GLUCOSE CLEARANCE STUDIES IN LACTATING COWS: INFLUENCE OF STAGE OF LACTATION AND RATION COMPOSITION

by

Melanie K. Gebhart

Thesis submitted to the Graduate Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Dairy Science

APPROVED:

D. H. Herbein, Chairman

C. E. Polan

F. C. Gwazdauskas

January, 1982
Blacksburg, Virginia

ACKNOWLEDGEMENTS

I am grateful for the assistance and friendship provided by the professors, fellow graduate students, lab technicians, and secretaries of the Dairy Science Department during my program at Virginia Tech.

I would like to extend a deep appreciation to Dr. Joe Herbein, my advisor, for his guidance, assistance, understanding, and enlightening discussions, in addition to all that he has taught me during my program.

Special thanks go to Dr. Carl Polan for his encouragement and guidance, Dr. Frank Gwazdauskas for his interest in my program, and Chuck Miller and the farm crew for helping make my collections at the barn run smoothly.

I would like to thank Larry Eckler for his friendship and all the assistance he has given me in the laboratory.

I appreciate the help provided by Twila Kenna and Bob Aiello with the collections for Study Two. In addition, I would like to thank Bob for being a good friend and providing many thought provoking discussions.

Special thanks go to Drs. Mike McGilliard, Ron Pearson, and Bill Vinson for statistical advice.

Special thanks go to Louis Tribble for his help with the collections for Study One and for his encouragement and moral support throughout my program.

I am grateful to my parents and family for their support and encouragement throughout my academic career.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS ii

Chapter page

I. INTRODUCTION 1

Objectives 2
Study One 2
Study Two 3

II. LITERATURE REVIEW 4

Methodology 4
In Vivo Kinetics Terminology 4
Intravenous Tolerance Test: A Method for Estimating Utilization 6
Feeding Regimes in Blood Metabolite Studies . 15
Glucose Metabolism in Ruminants 18
Changes with Growth 18
The Importance of Gluconeogenesis 18
Glucose Utilization during Pregnancy 23
Glucose Utilization during Lactation 25
Mammary glucose uptake 25
Glucose utilization in the mammary gland . 28
The Role of Insulin in Ruminant Metabolism . . . 37
Acetate in Ruminant Metabolism and Milk Synthesis 46

III. MATERIALS AND METHODS 52

Design of Study One 52
Experimental Conditions 53
Feeding Regime 53
Routine Procedure 55
Catheterization Procedure 55
Solutions and Doses used for Injections . . . 56
Injection Procedure 56
Sample Analysis 58
Milk Analysis 58
Blood Analysis 58
Determination of Plasma Glucose Concentration 59
Calculating Glucose Parameters 59
Determination of Plasma Insulin Concentration 61
Calculating Insulin Parameters 61

Determination of Extracellular Fluid and Blood Volumes 62
Statistical Procedure 64
Design of Study Two 65
Experimental Conditions 66
Feeding Regime 66
Routine Procedure 66
Catheterization Procedure 66
Solutions and Doses used for Injections . . . 68
Injection Procedure 68
Sample Analysis and Calculating Parameters . . . 68
Statistical Procedure 68

IV. RESULTS AND DISCUSSION - STUDY ONE 70

Body Weight, Dry Matter Intake, and Milk Production 70
Data and Statistical Analysis of Plasma Glucose Parameters 73
Plasma Glucose 73
Glucose Clearance Rate 76
Pool Size 79
Pool Space 81
Data and Statistical Analysis of Plasma Insulin Parameters 82
Plasma Insulin 83
Insulin Peak, Insulin Peak above Basal, and Insulin Peak Fold Increase above Basal 85
Insulin Response Curve Area 87
Insulin Clearance 90
Milk Yield and Composition Following Glucose and Acetate Loading 92

V. RESULTS AND DISCUSSION - STUDY TWO 103

Data and Statistical Analysis of Plasma Glucose Parameters 103
Data and Statistical Analysis of Plasma Insulin Parameters 107
Comparison of 60 day, Study Two Parameters with 90 day, Study One Parameters 109

VI. SUMMARY AND CONCLUSIONS 112

VII. EPILOGUE 115

REFERENCES 118

APPENDIX 129

VITA 144

LIST OF TABLES

Table		page
1.	Ration composition for Study One	54
2.	Ration compositions and analysis	67
3.	Cow status during sampling periods - Study One	71
4.	Plasma glucose parameters by lactation stage - Study One	74
5.	Plasma insulin parameters by lactation stage - Study One	84
6.	Effect of glucose loading on milk parameters - Early Stage	93
7.	Effect of glucose loading on milk parameters - Late Stage	94
8.	Effect of glucose loading on milk parameters - Early and Late data, combined	95
9.	Effect of acetate loading on milk parameters - Early Stage	96
10.	Effect of acetate loading on milk parameters - Late Stage	97
11.	Effect of acetate loading on milk parameters - Early and Late data, combined	98
12.	Cow status during sampling periods - Study Two	104
13.	Plasma glucose parameters by lactation stage and diet - Study Two	105
14.	Plasma insulin parameters by lactation stage and diet - Study Two	108
15.	Comparison of parameters at 60 days, Study Two with 90 days, Study One	110
16.	Plasma glucose parameter data - Early Stage - Study One	130

17. Plasma glucose parameter data - Late Stage - Study One 128

18. Plasma glucose parameter data - Dry Stage - Study One 129

19. Statistical summary of plasma glucose parameters - Study One 130

20. Insulin parameter data - Early Stage - Study One . 131

21. Insulin parameter data - Late Stage - Study One . 132

22. Insulin parameter data - Dry Stage - Study One . . 133

23. Statistical summary of insulin parameters - Study One 134

24. Statistical summary of milk parameters following glucose injection - Study One 135

25. Statistical summary of milk parameters following acetate injection - Study One 136

26. Plasma glucose parameter data - Study Two 137

27. Statistical summary of plasma glucose parameters - Study Two 138

28. Insulin parameter data - Study Two 139

29. Statistical summary of insulin parameters - Study Two 140

LIST OF FIGURES

Figure		page
1.	Glucose Tolerance Curve	9
2.	Two Methods of Evaluating Tolerance Curve	12
3.	Lactose Synthesis	29
4.	Blood Glucose and Insulin Cycle	37
5.	Glucose stimulated insulin response for Early, Late, and Dry data -Study One	85

Chapter I

INTRODUCTION

Ruminants derive most of their energy from volatile fatty acids formed from carbohydrate fermentation. Unlike nonruminants, ruminants absorb little glucose from the digestive tract and must rely largely on gluconeogenesis for their glucose supply.

During high production, the demands for glucose in the lactating ruminant greatly increase. It is generally accepted that glucose and acetate are the major sources of energy for the mammary gland. Glucose is the major precursor for lactose, and acetate is a major precursor for milk fat synthesis. Since lactose is considered the major osmole of milk, it has been proposed that changes in plasma glucose availability could affect milk volume.

In order to better understand metabolism and nutrient requirements of the lactating dairy cow, the availability and utilization rate of milk precursors at different stages of lactation need to be determined. In addition, hormonal and dietary interactions need to be elucidated.

OBJECTIVES

The overall purpose of the study was to investigate the availability and clearance rate of glucose in lactating and non-lactating dairy cattle. In addition, a preliminary study was conducted to observe whether ration type had an effect on the above mentioned blood parameters.

Study One

1. Objective one was to estimate glucose pool size, pool space, and clearance rate at three stages of lactation in first-lactation dairy cows.

2. The second objective was to determine if glucose and acetate loading by intravenous injection alter milk yield and milk fat, protein, and total solids content.

3. The third objective was to determine if diurnal variation in milk production exists in cows fed equal size meals every 4 hr.

4. The fourth objective was to determine whether intravenous glucose loading elicits an insulin response in first-lactation cows, and if the response varies depending on stage of lactation.

Study Two

1. Objective one was to determine whether high-concentrate feeding effects glucose pool size, pool space, and clearance rate in lactating dairy cows.

2. The second objective was to determine whether intravenous glucose loading elicits an insulin response in lactating dairy cows, and if the response varies with ration type.

Chapter II

LITERATURE REVIEW

This review consists of four major sections. The first is a consideration of methodology used to design the experiments. The remaining sections review several aspects of glucose, insulin and acetate in ruminant metabolism.

METHODOLOGY

In Vivo Kinetics Terminology

Common terms used in blood metabolite kinetic studies are as follows:

1. Pool refers to the quantity of a metabolite readily available (in the circulation) for utilization by the body. Kronfeld and Raggi (1964) stated that the molecules of a pool must be in dynamic equilibrium with the molecules entering and leaving the pool.

2. Pool Size is the mass of a metabolite pool.

3. Pool Space is the volume of distribution of a pool. Glucose pool space is equivalent to the volume of blood and extracellular fluid (Kronfeld et al., 1959; Bergman, 1963; White et al., 1969).

4. Clearance Rate is the rate of removal of a metabolite from a pool, expressed as % disappearance per minute.

5. Total Entry Rate is a kinetic parameter representing the rate at which a metabolite enters a pool.

6. Irreversible Loss is a kinetic parameter representing the rate at which a portion of the total entry rate leaves a pool and never returns, or the quantity of metabolite utilized per unit time.

7. Recycling Rate is a kinetic parameter representing the rate at which a metabolite leaves and re-enters the pool. Recycling occurs as a result of the end products of glucose catabolism entering the gluconeogenic pathway and represents the rate of glucose resynthesis (White et al., 1969; White and Leng, 1980).

8. Turnover Time refers to the time required to replace the glucose pool by all sources of glucose (White and Leng, 1980).

9. Turnover Rate refers to the rate at which the pool is replaced and is the movement of glucose carbon into and out of the glucose pool at a constant glucose concentration (Bergman, 1963).

Intravenous Tolerance Test: A Method for Estimating Utilization

Tolerance testing is a procedure involving the sudden loading of the blood system with a substance and the subsequent determination of its rate of disappearance from the blood. The disappearance rate is considered an estimate of the animal's ability to utilize or dispose of the substance. Glucose tolerance is commonly used as a diagnostic test for diabetes mellitus in humans. Since most of the interest regarding tolerance testing procedures has been associated with glucose, the literature reviewed in this section deals primarily with glucose tolerance tests in humans and ruminants.

There are two methods by which glucose can be administered. The dose can be taken orally or injected intravenously. The oral glucose tolerance test is preferred in diagnosing carbohydrate metabolic disorders in humans, particularly liver disease and diabetes, because the majority of the absorbed sugar passes directly through the liver (Holmes, 1951a). In ruminant animals, however, tolerance tests are conducted by intravenous (IV) injection to avoid alteration of glucose during rumen fermentation. Since this thesis involves ruminant animals, the discussion of tolerance tests will primarily be in reference to the intravenous glucose tolerance test (IVGTT).

Prior to the 1960s, the IVGTT received considerable attention in an effort to develop standard procedures. Since 1960, interest has shifted to the development of radioisotope techniques for kinetic studies of glucose and other metabolites. With increasing restrictions being placed on isotope usage, interest has re-developed in the use of tolerance tests to estimate in vivo metabolite utilization parameters.

The literature indicates that there have been a number of procedures used to conduct IVGTT. There is disagreement among researchers concerning the preferred dose, the concentration of the dose, timing the test, and in the interpretation of the test results (West and Wood, 1959). This has led to difficulties in comparing results of different studies.

The test involves injecting a known dose (mass) of a metabolite into the bloodstream, followed by collection of blood samples over a period of time. The dose must be sufficient to produce an elevation in blood concentration that can be measured precisely. Zero time is set as the beginning or end of the injection (West and Wood, 1959). West and Wood (1959) reported that although there exists variations in techniques used to conduct IVGTT, for the most part, these differences are insignificant.

The major area of disagreement concerning IVGTT is evaluation of test results, specifically interpretation of the disappearance curve obtained when blood glucose is plotted against time after injection. Figure 1a depicts a typical disappearance curve following an IV glucose injection (taken from Greville, 1943).

Greville (1943) conducted a study using the results of 35 IVGTT from human subjects to develop an equation that would best describe the tolerance curve. The disappearance of glucose followed an exponential function with respect to time. The rate of fall of blood sugar at any time (t) following injection was approximately proportional to the blood sugar (y) at that time. When the log of y was plotted against t a straight line was obtained. Figure 1b depicts the resulting plot obtained when y values from Figure 1a are converted to natural logarithm values. The slope of the line estimates the rate of fall of blood sugar as % disappearance per minute. Rather than using the log of observed glucose concentration, Greville (1943) proposed that the disappearance rate would be best estimated using the log of the glucose excess. Glucose excess was determined by subtracting "the limiting value of y" from the observed blood sugar concentrations. The "limiting value of y" was the hypoglycemic concentration the disappearance curve approaches as t becomes large.

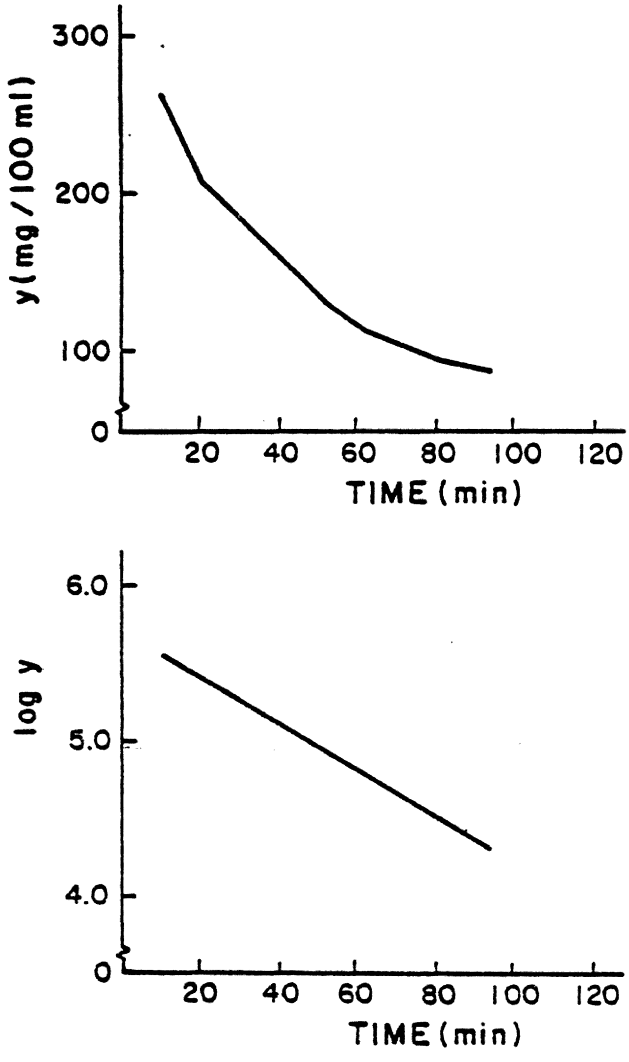

Figure 1. Glucose tolerance curve following a 25 g IV glucose load given to human subjects. a) Plot of blood glucose (y) vs. time. b) Semi-log plot of y vs. time. (Greville, 1943).

Amatuzio et al. (1953) used a modified version of Greville's method to study the repeatability of the IVGTT in human subjects. Glucose excess was calculated by subtracting basal glucose from the observed blood glucose concentrations. Twenty-five g of glucose were administered IV to 20 normal subjects. One to 3 months later the test was repeated. They (Amatuzio et al., 1953) observed the disappearance rates to be reproducible. Thirty-five g were administered to 13 subjects to determine if disappearance varied with dose, and they (Amatuzio et al., 1953) observed that the rates were similar.

In addition to Greville's method, another method has been used to evaluate glucose tolerance curves. This method involves evaluation of the log plot of observed glucose concentrations over time, with the slope of the line equal to disappearance. West and Wood (1953) reviewed the factors influencing the rate of blood glucose disappearance and attempted to determine which of the two methods best estimated disappearance. Their (West and Wood, 1953) observations were based on their own data and the re-examination of data reported by other researchers. Renal excretion of glucose in human subjects following a glucose load was small but had a significant effect on glucose clearance. Excretion was estimated to be about 4% of a 25 g dose. It

occured at a non-uniform rate, affecting predominately the early part of the curve. In addition, it was proposed the equilibration of the dose in the blood and extracellular fluid would also contribute to a greater disappearance rate in the early portion of the curve.

Figure 2 (taken from West and Wood, 1953) depicts the tolerance curves obtained from normal human subjects following 25 g IV glucose loading using the two evaluation methods previously mentioned. They observed that the glucose excess curve fell at a relatively constant rate in the middle of the curve, but not at the extremes. It was more rapid initially, which was probably due to renal excretion and mixing, and even more rapid in the later part of the curve. West and Wood (1953) concluded that although the rate of disappearance was observed to be relatively constant over a 1-hr time period, it was not constant for more than a few minutes at any one time. Conversely, the disappearance rate obtained from the absolute glucose curve remained relatively constant during the first hour after injection. The curve declined more rapidly in the initial segment and had a slight tendency to decrease during the last few minutes of the test. This can be seen in Figure 2. They concluded that the absolute glucose curve better estimated glucose disappearance rate. They recommended this method over Greville's (1943) method because the glucose excess curve

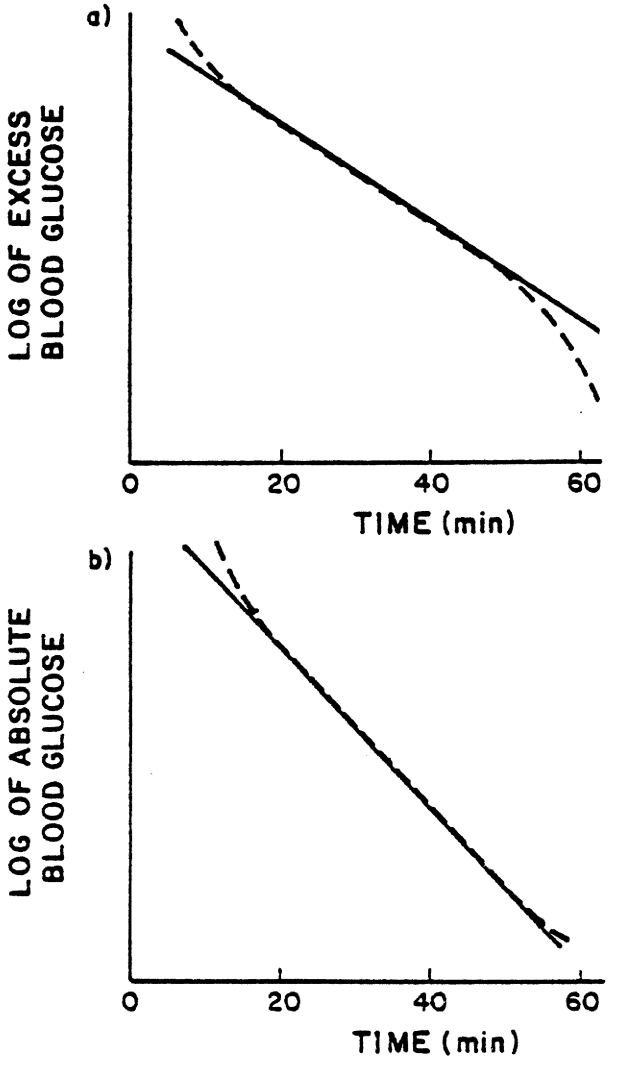

Figure 2. Semi-log plots of a tolerance curve using a) excess blood glucose or b) absolute blood glucose as the y-axis. (actual---, fitted line——). (West and Wood, 1953).

was less effective in determining glucose disappearance at physiologic levels of hyperglycemia (30 to 50 mg/dl above basal levels), which is the time when disappearance rate is closest to glucose utilization rate. The initial portion of the curve should be disregarded in the analysis, as it tends to overestimate disappearance.

Glucose tolerance has been studied to a limited degree in ruminant animals. McCandless and Dye (1950) conducted IVGTT on different ruminant species using a 1 g/kg body weight dose to determine the effect of age and ration type on tolerance time. Tolerance time was defined as the time required for elevated blood glucose to return to pre-injection concentration. This manner of expressing tolerance is too simplified to make accurate comparisons, but prior to the 1960s it was commonly used to express test results. McCandless and Dye (1950) observed that tolerance time in lambs increased with increasing age from 32 to 120 days and when rations were changed from hay ad libitum plus a grain supplement to strictly hay.

Holmes (1951a) conducted IVGTT on six pregnant cows, four of which were Ayrshires and two were beef cows. They were interested in determining a dose which would allow for precise measurement and the fraction of that dose that would

appear in urine. The dose adopted was .3 g/kg body weight. Tolerance time averaged 1 1/2 to 2 hr and renal excretion averaged 10.3% of the amount injected.

Holmes (1951b) conducted a subsequent study based on results obtained from the study cited above, to determine the effect of clinical ketosis in dairy cows on glucose tolerance. Of the three cases studied, glucose tolerance appeared to be normal. This is in agreement with the findings of Kronfeld et al. (1959) who observed similar glucose entry rates in normal and ketotic cows when a radioactive tracer was used.

Reid (1958) conducted both glucose and acetate tolerance tests on Merino sheep using the "excess above basal" method to evaluate the clearance curves. They observed reduced tolerance in sheep fed wheaten chaff as compared to a ration containing 20% wheaten chaff, 30% lucerne chaff, and 50% cracked maize. This is in agreement with the findings of McCandless and Dye (1950). Reid (1958) observed that the disappearance rates of acetate (at a dose of .25 g/kg body weight) were considerably higher than glucose disappearance rates (at a dose of .4 g/kg body weight). Sheep fed the ration containing 50% cracked maize had an average glucose clearance of 3.7% and an average acetate clearance of 8.2% disappearance/min.

In conclusion, tolerance testing appears to be a suitable method for estimating the utilization rates of glucose *in vivo*. The test has been observed to be reproducible and is relatively simple to conduct. In addition to utilization rate, this method can also be used to estimate pool size and pool space. Tolerance tests were conducted in the present study to estimate glucose clearance rate, pool size, and pool space in first-lactation and mature lactating cows.

Feeding Regimes in Blood Metabolite Studies

It is common practice to feed dairy cattle two large meals per day, usually in the morning and evening. When feeding in this manner, maximum absorption of volatile fatty acids from the rumen occurs from 1 to 3 hr following consumption of each meal (Sutton, 1971), and results in a non-uniform rate of flow of nutrients from the gut over a 24-hr period. This must be considered when conducting *in vivo* studies. These studies often involve estimation of blood metabolite parameters that are highly dependent on nutritional state. For example, Hara and Saito (1980) looked at diurnal variations in plasma glucose and insulin responses following oral glucose loads given to rats. Feeding only during the night resulted in greater insulin responses during the night as compared to daytime. Similar observations were made for daytime feeding.

