Gluconeogenesis in the liver of growing and lactating ruminants: The influence of ration composition and stage of lactation

by

Twila M. Kenna

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APPROVED:

Joseph H. Herbein, Chairman

Carl E. Polan

Mark L. Failla

Forrest M. Thye

William E. Vinson

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS ii

Chapter page

I. INTRODUCTION 1

II. LITERATURE REVIEW 3

GLUCONEOGENESIS 3
BIOCHEMISTRY 6
GLUCONEOGENIC PRECURSORS 13
Volatile Fatty Acids 13
Amino Acids 15
Pyruvate and Lactate 17
Glycerol 19
PROPIONATE METABOLISM 19
Propionate production 20
Propionate Absorption 21
Liver Metabolism 21
PHYSIOLOGICAL AND NUTRITIONAL INFLUENCES ON RUMINANT GLUCONEOGENESIS 23
Lactation and Pregnancy 23
Ration 27
Fiber 28
Ammonia 30
SUMMARY 32

III. EXPERIMENT 1 33

INTRODUCTION 33
MATERIALS AND METHODS 34
Animals and Diets 35
Samples and Incubation Procedure 35
Assays 37
RESULTS AND DISCUSSION 38

IV. EXPERIMENT 2 51

INTRODUCTION 51
MATERIALS AND METHODS 52
Animals and Diets 52
Samples and Incubation Procedure 55
Assays 57
RESULTS AND DISCUSSION 59

V. SUMMARY AND CONCLUSIONS 74

CALF AND COW COMPARISONS 74
SUBSTRATE CONCENTRATION EFFECTS IN INCUBATION MEDIA 83
SUMMARY 86

VI. EPILOGUE 90

REFERENCES 100

Appendix page

A. APPENDIX 1 — COW AND CALF DATA 116

B. APPENDIX 2 — SURFACE RESPONSE STUDIES 141

Methods and Materials 141
Assays 142
Results 142
Discussion 142

VITA 153

LIST OF TABLES

Table		page
1.	Body weight, liver weight and liver analysis - Experiment 1.	40
2.	Carbon dioxide production from propionate and lactate.	42
3.	Labelled glucose production from propionate and lactate.	43
4.	Ration composition and proximate analysis - Experiment 2.	54
5.	Milk yield and composition, body weight and feed intake.	61
6.	Rumen VFA, blood glucose and insulin, and liver protein.	63
7.	Carbon dioxide and glucose production on a protein basis.	66
8.	Carbon dioxide, glucose and carbon dioxide/glucose ratio.	72
9.	Carbon dioxide/Glucose ratios for Experiments 1 and 2.	76
10.	Labelled carbon dioxide production for Experiments 1 and 2.	77
11.	Labelled glucose production for Experiments 1 and 2.	78
12.	Glucose production and requirements for calves and cows.	84
13.	List of ruminant hormone studies.	97
14.	^{14}C-Carbon dioxide from [1-^{14}C]propionate - Experiment 1.	117
15.	^{14}C-Carbon dioxide from [2-^{14}C]propionate - Experiment 1.	118

16. ^{14}C-Carbon dioxide from [U-^{14}C]lactate - Experiment 1. 119

17. ^{14}C-Glucose from [1-^{14}C]propionate - Experiment 1. 120

18. ^{14}C-Glucose from [2-^{14}C]propionate - Experiment 1. 121

19. ^{14}C-Glucose from [U-^{14}C]lactate - Experiment 1. . 122

20. Total glucose - Experiment 1. 123

21. Calf body weight - Experiment 1. 124

22. Calf liver weight - Experiment 1. 125

23. Liver composition - dry matter - Experiment 1. . . 126

24. Liver composition - protein - Experiment 1. . . . 127

25. Liver composition - glycogen - Experiment 1. . . . 128

26. Liver composition - ether extract - Experiment 1. 129

27. Liver composition - ash - Experiment 1. 130

28. Rumen fluid, blood and transport data by %RDN - Experiment 1. 131

29. Rumen fluid, blood and transport data by physical form. 132

30. Ration composition and analysis - Experiment 1. . 133

31. ^{14}C-Carbon dioxide production - Experiment 2. . . 134

32. ^{14}C-Glucose production - Experiment 2. 135

33. Total glucose - Experiment 2. 136

34. Carbon dioxide, glucose and total glucose in dry cows. 137

35. Plasma glucose - Experiment 2. 138

36. Plasma insulin - Experiment 2. 139

37. Liver protein - Experiment 2. 140

38. Calf surface response equations. 145

39. Cow surface response equations. 146

LIST OF FIGURES

Figure		page
1.	Pathways of rumen carbohydrate fermentation.	8
2.	Major metabolic pathways in the liver and kidneys in ruminants.	9
3.	Glucose from [1-^{14}C]propionate by %RDN and physical form.	44
4.	Glucose from [2-^{14}C]propionate by %RDN and physical form.	45
5.	Glucose from [U-^{14}C]lactate by %RDN and physical form.	46
6.	Carbon dioxide production by stage of lactation and ration.	65
7.	Glucose production by stage of lactation and ration.	68
8.	Carbon dioxide/Glucose ratios by stage of lactation.	70
9.	Central composite design for three factors. . . .	144
10.	Effects of propionate concentration - Calves. . .	147
11.	Effects of glucose concentration - Calves.	148
12.	Effects of ammonium chloride concentration - Calves.	149
13.	Effects of propionate concentration - Cows. . . .	150
14.	Effects of glucose concentration - Cows.	151
15.	Effects of ammonium chloride concentration - Cows.	152

Chapter I

INTRODUCTION

Gluconeogenesis is an anabolic pathway that produces glucose. Glucose is an important metabolic substrate in all animals. In nonruminants it is the major source of energy. Although glucose is not a major energy supplier in ruminants, it is still a key metabolic intermediate. While maintenance, growth and production processes all require glucose, lactation places a high glucose demand on the ruminant. Gluconeogenesis enables the ruminant to meet these needs.

