PROJECT NUMBER: NE-148 (Revised)

TITLE: REGULATION OF NUTRIENT USE IN FOOD-PRODUCING ANIMALS

DURATION: Oct. 1, 1995 to Sept. 30, 2000

OFFICIAL

STATEMENT OF THE PROBLEM:

The cellular and molecular mechanisms that regulate nutrient use for the productive functions of growth, pregnancy and lactation in food-producing animals are not well known. These mechanisms involve endocrine, paracrine and(or) autocrine responses to changes in physiological state and nutrient availability, and associated effects on tissue and organ differentiation, growth and function. It is imperative that we better understand the mechanisms controlling nutrient partitioning if we are to improve the sustainability, efficiency, quality and profitability of these food-producing systems.

JUSTIFICATION:

Animal production continues to contribute approximately 55% of the total cash receipts derived from the agricultural economy in the U.S. (Agricultural Outlook, 1993). Although this represents a significant positive economic impact, concerns have been expressed that animal production systems sequester a disproportionate amount of natural and renewable resources, and contribute significantly to environmental accumulation of nitrogen and other compounds in the soil and ground water. Growth of the U.S population continues, and consumers are demanding that foods with improved quality and nutritional composition be produced with good environmental stewardship. Conservation of nonrenewable resources and reduction or removal of detrimental environmental impacts of animal production systems require that efficiency of agricultural production systems be enhanced. Alternative management strategies may need to be developed. Economic competitiveness and profitability of these systems must also be maintained or improved so that sustainability of those systems can be assured. All of these objectives must be achieved if consumers are to be afforded continued access to the bountiful supply and extensive variety of nutritious foods to which they have grown accustomed.

These societal goals can only be met through continued acquisition of new information and development of new technologies that will allow producers to increase productivity, not total production. Tremendous opportunity exists for enhancing productivity through manipulation of nutrient use for the productive functions of growth, lactation and pregnancy in food producing animals. Great strides toward this end were made during the current period of this Regional Research Project. We have identified specific metabolic hormones and growth factors that play key roles in coordinating changes in nutrient use by various organs and tissues in these physiological processes. Chief among these are somatotropin (ST) and the insulin-like growth factor (IGF) axis, select β-adrenergic agonists and anabolic steroids. However, we have yet to delineate the cellular and molecular mechanisms by which ST, the IGFs and other metabolism modifiers or growth factors exert their influences on critical tissues and organs (including; muscle, adipose, mammary gland, liver and placenta). Techniques have been developed (and are continually being developed) and shared by members of NE-148, which allow us to investigate the regulation of nutrient partitioning at the molecular level. Development, validation

and use of these techniques was begun during the last period of this project, but relatively little information regarding the molecular mechanisms by which nutrient partitioning is regulated in food-producing animals is currently available. Now, with these techniques, focus of the current revision is on the physiological and molecular events responsible for the regulation of nutrient partitioning in growth, pregnancy and lactation.

Nutritional modulation of key metabolic hormones such as insulin, ST and the IGFs is also of paramount importance in coordinating an animal's ability to adapt to changing physiological states and environments. We need to develop a better understanding of how levels of nutrient availability alter endocrine, paracrine and(or) autocrine influences on cell differentiation, growth and function in order to understand the basis for genetic superiority in productive functions among animal populations. *Examples of these animal models unique to this project are the genetically diverse lines of dairy cows at the Minnesota Station and the availability of AI sires to the Connecticut Station.*

Improved animal performance achieved by genetic selection involves the somatotropic axis. For example, members of NE-148 have reported that alterations in the somatotropic axis are associated with genetic merit (1-4). Exogenous administration of ST or its natural secretagogue, growth hormone releasing factor (GRF), increases lactation performance in dairy cows and sheep, and enhances growth performance, carcass composition and nutrient composition of meat in growing pigs, sheep and cattle. Participants in the Regional Project have made major contributions toward defining the dose-dependent physiological changes and magnitude of these response(s) (see reviews by Boyd and Bauman, 1989; Beermann and DeVol, 1991; Bauman and Vernon, 1993; Pettitgrew et al., 1993; Boyd et al., 1995; Zinn and Bravo-Ureta,1995; 5-10). Participants have also played a key role in discovering and defining direct effects of ST administration on various tissues and organs (including muscle, adipose and liver). We and others have shown that a portion of the effects of ST are indirect, mediated in some manner through IGF-I and -II, and their binding proteins (IGFBP). Nutritional regulation of the IGF-complex has also been shown to be a critical component of the chronic modulation of nutrient partitioning that occurs with changes in physiological state or ST administration. However, the mechanisms whereby nutrient availability regulates the IGF-complex and the mechanism(s) by which the IGF-complex mediates these responses in various tissues and organs have not been determined.

Other metabolism modifiers such as the β-adrenergic agonists and anabolic steroids also provide useful experimental models for studying the regulation of nutrient use by food producing animals, as has been shown by participants in this project. We have demonstrated unprecedented rates of amino acid uptake and protein deposition resulting from oral and direct "close-arterial" administration of select β-adrenergic agonists, and have shown that these compounds apparently differ from ST in their mode of anabolic action. Specific mode of action of β-agonists on skeletal muscle have not been determined. Likewise, the mechanism(s) by which anabolic steroids enhance rates of protein synthesis and deposition, and the potential impact they may have on nutrient requirements are not known.

The complexity of these systems and their importance in the regulation of nutrient use in food producing animals are justification for the continuation of this regional project. The overall objectives of this Regional Research Project are to provide information that will enhance our

ability to meet consumer needs, enhance the positive contributions provided by food-producing animals to our society, and to reduce the negative impact(s) they have on our environment. These goals are consistent with those enumerated in "FAIR '95". Results of the planned research should lead to greater availability of safe and wholesome foods derived from animals, and will further improve the efficiency with which they are produced. Information obtained will contribute to achieving the goals of sustainable agriculture: "to satisfy human food needs; make the most efficient use of nonrenewable resources and on-farm resources; enhance environmental quality and the natural resource base upon which the agricultural economy depends; sustain the economic viability of farm operations; and enhance the quality of life for farmers and society as a whole" (Food, Agriculture, Conservation, and Trade Act of 1990, Public Law 101-624, Title XVI, Subtitle A, Section 1603). Accordingly, because feed consumption constitutes a major component of the cost and environmental impact of animal-derived food production, we seek to continue our collaborative investigations into the cellular and molecular mechanisms by which nutrient use for growth, lactation and pregnancy are regulated in food-producing animals.

It should be noted that completion of the planned work described in this proposal, and expansion of the objectives, based on results obtained from these studies, requires supplemental or complementary financial support from other sources. Many of the projects described are co-supported by funding from Federal agencies, industry and other competitive granting sources.

RELATED CURRENT AND PREVIOUS WORK:

A major key to improving the efficiency of animal production is the development of fundamental knowledge of the regulation of nutrient use for productive functions. In growing animals, the physiological basis for variation in productive efficiency is related to differences in nutrient partitioning between the accretion of lean tissue and body fat (11). Although substantial progress has been made, over 1 billion kg/yr of carcass fat is trimmed from slaughtered meat animals in the USA. Similarly, lactating dairy cows with the highest productive efficiency are those which direct a larger portion of absorbed nutrients toward milk synthesis (12); genetically superior animals produce 300 to 400% more milk than the national average. However, the tremendous potential for improvement is dependent on developing an understanding of the regulation of nutrient utilization for productive functions. This information is currently lacking.

