National Library of Canada

Acquisitions and Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale du Canada

Direction des acquisitions et des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Canada

UNIVERSITY OF ALBERTA

PHYSIOLOGICAL CHANGES, TISSUE AND VISCERAL ORGAN GROWTH, AND MAMMARY GROWTH AND DEVELOPMENT IN BEEF HEIFERS UNDERGOING COMPENSATORY GROWTH

BY

E. S. K. YAMBAYAMBA

A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

ANIMAL GROWTH AND DEVELOPMENT

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

SPRING 1996

ISBN 0-612-10655-1

UNIVERSITY OF ALBERTA

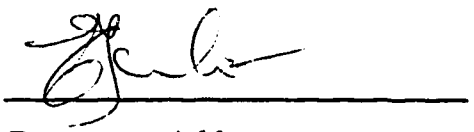
LIBRARY RELEASE FORM

NAME OF AUTHOR: **EGBERT KAVWANGA SIKAZWE YAMBAYAMBA**

TITLE OF THESIS: **PHYSIOLOGICAL CHANGES, TISSUE AND VISCERAL ORGAN GROWTH, AND MAMMARY GROWTH AND DEVELOPMENT IN BEEF HEIFERS UNDERGOING COMPENSATORY GROWTH**

DEGREE: **DOCTOR OF PHILOSOPHY**

YEAR THIS DEGREE GRANTED: **1996**



Permanent Address:
Department of Animal Sciences
The University of Zambia
P. O. Box 32379, UNZA
Lusaka, ZAMBIA

Dated: January 29 1996

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **PHYSIOLOGICAL CHANGES, TISSUE AND VISCERAL ORGAN GROWTH, AND MAMMARY GROWTH AND DEVELOPMENT IN BEEF HEIFERS UNDERGOING COMPENSATORY GROWTH** submitted by **EGBERT KAVWANGA SIKAZWE YAMBAYAMBA** in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in ANIMAL GROWTH AND DEVELOPMENT.

Dr. M. A. Price

Dr. G. R. Foxcroft

Dr. S. D. M. Jones

Dr. B. Fisher

Dr. D. C. Cumming

Dr. J. McKinnon
(External Examiner)

DEDICATION

To my late father, a no-nonsense man who had great vision for his children and provided the necessary environment for good education

To my late grand mother Nachangwa, whose caring love was a great inspiration in my entire life

To the great African Thinkers, especially the late Dr. Anta Diop, whose great ideas have entirely opened up my mind and have renewed my life

To my family: wife Monica, son Kauzya-nzila, and daughters Nachiilila and Mwaaya, whose love and encouragement continually provide a peace of mind

ABSTRACT

A series of studies was conducted to investigate physiological responses, tissue and visceral organ regrowth patterns, skeletal muscle and hepatic glucidic potential, and mammary growth and development in Hereford crossbred heifers undergoing compensatory growth. The heifers were allotted in equal numbers to either ad libitum feeding (ADLIB) or maintenance feeding for 3 mo followed by realimentation (REST). Study 1 used 12 heifers (227 kg, 12 kg SD; 230 d, 8 d SD on d 0) from which plasma was collected on d 0, 20, and 48 (feed restriction period) and d 104, 125, 153, and 195 (realimentation period) and analyzed for growth hormone (GH), insulin-like growth factor I (IGF-I), insulin, thyroid hormones, and various metabolites. Feed restriction was associated with higher ($P < .05$) concentrations of GH and non-esterified fatty acids, and lower ($P < .05$) concentrations of IGF-I, insulin, thyroid hormones, and glucose, than was ad libitum feeding. During realimentation, average daily gain for REST heifers was higher ($P = .001$) than that for ADLIB heifers, and the endocrine and metabolic changes were reversed, but not until after d 10 of realimentation.

Studies 2, 3, and 4 used 28 heifers (222 kg, 22 kg SD; 226 d, 10 d SD on d 0), drawn randomly from the same original population as those in study 1. They were slaughtered over a wide range of live weights, beginning d 1 of feed restriction (about 200 kg) through realimentation to the final slaughter weight of about 450 kg. Various tissue components and visceral organs were weighed, muscle was analyzed for glycogen content, and the mammary tissue was analyzed for nucleic acids. Feed restriction reduced ($P < .05$) the weights of muscle, fat, and visceral organs compared to ad libitum feeding. During realimentation, the affected tissues and organs grew faster ($P < .05$) relative to empty body weight in REST than

in ADLIB heifers. Body composition was similar in both treatments at equal live weight, and muscle glycogen content was also similar. Mammary tissues from REST heifers tended to have higher concentrations of nucleic acids than those from ADLIB heifers. These results indicate that enhanced growth rates during compensatory growth are controlled, in part, by the endocrine system, and that final body composition is not affected by growth pattern in beef heifers. Mammary development appears to be enhanced in compensating heifers.

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to my Supervisor, Dr. Mick Price, who has patiently led me through my Doctoral program. His confidence in me and my work was a great inspiration in my graduate studies life. I would also like to thank members of my Supervisory Committee Dr. George Foxcroft and Dr. Steve Morgan-Jones whose time and suggestions led to a thorough work of this huge research project. I thank Drs. John McKinnon (external), David C. Cumming (Obstetrics and Gynaecology), and Brian Fisher (Phys. Ed.) for taking their time to serve on the examining committee.

Thanks are also due to Laraine Strafford (Director, Off-Campus Research) and Terry Fenton (Laboratory Supervisor) who made the necessary arrangements to get started and proceed smoothly through my research. Their willingness to pay attention to all details is greatly appreciated.

At the Metabolic Unit, special acknowledgements go to Paul Gregory (Unit Supervisor), Brenda Tchir, John Clark, and Charlane Gorsak, who tirelessly helped with feeding the animals and blood sampling. The Ellerslie Research Station staff Steve Melnyk (Supervisor), Paul Hansen, and Scott Sylvester are similarly thanked for feeding the animals and arranging for slaughter with Lacombe Station. At the Agriculture and Agri-Food Canada Research Centre in Lacombe, I would like to sincerely thank Dr. Steve Morgan-Jones for allowing me to use their facilities for slaughter of animals and dissections. I thank Don Brereton (Plant Supervisor) and Wayne Robertson for taking the lead to ensure that all the work at the slaughter house went smoothly. I also thank Dr. Jennifer Aalhus, Dave Best, and other staff in the Chemical Analysis Lab for their help with the analysis of glucidics.

In the Endocrinology Lab, very special thanks go to Brian Turner, Shirley Shostak, and Dorothy Payne for their excellent technical advise. Of course I cannot forget to thank Dr. A. F. Parlow who kindly donated the bovine growth hormone for label, and the antiserum, and Dr. S. Raiz who donated the antiserum for IGF-I. In the Biotechnology Lab, I thank Renate Meuser for her technical advise on DNA and RNA isolation, and Dr. Walter Dixon for allowing me to use most of his facilities. Dr. H. Allen Tucker is thanked for taking time to personally send me some protocols regarding mammary DNA and RNA isolation. Gary

Sedgwick in the HPLC Lab, and Margaret Micko and Pat Marceau in the Nutrition Lab, are greatly thanked for their help in the analysis of 3-methyl histidine and protein, respectively.

On the computer, my heart-felt thanks go to Ray Weingardt who patiently and tirelessly helped me sort out the volumes of data that I had accumulated during the course of my research. Gavin Godby is thanked for the many times he responded to the technical problems I encountered on the computer. I also thank the many fellow graduate students who took time to help out in one way or another: Helen Miller, Eva Zurek, Sigrid Hofacker, Rajesh Jindal, David Ekpe, Jacob Miaron, Doo-soek Nam, Pang Hang, and MingFu Liu. It would not have been possible to do work of this magnitude without their help.

I wish to take this opportunity to thank the following organizations for their financial assistance: Canadian International Development Agency, who have wholly supported my entire stay in Canada, and also part of research funding; Natural Sciences and Engineering Research Council; Agriculture and Agri-Food Canada, and the Alberta Cattle Commission. Mr. Michel Parent, Ms Lise Lamontagne, and the now retired Prof. Len Siemens who have coordinated my scholarship are greatly acknowledged.

Indeed I cannot forget to pay special gratitude to my family: wife, Monica; son, Kauzya-nzila; daughters, Nachiilila and Mwaaya. Their tolerance during the many hours of my absence is greatly appreciated. I thank all members of the Southern Africa Cultural and Development Association (SACUDA) for the great friendship I have enjoyed during my stay in Edmonton. Finally, I wish to thank all friends and relatives back in Zambia for their moral and spiritual support during these many years of thousands of miles of separation.

Kavwanga E. Yambayamba

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS NOT DEFINED IN THE TEXT

Abbreviation	Definition
BSA	bovine serum albumin
BW	body weight
cpm	counts per minute
°C	degrees celcius
CV	coefficient of variation
d	day
dL	deciliter
DNA	deoxyribonucleic acid
g	gram
g	gravity
GIT	gasrointestinal tract
h	hour
HPLC	high performance liquid chromatography
kg	kilogram
kJ	kilojoule
µIU	microinternational unit
µL	microliter
mEq	milli equivalent
mg	milligram
min	minute
mL	milliliter
mRNA	messenger RNA
M	molar (concentration)
n	number
ng	nanogram
nm	nanometer

N	normal (concentration)
NEFA	non-esterified fatty acids
P	probability
PBS	phosphate buffered saline
RNA	ribonucleic acid
sec	second
TCA cycle	tricarboxylic acid cycle
TRIS	tris (hydroxymethyl) aminomethane
wk	week

CHAPTER ONE

1. General Introduction

1.1. What is Compensatory Growth?

Compensatory growth, sometimes referred to as "catch-up growth", refers to rapid growth rates that are exhibited by animals during realimentation following a period of undernutrition (Wilson and Osbourn, 1960). It is a complex physiological mechanism that has been studied in virtually all farm animals (Asghar et al., 1981 for rabbits; Porkniak and Cornejo, 1982 for chickens; Ferrell et al., 1986 for sheep; Pond and Mersmann, 1990 for pigs; Carstens et al., 1991 for cattle), rats (Mosier and Jansons, 1976; Mosier et al., 1985a,b), and humans (Delgado et al., 1987; Georgieff et al., 1989). In cattle, it is not uncommon to gain as much as 2 kg/d (Carstens et al., 1991; Yambayamba and Price, 1991). Similarly in humans, Ashworth and Millward (1986) stated that it is not uncommon for children undergoing compensatory growth to exhibit growth rates 20 times faster than normal healthy growing children. One important question often asked is whether compensatory growth can be used to advantage in animal production. It is difficult to adequately address this question unless we understand the underlying mechanisms of this growth process (Hogg, 1991). Despite decades of observations, there has been no conclusive explanation about the physiological mechanisms behind compensatory growth; this is basically because most studies have been done to merely

refine the detail rather than look into the mechanisms behind compensatory growth.

In cattle, the major focus of several studies has been on carcass composition in castrated males destined for meat production. Very little attention has been paid to young growing beef heifers despite their economic importance in herd dynamics. Given the importance of heifers, it is paramount to understand the physiological events occuring as a result of nutritional manipulations and how these affect the growth process and ultimate body composition. Other major areas worth exploring are the effects of compensatory growth on muscle glycogen stores, and mammary growth and development, particularly whether there is potential for influencing mammary development to improve productivity in beef operations.

However, to appreciate the mechanisms of compensatory growth and its effects, some understanding of the concepts of "normal growth" of various body components is important. This understanding would provide the basis of explaining the physiological changes in animals undergoing compensatory growth and how this would ultimately affect various tissues and organs.

1.2. What is Growth?

Growth can be defined in several ways, but in general, it is an increase in size of an individual. It is a complex process, familiar to all of us but clearly understood by none (Price and White, 1985). An individual will normally follow a predetermined growth curve if conditions are favourable. Van den Brande (1986) put it this way: "When considering the many possible interfering factors, the path followed towards adult size and shape must be

under tight control; moreover, the controlling mechanism is likely to be one with different hierachies: overall, regional, organ-bound, and local within tissues." Prader et al. (1963) and Van den Brande (1986) have called this narrow and predictable path followed by an individual during the course of growth, "canalization".

The process of growth encompasses several mechanisms including intricate interactions between the genome and many mediators of metabolism (e.g., the endocrine system), nutritional, and environmental factors. An individual's genetic constitution and endocrine system constitute intrinsic factors, while the quality and quantity of nutritional intake, and degree of environmental stimulation constitute extrinsic factors (Karniski et al., 1987).

In farm animals, particular attention has been paid to the growth of the major body components: bone, muscle, and fat. Maximum muscle, minimum bone, and an optimal amount of fat is the desired goal in animal production (Berg and Butterfield, 1976).

The process of growth itself takes place by hyperplasia (increase in cell number) and hypertrophy (increase in cell size). Measurements of tissue DNA content (index of cell number), tissue protein:DNA ratio (index of cell size), RNA:protein ratio (index of capacity for protein synthesis), and protein:RNA ratio (index of efficiency of protein synthesis), have been used in describing the relative contributions of hyperplasia and hypertrophy to growth in a number of tissues under various physiological conditions (Burleigh, 1980; DiMarco et al., 1987). The very early stages of growth of most tissues and organs are accomplished mainly by hyperplasia (Grant and Helferich, 1991) while later stages are mainly accomplished by hypertrophy.

1.2.1. Growth and Development of Bone, Muscle, and Accretion of Fat in Adipose Tissue

1.2.1.1. Bone

Longitudinal growth of long bones takes place at the epiphyseal growth plates located between the epiphysis and the metaphysis (Pines and Hurwitz, 1991; Nilsson et al., 1994). The various cells involved in bone growth include the osteoblasts that synthesize the organic matrix, the osteoclasts that are involved in bone resorption, and the chondroblasts which are responsible for the growth of cartilage (Grant and Helferich, 1991). Undifferentiated mesenchymal cells give rise to preosteoblasts which subsequently differentiate into osteoblasts; these then mature into osteocytes, and are calcified.

The actual bone growth is the result of the proliferation and hypertrophy of the chondrocytes (mature chondroblasts), with subsequent endochondral ossification in the epiphyseal growth plates (Nilsson et al., 1994). Endochondral ossification proceeds from the centre of the diaphysis toward each epiphysis of the bone (Grant and Helferich, 1991). Continuous ossification on the diaphyseal side of the epiphyseal cartilage, coupled with continuous growth of cartilage on the epiphyseal side of the epiphyseal cartilage, contributes to postnatal bone growth. At maturation, the epiphyseal cartilage plate closes and longitudinal bone growth ceases (Pines and Hurwitz, 1991). This means that an animal subjected to various environmental insults leading to zero or even negative growth, can resume normal growth as long as the epiphyseal plates have not closed.

1.2.1.2. Muscle

Muscle is made up of muscle fibers which are formed from mononucleated cells, the myoblasts (Grant and Helferich, 1991). During the early stages of development, embryonic mononucleated myoblasts differentiate and fuse to form primary multinucleated myotubes. Later, fetal myoblasts, surrounding the primary myotubes, generate secondary myotubes (Mascarello et al., 1992). The myotubes eventually differentiate to form fully functional muscle fibers.

During prenatal growth, muscle fibers increase by both hyperplasia and hypertrophy (Marple, 1983). Muscle growth via these processes continues through the time of birth with hyperplastic growth ceasing within a few days after birth. Postnatal growth is basically accomplished by the process of hypertrophy of the existing cells, and involves the synthesis of both contractile and cytoplasmic proteins (Marple, 1983). Growth in diameter of muscle fibers takes place through a process of longitudinal splitting of existing myofibrils (Goldspink, 1970; 1971). The resulting smaller myofibrils grow until they reach a mature size and split again to form even more myofibrils. This process continues until the animal reaches its mature size. Devlin and Emerson (1979) and Affara et al. (1980) noted that the process of myofibrillar protein synthesis is a highly synchronous and organized process. In terms of length, muscle fibers are known to increase by the addition of whole sarcomere units at the ends of the muscle fibers (Griffin et al., 1971).

Although muscle fibers are multinucleated cells, the nuclei within the cells do not divide during growth. However, Trenkle et al. (1978) demonstrated that the number of nuclei per muscle increases dramatically after birth in cattle. Apparently the nuclei are contributed by

the satellite cells which are located between the plasmalemma and basement membrane of the muscle fibers (Moss and Leblond, 1971). Young et al. (1978) showed that the rate of incorporation of nuclei and, ultimately, additional DNA into the muscle decreases as the animal matures. Anything which stimulates the rate of mitosis of satellite cells could give increased number of nuclei per muscle fiber, and this could result in greater potential for muscle growth.

1.2.1.3. Adipose Tissue and Fat Accretion

Adipose tissue basically consists of cells that accumulate fat. During embryogenesis, mesenchymal cells give rise to adipoblasts which are small, undifferentiated cells that are able to proliferate in the stromovascular region of the adipose tissue (Leat and Cox, 1980). The adipoblasts eventually differentiate into preadipocytes at which stage, they begin to accumulate lipid droplets as a result of lipogenic enzyme synthesis (Grant and Helferich, 1991). With the accumulation of lipid droplets, the preadipocytes evolve into immature adipocytes which have a great capacity for fat storage.

Changes in adipocyte number may play some role in postnatal adipose tissue growth but fat accretion is the major process of this tissue growth in later stages of growth in meat animals. Fat accretion per se is not true growth since it is more environmentally controlled. This means that fattening does not necessarily follow the rules of growth, such as of allometry. However, although fat accretion is dominant in later stages of animal growth, there is evidence that there may be induction of adipose cell proliferation among mature animals as the cells reach a mature size (Bertrand et al., 1978; Johnson et al., 1978). Enser

and Wood (1978) reported that growing and adult animals can actually recruit undifferentiated mesenchymal cells, which exist extensively throughout the connective tissue, to become adipocytes. Kirtland and Harris (1980) who underfed a group of rats similarly reported that following realimentation of the animals, there was a stimulation of fat cell proliferation. The authors interpreted the findings to mean that there is no finite period of hyperplasia of the adipose tissue. This means that proliferation could be initiated at any time in life, not only by a sudden excess of food, but by other circumstances such as "times of physiological deposition' of fat. Thus the ability to store fat in adipocytes combined with the capacity to "recruit" cells, makes the animal's ability to store fat effectively limitless. With this in mind, growth physiologists face the challenge of identifying means to reduce the amount of carcass fat while promoting the production of muscle in meat animals.

1.2.2. Nutritional Control of Growth and Development

Nutrition is by far the most important environmental influence on growth and development in both prenatal and postnatal stages (Widdowson and Lister, 1991). It is the ultimate source of the energy whose transformations constitute the essential phenomena of physical life (Emmans, 1994). Thus proper nutrition must be provided if maximum potential for growth is to be realized. In fact, total feed intake actually determines the size to which an animal may grow and the proportions of tissues deposited in the body (Widdowson and Lister, 1991). This implies that an animal given access to ad libitum feeding will grow to a larger size than one given restricted feeding for the same period of time.

While feed intake is important for the growth of an animal, the actual process of growth is accomplished by the nutrients present in the feed. Widdowson and Lister (1991) noted that provided the body's requirements for minerals, vitamins, essential amino acids, and fatty acids are met, it is the adequacy of protein and energy intake which determine how animals grow. Maximum rates of gain do not occur if either protein or energy is limiting, or if they are not in the right proportions. Since all feeds commonly fed to ruminants as sources of energy also contain protein, increasing energy intake results in more synthesis of microbial protein in the rumen and stimulation of protein synthesis in the animal (Trenkle, 1983). Apart from merely increasing energy intake to stimulate protein synthesis, any means of nutritional manipulation that would result in more efficient utilization of dietary energy and protein would result in higher rates of gain than normal.

Specific effects of nutrition on the growth of the major carcass tissues vary because of the differences in the relative growth of these tissues. Trenkle (1983) reported that in young animals, more of the absorbed nutrients are used for growth of bone and muscle. However, as the animals mature, more nutrients are directed to fat rather than to lean tissue. Any attempt to manipulate feeding levels can significantly affect how these tissues, particularly fat and muscle, grow. In cattle, Trenkle et al. (1978) observed that feed restriction resulted in slower muscle growth and reduced fat accumulation in the carcass. Bone growth is, however, not significantly affected by nutritional manipulation (Price, 1977; Yambayamba and Price, 1991). These responses of the different tissues to nutrition can have major implications in animals that are subjected to feed restriction for a period of time followed by realimentation.

1.2.3. Energy and Protein Status as Influenced by Nutrition

1.2.3.1. Energy Status

Plasma glucose and NEFA concentrations are indications of energy status of an animal. Primarily, glucose is the major substrate of oxidative metabolism in mammals (Reynolds et al., 1990; Coyle, 1992). This means that maintenance of normal plasma glucose levels is critical to the energy status of the animal. Ruminant animals do not absorb any significant amounts of glucose from the gut since the ingested carbohydrates are fermented to volatile fatty acids (VFA), the major ones being acetate, propionate and butyrate (Armentano and Young, 1983). Thus, the synthesis of glucose in ruminants is mainly through gluconeogenesis from VFA, particularly propionate (Foster and Blight, 1987), glucogenic amino acids (Anthony et al., 1986; Brockman and Laarveld, 1986), and lactate (Huntington et al., 1981). It would be expected that propionate would be the principal substrate for gluconeogenesis in the fed ruminant while glucogenic amino acids would be more predominant in the fasted or restricted animal.

Non-esterified fatty acids are mobilized from adipose tissue, and they are the major source of hepatic long chain fatty acids (LCFA) for hepatic oxidation during fasting (Jesse et al., 1986). Jesse et al. (1986) have characterized two major functions of hepatic LCFA oxidation as follows: 1) provision of an alternative energy source in the form of ketone bodies to extrahepatic tissues, and 2) action to allow maximum gluconeogenic rates during glucose shortage, such as in fasting. According to those authors, hepatic LCFA oxidation and ketogenesis is a continuous process regardless of the metabolic state, and is associated with

continuous gluconeogenesis.

1.2.3.2. Protein Status

The level of blood urea nitrogen (BUN) is indicative of the protein status of the animal (Ellenberger et al., 1989). A positive nitrogen retention in tissues indicates a higher rate of synthesis of nitrogenous compounds than degradation (Eisemann et al., 1989). In such a situation, a lower BUN concentration would be detected in the blood or urine than if degradation exceeded synthesis. Thus under conditions of restricted feeding, an elevation of BUN concentration would be expected from the catabolism of nitrogenous compounds. The synthesis of urea takes place in the liver as a mechanism of removing and detoxifying ammonia that arises from the oxidation of amino acids, nucleic acids and other nitrogenous compounds (Reynolds, 1992). The ammonia so produced combines with carbon dioxide to form urea.

1.2.4. Endocrine Control of Growth

Metabolically, the regulation of growth primarily depends on altering the kinetics of biochemical reactions (Black, 1988). The mediators that influence the rate of metabolic reactions in animals include hormones, peptides, growth factors, prostaglandins, catecholamines, metabolites, free ions, toxins, and other substances (Black, 1988). Among the potentially anabolic peptide hormones and growth factors are growth hormone (GH), insulin, insulin-like growth factors I and II (IGF-I and IGF-II), and epidermal growth factor

(EGF). Other hormones include thyroid hormones (Di Liegro et al., 1987; Nanto-Salonen et al., 1991) and steroids (Davis, 1988). For the purpose of the present work, only GH, IGF-I, insulin, and the thyroid hormones (T_3 and T_4) will be considered.

1.2.4.1. Growth Hormone

Growth Hormone is considered the principal hormone in growth stimulation (Borer, 1987; Davis, 1988); its actions on somatic growth are mostly mediated by IGF-I (Ashworth and Millward, 1986; Bruno et al., 1991). Growth hormone is a large polypeptide mostly consisting of 190 or 191 amino acids in the bovine (Lucy et al., 1993). It circulates in part as a complex in association with a binding protein (BP) both in humans (Bauman et al., 1988) and in domestic animals including sheep, cattle, pigs, and chickens (Davis et al., 1992). Bauman et al. (1988) have speculated on the biological role of complexed GH as follows:

1. The BP may act to prolong the persistence of GH in the body by restricting access to degradation sites.
2. The BP may buffer plasma GH levels and, by complexing GH, act as a circulating hormone reservoir during periods of variable pituitary secretion.
3. The circulating BP may interfere with receptor binding of GH, as both the receptor and BP have similar affinity for GH. By inhibiting receptor binding of GH, the BP may modulate GH action.
4. Conversely the presence of BP may enhance GH action by acting as a co-factor for receptor binding, by directing GH to tissues bearing receptors for the BP, or by

otherwise facilitating delivery of GH to the site of action.

1.2.4.1.1. Growth Hormone Secretion and Control

The secretion of GH by the anterior pituitary in adult animals is pulsatile in nature. In rats, Gluckman et al. (1987) observed that secretion is characterized by a synchronous endogenous ultradian rhythm, with high amplititude GH secretory peaks occuring at 3 to 4 h intervals throughout the day. In cattle, however, the pattern of GH secretion appears to be asynchronous and episodic (Anfinson et al., 1975), and the pulses do not appear to have a consistent relationship to the time of feeding or time of the day (Gluckman et al., 1987).

The secretion of GH is basically regulated by a dual system of the hypothalamic regulatory hormones: growth hormone releasing factor - GRF, and somatostatin - SRIF (Gluckman et al., 1987, Frohman, 1990). While GRF stimulates the release of GH, somatostatin has the opposite effect. The release of these hypothalamic hormones is in turn influenced by a network of monoaminergic neurons (Gluckman et al., 1987). Thus a variety of neurotransmitters such as norepinephrine, dopamine, serotonin, and many neuropeptides play a role in the neuroendocrine regulation of GH secretion.

It is believed that the initial event in the generation of a GH pulse is a diminished release of somatostatin from the hypothalamus, thus allowing a greater response to and possibly greater release of GRF (Frohman, 1990). The pulse may then be terminated by increased somatostatin release (Gluckman et al., 1987). According to Abe et al. (1983), there is also evidence that the negative feedback of GH secretion operates within the somatotrophic axis; both IGF-I and GH when administered centrally inhibit pituitary GH release (Abe et al.,

1983). Insulin-like growth factor I has been shown to inhibit GH release at both the hypothalamic and pituitary levels (Berelowitz et al., 1981; Abe et al., 1983). On the other hand, increased GH stimulates somatostatin release (Berelowitz et al., 1981), which in turn regulates the secretion of GH.

1.2.4.1.2. Growth Hormone Action and Influence on Growth Rate

The first step in GH action is binding to its receptor, and tissue sensitivity may be modulated by changes in the number and/or affinity of the binding sites (Breier et al., 1988). Breier et al. (1988) have observed that specific binding of bovine GH (bGH) to hepatic membranes is greater in well-fed ruminants than in the underfed ruminants. They have also observed a high affinity binding site under high planes of nutrition and a low affinity binding site under low planes of nutrition. The authors have subsequently proposed an active role for this somatotrophic receptor in regulating the state of the somatotrophic axis and the growth rate in the postnatal ruminant.

Underfed ruminants have diminished growth rates despite elevated GH levels (Driver and Forbes, 1981; Blum et al., 1985). Meanwhile, IGF-I concentration falls significantly during underfeeding (Breier et al., 1986). This changing relationship between circulating concentrations of GH and IGF-I suggests that nutrition-dependent changes in GH responsiveness are dominant influences on the growth rate of the ruminant animal.

1.2.4.2. Insulin-like Growth Factor

There are two important insulin-like growth factors namely IGF-I and IGF-II. These are

single-chain polypeptides of molecular weight of approximately 7500 daltons (Rechler, 1988). Amino acid sequences of the two IGFs are 60% identical to those of the B and A chains of insulin, but despite their chemical similarities, IGF-I and IGF-II seem to exhibit different physiological roles (Rechler, 1988). While IGF-I mediates many of the somatogenic effects of GH on postnatal growth (Leung et al., 1987; Evock et al., 1990), IGF-II has little effect on postnatal growth but it does play a major role in regulating fetal growth (Evock et al., 1990; Godfredson et al., 1991). Also IGF-I is mainly regulated by GH (Davis, 1988; Rechler, 1988) while IGF-II shows lesser GH dependence (Rechler, 1938).

Insulin-like growth factor I circulates in plasma and other body fluids complexed to specific binding proteins (IGFBP) (Rechler, 1988). In ruminants (Skaar et al., 1991) and swine (McCusker et al., 1991), four IGFBPs have been identified and they have been named as IGFBP-1, IGFBP-2, IGFBP-3, and IGFBP-4. The IGFBP-3 is the major GH-dependent form of IGFBP in postnatal serum (McCusker et al., 1991) and has a high affinity for IGF-I (Nanto-Salonen et al., 1991). According to McCusker et al. (1991), the IGF-IGFBP-3 complex has a relatively long half-life of about 3 to 6 h and the proposed functions of this complex are to protect IGF-I from degradation and to control the rate of efflux of the IGF from the vascular compartments. In contrast, the non-GH dependent IGF-IGFBP complexes (IGFBP-1, IGFBP-2 and IGFBP-4) turn over much more rapidly, with a half life of 10 to 40 min (Hodgkinson et al., 1987; McCusker et al., 1991).

1.2.4.2.1. Secretion and Actions of IGF-I

As stated earlier, the production of IGF-I is dependent on GH. Therefore plasma IGF-I

is regulated primarily by the somatotrophic axis. According to the model proposed by Davis (1988), the signals involved in the negative feedback regulation of IGF-I are at both the hypothalamic and pituitary levels.

Although IGF-I is principally produced by the liver (Russel et al., 1985; Murphy et al., 1987; VandeHaar et al., 1990), there are several other tissues from which this polypetide has been isolated (Murphy et al., 1987; Davis, 1988). Murphy et al. (1987) examined several tissues from rats and observed that IGF-I transcripts were detectable in each of the normal tissues examined; the relative order of abundance in tissues was: liver > lung > kidney > thymus > spleen > heart > skeletal muscle > testes > brain. Thus apart from IGF-I acting in an endocrine fashion, there is evidence that it can also act in an autocrine or paracrine fashion. Murphy et al. (1987) confirmed this in rats by demonstration of the IGF-I gene expression in the non-hepatic tissues. VandeHaar et al. (1990) also concluded in their experiment with rats that the decreased skeletal muscle IGF-I mRNA during protein restriction was consistent with the autocrine/paracrine action of IGF-I in muscle.

1.2.4.3. Insulin

Insulin, together with the IGFs comprise a family of polypeptide hormones that exhibit a similar spectrum of biological activities and show a high degree of homology in their sequences (King et al., 1982). Insulin and IGFs, however, have different potencies for various biological effects: while IGFs are more potent for growth-promoting effects, insulin is more potent for metabolic effects (King et al., 1982).

Insulin is a 6000 dalton peptide produced by β-cells of the pancreas (Leffert et al., 1979).

It has its major effects on nutrient metabolism (Sano et al., 1991), particularly favouring the uptake of glucose (Beaver et al., 1989) and amino acids (Ahmed et al., 1983; Beaver et al., 1989). Glucagon, produced by the α-cells of the pancreas acts in the opposite direction to insulin (Brockman and Laarveld, 1986).

1.2.4.3.1. Control of Insulin Secretion

Unlike several other hormones whose secretion by the glands is controlled by the hypothalomo- (hypothalamic releasing and inhibiting hormones/factors) hypophysial axis, insulin secretion seems to involve direct neural control (Woods and Porte, 1974). The endocrine pancreas is innervated by a branch of the vagus nerve and a branch of the splanchnic nerve (Niijima, 1989). Electrical stimulation of either nerve changes insulin and glucagon output from the pancreas. In association with this anatomical arrangement are the liver and adrenal medulla, also innervated by both nerves in order to coordinate the secretion of insulin and the regulation of blood glucose and synthesis of hepatic glycogen by insulin (Edwards and Silver, 1970). Experiments indicate that excitation of the vagus nerve facilitates insulin secretion from the β-cells of the pancreas and glycogen synthesis in the liver, and that excitation of the splanchnic nerve elicits glucagon secretion from the α-cells of the pancreas and glucose release from the liver (Niijima, 1989).

Glucose-responsive neurons in the hypothalamic region may, therefore, be involved in important ways in the sending of signals to the pancreas and adrenal medulla through the vagi and splanchnic pathways. Apart from the central glucose-responsive neurons, the activity of the efferent fibers is also modulated by the peripheral (gustatury, intestinal and hepatic)

glucose sensors (Niijima, 1989). In the event of hyperglycaemia, this facilitates efferent activity of the pancreatic and hepatic branches of the vagus nerve which in turn stimulates insulin secretion from the pancreas and glycogen synthesis in the liver. On the other hand, hypoglycaemia activates efferent activity of the pancreatic, hepatic and adrenal branches of the splanchnic nerve. This results in increased glucagon secretion from the pancreas and catecholamines from the adrenal medulla, all of which stimulate hepatic glycogen breakdown to glucose which is subsequently released into the blood.

1.2.4.3.2. Actions of Insulin on Amino Acids and Glucose Uptake

Among the major functions of insulin are promotion of amino acid uptake by tissues and regulation of blood glucose levels. The mechanisms to account for the insulin-enhancing effect on net amino acid uptake by muscle include, stimulation of amino acid transport, a depression of protein degradation, a slowing in amino acid oxidation, and a direct effect on protein synthesis (Ahmed et al., 1983). Insulin acts synergistically with GH to favour the uptake of glucose and amino acids by non-hepatic organs for synthesis of new tissue (Beaver et al., 1989).

1.2.4.4. Thyroid Hormones

Triidothyronine (T_3) and thyroxine (T_4) are synthesized in the thyroid gland from which their release involves the pituitary glycoprotein, thyroid stimulating hormone (TSH) (Di Liegro et al., 1987). Thyroid stimulating hormone is in turn positively regulated by the hypothalamic TSH-releasing hormone (TRH). Both TSH and TRH are under a feedback

control exerted by the thyroid hormones (Menezes-Ferreira et al., 1986). Although T_4 is the main form of circulating thyroid hormones, T_3 is the more potent hormone (Frandson, 1986; Di Liegro et al., 1987; Beaver et al., 1989). It is, in fact, postulated that T_4 may only be a prohormone or blood source of more T_3. Once in the cells, T_3 and T_4 facilitate cellular metabolism, whereupon they are converted to disposable metabolites. This takes place mainly by deiodination, as occurs in skeletal muscle and liver, but also by deamination and by sulfate conjugation, as occurs in the liver (Frandson, 1986). About 85% of T_4 is deiodinated in the target tissues at either the 5 or 5' position to give reverse T_3 (rT_3) or T_3, respectively (Oppenheimer, 1979). These conversions are catalyzed by different enzymes, namely 5-deiodinase (5-D) and two forms of 5'-deiodinase (5'-D). The ester products are mostly excreted via the bile into the intestine, where they are degraded, and much of the iodine is reabsorbed into the blood circulation.

Thyroid hormones circulate complexed with protein carriers that transport them to their sites of action. Two main carriers for the thyroid hormones have been identified (Di Liegro et al., 1987). These are thyroxine-binding globulin (TBG) and thyroxine-binding prealbumin (TBPA). Thyroxine-binding globulin has the highest affinity for the hormones, especially T_4.

1.2.4.4.1. Thyroid Hormone Actions and Metabolic Effects

A large volume of evidence suggests that hormone entry into target cells is mediated by interaction with specific binding proteins localized on the plasma membrane (Krenning et al., 1978; Alderson et al., 1985). It has been shown that plasma-membrane binding sites are most probably involved not only in hormone internalization but also triggering early hormonal

effects such as increased uptake of amino acids, nucleosides and glucose (Goldfine et al., 1975; Segal and Gordon, 1977); stimulation of cooperative behaviour of the two membrane-bound enzymes acetylcholinesterase and (Na^+,K^+)-ATPase (de Mendoza et al., 1977), and finally stimulation of (Ca^{2+},Mg^{2+})-ATPase (Golo et al., 1981).

Other thyroid hormone binding sites are present in the cytosol (Oppenheimer, 1979). Francon et al. (1985) have proposed that these cytosolic sites may play a regulatory role in the storage and supply of the hormone to other cellular compartments. Although binding sites for thyroid hormones have been identified on cell membranes and cytosol, evidence demonstrates that thyroid hormones predominantly act through interaction with nuclear receptors (Kolodny et al., 1985; Oppenheimer and Samuels, 1983). These receptors, which have been demonstrated in many tissues, have been reported to exhibit higher affinity for T_3 than for T_4 (T_3 being bound about 10- to 20-fold more tightly than T_4). Thus apart from the early responses, which are probably induced in the target cells via plasma-membrane receptors, the great majority of thyroid-hormone-induced responses are likely to be mediated by the nuclear T_3-receptors and result in the stimulation or inhibition of the expression of specific classes of proteins. Thyroid hormones exert their effects mainly by directly regulating gene expression, on association with specific chromatin-bound receptors (Di Liegro et al., 1987).

Thyroid hormones influence cellular processes throughout the body (Frandson, 1986). These hormones are found in all tissues and cells of the body, corresponding to their ubiquitous effects. However, the major store of T_3 and T_4 outside of the thyroid gland and the blood, is in skeletal muscle, probably because of the large mass of body muscle (Frandson,

1986). Metabolically, thyroid hormones increase absorption of glucose and its utilization by cells. Other effects include increased rates of lipolysis (Symonds et al., 1989), increased glycogenolysis, and increased synthesis of proteins in all cells, along with greater activity of nuclear and ribosomal RNA. The calorigenic (heat-producing) activity of thyroid hormones accounts for about one-half of the basal metabolic rate of the normal animal. This is due to the fact that these hormones increase the rate of oxygen consumption in all cellular metabolism and stimulate cytoplasmic protein synthesis (Frandson, 1986). However, too much thyroid hormone may result in impaired growth (Rosebrough et al., 1992).

1.2.5. Energy Expenditure and its Relation to Growth

Energy expenditure is basically measured as metabolic rate or heat production. Metabolic rate can be measured in various states of the animal, and terms have been adopted to precisely describe the conditions under which the measurements were taken. These are 1) basal metabolic rate (BMR), which is defined as heat production of an animal that is in a state of rest, postabsorptive, within a thermoneutral environment, in the absence of disease or stress, and following a prolonged period of feeding at maintenance levels (Kleiber, 1975); it represents the minimal energy expenditure compatible with life; 2) fasting metabolic rate (FMR), which refers to heat production in a fasted animal, and 3) resting metabolic rate (RMR), which provides a measure of energy expenditure of non-fasted animals. Resting metabolic rate merely requires that animals be at rest in a defined thermal environment. Metabolic rate is usually expressed on the basis of metabolically active mass ($BW^{.75}$), an

empirical base derived from comparison of many species (Blaxter and Boyne, 1982; Hudson and Christopherson, 1985).

1.2.5.1. Components of Metabolic Rate

Basal energy expenditure by an animal has been categorized into costs for service functions essential to the whole animal and costs to support the existence of a particular cell or tissue (Baldwin et al., 1980). Since basal metabolism accounts for a large portion of animal energy expenditure, Baldwin et al. (1980) and Summers et al. (1988) have suggested that improvement in animal efficiency may be achieved through the reduction of energy expenditure to support basal metabolism. Energy-using processes include turnover of macromolecules (e.g., proteins, nucleic acids, and lipids); transport of ions, (e.g., Ca^{2+}, K^{+}, Na^{+}), amino acids, and other molecules across membranes; endocytosis and exocytosis.

1.2.5.1.1. Turnover of Macromolecules

Energy expenditure incurred in protein turnover is normally emphasized in terms of ATP (or equivalent) expenditure in the synthetic process (Summers et al., 1988). Estimates from some studies (MacRae and Lobley, 1986; Reeds et al., 1987) suggest that about 15-25% of body energy expenditure supports protein synthesis. But although protein synthesis has been given more emphasis in energetic costs, Summers et al. (1988) noted that protein breakdown (proteolysis) also exerts a direct demand for energy, independent of the cost for synthesis. Proteolysis involves the breakdown of structurally normal proteins including enzymes, and rapid degradation of newly synthesized aberrant proteins. Each of these processes exerts a

demand for metabolic energy. Phospholipids also undergo rapid energy-requiring synthesis and breakdown, and the energy going into these processes may be of quantitative significance (Summers et al., 1988).

1.2.5.1.2. Transport Processes

There are a number of specific processes through which macromolecules and most peptide hormones get transported. These processes are normally linked to nucleotide 5'-triphosphatase (NTPase) and by the ubiquitous cell functions of endocytosis, exocytosis, and secretion (Summers et al., 1988). Pearse and Bretscher (1981) observed that the uptake of most peptide hormones and macromolecules occurs by way of endocytic adsorption of clathrin coated vesicles. Also endocytosis and exocytosis have been implicated in the fast, reversible hormone induced modulation of substrate movement by insertion of pre-existing cytoplasmic transporters into the plasma membrane or their removal therefrom. Proton pumps appear to be very important in endocytic and secretory pathways (Van Dyke et al., 1985), and their presence has been demonstrated in coated vesicles, endosomes, lysosomes, Golgi apparatus, endoplasmic reticulum, and vesicular bodies. While the total expenditure of ATP to support the endocytic, exocytic and secretory pathways is not known, Summers et al. (1988) noted that because of the involvement of several energy-linked processes, including H^+ pumping, this expenditure could be quantitatively significant.