White et al. (1969) compared three methods of administering ^{14}C glucose in sheep. They fed 800 g of lucerne chaff in 12 equal meals between 0700 and 1800 hr. Therefore, the animals had gone 13 hr without feed at 0700 hr. Isotope administration time was at 1000 hr. This corresponded to the time of maximum nutrient absorption from the gut following the 0700 hr meal. Parameters measured under these conditions may not be a reliable estimate of steady state conditions. Steady state would result in a constant rate of influx of gluconeogenic substrate into the pool or a relatively constant pool over time.

Young (1977) recommends continuous feeding or feeding intervals of 2 hr or less to have animals in steady state. A number of researchers have used this practice in glucose kinetic studies (Judson and Leng, 1973; Bickerstaffe et al., 1974; Young et al., 1974; Herbein et al., 1978; Prior and Christenson, 1978).

Bickerstaffe et al. (1974) conducted a study with two Jersey and two Fresian cows. They fed hay twice daily and delivered the concentrate portion of the diet by automatic feeder every 2 hr. Cows were milked hourly. They found that milk production varied little from hour to hour, and concluded the animals were in steady state.

It must be kept in mind that although 1- to 2-hr feeding intervals are desirable to achieve steady state, this is not the typical nutritional state of the ruminant.

Feeding intervals of 4-hr were used in this thesis project in an attempt to achieve steady state and still allow for normal management procedures and milking schedules. Steady state was asessed using twice daily milk production data.

GLUCOSE METABOLISM IN RUMINANTS

Changes with Growth

Digestion in a young ruminant, lacking a functioning rumen, is similar to that in nonruminant animals. At this time, the diet consists solely of milk. Dietary carbohydrates are absorbed in the form of simple sugars in the small intestine. It has been shown that blood glucose levels in calves and lambs are approximately twice that of the adult animal (McCandless and Dye, 1950).

When solid food is incorporated into the diet, rumination starts and rumen development commences. In a study with lambs, McCandless and Dye (1950) observed that when the animals' diets were changed from milk to hay, rumination started and the primary decrease in blood glucose occurred at this time.

The Importance of Gluconeogenesis

Unlike nonruminants, adult ruminants rely largely on gluconeogenesis to supply glucose to meet their metabolic needs. In ruminant digestion, dietary carbohydrate is fermented in the rumen to yield short-chain fatty acids (acetate, propionate, and butyrate), with little carbohydrate escaping the fermentation process. The amount of starch bypass depends on level of intake, physical form and starch

content of the diet (Bensadoun et al., 1962; Topps et al., 1968).

Studies have been conducted, largely with sheep, to estimate the amount of glucose absorbed from the small intestine. Bensadoun et al. (1962) obtained blood samples from the carotid artery and portal vein of sheep, while measuring portal blood flow rate, to estimate glucose absorption differences in animals fed either chopped or pelleted finely ground hay at different levels of intake. They (Bensadoun et al., 1962) estimated that essentially no glucose was absorbed with either diet or at three different feeding levels (46, 56, and 70 g/kg body weight). Blood glucose was higher at higher levels of intake and with the pelleted ration. They (Bensadoun et al., 1962) concluded this was the result of an increase in the availability of gluconeogenic precursors, namely propionic acid, with higher intakes and the finely ground pelleted ration. This is in agreement with the findings of Bergman et al. (1970), who estimated negligible glucose absorption in sheep fed either a pelleted hay or a 50% concentrate, 50% hay diet. It has been estimated that glucose absorption in sheep averages 5 to 13 g/day on hay diets (Heald, 1951; Topps et al., 1968) and 6 to 35 g/day, or 6% of the dietary starch intake, on pelleted concentrate diets (Topps et al., 1968). Therefore,

glucose absorption from the ruminant gut, when compared to the animal's daily glucose requirement, is considered to be negligible and the animal must derive its glucose supply largely from endogenous synthesis.

Although both the liver and kidney have high rates of gluconeogenesis in nonruminants, hepatic gluconeogenesis is thought to be the predominant source of glucose for the ruminant due to the availability of substrates in the portal circulation (Ballard et al., 1969). Studies conducted with sheep and involving the measurement of arterio-venous glucose differences between the portal vein, hepatic vein, and a splanchnic artery have led to the conclusion that hepatic gluconeogenesis accounts for about 85% of the endogenously synthesized glucose (Bergman et al., 1970). In sheep, hepatic glucose production has been estimated to be about .25 g/hr/kg metabolic body weight (Bergman et al., 1970). Renal glucose production has been estimated to account for 8 to 10% of the whole body's glucose turnover (Kaufman and Bergman, 1971). Therefore, the liver and, to a lesser extent, the kidney are responsible for maintaining a constant supply of glucose for the ruminant.

The metabolites that serve as the major precursors for gluconeogenesis are propionate and glucogenic amino acids.

Propionate is considered to be the major VFA involved in gluconeogenesis. Acetate and butyrate cannot contribute to a net synthesis of glucose due to the loss of two carbon atoms per acetate entering the TCA Cycle (Bergman, 1973), but it has been observed that they do aid in sparing glucose by being preferentially utilized for oxidative energy at the tissue level (Head et al., 1964).

The percentage of circulating propionate that is converted to glucose has been estimated to be from 19 to 65% (Bergman et al., 1966; Leng et al., 1967; Wiltrout and Satter, 1972; Bergman, 1973). The amount of propionate available for gluconeogenesis varies with type of diet fed and level of intake and their associated effects on rumen fermentation and, thus, propionate production.

The contribution of amino acids to gluconeogenesis has been shown to vary not only with diet but also with physiologic state (Bergman, 1973). It has been proposed that as much as 30% of the glucose synthesized may be from amino acid carbon (Hunter and Millson, 1964). During lactation, the availability of plasma amino acids is more limited because of their incorporation into milk protein in the mammary gland. Brukental et al. (1980) estimated that amino acids accounted for no more than 2% of glucose irreversible

loss in the high-yielding dairy cow. Amino acids are important precursors for glucose synthesis but their involvement is quite variable.

Glycerol, pyruvate, and lactate also contribute, to a lesser degree, to glucose synthesis. In the sheep, in the normal fed state, glycerol accounts for about 5% of the synthesized glucose, and under fasting conditions the percentage is even higher (Bergman et al., 1968). This is most likely the result of an increased availability of glycerol due to increased rates of lipolysis.

The contribution of pyruvate and lactate to gluconeogenesis is highly dependent on physiological status. Baird (1981) reviewed the involvement of these two metabolites in glucose synthesis in the ruminant. In the non-lactating, fed state, the liver takes up lactate and releases pyruvate. The gut output of lactate is higher than the liver uptake, and thus there is a net output of both lactate and pyruvate from the splanchnic area. However, in the non-lactating, fasted and the lactating, fed states there is a net uptake of both lactate and pyruvate by liver. Therefore, the involvement of lactate and pyruvate in glucose synthesis is dependent on the carbohydrate status of the animal.

In conclusion, the glucose requirements of ruminants must be met largely by gluconeogenesis in the liver, with propionate and amino acids as the major precursors for the synthetic process. The overall carbohydrate status of the animal varies largely, depending on the supply of glucogenic precursors and the physiological status of the animal.

Glucose Utilization during Pregnancy

Glucose is the main source of energy for the developing fetus (Leat, 1971). It has been estimated (Bauman and Currie, 1980) that glucose accounts for 50 to 70% of the fetal requirements for oxidative metabolism, or 4 to 6 g/day/kg fetal weight. This is a considerable amount during late pregnancy, when the size of the fetus approaches birth weight. Prior and Christenson (1978) estimated that the uterus accounted for 43% of the total glucose turnover during late pregnancy in sheep (109 days of gestation).

Bergman (1963) observed that overall glucose oxidation in pregnant and non-pregnant ewes is comparable. However, in order to provide glucose to the fetus, glucose utilization by maternal body tissues must be reduced. Setchell (1964) estimated that oxidation in extra-uterine tissue went from 10% down to 2% during late pregnancy in sheep.

Towards the end of gestation, fetal glucose requirement increases with increasing fetal size. During the period of maximal fetal growth, maternal mammary gland development is also most pronounced (Bauman and Currie, 1980). Therefore, the developing fetus and mammary gland place considerable glucose demands on the ruminant mother.

Fetal number has been shown to increase conceptus glucose requirement (Hayden et al., 1979). Pregnancy in sheep is characterized by a high incidence of severe carbohydrate insufficiency (pregnancy toxemia), which is associated with multiparous pregnancies.

In conclusion, carbohydrate metabolism is altered during pregnancy in ruminants. As gestation progresses, the glucose demands of the growing fetus and developing mammary gland continue to increase until parturition. Most of the available data on ruminant fetal glucose requirements are from studies conducted with sheep. This thesis will attempt to provide data on glucose clearance rates and pool sizes during late gestation, and compare them to observations made with the same cows at two different stages of lactation.

Glucose Utilization during Lactation

Glucose is an important precursor of milk synthesis in the ruminant mammary gland. The magnitude of glucose involvement in mammary metabolism was shown by Linzell (1960), when he observed that mammary oxygen consumption doubled, acetate consumption tripled, and glucose uptake increased ten-fold during lactation in goats. Some researchers have hypothesized that glucose could be the limiting factor in milk production (Linzell, 1967; Linzell and Peaker, 1971; Tindal et al., 1978).

Studies involving the use of perfused mammary glands, mammary tissue slices, and radioactive tracers, in addition to gross mammary nutrient uptake by metabolite arteriovenous differences, have resulted in a better understanding of the important contribution glucose makes to milk synthesis. This section reviews the involvement of glucose in milk synthesis. The major areas to be considered are mammary glucose uptake and glucose utilization in the mammary gland.

Mammary glucose uptake

During lactation, the ruminant mammary gland is capable of extracting appreciable amounts of glucose from the circulating plasma. The mechanisms involved with the transport

of glucose across mammary gland cellular membranes have not been resolved. Unlike glucose uptake in other body tissues, it has been observed that insulin is not required for glucose transport across mammary gland membranes in cows (Kronfeld et al., 1963; Schmidt, 1966) and sheep (Hove, 1978). However, it has been shown that prolonged insulin deficiency leads to drastic reductions in milk production in goats (Nowak and Dzialoszynski, 1967) and rats (Martin and Baldwin, 1971; Wilde and Kuhn, 1979). Martin and Baldwin (1971) suggest the primary role of insulin on glucose metabolism in the mammary gland is in regulation of intracellular processes, rather than metabolite transport. The most common theory held on glucose uptake is the existence of a glucose concentration gradient across the plasma membrane that promotes glucose entry into mammary cells (Linzell and Peaker, 1971).

Bergman reported in a review on glucose metabolism in ruminants (Bergman, 1973) that lactose synthesis alone could require as much as 1000 g of glucose per day in high-producing dairy cows. Kronfeld et al. (1968) observed that mammary glucose uptake in lactating dairy cattle was highly correlated with milk production, whereas the net transfers of acetoacetate, beta-hydroxybutyrate, free fatty acids, and acetate were not related to milk production. Glucose uptake

by the mammary gland has been estimated using radio-isotope tracers. Annison and Linzell (1964) observed that a fully lactating goat udder was capable of using 60 to 85% of the total glucose produced in the body. In Fresian cows the proportion has been observed to be as high as 70 to 90% (Lindsay, 1971). It appears that glucose uptake by the gland is a key factor in milk production.

In order to supply large quantities of glucose for lactation, the body must undergo alterations in carbohydrate metabolism. Glucose entry rate is greatly increased during lactation (Bergman and Hogue, 1967; Annison and Linzell, 1964) and it has been reported that there is a positive correlation between glucose entry and milk yield (Kronfeld et al., 1968; Annison et al., 1974; Bickerstaffe et al., 1974; Paterson and Linzell, 1974). Reid hypothesized (as cited in Kronfeld et al., 1968) that the ability to synthesize glucose is a key factor in a dairy cow maintaining maximum production. Although gluconeogenesis plays an important role in supplying glucose for milk synthesis, body metabolism must be coordinated to accomodate the increased glucose entry. The concentration of blood glucose must be maintained within a moderately narrow range to maintain homeostasis. This is in agreement with Bergman and Hogue's (1967) findings that mean glucose pool sizes remain rela-

tively constant regardless of milk production or glucose turnover in sheep. Therefore, something other than blood glucose concentration regulates milk synthesis.

Even though a considerable amount of glucose is being taken up by the mammary gland, there appears to be no change in non-mammary tissue utilization of glucose during lactation (Paterson and Linzell, 1974; Brukental et al., 1980). The mammary gland is somehow capable of extracting large quantities of glucose from plasma without depriving other body tissues. This ability is thought to be dependent on mammary blood flow. Paterson and Linzell (1974) and Bickerstaffe et al. (1974) observed that mammary glucose uptake was highly correlated with milk yield in dairy cows, even though the mammary arterio-venous glucose concentration difference was relatively constant. A high correlation between mammary blood flow and milk yield has been shown in cows (Kronfeld et al., 1968; Bickerstaffe et al., 1974) and sheep (Linzell, 1960).

Glucose utilization in the mammary gland

The major role of glucose in milk synthesis is considered to be the formation of lactose. Several review articles have been written on lactose synthesis (Rook and Wheelock, 1967; Ebner and Brodbeck, 1968; Larson, 1969;

Linzell and Peaker, 1971). It has been estimated that about 50% of the glucose taken up by the mammary gland is incorporated into lactose in goats, and it could be higher (60 to 80%) in cows (Kleiber et al., 1955; Wood et al., 1965; Lindsay, 1971; Bickerstaffe et al., 1974). Lactose synthesis has been estimated to account for 60% of the glucose entry in both cows (Bickerstaffe et al., 1974) and sheep (Bergman and Hogue, 1967). Figure 3 (adapted from a figure from Wood et al., 1957) depicts the scheme of lactose synthesis which was proposed by Watkins and Hassid (1962) and is now accepted as correct (Linzell and Peaker, 1971).

Glucose is not an essential precursor of all lactose carbon (Lindsay, 1971), but it has been found to supply approximately 80 to 90% of the carbon in lactose (Kleiber et al., 1955; Annison et al., 1974; Bickerstaffe et al., 1974). The formation of lactose involves combining beta-glucose with UDP-galactose in the presence of the enzyme lactose synthetase. Studies with radioisotopes indicate that virtually all beta-glucose comes from plasma glucose. UDP-galactose is also derived largely from glucose, even though it may originate from other compounds, such as acetate and glycerol (Kleiber et al., 1955; Hansen et al., 1962; Carubelli et al., 1964; Bartley et al., 1966).

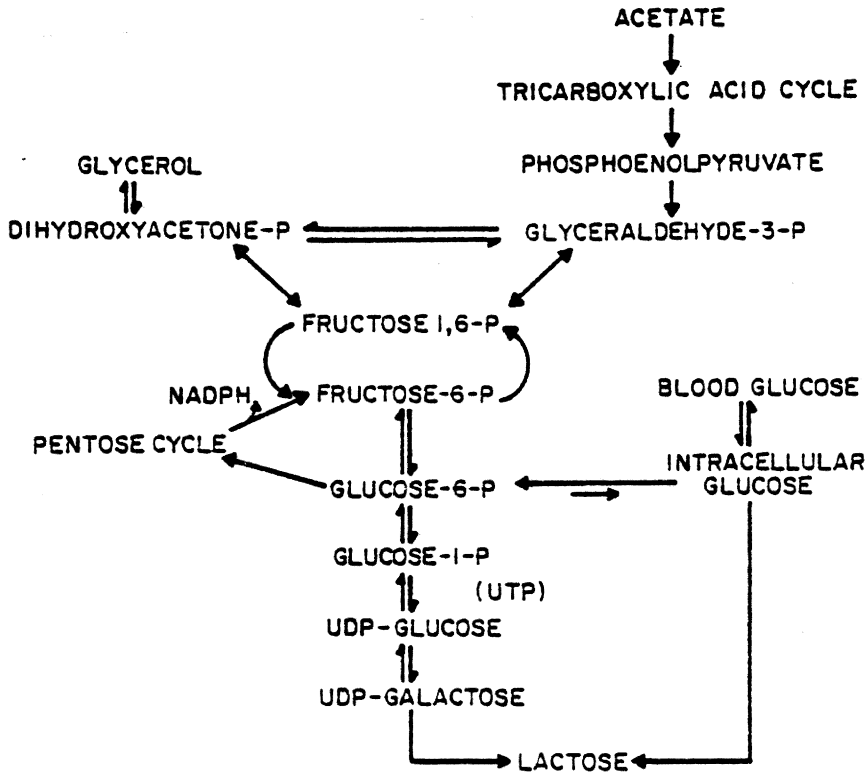

Figure 3. Scheme of lactose synthesis from glucose and UDP-galactose (adapted from a figure from Wood et al., 1957). The pathways in which glycerol and acetate contribute carbon have also been included.

Until recently, both the glucose and galactose moieties of lactose were thought to be derived from a single pool of glucose-1-P in the mammary gland. Wood et al. (1965) looked at the distribution of ^{14}C in the glucose and galactose moieties of lactose synthesized in perfused cow udders that were administered (1-^{14}C)-, (2-^{14}C)-, and (6-^{14}C)-glucose. The ^{14}C of the glucose moiety was identical to the glucose in the perfusion media. Therefore, they concluded that the galactosyl acceptor came directly from plasma glucose. On the other hand, when (2-^{14}C)-glucose was administered the galactose moiety was extensively randomized in positions 1 and 3. They concluded that the randomization in the galactose moiety was the result of reactions occuring in the pentose cycle. They estimated that about 23 to 30% of the glucose in the lactose synthesizing cells was metabolized in the pentose cycle. Similar observations have been made in rat mammary gland slices (Bartley et al., 1966).

The importance of lactose in milk is its effect on fluid secretion. The concentration of lactose remains relatively constant in order to maintain milk isotonic with blood (Rook et al., 1965). In addition to lactose, potassium, sodium, and chlorine are responsible for the osmotic pressure of milk (Rook and Wheelock, 1967). Unlike the minerals involved, lactose is unique in that it is secreted

into milk by exocitosis and is unable to penetrate the milk secreting cells once it is released into milk (Linzell et al., 1976). Therefore, lactose is considered to be the major osmole of milk (Linzell and Peaker, 1971) and can affect milk volume by drawing water into the alveolar lumens of the mammary gland.

During fasting, lactose synthesis is depressed more than any other synthetic pathway in the mammary gland (Linzell, 1967). Wilde and Kuhn (1979) observed a decrease in lactose synthesis in rats fed 76% of their regular intake. The percent lactose in the milk was unaffected, and the decreased lactose synthesis was accompanied by a decrease in fluid milk volume.

It has been proposed (Rook et al., 1965; Kuhn et al., 1980) that small changes in plasma glucose availability to the mammary gland could affect milk volume. Hardwick et al. (1961) observed, using a goat perfused mammary gland, that when glucose was not available in the perfusion media, milk synthesis did not occur. Milk synthesis ceased even though an adequate supply of acetate and amino acids was available. Hypoglycemia, resulting from insulin treatment, has been observed to depress milk yield and lactose secretion, whereas milk production was restored to normal when glucose

treatments were given in addition to insulin (Kronfeld et al., 1963; Rook et al., 1965; Schmidt, 1966; Linzell, 1967; Hove, 1978). Intravenous glucose infusions (Fisher and Elliot, 1966; Linzell, 1967; Mepham and Linzell, 1974) and abomasal infusions of glucose (Frobish and Davis, 1977) can elevate the concentration of plasma glucose and increase milk yield and lactose production.

One problem that has been associated with intravenous glucose infusions is milk fat depression (Hardwick et al., 1961; McClymont and Vallance, 1962; Fisher and Elliot, 1966; Fisher et al., 1967). McClymont and Vallance (1962) proposed that elevated plasma glucose could reduce milk fat by suppressing fat mobilization from adipose tissue. The reduction in fat mobilization could be the result of an increased concentration of circulating insulin. Insulin is one of the major hormones involved in deposition of body fat reserves. This will be discussed in more detail in the insulin section of this literature review.

It is believed that mammary gland processes involved with synthesizing milk components are pathways independent of each other, with glucose the main precursor in lactose synthesis, acetate, beta-hydroxybutyrate, and plasma fatty acids the precursors for milk fat, and plasma amino acids

the precursors for milk protein. However, all the pathways are indirectly dependent on lactose synthesis because in the absence of lactose synthesis, milk production ceases completely (Hardwick et al., 1961). Hardwick et al. (1963) estimated that a mammary gland empty of milk had sufficient fat reserves to form milk fat for 15 hr, whereas lactose reserves would only last 1 hr. This supports the theory of glucose being a major limiting factor in milk synthesis.

Lactose synthesis does not account for all glucose taken up by mammary gland. Hardwick et al. (1963) perfused isolated goat mammary glands with a mixture of glucose, acetate, and amino acids in proportions similar to _in vivo_ uptake by the gland. Using (U-^{14}C)-glucose (fatty acids were not present in the perfusion media), they observed that 39% of the mammary carbon dioxide production, 23% of milk triglyceride glycerol, 41% of milk citrate, and .5% of the milk fatty acids were derived from glucose.

Annison and Linzell (1964) used isotope dilution techniques with lactating and dry goats to estimate oxidation in the mammary gland during different stages of production. They estimated that glucose accounted for 9% of the total body carbon dioxide production. This is similar to the 8 to 11% range reported in other studies involving dairy cattle

(Annison et al., 1974; Bickerstaffe et al., 1974). In addition, the mammary gland was found to oxidize more glucose than the remainder of the body. Thirty-nine percent of the mammary carbon dioxide was found to be derived from glucose when lactating and only 20% when dry. Glucose uptake by the mammary gland also increases when milk is being synthesized. During lactation, 25% of glucose uptake was oxidized as compared to 76% during the dry period. The lower percent during lactation could be due to glucose utilization for milk synthesis.

Bickerstaffe et al. (1974) looked at mammary glucose uptake in Jersey and Fresian cows using udder blood flow and arterio-venous difference measurements. Their estimate of only 10 to 20% of glucose uptake being oxidized is considerably lower than the previously mentioned estimates in goats.