Unlike nonruminant carbohydrate digestion where glucose units are released and absorbed, ruminants obtain very little glucose from their diet. This is due to the activity of rumen microbes which degrade carbohydrates into volatile fatty acids. This absence of available glucose makes gluconeogenesis very important to the ruminant. While acetate, propionate and butyrate are the major volatile fatty acids produced, it is propionate that serves as the primary precursor for hepatic glucose synthesis.

Major precursors of ruminant gluconeogenesis include propionate, amino acids, lactate, pyruvate and glycerol. Glucose production is affected by the supply and/or availabil-

ity of these substrates as well as the physiological state of the animal. When lactating, the dairy cow has a large physiological demand for glucose for lactose synthesis. The ration fed regulates the substrates which will be present for metabolism. Since even when present, not all dietary substrates can necessarily be used, 'bioavailability' is important. Metabolic regulation is also affected by hormones. To increase the understanding of ruminant gluconeogenesis it is necessary to examine influences of such factors.

The objectives of these experiments were as follows: In Experiment 1, to determine the effects of a) ration physical form, b) ration rumen degradable nitrogen, and c) their interaction on in vitro calf liver propionate and lactate metabolism. In Experiment 2, to determine the effects of a) stage of lactation and b) ration fiber content on in vitro dairy cow liver propionate metabolism.

Chapter II

LITERATURE REVIEW

2.1 GLUCONEOGENESIS

Gluconeogenesis has long been recognized as an important part of nonruminant and ruminant metabolism. Many articles have reviewed in detail the concepts and significance of gluconeogenesis (16,30,31,99,100,175). This review of literature will briefly discuss basic points with emphasis on influences, both physiological and nutritional, that effect ruminant glucose production.

In the nonruminant complex carbohydrates are degraded into glucose units which are absorbed in the small intestine. Glucose is plentiful after feeding and gluconeogenesis is not needed. But in times of fasting and starvation or anaerobic conditions (i.e. exercise) gluconeogenesis is needed to provide glucose for energy.

Ruminant animals have the same gluconeogenic pathway as nonruminants with the same endproducts (86). Ruminant gluconeogenesis is active at all times, especially after meals (16). Dietary carbohydrates are digested in the ruminant by rumen microbes. The endproducts of the microbial fermentation are volatile fatty acids (VFA). The three major VFA are acetate, propionate and butyrate. The VFA produced

account for 80% of the energy requirements of the animal (33). The proportions of VFA that are produced change with the ration fed. Changing from a high roughage to a low roughage diet does not change the concentration of acetate in the rumen but the molar percent of propionate may double (2). Little interconversion occurs between VFA (33). The VFA are absorbed from the rumen, but reducing sugars are not (159).

Because of rumen fermentation, little glucose escapes the rumen (32). In work done with sheep, no evidence of glucose absorption into the blood was found (31,137) but in cows it is known that reducing sugar is continually being absorbed from the small intestine (159). Absorption from the digestive tract accounts for less than 10% of the animal's glucose requirement (32). This requires the ruminant to supplement the dietary glucose by gluconeogenesis and to conserve glucose when possible. One way the ruminant conserves glucose is by using acetate as its major energy source (40) instead of glucose. Acetate breakdown provides from 32-50% of the carbon dioxide while glucose only 4-23% (5,40), demonstrating the importance of acetate as a substrate for energy production via oxidation in the tricarboxylic acid (TCA) cycle. Another method of conservation is decreased glucose production when an exogenous glucose load

is supplied (5,18,55,143). In monogastrics, the decreased glucose production when exogenous glucose is increased is known to be caused by decreased activity of gluconeogenic enzymes (160).

Glucose turnover or utilization is increased greatly by lactation and pregnancy (31). The uptake of glucose is closely related to the output of lactose in milk (2). In the udder 50-85% of the glucose taken up is secreted as lactose (2,40) with only 10-20% oxidized (40). As much as 90% of the carbon in lactose is derived from glucose (40). Gluconeogenesis increases during pregnancy (152). The fetal uptake of glucose from the ewe accounts for 70% of the fetal requirement. Glucose recycling is high in the fetus (82).

The liver is the major site of gluconeogenesis in both ruminants and nonruminants. Hepatic production is responsible for 85% of the glucose turnover in ruminants (31,32) with kidneys providing 10-15% of the glucose (31). Unlike most other tissues in the body, hepatic use of glucose is minimal, e.g., in sheep 18% of hepatic production (31). The liver oxidizes glucose more rapidly than other tissues (18). In dry cows glucose oxidattion is greater than in lactating cows (18) demonstrating an adaptation occurring during lactation of increased mammary priority for glucose utilization. Using liver slices from beef animals, Atwal and Sauer

(9) demonstrated that the major limiting factor for hepatic gluconeogenesis was substrate concentration.

Lipogenesis and ketogenesis are metabolic pathways closely related to gluconeogenesis. Lipogenesis is synthesis of fatty acids and occurs primarily in the adipocytes. Hepatic fat synthesis is minimal being less than 1% of the total body production (105). The major substrates are acetate and lactate in ruminants (105,132,133) and glucose in nonruminants (76). Prior and Scott (133) suggested that increased availability of glucose or gluconeogenic precursors may be responsible for induction of lipogenesis in steers fed high concentrate diets. Ketogenesis is the production of ketone bodies which occurs in the gut and liver. Ketones are always present in the blood but during starvation or early lactation when blood sugar decreases an excessive produuction of ketone bodies occurs known as ketosis. During ketosis, hepatic ketogenesis increases (12). Both of these pathways involve and affect precursors for gluconeogenesis.