The other transport process involves active transport of ions, particularly Na^+, K^+, and Ca^{2+}. This accounts for a significant portion of basal energy expenditure (Milligan and Summers, 1986). Several workers maintain that energy expenditure associated with Na^+, K^+

transport could be perceived as the metabolic cost of maintaining ionic homeostasis, membrane potentials, or the ionic gradient necessary to sustain Na^+ flux-dependent nutrient uptake. McBride and Milligan (1985) stated that although the majority of estimates for the cost of Na^+, K^+ transport have been conducted with isolated tissues, similar estimates would be expected in vivo provided that a high viability is maintained in the isolated tissue or cell preparation. So far, results from several studies have convincingly shown that elevated Na^+, K^+ ATPase activity within various tissues is highly correlated with the productive capacity of an animal. Vandenburgh (1984) concluded that activation of Na^+, K^+ ATPase appears to be an important event in both hypertrophic and hyperplastic growth.

Apart from the support functions discussed thus far, there are energy costs incurred for other service functions that any consideration of whole-animal maintenance must account for. These service functions are basically those that are considered to be essential to the integrated organism and include heart, kidney, nervous, and liver functions, and respiration, accounting for about 36 - 50 % of basal energy expenditure (Baldwin et al., 1980).

1.2.5.2. Contribution of Visceral Organs to Energy Expenditure

Organs perform service functions that are essential to the integrated organism, and the energetic cost of these organs is substantial. Considerable data support suggestions that animal energy expenditures are related to the mass of visceral organs (Ferrell, 1988). Ferrell et al. (1986) in a feeding experiment with lambs observed that when comparisons were made among groups of similar weights, those lambs that had been fed to gain more rapidly had greater weights or proportions of liver, kidney, large intestine, small intestine, and stomach

than those that were fed to gain less rapidly. Also data with cattle (Murray et al., 1977) and pigs (Koong and Nienaber, 1987) support observations that weights of visceral organs, particularly weights of the liver and the GIT, vary in response to nutritional treatment. These two organs constitute a major proportion of total animal energy expenditure. According to Ferrell (1988), conservative estimates indicate that these tissues each account for 20-25% (or 40-50% if combined) of total animal energy expenditure, even though they constitute less than 10% of body mass. He concluded that the substantial contribution of liver and GIT tissues to whole-body energy expenditure is, partly related to the very high protein synthetic activity in these tissues.

Summers et al. (1988) summarized some major metabolic events resulting in high energy expenditures of the liver as: 1) macromolecule turnover; 2) transport processes of macromolecules and ions; 3) substrate recycling (glucose-glycogen, lactate-glucose); 4) detoxification; and 5) gluconeogenesis. As far as the GIT is concerned, the energy expenditure is through its regulation of exogenous nutrient supply through 1) its absorptive function; 2) its own metabolic processes; and 3) secretion of a variety of hormones that affect the function of visceral and peripheral tissues.

1.2.6. Growth and Development of the Mammary Gland

1.2.6.1. Early Development

The development of the mammary gland begins very early in life - as early as the embryonic stage (McGrath, 1987; Sheffield, 1988). According to Anderson (1978), the

mammary epithelial cells, quite early in the developmental process, proliferate into relatively undifferentiated embryonic mesenchyme. In many species such as cattle and rats, the mesenchyme differentiates into a well-defined fat pad, consisting mainly of adipose cells. Other components associated with the stroma include fibroblasts, blood vessels and nerve cells. The earliest signs of definitive fat pad formation are about 80 d of gestation in cows.

1.2.6.2. Postnatal Growth and Development

The development of the mammary gland consists of a series of very highly ordered events involving interactions among a number of distinct cell types (Sheffield, 1988; Akers, 1990). Of major importance in mammary gland development is the concept that the mammary gland consists of a fat pad of mesodermal origin into which epithelial cells of ectodermal origin proliferate (Tucker, 1987; Sheffield, 1988). Thus the organization of the cells is such that the epithelial cells together with the ductular cells, form the parenchyma. These are embraced in the stroma, a heterogenous matrix of other cell types including myoepithelial cells, adipocytes, fibroblasts and smooth muscle cells (Tucker, 1987).

The ducts elongate into the stromal portion as puberty approaches, and meanwhile there is an accompanying increase in the stroma (Reece, 1958). The exact role of fibroblasts and adipocytes in the mammary gland is not well defined, but data from co-culture experiments (Akers, 1990) suggest that the presence of fibroblasts enhance estrogen-dependent increases in epithelial cell progesterone receptors via the fibroblast production of a collagen substratum. Akers (1990) also noted that the presence of metabolically active fibroblasts in close contact with the epithelium is required for estrogen induction of epithelial proliferation. Regarding

the adipocytes, Levine and Stockdale (1984) reported that growth of isolated mammary gland cells was stimulated if cultured on the feeder layer of adipocytes. Thus, these cells may play an important role in the stimulation of other mammary cells in vivo.

1.2.6.3. Isometric and Allometric Growth of the Mammary Gland

In dairy cattle, Anderson (1978) and Sheffield (1988) reported that mammogenesis is isometric in utero and up to 3 mo of age, while growth from then on to puberty and during pregnancy is allometric. On the other hand, Tucker (1987) reported that mammogenesis is isometric until just before puberty when the mammary gland begins to grow at a relatively faster rate than whole body. It is probably not very clear when exactly this accelerated growth of the mammary gland commences. But certainly what is clear is that it begins before puberty. The allometric growth pattern is maintained for several estrous cycles and then returns to an isometric pattern until conception (Tucker, 1987).

The allometric growth of the mammary gland returns at conception, when the true alveoli are formed (Tuker, 1987). At this time, the mammary ducts elongate further and alveoli form and begin to replace the lipid portion of the mammary fat pad (Tucker, 1969). It is actually the size of the fat pad that limits parenchymal growth (Hoshino, 1964). It is therefore of major importance to feed the animal such that promotion of the fat pad will be limited, and instead promote parenchymal growth. Under normal conditions, the parenchymal cell numbers continue to proliferate throughout pregnancy while there is a compression of the connective tissue stroma. Depending upon species, between 49% and 94% of the total mammary growth occurs during gestation (Tucker, 1969; Kight and Peaker, 1982). The

proliferation of cells continues during lactation, particularly during the early stages (Tucker, 1987).

Eventually, the mammary cell numbers start declining as lactation progresses, with a decline in milk yield. Finally the cessation of suckling or milking leads to a rapid loss of mammary epithelial cells (Lascelles and Lee, 1978), with lysosomal enzymes being implicated in the destruction of the epithelial cells (Helminen et al., 1968). The myoepithelial cells remain in the gland during involution and maintain the structure of the remaining epithelial cells. In the process, the adipocytes start regaining lipid and, in fact, the animals subjected to long dry periods have mammary glands that resemble those of virgin heifers (Tucker, 1987).

1.2.6.4. Effect of Nutrition on Mammary Development

Plane of nutrition has significant effects on the development of the mammary gland in dairy and beef heifers. Feeding heifers a high plane of nutrition is associated with poor milk yields (Harrison et al., 1983; Stelwagen and Grieve, 1990). It has generally been found that a high plane of nutrition promotes fat deposition rather than the development of parenchymal tissue in the mammary gland. Petitclerc et al. (1984) demonstrated that animals reared at daily gains of .78 kg/d or less had better mammary gland growth during prepubertal stage than their contemporaries reared at daily gains of 1.0 kg/d or greater.

Stelwagen and Grieve (1990) have investigated the effects of three planes of nutrition in dairy heifers: low (.6 kg/d), medium (.75 kg/d), and high (.9 kg/d). Chemical analysis of the dissected mammary glands from the three groups of heifers revealed that the increase in

mammary DNA per unit increase in body weight was greatest for heifers on the low plane of nutrition. Since DNA is used to estimate mammary cell numbers, its decline with increasing feed intake suggested a potential adverse effect of feeding on mammogenesis. Although the prepubertal period during which mammogenesis may be adversely affected by plane of nutrition has not been clearly defined (Stelwagen and Grieve, 1990), data support the hypothesis that bovine mammogenesis is sensitive to high planes of nutrition during a critical period of rearing; this period is when the gland is undergoing a phase of rapid allometric growth.

Having established the general concepts about growth of tissues and the mammary gland, and how nutrition affects these processes, it is then possible to briefly review some physiological events associated with compensatory growth, and what effects compensatory growth may have on body composition and meat quality as measured by tissue glycogen concentration.

1.2.7. Physiological Changes in Compensatory Growth

The events that occur during compensatory growth are all manifestations of the animal's homeostatic mechanisms attempting to return to its genetically programmed path (Hogg, 1991). There are certainly complex physiological changes that take place during growth check. The return of favourable conditions must, therefore, result in well organized mechanisms that must lead to higher body weight gains than normal in order to put the animal

back into its growth channel. As Van den Brande (1986) stated, these physiological events must be occuring at all levels including whole animal, organ, tissue, and cell. Not much work has been done, particularly in ruminants, to establish the underlying mechanisms of compensatory growth. However, studies done in rats (Mosier et al., 1983, 1985a,b) indicate that the endocrine system and other physiological factors can be implicated in compensatory growth.

1.2.7.1. Hormonal Control of Compensatory Growth

To a large extent, hormonal factors have been implicated in compensatory growth, at least in rats (Sinha et al., 1973; Mosier et al., 1985a,b). Mosier et al. (1985a) observed increased levels of GH during feed restriction of rats and the levels remained elevated during the refeeding period. Increased plasma levels of GH during feed restriction have also been observed in pigs (Buonomo and Baile, 1991), sheep (Driver and Forbes, 1981) and cattle (Blum et al., 1985; Breier et al., 1986). While Mosier et al. (1985a) observed continued elevated plasma GH levels during compensatory growth in rats, Driver and Forbes (1981) and Blum et al. (1985) observed decreased levels in sheep and cattle, respectively. Thus GH seems to play an important role in compensatory growth of rats. The picture is, however, less clear in ruminants. Since the GH-IGF-I axis is functionally closely associated with insulin and thyroid hormones, it may be assumed that an interaction among these hormones, in some special way, during realimentation may play a role in compensatory growth.

But although GH and IGF-I seem to be the principal hormones in the mechanism of compensatory growth in rats, Van den Brande (1986) proposed that there is a possibility of

another mechanism. The author, in this context, suggested the possibility of an involvement of local stimulatory factors such as cartilage-derived factors, as well as a diminished effect of local inhibitors: the chalones. Chalones are tissue-specific substances secreted by tissues in quantities related to the mass of the tissue and having a negative effect on the growth of that particular tissue (Bullough, 1975), thus providing each tissue with a feed back mechanism regulating its own mass (Van den Brande, 1986). Such substances may play a vital role in keeping the organs on the predetermined track or bringing them back to it after deviation.

1.2.7.2. Neurological Control of Compensatory Growth

Data in the rat model suggest that compensatory growth is governed by the possible existence of a set-point for body size appropriate for age in the central nervous system (CNS) (Mosier and Jansons, 1970; Mosier et al., 1983). Mosier (1986) suggested the dorsomedial nuclei as the possible site harbouring the set-point, although this is still to be confirmed. Mosier et al. (1983) have demonstrated the existence of such a set-point in male and female rats through x-irradiation of the heads of 2-d old neonates followed by a 48-h fasting at age 39 d, and then followed by realimentation. From the results of these studies, Mosier (1986) has proposed an attractive hypothetical model of the CNS control of compensatory growth. In this model, the speculation is that the growth deficit of an individual is detected by a sensor which then relates the perceived body size to the set-point for body size appropriate for age. The set-point signals a stimulator to send out a stimulus to the hypothalamic system, resulting in the release of GRF while inhibiting the release of somatostatin. Growth hormone releasing factor then stimulates the pituitary gland to secrete GH which then carries out its actions of

growth in various tissues. The stimulator can also send the stimulus directly to stimulate production of cartilage-derived growth factors, and possibly to lower the production of chalones in other tissues. An integration of all these events is what is assumed to lead to accelerated growth during refeeding of a previously undernourished individual. So far, no attempt has been made to do any such intensive study in farm livestock.

1.2.8. Body Composition, Muscle Glycogen Stores, and Mammary Development in Compensatory Growth

As stated earlier, in studies of compensatory growth in cattle, more attention has been paid to castrated males destined for meat production. However, results have been variable; carcasses from steers which have undergone compensatory growth have been shown to be leaner (Smith et al., 1977; Mader et al., 1989), not different (Rompala et al., 1985), or fatter (Abdalla et al., 1988) than conventionally fed steers slaughtered at equal live weight. These results are, nevertheless, not surprising considering that the experiments have been conducted under different conditions, different designs, and using animals of differing age. The basic problem is the lack of understanding of physiological mechanisms in association with environmental factors to fully explain compensatory growth.

Having looked at body composition, it is worthwhile to consider any significant effects on muscle glycogen content since the pH of meat is dependent on this substrate (Henckel et al., 1992). In other words, can feed restriction followed by realimentation have major effects on some meat quality aspect associated with muscle glycogen stores? Muscle glycogen

content can be estimated by glucidic potential (GP), a measure of glycogen concentration and its metabolites. Since the amount of glucose (and subsequently glycogen) is quite limited in ruminants (Baird, 1981), any treatment resulting in the depletion of glycogen stores in muscle could lead to dark, firm, and dry (DFD) meat. Some factors known to deplete muscle glycogen stores include stress and muscular activity (Fernandez et al., 1992), but data on GP in beef cattle, particularly regarding nutritional effects, are limited.

In terms of mammary growth and development, there is no information available specifically about beef heifers undergoing compensatory growth. However, there is some information about dairy cattle (Park et al., 1989) and rats (Park et al., 1988). In these studies, it was observed that the compensating animals had more developed mammary tissues, and produced more milk during lactation than the conventionally fed animals. This positive effect of compensatory growth is, undoubtedly, worth investigating in beef cattle because it could be used as a tool to improve productivity in cow-calf operations.

With this background on the physiology of growth and compensatory growth, and some effects of the latter on body composition, glucidic potential, and mammogenesis, a series of studies was designed to investigate the following general areas in Hereford crossbred heifers undergoing compensatory growth:

1. Some important physiological and metabolic events.
2. Regrowth patterns of tissues and visceral organs following realimentation, and ultimate body composition.
3. Effects on muscle and hepatic glycogen stores.
4. Effects on mammary growth and development.

Although different heifers were used in study 1 (chapter 2) from the other studies, the major aim was to correlate the physiological mechanisms with the ultimate effects of compensatory growth on various components of the body. The experiments were, therefore, designed to be similar.

1.3. Literature Cited

Abdalla, H. O., D. G. Fox, and M. L. Thonney. 1988. Compensatory gain by Holtein calves after underfeeding protein. J. Anim. Sci. 66:2687.

Abe, H., M. E. Moilitch, J. J. Van Wyk, and L. E. Underwood. 1983. Human growth hormone and somatomedin C suppress the spontaneous release of growth hormone in unanesthetized rats. Endocrinology 113:1319.

Affara, N., P. Baubas, and A. Weydert. 1980. Changes in gene expression during myogenic differentiation. II. Identification of the proteins encoded by myotube-specific complementary DNA sequences. J. Mol. Biol. 140:459.

Ahmed, B. M., W. G. Bergen, and N. K. Ames. 1983. Effect of nutritional state and insulin on hind-limb amino acid metabolism in steers. J. Nutr. 113:1529.

Akers, R. M. 1990. Lactation physiology: A ruminant animal perspective. Protoplasma 159:96.

Alderson, R., I. Pastan, and S. Y. Cheng. 1985. Characterization of the 3,3'5-triiodothyronine-binding site on plasma membranes from human placenta. Endocrinology 116:2621.

Anderson, R. R. 1978. Embryonic and fetal development of the mammary apparatus. In: B. L. Larson (Ed.) Lactation: A comprehensive treatise; Vol. 4. Academic Press, New York, N.Y.

Anfinson, M. S., S. L. Davis, E. Christian, and D. O. Everson. 1975. Episodic secretion of growth hormone in steers and bulls. An analysis of frequency and magnitude of secretory

spikes occuring in a 24 hour period. Proc. West Sect. Am. Soc. Anim. Sci. 26:175.

Anthony, R. V., R. A. Bellows, R. E. Short, R. B. Staigmiller, C. C. Kaltenbach, and T. G. Dunn. 1986. Fetal growth of beef calves. I. Effect of prepartum dietary crude protein on birth weight, blood metabolites and steroid hormone concentrations. J. Anim. Sci. 62:1363.

Armentano, L. E. and J. W. Young 1983. Production and metabolism of volatile fatty acids, glucose and CO_2 in steers and the effects of monensin on volatile fatty acid kinetics. J. Nutr. 113:1265.

Asghar, A., A. M. Pearson, W. T. Magee, and M. A. Tahir. 1981. Effects of ad libitum, maintenance, and sub-maintenance feeding and of compensatory growth on some biochemical properties of muscle from weanling rabbits. J. Nutr. 111:1343.

Ashworth, A. and D. J. Millward. 1986. Catch-up growth in children. Nutr. Rev. 44:157.

Baird, G. D. 1981. Metabolic modes indicative of carbohydrate status in the dairy cow. Fed. Proc. 40:2530.

Baldwin, R. L., N. E. Smith, J. Taylor, and M. Sharp. 1980. Manipulating metabolic parameters to improve growth rate and milk secretion. J. Anim. Sci. 51:1416.

Baumann, G., K. Amburn, and M. A. Shaw. 1988. The circulating growth hormone (GH)-binding protein complex: A major constituent of plasma GH in man. Endocrinology 122:976.

Beaver, E. E., J. E. Williams, S. J. Miller, D. L. Hancock, S. M. Hannah, and D. L. O'Connor. 1989. Influence of breed and diet on growth, nutrient digestibility, body composition and plasma hormones of Brangus and Angus steers. J. Anim. Sci. 67:2415.

Berelowitz, M., M. Szabo, L. A. Frohman, S. Firestone, and L. Chu. 1981. Somatomedin-C mediates growth hormone negative feedback by effects on both the hypothalamus and the pituitary. Science 212:1279.

Berg, R. T., and R. M. Butterfield. 1976. New Concepts of cattle growth. Sydney University Press.

Bertrand, H. A., E. J. Masoro, and B. P. Yu. 1978. Increasing adipocyte number as the basis for perirenal depot growth in adult rats. Science 201:1234

Black, J. L. 1988. Animal growth and its regulation. J. Anim. Sci. 66(Suppl. 3):1.

Blaxter, K. L., and A. W. Boyne. 1982. Fasting and maintenance metabolism of sheep. J. Agric. Sci., Camb. 99:611.

Blum, J. W., W. Schnyder, P. L. Kunz, A. K. Blom, H. Bickel, and A. Schurch. 1985. Reduced and compensatory growth: Endocrine and metabolic changes during food restriction and refeeding in steers. J. Nutr. 115:417.

Borer, K. T. 1987. Hormonal and nutritional determinants of catch-up growth in hamsters. Growth 51:103.

Breier, B. H., J. J. Bass, J. H. Butler, and P. D. Gluckman. 1986. The somatotrophic axis in young steers: Influence of nutritional status on pulsatile release of growth hormone and circulating concentrations of insulin-like growth factor 1. J. Endocrinol. 111:209.

Breier, B. H., P. D. Gluckman, and J. J. Bass. 1988. The somatotrophic axis in young steers: Influence of nutritional status and oestradiol-17ß on hepatic high- and low-affinity somatotrophic binding sites. J. Endocrinol. 116:169.

Brockman, R. P., and B. Laarveld. 1986. Hormonal regulation of metabolism in ruminants:

A review. Livest. Prod. Sci. 14:313.

Bruno, J. F., J. Song, and M. Berelowitz. 1991. Regulation of rat hypothalamic preprogrowth hormone-releasing factor messenger ribonucleic acid by dietary protein. Endocrinology 129:1226.

Bullough, W. S. 1975. Chalone control mechanisms. Life Sci. 16:323.

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

Burleigh, I. G. 1980. Growth curves in muscle nucleic acid and protein: Problems of interpretation at the level of the muscle cell. In: T. L. J. Lawrence (Ed.) Growth in animals. Butterworths, London.

Carstens, G. E., D. E. Johnson, M. A. Ellenberger, and J. D. Tatum. 1991. Physical and chemical components of the empty body during compensatory growth in beef steers. J. Anim. Sci. 69:3251.

Coyle, E. F. 1992. Carbohydrate supplementation during exercise. J. Nutr. 122:788.

Davis, S. L. 1988. Recent concepts in regulation of growth by GH and IGF. J. Anim. Sci. 66(Suppl. 3):84.

Davis, S. L., M. Graf, C. A. Morrison, T. R. Hall, and P. J. Swift. 1992. Identification and partial purification of serum growth hormone binding protein in domestic animal species. J. Anim. Sci. 70:773.

de Mendoza, D., H. Moreno, E. M. Massa, M. D. Morero, and R. N. Farias. 1977. Thyroid hormone action and membrane fluidity: Blocking action of thyroxine on triiodothyronine effect. FEBS Lett. 84:199.

Delgado, H. L., R. Sibrian, and M. E. Delgado. 1987. Evidence for catch-up growth in adolescence. Lancet 1:1021.

Devlin, R. B., and C. P. Emerson, 1979. Coordinate accumulation of contractile protein mRNAs during myoblast differention. Dev. Biol. 69:202.

Di Liegro, I., G. Savettieri and A. Cestelli. 1987. Cellular mechanism of action of thyroid hormones. Differentiation 35:165.

DiMarco, O. N., R. L. Baldwin, and C. C. Calvert. 1987. Relative contribution of hyperplasia and hypertrophy to growth in cattle. J. Anim. Sci. 65:150.

Driver, P. M., and J. M. Forbes. 1981. Episodic growth hormone secretion in sheep in relation to time of feeding, spontaneous meals and short term fasting. J. Physiol. 317:413.

Edwards, A. V., and M. Silver. 1970. The glycogenolytic response to stimulation of the splanchnic nerves in adrenalectomized calves. J. Physiol. 211:109.

Eisemann, J. H., A. C. Hammond, and T. S. Rumsey. 1989. Tissue protein synthesis and nucleic acid concentrations in steers treated with somatotropin. Br. J. Nutr. 62:657.

Ellenberger, M. A., D. E. Johnson, G. E. Carstens, K. L. Hossner, M. D. Holland, T. M. Nett, and C. F. Nockels. 1989. Endocrine and metabolic changes during altered growth rates in beef cattle. J. Anim. Sci. 67:1446.

Emmans, G. C. 1994. Effective energy: A concept of energy utulization applied across species. Br. J. Nutr. 71:801.

Enser, M., and J. D. Wood. 1978. The development of adipose tissue in cattle. In: H. De Boer and J. Martin (Eds.) Patterns of growth and development in cattle. Martinus Nijhoff, The Hague.

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

Fernandez, X., M. Mågård, and E. Tornberg. 1992. The variation in pig muscle glycolytic potential during lairage: An in vivo study. Meat Sci. 32:81.

Ferrell, C. L., L. J. Koong, and J. A. Nienaber. 1986. Effect of previous nutrition on body composition and maintenance energy costs of growing lambs. Br. J. Nutr. 56:595.

Ferrell, C. L. 1988. Contribution of visceral organs to animal energy expenditures. J. Anim. Sci. 66(Suppl. 3):23.

Foster, A. H., and G. W. Blight. 1987. Change in metabolite levels and liveweight of grazing cattle when supplemented with Leucaena leucocephala or urea-molasses. Queensl. J. Agric. Anim. Sci. 44:59.

Francon, J., J. Osty, F. Chantoux, and A. M. Lennon. 1985. Cellular location of cystolic triiodothyronine binding protein in primary cultures of fetal rat brain. Mol. Cell Endocrinol. 39:197.

Frandson, R. D. 1986. In: Anatomy and Physiology of farm animals. 4th Edition. Lea & Febiger, Philadelphia.

Frohman, L. A. 1991. Endocrine and neuroendocrine regulation of cell function and growth. J. Anim. Sci. 69(Suppl. 3):74.

Georgieff, M. K., M. M. Mills, C. E. Zempel, and P. Chang. 1989. Catch-up growth, muscle and fat accretion, and body proportionality of infants one year after newborn intensive care. J. Pediatr. 114:288.

Gluckman, P. D., B. H. Breier, and S. R. Davis. 1987. Physiology of the somatotropic axis with particular reference to the ruminant. J. Dairy Sci. 70:442.

Godfredson, J. A., M. D. Holland, K. G. Odde, and K. L. Hossner. 1991. Hypertrophy and hyperplasia of bovine fetal tissues during development: Fetal liver insulin-like growth factor I mRNA expression. J. Anim. Sci. 69:1074.

Goldfine, I. D., G. G. Simons, G. H. Smith, and S. H. Ingbar. 1975. Cycloleucine transport in isolated rat thymocytes: In vitro effects of triiodothyronine and thyroxine. Endocrinology 96:1030.

Goldspink, G. 1970. The proliferation of myofibrils during muscle fiber growth. J. Cell Sci. 6:593

Goldspink, G. 1971. Changes in striated muscle fibers during contraction and growth with particular reference to myofibril splitting. J. Cell Sci. 9:123

Golo, M. G., L. E. Unates, and R. N. Farias. 1981. Effect of membrane fatty acid composition on the action of thyroid hormones on (Ca^{2+}-Mg^{2+})-adenosine triphosphatase from the erythrocyte. J. Biol. Chem. 256:7113.

Grant, A. L., and W. G. Helferich. 1991. An overview of growth. In: A. M. Pearson and T. R. Dutson (Eds.) Growth regulation in farm animals, Vol. 7. Elsevier Applied Science, London.

Griffin, G., P. E. Williams, and G. Goldspink. 1971. Region of longitudinal growth in striated muscle fibers. Nature New Biol. 232:28.

Harrison, R. D., I. P. Reynold, and W. Little. 1983. A quantitative analysis of mammary glands of dairy heifers reared at different rates of live weight gain. J. Dairy Res. 50:405.

Helminen, H. J., J. L. E. Ericsson, and S. Orrenius. 1968. Studies on mammary gland involution. IV. Histochemical and biochemical observations on alterations in lysosomes and lysosomal enzymes. J. Ultrastruct. Res. 25:240.

Henckel, P., P. F. Jorgensen, and P. Jensen. 1992. Glycogen content, buffering capacity and resting pH in live muscles of pigs of different halothane genotypes (a pilot project). Meat Sci. 32:131.

Hodgkinson, S. C., S. R. Davis, B. D. Bureleigh, H. V. Henderson, and P. D. Gluckman. 1987. Metabolic clearance of protein bound and free insulin-like growth factor-I in fed and starved sheep. J. Endocrinol. 115:233.

Hogg, B. W., 1991. Compensatory growth in ruminants. In: A. M. Pearson and T. R. Dutson (Eds.) Growth Regulation in Farm Animals. Vol. 7. Elsevier Applied Science, London.

Hoshino, K. 1964. Regeneration and growth of quantitatively transplanted mammary glands of normal female mice. Anat. Record. 150:221.

Hudson, R. J., and R. J. Christopherson. 1985. Maintenance and Metabolism. In: R. J. Hudson and R. G. White (Eds.) Bioenergetics of wild herbivores. CRC Press, Inc. Boca Raton, Florida.

Huntington, G. B., R. L. Prior, and R. A. Britton. 1981. Glucose and lactate absorption and metabolic interrelationships in steers changed from low to high concentrate diets. J. Nutr. 111:1164.

Jesse, B. W., R. S. Emery, and J. W. Thomas. 1986. Control of bovine hepatic fatty acid oxidation. J. Dairy Sci. 69:2290.

Johnson, P. R., J. S. Stern, M. R. C. Greenwood, and J. Hirsch. 1978. Adipose tissue hyperplasia and hyperinsulinemia in Zucker obese female rats. A developmental study. Metabolism 27 (Suppl. 2):1941.

Karniski, W., C. Blair, and J. S. Vitucci. 1987. The illusion of catch-up growth in premature infants. AJDC 141:520.

King, G. L., M. M. Rechler, and C. R. Kahn. 1982. Interactions between the receptors for insulin and the insulin-like growth factors on adipocytes. J. Biol. Chem. 257:10001.

Kirtland, J., and P. M. Harris 1980. Changes in adipose tissue of the rat due to early undernutrition followed by rehabilitation. 3. Changes in cell replication studied with tritiated thymidine. Br. J. Nutr. 43:33.

Kleiber, M. 1975. In: The fire of life: An introduction to animal enegertics. Robert E. Krieger Publ. Co., Huntington, N.Y.

Knight, C. H., and M. Peaker. 1982. Development of the mammary gland. J. Reprod. Fertil. 65:521.

Kolodny, J. M., P. R. Larsen, and J. E. Silva. 1985. In vitro 3,5,3'-triiodothyronine binding to rat cerebrocortical neuronal and glial nuclei suggests the presence of binding sites unavailable in vivo. Endocrinology 116:2019.

Koong, L. J., and J. A. Nienaber. 1987. Changes of fasting heat production and organ size of pigs during prolonged weight maintenance. In: P. W. Moe, H. F. Tyrrell, and P. J. Reynolds (Eds.) Energy metabolism of farm animals. EAAP Publ. No. 32. Rowman and Littlefield, Totowa, N.J.

Krenning, E. P., R. Docter, H. F. Bernard, T. J. Visser, and G. Hennemann. 1978. Active transport of triiodothyronine (T3) into isolated liver cells. FEBS Lett. 91:113.

Lascelles, A. K., and C. S. Lee. 1978. Involution of the mammary gland. In: B. L. Larson (Ed.) Lactation: A comprehensive treatise, Vol. 4. Academic Press. New York, N.Y.

Leat, W. F. M., and R. W. Cox. 1980. Fundamental aspects of adipose tissue. In: T. L. J. Lawrence (Ed.) Growth in animals. Butterworths, London.

Leffert, H. L., K. S. Koch, T. Moran, and B. Rubalcava. 1979. Hormonal control of rat liver regeneration. Gastroenterology 76:1470.

Leung, D. W., S. A. Spencer, G. Cachianes, R. G. Hammonds, C. Collins, W. J. Henzel, R. Barnard, M. J. Waters, and W. I. Wood. 1987. Growth hormone receptor and serum binding protein: Purification, cloning and expression. Nature 330:537.

Levine, J. F., and F. E. Stockdale. 1984. 3T3-L1 adipocytes promote the growth of mammary epithelium. Exp. Cell Res. 151:112.

Lucy, M. C., S. D. Hauser, P. J. Eppard, G. G. Krivi, J. H. Clark, D. E. Bauman, and R. J. Collier. 1993. Variants of somatotropin in cattle: Gene frequencies in major dairy breeds and associated milk production. Domestic Anim. Endocrinol. 10:325.

MacRae, J. C., and G. E. Lobley. 1986. Interactions between energy and protein. In: L. P. Milligan, W. L. Grovum, and A. Dobson (Eds.) Control of digestion and metabolism in ruminants. Prentice-Hall, Englewood Cliffs, N.J.

Mader, T. L., O. A. Turgeon Jr., T. J. Klopfenstein, D. R. Brink, and R. R. Oltjen. 1989. Effects of previous nutrition, feedlot regimen and protein level on feedlot performance of beef cattle. J. Anim. Sci. 67:318.

Marple, D. N. 1983. Principles of growth and development. Proc. Growth Management Conference. pp1-6. Terre Haute, IN.

Mascarello, F., M. L. Stecchini, A. Rowlerson, and E. Ballochi. 1992. Tertiary myotubes in postnatal growing pig muscle detected by their myosin isoform composition. J. Anim. Sci. 70:1806.

McBride, B. W., and L. P. Milligan. 1985. Magnitude of ouabain-sensitive respiration of lamb hepatocytes. Int. J. Biochem. 17:43.

McCusker, R. H., W. S. Cohick, W. H. Busby, and D. R. Clemmons. 1991. Evaluation of the developmental and nutritional changes in porcine insulin-like growth factor-binding protein-1 and -2 serum levels by immunoassay. Endocrinology 129:2631.

McGrath, M. F. 1987. A novel system for mammary epithelial cell culture. J. Dairy Sci. 70:1967.

Menezes-Ferreira, M. M., P. A. Petrick, and B. D. Weintraub, 1986. Regulation of thyrotropin (TSH) bioactivity by TSH- releasing hormone and thyroid hormone. Endocrinology 118:2125.

Milligan, J. D., and M. Summers. 1986. The biological basis of maintenance and its relevance to assessing responses to nutrients. Proc. Nutr. Soc. 45:185.

Mosier, H. D., Jr. 1986. The control of catch-up growth. Acta Endocrinol. 113 (S279):1.

Mosier, H. D., Jr., C. B. Good, R. A. Jansons, C. A. Sondhaus, L. C. Dearden, M. Alpizar-S, and O. F. Zuniga. 1983. The effect of neonatal head-irradiation and subsequent fasting on the mechanisms of catch-up growth. Growth 47:13.

Mosier, H. D., Jr., and R. A. Jansons. 1970. Effect of x-irradiation of selected areas of the head of the newborn rat on growth. Radiat. Res. 43:92.

Mosier, H. D., Jr., and R. A. Jansons. 1976. Growth hormone during catch-up growth and failure of catch-up growth in rats. Endocrinology 98:214.

Mosier, H. D., Jr., R. A. Jansons, and L. C. Dearden. 1985a. Increased secretion of growth hormone in rats undergoing catch-up growth after fasting. Growth 49:346.

Mosier, H. D., Jr., R. A. Jansons, K. F. Swingle, C. A. Sondhaus, L. C. Dearden, and L. C. Halsall. 1985b. Growth hormone secretion in the stunted head-irradiated rat. Pediatr. Res. 19:543.

Moss, E. P., and C. P. Leblond. 1971. Satellite cells as the source of nuclei in muscles of growing rats. Anat. Rec. 170:421.

Murphy, L. J., G. I. Bell, M. L. Duckworth, and H. G. Friesen. 1987. Identification, characterization, and regulation of a rat complimentary deoxyribonucleic acid which encodes insulin-like growth factor-I. Endocrinology 121:684.

Murray, D. M., N. M. Tulloh, and W. H. Winter. 1977. The effect of three different growth rates on some offal components of cattle. J. Agric. Sci. (Camb.) 89:119.

Nanto-Salonen, K., G. G. Glasscock, and R. G. Rosenfeld. 1991. The effects of thyroid hormone on insulin-like growth factor (IGF) and IGF-binding protein (IGFBP) expression in the neonatal rat: Prolonged high expression of IGFBP-2 in metimazole-induced congenital hypothyroidism. Endocrinology 129:2563.

Niijima, A. 1989. Neural mechanisms in the control of blood glucose concentration. J. Nutr. 119:833.

Nilsson, A., C. Ohlsson, O. G. P. Isakkson, A. Lindahl, and J. Isgaard. 1994. Hormonal regulation of longitudinal bone growth. Eur. J. Clin. Nutr. 48 (Suppl. 1) S150.

Oppenheimer, J. H. 1979. Thyroid hormone action at the cellular level. Science 203:971.

Oppenheimer, J. H., and H. Samuels. 1983. In: Molecular biology of the thyroid hormone action. Academic Press, New York, N.Y.

Orskov, E. R. 1990. Energy metabolism of the host animal. In: Energy nutrition in ruminants. Elsevier Science Publishers, New York, N.Y.

Park, C. S., Y. J. Choi, W. L. Keller, and R. L. Harrold. 1988. Effects of compensatory growth on milk gene expression and mammary differentiation. FASEB J. 2:2619.

Park, C. S., M. G. Baik, W. Keller, I. E. Berg, and G. M. Erickson. 1989. Role of compensatory growth in lactation: A stair-step nutrient regimen modulates differentiation and lactation of bovine mammary gland. Growth, Dev. Aging-GDA. 53:159.

Pearse, B. M. F., and M. S. Bretscher. 1981. Membrane recycling by coated vesicles. Ann. Rev. Biochem. 50:85.

Petitclerc, D., L. T. Chapin, and H. A. Tucker. 1984. Carcass composition and mammary development responses to photoperiod and plane of nutrition in Holstein heifers. J. Anim. Sci. 58:913.

Pines, M., and S. Hurwitz. 1991. The role of the growth plate in logitudinal bone growth. Poult. Sci. 70:1806.

Porkniak, J. A., and S. M. Cornejo. 1982. Effects of energy and protein undernutrition on productive performance and carcass, liver and digestive tract composition of broiler males. Nutr. Rep. Int. 26:319.

Pond, W. G., and H. J. Mersmann. 1990. Differential compensatory growth in swine following control of feed intake by high-alfalfa diet fed ad libitum or by limited feed. J. Anim. Sci. 68:352.

Prader, A., J. M. Tanner, and G. A. Harnack. 1963. Catch-up growth follwing illness or starvation. J. Pediatr. 62:646.

Price, M. A., 1977. The effects of severe feed restriction on bulls and steers. II. Carcass composition. Aust. J. Agric. Res. 28:529.

Price, M. A., and R. G. White. 1985. Growth and development. In: R. J. Hudson and R. G. White (Eds.) Bioenergetics of wild herbivores. CRC Press, Inc. Boca Raton, Florida.

Rechler, M. M. 1988. Molecular insights into insulin-like growth factor biology. J. Anim. Sci. 66(Suppl. 3):76.

Reece, R. P. 1958. Mammary gland development and function. In: J. T. Velardo (Ed.) The endocrinology of reproduction. Oxford University Press, New York, N.Y.

Reeds, P. J., B. A. Nicholson, and M. F. Fuller. 1987. Contribution of protein synthesis to energy expenditure in vivo and in vitro. In: P. W. Moe, H. F. Tyrrell, and P. J. Reynolds (Eds.) Energy metabolism of farm animals. EAAP Publ. No. 32. Rowman and Littlefield, Totowa, N.J.

Reynolds, L. P., C. L. Ferrell, D. A. Robertson, and J. Klindt. 1990. Growth hormone, insulin and glucose concentrations in bovine fetal and maternal plasmas at several stages of gestation. J. Anim. Sci. 68:725.

Reynolds, C. K. 1992. Metabolism of nitrogenous compounds by ruminant liver. J. Nutr. 122:850.

Rompala, R. E., S. D. M. Jones, J. G. Buchanan-Smith, and H. S. Bayley. 1985. Feedlot performance and composition of gain in late-maturing steer and exhibiting normal and compensatory growth. J. Anim. Sci. 61:637.

Rosebrough, R. W., J. P. McMurty, and R. Vasilatos-Younken. 1992. In vitro lipid metabolism, growth and metabolic hormone concentrations in hyperthyroid chickens. Br. J. Nutr. 68:667.

Russel, W. E., A. J. D'Ercole, and L. E. Underwood. 1985. Somatomedin C/insulinlike growth factor I during liver regeneration in the rat. Am. J. Physiol. 248:E618.

Sano, H., M. Nakai, T. Kondo, and Y. Terashima. 1991. Insulin reponsiveness to glucose and tissue responsiveness to insulin in lactating, pregnant, and nonpregnant, nonlactating beef cows. J. Anim. Sci. 69:1122.

Segal, J., and A. Gordon. 1977. The effects of actomysin D, puromycin, cycloheximide and hydroxyurea on 3',5,3-triiodo-L-thyronine stimulated 2-deoxy-D-glucose uptake in chick embryo heart cells in vitro. Endocrinology 101:150.

Sheffield, L. G. 1988. Organization and growth of mammary epithelia in the mammary gland fat pad. J. Dairy Sci. 7:2855.

Sinha, Y. N., J. N. Wilkins, F. Selby, and W. P. VanderLaan, 1973. Pituitary and serum growth hormone during undernutrition and catch-up growth in young rats. Endocrinology 92:1768.

Skaar, T. C., J. R. Vega, S. N. Pyke, and C. R. Baumrucker. 1991. Changes in insulin-like growth factor-binding proteins in bovine mammary secretions associated with pregnancy and parturition. J. Endocrinol. 131:127.

Smith, G. M., J. D. Crouse, R. W. Mandigo, and K. L. Neer. 1977. Influence of feeding regime and biological type on growth, composition and palatability of steers. J. Anim. Sci. 45:236.

Stelwage . K., and D. G. Grieve. 1990. Effect of plane of nutrition on growth and mammary gland development in Holstein heifers. J. Dairy Sci. 73:2333.

Summers, M., B. W. McBride, and L. P. Milligan. 1988. Components of basal expenditure. In: A. Dobson and M. J. Dobson (Eds.) Aspects of digestive physiology in ruminants. Comstock Publ. Assoc., Cornell University Press, Ithaca and London.

Symonds, M. E., M. J. Bryant, and M. A. Lomax. 1989. Lipid metabolism in shorn and unshorn pregnant sheep. Br. J. Nutr. 62:35.

Trenkle, A. 1983. The influence of nutrition on growth of beef cattle. Proc. Growth Management Conf. pp18-25. Terre Haute, IN.

Trenkle, A., D. L. DeWitt, and D. G. Topel. 1978. Influence of age, nutrition, and genotype on carcass traits and cellular development of the M.longissimus of cattle. J. Anim. Sci. 46:1597.

Tucker, H. A. 1969. Factors affecting mammary gland cell numbers. J. Dairy Sci. 52:721.

Tucker, H. A. 1987. Quantitative estimates of mammary growth during various physiological states: A review. J. Dairy Sci. 70:1958.

Van den Brande, J. L. 1986. Catch-up growth. Possible mechanisms. Acta Endocrinol. 113 (S279):13.

Van Dyke, R. W., C. A. Hornick, J. Belcher, B. Scharschmidt, and R. J. Havel. 1985. Identification and characterization of ATP-dependant proton transport by rat liver

multivesicular bodies. J. Biol. Chem. 260:11021.