Glucose serves as a source of glycerol-3-P in milk fat synthesis. Unlike nonruminant animals, ruminants incorporate only negligible amounts of glucose into fatty acids because of low levels of citrate cleavage enzyme and NADP-malate dehydrogenase (Ballard et al., 1969; Bauman et al., 1970; Baldwin and Smith, 1971). As mentioned previously, Hardwick et al. (1963) estimated that as much as 23% of glyceride glycerol was formed from glucose in perfused goat

udders. This high percentage could be an over-estimate due to the lack of fatty acids in the perfusion media. Kleiber et al. (1955) observed that 11 to 13% of glucose uptake by the mammary gland was incorporated into milk fat in Jersey cows.

In addition to glycerol synthesis, plasma glucose provides reducing equivalents (NADPH) needed to reduce acetate in fatty acid synthesis. Wood et al. (1965) estimated that 30% of glucose taken up by the perfused cow mammary gland was utilized via the pentose cycle, a cycle which generates NADPH.

As shown from the work cited, glucose is a major precursor of milk synthesis. It is required for milk secretion and lactose synthesis. In addition, glucose is an excellent source of energy for the mammary gland and has an important involvement in fat synthesis. In the present study, glucose clearance will be estimated in lactating and dry cows to determine if the physiological status of the animal affects exogenous glucose utilization. Milk obtained from lactating cows following glucose injections was analyzed to determine the effect of glucose loading on milk synthesis. The effect of optimal fiber vs low-fiber (high-grain) feeding on glucose clearance was also investigated.

THE ROLE OF INSULIN IN RUMINANT METABOLISM

Insulin is considered a major regulatory hormone of carbohydrate metabolism. An increase in circulating insulin is associated with an increase in glucose uptake by tissue, increase in glycogen synthesis and a decrease in gluconeogenesis and glycogenolysis. Figure 4 shows the proposed scheme of blood glucose regulation by insulin at the tissue level (Goldfine, 1978; Kahn, 1980).

As mentioned previously in this review, nonruminant absorption from the gut differs markedly from ruminant absorption. Nonruminants absorb considerable amounts of glucose from the gut, and they are dependent on insulin to maintain blood glucose homeostasis. Ruminants, on the other hand, absorb little glucose directly from the gut and rely heavily on gluconeogenesis to meet their glucose needs. Many researchers have proposed that glucose may not be very insulinogenic in ruminant animals (Manns and Boda, 1967; Ambro et al., 1973; Kamalu and Trenkle, 1978). Fatty acids containing three to eight carbon atoms stimulate insulin secretion in ruminants but are not effective in nonruminant animals (Horino et al., 1968). Studies have indicated that fatty acids may play a greater role in the regulation of insulin secretion than glucose in the ruminant animal.

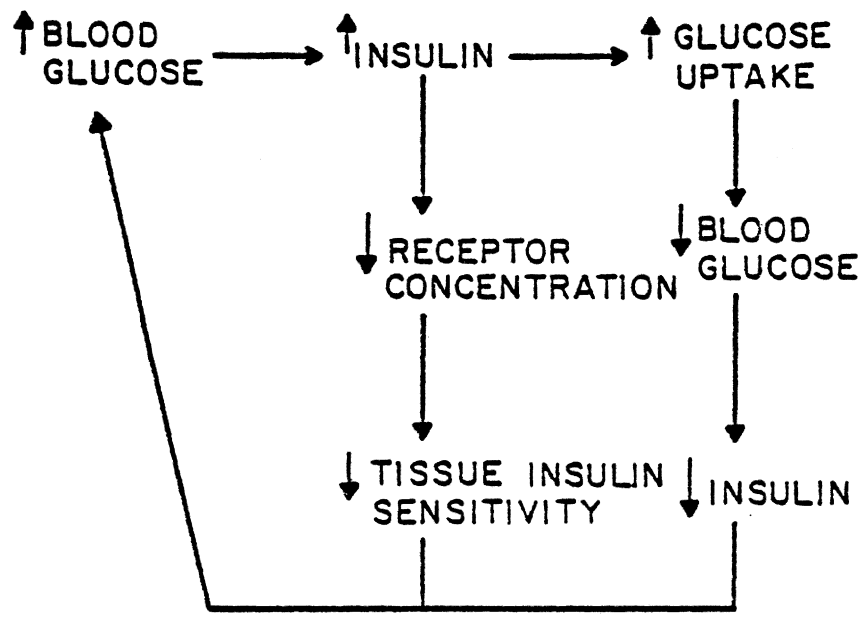

Figure 4. Proposed insulin cycle (Goldfine, 1978; Kahn, 1980).

Most of the studies looking at insulin stimulation by fatty acids and glucose in ruminant animals have been performed using sheep. Ambro et al. (1973) injected each of the following into crossbred ewes at a dose of 1.25 mmoles/kg body weight: glucose, lactate, acetate, propionate, butyrate, iso-valerate, and capronate. Glucose injection elevated blood glucose higher than the fatty acids but had less of an insulin response, with the exception of acetate. Acetate injection stimulated only a small insulin response even though blood glucose was not affected.

Horino et al. (1968) infused propionate, butyrate, and acetate into sheep at .025 mmoles/kg body weight for a period of 20 min. They observed that propionate increased plasma insulin from 32 to 152 uU/ml in 5 min. The insulin response of butyrate was 1.8 times as great as propionate. Both fatty acids stimulated insulin secretion even though blood glucose was not significantly elevated. Infusion of acetate did not result in a change in plasma glucose or insulin levels. They also administered single injections of propionate, butyrate, acetate, and glucose at .125 mmoles/kg body weight. Acetate again showed no affect on insulin and plasma glucose. The insulin response observed following glucose injection was significantly less than that resulting from propionate and butyrate injections even though circu-

lating plasma glucose was three to 15 times greater than the fatty acid-induced glucose responses. This is in agreement with the findings of other studies using sheep (Manns and Boda, 1967; Kamalu and Trenkle 1978) and cattle (McAtee and Trenkle, 1971).

Kamalu and Trenkle (1978) conducted a study with sheep injecting different concentrations of glucose intravenously. They (Kamalu and Trenkle, 1978) observed an increase of only .74 ng/ml in insulin concentration when glucose was injected at .6 mmoles/kg (or .108 g/kg) body weight. These results were considered when the present study was designed. A glucose dose of .1 g/kg body weight was expected to stimulate only a negligible insulin response in dairy cattle.

Ruminants do not appear to be as sensitive as nonruminants to insulin. Reid (1952) observed that the arteriovenous glucose difference in sheep was not significantly increased following intravenous glucose injection or insulin administration. Prior and Christenson (1978) proposed that a decrease in insulin sensitivity may aid ruminant animals in conserving glucose.

Insulin insensitivity and glucose sparing may be key factors in dairy cattle metabolism during peak production periods. In cows during early lactation, low glucose and

insulin concentrations have been observed (Koprowski and Tucker, 1973; Smith et al., 1976) and insulin concentration has been shown to be positively correlated to feed intake (Smith et al., 1976). This could be due to the negative energy state of the animal at this time. Low insulin concentrations would be advantageous in allowing the mobilization of energy stores to meet the requirements for milk production. In addition, low glucose levels could be a benefit to the animal in stimulating gluconeogenesis in the liver. Work done by Sacca et al. (1979) with conscious dogs indicated that a fall in blood glucose may reverse insulin-induced inhibition of glucose production independent of counterregulatory hormone action.

Insulin has been shown to be positively correlated with days into lactation and negatively correlated with milk yield (Koprowski and Tucker, 1973; Walker and Elliot, 1973; Smith et al., 1976; Walsh et al., 1980; Vasilatos and Wangsness, 1981). The increase in circulating insulin during late lactation could be associated with positive energy balance, as insulin has been shown to be positively correlated with weight gain (Hart et al., 1979).

Food intake is followed, after a few hours, by a significant increase in plasma insulin in cows (McAtee and

Trenkle, 1971; Hove and Blom, 1973) and sheep (Horino et al., 1968; Trenkle, 1971). The rise in plasma insulin is thought to correspond to the absorption of glucogenic precursors from the gut (Trenkle, 1978). Hove and Blom (1973) conducted a study with dairy cattle and observed an increase in insulin and acetoacetate and a decrease in blood sugar during the first few hours after eating. They suggested the decline in blood sugar was probably due to the increase in circulating insulin.

In addition to intake, the physiological status of the animal has been found to affect circulating insulin levels. Lomax et al. (1979) observed reduced insulin responses following infusion of glucose and propionate in lactating dairy cows as compared to dry cows. Koprowski and Tucker (1973) observed a decrease in serum insulin in dry cows as they advanced in pregnancy; opposite the trend for insulin to increase as lactation progressed. This shift in insulin during the dry period is probably the result of increasing demands to supply nutrients to the fetus and the developing mammary gland (Bauman and Currie, 1980).

Short-acting insulin injections (Schmidt, 1966) and insulin infusions (Rook et al., 1965) administered to dairy cattle result in decreased milk yields and lactose percen-

tages and increased fat and protein percentages. Hove (1978) infused large doses of insulin into lactating goats and observed a 20 to 30% reduction in lactose secretion rates. Kronfeld et al. (1963) injected insulin into dairy cows and observed a reduction in milk yield. When glucose was given in addition to insulin, milk production was restored to normal. They concluded that the depression in milk yield and lactose production was the result of hypoglycemia rather than a direct affect of insulin on the mammary gland.

It has been proposed that insulin may be involved in milk fat depression in dairy cows (McClymont and Vallance, 1962). Milk fat depression commonly occurs when the level of concentrate feeding is increased during lactation (Jorgensen et al., 1965; Murdock and Hodgson, 1967; Opstvedt et al., 1967; Evans et al., 1975). Plasma insulin and glucose levels are elevated during high concentrate feeding, and insulin has been shown to be negatively correlated to milk fat percent and fat production (Walker and Elliot, 1973; Jenny et al., 1974; Jenny and Polan, 1975). McClymont and Vallance (1962) suggested that the increased blood glucose concentration associated with high concentrate feeding suppresses lipolysis in adipose tissue. Other researchers (West and Passey, 1967; Prior and Scott, 1980) have con-

ducted studies which indicate the effect of glucose on adipose tissue is to increase lipogenesis. Either of these alternatives would result in a reduction in the plasma lipids available for milk fat synthesis.

Annison et al. (1974) observed that low-roughage (high-concentrate) diets caused a decrease in mammary triglyceride extraction from the blood. Work conducted with goats (West et al., 1972) has shown that hydrolysis of triglycerides by mammary lipoprotein lipase in the mammary capillaries is necessary for mammary uptake. This suggested that mammary lipoprotein lipase activity could be limiting during milk fat depression.

Rao et al. (1973) conducted a study using Holstein cows to look at the effect of glucose and insulin on lipoprotein lipase activity in adipose and mammary tissue. The infusion of glucose increased blood glucose and lipoprotein lipase activity in adipose tissue but did not affect mammary lipase activity. Additions of glucose and, or, insulin to adipose tissue during *in vitro* incubations resulted in increased lipoprotein lipase activity, with greatest activity when in combination as compared to either alone. Therefore, it appears that lipase activity in the mammary gland is not decreased, but rather adipose activity is increased.

Opstvedt et al. (1967) looked at the effect of only hay or concentrate ad libitum feeding on enzyme activity in adipose and mammary tissue of lactating dairy cattle. Concentrate feeding depressed milk fat percentage to 60 to 70% of control values. No large differences in mammary enzyme activities were observed; however, fatty acid synthetase of the concentrate group was only one-half that of the hay group. The adipose enzyme activity of glucose-6-P dehydrogenase, 6-P gluconate dehydrogenase, glyceride-3-P dehydrogenase, alpha-glycerol-P dehydrogenase, citrate cleavage enzyme, and malate dehydrogenase were 4, 2, 3, 3, 3, and 2 times greater, respectively, in the concentrate group as compared to the hay group. These enzymes are involved in lipogenesis. Since high concentrate diets increase blood glucose and insulin concentrations, insulin may be acting to divert nutrients to adipose lipid synthesis and thereby reduce the availability of fatty acids to the mammary gland.

As indicated by the literature cited, insulin is an important hormone involved in directing nutrients toward tissue growth and away from the lactating mammary gland. This study will attempt to accumulate additional data on insulin concentrations and glucose-induced insulin responses in lactating and non-lactating dairy cattle, with special interest in variations due to stage of lactation and feeding regimes.

ACETATE IN RUMINANT METABOLISM AND MILK SYNTHESIS

Acetate is the major volatile fatty acid produced during rumen fermentation and serves as a major source of energy for the ruminant animal. Annison and Linzell (1964) conducted a study with lactating sheep and estimated that 28% of total circulating carbon dioxide came from acetate whereas only 9% came from glucose. The oxidation of acetate has been reported higher in dairy cattle. Bickerstaffe et al. (1974) observed that acetate contributed 32 to 50% of total body carbon dioxide production in lactating dairy cows, and glucose accounted for only 4 to 11%.

Glucose is a major precursor for milk synthesis and has been proposed to be the major limiting factor for milk production (Linzell, 1967; Linzell and Peaker, 1971; Tindall et al., 1978). Since its availability depends on the animal's gluconeogenic capacity, acetate may be used preferentially when possible in order to spare glucose. Head et al. (1964) observed that an acetate infusion of one mole per hour decreased glucose turnover 75 to 79% in non-lactating Holstein cows. Their (Head et al., 1964) results indicate there is preferential acetate utilization by peripheral tissues when acetate availability increases.

Although acetate is oxidized to a greater extent in the body, glucose is the major source of oxidative energy for the mammary gland (Annison and Linzell, 1964). This is the result of greater glucose uptake, as compared to acetate. Annison and Linzell (1964) estimated that 60 to 85% of glucose entering the circulation was utilized by the mammary gland in lactating sheep, whereas only 14 to 15% of the circulating acetate was taken up by the gland. Of the acetate uptake, 46% was oxidized during lactation and it increased to 57% after the animals were dried off. Overall, acetate accounted for 28% of the mammary carbon dioxide production during lactation. This is considerably higher than the 14% estimate of Hardwick et al. (1963), but their low estimate could be due to using perfused goat mammary glands rather than sampling from intact animals.

Acetate contributes more to mammary oxidative energy than does any other fatty acid available to the gland. Dimenna and Emery (1980) studied the oxidation of fatty acids in bovine mammary tissue slices and observed that the rates of fatty acid oxidation decreased with increasing chain length. This is in agreement with the findings of Bickerstaffe et al. (1974) using arterio-venous difference and mammary blood flow measurements in dairy cattle.

In addition to its important contribution to energy metabolism, acetate is a major precursor for milk fat synthesis in the mammary gland. Unlike nonruminants, ruminants are unable to utilize glucose for fatty acid synthesis (Ballard et al., 1969; Bauman et al., 1970; Baldwin and Smith, 1971). Plasma fatty acids, acetate, and beta-hydroxybutyrate are the major precursors of milk fat, with plasma fatty acids contributing about 50% of the fatty acids in milk (Emery, 1973). De novo fatty acid synthesis in the mammary gland supplies the remaining milk fatty acids. Beta-hydroxybutyrate is the preferred chain initiator and acetate provides carbon for chain elongation (Emery, 1973). It has been shown that predominately acetate and beta-hydroxybutyrate contribute the carbon for the synthesis of fatty acids up to a chain length of C-14 and about 50% of the palmitate (C-16), and plasma fatty acids make up the remaining palmitate and all of the long chain fatty acids (Linzell and Peaker, 1971). Smith (unpublished data cited in Baldwin and Smith, 1971) estimated that 2.4 moles of blood triglyceride fatty acid, 10.4 moles of acetate, and 1 mole of beta-hydroxybutyrate are required to provide the carbon for a lactating dairy cow to produce 30 kg of milk and 1.11 kg of milk fat.

Acetate has been shown to account for 25 to 50% of milk triglyceride in lactating goats (Annison and Linzell, 1964) and dairy cows (Bickerstaffe et al., 1974). Hardwick et al. (1963), using isolated perfused goat udders, estimated that acetate accounted for 48% of the fatty acids, .3% of the lactose, 2% of the triglyceride glycerol, and 33% of the citrate in milk.

It has been proposed that milk fat depression in dairy cows fed high grain diets could be the result of reduced acetate availability for milk fat synthesis. A second theory proposes that milk fat depression could be the result of increased rumen propionate production rather than a decrease in acetate production, or a decrease in the acetate:propionate ratio (Van Soest, 1963). This theory has been supported by the results of VFA studies with cows fed either a control or low-fiber, high-grain diet (Davis, 1967; Bauman et al., 1971).

Jorgensen et al. (1965) studied the alterations in bovine rumen fermentation following the feeding of high-concentrate rations. They (Jorgensen et al., 1965) observed a slight decrease in acetate production and a significant increase in propionate production. When they (Jorgensen et al., 1965) administered 1 pound of sodium acetate per day

intrarumenally to a lactating dairy cow concurrently with feeding the high-concentrate diet, they did not observe an increase in milk fat production. If acetate had been limiting, an increase in milk fat would have been expected. This is in disagreement with the findings of Rook and Balch (1961) who intrarumenally infused a similar load of acetic acid in lactating cows and observed an increase in milk production and milk fat percentage; however, their animals were fed a standard dairy ration with a 60:40 forage to concentrate ratio.

Diets causing high propionate fermentation lead to an increase in circulating glucogenic metabolites (Jorgensen et al., 1965). An increase in circulating blood glucose causes metabolism to shift toward adipose deposition. This results in a decrease in lipid mobilization. Therefore, milk fat depression could be the result of a reduced availability of plasma fatty acids rather than decreased de novo fatty acid synthesis from acetate.

In conclusion, acetate is a major source of energy for the ruminant animal. Its availability indirectly has a glucose sparing effect on ruminant metabolism. It is also a major precursor in milk fat synthesis, providing the necessary carbon for chain elongation in mammary de novo fatty

acid synthesis. As part of the thesis project, the effect of acetate loading on milk production and composition was investigated.

Chapter III

MATERIALS AND METHODS

DESIGN OF STUDY ONE

Study One involved conducting both glucose and acetate tolerance tests on eight first-lactation Holstein cows. The project covered each cow's first lactation and dry period. The tests were conducted at three different time periods, during: early lactation (E), late lactation (L), and the dry period (D). Early and L periods were within 75 to 100 and 205 to 250 days postpartum, respectively. Dry period was within 22 to 44 days after drying off.

During each sampling period, three glucose and three acetate injections were administered to each animal. The three injections of each substance served as replicates. Injections during E and L were separated by at least 48 hr. During this time period, milk was collected at 15 and 3 hr pre-injection and 9 and 21 hr post-injection for analysis to determine the effect of glucose and acetate loading on milk composition. Injections during D were separated by at least 24 hr. The order in which the injections were given was chosen randomly, with no more than two successive injections of glucose or acetate given.

EXPERIMENTAL CONDITIONS

Feeding Regime

The animals were fed a corn silage-based ration during the study. The ration composition is listed in Table 1. Feed samples were cclllected biweekly for analysis.[1] The feed averaged 55% DM, and contained 15% CP and 22% ADF on a DM basis.

During each sampling period, the cows were housed in a stanchion barn and fed by automatic feeder. The feeder provided one-sixth of the daily intake every 4 hr. The cows entered the barn at least one week prior to the first injection to adjust to the feeder (adjustment period). The amount fed during E and L was based on average *ad libitum* intake during the adjustment period. During D, the cows were fed at maintenance, based on NRC recommendations (1979), with no allowance made for growth.

The animals adapted well to barn confinement and the feeding system. Meal consumption was completed within 1/2 hr. Water was available at all times in automatic water bowls.

---

[1]Virginia Polytechnic Institute and State University Forage Testing Laboratory, Blacksburg, Virginia.

Table 1. Ration composition and analysis[1]

Ingredient	
Corn Silage	68.0%
Corn Grain	14.0%
Protein Supplement (35%)	12.0%
Chopped Orchardgrass Hay	6.0%
Analysis:	
Dry Matter (%)	55.2
ADF (%)	22.4
Crude Protein (%)	14.5
NE_l (Mcal/kg)	1.65

[1]calculated on dry matter basis.

Routine Procedure

Body weights were obtained on three separate days during the adjustment period and the last day of the sampling period. Daily feed refusals and twice daily milk production data were recorded during the adjustment and sampling periods. During E and L, cows were taken to the milking parlor each day at 0100 and 1300 hr to be milked. A milk sample was collected at each milking for analysis.

Catheterization Procedure

At least two days prior to the first injection, two jugular catheters were surgically implanted contra-laterally. Xylazine hydro-chloride[2] (from 7.5 to 30 mg, in sterile saline) was administered IM 5 min prior to implantation to reduce animal stress.

The catheters were implanted using an 8 gauge needle (.319 cm ID, .396 cm OD). Once the needle was placed in the vein, a 20 cm long Silastic polyethelene tubing (.157 cm ID, .318 cm OD)[3] was fed through the needle into the vein. The exteriorized tube end was fitted with a one-way stopcock valve to facilitate blood collection. The valve was sutured

---

[2]Haver-Lockhart, Bayvet Division, Cutter Laboratories, Inc., Shawnee, Kansas.

[3]Dow Corning Corp., Midland, Michigan.

to the animals neck and the neck was wrapped with gauze tape to avoid possible loss of the catheters. Occasionally both catheters would become nonfunctional, and a 16 gauge over-the-needle catheter[4] was implanted as a replacement. The catheters were flushed each day with 10 ml of both saline and heparinized (50 U/ml) saline to prevent clotting in the tubing.

Solutions and Doses used for Injections

A 50% sterile glucose sclution[5] was injected at a dose of .1 g/kg body weight. A 2.5 M sodium acetate[6] solution was prepared in our laboratory. The pH of the solution was adjusted to 7.4 using 5 N HCl. The solution was autoclaved prior to use. Acetate was injected at a dose of 1 mmole/kg body weight.

Injection Procedure

Injections were conducted in a stall that had no acsess to food or water, and the animals remained standing throughout the sampling period. Both catheters were fitted with 30 cm long polyethylene extension catheters, which allowed for

---

[4]Bard Biomedical, a Division of C. R. Bard, Inc., Murray Hill, New Jersey.

[5]Travenol Laboratories, Inc., Deerfield, Illinois.

[6]Mallinkrodt, Inc., Paris, Kentucky.

the animals to be sampled undisturbed. The right jugular catheter was used for administering the injection, and the left was used for collecting blood samples. The injections were conducted at approximately 1500 hr, between the 1400 and 1800 hr feedings.

For each glucose injection, blood samples were collected at 15, 10, and 5 min pre-injection (or -15, -10, and -5) to determine basal glucose concentration. An additional sample was collected at -15 and -10 for hematocrit determination by the microcapillary method. Following injection, the catheter was rinsed with 10 ml of saline. Zero time was considered to be the initiation of the injection. Injection administration averaged 30 sec. Blood samples were collected at 2, 5, 7, 10, 15, 20, 25, 30, and 40 min post-glucose dosing.