Metabolic processes underlying growth and lactation are carefully orchestrated and controlled by complex interactions among a multiplicity of hormones and growth factors (11,13). ST plays a central role in the coordination of many of these key physiological processes. Studies have demonstrated that ST treatment of growing and lactating animals results in unprecedented gains in productive efficiency (5,14-17). The Food and Drug Administration (FDA) has approved commercial use of ST in lactating dairy cows, and approval for use in growing swine is pending. More importantly, ST represents a unique tool to aid in understanding the basic biology of tissue growth, metabolism and function. We and others have elucidated many aspects of the actions of ST in growth and lactation (7,18-22). Mechanisms involve both direct effects on some tissues and indirect effects which are apparently mediated by ST-dependent IGFs in other tissues. Adipose tissue is a direct target of ST, and this area of work represents a portion of the focus in previous work conducted by participants in the project (23-28). The net effect of ST in growing animals is to markedly reduce substrate uptake, de novo synthesis of fatty acids and triacylglycerol accumulation in adipose cells.

Focus of this revision will be on the IGF portion of the mechanism(s) by which ST exerts its effects, and on the role of the IGF axis in regulating nutrient partitioning in response to changes in nutrient availability. To date several groups have shown that chronic administration of ST or GRF causes elevated insulin and IGF-I concentrations in growing and lactating animals (29-34). The somatomedin hypothesis ascribed an endocrine role to the IGFs wherein ST stimulated IGF production by the liver and the IGFs were transported through the blood to target tissues. More recent work demonstrated that many tissues express mRNA for the IGFs, although at lower levels than observed in the liver (35-37), and this led to the speculation that the IGFs may exert paracrine or autocrine effects in various tissues. It is difficult to separate the effects of locally produced IGFs and IGFBPs from effects of those in the general circulation. Therefore, in vitro assays provide a useful tool, to supplement in vivo studies, for studying effects of the IGFs and IGF binding proteins on proliferation, differentiation and metabolism of cells or tissues of interest. For example, IGF-I is known to stimulate proliferation and differentiation of satellite cells in vitro, but in only one in three studies was muscle IGF-I mRNA abundance increased in response to pST administration in growing pigs (38-40).

Changes in physiological state and in nutrient availability also cause coordinated shifts in partitioning of nutrients to meet the dominant physiological function. Insulin is thought to be involved with mediation of the IGF-complex because associated changes have been observed. The classical changes in circulating insulin concentration that occur with altered levels of energy intake are well known, but how changes in availability of specific nutrients alter endocrine, paracrine and(or) autocrine regulation of tissue metabolism and growth has only recently received attention. Most of this work has been conducted in laboratory species and in humans (41). Most work with food-producing animals has used total feed intake, energy intake or protein intake as treatments (34,42-48). This recent work demonstrates that changes in energy and protein intake alter circulating concentrations of the IGFs in some but not all cases, and that uncoupling of the ST-IGF axis can occur under conditions of nutrient deprivation or inadequacy. This helps to explain the paradoxical situation where fasting or severe restriction of nutrient intake elevates ST concentrations, but not the IGFs. Elevation of circulating IGF and IGFBP concentrations by exogenous ST does not occur when animals experience significant negative energy balance, but these relationships have not been characterized in a detailed manner, particularly in nonruminants. Therefore, work will be conducted to assess if nutritional modulation of circulating concentrations of metabolites, metabolic hormones, IGFs and IGFBPs affect tissue (eg., muscle, mamary gland, liver) expression of the IGFs and IGFBPs in growth and lactation. The IGFs and IGFBPs are also thought to be involved in regulation of nutrient partitioning during pregnancy, and may mediate effects of nutritional status of the mother on fetal growth and development (48).

A CRIS search was conducted based on the keywords (β-agonists; growth hormones releasing factor; insulin-like growth factors and insulin-like growth factor binding proteins; nutrient partitioning; somatotropin) and statements of area of interest that emphasized nutrient regulation in domestic species, control by humoral and local factors, growth, lactation and pregnancy, molecular and cellular pathways and mechanisms, and model development. Not unexpectedly, the CRIS search identified a large number of projects well known to the technical committee from individual knowledge of the literature and the networking common in active research areas. The CRIS search demonstrated that a number of these research efforts, as well as unrelated projects triggered by the search criteria, are components of other existing regional research

projects. These regional projects for the most part are single species directed or concerned with reproduction, or some combination of both. Regional projects reviewed but deemed insufficiently relevant include: NC-111, NC-119, NC-185, NC-187, NC-204, NC-206, NE-127, S-213, S-233, S-248, W-112, W-171. Although more limited in scope, NC-131 (Factors regulating protein synthesis, degradation and growth in skeletal muscle) and W-181 (Modifying milk fat composition for improved manufacturing qualities and consumer acceptability) have some work and specific aims in common with NE-148. NC-131 focuses on cellular mechanisms involved in muscle development and protein accretion. W-181 focuses on lipid metabolism in high producing dairy cows. Overlap in technical committee membership between NE-148 and NC-131 will serve to keep each group informed of the other's efforts. Personal contacts and reports from the CSRS representative for the two projects should play a similar role for NE-148 and W-181. Unwarranted duplication of effort will be avoided. As regional projects begin to focus on more basic mechanisms and rely on growth and other factors which genetic analyses are increasingly grouping into molecular families and superfamilies, one can anticipate that future CRIS searches will identify greater numbers of projects with the potential for overlap, but inherent interactions and funding competition among researchers will keep substantive overlap to a minimum.

OBJECTIVES:

The overall goal of this project is to determine the factors which affect nutrient utilization for the productive functions of growth, pregnancy and lactation in farm animal species and to provide the basic knowledge necessary for further development of management strategies which optimize these functions. The primary emphasis of the project is to elucidate the mechanisms by which endocrine and hormone-like factors influence cellular differentiation, growth and metabolism to coordinate nutrient partitioning for tissue growth, conceptus development and lactation. The knowledge gained will lead to the development of integrative concepts and provide a sound basis for developing rational methods for increasing the productive efficiency of food producing animals.

Specific project objectives are:

1. To assess the autocrine, paracrine and endocrine mediation of humoral agents affecting proliferation, differentiation and metabolism in critical cells and tissues.

2. To characterize the coordination of physiological actions of humoral factors that accommodate adaptation and influence the efficiency of nutrient utilization in food producing animals.

3. To develop integrative concepts that describe the regulation of nutrient utilization and result in enhanced biological efficiency in the production of high-quality products that meet changing consumer demands.

PROCEDURES:

The specific collaboration on this regional project involves two types. First it involves direct collaboration between project members on a given experiment. In this instance the individuals each provide unique expertise needed to address the particular hypothesis. Second, the collaboration involves complementary studies by individual members. In this instance each individual study provides an important facet, complementing studies by other project members and redundancy is avoided. Overall, when results of these collaborations are combined, a more complete pattern emerges for the development of integrated concepts. The availability of unique animal models, the development of highly specialized protocols and experimental systems, and the degree of difficulty associated with some techniques precludes, in some cases, more than one station or participant being able to conduct certain experiments or procedures. Therefore, certain projects described below will seem to stand independent of regional effort or collaboration, but it is the sharing of the techniques (trouble-shooting and validation) and the results of these experiments which is critical for developing the integrated concepts which can be applied to production animal agriculture. The latter embraces the primary emphasis of objective 3 in this regional project.

The following represents specific procedures grouped by objective number.

OBJECTIVE 1: To assess the autocrine, paracrine and endocrine mediation of humoral agents affecting proliferation, differentiation and metabolism in critical cells and tissues.

