TITLE	17. REF.
N C	H-29	Anim Indus	Study of Essentials of Ruminant Diet	

18. RECOMMENDED FOR APPROVAL

TITLE	SIGNATURE	DATE

SECTIONS 18, 19, AND 20 NOT APPLICABLE FOR PROJECTS PREVIOUSLY APPROVED BY SESD

19. APPROVAL OF DIRECTOR, AGRICULTURAL EXPERIMENT STATION

SIGNATURE

DATE

20. FEDERAL-GRANT PROJECTS ONLY—TO BE APPROVED BY STATE EXPERIMENT STATIONS DIVISION, WASHINGTON, D. C.

SIGNATURE

DATE

INSTRUCTIONS: Complete Items 1, 2, 3, 7, 8, 9, 10, 18, and 19. Under Item 7, show title in CAPS, itemize objectives and leave space between the objectives and description of work proposed. Forward original of this form with required number of project outlines to State Experiment Stations Division, Washington, D. C. *(See reverse side for Essentials of an Experiment Station Project Outline.)*

SES Form 20
Dec 1960

U. S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
STATE EXPERIMENT STATIONS DIVISION

COVER-ABSTRACT-SIGNATURE PAGE FEDERAL-GRANT & NON-FEDERAL PROJECTS

SES—Form 8
(June 12, 1957)

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, FEDERAL-GRANT PROJECTS, 1959

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): HATCH H-29, A STUDY OF THE ESSENTIALS OF THE RUMINANT DIET.

2. DEPARTMENTS AND COOPERATING AGENCIES: Animal Industry

3. PERSONNEL: G. Matrone, G. H. Wise, H. A. Ramsey, and J. J. McNeill

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

Studies of a roughage-free purified diet containing salts of volatile fatty acids (Proc. Soc. Exper. Biol. and Med. 95:731, 1957) show that the Na and K cations associated with the acetates were the important factors of the diet rather than the volatile fatty acids per se. Purified diets, previously found unsuitable, in which glucose and starch, triacetin, or cellulose and starch, were substituted for the salts of the volatile fatty acids were equal to the volatile fatty acid diet when adequately supplemented with Na and K cations in the form of bicarbonate. Growth of lambs fed another diet containing glycerol with the salts of propionic and acetic acids was superior to those fed the volatile fatty acids diet. Analysis of rumen fluid samples suggested that the Na and K bicarbonates with glycerol favored propionate fermentation. Examination of the rumen fluid revealed a specialized flora of large, gram positive streptococci and diplococci for the cellulose-free diets. The flora of the cellulose diet more nearly resembled the flora of a hay-grain diet. Fermentation gas analyses showed CO_2, CH_4 and no H_2 for all diets.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science):

Too early for specific recommendations.

6. WORK PLANNED FOR NEXT YEAR:

Continue attempts to improve volatile fatty acid diet and to evaluate cause of differences encountered.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

Gennard Matrone, H. A. Ramsey and G. H. Wise. Effect of Volatile Fatty Acids, Sodium and Potassium Bicarbonate in Purified Diets for Ruminants. Proc. Soc. Exptl. Biol. Med. 100: 8-11. 1959.

8. Prepared by G. Matrone Approved _____ (Director).

Date February 20, 1959 Date _____

SES—Form 8
(June 12, 1957)

North Carolina AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, FEDERAL-GRANT PROJECTS, 1958

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): Hatch H-29, A STUDY OF THE ESSENTIALS OF THE RUMINANT DIET.

2. DEPARTMENTS AND COOPERATING AGENCIES: Animal Industry

3. PERSONNEL: G. Matrone, G. H. Wise, and H. A. Ramsey

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

Lambs fed a diet containing glucose and 31.9% of salts of the volatile fatty acids, acetic, butyric and propionic grew better than lambs fed glucose and starch or glucose, starch and cellulose. Animals receiving the acids attained mature weights and are normal in appearance. Preliminary results from another experiment indicate that Triacetin does not replace Na and K acetates and that butyric may be omitted from the volatile fatty acid diet. Values of the C_2, C_3, C_4, C_5, and C_6 and above acids of samples of rumen juice taken from lambs receiving the complete fatty acid diet and those receiving the diet without butyrate were similar.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science):

Too early for specific recommendations.

6. WORK PLANNED FOR NEXT YEAR:

Continue attempts to improve volatile fatty acid diet and to evaluate cause of differences encountered.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

G. Matrone, H. A. Ramsey and G. H. Wise. Purified Diets for Ruminants. Proc. Soc. Exptl. Biol. and Med., 95:731 (1957).
G. Matrone, H. A. Ramsey and G. H. Wise. Volatile Fatty Acids in Purified Diets for Ruminants. Abstract for Federation Proceedings, April, 1958.

8. Prepared by G. Matrone Approved _____ (Director).

Date March 5, 1958 Date _____

SES—Form 8
(June 12, 1957)

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

MISC. GIFTS

ANNUAL PROGRESS REPORT, FEDERAL-GRANT PROJECTS, 19 60

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): FRASCH MOORMAN, INVESTIGATIONS OF MICRONUTRIENT ELEMENTS OF NUTRITION IN FARM ANIMALS

2. DEPARTMENTS AND COOPERATING AGENCIES: Department of Animal Industry

3. PERSONNEL: G. Matrone, Clara Bunn, Eddie Thomason, Joe Judd, Aden Magee, and C. H. Hill

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

See H-29 and H-193.

By means of glycine-C-14 the life span of sheep erythrocytes was found to range from 131 to 157 days. From radio-copper studies it was found that excess dietary zinc affects copper metabolism by increasing copper excretion in urine. Zinc toxicity affects iron metabolism as well as copper metabolism. The active factor(s) in liver extract which alleviates the growth depression of zinc toxicity appears to be organic in nature but not one of the known vitamins.

The copper and iron requirement of the chick is estimated to be approximately 4 and 40 ppm of dry matter intake, respectively. Copper anemia in chicks is normocytic and normochromic rather than microcytic and hypochromic as is the case for iron anemia.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science):

Better understanding of mineral metabolism of animals.

6. WORK PLANNED FOR NEXT YEAR:

Continuation of investigation along present lines.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

Gennard Matrone, R.H.Hartman and A.J.Clawson. Studies of a Manganese-Iron Antagonism in the Nutrition of Rabbits and Baby Pigs. J. Nutrition, 67: 309-317. 1959.

Gennard Matrone. Interrelationship of Iron and Copper in the Nutrition and Metabolism of Animals. Symposium sponsored by Am. Inst. of Nutr. 1960.

C. H. Hill and Gennard Matrone. Iron and Copper Metabolism of Chick. In

8. Preparation. G. Matrone Approved (Director).

Date March 15, 1960 Date

SES—Form 8
(June 12, 1957)

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, FEDERAL-GRANT PROJECTS, 19 60

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): HATCH, H-35, BIOCHEMICAL FACTORS THAT AFFECT THE APPARENT NUTRITIONAL QUALITIES OF FORAGES

2. DEPARTMENTS AND COOPERATING AGENCIES: Animal Industry, Field Crops, and Experimental Statistics

3. PERSONNEL:

W. W. G. Smart, Jr., G. Matrone, H. L. Lucas, and W. B. Gilbert

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

The major part of the work of this project has been devoted to trying to predict digestibility from chemical composition. A quantitative theory was developed to test what progress is being made:

$y = a + bx$

y = digestible amount of the chemical fraction in the feed.
a = intercept; can represent the fecal material not arising from feed.
b = slope; can represent the "true" digest in coefficient of the fraction.

Many fractions have been tried from different species of forages of widely varying chemical contents. Good predictors have been protein, water soluble sugars, ether extracts, and 4% H_2SO_4 hydrolyzable carbohydrates. Other fractions need refinement.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science):

If chemical predication equations can be developed, expensive large animal experiments on forage evaluations can be kept to a minimum.

6. WORK PLANNED FOR NEXT YEAR:

Different trials with forages of widely different chemical compositions will be made. New chemical fractionations are being tried.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

H. L. Lucas, W. W. G. Smart, Jr., Mary Ann Cipolloni, W. B. Gilbert, and H. D. Cross. Chemical Composition and Digestibility of Forages. Presented at the 10th Southern Pasture and Forage Crop Improvement Conference, June 9-12, 1959.

8. Prepared by W. W. G. Smart, Jr. Approved _____ (Director).

Date March 15, 1960 Date _____

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North Carolina AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, FEDERAL-GRANT PROJECTS, 1958

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): Hatch H-35, BIOCHEMICAL FACTORS THAT AFFECT THE APPARENT NUTRITIONAL QUALITIES OF FORAGES.

2. DEPARTMENTS AND COOPERATING AGENCIES: Animal Industry, Field Crops, and Experimental Statistics

3. PERSONNEL:
G. Matrone, W. W. G. Smart, Jr., H. D. Gross and H. L. Lucas

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

Studies to determine the correlation of changes of the carbohydrate fraction of fescue and alfalfa during lignification with digestibility indicate lignin content of the forage is a better estimator of digestibility than is lignin content of the feces. "Cellusans" content of the forage is also a promising predictor of digestibility. Nitrogen content of feed or feces is a good predictor for alfalfa but not for fescue.

The factor(s) responsible for the reproductive failures of rabbits fed soybean hay and soybean meal appears to reside primarily in the forage. Moreover, preliminary results indicate the factor(s) is not estrogenic in nature.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science):

Insufficient data for specific recommendations at this time.

6. WORK PLANNED FOR NEXT YEAR:

Fescue and alfalfa hays of different stages of maturity (1957 crops) are being fed in digestion trials at present. Work will continue on identification of factor(s) responsible for poor reproduction in rabbits.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

Aden C. Magee and Gennard Matrone. Estrogenic Activity of Soybean Forage. In hands of Editors of the Journal of Animal Science.

8. Prepared by G. Matrone Approved _____ (Director).

Date March 5, 1958 Date _____

SES—Form 8
(June 12, 1957)

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, FEDERAL-GRANT PROJECTS, 1959

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): HATCH H-35, BIOCHEMICAL FACTORS THAT AFFECT THE APPARENT NUTRITIONAL QUALITIES OF FORAGES.

2. DEPARTMENTS AND COOPERATING AGENCIES:
Animal Industry, Field Crops, and Experimental Statistics

3. PERSONNEL:
W. W. G. Smart, Jr., G. Matrone, H. L. Lucas, and W. B. Gilbert

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

The major part of the work of this project has been devoted to trying to predict digestibility from chemical composition. Digestion trials were conducted with sheep, on forages of different chemical composition, harvested at many stages of maturity. Statistical analyses of the results have not been completed. Several of the chemical constituents were found to be satisfactory single predictors for the apparent digestibility of the organic matter of the alfalfa. The correlation coefficients of these constituents are crude fiber, -0.95; cellulose, -0.91; crude protein, 0.88; chromogen, 0.87; and lignin, -0.93. For the fescue samples, none of the single predictors were satisfactory. Use of an index with more than one predictor will be explored.

Progress in the study of the factor(s) responsible for the reproductive failure of rabbits fed soybean hay and soybean meal have been hampered by the poor reproductive performance of the entire rabbit colony.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science):

If chemical prediction equations can be developed, expensive large animal experiments on forage evaluation can be kept to a minimum.

6. WORK PLANNED FOR NEXT YEAR:

Digestion trials with forages of widely different chemical composition will be made. Other methods of chemical fractionation of the feed will be tried.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

H. L. Lucas, W. W. G. Smart, Jr., Mary Ann Cippolloni, and H. D. Gross. Relationship of Digestibility of Chemical Composition. Abstract presented at meeting of 15th Southern Pasture and Forage Crop Improvement Conference.
Aden C. Magee and Gennard Matrone. Estrogenic Activity of Soybean Forage. J. An. Sci. 17(3): 787-791.

8. Prepared by W. W. G. Smart, Jr. Approved _____ (Director).

Date February 20, 1959 Date _____

SES—Form 8
(June 12, 1957)

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, FEDERAL-GRANT PROJECTS, 1961

(Three copies to be given to the SES examiner)

HATCH,

1. PROJECT (Fund, number, and title): H-35, BIOCHEMICAL FACTORS THAT AFFECT THE APPARENT NUTRITIONAL QUALITIES OF FORAGES.

2. DEPARTMENTS AND COOPERATING AGENCIES: Department of Animal Industry, Department of Experimental Statistics, Department of Field Crops

3. PERSONNEL: W. W. G. Smart, Jr., Gennard Matrone, W. B. Gilbert, Henry L. Lucas, Nancy W. Stanley

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked): Work has continued on the various ways of analyzing feeds which give better prediction of animal response (apparent digestibility). A cellulase inhibitor has been isolated from sericea lespedeza. It has been concentrated but not completely identified. It is polyphenol in nature and can be overcome by a polyphenol oxidase isolated from mushrooms.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science): Though these findings are not directly useful to the general public, in time application for these findings will be useful.

6. WORK PLANNED FOR NEXT YEAR: Work will continue in the development of better ways of analyzing forages and in the identification of the polyphenol in forage which affects digestibility and nutritive value.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

M. W. Carter, Gennard Matrone and W. W. G. Smart, Jr. The effect of genistin and its aglycone on weight gain in the mouse. British Journal of Nutrition, 14: 301-304. 1960.

8. Prepared by W. W. G. Smart, Jr. Approved _____ (Director).

Date March 28, 1961 Date _____

N C	H-35	AnimIndus	Biochemical Factors Affecting Forages

1. STATE 2. PROJ. NO. 3. DEPT. 4. ABBREV. TITLE 5. REF.
7. TITLE, PROJECT OBJECTIVES AND DESCRIPTION OF WORK PROPOSED 6. X-REF.

BIOCHEMICAL FACTORS THAT AFFECT THE APPARENT NUTRITIONAL QUALITIES OF FORAGES -- Identify and to biologically characterize organic compounds in forages that have a physiological action (e.g., hormone-like substances, saponins, tannins). Identify factors in forages that cause differences in animal responses associated with digestibility of feed consumed (e.g., degree of lignification and cellulose formation).

DESCRIPTION OF WORK

Forages of widely varying chemical composition and stages of maturity were fed to sheep in digestion trials. From chemical analyses predictions of digestibility from chemical composition were made. The major fraction of feed which gives poor prediction of digestibility from chemical composition is the complex carbohydrate fraction. An estrogen-like compound was isolated from soybeans and identified as genistein. A tannin-like compound has been isolated from lespedeza which is a cellulase and pectinase inhibitor.

8. INDICATE TYPE OF PROJECT	HATCH	RRF	AMA	NON-FED.	NEW	REV.	MARKETING	9. DURATION	10. COOPERATION	11. APPROVAL DATES	12. X-REF.
	X							61		6-8-56	

13. STATE	14. PROJ. NO.	15. DEPT.	16. ABBREV. TITLE	17. REF.
N C	H-35	AnimIndus	Biochemical Factors Affecting Forages	

18. RECOMMENDED FOR APPROVAL

TITLE SIGNATURE DATE

SECTIONS 18, 19, AND 20 NOT APPLICABLE FOR

PROJECTS PREVIOUSLY APPROVED BY SESD

19. APPROVAL OF DIRECTOR, AGRICULTURAL EXPERIMENT STATION

SIGNATURE DATE

20. FEDERAL-GRANT PROJECTS ONLY--TO BE APPROVED BY STATE EXPERIMENT STATIONS DIVISION, WASHINGTON, D. C.

SIGNATURE DATE

INSTRUCTIONS: Complete items 1, 2, 3, 7, 8, 9, 10, 18, and 19. Under item 7, show title in CAPS, itemize objectives and leave space between the objectives and description of work proposed. Forward original of this form with required number of project outlines to: State Experiment Stations Division, Washington, D. C. *(See reverse side for Essentials of an Experiment Station Project Outline.)*

SES Form 20
Dec 1960

U. S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
STATE EXPERIMENT STATIONS DIVISION

COVER-ABSTRACT-SIGNATURE PAGE FEDERAL-GRANT & NON-FEDERAL PROJECTS

SES—Form 8
(June 12, 1957)

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, FEDERAL-GRANT PROJECTS, 19 60

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): HATCH, 193, NUTRITION AND DISEASES OF THE NEONATAL PIG
   H-23, NUTRITIONAL REQUIREMENTS OF THE SUCKLING PIG
2. DEPARTMENTS AND COOPERATING AGENCIES:
   Department of Animal Industry
3. PERSONNEL:
   G. Matrone, J. G. Lecce, Clara R. Bunn, and Eddie Thomason
4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

   Study of iron requirements of baby pigs, Project H-23, is completed. The minimum iron requirement of baby pigs is approximately 60 ppm of the dry matter intake. From radio-iron studies it was found that pigs fed either 40 or 80 ppm of Fe absorb approximately 20% of orally fed iron. Baby pigs fed 10 and 20 ppm of iron utilize about 40% of the dietary iron. Body weight was depressed significantly when the diet contained less than 20 ppm of iron. Heart weight, which increased with increasing levels of iron, appears to be more sensitive than either body weight or hemoglobin.

   Studies have been initiated on the nutrition and diseases of colostrum-free neonatal pigs, Project H-193.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science):

   Better understanding of baby pig nutrition and diseases.

6. WORK PLANNED FOR NEXT YEAR:

   Project was revised during year. Investigation during next year will emphasize protein nutrition and metabolism and its relationship to diseases of neonatal pig.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:
   Lecce, James G., and Gennard Matrone. Porcine Neonatal Nutrition: The Effect of Diet on Blood Serum Proteins and Performance of the Baby Pig. J. Nutrition, 70: 13-20. 1960.

8. Prepared by G. Matrone Approved (Director).
   Date March 15, 1960 Date

FINAL REPORT, COMPLETED OR REVISED PROJECTS

NORTH CAROLINA Agricultural Experiment Station

1. Project title, number, and fund:
   Maximum roughage for fattening beef cattle. Purnell No. 2

2. Departments and cooperating agencies:
   Animal Husbandry Bureau of Animal Industry, U. S. D. A.

3. Major personnel:
   Earl H. Hostetler, J. E. Foster, N. C., O. G. Hankins and W. H. Black, B. A. I.

4. Date begun: Fall 1935 Date completed: April 24th, 1941

5. Estimated total cost by funds (salaries and maintenance):
   Purnell - $22,000
   B. A. I.- 13,000
   TOTAL $35,000

6. The problem (briefly restate its nature, importance, and economic significance):
   Beef cattle production in the South East and especially in that portion of North Carolina had been expanding for some years before this project was inaugurated and is still rapidly increasing. Lespedeza is the most extensively grown pasture and hay crop in Piedmont and Eastern North Carolina. Abundant pasture and roughage could be provided but could satisfactory and economical beef for slaughter be produced without the use of concentrates in the finishing period.

   Grade Hereford cattle were carried principally on pasture (Italian rye grass and Crimson clover in the winter and lespedeza in the summer) and lespedeza hay until they were about 20 months old and weighed approximately 800 pounds each. They were then divided into two groups, paired, and individually stall-fed until one of the pair reached 1,000 pounds at which time they were slaughtered. Group 1 was full fed shelled corn and lespedeza hay, but Group 11 was full fed lespedeza hay alone.

7. Major Results and Conclusions:
   The results of four complete trials which finished from 9 to 10 steers and or spayed heifers to the group each trial, showed that satisfactory beef can be produced on lespedeza hay alone. However, a ration of hay alone was not as economical and did not produce as rapid gains or as palatible beef as one which contains both grain and hay. The animals that were finished on grain and hay were

superior to those finished on hay alone, primarily, because their carcasses carried more finish or fat.

8. Practical applications and public benefits achieved or in prospect:

The comparative results obtained by finishing yearling beef cattle on lespedeza hay and corn or on lespedeza hay alone will have practical application in the investigation of other legume hays and carbonaceous concentrates. Although a higher quality beef can be produced by supplementing a full feed of roughage with a full feed of concentrates, these trials brought out that, a satisfactory product that is highly nutritious may be produced without the use of concentrates in the fattening ration. Under the conditions which this project was conducted the ration containing a concentrate was more economical than hay alone, but if concentrates become at a premium, as in war times, or as in some localities, the reverse might be true. Then too, only the two extremes were compared in these investigations and it is quite likely that even now a ration somewhere between these two extremes might be more practical than either of those studied.

9. Publications:

None

Director

FINAL REPORT, COMPLETED OR REVISED PROJECTS

North Carolina Agricultural Experiment Station

1. Project title, number, and fund: Methods of Supplementing Soybean Rations for Growing Pigs; P13-A12 and P15-A14—Purnell.

2. Departments and cooperating agencies: Animal Industry Department and Institute of Statistics.

3. Major personnel: Earl H. Hostetler, Walter J. Peterson, D. E. Brady, and F. H. Smith.

4. Date begun: 1943 Date revised/completed: January 1, 1947.
If discontinued without completion state reasons:

5. Estimated total cost by funds (salaries and maintenance): $29,407

6. The problem (briefly restate its nature, importance, and economic significance): This study was undertaken because of repeated failure to find practical protein feeds or methods of feeding them which would adequately supplement the diet of young pigs gleaning harvested fields and consuming a large preponderance of raw soybeans. Similar failures had resulted in dry lot under conditions simulating the grazing diet. In view of this, it seemed desirable to test the supplemental values of cystine as well as a mixture of the common B-vitamins on diets consisting principally of raw soybeans.

7. Major results and conclusions: Seventy-two pigs, weighing 30 to 45 pounds each, were used in a balanced lattice experiment to test the supplemental value of cystine and vitamins of the B-complex in rations containing raw soybeans as the only protein ingredient. Basal Ration I was made up of 49% ground raw soybeans, 49% starch, and 2% of an adequate mineral mixture. Basal Ration II contained 98% ground raw soybeans and 2% minerals. The supplemental values of cystine (0.3% of the ration) and a mixture of thiamin, riboflavin, niacin, pyridoxine and calcium pantothenate, alone and in combination, were tested on both basal rations. The vitamins were provided at a level which gave a margin of safety of at least 100%. Vitamin A was provided in all rations in the form of cod-liver oil. After 70 days on feed, average daily gains, adjusted for block and litter differences, and feed consumed per cwt. gain (the latter indicated in parentheses) were as follows (in lbs.): B.R. I 0.48 (422), with cystine 0.72 (349), with B-complex 0.75 (378), with B-complex and cystine 0.86 (326); B.R. II 0.90 (376), with cystine 0.98 (250), with B-complex 0.88 (366), with cystine and B-complex 0.92 (350). The effect of cystine was significant ($p < .05$) under all conditions of the experiment, and this effect was not greatly changed by the basal diet which it supplemented or by the presence or absence of B-complex. The average response to cystine addition was 0.12 lbs. per day. B-complex supplement increased growth by 0.21 lbs. per day on B.R. I, but had little effect on the all-soybean ration. Additions of both B-complex and cystine to B.R. I made it quite comparable to B.R. II.

(over)

* Two copies to be sent to the Office of Experiment Stations.

8. Practical applications and public benefits achieved or in prospect:
It is apparent that raw soybeans are deficient in available cystine and/or methionine. Where possible, it is, therefore, advisable to use processed soybean meals in preference to the raw product since it has been well-established that heat treatment liberates these amino acids. If raw soybeans are used, the protein portion of the diet should be supplemented with proteins rich in methionine, and the pigs should not be kept on raw soybean diets after they reach 100 pounds.

9. Publications:

Peterson, W. J., Hostetler, R. H., and Shaw, A. O. 1942. Studies in Feeding Soybeans to Pigs. J. Animal Sci. 1, 360. Proc.

Hostetler, R. H., Peterson, W. J., and Shaw, A. O. 1943. Feeding Soybeans to Pigs. Research and Farming, March, Vol. 1, Progress Report No. 2, p. 6.

Manuscript prepared to be submitted to the Society of Animal Production.

_____
Director

FINAL REPORT, COMPLETED OR REVISED PROJECTS

North Carolina Agricultural Experiment Station

1. Project title, number, and fund: Methods of Supplementing Soybean Rations for Growing Pigs; P13-Ai2 and P15-Ai4--Purnell.

2. Departments and cooperating agencies: Animal Industry Department and Institute of Statistics.

3. Major personnel: Earl H. Hostetler, Walter J. Peterson, D. E. Brady, and F. H. Smith.

4. Date begun: 1943 Date revised/completed: September 1, 1947.
If discontinued without completion state reasons:

5. Estimated total cost by funds (salaries and maintenance): $30,652

6. The problem (briefly restate its nature, importance, and economic significance): This study was undertaken because of repeated failure to find practical protein feeds or methods of feeding them which would adequately supplement the diet of young pigs gleaning harvested fields and consuming a large preponderance of raw soybeans. Similar failures had resulted in dry lot under conditions simulating the grazing diet. In view of this, it seemed desirable to test the supplemental values of cystine as well as a mixture of the common B-vitamins on diets consisting principally of raw soybeans.