VandeHaar, M. J., B. M. Moats-Staats, M. L. Davenport, J. -M. Ketelslegers, B. K. Sharma, and L. E. Underwood. 1991. Reduced serum concentrations of insulin-like growth factor-I (IGF-I) in protein-restricted growing rats are accompanied by reduced IGF-I mRNA levels in liver and skeletal muscle. J. Endocrinol. 130:305.

Vandenburgh, H. H. 1984. Relationship of muscle growth in vitro to sodium pump activity and transmembrane potential. J. Cellular Physiol. 119:283.

Wilson, P. N., and D. F. Osbourn. 1960. Compensatory growth after undernutrition in mammals and birds. Biol. Rev. 35:324.

Widdowson, E. M., and Lister, D. 1991. Nutritional control of growth. In: A. M. Pearson and T. R. Dutson (Eds.) Growth regulation in farm animals, vol. 7. Elsevier Applied Science, London.

Woods, S. C., and D. Porte Jr. 1974. Neural control of endocrine pancreas. Physiol. Rev. 54:596.

Yambayamba, E., and M. A. Price. 1991. Growth performance and carcass composition in beef heifers undergoing catch-up (compensatory) growth. Can. J. Anim. Sci. 71:1021.

Young, R., T. Miller, and R. Merkel. 1978. Clonal analysis of satellite cells in growth of mice. J. Anim. Sci. 45:1241.

CHAPTER TWO

2. Hormonal Status, Metabolic Changes, and Resting Metabolic Rate in Beef Heifers Undergoing Compensatory Growth

2.1. Introduction

The study of compensatory growth, particularly in ruminants, has been a major focus in livestock growth and development, with more attention on body composition in castrated males. In the last decade, some studies (Blum et al., 1985; Breier et al., 1986; Hayden et al., 1993) have evaluated endocrine and metabolic changes, and their relationships to compensatory growth. However, these studies have also mainly focused on castrated male animals. Moreover, these studies have concentrated only on specific aspects of physiology rather than attempting a broad understanding of the mechanisms underlying compensatory growth.

Biological factors that may account for compensatory growth include changes in feed intake, efficiency of energy and protein utilization, maintenance energy requirement, and change in composition of gain (Carstens et al., 1989). The present comprehensive study was undertaken to further investigate the above factors including endocrine changes, metabolic changes, the extent of skeletal muscle breakdown during feed restriction, and resting metabolic rate as an index of maintenance energy requirement in beef heifers.

2.2. Materials and Methods

2.2.1. Animals and Feeding

The experiment used 12 Hereford crossbred heifers weighing 227 kg (12 kg SD) and aged 230 d (8 d SD) on d 0. The heifers were born at the University of Alberta Ranch, Kinsella, in the spring of 1992. The heifers were weaned in early October, and 2 wk later, they were trucked 150 km to the University of Alberta's Edmonton Research Station 30 d before the beginning of the experiment. During the 30-d adjustment period to the experimental diet (Table 2-1), the heifers were kept indoors as a single group and they were fed to appetite. Fresh water was always available and they were handled every day and halter trained. On d -1 they were randomly allotted in equal numbers to two treatments as follows: ad libitum feeding (ADLIB), and feed restriction for 95 d followed by realimentation (REST). They were put in individual stalls with wood shavings as bedding material.

The heifers were offered a weighed amount of feed each morning between 0800 and 0900. The feed for the ADLIB group was in excess of their appetites, and the REST heifers were fed on a calculated maintenance ration based on their metabolic body weights (i.e. 77 kcal/kg metabolic body weight) to ensure zero weight-change over the 95-d period. The left-over feed for ADLIB heifers was weighed back daily before fresh feed was supplied. All heifers were weighed weekly for the first 6 wk, and every 2 wk thereafter. Starting on d 95 of the experiment, the restricted heifers had their feed level increased by 400 g/d until they were being fed on an ad libitum basis, a process that was completed in 2 to 3 wk. One heifer from

Table 2-1. Composition of experimental diet (as fed)

	Diet composition, g/kg
Barley grain	754.5
Alfalfa grass hay	200.0
Canola meal	30.0
Calcium carbonate	8.0
Fortified salt	5.0
Vitamin ADE	2.5
Calculated Nutrient composition	
Dry matter (DM), %	89.0
Crude protein, % of DM	12.86
DE, MJ/kg of DM	13.79

the ADLIB group died of bloat on d 148, and was not replaced.

2.2.2. Blood Sampling and Plasma Collection

Blood samples were collected from individual heifers on d 0 (base line), 20, 48, 104, 125, 153, and 195 (common live weight, 420 kg) (Figure 2-1). Days 104, 125, 153, and 195, respectively, were referred to as d 10, 31, 59, and 101 of realimentation. Temporary indwelling catheters (Tygon microbore tubing; Fisher Scientific, Pittsburg, PA) were inserted into the jugular veins of the heifers the day prior to blood sampling. The remaining feed from heifers was removed on the night before blood collection. On the following day, blood sampling began at 0830; animals were not fed before and during blood collection, but they had access to water. Two heifers (one from each treatment) were not bled on d 0 due to problems with catheters.

The first blood sample was collected in two parts: 7 mL into heparinized vacuum tubes containing sodium fluoride for the analysis of glucose; 20 mL into heparinized 16-mm x 100-mm tubes for the analysis of GH, IGF-I, thyroid hormones (T_4 and T_3), insulin, glucose, NEFA, BUN, and 3-methyl histidine (3-MH) concentrations. Thereafter, additional 10-mL samples (for GH analysis only) were collected every 20 min for 7 h.

The blood samples were placed on ice immediately after collection, and were centrifuged at 3,000 x *g* within 2 h of collection. Plasma was harvested in mini-vials and stored in duplicate (triplicate for first sample) at -40°C until it was analyzed.

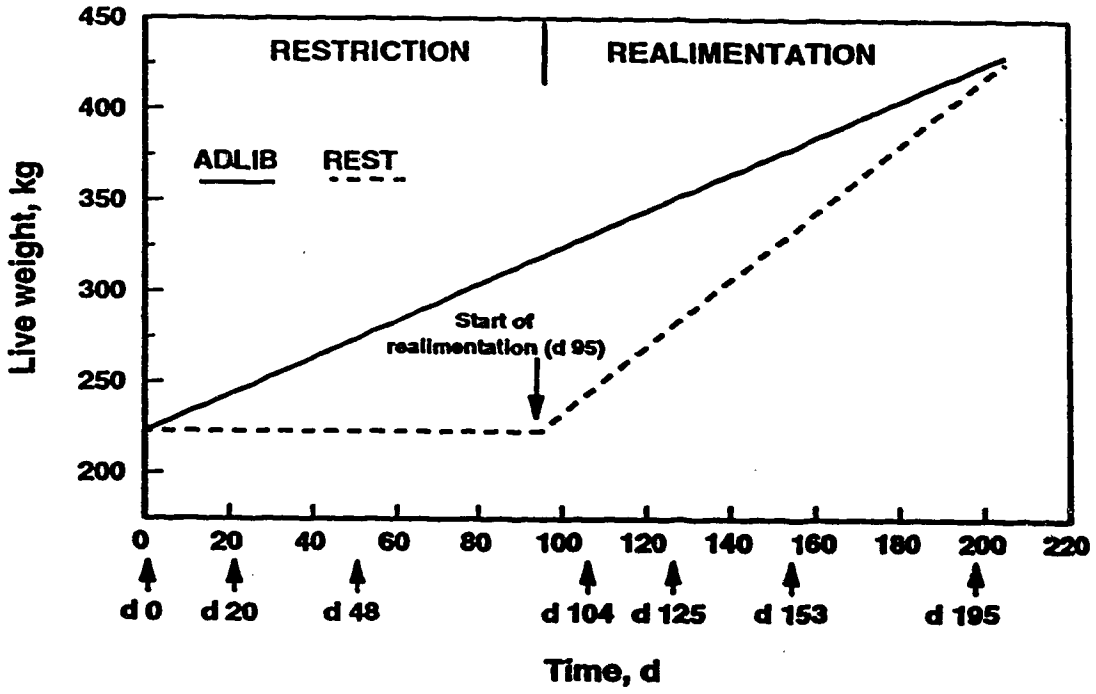


Figure 2-1. Schematic representation of growth paths of ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers. Arrows indicate the day of blood sampling; d 104, 125, 153, and 195, respectively, were referred to as d 10, 31, 59, and 101 of realimentation.

2.2.3. Hormone Assays

2.2.3.1. Growth Hormone

The GH was analyzed by the method of de Boer and Kennelly (1989) with the following modifications. Standard curves were developed with bGH (AFP5340B) in the range from .0625 to 4.0 ng/tube. Standards, prepared in 1% BSA/PBS assay buffer, were assayed in quintuplicates and plasma was assayed in duplicate in 12-mm x 75-mm glass disposable tubes. On d 1, 100 μL of standard or plasma was diluted to 300 μL by the addition of 200 μL of the assay buffer. A further 200 μL of the assay buffer containing antiserum (AFP55; A. F. Parlow, Pituitary Hormones and Antisera Centre, Harbor-UCLA Medical Centre, CA) diluted 1:50,000 was added to the tubes. At this dilution, specific binding in the absence of unlabeled protein was 42%. The samples were incubated for 24 h at 4°C. Concentrated label was diluted with assay buffer to give approximately 10,000 cpm in 200 μL that was added to all tubes. The samples were further incubated for 24 h at 4°C. Separation of bound from free [^{125}I]bGH was done by addition of 200 μL of the second antibody (Anti-monkey gamma globulin, lot P4, Antibodies Inc., Davis, CA) diluted 1:8.9. The samples were incubated for another 14 h. Before centrifugation, 1 mL of cold deionized water was added to each tube and centrifuged at 3,200 x *g* for 30 min. The supernatant was aspirated and the pellet was counted on a COBRA auto-gamma counter (model B5010, Packard Instrument Co., Downers Grove, IL) for 2 min. Intra- and interassay CV were 7.9 and 13.9%, respectively. The mean assay sensitivity (n = 5 assays) was .062 ng/tube. Serial dilutions of pooled bovine plasma demonstrated parallelism to standard curves.

2.2.3.2. *Insulin-like Growth Factor I*

Plasma samples were acid-ethanol extracted and TRIS-neutralized as described by Cosgrove et al. (1992) and analyzed in a single RIA as described by Glimm et al. (1988) and modified by Cosgrove et al. (1992). Recombinant human IGF-I (Bachem, cat. no. DGR012) was used for both iodination and standards. One hundred microliter replicate standards (n = 5) and duplicate plasma samples were diluted with 200 µL of assay buffer, and a further 100 µL antiserum (Anti-hIGF, USDA-UB3-189; provided by Dr. Salvatore Raiti, Director, National Hormone and Pituitary Program, Baltimore, MD), diluted 1:360 with assay buffer, were added prior to incubation at 4°C for 24 h. One hundred microliters of labeled IGF-I diluted in assay buffer to give approximately 10,000 cpm, was then added to all tubes and incubated at 4°C for a further 24 h. Antigen-antibody complexes were precipitated by addition of 200 µL of a goat anti-rabbit gamma globulin (CalBiochem, cat. no. 539845) diluted 1:140 in 1:600 normal rabbit serum (purchased from Animal Services, University of Alberta) and incubated for 16 h. One milliliter of deionized water was then added and the tubes were centrifuged at 3,000 x *g* for 30 min. The supernatant was aspirated and the pellet was counted for 2 min. Validation of the assay for bovine sera was accomplished by establishing parallel displacement curves between serially diluted, acid-ethanol extracted bovine serum with recombinant human IGF-I. Accuracy was demonstrated by a 92% recovery of IGF-I after spiking a sample of bovine plasma and serially diluting it with the same plasma to give three concentrations prior to acid-ethanol extraction. The intraassay CV was 9.4%; sensitivity of the assay was .155 ng/tube.

2.2.3.3. *Thyroid Hormones and Insulin*

Plasma total T_4, total T_3 , and insulin were analyzed using Coat-A-Count assay kits (Diagnostic Products Corporation, Los Angeles, CA) in which labeled ^{125}I competes with T_4, T_3, and insulin in the plasma for specific sites on antibody-coated tubes. The kits were validated by performing curve displacement and parallelism (Williams et al., 1987). The assay procedures were carried out without any modifications. The intraassay CV for total T_4, total T_3, and insulin were 5.7, 5.3, and 9.2%, respectively. Assay sensitivity for T_4 and T_3 was 2.4 and .06 ng/mL, respectively, whereas that for insulin was .096 μIU/mL.

2.2.4. Metabolite Assays

Plasma glucose was determined using commercial kits (Sigma Diagnostics, St. Louis, MO) by the method of Trinder (1969). The BUN concentration was determined using the Technicon autoanalyzer (Technicon Instruments Corporation, Tarrytown, NY) by the modified method of Marsh et al. (1965). The NEFA concentration was measured using WAKO kits (WAKO Chemicals USA, Richmond, VA) with modifications. Twenty five microlitres of standards and plasma samples was pipetted in duplicate into 12-mm x 75-mm disposable glass tubes, following which 350 μL of diluted color reagent A was added. The tubes were vortexed and incubated at 37°C for 20 min, after which 800 μL of diluted reagent B was added. The tubes were incubated at 37°C for another 20 min, and the optical density was read at 550 nm.

Plasma 3-MH concentration, as an index of muscle breakdown (Harrison and Milne,

1981; Nishizawa et al., 1989), was determined by HPLC using the method described by Scott et al. (1993) with some modifications. Briefly, 100 μL of internal standard (.1 mM histidinol) was added to 200 μL of standard (.1 mM 3-MH) or 150 μL of plasma samples. The samples were deproteinized with 100 μL of 3.0 M $HClO_4$ and centrifuged at 3,000 x *g* for 15 min. A 200-μL portion of the supernatant was collected and mixed with 400 μL of water, 115 μL of 1.5 M NaOH, and 400 μL of .2 M $Na_2B_4O_7$ (pH 9.0). While being vortexed, the samples were derivatized with 250 μL of fluorescamine solution (160 mg of fluorescamine/100 mL of acetonitrile). Samples were allowed to sit for a few seconds to use up the excess fluorescamine and then 400 μL of 2.0 M HCl was added. The samples were then incubated at 90°C for 45 min and extracted twice with diethyl ether (Fisher Scientific, Fair Lawn, NJ). The samples were analyzed using a Varian Model 5000 Liquid chromatograph with a Varian 2050 spectrofluorometer detector and a Varian 9090 autoanalyzer (Varian Instruments, Walnut Creek, CA). The binary gradient used in the sample analysis was as follows: solvent A was 2.5 mM cetylnitrimethylammonium bromide and .1 M sodium acetate buffer (pH 6.5), and solvent B was 2.5 mM cetylnitrimethylammonium bromide in 90% acetonitrile (pH 6.5). The gradient rose from 25 to 80% of solvent B over 12 min and held at that level for 2 min and then back to 25% in .5 min. Total analysis time was 18 min per sample.

2.2.5. Resting Metabolic Rate

Four heifers were selected at random from each treatment to investigate the pattern of change in RMR during feed restriction and realimentation. The selected heifers had their feed removed the previous night but had access to water. Oxygen consumption (VO_2) was measured on three occasions: 5 d before the beginning of realimentation and 15 and 36 d into realimentation. This was done using an open-circuit respiratory apparatus (Young et al., 1975). The double-channel apparatus was connected to ventilated hoods that were placed in individual pens on the day of measurement. The VO_2 was measured every 5 sec and averaged every 3 min during a 4 h period from 0900 to 1300. Air flow rates were measured using a flowmeter (Rotometer, Fisher and Porter, Warmister, PA). Wet and dry bulb temperatures, manometer, and barometer measurements were obtained to adjust air flow rates to a standard dry, temperature, pressure basis. Oxygen concentrations of incoming and outgoing air were measured using a paramagnetic oxygen analyzer (Servomex 540A, Sussex, U.K.), and the VO_2 was determined by difference. The open-circuit system was calibrated with nitrogen as zero gas (Young et al., 1984).

2.2.6. Statistical Analysis

Regression of individual live weights on time was done to compute individual average daily gains (ADG). Analysis of variance was then performed using GLM procedures (SAS, 1990) to evaluate treatment effects on ADG, average feed intake, feed efficiency, and total

feed consumption. One heifer in the ADLIB group died and her performance data were excluded from the analysis.

Because of the variable but not clearly defined episodic nature of GH secretion observed, analysis of episodic characteristics was considered inappropriate. Therefore, the GH profiles were initially characterized using the "sliding windows" program of Shaw and Foxcroft (1985) to generate individual animal estimates for mean GH concentration, profile maxima and minima, and area under the curve for each sampling day. The generated means were subsequently analyzed as a split-plot design using the GLM procedures (SAS, 1990) to compare the treatments within each sampling day and across time. The data for other hormones and metabolites were analyzed in the same fashion as a split-plot design. The 3-MH chromatogram data were initially processed using Shimadzu EZChrom Chromatography system software (Shimadzu Scientific Instruments, Columbia, MD) to generate concentration values for each sample. The data were then analyzed as above. Heat production was calculated from VO_2 data according to McLean (1972) on the basis of metabolic body weight ($BW^{.75}$). The data were then analyzed using a repeated measures test (SAS, 1990) to assess treatment effects within each day and across time.

2.3. Results

2.3.1. Body Weight Gains, Feed Intake, and Feed Efficiency

The ADG, feed intake, and feed efficiency data of heifers are presented in Table 2-2. The body weights of REST heifers were maintained relatively constant during the feed restriction period, whereas ADLIB heifers were gaining .97 ± .09 kg/d. During the realimentation period, the ADG of REST heifers was higher (P = .001) than that of ADLIB heifers (1.54 ± .05 vs 1.08 ± .03 kg/d). The ADG of the realimented heifers was particularly high during the first half (8 wk) of the realimentation period, with some individual heifers gaining more than 2 kg/d.

Average feed intake per heifer was maintained at 3.2 ± .1 kg/d for REST heifers during feed restriction, whereas ADLIB heifers consumed 5.8 ± .3 kg/d. The REST heifers were gradually introduced to higher feed intakes during realimentation, and by the end of the 3rd wk all the heifers were eating to appetite. By the end of the 4th wk, their feed intake was higher than that of ADLIB heifers. Considered over the whole realimentation period, the REST heifers' feed intake was slightly higher but not significantly different (P = .40) from that of ADLIB heifers (9.4 ± .3 vs 8.7 ± .7 kg/d). Feed efficiency of REST heifers was higher (P = .01) than that of ADLIB heifers during this period. Over the whole experimental period, total feed intake per heifer for REST and ADLIB heifers (1,401 vs 1,441 kg) was similar (P = .55).

Table 2-2. Least squares means of body weight (BW[a]), feed intake (FI), ADG, and feed efficiency (FE) of ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers during feed restriction and realimentation periods

	ADLIB (n = 6)	REST (n = 6)	P
Baseline (d 0)			
BW	223 ± 4	232 ± 12	.60
Restriction period			
BW, kg	305 ± 6	239 ± 5	.001
FI, kg	5.8 ± .3	3.2 ± .1	.01
ADG, kg/d	.97 ± .09	.07 ± .01	.001
FE, gain/feed	.17 ± .01	-	-
Realimentation period[b]			
BW, kg	419 ± 7	414 ± 5	.40
FI, kg	8.7 ± .7	9.4 ± .3	.18
ADG, kg/d	1.08 ± .03	1.54 ± .05	.001
FE, gain/feed	.13 ± .01	.17 ± .01	.01

[a]BW for restriction (d 0 to d 95) and realimentation (d 95 to d 195) periods was that taken at the end of each period.

[b]One heifer from the ADLIB group died on d 148, and was not replaced.

2.3.2. Hormonal Status

2.3.2.1. *Plasma Growth Hormone, Insulin-like Growth Factor I, and Insulin Profiles*

The data for GH, IGF-I, and insulin concentrations are presented in Figure 2-2. There were significant ($P < .01$) effects of treatment, time, and treatment x time interactions for all the hormone profiles. Mean plasma GH concentration, profile maxima and minima, and area under the curve showed a similar pattern of change over time; therefore, only the mean GH values are reported here. Feed restriction for the first 20 d did not affect ($P > .05$) GH secretion. However, on d 48, REST heifers had higher ($P = .01$) plasma GH concentration than ADLIB heifers (13.88 ± .95 vs 8.52 ± .17 ng/mL). Realimentation of the restricted heifers did not alter GH status immediately; it was observed that 10 d into realimentation the GH concentrations were still significantly ($P = .01$) elevated in these heifers compared with those in the ADLIB heifers (16.26 ± 1.79 vs 8.70 ± .89 ng/mL). By 31 d of realimentation, GH concentrations in REST heifers had fallen to a level similar to that of ADLIB heifers, and no further differences were observed.

Unlike GH, plasma IGF-I concentrations responded more rapidly to the low nutritional plane. On d 20 of feed restriction, mean plasma IGF-I concentration in REST heifers was lower ($P = .04$) than in ADLIB heifers (131.85 ± 8.33 vs 167.02 ± 12.28 ng/mL). This trend continued throughout the feed restriction period; IGF-I concentration in REST heifers further fell to 106.18 ± 12.04 ng/mL and that in ADLIB heifers rose to 188.87 ± 11.45 ng/mL on d 48. On realimentation of the REST group, the IGF-I concentration rose to ADLIB levels in a shorter time than did GH. No differences ($P = .92$) existed between REST and ADLIB

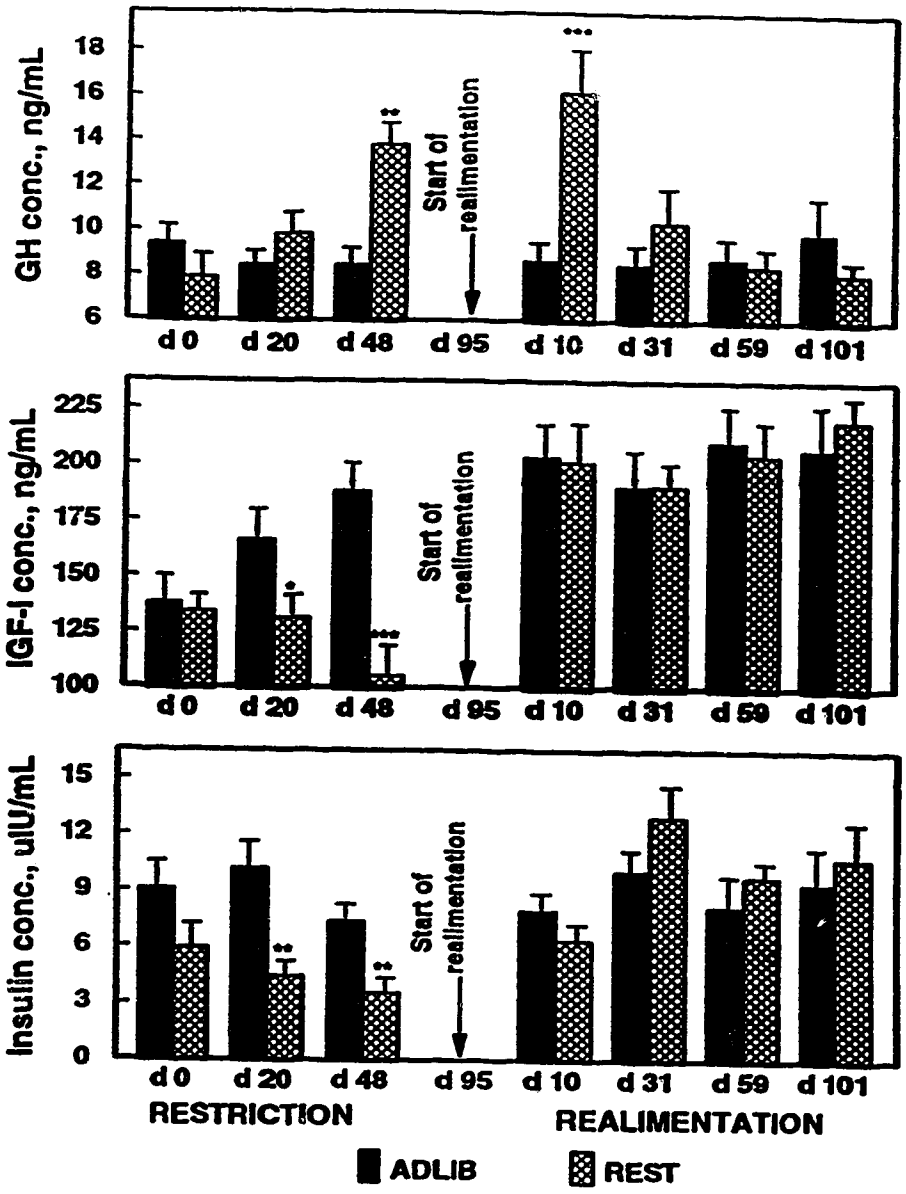


Figure 2-2. Mean plasma growth hormone (GH), insulin-like growth factor I (IGF-I), and insulin concentrations of ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers. *P < .05; **P < .01; ***P < .001.

heifers by d 10 of realimentation, and this trend continued for the rest of the experimental period. There was also a gradual rise in plasma IGF-I concentration in ADLIB heifers over time, although this seemed to peak off in the early phase of realimentation.

Insulin responded to feed restriction and realimentation in a similar manner to that of IGF-I. Feed restriction resulted in reduced (P = .01) circulating insulin in REST heifers compared with ADLIB heifers (4.52 ± .57 vs 10.25 ± 1.45 μIU/mL) on d 20. Similarly, plasma insulin concentrations on d 48 were lower (P = .01) in REST than in ADLIB heifers (3.66 ± .61 vs 7.47 ± .92 μIU/mL). Realimentation resulted in a rapid insulin response, and by d 10 there were no significant differences (P = .12) between treatments.

2.3.2.2. *Episodic Nature of Growth Hormone Secretion*

Although the pattern of change in GH secretion varied among heifers, Figure 2-3 illustrates the differences between REST and ADLIB heifers on d 48 of feed restriction and d 10 and 31 of realimentation that contributed to the overall effects of treatment. The heifer from the REST group in Figure 2-3 was still exhibiting an elevated GH concentration on d 31 of realimentation, although overall there was no treatment difference at this time.

2.3.2.3. Thyroid Hormones and Resting Metabolic Rate

There was no overall treatment effect (P = .15) for total T_4 concentration, but there were time effects (P = .001) and a treatment x time interaction (P = .02). On the other hand, the effects of treatment, time, and the treatment x time interaction were all significant (P < .01) for total T_3 concentration. Neither total T_4 nor total T_3 concentration was affected by feed

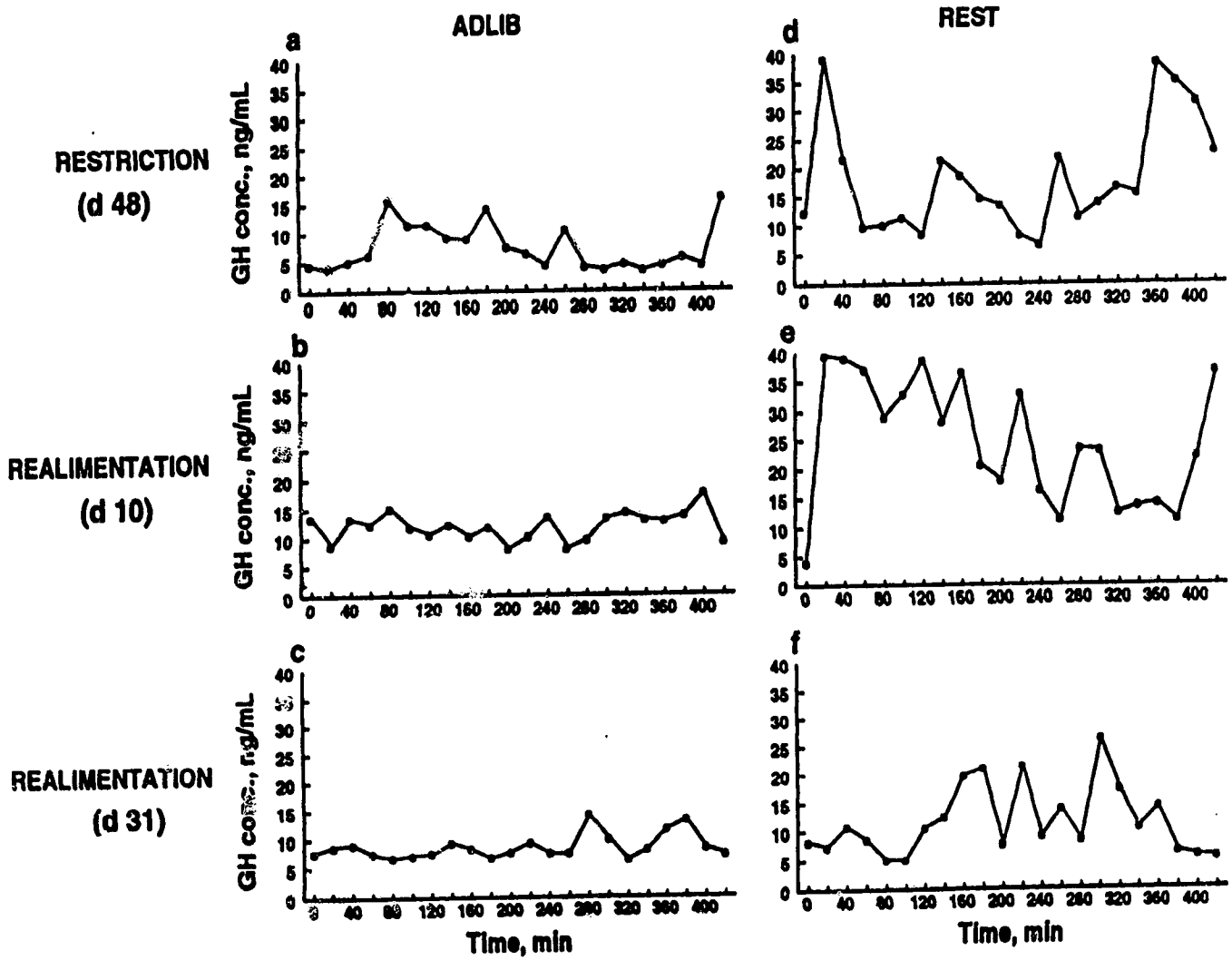


Figure 2-3. Plasma growth hormone (GH) profiles of two random heifers on different planes of nutrition. Graphs a, b, and c show GH profiles of one heifer from the ad libitum fed (ADLIB) group, whereas graphs d, e, and f show profiles of one heifer from the feed restricted-refed (REST) group. Plasma samples were collected on d 48 of feed restriction, and d 10 and 31 of realimentation.

restriction during the first 20 d of feed restriction (Figure 2-4). On d 48, both T_4 (P = .01) and T_3 (P = .001) concentrations were lower in REST than in ADLIB heifers (52.52 ± 1.83 vs 65.07 ± 3.40 ng/mL, and .93 ± .05 vs 1.25 ± .05 ng/mL, respectively). The differences between treatments persisted through d 10 of realimentation (52.43 ± 3.82 vs 68.03 ± 5.96 ng/mL, and .94 ± .04 vs 1.45 ± .09 ng/mL, respectively). By the 31st d of realimentation, the concentration of both thyroid hormones in REST heifers had risen to ADLIB levels and remained similar for the rest of the experimental period.

The resting metabolic rate, estimated by computed daily HP, is presented in Figure 2-5. There was no overall treatment effect (P = .44) for daily HP; however, the effects of time (P = .001) and treatment x time interaction (P = .02) were significant. There was a gradual increase in daily HP by both groups of heifers over the period of the study.

During feed restriction (5 d before realimentation), daily HP was lower (P = .03) in REST than in ADLIB heifers (525.5 ± 15.4 vs 617.6 ± 28.3 kJ/d/$BW^{.75}$). Fifteen days after the beginning of realimentation, there was a rise in daily HP but it was still lower (P = .02) in these heifers than in ADLIB heifers (600.3 ± 19.7 vs 683.6 ± 21.1 kJ/d/$BW^{.75}$). By the 36th d of realimentation, REST heifers had recovered their metabolic rates to those of ADLIB heifers.

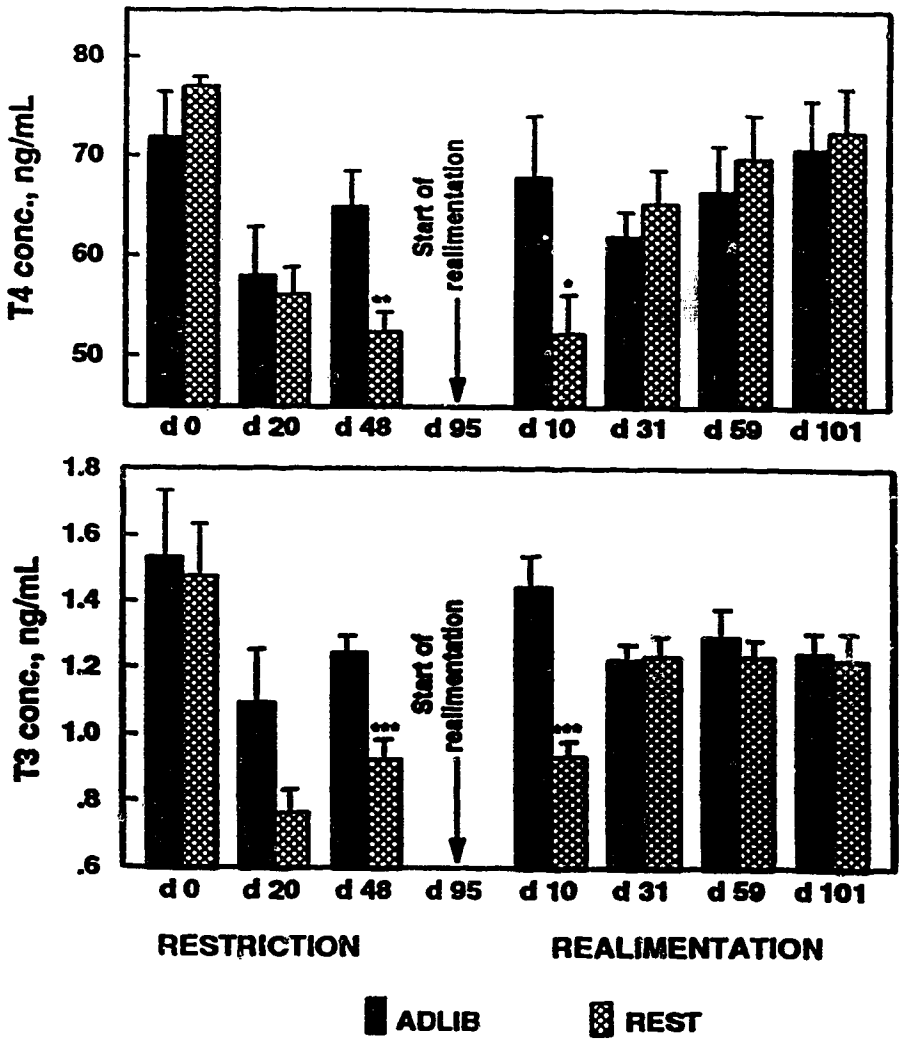


Figure 2-4. Mean plasma total thyroxine (T_4) and total triiodothyronine (T_3) concentrations of ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers. *P < .05; **P < .01; ***P < .001.

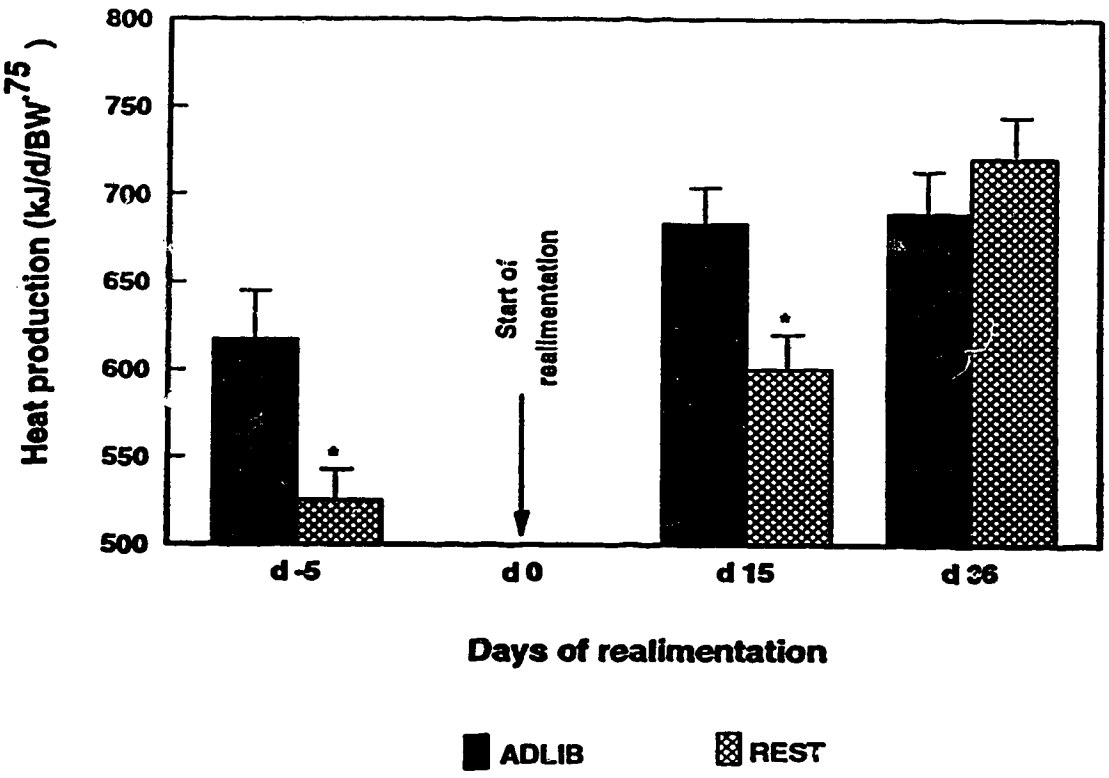


Figure 2-5. Daily heat production of ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers, measured 5 d before the beginning of realimentation, and 15 and 36 d after the beginning of realimentation of REST heifers. *P < .05.

2.3.3. Metabolite Status

2.3.3.1. *Plasma Glucose and Non-esterified Fatty Acid Concentrations*

Plasma glucose and NEFA concentrations are presented in Figure 2-6. There were significant effects ($P < .01$) of treatment, time, and treatment x time interactions for both glucose and NEFA concentrations. Both metabolites were significantly affected by feed restriction, though in opposite directions. On d 20, glucose concentration was lower ($P = .03$) in REST than in ADLIB heifers (88.57 ± 2.61 vs 103.27 ± 5.04 mg/dL). The trend was the same on d 48 as the glucose concentration was still lower ($P = .01$) in REST than in ADLIB heifers (87.30 ± 3.08 vs 104.37 ± 3.92 mg/dL). There was, however, a rapid response in the glucose concentration during realimentation, and by d 10 of this period the concentration in REST heifers was not different ($P = .94$) from that in ADLIB heifers.

Meanwhile, plasma NEFA concentration on d 20 of feed restriction was higher ($P = .01$) in REST than in ADLIB heifers (474.98 ± 37.19 vs 288.56 ± 42.43 mEq/mL), an indication of lipolysis in the feed restricted animals. On d 48, the concentration was even higher ($P = .001$) in REST than in ADLIB heifers (538.22 ± 44.64 vs 257.42 ± 24.74 mEq/mL). Realimentation of the REST group reversed the trend, and by d 10 no significant differences ($P = .57$) between treatments were found.