A. Growth

Previous work centered on determining the effects of exogenous ST and β-adrenergic agonists on skeletal muscle growth, protein and lipid metabolism, body composition, changes in endocrine and metabolic status, and effects on nutrient requirements of growing animals. Our future efforts will provide a better balance between descriptive studies and more mechanistic approaches, with specific attention to the IGF system and the ST-IGF axis. The overall thrust is to determine the pathways by which nutritional and endocrine modulation of skeletal muscle, adipose tissue and organ growth are mediated in growing animals. Attention to experimental systems in pigs, cattle and sheep will facilitate development of broadly applicable generalizations.

Measurement of tissue expression of IGFs and IGFBPs must be conducted in conjunction with measurement of their circulating concentrations to determine the relative importance of each in regulating proliferation, differentiation and growth of cells skeletal muscle, liver and adipose. Influences of quantity of absorbed nutrients (amino acids and energy substrates) on skeletal muscle and liver expression of IGFs and IGFBPs will be studied in growing animals. Collaborative experiments will be conducted by NY-C, PA, North Carolina and USDA-BARC to address these objectives.

Temporal patterns of expression of the IGFs and IGFBPs will be determined in skeletal muscle and liver in response to change in amino acid absorption and availability. Initial studies will define the time-course of change in circulating concentrations of IGFs, IGFBPs, ST, insulin, PUN, glucose and NEFA in response to abomasal casein infusion in growing steers. Steers

weighing 200 kg will be housed in metabolism stalls and fed hourly a mixed concentrate diet formulated to provide levels of protein intake approximating 75% of NRC requirements for optimal growth rates (equivalent to 20 g/d N balance). Energy content of the diet will be adequate to support a nitrogen balance of 65 g/d. Following blood and tissue biopsy sample collection under basal diet conditions, abomasal casein infusion will be initiated at a level to increase N balance from 20 to 25 g/d to approximately 65 g/d. Sequential biopsies of skeletal muscle tissue will be obtained before abomasal casein infusion and at 8, 16, 24 and 48 h, and 7 and 14 d after initiating casein infusion. A liver biopsy will be obtained prior to casein infusion. Skeletal muscle and liver samples will be collected immediately postmortem. Temporal pattern of change of skeletal muscle mRNA abundance for IGF-I and -II, IGFBPs, α-actin and myosin heavy-chain will be determined.

This collaborative study will involve conducting the live-animal work at NY-C and a commitment by the PA Station to provide assessment of circulating concentrations of the IGFBPs and measurement of mRNA abundance for the IGFBPs in skeletal muscle and liver. Circulating concentrations of the IGFs and other hormones and metabolites will be assayed at NY-C, with assistance by Aidan Moloney, TEAGASC, Grange Research Institute, Ireland. Assistance with measurement of muscle and liver IGF mRNA will be provided by David Gerrard, University of Missouri. Previous studies conducted at NY-C have provided quantitative estimates of amino acid net flux changes across the hind limb using this protocol and the arterio-venous preparation and a transit-time ultrasound blood flow probe on the external iliac artery. Net flux of essential amino acids is doubled, and whole body N balance is increased from 20 to 25 to 65 g/d, with corresponding increases in circulating concentrations of IGF-I.

Studies to determine the effects of altering the level of energy and protein intake (amino acid absorption) on the ST-IGF axis will be conducted with growing pigs (NY-C). Because definitive information on energy and protein requirements has been obtained in previous studies, quantity and quality of nutrients absorbed from the digestive tract can be accurately predicted. However, we have also completed direct measurements of mesenteric absorption of nutrients in lambs and cattle administered incremental abomasal infusion of protein, and have documented that conventional diets do not appear to accommodate total tissue amino acid requirements in growing ruminants. Therefore, nutritional modulation of humoral factors and nitrogen metabolism will be used to determine associated changes in IGF's and IGFBPs in growing cattle.

Impact of quantity and type of dietary energy on the IGF-complex will be studied in 60 kg castrate male pigs under basal conditions and during ST challenge. Four levels of energy intake representing 100, 80, 60 and 40% of ad libitum intake will be assessed while maintaining a constant adequate level of intake of protein in a 14 d treatment period. Concentrations ST, insulin, IGF-I and -II, and IGFBP (-1, -2, -3 and -4) will be determined. Concentrations of PUN, glucose and NEFA will be measured to assess changes in metabolic status. The ST challenge will be conducted on d11 to 14. Similar measurements will be made to determine the effects of quantity and quality of dietary protein in pigs under basal conditions and during a ST challenge. Four levels of protein intake will be used ranging from dietary levels that achieve adequate amino acid availability (requirement) with pST administration and without, to levels that will meet maintenance requirements, but are inadequate for protein deposition. The ratio of EAA and NEAA will be varied while maintaining isonitrogenous diets to assess effects of protein quality on the IGF-complex. Experimental protocol will be the same as described above.

Delineation of nutritional modulation of tissue expression of the IGF's and IGFBPs will be conducted in growing pigs under basal conditions, during a ST challenge, and in response to alteration of dietary energy and protein intake. Relative levels of mRNA for the IGFs and IGFBPs in skeletal muscle, liver, kidney, perirenal and subcutaneous adipose tissue, heart and brain will be determined. Results will provide information regarding whether the autocrine/paracrine effects of IGF-I may be involved in enhancing protein deposition, and to what extent associated changes in circulating concentrations of ST, insulin and IGFs and IGFBP occur.

Subsequently, independent and interdependent effects of abomasal infusion of casein and administration of trenbolone acetate (TBA) will be studied in growing cattle using a similar approach, but with fewer sampling intervals (NY-C). We have previously shown that the β-agonist cimaterol progressively increases hind limb amino acid net uptake by up to 230% at 14 days of close arterial infusion, but have not addressed the question of effects of anabolic steroid administration on amino acid uptake and protein deposition. Similar steers and diet will be used with treatments administered in a Latin Square design. Pre-treatment muscle biopsies will be obtained from the semimembranosus muscle. Additional biopsies will be obtained at appropriate time intervals after initiating abomasal protein infusion and TBA administrations.

Acute direct effects of a β-agonist (clenbuterol), as well as of ST and IGF-1, on liver parenchymal cells isolated from normal growing lambs will be investigated using cells maintained in short-term incubations (NC). Dose response studies will be conducted for each metabolism modifier. Indirect and chronic effects will be evaluated with similar preparations from animals that have received chronic administration of the metabolism modifiers or an excipient. Pending positive effects being observed with the β-agonist treatments in the initial studies, a close-arterial infusion of the β-agonist will be used to assess direct chronic effects (after 5 d treatment) on metabolite net-flux across the liver in vivo and on the same parenchymal cell incubations from control and treated livers. Variables measured in the parenchymal cell studies will include incorporation of propionate, lactate, alanine and glutamine into glucose and glycogen vs CO2 using ^{14}C radiolabeled substrates. Urea production and activities of glutaminase and glutamine synthase will also be measured. Initial work will be with the β-agonist, and results may indicate modification of the variables to be measured using ST or IGF-1 treatments. Effects of insulin and glucagon will also be assessed.

Gene expression in the IGF system will also be studied. Size of the IGF-I transcripts in pig and steer tissues will be evaluated using Northern hybridization; IGF-I and -II mRNA will be quantified by use of a solution hybridization assay; IGF-1 and -II transcripts will be localized in situ in bovine skeletal muscle (NY-C). Probes for the myogenic regulatory factors, which are also classed as transcription factors, will be used to determine whether the effects of enhanced amino acid absorption and amino acid uptake by muscle (to facilitate marked increase in muscle protein synthesis) in cattle involves these transcription factors (NY-C, USDA-BARC and Dave Gerard at the University of Missouri). Differential gene expression will be investigated by examining skeletal muscles that exhibit differential response to testosterone (ie., splenius muscle in the neck vs the semitendinosus and triceps brachii in proximal limbs) in growing sheep (NY-C). A combination approach will be taken to use probes for the IGFs and the myogenic regulatory factors and to use differential display to identify unknown genes that may be involved in steroid-induced skeletal muscle hypertrophy.