2.2 BIOCHEMISTRY

As previously mentioned, the metabolism of rumen microbes plays a major role in ruminant nutrition. The fermentation of carbohydrates by the microbes provides the ruminant with

VFA. In Figure 1 (86) the pathways leading to the formation of the principal products are summarized. From the intermediate pyruvate all of the volatile fatty acids are formed. Acetate, ethanol and butyrate are all produced from acetyl-CoA. Propionate can be formed via two methods. The pathway via lactate and acrylate is called the nonrandomizing or direct reductive pathway. The randomizing or dicarboxylic acid pathway involves oxaloacetate, malate, fumarate, succinate and methylmalonyl-CoA. This pathway is the major route for propionate synthesis as indicated by the high turnover of ruminal succinate (62).

The major metabolic pathways in the liver and kidneys in ruminants are summarized by Figure 2 (30). Glycogen stores in the body provide a readily accessible supply of glucose in times of immediate need. In fasted rats it has been shown that exogenous glucose will accelerate the formation of glycogen from glucose (via gluconeogenesis) and cause an increase in the enzyme glycogen synthetase (144). Ballard (15) showed that in sheep and cow liver the incorporation of glucose into glycogen is very low in relation to the activity in other animals such as the rat, mouse, guinea pig, rabbit, cat, dog and possum.

Glycolysis is the catabolism of glucose to pyruvate. The enzymes glucokinase, which is specific for glucose, and hex-

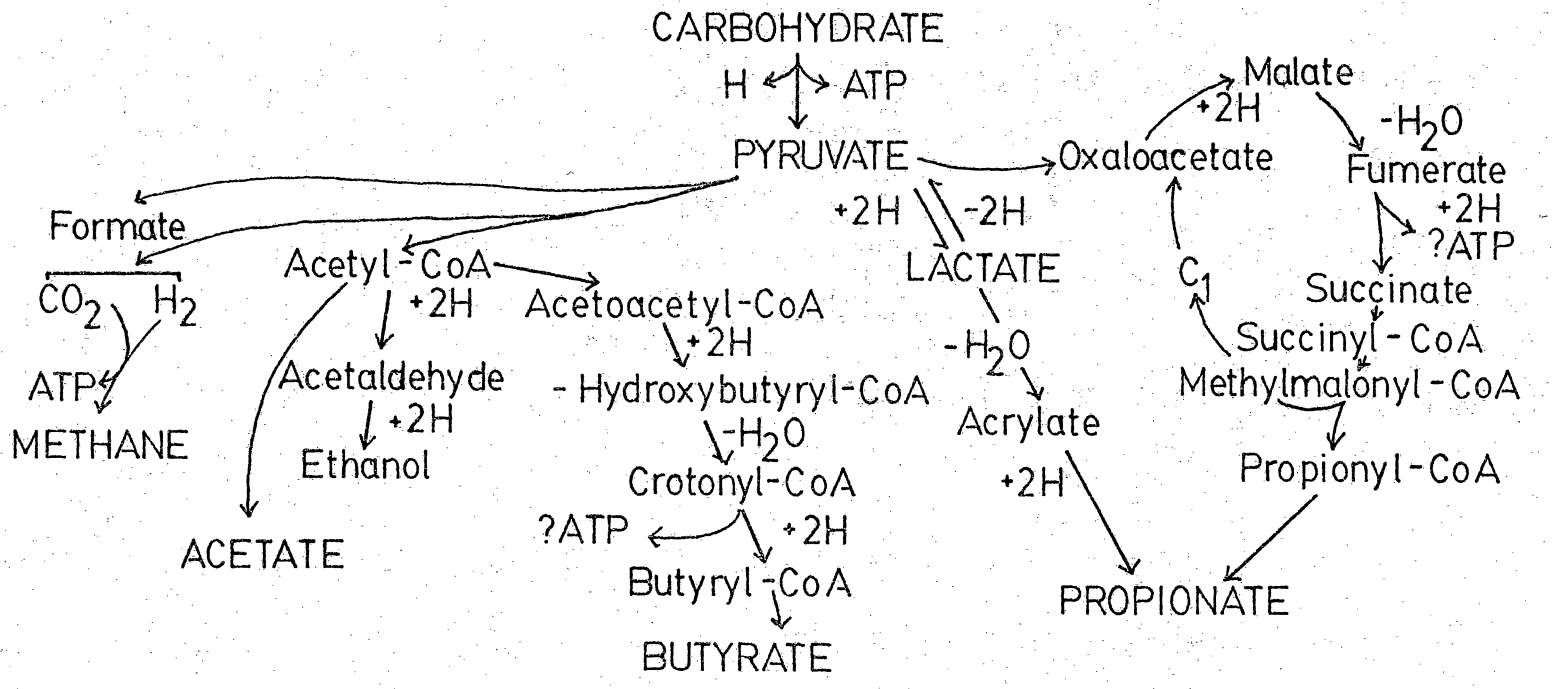

Figure 1. Pathways leading to the principal products of the carbohydrate fermentation in the rumen (Hungate, 86).