7. Major results and conclusions: Seventy-two pigs, weighing 30 to 45 pounds each, were used in a balanced lattice experiment to test the supplemental value of cystine and vitamins of the B-complex rations in containing raw soybeans as the only protein ingredient. Basal Ration I was made up of 49% ground raw soybeans, 49% starch, and 2% of an adequate mineral mixture. Basal Ration II contained 98% ground raw soybeans and 2% minerals. The supplemental values of cystine (0.3% of the ration) and a mixture of thiamin, riboflavin, niacin, pyridoxine and calcium pantothenate, alone and in combination, were tested on both basal rations. The vitamins were provided at a level which gave a margin of safety of at least 100%. Vitamin A was provided in all rations, in the form of cod-liver oil. After 70 days on feed, average daily gains, adjusted for block and litter differences, and feed consumed per cwt. gain (the latter indicated in parentheses) were as follows (in lbs.): B. R. I 0.48 (422), with cystine 0.72 (349), with B-complex 0.75 (378), with B-complex and cystine 0.86 (326); B.R. II 0.90 (276), with cystine 0.98 (250), with B-complex 0.88 (266), with cystine and B-complex 0.92 (250). The effect of cystine was significant ($p < .05$) under all conditions of the experiment, and this effect was not greatly changed by the basal died which it supplemented or by the presence or absence of B-complex. The average response to cystine addition was 0.12 lbs. per day. B-complex supplement increased growth by 0.21 lbs. per day on B. R. I, but had little effect on the all-soybean ration. Additions of both B-complex and cystine to B.R. I made it quite comparable to B.R. II.

8. Practical applications and public benefits achieved or in prospect: It is apparent that raw soybeans are deficient in available cystine and/or methionine. Where possible, it is, therefore, advisable to use processed soybean meals in preference to the raw product since it has been well-

established that heat treatment liberates these amino acids. If raw soybeans are used, the protein portion of the diet should be supplemented with proteins rich in methionine, and the pigs should not be kept on raw soybean diets after they reach 100 pounds.

9. Publications:

Peterson, W. J., Hostetler, E. H., and Shaw, A. O. 1942. Studies in Feeding Soybeans to Pigs. J. Animal Sci. 1, 360. Proc.

Hostetler, E. H., Peterson, W. J., and Shaw, A. O. 1943. Feeding Soybeans to Pigs. Research and Farming, March, Vol. 1, Progress Report No. 2, p. 6.

Manuscript prepared to be submitted to the Society of Animal Production.

FINAL REPORT, COMPLETED OR REVISED PROJECTS

North Carolina Agricultural Experiment Station

1. Project title, number, and fund: Methods of Supplementing Soybean Rations for Growing Pigs; P13-Ai2 and P15-Ai4--Purnell.

2. Departments and cooperating agencies: Animal Industry Department and Institute of Statistics.

3. Major personnel: Earl H. Hostetler, Walter J. Peterson, D. E. Brady, and F. H. Smith.

4. Date begun: 1943 Date revised/completed: September 1, 1947.
If discontinued without completion state reasons:

5. Estimated total cost by funds (salaries and maintenance): $30,652

6. The problem (briefly restate its nature, importance, and economic significance): This study was undertaken because of repeated failure to find practical protein feeds or methods of feeding them which would adequately supplement the diet of young pigs gleaning harvested fields and consuming a large preponderance of raw soybeans. Similar failures had resulted in dry lot under conditions simulating the grazing diet. In view of this, it seemed desirable to test the supplemental values of cystine as well as a mixture of the common B-vitamins on diets consisting principally of raw soybeans.

7. Major results and conclusions: Seventy-two pigs, weighing 30 to 45 pounds each, were used in a balanced lattice experiment to test the supplemental value of cystine and vitamins of the B-complex rations in containing raw soybeans as the only protein ingredient. Basal Ration I was made up of 49% ground raw soybeans, 49% starch, and 2% of an adequate mineral mixture. Basal Ration II contained 98% ground raw soybeans and 2% minerals. The supplemental values of cystine (0.3% of the ration) and a mixture of thiamin, riboflavin, niacin, pyridoxine and calcium pantothenate, alone and in combination, were tested on both basal rations. The vitamins were provided at a level which gave a margin of safety of at least 100%. Vitamin A was provided in all rations, in the form of cod-liver oil. After 70 days on feed, average daily gains, adjusted for block and litter differences, and feed consumed per cwt. gain (the latter indicated in parentheses) were as follows (in lbs.): B. R. I 0.48 (422), with cystine 0.72 (349), with B-complex 0.75 (378), with B-complex and cystine 0.86 (326); B.R. II 0.90 (276), with cystine 0.98 (250), with B-complex 0.88 (266), with cystine and B-complex 0.92 (250). The effect of cystine was significant ($p < .05$) under all conditions of the experiment, and this effect was not greatly changed by the basal died which it supplemented or by the presence or absence of B-complex. The average response to cystine addition was 0.12 lbs. per day. B-complex supplement increased growth by 0.21 lbs. per day on B. R. I, but had little effect on the all-soybean ration. Additions of both B-complex and cystine to B.R. I made it quite comparable to B.R. II.

8. Practical applications and public benefits achieved or in prospect: It is apparent that raw soybeans are deficient in available cystine and/or methionine. Where possible, it is, therefore, advisable to use processed soybean meals in preference to the raw product since it has been well-

established that heat treatment liberates these amino acids. If raw soybeans are used, the protein portion of the diet should be supplemented with proteins rich in methionine, and the pigs should not be kept on raw soybean diets after they reach 100 pounds.

9. Publications:

Peterson, W. J., Hostetler, E. H., and Shaw, A. O. 1942. Studies in Feeding Soybeans to Pigs. J. Animal Sci. 1, 360. Proc.

Hostetler, E. H., Peterson, W. J., and Shaw, A. O. 1943. Feeding Soybeans to Pigs. Research and Farming, March, Vol. 1, Progress Report No. 2, p. 6.

Manuscript prepared to be submitted to the Society of Animal Production.

SES—Form 8
(June 12, 1957)

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

MISC. GIFTS

ANNUAL PROGRESS REPORT, FEDERAL-GRANT PROJECTS, 19 60

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): PHS-Ramsey THE NUTRITIONAL SIGNIFICANCE OF PREGASTRIC ESTERASE.

2. DEPARTMENTS AND COOPERATING AGENCIES: Department of Animal Industry

3. PERSONNEL: H. A. Ramsey, D. E. Otterby, J. W. Young, and G. H. Wise.

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked): The effects of the following items on the secretion of pregastric esterase by calves were investigated: continuous nursing, length of nursing period, and rate of milk consumption. When 50 pounds of milk were fed continuously, as 10 successive 5-pound increments, the enzyme content of each increment decreased progressively. The quantity of esterase secreted into a single unit of milk was increased by increasing the length of the nursing period. Increasing the rate of milk consumption stimulated the output of pregastric esterase slightly. Enzyme secretion was higher in the morning than in the afternoon.

The abomasal hydrolysis of milk fat by pregastric esterase was also studied. When whole milk was fed orally, the concentration of free fatty acids in abomasal contents (removed 1, 2, and 3 hours after feeding) was 4 to 5 times higher than in contents where the milk was introduced directly into the abomasum. Butyric acid comprised 37% of the free fatty acids in the oral-fed samples removed at one hour, thus indicating a relatively high specificity of pregastric esterase for the glycerides of short-chain fatty acids.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science):

The project provides basic information on the utilization of dietary fat, one of the major energy sources of the young calf.

6. WORK PLANNED FOR NEXT YEAR:

Investigate the role of pregastric esterase in the alimentary hydrolysis of dietary fat and the possible effect of this enzyme on protein digestion. The electrophoretic separation of esterases from various tissues and fluids will be studied.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

J. W. Young, H. A. Ramsey, and G. H. Wise. Effects of Age and Diet on the Secretion of Pregastric Esterase in Calves. Submitted for publication.

H. A. Ramsey, J. W. Young, and G. H. Wise. Effects of Continuous Nursing, Length of the Nursing Period, and Rate of Milk Consumption on the Secretion of Pregastric Esterase by Calves. Submitted for publication.

8. Prepared by H. A. Ramsey Approved (Director).

Date March 15, 1960 Date

PHS Ramsey

ANNUAL PROGRESS REPORT FOR STATE SUPPORTED PROJECTS
OF THE
NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION
(Three copies to be submitted to Director's Office)

1. PROJECT: (Fund, number, and title): **P.H.S. (Ramsey) - The Nutritional Significance of Pregastric Esterase.**

2. DEPARTMENTS AND COOPERATING AGENCIES: **Department of Animal Industry**

3. PERSONNEL: **H. A. Ramsey, G. H. Wise and J. W. Young**

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

The secretion of pregastric esterase (PGE) was studied in 2 groups of calves from 16 through 180 days of age. Group I was fed only whole milk, whereas group II was fed hay and grain. PGE secretion was determined by feeding 5 lb. non-fat milk, collecting the milk-saliva mixture from the cardial end of the esophagus via a rumen fistula, and measuring the lipolytic activity thereof. Milk fat was used as the substrate. PGE secretion was unaffected by either diet or age of animal. Occasionally, 50 lb. non-fat milk was sham-fed as 10 successive 5-lb. increments. PGE was highest in the first increment and decreased markedly in subsequent increments. The substrate specificity of PGE is such that butyric acid comprises about 50 molar % of the acids liberated from milk fat. The hydrolysis of 2-naphthyl laurate by PGE in a colorimetric assay technique is accelerated greatly by adding small quantities of non-fat milk solids, casein or egg albumen, whereas that by pancreatic lipase or liver esterase is not. The hydrolysis of milk fat by PGE, however, does not seem to be stimulated by non-fat milk solids.

5. USEFULNESS OF FINDINGS(Benefits to Agriculture and the general public and contributions to science):

This project provides basic information on the utilization of dietary fat, one of the major energy sources of the young calf.

6. WORK PLANNED FOR NEXT YEAR:
Studies will be initiated to determine the extent to which pregastric esterase is responsible for lipolysis of dietary fat in the alimentary tract of the calf. Saliva, containing pregastric esterase, will be subjected to electrophoretic analysis to determine if more than one enzyme is present.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR: **The following abstracts have been submitted ~~in~~ for presentation at the 1959 meeting of the American Dairy Science Association. 1. Effects of age and diet on the secretion of pregastric esterase in the calf. J. W. Young, H. A. Ramsey and G. H. Wise. 2. Effects of non-fat milk solids and other compounds on the activity of pregastric esterase, liver esterase and pancreatic lipase. Ramsey.**

8. Prepared by **H. A. Ramsey** Approved _____
Director

Date **February 20, 1959** Date _____

PHS Tove

ANNUAL PROGRESS REPORT FOR STATE SUPPORTED PROJECTS
OF THE
NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION
(Three copies to be submitted to Director's Office)

1. PROJECT: (Fund, number, and title): **P.H.S. (Tove) - Metabolism of Unsaturated Fatty Acids of Depot Fat.**

2. DEPARTMENTS AND COOPERATING AGENCIES:
Department of Animal Industry and U. S. Public Health Service

3. PERSONNEL:
S. B. Tove and F. H. Smith

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

Mice were fed various fatty acids and the fatty acid composition of depot fat determined by gas chromatography. When linoleic, oleic or myristic acids are fed, the depot fat levels of these acids increase. There is no increase when palmitic or stearic acids are fed. With an increase in depot fat linoleic acid, palmitoleic acid is the only acid that decreases, and palmitic and stearic acids increase. With an increase in depot fat oleic acid only palmitic acid decreases, and there is an increase in myristic acid. Feeding tripropion results in the deposition of odd carbon fatty acids.

5. USEFULNESS OF FINDINGS(Benefits to Agriculture and the general public and contributions to science): **These studies will provide information on the factors that affect the fatty acid composition of animal products. The fatty acid composition may be of importance to the health of the population consuming these products.**

6. WORK PLANNED FOR NEXT YEAR:
Continuation of the studies using other fatty acids. Studies on the mechanism of synthesis of unsaturated fatty acids by animals.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:
Kinetics of the Depletion of Linoleic Acid in Mice. (Being reviewed)
Apparatus for Separation of Fatty Acid Methyl Esters by Gas Chromatography. (Submitted to Analytical Chemistry)

8. Prepared by **S. B. Tove** Approved _____
Director

Date **February 20, 1959** Date _____

SES—Form 8
(June 12, 1957)

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

MISC. GIFTS

ANNUAL PROGRESS REPORT, FEDERAL GRANT PROJECTS, 19 60

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): PHS-Tove METABOLISM OF UNSATURATED FATTY ACIDS OF DEPOT FAT.

2. DEPARTMENTS AND COOPERATING AGENCIES: Department of Animal Industry and U. S. Public Health Service.

3. PERSONNEL: S. B. Tove and F. H. Smith.

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked): Studies on the effect of dietary fat on the fatty acid composition of depot fat were continued. Previous results had shown that there were specific patterns of substitution for the various fatty acids. For example, an increase in linoleic results in a decrease in palmitoleic and an increase in stearic acid. This work has been extended to the different positions of the glycerol moiety. These results show that at low levels of linoleic acid in depot fat, the 2 position of the glycerol is richer in linoleic acid than the alpha positions. As the dietary level of linoleic acid increased, there was an increase of linoleic acid in the alpha positions, whereas the 2 position remained fairly constant. The shape of the curves obtained suggests that different specificity patterns may be associated with the two different alpha positions. In contrast, studies with oleic acid showed oleate was deposited equally in both the inner and outer positions of the glycerol skeleton, but the maximum level of oleate attained suggests that trioleic may not be synthesized.

Work on odd-carbon fatty acid formation was also continued. When acids of 3, 5, 7 or 9 carbons are fed, a C-15 saturated acid and a C-17 acid are found in the fat showing that the odd acids fed are probably broken down to a 3-carbon chain and the odd acids in the depot fat synthesized from this.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science):

These studies will provide information on the factors that affect the fatty acid composition of animal products. The fatty acid composition may be of importance to the health of the population consuming these products.

6. WORK PLANNED FOR NEXT YEAR:

Continue studies on the distribution of fatty acids in the 3 positions of the glycerol. Attempts will be made to differentiate between the 2 alpha positions. Studies on the effect of dietary fat on the depot fat and milk fat of ruminants will be conducted.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

S. B. Tove and F. H. Smith. Kinetics of the Depletion of Linoleic Acid in Mice. Arch. Biochem. Biophys., 85: 352-365. 1959.

S. B. Tove. Production of Odd-Numbered Carbon Fatty Acids from Propionate in Mice. Nature, 184: 1647. 1959.

S. B. Tove and F. H. Smith. Changes in the Fatty Acid Composition of the Depot Fat of Mice Induced by Feeding Oleate and Linoleate. J. Nutrition. In press.

8. Prepared by _____ (Director).

Date March 15, 1960 S. B. Tove

Date _____

SES—Form 8
(June 12, 1957)

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, ~~FEDERAL GRANT~~ MISC. GIFTS PROJECTS, 1960

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): Nutrition Foundation, CHEMICAL NATURE AND MECHANISM OF ACTION OF A DIETARY FACTOR AFFECTING COMPOSITION OF FAT

2. DEPARTMENTS AND COOPERATING AGENCIES:

3. PERSONNEL:

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

Work coordinated with PHS-Tove, Metabolism of Unsaturated Fatty Acids of Depot Fat.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science):

6. WORK PLANNED FOR NEXT YEAR:

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

8. Prepared by S. B. Tove Approved _____ (Director).

Date March 15, 1960 Date _____

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

PROJECT OUTLINE

1953-54
$18,000
4000 travel
7000 - N.C. State Exp Stat.
7000 - Ga + Ky indicators

Project No. S-12
North Carolina Supporting Project
Date Submitted _____
Approved _____
Revised _____

1. Title

Statistical Studies of techniques used in pasture evaluation.

2. Objectives:

(a) To improve pasture evaluation techniques through a study of the nature and the magnitude of the errors in

(i) grazing trials
(ii) mechanically-harvested small plots
(iii) cage and mower-strip methods
(iv) indicator methods

(b) To provide statistical consultant services to the other S-12 pasture technique studies.

3. Reasons for undertaking Investigations:

Objective (a-i): An ever-increasing number of grazing trials is being initiated, even though they are cumbersome and expensive. Any economies that can be effected in the conduct of these trials will permit a proportional increase in the number of tests that can be made. A logical first step in the refinement of grazing experiments is to ascertain the magnitude and the nature of the experimental errors, and to discern how these errors are related to factors such as (i) kind of animal, (ii) grazing procedure, (iii) unit of measure, (iv) pasture size and number of animals per pasture, and (v) general type of pasture.

Objective (a-ii): The cumbersome nature and the great expense of grazing trials makes it necessary that new species and mixtures, alternate practices with respect to fertilization, and certain other aspects of management be screened by agronomic means. Hence, a good share of the knowledge regarding pastures has been and probably always will be based on the results of small-plot agronomic experiments in which mechanical harvesting is employed.

Nevertheless, even among investigators who have used the agronomic methods routinely, there is serious question as to the adequacy of the small-plot results. Especially difficult is the evaluation of forage quality by these methods. The first logical step in determining the adequacy of and means of improving the agronomic studies is to ascertain the magnitudes of the correlations which exist between the small-plot measurements (yield, botanical and chemical compositions) and the per-acre and per-animal yields of animal products under grazing.

Objective (a-iii): There is considerable conjecture as to the value of the cage and the mower-strip methods. These methods, by their nature, are of use only in conjunction with grazing trials. A primary objective of them usually has been to estimate the amount of forage available for grazing and the amount consumed by the animals, both per acre and per animal, but if proper measurements are taken they can also provide indices of the intensity of grazing of a sward and of the reactions of a sward to grazing. It is well known, however, that the cage and the mower-strip methods are subject to large errors of both the random and the bias types. The occurrence of large random errors requires that relatively large numbers of cages or mower-strips must be used to obtain precision and the biases can result in unreasonable and misleading data. There is a possibility that the cage and mower-strip methods can be replaced by "indicator" methodsto obtain data on forage consumption and by eye-estimation, point-quadrat or related procedures to obtain data on intensity of grazing and sward reaction. Although at least two Stations in the Southern Region have ceased using cage and mower-strip methods, these methods are used widely. It is important, therefore, to assess the errors associated with the cage and the mower-strip methods under a variety of conditions, to ascertain possible means of reducing these errors, and to compare cage and mower-strip results with the results of the "indicator", point quadrat and related methods.

Objective (a-iv): Methods that utilize certain components of the feed or feces (lignin, chromogen, nitrogen, etc.) as indicators of the digestibility of grazed forage have been developed to a state of great promise. The digestibility figures so obtained, combined with estimates of fecal output, permit estimation of amounts of forage consumed. Yet the nature and the magnitude

of the errors in these procedures are not known, except perhaps under special conditions. In addition to the use of "internal" indicators to estimate digestibility, "external" indicators (chromium oxide, dyes, etc.) are being used with periodic fecal sampling in order to circumvent total collection of feces. However, diurnal fluctuations in the fecal content of external indicators can introduce large errors into the procedures. In order to minimize the errors due to the diurnal variations, statistical studies of various fecal sampling schemes need to be made.

Objective (b): At least two and probably four or more Stations other than North Carolina will be concerned in other S-12 technique studies. Certain investigators who possibly will be involved have indicated a desire for personnel from North Carolina State College to consult with them regarding the statistical aspects of their studies.

4. Previous work and present status of investigations in the field of this project:

Objective (a-i): There is considerable material in the literature which can be brought to bear on the matter of errors in grazing trials. The general sizes of between-plot variability in yield of crops harvested by mechanical means, between-animal variability in performance, and weighing errors, are quite well known. Outside of preliminary information from this station, however (Shepherd et al, Grazing and fire influences in pond pine forests, N. C. Tech. Bul. 97, 1951; Lucas, paper presented at Animal Science Meeting*, 1952), and one other reference (Williams, Proc. V Int. Grassland Congress, 1949), no estimates of overall errors for grazing trials have been found. Estimates of portions of the errors have been published, e.g. Kincaid using steers (J. Anima. Sci. 4:164-173, 1945) and Nevens using dairy heifers (J. Dairy Sci., 27:1011-1014, 1944) give between-animal variabilities for rate of gain of grazing animals. Furthermore, outside of the preliminary work done at this station, there has been found little information relative to the nature of the overall errors, i.e. the manner in which different components of the errors (plot variability, animal variability, weighing errors, etc.) enter into the over-all errors, and on the question of how the magnitudes and the natures of the errors vary for different units of measure and for dif-

*Copy attached.

ferent methods of computing the units.

Objective (a-ii): No published work bearing directly on objective (a-ii) has been found. A little work on this question has been done at North Carolina State College, but the results are inconclusive.

Objective (a-iii): Considerable work has been published regarding cage methods and some regarding mower-strip methods. Particularly outstanding are the papers of Linehan et al (J. Brit. Grassl. Soc. 1:1-29, 1946; Ibid 2:145-168, 1947; Ibid 7:73-98, 1952). Wagner et al. (J. Amer. Soc. Agron. 42:487-491, 1950) has published a paper dealing with the relative sizes of the errors of the cage methods and of the mower-strip methods. Linehan's work provides a good account of the random errors and the biases under English conditions and of how the biases might be reduced. The effect on errors of factors such as size and shape of cage or mower-strip have been studied to some extent (e.g. G. W. Naylor, N. C. State College Ph.D. thesis, 1953). There is need, however, for similar studies under a greater variety of conditions, and critical attention needs to be given especially to the proper place of cage and mower-strip procedures in pasture research programs.

Objective (a-iv): Work with the "internal" indicators (especially lignin, chromogen and nitrogen) shows that these procedures for estimating digestibility can serve well for humid region forages (e.g. Reid et al, J. Nutr. 46:255-269, 1952; Forbes, J. Anim. Sci. 9:231-237, 1950), but difficulties have been encountered in applying the chromogen method to certain desert range species (Cook and Harris, J. Anim. Sci. 10:565-573, 1951). Quantitative information on the errors to be expected under various divergent experimental conditions is, however, lacking. Several studies have been made on the use of external indicators (especially Cr_2O_3) with "grab" sampling of feces in order to estimate total fecal output. Particular attention has been given to the diurnal fluctuations in fecal content of the external indicators (e.g. Kane et al, J. Nutr. 47:263-273, 1952; Hardison et al, 36:583, 1953), and certain sampling schemes have been proposed to overcome the errors which the diurnal fluctuations can cause. Unpublished experience of investigators in the Southern region has shown, however, that the porposed schemes often can result in high errors. No methods of controlling these errors with certainty are known.

Objective (b): Statistical personnel of North Carolina State College who are associated with this project, have the following experience: (i) several years of consulting with investigators in the Southern Region regarding problems in pasture evaluation, (ii) a tour of the Southern Region to study pasture evaluation techniques, and (iii) Organizer and Editor for Section K (Experimental Procedures in Grassland Research) of the VI International Grassland Congress. In addition this group has training and experience in Statistics, Mathematics, Agronomy, Animal Husbandry, Animal Nutrition, and the associated sub-fields and requisite more basic sciences, such as Biochemistry, Animal and Plant Physiology, and Soils. In this group one Ph.D. degree is held in Agronomy and two in Animal Nutrition.

5. Outline of Procedure:

Collection of data: Data for these studies will be obtained primarily from Stations in the Southern Region, but if such prove inadequate for reliable results, data also will be sought from Stations in other regions. Although much of the data required for this study can be obtained by correspondence, experience has shown that long delays in obtaining data can be prevented by having one of the persons in charge of the project visit the Station from which data are to be obtained. Such visits are especially advantageous in some instances for other reasons. They prevent confusion regarding the type of data desired and yield proper acquaintance with the manner in which the experiments were done.

Objective (a-i): Data will be obtained from grazing trials in which two or more pastures (field replicates) have been used on each treatment, and which have been continued for two or more consecutive years. Field replication is necessary in order that the errors estimated include both major components, i.e. the between-animal variability and the between-pasture variability. Experiments which continue through two or more years are required to permit the estimation of the interactions of years with treatments and of years with individual pastures. In the Southern region there are data already available from a considerable number of experiments of the type desired, and much additional data will become available during the next few years. Several

Stations have already furnished data bearing on Objective (a-i) to North Carolina and several others have indicated a willingness to do so. The data obtained will be analyzed statistically using appropriate methods for each experiment. Attempts will be made to explain the observed variations in size of error by the development of quantitative unifying theory which relates error size to the definable factors which distinguish one set of experimental conditions from another.

Objective (a-ii): Data will be sought which permit direct correlation of the results from small-plot trials with those from grazing trials. In the Southern region there have been initiated a few studies in which grazing experiments and comparable small-plot experiments are being done side by side. Other sets of data involving the same forage species, managements and soils also will be useful even though not obtained in adjacent areas or in the same years. Although field replication is desirable in both the small-plot trials and the grazing trials, it is not necessary. The statistical analyses basically will be of the simple and multiple regression types in order to relate the results from the two types of trials. Both linear and non-linear relations will be investigated, and attempts will be made to explain quantitatively any between-experiment variations in the observed relations.