The **Maryland Station** will study the impact of gonadal steroids (estradiol and testosterone) on the central role of the ST axis in sheep. Using a unique surgical approach (49), hypophyseal portal blood will be collected to enable direct quantification of the hypothalamic hormones (eg., GRF and SRIF) that regulate ST release. Using this animal system, effects of gonadal steroids on the dynamics of neuropeptide release can be evaluated directly.

Additional studies at the **New York Station** will investigate nutritional regulation of fetal and neonatal muscle growth in lambs during late fetal and early neonatal life, and its possible mediation by the IGF system at a time when ST is not a dominant influence on growth of lean tissues. An initial study will focus on early postnatal growth. Newborn lambs of either high (>4.5 kg) or low (<2.5) birthweight will be slaughtered at birth, or artificially raised to different target weights up to 20 kg at two different rates: >350 g/d (ad lib milk replacer) or ≈150 g/d (restricted milk replacer). Total fiber number of several skeletal muscles, with varying growth impetus characteristics will be counted by rigorous modification of existing techniques, developed in the laboratory of intramural collaborator, Dr. J. Hermanson. Effects of birth weight and nutrition on muscle cellularity and capacity for hyperplastic and hypertrophic growth will be related to plasma and tissue concentrations, and tissue mRNA abundance of IGF-I, IGF-II and IGFBP-2 and -3. This descriptive study will provide the basis for design of subsequent, more mechanistic studies of the nutritional regulation of post-primary myoblast proliferation and myofiber differentiation during late-gestation fetal growth.

Work at the **New Jersey Station** will investigate the regulation of neonatal growth by the IGFs and IGFBPs in piglets subjected to intrauterine growth retardation. In the first set of experiments, runt and same-sex littermates of average body weight will be removed from the sow 24 h after parturition and reared on milk replacer at levels of intake of either 7% or 12% of body weight. These levels of intake represent restriction equivalent to that expected if left with the sow, and unrestricted intakes, respectively. Daily intakes and body weight gain, and alternate day jugular venipuncture blood samples will be collected over the 14 d treatment period. Blood concentrations of IGF-I, IGFBPs, glucose, insulin and thyroid hormones will be measured. Tissues will be collected after slaughter on d 14 to determine content and mRNA abundance for IGF-I and protein and DNA concentration and content in the liver, brain, spleen, kidney, diaphragm and semitendinosus muscle.

In subsequent experiments, the effects of IGF-I infusion on ST secretion pattern will be determined in the neonatal piglet. Pigs will be removed from the sow at 6 h or 14 days post parturition and blood samples will be collected at 15-minute intervals to characterize the pattern of ST secretion. The newborn pigs will be catheterized via the umbilical artery, and the 14 day-old pigs via the jugular vein. Newborn pigs will be hand-fed milk replacer, and the older pigs will be trained to drink milk replacer from a bowl. The characterization data will be used to determine the length of sampling required to evaluate the effects of IGF infusion on ST secretion in pigs of the same ages reared under the same nutritional management scheme.

B. Lactation:

Evidence suggests that mammary cells proliferate and prepare for milk synthesis, in part, as a consequence of autocrine and paracrine growth factor interactions in mammary tissue. Growth and development of mammary glands are thought to be mediated in part by IGFs and their

interactions with IGFBPs, but other growth factors are also involved. The IGF system, including the IGFPs, will provide a major focus for our studies in this area, along with TGF-β_1, a putative regulator of mammary epithelial development, and ST. The **Pennsylvania Station** will continue investigation of the impact the IGF-system in vitro. The mouse mammary epithelial cell line (COMMA-D/MME) and the parental COMMA-D will be utilized as model mammary cell systems. Additionally, frozen preserved bovine mammary cells will also be analyzed. Mammary tissue from bovine mammary acini will be evaluated for changes in IGFBP and casein synthesis and secretion. A total of 9 lactating Holstein dairy cows (three each from early, mid and late lactation) will be killed to prepare mammary acini preparations for fresh assays and preservation for later assays. Cells are cultured on plastic or collagen gels with various treatments including serum free media (SFM), insulin, cortisol and prolactin. Specifically, the regulation, synthesis, and secretion of IGF-II, and the IGFBPs (1-4) will be evaluated by Western Blots and Northern Analysis.

The **New York Station** will determine the effects of altering the dietary level of energy and protein intake (amino acid absorption) on the ST-IGF axis in lactating dairy cows. Because energy and protein intake cannot be varied independently, total diet intakes of 120%, 80% and a short-term fast will be used. Circulating levels of the IGFs and the IGFBPs will be determined under these conditions with basal diet alone and during a 4d bST challenge. A single day ST challenge will be used in the fasted treatment. Impact of level of dietary intake on tissue specific mRNA expression of the IGFs and IGFBPs will also be examined. Treatments will consist of ad libitum intake, 50% of ad libitum intake and a 3d fast. Circulating concentration of IGFs, IGFBPs, ST, insulin and metabolites will be measured before the cows are slaughtered to harvest the same tissue studied in the pig studies(see above), but will include mammary tissue as well. Tissue concentrations and mRNA abundance for the IGFs and IGFBPs will be performed using similar procedures as those described above.

Other studies focusing on the IGF system and combining traditional with molecular approaches are being carried out by the **Connecticut Station**. In conjunction with Petitclerc at Agriculture Canada (Lennoxville) investigations into the expression of growth factors in the bovine mammary gland at different stages of development continue. These investigations are geared toward studying development during gestation. The initial steps will be to develop bovine probes for the genes of these growth factors and then determine expression in the mammary glands of pregnant beef and dairy heifers. They will also determine concomitant changes in the composition of the gland and in cell type at several stages of gestation. A collaboration with Kris Sejrsen in the Department of Research in Cattle and Sheep at the National Institute of Animal Science in Denmark is being established that will focus on the expression of IGF-I and -II, and TGFα during development in prepubertal dairy heifers. Typically, feeding animals a diet with greater energy increases their growth rate and brings an animal to its mature size at an earlier age. However, if prepubertal heifers grow too rapidly, they will not produce as much milk in their lifetime as animals which grow more slowly. The loss in milk is associated with reduced mammary development in these animals. Factors that control the reduction in mammary development are unknown. Therefore, we will investigate the role of specific growth factors and hormone receptors within the developing mammary gland in rapidly growing heifers will be investigated. Beginning to understand the mechanism whereby high feed intake levels inhibit mammary gland development may make it possible to eliminate the negative impact of rapid growth on lifetime milk production and maintain the benefits of increased body size.

The **Vermont Station** has developed a method to determine TGF-β_1 binding to mammary tissue during different physiological states which is of interest to several Stations (**NY-C, . . , USDA-BARC**). Mammary tissue from pregnant and lactating cows will be used to determine changes in receptor number and affinity during different physiological states. The **New York Station** will cooperate by providing mammary tissue from animals in their ongoing studies Since TGF-β_1 may be an important regulator of mammary epithelial development, these studies will allow for a better understanding of when TGF-β_1 can act upon the mammary gland. In addition, studies will be carried out to determine if TGF-β_1 receptors are altered during different stages of sheep placental development (**NY-C, VT**).