2.3.3.2. *Blood Urea Nitrogen and 3-Methyl Histidine Concentrations*

The BUN and 3-MH data are presented in Figure 2-7. There were no overall treatment effects ($P > .05$) for either BUN or 3-MH concentrations. The time and treatment x time

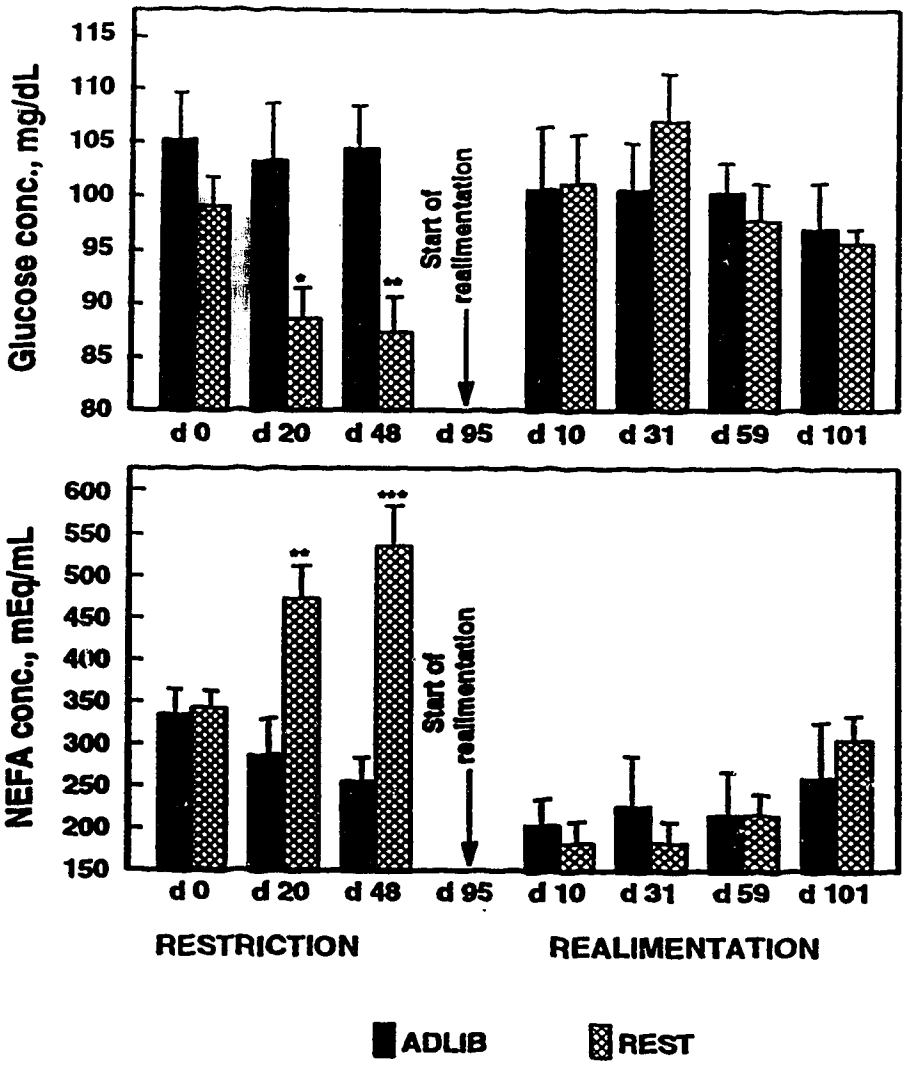


Figure 2-6. Mean plasma glucose and non-esterified fatty acids (NEFA) concentrations of ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers. *P < .05; **P < .01; ***P < .001.

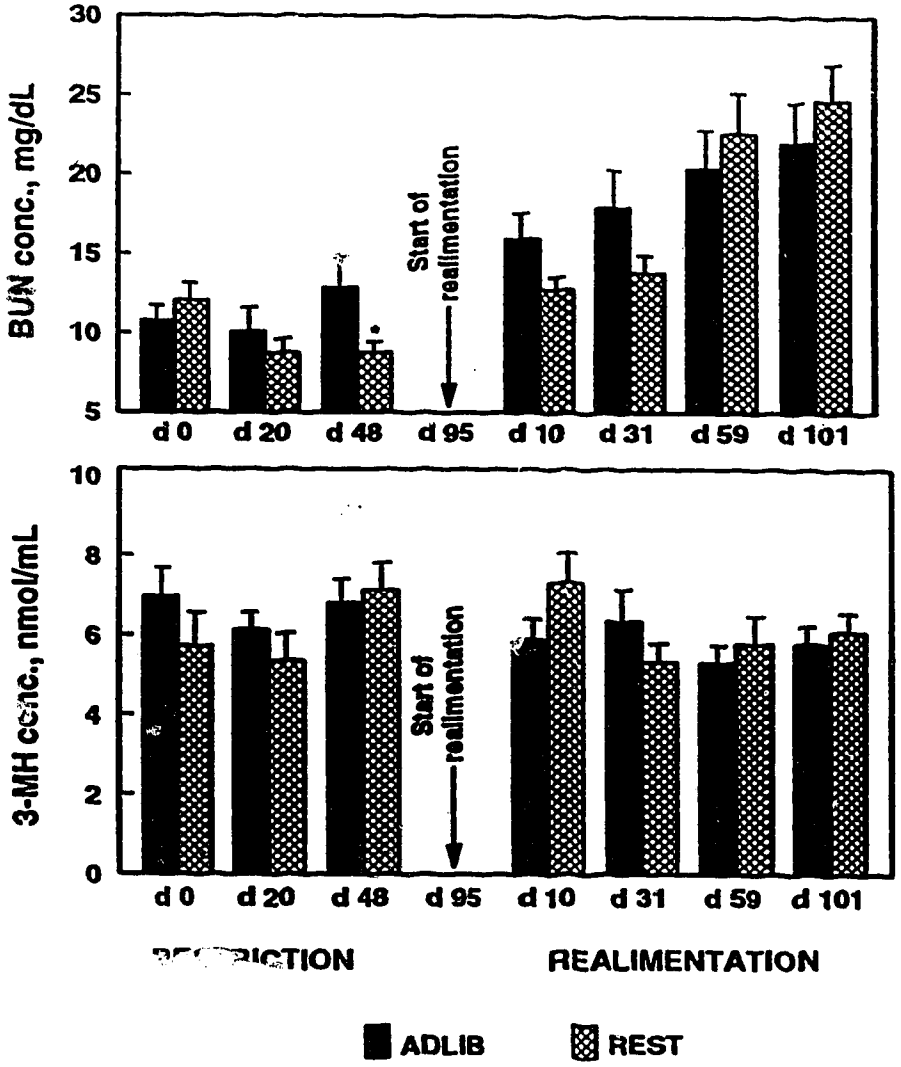


Figure 2-7. Mean blood urea nitrogen (BUN) and 3-methyl histidine (3-MH) concentrations of ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers. *P < .05.

interaction were significant ($P < .05$) for BUN concentration, whereas only the time effect was significant ($P = .02$) for 3-MH. On d 48 of feed restriction, BUN concentration was lower ($P = .05$) in the REST than in the ADLIB group (8.75 ± .64 vs 12.85 ± 1.78 mg/dL). During realimentation, although no significant difference was detected between treatments on d 10 and 31, there was a tendency ($P = .11$) toward lower BUN concentration in REST than in ADLIB heifers (12.77 ± .74 vs 15.95 ± 1.65 mg/dL on d 10; 13.80 ± 1.0 vs 17.92 ± 2.30 mg/dL on d 31). The BUN concentration increased over time in both treatments.

In contrast to other measured metabolites, the concentration of 3-MH was not affected by nutritional treatment. The 3-MH concentration remained similar in both treatments throughout the experimental period.

2.4. Discussion

2.4.1. Growth Performance

By design, the body weights of REST heifers were maintained relatively constant during the feed restriction period, whereas ADLIB heifers were gaining .97 kg/d. During the realimentation period, the REST heifers gained significantly faster than the ADLIB heifers, particularly during the first-half (8 wk) of the period. This pattern of growth in cattle undergoing compensatory growth has been reported in other studies (Carstens et al., 1991; Yambayamba and Price, 1991; Henricks et al., 1994). The high growth rates during ad libitum feeding of previously restricted cattle are mainly attributed to increased efficiency of

energy and protein utilization (Fox et al., 1972), increased feed intake, and decreased maintenance requirement (Carstens et al., 1989). The current results confirmed both increased feed intake and feed efficiency in the realimented heifers compared to those on ad libitum feeding.

Hicks et al. (1990) and Murphy et al. (1994) showed that feed restriction led to improved feed efficiency in beef cattle and lambs, respectively. Although no direct evidence has been reported for the mechanisms by which feed efficiency is improved with restricted feeding, Murphy and Loerch (1994) have suggested factors such as reduced size of metabolically active organs leading to lower maintenance energy expenditures, reduced physical activity, and increased diet digestibility. Such animals, when realimented, would slowly raise their basal metabolism. Thus their feed efficiency would remain high, at least in the initial phase of compensatory growth. Carstens et al. (1989) reported that the efficiency of metabolizable energy utilization in their realimented steers remained high for at least 4 wk, and then decreased steadily to control levels. Data from the present study showed high growth rates in the realimented heifers during the first 8 to 9 wk; some individual heifers gained more than 2 kg/d. The ADG was 2.11 kg/d during the first 5 wk of realimentation, and slowly dropped to 1.53 kg/d during the second 5 wk. Feed efficiency certainly remained high during this period, and the overall growth performance remained higher than that of the ADLIB heifers during the period. Having confirmed that both increased feed efficiency and feed intake, and a decrease in maintenance requirements contributed to compensatory growth, it is then possible to discuss the physiological mechanisms associated with factors contributing to compensatory growth in this experimental model.

2.4.2. Hormone Profiles and Resting Metabolic Rate

2.4.2.1. *Growth Hormone, Insulin-like Growth Factor I, and Insulin*

Although IGF-I and insulin concentrations were significantly lower in REST than in ADLIB heifers throughout the feed restriction period, mean plasma GH during the first 20 d of this period was not significantly altered. Feed restriction is generally associated with higher plasma GH and lower IGF-I (Breier et al., 1986, 1988; Breier and Gluckman, 1991) and insulin (Brockman and Laarveld, 1986; McCann and Hansel, 1986; Lobley, 1992) concentrations in ruminants. The synthesis and secretion of IGF-I are principally dependent on GH (Davis, 1988), although insulin is also known to modulate the same (Daughaday et al., 1976). As would be expected, the most immediate response to feed restriction was a reduced plasma insulin concentration, reflecting the lower energy and protein supply. This subsequently led to an immediate decrease in the IGF-I concentration despite the unaltered GH concentration during the first 20 d of feed restriction. Continued feed restriction eventually resulted in higher GH concentrations in REST heifers than in ADLIB heifers. Breier et al. (1986), who fed three planes of nutrition to Angus steers, found that medium (1.8% DM of live weight) and low (1% DM of live weight) planes of nutrition significantly increased mean plasma GH concentration, the amplitude of GH pulses and area under the curve. From these results and those of Ellenberger et al. (1989), Hayden et al. (1993) concluded that the enhanced GH concentration during low nutrition seemed to result from an increase in the amplitude rather than the frequency of the pulse, and that this increase is related to a decrease in the negative feedback control of hypothalamic somatostatin. The

increase in GH concentration has also been attributed to reduced metabolic clearance rate (Mosier et al., 1980) or increased half-life (Trenkle, 1976). Other than the increased plasma GH concentration in feed-restricted heifers, the present study also demonstrated that the pattern of GH secretion in all heifers was asynchronous and episodic, as shown by the two heifers in Figure 2-3. Some heifers showed more pronounced episodes of GH release than others. Similar results of GH secretion have been reported in steers (Breier et al., 1986) and bulls (Anfinson et al., 1975).

Although IGF-I is produced locally in several tissues of the body in which it can act in a paracrine or autocrine fashion (Murphy et al., 1987; Davis, 1988), peripherally circulating IGF-I is principally produced by hepatocytes (VandeHaar et al., 1990; Hannon et al., 1991). Despite the elevated plasma GH concentration during low nutrition, the hepatocytes and extra-hepatic tissues are known to become insensitive to GH action, most probably as a result of reduced numbers of GH receptors (Van den Brande, 1986; Breier et al., 1988) or reduced activity of the receptors (Davis, 1988). As a consequence, feed restriction is also associated with reduced IGF-I mRNA in hepatic tissues (Emler and Schalch, 1987) and in extra-hepatic tissues (Lowe et al., 1989). These effects are related to the hypoinsulinemic state of the feed-restricted animal and lead to impairment of the synthesis and secretion of IGF-I. Breier and Gluckman (1991) have also proposed that the lower plasma IGF-I levels during low nutrition may result from enhanced clearance of the IGF-I bound to the IGF-binding proteins. Because of this uncoupling of the GH-IGF-I axis, the somatic effects that GH has on postnatal growth are impaired because many of them are mediated by IGF-I (Evock et al., 1990; Godfredson et al., 1991).

The time course of change in the GH-IGF-I-insulin axis during realimentation was similar to that seen during feed restriction. Although insulin and IGF-I concentrations in the realimented heifers rose to control levels by d 10 of realimentation, GH concentrations were still significantly elevated in these heifers, only falling to control levels by d 31. Plasma GH concentrations in feed-restricted ruminants have been reported to fall to control levels within 4 d of realimentation (Blum et al., 1985) but the present study contradicts this and confirms a previous study (Yambayamba and Price, unpublished data) in which heifers restricted for 4 mo still had elevated plasma GH concentrations after 25 d of realimentation. The present results are in agreement with Breier et al. (1986), who found that steers that had been moderately restricted for 4 wk had normal GH concentrations after 10 d of realimentation, whereas severely restricted steers still exhibited increased GH concentrations at this time. In rats, Mosier et al. (1985) also reported elevated plasma GH for at least 2 wk after the beginning of realimentation, thus implicating GH in the physiological mechanisms of compensatory growth.

Although there is no conclusive experimental evidence to associate GH with compensatory growth in ruminants, results of the present study suggest a strong correlation between the elevated GH concentrations and the high growth rates in beef heifers, at least in the initial phase of compensatory growth. The lag in the response of GH to realimentation in one way implied that the functions of GH are chronic rather than acute (Etherton, 1994). This lag in response coupled with the faster response of insulin and IGF-I could have major implications on somatic responses during realimentation. Van den Brande (1986) concluded that a possible explanation for the high growth rates during realimentation could be that the

tissues become more sensitive to IGF-I as a consequence of deprivation during the period of growth retardation. Thus during realimentation, a normal IGF-I concentration would induce a larger response. In the present study, plasma IGF-I concentration increased over time both in ADLIB and REST heifers. This suggests hepatic sensitivity was changing as feeding progressed, but there was a larger somatic response in the realimented heifers possibly due to the above reasons suggested by Van den Brande (1986).

Associated with the high hepatic sensitivity is the nature of GH binding sites. Breier et al. (1988) observed that the specific binding of bovine GH in hepatic membranes was greater in well-fed than underfed ruminants. They observed high-affinity binding sites at a high plane of nutrition as opposed to low-affinity binding sites at a low plane of nutrition. Thus in compensatory growth, it may be concluded that as the level of nutrition increases during realimentation, the high affinity binding sites for GH become more abundant both in the hepatic and extra-hepatic tissues, leading to high responses in the somatic effects of the previously deprived tissues.

The rapid increase in insulin levels in REST heifers following realimentation would be functionally related to a stimulation of amino acid transport, slowing of amino acid oxidation, depression of protein degradation, and a direct effect on protein synthesis (Ahmed et al., 1983); it may therefore be postulated that insulin played a major role in the physiological mechanism of compensatory growth in beef cattle. In fact Hayden et al. (1993) observed in their steers that the insulin level in the compensatory animals exceeded that of controls in the initial phase of realimentation. Blum et al. (1985) reported similar results in steers and they proposed that the transient increase of insulin during realimentation may have functioned as

a signal for the initiation of anabolic processes. Moreover, despite the fact that insulin is hypoglycaemic and GH is hyperglycaemic (Brockman and Laarveld, 1986), these hormones act synergistically to favor the uptake of glucose and amino acids by non-hepatic organs for the synthesis of new tissue (Beaver et al., 1989). As discussed earlier, insulin is also known to affect the production of IGF-I, and these interactions with IGF-I and GH can partly explain the high growth performance in ruminants undergoing compensatory growth.

2.4.2.2. *Thyroid Hormones and Resting Metabolic Rate*

Both total T_4 and total T_3 were affected in the same manner by feed restriction and realimentation. The results obtained on d 20 were not expected because the peripheral thyroid status in ruminants is regulated by energy availability (Eales, 1988). However, although feed restriction is associated with decreased thyroid activity (Blake et al., 1991; Murphy and Loerch, 1994), some workers (Ellenberger et al., 1989) have observed that both T_4 and T_3 were unaltered in feed-restricted steers, whereas Beaver et al. (1989) actually observed increased T_3 levels in steers fed low energy. The propositions advanced for these unexpected results are a matter of speculation.

By d 48, however, the thyroid hormone concentrations in REST heifers were significantly lower than in ADLIB heifers, and this was expected. Because thyroid hormones are associated with basal metabolic rate (Murphy and Loerch, 1994), it is deduced that the metabolic rate of REST heifers was lower than that of ADLIB heifers by d 48. The metabolic rate in REST heifers remained lower during the feed restriction period and this was confirmed by the RMR results obtained 5 d before realimentation (Figure 2-5). These results are in

agreement with those of Carstens et al. (1989) and Lapierre et al. (1992), who observed lower heat production and oxygen consumption, respectively, in feed restricted steers.

During realimentation, plasma concentrations of both thyroid hormones were still lower in REST heifers on d 10 but rose to ADLIB levels by d 31. The rise in plasma T_3 concentrations during realimentation is in agreement with the observations of Hayden et al. (1993) in steers. However, the rise in plasma T_4 concentration does not agree with Hayden et al. (1993), who observed that this hormone concentration in the realimented animals remained lower than in the ad libitum fed animals throughout the realimentation period. These conflicting results may be due to the differences in the severity of feed restriction and the pattern of realimentation. In the present study, the heifers' live weights were maintained relatively constant during feed restriction, whereas Hayden et al. (1993) allowed their restricted steers to gain .2 kg/d. Also the diet was maintained the same in the present study whereas Hayden et al. (1993) changed the diet of their steers during realimentation. Results in the present study are, however, in agreement with those of Fox et al. (1974), who observed lower thyroid secretion in steers fed to maintain body weights for 5 or 6 mo. The reduced thyroid secretion was persistent through the first part of the realimentation period, slowly increasing to or above the levels of controls as realimentation progressed. The authors concluded that such changes in thyroid secretion rates suggested that there was a reduced nergy requirement for maintenance during the period of energy restriction and the first part of the realimentation period, thus contributing to the ability of the cattle to utilize protein and energy more efficiently when placed on full feed.

The concept of reduced energy requirement during the initial phase of compensatory

growth was supported by the present results of RMR. It was observed that realimentation of the REST heifers did not result in an abrupt increase in RMR. This was evident from the measurements taken after 15 d of realimentation. However, RMR in REST heifers had risen to ADLIB levels by d 36 of realimentation. Again this mirrored the responses in thyroid hormone concentrations. Although the thyroid hormone concentrations and RMR were not measured on the same days, there is an obvious indication of the association between them and the slow recovery of RMR during realimentation. In fact, Carstens et al. (1989) found that it took their restricted steers 3 wk of realimentation to increase heat production to control levels.

It is concluded from the present study that the changes in thyroid secretion rates and the pattern of RMR recovery in the REST heifers were an indication of a reduced energy requirement for maintenance during the restriction period and the initial phase of the realimentation period. As concluded by Fox et al. (1974), such changes contributed to the ability of the heifers to utilize energy and protein more efficiently during realimentation. Moreover, a reduced energy requirement would result in the cost of maintaining an animal during initial compensatory growth being less than would be expected. Fox et al. (1974) further noted that lower maintenance requirement during feed restriction and the first part of realimentation would result in a greater proportion of the total energy consumed being used for growth than would normally be expected, thus contributing to compensatory growth.

2.4.3. Metabolite Concentrations

2.4.3.1. *Glucose and Non-esterified Fatty Acid Concentrations*

The lower and higher plasma concentrations of glucose and NEFA, respectively, in REST than in ADLIB heifers during the feed restriction period, was expected. Other studies have demonstrated similar results for glucose (Anthony et al., 1986; Foster and Blight, 1987) and NEFA (DiMarco et al., 1981; Hayden et al., 1993). During feed restriction, there is a shift in the energy balance due to low levels of blood glucose and at this point, the utilization of fatty acids becomes the major source of energy (Brockman and Laarveld, 1986). It was quite apparent in the present study that mobilization of NEFA from the adipose tissue was positively correlated with increased plasma level of GH, suggesting a lipolytic activity of GH during low nutrition (Hayden et al., 1993; Schwarz et al., 1993). In doing so, the use of protein as an energy source was spared (Eisemann et al., 1986).

One important observation in the present study was that the concentration of NEFA increased as feed restriction progressed, suggesting a higher negative energy balance and therefore more need to mobilize fat. Coincidentally, the GH concentration in the restricted heifers was also rising during this period. DiMarco et al. (1981) reported an eightfold increase in NEFA concentration within 6 d of fasting steers. In the present study in which the heifers were underfed rather than fasted, there was a 1.6-fold increase in 48 d.

Realimentation of REST heifers resulted in the rise and fall of plasma glucose and NEFA concentrations, respectively, and by d 10 no differences between treatments were apparent. Hayden et al. (1993) observed similar trends in both metabolites in their steers. DiMarco et

al. (1981) found that the realimentation period necessary to decrease the NEFA concentration to basal or prefasting levels was approximately 8 d. It would seem from these observations and the present results that the shift in the metabolic status with realimentation is not abrupt. It takes some days, and while this is happening, there is considerable increase in the efficiency of energy utilization because maintenance requirements are still low.

2.4.3.2. *Blood Urea Nitrogen and 3-Methyl Histidine Concentrations*

The BUN concentration, which is indicative of protein balance (Ellenberger et al., 1989), was surprisingly found to be lower in REST than in ADLIB heifers on d 48 of feed restriction. Hayden et al. (1993) and Cole et al. (1994) have reported higher BUN concentrations in feed-restricted animals than in the ad libitum fed animals. In the present study, restricted heifers may not have reached a catabolic state for protein by d 48. Because no further blood samples were taken before realimentation, it is not known whether they ever reached this state. However, the high concentrations of plasma GH and NEFA during the feed restriction period may have considerably spared protein mobilization. Similarly, Ellenberger et al. (1989) did not observe higher BUN concentrations in their restricted steers and they suggested that dietary restriction was probably not severe enough to cause such effects. In the context of the present study, it is suggested that as reported by Hicks et al. (1990), the REST heifers had become more efficient in nutrient utilization and nitrogen retention.

During realimentation, although no significant differences between treatments were observed, BUN concentration tended to be lower in REST than in ADLIB heifers, particularly in the initial phase of compensatory growth. There was a gradual rise in BUN concentration

in both treatment groups over time, possibly reflecting the age effect, as reported by Peterson and Waldern (1981). Similar to the present results, Ellenberger et al. (1989) reported a decline of BUN concentration in the early phase of realimentation, and concluded that this was an indication of the efficient use of nutrients during early realimentation. In the present study, it may also be concluded that because GH concentration was still elevated in REST heifers during early realimentation, this may have resulted in more nitrogen retention and a reduction in the whole-body oxidation of amino acids.

Like BUN concentration, results of 3-MH in the present study did not indicate any significant muscle (or possibly gut tissue) mobilization for energy during the first 48 d of feed restriction. Again, because no blood samples were taken between d 48 and the end of the feed restriction period, it is not known whether significant muscle or gut tissue breakdown took place during this period. However, during realimentation, no differences between treatments were observed by d 10. Thus, whether significant muscle mobilization during the later part of feed restriction took place or not, the present results indicate efficient nitrogen retention and reduction in amino acid oxidation during realimentation of the heifers.

2.5. Conclusions

Overall, results of the present study indicate that feed restriction followed by realimentation was associated with significant endocrine and metabolic changes. There was a lag between nutritional treatment and some endocrine and metabolic responses. The quick response of insulin and IGF-I during realimentation coupled with the continued elevation of

GH and lower thyroid hormone concentrations and metabolic rate in the initial phase of realimentation can be associated with the observed higher growth performance of the restricted-refed heifers compared with the ad libitum fed heifers. From the present BUN and 3-MH data, there seems to be a protein sparing mechanism during a large part of feed restriction, and a high efficiency of nitrogen retention during realimentation. It is concluded that these differential responses, in coordination with other physiological changes, are associated with compensatory growth and explain why more severely restricted ruminants compensate more rapidly.

2.6. Literature Cited

Ahmed, B. M., W. G. Bergen, and N. K. Ames. 1983. Effect of nutritional state and insulin on hind-limb amino acid metabolism in steers. J. Nutr. 113:1529.

Anfinson, M. S., S. L. Davis, E. Christian, and D.O. Everson. 1975. Episodic secretion of growth hormone in steers and bulls. An analysis of frequency and magnitude of secretory spikes occuring in a 24 hour period. Proc. West Sect. Am. Soc. Anim. Sci. 26:175.

Anthony, R. V., R. A. Bellows, R. E. Short, R. B. Staigmiller,C. C. Kaltenbach, and T.G. Dunn. 1986. Fetal growth of beef calves. I. Effect of prepartum dietary crude protein on birth weight, blood metabolites and steroid hormone concentrations. J. Anim. Sci. 62:1363.

Beaver, E. E., J. E. Williams, S. J. Miller, D. L. Hancock, S. M. Hannah, and D. L. O'Connor. 1989. Influence of breed and diet on growth, nutrient digestibility, body composition and plasma hormones of Brangus and Angus steers. J. Anim. Sci. 67:2415.

Blake, N. G., D. J. A. Eckland, O. J. F. Foster, and S. L. Lightman. 1991. Inhibition of hypothalamic thyrotropin-releasing hormone messenger ribonucleic acid during food deprivation. Endocrinology 129:2714.

Blum, J. W., W. Schnyder, P. L. Kunz, A. K. Blom, H. Bickel, and A. Schurch. 1985. Reduced and compensatory growth: Endocrine and metabolic changes during food restriction and refeeding in steers. J. Nutr. 115:417.

Breier, B. H., J. J. Bass, J. H. Butler, and P. D. Gluckman. 1986. The somatotrophic axis in young steers: Influence of nutritional status on pulsatile release of growth hormone and

circulating concentrations of insulin-like growth factor 1. J. Endocrinol. 111:209.

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

Breier, B. H., P. D. Gluckman, and J. J. Bass. 1988. The somatotrophic axis in young steers: influence of nutritional status and oestradiol-17ß on hepatic high- and low-affinity somatotrophic binding sites. J. Endocrinol. 116:169.

Brockman, R. P., and B. Laarveld. 1986. Hormonal regulation of metabolism in ruminants: A review. Livest. Prod. Sci. 14:313.

Carstens, G. E., D. E. Johnson, and M. A. Ellenberger. 1989. Energy metabolism and composition of gain in beef steers exhibiting normal and compensatory growth. In: Energy Metabolism of Farm Animals. Eur. Assoc. Anim. Prod. Publ. No. 43. Pudoc, Wangeningen.

Carstens, G. E., D. E. Johnson, M. A. Ellenberger, and J. D. Tatum. 1991. Physical and chemical components of the empty body during compensatory growth in beef steers. J. Anim. Sci. 69:3251.

Cole, N. A., R. H. Gallavan, S. L. Rodriguez, and C. W. Purdy. 1994. Influence of triiodothyronine injections on calf immune response to an infectious bovine rhinotracheitis virus challenge and nitrogen balance of lambs. J. Anim. Sci. 72:1263.

Cosgrove, J. R., J. E. Tilton, M. G. Hunter, and G. R. Foxcroft. 1992. Gonadotropin independent mechanisms participate in ovarian responses to realimentation in feed restricted prepubertal gilts. Biol. Reprod. 47:736.

Daughaday, W. H., M. C. Mueller, and M. S. Phillips. 1976. The effect of insulin and growth hormone on the release of somatomedin by the isolated rat liver. Endocrinology 98:1214.

Davis, S. L. 1988. Recent concepts in regulation of growth by GH and IGF. J. Anim. Sci. 66(Suppl. 3):84.

De Boer, G., and J. J. Kennelly. 1989. Effect of somatotropin injection and dietary protein concentration on milk yield, and kinetics of hormones in dairy cows. J. Dairy Sci. 72:419.

DiMarco, N. M., D. C. Beitz, and G. B. Whitehurst. 1981. Effect of fasting on free fatty acid, glycerol and cholesterol concentrations in blood plasma and lipoprotein lipase activity in adipose tissue of cattle. J. Anim. Sci. 52:75.

Eales, J. G. 1988. The influence of nutritional state on thyroid function in various vertebrates. Am. Zool. 28:351.

Eisemann, J. H., H. F. Tyrrell, A. C. Hammond, P. J. Reynolds, D. E. Bauman, G. L. Haaland, J. P. McMurtry, and G. A. Varga. 1986. Effect of bovine growth hormone administration on metabolism of growing Hereford heifers: Dietary digestibility, energy and nitrogen balance. J. Nutr. 116:157.

Ellenberger, M. A., D. E. Johnson, G. E. Carstens, K. L. Hossner, M. D. Holland, T. M. Nett, and C. F. Nockels. 1989. Endocrine and metabolic changes during altered growth rates in beef cattle. J. Anim. Sci. 67:1446.

Emler, C. A., and D. S. Schalch. 1987. Nutritionally induced changes in hepatic insulin-like growth factor (IGF-I) gene expression in rats. Endocrinology 120:832.

Etherton, T. D. 1994. Metabolic modifiers in animal production. In: P.A. Thacker (Ed.) Livestock Production for the 21st Century: Priorities and Research Needs. pp 73-92.

University of Saskachewan, Saskatoon.

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

Foster, A. H., and G. W. Blight. 1987. Change in metabolite levels and liveweight of grazing cattle when supplemented with Leucaena leucocephala or urea-molasses. Queensl. J. Agric. Anim. Sci. 44:59.

Fox, D. G., R. P. Johnson, R. L. Preston, T. R. Dockerty, and E. W. Klosterman. 1972. Protein and energy utilization during compensatory growth in beef cattle. J. Anim. Sci. 34:310.

Fox, D. G., R. L. Preston, B. Senft, and R. R. Johnson. 1974. Plasma growth hormone levels and thyroid secretion rates during compensatory growth in beef cattle. J. Anim. Sci. 38:437.

Glimm, D. R., V. E. Baracos, and J. J Kennelly. 1988. Effect of bovine somatotropin on the distribution of immunoreactive insulin-like growth factor-I in lactating bovine mammary tissue. J. Dairy Sci. 7:2923.

Godfredson, J. A., M. D. Holland, K. G. Odde, and K. L. Hossner. 1991. Hypertrophy and hyperplasia of bovine fetal tissues during development: Fetal liver insulin-like growth factor I mRNA expression. J. Anim. Sci. 69:1074.

Hannon, K., A. Gronowski, and A. Trenkle. 1991. Relationship of liver and skeletal muscle IGF-1 mRNA to plasma GH profile, production of IGF-1 by liver, plasma IGF-1 concentrations, and growth rates of cattle. P.S.E.B.M. 196:155.

Harrison, C. I., and G. Milne. 1981. The urinary excretion of N^{τ}-methyl histidine by cattle: Validation as an index of muscle protein breakdown. Br. J. Nutr. 45:411.

Hayden, J. M., J. E. Williams, and J. J. Collier. 1993. Plasma growth hormone, insulin-like growth factor, insulin, and thyroid hormone association with body protein and fat accretion in steers undergoing compensatory gain after dietary energy restriction. J. Anim. Sci. 71:3327.

Henricks, D. M., T. C. Jenkins, J. R. Ward, C. S. Krishnan, and L. Grimes. 1994. Endocrine responses and body composition changes during feed restriction and realimentation in young bulls. J. Anim. Sci. 72:2289.

Hicks, R. B., F. N. Owens, D. R. Gill, J. J. Martin, and C. A. Strasia. 1990. Effects of controlled feed intake on performance and carcass characteristics of feedlot steers and heifers. J. Anim. Sci. 68:233.

Lapierre, H., H. F. Tyrrell, C. K. Reynolds, T. H. Elsasser, P. Gaudeau, and P. Brazeau. 1992. Effects of growth hormone-releasing factor and feed intake on energy metabolism in growing beef heifers: Whole-body energy and nitrogen metabolism. J. Anim. Sci. 70:764.

Lobley, G. E. 1992. Control of the metabolic fate of amino acids in ruminants: A review. J. Anim. Sci. 70:3264.

Lowe, W. L. Jr., M. Adamo, H. Werner, T. C. Roberts, Jr., and D. LeRoith. 1989. Regulation by fasting of rat insulin like growth factor I and its receptor. J.Clin. Invest. 84:619.

Marsh, W. H., B. Fingerhut, and H. Miller. 1965. Automated and manual direct methods for the determination of blood urea. Clin. Chem. 11:624.

McCann, J. P., and W. Hansel. 1986. Relationships between insulin and glucose metabolism and pituitary-ovarian functions in fasted heifers. Biol. Reprod. 34:630.

McLean, A. 1972. On the calculation of heat production from open-circuit calorimetric measurements. Br. J. Nutr. 27:597.

Mosier, H. D., Jr., R. A. Jansons, and L. C. Dearden. 1985. Increased secretion of growth hormone in rats undergoing catch-up growth after fasting. Growth 49:346.

Mosier, H. D., Jr., R. A. Jansons, C. S. Biggs, S. M. Tanner, and L. C. Dearden. 1980. Metabolic clearance rate of growth hormone during experimental growth arrest and subsequent recovery in rats. Endocrinology 107:744.

Murphy, J. L., G. I. Bell, M. L. Duckworth, and H. G. Friesen. 1987. Identification, characterization, and regulation of a rat complementary deoxyribonucleic acid which encodes insulin-like growth factor-I. Endocrinology 121:684.

Murphy, T. A., and S. C. Loerch. 1994. Effects of restricted feeding of growing steers on performance, carcass characteristics, and composition. J. Anim. Sci. 72:2497.

Murphy, T. A., S. C. Loerch, and F. E. Smith. 1994. Effects of feeding high-concentrate diets at restricted intakes on digestibility and nitrogen metabolism in growing lambs. J. Anim. Sci. 72:1583.

Nishizawa, N., S. Igarashi, H. Itabashi, and S. Hareyama. 1989. Urinary excretion of N^{ε}-methylhistidine in goat. Nutr. Rep. Int. 40:567.

Peterson, R. G., and D. E. Waldern. 1981. Repeatabilities of serum constituents in Holstein-Friesians affected by feeding, age, lactation, and pregnancy. J. Dairy Sci. 64:822.

SAS, 1990. SAS User's Guide: Statistics (Version 6.06 Ed.). SAS Inst. Inc., Cary, NC.

Schwarz, F. J., D. Schams, R. Ropke, M. Kirchgessner, J. Kogel, and P. Matzke. 1993. Effects of somatotropin treatment on growth performance, carcass traits, and the endocrine system in finishing beef heifers. J. Anim. Sci. 71:2721.

Scott, S. L., R. J. Christopherson, J. R. Thompson, and V. E. Baracos. 1993. The effect of a cold environment on protein and energy metabolism in calves. Br. J. Nutr. 69:127.

Shaw, H. J., and G. R. Foxcroft. 1985. Relationships between LH, FSH and prolactin secretion and reproductive activity in the weaned sow. J. Reprod. Fertil. 75:17.

Trenkle, A. 1976. Estimates of the kinetic parameters of growth hormone metabolism in fed and fasted calves and sheep. J. Anim. Sci. 43:1035.

Trinder, P. 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem. 6:24.

Van den Brande, J. L. 1986. Catch-up growth. Possible mechanisms. Acta Endocrinol. 113 (S279):13.

VandeHaar, M. J., B. M. Moats-Staats, M. L. Davenport, J. -M. Ketelslegers, B. K. Sharma, and L. E. Underwood. 1991. Reduced serum concentrations of insulin-like growth factor-I (IGF-I) in protein-restricted growing rats are accompanied by reduced IGF-I mRNA levels in liver and skeletal muscle. J. Endocrinol. 130:305.

Williams, J. E., S. J. Miller, T. A. Mollett, S. E. Grebing, D. K. Bowman, and M. R. Ellersieck. 1987. Influence of frame size and zeranol on growth, compositional growth

and plasma hormone characteristics. J. Anim. Sci. 65:1113.

Yambayamba, E., and M. A. Price. 1991. Growth performance and carcass composition in beef heifers undergoing catch-up (compensatory) growth. Can. J. Anim. Sci. 71:1021.

Young, B. A., T. W. Fenton, and J. A. McLean. 1984. Calibration methods in respiratory calorimetry. J. Appl. Physiol. 56:1120.

Young, B. A., B. Kerrigan, and R. J. Christopherson. 1975. A versatile respiratory pattern analyzer for studies of energy metabolism of livestock. Can. J. Anim. Sci. 55:17.

CHAPTER THREE

3. Compensatory Growth of Carcass Tissues and Visceral Organs in Beef Heifers

3.1. Introduction

For years, the major aspect of investigation on compensatory growth has been its effect on the ultimate carcass composition in castrated male animals destined for meat production. Results have been variable. Some studies (Murray et al., 1977; Murray and Slezacek, 1988; Aziz et al., 1993) have also investigated the response of the non-carcass components during compensatory growth. Results have also been variable, basically due to differences in experimental designs. Little attention has been paid to young growing female livestock, although Yambayamba and Price (1991) have reported some preliminary results of carcass composition from 3-rib joint dissection in realimented heifers.

In order to understand the recovery patterns of the carcass tissues during compensatory growth, serial slaughter with full side carcass dissection is the most appropriate technique to employ (Nour and Thonney, 1987). Since feed utilization and the partitioning of nutrients to various tissues is influenced by visceral organs, it is equally important to understand their recovery patterns in order to explain the ultimate body composition of the animal. The objective of this study was to investigate the recovery patterns of muscle, bone, and fat; the

recovery patterns of various visceral organs, and their association with compensatory growth; and the contribution of gut fill to compensatory growth in beef heifers.

3.2. Materials and Methods

3.2.1. Animals and Experimental Design

Twenty-eight Hereford crossbred heifers weighing 222 kg (22 kg SD) and aged 226 d (10 d SD) on d 0 were used in this study. These heifers came from the same general pool as those described in study 1 (Chapter 2). After weaning, they were trucked to Ellerslie Research Station near Edmonton on the same day as those in study 1. During the period of adjustment (35 d) to the experimental diet (see Table 2-1), all the heifers were fed to appetite and fresh water was always available. On d 0, a pair of heifers was randomly selected for slaughter. Of the remaining 26 heifers, 12 were allotted to ad libitum feeding (ADLIB), and 14 to a maintenance ration for 92 d followed by realimentation (REST). They were housed in groups of seven in open-front pens with concrete floors, and wood shavings were used as bedding material. The heifers were weighed on d 0, and the ADLIB heifers continued on ad libitum feeding while the REST heifers received a maintenance ration to achieve zero gain over the 92-d period. They were fed once daily at 0900, and the left-over feed from the ADLIB pens was weighed before fresh feed was provided. The heifers were weighed weekly for the first 6 wk and thereafter every 2 wk. The ration for the ADLIB heifers was increased based on their consumption while that for the REST heifers was adjusted as necessary to maintain

constant live weight. On d 92, the REST heifers were introduced to ad libitum feeding by increasing the ration by 400 g/head/d until they were receiving feed in excess of appetite. This process took 2 to 3 wk.

3.2.2. Slaughter and Carcass Dissections

The heifers were trucked 130 km for slaughter and dissection at Agriculture and Agri-Food Canada Research Centre, Lacombe, Alberta. They arrived the day before slaughter and were fasted overnight. Two heifers were slaughtered on d 1 (INIT); the remaining heifers were randomly allocated to slaughter dates, with two heifers being slaughtered from each treatment on each occassion (Figure 3-1). The heifers were slaughtered over a wide range of live weights (200 to 450 kg), with the last pair from each group being slaughtered at equal live weight (about 450 kg).

Following stunning (captive bolt) and bleeding on the kill floor, the head, feet, skin, and tail were removed through normal commercial practice. After evisceration, kidney fat, channel fat, and omental fat were removed and weighed. The stomach (reticulo-rumen, omasum, and abomasum) and the intestines were separated and weighed full and empty. The other visceral organs were also weighed individually. Empty body weight (EBW) was taken as the weight of the animal before slaughter (full body weight, FBW) minus gut fill (weight of gut contents). The carcasses were split sagittally, weighed and chilled overnight at 3°C . The left sides were quartered between the 12th and 13th ribs, and each quarter was physically separated into muscle, bone and fat. The fat was further separated into subcutaneous fat

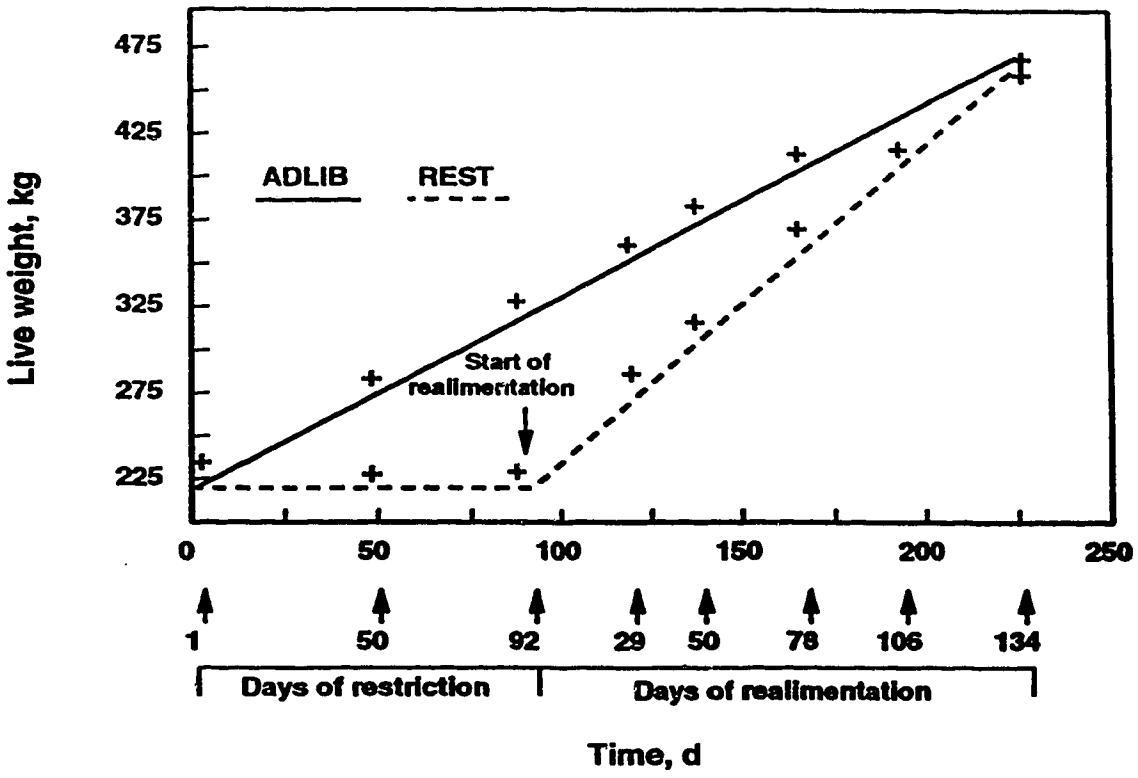


Figure 3-1. Schematic representation of the experimental design showing planned growth paths for ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers. The restricted heifers were realimented on d 92 (arrow); +: slaughter group of two heifers.