Objective (a-iii): In a large number of the grazing trials in the Southern Region, cage or mower-strip data have been or are taken routinely. Adequately representative sets of such data will be obtained. Field replication of the pasture treatments is desirable, but not necessary. A wide variety of types of statistical analyses are possible for data bearing on Objective (a-iii). Judgement will have to be made regarding the most effective analyses after the data are in hand. Particular attention will be given to the testing of the various procedures proposed by Linehan (previously cited) for removing bias when computing results.

Objective (a-iv): Two Stations other than North Carolina in the Southern region are receiving S-12 funds especially to further the work on indicator methods. Several other Stations including North Carolina are also doing indicator work. Data which already are or will become available bearing on the errors in using either "internal" or "external" indicator will be analyzed as is appropriate. The types of data and analyses needed are not

clearly known at this time. It is anticipated that special studies will have to be designed and completed to provide proper data for some phases of this work.

Objective (b): Statistical consultant services will be provided for the other technique studies receiving S-12 support.

6. Probable Duration of project: Five years.

7. Date of initiation: July 1, 1953

8. Personnel:

Name	Department and Station	Relation to Project
H. L. Lucas	Statistics, N. C. State	Leader
R. G. Petersen	Statistics, N. C. State	Co-leader
W. W. G. Smart, Jr.	Statistics and Nutrition, N. C. State	Co-leader
W. W. Woodhouse, Jr.	Agronomy, N. C. State	cooperator

The Technical Committee for Regional Project S-12, and pasture personnel at North Carolina and other Stations in the Southern Region and in the U.S.D.A. will serve in advisory capacities.

9. Cooperation:

(a) Interdepartmental (North Carolina):

Agronomy, Animal Industry, Statistics

(b) Other agencies:

State and Federal Stations and Agencies especially in the Southern Region.

10. Financial Support:

Proposed Budget July 1953 to July 1954:

	Total	Institute of Statistics	963
H. L. Lucas (1/4 time)	$2500		$2500
R. G. Petersen (1/2 time)	2640	$2640	
W. W. G. Smart, Jr. (1/4 time)	1375	675	700
Secretarial, stenographic and clerical help	1400	400	1000
Computing help	2600	600	2000
Travel*	835	235	600
Supplies and equipment	300	100	200
Total	$11650	$4650	$7000

July 1954 and on:

The Institute of Statistics cannot supply funds in support of this project after 1953-54. This means the 963 S-12 funds will have to supply the whole budget for 1954-55 and thereafter. The total budget should be expanded about 30% in 1954-55 and thereafter.

*Travel for Objective (b) will be paid from the 963 S-12 trust fund.

ANNUAL PROGRESS REPORT FOR STATE SUPPORTED PROJECTS
OF THE
NORTH CARCLINA AGRICULTURAL EXPERIMENT STATION
(Three copies to be submitted to Director's Office)

1. PROJECT: (Fund, number, and title): **S-79, Investigations in Animal Nutrition. Phase A - Cottonseed Meal Investigations**

2. DEPARTMENTS AND COOPERATING AGENCIES: **Department of Animal Industry (Nutrition) and USDA, ARS, FCRB Cotton Division**

3. PERSONNEL: **F. H. Smith, Virginia W. Smart, Claude Rhyne and P. A. Miller**

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

Seed progeny from various crosses of experimental genetic lines of cotton were assayed for total gossypol content and were found to range from 0.08 to 1.43%.

Cottonseed meals prepared from 8 genetically different seed lines were assayed by feeding them to weanling rats at the 10% protein level with an ether-extracted commercial seed serving as a control. The gossypol content of the meals ranged from 0.035 to 0.636%. The meal with the lowest gossypol content produced the greatest gains and was followed by the meal with the next lowest gossypol content. All of the meals were superior to the control.

It was found in feeding trials with rats that diets containing about 4% less protein supplied by cottonseed meal containing 0.47% total gossypol would produce gains approximately equal to those obtained from the same meal to which 1.34% gossypol was bound.

Lysine content, by the dinitrofluorobenzene method, was not different for two meals producing significant differences in gains.

5. USEFULNESS OF FINDINGS(Benefits to Agriculture and the general public and contributions to science): **These studies indicate that improvement in the nutritive quality of cottonseed meal may result from the development of genetic lines of cotton having a low gossypol content.**

6. WORK PLANNED FOR NEXT YEAR:
The continuation of the assaying experimental genetic lines of cotton for gossypol content and to further study the causes of differences in growth responses.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:
Spectrophotometric Determination of Total Gossypol in Cottonseed Meal and Cottonseed Meats. J. Am. Oil. Chem. Soc. 35: 261-265 (1958).
The Effects of Bound Gossypol on the Growth Promoting Properties of Cottonseed. J. Nutr. 66:393-409 (1958).

8. Prepared by **F. H. Smith** Approved _____ Director

Date **February 20, 1959** Date _____

ANNUAL PROGRESS REPORT FOR STATE SUPPORTED PROJECTS
OF THE
NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION
(Three copies to be submitted to Director's Office)

1. PROJECT: (Fund, number, and title): S-79, Investigations in Animal Nutrition. Phase B - Microbiological Studies.

2. DEPARTMENTS AND COOPERATING AGENCIES: Department of Animal Industry

3. PERSONNEL: J. J. McNeill

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

Results have been obtained concerning the formation of volatile fatty acids by bovine rumen bacteria from carbon dioxide and molecular hydrogen. Washed suspensions of rumen bacteria produce greatly increased amounts of acetic, propionic, and butyric acids from carbon dioxide and hydrogen compared to controls wherein hydrogen is replaced by nitrogen.

5. USEFULNESS OF FINDINGS(Benefits to Agriculture and the general public and contributions to science):

a. The above findings are of fundamental importance because the fixation of carbon dioxide using energy derived from the oxidation of molecular hydrogen has been reported only once before, some 20 years ago. Such a reaction has not been known to occur in the rumen.

b. This finding is of interest to agriculture because the reaction described competes with the methane reaction in the rumen, since both systems utilize carbon dioxide and hydrogen. The formation of fatty acids is a more desirable reaction because it results in the production of utilizable end-products, while methane formation results in the formation of an inert gas containing considerable energy which is no longer available to the animal. Knowledge of this fatty-acid formation system may make it possible to devise methods for favoring this reaction at the expense of the methane system.

6. WORK PLANNED FOR NEXT YEAR:

The isolation of the organism(s) responsible for this reaction will be attempted. If a pure culture is obtained, characterization of the organism and the enzyme systems responsible for the reaction will be carried out. With such information it should be possible to determine whether this system is of quantitative importance in the rumen.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

None

8. Prepared by J. J. McNeill

Approved _____
Director

Date February 20, 1959

Date _____

SES—Form 8
(June 12, 1957)

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, FEDERAL-GRANT PROJECTS, 1960

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): S-79, INVESTIGATIONS IN ANIMAL NUTRITION
(A) Microbiological Formation of Methane in Ruminants

2. DEPARTMENTS AND COOPERATING AGENCIES: Department of Animal Industry

3. PERSONNEL: J. J. McNeill

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

Experiments were conducted to determine if rumen bacteria carried out the reaction $2CO_2 + 4H_2 \rightleftharpoons CH_3COOH + 2H_2O$ using resting cell suspensions. It does not appear that this reaction occurs in the rumen.

The gases produced by the fermentation of rumen contents from animals receiving purified diets were analyzed. Carbon dioxide and methane were produced in all cases, and in a ratio of 90% CO_2 and 10% CH_4. This ratio was remarkably constant.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science):

It may be possible to influence the rumen fermentation to favor acetate formation at the expense of methane formation, with considerable benefit to the energy metabolism of the animal.

6. WORK PLANNED FOR NEXT YEAR:

More sensitive methods, with C-14, will be applied to detect the occurrence of acetate formation from CO_2 and H_2.

Attempts will be made to inhibit the methane reaction to ascertain whether the magnitude of the acetate formation can be favored by preventing the reaction from going rapidly to completion.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

8. Prepared by J. J. McNeill Approved _____ (Director).

Date March 16, 1960 Date _____

ANNUAL PROGRESS REPORT

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION STATE PROJECTS

1. PROJECT: (Fund, number, and title): S-79 - Investigations in Animal Nutrition. Phase G - Microbial Methane Formation in the Rumen and Effects of the Methane Reaction on the Overall Rumen Fermentation

2. DEPARTMENTS AND COOPERATING AGENCIES:
Department of Animal Industry

3. PERSONNEL:
J. J. McNeill

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

Two phases of the microbial formation of rumen methane have been studied:
a. Mechanism of methane formation
b. Influence of methane formation upon the rate of fermentation in the rumen.

The principal results may be summarized as follows:
a. Formate appears to be the major precursor of methane. Rumen bacteria were shown to carry out a rapid formation of methane from carbon dioxide and molecular hydrogen. Enrichment cultures of methanogenic bacteria have been obtained using carbon dioxide as sole carbon source and molecular hydrogen as sole energy source. D-limonene, claimed to be a specific inhibitor of the methane reaction, was found to be without effect on the production of methane from formate.
b. The rate of utilization of glucose, pyruvate, and formate by rumen bacteria was found to be accelerated by those factors which also favor the methanogenic reaction.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science.):
a. The absence of an effect of D-limonene upon methane formation from formate casts doubt upon the feasibility of using such compounds as a means of avoiding the energy loss involved in the methanogenic reaction.
b. The finding that the methane reaction accelerates the microbial utilization of other metabolically important compounds resulting from the fermentation of materials rich in cellulose and starch, indicates that the methane reaction may not be entirely an undesirable reaction in regard to conversion of feed to utilizable energy.

6. WORK PLANNED FOR NEXT YEAR:
Work will be continued as is from the present year with increased emphasis upon the mechanism of methane formation from formate.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:
None

8. Prepared by J. J. McNeill Approved _____

Date March 5, 1958 Date _____

ANNUAL PROGRESS REPORT

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION STATE PROJECTS

1. PROJECT: (Fund, number, and title): S-79 - Investigations in Animal Nutrition. Phase B - Enzymes of the Alimentary Tract and Related Areas.

2. DEPARTMENTS AND COOPERATING AGENCIES:
Department of Animal Industry

3. PERSONNEL:
H. A. Ramsey and G. H. Wise

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

This study pertains to esterases that are known to be secreted in the pregastric portion of the alimentary tract of the young calf. Because of inadequate funds for technical assistance, however, work in this area has been dormant during the past year.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science.):

6. WORK PLANNED FOR NEXT YEAR:

A study will be initiated to ascertain the effects of age of animal and of diet on the secretion of pregastric esterase by the calf.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

Ramsey, H. A. Photometric Procedure for Determining Esterase Activity. Clin. Chem., 3:185 (1957).

8. Prepared by H. A. Ramsey Approved _____
(Director)

Date March 5, 1958 Date _____

ANNUAL PROGRESS REPORT

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION STATE PROJECTS

1. PROJECT: (Fund, number, and title): S-79 - Investigations in Animal Nutrition. Phase A - Cottonseed Meal Investigations.

2. DEPARTMENTS AND COOPERATING AGENCIES: Department of Animal Industry (Nutrition Section), and U.S.D.A., A.R.S., F.C.R.B., Cotton Division

3. PERSONNEL:

F. H. Smith, Virginia W. Smart, P. A. Miller and Claude Rhyne

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

The gossypol content of cottonseed produced in a breeding project in which reduction of gossypol was a factor studied was assayed chemically and biologically (rat growth). The nutritive value of some of these low gossypol lines, grown in Mexico, have been studied by feeding to rats, at the 10% protein level, after hexane extraction and binding the residual gossypol to the meal. The rats made excellent growth on all meals except one which had a relatively high gossypol content. The growth from this meal was significantly lower than that from another meal having about the same gossypol content.

A second feeding trial was conducted on meals (ranging from 0.32 to 0.93% gossypol) prepared from seed from low gossypol lines grown in North Carolina. All the meals produced excellent growth.

Preliminary studies have been made on the oils from some of the low-gossypol-line seed.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science.):

The development of a variety of cotton containing a low level of gossypol with other desirable agronomic characteristics should result in higher quality cottonseed meal and cottonseed oil.

6. WORK PLANNED FOR NEXT YEAR:

To test some of the better low-gossypol lines for gossypol content and to evaluate the seed protein by feeding studies.

To attempt to determine the cause of the differences in growth from the meals with about the same gossypol content.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

Spectrophotometric Determination of Total Gossypol in Cottonseed Meal and Cottonseed Meats (Accepted for publication by the Journal of the American Oil Chemists Society)

Effect of Bound Gossypol on the Growth Promoting Properties of the Protein in Cottonseed, Soybean and Peanut Meals (Being reviewed)

8. Prepared by F. H. Smith Approved ~~(Director)~~

Date March 5, 1958 Date

SES—Form 8
(June 12, 1957)

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

STATE
~~FEDERAL GRANT~~

ANNUAL PROGRESS REPORT, ~~FEDERAL GRANT~~ PROJECTS, 19 61

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): STATE, S-79, INVESTIGATIONS IN ANIMAL NUTRITION.

2. DEPARTMENTS AND COOPERATING AGENCIES: Department of Animal Industry

3. PERSONNEL: J. J. McNeill, W. C. Heiser, E. Haywood, J. B. Murray, J. T. Farlowe

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked): See S-235.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science): See S-235.

6. WORK PLANNED FOR NEXT YEAR: Funds to be redistributed, and project closed.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR: None.

8. Prepared by J. J. McNeill Approved _____ (Director).

Date March 29, 1961 Date _____

SES—Form 8
(June 12, 1957)

North Carolina STATE AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, FEDERAL GRANT PROJECTS, 1960

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): S-163, GENETIC STUDY AND EVALUATION OF CYTOGENETIC COTTON MATERIALS DERIVED FROM RELATED COTTON SPECIES, WHICH CAN BE USEFUL IN APPLIED COTTON BREEDING (a) Study of Factors Affecting the Growth Promoting Properties of Cottonseed Obtained Through the Plant Breeding Program
2. COOPERATING AGENCIES: Department of Animal Industry, Department of Field Crops and USDA, ARS, FCRB Cotton Division
3. PERSONNEL:

F. H. Smith, Virginia W. Smart and Claude Rhyne

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

Seed progeny of various crosses of low-gossypol lines designed to unravel the genetics of gossypol heritability were analyzed. Out of 77 samples analyzed, 4 samples contained less than 0.1%, 16 less than 0.3%, and 32 less than 0.5% gossypol.

Cottonseed meals from experimental genetic lines were compared in rat-feeding studies with a meal prepared from a commercial variety after the gossypol was extracted. In three feeding trials, 12 experimental meals ranging in total gossypol content from 0.38% to 1.19% were fed. None of the experimental meals were inferior to the extracted control, thus all were significantly superior to an unextracted control.

The deleterious effects of bound gossypol in cottonseed meal experimentally prepared and fed at the 10% protein level were slight when concentrations were about 0.85% or less and were marked as the level was increased to 1.7%.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science):

These studies give additional evidence that the nutritional value of cottonseed meal may be improved by reducing the gossypol genetically, and that the greatest improvement will be secured by lowering the gossypol content of the seed to 0.5% or less.

6. WORK PLANNED FOR NEXT YEAR:

The continuation of assaying experimental lines of cotton for gossypol content, and bioassaying some of the meals for growth promoting properties.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

F. H. Smith. Preparation of Pure Gossypol from Dianilinogossypol. Journal of Amer. Oil Chem. Soc. In press.

F. H. Smith, C. L. Rhyne and V. W. Smart. Dietary Evaluation of Cottonseed Protein from Cotton Bred for Low Gossypol Content. Submitted for publication in J. Agr. and Food Chem.

8. Prepared by F. H. Smith Approved _____ (Director).

Date March 15, 1960 Date _____

ANNUAL PROGRESS REPORT FOR STATE SUPPORTED PROJECTS
OF THE
NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION
(Three copies to be submitted to Director's Office)

1. PROJECT: (Fund, number, and title): S-154, The Evaluation of Feed Intake and Digestibility in the Utilization of Forage by Grazing Meat Animals.

2. DEPARTMENTS AND COOPERATING AGENCIES: Department of Animal Industry, Department of Experimental Statistics, and Animal Husbandry Research Division, ARS, USDA

3. PERSONNEL: W. W. G. Smart, Jr., G. Matrone, F. H. Smith, E. R. Barrick, and H. L. Lucas

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

To be able to use easily any indicator for a predictor of feed consumption and digestibility it is necessary either that it be excreted uniformly in the feces, or to make total fecal collections. To study diurnal variation of monastral blue as an indicator, the dye was mixed thoroughly with ground alfalfa. After a conventional digestion trial with sheep, fecal samples were collected every four hours for a 24-hour period. There was a diurnal pattern in the excretion of the dye. By multiple covariance it was shown that approximately 40% (R^2) of the variations in dye concentration could be accounted for by variation in the concentration of the proximate constituents. Protein was the most highly correlated constituent with an r = +6009, crude fiber was next with r = -.4637.

The chromogen, copper-chlorophyll, and conventional digestion trials were compared with beef steers.

5. USEFULNESS OF FINDINGS(Benefits to Agriculture and the general public and contributions to science):

If indirect methods of estimating digestibility and consumption could be developed, many expensive conventional trials might be eliminated.

6. WORK PLANNED FOR NEXT YEAR:

Further testing of monastral blue and chromogens will be made, and, since there is some question as to whether monastral blue might in part enter the animal body, the Cu in the dye will be made radioactive to test this.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:
Smart, et al. A Study of the Comparative Composition and Digestibility of Cane Forage. Submitted to Journal of Animal Science.

8. Prepared by W. W. G. Smart, Jr. Approved _____
Director

Date February 20, 1959 Date _____

ANNUAL PROGRESS REPORT

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION STATE PROJECTS

1. PROJECT:(Fund, number, and title):
S-154 - The Evaluation of Feed Intake and Digestibility in the Utilization of Forage by Grazing Meat Animals

2. DEPARTMENTS AND COOPERATING AGENCIES: Depts. of Animal Industry and Statistics (N.C. Agri. Expt. Sta.), Animal and Poultry Husbandry Res. Branch, Agri.Res.Ser. (U.S.D.A.)

PERSONNEL:
W. W. G. Smart, Jr., G. Matrone, F. H. Smith, E. R. Barrick and H. L. Lucas

RESEARCH ACCOMPLISHMENTS OF THE YEAR(Confidential information should be so marked):

In digestion trials with sheep fed fescue and alfalfa of different stages of maturity, chromogen or Cu-chlorophylls proved to be a good indicator of digestibility from either feed or fecal composition. In the alfalfa, crude protein also was a good indicator of digestibility from feed or fecal composition. Crude protein in the fescue did not prove a good indicator. True protein determinations did not improve the prediction of digestibility using protein.

Digestion trials with 1957 crops of alfalfa and fescue are now being conducted.

5. USEFULNESS OF FINDINGS(Benefits to agriculture and the general public and contributions to science.):

The findings contribute to possible means of measuring forage yields by grazing animals, which measurements are of primary importance in ascertaining the economy of livestock production.

6. WORKED PLANNED FOR NEXT YEAR:
Further testing of monastral blue and chromogens will be made, since there is some question as to whether monastral blue might in part enter the animal body. Cu, in dye, will be made radio-active to test this.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:
None

8. Prepared by W. W. G. Smart, Jr. Approved _____
(Director)

Date March 5, 1958

SES—Form 8
(June 12, 1957)

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, ~~FEDERAL~~ STATE GRANT PROJECTS, 1960

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): S-154, EVALUATION OF FEED INTAKE AND DIGESTIBILITY IN THE UTILIZATION OF FORAGE BY GRAZING MEAT ANIMALS

2. DEPARTMENTS AND COOPERATING AGENCIES: Department of Animal Industry, Department of Experimental Statistics, and Animal Husbandry Research Division, ARS, USDA

3. PERSONNEL: W. W. G. Smart, Jr., G. Matrone, F. H. Smith, H. L. Lucas, and M. B. Wise

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

To be useful any indirect indicator for prediction of consumption and digestibility must be excreted uniformly. Studies have been made on the diurnal variation in excretion. Tests have been made on the use of lignin and chromogen as indicators of digestibility. Both leave something to be desired since they are only about 85% recoverable from forages covering a wide range of chemical composition.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science):

If indirect methods of consumption could be developed, many expensive conventional trials could be eliminated.

6. WORK PLANNED FOR NEXT YEAR:

New methods for determining lignin and chromogens will be tested. Predictions of consumption will be tested against soiling procedures where consumption is known.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

Smart et al. A Study of the Comparative Composition and Digestibility of Cane Forage. N. C. Experiment Station Tech. Bulletin No. 140 (In press).

8. Prepared by W. W. G. Smart, Jr. Approved _____ (Director).

Date March 15, 1960 Date _____

SES—Form 8
(June 12, 1957)

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, ~~FEDERAL GRANT~~ STATE PROJECTS, 19 61

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): STATE S-154, THE EVALUATION OF FEED INTAKE AND DIGESTIBILITY IN THE UTILIZATION OF FORAGE BY GRAZING MEAT ANIMALS.

2. DEPARTMENTS AND COOPERATING AGENCIES: Department of Animal Industry, Department of Experimental Statistics, Department of Field Crops, Animal Husbandry Research Division, ARS, USDA

3. PERSONNEL: W. W. G. Smart, Jr., Gennard Matrone, W. B. Gilbert, Nancy W. Stanley

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked): Major efforts have been devoted to the study of factors which affect digestibility. Three species of forages, at several stages of maturity, were studied over several years. Many chemical analyses were studied to determine how these might predict digestibility. The major trouble still remains in the N.F.E. and cellulose fractions. Some progress was made with acid fractionations of the complex carbohydrates. Water soluble 1.25% H_2SO_4 and 4% H_2SO_4 hydrolyzable fractions are good predictors, while 10% and 72% H_2SO_4 still leave better predictions to be desired.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science): The ultimate goal is to determine the value of a pasture in terms of herbage digestibility and in the amount the animal harvests. The attainment of these goals would be of great value to pasture research.

6. WORK PLANNED FOR NEXT YEAR: Work will continue along present lines. More effort will be expended toward methods of determining consumption of pasture and factors which affect this.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR: None

8. Prepared by W. W. G. Smart, Jr. Approved _____ (Director).

Date March 28, 1961 Date _____

SES—Form 8
(June 12, 1957)

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, FEDERAL-GRANT PROJECTS, 1961

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): 193, NUTRITION AND DISEASES OF THE NEONATAL PIG.

2. DEPARTMENTS AND COOPERATING AGENCIES: Animal Nutrition Section, Dept. of Animal Industry, North Carolina State College

3. PERSONNEL: J. G. Lecce, G. Matrone

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

Neonatal serum proteins from unfed pigs were compared with serum from mature pigs. This yielded an immature and mature serum protein profile. The capacity of diets to augment and influence changes from the immature profile to the mature profile were determined. It was found that pigs nursing the sow not only had intense and immediate changes in serum protein, but also continuous and rapid alterations toward the mature serum profile. Pigs fed other diets such as cows milk experienced a delay in the development of their serum proteins. It was determined further that piglets had to nurse the sow for between 2 and 4 days before they were sufficiently mature physiologically so that no delay was experienced in serum protein development.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science):

Learn ways and means to efficiently wean pigs at the time of their birth.

6. WORK PLANNED FOR NEXT YEAR:

Learn how the sows mammary secretions influences vigor in the piglet.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

Lecce, J. G., & Matrone, G., "Porcine Neonatal Nutrition: Effect of Weaning Time on the Maturation of the Serum Protein Profile." J. Nutrition, Vol. 73, No. 2, Feb., 1961, pp. 167-171. - Lecce, J. G. & Matrone, G., "Porcine neonatal nutrition: the effect of diet on blood serum proteins and performance of the baby pig." J. Nutrition, Vol. 70, No. 1, Jan., 1960, pp. 13-20.

8. Prepared by _____ Approved _____ (Director).

Date March 27, 1961 Date _____

N.C. 193 Anim Indus Nutrition and Diseases of the Neonatal Pig

1. STATE | 2. PROJ. NO. | 3. DEPT. | 4. ABBREV. TITLE | 5. REF. | 6. X-REF.

7. TITLE, PROJECT OBJECTIVES AND DESCRIPTION OF WORK PROPOSED:

NUTRITION AND DISEASES OF THE NEONATAL PIG --Study the effects of diet on serum protein synthesis in the neonatal pig; investigate the mechanism of serum protein maturation in the neonatal pig; determine interrelationships between nutrition and microbial diseases of the neonatal pig.

DESCRIPTION OF WORK

Neonatal serum proteins from unfed pigs were compared with serum from mature pigs. This yielded an immature and mature serum protein profile. The capacity of diets to augment and influence changes from the immature profile to the mature profile were determined. It was found that pigs nursing the sow not only had intense and immediate changes in serum protein, but also continuous and rapid alterations toward the mature serum profile. Pigs fed other diets such as cows milk experienced a dealy in the development of their serum proteins. It was determined further that piglets had to nurse the sow for between 2 and 4 days before they were sufficiently mature physiologically so that no delay was experienced in serum protein development.