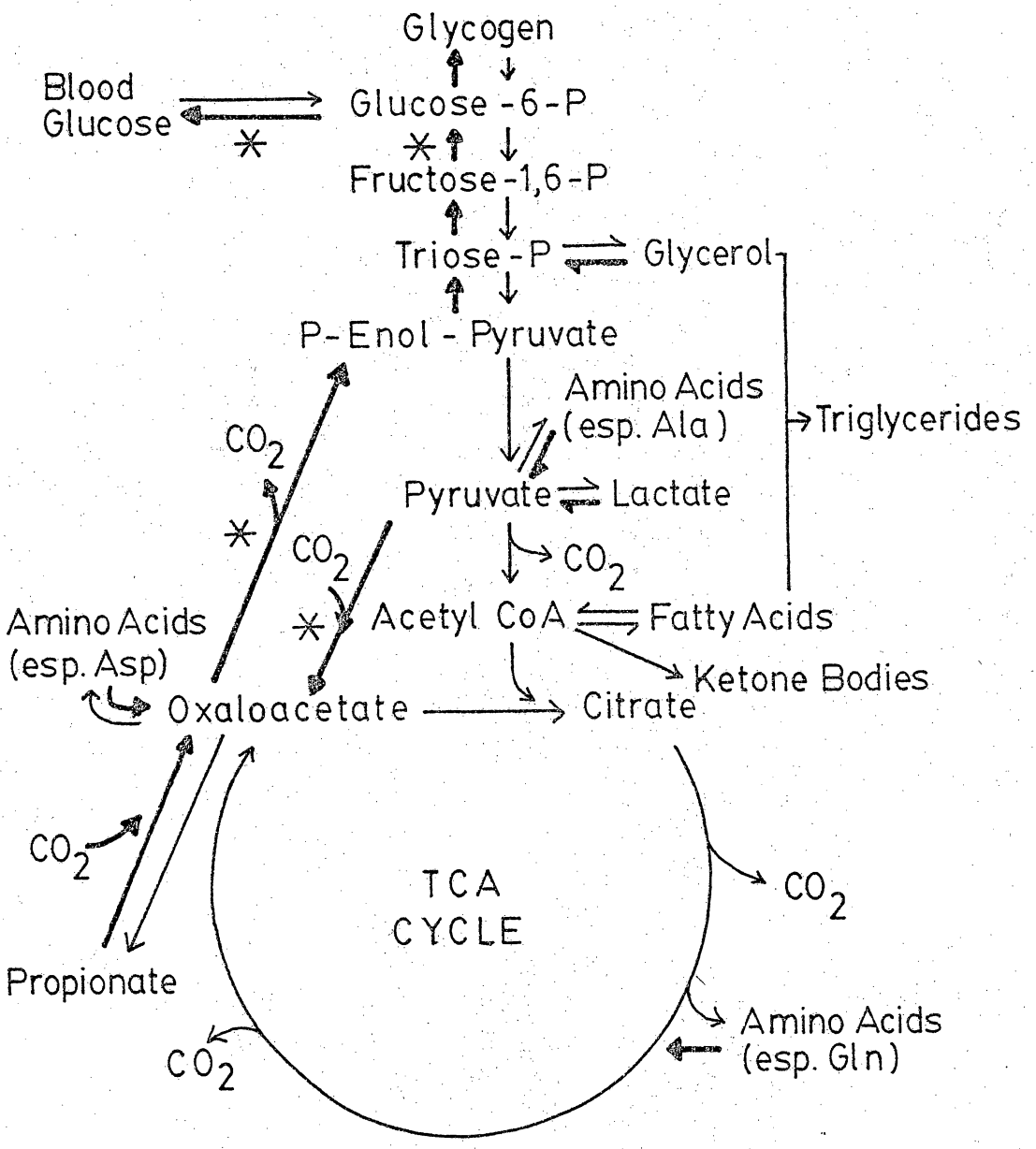

Figure 2. Major metabolic pathways in the liver and kidneys in ruminants. Pathways for gluconeogenesis are shown as heavy arrows and its 4 major pacemaker reactions are indicated by asterisks (Bergman, 30).

okinase, which will react with any hexose, cause the phosphorylation of hexoses. In ruminants no liver glucokinase has been found but hexokinase is present (15). This shows an adaptation of the animal to its normal 'low' glucose concentration and its ability to use other hexoses to provide intermediates via glycolysis. Glycerol is needed to form triglycerides for lipogenesis. Induction of lipogenesis in steers was found to be caused by glucose, propionate, lactate and acetate with maximal induction by glucose and minimal induction by acetate (133).

The TCA cycle is important in producing energy for the cell. Williamson and Cooper (169) have recently reviewed the regulation of the cycle in mammalian systems. Bartley et al. (19) found that glucose-6-phosphatase and phosphorylase activity in calves started to change between 8 and 16 weeks of age to levels of normal adult cows. They suggested that carbohydrate metabolism in young ruminants is similar to nonruminants before development of rumen function. Three enzymes related to the TCA cycle have been examined in both fetal and adult bovine liver (76). Using the citrate cleavage pathway both monogastrics and fetal ruminants can produce fatty acids from glucose. In the adult ruminant this pathway is not active as shown by the low activities of ATP-citrate lyase and NADP-malate dehydrogenase. However,

aspartate aminotransferase, has a higher activity in the adult ruminant (76) and demonstrates the importance of aspartate for transport of oxaloacetate from the mitochondria to the cytoplasm (aspartate shuttle).