Other studies will focus on the role of TGFs in the regulation of mammary gland metabolism. Since it is likely that the TGFs are, in part, modulated by endocrine regulators, these studies will be carried out with mammary tissue from cows treated with bST or estrogen will determine growth factor expression and concentration differences (**VT**). Concentrations of TGF-α and TGF-β_1 will be determined by a quantitative bioassay using A431 human epidermoid carcinoma cells and Mv1Lu mink lung epithelial cells for TGF-α and TGF-β_1, respectively. Messenger RNA expression for TGF-α and TGF-β_1 will be determined. Physiological effects of TGF-β_1 on expression of β-casein mRNA and protein production will be examined using mammary tissue slices or explants from lactating cows.

It is clear from a variety of studies that mammary development and lactation are controlled by a complex array of local growth factors that interact at the mammary gland and respond to endocrine signals. Specific receptors and binding proteins also appear to be essential in these processes, and will occupy the attention of several groups. ST receptor transcript levels during late pregnancy and lactogenesis will be quantified in porcine mammary gland using solution hybridization/ribonuclease protection assay (**PA**). IGFBPs, shown to act as carrier proteins that prolong the half-lives of IGFs and to interact with IGFs to inhibit or stimulate IGF biological activity, are present in mammary secretions and change with stages of lactation. IGFBPs are differentially synthesized and secreted by the MME cell line in culture, providing an excellent model to assess their effects as well as the occurrence of IGFBP specific proteases that appear in mammary cells as they differentiate into lactation. Cells from the mouse mammary cell line COMMA-D express β-casein mRNA under the influence of three lactogenic hormones; insulin, cortisol and prolactin. Application of exogenous IGF and the immunoneutralization of autocrine produced IGF system components will allow for assessment of the IGF-System on mammary cell differentiation. The **Pennsylvania Station** will also evaluate the effects of the IGF system on expression of β-casein mRNA and β-casein synthesis in mouse mammary cells as well as in preserved bovine mammary acini preparations. Culture treatments will include IGF analogues and IGFBPs as well as immunoneutralization of the autocrine IGF system components to assess the impact of endogenous and exogenous IGF system upon mammary growth and development.

The **Connecticut** and the **Minnesota Stations** will conduct collaborative experiments to identify physiologic and genetic markers associated with increased production traits in dairy cattle. The **Connecticut** and the **Minnesota Stations** will utilize AI dairy sires and genetically diverse dairy herd as animal models, respectively. The goal is to obtain information required to enhance development of marker-assisted selection programs. The approach is both targeted, with focus on loci known to be involved in energy partitioning, and broad (i.e., use of random oligonucleotides and primers to screen to entire genome for the presence of polymorphisms,

RAPD). The utility of any polymorphism discovered in three-generation families in the University of Connecticut herd will be examined so that both maternal and paternal contributions to subsequent generations can be followed (CT). Holsteins from the Minnesota herd will be examined by each station for differing genetic merit, and evidence of differential gene expression (e.g., abundance of mRNA for IGFs and ST) will be sought. Samples and techniques will be shared to expand the knowledge base between these two stations (MN, CT).

A second approach will be to determine if ST response to a regimen of SRIF and GRF administration can be used to evaluate genetic merit in young dairy bulls (9 to 12 months of age), and to investigate the mechanisms regulating differential response among bulls of differing genetic merit (CT). After progeny test information becomes available several years hence, they will be able to determine whether ST response to exogenous challenge is related to genetic merit for production traits in dairy cattle. Mature bulls will be used to investigate possible differences in pituitary gland sensitivity between superior and inferior sires. Specifically, they will address the hypothesis that the greater ST-response to GRF in superior bulls may be due to greater numbers of somatotropes in the pituitary gland and(or) a differential rate of secretion of ST from somatotropes in response to GRF or SRIF. To investigate this they will use the reverse hemolytic plaque assay of Neill and Frawley (50). In addition, the area of the plaque will be determined as a measure of rate of hormone released from individual somatotropes. Zinn et al. (51) have modified the original method for use with dispersed bovine pituitary cells. Due to specialized slaughter and cell culture facilities, these studies will be conducted in cooperation with the **Pennsylvania Station**. Parallel studies will be conducted at the **Minnesota Station** with young bulls (1 to 9 months of age) from genetically diverse lines of cattle. Overall, with the cooperation between the **Connecticut and Minnesota Stations**, the response to GRF and SRIF in vivo and in the plaque assay can be evaluated in dairy bulls from birth through maturity.

C. Pregnancy

Regulation of glucose partitioning in late pregnancy with focus on the role of placental glucose transport proteins in the regulation of glucose uptake by the growing conceptus in late pregnancy will be investigated at the **New York Station**. Initial studies have identified GLUT1 as the most abundant transport protein in the ovine placenta. Future studies will determine the quantitative role of this isoform in determining facilitated diffusion of glucose across the placenta, using a combination of in vitro (Cytochalasin B binding, immunoblotting, immunolocalization, Northern blotting, in situ hybridization) and in vivo (compartmental modelling of 3-0-methylglucose maternal-fetal transfer kinetics) techniques. Effects of gestational development and maternal nutrition on placental expression and functional activity of GLUT1 will be examined.

OBJECTIVE 2: To characterize the coordination of physiological actions of humoral factors that accommodate adaptation and influence the efficiency of nutrient utilization in food producing animals.

A. Growth

Results from previous studies clearly demonstrate that conventional complete mixed concentrate diets do not provide adequate nutrient availability to achieve maximum growth rates in lambs and cattle. Our studies center around ways to achieve adequate nutrient availability and utilization to support maximum growth rates in cattle and lambs, with attention directed to the portal for nutrient entry, the small intestine, as well as to important end organs and structures.

Newborn rat pups will be used as models to test the impact of milk- (colostrum) borne hormones and growth factors upon the early neonate's intestinal development (PA). Newborn rat pups are used in a pup-in-a-cup (PIC) experimental system. Pups may be fed rat milk replacer (RMR), RMR plus rat milk infranatant (aqueous phase of rat milk), or RMR plus various endocrine/growth factors. We will focus initially on the IGF-system and its impact upon the growth and development of the small intestine. Histocytochemistry, in situ hybridization and Western and Northern Analysis techniques will be employed to detect growth or developmental alterations induced by the IGF-System.

Studies with growing cattle will quantify appearance of amino acids in the small intestine, net flux of amino acids and other metabolites across the portal-drained viscera, net flux of amino acids across the hind limb and nitrogen balance in growing steers in response to increments of dietary supplementation of a mixture of protein sources that show lower degradability in the rumen (NY-C). These data will be incorporated into the Cornell Net Carbohydrate and Protein System Model to provide information that is lacking (but needed) to accurately estimate net amino acid balance in diets of growing cattle. Incorporation of data obtained from these and other experiments conducted with administration of metabolism modifiers will enhance our ability to formulate diets to meet nutrient requirements for growing cattle. Efficiency of nutrient utilization in growth will be evaluated in genetically diverse lines of cattle at the Minnesota Station that have current body size differences of approximately 150 kg after the first calving. The hypothesis to be tested is that selective breeding has resulted in endocrine alterations in the higher producing cows. Studies will include comparison of effects of metabolic modifiers and their potential interactions with the genetic lines (MN).