8. INDICATE TYPE OF PROJECT	HATCH	RRF	AMA	NON-FED.	NEW	REV.	MARKETING	9. DURATION	10. COOPERATION	11. APPROVAL DATES	12. X-REF.
	X							64		11-30-59	

13. STATE	14. PROJ. NO.	15. DEPT.	16. ABBREV. TITLE	17. REF.
N.C.	193	Anim Indus	Nutrition and Diseases of the Neonatal Pig	

18. RECOMMENDED FOR APPROVAL

TITLE SIGNATURE DATE

SECTIONS 18, 19, AND 20 NOT APPLICABLE FOR PROJECTS PREVIOUSLY APPROVED BY SED

19. APPROVAL OF DIRECTOR, AGRICULTURAL EXPERIMENT STATION

SIGNATURE DATE

20. FEDERAL-GRANT PROJECTS ONLY--TO BE APPROVED BY STATE EXPERIMENT STATIONS DIVISION, WASHINGTON, D. C.

SIGNATURE DATE

INSTRUCTIONS: Complete Items 1, 2, 3, 7, 8, 9, 10, 18, and 19. Under Item 7, show title in CAPS, itemize objectives and leave space between the objectives and description of work proposed. Forward original of this form with required number of project outlines to State Experiment Stations Division, Washington, D. C. *(See reverse side for Essentials of an Experiment Station Project Outline.)*

SES Form 20
Dec 1960

U. S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
STATE EXPERIMENT STATIONS DIVISION

COVER-ABSTRACT-SIGNATURE PAGE FEDERAL-GRANT & NON-FEDERAL PROJECTS

NORTH CAROLINA STATE COLLEGE
SCHOOL OF AGRICULTURE

Office of Director of Research

PROJECT ACTION NOTIFICATION

DEPARTMENT: Animal Industry

PROJECT NO.: S-232(MG NIH Grant E-3209)

PROJECT TITLE: The Nutritional Significance of Pregastric Esterase

ACTION TAKEN: Approved as State project May 31, 1960

DATE: June 1, 1960

COMMENT: This project was approved and supported by National Institute of Health funds for four years in 1958. It also has State money budgeted to it. In order to simplify our project budgeting and numbering we are now designating this study as Project S-233 (MG NIH 3209).

R. L. Lovvorn
Director of Research

cc: H. A. Stewart
Accounting Office
Department Head
Director's Office

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION
PROJECT OUTLINE

Project No. S-232(NIH E-3209)
Date
Submitted
Approved May 31, 1960
Revised

1. Title

The Nutritional Significance of Pregastric Esterase

2. Objective(s)

a. To investigate the origin of pregastric esterase and to study factors affecting its secretion.

b. To investigate the nutritional importance of pregastric esterase to the animal at different stages of postnatal development.

c. To study various biochemical properties of pregastric esterase.

3. Reasons for undertaking Investigations*

Alimentary lipolysis is recognized as a prerequisite to the absorption of dietary fat. Although pregastric esterase, a lipolytic enzyme present in saliva, is known to hydrolyze fat in vitro, its importance in the digestive processes of the intact animal have not been established. As a source of energy, fat is a particularly important constituent in the diets of young mammals, thus accentuating the need for studying enzymes involved in the digestion of fat. This need is further emphasized by the diversity of fats being added to feeds and food.

* Including economic justification

4. Previous work and present status of investigations in the field of this project:

See National Institutes of Health Project No. A-2230, pages 7-8.

5. Outline of Procedure:

See National Institutes of Health Project No. A-2230, pages 4-6.

6. Probable Duration of Project: 3 years

7. Date of Initiation: January 1, 1960

8. Personnel:

Name	Department	Relation to Project
H. A. Ramsey	Animal Industry	Leader

9. Coöperation:

a. Interdepartmental

b. Other Agencies

National Institutes of Health of U. S. Public Health Service

10. Financial Support:

a. Proposed Budget 7/1/60 to 6/30/61

Items	ALLOCATION OF FUNDS				
	Hatch	Regional Research	State	Other	Total
1. Salaries				PHS	
H. A. Ramsey			$7,000.		
E. Barnes				$3,312.	
G. H. Wise			1,256.		
2. Labor					
3. Travel				150.	
4. Equipment & Supplies				2,788.	
5. All Other (Contractual and Printing)				400.	
Total			$8,256.	$6,650.	

b. Proposed Future Budgets:

Year	Salaries	Total Expenditures	Estimated Income
1961-1962	$11,568.	$14,906.	

11. General Remarks:

SIGNATURES OF APPROVAL

1. Approval of Project Leaders

Date December 2, 1959 ..... Harold A. Ramsey .....

Title Research Assistant Professor .....

Date ....

Title .....

Date ....

Title .....

2. Approval of Heads of Departments or Coöperating Agencies

Date May 24/60 ..... George Hyatt Jr .....

Head, Dept of Animal Industry .....

Date ....

Head, ...

Date ....

Head, ...

3. Approval of Director

Date June 7, 1960 ..... R. H. Brown .....

Director, North Carolina Agricultural Experiment Station

4. Approval of U. S. D. A.

Date ....

Chief, Office of Experiment Stations

SES—Form 8
(June 12, 1957)

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATE

ANNUAL PROGRESS REPORT, FEDERAL GRANT PROJECTS, 1961

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): STATE, S-232 THE NUTRITIONAL SIGNIFICANCE OF PREGASTRIC ESTERASE.
2. DEPARTMENTS AND COOPERATING AGENCIES: Department of Animal Industry, Public Health Service
3. PERSONNEL: H. A. Ramsey, G. H. Wise, Carolyn Nash.
4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

Extracts of various abmentary and related tissues from the calf were subjected to electrophoresis in starch gel. The different esterases in each tissue then were located by a special staining procedure. Two esterases, each possessing a characteristic electrophoretic mobility, were found in certain oral and pharyogeal tissues and in the parotid and submaxillary salivary glands. These two esterases were also found in the liver, but they were not present among the esterases found in extracts of the rumen, reticulum, omasum, abomasum, duodenum or pancreas. These two enzymes are believed to be the components of pregastric esterase, though attempts to confirm this by examining the saliva have been unsuccessful. Preliminary evidence suggests that these two esterases differ in their substrate specificity and that they are not present in the same ratio in different tissues use.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science):

The project provides basic information on some of the enzymes involved in the utilization of dietary fat, one of the major energy sources of the young calf.

6. WORK PLANNED FOR NEXT YEAR:

To study the substrate specificity of each enzyme in pregastric esterase from the calf. To observe electrophoretically the distribution of esterases in similar tissues from the lamb and the kid. To continue investigations of the quantitative role of pregastric esterase in abomasal hydrolysis of milk fat by the calf.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

1. Effects of Age and Diet on the Secretion of Pregastric Esterase in Calves. J. W. Young, H. A. Ramsey and G. H. Wise. J. Dairy Sci. 43: 1068 :(1960).
2. Effects of Continuous Nursing, Length of the Nursing Period, and Rate of Milk Consumption on the Accretion of Pregastric Esterase by Calves.*

8. Prepared by [signature] (Director).

Date _____ Date _____

* H. A. Ramsey, J. W. Young, and G. H. Wise. J. Dairy Sci. 43: 1076 (1960)

SES—Form 8
(June 12, 1957)

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION
STATE

ANNUAL PROGRESS REPORT, ~~FEDERAL GRANT~~ PROJECTS, 19 61

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): STATE, S-233, METABOLISM OF UNSATURATED FATTY ACIDS OF DEPOT FAT (THE CHEMICAL NATURE AND MECHANISM OF ACTION OF A DIETARY FACTOR AFFECTING THE COMPOSITION OF FAT - Nutrition Foundation)
2. DEPARTMENTS AND COOPERATING AGENCIES: Department of Animal Industry, Public Health Service, Nutrition Foundation
3. PERSONNEL: Samuel B. Tove, Ann J. Hawkins, Anne W. Cannon, F. H. Smith
4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked): Previous work has shown that the synthesis of the depot fat of animals is far from a random process. Not only does one fatty acid exert a specific influence on the deposition of the other fatty acids, but also there are differences with respect to position in the triglyceride molecule. It had been previously observed that there are two rates of linoleate disappearance from mice given a fat-free diet after having been on a high linoleic acid regimen. Recent work shows that the fast rate of disappearance is associated with one of the alpha positions, and that the acids of the beta position are metabolized more slowly.

Experiments on the synthesis of lipid from palmitoyl-CoA by enzyme preparations of rat adipose tissue are being conducted in an effort to account for some of the specificity effects observed with the intact animals.

Tripalmitin containing C^{14}-palmitic acid in one of the α positions (stereospecific) and in the β position have been synthesized. Using these substrates we are currently searching for a lipase that will hydrolyze the α positions of triglycerides stereospecifically.

Work in collaboration with project H-29 has shown that less hydrogenation of dietary fatty acids by the rumen microflora occurs on purified diets than on normal diets.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science):

These studies will provide information on the factors that affect the fatty acid composition of animal products. The fatty acid composition may be of importance to the health of the population consuming these products.

6. WORK PLANNED FOR NEXT YEAR: Continue search for a stereospecific lipase and studies on lipid synthesis by enzyme systems of adipose tissue. Initiate an investigation of the hydrogenation system of the rumen microflora. Continue studies on the metabolism of odd-numbered carbon fatty acids.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

S. B. Tove. The Origin of Depot Fat. J. Dairy Sci., 43: 1354-1360, 1960.

8. Prepared by Samuel B. Tove Approved _____ (Director).

Date March 29, 1961 Date _____

NORTH CAROLINA STATE COLLEGE
SCHOOL OF AGRICULTURE

Office of Director of Research

PROJECT ACTION NOTIFICATION

DEPARTMENT: Animal Industry

PROJECT NO.: S-233 (Misc. Gifts, NIH Grant No. A2483)

PROJECT TITLE: Metabolism of Unsaturated Fatty Acid of Depot Fat

ACTION TAKEN: Approved as State project on May 31, 1960

DATE: June 1, 1960

COMMENT: This project was approved and supported by National Institute of Health funds for four years in 1958. It also has State money budgeted to it. In order to simplify our project budgeting and numbers we are now designating this study as Project S-233(MG NIH).

R. L. Lovvorn
Director of Research

cc: H. A. Stewart
Accounting Office
Department Head
Director's Office

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION
PROJECT OUTLINE

Project No. S-233(NIH Grant No. A-2483
Date
Submitted .....
Approved May 31, 1960
Revised .....

1. Title

Metabolism of Unsaturated Fatty Acids of Depot Fat

2. Objective(s)

a. Determine the biochemical factors that influence the deposition and depletion of unsaturated fatty acids in depot fat.

b. Determine the mechanism of action of the factors.

(See National Institutesof Health Project No. A-2483, page 4, for detailed objectives.)

3. Reasons for undertaking Investigations*

The keeping quality, flavor and nutritional value of animal products depend in a large measure on the fatty acid composition of the fat. Consequently it is of importance to understand the process whereby the fatty acids are deposited in an animal fat with the view of its ultimate control by man. The recent implication of linoleic acid in the control of atherosclerosis makes this problem even more timely.

* Including economic justification

4. Previous work and present status of investigations in the field of this project:

See National Institutes of Health Project No. A-2483, page 7.

5. Outline of Procedure:

See National Institutes of Health Project No. A-2483, pages 4-6.

6. Probable Duration of Project: 3 years

7. Date of Initiation: January 1, 1960

8. Personnel:

Name	Department	Relation to Project
S. B. Tove	Animal Industry	Leader
F. H. Smith	Animal Industry	Co-Leader

9. Coöperation:

a. Interdepartmental

b. Other Agencies

National Institutes of Health of U. S. Public Health Service.

10. Financial Support:

a. Proposed Budget 7/1/60.... to .6/30/61

Items	ALLOCATION OF FUNDS				
	Hatch	Regional Research	State	Other	Total
1. Salaries				PHS	
S. B. Tove			$6,980.		
Ann Cannon				$3,720.	
Ag. Res. Asst. III			3,312.		
F. H. Smith			2,000.		
G. H. Wise			1,000.		
R. L. Anderson			1,500.		
2. Labor					
3. Travel				150.	
4. Equipment & Supplies				5,480.	
5. All Other (Contractual and Communications)				650.	
Total			$14,792.	$10,000.	

b. Proposed Future Budgets:

Year	Salaries	Total Expenditures	Estimated Income
1961-1962	$18,512.	$24,792.	

11. General Remarks:

SIGNATURES OF APPROVAL

1. Approval of Project Leaders

Date November 24, 1959 ..... Samuel B Tove .....

Title Research Associate Professor .....

Date November 24, 1959 ..... F. H. Smith .....

Title Research Associate Professor .....

Date ....

Title .....

2. Approval of Heads of Departments or Coöperating Agencies

Date ....

Head, ...

Date ....

Head, ...

Date ....

Head, ...

3. Approval of Director

Date June 2, 1960 ..... R. H. Lossoon .....

Director, North Carolina Agricultural Experiment Station

4. Approval of U. S. D. A.

Date ....

Chief, Office of Experiment Stations

R'cd date	10-17-60
Council	March/61
Action	

U. S. Department of
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

APPLICATION FOR RESEARCH GRANT
(A PRIVILEGED COMMUNICATION)

(Leave Blank)

No.	A-2483 (C2S1)
SS	NUTR (3)
Formerly	

NRP (NO)

Application is hereby made for a grant in the amount and for the period stated, for the purpose of conducting research as described herein, in accord with the Agreement signed below.

A. AMOUNT REQUESTED: $ 19,250 (Same as total of itemized budget, page 2, item A8.)

B. PERIOD DATES: September 1, 1960 (Mo. Day Year) thru August 31, 1961 (Mo. Day Year) (Normally 12 months. See instructions.)

C. TITLE OF RESEARCH PROPOSAL (Do not exceed 53 typewriter spaces)
METABOLISM OF UNSATURATED FATTY ACIDS OF DEPOT FAT

x

A-2483 (C2)

E. PRINCIPAL INVESTIGATOR:
Name Samuel B. Tove Telephone No. TE 4-5211 Extension 241
Title Professor Department or Service Animal Industry
Mailing address of Research office North Carolina State College
Raleigh, North Carolina
Institution North Carolina State College Major Sub Division School of Agriculture

F. CO-PRINCIPAL INVESTIGATOR, if any. (Name and title only)
Frank H. Smith, Associate Professor

G. INSTITUTION SPONSORING REQUEST
Name N.C.State College of Agriculture and Engineering of the University of N rth Carolina
Mail address Raleigh, N. C.
Name & title of official authorized to sign application on behalf of institution W. D. Carmichael, Jr., Vice Pres. & Finance Officer, Univ. of North Carolina, Chapel Hill, N. C.

H. NAME, TITLE, AND ADDRESS OF FINANCIAL OFFICER:
J. G. Vann, Business Manager
N. C. State College
Raleigh, N. C.
Manner in which check(s) should be drawn: (746408)
N. C. Agricultural Experiment Station

I. AGREEMENT: It is understood and agreed by the undersigned that any grant received as a result of this application is subject to the following terms: (1) Funds granted as a result of this request are to be expended for research or related purposes as governed by Public Health Service and grantee institution policies; (2) the grant may be revoked in whole or in part at any time by the Surgeon General of the Public Health Service, provided that a revocation shall not include any amount obligated previous to the effective date of the revocation if such obligations were made solely for the purposes of research; (3) all reports of original investigations supported by the grant shall acknowledge such support; (4) if any invention arises or is developed in the course of the work aided by the grant, the undersigned will either (a) refer to the Surgeon General for determination, or (b) determine in accordance with grantee institution's own policies as formally stipulated in a separate supplementary agreement entered into between the Surgeon General and the grantee institution, whether patent protection on such invention shall be sought and how the rights in the invention, including rights under any patent issued thereon, shall be disposed of and administered, in order to protect the public interest.

J. PERSONAL SIGNATURES (in ink)
(1) Principal Investigator _____ (Same as shown in "E" above) _____ (date)
(2) Authorized official of applicant institution W. D. Carmichael, Jr. (Same as shown in "G" above) 10/10/60 (date)

Mail completed application to:
Division of Research Grants
National Institutes of Health
Bethesda 14, Md.

A. BUDGET REQUEST (for the period shown on page 1) A2483 C2S1

(1)	(2)	(3)
1. PERSONNEL List all positions, including Principal and Co-investigator. Amounts requested must not exceed proportion of total salary computed from % of time spent.	% time on this project	Requested from PHS (omit cents)
None	%	$
	%	
	%	
	%	
	%	
	%	
	%	
2. PERMANENT EQUIPMENT, itemize (see instructions)		
Model 314X Packard Automatic Tri-Carb Liquid Scintillation		$
Spectrometer		12,500
Beckman/Spinco Model L Preparative Ultracentrifuge		6,000
3. CONSUMABLE SUPPLIES, itemize (see instructions)		
None		$
4. TRAVEL, itemize (see instructions)		
None		$
5. OTHER EXPENSE, itemize (see instructions)		
None		$
6. TOTAL DIRECT COST REQUIREMENTS		$ 18,500
7. INDIRECT COST ALLOWANCE (The administrative official signing this application may request an amount for indirect costs. Review detailed instructions) (Round to low dollar)		$ 750
8. TOTAL BUDGET (Same as amount shown in item A, page 1)		$ 19,250

B. ESTIMATE OF SUPPORT REQUESTED FOR THE YEAR FOLLOWING THE BUDGET PERIOD ITEMIZED ABOVE. Applicants for 1-year grants should type the word "None" in space for TOTAL BUDGET shown below.

Personnel	Equipment	Supplies	Travel	Other	Total Direct Cost	Indirect Cost	TOTAL BUDGET
$ None	$ XXXXXX None	$ None	$ None	$ None	$ XXXXXX None	$ XXX None	$ XXXXXX None

C. ADDITIONAL YEARS OF SUPPORT, beyond the 2 years covered above, if requested. Please show the TOTAL AMOUNTS required for each such additional year, including indirect cost allowance.

3. $ None 4. $ None 5. $ None 6. $ None 7. $ None

A2483 C2S1

RESEARCH SUPPORT

List all other research support of the Principal Investigator, including that from own institution, and applications that are pending. Use continuation page if necessary. See instructions.

A. PUBLIC HEALTH SERVICE SUPPORT:

GRANT NUMBER	TITLE OF PROJECT	AMOUNT	PERIOD OF SUPPORT
(1) Active or approved:			
A-2483	Metabolism of Unsaturated Fatty Acids of Depot Fat	$11,500	9/1/60 to 8/31/61
(2) Applications submitted, awaiting decision:			
	None		

B. ALL OTHER RESEARCH SUPPORT:

SOURCE	TITLE OF PROJECT	AMOUNT	PERIOD OF SUPPORT
(1) Active or approved:			
NSF	Interrelationships in the Metabolism of Short Chain Fatty Acids	$21,600	7/1/60 to 6/30/63
(2) Applications submitted, awaiting decision:			
	None		

BIOGRAPHICAL SKETCHES

Provide brief sketches for professional personnel already selected who are to be actively engaged in this project. The following format should be used for each person, with Co-investigator (if any) immediately following Principal Investigator, then other professional personnel, lettered consecutively.

A. Principal Investigator: Samuel B. Tove, Professor
(Name and title)

1. Date of birth: 7/29/21 ; Place of birth: Baltimore, Maryland ;
Present nationality: United States ; Male ☒; Female ☐.

2. Educational experience:

a. Degrees conferred (Begin with baccalaureate degree. Identify honorary degrees under field.):

DEGREE	INSTITUTION CONFERRING	FIELD(S)	YEAR
B.S.	Cornell University	Nutrition	1943
M.S.	University of Wisconsin	Biochemistry	1948
Ph.D.	University of Wisconsin	Biochemistry	1950

b. Other research training and experience, especially that establishing research qualifications in area covered by this application:

WHERE	NATURE	YEAR
Oak Ridge, Tennessee	Isotopes Technique Course	1951

3. Fields of present major scientific interest, in order of choice:
Biochemistry, Nutrition, Bacteriology

4. Supplemental information: Societies: American Association for the Advancement of Science, American Chemical Society, American Oil Chemists Society, American Institute of Nutrition, Society of the Sigma Xi, Phi Lambda Upsilon, Gamma Sigma Delta

B. Frank H. Smith, Associate Professor
(Name and title)

1. Date of birth: 5/18/03 ; Place of birth: Cornelius, North Carolina ;
Present nationality: United States ; Male ☒; Female ☐.

2. Educational experience:

a. Degrees conferred (Begin with baccalaureate degree. Identify honorary degrees under field.):

DEGREE	INSTITUTION CONFERRING	FIELD(S)	YEAR
B.S.	Davidson College	Chemistry	1926
M.S.	North Carolina State College	Chemistry	1931

b. Other research training and experience, especially that establishing research qualifications in area covered by this application:

WHERE	NATURE	YEAR
University of North Carolina	Course in Instrumentation	1941

3. Fields of present major scientific interest, in order of choice:
Biochemistry, Analytical Chemistry, Nutrition

4. Supplemental information:
Societies: American Chemical Society, American Oil Chemists Society, North Carolina Academy of Science, Society of the Sigma Xi

RESEARCH PLAN AND SUPPORTING DATA

Details of the proposed plan and other necessary data should be typed (single spaced) in accord with the outline below, which is suggestive only. See instructions. Please continue numbering pages in sequence for entire application. Additional continuation sheets, if needed, may be requested from the Division of Research Grants.

1. **RESEARCH PLAN**
   - A. Specific Aims - Provide a concise statement of the aims of the work immediately proposed, and relate these to your long-term goal.
   - B. Method of Procedure - Give details of your research plan, including how results will be analyzed. For each specific aim mentioned in "A" show how your plan is expected to fulfill the aim.
   - C. Significance of this Research - Explain why the results of the proposed work may be important.
   - D. Facilities Available - Describe the general facilities at your disposal. List the major items of permanent equipment.
2. **SUPPORTING DATA**
   - A. Previous Work Done on this Project - Describe briefly any work you have done to date that is particularly pertinent.
   - B. Results Obtained by Others - Summarize important results to date obtained by others on this problem, citing publications. Select no more than five.
   - C. Personal Publications - Cite your most important publications on this or closely related work. List no more than five.
   - D. Justification of Budget - Defend itemized budget for the initial period (A, page 2) where you feel it necessary, and delineate reasoning basic to budget estimates for continuation years.

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1. **RESEARCH PLAN**

A. Specific Aims — The overall objectives of this project are the determination of the biochemical factors that influence the depletion and deposition of fatty acids in depot fat and the determination of their mechanism of action.

The specific aims for the future are detailed in the request for renewal that has been submitted with this request. Those aims specifically concerned with the equipment requested herein are:

1. Determination of the mechanism whereby one fatty acid exerts a specific influence on the deposition of other fatty acids, and the determination of the mechanism of positional specificity of glyceride structure.
2. The determination of the specificity of lipases in general with the hope of finding a lipase exhibiting stereospecificity in the hydrolysis of one of the alpha positions of the glyceride.

Work on the solution of both of these problems has been initiated. The equipment requested is needed for the solution of these problems in that both involve enzyme systems requiring high speed centrifugation in their preparation and that both require the measurement of C^{14} in lipids.

B. Method of Procedure - Procedure for specific aim 1: A microsomal system from rat adipose tissue has been found that will synthesize glycerides from palmitoyl-CoA and either L-α-glycerolphosphate, phosphatidic acids, or 1,2 diglycerides. Attempts will be made to solubilize, fractionate, and resolve this system into the individual enzymes that comprise the complex. Glyceride synthesis will be determined by measuring the incorporation of labeled fatty acids into glycerides that will be isolated from the incubation medium by chromatography on Florosil or silicic acid.

The microsomal system is prepared from homogenates of rat adipose tissue by differential centrifugation, the final step being sedimentation

at 109,000 g. Attempts will be made to break the particulate complex by means of techniques such as solvent extraction (e.g., butanol), detergent extraction (e.g., sodium cholate), aqueous extraction of acetone powders or sonicates of the microsomes. If the complex can be broken and solution of the proper enzymes obtained, attempts will be made to purify the enzymes by the usual protein fractionation techniques.

Initial emphasis will be placed on the synthesis of triglycerides from an acyl-CoA ester and a 1,2 diglyceride. The coenzyme A esters of various C^{14} labeled fatty acids will be prepared by means of a microsome preparation from guinea pig liver (J. Biol. Chem., 204: 329, 1954). Diglycerides containing various fatty acids in different combinations and configurations (e.g., 1 oleyl-2 palmitoyl diglyceride and 1 palmitoyl-2 oleyl diglyceride) will be prepared by esterification of the 2 position of symmetrical 1,3 diglycerides. The mixed 1,2 diglycerides will be isolated following hydrolysis of the triglycerides by pancreatic lipase.

Subsequently it is expected to study the synthesis of phosphatidic acids and the lipase catalyzed exchange of one fatty acid by another by the adipose tissue enzyme systems.