(SCF), intermuscular fat (IMF), and carcass cavity fat (CCF). Carcass cavity fat was defined as the fat on the internal surface of the rib cage.

3.2.3. Statistical Analysis

Because of the nature of the experiment (serial slaughter), the heifers in each treatment were assumed to be nested within period in order to meaningfully compute the ADG. Dummy variables were assigned to each animal (Overall and Klett, 1972) and regression analysis of live weights on time was done to compute the ADG. Paired *t-test* using the GLM procedures (SAS, 1990) was done to assess the difference between the regressions during the restriction and realimentation periods.

The weight of the carcass side was taken as the sum of the weights of its separated components and, similarly, total carcass fat weight was the sum of individual fat depot weights. Abdominal fat weight was the sum of kidney fat, channel fat, and omental fat weights. The weights of individual carcass tissues and fat depots were expressed as proportions of side weights and total fat weight, respectively. The visceral organ and abdominal fat weights were expressed as proportions of EBW while gut fill was expressed as a proportion of full body weight. These data were subjected to a two-way analysis of variance (SAS, 1990) to compare the treatment effects within period and treatment x time interactions. Comparison of body composition between INIT heifers and the other groups was done only during the restriction period to monitor the changes that took place, particularly in REST heifers.

The carcass and non-carcass data were transformed to logarithms in order to transform the allometric equation, $Y = aX^b$, to a linear relationship: log Y = log a + b log X, for representing changes in growth and development during compensatory growth. The visceral organ weights, individual side carcass component weights, and fat depot weights (Y) were regressed on EBW, side weight, and total side fat weight (X), respectively, to compute growth coefficients (b values). The b values were analyzed to determine whether they differed significantly from 1.0. The b values of the two regressions were then compared using the paired *t-test* GLM procedures (SAS, 1990) to assess the treatment effects.

3.3. Results

3.3.1. Growth Performance

By design, the body weights of REST heifers were maintained relatively constant during the feed restriction period while ADLIB heifers were gaining at 1.37 ± .04 kg/d (Figure 3-2). During realimentation, the ADG of REST heifers was higher (P = .001) than that of ADLIB heifers (1.99 ± .09 vs .96 ± .09 kg/d). By d 134 of realimentation, the REST heifers had caught up to the ADLIB heifers in live weight. The average feed consumption by the REST group was also higher than that of the ADLIB group during this period. However, overall the REST heifers consumed less feed (P = .001) than the ADLIB heifers (5,436 vs 6,780 kg).

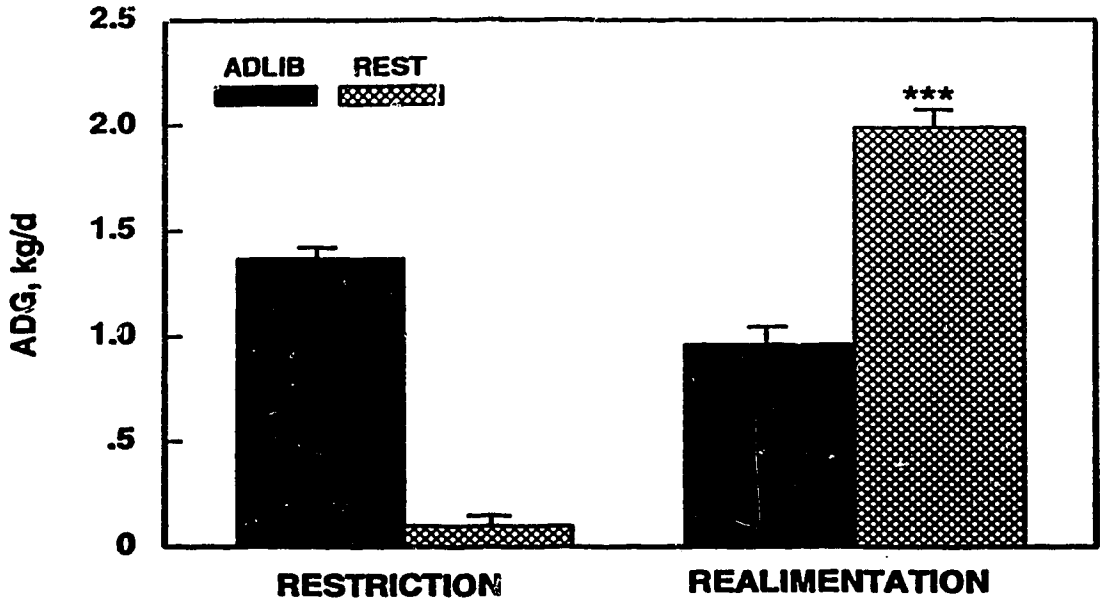


Figure 3-2. Average daily gains (ADG) of ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers. The feed restriction period lasted 92 d, followed by realimentation. ***P < .001.

3.3.2. Body Composition

3.3.2.1. Carcass Tissues

The carcass data are presented in Table 3-1. There were significant effects of treatment ($P < .001$) and time ($P < .01$) on the proportions of the carcass tissues. There were also treatment effects ($P < .001$) on the proportions of the fat depots except CCF. There was no significant treatment x time interaction on any of the tissues. During feed restriction, there was no significant effect ($P > .05$) on the proportion of side muscle. However, the proportion of side bone was higher ($P < .05$) in the REST than in the INIT group, which was higher than in the ADLIB group. Conversely, the proportion of total side fat was lower ($P < .05$) in the REST than in the INIT group, which was lower than in the ADLIB group on both slaughter days. The SCF proportion tended to be lower ($P < .10$) in REST than in INIT and ADLIB heifers on both days; the IMF proportion tended to be higher ($P = .08$) in REST than in INIT and ADLIB heifers on d 50, and it was significant ($P = .02$) on d 92. The proportion of CCF was unaffected by feed restriction.

During realimentation, side muscle proportion did not exhibit any significant differences between REST and ADLIB heifers, although there was a tendency ($P < .10$) towards a higher proportion in the former than in the latter on d 50 and 78. The effects of feed restriction on side bone proportion were still evident 29 d into realimentation, as the proportion was higher ($P = .04$) in REST than in ADLIB heifers. The difference, however, slowly disappeared through the course of realimentation and by d 134, no difference between treatments was apparent. Conversely, side fat proportion was lower ($P = .05$) in REST than in ADLIB

Table 3-1. Least squares means ± SE of carcass tissue weights relative to side weight (g/kg), and fat depot weights relative to total side fat weight (g/kg) of heifers slaughtered on d 1 (INIT), ad libitum fed (ADLIB), and feed restricted-refed (REST) heifers

Day of slaughter	INIT[a] (n = 2)	ADLIB (n = 2)	REST (n = 2)	P
Restriction period				
d 50 (d 1 for INIT)				
Side muscle	614 ± 14	573 ± 30	635 ± 28	.17
Side bone	208 ± 4[c]	182 ± 8[b]	227 ± 4[d]	.05
Side fat	178 ± 10[c]	246 ± 19[d]	138 ± 14[b]	.05
Subcutaneous fat	296 ± 13	366 ± 33	268 ± 38	.09
Intermuscular fat	607 ± 14	532 ± 31	622 ± 28	.08
Carcass cavity fat	97 ± 27	103 ± 5	109 ± 9	.31
d 92 (d 1 for INIT)				
Side muscle	614 ± 14	583 ± 25	639 ± 37	.17
Side bone	208 ± 4[c]	177 ± 9[b]	243 ± 18[d]	.04
Side fat	178 ± 10[c]	239 ± 24[d]	119 ± 1[b]	.04
Subcutaneous fat	296 ± 13	351 ± 28	260 ± 19	.06
Intermuscular fat	607 ± 14[bc]	554 ± 18[b]	644 ± 10[c]	.02
Carcass cavity fat	97 ± 27	94 ± 9	96 ± 9	.44
Realimentation period				
d 29				
Side muscle	-	577 ± 42	650 ± 18	.13
Side bone	-	168 ± 7	204 ± 9	.04
Side fat	-	255 ± 35	145 ± 9	.05
Subcutaneous fat	-	353 ± 12	250 ± 15	.02
Intermuscular fat	-	548 ± 3	625 ± 50	.13
Carcass cavity fat	-	99 ± 15	125 ± 35	.28
d 50				
Side muscle	-	554 ± 16	615 ± 20	.07
Side bone	-	168 ± 9	180 ± 1	.16
Side fat	-	277 ± 24	205 ± 20	.07
Subcutaneous fat	-	355 ± 2	332 ± 7	.04
Intermuscular fat	-	540 ± 14	550 ± 4	.28
Carcass cavity fat	-	104 ± 16	118 ± 3	.24
d 78				
Side muscle	-	531 ± 24	593 ± 10	.07
Side bone	-	153 ± 1	181 ± 13	.08
Side fat	-	316 ± 24	225 ± 3	.03
Subcutaneous fat	-	361 ± 16	318 ± 13	.09
Intermuscular fat	-	540 ± 13	578 ± 8	.06
Carcass cavity fat	-	99 ± 4	105 ± 4	.20
d 134				
Side muscle	-	518 ± 2	528 ± 17	.31
Side bone	-	146 ± 9	150 ± 10	.40
Side fat	-	336 ± 11	322 ± 27	.34
Subcutaneous fat	-	350 ± 12	358 ± 8	.32
Intermuscular fat	-	564 ± 17	553 ± 4	.30
Carcass cavity fat	-	86 ± 5	89 ± 5	.36

[a]Comparisons between INIT heifers and the other groups were done only during the restriction period.
[b,c,d]Means within a row with different superscripts differ.

heifers on d 29, 50, and 78. The proportion of side fat slowly increased in both treatments, and at equal live weight (d 134), there was no difference between treatments. The proportion of side SCF was lower ($P < .05$) in REST than in ADLIB heifers on d 29 and 50, and to a smaller degree ($P = .09$) on d 78 of realimentation; complete recovery had taken place by d 134. The side IMF proportion in REST heifers decreased gradually and no significant differences were detected between treatments at any time during realimentation. The side CCF proportion was not affected by treatment to any significant degree.

3.3.2.2. Visceral Organs

The data on visceral organ proportions are presented in Table 3-2. There were significant treatment and time effects ($P < .01$) on the proportions of liver, kidneys, spleen, and pancreas. The proportion of liver was lower ($P = .05$) in REST than in ADLIB heifers on d 50 of feed restriction, while INIT heifers were intermediate. The proportions of heart, kidneys, lungs + trachea, spleen, pancreas, and adrenal glands were not significantly affected by feed restriction. On d 92, the proportions of liver ($P = .04$) and spleen ($P = .01$) were lower in REST than those in INIT and ADLIB heifers. No treatment differences were observed in the proportions of the other organs during this period.

During realimentation, the proportions of liver and spleen in REST heifers recovered rapidly and no differences between these heifers and ADLIB heifers existed by d 29. On d 50 and 78, the proportion of liver in REST heifers was higher ($P = .01$) than that of ADLIB heifers. By d 134, however, the proportions were similar. This pattern of recovery was also evident in the spleen. No significant effect was observed in the other organs except for the

Table 3-2. Least squares means ± SE of visceral organ weights relative to empty body weight (g/kg) of heifers slaughtered on d 1 (INIT), ad libitum fed, (ADLIB) and feed restricted-refed (REST) heifers

Day of slaughter	INIT[a] (n = 2)	ADLIB (n = 2)	REST (n = 2)	P
Restriction period				
d 50 (d 1 for INIT)				
Liver	17.1 ± .7[bc]	19.4 ± 1.3[c]	15.3 ± 1.1[b]	.05
Heart	5.4 ± .4	5.9 ± .6	6.2 ± .6	.38
Kidneys	2.6 ± 0.0	3.0 ± .3	2.9 ± .3	.39
Lungs + Trachea	21.4 ± .4	19.3 ± 2.1	16.8 ± 1.0	.06
Spleen	2.04 ± .1	2.2 ± 0.0	2.1 ± .2	.40
Pancreas	.85 ± .2	.94 ± .1	1.3 ± .1	.06
Adrenal glands	.05 ± .01	.06 ± 0.0	.07 ± .01	.33
d 92 (d 1 for INIT)				
Liver	17.1 ± .7[c]	15.7 ± .3[c]	13.7 ± .7[b]	.04
Heart	5.4 ± .4	4.8 ± .4	5.4 ± .9	.30
Kidneys	2.6 ± .04	2.2 ± 0.0	2.5 ± .05	.11
Lungs + Trachea	21.4 ± 1.4	17.5 ± 1.1	17.7 ± 2.8	.18
Spleen	2.04 ± .1[c]	1.7 ± .3[c]	1.0 ± .1[b]	.01
Pancreas	.85 ± .2	.69 ± .1	.68 ± .02	.23
Adrenal glands	.05 ± 0.0	.06 ± 0.0	.05 ± .03	.43
Realimentation period				
d 29				
Liver	-	13.8 ± .6	15.2 ± 1.0	.18
Heart	-	4.9 ± .3	5.0 ± .3	.48
Kidneys	-	2.5 ± 0.0	2.8 ± .3	.21
Lungs + Trachea	-	16.3 ± 1.7	15.9 ± 1.5	.44
Spleen	-	1.7 ± .1	1.7 ± .1	.50
Pancreas	-	.82 ± .2	.63 ± .1	.08
Adrenal glands	-	.06 ± 0.0	.05 ± .01	.37
d 50				
Liver	-	15.3 ± .1	16.4 ± .1	.01
Heart	-	5.1 ± .6	5.3 ± .9	.44
Kidneys	-	2.1 ± .3	2.4 ± .01	.21
Lungs + Trachea	-	16.0 ± .7	14.6 ± 1.7	.26
Spleen	-	1.7 ± .01	1.7 ± .1	.50
Pancreas	-	.64 ± .3	.74 ± .3	.42
Adrenal glands	-	.07 ± 0.0	.06 ± .01	.24
d 78				
Liver	-	12.8 ± .1	15.7 ± .9	.04
Heart	-	4.7 ± .1	4.7 ± .6	.50
Kidneys	-	2.0 ± .1	2.6 ± .3	.10
Lungs + Trachea	-	16.4 ± 1.2	16.4 ± .01	.50
Spleen	-	1.3 ± .1	1.9 ± .1	.03
Pancreas	-	.59 ± 0.0	.92 ± .1	.05
Adrenal glands	-	.05 ± 0.0	.05 ± .03	.47
d 134				
Liver	-	14.4 ± 1.4	14.6 ± .9	.46
Heart	-	4.5 ± 0.0	4.5 ± .01	.50
Kidneys	-	1.9 ± .1	1.9 ± .1	.50
Lungs + Trachea	-	17.5 ± 1.8	16.2 ± .01	.27
Spleen	-	1.5 ± .1	1.8 ± .1	.08
Pancreas	-	1.1 ± .2	.99 ± .1	.34
Adrenal glands	-	.05 ± 0.0	.05 ± .01	.48

[a]Comparisons between INIT heifers and the other groups were done only during the restriction period.
[b,c]Means within a row with different superscripts differ.

proportion of the pancreas which was higher (P = .05) in REST than in ADLIB heifers on d 78. Interactions between nutritional treatment and time were significant for the proportions of liver (P = .02) and spleen (P = .01), and these are presented in Figures 3-3 and 3-4, respectively.

3.3.2.3. Gastrointestinal Tract and Abdominal Fat

The GIT was divided into stomach (reticulo-rumen, omasum and abomasum) and intestines. Results are presented in Table 3-3. There were significant treatment and time effects (P < .01) on the proportions of empty stomach and intestines. The proportion of empty stomach was higher (P = .03) in ADLIB and REST than in INIT heifers on d 50 of feed restriction; on d 92, its proportion in REST heifers had fallen to that of INIT heifers, subsequently being lower (P = .04) than in ADLIB heifers. The proportion of empty intestines was similar among the groups on d 50, but it was lower (P = .04) in REST than in ADLIB heifers on d 92 of feed restriction. The proportion of abdominal fat was lower (P < .05) in REST and INIT than in ADLIB heifers on both d 50 and 92 of feed restriction.

During realimentation, the proportions of stomach and intestines in REST heifers recovered very rapidly and by d 29, no differences between these heifers and ADLIB heifers were apparent. The proportion of stomach in REST heifers actually recovered above the controls and a significant difference (P = .02) was observed on d 78. This significant treatment x time interaction (P = .01) for stomach is illustrated in Figure 3-5. The proportion of intestines was lower (P = .03) in REST than in ADLIB heifers on d 134. Unlike the GIT, abdominal fat recovered slowly during realimentation, remaining lower (P < .05) in REST

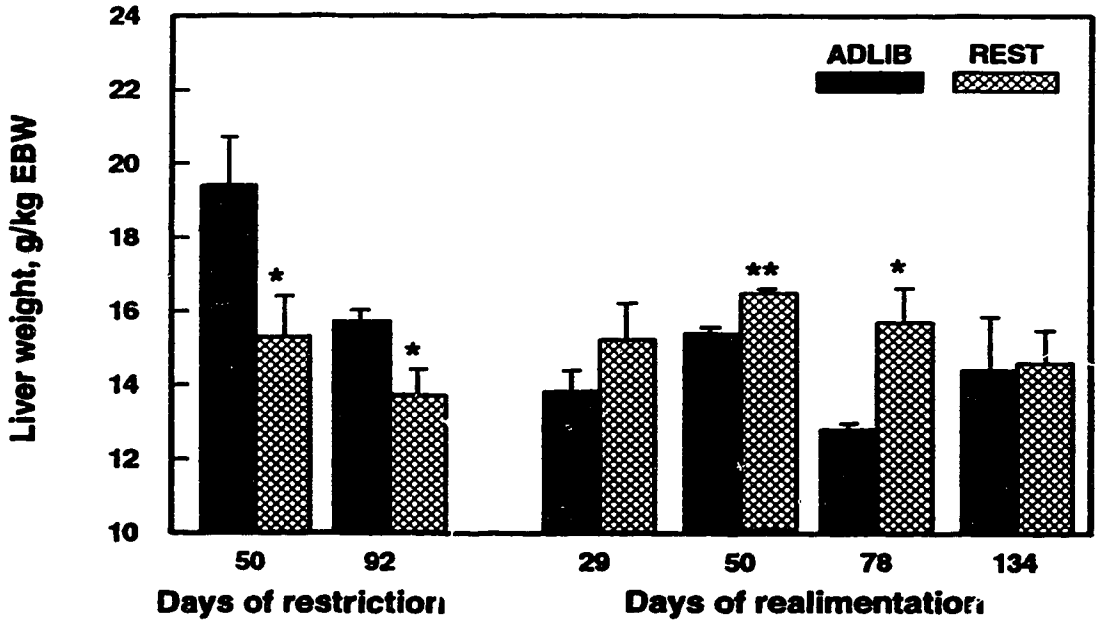


Figure 3-3. Liver weight expressed as a proportion of empty body weight (EBW) of ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers. *P < .05, **P < .01.

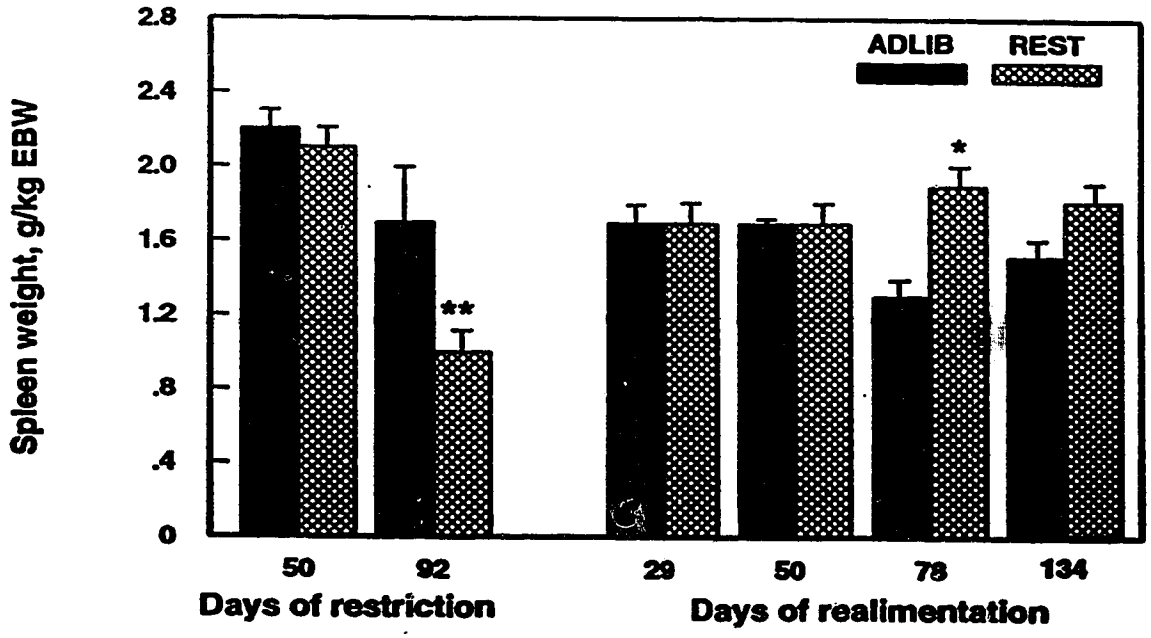


Figure 3-4. Spleen weight expressed as a proportion of empty body weight (EBW) of ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers. *P < .05, **P < .01.

Table 3-3. Least squares means ± SE of gastrointestinal tract and abdominal fat weights relative to empty body weight (g/kg) of heifers slaughtered on d 1 (INIT), ad libitum fed (ADLIB), and feed restricted-reted (REST) heifers

Day of slaughter	INIT[a] (n = 2)	ADLIB (n = 2)	REST (n = 2)	P
Restriction period				
d 50 (d 1 for INIT)				
Empty stomach	37.0 ± .6[b]	47.6 ± 2.7[c]	47.7 ± 2.3[c]	.03
Empty intestines	40.2 ± 3.4	39.5 ± 2.9	37.3 ± 2.7	.32
Abdominal fat	25.8 ± 1.6[b]	50.8 ± 5.7[c]	28.7 ± 4.1[b]	.04
d 92 (d 1 for INIT)				
Empty stomach	37.0 ± .6[b]	45.4 ± 3.0[c]	32.6 ± 2.1[b]	.04
Empty intestines	40.2 ± 3.4[bc]	46.1 ± 2.8[c]	34.3 ± 2.1[b]	.04
Abdominal fat	25.8 ± 1.6[b]	40.3 ± 4.3[c]	17.7 ± 3.9[b]	.03
Realimentation period				
d 29				
Empty stomach	-	38.1 ± 2.8	43.2 ± 2.7	.16
Empty intestines	-	41.7 ± 2.8	40.2 ± 2.5	.36
Abdominal fat	-	47.2 ± 5.1	27.2 ± 4.0	.05
d 50				
Empty stomach	-	39.9 ± 2.5	45.7 ± 3.0	.14
Empty intestines	-	51.0 ± 3.1	42.7 ± 2.6	.09
Abdominal fat	-	45.4 ± 2.9	30.6 ± 2.5	.03
d 78				
Empty stomach	-	33.0 ± 2.6	52.1 ± 2.9	.02
Empty intestines	-	47.1 ± 2.2	43.5 ± 2.3	.19
Abdominal fat	-	56.1 ± 3.5	32.9 ± 2.8	.02
d 134				
Empty stomach	-	31.6 ± 2.4	34.1 ± 2.7	.28
Empty intestines	-	54.6 ± 2.7	39.1 ± 2.7	.03
Abdominal fat	-	63.6 ± 7.0	50.2 ± 7.0	.15

[a]Comparisons between INIT heifers and the other groups were done only during the restriction period.
[b,c]Means within a row with different superscripts differ.

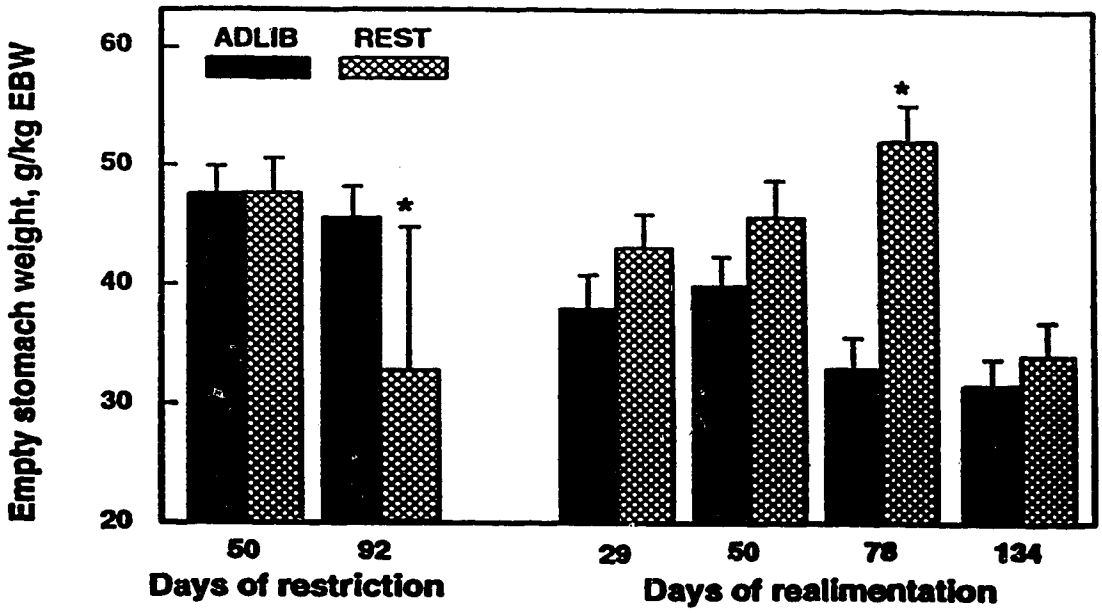


Figure 3-5. Empty stomach (reticulo-rumen + omasum + abomasum) weight expressed as a proportion of empty body weight (EBW) of ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers. *P < .05.

than in ADLIB heifers through most of the period. However, at the final slaughter weight, abdominal fat level was similar in both treatments.

3.3.3. Gut Fill

There were significant treatment and time effects ($P < .01$) on the proportion of gut fill. The treatment x time interaction was marginally significant ($P = .06$). On d 50 and 92 of feed restriction, the proportion of gut fill in REST heifers was higher ($P = .03$) than that of ADLIB heifers (Figure 3-6). As the experiment progressed during realimentation, there was a general fall in the gut fill proportion in both REST and ADLIB heifers but it was still higher ($P < .05$) in the former than in the latter except at equal live weight (d 134). In absolute terms, gut fill was larger ($P = .03$) in REST than in ADLIB heifers on d 50 of feed restriction (36.8 ± .2 vs 28.4 ± 2.2 kg). However, the amounts were similar on d 92 and through the whole realimentation period.

3.3.4. Allometry

Regression coefficients for carcass and non-carcass data are presented in Table 3-4. The growth coefficients for side muscle were not significantly affected by treatment; however, the *b* value for the REST group (.90 ± .07) was not different from 1.0 ($P = .12$), while that for the ADLIB group (.83 ± .06) was significantly less than 1.0 ($P = .02$). The *b* values for side bone were not different between treatments although that for REST heifers tended to be

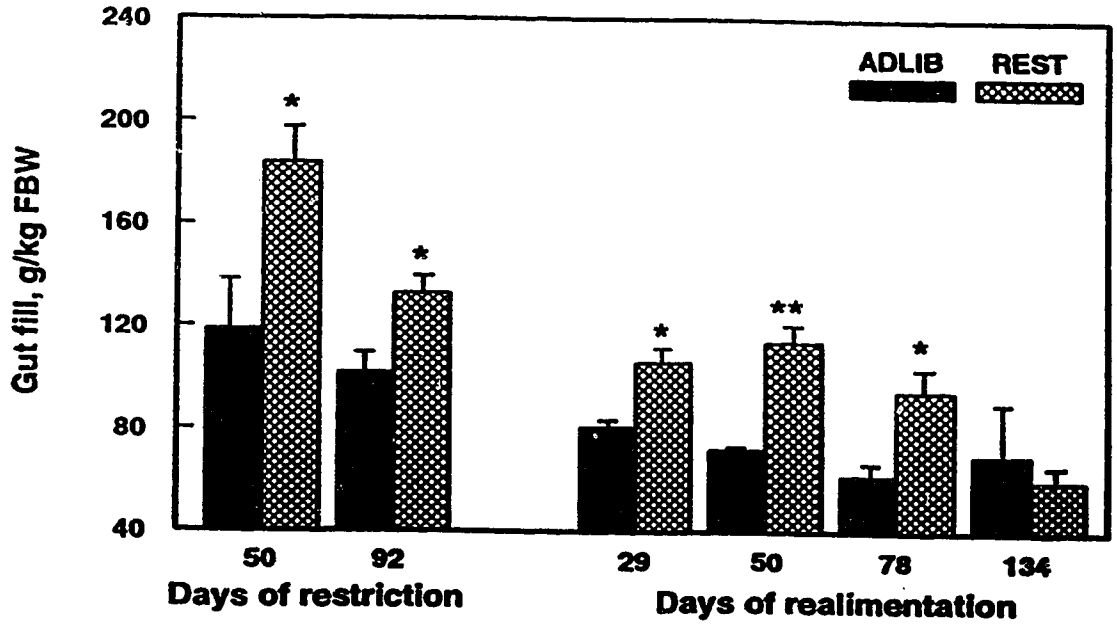


Figure 3-6. Gut fill weight expressed as a proportion of full body weight (FBW) at slaughter of ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers. *P < .05, **P < .01.

Table 3-4. Growth of side carcass tissues, side carcass fat depots, and visceral organs (Y, kg) relative to side weight, total side fat weight, and empty body weight (X, kg), respectively, in ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers[a]

	ADLIB (n = 14)			REST (n = 12)			
Dependent variable	*a*	*b* ± SE	R^2	*a*	*b* ± SE	R^2	P[b]
Carcass tissues							
Log muscle wt	.08	.83 ± .06	.94	.01	.90 ± .07	.93	.23
Log bone wt	.03	.59 ± .06	.89	.37	.43 ± .08	.73	.06
Log fat wt	-1.90	1.66 ± .16	.90	-2.91	2.14 ± .17	.94	.03
Fat depots							
Log subcutaneous fat wt	-0.62	1.12 ± .04	.94	-0.77	1.21 ± .04	.99	.01
Log intermuscular fat wt	-0.18	.94 ± .03	.99	-0.13	.91 ± .03	.99	.03
Log carcass cavity fat wt	-0.96	.96 ± .10	.87	-0.86	.91 ± .08	.93	.33
Visceral organs							
Log liver wt	-0.81	.60 ± .08	.80	-1.97	1.06 ± .10	.92	.01
Log heart wt	-1.66	.74 ± .10	.81	-1.65	.73 ± .13	.73	.48
Log kidneys wt	-1.42	.51 ± .11	.61	-1.81	.67 ± .14	.68	.19
Log lungs + trachea wt	-1.03	.71 ± .11	.77	-1.59	.91 ± .14	.78	.13
Log spleen wt	-1.59	.53 ± .14	.51	-3.0	1.07 ± .24	.45	.03
Log pancreas wt	-2.41	.72 ± .34	.41	-4.98	1.78 ± .42	.60	.03
Log adrenals wt	-4.06	.92 ± .18	.65	-4.22	.97 ± .15	.79	.42
Log abdominal fat wt	-3.07	1.69 ± .27	.74	-4.34	2.15 ± .17	.93	.09
Log stomach wt	-0.55	.65 ± .10	.73	-1.81	1.18 ± .21	.73	.01
Log intestines wt	-2.08	1.30 ± .11	.91	-1.65	1.10 ± .12	.88	.24

[a]The parameter estimates were derived from the allometric equation $Y = aX^b$ in its linear form $\log_{10}Y = \log_{10}a + b.\log_{10}X$.
[b]Probability of treatment difference in *b* values.

greater (P = .06) than that for ADLIB heifers; both values were significantly less than 1.0 (P < .001). The *b* value for side fat was greater (P = .03) in REST than in ADLIB heifers, and both values were significantly greater than 1.0 (P = .001).

The growth coefficient for SCF was greater (P = .01) in the REST group than in the ADLIB group. Also the SCF *b* value for REST heifers was greater than 1.0 (P = .001) while that for the ADLIB group was not different from 1.0 (P = .60). On the other hand, the growth coefficient for side IMF was smaller (P = .03) in the REST than in the ADLIB group, although neither value was significantly different from 1.0 (P > .05). The growth coefficients for side CCF were similar between treatments and were not different from 1.0 (P > .05).

The *b* values for liver, spleen, pancreas and stomach weights were greater (P < .05) in REST than in ADLIB heifers, while no significant differences (P > .05) between treatments were observed in the other organs. The *b* values for liver and spleen weights were not different from 1.0 (P > .05) in the REST heifers but they were less than 1.0 (P < .05) in the ADLIB heifers. On the other hand, the *b* values for pancreas and stomach weights were greater than 1.0 (P < .05) in REST heifers and less than 1.0 (P < .05) in ADLIB heifers. The *b* values for heart, kidneys, and lungs + trachea weights were generally less than 1.0 (P < .05) except in REST heifers where the *b* value for lungs + trachea weight was not different from 1.0 (P = .57). The *b* values for adrenal gland weights were not different from 1.0 (P > .05) in either treatment, while those for abdominal fat weight were greater than 1.0 (P < .05). For the intestines, the *b* value was not different from 1.0 (P = .44) in the REST group but was greater than 1.0 (P = .02) in the ADLIB group.

3.4. Discussion

3.4.1. Growth Rates

By design, the body weights of REST heifers were maintained relatively constant during feed restriction while ADLIB heifers gained 1.37 kg/d. During the realimentation period, the ADG of REST heifers was higher than that of ADLIB heifers (1.99 vs .96 kg/d), particularly during the first 6 to 8 wk, with some individual heifers gaining up to 2.8 kg/d. Similar results have been reported in beef heifers (Yambayamba and Price, 1991) and steers (Carstens et al., 1989).

The extent of compensatory growth depends on several factors including nature and severity of feed restriction, duration of the feed restriction period, age of the animal when feed restriction was imposed, and pattern of realimentation (Wilson and Osbourn, 1960). Although gut fill has been reported to contribute to apparent compensatory growth in cattle (Baker et al., 1985; Carstens et al., 1989), the high growth rates during realimentation have been largely attributed to increased efficiency of energy and protein utilization (Fox et al., 1972), increased feed intake, decreased maintenance requirement, and changes in the composition of gain (Carstens et al., 1989). Studies of hormonal and metabolic shifts during compensatory growth have somewhat clarified some mechanisms controlling compensatory growth (see Chapters 1 and 2). Considering the differences in the experimental designs and environmental conditions, it is not surprising that results obtained by different workers have been variable. Results from the present study clearly indicate the occurence of compensatory

growth in beef heifers.

3.4.2. Body Composition

3.4.2.1. Carcass Composition

Fat, being the most variable tissue in the carcass (Berg and Butterfield, 1976), has a large influence on the proportions of bone and muscle. In the present study, the lower proportion of total side fat and the consequent higher proportion of side bone in REST heifers compared to ADLIB and INIT heifers was a reflection of the utilization of fat reserves for energy during feed restriction. On the other hand, there was no significant effect on the proportion of side muscle during this period. Price (1977) observed the same trend in restricted steers: while those steers exhibited a significant decrease and increase in the fat and bone proportion, respectively, there was a smaller, though significant increase in the muscle proportion. According to Price (1977), both muscle and fat are lost in more or less equal absolute amounts during feed restriction, but the proportion of fat lost is much higher because it is a smaller tissue. Since muscle has a primary functional role, manifested in its allometric relationship with live weight, the absolute weight of muscle is closely related to the bulk that it has to support, a concept that Price (1977) has called "homeomyosis".

Within the fat tissue, fat depots were affected differentially by feed restriction. Berg and Butterfield (1976) observed that fat depots follow an order of depletion during feed restriction, opposite to the direction of their development. In this situation, the undernourished animal would first utilize the SCF depot, which was the tendency in the

present study. The higher proportion of side IMF observed in REST heifers compared to ADLIB heifers and the non-significance between treatments in terms of side CCF proportion were a reflection of the greater mobilization of the SCF depot in the restricted animals.

During realimentation of the REST heifers, there was an increase in the proportion of total side fat over time, causing side muscle and bone proportions to decrease as full feeding progressed. By d 29 of realimentation, the proportion of total side fat in REST heifers had increased by 22% from that in heifers slaughtered at the end of the restriction period. By d 134, there were no differences in the side carcass composition between treatments. These results agree with the previous study (Yambayamba and Price, 1991), in which carcass composition was estimated from 3-rib joint dissections.

The effect of realimentation on fat depots was an increase in the proportion of SCF and a decrease in the proportion of IMF in REST heifers, opposite to the trends observed during feed restriction. Realimentation, however, did not significantly affect CCF proportion. These results are consistent with those reported in steers (Tatum et al., 1986) in which as total side fat increased, the proportion of IMF declined slightly while the proportion of SCF increased and that of internal fat remained relatively constant.

3.4.2.2. Visceral Organs

Among the visceral organs studied, only the liver and the spleen were significantly affected by feed restriction. Since the liver is the centre for intermediary metabolism (Stangassinger and Giesecke, 1986), feed restriction would be expected to decrease its metabolic activity and size (Burrin et al., 1990). Several studies in ruminants (Reid et al.,

1980; Ferrell et al., 1986; Aziz et al., 1993) have reported decreases in the absolute and relative size of the liver. The lower proportion of spleen in REST heifers on d 92 of feed restriction was also expected. Seebeck (1967) found similar results in steers, and since one of the functions of the spleen is to store blood for release under stressful conditions, he suggested that the lower spleen weight may have been related to a concomitant reduction in blood weight.

It was not clear why feed restriction did not have a significant effect on the proportions of kidneys. These results do not agree with those reported for lambs (Ferrell et al., 1986; Aziz et al., 1993) but do agree with those observed in beef heifers (Yambayamba and Price, 1992) subjected to 2 and 4 mo of feed restriction. It may be speculated that feed restriction in the present study was not severe enough to cause any significant effect on these organs.

The proportions of heart and lungs + trachea were not altered by feed restriction. This was not surprising, since the work performed by these organs is proportional to the size of the body. The proportion of pancreas was also unaffected by feed restriction, contrary to the results observed by Aziz et al. (1993) in lambs. Aziz et al. (1993) interpreted their finding in terms of the role the pancreas plays in the secretion of digestive fluids. They noted that the reduction in this organ's weight during weight loss seemed logical since feed intake normally declines during weight loss. The finding in the present study suggests that feed restriction was not severe enough to influence digestive function. The same explanation could account for the lack of effect of feed restriction on the adrenal glands.

During realimentation, there was a rapid response in the proportions of the liver and spleen of REST heifers. It can be seen in Figure 3-3 that by d 29 of realimentation, the liver

proportion in REST heifers had already caught up with that of ADLIB heifers, and was significantly higher by d 50. The higher liver proportion in REST heifers was observed for a greater part of the realimentation period. A similar pattern of recovery was observed in the proportion of spleen (Figure 3-4); by d 29 of realimentation, full recovery had taken place and there was no significant difference between treatments. Since the spleen stores blood, and since there is an increased blood flow to the portal drained viscera during feed intake (Lapierre et al., 1992), it may be concluded that the rapid response of the spleen during realimentation of REST heifers was due to increased cardiac output and increased capacity to store blood by this organ. The other organs were not affected by treatment during realimentation, except for the pancreas proportion which was higher in REST than in ADLIB heifers on d 78.

3.4.2.3. Gastrointestinal Tract and Abdominal Fat

While the proportion of empty stomach was higher in REST and ADLIB than in INIT heifers on d 50 of feed restriction, there was a significant fall in its proportion in REST heifers by d 92. Similarly, the effect of feed restriction on the proportion of empty intestines was not observed until d 92. This lag in the response to nutrition was unexpected since the GIT, like liver, normally responds very rapidly to nutritional demands (Murray et al., 1977). The proportion of abdominal fat in REST heifers stayed relatively constant during the 50-d period of feed restriction while that in ADLIB heifers increased significantly above the baseline (INIT). By d 92, however, the proportion in REST heifers had marginally fallen, but this was not significantly different from the baseline. This lack of significant effect by feed restriction

on abdominal fat agrees with the concept that internal fat is least affected by feed restriction (Berg and Butterfield, 1976).