B. Lactation:

One of the unique resources of the NE-148 regional research group is the availability of genetically diverse lines of dairy cattle at the Minnesota Station. Establishment and maintenance of these lines has resulted in a production difference of 4,000 kg of milk per lactation between the two lines. However, the physiological and molecular differences between these genetically diverse animals has not been well characterized. These animals provide a unique model and opportunity to investigate specific humoral and molecular genetic factors associated with differences in nutrient utilization, mammary gland development and milk production. That is, what differences has selection of these lines caused, if any, in humoral and(or) genetic factors associated with nutrient utilization, mammary development and milk

production? Based on previous work by members of NE-148, we hypothesize that differences in humoral factors associated with nutrient utilization, mammary gland development and milk production are responsible for the phenotypic differences in milk production, including, ST, prolactin, IGF I and II, placental lactogen (PL), estrogens and estrone sulfate in blood, ST response to GRF, IGFBPs in milk secretions and expression of growth factors in the mammary gland. Moreover, we anticipate that differences in loci-specific and RAPD polymorphisms exist. Expertise from several stations will be utilized to characterize potential phenotypic and genetic differences in these two lines of cattle. Members of NE-148 will utilize this information to develop hypotheses and experiments to test those hypotheses that focus on the involvement of specific tissues (eg., mammary gland, muscle) and organs (eg., liver, hind limb) that result in the reported differences in milk production.

Samples, blood, secretions (when possible) and mammary gland secretory tissue, will be collected from these animals beginning with the prepubertal allometric growth phase of the mammary gland until peak lactation. Specific commitments for these initial characterization studies include: animals, collection of samples (MN), IGFBPs and β-casein in secretions and, estrogens and estrone sulfate in blood (PA), PL in blood (Maine), IGFs in blood (NY-C), RFLP and RAPD characterization (CT, MN). and expression of growth factors in the mammary gland (CT, VT).

Regulatory factors other than the IGFs system,viz., the ST axis and lactogenic hormones, will be examined. Behavior of mammary cells in vitro will be examined at the **Pennsylvania Station,** with attention directed to porcine β-casein mRNA expressions a measure of metabolic differentiation. The experimental system will examine the magnitude of effects exerted by of lactogenic hormones (insulin, cortisol and prolactin) and also compare the degree of metabolic differentiation which can be achieved in vitro and in vivo. **Agriculture Canada** will concentrate on the regulation of splanchnic metabolism in lactating dairy cows and experimental variables that include stage of lactation, level of protein intake, GRF administration and level of productivity.

The **Maryland Station** in cooperation with **USDA-BARC** will expose cows to different photoperiods to induce increases in milk production independent of exogenous ST administration. The hypothesis is that photoperiod-induced galactopoietic effects are associated with changes in the secretion of IGFs and IGFBPs independent of ST secretion.

C. Pregnancy:

To enhance our understanding of protein nutrition and regulation of nitrogen partitioning in late pregnancy, the **New York Station** will investigate the tissue origins and capacity for amino acid net uptake and release of so-called "labile protein reserve" in ewes at 110 to140 d pregnancy fed energy-sufficient diets containing 8, 12 or 16% crude protein. Conceptus tissue responses will also be measured. A secondary objective is to relate influences of protein nutrition on tissue amino acid fluxes to plasma and tissue concentrations, and tissue mRNA abundance of the IGFs and IGFBPs. In the longer term, these studies will be extended to examine the influence of prepartum protein nutrition on early postparturient health and production of dairy cows.

Regulation of metabolic adaptations to pregnancy will also be studied by examining the role of estrogens as homeorhetic mediators of the predisposition towards fat mobilization in late pregnancy (NY-C). In these investigations nonpregnant ewes will be injected with a slow-release preparation of microspheres impregnated with estradiol 17β. Preliminary studies have defined a treatment regimen which mimics plasma concentrations similar to that observed in late-pregnant ewes. Treatment effects on basal rates of entry of plasma metabolites, including NEFA and glycerol, and on whole-body and adipose-specific responses to lipolytic agonists (eg., epinephrine) and antagonists (eg., insulin) will be studied.

The **Maine Station** will investigate the effects of circulating concentrations of bPL on nitrogen metabolism in the pregnant bovine. In previous experiments both intravenous arginine infusion and feeding an all-forage diet during d 150 to 230 of gestation have been shown to increase plasma bPL concentrations. One or both treatments will be used to assess the effects of elevating bPL on nitrogen balance at several intervals during this portion of gestation.

OBJECTIVE 3: To develop integrative concepts that describe the regulation of nutrient utilization and result in enhanced biological efficiency in the production of high-quality products that meet changing consumer demands.

This objective epitomizes the desired outcome from the completion of productive collaborative research planned for this project. During the last period we devoted time to "round table" discussions in partial fulfillment of this objective. We asked for a one year extension of the last project period to formalize a written summary of knowledge gained to date and how it can be applied to this end (ie., objective 3). This effort is in progress. One important outcome of previous work in the project was the demonstration of the increased efficiency of protein use for growth in pigs, sheep and cattle administered ST, and characterization of the effects of ST on nutrient requirements. Several participants contributed directly to the publication of the NRC document "Metabolic Modifiers-Effects on Nutrient Requirements of Food-Producing Animals".

For the current project period we again plan to use the basic information obtained from experimentation planned under objectives 1 and 2 to synthesize integrated concepts that can be applied to improve efficiency of nutrient use for growth, lactation and pregnancy in food-producing animals. One example is the work planned for measuring net nutrient flux across the portal drained viscera and hind limb in cattle fed increments of a mixed undegraded protein source in the diet. Abomasal protein infusion work demonstrated that conventional diets do not provide adequate availability of nutrients for maximum nitrogen balance in growing ruminants, with or without metabolism modifier administration. Quantitative measurements will now be made with a more practical approach That is, extent to which amino acid absorption and availability can be enhanced by diet formulation, and associated endocrine, paracrine and(or) autocrine changes that occur with increased N balance will be determined in pigs and ruminants (NY-C, NC). Integration of these results will allow us to enhance the accuracy and completeness of our modelling approaches to predicting nutrient requirements in growing animals.

Annual committee meetings will include discussion sessions centered on "integration" of new information. In addition, utilizing the New England Animal Biotechnology Conference as a forum, scientists that are not members of NE-148, but that have made significant contributions to mechanisms involved in nutrient utilization will be invited to present their data and assist in development of unifying concepts. This type of activity will provide the greatest opportunity for planned cooperation between participants and non-participants within a circle of expertise. During the project period, written formalization of new concepts will be initiated. Our previous efforts have demonstrated the interest and willingness of participants to collaborate, and the productivity and value of the research that results.

ORGANIZATION

The technical committee will consist of one voting representative from each participating Experiment Station. Officers will include a Chairperson, Vice Chairperson and a Secretary, and term of office will be one year. The Secretary will be selected by majority vote at the annual meeting. The Secretary will proceed to the offices of Vice Chair and Chair in successive years. Special committees will be formed as necessary by consensus of the voting members of the technical committee.

REGIONAL PROJECT TITLE:

REGULATION OF NUTRIENT USE IN FOOD-PRODUCING ANIMALS

Administrative Advisor	7/21/95 **Date**
Chair, Regional Association of Directors	8/7/95 **Date**
Chair, Committee of Nine	9/28/95 **Date**
Administrator, Cooperative Research, Education and Extension Service	9/28/95 **Date**

REFERENCES:

1. Kazmer, G. W., Zinn, S.A., Rycroft, H., Campbell, R.M. 1992. Serum growth hormone in and semen characteristics of proven AI dairy sires after administration of growth hormone-releasing factor. *Canadian J. Anim. Sci.* 72:959-963.

2. Reinecke, R.L., Barnes, M.A., Akers, R.M., Pearson, R.E. 1993. Effect of selection for milk yield on lactational performance and plasma growth hormone, insulin and IGF-I in first lactation Holstein cows. *J. Dairy Sci.* 76 (Suppl. 1): 286.