Procedure for specific aim 2: The curve depicting the increase in linoleic acid in the alpha positions of mouse depot fat with an increase in dietary level showed two slopes. This finding coupled with the fact that different enzyme systems are involved in the esterification of the two alpha-hydroxyl groups of glycerol suggests that the two alpha positions of the triglyceride may have different fatty acid distributions. This idea could be tested if there were available a lipase capable of stereospecific hydrolysis of one of the alpha positions. We have therefore initiated a search for a stereospecific lipase, for in addition to testing the notion above, such a lipase, if found, would be an extremely valuable tool in the study of glyceride structure. Even if not found, the search will provide data on the specificity of the various lipases toward hydrolysis of α or β esterified fatty acids.

The general procedure used will be that of incubating a purified lipase with an appropriately labeled substrate and determining the specific activity of the hydrolysis products. Tripalmitin containing C^{14} labeled palmitic acid in the 1 position of the triglyceride has been prepared from D-dipalmitin and carboxyl-labeled palmitoyl chloride. Tripalmitin containing labeled palmitic acid esterified at the 2 position of the glyceride will be prepared from 1,3 dipalmitin and C^{14} labeled palmitoyl chloride. Purified lipases will be prepared from various sources such as seeds (e.g., oats, wheat, castor bean), molds, and animal tissues (e.g., adipose tissue, gastric mucosa, liver) and examined for specificity of hydrolysis.

C. Significance of This Research - With the recent involvement of unsaturated fatty acids in atherosclerosis, knowledge of the metabolism of unsaturated fatty acids of depot fat is of immediate concern to the understanding of this disease that is vital in the achievement of measures for its prevention and cure.

D. Facilities Available - The following are the major facilities available: chemical laboratories and associated general equipment; air conditioned animal room with facilities for housing 280 mice and 120 rats individually; radioisotope equipment such as γ spectrometer, gas flow counter with automatic sample changer. Major items of laboratory equipment include spectrophotometers, refrigerated centrifuge, fraction collector, and gas chromatography apparatus.

2. SUPPORTING DATA

A. Previous Work Done on this Project - See Request for Renewal of Grant No. A-2483 entitled "Metabolism of Unsaturated Fatty Acids of Depot Fat".

B. Results Obtained by Others - See Request for Renewal of Grant No. A-2483 entitled "Metabolism of Unsaturated Fatty Acids of Depot Fat".

C. Personal Publications - See Request for Renewal of Grant No. A-2483 entitled "Metabolism of Unsaturated Fatty Acids of Depot Fat".

D. Justification of Budget - The first phase of this research project, namely the investigations involving the use of the whole animal, is nearing a terminal stage, and the second phase, that of accounting for the observations of fatty acid specificity in depot fat, has begun. During this phase much of the work will involve enzyme fractionation and the assay of C^{14} labeled lipids in which the two items of equipment requested herein will be needed.

Spinco preparative centrifuge: The preparation of the adipose tissue system synthesizing triglycerides requires isolation of microsomes by high speed centrifugation. The work presently under way requires frequent (every other day) use of a high speed centrifuge, and it is expected that the need for this instrument will increase even more in the future. The only centrifuge available capable of achieving the gravitational fields necessary is located across the campus in the Biological Science building. Since this instrument is used extensively by personnel in that building, conflicts of scheduling have already arisen causing delay in the progress of the work. As our requirements for the centrifuge increase, the problem will become more acute.

Liquid scintillation spectrometer: The apparatus used at present for the counting of radioactive samples is a geiger counter equipped with an ultra-thin window and an automatic sample changer. Although this apparatus is very satisfactory for most radioactive samples, it leaves much to be desired in counting C^{14} labeled lipids.

In order to maximize the reproducibility of counting C^{14} labeled lipids, we have had to use infinitely thin samples containing only a portion of the total sample available. In addition, the marked tendency for the lipid to creep over the walls of the planchet necessitates the use of deep walled planchets. Because of these two factors and the geometry loss in using a geiger tube, we are able to measure only about 1% of C^{14} disintegrations in a typical sample. Moreover, the reproducibility of counting duplicate samples is still poor.

In contrast, the use of a liquid scintillation spectrometer would permit the counting of about 90% of the C^{14} disintegrations in a sample with far greater precision and accuracy. This increase in counting efficiency and precision is especially desirable as it would greatly increase the number of experiments that could be conducted with the limited amounts of purified enzymes and/or radioactive substrates that require considerable time and effort to prepare (stereoisomerically labeled triglycerides, fatty acyl-CoA esters).

Inasmuch as a liquid scintillation counter is not located on our campus, it is requested that funds be provided for its purchase. Because a large number of samples will require counting (e.g., about 60 chromatographic fractions would accrue in a single experiment on glyceride synthesis) and because it is anticipated that this instrument would receive extensive campus-wide use, the automatic feature is requested.

Although the two items of equipment are requested as a supplement to Grant No. A-2483, further justification for their purchase comes from the fact that they will receive extensive use in other research projects, some of which are listed below:

1. "Interrelationships in the Metabolism of Volatile Fatty Acids." Principal Investigator: S. B. Tove, Professor, Department of Animal Industry. 1960-61 budget, $7,000, from National Science Foundation.

In ruminant liver slices it has been found that monocarboxylic acids increase propionate metabolism, propionate inhibits acetate oxidation and citrate increases butyrate oxidation (Pritchard and Tove, Biochim. Biophys. Acta, 41:130, 1960). Studies on the mechanism of these interrelationships will require the centrifuge for the purification of the enzyme systems that has been initiated. The liquid scintillation spectrometer would greatly simplify and speed up the assay of these systems as the formation of C^{14} labeled products forms the basis of assay in all cases.

2. "Interaction of Nutrition and Disease in the Neo-Natal Pig." Principal Investigator: J. G. Lecce, Associate Professor, Department of Animal Industry. 1960-61 budget, $20,000, from N. C. Agricultural Experiment Station.

Newborn pigs are unable to synthesize serum proteins unless given colostrum (Lecce and Matrone, J. Nutrition, 70:13, 1960). It appears that colostrum contains a factor that activates protein synthesis in the newborn pig (Lecce and Matrone, J. Nutrition, in press). The centrifuge would be used in the fractionation and isolation of the colostrum factor. Attempts to study the synthesis of serum proteins using C^{14} labeled amino acids have been thwarted because of the inefficiency of the counting equipment available. The high counting efficiency of the liquid scintillation spectrometer would permit this study.

3. "The Effect of Univalent Cations on the Volatile Fatty Acid Production by Ruminant Microorganisms." Principal Investigator: G. Matrone, Professor, Department of Animal Industry. 1960-61 budget, $11,000, Frasch Foundation, E. I. du Pont de Nemours and Company, and N. C. Agricultural Experiment Station.

In the presence of univalent cations the production of propionate is increased, the conversion of acetate to butyrate is increased as is the fixation of CO_2 (Van Campen and Matrone, J. Nutrition, in press). One phase of this study is the investigation of the interconversion of volatile fatty acids as influenced by univalent cations. Counting these volatile products in solution, using a liquid scintillation counter, would greatly facilitate this work.

A second phase is the investigation of specific enzyme systems found to require univalent cations (e.g., fatty acid activation). The centrifuge will be used in the purification of these enzymes.

4. "Organic Insecticidal Residues in Cigarette Smoke." Principal Investigator: T. G. Bowery, Professor, Chemistry Department. 1960-61 budget, $6,100, from NIH grant RG5832.

The objectives of this project are the isolation and identification of the major insecticidal residues in cigarette smoke and the determination of the site and level of deposition of these residues in animals. Of the two insecticides to be used in this work, one (TDE; 1,1 dichloro-2, 2 bis p-chlorophenylethane) has been obtained labeled with C^{14} only in the 2 position and hence only residues containing carbon from this position can be studied. The other (endrin; 1,2,3,4,10,10-hexachloro 6,7 epoxy-1,4,4a,5,6,7,8,8a octahydro 1,4 endo, endo 5,8 dimethanonaphthalene) labeled with C^{14} or Cl^{36} is presently unobtainable. Both of the insecticides as well as others, however, could be readily labeled uniformly with tritium by the Wilzbach technique. The use of this procedure is limited by the absence of suitable counting equipment. The liquid scintillation spectrometer would be used in this investigation and would thus bypass a serious obstacle to the progress of this research.

DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

(Leave blank)

APPLICATION FOR PREVIOUSLY RECOMMENDED YEAR OF RESEARCH GRANT SUPPORT

Date June 25, 1959

Application is hereby made for a renewal grant in the amount of $_____ for the period from September (Month) 1, (Day) 1959 (Year) through August (Month) 31, (Day) 1960 (Year) inclusive for the purpose of continuing a research project on the following subject:

(Repeat title used on previous application)

TITLE OF PROJECT: Metabolism of Unsaturated Fatty Acids of Depot Fat

Name, title & mailing address of principal Investigator:

Samuel B. Tove, Research Associate Prof.
Animal Nutrition Section
Department of Animal Industry
North Carolina State College
Raleigh, North Carolina

Name & title of any co-investigator (s):

Frank H. Smith, Research Associate Prof.

Name, title & mailing address of Financial Officer to whom check should be mailed:

J. G. Vann, Business Manager
North Carolina State College
Raleigh
North Carolina

PROPOSED BUDGET	No.	AMOUNT
Personnel: a. Professional		$
b. Other	2	6,000.
Permanent Equipment		1,500.
Consumable Supplies		2,500.
Travel		-
Other Expenses		-
Sub Total		$ 10,000.
Indirect Cost (Overhead)		1,500.
TOTAL REQUEST		$ 11,500.

AGREEMENT

It is understood and agreed by the applicant: (1) That funds granted as a result of this request are to be expended for the purposes set forth herein; (2) that the grant may be revoked in whole or part at any time by the Surgeon General of the Public Health Service, provided that a revocation shall not include any amount obligated previous to the effective date of the revocation if such obligations were made solely for the purposes set forth in this application; (3) that all reports of original investigations supported by any grant made as a result of this request shall acknowledge such support; (4) that, if any invention arises or is developed in the course of the work aided by any grant received as a result of this application, the applicant institution will either (a) refer to the Surgeon General for determination, or (b) determine in accordance with its own policies, as formally stipulated in a separate supplementary agreement entered into between the Surgeon General and the grantee institution, whether patent protection on such invention shall be sought and how the rights in the invention, including rights under any patent issued thereon, shall be disposed of and administered, in order to protect the public interest.

(Leave blank) DRG record of additional years of support	
1st	$
2nd	
3rd	
4th	

NAME OF INSTITUTION: North Carolina State College of Agriculture and Engineering of the University of North Carolina

NAME AND TITLE OF OFFICIAL AUTHORIZED TO SIGN FOR INSTITUTION (Please Type): W. D. Carmichael, Jr.
Vice-President and Finance Officer

PHS-2590
REV. 8-56

Form Approved
Budget Bureau No. 68-R249.7

PROGRESS REPORT

Grant No. A-2483

Principal Investigator
or
Program Director Samuel B. Tove Institution North Carolina State College

Title of project Metabolism of Unsaturated Fatty Acids of Depot Fat
(Repeat title shown on page 1)

STATEMENT OF ACCOMPLISHMENT COVERING PERIOD 9/1/58 thru 6/30/59

(Limit to one page. If you wish to submit a more detailed report use this page for a summary and provide seven copies of any additional material.)

A gas chromatography apparatus has been designed and built. With this apparatus the methyl-esters of the higher fatty acids can be separated and determined; hence, this technique has been applied to the study of the factors influencing the fatty acid composition of animal depot fat.

When high depot fat levels of linoleic or oleic acids were produced in mice by dietary means, the only acids to decrease were those with the same solubility properties as the acid fed, i.e., palmitoleic and palmitic acids respectively. In addition, there was an increase in a saturated acid; stearic acid increased with linoleic and myristic increased with oleic. These results indicate that the deposition of fatty acids is not a random process but rather there is a specific replacement of one fatty acid by another. Although undoubtedly several factors play a role in this process, present results indicate that solubility of a given acid is one of these.

Previous work with linoleate had raised the question whether the slow rate of linoleate decline was specific for an essential fatty acid or represented a general lipid pool. Results of recent studies in which the decline of myristic acid gave the same curves as previously observed with linoleic acid indicates that the slow pool hypothesis is probably correct. The speed with which myristic acid was initially lost from the depots was startling. It was found that the depot fat level of myristate fell from 39% to 9% in less than 2 days. Associated with an increase in myristic acid was a concomitant increase in myristoleic acid. High levels of myristate were found to drastically inhibit fat deposition.

The production of odd carbon fatty acids in the depot fat of mice was achieved by feeding diets containing 40% tripropionate. Although less than 1% of the total fatty acids were the odd carbon acids, it is significant the predominating odd acids were pentadecanoic acid and heptadecanoic acid. These acids are one carbon less than the predominating even carbon acids (palmitic and oleic) of normal mouse fat.

☆ U.S. GOVERNMENT PRINTING OFFICE: 1958—462009

Form Approved
Budget Bureau No. 68-R 249.7

SES—Form 8
(June 12, 1957)

NORTH CAROLINA STATE AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, ~~FEDERAL GRANT~~ PROJECTS, 1961

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): STATE, S-235, METHANE FORMATION AND RELATED FERMENTATIONS IN THE RUMEN.

2. DEPARTMENTS AND COOPERATING AGENCIES: Department of Animal Industry

3. PERSONNEL: John J. McNeill

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked): A study was initiated on the interactions between the two major ruminal fermentations, i.e., the acetic-butyric, and propionic acid fermentations, employing washed, resting rumen bacterial cell suspensions. The effect of succinate - a precursor of propionate - on the formation of volatile fatty acids from glucose was studied. The influence of substrate concentration, carbon dioxide, hydrogen, etc., on the fermentation of glucose to volatile fatty acids was determined also. The principle findings were as follows:

a. The presence of succinate greatly accelerates the utilization of glucose while having but slight effect on the proportions of the volatile fatty acids produced.

b. The fermentation of glucose under a carbon dioxide-hydrogen atmosphere results in a 25% increase in the amount of substrate converted to volatile fatty acids.

c. When lower concentrations of substrate are used, a greater percentage of the substrate is converted to volatile fatty acids than at higher substrate concentrations.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science): Results yield information which may make it possible to alter the proportions of the end-products of the ruminal fermentation with accompanying benefit to the host animal.

6. WORK PLANNED FOR NEXT YEAR: The bacterial interactions between the cellulose and starch fermentations will be studied at the specie and molecular level by studying the rates of fermentation and the types and proportions of end-products formed when the two substrates are fermented separately and when mixed together.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR: None

8. Prepared by J. J. McNeill Approved _____ (Director).

John J. McNeill

Date March 29, 1961 Date _____

SES—Form 8
(June 12, 1957)

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION
STATE

ANNUAL PROGRESS REPORT, ~~FEDERAL GRANT~~ PROJECTS, 1961

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): STATE, S-236, INVESTIGATION OF FACTORS AFFECTING THE CHEMICAL AND NUTRITIONAL PROPERTIES OF COTTONSEED PRODUCTS FROM SEED PRODUCED IN COTTON BREEDING PROGRAM.
2. DEPARTMENTS AND COOPERATING AGENCIES: Department of Animal Industry, Department of Field Crops, U.S.D.A.-A.R.S., and National Cottonseed Products Association, Inc.
3. PERSONNEL: F. H. Smith, V. W. Smart, P. F. Heinstein, J. A. Lee, C. L. Rhyne
4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked): A number of experimental cottonseed were analyzed for total gossypol. The gossypol values ranged from very low for the glandless to relatively high for the glanded strains.

   Meals were prepared from the seed of several experimental and normal strains of cotton, and bioassayed by rat-feeding trials. In general, the meals having the lowest levels of total gossypol produced the best gains. The meals having the highest total gossypol showed lower amounts of free epsilon amino lysine by the dinitrofluorobenzene method.

   It was found that glandless cotton does produce gossypol. Root cultures indicate that gossypol is synthesized by the roots.

   Gossypol was isolated from the liver of pigs which had consumed gossypol.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science): These studies show that gossypol can be reduced to a very low level in glandless cottonseed which yield meals of superior nutritional quality.

6. WORK PLANNED FOR NEXT YEAR: To continue some further work with genetic strains of cotton, and also study the metabolism of gossypol by animals.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

F. H. Smith. Preparation of Pure Gossypol from Dianilinogossypol. J. Amer. Oil Chemists' Soc., 37: 286-288, 1960.

F. H. Smith, C. L. Rhyne, and V. W. Smart. Dietary Evaluation of Cottonseed Protein from Cotton Bred for Low Gossypol Content. Agr. and Food Chem., 9: 82-84, 1961.

8. Prepared by F. H. Smith — Approved _____ (Director).

   Frank H. Smith

   Date March 29, 1961 — Date _____

SES—Form 8
(June 12, 1957)

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, ~~FEDERAL GRANT~~ STATE PROJECTS, 1961

(Three copies to be given to the SES examiner)

1. PROJECT (Fund, number, and title): STATE, S-245, METABOLISM OF VOLATILE FATTY ACIDS.

2. DEPARTMENTS AND COOPERATING AGENCIES: Department of Animal Industry, National Science Foundation

3. PERSONNEL: Samuel B. Tove

4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked): One of the primary objectives of this project had been the study of a butyryl-CoA propionate transferase. The presence of an active propionate thiolkinase in extracts of sheep liver mask the presence of the transferase. Hence studies on the purification and properties of the activation system have been initiated.

Using acetate, propionate and butyrate, doubly labeled in the carboxyl group (O^{18} and C^{14}) evidence has been obtained that there is a rapid activation and deactivation of acetate in sheep liver. This is not found for propionate and butyrate, even though these acids are metabolized more rapidly than acetate. These findings (one experiment thus far) would provide an explanation of the mechanism for the specific dynamic action of acetate.

5. USEFULNESS OF FINDINGS (Benefits to agriculture and the general public and contributions to science): Provide basic information on the metabolism of the real energy supply of the ruminant animal, and from this knowledge, of how to alter and improve the efficiency of production of meat and milk by ruminants.

6. WORK PLANNED FOR NEXT YEAR: Continue the studies on the propionyl-thiolkinase, and repeat the experiments with doubly-labeled fatty acids.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR: None

8. Prepared by Samuel B. Tove Approved _____ (Director).

Date Samuel B. Tove March 29, 1961 Date _____

ANNUAL PROGRESS REPORT

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION STATE PROJECTS

1. PROJECT:(Fund, number, and title): Miscellaneous Gifts - Nutrition Foundation Grant No. 248. The Chemical Nature and Mechanism of Action of a Dietary Factor Affecting the Composition of Fat Deposited by Animals.

2. DEPARTMENTS AND COOPERATING AGENCIES:

Department of Animal Industry

PERSONNEL:

S. B. Tove and F. H. Smith

RESEARCH ACCOMPLISHMENTS OF THE YEAR(Confidential information should be so marked):

The factor alluded to in the title has been identified as linoleic acid and hence the work on this project has been directed toward a study of the kinetics of unsaturated fatty acid deposition and depletion in the carcass fat of animals. Results of these studies show that a common rate of linoleate depletion from the carcass fat of mice occurs in females, immature males and mature males at relatively high tissue levels of linoleate. However, a slower rate (one-fourth that of the common) is observed only in mature males and only when the level of carcass fat linoleate is 12% or below. Furthermore, all changes observed in carcass linoleate levels are completely counterbalanced by changes in the mono-unsaturated acids.

5. USEFULNESS OF FINDINGS(Benefits to agriculture and the general public and contributions to science.):

Little is known about the time trends in altering the fatty-acid composition of an animal's fat. Since the fatty acid composition of an animal's fat is an important factor in consumer demand and in keeping quality of the meat, it is important to know what are the factors that control the carcass fat composition. In addition, this study should shed some light on the interplay between the various fat depots and other sources of energy available to the animal.

6. WORKED PLANNED FOR NEXT YEAR:

To attempt to determine if the slower rate of linoleate depletion is caused by sex hormones and to determine what the slow rate of linoleate represents.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

Tove, S. B. and F. H. Smith. Relationship of Dietary Dienoic Acid Content to That in Mouse Carcass Fat. Proceedings of the Society for Experimental Biology and Medicine, 97:92-95 (1958).

8. Prepared by S. B. Tove Approved _____ (Director)

Date March 5, 1958

Not Found

ANNUAL PROGRESS REPORT FOR STATE SUPPORTED PROJECTS
OF THE
NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION
(Three copies to be submitted to Director's Office)

1. PROJECT: (Fund, number, and title): **Nutrition Foundation - The Chemical Nature and Mechanism of Action of a Dietary Factor Affecting the Composition of Fat Deposited by Animals.**
2. DEPARTMENTS AND COOPERATING AGENCIES: **Department of Animal Industry**
3. PERSONNEL: **S. B. Tove and F. H. Smith**
4. RESEARCH ACCOMPLISHMENTS OF THE YEAR (Confidential information should be so marked):

5. USEFULNESS OF FINDINGS(Benefits to Agriculture and the general public and contributions to science):

6. WORK PLANNED FOR NEXT YEAR:
This project should be closed out. Work to be incorporated in P.H.S. (Tove)- Metabolism of Unsaturated Fatty Acids.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

8. Prepared by **S. B. Tove** Approved _____
Director

Date **February 20, 1959** Date _____

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION
PROJECT OUTLINE

Project No.	
Date Submitted	
Approved	
Revised	

1. Title Microbiological Formation of Methane in Ruminants

2. Objective(s)

   A. To investigate the terminal metabolic systems whereby methane is produced by rumen bacteria.

   B. To study the effects of various chemical and biological factors upon the formation of methane by rumen bacteria.

3. Reasons for undertaking Investigations*

Recent adjustments in the agricultural pattern in the Southeast have resulted in increased emphasis on the dairy and the beef cattle industries. Research groups concerned with the nutrition of cattle undoubtedly will be called upon to contribute, in a large measure, toward a realization of the maximum potential benefits that may be derived from these expanding industries. One of the most important unexplored areas of ruminant nutrition that gives promise of yielding information of both fundamental and applied value is in the study of bacterial formation of methane in the rumen.

Approximately 10% of the total energy available in feed is lost as methane. As much as 500 liters of this inert gas may be produced daily in the rumen of an adult cow. Characterization of the metabolic systems by which methane is produced by rumen bacteria may yield information that would make it possible to modify the metabolism of the methane bacteria in such a manner that an increased efficiency in the conversion of gross energy of feeds to animal products would result.

*Including economic justification

4. Previous work and present status of investigations in the field of this project:

Pilgrim (1948) was probably the first to describe a definite role of rumen bacteria in methane formation. He postulated that the action involved two groups of organisms, one producing molecular hydrogen and the other oxidizing this hydrogen for the reduction of carbon dioxide to methane. Beijer (1952) reported that methane was formed by the action of whole rumen contents on succinate and formate. Carroll and Hungate (1955) found that formate was degraded to carbon dioxide and hydrogen by rumen fluid, followed by the immediate reduction of carbon dioxide to methane. McNeill (1957), using washed suspensions of rumen bacteria, found that methane was formed from carbon dioxide and molecular hydrogen. He also noted that methane formation was decreased in the presence of hydrogen acceptors (nitrate, sulfate, DPN) and increased by hydrogen donors (molecular hydrogen, formate), thus indicating that available hydrogen is a limiting factor in methane formation.

5. Outline of Procedure:

Before a rational approach to the alteration of methanogenesis can be planned, information is needed on the characteristics of the metabolic pathways operating in rumen bacteria involved in the formation of methane. Concurrent with this basic approach, preliminary studies of factors influencing methane formation by rumen bacteria will be conducted.

Washed cell suspensions of rumen bacteria will be used exclusively, unless circumstances necessitate the use of pure cultures, cell-free extracts, etc.

A. Characterization of metabolic pathways leading to methane formation.

1. Principal mechanism of methane formation.

a. The primary mechanism of biological methane formation is the carbon dioxide reduction mechanism, according to the classical equation:

$$CO_2 + 4\ H_2 \longrightarrow CH_4 + 2\ H_2O$$

In order to ascertain whether or not this mechanism operates in the rumen, a carbon and hydrogen balance experiment will be conducted. Washed cell suspensions of rumen bacteria will be incubated in the presence of carbon dioxide and of molecular hydrogen and in the absence of other substrates. Measurements will be made for methane formation and of hydrogen and carbon dioxide changes, using manometric techniques and gas chromatography.

5. Outline of Procedure (continued)

b. In order to determine the effect of specific substrates on methane formation, rumen bacteria will be incubated in the presence of glucose, pyruvate, formate, and other metabolic intermediates. Methane, carbon dioxide, and fatty acid determinations will be made.

It is assumed that the resting cell suspensions will not incorporate significant amounts of substrate carbon and that the metabolic end products listed constitute 100% of the substrate carbon. Certain substrates characteristically give rise to higher yields of methane than others, the reasons for which are not known. Carbon balance experiments, using rumen bacteria, should yield data making it possible to relate variations in the yields of methane, carbon dioxide, and volatile fatty acids to the operation of different pathways in the dissimilation of the substrates.