For each sample, 10 ml of blood was collected and transferred into a tube containing 200 U heparin and approximately 2 mg sodium fluoride to prevent clotting and glycolysis. After collecting each sample, the catheter was flushed with 10 ml of saline. The samples were spun for 10 min at a relative centrifical force of 6,785 x g using a tabletop centrifuge. Plasma was pipetted off and stored in vials at -15 deg C until analyzed.

SAMPLE ANALYSIS

Milk Analysis

Milk samples were analyzed for total solids, fat, and protein content. Results were expressed as percentages to the nearest one-hundreth decimal place.

Fat content was determined[7] by a light diffusion technique using a Milk Tester Automatic.

Protein content was determined by an amido black dye binding method using a Foss Pro-Milk II Semiautomatic Tester[8].

To determine total solids, a 2 ml aliquot of milk, of known weight, was dried for 3 hr in a 100 deg C forced-air oven. Dried samples were weighed to determine the dry milk weight. The percent total solids was determined by dividing the dry milk weight by the fluid milk weight.

Blood Analysis

With the exception of the Evan's blue dye and insulin assays, blood assays were conducted using deproteinized plasma. Plasma samples were deproteinized prior to analysis

---

[7]Fat analysis performed by the Virginia Federation of DHIA's, Blacksburg, Virginia.

[8]Foss Electric, Hillerod, Denmark.

using a modification of the Somogyi-Nelson Method (Reinhold, 1953). A 1 ml aliquot of plasma was combined with 2 ml of 5% zinc sulfate , 2 ml of 0.3 N barium hydroxide, and 5 ml of distilled water. The sample was centrifuged at a relative centrifical force of 4,640 x g for 15 min using a Beckman J2-21 Centrifuge[9]. The deproteinized plasma, contained in the supernate fraction, was pipetted off and stored in vials at -15 deg C until further analysis.

Determination of Plasma Glucose Concentration

Deproteinized plasma samples from the glucose injections were assayed for glucose in duplicate using a glucose oxidase enzyme kit[10]. Color intensity was determined using a Beckman Model 35 Spectrophotometer[11]. The concentration of glucose in the plasma samples was recorded in mg/dl, to the nearest one-tenth decimal place.

Calculating Glucose Parameters

Glucose tolerance curves were constructed by plotting post-injection concentration (y) against time (t). The basal concentration was estimated by averaging the -15, -10,

---

[9]Beckman Instruments, Inc., Fullerton, California.

[10]Sigma Chemical Company, Saint Louis, Missouri.

[11]Beckman Instruments, Inc., Fullerton, California.

and -5 samples. The 2-min sample was eliminated because it consistently appeared to overestimate the initial portion of the curve. The 40-min sample was eliminated because the concentration had returned to basal levels by this time. Semilog plots were constructed, with the log of y plotted against t. Data fit a single compartment kinetic model. Regression analysis was conducted to determine the slope and y intercept of each curve. The slope of the curve estimated glucose clearance rate and was expressed as % disappearance/min and % disappearance/min/100 kg body weight to the nearest one-hundreth decimal place. The y intercept estimated the concentration immediately following the injection (zero time). The change in concentration due to the dose was determined by subtracting the basal concentration from the zero time concentration. Glucose pool space was estimated by dividing the dose by the change in concentration and was expressed in liters and ml/kg body weight to the nearest tenth decimal place. Glucose pool size was estimated by multiplying pool space by the basal concentration and was expressed in g and mg/kg body weight to the nearest one-hundreth decimal place.

Determination of Plasma Insulin Concentration

Plasma samples from the glucose injections were analyzed for insulin using a double antibody radioimmunoassay. Guinea pig anti-Bovine insulin (no. 500015)[12] was used as the first antibody, and Goat anti-Guinea pig insulin (no. 500085)[13] was used as the second antibody. Bovine insulin[14] was used for the standard. Iodinated insulin[15] was used as the tracer.

Calculating Insulin Parameters

Basal insulin concentration was estimated by averaging the -15, -10, and -5 min sample concentrations. Peak response was the highest concentration post-injection. The peak response above basal was determined by subtracting the basal concentration from the peak response. The peak fold increase is the peak response expressed as a multiple of the basal concentration, and was determined by dividing the peak response by the basal level.

---

[12]Research Products International, Mt. Prospect, Illinois.

[13]Ibid.

[14]A gift from Eli Lilly Company, Indianapolis, Indiana.

[15]New England Nuclear, Boston, Massachusetts.

Linear regression analysis was conducted on the semi-log plots of insulin concentration against time, from peak response to 40 min post-injection. The slope of the curve estimated insulin clearance and was expressed as % disappearance/min to the nearest one-hundreth decimal place. Cubic regression analysis was conducted on the insulin data from each glucose injection, using the model Insulin = Time + $Time^2$ + $Time^3$ + Error. Resulting curves were integrated to estimate the response curve area above basal concentration.

Determination of Extracellular Fluid and Blood Volumes

In addition to the glucose and acetate injections given during D, cows 1236 and 1253 received single injections of xylose and Evan's blue after completing the injection series to estimate extracellular fluid volume and blood volume, respectively. Injections were conducted to determine the comparability of glucose pool space estimates to extracellular fluid volume.

Extracellular fluid volume was determined using a modification of the blood xylose assay of Benson et al. (1957). A 37.5% (w/v) aqueous solution of D-xylose[16] was injected at a dose of 60 g, based on the recommendations of Dr. R.

[16]Aldrich Chemical Company, Inc., Milwaukee, Wisconsin.

Russell[17] (personal communication). Blood samples (10 ml) were collected at 5 min intervals from 15 min pre-injection to 55 min post-injection. A hematocrit was determined for each sample by the micro-capillary method. Plasma was obtained using the method previously described. The deproteinized plasma samples were analyzed in duplicate using a Beckman Model 35 Spectrophotometer[18].

The semi-log plots of the xylose tolerance curves appeared to approximate straight lines. Xylose space was determined using the method used to calculate glucose pool space. The 5 min sample was eliminated to avoid overestimating the initial portion of the curve. Results were expressed in liters, to the nearest tenth decimal place.

Blood volume was determined using an adaptation of Mackie's (1976) Evan's blue dye method. A single injection of Evan's blue was administered after the xylose injection was completed. A 1.5% solution of Evan's blue[19] (in sterile saline) was used for the injection of .5 mg Evan's blue/kg body weight. A 10 ml blood sample was collected at -10 and -5 min pre-injection and 5, 10, 15, 20, 25, and 30 min

---

[17]Iowa State University, Ames, Iowa.

[18]Beckman Instruments, Inc., Fullerton, California.

[19]Sigma Chemical Company, St. Louis, Missouri.

post-injection. Hematocrits were conducted on pre-injection samples by the micro-capillary method. Plasma was obtained using the method previously described. Plasma samples were analyzed for Evan's blue in duplicate using a Bausch & Lomb Spectronic 20[20]. The concentration of Evan's blue in the 10 min sample was used for calculating blood volume.

STATISTICAL PROCEDURE

All blood data was subjected to analysis of variance using the appropriate error mean square and degrees of freedom. Differences between stages were tested by Contrast Analysis using the Modified Bonferroni F Test. Means were tested using 6.210 at the .05 level of significance. The model was Y = Stage + Cow + Stage x Cow + Error, for each blood parameter.

Milk data were subjected to analysis of variance using the appropriate error mean square and degrees of freedom. Differences between pre- and post-injection milk samples were tested by Contrast analysis using the Modified Bonferroni F Test. The model was Y = Stage + Cow + Time + Stage x Cow + Day(Time) + Error, for each milk parameter. Differences in pre-injection milk samples due to previous injections was tested by contrast analysis using the model Y =

---

[20]Bausch & Lomb, Rochester, New York.

Stage + Cow + StagexCow + Pretreatment + Error, for each milk parameter. Milk parameter differences were tested using 6.396 at the .05 level of significance.

DESIGN OF STUDY TWO

Study Two was a preliminary study conducted to investigate the effect of ration type and stage of lactation on glucose and insulin blood parameters in lactating Holstein cows. Nine cows were involved in another study and were available for our use to collect data similar to Study One. The ration compositions and milk parameters, intake, and body weight data have been previously reported (Kenna, 1981). Study Two involved conducting glucose tolerance tests at 60, 90, and 120 days of lactation. During each sampling period, one glucose injection was administered to each animal.

Initially, all cows were fed a corn silage-based (control) ration. After the 60 day sampling period, five of the cows, chosen randomly, were fed a high-concentrate ration, and four remained on the initial diet. They were fed these rations for the remainder of the study.

EXPERIMENTAL CONDITIONS

Feeding Regime

The cows were fed a corn silage-based (control) ration when the 60 day sampling was conducted. After the 60 day sampling, five cows were changed to a high-concentrate, low-fiber ration. Table 2 lists the compositions of the control and high-concentrate (HC) rations.

All animals were fed *ad libitum*, and daily feed refusal data were recorded. The cows were housed in a stanchion barn and fed twice daily, at 0700 and 1400 hr. Water was available at all times in automatic water bowls.

Routine Procedure

Body weights were obtained prior to administering the injection. Cows were taken to the milking parlor each day at 0100 and 1300 hr to be milked. Twice daily milk production data were recorded.

Catheterization Procedure

On the day prior to injection, a 16 gauge over-the-needle catheter[21] was implanted in the jugular vein. The catheter was sutured to the animal's neck and the neck was wrap-

---

[21]Bard Biomedical, a Division of C. R. Bard, Inc., Murray Hill, New Jersey.

Table 2. Compositions and analyses[1] of control and high-concentrate (HC) rations

Ingredient	Control	
Corn Silage	62.6%	--
Ground Shelled Corn	--	46.6%
Ground Orchardgrass Hay	7.9%	23.3%
Wet Molasses	--	4.5%
Supplement HC[2]	29.5%	--
Supplement HH[2]	--	25.6%
Analysis:		
Dry Matter (%)	45.4	84.1
ADF (%)	24.4	13.0
Crude Protein (%)	16.3	16.8
NE_1 (Mcal/kg)	1.6	1.7

[1]calculated on dry matter basis.

[2]contained: soybean meal, molasses, dist. dried grains, wheat, ground shelled corn, vitamins, and minerals.

ped with gauze tape. Each day, the catheter was flushed with 10 ml of heparinized (50 U/ml) saline.

Solutions and Doses used for Injections

Solutions and doses were the same as those used in Study One.

Injection Procedure

The procedure was the same as that used in Study One.

SAMPLE ANALYSIS AND CALCULATING PARAMETERS

Plasma samples from the glucose injections were assayed for glucose and insulin using the methods previously described for Study One.

Glucose and insulin parameters were calculated in the same manner as Study One.

STATISTICAL PROCEDURE

All blood data were subjected to analysis of covariance using the appropriate error mean square and degrees of freedom. Covariate analysis was used because the cows fed the HC ration at the 90 and 120 day sampling periods produced more milk at 60 days than the control group. Least square means at 90 and 120 days of lactation were tested using the

F-Test at the .05 level of significance. The model was Y = Diet + Stage + Diet x Stage + Cow(Diet) + Covariate + Error, for each blood parameter. The covariate was an adjustment based on the parameter at 60 days.

Chapter IV

RESULTS AND DISCUSSION - STUDY ONE

BODY WEIGHT, DRY MATTER INTAKE, AND MILK PRODUCTION

The body weight, dry matter intake, and milk production data are listed by lactation stage and cow in Table 3. Body weight increased from 482 to 591 kg and dry matter intake decreased from 17.8 to 8.1 kg from E to D, respectively, with L means intermediate (Table 3). These trends are typical of a normal lactation.

Milk production was atypical, with a non-significant ($P>.05$) difference between E and L. Cows 1231 and 1252 produced slightly more milk during L as compared to E. Low production during E was probably the result of a deficiency in energy intake. The animals were allowed to eat *ad libitum* during the two lactation stages, but the ration did not contain sufficient energy for early lactation cows. As a result, the lactation curves were atypical, having very persistent production from E to L.

As previously mentioned, body weight increased, dry matter intake decreased, and milk production remained unchanged from E to L. As a whole, these trends appear abnormal. The source of energy for high milk production during L is not readily apparent. However, since these ani-

Table 3. Body weight, dry matter intake, fat-corrected milk, and days postpartum of each cow at initiation of each injection period

Stage	Cow No.	Body Weight (kg)	DM Intake (kg)	FCM[1] (kg)	Days Post-partum
Early	1223	477	16.5	22.4	89
	1231	516	17.1	19.9	75
	1233	486	17.6	23.0	84
	1236	509	17.6	20.3	80
	1245	502	18.2	25.8	80
	1252	433	17.6	18.9	83
	1253	488	18.2	23.2	79
	1254	448	19.3	20.9	83
Mean		482	17.8	21.8	82
SE		29	.8	2.2	4
Late	1223	519	14.3	19.4	216
	1231	605	14.9	20.2	229
	1233	523	14.9	22.0	215
	1236	557	14.3	16.7	220
	1245	552	16.5	19.9	203
	1252	507	14.9	19.9	235
	1253	532	14.9	20.2	223
	1254	509	13.8	20.1	209
Mean		538	14.8	19.8	219
SE		33	.8	1.5	10
Dry	1223	595	8.3	--	404 (36)[2]
	1231	659	7.7	--	389 (27)
	1233	595	8.8	--	373 (26)
	1236	592	7.7	--	313 (27)
	1245	632	8.8	--	364 (24)
	1252	573	7.7	--	397 (22)
	1253[3]	530	7.7	--	384 (29)
	1254	555	7.7	--	355 (28)
Mean		591	8.1	--	372
SE		41	.5	--	29

[1]FCM = 4% fat-corrected milk
= (kg milk x .4) + (kg fat x 15)

[2]The number in parentheses indicates days from drying off.

[3]1253 was the only non-pregnant cow during dry period.

mals were in their first lactation, growth could be an important factor. During E the demands for growth could have been sufficient to result in low milk yields. These animals were small in body size and there was probably only a limited amount of body reserves available for mobilization. If growth was less pronounced during L, more nutrients would have been available for milk production.

Higher milk production during E would have been more desirable, but when the study was designed it was necessary to select a ration that could be fed throughout the entire study so that comparisons among stages could be made. A high-fiber ration was chosen to avoid metabolic disorders during L and D.

Table 3 lists the days postpartum at initiation of each sampling period by cow. All cows were pregnant during D, with the exception of 1253. She was dried off and removed from the milking herd in a similar manner as the other cows. The data obtained from 1253's glucose tolerance tests during D were comparable to the other cow data and were included in the statistical analysis.

DATA AND STATISTICAL ANALYSIS OF PLASMA GLUCOSE PARAMETERS

Overall means for plasma glucose parameters averaged by cow and lactation stage are given in Table 4. In addition, an adjustment for body weight was made for clearance rate, pool size, and pool space, and these means are also included in Table 4. Basal glucose, pool size, and pool space increased with each successive lactation stage, whereas clearance rate decreased (Table 4). Correcting for body weight affected glucose pool size and pool space, with the adjusted parameters showing no difference across the lactation stages. Clearance rate, however, had a similar trend both before and after adjustment. The glucose parameters will be discussed in detail in the following subsections with regards to lactation and cow differences determined by statistical analysis. No difference ($P>.05$) in replicates was observed for basal plasma glucose and plasma insulin and glucose clearance rate post-dosing. Therefore, cow replicates were treated as three observations in the statistical analysis.

Plasma Glucose

The overall mean values of basal plasma glucose given in Table 4 are summarized from Appendix Tables 16-18, which contain individual cow data for plasma glucose expressed as

Table 4. Glucose parameter means ± SE by stage of lactation for first-lactation cows

Stage	n	Plasma Glucose (mg/dl)	Clearance Rate (%/min)	Clearance Rate (%/min/100 kg)	Pool Size (g)	Pool Size (mg/kg)	Pool Space (l)	Pool Space (ml/kg)
Early	24	57.4 ± 1.1[a]	2.43 ± .11[a]	.504 ± .021[a]	39.2 ± 1.7[a]	81.4 ± 3.5	68.4 ± 2.7[a]	142.2 ± 5.8
Late	24	60.1 ± 1.1[ab]	2.08 ± .06[b]	.388 ± .012[b]	45.8 ± 1.4[b]	85.4 ± 2.7	76.3 ± 2.0[ab]	142.1 ± 3.8
Dry	24	62.3 ± 0.9[b]	1.71 ± .09[c]	.290 ± .017[c]	49.2 ± 1.3[b]	83.2 ± 2.0	79.3 ± 2.2[b]	134.0 ± 3.2

[abc]Means in the same column which do not have a common letter in the superscript are different ($P<.05$).

mg/dl. Appendix Table 19 provides the statistical summary and indicates there was variation ($P<.05$) among lactation stages and cows.

Plasma glucose increased ($P<.05$) from 57.4 to 62.3 mg/dl from E to D, respectively (Table 4). Plasma glucose during L was intermediate at 60.1 mg/dl (Table 4) but not different from E and D ($P>.05$).

The overall trend of plasma glucose to increase with increasing days into lactation is consistent with the findings of other researchers (Koprowski and Tucker, 1973; Smith et al., 1976; Hart et al., 1978), and is indicative of the large glucose demand of the mammary gland during lactation. During D the animals were in a more positive energy state (indicated by the increase in body weight, Table 3) even though intake was lower than during E and L. The low plasma glucose concentration during E and L could be the result of high glucose uptake by the mammary gland during lactation.

Plasma glucose was negatively correlated with dry matter intake ($r = -.45$, $P<.05$). Even though plasma glucose was higher during D than L (Table 4), the levels were not significantly different. Intake was restricted during D to meet only maintenance requirements. If the level of feeding had been increased during D, a greater difference in plasma glucose between D and L probably would have been observed.

Our plasma glucose data is similar to observations of Hart et al. (1978) who observed an increase in plasma glucose from 68.2 to 73.5 mg/dl from early lactation (mean of 60 days) to the dry period, respectively. In addition they (Hart et al., 1978) observed an 18% increase in glucose concentration when the animals were dry as compared to values during early lactation, whereas in the present study only a 9% increase was obtained. The higher plasma glucose levels reported in their study (Hart et al., 1978) is probably the result of feeding a 10:90 hay:concentrate ration.

Glucose Clearance Rate

The overall mean values of glucose clearance rate given in Table 4 are summarized from Appendix Tables 16-18, which contain individual cow data for glucose clearance expressed as % disappearance/min and % disappearance/min/100 kg body weight. Appendix Table 19 provides the statistical summaries and indicates there was variation ($P<.05$) among lactation stages and cows.

Glucose clearance decreased ($P<.05$) from E to D from 2.43 to 1.71% disappearance/min, respectively, with L intermediate at 2.08% and different ($P<.05$) from the other two stages. Glucose clearance was negatively correlated with body weight ($r = -.51$, $P<.05$). When adjusted for body

weight, glucose clearance exhibited the same trend, going from .504% disappearance/min/100 kg body weight during E to .290% during D, with L intermediate at .388%. There was a decrease ($P<.05$) in body weight adjusted-glucose clearance with each successive lactation stage. Therefore, it can be concluded that something other than body weight is affecting glucose clearance. In addition to body weight, glucose clearance was positively correlated with dry matter intake ($r = .70$, $P<.01$). This is comparable to the observations of Herbein et al. (1978) that a linear relationship exists between glucose turnover and digestible energy intake.

As mentioned in the literature review, clearance estimates the animal's ability to utilize glucose. From the data mentioned above, it can be concluded that cows utilize glucose more rapidly when lactating as compared to when dry and more rapidly during early lactation as compared to late lactation. Since milk production was not different between E and L, dry matter intake may be a key factor affecting glucose clearance.

Plasma glucose returned to basal concentration 30 min post-injection. Comparable results were reported by Holmes (1951a) when plasma glucose returned to normal 1 1/2 to 2 hr post-dosing in Ayrshire cows following a glucose dose 300%

(.3 g/kg) of that used in this study. In contrast, Reid (1952) reported that plasma glucose returned to normal 2 to 4 hr post-dosing in Merino ewes following a glucose dose 200% (.2 g/kg) of that used in this study.

In the literature review, two methods of calculating glucose clearance were discussed. The clearance data reported in this study was calculated by the method of least squares using natural logarithms of the actual glucose values. With this method, the intraclass correlation among samples from the same cow and lactation stage was 42%, which represents the repeatability of clearance rate replicates of cows during each lactation stage. In contrast, the intraclass correlation of our data was only 36% using Greville's log of glucose excess method. This is in agreement with the findings of West and Wood (1953), who observed more variability using Greville's method as compared to using the actual glucose concentration method. The repeatability of the IVGTT has been reported as high as 50% by Ganda et al. (1978), but the validity of their test for repeatability is questioned by Dr. M. L. McGilliard[22] (personal communication).

---

[22]Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Verdonk et al. (1981) reported that it is not valid to use glucose clearance to evaluate utilization at differing basal plasma glucose concentrations. They observed that glucose clearance in human subjects decreased as a function of plasma glucose concentration ($r = -.85$), while insulin concentration was maintained constant. This is similar to the findings of Ferrannini and Pilo (1979), who observed a negative correlation ($r = -.59$) between plasma glucose level and glucose disappearance rate in human subjects when insulin secretion was allowed to vary. In contrast, Defronzo and Ferrannini (1981) found glucose clearance to be independent of plasma glucose concentration when insulin secretion is allowed to vary. In our study glucose clearance was not significantly correlated with basal glucose level ($r = -.23$, $P>.05$). This will be discussed further in the insulin section of the results and discussion.

Pool Size

The overall mean values of glucose pool size given in Table 4 are summarized from Appendix Tables 16-18, which contain individual cow data for glucose pool size expressed as g and mg/kg body weight. Appendix Table 19 provides the statistical summaries and indicates there was variation ($P<.05$) among lactation stages and cows when pool size was

expressd as g, but only cow effects were significant (P<.05) when pool size was expressed as mg/kg body weight.

Glucose pool size increased significantly (P<.05) from E to L from 39.19 to 49.6 g, respectively (Table 4). The L value was intermediate at 45.84 g (Table 4), and was greater (P<.05) than E but not different from D. Glucose pool size (g) was positively correlated with body weight (r = .59, P<.01) and dry matter intake (r = -.57, P <.01). When adjusted for body weight, glucose pool size showed no difference between stages (Table 4). This is in agreement with the findings of Bergman and Hogue (1967) that mean pool sizes remain relatively constant regardless of milk production or glucose turnover in sheep. It can be concluded from these results that glucose pool size, or the mass of glucose in the circulation, is highly dependent on body weight and intake.