Gluconeogenesis is a pathway with two special features: 1) there are wide variations in its activity determined by physiological situations; and, 2) the involvement of two directional enzymes (99). Several reviews (99,101) have discussed the carbon flow through gluconeogenesis and its relationship with other pathways. Gluconeogenesis from pyruvate involves the TCA cycle via formation of oxaloacetate by pyruvate carboxylase and then conversion of oxaloacetate to malate. When lactate is a precursor it is oxidized first to pyruvate, which is in turn carboxylated to oxaloacetate. Pyruvate gluconeogenesis is characterized by malate efflux from the mitochondria while lactate gluconeogenesis is facilitated by aspartate efflux (116).

Propionate enters the gluconeogenic pathway by a reaction involving the enzyme propionyl-CoA synthetase, which has been found to be higher in liver tissue than rumen epithelium (7). Once in the TCA cycle as succinate, propionate carbon can either stay in the cycle or leave as malate. The breakdown of propionate has been studied using ^{14}C-labelled tracer molecules. When [1-^{14}C]propionate is oxidized in the

TCA cycle all the ^{14}C is lost as ^{14}C-carbon dioxide during one turn of the cycle (i.e. succinate to succinate). If propionate is labelled in the second carbon no ^{14}C-carbon dioxide is produced by the first turn of the cycle but each additional turn causes the loss of 50% of the remaining radioactivity. Direct metabolism of propionate to glucose causes no loss of ^{14}C (103,170). Using these isotopes and measuring labelled carbon dioxide and glucose production from each enables one to follow the pathways involved in propionate metabolism.

As mentioned above these precursors can leave the TCA cycle and mitochondria as either malate or aspartate. In the cytoplasm oxaloacetate is again reformed in reactions involving the enzyme malate dehydrogenase (malate to oxaloacetate) or aspartate aminotransferase (aspartate to oxaloacetate). The conversion of oxaloacetate to phosphoenolpyruvate is regulated by the enzyme phosphoenolpyruvate carboxykinase (PEPCK). It has been shown in rat hepatocytes to be a rate limiting enzyme (138). Although this has not yet been proven for the ruminant it is known that PEPCK activity is high in the liver (176) and positively correlated with digestible dry matter intake as well as crude protein intake, milk production and body weight (50).

2.3 GLUCONEOGENIC PRECURSORS

Many compounds can be used as gluconeogenic precursors. Substrates of major importance include amino acids, pyruvate, lactate and glycerol. While all of these metabolites are used by both nonruminants and ruminants, propionate is of special importance to the ruminant. The utilization of these various precursors depends upon their concentration and availability as well as the presence of other substrates which may stimulate or inhibit their use.

2.3.1 Volatile Fatty Acids

Volatile fatty acids, the fermentation endproducts of the rumen microbes, have a central role in ruminant gluconeogenesis. Two of these, acetate and butyrate, do not yield net synthesis of glucose (3,43,102) but affect the metabolism of other compounds. Propionate, which directly affects gluconeogenesis and produces a net yield of glucose, can account for up to 65% of ruminant glucose synthesis (34). A more detailed discussion of propionate metabolism follows this section.

Acetate's major role in ruminant metabolism is energy production (40). The liver, the primary site of gluconeogenesis, removes only small amounts of acetate from the portal blood (36). In sheep hepatic utilization of acetate is low (3) and only one fifth of endogenous acetate can be

accounted for by the liver mostly by oxidation (36). Using liver slices from cows, Chung et al. (54) found the endproducts of acetate metabolism to include B-hydroxybutyrate, glutamine, citrate, and small amounts of glucose and lactate. Other workers (75), using both cow and sheep liver slices showed production of lipid from acetate. So while providing an energy source and a carbon source for the TCA cycle, acetate is not gluconeogenic itself but is involved in lipogenesis.

Butyrate, like acetate, is not gluconeogenic but it is metabolized by the rumen epithelium and liver. Between 80 and 100% is removed from the portal blood by the liver (36). Its metabolism by lactating cows is similar to that in other animals (43). In the study by Chung et al. (54) cow liver slices converted butyrate to B-hydroxybutyrate, glutamate and glutamine. The major effects of butyrate on gluconeogenesis are indirect. First, it affects gluconeogenic enzymes. In the rumen epithelium, butyrate inhibits propionyl-CoA synthetase (7). In perfused rat liver (53), the inhibition of this enzyme inhibits gluconeogenesis from propionate. Conversely, work by Leng and Annison (102) with sheep liver slices demonstrated that butyrate stimulated propionate conversion to glucose. This stimulation could mean that liver propionyl-CoA synthetase is not inhibited

and/or that butyrate is acting to spare propionate carbon for gluconeogenesis. This sparing action of butyrate, the second indirect effect, has also been shown in pyruvate metabolism. In an in vivo experiment (44) with lactating cows butyrate increased pyruvate conversion to oxaloacetate and decreased pyruvate oxidation. An in vitro experiment, Atwal and Sauer (9) showed a decrease in glucose production from pyruvate when butyrate was added to beef liver slice incubations. Butyrate is not gluconeogenic but does seem to affect enzymes involved and in some cases shows a sparing action on other compounds to increase their utilization.

2.3.2 Amino Acids

Most amino acids are glucogenic with the major exceptions being lysine and leucine (30). Amino acids are stored in muscle as protein and can be released for metabolism. When used for gluconeogenesis the nitrogen is removed to form urea which can be excreted or recycled in the ruminant animal. The carbon skeleton then enters the TCA cycle for utilization. Two of the major amino acids for transport of both nitrogen and carbon to the liver in the ruminant are alanine and glutamate which are also the principle amino acids taken up by the liver (30).