2. Alternate mechanisms in the formation of methane.

a. Alternative mechanisms of methane formation, not involving carbon dioxide reduction, have been described for several non-rumen methane bacteria (Barker, 1956). These mechanisms operate through the intact transfer of the methyl groups of methanol and acetate to methane, without prior oxidation of the methyl group to carbon dioxide. To find out if such mechanisms occur in the rumen, washed suspensions of rumen bacteria will be incubated in the presence of methanol, formate, and other potential one-carbon precursors of methane, together with $C^{14}O_2$. Any methane produced will be measured; it then will be oxidized to carbon dioxide by slow combustion, precipitated as barium carbonate, and counted for specific activity. By comparing the activity of the methane with the activity of the carbon dioxide, it will be possible to determine whether or not any methane was formed directly from the methyl groups of the substrates (Stadtman and Barker, 1949).

B. Influence of various chemical and biological factors on methane formation.

While the aforedescribed work is in progress, preliminary experiments will be initiated on the possibility of altering the methanogenic reaction.

1. The influence of various additives on methane formation.

Various compounds will be studied to ascertain their influence on methane formation. These compounds may be conveniently separated into two groups: compounds, such as d-limonene, which depress methane formation by enzymatic inhibition, and those (sulfate, riboflavin, etc.) which decrease methane formation by functioning as competitive hydrogen acceptors.

5. Outline of Procedure (continued)

a. The effect of d-limonene on methane formation.

Limonene, an ingredient of citrus peel oil, has been shown to decrease methane formation in sewage sludge fermentations (McNary et al., 1951). In addition to the effect of limonene on methane formation, its effect on the rate of volatile fatty acid production by rumen bacteria from glucose and other substrates will be determined, by chromatographic methods, to see if other essential metabolic systems are being affected.

If sufficient supplies of limonene can be obtained, feeding experiments with sheep will be conducted. The metabolic activity of rumen bacterial suspensions from these animals will be studied and compared with the activity of the rumen bacteria from control animals to see if prolonged contact with limonene has altered the enzymatic activities of the bacterial cells with respect to their ability to attack various substrates with the formation of carbon dioxide, methane and volatile fatty acids.

b. Other compounds have been suggested for such studies, but these will not be tried until the results of the aforementioned experiments with limonene are obtained.

References:

1. Barker, H. A. The biological formation of methane. Industrial and Engineering Chemistry, 48:1438. 1956.

2. Beijer, W. H. Methane fermentation in the rumen of cattle. Nature, 170:576. 1952.

3. Carroll, E. J. and Hungate, R. E. Formate dissimilation and methane production in bovine rumen contents. Arch. Biochem. and Biophys., 56:525. 1955.

4. McNary, R. R., Walford, R. W., and Patton, V. D. Experimental treatment of citrus waste water. Food Technology, 5:320. 1951.

5. McNeill, J. J. The methane formation and hydrogenase activity of bovine rumen bacteria. J. Dairy Sci. In Press. 1957.

6. Pilgrim, A. F. The production of methane and hydrogen by the sheep. Australian Jour. Scientific Research, Ser. B., Biol. Sci., 1:130. 1948.

7. Stadtman, T. C. and Barker, H. A. Studies on the methane fermentation. VII. Tracer experiments on the mechanism of methane formation. Arch. Biochem., 21:256-264. 1949.

6. Probable Duration of Project: 5 years

7. Date of Initiation: July 1, 1957

8. Personnel:

Name	Department	Relation to Project
J. J. McNeill	Animal Industry	Leader
S. B. Tove	Animal Industry	Co-operator
J. G. Lecce	Animal Industry	Advisor
M. L. Speck	Animal Industry	Advisor
W. E. Thomas	Animal Industry	Advisor

9. Coöperation: -

a. Interdepartmental

b. Other Agencies

10. Financial Support:

a. Proposed Budget ...1957... to ..1958.

Items	ALLOCATION OF FUNDS				
	Hatch	Regional Research	State	Other	Total
1. Salaries Technician	$1500.				
2. Labor	500.				
3. Travel	-				
4. Equipment & Supplies	5500				
5. All Other	-				
Total	$7500.				

b. Proposed Future Budgets:

Year	Salaries	Total Expenditures	Estimated Income
1958-59	$3500.	$7500.	
1959-60	$3500.	$7500.	

11. General Remarks:

SIGNATURES OF APPROVAL

1. Approval of Project Leaders

Date 4-17-57 John J. McNeill

Title Assistant Professor

Date April 17, 1957 L. B. Tove by J.S.H.W.

Title Associate Professor

Date.....

Title.....

2. Approval of Heads of Departments or Coöperating Agencies

Date 1 May 57 J. W. Goodman

Head, DEPARTMENT OF ANIMAL INDUSTRY

Date.....

Head,.....

Date.....

Head,.....

3. Approval of Director

Date.....

Director, North Carolina Agricultural Experiment Station

4. Approval of U. S. D. A.

Date.....

Chief, Office of Experiment Stations

AN APPLICATION

to

NATIONAL INSTITUTES OF HEALTH

for

Research Grant Support

METABOLISM OF UNSATURATED FATTY ACIDS IN DEPOT FAT

Covering the period September 1, 1961, through August 31, 1962

Requesting support in the amount of $14,663

APPROVED:

George Hyatt Jr.

George Hyatt, Jr., Head
Department of Animal Industry
North Carolina State College

R. L. Lovvorn, Director of Research
School of Agriculture
North Carolina State College

J. G. Vann, Business Manager
North Carolina State College

John T. Caldwell, Chancellor
North Carolina State College

D. B. Anderson, Vice-President
Consolidated University of North Carolina

NORTH CAROLINA STATE COLLEGE OF AGRICULTURE AND ENGINEERING OF
THE UNIVERSITY OF NORTH CAROLINA
Raleigh, N. C.

Date

(Leave Blank)

B'cd date
Council
Action

U. S. Department of
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

APPLICATION FOR RESEARCH GRANT

(A PRIVILEGED COMMUNICATION)

(Leave Blank)

No.
SS
Formerly

Application is hereby made for a grant in the amount and for the period stated, for the purpose of conducting research as described herein, in accord with the Agreement signed below.

A. AMOUNT REQUESTED: $ 14,663 (Same as total of itemized budget, page 2, item A8.)

B. PERIOD DATES: September 1, 1961 thru August 31, 1962 (Normally 12 months. See instructions.)
Mo. Day Year Mo. Day Year

C. TITLE OF RESEARCH PROPOSAL (Do not exceed 53 typewriter spaces)
METABOLISM OF UNSATURATED FATTY ACIDS IN DEPOT FAT

D. TYPE OF APPLICATION (please check one only, and add No. if applicable): ☐ New Project Proposal; or ☐ Revision of, ☐ Supplement to, or ☒ Renewal of PHS application or grant No. A-2483 (C2) Nutr.

E. PRINCIPAL INVESTIGATOR:
Name Samuel B. Tove Telephone No. TE 4-5211 Extension 241
Title Professor Department or Service Animal Industry
Mailing address of Research office North Carolina State College
Raleigh, North Carolina
Institution North Carolina State College Major Sub Division School of Agriculture

F. CO-PRINCIPAL INVESTIGATOR, if any. (Name and title only)

G. INSTITUTION SPONSORING REQUEST
Name N. C. State College of Agriculture and Engineering of the University of
Mail address North Carolina, Raleigh, N. C.
Name & title of official authorized to sign application on behalf of institution W. D. Carmichael, Jr., Vice Pres. & Finance Officer, Univ. of North Carolina, Chapel Hill, N. C.

H. NAME, TITLE, AND ADDRESS OF FINANCIAL OFFICER:
J. G. Vann, Business Manager
N. C. State College
Raleigh, N. C.
Manner in which check(s) should be drawn:
N. C. State College

I. AGREEMENT: It is understood and agreed by the undersigned that any grant received as a result of this application is subject to the following terms: (1) Funds granted as a result of this request are to be expended for research or related purposes as governed by Public Health Service and grantee institution policies; (2) the grant may be revoked in whole or in part at any time by the Surgeon General of the Public Health Service, provided that a revocation shall not include any amount obligated previous to the effective date of the revocation if such obligations were made solely for the purposes of research; (3) all reports of original investigations supported by the grant shall acknowledge such support; (4) if any invention arises or is developed in the course of the work aided by the grant, the undersigned will either (a) refer to the Surgeon General for determination, or (b) determine in accordance with grantee institution's own policies as formally stipulated in a separate supplementary agreement entered into between the Surgeon General and the grantee institution, whether patent protection on such invention shall be sought and how the rights in the invention, including rights under any patent issued thereon, shall be disposed of and administered, in order to protect the public interest.

J. PERSONAL SIGNATURES (in ink)
(1) Principal Investigator Samuel B Tove 7/21/60
(Same as shown in "E" above) (date)

(2) Authorized official of applicant institution
(Same as shown in "G" above) (date)

Mail completed application to:
Division of Research Grants
National Institutes of Health
Bethesda 14, Md.

PHS 398
Rev. 7-59

APPLICANT'S COPY

Form Approved
Budget Bureau No. 68-R.248.8

A. BUDGET REQUEST (for the period shown on page 1)

(1)	(2)	(3)
1. PERSONNEL List all positions, including Principal and Co-Investigator. Amounts requested must not exceed proportion of total salary computed from % of time spent.	% time on this project	Requested from PHS (omit cents)
S. B. Tove, Principal Investigator	50 %	$ -
Anne Cannon, Technician	100 %	4,500
Ann Hawkins, Technician	67 %	-
Graduate Assistant	50 %	2,400
Misc. Labor	100 %	100
	%	
	%	
2. PERMANENT EQUIPMENT (Itemize (see instructions)		
None		$
3. CONSUMABLE SUPPLIES (Itemize (see instructions)		
Misc. Equipment (expendable)		$ 2,500
Glassware, Chemicals, Misc. Supplies		2,000
Labeled Compounds		1,000
4. TRAVEL (Itemize (see instructions)		
Attendance at Scientific Meetings		$ 250
5. OTHER EXPENSE (Itemize (see instructions)		
None		$
6. TOTAL DIRECT COST REQUIREMENTS		$ 12,750
7. INDIRECT COST ALLOWANCE (If not authorized, official signing this application may request an amount for indirect costs. Review detailed instructions). (Round to nearest dollar.)		$ 1,913
8. TOTAL BUDGET (Item 6 plus amount shown in Item 7) (Enter on page 1)		$ 14,663

B. ESTIMATE OF SUPPORT REQUESTED FOR THE YEAR FOLLOWING THE BUDGET PERIOD ITEMIZED ABOVE. Applicants for 1-year grants should type the word "None" in space for TOTAL BUDGET shown below.

Personnel	Equipment	Supplies	Travel	Other	Total Direct Cost	Indirect Cost	TOTAL BUDGET
$ 7,000	$ None	$ 5,500	$ 250	$ None	$ 12,750	$ 1,913	$ 14,663

C. ADDITIONAL YEARS OF SUPPORT beyond the 2 years covered above, if requested. Please show the TOTAL AMOUNTS required for each such additional year, including indirect cost allowance.

3. $ 14,663 4. $ 14,663 5. $ 14,663 6. $ None 7. $ None

Prepared for the Bio-Sciences Information Exchange.
Not for publication or publication reference.

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

NOTICE OF RESEARCH PROJECT

PROJECT NO. (Do not use this space)

SUBMITTED TO: Public Health Service, National Institutes of Health, Division of Research Grants, Bethesda 14, Md.

TITLE OF PROJECT:

Metabolism of Unsaturated Fatty Acids in Depot Fat

Give names, departments, and official titles of PRINCIPAL INVESTIGATORS and ALL OTHER PROFESSIONAL PERSONNEL engaged on the project.

Samuel B. Tove
Department of Animal Industry, North Carolina State College, Raleigh, N. C.
Professor

NAME AND ADDRESS OF APPLICANT INSTITUTION:

North Carolina State College, Raleigh, N. C.

SUMMARY OF PROPOSED WORK — (200 words or less — Omit Confidential data.)

In the Bio-Sciences Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

Studies on the biochemical mechanism whereby one fatty acid exerts a specific effect on the distribution of the other fatty acids of the depot fat of animals, will be conducted. In this investigation the effect of a given fatty acid on the incorporation of other fatty acids into glycerides by enzyme systems of adipose tissue will be studied. In addition, the influence of fatty acid composition and structure of the diglycerides on the formation of particular triglycerides will be studied.

Various lipases will be investigated with regard to their specificity of action in respect to the glyceride position of the ester bond hydrolyzed. A special search for a lipase capable of stereospecific hydrolysis of one of the alpha glyceride positions will be conducted.

Previous studies on the deposition of fatty acids containing an odd number of carbon atoms will be continued.

SIGNATURE OF
PRINCIPAL
INVESTIGATOR

Identify the Professional School (medical, dental, public health, graduate, or other) with which this project should be identified:

SCHOOL

INVESTIGATOR — DO NOT USE THIS SPACE

PHS 766-1
PHS 1-60

Form Approved
Budget Bureau No. 68-R002

ANIMAL NUTRITION SECTION - Projects 1960-1961

		Number
Effect of Fertilization and Management on the Composition and Nutritive Value of Plants Commonly Used for Livestock Feeds		H-17 St-H-17
A Study of the Essentials of the Ruminant Diet		H-29
	Du Pont	Misc. Gifts
Biochemical Factors that Affect the Apparent Nutritional Qualities of Forage		H-35
Nutrition and Diseases of the Neonatal Pig (Cooperation with Veterinary Section)		St-H-193 H-193
	MR H0193	Misc. Recpts.
Investigations in Animal Nutrition		S-79
	Borden	Misc. Gifts
	NSF (Res.)	Misc. Gifts
The Evaluation of Feed Intake and Digestibility in the Utilization of Forage by Grazing Meat Animals		S-154
The Nutritional Significance of Pregastric Esterase		S-232
	PHS-Ramsey	Misc. Gifts
Metabolism of Unsaturated Fatty Acids of Depot Fat (PHS-Tove) and The Chemical Nature and Mechanism of Action of a Dietary Factor Affecting the Composition of Fat (Nutrition Foundation)		S-233
	PHS-Tove	Misc. Gifts
	Nutr. Found.	Misc. Gifts
Methane Formation and Related Fermentations in the Rumen		S-235
	NDEA (Botony)	Misc. Gifts
Investigation of Factors Affecting the Chemical and Nutritional Properties of Cottonseed Products from Seed Produced in Cotton Breeding Program		S-236
	N.C.P.A.	Misc. Gifts
Micro-Nutrient Elements in Animal Nutrition (Frasch) and Investigations of Micro-Nutrient Elements in Farm Animals (Moorman)		
	Frasch	Misc. Gifts
	Moorman	Misc. Gifts
Metabolism of Volatile Fatty Acids		
	NSF-Tove	Misc. Gifts

ANIMAL NUTRITION SECTION - Projects 1962-1963

		Number
Effect of Fertilization and Management on the Composition and Nutritive Value of Plants Commonly Used for Livestock Feeds (H-17) and Mineral Elements in Animal Nutrition (Frasch)		H-17
		St-H-17
	Frasch	Misc.Gifts
A Study of the Essentials of the Ruminant Diet		H-29
Biochemical Factors that Affect the Apparent Nutritional Qualities of Forage		H-35
Nutrition and Diseases of the Neonatal Pig (Cooperation with Veterinary Section)		H-193
		St-H-193
	MR-H-193	Misc.Recpts.
The Evaluation of Feed Intake and Digestibility in the Utilization of Forage by Grazing Meat Animals		St-154
The Nutritional Significance of Pregastric Esterase		St-232
	ADA	Misc. Gifts
	Borden	Misc. Gifts
	PHS-Ramsey	Trust Fund
Metabolism of Unsaturated Fatty Acids of Depot Fat		St-233
	PHS-Tove	Trust Fund
Methane Formation and Related Fermentations in the Rumen		St-235
	NDEA (Botany)	Misc. Gifts
Investigation of Factors Affecting the Chemical and Nutritional Properties of Cottonseed Products from Seed Produced in Cotton Breeding Program (St-236) and A Study of Factors in Cottonseed Products Affecting the Physiological Response in Animals (N.C.P.A.)		St-235
	N.C.P.A.	Misc. Gifts
Metabolism of Volatile Fatty Acids		St-245
	NSF-Tove	Trust Fund

ANIMAL NUTRITION PROJECTS - 1963-64

H-29 Grace	A STUDY OF THE ESSENTIALS OF THE RUMINANT DIET
H-35	BIOCHEMICAL FACTORS THAT AFFECT THE APPARENT NUTRITIONAL QUALITIES OF FORAGES
H-252 PHS Trust	A STUDY OF BACTERIA AND ENZYMES INVOLVED IN THE DIGESTION OF CELLULOSE IN THE RUMEN
H-256 Frasch Moorman St-H-256	MINERAL ELEMENTS IN ANIMAL NUTRITION
S-154	THE EVALUATION OF FEED INTAKE AND DIGESTIBILITY IN THE UTILIZATION OF FORAGE BY GRAZING MEAT ANIMALS
S-232 PHS Trust ADA Borden	THE NUTRITIONAL SIGNIFICANCE OF PREGASTRIC ESTERASE
S-233 PHS Trust	METABOLISM OF UNSATURATED FATTY ACIDS OF DEPOT FAT
S-235	METHANE FORMATION AND RELATED FERMENTATIONS IN THE RUMEN
S-236 PHS Trust NCPA	INVESTIGATION OF FACTORS AFFECTING THE CHEMICAL AND NUTRITIONAL PROPERTIES OF COTTONSEED PRODUCTS FROM SEED PRODUCED IN COTTON BREEDING PROGRAM
S-245 NSF Trust	METABOLISM OF VOLATILE FATTY ACIDS
H-193 St-H-193	NUTRITION AND DISEASES OF THE NEONATAL PIG (Coop with Animal Disease)

NORTH CAROLINA STATE COLLEGE
SCHOOL OF AGRICULTURE
RALEIGH

Department of Animal Industry

March 7, 1960

MEMORANDUM TO: Dr. R. L. Lovvorn, Director of Research
Dr. H. A. Stewart, Assistant Director of Research

FROM: George H. Wise

Subject: Request for Renewal of Grant-in-Aid from National Cottonseed Products Association, Inc.

Attached is a copy of a request for renewal of grant-in-Aid to support cottonseed meal research currently being conducted by Mr. F. H. Smith. If this request does not meet with your approval, please let us know as soon as possible, inasmuch as the renewal request must be mailed tomorrow.

The attached copy may be retained for your files.

msb

Attachment

Approved: George Hyatt Jr.
G. Hyatt, Jr., Head
Department of Animal Industry

Animal Nutrition Section
Department of Animal Industry
North Carolina Agricultural Experiment Station
N. C. State College
Raleigh, N. C.

Request for Renewal of Grant-in-Aid to Support
Cottonseed Meal Research

A. Project Title: Study of Factors Affecting the Growth Promoting Properties of Cottonseed Products Obtained from Seed Produced in Cotton Breeding Programs.

B. Objectives:

1. To determine total gossypol of cottonseed from various genetic lines of cotton to aid the geneticist in ascertaining progress achieved in lowering gossypol content.

2. To test the growth promoting properties of cottonseed protein obtained from genetic strains selected for low gossypol content.

3. To investigate the primary factors, other than gossypol, responsible for differences in growth response of rats fed meals from cottonseed produced in various experimental genetic lines of cotton.

4. To test oils extracted from experimental cottonseed used in feeding studies for fatty acid composition.

C. Personnel:

F. H. Smith, Project Leader
V. W. Smart
G. H. Wise
P. F. Heinstein

D. Cooperating Institutions or Agencies:

N. C. Agricultural Experiment Station and the National Cottonseed Products Association, Inc., in cooperation possibly with other Experiment Stations engaged in a program of breeding for low gossypol.

E. Significance of Project:

The detrimental effects of bound gossypol on the nutritive quality of cottonseed meal have been demonstrated (1,2,5) and related to the amount occurring in the meal (N. C. Agric. Expt. Sta., unpublished data). It has been postulated that gossypol combines with protein at the free E-amino group of lysine (3), thus making it less available as a nutrient.

The detrimental effect of bound gossypol has been partially corrected by lysine supplementation (5). Accummulated data (N. C. Agric. Expt. Sta., unpublished) have shown that the rate at which the nutritive quality of cottonseed meal is lowered by bound gossypol becomes much greater after the latter exceeds 0.85%. The information indicates that the elimination of gossypol, or even reducing it to a low level, would result in greatly improved cottonseed meal.

Furthermore, it has been noted that in feeding certain cottonseed meals prepared from experimental genetic strains of cotton, that some factor or factors other than gossypol may be affecting the growth promoting properties of the protein. Although these effects are less marked and less common than those from gossypol, they should be studied.

F. Summary of Progress and Present Status:

Considerable progress has been made towards unraveling the genetics of gossypol heritability through relating plant characteristics with the gossypol content of the seed. From various crosses made by a geneticist at the North Carolina Agricultural Experiment Station 8 genotypes were isolated and some strains having 0.3 to 0.8% total gossypol were developed. The results appear to be promising from an agronomic standpoint. In this heritability study the seed from two selections had a total gossypol content of less than 0.03%, and from two others the seed had less than 0.1%. These developments are important in making gossypol-free cotton a reality.

Meals prepared from experimental strains of cotton performed, in feeding trials with rats, as well as, and in some cases better than, meals prepared from ether-extracted cottonseed, which process yields meals low in gossypol and high in quality. Free gossypol, as well as the total, was low in the meals prepared from cottonseed having a low gossypol content. The performance from 36 experimental meals fed in studies involved with the development of cotton having a low gossypol content has been very gratifying.

It appears that a greater improvement from a nutritional aspect will result from reducing the gossypol content of cottonseed from 1% to 0.3% than will occur from reducing it from 0.3% to zero. Nevertheless, the zero level is the desired goal.

G. Institutional Facilities Available:

Animal facilities are available for animal (rat) assays to the extent of about 16 meals per year. Laboratory equipment and facilities are available for the preparation and analysis of experimental meals as well as for the estimation of either free or total gossypol on small samples of decorticated cottonseed.

H. Experimental Plan:

1. In the chemical assay, decorticated seed from various genetic lines of cotton will be analyzed for total gossypol.

2. Cottonseed meals prepared from hexane extracted seeds obtained from low gossypol strains of cotton will be individually fed to weanling rats to assay the growth promoting properties.

3. After eliminating the physiological effects of bound gossypol from diets containing cottonseed meal (this can be achieved more effectively with the near zero lines than by chemical treatment of seed having high levels), differences in the growth of weanling rats will be investigated. The sequence of studying probable factors involved will be:

   a. Determinations of lysine content by the 2,4-dinitrofluorobenzene method.

   b. If the differences are not explainable by (a), the meal will be analyzed for amino acids by the ion-exchange chromatographic method (4).

   c. Should neither (a) nor (b) explain the differences, the meals will be analyzed for inorganic elements.

4. Analyze oils from experimental seeds extracted for feeding by gas chromatography for fatty acids.

I. Anticipated Duration:

Two years

J. Estimated Cost:

1. Estimated total annual cost	$10,000
2. Institution's contribution in equipment, supplies and salaries	6,000
3. Grant-in-aid requested	4,000

K. Literature Cited:

1. Baliga, B. P. and Lyman, C. M. 1957. Preliminary report of the nutritional significance of bound gossypol in cottonseed meal. J. Am. Oil Chemists' Soc. 34, 21-24.

2. Lyman, C. M., Chang, Wan Yuin and Couch, J. R. 1953. Evaluation of protein quality in cottonseed meal by chick growth and by a chemical index method. J. Nutrition 47: 679-690.

3. Lyman, C. M., Baliga, B. P. and Slay, M. W. 1959. Reactions of gossypol with protein. Arch. Biochem. Biophys. 84: 486-497.

4. Moore, S., Spackman, D. H. and Stein, W. H. 1958. Chromatography of amino acids on sulfonated polystyrene resins. Anal. Chem. 30: 1185-1190.

5. Smith, F. H., Young, C. T. and Sherwood, P. W. 1958. The effect of bound gossypol on the growth-promoting properties of cottonseed, soybean, and peanut meals. J. Nutrition 66: 393-408.

Department of
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE

APPLICATION FOR GRADUATE TRAINING GRANT
UNDER THE PUBLIC HEALTH SERVICE ACT

Rec'd 2/2/60

(LEAVE BLANK)
2G-482(R1)
Special (1)

June '60 Council

PRIVILEGED COMMUNICATION

ATTENTION:

Date February 15, 1960

- ☐ NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES
- ☐ NATIONAL HEART INSTITUTE
- ☐ NATIONAL CANCER INSTITUTE
- ☐ NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND BLINDNESS
- ☐ NATIONAL INSTITUTE OF DENTAL RESEARCH
- ☒ DIVISION OF GENERAL MEDICAL SCIENCES
- ☐ DIVISION OF NURSING RESOURCES PHS
- ☐ NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
- ☐ _____

AREA (see Instruction Sheet)
Experimental Nutritional Sciences

Application for a training grant is hereby made by the institution named below for the subject and purpose indicated. The amount requested is based on estimated requirements for a one year period, but not limited in availability to such period. (See Instructions.)