3. Weber, W.J., Crooker, B.A., Frey, R.S., Chester-Jones, H., Dayton, W.R., Hansen, L.B. 1993. Effect of selection for milk yield on circulating insulin-like growth factor I (IGF-I) in Holstein heifers. *J. Dairy Sci.* 76 (Suppl. 1): 287.

4. Zinn, S. A., G. W. Kazmer, H. Rycroft and R. A. Campbell. 1993. Growth hormone response after administration of growth hormone-releasing factor to proven dairy sires. Livestock Prod. Sci. 40:157-164.

5. Boyd, R. D., Bauman, D. E. 1989. Mechanisms of action for somatotropin in growth. In *Current Concepts of Animal Growth Regulation*, eds. D. R. Campion, G. J. Hausman, R. J. Martin, pp. 257-293. New York: Plenum Publishing Corp..

6. Beermann, D. H. and D. L. DeVol. 1991. Effects of somatotropin, somatotropin-releasing factor and somatostatin on growth. In Pearson, A. M. and T. R. Dutson (Ed.) *Growth Regulation in Farm Animals* (Advances in Meat Research). pp 373-426. Elsevier Publishing, Essex, England.

7. Bauman, D. E., Vernon, R. G. 1993. Effects of exogenous bovine somatotropin on lactation. *Annu. Rev. Nutr.* 13:437-461.

8. Pettitgrew, J.E., McNamara, J.P., Tokach, M.D., King, R.H., Crooker, B.A. 1993. Metabolic connections between nutrient intake and lactational performance in the sow. *Livest. Prod. Sci.* 35:137-152.

9. Boyd, R. D., Kensinger, R. S., Harrell, R. J., Bauman, D. E.. 1995. Nutrient uptake and endocrine regulation of milk synthesis by mammary tissue of lactating sows. *J. Animal Sci.* 73: (In Press).

10. Zinn, S.A., Bravo-Ureta,. B. 1995. The effect of using recombinantly derived bovine somatotropin on dairy production, heath and economics. In Phillips, C.J.C. (Ed.) *Progress in Dairy Science*. (In Press).

11. Bell, A. W., Bauman, D. E., Currie, W. B. 1987. Regulation of nutrient partitioning and metabolism during pre-and postnatal growth. *J. Anim. Sci.* 65(Suppl. 2):186-212.

12. Bauman, D. E., McCutcheon, S. N., Steinhour, W. D., Eppard, P. J., and Sechen, S. J. 1985. Sources of variation and prospects for improvement of productive efficiency in the dairy cow: A review. *J. Anim. Sci.* 60:483-492.

13. Breier, B. H., Gluckman, P. D. 1991. The regulation of postnatal growth: nutritional influences on endocrine pathways and function of the somatotrophic axis. *Livest. Prod. Sci.* 27:77-94.

14. Etherton, T. D., Wiggins, J. P., Evock, C. M., Chung, C. S., Rebhun, J. F., et al. 1987. Stimulation of pig growth performance by porcine growth hormone: determination of the dose-response relationship. *J. Anim. Sci.* 64:433-443

15. Beermann, D. H., Hogue, D. E., Fishell, V. K., Aronica, S., Dickson, H. W. and Schricker, B. R. 1990. Exogenous human growth hormone-releasing factor and ovine somatotropin improve growth performance and composition of gain in lambs. *J. Anim. Sci.* 68:4122-4133.

16. Etherton, T. D., Smith, S. B. 1991. Somatotropin and β-adrenergic agonists: their efficacy and mechanisms of action. *J. Anim. Sci.* 69(Suppl. 2):2-26.

17. Moseley, W. M., Paulissen, J. B., Goodwin, M. C., Alainz, G. R. and Claflin, W. H. 1992. Recombinant bovine somatotropin improves growth performance in finishing steers. *J. Anim. Sci.* 70:412-425.

18. Eisemann, J.H., Hammond, A.C., Rumsey, T.S. & Bauman, D.E. 1989. Nitrogen and protein metabolism, and metabolites in plasma and urine of beef steers treated with somatotropin. *J. Anim. Sci.* 67: 105-115.

19. Boyd, R. D., Bauman, D. E., Fox, D. G., Scanes, C. G. 1991. Impact of metabolism modifiers on protein accretion and protein and energy requirements of livestock. *J. Anim. Sci.* 69(Suppl. 2):56-75.

20. Wray-Cahen, D., Ross, D. A., Bauman, D. E., Boyd, R. D. 1991. Metabolic effects of porcine somatotropin: nitrogen and energy balance and characterization of the temporal pattern of blood metabolites and hormones. *J. Anim. Sci.* 69:1503-1514

21. Krick, B. J., Boyd, R. D., Roneker, K. R., Beermann, D. H., Bauman, D. E., Ross, D. A. and Meisinger, D. J. 1993. Porcine somatotropin impacts the dietary lysine requirement and net lysine utilization for growing pigs. *J. Nutr.* 123:1913-1922.

22. Dunshea, F.R., Bauman, D.E., Boyd, R.D. & Bell, A.W. 1992. Temporal response of circulating metabolites and hormones during somatotropin treatment of growing pigs. *J. Anim. Sci.* 70: 123-131.

23. Walton, P.E. and Etherton, T.E. 1986. Stimulation of lipogenesis by insulin in swine adipose tissue:Antagonism by porcine growth hormone. *J. Anim. Sci.* 62:1584-1595.

24. Walton, P.E., Etherton, T.E. and Chung, C.S. 1987. Exogenous pituitary and recombinant growth hormones induce insulin and insulin-like growth factor resistance in pig adipose tissue. *Domest. Anim. Endocrinol.* 4:183-189.

25. Magri, K.A., Adamo, M., LeRoith, D. and Etherton, T.D. 1990. The inhibition of insulin action and glucose metabolism by porcine growth hormone in porcine adipocytes is not the result of any decrease in insulin binding or insulin receptor kinase activity. *Biochem. J.* 266:107.

26. Dunshea, F.R., Harris, D.M., Bauman, D.E., Boyd, R.D. & Bell, A.W. 1992. Effect of somatotropin on nonesterified fatty acid and glycerol metabolism in growing pigs. *J. Anim. Sci.* 70: 132-140.

27. Dunshea, F.R., Harris, D.M., Bauman, D.E., Boyd, R.D. & Bell, A.W. 1992. Effect of porcine somatotropin on in vivo glucose kinetics and lipogenesis in growing pigs. *J. Anim. Sci.* 70: 141-151.

28. Harris, D.M., Dunshea, F.R., Bauman, D.E., Boyd, R.D., Wang, S.-Y., Johnson, P.A. and Clarke, S.D. 1993. Effect of in vivo somatotropin treatment of growing pigs on adipose tissue lipogenesis. *J. Anim. Sci.* 71:3293-3300.