In the monogastric animal the importance of glucose production from amino acids has clearly been demonstrated. Exton and Park (67) showed that as much as 53% of the blood glucose could be produced from amino acids. Alanine alone can account for between 14-18% of the blood glucose (97). Alanine conversion to glucose shows the typical feedback inhibition by glucose (101) and increased rate of conversion when alanine concentration increases (146). Gluconeogenesis from alanine and aspartate has also been demonstrated using liver slices from pigs (58). Rate of glucose synthesis also varies with individual amino acids. In rats, aspartate, glutamate and alanine injections raised glucose levels faster than injections of serine, glutamine, glycine, or histidine (1).

In ruminant animals research on gluconeogenesis from amino acids has been more limited and less conclusive. Clark et al. (55) found increased gluconeogenesis when casein was infused postruminally. When comparing utilization of alanine for glucose production, the sheep fetus has been shown to be greater than that of the nonruminant fetus (131). An in vitro experiment Morton et al. (119) demonstrated glucose synthesis from amino acids by isolated sheep hepatocytes.

The actual importance of amino acids to gluconeogenesis in the ruminant is not definitely known. Estimates of the total glucose derived from amino acids range from a high of 30 to 50% (42), similar to that in rats, to a low of less than 2% (48). These values of course are dependent upon nutritional and physiological status of the animal. In lactating cows glutamate is known to contribute more to gluconeogenesis than to protein synthesis (61). A large amount of ^{14}C, 46 to 54% of the total found in milk constituents, from [U-^{14}C]glutamate injections was found in the milk lactose compared with 25 to 37% in casein (61). Calculations using data from Wolff and Bergman (30) showed that at least 11% and a maximum of 30% of the total glucose originated from alanine, glutamine, glutamate, asparagine, and aspartate. Glutamate alone accounted for 8% of the total glucose in lactating cows (61).

2.3.3 Pyruvate and Lactate

Pyruvate and lactate also are important gluconeogenic precursors. In rats between 5 and 7% of blood glucose is derived from pyruvate (67,97). In both in vivo (44) and in vitro (9) experiments with ruminants pyruvate has been shown to be gluconeogenic. During anaerobic metabolism lactate is formed from pyruvate. The recycling of this lactate to glu-

cose is called the Cori cycle. Glucose produced by this method does not yield a net synthesis of glucose. In ruminants, sources of lactate include the diet, rumen fermentation and endogenous production (pyruvate to lactate). Early work (103) suggested that large amounts of lactate were produced from propionate by the rumen mucosa but studies (166,176) since then have found limited (2 to 6%) conversion of propionate to lactate.

Lactate has been shown to be a glucogenic substrate in rats (1,67,97), pigs (58), chickens (45), adult and fetal sheep (4,131,134) and cows (13). Its importance as a gluconeogenic precursor depends upon its availability (134), as well as the availability of glucose and other glucose precursors (13). In the sheep fetus as much as 48.9% of the total glucose was derived from infused lactate (131). Adult sheep have shown lower utilization of lactate with only 14% of blood glucose being lactate derived (4). Higher utilization is found when a deficiency of other precursors occurs (13).

Lactate also is involved in lipogenesis. In sheep its utilization for fatty acid production is only 38% of the rate of acetate (130). Besides fatty acid synthesis lactate also can be used in glycerogenesis which is needed for triglyceride production (168). Finally excess lactate also can

indirectly affect fatty acid synthesis by stimulating the rate of acetate utilization (132). This means that lactate is a substrate for two opposing pathways and its fate depends upon other precursors availability.

2.3.4 Glycerol

In the rat gluconeogenesis from glycerol has been shown to supply between 10 and 30% of the total blood glucose (67,97). Atwal and Sauer (9) found that in vitro glucose production in beef liver slices from glycerol was only 78% of the rate from pyruvate. The effect of starvation was studied using sheep (35). In well fed sheep less than 5% of the total glucose produced was from glycerol. With fasting, glycerol turnover increased and the percent of glucose from glycerol increased to 40%. The reason for this increase with starvation is that adipose tissue is the major source of glycerol so fasting increases the concentration of glycerol available. In the lactating cow the importance of glycerol for gluconeogenesis will increase early in lactation during fat mobilization.

2.4 PROPIONATE METABOLISM

2.4.1 Propionate production

Propionate is produced in the rumen by bacteria (Figure 1). The amount and proportion of production depends upon composition and amount of the diet fed. When sheep were fed hourly either 500 g lucerne and wheaten hay or 800 g lucerne hay, propionate productions were 34 g/day and 62 g/day respectively (153). Leng et al. (103) fed sheep these same rations in twelve equal amounts hourly and found higher propionate production rates. This demonstrates that the effect of increased feed intake at a given feed interval is one of increased propionate production. In lactating cows the proportion of rumen propionate increases and rumen acetate and butyrate decreases compared to dry cows due to the decrease in ruminal pH caused by the increased feed intake (170). Changing the composition of the diet also affects production. The acetate:propionate ratio for cows on a control ration was 3:1 and changed to 1:1 when a high grain low fiber diet was fed (26). This change in ration increased rumen propionate concentration threefold. Both the work of Bauman et al. (26) and a study conducted by Yost et al. (174) shows that propionate production is a direct function of digestible energy intake.

2.4.2 Propionate Absorption

Propionate produced in the rumen is absorbed through the rumen wall into the portal blood. Weekes and Webster (166) infused propionate into the rumen of sheep and found between 86 and 91% absorbed. The absorption also increased when propionate concentration was increased. It was estimated by Leng et al. (103) that 70% of the glucose derived from propionate in the liver was from lactate formed from propionate in the rumen mucosa. Later workers (166,167) have found limited conversion of propionate to lactate. Using bull calves Weigand et al. (167) found only 2.3% of the propionate directly metabolized to lactate by the rumen mucosa. Lactate that was formed from propionate was derived first from glucose via the glycolytic pathway.