PERIOD	AMOUNT REQUESTED (from page 2, item 18)
July 1, 19 60 through June 30 19 61	$22,896.

PURPOSE (check appropriate SINGLE item)

A ☐ Establishing new training program
B ☒ Expanding present training program
C ☐ Improving present training program
D ☐ Continuing grant-supported training program

NAME AND TITLE OF TRAINING PROGRAM DIRECTOR Samuel B. Tove Research Associate Professor	MAILING ADDRESS OF TRAINING PROGRAM DIRECTOR Nutrition Section, Department of Animal Industry, N.C. State College, Raleigh, N.C.
NAME OF INSTITUTION North Carolina State College of Agriculture and Engineering of the University of N. C.	ADDRESS OF INSTITUTION Raleigh, N. C.
NAME OF DEPARTMENT Animal Nutrition Section Department of Animal Industry	ADDRESS OF DEPARTMENT North Carolina State College Raleigh, N. C.
NAME AND TITLE OF DEPARTMENT HEAD George Hyatt, Jr., Professor	MAILING ADDRESS OF DEPARTMENT HEAD North Carolina State College Raleigh, N. C.
NAME AND TITLE OF FINANCIAL OFFICER J. G. Vann, Business Manager	MAILING ADDRESS OF FINANCIAL OFFICER North Carolina State College Raleigh, N. C.
NAME AND TITLE OF OFFICIAL AUTHORIZED TO SIGN FOR INSTITUTION W. D. Carmichael, Jr. Vice President and Finance Officer	MAILING ADDRESS OF OFFICIAL AUTHORIZED TO SIGN FOR INSTITUTION Consolidated University of North Carolina Chapel Hill, N. C.

AGREEMENT

It is understood and agreed by the applicant: (1) that funds granted as a result of this request are to be expended for the purposes set forth herein; (2) that the grant may be revoked in whole or in part at any time by the Surgeon General of the Public Health Service in the event that the funds are not utilized in accordance with the purposes set forth in this application; (3) that the training center will comply with the current requirements established by the Surgeon General to qualify for training grants; and (4) that, if any invention arises or is developed in the course of the work aided by any grant received as a result of this application, the applicant institution will either (a) refer to the Surgeon General for determination, or (b) determine in accordance with its own policies, as formally stipulated in a separate supplementary agreement entered into between the Surgeon General and the grantee institution, whether patent protection on such invention shall be sought and how the rights in the invention, including rights under any patent issued thereon, shall be disposed of and administered in order to protect the public interest.

PERSONAL SIGNATURE W D Carmichael Jr

(This agreement must carry the actual signature of the official authorized to sign for institution.)

(Sign original only in ink)

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2C-482(R1)

These dates to be the same as period on page 1.

1A. Budget Proposed for the Year July 1, 19 60 through June 30, 19 61

CATEGORY AND ITEM (Use additional blank sheets, if needed)	BUDGET REQUESTED FROM P.H.S.	BUDGET OTHER SOURCES
PERSONNEL (Itemize)		
S.B.Tove, Associate Professor and Director of Project	-	2,600.
G.H.Wise, Professor and Head of Section	-	3,000.
G.Matrone and C.H.Hill, Professors	-	4,500.
F.H.Smith, Associate Professor	-	2,100.
J.J.McNeill, H.A.Ramsey and W.W.G.Smart, Jr., Assistant Professors	-	5,100.
Secretary (1/4 time)	800.	2,500.
Technical Assistants (3 full-time; chem. and animal labs)	1,000.	10,000.
TRAINING STIPENDS		
16 predoctoral trainees	14,400.	24,000.
CATEGORY TOTAL	16,200.	53,800.
PERMANENT EQUIPMENT (Itemize)		
None		
CATEGORY TOTAL		
CONSUMABLE SUPPLIES (Itemize)		
Secretarial supplies	500.	500.
Laboratory supplies	2,500.	2,000.
Miscellaneous laboratory equipment	1,500.	2,500.
CATEGORY TOTAL	4,500.	5,000.
TRAVEL		
Staff attendance to scientific meetings	-	1,000.
Visiting lecturers	500.	500.
CATEGORY TOTAL	500.	1,500.
OTHER EXPENSE (Itemize)		
CATEGORY TOTAL		
SUBTOTAL	21,200.	
INDIRECT COSTS (Not to exceed 8% of subtotal)	1,696.	
1B. TOTAL FOR THE YEAR	22,896.	

1C. ESTIMATE OF FUTURE REQUIREMENTS

FIRST ADDITIONAL YEAR $ 22,896. THIRD ADDITIONAL YEAR $ 38,300.

SECOND ADDITIONAL YEAR $ 38,300. ($24,000. in stipends) FOURTH ADDITIONAL YEAR $ 38,300.

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2G-482(R1)

2. List support for this training program from all sources, including the Public Health Service. Specify the source in each case. (Funds for research in which trainees are involved)

SOURCE		AMOUNT	PERIOD OF SUPPORT
PREVIOUS (within last five years)			
North Carolina Agricultural Experiment Station		91,430.	7/1/58-6/30/59 (1 yr)
Atomic Energy Commission		5,642.	1951-1958 (7 yr)
Tennessee Valley Authority		4,000.	1953-1956 (4 yr)
American Dairy Association		3,000.	1954-1956 (2 yr)
Nutrition Foundation		3,500.	1956-1957 (2 yr)
Abbott Laboratories, Inc.		2,000.	1955-1956 (2 yr)
CURRENT			
Grants	Research Leaders		
Herman Frasch Foundation	G. Matrone	10,000.	1/1/57-12/31/61
Moorman Manufacturing Company	G. Matrone	2,500.	7/1/58-6/30/61
Public Health Service (A-2230)	H. A. Ramsey	6,650.*	9/1/58-8/31/62
Public Health Service (A-2483)	S. B. Tove	10,000.*	9/1/58-8/31/61
E. I. DuPont de Nemours and Company	G. Matrone	3,000.	1/1/59-Indefinite
National Cottonseed Products Association	F. H. Smith	4,000.	6/15/59-Indefinite
Public Health Service (E-2598)	C. H. Hill	14,767.	9/1/59-8/31/62
N. C. Agricultural Experiment Station	Staff	91,430.	7/1/59-6/30/60
No grants exclusively for training active		*Net amount available for research	
PENDING			
National Science Foundation (B-8204)	S. B. Tove	39,600.	7/1/60-6/30/63
No grants exclusively for training pending			

TRAINING PLAN AND SUPPORTING DATA

On the continuation pages provided, give details of the proposed training plan and other necessary data in accordance with the outline below. Number each page consecutively. Additional continuation pages, if needed, may be requested from the appropriate Institute.

Before preparing this portion of the application, see the instruction sheet from the specific Institute to which you are applying.

I. PROPOSED PROGRAM

A. Purpose: provide a complete but concise statement of the training needs that the proposed training program will fulfill.

B. Training Plan:

1. Describe the current training program (if any) at your institution in this area of training.
2. Describe the proposed training program for which support is requested.
3. Provide a list of the categories of professional personnel and the estimated number of persons within each category for whom training will be provided annually.

II. STAFF AND FACILITIES

A. Staff: Provide brief biographical sketches of all professional staff having major responsibilities in this training program.

B. Facilities: Describe the training facilities at your disposal.

PROPOSED PROGRAM

A. Purpose

During the past 10 years, the Nutrition Section of the Department of Animal Industry has been participating in an active and progressive doctoral program designed to produce nutritional scientists soundly trained in the basic sciences and prepared to assume leadership in teaching and research in nutritional biochemistry. Since the fundamental nutritional and biochemical principles are common to all animal species, a college of agriculture is particularly suited to train nutritional biochemists. The ready availability of a wide variety of animal species and related physical facilities at agricultural institutions has provided an assortment of tools that have been used advantageously in the exploration of these basic nutritional and biochemical principles.

The professional reputation of the nutrition staff and the type of research and training program it is offering have received increasingly favorable recognition in recent years. Thus there is a growing demand for graduate student admission and support. With a well trained aggressive staff and increased support for basic research and the assurance of increased laboratory facilities in the near future, the primary limitation in advancing the training program is financial support for graduate students. Hence, to provide the needed growth financial aid is requested.

B. Training Plan

Administratively, the Nutrition Section is a unit of a technical department, but the functions of this Section and the interests of its staff are in fundamental areas of investigation. Research of the more applied or developmental nature is conducted by other sections, the personnel and program of which are not indicated herein.

Close cooperation exists between the Nutrition Section and the Departments of Chemistry and of Experimental Statistics as well as with the biochemists and physiologists of other departments, e.g., Botany and Zoology. The cooperation exists not only in research but also in teaching. Several of the courses taught by the training staff are cross-listed with those of other departments, and, in some cases, members of the Nutrition Section staff have served as major advisors for graduate students of these departments.

(1) Current training plan: Predoctoral training includes a program at the master of science level as well as at the doctoral. Funds are requested for the expansion of the total graduate student program.

The master's program, comprising the first two years of predoctoral study, serves to introduce the student to research and to aid the staff in screening prospects for the doctoral program. Emphasis is given to the selection of outstanding students interested in and capable of completing the doctoral program.

During the first year of graduate work, emphasis is on a formal course program, but exposure of research is sufficient to enable the student to select a thesis problem during the latter part of this period. In the succeeding years, proportionately more time is spent in research. By the final year the student is engaged almost entirely with work relating to his dissertation.

In order to meet the requirements for a doctorate in nutritional biochemistry the student must have had the equivalent of five years of chemistry (including inorganic, organic and physical), four years of biochemistry and nutrition, a year of experimental statistics, and a year of physiology. Students usually elect a minor in chemistry, experimental statistics, physiology or bacteriology, and thereby receive further instruction in these disciplines.

The student is given the privilege of choosing a research problem that is in line with his own interest and consistent with the facilities available on the campus and the research interests of the staff. Most often, however, the student chooses a problem related to one of the projects already under study in the Section. In the former case, support for the operational phase of the research comes entirely from Experiment Station funds, and in the latter case, the support is derived from the particular project grant involved. (It should be emphasized, however, that the stipends for current trainees come almost entirely from non-grant sources.) The research problems chosen in every instance concern basic problems in nutritional biochemistry.

Additional training comes from special lectures by outstanding researchers in the fields of biochemistry and nutrition. This program is made available through an informal association among the three institutions in the area (Duke University and the Chapel Hill and Raleigh branches of the Greater University of North Carolina). Each of the institutions provides three speakers annually, making a total of nine lectures during the academic year. These lectures are in addition to those sponsored by such organizations as Sigma Xi and American Chemical Society, which are also available.

(2) Future plans for training: The general plan for the immediate future is to expand the training within the system currently in effect. Improvement of quality, however, is a constant goal.

(3) Staff participation: All members of the staff listed herein are engaged in both formal teaching and research. The teaching duties normally occupy about one-fourth of the time in addition to the time spent in consultation with trainees. It is impossible to assess the time devoted to this phase of teaching because of the intimacy of the research and graduate training programs. With the expanded program the amount of time spent in graduate student consultation will be increased commensurate with the expansion in the number of trainees.

(4) Recruitment of trainees: Most of our graduate students have received under-graduate training either at colleges of agriculture in one of the land-grant institutions or at small private colleges. Because of the source of our students, we have the opportunity to produce nutritional scientists of individuals that normally would not be attracted to non-agricultural institutions. It is anticipated that in the future we would continue to attract students from the same sources. No difficulty is foreseen in recruiting the additional trainees for the expanded program, inasmuch as we consistently have had to deny admission to highly qualified students because of the limited funds budgeted for graduate stipends.

Although our doctoral program has been active for only 10 years, we have grown rapidly. We attribute this to our continued insistence on high standards of training and emphasis on quality of students selected for admission. We are proud of our growing reputation in research and student training and in the performance of our graduates.

II. STAFF AND FACILITIES

A. Staff

Charles H. Hill

Born: August 2, 1921 - Indianapolis, Indiana

Training:

B.S.	Animal Nutrition	Colorado A and M	1948
M.S.	Animal Nutrition	Cornell University	1949
Ph.D.	Nutrition, Biochemistry and Physiology	Cornell University	1951

Appointments:

1942-1945	U. S. Navy
1951-1952	Assistant Chemist, Agricultural Chemistry Department, University of Nebraska
1952-1958	Associate Professor, Poultry Nutrition, N. C. State College
1959-	Professor, Poultry Nutrition, N. C. State College

Professional Societies:

American Institute of Nutrition
Poultry Science Association
American Chemical Society

Honors:

Sigma Xi

Current research interests:

The major areas of interest at present are:

1. The role of nutrition in disease resistance. These studies include the role of vitamins, minerals, protein and unidentified factors in the resistance of chicks to Salmonella infection and to a transmissible lymphoid tumor.

2. Mineral nutrition in health and disease. Current emphasis is on iron and copper requirements and their metabolic interactions.

Bibliography: (21 publications)

Selected list:

1. Hill, C. H., Kelly, J. W. The effect of antibiotics on the growth of chicks raised in new quarters. J. Nutr. 51: 463-466, 1953.

2. Hill, C. H., Garren, H. W. The effect of high levels of vitamins on the resistance of chicks to fowl typhoid. Ann. N. Y. Acad. Sci. 63: 186-194, 1955.

3. Hill, C. H., Garren, H. W. The influence of choline on the transplantation of a typhoid tumor (RPL 12) into chicks. Cancer Research 16: 1019-1022, 1956.

4. Hill, C. H., Keeling, A. D., Kelly, J. W. Studies on the effects of antibiotics on the intestinal weights of chicks. J. Nutr. 62: 255-268, 1956.

5. Hill, C. H., Garren, H. W. Differentiation between a growth promoting factor and a tumor susceptibility factor in eggs. Nature 184: 823, 1959.

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John J. McNeill

Born: December 4, 1922 - Washington, D. C.

Training:

- .	Biological Sciences	George Washington Univ.	1947-1950
B.S.	Bacteriology	University of Maryland	1951
M.S.	Bacteriology	University of Maryland	1953
Ph.D.	Bacteriology and Biochemistry	University of Maryland	1956

Appointments:

1940-1944	U. S. Naval Gun Factory
1944-1946	U. S. Navy
1948-1949	Boy's Club of Washington
1951-1955	Graduate Assistant, Univ. of Maryland
1956-Present	Assistant Professor, N. C. State College

Professional societies:

Society of American Bacteriologists
American Dairy Science Association
North Carolina Academy of Science

Current research interests:

1. Mechanism of methane formation and metabolism of molecular hydrogen by rumen bacteria. Characterization of the environmental factors influencing bacterial production of methane, especially from formate, carbon dioxide, and hydrogen.

2. Formation of volatile fatty acids by rumen bacteria, especially mechanisms involving fixation of carbon dioxide utilizing energy from oxidation of molecular hydrogen.

3. Study of the role of biotin in the formation of long-chain fatty acids and composition of lipid fraction of bacteria requiring biotin.

4. Study of characteristics of bacteria in senescent phase of growth.

Bibliography: (7 publications)

Selected list:

1. McNeill, J. J., Doetsch, R. N. and Shaw, J. C. Some nutritional requirements of bovine rumen bacteria. J. Dairy Sci. 37: 81-88, 1954.

2. Jurtshuk, P., Doetsch, R. N., McNeill, J. J., and Shaw, J. C. In vitro studies of the effect of aureomycin and terramycin on mixed suspensions of bovine rumen bacteria. J. Dairy Sci. 37: 1468-1472, 1954.

3. McNeill, J. J. and Jacobson, D. R. Methane formation and hydrogen metabolism of bovine rumen bacteria. Md. Agr. Expt. Sta. Misc. Publ. No. 238, 1955.

4. Jacobson, D. R., Lindahl, I. L., McNeill, J. J. Shaw, J. C., Doetsch, R. N. and Davis, R. E. Feedlot bloat studies. II. Physical factors involved in the etiology of frothy bloat. J. Animal Sci. 16: 512-524, 1957.

5. Matrone, G., Ramsey, H. A. and McNeill, J. J. Sodium and potassium bicarbonates and glycerol in purified diets for ruminants. Federation Proceedings (Abs.) 18(1): 536, 1959.

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Gennard Matrone

Born: January 22, 1914 - Batavia, New York

Training:

B.S.	Dairy Chemistry	Cornell University	1938
M.S.	Biochemistry and Nutrition	Cornell University	1944
Ph.D.	Nutrition and Biochemistry	N. C. State College	1950

Appointments:

1938-1941	Research Assistant in Nutrition, Cornell University
1941-1952	Research Chemist, U. S. Plant, Soils Nutrition Laboratory, Ithaca, New York
1952-Present	Research Professor, N. C. State College

Professional societies:

American Chemical Society (Division of Biological Chemistry)
American Association for the Advancement of Science (Fellow)
American Institute of Nutrition
American Society of Animal Production
American Dairy Science Association
The Biometric Society
North Carolina Academy of Science

Honors:

Phi Kappa Phi; Sigma Xi; Fellow AAAS; Gamma Sigma Delta

Current research interests:

Mineral metabolism of animals with particular emphasis on soil-plant-animal relationships. The main area of interest is in comparative biochemistry of the mode of action of mineral elements in animals and plants. Currently, for example, the mode of action of a manganese x iron antagonism which is manifested in both animals and plants is under investigation. A second area of interest is the study of rumen metabolism by means of purified diets. Other areas of interest include the effect of plant estrogens on reproduction and serum protein synthesis of the neo-natal piglet.

Bibliography: (29 publications)

Selected list:

1. Matrone, G., Ramsey, H. A. and Wise, G. H. Purified diets for ruminants. Proc. Soc. Exptl. Biol. and Med. 95: 731, 1957.

2. Matrone, G., Hartman, R. H., and Clawson, A. J. Studies of a manganese-iron antagonism in the nutrition of rabbits and baby pigs. J. Nutr. 67: 309, 1959.

3. Matrone, G., Ramsey, H. A. and Wise, G. H. Effect of fatty acids and sodium and potassium bicarbonate in purified diets for ruminants. Proc. Soc. Exptl. Biol. and Med. 100: 8, 1959.

4. Lecce, J. G. and Matrone, G. Porcine Neonatal Nutrition: The effect of diet on blood serum proteins and performance of the baby pig. J. Nutr. 70: 13, 1960.

5. Matrone, G. Interrelationships of iron and copper in the nutrition and metabolism of animals. Paper to be presented at the Symposium on Interaction of Mineral Elements in Nutrition and Metabolism to be held at the Federation Meetings in Chicago, April 13-14, 1960.

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Harold A. Ramsey

Born: September 16, 1927 - Fort Scott, Kansas

Training:

B.S.	Dairy Husbandry	Kansas State College	1950
M.S.	Nutrition and Biochemistry	N. C. State College	1953
Ph.D.	Nutrition and Biochemistry	N. C. State College	1955

Appointments:

1950-1951	Graduate Assistant, N. C. State College
1951-1954	Ralston Purina Research Fellow, N. C. State College
1954-1955	Graduate Assistant, N. C. State College
1955-Present	Assistant Professor, N. C. State College

Professional societies:

American Dairy Science Association
American Society of Animal Production
North Carolina Academy of Science

Honors:

Sigma Xi; Phi Kappa Phi; Alpha Zeta; Gamma Sigma Delta

Current research interests:

1. Enzymes of the digestive system. Emphasis is being placed currently on pregastric esterase, the effects of diet, changes in age, and other factors on its secretion, the extent to which it participates in the alimentary lipolysis of dietary fat, its specificity for glycerides of different fatty acids, and the extent to which this enzyme is secreted by various species.

2. Dietary essentials for the normal metabolism of the ruminant. A study, utilizing purified diets, of the compounds that must be present in the ruminant diet to permit normal growth and function.

Bibliography: (10 publications)

Selected list:

1. Ramsey, H. A., Wise, G. H., Tove, S. B. and Waugh, R. K. Effects of various diets on the diurnal patterns of blood plasma lipides of dairy calves. J. Dairy Sci. 37: 1357-1367, 1954.

2. Ramsey, H. A., Wise, G. H. and Tove, S. B. Esterolytic activity of certain alimentary and related tissues from cattle in different age groups. J. Dairy Sci. 39: 1312-1322, 1956.

3. Ramsey, H. A. Photometric procedure for determining esterase activity. Clin. Chem. 3(3): 185-193, 1957.

4. Martin, W. G., Ramsey, H. A., Matrone, G. and Wise, G. H. Responses of young calves to a diet containing salts of volatile fatty acids. J. Dairy Sci. 42: 1377-1386, 1959.

5. Ramsey, H. A., Young, J. W., and Wise, G. H. Effects of continuous nursing, length of the nursing period, and rate of milk consumption on the secretion of pregastric esterase by calves. J. Dairy Sci. In press.

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W. W. G. Smart, Jr.

Born: October 13, 1920 - Mooresboro, N. C.

Training:

B.S.	Chemistry	Clemson College	1942
M.S.	Agricultural and Biological Chemistry	N. C. State College	1949
Ph.D.	Nutrition, Biochemistry and Statistics	N. C. State College	1952

Appointments:

1942-1946	Army Chemical Corps
1947-1949	Graduate Assistant, N. C. State College
1949-1953	Research Instructor, N. C. State College
1953-Present	Assistant Professor, N. C. State College

Professional societies:

American Chemical Society
American Society of Animal Production
American Association for the Advancement of Science
American Dairy Science Association
Biometrics Society

Honors:

Sigma Xi

Current research interests:

1. The relationships of animal responses to chemical composition of plants. Better methods of chemically defining plant material are being sought which will more precisely predict animal performance.

2. The study of direct methods of predicting feed intake of animals.

Bibliography: (9 publications)

Selected list:

1. Smart, W. W. G., Jr., Sherwood, F. W., Matrone, G. and Wise, G. H. Pigments involved in the chromogen(s) ratio method. Agr. and Food Chem. 1: 318-321, 1953.

2. Carter, M. W., Smart, W. W. G., Jr. and Matrone, G. Estimation of estrogenic activity of genistein obtained from soybean meal. Proc. Soc. Exp. Biol. and Med. 84: 506-507, 1953.

3. Peterson, W. J., Bell, T. A., Etchells, J. L. and Smart, W. W. G., Jr. A procedure for demonstrating the presence of carotenoid pigments in yeasts. J. Bacter. 67: 708-713, 1954.

4. Smart, W. W. G., Jr., Matrone, G. and Smart, V. W. Use of copper derivatives of chlorophylls in ratio method for estimating digestibility of forages. Agr. and Food Chem. 2: 1331-1332, 1954.

5. Carter, M. W., Matrone, G. and Smart, W. W. G., Jr. Effect of genistin on reproduction of the mouse. J. Nutr. 55: 639-646, 1955.

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F. H. Smith

Born: May 18, 1903 - Cornelius, N. C.

Training:

B.S.	Chemistry	Davidson College	1926
M.S.	Chemistry	N. C. State College	1931

Appointments:

1926	Mathematics Teacher, Harrisburg High School
1927-1928	Jr. Chemist, N. C. Dept. of Agriculture
1928-1946	Assistant Professor, N. C. State College
1946-Present	Associate Professor, N. C. State College

Professional societies:

American Chemical Society
American Oil Chemists Society
North Carolina Academy of Science

Honors:

Sigma Xi

Current research interests:

1. A study of the manner or mechanism by which gossypol bound to cottonseed protein interferes with the utilization of the amino acids, thus preventing the most efficient utilization of cottonseed protein.

2. Cooperating with the geneticist in an attempt to develop, through breeding, a variety of cotton in which the gossypol is eliminated or greatly reduced.

Bibliography: (36 publications)

Selected list:

1. Smith, F. H., Blumer, T. N., Williams, Alexa C. and Tyler, W. E. Effect of crystal size on salt penetration of pork loins. Food Research 16: 166-168, 1951.

2. Smith, F. H., Beeson, K. C. and Price, W. E. Chemical composition of herbage browsed by deer in two wildlife management areas. J. Wildlife Mgmt. 20: 359-367, 1956.

3. Smith, F. H. Spectrophotometric determination of total gossypol in cottonseed meal and cottonseed meats. J. Am. Oil Chem. Soc. 35: 261-265, 1958.

4. Smith, F. H., Young, C. T. and Sherwood, F. W. Effect of bound gossypol on the growth-promoting properties of cottonseed, soybean and peanut meals. J. Nutr. 66: 393-409, 1958.

5. Smith, F. H. Preparation of Pure Gossypol from Dianilinogossypol. J. Am. Oil Chem. Soc. In press.

S. B. Tove

Born: July 29, 1921 - Baltimore, Maryland

Training:

B.S.	Animal Husbandry	Cornell University	1943
M.S.	Biochemistry	Univ. of Wisconsin	1948
Ph.D.	Biochemistry	Univ. of Wisconsin	1950

Appointments:

1943-1946	U. S. Armed Forces
1946	Chemist, Laboratory of Industrial Hygiene
1947-1950	Graduate Assistant, University of Wisconsin
1950-Present	Assistant Professor, Associate Professor, N. C. State College

Professional societies:

American Chemical Society
American Association for the Advancement of Science
American Institute of Nutrition
New York Academy of Science
North Carolina Academy of Science
American Oil Chemists Society

Honors:

Sigma Xi

Current research interests:

1. Lipide metabolism, with current interest on the metabolism of fatty acids in depot fat and the physiological and biochemical factors that influence it.