Previously reported values for body weight-adjusted glucose pool size in normal lactating cattle, using labelled glucose (Baxter et al., 1955; Kronfeld et al., 1959; Kronfeld and Raggi, 1964), have been considerably higher (146 mg/kg body weight, average value) than those observed in this study (83 mg/kg body weight, average value). Milk production and intake data from these studies were not

available for comparison. Kronfeld et al. (1959) observed a glucose pool size of 92 mg/kg in a 96-hr fasted, non-lactating cow, which is similar to the average value of 83 mg/kg in this study. The low glucose pool sizes observed in this study could be due to low energy intake.

Pool Space

The overall mean values of glucose pool space given in Table 4 are summarized from Appendix Tables 16-18, which contain individual cow data for glucose pool space expressed as liters and ml/kg body weight. Appendix Table 19 provides the statistical summaries and indicates there was variation ($P<.05$) among lactation stages and cows when pool space was expressed as liters, but only cow effects were significant ($P<.05$) when pool space was expressed as ml/kg body weight.

Glucose pool space increased ($P<.05$) from E to D from 68.4 to 79.3 liters, respectively (Table 4). The L value was intermediate at 76.3 liters (Table 4) and not different from E and D ($P>.05$). When adjusted for body weight, glucose pool space showed no difference between lactation stages, with an average value of 139 liters.

Cow 1236 and 1253 had average glucose pool spaces of 83.7 and 71.4 liters, respectively and average xylose pool spaces of 88.4 and 59.9 liters, respectively, during D.

Blood volumes, as a percent of body weight, of 1236 and 1253 during D were 7.45 and 5.89%, respectively. Xylose estimated extracellular fluid to be 15 and 14% of body weight for cows 1236 and 1253, respectively. This is comparable to the 15% estimate in cows reported by Anderson and Mixner (1959), using phenolsulfonphthalein.

Kronfeld and Raggi (1964) injected C^{14}-glucose into cows and estimated glucose space to be 27% of body weight. This is comparable to the 26% estimate reported by Bergman and Hogue (1967) in sheep, but considerably higher than our 12 and 16% estimates and the 18% estimate reported by Head et al. (1964) in open non-lactating Holstein cows using constant infusion and single injection techniques with C^{14}-glucose. It has been reported that body water can constitute as much as 12 to 28% of the total body weight (Hansard, 1964). Our estimate, and the estimates for glucose pool space mentioned above fall within this range.

DATA AND STATISTICAL ANALYSIS OF PLASMA INSULIN PARAMETERS

Overall means for plasma insulin parameters averaged by cow and lactation stage are given in Table 5. Basal plasma insulin and insulin response curve area above basal level increased with each successive lactation stage; whereas,

insulin clearance rate decreased. Insulin peak concentration (PC) and peak increase above basal level (PCAB) were lower and insulin peak fold increase above basal concentration (PFI) was higher at E as compared to D, but these differences were not significant ($P>.05$). The insulin parameters will be discussed in the following subsections with regards to lactation and cow differences determined by statistical analysis.

Plasma Insulin

The overall mean values of basal insulin concentration given in Table 5 are summarized from Appendix Tables 20-22, which contain individual cow data for plasma insulin expressed as ng/ml. Appendix Table 23 provides the statistical summary and indicates there was significant variation ($P<.05$) among lactation stages and cows.

Plasma insulin increased ($P<.05$) from E to D from .78 to 1.24 ng/ml, respectively (Table 5). Plasma insulin during L was intermediate at .97 ng/ml and not different from E and D ($P>.05$). As would be expected, basal insulin was significantly correlated with plasma glucose ($r = .53$, $P<.01$). Low insulin levels during early lactation could be advantageous to stimulate gluconeogenesis in the liver and allow maximum nutrient uptake by the mammary gland.

Table 5. Insulin parameter LS Means ± SE by stage of lactation for first-lactation cows

Stage	n	Plasma Insulin (ng/ml)	Peak Conc. (ng/ml)	Peak Increase[1] (ng/ml)	Peak Fold Increase[1]	Response Curve[1] Area	Clearance Rate[2] (%/min)
Early	24	0.78 ± .07[a]	4.30 ± .21	3.51 ± .18	5.41 ± .30	65.3 ± 4.8[a]	5.65 ± .24[a]
Late	24	0.97 ± .07[ab]	4.56 ± .21	3.59 ± .18	4.06 ± .30	72.5 ± 4.8[ab]	4.57 ± .24[b]
Dry	22	1.24 ± .08[b]	5.26 ± .22	4.02 ± .19	3.74 ± .32	93.9 ± 5.0[b]	2.98 ± .26[c]

[1]above basal concentration.

[2]calculated from peak concentration to 40 min post-injection.

[abc]Means in the same column which do not have a common letter in the superscript are different ($P<.05$).

The overall trend of basal insulin to increase with increasing days into lactation is consistent with the findings of other researchers (Koprowski and Tucker, 1973; Hart et al., 1980; Vasilatos and Wangsness, 1981). Hart et al. (1980) observed average plasma insulin values of 15.2 and 32.8 uU/ml in four high yielding Fresian cows at 90 and 150 days into lactation, respectively. This is comparable to our .78 ng/ml (18.7 uU/ml) average during E and .97 ng/ml (23.3 uU/ml) average during L.

Insulin Peak, Insulin Peak above Basal, and Insulin Peak Fold Increase above Basal

The overall mean values for insulin peak concentration (PC), peak concentration above basal (PCAB), and peak fold increase (PFI) given in Table 5 are summarized from Appendix Tables 20-22, which contain individual cow data for insulin PC, PCAB, and PFI. Appendix Table 23, provides the statistical summaries and indicates there was significant variation ($P<.05$) among cows for each insulin peak parameter, but there was no variation due to lactation stage.

Peak concentration tended to increase from 4.30 to 5.26 ng/ml from E to D, respectively (Table 5) with L intermediate at 4.56 ng/ml; however, these differences were not significant ($P>.05$). Insulin peak increase above basal concen-

tration tended to increase from 3.51 to 4.02 ng/ml from E to D (Table 5), respectively, with L intermediate at 3.59; however, these differences were not significant (P>.05). The insulin PFI tended to decrease from E to D from 5.41 to 3.74 (Table 5), respectively, with L intermediate at 4.06; however, these differences were not significant (P>.05).

Since insulin levels were higher at successive lactation stages from E to D, an increase in PC from E to D would be expected. The parameter PCAB estimates the magnitude of the actual increase in insulin at peak time, which varied with each injection. Analysis shows that there was no difference in the magnitude of change (Table 5). However, the actual change in concentration may not be indicative of the body's insulin response. For this reason, insulin peak was also expressed as a fold increase above basal level (PFI). Table 5 shows that insulin peak during E was 5.41 times greater than basal, whereas insulin peak during D was only 3.74 times greater than basal; however, these values were not significantly different (P>.05). Appendix Table 23 indicates there was variation (P<.05) in PC and PFI among the cows at different stages. Therefore, cow variation overrides any variation due to lactation stage.

These peak results are not comparable to results found in sheep (Kamalu and Trenkle, 1978). Kamalu and Trenkle (1978) observed an increase of only .74 ng/ml in insulin concentration when glucose was injected into sheep at a dose similar to the one used in this study. Felig and Wahren (1971) observed a 60 to 100% increase in peripheral insulin in humans when blood glucose rises 10 to 15 mg/dl. Using their estimates and the difference in plasma glucose pre- and post-dosing, insulin peaks during E would be expected to be within 4.25 to 4.69 ng/ml, during L within 5.11 to 5.64 ng/ml, and during D within 7.6 and 7.63 ng/ml. Our insulin peak responses were 4.30, 4.56, and 5.27 for E, L, and D respectively, which are comparable to Felig and Wahren's (1971) human insulin responses. The insulin responses observed in this study cast doubt on the "insulin-insensitive" classification placed on ruminant animals. It could be possible that lactating dairy cattle metabolism differs from that of other ruminant animals, as a result of their high production demands.

Insulin Response Curve Area

The overall mean values of insulin response curve area given in Table 5 are summarized from Appendix Tables 20-22, which contain individual cow data for curve area. Appendix

Table 23, provides the statistical summary and indicates there is variation ($P<.05$) among lactation stages and cows.

Response curve area increased ($P<.05$) from E to D from 65.3 to 93.9, respectively, with L intermediate at 72.5 but not different from E and D ($P>.05$). Figure 5 depicts the predicted response curve for each stage, based on actual data from 2 to 40 min following glucose dosing. The 95% confidence intervals for D and the lactation stages (E and L) do not overlap at 15, 20, 25, 30, and 40 min post-dosing. This is further evidence that the total insulin response during D was higher than the lactation responses.

As mentioned in the plasma glucose parameter subsection of the results, Verdonk et al. (1981) questions the validity of glucose clearance to estimate glucose utilization. However, in their study (Verdonk et al., 1981) they manipulated insulin during sampling to maintain a constant insulin concentration. It is unknown whether glucose dosing saturates the glucose transport system. If it does, clearance could well be dependent on initial basal glucose levels. But, the author questions the validity of glucose clearance at constant insulin concentrations.

During early lactation a cow is rapidly synthesizing glucose in the liver to provide glucose for milk synthesis.

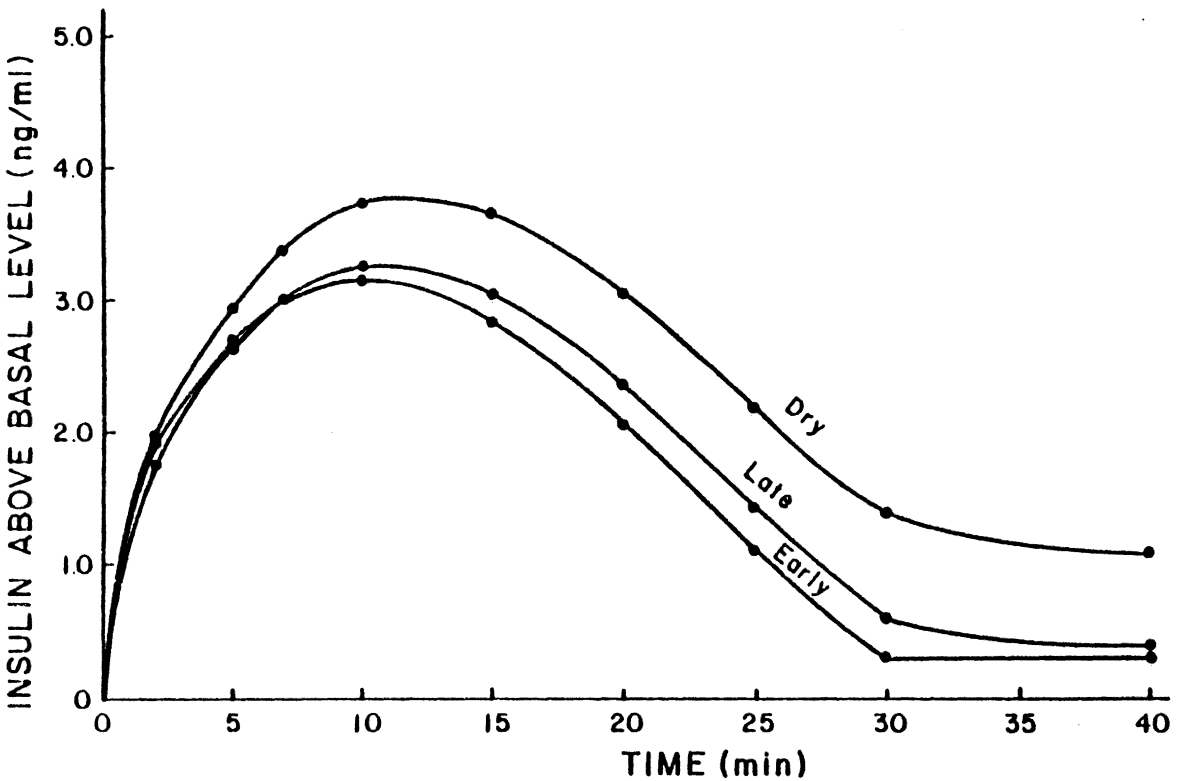

Figure 5. Predicted insulin response curves following glucose injection for each lactation stage, based on actual data from 2 to 40 min post-injection.

The cow's ability to clear glucose from the blood is indicated by the low circulating glucose level during this time. In the present study, the lactation stage with the fastest glucose clearance (E) did have the lowest plasma glucose level, but also had the smallest insulin response (Figure 5). In contrast, the slowest glucose clearance stage (D) corresponded to the largest insulin response. The author interprets the data to indicate that cows during early lactation have a greater ability to remove glucose from the circulation, and the low plasma glucose levels at this time could be the result of the high rates of removal.

Insulin Clearance

The overall mean values of insulin clearance given in Table 5 are summarized from Appendix Tables 20-22, which contain individual cow data for clearance expressed as % disappearance/min. Appendix Table 23, provides the statistical summary and indicates there was variation ($P<.05$) among lactation stages and cows.

Insulin clearance rate decreased significantly ($P<.05$) from E to D from 5.65 to 2.98% disappearance/min, respectively, and L was intermediate at 4.57% disappearance/min and significantly different ($P<.05$) from both E and D. The faster return of insulin to basal levels during E would be

advantageous because of the importance of gluconeogenesis for milk production during this time and the adverse effect insulin would have on this. The clearance of glucose and insulin from the circulation mimicked each other. This is further support that lactating dairy cows may be more affected by glucose and insulin than is currently thought.

Trenkle (1971) injected labelled insulin into sheep, and estimated insulin secretion based on his estimates of insulin disappearance rate. Average secretion in fed sheep was estimated at .66 ug/kg/hr. An attempt was made to estimate insulin secretion during the three lactation stages using the insulin data from our study. Calculations were as follows: 1) A sample representing the average insulin concentration from peak response back to basal level was chosen to represent the average insulin concentration during the insulin clearance portion of the curve, or the downslope (Figure 5). The 20 min sample was chosen because it fell at the mid-point of the downslope. 2) The sample concentration was multiplied by the estimated glucose pool space for the injection to estimate the total ng of circulating insulin. 3) The total circulating insulin was then multiplied by insulin clearance to estimate the total ng of insulin cleared/min. 4) Additional calculations were made to adjust insulin clearance for body weight and to have the resulting

secretion units the same as Trenkle's (1971). It was estimated that insulin secretion was .81 ug/kg/hr (13.5 ng/kg/min) during D and 1.2 ug/kg/hr (20.0 ng/kg/min) during lactating stages (E and L data combined). The secretion rate estimated for D was comparable to Trenkle's (1971) .66 ug/kg/hr (11 ng/kg/min) in non-lactating sheep. The faster secretion calculated for the lactating stages could be indicative of the animal's ability to respond to glucose quickly when it is in such great demand.

MILK YIELD AND COMPOSITION FOLLOWING GLUCOSE AND ACETATE LOADING

Tables 6-8 and Tables 9-11 list the glucose and acetate, respectively, least squares means ± SE and contrasts by time (corresponding to injection time) for E, L, and E and L data combined, respectively. Zero time refers to the injection time. The injections were given at 1500 hr. Times -15 and -3 correspond to the night and noon milk samples collected prior to injection, respectively, and times +9 and +21 correspond to the noon and night milk samples collected post-injection. The -15 and -3 milk samples were not affected by previous injections of either glucose or acetate ($P>.05$).

Table 6. Glucose milk parameter LS Means ± SE and contrasts by time for early lactation data

Time[1]	Yield (kg)	Fat (%)	Fat (kg)	Protein (%)	Protein (kg)	Total Solids (%)	Total Solids (kg)
-15	12.0 ± .2	3.26 ± .07	.39 ± .01	3.21 ± .04	.39 ± .01	11.97 ± .06	1.43 ± .02
-3	10.8 ± .2	3.57 ± .07	.39 ± .01	3.26 ± .04	.36 ± .01	12.32 ± .07	1.34 ± .02
+9	12.0 ± .2	3.30 ± .07	.39 ± .01	3.22 ± .04	.38 ± .01	12.04 ± .06	1.44 ± .02
+21	11.0 ± .2	3.67 ± .07	.40 ± .01	3.25 ± .04	.36 ± .01	12.29 ± .07	1.34 ± .02
Contrasts[2]:							
-15 vs. +9	0.0	-.04	.00	-.01	.00	-.06	.00
-3 vs. +21	-.1	-.10	-.01	.01	.00	.03	.00
-15 vs. -3	1.2*	-.32	.00	-.05	.03	-.35	.09
+9 vs. +21	1.0	-.38*	-.01	-.03	.03	-.25	.09

[1]Zero time represents injection time.

[2]Corresponding numbers represent estimated differences.

*$P < .05$.

Table 7. Glucose milk parameter LS Means ± SE and contrasts by time for late lactation data

Time[1]	Yield (kg)	Fat (%)	Fat (kg)	Protein (%)	Protein (kg)	Total Solids (%)	Total Solids (kg)
-15	11.4 ± .2	3.36 ± .11	.37 ± .01	3.15 ± .03	.36 ± .01	11.99 ± .08	1.36 ± .02
-3	10.0 ± .2	3.40 ± .11	.34 ± .01	3.19 ± .03	.32 ± .01	12.43 ± .08	1.23 ± .02
+9	11.6 ± .2	3.04 ± .11	.35 ± .01	3.12 ± .03	.36 ± .01	11.95 ± .08	1.38 ± .02
+21	10.2 ± .2	3.30 ± .11	.33 ± .01	3.17 ± .03	.32 ± .01	12.51 ± .08	1.26 ± .02
Contrasts[2]:							
-15 vs. +9	-.2	.32	.02	.03	.00	.04	-.02
-3 vs. +21	-.2	.10	.00	.02	.00	-.08	-.03
-15 vs. -3	1.4*	-.04	.04	-.04	.04	-.45*	.12
+9 vs. +21	1.4*	-.26	.02	-.05	.04	-.56*	.12

[1]Zero time represents injection time.

[2]Corresponding numbers represent estimated differences.

*P<.05.

Table 8. Glucose milk parameter LS Means ± SE and contrasts by time for early and late lactation data, combined

Time[1]	Yield (kg)	Fat (%)	Fat (kg)	Protein (%)	Protein (kg)	Total Solids (%)	Total Solids (kg)
-15	11.7 ± .1	3.30 ± .07	.38 ± .01	3.18 ± .03	.37 ± .01	11.98 ± .05	1.39 ± .01
-3	10.4 ± .1	3.49 ± .07	.36 ± .01	3.23 ± .03	.34 ± .01	12.39 ± .05	1.29 ± .01
+9	11.8 ± .1	3.17 ± .07	.37 ± .01	3.17 ± .03	.37 ± .01	11.99 ± .05	1.41 ± .01
+21	10.6 ± .1	3.49 ± .07	.37 ± .01	3.21 ± .03	.34 ± .01	12.40 ± .05	1.30 ± .01
Contrasts[2]:							
-15 vs. +9	-.1	.13	.01	.01	.00	-.01	-.02
-3 vs. +21	-.2	.00	.00	.02	.00	-.01	-.01
-15 vs. -3	1.29*	-.19	.02	-.05	.04	-.41*	.10
+9 vs. +21	1.23*	-.32*	.00	-.04	.03	-.41*	.11

[1]Zero time represents injection time.

[2]Corresponding numbers represent estimated differences.

*P<.05.

Table 9. Acetate milk parameter LS Means ± SE and contrasts by time for early lactation data

Time[1]	Yield (kg)	Fat (%)	Fat (kg)	Protein (%)	Protein (kg)	Total Solids (%)	Total Solids (kg)
-15	12.0 ± .2	3.29 ± .07	.39 ± .01	3.16 ± .03	.38 ± .01	12.00 ± .06	1.44 ± .02
-3	11.0 ± .2	3.49 ± .07	.38 ± .01	3.19 ± .03	.35 ± .01	12.14 ± .05	1.33 ± .02
+9	12.1 ± .2	3.28 ± .07	.40 ± .01	3.23 ± .03	.39 ± .01	11.90 ± .06	1.44 ± .02
+21	11.0 ± .2	3.47 ± .07	.38 ± .01	3.25 ± .03	.36 ± .01	12.14 ± .06	1.33 ± .02
Contrasts[2]:							
-15 vs. +9	-.1	-.01	.00	-.07	-.01	.10	.00
-3 vs. +21	0.0	.02	.00	-.07	-.01	.00	.00
-15 vs. -3	1.1	-.20	.01	-.03	.03	-.14	.11
+9 vs. +21	1.2	-.20	.01	-.02	.03	-.24	.11

[1]Zero time represents injection time.

[2]Corresponding numbers represent estimated differences.

*P<.05.

Table 10. Acetate milk parameter LS Means ± SE and contrasts by time for late lactation data

Time[1]	Yield (kg)	Fat (%)	Fat (kg)	Protein (%)	Protein (kg)	Total Solids (%)	Total Solids (kg)
-15	11.5 ± .2	3.23 ± .12	.36 ± .01	3.19 ± .03	.37 ± .01	12.19 ± .08	1.41 ± .02
-3	10.1 ± .2	3.31 ± .12	.33 ± .01	3.16 ± .03	.32 ± .01	12.47 ± .08	1.26 ± .02
+9	11.3 ± .2	3.13 ± .12	.35 ± .01	3.16 ± .03	.36 ± .01	11.98 ± .08	1.35 ± .02
+21	10.1 ± .2	3.31 ± .12	.33 ± .01	3.17 ± .03	.32 ± .01	12.43 ± .08	1.25 ± .02
Contrasts[2]:							
-15 vs. +9	0.2	.10	.02	.04	.01	.21	.05
-3 vs. +21	0.1	.00	.00	.00	.00	.04	.01
-15 vs. -3	1.5*	-.08	.03	.03	.05	-.28	.14*
+9 vs. +21	1.2*	-.18	.02	-.01	.04	-.45*	.10

[1]Zero time represents injection time.

[2]Corresponding numbers represent estimated differences.

*P<.05.