Realimentation of REST heifers was characterized by a rapid weight recovery in both empty stomach and empty intestines. By d 29 of realimentation, the proportions of these organs in REST heifers were similar to those of ADLIB heifers, and stayed so throughout the realimentation period. Although the proportion of intestines was lower ($P = .03$) in REST than in ADLIB heifers on d 134, this was considered to happen by chance, since full recovery had been observed in REST heifers slaughtered on d 29, 50, and 78. Nutritional treatment x time interactions were significant for the proportion of stomach, suggesting a temporary over compensation in this organ (Figure 3-5). Considering the active role that the GIT plays in metabolism, the rapid response observed during realimentation was expected. On the other hand, the response of abdominal fat to realimentation was slow, with its proportion in REST heifers being lower than in ADLIB heifers for a greater part of the period. From these results, it would seem that nutritional treatment did not have a significant effect on internal fat until later.

3.4.5. Gut Fill

The larger amount and proportion of gut fill observed in REST heifers during the restriction period was an interesting finding. All things being equal, one would expect that feed restriction would be associated with significantly smaller amounts of gut fill compared with ad libitum feeding (Carstens et al., 1989). However, Okine and Mathison (1991) and

Luginbuhl et al. (1994) found that low feed intakes in cattle were associated with reduced passage rate of digesta from the reticulo-rumen to the lower gut. Thus, the decreased passage rate of digesta in the feed restricted animals would result in increased retention of the ingested feed, supposedly for maximum utilization under such conditions (Hicks et al., 1990).

The weight of gut fill relative to live weight in REST heifers continued to decline during realimentation, suggesting that compensatory growth was not due to gut fill, contrary to some suggestions (Baker et al., 1985; Carstens et al., 1989). In his review, Hogg (1991) concluded that the rapidly changing gut fill in restricted-refed ruminants could make a substantial contribution to the apparent increase in live weight gain. Results in the present study show that the absolute amount of gut fill in the early stages of realimention when compensatory growth was supposedly taking place, was not different from the control animals. Thus, compensatory growth in the present study represented the actual rapid hypertrophy of muscle and other tissues.

3.4.6. Allometry

3.4.6.1. Carcass Tissues and Fat Depots

The present growth coefficients for side muscle are similar to those reported in heifers restricted for 2 mo followed by realimentation (Yambayamba and Price, 1991). Similarly, Murray et al. (1974) found a higher *b* value for side muscle growth in the low fed steers (*b* = .844) compared to the ad libitum fed steers (*b* = .573). In the present study although no significant differences were found between the two *b* values for muscle, the *b* value for the

ADLIB heifers was significantly less than 1.0 while that for the REST heifers was not different from 1.0. These results suggest that muscle may have been growing relatively faster in the realimented heifers than in the ADLIB heifers. If so, this would confirm the claim that compensatory growth was real rather than being attributable to gut fill. Others have shown significant increases in muscle protein (Hayden et al., 1993), RNA (Howarth and Baldwin, 1971), and DNA (Trenkle et al., 1978). In all, the present results on muscle recovery pattern can be logically interpreted in terms of rebalancing the muscle:bone ratio which is "deflected" during feed restriction (Berg and Butterfield, 1976).

The growth coefficients for side bone relative to side weight were similar in both treatments and significantly less than 1.0. Nutritional restriction has been shown to have little effect on bone growth in cattle (Murray et al., 1974, Price, 1977, Yambayamba and Price, 1991). The growth coefficients for total side fat relative to side weight in both treatments were significantly greater than 1.0. However, the *b* value for the realimented heifers was significantly greater than that of the ad libitum fed heifers, as expected, considering that fat is the most labile carcass tissue. This means that during realimentation, both side muscle and side fat were growing relatively faster in REST than in ADLIB heifers. This would move them towards rebalancing the muscle:bone ratio and attaining the fat level appropriate for their age. Despite the higher growth coefficients for side fat relative to side weight in REST heifers during realimentation, the carcass composition was similar to ADLIB heifers at equal live weight, presumably because the period of fat deposition was so shortened that total fat deposition did not exceed that in the ADLIB heifers.

Considering the relative growth of side fat depots, the SCF depot was accumulating

relatively faster in REST heifers (b > 1.0) than in ADLIB heifers (*b* not different from 1.0). No differences between the *b* values in the other two depots were found, and these values were not different from 1.0. The growth coefficients found for SCF and IMF in the unrestricted heifers of the present study are similar to those reported by Tatum et al. (1986). It can be concluded from these results that during realimentation, most of the fat accumulation in REST heifers was stored in the subcutaneous depot.

3.4.6.2. Visceral Organs and Abdominal Fat

The greater growth coefficients for liver, spleen, pancreas, and stomach weights in REST than in ADLIB heifers during the realimentation period indicated a great capacity for recovery of these organs. While the *b* values for liver and spleen in the realimented heifers were not different from 1.0, they were greater than 1.0 for stomach and pancreas. The *b* values for these organs in ADLIB heifers were all less than 1.0. These results suggest a higher metabolic activity in these organs in the realimented heifers compared to the control heifers.

The *b* values for heart, kidneys, and lungs + trachea were similar in both treatments and were all less than 1.0, except for lungs + trachea (*b* not different from 1.0) in the realimented heifers. These results suggest that these organs do not necessarily respond to nutritional demands significantly as in the case of the liver and GIT. Similar results were reported for steers (Seebeck, 1967). While the *b* values for abdominal fat were greater than 1.0 in both treatments, its accumulation tended to be faster in the realimented heifers than in ADLIB heifers, eventually catching up by d 134.

3.5. Conclusions

Feed restriction followed by realimentation had no effect on the ultimate body composition of beef heifers in the present study. While feed restriction resulted in significant changes in the proportions of some carcass tissues and visceral organs, realimentation was associated with rapid recovery of these tissues and organs. The relative growth of muscle was probably higher in the realimented than in the unrestricted heifers. Although the *b* values were similar between treatments, this conclusion would be drawn from the fact that at equal live weight, carcass composition was similar in both treatments. Fat accumulation was faster in the realimenting than in the unrestricted heifers but the time of complete recovery was so shortened that there was no significant difference between treatments at equal live weight. Regarding the visceral organs, compensatory growth has been associated with very rapid responses of the liver and the GIT. Although these two organs constitute a major proportion of total animal energy expenditure even though they constitute less than 10% of body mass (Ferrell, 1988), their accelerated growth during compensatory growth is associated with greater feed intake and higher efficiency of feed utilization. Anugwa and Pond (1989) stated that an increased rate of growth of these metabolically active organ systems, despite the associated obligatory increase in energy expenditure to accomodate their increased size, is suggested as a possible underlying requisite for compensatory growth of the whole body during realimentation following feed restriction.

Finally, although some studies have shown that gut fill substantially contributes to compensatory growth in ruminants, the present study showed that there was no contribution

from gut fill. In fact, feed restriction probably resulted in a slower passage rate of digesta from the reticulo-rumen, leading to greater gut fill in the restricted than in the unrestricted heifers. No further increase in gut fill was observed following realimentation; thus most of the weight gain was attributed to the actual body weight gain. It is concluded that compensatory growth is a function of actual tissue growth and has no negative effects on body composition.

3.6. Literature Cited

Anugwa, F. I. O., and W. G. Pond. 1989. Growth of whole body and organs of growing rats during feed restriction and subsequent realimentation. Growth, Development & Aging 53:167.

Aziz, N. N., D. M. Murray, and R. O. Ball. 1993. The effect of live weight gain and live weight loss on body composition of Merino wethers: Non-carcass organs. J. Anim. Sci. 71:400.

Baker, R. D., N. E. Young, and J. A. Laws. 1985. Changes in the composition of cattle exhibiting compensatory growth and the modifying effects of grazing management. Anim. Prod. 41:309.

Berg, R. T., and R. M. Butterfield. 1976. New Concepts of cattle growth. Sydney University Press.

Burrin, D. G., C. L. Ferrell, R. A, Britton, and M. Bauer. 1990. Level of nutrition and visceral organ size and metabolic activity in sheep. Br. J. Nutr. 64:439

Carstens, G. E., D. E. Johnson, and M. A. Ellenberger. 1989. Energy metabolism and composition of gain in beef steers exhibiting normal and compensatory growth. In: Energy Metabolism of Farm Animals. EAAP Publ. No. 43. Pudoc, Wageningen.

Ferrell, C. L., L. J. Koong, and J. A. Nienaber. 1986. Effect of previous nutrition on body composition and maintenance energy costs of growing lambs. Br. J. Nutr. 56:595.

Ferrell, C. L., 1988. Contribution of visceral organs to animal energy expenditures. J. Anim. Sci. 66(Suppl. 3):23.

Fox, D. G., R. R. Johnson, R. L. Preston, T. R. Dockerty, and E. W. Klosterman. 1972. Protein and energy utilization during compensatory growth in beef cattle. J. Anim. Sci. 34:310.

Hayden, J. M., J. E. Williams, and J. J. Collier. 1993. Plasma growth hormone, insulin-like growth factor, insulin, and thyroid hormone association with body protein and fat accretion in steers undergoing compensatory gain after dietary energy restriction. J. Anim. Sci. 71:3327.

Hicks, R. B., F. N. Owens, D. R. Gill, J. J. Martin, and C. A. Strasia. 1990. Effects of controlled feed intake on performance and carcass characteristics of feedlot steers and heifers. J. Anim. Sci. 68:233.

Hogg, B. W., 1991. Compensatory growth in ruminants. In: A. M. Pearson and T. R. Dutson (Eds.) Growth Regulation in Farm Animals. Vol. 7. Elsevier Applied Science, London.

Howarth, R. E., and R. L. Baldwin. 1971. Synthesis and accumulation of protein and nucleic acid in rat gastrocnemius muscle during normal growth, restricted growth, and recovery from restricted growth. J. Nutr. 101:477.

Lapierre, H., C. K. Reynolds, T. H. Elsasser, P. Gaudreau, P. Brazeau, and H. F. Tyrrell. 1992. Effects of growth hormone-releasing factor and feed intake on energy metabolism in growing beef steers: Net hormone metabolism by portal-drained viscera and liver. J. Anim. Sci. 70:742.

Luginbuhl, J.-M., K. R. Pond, and C. C. Burns. 1994. Whole-tract digesta kinetics and comparison of the techniques for the estimation of fecal output in steers fed coastal

Bermudagrass hay at four levels of intake. J. Anim. Sci. 72:201.

Murray, D. M., N. M. Tulloh, and W. H. Winter., 1977. The effect of three different growth rates on some offal components of cattle. J. Agric. Sci. (Camb.) 89:119.

Murray, D.M., and O. Slezacek. 1988. The effect of weight stasis on the non-carcass components of crossbred sheep. Aust. J. Agric. Res. 39:653.

Murray, D. M., N. M. Tulloh, and W. H. Winter. 1974. The effect of three different growth rates on empty body weight, carcass weight and dissected carcass composition of cattle. J. Agric. Sci. 82:535.

Nour, A. Y. M, and M. L. Thonney, 1987. Carcass, soft tissue and bone composition of early and late maturing steers fed two diets in two housing types and serially slaughtered over a wide weight range. J. Agric. Sci., Camb. 109:345.

Okine, E. K., and G. W. Mathison. 1991. Effects of feed intake on particle distribution, passage of digesta, and extent of digestion in the gastrointestinal tract of cattle. J. Anim. Sci. 69:3435.

Overall, J. E., and C. J. Klett. 1972. Applied multivariate analysis. McGraw-Hill Book Co., N.Y.

Price, M. A., 1977. The effects of severe feed restriction on bulls and steers. II. Carcass composition. Aust. J. Agric. Res. 28:529.

Reid, I. M., J. G. Roberts, and G. D. Baird. 1980. The effects of underfeeding during pregnancy and lactation on structure and chemistry of bovine liver and muscle. J. Agric. Sci. 94:239.

SAS. 1990. SAS User's Guide: Statistics (Version 6.06 Ed.). SAS Inst. Inc., Cary, NC.

Seebeck, R. M., 1967. Developmental growth and body weight loss of cattle. I. Experimental design, body weight growth, and the effects of developmental growth and body weight loss on the dressed carcass and the offal. Aust. J. Agric. Res. 18:1015.

Stangassinger, M., and D. Giesecke. 1986. Splanchnic metabolism of glucose and related energy substrates. In: L. P. Milligan, W. L. Grovum and A. Dobson (Eds.) Control of digestion and metabolism in ruminants. Prentice-Hall, Englewood Cliffs, N.J.

Tatum, J. D., F. L. Williams Jr., and R. A. Bowling. 1986. Effects of feeder-cattle frame size and muscle thickness on susequent growth and carcass development. III. Partitioning of separable carcass fat. J. Anim. Sci. 62:132.

Trenkle, A., D. L. DeWitt, and D. G. Topel. 1978. Influence of age, nutrition and genotype on carcass traits and cellular development of the *M. longissimus* of cattle. J. Anim. Sci. 46:1597.

Wilson, P. N., and D. F. Osbourn. 1960. Compensatory growth after undernutrition in mammals and birds. Biol. Rev. 35:324.

Yambayamba, E., and M. A. Price. 1991. Growth performance and carcass composition in beef heifers undergoing catch-up (compensatory) growth. Can. J. Anim. Sci. 71:1021.

Yambayamba, E. S. K., and M. A. Price. 1992. Compensatory growth of livers, kidneys, and offal fat in beef heifers. Can J. Anim. Sci. (Abstr) 72:997.

CHAPTER FOUR

4. Skeletal Muscle and Hepatic Glucidic Potential in Feed Restricted-refed Beef Heifers

4.1. Introduction

Glucidic potential (GP) is a term applied to describe the measure of glycogen, glucose, glucose-6-phosphate (G-6-P), and lactate concentrations in a tissue. It is an index of ultimate pH of meat following slaughter of an animal (Guignot et al., 1992), glycogen level being the main determinant of the pH (Warris et al., 1989). Glycogen depletion during the handling of animals prior to slaughter can lead to dark, firm and dry (DFD) meat, a condition resulting from elevated pH. Thus a low GP at slaughter is indicative of a high likelihood of DFD meat. On the other hand, a higher than normal GP may lead to pale, soft exudative (PSE) meat (Henckel et al., 1992).

While preslaughter handling is perhaps the most significant factor influencing DFD or PSE, feeding level may have some influence on meat quality although the effect is minor (Fernandez et al., 1992). Briskey et al. (1960) observed that feeding a high-carbohydrate diet to pigs increased muscle glycogen content. On the other hand, fasting bulls for 4 d led to depletion of skeletal muscle glycogen (Crouse et al., 1984).

While skeletal muscle GP has a direct effect on meat quality, the liver, being the centre

of intermediary metabolism, can also be involved. Unlike skeletal muscle, the liver responds very rapidly to nutritional levels; in dairy cows, for example, feed restriction has been associated with significant depletion of hepatic glycogen content (Drackley et al., 1991; Veenhuizen et al., 1991).

Not much attention has been paid to beef heifers in this regard. Moreover, data are lacking on the effects of long-term feed restriction followed by realimentation on either muscle or hepatic GP in beef cattle. The objective of this study was to investigate the effects of a 92 d maintenance ration followed by realimentation on the GP of longissimus muscle and liver in Hereford crossbred heifers.

4.2. Materials and Methods

4.2.1. Animals, Slaughter, and Tissue Collection

The heifers used in this study were those described in section 3.2.1., with the exception of d 1 data which were discarded because of collection errors. Within 5 min following bleeding, a small muscle sample (40-50 g) was obtained from the 3rd-4th lumbar region of the longissimus muscle, using a 20 mm diameter corer. The sample was immediately frozen in liquid nitrogen (-70°C) and stored at -35°C until analyzed. Another sample (50-100 g) was obtained from the caudate lobe of the liver, immediately frozen in liquid nitrogen and stored at -35°C for later analysis.

4.2.2. Determination of Muscle and Hepatic Glucidic Potential

The metabolites were extracted from frozen muscle and liver samples by first pulverizing the sample with a chilled (-70°C) mortar and pestle using liquid nitrogen to keep the sample frozen. About 1 g of the crushed sample was weighed into a 15 mL disposable, conical centrifuge tube. While in the ice bath, 5 mL .6 N cold perchloric acid was added to the tube and the sample was homogenized with a Polytron homogenizer (Kinematica GmbH, Switzerland) for 45 sec. The homogenate (.2 mL) was pipetted into a centrifuge tube for the amyloglucosidase procedure as described by Dalrymple and Hamm (1973). The remaining homogenate was centrifuged (3000 x *g*) for 20 min at 2°C following which the supernatant was decanted into a 15 mL centrifuge tube. The extracts were then neutralized as described by Dalrymple and Hamm (1973) except that the supernatant was not filtered but centrifuged as above. The neutralized extract was stored at 0°C until assayed for glucose, lactate, and G-6-P. All spectrophotometric readings were performed at 340 nm on a Philips model PU 8625 UV/Vis spectrophotometer.

4.2.3. Glycogen and Metabolite Assays

Since the assay for glycogen required a glucose blank (perchloric acid extract) from the same sample, both glycogen and glucose were determined using the same glucose blank. The assays were performed as described by Keppler and Decker (1974) using the homogenate incubated with amyloglucosidase (for glycogen) and the supernatant from the perchloric acid

extraction assay (for glucose). Liver samples required an additional 1:5 dilution for glucose assay.

The lactate assay was performed as described by Gutmann and Wahlefeld (1974) using the supernatant from the perchloric acid extraction. In some cases it was necessary to dilute the supernatant (usually 1:10) with neutralized perchloric acid (.6 N neutralized to a methyl orange end point with 5.4 N KOH) to obtain absorbance readings in acceptable ranges (i.e. <1.000). These dilutions were accounted for in the calculations.

The G-6-P assay was performed as described by Lang and Michal (1974) using the supernatant from the perchloric acid extraction except that Fructose-6-Phosphate was not assayed after completion of the G-6-P assay.

4.2.4. Statistical Analysis

Glucidic potential was calculated using the following formula:

GP = ([glycogen] + [glucose] + [G-6-P]) + ½[lactate],

expressed in µmol glycogen equivalent per gram of fresh tissue, assuming a dry matter content of 25%. The data were then analyzed as a split-plot design using the GLM procedures (SAS, 1990) to assess effects of treatment, slaughter date, and their interaction. A paired *t-test* was used to test significance between treatments within each slaughter date. The model used in the analysis was:

$Y_{ijk} = \mu + T_i + D_j + TD_{ij} + \epsilon_{k(ij)}$

where:

Y_{ijk} = glucidic parameter,

T_i = nutritional treatment,

D_j = date of slaughter,

Td_{ij} = nutritional treatment x slaughter date interaction,

$\epsilon_{k(ij)}$ = random error term.

4.3. Results

4.3.1. Muscle Glucidic Potential

Results for muscle GP are presented in Table 4-1. There was an overall significant effect ($P < .05$) of slaughter date on glucose, lactate, and G-6-P concentrations in the longissimus muscle. However, there was no significant treatment x slaughter date interaction for any of the parameters. On both slaughter dates during the feed restriction period, no significant treatment differences ($P > .05$) were found in any of the parameters except for lactate which was lower ($P = .02$) in REST than in ADLIB longissimus muscle. During the realimentation period, none of the parameters exhibited any significant difference ($P > .05$) between treatments.

Table 4-1. Least squares means of carbohydrate stores and the computed glucidic potential (μmol/g) of longissimus muscle in ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers

Day of slaughter	ADLIB (n = 2)	REST (n = 2)	SEM	P
Restriction period				
d 50				
Glycogen	35.66	45.63	16.33	.67
Glucose	1.60	1.24	.26	.36
Lactate	49.33	32.80	4.52	.02
Glucose-6-phosphate	.04	.07	.18	.92
Glucidic potential	71.93	53.37	15.14	.40
d 92				
Glycogen	77.11	61.52	16.33	.51
Glucose	.71	.66	.26	.90
Lactate	20.53	18.06	4.52	.71
Glucose-6-phosphate	.13	.06	.18	.81
Glucidic potential	88.22	71.28	15.14	.44
Realimentation period				
d 29				
Glycogen	79.34	90.40	16.33	.64
Glucose	.45	.58	.26	.72
Lactate	13.12	12.71	4.52	.95
Glucose-6-phosphate	.07	.07	.18	.99
Glucidic potential	86.42	97.41	15.14	.62
d 50				
Glycogen	56.00	63.34	16.33	.76
Glucose	1.03	.98	.26	.90
Lactate	15.06	16.78	4.52	.79
Glucose-6-phosphate	.11	.05	.18	.83
Glucidic potential	64.66	72.76	15.14	.71
d 78				
Glycogen	80.03	86.07	16.33	.80
Glucose	.68	.51	.26	.65
Lactate	17.47	13.79	4.52	.58
Glucose-6-phosphate	.40	.34	.18	.98
Glucidic potential	89.85	93.86	15.14	.86
d 134				
Glycogen	73.90	84.32	16.33	.66
Glucose	.52	1.10	.26	.15
Lactate	12.76	23.55	4.52	.12
Glucose-6-phosphate	.61	.72	.18	.68
Glucidic potential	81.42	97.92	15.14	.46

4.3.2. Hepatic Glucidic Potential

Results for hepatic GP are presented in Table 4-2. There was an overall significant treatment effect (P = .01) on hepatic glycogen concentration, and there was also a treatment x slaughter date interaction (P = .02) for G-6-P concentration. During the feed restriction period, no significant differences (P > .05) between treatments were found in any of the parameters. During realimentation, hepatic glycogen concentration was lower (P = .04) in REST than in ADLIB livers on d 29. There were no treatment differences in glycogen concentration on d 50 and 78, but again lower concentration levels (P = .01) were observed in REST than in ADLIB heifers on d 134. Glucose concentration was not significantly different between treatments on any of the slaughter dates. Lactate and G-6-P concentrations were lower (P = .01) in REST than in ADLIB livers on d 29 of realimentation, but there were no significant treatment effects (P > .05) on any other slaughter date. Glucidic potential was similar in both treatments except on d 134 when the value was smaller (P = .04) in REST than in ADLIB livers.

Table 4-2. Least squares means of carbohydrate stores[a] and the computed glucidic potential (μmol/g) of liver in ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers

Day of slaughter	ADLIB (n = 2)	REST (n = 2)	SEM	P
Restriction period				
d 50				
Glycogen	104.6	101.2	21.8	.92
Glucose	82.1	76.5	24.4	.87
Lactate	15.0	13.0	1.9	.47
Glucose-6-phosphate	1.9	1.9	30.0	.99
Glucidic potential	190.8	187.5	43.7	.96
d 92				
Glycogen	135.4	120.1	21.8	.63
Glucose	102.5	96.2	24.4	.86
Lactate	11.4	10.7	1.9	.80
Glucose-6-phosphate	18.8	49.6	30.0	.48
Glucidic potential	228.4	237.0	43.7	.89
Realimentation period				
d 29				
Glycogen	178.8	109.8	21.8	.04
Glucose	101.6	95.0	24.4	.85
Lactate	19.8	11.7	1.9	.01
Glucose-6-phosphate	208.0	9.5	30.0	.01
Glucidic potential	290.5	210.7	43.7	.22
d 50				
Glycogen	180.3	124.2	21.8	.09
Glucose	99.6	77.6	24.4	.54
Lactate	14.0	11.6	1.9	.38
Glucose-6-phosphate	79.7	62.8	30.0	.69
Glucidic potential	286.9	207.6	43.7	.22
d 78				
Glycogen	127.2	91.6	21.8	.27
Glucose	74.22	79.1	24.4	.89
Lactate	14.1	12.6	1.9	.59
Glucose-6-phosphate	9.4	7.5	30.0	.88
Glucidic potential	208.4	177.0	43.7	.62
d 134				
Glycogen	176.9	81.3	21.8	.01
Glucose	300.1	250.3	24.4	.17
Lactate	2.0	4.3	1.9	.39
Glucose-6-phosphate	9.4	2.8	30.0	.88
Glucidic potential	478.0	333.7	43.7	.04

[a]Glucose-6-phosphate values = x 10^3

4.4. Discussion

4.4.1. Glucidic Potential of Muscle

Since the ultimate pH of meat is mainly determined by the amount of muscle glycogen at slaughter (Crouse and Smith, 1986; Warris et al., 1989), any management tools that increase muscle glycogen content in ruminants could reduce DFD incidents in ruminants. In the present study, feed restriction had no significant effect on the glucidic parameters except for lactate concentration which was lower (P = .02) in REST than in ADLIB longissimus muscle on d 50. Muscle glycogen reserves are depleted during either fasting (Crouse et al., 1984) or feed restriction (McVeigh and Tarrant, 1982). Therefore in the present study, it would be expected that the restricted heifers would have lower glycogen and glucose concentrations. Moreover, since lactate and glucogenic amino acids predominate in gluconeogenesis in the underfed ruminants (Baird, 1981), the lower concentration of lactate in REST heifers in the present study would have been expected to yield lower glucose and, consequently, lower glycogen concentrations. Crouse et al. (1984) fasted bulls for 96 h and this led to a significant depletion of muscle glycogen content. Similarly, Calder and Geddes (1992) reported a decrease of 55% in skeletal muscle glycogen concentration in rats that were fasted for 40 h. The heifers in the present study were not fasted; however, Yambayamba and Price (1991) have shown that feed restriction of beef heifers for extended periods results in conversion of intermediate (αR) myofibers to red (βR) myofibers which contain less glycogen (Monin, 1981). Thus it would be logical to expect a lower concentration of glycogen in the skeletal

muscle of feed restricted heifers.

In the present study, while the utilization of glucogenic amino acids for gluconeogenesis may have increased in the underfed heifers, other factors may have influenced the nonsignificant effect of feed restriction. Minnassian et al. (1994) reported that liver glycogen stores were depleted in rats fasted for 24 or 48 h, but there was a rebound of the glycogen stores when the rats were fasted for longer periods (72 or 96 h). The authors postulated that prolonged fasting possibly resulted in the activation of the enzyme glycogen synthase, and/or inhibition of glycogen phosphorylase. They also contended that there may have been an increase of the glycogen precusor substrate, G-6-P, through an enhanced supply (from gluconeogenesis) or from a decreased utilization. In beef heifers fed either barley or hay, McVeigh and Tarrant (1982) reported higher glycogen concentrations in the longissimus muscle of the former than the latter, and a higher activity of the enzyme, glycogen synthase, in the glycogen-depleted heifers compared to those not depleted. These observations support the present findings in the feed restricted heifers, although data variability between animals may also have influenced nonsignificance (Fernandez et al., 1992).

During the realimentation period, no differences between treatments were observed on any glucidic parameter. This was expected since a rebound had possibly already occured. Overall, there was a significant effect of slaughter date on the concentrations of glucose, lactate, and G-6-P. The variation of these parameters with time could be due to various factors such as physiological status at the time of slaughter, handling of the animals, and weather conditions. The significant effect of slaughter date may also explain why there was no overall treatment effect. On the whole, however, GP was not significantly affected by

treatment, and this is in agreement with the findings of Fernandez et al. (1992) in pigs.

4.4.2. Glucidic Potential of Liver

Like with skeletal muscle, the nonsignificant effect of feed restriction on liver was not expected. Veenhuizen et al. (1991) who restricted dairy cows by 20% of ad libitum feeding observed a significant decrease in the liver glycogen content within 5 d; the values reached nearly zero by d 35. Similar results have been reported in rats (Calder and Geddes, 1992) where after 40 h of fasting, liver glycogen concentration was decreased by 95%. However, since the heifers in the present study had been restricted for a long period, the speculation by Minassian et al. (1994) and the results of McVeigh and Tarrant (1982) may support the present data.

Surprisingly on d 29 of realimentation, glycogen, lactate, and G-6-P concentrations were lower in the livers of REST than ADLIB heifers. Since the assumption is that a rebound had taken place during the feed restriction period, it is not known what may have caused the drop in the concentrations during early realimentation. Of course, preslaughter handling would be an obvious speculation. No differences between treatments were observed on other slaughter days during realimentation except on d 134 when glycogen concentration and the ultimate GP were lower in REST than in ADLIB heifers. This result was surprising but again may reflect preslaughter handling of the animals. Thus overall, there was a significant treatment effect for glycogen and GP, with REST heifers having lower hepatic glycogen concentration and lower hepatic GP than ADLIB heifers. There was a significant time effect on glucose, lactate,

and G-6-P concentrations, and GP. It is suspected that apart from preslaughter handling of the animals, there may have been variations in the elapsed time before the liver was removed following exanguination.

4.5. Conclusions

It is concluded from the present data that long periods of feed restriction in beef heifers have no consequences on skeletal muscle glucidic potential. It is postulated that there may be a rebound of glycogen stores in muscle if the feed restriction period is long. Similarly, although liver responds very rapidly to nutritional demands, long-term feed restriction does not appear to have significant effects on hepatic glucidic potential. On the whole, feed restriction followed by realimentation does not affect skeletal muscle and hepatic glycogen stores.

4.6. Literature Cited

Baird, G. D. 1981. Metabolic modes indicative of carbohydrate status in the dairy cow. Fed. Proc. 40:2530.

Briskey, E. J., R. W. Bray, W. G. Hoekstra, P. H. Phillips, and P. H. Grummer. 1960. Effect of high protein, high fat and high sucrose rations on the water-binding and associated properties of pork muscle. J. Anim. Sci. 19:404.

Calder, P. C., and R. Geddes. 1992. Heterogeneity of glycogen synthesis upon refeeding following starvation. Int. J. Biochem. 24:71.

Crouse, J. D., and S. B. Smith. 1986. Bovine longissimus muscle glycogen concentration in response to isometric contraction and exogenous epinephrine. Am. J. Vet. Res. 47:939.

Crouse, J. D., S. B. Smith, and R. L. Prior. 1984. Bovine muscle glycogen as affected by fasting and refeeding. J. Anim. Sci. 59:384.

Dalrymple, R. H., and R. Hamm. 1973. A method for the extraction of glycogen and metabolites from a single muscle sample. J. Fd. Technol. 8:439.

Drackley, J. K., J. J. Veenhuzein, M. J. Richard, and J. W. Young. 1991. Metabolic changes in blood and liver of dairy cows during either feed restriction or administration of 1,3-butanediol. J. Dairy Sci. 74:4254.

Fernandez, X., E. Tornberg, M. Mågård, and L. Göransson. 1992. Effect of feeding a high level of sugar in the diet for the last 12 days before slaughter on muscle glycolytic potential and meat quality traits in pigs. J. Sci. Food Agric. 60:135.

Guignot, F., Y. Quilichini, M. Renerre, A. Lacourt, and G. Monin. 1992. Relationship between muscle type and some traits influencing veal colour. J. Sci. Food Agric. 58:523.

Gutmann, I., and A. W. Wahlefeld. 1974. L-(+)-Lactate determination with lactate dehydrogenase and NAD. In: H. U. Bergmeyer (Ed.) Methods of Enzymatic analysis. Academic Press, Inc., N.Y.

Henckel, P., P. F. Jorgensen, and P. Jensen. 1992. Glycogen content, buffering capacity and resting pH in live muscles of pigs of different halothane genotypes (a pilot project). Meat Sci. 32:131.

Keppler, D., and K. Decker. 1974. Glycogen determination with amyloglucosidase. In: H. U. Bergmeyer (Ed.) Methods of Enzymatic analysis. Academic Press, Inc., N.Y.

Lang, G., and G. Michal. 1974. D-glucose-6-phosphate and D-fructose-6-phosphate. In: H. U. Bergmeyer (Ed.) Methods of Enzymatic analysis. Academic Press, Inc., N.Y.

McVeigh, J. M. and P. V. Tarrant. 1982. Glycogen content and repletion rates in beef muscle, effect of feeding and fasting. J. Nutr. 112:1306.

Minnassian, C., A. Ajzannay, J. P. Riou, and G. Mithieux. 1994. Investigation of the mechanism of glycogen rebound in the liver of 72-hr fasted rats. J. Biol. Chem. 269:16585.

Monin, G. 1981. Muscle metabolic type and the DFD condition. In: D. E. Hood and P. V. Tarrant (Eds.) The Problem of Dark-cutting in Beef: Current Topics in Veterinary Medicine and Animal Science, Vol. 10. Martinus Nijhoff, The Hague.

SAS. 1990. SAS User's Guide: Statistics (Version 6.06 Ed.). SAS Inst. Inc., Cary, NC.

Veenhuizen, J. J., J. K. Drackley, M. J. Richard, T. P. Sanderson, L. D. Miller, and J. W. Young. 1991. Metabolic changes in blood and liver during development and early treatment of experimental fatty liver and ketosis in cows. J. Dairy Sci. 74:4238.

Warris, P. D., E. A. Bevis, and P. J. Ekins. 1989. The relationships between glycogen stores and muscle ultimate pH in commercially slaughtered pigs. Br. Vet. J. 145:378.

Yambayamba, E., and M. A. Price. 1991. Fiber-type proportions and diameters in the longissimus muscle of beef heifers undergoing catch-up (compensatory) growth. Can. J. Anim. Sci. 71:1031.

CHAPTER FIVE

5. Effect of Compensatory Growth on Mammary Growth and Development in Beef Heifers

5.1. Introduction

It is well established in dairy cattle (Harrison et al., 1983; Park et al., 1989; Stelwagen and Grieve, 1990) and rats (Park et al., 1988) that high planes of nutrition during prepuberty have adverse effects on mammary development. Petitclerc et al. (1984) found that a high level of feed intake by dairy heifers during the prepubertal stage permanently affected lactation performance by increasing mammary fat deposition at the expense of parenchymal tissue. Sejrsen et al. (1982) reported that dairy heifers fed to grow at .6 kg/d had 32% more parenchymal DNA and 64% less fat in the mammary gland than those fed to gain 1.2 kg/d. Basically, a larger fat pad displaces the secretory cells (Owens et al., 1993), and this is most likely to happen in the prepubertal stage when mammary growth is rapid (Akers, 1990).

The economic value of rapid growth in beef cattle is well known (Christian et al., 1965). However, this rapid increase in skeletal size of a calf can be masked by variation in the milk production of the dam (Holloway and Totusek, 1973; Martin et al., 1981). Often, beef replacement heifers are selected for rapid preweaning growth (Owens et al., 1993); it is not known whether this would limit potential milk production, leading to low productivity in a

beef operation.

To date, there is very little information on mammary development in beef heifers. This study was conducted to investigate the effects of feed restriction followed by realimentation on mammary development in Hereford crossbred heifers.

5.2. Materials and Methods

5.2.1. Animals, Feeding, and Collection of Mammary Tissue

The heifers used in this study were those described in section 3.2.1. Following stunning (captive bolt) and bleeding on the kill floor, the abdominal hide was reflected and the mammary gland was removed from the inguinal region within 15 to 20 min. The gland was cut into halves and the left half was immediately frozen in liquid nitrogen (-70°C) and stored at -40°C until assayed for DNA, RNA, protein, and lipid content.

5.2.2. Sampling the Parenchymal Tissue

While still frozen, the mammary glands were placed on dry ice and chopped transversely to expose the parenchymal tissue. The separable fat was removed and the remaining tissue was thinly sliced, immersed in liquid nitrogen and pulverized using a sterile prechilled (-70°C) mortar and pestle. The samples were transferred to 50 mL falcon tubes and stored at -70°C.

5.2.3. Isolation of RNA

The RNA isolation was done using the TRIzol method as described by Chomczynski (1993) with a few modifications. Briefly, between 50 and 100 mg of parenchymal tissue was weighed into a prechilled polypropylene tube, to which 1 mL of TRIzol solution was added. The sample was homogenized for 30 sec using a polytron homogenizer followed by a 5 min incubation period at room temperature. Chloroform (.2 mL) was added, followed by a further 2 to 5 min incubation at room temperature and centrifugation at 12,000 x *g* for 15 min. This procedure separated the solution into an aqueous phase and an organic phase, with RNA remaining exclusively in the former. The aqueous phase (.5 to .6 mL) was transferred to a new tube, and the RNA was recovered by overnight precipitation with .5 mL isopropanol at -70°C. The samples were centrifuged at 12,000 x *g* for 10 min and the supernatant was discarded. The pellet was washed twice with 1 mL 75% ethanol, air-dried for 5 to 10 min and finally dissolved in 100 µL RNase-free water by gentle vortexing and incubating for 5 to 10 min at 55°C. The RNA concentration was measured by diluting 20 µL in 1 mL of water, and reading the optical density on a GeneQuant spectrophotometer (Pharmacia LKB Biochrom Ltd., Cambridge, U.K.) at 260 nm.

5.2.4. Isolation of DNA

DNA was isolated according to Gross-Bellard et al. (1972) with a few modifications. Briefly, about 200 mg of ground sample was weighed and suspended in 2.4 mL digestion

buffer in 20 mL polypropylene tubes. The samples were incubated at 50°C for 18 h and extracted with an equal volume of 25:24:1 phenol:chloroform:isoamyl alcohol. They were centrifuged at 1,700 x *g* for 10 min and the top aqueous phase was transferred to a new tube. Half volume of 7.5 M ammonium acetate and 2 volumes of 100% ethanol were added to the samples and centrifuged at 12,000 x *g* for 15 min. The supernatant was discarded and the pellet was washed with 75% ethanol, air-dried, and resuspended in 1 mL diethylpyrocarbonate-treated water. To facilitate dissolving the DNA pellet, the samples were incubated overnight at 50°C. Absorbance was read on the GeneQuant spectrophotometer at 260 nm.

5.2.5. Determination of Protein and Lipid Concentrations

Protein concentration was determined using the Leco nitrogen analyzer (Model FP-428, Leco Corporation, St. Joseph, MI). In this system, a sample is rapidly burned in pure oxygen and the products of combustion (mainly carbon dioxide, water, nitrates, and nitrogen) are passed through the thermoelectric cooler to remove most of the water. Eventually, oxygen, carbon dioxide and the rest of the water are removed and the nitrates are converted to nitrogen. Nitrogen is then measured by the thermal conductivity cell.

Lipid concentration was determined using the goldfish extraction apparatus (Labconco Corporation, Kansas City, MO) with petroleum ether as the extraction solvent. The samples were extracted for 6 h, and the beakers were dried at 100°C for 30 min before cooling to room temperature and weighing.

5.2.6. Statistical Analysis

Mammary weights were transformed to logarithms in order to use the allometric equation, $Y = aX^b$, in its linear form: $\log Y = \log a + b \log X$. The mammary weights (Y) were then regressed on empty body weight (EBW) (X) to compute growth coefficients (*b* values) which were analyzed (*t-test*) to determine if they were significantly different from 1. The *b* values of the two regressions were then compared using the paired *t-test* GLM procedures (SAS, 1990) to assess the treatment effects.

The mammary parenchymal data, expressed on dry matter basis, were subjected to a two-way analysis using the GLM procedure (SAS, 1990) to assess the treatment effects and treatment x time interactions. A paired *t-test* was used to test significance between treatments at each slaughter date. The model used in the analysis was:

$$Y_{ijk} = \mu + T_i + D_j + TD_{ij} + \epsilon_{k(ij)}$$

where:

Y_{ijk} = mammary parenchymal parameter,

T_i = nutritional treatment,

D_j = date of slaughter,

Td_{ij} = nutritional treatment x slaughter date interaction,

$\epsilon_{k(ij)}$ = random error term.

5.3. Results

5.3.1. Growth of Mammary Glands

The weight gains of the mammary glands are presented in Figure 5-1. There were significant effects of treatment (P = .001) and time (P = .001) but no significant treatment x time interaction for mammary weights. For most of the experimental period, the mammary glands of REST heifers were lighter (P < .05) than those of ADLIB heifers. At the end of the feed restriction period (d 92), the mammary glands were lighter (P = .04) in REST than in ADLIB heifers (1.1 ± .4 vs 3.1 ± .4 kg); this persisted through d 29 (1.3 ± .4 vs 4.8 ± .7 kg; P = .02), 50 (2.1 ± .2 vs 3.5 ± .02 kg; P = .01), and 78 (3.1 ± .3 vs 5.7 ± .5 kg; P = .02) of realimentation. However, by d 134 into realimentation when live weight was fully recovered, the difference between treatments was relatively small and non-significant (5.1 ± .2 vs 6.2 ± .4 kg; P = .07).

Regression of the mammary weights on EBW revealed that the mammary growth coefficient for REST heifers was greater (P = .001) than that for ADLIB heifers (2.2 ± .16 vs 1.35 ± .19); both *b* values were greater than 1 (P < .01).