2. Metabolism of volatile fatty acids, with special emphasis on the metabolism of propionate, its relationship to other short chain acids and the production of odd carbon fatty acids.

Bibliography: (18 publications)

Selected list:

1. Tove, S. B., Andrews, J. S., Jr. and Lucas, H. L. The turnover of palmitic, stearic and unsaturated fatty acids in rat liver. J. Biol. Chem. 218: 275-281, 1956.

2. Anderson, R. L. and Tove, S. B. Effect of copper deficiency on synthesis of haem. Nature 182: 315, 1958.

3. Tove, S. B. and Smith, F. H. Kinetics of linoleic acid depletion. Arch. Biochem. Biophys. 85: 352-365, 1959.

4. Pritchard, G. I. and Tove, S. B. Stimulation of propionate metabolism by monocarboxylic acids. Biochem. Biophys. Acta. In press.

5. Pritchard, G. I. and Tove, S. B. Interrelationships between the metabolism of short-chain fatty acids by ruminant liver slices. Biochem. Biophys. Acta. In press.

. . . . .

George H. Wise

Born: July 7, 1908 - Saluda County, South Carolina

Training:

B.S.	Dairying	Clemson College	1930
M.S.	Dairy Nutrition and Biochemistry	Univ. of Minn.	1932
Ph.D.	Dairy Nutrition and Biochemistry	Univ. of Minn.	1936

Appointments:

1933-1936	Graduate Assistant, Univ. of Minnesota
1937-1944	Associate Professor, Clemson College
1944-1947	Associate Professor, Kansas State College
1947-1949	Associate Professor, Iowa State College
1949-Present	Professor and Head, Animal Nutrition Section, N. C. State College

Professional societies:

American Association for the Advancement of Science
American Chemical Society
American Society of Animal Production
American Dairy Science Association
American Institute of Nutrition
Society Experimental Biology and Medicine
North Carolina Academy of Science

Honors:

Sigma Xi; Phi Kappa Phi; Fellow AAAS; American Feed Manufacturers' Award (research in dairy nutrition), 1948; Borden Award (research in dairy nutrition and physiology), 1949; William Neal Reynolds Professorship (N. C. State College)

Current research interests:

Research interests are integrated with the various programs of the respective members of the animal nutrition staff. His role, as section head, is more directive or advisory than active participation in research. The current general areas of investigation to which he is in position to make the greatest direct contributions are (a) studies of factors affecting the utilization of various nutrients and (b) the determination of the nutritive requirements of animals. The present specific phases

of the broad areas indicated are the exploration of the role of saliva in nutrient metabolism and the development of the "purified" diet system in the determination of nutrient needs of large animals.

Bibliography: (53 publications)

Selected list:

1. Wise, G. H., Miller, P. G. and Anderson, G. W. Changes observed in milk sham-fed to dairy calves. J. Dairy Sci. 23: 997-1011, 1940.

2. Wise, G. H., Caldwell, M. J. and Hughes, J. S. The effect of the prepartum diet of the cow on the vitamin reserves of her newborn offspring. Science 103: 616-618, 1946.

3. Parrish, D. B., Wise, G. H. and Hughes, J. S. The state of vitamin A in colostrum and in milk. J. Biol. Chem. 167: 673-678, 1947.

4. Wise, M. B., Barrick, E. R., Wise, G. H. and Osborne, J. C. Effects of substituting xylose for glucose in a purified diet for pigs. J. Ani. Sci. 13: 365-374, 1954.

5. Wise, G. H., Jacobson, N. L., Allen, R. S. and Yang, S. P. Rate of absorption of carotene and vitamin A from the alimentary tract of dairy calves. II. Effects of methods of dispersion and of administration. J. Dairy Sci. 41: 143-151, 1958.

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B. Facilities

There are 12 well-equipped chemical laboratories available in which the trainees will conduct their research programs. In addition, there is a small animal laboratory with facilities for handling mice, rats and rabbits; a large animal laboratory with facilities for handling sheep, swine and cattle; and laboratories for handling poultry. Money has been appropriated for a major addition to the building in which the Nutrition Section is housed. With this addition the floor space occupied by the Section will be markedly increased. In addition to expanded laboratory space, the new building will provide facilities of more special nature, such as cold rooms, animal laboratories, and instrument rooms. It is anticipated that this building will be completed by 1962.

The major items of equipment in the Nutrition Section include a Beckman spectrophotometer, Warburg apparatus, complete radioisotope equipment for measuring both beta and gamma emitters, gas chromatography apparatus, bomb calorimeter, freeze-drying apparatus, fraction collector, and refrigerated centrifuge. In addition, the following major items of

equipment are on the campus and available for use: mass spectrometer, emission spectrograph, recording spectrophotometer, automatic 200-tube counter current apparatus, infra-red spectrophotometers, high speed electronic computors, electrophoresis apparatus, controlled temperature and humidity chambers, electron microscope, X-ray diffraction apparatus, Spinco preparative centrifuge, nuclear reactors and associated radiation facilities.

III. PAST TRAINING RECORDS

Since the initiation of the graduate program in 1950, 11 doctorate degrees and 10 master's degrees have been awarded. Five of the 10 who earned master's degrees are currently engaged in the doctoral program in the Nutrition Section, and an additional 4 entered doctoral programs at other institutions.

A. The following are individuals who terminated their graduate programs at N. C. State College at the Master of Science level. (Students continuing in the Nutrition Section are not listed)

Virginia Weldon Smart, M.S. 1952
Initial and present position - Research assistant, Nutrition Section, N. C. State College

Chuan-Tao Yu, M.S. 1955
Initial position - Research assistant in doctorate program, Department of Biochemistry, University of California at Berkeley.
Present position - Postdoctorate in Biochemistry, Harvard University Medical School.

Clyde Thomas Young, M.S. 1955
Initial position - Military service.
Present position - Research assistant in doctorate program, Georgia Tech.

Joseph Alexander Orlando, M.S. 1956
Initial position - Research assistant, doctorate program in Biochemistry, University of California at Berkeley.
Present position - Postdoctorate in Biochemistry, Brandeis University.

William Gilbert Martin, M.S. 1958
Initial and present position - Research assistant, doctorate program in Biochemistry, University of West Virginia.

B. The following received the Doctor of Philosophy degree:

Gennard Matrone 1950
Initial position - Research chemist, U. S. Plant, Soils, Nutrition Laboratory (stationed at Raleigh).
Present position - Professor in Nutrition Section, N. C. State College.

George Elliot Hawkins, Jr. 1952
Initial and present position - Assistant professor, Dairy Nutrition, Alabama Polytechnic Institute.

William Wesley Garry Smart, Jr. 1952
Initial and present position - Assistant professor, Nutrition Section, N. C. State College.

Cecil Conley 1954
Present position - Assistant professor, Dairy Nutrition, Clemson College.

Gordon Clemence Ashton 1955
Initial position - Assistant professor, Iowa State University.
Present position - Associate professor of statistics in the area of biochemistry and nutrition, Ontario Agricultural College.

John Stevens Andrews, Jr. 1955
Initial position - Postdoctorate, Department of Biochemistry, University of California at Los Angeles.
Present position - Research associate, Howe Laboratory of Ophthalmology, Harvard Medical School.

Harold Arch Ramsey 1955
Initial and present position - Assistant professor, Nutrition Section, N. C. State College.

Robert Hall Hartman 1956
Initial and present position - Nutrition research, Swift and Company, Chicago, Illinois.

Melvin Windsor Carter 1956
Initial position - Assistant professor, Purdue University, Lafayette, Indiana.
Present position - Assistant professor in Experimental Statistics in the area of biochemistry and nutrition, N. C. State College.

Gerald Ian Pritchard 1958
Initial and present position - Research Officer, Biochemistry and Nutrition Unit, Animal Research Institute, Canada Department of Agriculture, Ottawa.

William Preston Ingram 1959
Initial and present position - Assistant professor, Chemistry Department, N. C. State College.

C. Ratio of staff to trainees

The present ratio of training staff to predoctoral trainees is 1.38 (11 trainees and 8 staff). The expanded training program would increase this ratio to 2.0 (16 trainees and 8 staff). No postdoctorate trainees are now in residence, but it is anticipated that in the near future such a program will be inaugurated.

Since the new building will greatly increase physical facilities, it is proposed to expand the training program coincident with the completion of the building. Additional funds are requested to provide for this expansion. During the first additional year the budget will be the same as on page 2. In the succeeding years the increased funds will be apportioned on the same basis with $24,000 of the $38,300 for training stipends.

Present and future graduate student assignments would be approximately as follows:

Staff Member	Present	Future
Dr. Hill	1	2
Dr. McNeill	1	2
Dr. Matrone	4	4
Dr. Ramsey	2	2
Dr. Smart	1	2
Prof. Smith	1	1
Dr. Tove	1	2
Dr. Wise	0	1

IV. STATEMENT OF PROGRAM DIRECTOR

The strength of our training program rests largely with our staff. We are a young enthusiastic group unhampered by conventionalism and recognized by the college faculty as the campus leaders in modern nutritional biochemistry. The diversity of interest and talent of our staff coupled with the wide variety of facilities and experimental material available make us especially qualified to study a problem from the molecular level to the whole organism. In addition to a strong training in nutritional biochemistry and related areas, we are able, through our Institute of Statistics, to build in our students a foundation in research logic and philosophy that is unique among institutions giving training in nutritional biochemistry.

PROGRESS REPORT OF RESEARCH PROJECT

MICRO-NUTRIENT ELEMENTS IN ANIMAL NUTRITION

October 1, 1958 - September 30, 1959

submitted by

The Animal Nutrition Section of
The North Carolina Agricultural Experiment Station

for review by

The Frasch Foundation Award Committee of
The American Chemical Society

The subject of this report deals primarily with progress made in investigations outlined in the 1958 report. During the year two papers and two abstracts were published, preparation of one manuscript has been completed and submitted, and several more are in preparation.

EXPERIMENTAL WORK

Micro-nutrient interrelationships

Mn x Fe. Last year's work concerning the characterization of the Mn-Fe antagonism in baby pigs and rabbits was published in the Journal of Nutrition[1/]. A manuscript showing that the site of Mn interference is with Fe absorption rather than with hemoglobin formation is near completion. Initial studies on the mechanism of the Mn antagonism have been carried out with liver slices. Briefly, the technique involves incubating liver slices in Krebs-Ringer solution with varying levels of manganese and recording the uptake of radio-iron, Fe^{59}. Preliminary results suggest a competitive type of inhibition. The premise tentatively held is that the system inhibited is the one involving formation of liver ferritin, the storage form of body iron. Ferritin is also a participant in the mechanism of Fe absorption from the gut. Further work is necessary to establish the type of inhibition and to determine directly whether or not the ferritin system is involved. *In vitro* studies with liver slices and *in vivo* studies with rabbits and guinea pigs will be carried out. The *in vivo* studies will be concerned with the effect of Mn on the formation of intestinal mucosa ferritin.

1/ G. Matrone, R. H. Hartman and A. J. Clawson. Studies of a Manganese-Iron Antagonism in the Nutrition of Rabbits and Baby Pigs. J. Nutrition, 67, 309-318 (1959).

Zn x Cu. A paper[2/] on the effects of excessive dietary zinc was presented at the 1959 fall meetings of the American Chemical Society at Atlantic City, New Jersey. During the past year the studies of zinc toxicity centered on elucidating (1) the factor(s) in liver extract which alleviates the growth inhibition and (2) the interference with copper metabolism. The experiments show that the active factor(s) in liver is organic in nature rather than inorganic. A radioisotope experiment with Cu^{64} indicated that Zn does not interfere appreciably with copper absorption. Copper utilization, however, is affected in that a smaller percent of the dose of radio copper was found in the tissues and a greater percent was found in the urine of the Zn-fed rats. These experiments also show that Zn toxicity interferes directly with iron metabolism. A radio-iron balance experiment designed to determine whether or not Zn interferes with absorption and/or utilization has been completed and evaluation of the data is in progress. Depression of cytochrome oxidase activity of the heart in zinc toxicity is prevented with supplemental dietary copper but supplemental iron is without effect, suggesting that cytochrome oxidase may contain a copper prosthetic group.

Micro-nutrient element requirements

Physiological Fe requirements. For the concept of "physiological Fe requirement" the reader is referred to the 1958 progress report. A manuscript on the life span of the red blood cells of sheep is in preparation. An experiment with sheep utilizing double labels, C^{14}-glycine and Fe^{59}, is currently underway. The object of this experiment is to obtain an estimate of the amount of hemoglobin

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2/ A. C. Magee, III and G. Matrone. Zinc Toxicity Studies with Rats. Paper 180, page 81C, Abs. of 136th Meeting of the A.C.S., September 1959.

iron reutilized, in conjunction with estimates of the life span of red blood cells. Formulas for expressing the response curves mathematically in order to estimate the parameters of interest are being developed in cooperation with the Department of Statistics.

Fe requirements of baby pigs. The experimental work on the Fe requirement of the baby pig (See progress report of 1958) has been completed. The minimum requirement for the baby pig (0-6 weeks of age) is estimated to be approximately 60 ppm of Fe of the dry matter intake. These data and data obtained with Fe^{59}, estimating the rate of synthesis and total production of hemoglobin for determining the physiological Fe requirement, are being prepared for publication.

Fe and Cu studies with chicks. Data from preliminary experiments conducted last year indicated that the dietary requirement of chicks was approximately 40 ppm of Fe and 3 ppm of copper of the dry matter intake. In six subsequent experiments, however, we have been unable to obtain uncomplicated copper deficiency in chicks as measured by hemoglobin response. Chicks fed diets deficient in Fe and Cu manifest anemia more quickly and more severely than those fed Fe deficient diets, but those fed Cu deficient diets maintain their hemoglobin at a level similar to that of the positive controls. The average mortality rate of the chicks on the copper deficient diet is higher than that of the chicks on the Fe deficient diet and equal to that of the chicks on the Fe and Cu deficient diet. The possibilities we have considered and tested are too numerous to record here. Currently we are entertaining the idea that Cu functions in a vital process that is manifested, under our conditions, before anemia. Since our basal diet is lower in Cu than it was in

the earlier studies, it may be that if a basal diet contained as much Cu as did the basal diet in our earlier studies survival would be improved sufficiently for anemia to develop. This and other possibilities are currently being tested.

The difficulty of obtaining Cu deficient chicks has also hampered the studies concerned with the function of copper in heme synthesis.

Macro-nutrient element studies

Last year (1958 report) we reported that our results suggested that bulk or roughage is not an essential in the diet of ruminants and that a purified diet devised for simple-stomach animals is suitable for ruminants provided that it is adequately supplemented with Na and K cations in the form of bicarbonates. Studies during the current year have been concerned primarily with the role of these cations in the rumen. By means of in vivo and in vitro experiments and the use of C^{14}-bicarbonate and methyl-labeled C^{14}-acetate, the following results were obtained: Na and K bicarbonates in the diet appear to shift the ratios and quantities of ruminal end products from acetic to the propionic side. CO_2 fixation in propionate by rumen microorganisms was increased $2\frac{1}{2}$ times in the presence of the Na and K cations. Acetate is a primary precursor of butyrate in the rumen. Product-precursor analysis of the data suggests that these cations may act as cofactors in the acetate $\longrightarrow$ butyrate reaction. Preliminary enzymatic studies suggest that potassium may be the effective cation cofactor. Future work will center on product-precursor studies and cation activation of reactions catalyzed by enzymes isolated from the rumen.

During the year a paper[3/] was presented at the April meetings of the Federation Societies at Atlantic City, New Jersey. In addition, a paper[4/] was prepared, by invitation, for the Cornell Nutrition Conference to be held in Buffalo, New York in November 1959.

Soil, plant, animal relationships

Mo x lime study. Bioassay of the 1958 soybean forage crop has not been completed. The 1956 and 1957 results (See 1958 progress report) are being prepared for publication.

FUTURE EXPERIMENTAL WORK

Work in progress will be continued and new leads will be investigated as soon as possible.

3/ G. Matrone, H. A. Ramsey and J. J. McNeill. Sodium and Potassium Bicarbonates and Glycerol in Purified Diets for Ruminants. Fed. Proc., 18, paper 2111, March 1959.

4/ G. Matrone, H. A. Ramsey and G. H. Wise. Role of Sodium and Potassium Cations in Volatile Fatty Acid Metabolism of Ruminants. Proceedings of Cornell Nutrition Conference to be held November 11-13, 1959.

AN APPLICATION

to

PUBLIC HEALTH SERVICE

NATIONAL INSTITUTES OF HEALTH

for Research Grant Support

Role of Various Proteases in Neonatal Nutrition

Covering the period from September 1, 1962, through August 31, 1963

Requesting support in the amount of $12,715

APPROVED:

R. L. Lovvorn, Director of Research
School of Agriculture
North Carolina State College

J. G. Vann, Business Manager
North Carolina State College

John T. Caldwell, Chancellor
North Carolina State College

D. B. Anderson, Vice-President
Consolidated University of North Carolina

NORTH CAROLINA STATE COLLEGE OF AGRICULTURE AND ENGINEERING OF THE
UNIVERSITY OF NORTH CAROLINA
Raleigh, North Carolina

FEB 6 1962

Date

(Leave Blank)
R'cd date
Council
Action

U. S. Department of
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

APPLICATION FOR RESEARCH GRANT

(A PRIVILEGED COMMUNICATION)

(Leave Blank)
No.
SS
Formerly

Application is hereby made for a grant in the amount and for the period stated, for the purpose of conducting research as described herein, in accord with the Agreement signed below.

A. AMOUNT REQUESTED: $ 12,715 (Same as total of itemized budget, page 2, item A8.)

B. PERIOD DATES: September 1, 1962 thru August 31, 1963 (Normally 12 months. See instructions.)
Mo. Day Year Mo. Day Year

C. TITLE OF RESEARCH PROPOSAL (Do not exceed 53 typewriter spaces)

Role of Various Proteases in Neonatal Nutrition

D. TYPE OF APPLICATION (please check one only, and add No. if applicable): ☒ New Project Proposal;
or ☐ Revision of, ☐ Supplement to, or ☐ Renewal of PHS application or grant No. _____

E. PRINCIPAL INVESTIGATOR:
Name Harold Arch Ramsey Telephone No. TE 4-5211 Extension 241 or 574
Title Research Associate Professor Department or Service Dept. of Animal Industry
Mailing address of Research office Animal Nutrition Section, Department of Animal Industry, North Carolina State College, Raleigh, North Carolina
Institution N. C. State College of Agriculture and Engineering, Raleigh, N. C. Major Sub Division N. C. Agricultural Experiment Station

F. CO-PRINCIPAL INVESTIGATOR, if any. (Name and title only)

None

G. INSTITUTION SPONSORING REQUEST
Name N. C. State College of Agriculture
Mail address and Engineering of the University of North Carolina, Raleigh, N. C.
Name & title of official authorized to sign application on behalf of institution A. H. Shepard, Bus. Off. and Treas., Consolidated Univ. of N. C., Chapel Hill, N. C.

H. NAME, TITLE, AND ADDRESS OF FINANCIAL OFFICER:
J. G. Vann, Business Manager
105 Holladay Hall
N. C. State College of Agr. and Eng.
Raleigh, N. C.
Manner in which check(s) should be drawn:
N. C. Agricultural Experiment Station

I. AGREEMENT: It is understood and agreed by the undersigned that any grant received as a result of this application is subject to the following terms. (1) Funds granted as a result of this request are to be expended for research or related purposes as governed by Public Health Service and grantee institution policies; (2) the grant may be revoked in whole or in part at any time by the Surgeon General of the Public Health Service, provided that a revocation shall not include any amount obligated previous to the effective date of the revocation if such obligations were made solely for the purposes of research; (3) all reports of original investigations supported by the grant shall acknowledge such support; (4) if any invention arises or is developed in the course of the work aided by the grant, the undersigned will either (a) refer to the Surgeon General for determination, or (b) determine in accordance with grantee institution's own policies as formally stipulated in a separate supplementary agreement entered into between the Surgeon General and the grantee institution, whether patent protection on such invention shall be sought and how the rights in the invention, including rights under any patent issued thereon, shall be disposed of and administered, in order to protect the public interest.

J. PERSONAL SIGNATURES (in ink)
(1) Principal Investigator Harold Arch Ramsey (Same as shown in "E" above) Feb. 6, 1962 (date)
(2) Authorized official of applicant institution A H Shepard Jr (Same as shown in "G" above) " " " (date)

Mail completed application to:
Division of Research Grants
National Institutes of Health
Bethesda 14, Md.

A. BUDGET REQUEST (for the period shown on page 1)

(1) 1. PERSONNEL List all positions, including Principal and Co-Investigator. Amounts requested must not exceed proportion of total salary computed from % of time spent.	(2) % time on this project	(3) Requested from PHS (omit cents)
Principal investigator	50%	$ -
Laboratory technician	100%	4,000
Matching contribution for state retirement and social security (6.8% of $4,000)	%	272
Graduate research assistant	50%	1,800
	%	
	%	
2. PERMANENT EQUIPMENT, itemize (see instructions)		
Photovolt recording-integrating densitometer and accessories		$ 1,800
Starch gel electrophoresis apparatus, Buchler Instruments, Inc.		185
Miscellaneous laboratory equipment		400
3. CONSUMABLE SUPPLIES, itemize (see instructions)		
Laboratory glassware		$ 300
Chemicals		700
Ingredients for experimental diets		500
Miscellaneous supplies		300
4. TRAVEL, itemize (see instructions)		
To attend scientific meetings		$ 200
5. OTHER EXPENSE, itemize (see instructions)		
Reprints of published manuscripts		$ 100
Purchase of experimental animals		200
Contractual labor		300
6. TOTAL DIRECT COST REQUIREMENTS		$ 11,057
7. INDIRECT COST ALLOWANCE (The administrative official signing this application may request an amount for indirect costs. Review detailed instructions) (Round to low dollar)		$ 1,658
8. TOTAL BUDGET (Same as amount shown in item A, page 1)		$ 12,715

B. ESTIMATE OF SUPPORT REQUESTED FOR THE YEAR FOLLOWING THE BUDGET PERIOD ITEMIZED ABOVE. Applicants for 1-year grants should type the word "None" in space for TOTAL BUDGET shown below.

Personnel	Equipment	Supplies	Travel	Other	Total Direct Cost	Indirect Cost	TOTAL BUDGET
$ 6,072	$ 400	$ 1,800	$ 200	$ 600	$ 9,072	$ 1,360	$ 10,432

C. ADDITIONAL YEARS OF SUPPORT, beyond the 2 years covered above, if requested. Please show the TOTAL AMOUNTS required for each such additional year, including indirect cost allowance.

3. $ 10,432 4. $ None 5. $ None 6. $ None 7. $ None

Prepared for the Bio-Sciences Information Exchange.

Not for publication or publication reference.

U. S. Department of
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

SUBMITTED TO: Public Health Service, National Institutes of Health, Division of Research Grants, Bethesda 14, Md.

TITLE OF PROJECT:

Role of Various Proteases in Neonatal Nutrition.

Give names, departments, and official titles of PRINCIPAL INVESTIGATORS and ALL OTHER PROFESSIONAL PERSONNEL engaged on the project.

Principal Investigator:
Harold A. Ramsey, Research Associate Professor
Animal Nutrition Section
Department of Animal Industry

NAME AND ADDRESS OF APPLICANT INSTITUTION:

North Carolina State College of Agriculture and Engineering
Raleigh, North Carolina

SUMMARY OF PROPOSED WORK – (200 words or less – Omit Confidential data.)

In the Bio-Sciences Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

The major objective of this research project is to study the nutritional importance of various proteases during the neonatal period of life. Consideration will be given to both plant and animal sources of protein. The calf will be used as a test subject. Initially, particular emphasis will be given to certain changes in proteases that occur during this period: (a) in rate of secretion, as reflected by changes in concentration of enzyme in various tissues, and (b) in ability to hydrolyze various sources of protein. Subsequently, attention will be given to the physiological response of the neonatal calf to diets containing various sources of protein. This area of investigation will include observations on (a) the digestion of proteins in the stomach and the small intestine, (b) the effect of age on protein digestibility, and (c) changes in blood protein patterns.

SIGNATURE OF PRINCIPAL INVESTIGATOR Harold A. Ramsey

Identify the Professional School (medical, dental, public health, graduate, or other) with which this project should be identified:
SCHOOL School of Agriculture

INVESTIGATOR – DO NOT USE THIS SPACE

PHS 166
REV 10-60

FORM APPROVED
BUDGET BUREAU NO. 105-R002