Table 11. Acetate milk parameter LS Means ± SE and contrasts by time for early and late lactation data, combined

Time[1]	Yield (kg)	Fat (%)	Fat (kg)	Protein (%)	Protein (kg)	Total Solids (%)	Total Solids (kg)
-15	11.8 ± .1	3.26 ± .07	.38 ± .01	3.17 ± .02	.37 ± .00	12.09 ± .05	1.42 ± .02
-3	10.5 ± .1	3.40 ± .07	.36 ± .01	3.18 ± .02	.34 ± .00	12.30 ± .05	1.30 ± .02
+9	11.7 ± .1	3.19 ± .07	.37 ± .01	3.19 ± .02	.37 ± .00	11.94 ± .05	1.40 ± .01
+21	10.6 ± .1	3.39 ± .07	.35 ± .01	3.21 ± .02	.34 ± .00	12.28 ± .05	1.29 ± .01
Contrasts[2]:							
-15 vs. +9	-.1	.07	.01	-.02	.00	.16	.02
-3 vs. +21	0.0	.01	.00	-.03	.00	.02	.01
-15 vs. -3	1.3*	-.15	.02	.00	.04*	-.21*	.12*
+9 vs. +21	1.2*	-.20	.02	-.02	.04	-.35*	.11

[1]Zero time represents injection time.

[2]Corresponding numbers represent estimated differences.

*P<.05.

Contrast analysis was conducted on the -15 and +9 samples and also on the -3 and +21 samples to determine the effect of glucose and acetate loading on milk parameters. The former analyzes night differences and the latter analyzes noon differences. Contrast analysis was conducted on the -15 and -3 samples and also on the +9 and +21 samples to determine if steady state, with regards to milk production and composition, was achieved with 4-hr feeding intervals.

Tables 6 and 7 list the E and L glucose milk parameter least squares means ± SE and contrasts by time, respectively. Lack of significant differences ($P>.05$) in the -15 vs +9 and -3 vs +21 contrasts indicates there was no effect of glucose loading during E and L on milk yield and composition. However, there was a significant difference ($P<.05$) in noon and night milk yield and fat % during E and milk yield and total solids % during L, with higher milk yield and lower milk fat and total solids % for night as compared to noon milk.

Table 8 lists the glucose milk parameter least squares means ± SE and contrasts by time with E and L milk data, combined. Appendix Table 24 provides the statistical summaries for each milk parameter for E and L data, combined, and indicates overall cow effects were significant ($P<.05$)

for all parameters. Overall stage effects were significant ($P<.05$) for only kg of milk fat, kg milk protein, and kg of milk total solids (Appendix Table 24). Since milk yield was not different between E and L, the data were combined.

Lack of significant differences ($P>.05$) in the -15 vs +9 and -3 vs +21 contrasts (Table 8) indicates there was no effect of glucose loading on milk yield and composition during E and L. However there was a significant difference ($P<.05$) between noon and night milk yield and milk fat and total solids %, with higher milk yield and lower milk fat and total solids % for night as compared to noon milk. There was a slight tendency for milk to increase from 11.7 (-15) to 11.8 kg (+9) and milk fat % to decrease from 3.30 (-15) to 3.17% (+9); however, these differences were not significant ($P>.05$).

It was estimated that the glucose loads given in this study averaged about 3% of the daily glucose requirement for a cow weighing 500 kg and producing 22 kg milk daily, based on milk of 4.9% lactose content and 60% of glucose entry used for lactose synthesis (Bergman and Hogue, 1967). Since the insulin responses were large following glucose dosing in these animals, the insulin probably acted to divert nutrients away from the mammary gland, since this organ is

insensitive to insulin. Most likely, the glucose was taken up by the body tissues rather than appearing in the milk.

Table 9 and 10 lists the acetate milk parameter least squares means ± SE and contrasts by time for E and L milk data, respectively. The lack of significant differences in the -15 vs +9 and -3 vs +21 contrasts indicates there was no effect of acetate loading during E and L on milk yield and composition. However, there was a significant difference ($P<.05$) between noon and night milk yield and total solids (% and kg) during L, with higher milk yield and kg total solids and lower total solids % for night as compared to noon milk.

Table 11 lists the acetate milk parameter least squares means ± SE and contrasts by time for E and L milk data, combined. Appendix Table 25 provides the statistical summaries for each milk parameter for E and L data, combined, and indicates overall cow effects were significant ($P<.05$) for all milk parameters. Overall stage effects were significant ($P<.05$) for kg of milk fat and protein and total solids % (Appendix Table 25). Since milk yield was not different between E and L, the data were combined.

Lack of significant differences ($P>.05$) in the -15 vs +9 and -3 vs +21 contrasts indicates there was no effect of acetate loading on milk yield and composition. A

significant difference ($P<.05$) in the noon and night contrasts were observed for milk yield, kg of protein, and total solids (% and kg).

Both glucose and acetate loading, at the doses used in this study, did not affect milk yield or composition. One interesting point brought out by the data was a difference in yield and composition of noon and night milk. The 4-hr feeding intervals used in this study were used in an attempt to achieve steady state with regards to milk production and nutrient absorption from the gut. Bickerstaffe et al. (1974) observed steady state milk production when lactating cows were milked at hourly intervals, but the author is unaware of reports of equal milk production at noon and night milkings with 12-hr milking intervals. Dr. D. E. Bauman[23] (personal communication) questions whether steady state can be achieved with regards to milk production. In addition to nutrient supply, hormone levels, cow activity, and environmental temperature fluctuations throughout the day may influence milk production.

---

[23]Cornell University, Ithaca, New York.

Chapter V

RESULTS AND DISCUSSION - STUDY TWO

The body weight, dry matter intake, and fat-corrected milk data are listed by cow, diet, and lactation stage in Table 12. Cows that were changed to the high-concentrate diet (HC) produced more milk at 60 days than the control-fed animals, and for this reason covariate analysis was conducted, adjusting data using the 60 day parameters.

DATA AND STATISTICAL ANALYSIS OF PLASMA GLUCOSE PARAMETERS

Overall means for plasma glucose parameters averaged by lactation stage and diet are listed in Table 13. The values given in Table 13 are summarized from Appendix Table 26, which contains individual cow data. Appendix Table 27 provides the statistical summary for each glucose parameter. There was significant variation ($P<.05$) in body weight-adjusted pool space (ml/kg) due to a stage by diet effect, but there was no variation ($P>.05$) for any of the plasma glucose parameters due to diet or stage effects. This was the result of an insufficient number of cows in the study. Based on the analysis of variance results for plasma glucose (Appendix Table 27), for a plasma glucose difference of 8 mg/dl between stage by diet groups to have been significant ($P<.05$), with a power of test of .80, 12 cows would have been needed in each stage by diet group.

Table 12. Body weight, dry matter intake, and fat-corrected milk data by cow, stage of lactation, and diet for each injection period

Cow	Day	Diet[1]	BW (kg)	DM Intake (kg)	FCM[2] (kg)
1106	60	C	541	10	22.2
	90	HC	539	21	20.1
	120	HC	543	21	20.2
1178	60	C	530	21	27.3
	90	HC	541	22	19.2
	120	HC	520	23	21.7
1187	60	C	661	16	20.0
	90	C	661	19	18.5
	120	C	686	17	19.1
1191	60	C	641	18	29.9
	90	HC	630	26	29.1
	120	HC	627	22	32.8
1211	60	C	518	15	24.8
	90	C	523	19	24.0
	120	C	547	17	20.7
1222	60	C	523	18	27.0
	90	HC	493	22	27.9
	120	HC	522	21	27.9
1229	60	C	622	23	24.3
	90	HC	632	22	21.2
	120	HC	609	16	21.9
1230	60	C	590	18	23.9
	90	C	573	20	23.7
	120	C	586	21	23.5
1239	60	C	490	18	20.9
	90	C	500	16	22.2
	120	C	505	15	20.9

[1]C = control, HC = high-concentrate.

[2]FCM = 4% fat-corrected milk
= (kg milk x .4) + (kg fat x 15)

Table 13. Glucose parameter LS Means ± SE by stage of lactation and diet for lactating cows

Day	Diet	n	Plasma Glucose (mg/dl)	Clearance Rate (%/min)	Clearance Rate (%/min/ 100 kg)	Pool Size (g)	Pool Size (mg/kg)	Pool Space (l)	Pool Space (ml/kg)
90	C	4	66.5 ± 1.8	2.57 ± .23	.445 ± .040	48.9 ± 4.3	88.3 ± 8.2	73.7 ± 4.5	132.9 ± 8.6
	HC	5	67.9 ± 1.7	2.17 ± .20	.403 ± .035	46.9 ± 3.9	83.8 ± 7.3	69.0 ± 4.0	123.4 ± 7.7
120	C	4	68.2 ± 1.8	2.56 ± .23	.426 ± .040	44.8 ± 4.3	77.6 ± 8.2	66.3 ± 4.5	114.0 ± 8.6
	HC	5	69.7 ± 1.7	2.28 ± .10	.416 ± .035	51.9 ± 3.9	91.8 ± 7.3	73.5 ± 4.0	129.8 ± 7.7

Table 13 shows that there was a tendency for plasma glucose to be higher at 120 days as compared to 90 days and higher with the HC as compared to the control ration. However, these differences were not significant (P>.05). This is in agreement with the observations of Study One and reports from other studies (Walker and Elliot, 1973; Jenny et al., 1974; Jenny and Polan, 1975; Smith et al., 1976; Hart et al., 1978).

Glucose clearance had a tendency to be higher on the control ration than on the HC ration, averaging 2.57 and 2.17%/min, respectively, at 90 days and 2.56 and 2.28%/min, respectively, at 120 days. However, these differences were not significant (P>.05). There appeared to be no difference due to stage. Higher clearance would indicate a greater ability to utilize (remove) the glucose load more rapidly. The same trends were observed for body weight-adjusted glucose clearance; however, the differences were not significant (P>.05).

There was no difference in pool size (g) due to stage and/or diet. Pool space expressed as liters and ml/kg was similar (P>.05) between treatments (Table 13), averaging 70.6 liters and 125.0 ml/kg across the four treatment groups.

DATA AND STATISTICAL ANALYSIS OF PLASMA INSULIN PARAMETERS

Overall means for insulin parameters averaged by lactation stage and diet are listed in Table 14. The values given in Table 14 are summarized from Appendix Table 28, which contains individual cow data. Appendix Table 29 provides the statistical summary for each insulin parameter. None of the diet and stage effects were significantly different ($P>.05$) for any of the insulin parameters. This is the result of an insufficient number of cows in the study. Based on the analysis of variance results for plasma insulin (Appendix Table 29), for a plasma insulin difference of .4 ng/ml between stage by diet groups to be significant ($P<.05$), with a power of test of .80, ten cows would have been needed in each stage by diet group. There was a significant ($P<.05$) covariate effect for insulin peak concentration and insulin PCAB (Appendix Table 29) and there was variation ($P<.05$) among cows within diets for insulin PFI (Appendix Table 29).

There was a tendency for plasma insulin to be higher in HC-fed animals than control-fed and in cows at 120 days than 90 days, but these differences were not significant ($P>.05$). The trend of insulin to be higher as lactation progresses was observed in Study One.

Table 14. Insulin parameter LS Means ± SE by stage of lactation and diet for lactating cows

Day	Diet	n	Plasma Insulin (ng/ml)	Peak Conc. (ng/ml)	Peak Increase[1] (ng/ml)	Peak Fold Increase[1]	Response Curve[1] Area	Clearance Rate[2] (%/min)
90	C	4	.72 ± .10	4.08 ± .45	3.25 ± .39	5.17 ± 1.10	73.7 ± 13.3	5.17 ± .37
	HC	5	.83 ± .09	4.22 ± .40	3.47 ± .34	3.73 ± 0.96	71.9 ± 11.8	4.94 ± .37
120	C	4	.84 ± .10	4.42 ± .45	3.47 ± .39	4.88 ± 1.10	79.0 ± 13.3	4.94 ± .37
	HC	5	.95 ± .09	4.87 ± .40	4.01 ± .34	4.01 ± 0.96	75.6 ± 11.8	4.23 ± .32

[1]above basal concentration.

[2]calculated from peak concentration to 40 min post-injection.

Insulin response curve area had a tendency to be higher in cows at 120 days compared to 90 days, and in control-fed compared to HC-fed cows. However, these differences were not significant (P>.05). Insulin clearance had a tendency to be higher in control-fed cows compared to HC-fed cows and at 90 days compared to 120 days. However, these differences were not significant (P>.05).

COMPARISON OF 60 DAY, STUDY TWO PARAMETERS WITH 90 DAY, STUDY ONE PARAMETERS

Table 15 lists the mean glucose and insulin parameter means ± SE for 60 day data from Study Two and 90 day data from Study One. The diets fed to the two groups were similar, so the data was pooled for comparisons. Means will be discussed with respect to possible trends across stages of lactation. However, no statistical analysis was conducted on the data because of the small number of observations at 60 days.

Plasma glucose had a tendency to be higher at 60 days compared to 90 days (Table 15). This is inconsistent with reports from Hart et al. (1978) and Study One data. There was a trend for glucose clearance to be higher, as %/min and %/min/100 kg, at 90 days compared to 60 days. This could correspond to maximum intake occuring at 90 days of lacta-

Table 15. Glucose and insulin parameter means ± SE for 60 days (Study Two) and 90 days (Study One)

Parameter	60 Days	90 Days
Plasma Glucose (mg/dl)	64.8 ± 3.4	57.4 ± 1.1
Glucose Clearance Rate (%/min)	2.14 ± .47	2.43 ± .11
(%/min/100 kg)	.390 ± .10	.504 ± .021
Glucose Pool Size (g)	45.0 ± 6.7	39.2 ± 1.7
(mg/kg)	80.6 ± 8.9	81.4 ± 3.5
Glucose Pool Space (liters)	69.3 ± 9.0	68.4 ± 2.7
(ml/kg)	124.2 ± 10.8	142.2 ± 5.8
Plasma Insulin (ng/ml)	.67 ± .27	.78 ± .07
Peak Conc. (ng/ml)	3.65 ± .97	4.30 ± .21
Peak Increase[1] (ng/ml)	2.99 ± .92	3.51 ± .18
Peak Fold Increase[1]	4.81 ± 1.62	5.41 ± .30
Response Curve Area[1]	57.0 ± 19.5	65.3 ± .48
Insulin Clearance (%/min)	5.10 ± 1.25	5.65 ± .24

[1]above basal level.

tion. Pool size, as g and mg/kg, and pool space, as liters and ml/kg, appeared similar at 60 and 90 days.

Plasma insulin had a tendency to be lower at 60 days compared to 90 days. This agrees with the observations in Study One and reports from other studies (Koprowski and Tucker, 1973; Hart et al., 1980; Vasilatos and Wangsness, 1981). However, it seems unusual that plasma glucose was higher and plasma insulin was lower at 60 days. Insulin response curve area and clearance rate tended to be larger at 90 days compared to 60 days, and this follows the trends observed in Study One.

Chapter VI

SUMMARY AND CONCLUSIONS

Two studies were conducted to estimate glucose plasma parameters (clearance rate, pool size, and pool space) at different stages of lactation and under different feeding regimes. The effects of glucose loading on insulin response and glucose and acetate loading on milk production and composition were also investigated.

In study One, glucose intravenous tolerance tests were conducted on eight first-lactation Holstein cows at three stages of lactation [early (E)=75 to 100 days, late (L)=205 to 250 days, and dry period (D)]. Cows were fed a corn silage-based ration (15% CP, 22% ADF) ad libitum during E and L and at maintenance during D. Four-hour feeding intervals were used in an attempt to achieve steady state. A milk sample was collected twice daily for analysis. Body weight, intake, and milk production data were recorded.

Three glucose (.1 g/kg BW) and three acetate (1 mmole/kg BW) injections were administered during each stage. Blood was collected for glucose injections at at 5, 10, and 15 min pre-injection and from 2-40 min post-dosing. The plasma parameters were calculated by the method of least squares using natural logarithms of actual concentration

values and regression analysis. Glucose data fit a single compartment kinetic model. Glucose injection samples were assayed for insulin by a double antibody method.

Plasma glucose and insulin increased ($P<.05$) from E to D. There was a decrease ($P<.05$) in glucose clearance with each successive lactation stage, and clearance was correlated with dry matter intake ($r = .70$, $P<.01$). Glucose pool size (g) and space (liters) increased ($P<.05$) from E to D, but when adjusted for body weight there was no difference between stages.

Insulin response above basal level following glucose loading was greatest during D, with higher insulin peak values and slower rates of insulin clearance as compared to E and L.

There was no affect of glucose and acetate loading on milk production and % fat, protein, and total solids. Differences ($P<.05$) in noon and night milk yield with 4-hr feeding intervals were observed, with higher yields at night milkings.

In Study Two, single intravenous glucose tolerance tests were conducted on nine lactating cows at 60, 90, and 120 days postpartum. At 60 days, cows were fed a control

ration (16% CP, 24.4% ADF). After 60 days, five were fed a high-concentrate, low fiber ration (HC) (16.8% CP, 13.0% ADF). Injections and sample analysis were conducted similar to Study One.

No significant differences ($P>.05$) in plasma glucose and insulin parameters due to diet or stage were observed. This was the result of an insufficient number of cows in the study.

In conclusion, glucose clearance appears to decrease as lactation progresses, but pool size and space, when adjusted for body weight, show no difference between lactation stages (from E to D). Glucose stimulated insulin response above basal level increases and insulin clearance rate decreases as lactation progresses.

Chapter VII

EPILOGUE

The study described in this thesis presented data on glucose clearance in dairy cows under lactating and non-lactating conditions and under high concentrate versus control feeding conditions. On completion of analysis of the data, several questions surfaced that need to be researched in the future.

The use of glucose clearance as a method of estimating utilization is still in the preliminary stages. Based on the findings of Verdonk et al. (1981) and Defronzo and Ferrannini (1981), the effect of basal glucose level and insulin response on glucose clearance need to be determined. A clamp technique could be used to manipulate basal glucose and/or insulin response during the injection and sampling period. This would result in a better understanding of the controlling factors of glucose clearance. It would be interesting to see if the results from Study One would be altered if basal glucose was identical for all three lactation stages.

In addition, the effect of dose on glucose clearance needs further investigation. Prior to commencement of Study One, preliminary work was done in our lab to determine a

suitable dose to elevate plasma glucose sufficiently to estimate glucose clearance. Glucose doses of .05, .1, and .25 g/kg body weight were tested. Data indicated that the .05 g/kg body weight dose was insufficient to estimate clearance, but the .1 and .25 g/kg doses sufficiently elevated plasma glucose and had comparable clearance estimates in lactating and non-lactating cows. The data indicated clearance was not affected by dose, but additional research is needed to determine the suitable dose range and the repeatability of their clearance estimates.

Three stages were included in the investigation in Study One. In addition, glucose clearance needs to be determined in cows at parturition, at 60 days postpartum (corresponding to the peak production period), and late in the dry period. Changes in glucose clearance near parturition have been investigated by Bernal and Bauman (1981), and their data indicated that glucose clearance increases postpartum when compared to prepartum.

As shown from the milk data from Study One, milk yield was higher at night compared to noon milkings with 4-hr feeding intervals. It could be possible that milk production is not a good indicator of steady state conditions.

The effect of diet on glucose clearance was not elucidated in Study Two due to the small number of cows sampled. A repeat of Study Two using a comparable number of cows and replications as in Study One is necessary. As shown in the results from Study One, the tolerance test averages about 42% repeatable. Therefore, three test replications are required to attain a reliable clearance estimate. If three replicates were obtained per cow, the number required for each stage by diet group should drop below ten.

The purpose of this chapter was to briefly describe some of the areas that need further investigation concerning tolerance testing in ruminant animals. The present study provides data for a foundation for further work.

REFERENCES

Amatuzio, D. S., F. L. Stutzman, M. J. Vanderbilt, and S. Nesbitt. 1953. Interpretation of the rapid intravenous glucose tolerance test in normal individuals and in mild diabetes mellitus. J. Clin. Invest. 32:428.

Ambro, K., H. Takahashi, and T. Tsuda. 1973. Effects of feeding and infusion of short-chain fatty acids and glucose on plasma insulin and blood glucose levels in sheep. Tohoku J. Agric. Res. 24:54.

Anderson, R. R., and J. P. Mixner. 1959. Repeatability and precision of phenolsulfonphthalein clearance measures for renal function in dairy cattle. J. Dairy Sci. 42:545.

Annison, E. F., and J. L. Linzell. 1964. The oxidation and utilization of glucose and acetate by the mammary gland of the goat in relation to their over-all metabolism and to milk formation. J. Physiol. 175:372.

Annison, E. F., R. Bickerstaffe, and J. L. Linzell. 1974. Glucose and fatty acid metabolism in cows producing milk of low fat content. J. Agric. Sci., Camb. 82:87.

Baird, G. D. 1981. Lactation, pregnancy, and metabolic disorder in the ruminant. Proc. Nutr. Soc. 40:115.

Baldwin, R. L., and N. E. Smith. 1971. Intermediary aspects and tissue interactions of ruminant fat metabolism. J. Dairy Sci. 54:583.

Ballard, F. J., R. W. Hanson, and D. S. Kronfeld. 1969. Gluconeogenesis and lipogenesis in tissue from ruminant and nonruminant animals. Fed. Proc. 28:218.

Bartley, J. C., S. Abraham, and I. L. Chaikoff. 1966. Biosynthesis of lactose by mammary gland slices from the lactating rat. J. Biol. Chem. 241:1132.

Bauman, D. E., R. E. Brown, and C. L. Davis. 1970. Pathways of fatty acid synthesis and reducing equivalent generation in mammary gland of rat, sow, and cow. Archives Biochem. Biophys. 140:237.

Bauman, D. E., C. L. Davis, and H. F. Bucholtz. 1971. Propionate production in the rumen of cows fed either a control or high-grain, low-fiber diet. J. Dairy Sci. 54:1282.

Bauman, D. E., and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514.

Baxter, C. F., M. Kleiber, and A. L. Black. 1955. Glucose metabolism in the lactating dairy cow. Biochim. Biophys. Acta 17:354.

Bensadoun, A., O. L. Paladines, and J. T. Reid. 1962. Effect of level of intake and physical form of the diet on plasma glucose concentration and volatile fatty acid absorption in ruminants. J. Dairy Sci. 45:1203.

Benson, J. A., P. J. Culver, S. Ragland, C. M. Jones, G. D. Drummey, and E. Bougas. 1957. The d-xylose absorption test in malabsorption syndromes. New. Eng. J. Med. 256:335.

Bergman, E. N. 1963. Quantitative aspects of glucose metabolism in pregnant and nonpregnant sheep. Am. J. Physiol. 204:147.

Bergman, E. N. 1973. Glucose metabolism in ruminants as related to hypoglycemia and ketosis. Cornell Vet 63:341.

Bergman, E. N., and D. E. Hogue. 1967. Glucose turnover and oxidation rates in lactating sheep. Am. J. Physiol. 213:1378.

Bergman, E. N., M. L. Katz, and C. F. Kaufman. 1970. Quantitative aspects of hepatic and portal glucose metabolism and turnover in sheep. Am. J. Physiol. 219:785.