2.4.3 Liver Metabolism

In both sheep and cows the liver extensively removes 80 to 100% (36) and 80 to 85% (13) of the portal blood propionate, respectively. One of the major uses for propionate is gluconeogenesis (3,102,143). Other products have also been found by workers. Smith and Osbourne-White (148), using sheep liver homogenates, noted production of succinate, fumarate and malate, but no lactate. In a study by Chung et al. (54), isotopically labelled malate, aspartate and lac-

tate were produced from [1-^{14}C]propionate by cow liver slices. In both in vivo (160) and in vitro (143) experiments with cows, lipid derived from propionate increased with increased exogenous glucose. Prior and Scott (133) suggest that the increased availability of glucose or gluconeogenic precursors may be responsible for induction of lipogenesis.

Both propionate absorption through the rumen wall and glucose production from propinate are dependent upon propionate production (153). Yost et al. (174) found 60% of the absorbed propionate was metabolized into glucose in steers. In sheep 50% was converted into glucose and 25% oxidized into carbon dioxide (34). When expressed as a percent of total blood glucose Bergman et al. (34) found 65% to be propionate derived and ranging between 25 and 38% by Leng et al. (103) in sheep studies. Similar rates were found in cows with 32% in dry cows and 45% in lactating cows (170). These results and noted increases in percent lactose in milk when propionate infusions into the rumen were increased (68) demonstrate a response to the physiological stress of lactation.

2.5 PHYSIOLOGICAL AND NUTRITIONAL INFLUENCES ON RUMINANT GLUCONEOGENESIS

2.5.1 Lactation and Pregnancy

In a recent article Baird (11) reviewed carbohydrate status during lactation and pregnancy in ruminants. He defined three major factors which interplay to control ruminant physiology: 1) the priority of mammary gland and conceptus's demands, especially for glucose; 2) the limited voluntary feed intake that occurs in early lactation and late pregnancy that leads to an insufficient energy supply for maintenance and production; and, 3) the hormonal pattern which facilitates lipid mobilization. These three factors are responsible for most of the changes that occur metabolically during lactation and pregnancy. Lindsay (106) reviewed ruminant glucose metabolism during growth, pregnancy and lactation. Johnson and Vanjonack (92) also have emphasized the importance of ascertaining the effect of stress on hormonal patterns and interactions of environment. These articles point out the importance of determining how lactation and pregnancy affect the ruminant.

Lactation in the ruminant has a profound affect on glucose metabolism. The arterial concentration of blood glucose is higher in non-lactating than lactating cows, 59 and 49 mg % respectively (13). Glucose entry is almost doubled in lactating cows (170). These findings demonstrate the

higher glucose utilization during lactation. This increase in glucose demand occurs simultaneously with an increased output of glucose by the liver (13). Glucose is conserved by the liver in lactating cows. In dry cows glucose oxidation enzymes are saturated but not in lactating cows (13).

Nonmammary utilization of glucose does not change during lactation at least up to a milk production of 30 kg/day (48). This means that the increase in glucose requirements and metabolism is due to the mammary gland. In goats it was found that glucose uptake increased significantly between 2 and .5 days prepartum (59). This increase was caused by increased mammary extraction and not just increased blood flow. The increase in hepatic gluconeogenesis that occurs during lactogenesis is a demonstration of homeorhetic and homeostatic mechanisms regulating nutrient partitioning for milk production (25).

Level and stage of milk production also causes differences in glucose status. High producing cows have lower blood glucose, 65.7 mg %, when lactating and higher blood glucose, 75.3 mg %, when dry than low producing cows (79). In another study (90) with cows, at 45 days into lactation blood glucose was 60.9 mg% and rose to 72.8 mg % at 150 days postpartum. Early in lactation high producing cows have even lower blood glucose but no similar effect was found on low producing cows (79).

Glucose infusions have been used to try to increase glucose for lactose synthesis in an effort to increase milk production. In studies by Orskov et al. (127) and Clark et al. (55) glucose infusions did not affect production. Two possible reasons for this lack of response are 1) milk yield was not limited by glucose for lactose synthesis in the diets fed (127) and 2) the change in tolerance to exogenous glucose by lactating cows. The nonlactating cow responds to exogenous glucose in a manner similar to that in nonruminants, by increasing insulin secretion to effect an increase in peripheral utilization. The lactating cow, on the other hand, decreases hepatic glucose output to maintain glucose homeostasis (108).

Propionate and lactate metabolism also is affected by lactation. It was estimated by Mathias and Elliot (114) that 30-40% of the variation in the rate of propionate metabolism is caused by age and physiological status (i.e., pregnancy and lactation). The proportion of blood glucose derived from propionate increases in lactating cows (170) but work by Mathias and Elliot (114) with liver homogenates did not show an increase in propionate metabolism early in lactation. Other precursors must therefore be used to meet the lactational needs. Lactate is one precursor which does become more important during lactation. The flux of lactate

from the liver was found to change from net outflow in non-lactating cows to net utilization in lactating cows (11). While hepatic uptake increases, extraction also appears to be increasing. This may be caused by a larger liver (relative to body weight) in lactating cows (13).

The third factor Baird (11) mentioned was the hormonal pattern of the animal. Several reviews (77,147) have been written studying the correlations between hormones and metabolites. Insulin is a hormone which lowers blood glucose concentration and increases lipogenesis. The insulin concentrations in lactating cows were 14.3 and 19.7 microUnits/ml at 45 and 150 days postpartum respectively (90). Lactating cows and high producers have lower insulin concentration than dry and low producing cows, respectively (13,78). In agreement with Baird's suggestion (11) energy deficiency has been found to affect insulin concentration (79,90) as well as other hormones (147).