5.3.2. Nucleic Acids, Protein, and Lipid Concentrations

The mammary glands collected on d 1 (INIT) and 50 of feed restriction were unfortunately spoiled due to a malfunction of the meat laboratory freezer, and thus no

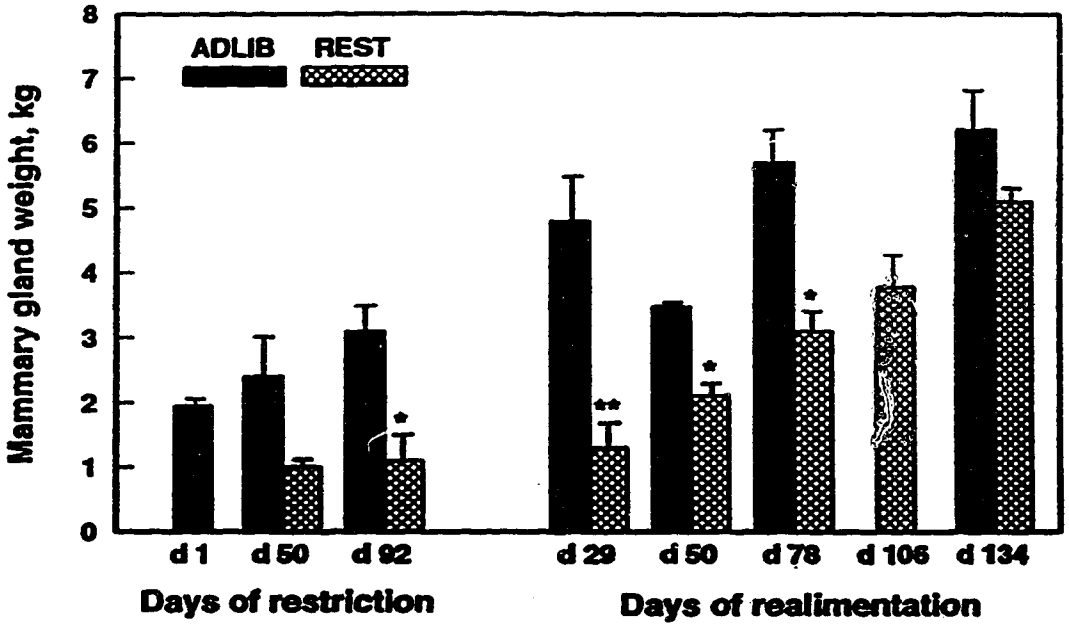


Figure 5-1. Mammary gland growth in ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers during feed restriction and realimentation periods. *P < .05; **P < .01.

chemical analysis data are available for this period. The DNA and RNA concentrations in the mammary parenchymal tissue are presented in Figure 5-2. There was a significant time effect (P = .01) on DNA concentration; the overall treatment effect tended to be significant (P = .09), but there was no significant treatment x time interaction (P = .41). The overall treatment, time, and treatment x time effects were not significant (P > .05) for RNA concentration. The trend for DNA concentration was such that it increased steadily in the mammary tissue of REST heifers during the realimentation period. Although the mammary tissue from ADLIB heifers also exhibited an increase in the DNA concentration over time, this appeared minimal compared to REST heifers. The values for DNA concentration were marginally larger in REST than in ADLIB heifers on d 29 (6.10 ± 0.58 vs 5.75 ± 1.63 mg/g; P = .43), 50 (7.47 ± .95 vs 6.80 ± .50 mg/g; P = .30), 78 (9.59 ± 1.93 vs 6.20 ± .37 mg/g; P = .10), and 134 (8.43 ± .91 vs 6.38 ± 1.15 mg/g; P = .15) of realimentation. The RNA concentration exhibited a similar trend on d 50 (1.31 ± .25 vs 1.06 ± .04 mg/g; P = .21) and 78 (1.90 ± .81 vs .72 ± .20 mg/g; P = .15) of realimentation.

Data for protein and lipid concentrations in the parenchyma are presented in Figure 5-3. Protein concentration was not significantly affected (P > .05) by treatment, time, or the treatment x time interaction. On the other hand, the time effect was significant (P = .02) for lipid content. Although not statistically significant, there was a general increase in the protein and decrease in the lipid concentration in both treatments over time. There tended to be less lipid in the mammary glands of the REST than the ADLIB heifers on d 50 (793.2 ± 9.1 vs 888.6 ± 8.0 mg/g), 78 (805.6 ± 61.7 vs 896.8 ± 40.8 mg/g) and 134 (756.7 ± 43.6 vs 787.4 ± 50.1 mg/g) of realimentation, although the difference was only significant (P = .02) on d 50.

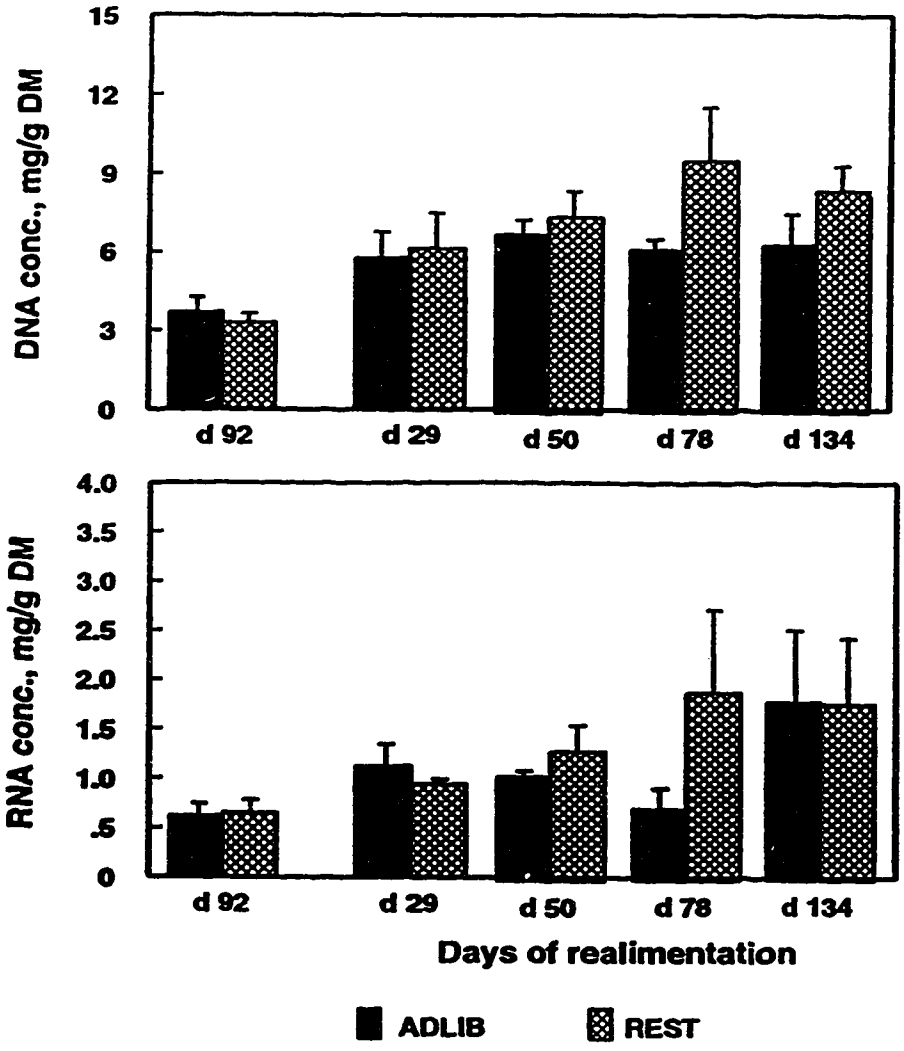


Figure 5-2. Mammary parenchymal DNA and RNA concentrations in ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers during the realimentation period; d 92 was the end of the restriction period.

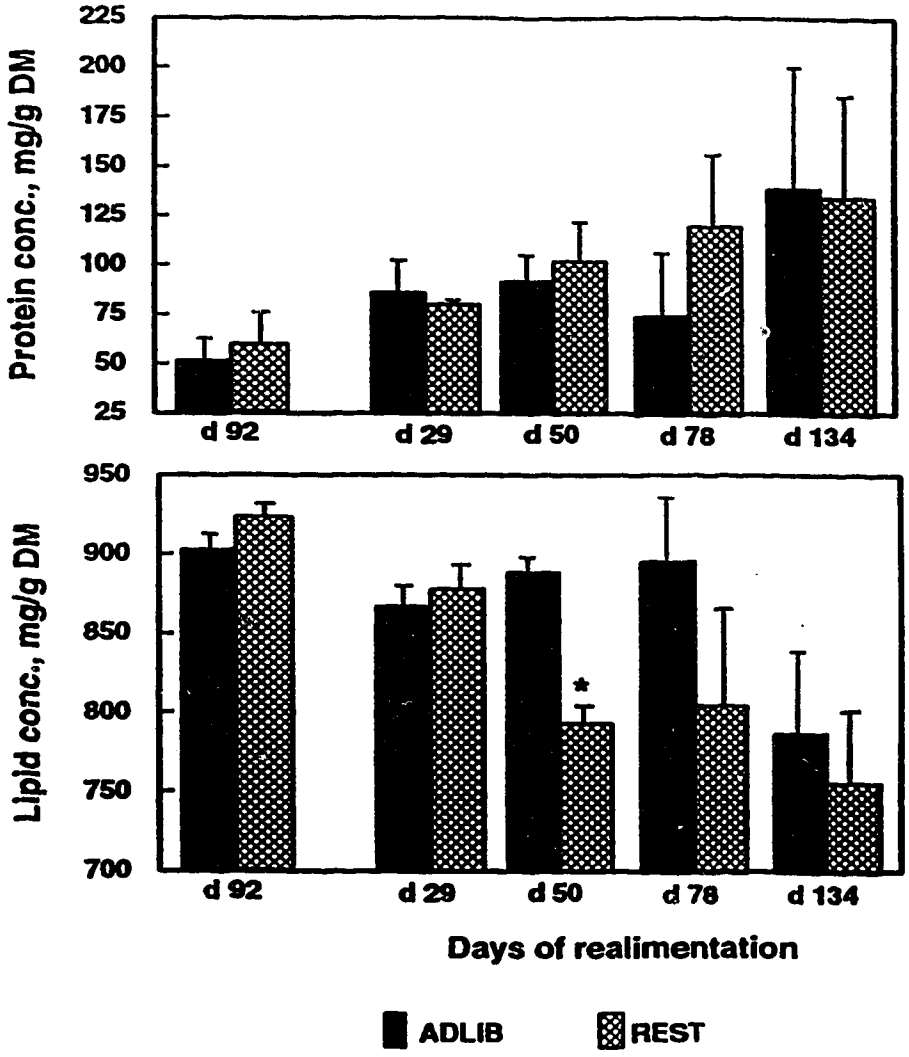


Figure 5-3. Mammary parenchymal protein and lipid concentrations in ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers during the realimentation period; d 92 was the end of the restriction period. *P < .05

The RNA:DNA ratio (functional activity of epithelial cells) and protein:DNA ratio (size of epithelial cells) were not significantly affected by treatment (Table 5-1). The overall effects of treatment, time, and treatment x time interaction were not significant ($P > .05$), and there did not seem to be consistent patterns in these parameters.

5.4. Discussion

5.4.1. Growth Performance and Mammary Growth

The body weights of REST heifers were maintained relatively constant during the feed restriction period. Feed restriction resulted in smaller mammary glands in REST than in ADLIB heifers. This was expected since most organs and tissues of the body are significantly affected by reduced feed intake. During realimentation, the ADG for REST heifers was greater ($P = .001$) than that for ADLIB heifers, and there was a significant response in mammary weight gains. The average age of the heifers was about 10.5 mo at the beginning of realimentation, and since mammary growth shows a positive allometric relationship with live weight through puberty (Anderson, 1978; Tucker, 1987; Sheffield, 1988), it was not surprising that the growth coefficients for both treatments were significantly greater than 1.0 during this period. However, the *b* value for REST mammary glands was significantly greater than that of ADLIB heifers. These results are in agreement with those found in sheep (Johnson and Hart, 1985) where the animals which received the lowest feeding regimen had the highest growth coefficients for mammary glands relative to live weight.

Table 5-1. RNA:DNA and protein:DNA ratios in ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers

Day of slaughter	ADLIB (n = 2)	REST (n = 2)	P
Restriction period			
d 92			
RNA:DNA	.18 ± .06	.20 ± .04	.40
Protein:DNA	14.27 ± 4.74	17.64 ± 4.40	.33
Realimentation period			
d 29			
RNA:DNA	.20 ± .03	.16 ± .01	.17
Protein:DNA	15.41 ± 1.74	13.16 ± .98	.19
d 50			
RNA:DNA	.16 ± .01	.17 ± .01	.28
Protein:DNA	13.38 ± .94	13.45 ± .85	.48
d 78			
RNA:DNA	.12 ± .03	.19 ± .05	.18
Protein:DNA	11.82 ± 4.24	12.33 ± 1.23	.46
d 134			
RNA:DNA	.31 ± .17	.22 ± .10	.35
Protein:DNA	24.38 ± 13.78	16.81 ± 7.88	.34

One important observation, however, was that despite the greater *b* value for mammary growth in REST heifers compared to ADLIB heifers, the mammary glands in the former were lighter than those from the latter throughout the period except for the heifers slaughtered at equal live weight. This could be due to less fat being deposited in the mammary glands of the REST heifers compared to the ADLIB heifers. In compensatory growth, a higher proportion of energy is channelled into true growth rather than fat accretion (Fox et al., 1974). This concept is supported by the greater proportion of fat in the heavier mammary glands of the ADLIB heifers compared to the REST heifers. It may, therefore, be logically assumed that most of the growth observed in the REST glands during realimentation was due to the parenchymal growth.

5.4.2. Nucleic Acid Concentrations

Since mammary gland weight or size alone does not give a true indication of mammogenesis, nucleic acids have been used as indices of the extent of mammary development. In the present study, there was an overall time effect (P = .01), and the overall treatment effect tended to be significant (P = .09) for DNA concentration. It is clear from Figure 5-2 that although there was a general increase in the parenchymal DNA concentration in both treatments, the REST glands appeared to have a greater DNA concentration than the ADLIB glands, with the peak concentration being reached on d 78 of realimentation. The tendency toward greater DNA concentration in the REST glands suggested greater mammary development compared to ADLIB glands. If so, these results could support the earlier

assumption that most of the weight gain of the REST mammary glands may have been due to the parenchymal tissue rather than the fat pad growth. The RNA concentration followed a more or less similar trend to that of DNA, although there was neither significant time nor treatment effect in this case.

Umberger et al. (1985) reported that ewes that were fed to gain more rapidly had reduced number of alveoli (and epithelial cells per alveolus) compared to those fed to gain less rapidly. Since mammary epithelial cell number is the ultimate limiting factor in milk production (McGrath, 1987), nutritional management of replacement female animals is of great importance. According to Tucker (1987) and Akers (1990), the most critical time for this in ruminants is during the pre- and peripubertal period. This is the period when tissue foundation is being established, and overfeeding the animals at this time could adversely interfere with cell proliferation, leading to a permanent reduction in lactation potential. In fact, Akers (1990) and Capuco and Akers (1990) noted that the basis of marked differences in milk yields between animals is failure of epithelial cells to proliferate in the low producing animals. In keeping with this, the present DNA and RNA results showed that ad libitum feeding could be associated with slower proliferation of cells in the parenchyma. On the other hand, feed restriction limited fat pad growth (hence the lighter mammary glands in REST heifers). During realimentation, however, it would be assumed that a higher proportion of nutrients went into parenchymal development rather than fat pad growth.

Compensatory growth can modulate mammary development, and the present results seem to be in line with those obtained in dairy cattle by Park et al. (1989) who evaluated the development and differentiation of the mammary gland as induced by a specific stair-step

nutrient regimen. Using 5.5 mo old Holstein heifers, Park et al. (1989) subjected the test group to a stair-step growth phase and fed according to an alternating 3-, 2-, 5-, 2-, 5-, and 2-mo schedule, beginning with the restricted ration followed by realimentation. This resulted in periods of compensatory growth when realimentation took place. The mammary tissues obtained by biopsy from pregnant heifers in the compensating group had significantly higher concentrations of DNA and RNA than the control group. Further, the animals in the compensating group produced 8 to 10% more milk than those in the control group. Similar results had been demonstrated in rats (Park et al., 1988).

The trends in the present study using beef cattle were similar to those reported by Park et al. (1989). On average, the DNA and RNA concentrations were 17.6% and 19.7%, respectively, higher in the REST than in the ADLIB glands. The DNA concentration was of particular importance in the present study since the number of epithelial cells is the most important factor in milk yield (McGrath, 1987). It is not clear what factors would trigger a higher mitotic activity of epithelial cells during compensatory growth, but Park et al. (1989) suggested that the changes in nutrient density may modulate hormone secretion and enzymatic activities. Tucker (1969) identified several hormones that are directly or indirectly associated with mammary cell proliferation; these include gonadotropins, prolactin, estradiol, progesterone, adrenal corticoids, thyroid hormones, and growth hormone (GH). Of course the activity of each of these hormones would depend on the animal's stage of development. Insulin-like growth factor I (IGF-I) which mediates most of GH actions (Davis, 1988) is equally important in mammogenesis (Glimm et al., 1988; Collier et al., 1993). It has been shown in ruminants that feed restriction followed by realimentation is associated with

significant changes in the endocrine system, particularly the GH-IGF-I axis (Breier et al., 1986). It may, therefore, be concluded that during compensatory growth, endocrine changes had significant influence on mammary cell mitotic activity in REST heifers.

5.4.3. Protein and Lipid Concentrations

The apparent increase in the protein concentration and significant decrease in the lipid concentration of the parenchyma over time was expected. One important observation (Figure 5-3) was that the protein and lipid contents of the parenchyma were similar between treatments at the end of feed restriction (d 92). However, as the realimentation period progressed, there was a shift in the concentration of protein and lipid. Notable in the observations was the sharp fall in the lipid concentration of the parenchyma of REST glands over time. This probably reflected the rapid parenchymal development, displacing fat. The fat pad, while essential for the differentiation and proliferation of the epithelial cells (Knight and Peaker, 1982), can greatly interfere with mammogenesis if not checked. According to Tucker (1969), fat resists the formation of ducts and alveoli. Thus the larger the fat pad, the smaller the proportion of the parenchyma. Stelwagen and Grieve (1990) reported similar mammary DNA content in dairy heifers fed on low, medium, or high plane of nutrition; however, the heifers receiving the medium and high planes had 57 and 129%, respectively, more mammary fat than those on the low plane. The authors concluded that the decrease in the adipose tissue with decreasing plane of nutrition indirectly indicated a larger proportion of parenchymal DNA for these animals. Although it is not practical to completely separate

parenchymal from extra-parenchymal tissue (Stelwagen and Grieve, 1990), the present results suggest that there may have been more parenchymal DNA in the REST than in the ADLIB glands. Also since GH is found to be elevated in feed restricted animals (Breier et al., 1986), Park et al. (1988) concluded that the low lipid content of mammary tissue found in the compensatory animals suggests a lipolytic role of this hormone in the mammary gland.

The RNA:DNA and protein:DNA ratios did not seem to provide much information in the present study. While the RNA:DNA and protein:DNA ratios in the ADLIB glands appeared to show variation over time, the REST glands did not appear to exhibit this trend. It is not clear whether such inconsistent results could be due to the fact that most heifers were slaughtered during the period before the secretory cells were fully functional. There also appear to be some conflicting data in the literature. Park et al. (1988), working with rats, found that the RNA:DNA and protein:DNA ratios increased from pregnant to lactating stages in both control and compensatory animals but the latter showed a greater increase in these parameters in lactating mammary tissues. Based on these results, the authors stated that compensatory growth may bring about hyperplasia, hypertrophy, and protein synthetic activity in epithelial cells. On the other hand, in dairy heifers, Stelwagen and Grieve (1990) observed increased mammary DNA concentration in low fed heifers but this was concurrently associated with decreased protein:DNA ratio. The authors postulated that this may indicate a more densely organized cell matrix, which may be due to an increased amount of smaller parenchymal secretory cells. Their data, however, did not provide conclusive evidence to support such an hypothesis.

On the whole, the present work suggests that compared to unrestricted feeding,

nutritional restriction followed by realimentation in beef heifers could be associated with increase in the parenchymal DNA concentration, suggesting more cells in the mammary tissue of these heifers compared to those on ad libitum feeding.

5.5. Conclusions

Although not conclusive, the present data seem to suggest that feed restriction followed by realimentation may lead to enhanced mammary development in beef heifers. The apparent difference in the mammary lipid concentration between REST and ADLIB glands could be an indication of the differences in fat accumulation. It is concluded that compensatory growth may modulate mammary growth, although the mechanism has not been elucidated. Such feeding manipulations could subsequently result in higher mitotic activity of the secretory cells, with a possible higher potential for milk production.

5.6. Literature Cited

Akers, R. M. 1990. Lactation physiology: A ruminant animal perspective. Protoplasma 159:96.

Anderson, R. R. 1978. Embryonic and fetal development of the mammary apparatus. In: Lactation: A comprehensive treatise. Vol.4. B.L. Larson (Ed.) Academic Press, New York, N.Y.

Breier, B. H., J. J. Bass, J. H. Butler, and P. D. Gluckman. 1986. The somatotrophic axis in young steers: Influence of nutritional status on pulsatile release of growth hormone and circulating concentrations of insulin-like growth factor 1. J. Endocrinol. 111:209.

Capuco, A. V., and R. M. Akers. 1990. Thymidine incorporation by lactating mammary epithelium during compensatory mammary growth in beef cattle. J. Dairy Sci. 73:3094.

Chomczynski, P. 1993. A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. BioTechniques 15:532.

Christian, L. L., E. R. Houser, and A. B. Chapman. 1965. Association of preweaning and postweaning traits with weaning weight in cattle. J. Anim. Sci. 24:652.

Collier, R. J., M. F. McGrath, J. C. Byatt, and L. L. Zurfluh. 1993. Regulation of bovine mammary growth by peptide hormones: Involvement of receptors, growth factors and binding proteins. Livest. Prod. Sci. 35:21.

Davis, S. L. 1988. Recent concepts in regulation of growth by GH and IGF. J. Anim. Sci. 66(Suppl. 3):84.

Fox, D. G., R. L. Preston, B. Senft, and R. R. Johnson. 1974. Plasma growth hormone levels and thyroid secretion rates during compensatory growth in beef cattle. J. Anim. Sci. 38:437.

Glimm, D. R., V. E. Baracos, and J. J. Kennelly. 1988. Effect of bovine somatotropin on the distribution of immunoreactive insulin-like growth factor-I in lactating bovine mammary tissue. J. Dairy Sci. 71:2923.

Gross-Bellard, M., P. Oudet, and P. Chambon. 1973. Isolation of high-molecular-weight DNA from mammlian cells. Eur. J. Biochem. 36:32.

Harrison, R. D., I. P. Reynold, and W. Little. 1983. A quantitative analysis of mammary glands of dairy heifers reared at different rates of live weight gain. J. Dairy Res. 50:405.

Holloway J. W., and R. Totusek. 1973. Relationship between preweaning nutritional management and subsequent performance of Angus and Hereford females through three calf crops. J. Anim. Sci. 37:807.

Johnson, I. D., and I. C. Hart. 1985. Pre-pubertal mammogenesis in the sheep. 1. The effects of level of nutrition on growth and mammary development in female lambs. Anim. Prod. 41:323.

Knight, C. H., and M. Peaker. 1982. Development of the mammary gland. J. Reprod. Fertil. 65:521.

Martin, T. G., R. P. Lemenager, G. Srinivasan, and R. Alenda. 1981. Creep feed as a factor influencing performance of cows and calves. J. Anim. Sci. 53:33.

McGrath, M. F. 1987. A novel system for mammary epithelial cell culture. J. Dairy Sci. 70:1967.

Owens, F. N., P.Dubeski, and C. F. Hanson. 1993. Factors that alter the growth and development of ruminants. J. Anim. Sci. 71:3138.

Park, C. S., Y. J. Choi, W. L. Keller, and R. L. Harrold. 1988. Effects of compensatory growth on milk gene expression and mammary differentiation. FASEB J. 2:2619.

Park, C. S., M. G. Baik, W. Keller, I. E. Berg, and G. M. Erickson. 1989. Role of compensatory growth in lactation: A stair-step nutrient regimen modulates differentiation and lactation of bovine mammary gland. Growth, Dev. Aging-GDA. 53:159.

Petitclerc, D., L. T. Chapin, and H. A. Tucker. 1984. Carcass composition and mammary development responses to photoperiod and plane of nutrition in Holstein heifers. J. Anim. Sci. 58:913.

SAS. 1990. SAS User's Guide: Statistics (Version 6.06 Ed.). SAS Inst. Inc., Cary, NC.

Sejrsen, K., J. T. Huber, H. A. Tucker, and R. M. Akers. 1982. Influence of plane of nutrition on mammary development in pre- and postpubertal heifers. J. Dairy Sci. 65:793.

Sheffield, L. G. 1988. Organization and growth of mammary epithelia in the mammary gland fat pad. J. Dairy Sci. 7:2855.

Stelwagen, K., and D. G. Grieve. 1990. Effect of plane of nutrition on growth and mammary gland development in Holstein heifers. J. Dairy Sci. 73:2333.

Tucker, H. A. 1969. Factors affecting mammary gland cell numbers. J. Dairy Sci. 52:721.

Tucker, H. A. 1987. Quantitative estimates of mammary growth during various physiological states: A review. J. Dairy Sci. 70:1958.

Umberger, S. H., L. Goode, E. V. Caruolo, R. W. Harvey, J. H. Britt, and A. C. Linnerud. 1985. Effects of accelerated growth during rearing on reproduction and lactation in ewes

lambing at 13 to 15 months of age. Theriogenology 23:555.

CHAPTER SIX

6. General Discussion and Conclusions

Overall, this comprehensive study in beef heifers gives an insight into some possible physiological mechanisms and how these relate to some effects of compensatory growth observed in beef cattle. The study makes it possible to look at compensatory growth from a wider perspective, and the exploitation of the phenomenon of compensatory growth can now be addressed more clearly.

6.1. Endocrine Factors in Compensatory Growth

The present work in beef heifers showed that there is a well coordinated endocrine response at the GH-IGF-I-insulin axis level. During feed restriction, there is a down-regulation of GH receptors in the tissues, resulting in the uncoupling of the GH-IGF-I axis. According to Thissen et al. (1990, 1991), this uncoupling may also be caused by a post-translational modification in the hepatic GH receptor, preventing the receptor signal from eliciting its action. In the event of this uncoupling, there is a decrease in cellular protein synthesis. Since muscle protein mass is the net result of the balance between synthesis and breakdown of protein (van den Hemel-Grooten et al., 1995), the latter exceeds the former resulting in overall low, zero, or negative body weight gains. It was observed in the present

work that the GH concentration was elevated in the REST heifers throughout the feed restriction period, resulting in elevated concentrations of NEFA during the same period. As Etherton (1994) noted, the role of GH in feed restricted animals changes from anabolism to another of its roles: adipose tissue mobilization to provide free fatty acids for energy.

During realimentation, the scenario completely changes, with IGF-I synthesis in tissues increasing and the concentration of plasma IGF-I being elevated rapidly (Wester et al., 1995). Plasma insulin concentration also increases rapidly during realimentation, and the role of GH changes to promoting protein synthesis. With the mediation of IGF-I, GH acts with insulin, resulting in the stimulation of protein synthesis in the cells. Trenkle et al. (1978) observed an increase in muscle RNA concentration in the realimented cattle, and this was a clear indication of increased protein synthesis. Hays et al. (1995) noted that cattle exhibiting compensatory growth following a period of feed restriction possess a great potential for lean tissue accretion due to the rapid and coordinated endocrine and metabolic responses that occur during the realimentation. This is not surprising in a truly compensating animal, since the physiological age has been affected, and the animal must regain its predermined growth channel. In particular, body weight gain is greatest during the early phase of realimentation.

Indeed, several other endocrine and metabolic responses may be implicated in compensatory growth. In fact, it may be speculated that the so-called chalones may be lowered in various tissues and organs as a result of the actions of the endocrine system. These well coordinated physiological events trigger the whole process of compensatory growth.

6.2. Metabolic Rate and Visceral Organs

In ruminants undergoing compensatory growth, there have been reports about increased feed efficiency (Hays et al., 1995) and increased protein and energy utilization (Fox et al., 1972). Apparently this efficiency in nutrient utilization is due to the fact that cellular maintenance requirements are still low, particularly during the early phase of compensatory growth (Fox et al., 1974). In general, reduced live weight is the ultimate factor associated with the reduced maintenance requirements during feed restriction and the early phase of realimentation. Among the visceral organs, the liver and GIT contribute significantly to the overall reduced maintenance requirements during this period. As Freetly et al. (1995) stated, it is reasonable to assume that metabolic activity of the portal-drained viscera (PDV) and liver is reduced and seeks a new steady-state following feed restriction.

The first study (Chapter 2) showed that feed restriction resulted in reduced resting metabolic rate and this persisted for several days into realimentation. Serial slaughter data in the second study (Chapter 3) showed that the PDV and liver weights relative to empty body weight decrease in young ruminants fed at maintenance. Since these organs contribute enormously to total energy expenditure, their reduced weights during feed restriction must contribute significantly to the overall reduction of metabolic rate. These data, put together, demonstrated that the PDV and liver do contribute significantly to energy expenditure.

During realimentation, the liver and GIT are known to respond very rapidly, as observed in the present work. The overall metabolic rate, however, increases slowly until a new level is established. During this time, the energy that would otherwise have been used for

maintenance is available for tissue growth, contributing significantly to the process of compensatory growth. This efficiency in the utilization of energy is a reflection of the physiological adjustments that the animal must make in order to regain its growth channel.

6.3. Carcass Composition and Glucidic Potential

The ultimate body composition following compensatory growth is influenced by several factors including age at which nutritional restriction was imposed, length and severity of restriction, nature of restriction, and pattern of realimentation (Wilson and Osbourn, 1960). Physiologically, however, it is the endocrine and metabolic changes that are effectively associated with the ultimate tissue proportions and composition.

In general, cattle deposit greater quantities of protein during compensatory growth (Hayden et al., 1993), basically because of the resulting imbalance in the muscle to bone ratio during feed restriction. This means during compensatory growth, one of the first priorities is to rebalance t... ...nd this can only happen if the synthesis of protein is higher than protein catabolism, at least in the initial phase. Since GH status is positively correlated with carcass muscle or protein synthesis (Trenkle and Topel, 1978; Eisemann et al., 1986), the GH data in the first study of this thesis and carcass composition in the second study could be interpreted to suggest a correlation between GH concentration and muscle recovery during compensatory growth. Thus the body weight gain during compensatory growth was mostly muscle rather than fat, resulting in carcass composition that was similar to the ad libitum fed heifers at equal weight. Although fat was also being deposited at a relatively high rate, the

period of fattening was so shortened that the proportion of fat in the compensating heifers did not exceed that in the ad libitum heifers. It must also be noted that the restricted heifers did not accumulate any fat during the whole period of feed restriction.

Muscle glycogen stores, as measured by glucidic potential, were unaffected by compensatory growth. This was to be expected, since full recovery had taken place by the final slaughter date. McVeigh and Tarrant (1982) have shown that starvation can seriously reduce muscle glycogen content and give rise to high ultimate pH, resulting in DFD meat. However, evidence suggests that as long as animal handling and slaughter conditions are well controlled, there will be no significant differences in any aspect of meat quality which could be attributed to the nutritional stress (Hogg, 1991). Thus it is not surprising to find that in animals which have been subjected to some degree of feed restriction and then realimented, their meat is of similar eating quality to that of normally grown animals (Morgan, 1972, 1979; Asghar and Yeates, 1979). From the present work, it is concluded that feed restriction and compensatory growth do not have any adverse effects on muscle glycogen stores.

6.4. Mammary Growth and Development

The mammary gland is very sensitive to nutrition. The present work (Chapter 5) seemed to suggest that the compensating heifers tended to have more developed mammary tissues than did their ad libitum fed counterparts. It is concluded that compensatory growth may enhance mitotic activity of the secretory cells, possibly through mechanisms such as increased hormonal and enzyme activity. In the present work, the increased concentrations of GH and

IGF-I during early compensatory growth may have possibly been involved in enhancing such mitosis. It is particularly important to note that the enhanced proliferation of the secretory cells could be possible mostly due to the apparent reduction of fat accumulation during feed restriction, thereby reducing physical restriction of the parenchymal tissue.

6.5. Perspective

Compensatory growth must be viewed as transitory; a time during which an animal's homeostatic mechanisms respond to an increased availability of food (Hogg, 1991). Significant physiological changes occur during this time, and as well the use and partitioning of energy and protein change towards higher efficiency. While the existing literature on compensatory growth shows that results have been variable, the phenomenon is real and can be used to advantage in various situations.

In tropical and subtropical climates where droughts are not uncommon, taking advantage of compensatory growth by restricting feeding for a period of time, could help alleviate some costs incurred in raising beef cattle. This is also true in temperate climates where there are long periods of winter. The present work in beef heifers demonstrates that imposing feed restriction soon after weaning (at age 6 to 7 mo) for 3 mo followed by realimentation does not cause any long term harm to the animals. Because of the "built-in" mechanism, the heifers are able to fully recover their live weights to their ad libitum fed counterparts. Yambayamba and Price (1991) have previously shown that early weaned beef heifers can, in fact, be subjected to feed restriction for up to 4 mo and still be able to recover fully when realimented,

without any deleterious effects. Quite evidently, ultimate body composition is unaffected.

In some situations, feed costs can be reduced significantly by exploiting compensatory growth. This was clearly demonstrated in the second study (Chapter 3) of the present work in which the overall total feed intake of the compensating heifers was significantly less than that of the ad libitum fed heifers. It must be appreciated that growth is an expensive process, and that growth can only take place after the maintenance requirements have been met. This means that animals must be fed above maintenance levels in order to grow, and depending on the prevailing conditions, this can turn out to be very costly. The present work demonstrated that feeding beef heifers a maintenance ration followed by realimentation resulted in significantly higher efficiency of feed and nutrient utilization, giving the animals a tremendous capacity to utilize a higher proportion of energy for the process of growth. It was this efficiency that ultimately was associated with quick body weight recovery and the significantly less total amount of feed consumed, compared to continuous ad libitum feeding.

Perhaps even more interesting in the present work was the fact that compensatory growth appeared to modulate mammary development in a positive way. Although not conclusive, the present work provides promising evidence that replacement beef heifers subjected to feed restriction followed by realimentation may accumulate less fat, thereby giving more opportunity to the parenchymal tissue to develop to its maximum potential. Although milk production is not as important in beef cattle as it is in dairy cattle, good mammary development is equally essential in the former, particularly in cow-calf beef operations. It is quite obvious that adequate milk production by the dam will lead to calves gaining rapidly during the suckling period, and ultimately achieving high productivity in such an operation.

Therefore, compensatory growth may just be the answer!

With the development of better techniques in molecular biology, a clearer understanding of the phenomenon of compensatory growth may be achieved soon, and it will occupy an important place in beef production.

6.6. Literature Cited

Asghar, A., and N. T. M. Yeates. 1979. Muscle characteristics and meat quality of lambs grown on different nutritional planes. IV. Effect on meat quality. J. Agric. Biol. Chem. 43:455.

Eisemann, J. H., A. C. Hammond, D. E. Bauman, P. J. Reynolds, S. N. McCutcheon, H. F. Tyrrell, and G. L. Haaland. 1986. Effect of bovine growth hormone administration on metabolism of growing Hereford heifers: Protein and lipid metabolism and plasma concentrations of metabolites and hormones. J. Nutr. 116:2504.

Etherton, T. D. 1994. Metabolic modifiers in animal production. In: P. A. Thacker (Ed.) Livestock Production for the 21st Century: Priorities and Research Needs. pp 73-92. University of Saskachewan, Saskatoon.

Fox, D. G., R. R. Johnson, R. L. Preston, T. R. Dockerty, and E. W. Klosterman. 1972. Protein and energy utilization during compensatory growth in beef cattle. J. Anim. Sci. 34:310.

Fox, D. G., R. L. Preston, B. Senft, and R. R. Johnson. 1974. Plasma growth hormone levels and thyroid secretion rates during compensatory growth in beef cattle. J. Anim. Sci. 38:437.

Freetly, H. C., C. L. Ferrell, T. G. Jenkins , and A. L. Goetsch. 1995. Visceral oxygen consumption during chronic feed restriction and realimentation in sheep. J. Anim. Sci. 73:843.

Hayden, J. M., J. E. Williams, and J. J. Collier. 1993. Plasma growth hormone, insulin-like growth factor, insulin, and thyroid hormone association with body protein and fat accretion in steers undergoing compensatory gain after dietary energy restriction. J. Anim. Sci. 71:3327.

Hays, C. L., G. M. Davenport, T. G. Osborn, and D. R. Mulvaney. 1995. Effect of dietary protein and estradiol-17β on growth and insulin-like growth factor I in cattle during realimentation. J. Anim. Sci. 73:589.

Hogg, B. W., 1991. Compensatory growth in ruminants. In: A. M. Pearson and T. R. Dutson (Eds.) Growth Regulation in Farm Animals. Vol. 7. Elsevier Applied Science, London.

Morgan, J. H. L. 1972. Effect of plane of nutrition in early life on subsequent live-weight gain, carcass and muscle characteristics and eating quality of meat in cattle. J. Agric. Sci. (Camb.) 78:417.

Morgan, J. H. L. 1979. The effects of supplementary feeding, pattern of growth and slaughter age on the carcass characteristics of Hereford steers. J. Agric. Sci. (Camb.) 92:299.

McVeigh, J. M., and P. V. Tarrant. 1982. Glycogen content and repletion rates in beef muscle, effect of feeding and fasting. J. Nutr. 112:1306.

Thissen, J. P., S. Triest, M. Maes, L. E. Underwood, and J. M. Ketelslegers. 1990. The decreased plasma concentration of insulin-like growth factor-I in protein restricted rats is not due to decreased numbers of growth hormone receptor in isolated hepatocytes. J. Endocrinol. 124:159.

Thissen, J. P., S. Triest, B. M. Moats-Staats, L. E. Underwood, T. Mauerhoff, D. Maiter, and J. M. Ketelslegers. 1991. Evidence that pretranslational and translational defects decrease serum insulin-like growth factor-I concentrations during dietary protein restriction. Endocrinology 129:429.

Trenkle, A., and D. G. Topel. 1978. Relationships of some endocrine measurements to growth and carcass composition of cattle. J. Anim. Sci. 46:1604.

Trenkle, A., D. L. DeWitt, and D. G. Topel. 1978. Influence of age, nutrition and genotype on carcass traits and cellular development of the *M. longissimus* of cattle. J. Anim. Sci. 46:1597.

Wester, T. J., R. A. Britton, T. J. Klopfenstein, G. A. Ham, D. T. Hickok, and C. R. Krehbiel. 1995. Differential effects of plane of protein or energy nutrition on visceral organs and hormones in lambs. J. Anim. Sci. 73:1674.

Wilson, P. N., and D. F. Osbourn. 1960. Compensatory growth after undernutrition in mammals and birds. Biol. Rev. 35:324.

van den Hemel-Grooten, H. N. A., M. Koohmaraie, J. T. Yen, J. R. Arbona, J. A. Rathmacher, S. L. Nissen, M. L. Fiorotto, G. J. Garssen, and M. W. A. Verstegen. 1995. Comparison between 3-methyhistidine production and proteinase activity as measures of skeletal muscle breakdown in protein-deficient growing barrows. J. Anim. Sci. 2272:2281.

Yambayamba, E., and M. A. Price. 1991. Growth performance and carcass composition in beef heifers undergoing catch-up (compensatory) growth. Can. J. Anim. Sci. 71:1021.

7. APPENDIX I

7.1. Details of Radioimmunoassay Procedures

7.1.1. BOVINE GROWTH HORMONE (RIA)

Reference:

De Boer, G., and J. J. Kennelly. 1989. Effect of somatotropin injection and dietary protein concentration on milk yield, and kinetics of hormones in dairy cows. J. Dairy Sci. 72:419.

Iodination

Column Preparation

1. Two days prior to iodination, swell sephadex G-75 in .05 M phosphate buffer (5 g in 200 mL). Leave sephadex in fridge.
2. A day prior to iodination, prepare the column: pack a small amount of glass wool in the bottom of a 1 cm x 30 cm glass column. Add 5 cm of tubing to the bottom and clamp.
3. Bring sephadex to room temperature (may use warm water), and degas it.
4. Fill the column with the swollen sephadex. Equilibrate (prime) the column with 20 mL PO_4.
5. Leave column in cold room overnight.
6. On iodination day, equilibrate with 1 mL 5% BSA/PO_4. Wash well with .05 M phosphate buffer (50 mL). Warm the column to room temperature before doing the iodination.

**Note: Steps 4 and 6 can also be done on the actual iodination day if desired.

Reagents for Iodination

1. Phosphate Buffer, pH 7.5, .5 M

   Na_2HPO_4 2.9812 g

   NaH_2PO_4 .5727 g

   Make the above in 50 mL deionized distilled H_2O;

   make large quantities, filter and freeze.

2. Phosphate Buffer, pH 7.5, .05 M

   Dilute (1) above 1:10

3. Chloramine T

   -9 mg/10 mL of .05 M phosphate buffer.

   -keep powder in dark, dissolve just before use.

4. Na-Metabisulfite

   -9 mg/10 mL of .05 M phosphate buffer.

   -keep powder in dark; dissolve just before use.

5. Potassium Iodide (KI)

   37.5 mg/10 mL of .05 M phosphate buffer.

6. BSA

   250 mg/5 mL of .05 M phosphate buffer (ie. 5% BSA/PO_4)

Iodination Procedure

1. Weigh 20-50 µg of bGH stock just before iodination. Solubilize this aliquot in .01 M $NaHCO_3$ at 100 µg/mL.
2. Add 50 µl of bGH.
3. Add 45 µl .5 M phosphate buffer.
4. Add .5 mCi I-125 in 5 µl (consider decay if not fresh).
5. Add 25 µl Chloramine T; vortex for **exactly** 30 sec.
6. Add 50 µl of Na-Metabisulfite; vortex.
7. Add 100 µl of KI
8. Add 100 µl 5% BSA/PO_4.
9. Add the reaction mixture to sephadex G-75 in a column.
10. Wash the column with .05 M phosphate buffer and collect 1 mL fractions.
11. Collect 25 fractions. Good iodinated hormone should appear in fractions 10 to 17. Free iodine should appear in fractions 18 to 25.
12. Pipette 10 µl from collected fractions and count. Predict the fractions containing good label. Add 50 µl 5% BSA/PO_4 to each tube to protect the protein.
13. Do a binding test for each fraction; pool those fractions that give good binding and aliquot. Freeze immediately. Label is good for 4 wk.