Bergman, E. N., W. E. Roe, and K. Kon. 1966. Quantitative aspects of propionate metabolism and gluconeogenesis in sheep. Am. J. Physiol. 211:793.

Bergman, E. N., D. J. Starr, and S. S. Reulein, Jr. 1968. Glycerol metabolism and gluconeogenesis in the normal and hypoglycemic ketotic sheep. Am. J. Physiol. 215:874.

Bernal, G., and D. E. Bauman. 1981. Changes in glucose homeostasis with onset of lactation. J. Dairy Sci. Supp. 1:159 (Abstr.).

Bickerstaffe, R., E. F. Annison, and J. L. Linzell. 1974. The metabolism of glucose, acetate, lipids, and amino acids in lactating dairy cows. J. Agric. Sci., Camb. 82:71.

Brukental, I., J. D. Oldham, and J. D. Sutton. 1980. Glucose and urea kinetics in cows in early lactation. Brit. J. Nutr. 44:33.

Carubelli, R., B. Taha, R. E. Trucco, and R. Caputto. 1964. Incorporation of (^{14}C)glucose into lactose and neuramin-lactose by rat mammary glands. Biochim. Biophys. Acta 83:224.

Davis, C. L. 1967. Acetate production in the rumen of cows fed either contrcl or low-fiber, high-grain diets. J. Dairy Sci. 50:1621.

Defronzo, R., and E. Ferrannini. 1981. Glucose clearance is constant throughout the physiologic range of plasma glucose concentration. Clin. Res. 29:404A.

Dimenna, G. P., and R. S. Emery. 1980. Factors affecting fatty acid oxidation in bovine mammary tissue. Lipids 15:497.

Ebner, K. E., and Urs Brodbeck. 1968. Biological role of alpha-lactalbumin: A review. J. Dairy Sci. 51:317.

Emery, R. S. 1973. Biosynthesis of milk fat. J. Dairy Sci. 56:1187.

Evans, E., J. G. Buchanan-Smith, G. K. MacLeod, and J. B. Stone. 1975. Glucose metabolism in cows fed low- and high-roughage diets. J. Dairy Sci. 58:672.

Ferrannini, E., and A. Pilo. 1979. Pattern of insulin delivery after intravenous glucose injection in man and its relation to plasma glucose disappearance. J. Clin. Invest. 64:243.

Fisher, L. J., and J. M. Elliot. 1966. Effect of intravenous infusion of propionate or glucose on bovine milk composition. J. Dairy Sci. 49:826.

Fisher, L. J., J. M. Elliot, and D. A. Corse. 1967. Fatty acid composition of bovine milk fat as influenced by intravenous infusion of propionate or glucose. J. Dairy Sci. 50:53.

Frobish, R. A., and C. L. Davis. 1977. Effects of abomasal infusions of glucose and propionate on milk yield and composition. J. Dairy Sci. 60:204.

Ganda, O. P., J. L. Day, J. S. Soeldner, J. J. Connon, and R. E. Gleason. 1978. Reproducibility and comparitive analysis of repeated intravenous and oral glucose tolerance tests. Diabetes 27:715.

Goldfine, I. D. 1978. Insulin receptors and the site of action of insulin. Life Sci. 23:2639.

Greville, G. D. 1943. The intravenous glucose tolerance equation. Biochem. J. 37:17.

Hansard, S. L. 1964. Total body water of farm animals. Am. J. Physiol. 206:1369.

Hansen, R. G., H. G. Wood, G. J. Peeters, B. Jacobson, and J. Wilken. 1962. Lactose synthesis: VI. Labelling of lactose precursors by glycerol-1,3-C^{14} and glucose-2-C^{14}. J. Biol. Chem. 237:1034.

Hara, E., and M. Saito. 1980. Diurnal changes in plasma glucose and insulin responses to oral glucose load in rats. Am. J. Physiol. 238:E463.

Hart, I. C., J. A. Bines, S. V. Morant, and J. L. Ridley. 1978. Endocrine control of energy metabolism in the cow: Comparison of the levels of hormones (prolactin, growth hormone, insulin, and thyroxine) and metabolites in the plasma of high- and low-yielding cattle at various stages of lactation. J. Endocrinol. 77:333.

Hardwick, D. C., J. L. Linzell, and T. B. Mepham. 1963. The metabolism of acetate and glucose by the isolated perfused udder. 2. The contribution of acetate and glucose to carbon dioxide and milk constituents. Biochem. J. 88:213.

Hardwick, D. C., J. L. Linzell, and S. M. Price. 1961. The effect of glucose and acetate on milk secretion by the perfused goat udder. Biochem. J. 80:37.

Hart, I. C., J. A. Bines, and S. V. Morant. 1979. Endocrine control of energy metabolism in the cow: Correlations of hormones and metabolites in high and low yielding cows for stages of lactation. J. Dairy Sci. 62:270.

Hayden, T. J., C. R. Thomas, and I. A. Forsyth. 1979. Effect of number of young born (litter size) on milk yield of goats: Role for placental lactogen. J. Dairy Sci. 62:53.

Head, H. H., J. D. Connolly, and W. F. Williams. 1964. Glucose metabolism in dairy cattle and the effect of acetate infusion. J. Dairy Sci. 47:1371.

Heald, P. J. 1951. The assessment of glucose-containing substances in rumen micro-organisms during a digestion cycle in sheep. Brit. J. Nutr. 5:84.

Herbein, J. H., R. W. Van Maanen, A. D. McGilliard, and J. W. Young. 1978. Rumen propionate and blood glucose kinetics in growing cattle fed isoenergetic diets. J. Nutr. 108:994.

Holmes, J. R. 1951a. Carbohydrate metabolism in the bovine: I. Intravenous glucose tolerance in the healthy cow. J. Comp. Path. 61:1.

Holmes, J. R. 1951b. Carbohydrate metabolism in the bovine: II. Intravenous glucose tolerance in various diseases, including clinical ketosis. J. Comp. Path. 61:15.

Horino, M., L. J. Machlin, F. Hertelendy, and D. M. Kipnis. 1968. Effect of short-chain fatty acids on plasma insulin in ruminant and nonruminant species. Endocrinol. 83:118.

Hove, K. 1978. Maintenance of lactose secretion during acute insulin deficiency in lactating goats. Acta Physiol. Scand. 103:173.

Hove, K., and A. K. Blom. 1973. Plasma insulin and growth hormone in dairy cows; diurnal variation and relation to food intake and plasma sugar and acetoacetate levels. Acta Endocrinol. 73:289.

Hunter, G. D., and G. C. Millson. 1964. Gluconeogenesis in the lactating dairy cow. Res. Vet. Sci. 5:1.

Jenny B. F., and C. E. Polan. 1975. Postprandial blood glucose and insulin in cows fed high grain. J. Dairy Sci. 58:512.

Jenny, B. F., C. E. Polan, and F. W. Thye. 1974. Effects of high grain feeding and stage of lactation on serum insulin, glucose, and milk fat percentage in lactating cows. J. Nutr. 104:379.

Jorgensen, N. A., L. H. Schultz, and G. R. Barr. 1965. Factors influencing milk fat depression on rations high in concentrates. J. Dairy Sci. 48:1031.

Judson, G. J., and R. A. Leng. 1973. Studies on the control of gluconeogenesis in sheep: effect of glucose infusion. Brit. J. Nutr. 29:159.

Kahn, C. R. 1980. Role of insulin receptors in insulin-resistant states. Metabolism 29:455.

Kamalu, T. N., and A. H. Trenkle. 1978. Insulin secretory response to increasing doses of glucose and volatile fatty acids in sheep. Nutr. Reports International 18:267.

Kaufman, C. F., and E. N. Bergman. 1971. Renal glucose, free fatty acid, and ketone body metabolism in the unanesthetized sheep. Am. J. Physiol. 221:967.

Kenna, T. M. 1981. Gluconeogenesis in the liver of growing and lactating ruminants: The influence of ration composition and stage of lactation. Ph. D. Dissertation. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Kleiber, M., A. L. Black, M. A. Brown, C. F. Baxter, J. R. Luick, and F. H. Stadtman. 1955. Glucose as a precursor of milk constituents in the intact dairy cow. Biochim. Biophys. Acta 17:252.

Koprowski, J. A., and H. A. Tucker. 1973. Bovine serum growth hormone, corticoids, and insulin during lactation. Endocrinol. 93:645.

Kronfeld, D. S., G. P. Mayer, J. McD. Robertson, and F. Raggi. 1963. Depression of milk secretion during insulin administration. J. Dairy Sci. 46:559.

Kronfeld, D. S., and F. Raggi. 1964. Glucose kinetics in normal, fasting, and insulin-treated cows. Am. J. Physiol. 206:109.

Kronfeld, D. S., F. Raggi, and C. F. Ramberg, Jr. 1968. Mammary blood flow and ketone body metabolism in normal, fasted, and ketotic cows. Am. J. Physiol. 215:218.

Kronfeld, D. S., E. G. Tombropoulos, and M. Kleiber. 1959. Glucose biokinetics in normal and ketotic cows. J. Appl. Physiol. 14:1026.

Kuhn, N. J., D. T. Carrick, and C. J. Wilde. 1980. Lactose synthesis: The possibilities of regulation. J. Dairy Sci. 63:328.

Larson, B. L. 1969. Biosynthesis of milk. J. Dairy Sci. 52:737.

Leat, W. M. F. 1971. Digestion and metabolism of carbohydrates in the foetal and neonatal ruminant. Proc. Nutr. Soc. 30:236.

Leng, R. A., J. W. Steel, and J. R. Luick. 1967. Contribution of propionate to glucose synthesis in sheep. Biochem. J. 103:785.

Lindsay, D. B. 1971. Changes in the pattern of glucose metabolism in growth, pregnancy, and lactation in ruminants. Proc. Nutr. Soc. 30:272.

Linzell, J. L. 1960. Mammary-gland blood flow and oxygen, glucose and volatile fatty acid uptake in the conscious goat. J. Physiol., Lond. 153:492.

Linzell, J. L. 1967. The effect of infusions of glucose, acetate, and amino acids on hourly milk yield in fed, fasted, and insulin-treated goats. J. Physiol. 190:347.

Linzell, J. L., T. B. Mepham, and M. Peaker. 1976. The secretion of citrate into milk. J. Physiol. 260:739.

Linzell, J. L., and M. Peaker. 1971. Mechanism of milk secretion. Physiological Reviews 52:564.

Lomax, M. A., G. D. Baird, C. B. Mallinson, and H. W. Symonds. 1979. Differences between lactating and non-lactating dairy cows in concentration and secretion rate of insulin. Biochem. J. 180:281.

Mackie, W. S. 1976. Plasma volume measurements in sheep using Evan's blue and continuous blood sampling. Res. Vet. Sci. 21:108.

Manns, J. G., and J. M. Boda. 1967. Insulin release by acetate, propionate, butyrate, and glucose in lambs and adult sheep. Am. J. Physiol. 212:747.

Martin, R. J., and R. L. Baldwin. 1971. Effects of insulin and anti-insulin serum treatments on levels of metabolites in rat mammary glands. Endocrinol. 88:868.

McAtee, J. W., and A. Trenkle. 1971. Metabolic regulation of plasma insulin levels in cattle. J. Anim. Sci. 33:438.

McCandless, E. L., and J. A. Dye. 1950. Physiological changes in intermediary metabolism of various species of ruminants incident to functional development of rumen. Am. J. Physiol. 162:434.

McClymont, G. L., and S. Vallance. 1962. Depression of blood glycerides and milk fat synthesis by glucose infusion. Proc Nutr. Soc. 21:xli.

Mepham, T. B., and J. L. Linzell. 1974. Effects of intramammary arterial infusion of non-essential amino acids and glucose in the lactating goat. J. Dairy Res. 41:111.

Murdock, F. R., and A. S. Hodgson. 1967. Milk production response of dairy cows fed high-moisture grass silage. I. Effect of varying levels of hay and concentrate. J. Dairy Sci. 50:57.

National Research Council. 1975. Nutrient requirements of domestic animals. No. 3. Nutrient requirements of dairy cattle. 5th ed. Nat. Res. Pub. ISBN 0-309-02749-7.

Nowak, J., and L. Dzialoszynski. 1967. Effect of experimental alloxan diabetes on secretion and composition of goat milk. Acta Physiol. Pol. 18:488.

Opstvedt, J., R. L. Baldwin, and M. Ronning. 1967. Effect of diet upon activities of several enzymes in abdominal adipose and mammary tissues in the lactating dairy cow. J. Dairy Sci. 50:108.

Paterson, J. Y. F., and J. L. Linzell. 1974. Cortisol secretion rate, glucose entry rate and mammary uptake during pregnancy and lactation in dairy cows. J. Endocrinol. 62:371.

Prior, R. L., and R. K. Christenson. 1978. Insulin and glucose effects on glucose metabolism in pregnant and nonpregnant ewes. J. Anim. Sci. 46:201.

Prior, R. L., and R. A. Scott. 1980. Effects of intravenous infusions of glucose, lactate, propionate, or acetate on the induction of lipogenesis in bovine adipose tissue. J. Nutr. 110:2011.

Rao, D. R., G. E. Hawkins, and R. C. Smith. 1973. Effect of glucose and insulin on lipoprotein lipase activity in adipose tissue and milk. J. Dairy Sci. 56:1415.

Reid, R. L. 1952. Studies of the carbohydrate metabolism of sheep: V. The effect of hyperglycaemia and of insulin on the rate of extrahepatic glucose assimilation. Aust. J. Agric. Res. 3:160.

Reid, R. L. 1958. Studies on the carbohydrate metabolism of sheep: VII. Intravenous glucose and acetate tolerance tests. Aust. J. Agric. Res. 9:788.

Reinhold, J. G. 1953. Glucose. Standard Methods Clinical Chemistry 1:65.

Rook, J. A. F., and C. C. Balch. 1961. The effects of intraruminal infusions of acetic, propionic, and butyric acids on the yield and composition of the milk of the cow. Brit. J. Nutr. 15:361.

Rook, J. A. F., J. E. Storry, and J. V. Wheelock. 1965. Plasma glucose and acetate and milk secretion in the ruminant. J. Dairy Sci. 48:745.

Rook, J. A. F., and J. V. Wheelock. 1967. The secretion of water and of water-soluble constituents in milk. J. Dairy Res. 34:273.

Sacca, L., P. E. Cryer, and R. S. Sherwin. 1979. Blood glucose regulates the effects of insulin and counterregulatory hormones on glucose production in vivo. Diabetes 28:533.

Schmidt, G. H. 1966. Effect of insulin on yield and composition of milk of dairy cows. J. Dairy Sci. 49:381.

Setchell, B. P., and G. M. H. Waites. 1964. Blood flow and uptake of glucose and oxygen in the testis and epididymus of the ram. J. Physiol., Lond. 171:411.

Smith, R. D., W. Hansel, and C. E. Coppock. 1976. Plasma growth hormone and insulin during early lactation in cows fed silage based diets. J. Dairy Sci. 59:248.

Sutton, J. D. 1971. The rate of carbohydrate fermentation in the rumen. Proc Nutr. Soc. 30:36.

Tindal, J. S., G. S. Knaggs, I. C. Hart, and L. A. Blake. 1978. Release of GH in lactating and non-lactating goats in relation to behavior, stages of sleep, electroencephalograms, environmental stimuli and levels of prolactin, insulin, glucose, and free fatty acids in the circulation. J. Endocrinol. 76:333.

Topps, J. H., R. N. B. Kay, and E. D. Goodall. 1968. Digestion of concentrate and of hay diets in the stomach and intestines in ruminants. I. Sheep. Brit. J. Nutr. 22:261.

Trenkle, A. 1971. Postprandial changes in insulin secretion rates in sheep. J. Nutr. 101:1099.

Trenkle, A. 1978. Relation of hormonal variations to nutritional studies and metabolism of ruminants. J. Dairy Sci. 61:281.

Van Soest, P. J. 1963. Ruminant fat metabolism with particular reference to factors affecting low milk fat and feed efficiency. A review. J. Dairy Sci. 46:204.

Vasilatos, R., and P. J. Wangsness. 1981. Diurnal variations in plasma insulin and growth hormone associated with two stages of lactation in high producing dairy cows. Endocrinol. 108:300.

Walker, C. K., and J. M. Elliot. 1973. Effect of roughage restriction on serum insulin in the dairy cow. J. Dairy Sci. 56:375.

Walsh, D. S., J. A. Vesely, and S. Mahadevan. 1980. Relationship between milk production and circulating hormones in dairy cows. J. Dairy Sci. 63:290.

Watkins, W. M., and W. Z. Hassid. 1962. Synthesis of lactose by particulate enzyme preparations from guinea pig and bovine mammary glands. J. Biol. Chem. 237:1432.

West, K. M., and D. A. Wood. 1953. The intravenous glucose tolerance test. Am. J. Med. Sci. 61:25.

White, R. G., and R. A. Leng. 1980. Glucose metabolism in feeding and postabsorptive lambs and mature sheep. Comp. Biochem. Physiol. 67:223.

White, R. G., J. W. Steel, R. A. Leng, and J. R. Luick. 1969. Evaluation of three isotope-dilution techniques for studying the kinetics of glucose metabolism in sheep. Biochem. J. 114:203.

Wilde, C. J., and N. J. Kuhn. 1979. Lactose synthesis in the rat, and the effects of litter size and malnutrition. Biochem. J. 182:287.

Wiltrout, D. W. and L. D. Satter. 1972. Contribution of propionate to glucose synthesis in the lactating and nonlactating cow. J. Dairy Sci. 55:307.

Wood, H. G., G. J. Peeters, R. Verbeke, M. Lauryssens, and B. Jacobson. 1965. Estimation of the pentose cycle in the perfused cow's udder. Biochem. J. 96:607.

Wood, H. G., P. Schambye, and G. J. Peeters. 1957. Lactose synthesis: II. The distribution of C^{14} in lactose of milk from the perfused isolated cow udder. J. Biol. Chem 226:1023.

Young, J. W. 1977. Gluconeogenesis in cattle: Significance and Methodology. J. Dairy Sci. 60:1.

Young, J. W., D. R. Trott., P. J. Berger, S. P. Schmidt, and J. A. Smith. 1974. Gluconeogenesis in ruminants: Glucose parameters in calves under standardized conditions. J. Nutr. 104:1049.

APPENDIX

Table 16. Plasma glucose parameters listed by cow for each injection during the early stage

Cow	Inj. No.	Plasma Glucose (mg/dl)	Glucose Dose (g)	Clearance Rate (%/min)	Clearance Rate (%/min/100 kg)	Pool Size (g)	Pool Size (mg/kg)	Pool Space (l)	Pool Space (ml/kg)
1223	1	60.1	56.4	2.36	.495	39.6	83.0	70.0	147
	2	63.1	56.5	1.63	.342	52.4	109.9	65.8	138
	3	60.0	56.4	1.82	.382	42.0	88.1	83.1	174
1231	1	67.6	55.9	1.66	.322	50.5	97.9	71.0	138
	2	62.8	50.9	2.65	.514	44.6	86.4	58.1	113
	3	60.5	51.1	2.85	.552	35.2	68.1	74.1	144
1233	1	54.8	47.8	1.79	.368	56.3	115.8	102.7	211
	2	56.9	47.8	2.33	.479	49.5	101.8	79.4	163
	3	55.0	48.7	2.86	.588	43.7	89.9	87.0	179
1236	1	53.0	50.1	2.16	.424	35.6	70.0	67.2	132
	2	57.7	50.3	2.69	.528	34.9	68.5	60.4	119
	3	54.7	50.1	1.98	.389	37.5	73.7	68.6	135
1245	1	52.5	49.5	3.10	.618	34.2	68.1	65.1	130
	2	50.3	49.7	2.98	.594	29.1	57.9	57.8	115
	3	50.0	49.1	2.68	.534	35.1	69.9	70.1	140
1252	1	46.0	43.1	2.30	.531	33.6	77.6	73.0	169
	2	52.5	42.7	2.24	.517	30.6	70.6	58.3	135
	3	55.0	43.3	2.17	.501	43.2	99.7	78.5	181
1253	1	56.6	48.0	2.37	.486	40.8	83.6	72.1	148
	2	60.0	48.2	2.65	.543	37.5	76.8	62.5	128
	3	64.3	48.8	3.91	.801	26.6	54.6	41.4	85
1254	1	58.0	45.0	2.68	.598	23.5	52.5	40.5	90
	2	63.6	45.0	2.43	.542	44.5	99.4	70.0	156
	3	62.7	45.2	2.01	.449	40.3	89.9	64.2	143

Table 17. Plasma glucose parameters listed by cow for each injection during the late stage

Cow	Inj. No.	Plasma Glucose (mg/dl)	Glucose Dose (g)	Clearance Rate (%/min)	Clearance Rate (%/min/ 100 kg)	Pool Size (g)	Pool Size (mg/kg)	Pool Space (l)	Pool Space (ml/kg)
1223	1	65.9	51.6	1.86	.358	49.5	95.4	75.1	145
	2	62.1	51.6	2.25	.434	49.3	94.9	79.3	153
	3	59.1	51.5	1.88	.362	45.6	87.9	77.2	149
1231	1	59.7	59.4	2.45	.405	48.5	80.1	73.2	121
	2	58.2	59.1	2.14	.354	42.6	70.4	79.0	131
	3	70.0	59.9	2.21	.365	55.3	91.4	82.9	137
1233	1	63.3	51.9	1.72	.329	61.1	116.9	96.6	185
	2	58.5	51.7	1.76	.337	58.2	111.3	99.5	190
	3	63.7	52.0	1.87	.358	44.4	84.9	67.9	130
1236	1	59.7	55.3	2.43	.436	38.1	68.4	63.8	115
	2	60.5	54.5	2.08	.373	40.6	72.9	67.1	121
	3	53.5	55.3	1.40	.251	43.9	78.7	82.0	147
1245	1	51.9	54.2	2.12	.384	43.4	78.6	83.6	151
	2	54.1	55.6	2.25	.408	46.8	84.8	86.5	157
	3	57.5	55.0	2.20	.399	50.0	90.5	86.9	157
1252	1	59.9	49.6	2.71	.535	39.0	77.0	65.1	128
	2	50.3	50.4	2.21	.436	34.5	68.1	68.7	135
	3	51.0	51.3	2.20	.434	35.3	69.7	69.3	137
1253	1	64.1	52.5	1.83	.344	49.6	93.3	77.4	146
	2	58.8	53.5	2.49	.468	37.6	70.6	63.9	120
	3	65.4	52.3	2.34	.440	53.5	100.6	80.5	151
1254	1	62.6	51.1	2.03	.399	42.2	82.9	67.4	132
	2	65.0	50.1	1.78	.350	44.4	87.3	68.3	134
	3	66.5	50.3	1.77	.348	46.8	91.9	70.4	138