29. Breier, B. H., Gluckman, P. D., Bass, J. J. 1988. Influence of nutritional status and oestradiol-17β on plasma growth hormone, insulin-like growth factors-I and -II and the response to exogenous growth hormone in young steers. *J. Endocrinol.* 118:243-250

30. Cohick, W. S., Plaut, K., Sechen, S. J., Bauman, D. E. 1989. Temporal pattern of insulin-like growth factor-I response to exogenous bovine somatotropin in lactating cows. *Domest. Anim. Endocrinol.* 6:263-274

31. Walton, P. E., Etherton, T. D. 1989. Effects of porcine growth hormone and insulin-like growth factor-I (IGF-I) on immunoreactive IGF-binding protein concentration in pigs. *J. Endocrinol.* 120:153-160

32. Evock, C. M., Walton, P. E., Etherton, T. D. 1990. Effect of GH status on IGF-I and IGF-II concentrations and serum IGF binding profiles in pigs. *J. Anim. Sci.* 68:1953-1964

33. Beermann, D. H., Robinson, T. F., Byrem, T. M., Hogue, D. E., Bell, A. W., et al. 1991. Abomasal casein infusion and exogenous somatotropin enhance nitrogen utilization by growing lambs. *J. Nutr.* 121:2020-2028

34. Vicini, J. L., Buonomo, F. C., Veenhuizen, J. J., Miller, M. A., Clemmons, D. R., et al. 1991. Nutrient balance and stage of lactation affect responses of insulin, insulin-like growth factors I and II, and insulin-like growth factor-binding protein 2 to somatotropin administration in dairy cows. *J. Nutr.* 121:1656-1664

35. Han, V. K. M., Lund, P. K., Lee, D. C., D'Ercole, A. J. 1988. Expression of somatomedin/insulin-like growth factor messenger ribonucleic acids in the human fetus: identification, characterization, and tissue distribution. *J. Clin. Endocrinol. Metab.* 66:422-429

36. Lund, P. K., Moats-Staats, B. M., Hynes, M. A., Simmons, J. G. ., Jansen, M., et al. 1986. Somatomedin-C/insulin-like growth factor-I and insulin-like growth factor-II mRNAs in rat fetal and adult tissues. *J. Biol. Chem.* 261:14539-14544

37. Möller, C., Arner, P., Sonnenfeld, T., Norstedt, G. 1991. Quantitative comparison of insulin-like growth factor mRNA levels in human and rat tissues analyzed by a solution hybridization assay. *J. Mol. Endo.* 7:213-222

38. Grant, A. L., Helferich, W. G., Kramer, S. A., Merkel, R. A., Bergen, W. G. 1991. Administration of growth hormone to pigs alters the relative amount of insulin-like growth factor-I mRNA in liver and skeletal muscle. *J. Endocrinol.* 130:331-338

39. Coleman, M.E., Russell, L. and Etherton, T.D. 1994. Porcine somatotropin (pST) increases IGF-I mRNA abundance in liver and adipose tissue but not in skeletal muscle of growing pigs. *J. Anim. Sci.* 72:918-924.

40. Ramsay, T.G., Caperna, T.J. Chung, I.B., Czerwinski, S.M., McMurtry, J.P., Rosebrough, R.W. and Steele, N.C. 1994. Metabolic and insulin-like growth factor-I responses by swine tissues to porcine somatotropin administration during the first 24 hours post injection. *Am. J. Physiol.* (in press).

41. Clemmons, D. R., Underwood, L. E. 1991. Nutritional regulation of IGF-1 and IGF binding proteins. *Annu. Rev. Nutr.* 11:393-412 32.

42. Elsasser, T. H., Rumsey, T. S., Hammond, A. C. 1989. Influence of diet on basal and growth hormone-stimulated plasma concentrations of IGF-I in beef cattle. *J. Anim. Sci.* 67:128-141

43. Bass, J. J., Oldham, J. M., Hodgkinson, S. C., Fowke, P. J., Sauerwein, H., et al. 1991. Influence of nutrition and bovine growth hormone (GH) on hepatic GH binding, insulin-like growth factor-I and growth of lambs. *J. Endocrinol.* 128:181-186

44. Buonomo, F. C., Baile, C. A. 1991. Influence of nutritional deprivation on insulin-like growth factor I, somatotropin, and metabolic hormones in swine. *J. Anim. Sci.* 69:755-760

45. Hodgkinson, S. C., Bass, J. J., Gluckman, P. D. 1991. Plasma IGF-I binding proteins in sheep: effect of recombinant growth hormone treatment and nutritional status. *Domest. Anim. Endocrinol.* 83:343-351.

46. McGuire, M. A., Bauman, D. E., Miller, M. A., Hartnell, G. F. 1992. Response of somatomedins (IGF-I and IGF-II) in lactating cows to variations in dietary energy and protein and treatment with recombinant n-methionyl bovine somatotropin. *J. Nutr.* 122:128-136

47. McGuire, M. A., Vicini, J. L., Bauman, D. E., Veenhuizen, J. J. 1992. Insulin-like growth factors and binding proteins in ruminants and their nutritional regulation. *J. Anim. Sci.* 70:2901-2910

48. Osborn, B. H., Fowlkes, J., Han, V. K. M., Freemark, M. 1992. Nutritional regulation of insulin-like growth factor-binding protein gene expression in the ovine fetus and pregnant ewe. *Endocrinology* 131:1743-1750.

49. Caraty, A., Locatelli, A., Moenter, S. M., Karsch, F. J. 1994. Sampling of hypophyseal portal blood of conscious sheep for direct monitoring of hypothalamic neurosecretory substances. In *Methods in Neurosciences*, ed., Conn, M., 9:162-183, Academic Press, San Diego.

50. Neill, J.D., Frawley, L. S.. 1983. Detection of hormone release from individual cells in mixed populations using a reverse hemolytic plaque assay. *Endocrinology* 112: 1123-1135.

51. Zinn, S.A., Chapin, L.T., Lookingland, K. J., Moore, K. E., Tucker H. A. 1991. Effects of photoperiod on lactotropes and on dopaminergic and 5-hydroxytryptaminergic neurons in bull calves. *J. Endocrinol.* 129: 141-148.

ATTACHMENT I. PROJECT LEADERS AND PARTICIPATING INSTITUTIONS

State, Agency, Institution	Project Leaders	Area of Expertise
Agriculture Canada Research Branch, Quebec	H. Lapierre*	Nutrition/Metabolism
Connecticut, Univ. of CT	S.A. Zinn*	Physiol/Endocrinol
	G.W. Kazmer	Physiol/Endocrinol
Maine, Univ. of Maine	C.R. Wallace*	Physiol/Endocrinol
Maryland, Univ. of Maryland	G.E. Dahl*	Physiol/Endocrinol
Minnesota, Univ. of Minnesota	B.A. Crooker*	Nutr/Metabolism
	W.R. Dayton	Muscle Biol/Biochem
	M.R. Hathaway	Muscle Biol
	J.E. Pettigrew	Nutrition
New Jersey, Rutgers Univ.	B. Jesse	Nutrition
	P.A. Schoknecht*	Nutr/Physiol
	J.E. Wohlt	Ruminant Nutr
New York, Cornell Univ.	D.E. Bauman	Nutr/Physiol/Biochem
	D.H. Beermann*	Muscle Biol/Physiol
	A.W. Bell	Nutritional Physiol
	M.L. Thonney	Nutrition/Growth
North Carolina, NC State Univ.	J.H. Eisemann*	Nutr/Metab/Physiol
Pennsylvania The Penn State Univ.	C.R. Baumrucker*	Cell Biol/Nutr
	R.S. Kensinger	Nutritional Physiol
USDA-BARC Beltsville, MD	S. Czerwinski	Muscle Biol/Physiol
	T.H. Elsasser*	Endocrinology
Vermont, Univ. of Vermont	K.I. Plaut*	Lactation/Physiol

*Designates voting members of the Technical Committee.

L.J. Pierro, University of Connecticut, Administrative Advisor.

ATTACHMENT II. RESOURCES

Station	SY	PY	TY
Agriculture Canada	.1	--	--
Connecticut(S)	.4	.3	--
Maine	. 4	--	.4
Minnesota	1.4	--	1.0
New Jersey	.4	--	.2
New York(C)	.55	.5	2.0
North Carolina	.2	--	.1
Pennsylvania	.33	.2	.4
Maryland	.3	--	.1
Vermont	.5	.5	.2
TOTALS	**4.58**	**1.5**	**4.4**