Radioimmunoassay

Reagents and Products

Buffer (PBS)

$NaH_2PO_4.H_2O$	.1167 g/L
Na_2HPO_4	1.3153 g/L
NaCl	8.182 g/L
Na-Azide	.200 g/L

pH 7.4; store at 4°C for maximum 2 mo.

Bovine growth hormone for label: AFP5340B, donated by Dr. A. F. Parlow, Director, Pituitary Hormones and Antisera Centre, Harbor-UCLA Medical Center, 1000 West Carson Street, Torrance, California, 90509; Tel. (310) 222-3537.

Antiserum: AFP55, donated by Dr. A. F. Parlow.

Anti-Monkey Gamma Globulin (AMGG), lot P4: Antibodies Inc., P. O. Box 442, Davis, California 95616; Tel. (916) 758-4400.

Procedure

1. Prepare 12 mm x 75 mm tubes as follows:

    15 replicates of Totals

    15 replicates of NSB tubes

    15 replicates of Bmax tubes

    15 replicates of each standard

    8 replicates of Control Plasma (CP)

2 replicates of unknowns

2. Prepare standards with the following concentrations:

Std #	*ng/tube*
11	4.00
10	2.00
9	1.50
8	1.00
7	.750
6	.500
5	.375
4	.250
3	.1875
2	.125
1	.0625

Serially dilute std **#11** by half to achieve stds **10, 8, 6, 4, 2, 1.** Std **#9** must be prepared separately and serially diluted to achieve stds **7, 5, 3.**

3. Accurately pipette 300, 150, 75, 37.5, 18.25, and 9.125 µL of CP in quadruplicate. Note: serial dilution works well.
4. Pipette 100 µL of plasma and 100 µL of standards. Make up to 300 µL by depositing

200 µL of assay buffer (1% BSA/PBS).

3. Pipette 300 µL of the serially diluted CP.
4. To BMax tubes, deposit 300 µL of assay buffer.
5. Deposit 500 µL assay buffer to NSB tubes.
6. Add 200 µL assay buffer containing AbI to BMax, plasma samples, CP tubes and stds. **Do not add to NSBs and Totals!**
7. Cover tubes with foil, vortex and incubate at 4°C for 25 h.
8. Add 200 µL label to all tubes. Note: the stock labeled GH solutionn should be diluted with 1% BSA/PBS to give approximately 10,000 cpm.
9. Deposit 200 µL label to 15 empty tubes to determine the total counts.
10. Vortex and incubate at 4°C for 24 h.
11. Add 200 µL AMGG diluted 1:8.9 (lot P4) with 1% BSA/PBS to all tubes **except Totals.**
12. Vortex and incubate at 4°C for 24 h.
14. Add 1 mL cold deionized distilled Water just before spinning. Centrifuge the tubes at 3,200 rpm at 4°C for 30 min (45 min for stds), and aspirate. Do not spin or aspirate totals. Count for 2 min.

7.1.2. INSULIN-LIKE GROWTH FACTOR I (RIA)

Reference:

Cosgrove, J. R., J. E. Tilton, M. G. Hunter, and G. R. Foxcroft. 1992. Gonadotropin independent mechanisms participate in ovarian responses to realimentation in feed restricted prepubertal gilts. Biol. Reprod. 47:736.

Iodination

Column Preparation

1. Prepare a 5 mL syringe for the column; park a bit of glass-wool at the bottom of the syringe, to be used as filter.
2. Fill in approximately 2 mL of anion-exchange resin (Biorad anion exchange resin AG 1 x 8; 50 - 100 mesh). Park, using plunger.
3. Flush column with the following (in that order):

   1 mL .5 M PO_4 buffer

   3 mL .05 M PO_4 buffer

   1 mL 1% BSA in .05 M PO_4 buffer

   3 mL .05 M PO_4 buffer
4. Pipette .5 mL of 1% BSA in .05 M PO_4 buffer into the collection tube.

Iodination Procedure

1. Add 15 µL (1.5 µg) of the aliquoted IGF-I.
2. Add 45 µL .05 M PO_4.
3. Add 5 µL I-125 (.5 mCi).
4. Add 25 µL Chloramine-T (12 mg/10 mL .05 M PO_4). Vortex for exactly 25 sec.
5. Add 50 µL Na-Metabisulfite (12 mg/10 mL .05 M PO_4).
6. Add 100 µL 12% BSA in .05 M PO_4.
7. Transfer the reaction mixture to the column; flush the column with 2 mL of 1% BSA in .05 M PO_4 buffer.
8. Collect label in the collection tube, and count 10 µL.

IGF-I Extraction

Reagents Required

Acid Ethanol - 12.5% (v/v) of 2.2 M HCL (analytical reagent grade) in 87.5% (v/v) distilled ethanol

Tris Base - .0855 M Trizma Base in IGF-I assay buffer, Sigma No. T-1503, reagent grade.

Neutralized Acid Ethanol Acid Ethanol and Tris Base solutions mixed in the ratio of 1 part Acid Ethanol and 4 parts Tris Base.

Procedure

1. Prepare 1 set of 13 mm x 100 mm tubes and 1 set of 12 mm x 75 mm tubes for control plasma (CP), cold recoveries (CR), and unknowns.

2. Serially dilute CP with assay buffer to make three concentrations (run at 100, 50, and 25 µL).
3. Spike 1 mL of plasma sample with a known amount of IGF-I stock, and serially dilute with plasma to make three concentrations. Prepare a blank by adding assay buffer to 1 mL of plasma sample. Incubate spiked plasma for 30 min to allow binding to IGF-I binding proteins.
4. Accurately pipette 100 µL of CP's, CR's, and plasma samples into 13 mm x 100 mm tubes. Add 3,000 µL of Acid Ethanol to the tubes, double parafilm, vortex, and incubate for 16 - 24 h.
5. Centrifuge for 30 min at 3,000 rpm, 4°C. Carefully pipette 200 µL of the supernatant into corresponding 12 mm x 75 mm tubes; neutralize by adding 800 µL of Tris Base, vortex, and store at 4°C until assayed.

Radioimmunoassay

Reagents and Products

Buffer

Sodium Phosphate (Monobasic .03 M)	4.14 g/L
EDTA-Na_2 (.01 M)	3.72 g/L
Protamine SO_4 (grade 2, .02%)	.20 g/L
Sodium Azide (.02%)	.20 g/L
Dissolve and then add Tween 20	.50 g/L

Make 1 litre, adjust pH to 7.5

Recombinant human IGF-I for label; Bachem Cat. No. DGR012

Antiserum: Anti-hIGF-I; USDA-UB3-189. Provided by Dr. Salvatore Raiti, Director, National Hormone and Pituitary Program, Suite 501-9, 210 West Pratt Street, Baltimore, MD 21201-3472, USA. Tel. (310) 837-2552

Normal Rabbit Serum (NRS) #71. University Animal Services

Goat anti-rabbit gamma globulin (GARGG); Calbiochem Cat. No. 539845, lot No. 073890

Procedure

1. Prepare 12 mm x 75 mm tubes for the assay as required.
2. Prepare standards with the following concentrations:

Std #	*ng/tube*
10	.5
9	.25
8	.125
7	.0625
6	.03125
5	.0156
4	.007825
3	.0039
2	.00195
1	.00097

3. Deposit 100 µL of standards, CP's, CR's, and unknowns to the appropriate tubes. Deposit 200 µL of assay buffer to each tube.
4. Dilute AbI 1:360 and deposit 100 µL to all tubes except Totals and NSB's. Note: Bmax tubes must orinally contain 300 µL assay buffer. Deposit 400 µL assay buffer to NSB tubes. Incubate all tubes for 24 h at 4°C.
5. Dilute concentrated label to give approximately 10,000 cpm in 100 µL. Deposit 100 µL into all tubes. Incubate for 24 at 4°C.
6. Dilute normal rabbit serum (NRS #71) 1:600; deposit 100 µL into all tubes except Totals. Dilute goat anti-rabbit gamma globulin (GARGG) 1:140; deposit 100 µL into all tubes except Totals. Incubate for 16 - 24 h.
7. Add 1 mL of cold deionized distilled water to all tubes (except Totals) immediately before spinning. Spin for 30 min at 3000 rpm, 4°C. Aspirate immediately and count pellet for 2 min. Do not aspirate Totals!

Calculation of Cold Recovery

$$\%CR = \frac{\text{Amount of IGF-I recovered}}{\text{Amount added + blank}} \times 100$$

7.1.3. 3-METHYL HISTIDINE DETERMINATION BY HPLC

Reference:

Scott, S. L., R. J. Christopherson, J. R. Thompson, and V. E. Baracos. 1993. The effect of a cold environment on protein and energy metabolism in calves. Br. J. Nutr. 69:127.

Reagents Required:

100 x 10^{-6} M standards (Histidine)

100 x 10^{-6} M internal standard (Histidinol)

3 M $HCLO_4$

1.5 M NaOH

.2 M Na-Borate

2 M Hcl

Preparation of Samples

1. Add 100 µL of internal standard (histidinol) to 200 µL of standard or 150 µL of plasma samples.
2. Deproteinize samples with 100 µL of 3 M $HCLO_4$, and centrifuge at 3,000 x *g* for 15 min.
3. Collect a 200-µL portion of the supernatant into a screw cap vial and add the following: 400 µL of water, 115 µL of 1.5 M NaOH, and 400 µL of Na-Borate (pH 9.0).
4. While being vortexed, add 250 µL of fluorescamine (160 mg of fluorescamine/100 mL acetonitrile) to the sample for derivatization.

5. Allow samples to sit for a few seconds to use up excess fluorescamine; then add 400 µL of HCl.
6. Incubate samples at 90°C for 45 min, and extract twice with diethyl ether.

HPLC Procedure

1. Analyze samples using a Varian Model 5000 Liquid Chromatograph with a Varian 2050 spectrofluometer detector and a Varian 9090 autoanalyzer.
2. Use the following binary gradient: solvent A - 2.5 mM acetylnitrimethylammonium bromide and .1 M sodium acetate buffer (pH 6.5); solvent B - 2.5 mM acetylnitrimethyl-ammonium bromide in 90% acetonitrile (pH 6.5).
3. The gradient should rise from 25 to 80% of solvent B over 12 min and be held at that level for 2 min and then back to 25% in .5 min. Total analysis time per sample is 18 min.

7.1.4. ISOLATION OF RNA (TRIzol Method)

Reference:

Chomczynski, P. 1993. A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. BioTechniques 15:532.

Reagents Required

TRIzol™ reagent (Gibco BRL, Cat. No. 155 96-026 / 100 mL)

Chloform BDH, analytical grade ACS 210

Isopropanol Sigma, Cat No. I-9516

Diethylpyrocarbonate (DEPC) treated water

75% ethanol in DEPC treated water

RNase free water (DEPC treated water)

Procedure

Unless otherwise stated, the procedure is carried out at room temperature and the reagents are also at room temperature.

1. Pulverize mammary tissue in a strile mortar with pestle prechilled to -70°C using liquid nitrogen and dry ice.
2. Weigh between 50 and 100 mg of sample into prechilled 6 mL sterile polypropylene falcon tube kept on dry ice.
3. Add 1 mL TRIzol solution and homogenize the sample using a Polytron homogenizer (30

sec, setting 10, small blade).

4. Transfer the sample into 1.5 mL eppendorf tube; incubate at room temperature for 5 min.
5. Add .2 mL of chloroform, and shake tubes vigorously for 15 sec. Incubate for another 2 to 5 min at room temperature.
6. Centrifuge the samples at < 12,000 x *g* for 15 min, 4°C.
7. Transfer the aqueous phase (.5 - .6 mL) to a fresh eppendorf tube (avoid any contact with interface).
8. Add .5 mL of isopropanol, vortex and let the RNA precipitate overnight at -70°C.
9. Centrifuge at < 12,000 x g for 10 min, 4°C; remove the supernatant.
10. Wash the pellet 2 times with 75% ethanol (at least 1 mL): mix sample by vortexing, and centrifuge at < 7,500 x g for 10 min, 4°C.
11. Air-dry the pellet for 5 to 10 min.
12. Dissolve RNA in 100 µL of RNase free water by gentle vortexing and incubating for 5 - 10 min at 55 - 60°C.
13. Dilute a small sample (5 - 10 µL) in 1 mL of Rnase free water and measure the concentration on a spectrophotometer (260 nm). Optical density (OD) readings should be within the range of .2 - .8.

Expected ratio 260/280 ~ 1.7 - 2.0. Low ratio indicates possible protein contamination.

7.1.5. ISOLATION OF DNA

Reference:

Gross-Bellard, M., P. Oudet, and P. Chambon. 1973. Isolation of high-molecular-weight DNA from mammlian cells. Eur. J. Biochem. 36:32.

Reagents Required

Digestion buffer

100 mM NaCl

10 mL Tris.Cl, pH 8.0

25 mM EDTA, pH 8.0

.5% SDS

.1 mg/mL proteinase K

25:24:1 phenol/chloroform/isoamyl alcohol

7.5 M ammonium acetate

100% and **75% ethanol**

Procedure

1. Weigh about 200 mg of pulverized sample and suspend in 2.4 mL digestion buffer in 20 mL polypropylene tubes.
2. Incubate samples, shaking, in tightly capped tubes, for 18 h at 50°C.
3. Extract with an equal volume of 25:24:1 phenol:chloroform:isoamyl alcohol and

centrifuge at 1,700 x *g* for 10 min. If phases do not resolve well, add another volume digestion buffer, omitting proteinase K, and repeat centrifugation. If thick white material appears at interface, repeat organic extraction. Transfer the top aqueous phase to a new tube.

4. Add half volume of 7.5 M ammonium acetate and 2 volumes of 100% ethanol, and centrifuge at 12,000 x *g* for 15 min.
5. Discard the supernatant, and wash the pellet with 75% ethanol. Air-dry and resuspend in 1 mL diethylpyrocarbonate-treated water. To facilitate dissolving the DNA pellet, incubate the samples overnight at 50°C.
6. Make appropriate dilution and read absorbance on the spectrophotometer at 260 nm. OD readings should be in the range of .2 - .8. Expected ratio (260/280) is 1.7 - 2.0.

8. APPENDIX II

8.1. Tabular Data From Study 1 (Chapter 2)

8.2. Tabular Data From Study 2 (Chapter 3)

8.3. Tabular Data From Study 4 (Chapter 5)

Table 8-1-1. Characteristics of growth hormone secretion (ng/mL) of ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers during feed restriction and realimentation periods

	ADLIB (n = 6)	REST (n = 6)	P
Restriction period			
d 0			
Minimum amplitude	6.96 ± .80	6.04 ± .80	.44
Maximum amplitude	12.94 ± 1.04	10.39 ± 1.06	.13
Mean concentration	9.45 ± .77	7.97 ± .96	.26
Area under curve	61.19 ± 6.11	51.27 ± 6.23	.29
d 20			
Minimum amplitude	6.29 ± .44	6.78 ± .69	.56
Maximum amplitude	11.10 ± .95	13.74 ± 1.43	.15
Mean concentration	8.51 ± .68	9.90 ± .90	.25
Area under curve	56.68 ± 4.04	61.79 ± 5.45	.47
d 48			
Minimum amplitude	5.75 ± .63	9.09 ± .76	.01
Maximum amplitude	12.24 ± .91	19.79 ± 1.49	.01
Mean concentration	8.52 ± .71	13.88 ± .95	.001
Area under curve	55.08 ± 5.68	93.45 ± 8.17	.01
Realimentation period			
d 10			
Minimum amplitude	6.45 ± .89	10.40 ± 1.42	.04
Maximum amplitude	11.45 ± .88	23.20 ± 1.95	.001
Mean concentration	8.70 ± .89	16.26 ± 1.79	.01
Area under curve	55.43 ± 6.26	110.76 ± 14.72	.01
d 31			
Minimum amplitude	6.09 ± .60	6.39 ± .53	.72
Maximum amplitude	11.65 ± 1.05	15.48 ± 2.91	.24
Mean concentration	8.54 ± .77	10.42 ± 1.59	.31
Area under curve	54.71 ± 5.71	66.42 ± 10.78	.36
d 59			
Minimum amplitude	6.29 ± .73	5.71 ± .60	.55
Maximum amplitude	11.94 ± 1.15	11.62 ± .84	.83
Mean concentration	8.79 ± .88	8.48 ± .74	.79
Area under curve	55.67 ± 6.10	54.45 ± 5.40	.88
d 101			
Minimum amplitude	7.53 ± 1.11	5.77 ± .16	.16
Maximum amplitude	12.76 ± 2.10	11.37 ± 1.08	.57
Mean concentration	9.93 ± 1.60	8.18 ± .54	.33
Area under curve	65.32 ± 11.20	51.34 ± 3.89	.28

Table 8-1-2. Plasma insulin-like growth factor I (IGF-I) and insulin concentrations of ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers during feed restriction and realimentation periods

	ADLIB (n = 6)	REST (n = 6)	P
IGF-I, ng/mL			
Restriction period			
d 0	138.72 ± 10.79	134.92 ± 6.21	.77
d 20	167.02 ± 12.28	131.85 ± 8.33	.04
d 48	188.87 ± 11.45	106.18 ± 12.04	.001
Realimentation period			
d 10	204.15 ± 13.97	202.07 ± 16.13	.92
d 31	191.32 ± 14.78	191.52 ± 8.67	.99
d 59	211.24 ± 15.10	205.43 ± 13.12	.78
d 101	207.72 ± 18.79	220.86 ± 9.42	.55
Insulin, μIU/mL			
Restriction period			
d 0	9.19 ± 1.28	6.0 ± 1.22	.11
d 20	10.25 ± 1.45	4.52 ± .57	.01
d 48	7.47 ± .92	3.66 ± .61	.01
Realimentation period			
d 10	8.05 ± .74	6.46 ± .57	.12
d 31	10.14 ± 1.11	13.01 ± 1.66	.18
d 59	8.23 ± 1.54	9.83 ± .64	.33
d 101	9.47 ± 1.79	10.84 ± 1.75	.60

Table 8-1-3. Plasma total thyroxine (T_4) and total triidothyronin (T_3) concentrations of ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers during feed restriction and realimentation periods

	ADLIB (n = 6)	REST (n = 6)	P
Total T_4, ng/mL			
Restriction period			
d 0	72.02 ± 5.14	77.14 ± .71	.35
d 20	58.15 ± 4.81	56.26 ± 2.52	.73
d 48	65.07 ± 3.40	52.52 ± 1.83	.01
Realimentation period			
d 10	68.03 ± 5.96	52.43 ± 3.82	.05
d 31	62.12 ± 2.46	65.43 ± 3.09	.42
d 59	66.66 ± 4.44	69.99 ± 4.23	.60
d 101	70.94 ± 5.87	72.68 ± 3.08	.80
Total T_3, ng/mL			
Restriction period			
d 0	1.54 ± .19	1.48 ± .15	.81
d 20	1.10 ± .15	.77 ± .06	.07
d 48	1.25 ± .05	.93 ± .05	.001
Realimentation period			
d 10	1.45 ± .09	.94 ± .04	.001
d 31	1.23 ± .04	1.24 ± .05	.88
d 59	1.30 ± .08	1.24 ± .04	.44
d 101	1.25 ± .07	1.23 ± .09	.82

Table 8-1-4. Oxygen consumption (VO_2) and the computed heat production (HP) of ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers during feed restriction and realimentation periods

	ADLIB (n = 4)	REST (n = 4)	P
Day of realimentation			
d -5			
Live weight, kg	309 ± 11	242 ± 4	.01
VO_2, mL/min	1545 ± 112	1123 ± 45	.01
HP, kJ/d	617.6 ± 28.3	525.5 ± 15.4	.03
d 15			
Live weight, kg	326 ± 15	267 ± 6	.01
VO_2, mL/min	1779 ± 109	1341 ± 39	.02
HP, kJ/d	683.6 ± 21.5	600.3 ± 19.7	.02
d 36			
Live weight, kg	352 ± 15	303 ± 6	.02
VO_2, mL/min	1900 ± 107	1782 ± 32	.30
HP, kJ/d	689.4 ± 26.6	720.8 ± 21.2	.40

Table 8-1-5. Plasma glucose and non-esterified fatty acids (NEFA) concentrations of ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers during feed restriction and realimentation periods

	ADLIB (n = 6)	REST (n = 6)	P
Glucose, mg/dL			
Restriction period			
d 0	105.18 ± 4.21	99.02 ± 2.65	.18
d 20	103.27 ± 5.04	88.57 ± 2.61	.03
d 48	104.37 ± 3.92	87.30 ± 12.04	.006
Realimentation period			
d 10	100.53 ± 5.59	101.08 ± 4.36	.94
d 31	100.50 ± 4.24	106.90 ± 4.22	.31
d 59	100.26 ± 2.56	97.67 ± 3.28	.56
d 101	96.94 ± 4.30	95.62 ± 1.10	.78
NEFA, mEq/mL			
Restriction period			
d 0	336.89 ± 24.15	344.65 ± 18.64	.81
d 20	288.56 ± 42.43	474.98 ± 37.19	.01
d 48	257.42 ± 25.74	538.22 ± 44.64	.001
Realimentation period			
d 10	205.62 ± 28.48	184.0 ± 24.03	.57
d 31	227.60 ± 57.41	184.96 ± 23.69	.49
d 59	218.53 ± 48.69	218.29 ± 23.04	.85
d 101	262.41 ± 64.37	307.30 ± 25.73	.50

Table 8-1-6. Plasma blood urea nitrogen (BUN) and 3-methyl histidine (3MH) concentrations of ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers during feed restriction and realimentation periods

	ADLIB (n = 6)	REST (n = 6)	P
BUN, mg/mL			
Restriction period			
d 0	10.82 ± .90	12.08 ± 1.14	.12
d 20	10.10 ± 1.44	8.78 ± .74	.44
d 48	12.85 ± 1.78	8.75 ± .64	.05
Realimentation period			
d 10	15.95 ± 1.65	12.77 ± .74	.11
d 31	17.92 ± 2.30	13.80 ± 1.0	.33
d 59	20.42 ± 2.45	22.65 ± 2.50	.54
d 101	22.08 ± 2.55	24.72 ± 2.23	.46
3MH, nmol/mL			
Restriction period			
d 0	6.98 ± .67	5.73 ± .77	.25
d 20	6.16 ± .40	5.37 ± .67	.33
d 48	6.83 ± .56	7.14 ± .66	.73
Realimentation period			
d 10	5.91 ± .37	7.34 ± .68	.10
d 31	6.36 ± .71	5.34 ± .42	.24
d 59	5.33 ± .39	5.79 ± .67	.59
d 101	5.81 ± .52	6.10 ± .48	.69

Table 8-2-1. Average daily gains (kg/d) of ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers during feed restriction and realimentation periods

	ADLIB (n = 12)	REST (n = 14)	P
Restriction period	1.37 ± .04	.10 ± .001	.001
Realimentation period	.96 ± .09	1.99 ± .09	.001

Table 8-2-2. Least squares means ± SE of carcass tissue and fat depot weights (kg) of heifers slaughtered on d 1 (INIT), ad libitum fed (ADLIB), and feed restricted-refed (REST) heifers

Day of slaughter	INIT[a] (n = 2)	ADLIB (n = 2)	REST (n = 2)	P
Restriction period				
d 50 (d 1 for INIT)				
Side muscle	37.5 ± 1.8^{bc}	42.2 ± 2.1^{c}	32.6 ± 1.7^{b}	.05
Side bone	12.7 ± .1^{c}	13.4 ± .6^{c}	11.6 ± .1^{b}	.05
Side fat	10.8 ± .3^{c}	18.2 ± 1.2^{d}	7.1 ± 1.1^{b}	.01
Subcutaneous fat	3.2 ± .1^{b}	6.6 ± .6^{c}	1.9 ± .6^{b}	.01
Intermuscular fat	6.6 ± .1^{c}	9.6 ± .3^{d}	4.4 ± .5^{b}	.01
Carcass cavity fat	1.1 ± .3^{bc}	1.9 ± .1^{c}	.8 ± .1^{b}	.03
d 92 (d 1 for INIT)				
Side muscle	37.5 ± 1.8	46.9 ± 4.5	38.2 ± 10.8	.27
Side bone	12.7 ± .1	14.2 ± 1.6	13.8 ± 1.1	.44
Side fat	10.8 ± .3^{b}	19.0 ± 1.6^{c}	7.0 ± 1.7^{b}	.01
Subcutaneous fat	3.2 ± .1^{b}	6.7 ± 1.1^{c}	1.9 ± .6^{b}	.01
Intermuscular fat	6.6 ± .1^{b}	10.5 ± .6^{c}	4.5 ± 1.0^{b}	.01
Carcass cavity fat	1.1 ± .3^{bc}	1.8 ± .2^{c}	.7 ± .1^{b}	.02
Realimentation period				
d 29				
Side muscle	-	69.9 ± 11.7	48.8 ± 4.0	.19
Side bone	-	18.0 ± 1.3	15.5 ± 2.4	.23
Side fat	-	27.1 ± .6	11.0 ± 1.9	.01
Subcutaneous fat	-	9.6 ± .6	2.7 ± .3	.01
Intermuscular fat	-	14.8 ± .4	6.7 ± 1.7	.02
Carcass cavity fat	-	2.7 ± .3	1.3 ± .2	.04
d 50				
Side muscle	-	56.6 ± 3.3	51.3 ± 1.3	.14
Side bone	-	17.1 ± .6	15.0 ± .1	.04
Side fat	-	28.6 ± 5.0	17.1 ± 1.7	.08
Subcutaneous fat	-	10.2 ± 1.8	5.7 ± .7	.07
Intermuscular fat	-	15.5 ± 3.1	9.4 ± .9	.10
Carcass cavity fat	-	2.9 ± .1	2.0 ± .2	.01
d 78				
Side muscle	-	63.5 ± 2.6	59.2 ± 3.1	.20
Side bone	-	18.3 ± .1	18.0 ± .6	.33
Side fat	-	37.7 ± 3.3	22.5 ± 1.1	.02
Subcutaneous fat	-	13.7 ± 1.8	7.2 ± .6	.04
Intermuscular fat	-	20.3 ± 1.3	13.0 ± .5	.02
Carcass cavity fat	-	3.7 ± .2	2.4 ± 0.0	.01
d 134				
Side muscle	-	65.3 ± 3.9	67.7 ± 3.0	.34
Side bone	-	18.5 ± 2.2	19.3 ± 1.5	.40
Side fat	-	42.3 ± 1.1	41.2 ± 3.0	.38
Subcutaneous fat	-	14.8 ± .1	14.8 ± 1.4	.50
Intermuscular fat	-	23.9 ± 1.3	22.8 ± 1.5	.32
Carcass cavity fat	-	3.6 ± .1	3.6 ± .1	.50

[a]Comparisons between INIT heifers and the other groups were done only during the restriction period.
[b,c,d]Means within a row with different superscripts differ.

Table 8-2-3. Least squares means ± SE of visceral organ weights (kg) of heifers slaughtered on d 1 (INIT), ad libitum fed (ADLIB), and feed restricted-refed (REST) heifers

Day of slaughter	INIT[a] (n = 2)	ADLIB (n = 2)	REST (n = 2)	P
Restriction period				
d 50 (d 1 for INIT)				
Liver weight	3.52 ± .16[c]	4.13 ± .07[d]	2.34 ± .07[b]	.01
Heart weight	1.12 ± .08[bc]	1.23 ± .06[c]	1.01 ± .05[b]	.05
Kidneys weight	.53 ± .01[b]	.63 ± .03[c]	.48 ± .04[b]	.05
Lungs + trachea weight	4.41 ± .31[c]	4.05 ± .27[c]	2.73 ± .03[b]	.02
Spleen weight	.46 ± .01[c]	.46 ± .02[c]	.35 ± .03[b]	.04
Pancreas weight	.18 ± .03	.20 ± .03	.21 ± .03	.38
Adrenal glands weight	.010 ± .001[b]	.013 ± .001[c]	.011 ± .001[bc]	.04
d 92 (d 1 for INIT)				
Liver weight	3.52 ± .16[c]	4.33 ± .07[c]	2.62 ± .40[b]	.03
Heart weight	1.12 ± .08	1.32 ± .16	1.0 ± .01	.09
Kidneys weight	.53 ± .01	.62 ± .04	.48 ± .10	.16
Lungs + trachea weight	4.41 ± .31[c]	4.81 ± .10[c]	3.27 ± .05[b]	.01
Spleen weight	.46 ± .01[c]	.46 ± .10[c]	.25 ± .03[b]	.02
Pancreas weight	.18 ± .03	.19 ± .01	.13 ± .03	.10
Adrenal glands weight	.010 ± .001[b]	.016 ± .001[c]	.010 ± .001[b]	.02
Realimentation period				
d 29				
Liver weight	-	5.01 ± .82	3.84 ± .41	.16
Heart weight	-	1.78 ± .30	1.22 ± .06	.10
Kidneys weight	-	.90 ± .10	.69 ± .01	.08
Lungs + trachea weight	-	5.89 ± .99	3.99 ± .87	.17
Spleen weight	-	.60 ± .12	.43 ± .09	.19
Pancreas weight	-	.29 ± .02	.13 ± .01	.01
Adrenal glands weight	-	.021 ± .001	.011 ± .001	.01
d 50				
Liver weight	-	5.31 ± .32	4.60 ± .07	.08
Heart weight	-	1.77 ± .11	1.50 ± .32	.25
Kidneys weight	-	.73 ± .05	.68 ± .02	.22
Lungs + trachea weight	-	5.57 ± .19	4.13 ± .65	.08
Spleen weight	-	.58 ± .04	.31 ± .03	.15
Pancreas weight	-	.21 ± .10	.21 ± .05	.49
Adrenal glands weight	-	.023 ± .005	.015 ± .003	.17
d 78				
Liver weight	-	5.20 ± .01	5.34 ± .32	.39
Heart weight	-	1.91 ± .03	1.56 ± .16	.08
Kidneys weight	-	.80 ± .08	.88 ± .08	.27
Lungs + trachea weight	-	6.62 ± .46	5.50 ± .20	.08
Spleen weight	-	.53 ± .09	.62 ± .04	.22
Pancreas weight	-	.24 ± .02	.31 ± .03	.10
Adrenal glands weight	-	.022 ± .002	.017 ± .001	.06
d 134				
Liver weight	-	6.21 ± .30	6.21 ± .71	.50
Heart weight	-	1.94 ± .10	1.93 ± .09	.47
Kidneys weight	-	.80 ± .01	.80 ± .01	.50
Lungs + trachea weight	-	7.49 ± .49	6.90 ± .26	.20
Spleen weight	-	.66 ± .06	.79 ± .11	.20
Pancreas weight	-	.47 ± .07	.42 ± .04	.27
Adrenal glands weight	-	.021 ± .003	.022 ± .004	.09

[a]Comparisons between INIT heifers and the other groups were done only during the restriction period.
[b,c,d]Means within a row with different superscripts differ.

Table 8-2-4. Least squares means ± SE of the gastrointestinal tract and abdominal fat weights (kg) of heifers slaughtered on d 1 (INIT), ad libitum fed (ADLIB), and feed restricted-refed (REST) heifers

Day of slaughter	INIT[a] (n = 2)	ADLIB (n = 2)	REST (n = 2)	P
Restriction period				
d 50 (d 1 for INIT)				
Full stomach weight	27.5 ± 1.7[c]	32.5 ± .1[d]	39.0 ± .3[b]	.01
Empty stomach weight	7.6 ± .1[b]	10.4 ± .1[c]	7.8 ± .2[b]	.01
Full intestines weight	10.7 ± .5[b]	14.4 ± 1.6[b]	22.7 ± .4[c]	.02
Empty intestines weight	8.3 ± .7	8.4 ± 1.6	6.1 ± .1	.14
Abdominal fat weight	5.3 ± .3[b]	10.5 ± 1.9[c]	4.7 ± .5[b]	.05
d 92 (d 1 for INIT)				
Full stomach weight	27.5 ± 1.7	38.2 ± 6.2	31.1 ± 6.1	.25
Empty stomach weight	7.6 ± .1[b]	12.5 ± .6[c]	6.2 ± .9[b]	.01
Full intestines weight	10.7 ± .5[b]	18.5 ± 1.8[c]	11.1 ± 2.8[c]	.03
Empty intestines weight	8.3 ± .7[b]	12.7 ± 0.0[c]	6.5 ± .8[bc]	.01
Abdominal fat weight	5.3 ± .3[b]	11.1 ± .6[c]	3.4 ± .8[b]	.01
Realimentation period				
d 29				
Full stomach weight	-	41.6 ± 4.2	35.2 ± 4.5	.20
Empty stomach weight	-	13.6 ± .7	10.7 ± 1.3	.09
Full intestines weight	-	18.4 ± .2	15.2 ± 3.5	.23
Empty intestines weight	-	14.8 ± .1	10.0 ± 1.6	.05
Abdominal fat weight	-	17.1 ± 3.9	6.7 ± .5	.05
d 50				
Full stomach weight	-	38.9 ± 3.1	45.9 ± .1	.08
Empty stomach weight	-	13.9 ± 1.4	12.9 ± .7	.30
Full intestines weight	-	19.9 ± .2	15.1 ± .1	.001
Empty intestines weight	-	17.7 ± .3	12.0 ± .1	.01
Abdominal fat weight	-	16.2 ± 5.7	8.6 ± .6	.16
d 78				
Full stomach weight	-	37.3 ± 2.2	49.8 ± 7.8	.13
Empty stomach weight	-	13.3 ± .7	17.6 ± 3.7	.19
Full intestines weight	-	21.6 ± .9	17.9 ± .1	.03
Empty intestines weight	-	19.0 ± .2	14.6 ± .1	.001
Abdominal fat weight	-	22.6 ± 2.0	11.1 ± 2.4	.03
d 134				
Full stomach weight	-	41.6 ± 9.2	38.7 ± 4.5	.40
Empty stomach weight	-	13.6 ± .6	14.6 ± 1.8	.33
Full intestines weight	-	28.1 ± .6	19.6 ± 2.6	.04
Empty intestines weight	-	23.4 ± 1.1	16.7 ± 1.9	.05
Abdominal fat weight	-	27.2 ± 4.2	21.4 ± 1.5	.22

[a]Comparisons between INIT heifers and the other groups were done only during the restriction period.
[b,c,d]Means within a row with different superscripts differ.

Table 8-2-5. Variation of gut fill (kg) and gut fill relative to full body weight (g/kg) of heifers slaughtered on d 1 (INIT), ad libitum fed (ADLIB), and feed restricted-refed (REST) heifers

Day of slaughter	INIT[a] (n = 2)	ADLIB (n = 2)	REST (n = 2)	P
Restriction period				
d 50 (d 1 for INIT)				
Gut fill, kg	22.3 ± 4.0[b]	28.4 ± 2.2[b]	36.8 ± .2[c]	.03
Gut fill proportion	98 ± 1[b]	119 ± 20[b]	184 ± 13[c]	.05
d 92 (d 1 for INIT)				
Gut fill, kg	22.3 ± 4.0	31.5 ± 3.8	29.6 ± 7.4	.12
Gut fill proportion	98 ± 1[b]	102 ± 8[b]	133 ± 6[c]	.04
Realimentation period				
d 29				
Gut fill, kg	-	31.6 ± 3.9	29.7 ± 4.9	.40
Gut fill proportion	-	81 ± 2	106 ± 6	.03
d 50				
Gut fill, kg	-	30.1 ± 1.6	35.1 ± 2.9	.14
Gut fill proportion	-	72 ± 1	114 ± 6	.01
d 78				
Gut fill, kg	-	29.6 ± 2.2	33.5 ± 4.2	.25
Gut fill proportion	-	62 ± 4	95 ± 8	.05
d 134				
Gut fill, kg	-	32.7 ± 8.2	27.1 ± 3.5	.30
Gut fill proportion	-	70 ± 20	60 ± 5	.05

[a]Comparisons between INIT heifers and the other groups were done only during the restriction period.
[b,c]Means within a row with different superscripts differ.

Table 8-2-6. Least squares means ± SE of the external components of offal weight (kg) of heifers slaughtered on d 1 (INIT), ad libitum fed (ADLIB), and feed restricted-refed (REST) heifers

Day of slaughter	INIT[a] (n = 2)	ADLIB (n = 2)	REST (n = 2)	P
Restriction period				
d 50 (d 1 for INIT)				
Hide weight	19.8 ± .8[b]	22.6 ± .5[c]	18.1 ± .7[b]	.02
Head weight	8.2 ± .2	9.6 ± .4	8.7 ± .3	.15
Feet weight	4.9 ± .2[bc]	5.3 ± .1[c]	4.7 ± .1[b]	.02
Tail weight	.56 ± .04	.52 ± .12	.50 ± .08	.45
d 92 (d 1 for INIT)				
Hide weight	19.8 ± .8[b]	25.4 ± 1.4[c]	17.5 ± 1.1[b]	.02
Head weight	8.2 ± .2	10.1 ± .1	8.9 ± 1.2	.24
Feet weight	4.9 ± .2	5.8 ± .7	5.4 ± .6	.35
Tail weight	.74 ± .06	.74 ± .06	.69 ± .07	.07
Realimentation period				
d 29				
Hide weight	-	32.0 ± 1.6	23.1 ± 1.8	.03
Head weight	-	11.6 ± .5	10.4 ± .9	.18
Feet weight	-	7.5 ± .5	6.3 ± .8	.17
Tail weight	-	.75 ± .03	.83 ± .05	.15
d 50				
Hide weight	-	30.8 ± 2.1	25.3 ± 2.9	.13
Head weight	-	11.8 ± .8	11.1 ± .3	.25
Feet weight	-	6.6 ± .1	6.5 ± .3	.16
Tail weight	-	.80 ± .02	.90 ± .12	.25
d 78				
Hide weight	-	36.5 ± 3.1	31.9 ± .8	.14
Head weight	-	13.1 ± .7	12.4 ± .4	.24
Feet weight	-	7.2 ± .1	7.2 ± .1	.50
Tail weight	-	.39 ± .01	.41 ± .01	.01
d 134				
Hide weight	-	36.7 ± 1.1	37.9 ± 1.5	.29
Head weight	-	13.1 ± .2	14.0 ± .6	.15
Feet weight	-	7.6 ± 1.0	7.5 ± .4	.47
Tail weight	-	.97 ± .03	.92 ± .04	.21

[a]Comparisons between INIT heifers and the other groups were done only during the restriction period.
[b,c]Means within a row with different superscripts differ.

Table 8-3-1. Least squares means ± SE of mammary weights, nucleic acid concentrations, protein, and lipid concentrations in ad libitum fed (ADLIB) and feed restricted-refed (REST) heifers

Day of slaughter	ADLIB (n = 2)	REST (n = 2)	P
Restriction period			
d 92			
Mammary weight, kg	3.1 ± .4	1.1 ± .4	.04
DNA conc., mg/g DM	3.70 ± .50	3.30 ± .07	.26
RNA conc., mg/g DM	.62 ± .11	.65 ± .13	.44
Protein conc., mg/g DM	50.35 ± 10.28	58.55 ± 15.57	.35
Lipid conc., mg/g DM	901.8 ± 7.8	922.4 ± 8.9	.40
Realimentation period			
d 29			
Mammary weight, kg	4.8 ± .7	1.3 ± .4	.02
DNA conc., mg/g DM	5.75 ± 1.63	6.10 ± .58	.43
RNA conc., mg/g DM	1.12 ± .20	.94 ± .01	.23
Protein conc., mg/g DM	85.80 ± 15.12	79.75 ± 1.65	.36
Lipid conc., mg/g DM	867.4 ± 13.6	878.2 ± 15.3	.30
d 50			
Mammary weight, kg	3.5 ± .02	2.1 ± .2	.01
DNA conc., mg/g DM	6.80 ± .50	7.47 ± .95	.30
RNA conc., mg/g DM	1.06 ± .04	1.31 ± .25	.21
Protein conc., mg/g DM	91.35 ± 13.07	101.25 ± 19.18	.36
Lipid conc., mg/g DM	888.6 ± 8.0	793.2 ± 9.1	.02
d 78			
Mammary weight, kg	5.7 ± .5	3.1 ± .3	.02
DNA conc., mg/g DM	6.20 ± .37	9.59 ± 1.93	.10
RNA conc., mg/g DM	.72 ± .20	1.90 ± .81	.15
Protein conc., mg/g DM	74.95 ± 30.89	120.55 ± 35.41	.22
Lipid conc., mg/g DM	896.8 ± 40.8	805.6 ± 61.7	.17
d 134			
Mammary weight, kg	6.2 ± .4	5.1 ± .2	.07
DNA conc., mg/g DM	6.38 ± 1.15	8.43 ± .91	.15
RNA conc., mg/g DM	1.80 ± .71	1.78 ± .65	.49
Protein conc., mg/g DM	139.75 ± 59.84	134.65 ± 51.22	.48
Lipid conc., mg/g DM	787.4 ± 50.1	756.7 ± 43.6	.34