Table 18. Plasma glucose parameters listed by cow for each injection during the dry period

Cow	Inj. No.	Plasma Glucose (mg/dl)	Glucose Dose (g)	Clearance Rate (%/min)	Clearance Rate (%/min/ 100 kg)	Pool Size (g)	Pool Size (mg/kg)	Pool Space (l)	Pool Space (ml/kg)
1223	1	61.8	59.0	1.48	.249	56.7	95.2	91.7	154
	2	64.0	60.0	1.48	.249	55.5	93.2	86.7	146
	3	59.3	59.1	1.64	.276	54.6	91.8	92.2	155
1231	1	63.9	66.4	2.24	.340	52.4	79.6	82.1	125
	2	65.2	64.8	1.24	.188	61.4	93.2	94.2	143
	3	63.6	65.0	1.86	.282	53.9	81.7	84.7	129
1233	1	59.2	59.2	1.46	.245	50.8	85.4	85.9	144
	2	61.0	59.0	1.54	.259	46.7	78.6	76.6	129
	3	62.9	58.7	1.46	.245	46.3	77.8	73.6	124
1236	1	58.3	58.6	1.26	.213	50.9	86.0	82.1	139
	2	59.7	58.1	1.06	.179	57.2	96.5	87.4	148
	3	52.8	59.0	1.03	.174	43.3	73.2	95.7	162
1245	1	60.1	62.9	2.14	.339	46.5	73.6	77.4	122
	2	59.9	62.2	2.13	.337	40.0	63.3	66.8	106
	3	56.7	62.9	2.26	.358	45.4	71.8	80.0	127
1252	1	61.9	57.1	1.56	.272	53.8	94.0	87.0	152
	2	57.3	56.7	1.44	.251	44.0	76.7	76.7	134
	3	60.3	57.0	1.54	.269	46.6	81.2	77.2	135
1253	1	65.3	53.4	2.27	.428	45.3	85.5	69.4	131
	2	71.3	53.1	2.50	.472	39.6	74.7	55.5	105
	3	69.4	52.9	2.64	.498	38.0	71.6	54.7	103
1254	1	71.8	54.7	1.77	.319	53.4	96.2	74.3	134
	2	66.2	54.8	1.25	.225	54.6	98.3	82.4	149
	3	62.9	55.0	1.69	.304	43.2	77.7	68.6	124

Table 19. Mean squares and tests of significance[1] of factors affecting plasma glucose and glucose clearance rate, pool size, and pool volume in first-lactation cows

Source	df	Plasma Glucose (mg/dl)	Clearance Rate (%/min)	Clearance Rate (%/min/100 kg)	Pool Size (g)	Pool Size (mg/kg)	Pool Space (liters)	Pool Space (ml/kg)
		MS[1]						
Stage	2	143.19*	3.14*	.274*	618.77*	93.92	761.55*	514.16
Cow	7	146.71*	.74*	.028*	174.65*	575.39*	417.99*	1420.15*
Stage x Cow	14	12.39	.16	.006	51.47	195.10	152.54*	578.85*
Error	48	10.87	.12	.004	32.32	124.79	76.37	295.67
Total	71							
Contrasts:								
Early vs Late	1	84.27	1.44*	.162*	530.47*	187.38	742.46	1.07
Early vs Dry	1	285.68*	6.28*	.547*	1193.31*	39.21	1427.14*	803.17
Late vs Dry	1	59.63	1.70*	.113*	132.53	55.15	104.81	733.78

[1]Stage x Cow mean square (MS) tested by Stage MS. Error MS tested Cow and Stage x Cow MS.

*P<.05.

Table 20. Plasma insulin parameters listed by cow for each injection during the early stage

Cow	Inj. No.	Plasma Insulin (ng/ml)	Peak Conc. (ng/ml)	Peak Increase[1] (ng/ml)	Peak Fold Increase[1]	Response Curve Area[1]	Clearance Rate (%/min)[2]
1223	1	0.50	4.84	4.34	8.62	65.8	6.81
	2	0.56	4.69	4.12	7.33	73.2	6.54
	3	1.16	4.48	3.32	2.85	82.4	7.32
1231	1	0.97	4.04	3.36	4.97	68.1	5.12
	2	0.68	5.09	4.12	4.27	78.8	5.87
	3	0.56	4.68	4.12	7.37	81.1	6.03
1233	1	0.89	4.28	3.40	3.84	61.3	6.55
	2	0.40	3.02	2.62	6.64	46.5	6.89
	3	0.65	3.97	3.32	5.14	74.0	6.74
1236	1	0.40	2.25	1.85	4.62	45.7	4.95
	2	0.86	3.48	2.62	3.03	63.2	5.54
	3	0.74	2.56	1.82	2.47	32.0	3.69
1245	1	0.36	5.00	4.65	13.02	85.3	6.28
	2	0.44	5.52	5.08	11.59	85.3	6.34
	3	0.46	4.70	4.23	9.14	100.7	7.02
1252	1	0.69	5.26	4.57	6.61	71.3	4.92
	2	0.54	4.09	3.54	6.52	61.2	5.40
	3	1.02	5.35	4.33	4.25	76.6	4.95
1253	1	1.31	4.90	3.59	2.75	72.8	4.49
	2	1.23	5.59	4.36	3.56	71.8	5.70
	3	0.98	5.69	4.71	4.83	72.8	4.78
1254	1	0.85	4.25	3.41	4.03	61.5	7.91
	2	1.44	2.40	0.96	0.67	20.2	1.74
	3	1.11	2.97	1.86	1.68	16.2	4.12

[1] above basal concentration.

[2] calculated from peak level to 40 min post-injection.

Table 21. Plasma insulin parameters listed by cow for each injection during the late stage

Cow	Inj. No.	Plasma Insulin (ng/ml)	Peak Conc. (ng/ml)	Peak Increase[1] (ng/ml)	Peak Fold Increase[1]	Response Curve[1] Area	Clearance Rate (%/min)[2]
1223	1	0.66	4.57	3.91	5.94	69.0	5.21
	2	0.77	3.96	3.18	4.12	57.8	4.87
	3	0.81	4.39	3.58	4.43	76.7	5.65
1231	1	0.75	4.66	3.92	5.23	77.5	5.52
	2	0.75	5.30	4.55	6.03	91.2	7.10
	3	1.53	5.55	4.02	2.62	79.0	4.74
1233	1	0.88	4.11	3.23	3.69	52.7	4.37
	2	0.81	4.21	3.40	4.22	70.0	5.37
	3	0.93	4.31	3.38	3.64	69.0	4.78
1236	1	0.68	1.57	0.90	1.33	24.0	1.29
	2	1.04	2.70	1.66	1.60	34.9	2.95
	3	0.85	2.62	1.77	2.10	43.2	1.94
1245	1	0.76	4.81	4.04	5.29	74.2	8.88
	2	0.70	6.00	5.30	7.61	112.0	5.55
	3	1.37	4.65	3.28	2.38	65.3	3.24
1252	1	0.51	5.46	4.95	9.64	80.9	8.02
	2	0.55	4.28	3.73	6.82	73.4	6.20
	3	0.95	5.68	4.73	4.97	119.9	4.55
1253	1	1.03	3.82	2.80	2.73	41.1	3.88
	2	1.59	7.19	5.60	3.52	131.2	4.18
	3	1.84	7.32	5.49	2.99	120.8	4.10
1254	1	0.91	1.96	1.06	1.17	18.1	1.29
	2	0.85	2.93	2.08	2.46	36.0	1.88
	3	1.84	7.32	5.49	2.99	121.0	4.10

[1] above basal concentration.

[2] calculated from peak level to 40 min post-injection.

Table 22. Plasma insulin parameters listed by cow for each injection during the dry period

Cow	Inj. No.	Plasma Insulin (ng/ml)	Peak Conc. (ng/ml)	Peak Increase[1] (ng/ml)	Peak Fold Increase[1]	Response Curve[1] Area	Clearance Rate (%/min)
1223	1	0.81	4.46	3.64	4.48	79.1	4.11
	2	0.74	4.77	4.03	5.44	73.7	2.93
	3	--	--	--	--	--	--
1231	1	2.14	6.13	3.99	1.86	71.8	2.67
	2	1.34	6.16	4.83	3.61	97.4	2.89
	3	1.19	6.41	5.22	4.41	125.4	2.74
1233	1	0.77	4.44	3.67	4.78	80.7	4.51
	2	0.66	3.94	3.28	4.99	74.6	2.75
	3	0.89	5.23	4.34	4.86	108.1	2.51
1236	1	1.55	4.24	2.96	2.31	63.8	2.81
	2	1.13	4.35	2.80	1.81	79.1	1.39
	3	1.28	3.08	1.95	1.73	63.5	1.17
1245	1	1.03	5.71	4.69	4.57	126.0	4.73
	2	0.55	3.86	3.31	6.00	73.2	2.99
	3	0.81	2.99	2.18	2.71	40.4	5.60
1252	1	--	--	--	--	--	--
	2	0.76	4.37	3.61	4.74	78.5	3.31
	3	0.73	4.07	3.33	4.55	83.5	2.35
1253	1	1.95	9.41	7.47	3.84	184.4	3.41
	2	2.75	8.98	6.23	2.26	152.3	3.25
	3	1.33	8.61	7.28	5.48	169.0	3.85
1254	1	1.85	5.13	3.29	1.78	76.3	2.31
	2	1.11	3.56	2.44	2.19	71.0	0.94
	3	2.85	7.61	4.76	1.67	125.2	1.88

[1]above basal concentration.

[2]calculated from peak level to 40 min post-injection.

Table 23. Mean squares and tests of significance[1] of factors affecting plasma insulin and insulin peak concentration, peak increase, peak fold increase, response curve area and clearance rate following glucose injection in first-lactation cows

Source	df	Plasma Insulin (ng/ml)	Peak Conc. (ng/ml)	Peak Increase[2] (ng/ml)	Peak Fold Increase[2]	Response Curve Area[2]	Clearance Rate (%/min)[3]
		MS[1]					
Stage	2	1.18*	5.58	1.70	18.24	4936.60*	440.71*
Cow	7	.96*	10.86*	8.57*	25.01*	3198.37*	9.29*
Stage x Cow	14	.14	2.01*	1.40	5.48*	983.18	1.66
Error	46	.13	1.05	.76	2.17	543.19	1.41
Total	69						
Contrasts:							
Early vs Late	1	.43	.82	.06	21.71	611.33	14.13*
Early vs Dry	1	2.33*	10.57	2.18	31.59	9245.14*	80.94*
Late vs Dry	1	.80	5.62	2.94	1.21	5207.76	28.63*

[1]Stage mean square (MS) tested by Stage x Cow MS. Error MS tested Cow and Stage x Cow MS.

[2]above basal concentration.

[3]calculated from peak level to 40 min post-injection.

*P<.05.

Table 24. Mean squares and tests of significance[1] of factors affecting milk yield and fat, protein and total solids content following glucose injection for early and late lactation data, combined

Source	df	Yield (kg)	Fat (%)	Fat (kg)	Protein (%)	Protein (kg)	Total Solids (%)	Total Solids (kg)
		MS[1]						
Stage	1	19.89	1.44	.092*	.27	.045*	.14	.31*
Cow	7	28.26*	1.88*	.011*	.59*	.019*	6.67*	.20*
Time	3	25.37*	1.13*	.002	.03	.018*	2.55*	.17*
Stage x Cow	7	4.49*	.53*	.006	.19*	.004*	.54*	.05*
Day(Time)	8	1.93*	.13	.002	.05	.005*	.07	.03*
Error	153	.73	.21	.003	.03	.001	.12	.01
Total	179							
Contrasts:								
-15 vs +9	1	.37	.41	.002	.00	.000	.00	.01
-3 vs +21	1	.77	.00	.001	.01	.000	.00	.00
-15 vs -3	1	39.49*	.82	.007	.05	.028	3.74*	.25
+9 vs +21	1	35.21*	2.35*	.000	.03	.026	3.91*	.24

[1]Stage mean square (MS) tested by Stage x Cow MS. Error MS tested Cow, Time, Stage x Cow, and Day(Time) MS.

*P<.05.

Table 25. Mean squares and tests of significance[1] of factors affecting milk yield and fat, protein and total solids content following acetate injection for early and late lactation data, combined

Source	df	Yield (kg)	Fat (%)	Fat (kg)	Protein (%)	Protein (kg)	Total Solids (%)	Total Solids (kg)
		MS[1]						
Stage	1	27.11	.94	.10*	.07	.037*	2.21*	.21
Cow	7	33.95*	4.32*	.03*	.61*	.027*	6.19*	.29*
Time	3	23.80*	.51	.01	.01	.020*	1.37*	.21*
Stage x Cow	7	8.90*	1.03*	.01*	.14*	.006*	.22	.10*
Day(Time)	8	2.65*	.43*	.00	.04	.003*	.06	.03*
Error	157	.75	.21	.88	.02	.001	.11	.01
Total	183							
Contrasts:								
-15 vs +9	1	.07	.11	.00	.01	.000	.53	.01
-3 vs +21	1	.01	.00	.00	.03	.000	.01	.00
-15 vs -3	1	37.86*	.48	.01	.00	.032*	.96*	.34*
+9 vs +21	1	33.53*	.97	.01	.01	.027	2.81*	.27

[1]Stage mean square (MS) tested by Stage x Cow MS. Error MS tested Cow, Time, Stage x Cow, and Day(Time) MS.

*P<.05.

Table 26. Plasma glucose parameters listed by cow, stage of lactation, and diet

Cow	Day	Diet	Plasma Glucose (mg/dl)	Glucose Dose (g)	Clearance Rate (%/min)	Clearance Rate (%/min/ 100 kg)	Pool Size (g)	Pool Size (mg/kg)	Pool Space (l)	Pool Space (ml/kg)
1187	60	C	63.3	66.1	2.71	.410	44.7	67.7	70.7	107
	90	C	68.2	66.1	2.65	.401	49.6	75.1	72.8	110
	120	C	65.7	69.8	2.38	.347	48.9	71.3	74.5	109
1211	60	C	63.3	62.2	2.97	.573	36.6	70.7	57.8	112
	90	C	64.8	52.3	2.50	.478	40.6	77.7	62.7	119
	120	C	70.7	52.2	2.51	.459	45.4	83.0	64.2	117
1230	60	C	69.7	57.5	1.89	.320	54.3	92.0	77.9	132
	90	C	68.2	56.0	2.70	.471	57.9	101.0	84.9	148
	120	C	66.7	58.9	2.70	.461	41.8	71.3	62.7	107
1239	60	C	64.1	48.0	2.44	.530	37.1	80.5	57.8	126
	90	C	65.5	50.3	2.31	.489	44.4	94.0	67.7	143
	120	C	70.2	51.4	2.52	.499	40.1	79.3	57.1	113
1106	60	C	70.0	49.8	1.90	.351	45.0	83.2	64.3	119
	90	HC	72.0	53.9	2.80	.519	44.0	81.6	61.1	113
	120	HC	83.2	54.7	1.69	.311	73.5	135.4	88.4	163
1178	60	C	65.9	52.4	1.74	.342	47.7	93.7	72.3	142
	90	HC	65.0	54.7	1.71	.316	45.7	84.6	70.4	130
	120	HC	68.1	52.2	1.97	.379	50.0	96.2	73.5	141
1191	60	C	63.6	64.1	1.60	.250	54.3	84.7	85.4	133
	90	HC	65.3	62.7	2.24	.369	52.4	86.4	80.3	132
	120	HC	65.3	62.2	2.40	.383	49.8	79.4	76.2	122
1222	60	C	58.9	52.7	2.14	.409	38.6	73.7	65.5	125
	90	HC	70.0	49.0	1.80	.365	44.9	91.0	64.1	130
	120	HC	66.1	52.6	2.71	.519	40.2	76.9	60.8	116
1229	60	C	64.5	60.3	1.90	.324	46.3	79.0	71.8	122
	90	HC	66.6	63.1	2.40	.383	50.6	80.7	76.0	121
	120	HC	65.2	60.8	2.74	.425	49.1	76.1	75.3	117

Table 27. Mean squares and tests of significance[1] of factors affecting plasma glucose and glucose clearance rate, pool size, and pool volume in lactating cows

Source	df	Plasma Glucose (mg/dl)	Clearance Rate (%/min)	Clearance Rate (%/min/100 kg)	Pool Size (g)	Pool Size (mg/kg)	Pool Space (liters)	Pool Space (ml/kg)
		MS[1]						
Diet	1	9.60	.24	.002	27.43	95.37	6.21	36.02
Stage	1	13.23	.01	.000	.94	8.23	9.68	174.91
Diet x Stage	1	.03	.02	.001	91.11	385.83	155.97	708.63*
Cow(Diet)	6	24.79	.10	.004	42.00	183.98	39.51	109.26
Covariate	1	59.53	.01	.005	122.09	249.27	315.69	348.81
Error	7	13.61	.17	.005	73.77	261.88	76.81	277.70
Total	17							

[1]Cow(Diet) mean square (MS) tested by Diet MS. Error MS tested Stage, Diet x Stage, Cow(Diet), and Covariate MS.

*P<.05.

Table 28. Insulin parameters listed by cow, stage of lactation and diet

Cow	Day	Diet	Plasma Insulin (ng/ml)	Peak Conc. (ng/ml)	Peak Increase[1] (ng/ml)	Peak Fold Increase[1]	Response Curve[1] Area	Clearance Rate (%/min)[2]
1187	60	C[3]	0.72	5.05	4.34	6.05	133.3	5.84
	90	C	1.05	6.65	5.60	5.33	89.8	4.75
	120	C	0.92	5.41	4.50	4.89	58.7	4.88
1211	60	C	0.71	4.99	4.28	6.01	51.6	7.03
	90	C	0.50	3.61	3.11	6.19	131.1	6.52
	120	C	0.80	5.92	5.12	6.39	56.1	4.45
1230	60	C	0.54	3.42	2.87	5.31	59.4	4.90
	90	C	0.65	3.51	3.06	4.71	51.8	4.73
	120	C	0.55	3.71	2.89	5.27	61.8	5.70
1239	60	C	0.49	3.43	3.02	6.20	68.6	6.45
	90	C	0.72	3.92	3.20	4.42	61.7	4.79
	120	C	1.14	4.49	3.35	2.95	70.0	4.86
1106	60	C[3]	0.64	3.28	2.65	4.16	65.8	3.18
	90	HC	0.90	4.03	3.13	3.50	76.3	4.17
	120	HC	1.21	4.61	3.40	2.81	27.7	4.49
1178	60	C	0.54	1.90	1.37	2.54	34.8	3.83
	90	HC	0.65	2.38	1.73	2.68	62.9	3.40
	120	HC	1.08	4.55	3.46	3.19	41.2	3.94
1191	60	C	1.34	3.89	2.55	1.90	59.3	4.48
	90	HC	0.75	4.12	3.38	4.52	62.4	4.15
	120	HC	0.73	4.55	3.82	5.25	34.6	3.85
1222	60	C	0.51	3.01	2.50	4.94	99.1	5.64
	90	HC	1.01	4.85	3.84	3.80	83.8	4.22
	120	HC	0.79	4.54	3.75	4.72	76.9	3.89
1229	60	C	0.54	3.89	3.35	6.18	82.0	4.53
	90	HC	0.80	4.12	3.33	4.17	74.2	4.52
	120	HC	0.89	4.54	3.65	4.13		4.87

[1] above basal concentration.

[2] calculated from peak level to 40 min post-injection.

[3] C = control; HC = high-concentrate.

Table 29. Mean squares and tests of significance[1] of factors affecting plasma insulin and insulin peak concentration, peak increase, peak fold increase, response curve area and clearance rate following glucose injection in lactating cows

Source	df	Plasma Insulin (ng/ml)	Peak Conc. (ng/ml)	Peak Increase[2] (ng/ml)	Peak Fold Increase[2]	Response Curve[2] Area	Clearance Rate (%/min)[3]
		MS[1]					
Diet	1	.05	.26	.37	3.57	25.43	1.61
Stage	1	.06	1.11	.64	.00	91.81	.01
Diet x Stage	1	.00	.11	.11	.37	3.03	.13
Cow(Diet)	6	.05	.58	.38	2.01*	767.58	.30
Covariate	1	.03	4.75*	4.65*	.00	1383.53	.01
Error	7	.04	.72	.51	.29	666.71	.41
Total	17						

[1]Diet mean square (MS) tested by Cow(Diet) MS. Error MS tested Stage Diet x Stage, Cow(Diet), and Covariate MS.

[2]above basal concentration.

[3]calculated from peak level to 40 min post-injection.

*P<.05.

The vita has been removed from the scanned document

GLUCOSE CLEARANCE STUDIES IN LACTATING COWS: INFLUENCE OF LACTATION STAGE AND RATION COMPOSITION

by

Melanie K. Gebhart

(ABSTRACT)

Glucose pool size (PS), pool space (PV) and clearance rate (CR) were determined for eight first-lactation Holstein cows at three lactation stages [early (E) = 75-100 days, late (L) = 205-250 days, and dry (D)]. A corn silage-based ration (15% CP, 22% ADF) was fed ad libitum during lactation and at maintenance during D, using 4-hr feeding intervals. Three intravenous glucose (.1 g/kg BW) injections were administered each period. Serial blood samples were collected for 40 min post-dosing. Parameters were calculated by the least squares method using natural logarithms of glucose concentration. Basal plasma glucose and insulin concentrations were 57.4, 60.1, and 62.3 mg/dl and .78, .97, and 1.24 ng/ml, respectively, for E, L, and D, respectively. PS and PV increased ($P<.05$) from E to D; however, when adjusted for body weight there was no difference across stages. CR decreased ($P<.05$) from E (2.43%/min) to D

(1.71%/min) and was correlated with dry matter intake (r = .70, P<.01). Glucose-induced insulin response was greater (P<.05) during D than during lactation due to higher peak (P<.05) and lower insulin clearance (P<.05) values.

Glucose parameters were determined by single injection in lactating cows fed a high-concentrate ration (16.8% CP, 13% ADF) (n=5) and a control ration (16.3% CP, 24.4% ADF) (n=4), at 90 and 120 days postpartum. No differences due to stage or diet were observed, due to insufficient observations. Plasma glucose and insulin tended to be higher with HC-feeding and at the later stage.