The influence of pregnancy has been studied most often with sheep. When pregnant, sheep exhibit increased gluconeogenesis (31,95) and a higher percent of ruminal propionate conversion to glucose (153). The increased demands of the fetus during the last two months of gestation have been found to be equal to that of producing 3-6 kg milk/day (25). The total needs of the pregnant animal are lower than those

of the lactating animal but by the end of pregnancy are 75% greater than those of the nonpregnant animal of the same size (25).

2.5.2 Ration

As already mentioned the ration fed plays a major role in ruminant metabolism. In his article (11) Baird describes carbohydrate insufficiency as playing a principal part in the effects of lactation and pregnancy. Feed intake then is important. When sheep were starved blood glucose was found to drop from 78.2 to 62.3 mg % (129) due to lack of substrate. But in fasting rats, the increased hepatic mobilization and oxidation of fatty acids leads to increased carbon dioxide fixation which could promote gluconeogenesis (115). The interaction between production in ruminants and their feed intake must be considered. Dairy cows in early lactation and pregnant sheep in late gestation, often with two fetuses, have the lowest intake as well as the highest need for glucose and, therefore, gluconeogenic precursors. In these circumstances animals are very susceptible to certain metabolic disorders. Ketosis which is an overproduction of ketone bodies, is often spontaneous in dairy cows during early lactation when a carbohydrate insufficiency and lipid mobilization occur. Pregnant sheep are subject to

pregnancy toxaemia that, like ketosis, is caused by an energy deficiency and affects animals under stress (11).

2.5.2.1 Fiber

Most of the research in the area of rations has dealt with low fiber or high concentrate diets. Normal feeding practices in dairy cows involves the increase in the proportion of concentrate fed a cow during lactation. One of the early effects of this type of high concentrate, low fiber ration (HCLF) is milk fat depression from a normal value, 3.5%, to a low of 2.5 or 1.9% (2,26,29,157,164). Recently more interest in the metabolical changes due to this kind of ration has been taken.

Blood glucose increases when a HCLF ration is fed (2,64,65,66,87,88,90). Increases in pool size, entry rate and total glucose absorbed also occur (2,64,66,87). An interaction with lactation was found by Jenny et al. (90) when no increase in plasma glucose occurred in cows in early lactation and negative energy balance. In the mammary gland of cows fed a 100% concentrate ration glucose oxidation and lipogenesis decreased (14). Gomila et al. (74) studied the effect of feeding schedule and found no concise pattern in serum glucose.

One possible reason for the effects caused by HCLR rations could be changes in enzymes and their activities. But Young et al. (176) found that the enzymes PEPCK, malic enzyme, citrate cleavage enzyme and glucose-6-phosphate dehydrogenase were not easily influenced by dietary changes. Hormones are another possible explanation. Cows fed a control ration had a plasma insulin concentration of 20.6 microUnits/ml which increased to 25 microUnits/ml when fed a restricted roughage ration (164). Other studies with cows and sheep found similar results (65,88,90). As with plasma glucose, insulin concentration did not increase in cows fed a HCLF ration until midlactation in the experiment by Jenny et al. (90). This also was thought to be caused by the large energy demand of early lactation. Information on other hormones is lacking.

Changing ration composition also changes the fermentation pattern in the rumen. The proportion of rumen propionate increases in sheep, cows and lambs when a HCLR ration is fed (26,60,65,157). Increasing roughage in the ration has the opposite effect, primarily by affecting the proportion of acetate produced (65,157). The acetate to propionate ratio in the rumen when feeding a ration of 60:40 roughage:concentrate is 3:1. This decrease to approximately 1:1 when feeding a HCLR (greater than 60% concentrate) (26).

Lactate production also increases when a HCLR ration is fed to sheep (130). Another effect is an increase in absorption of lactate from the portal blood (87).

2.5.2.2 Ammonia

Protein metabolism in the ruminant has received greater attention in recent years. Techniques have been designed to provide protein or amino acids to the small intestine of the ruminant. These methods are commonly referred to as 'by-passing' the rumen. In the rumen, microbes will digest proteins and amino acids (to different extents depending on source and processing of protein) and use the endproducts to synthesize their own proteins. one of these endproducts is ammonia. Ammonia is important for recycling nitrogen back to the rumen as urea in the ruminant. It is also known to play a role in intermediary metabolism in monogastrics. Gluconeogenesis appears to be either suppressed or stimulated by ammonia depending upon the precursor used. In isolated rat hepatocytes ammonium chloride has suppressed glucose production from pyruvate (116,178) and malate (172). Zahlten et al. (178) demonstrated that glucose production from pyruvate and malate involved the malate shuttle by using n-butylmalonate to inhibit malate transport from mitochondria. Lactate gluconeogenesis in rat hepatocytes has

been stimulated (178) and inhibited (116) by ammonium chloride. The stimulation is thought to be caused by increased transamination of oxaloacetate to aspartate. The reducing equivalents from lactate oxidation and ammonia increase glutamate synthesis for transamination. Aspartate transport out of the mitochondria is by aspartate shuttle. Competition for oxaloacetate between transamination and aspartate shuttle versus malate dehydrogenase and malate shuttle accounts for varied effects of ammonium chloride depending upon substrates involved.

In ruminants contradicting results have been obtained. Feeding urea in the diet to increase ruminant ammonia concentration caused a decrease in glucose concentration because of a reduction in gluconeogenesis (104). Infusions of ammonium chloride in sheep (17,70) has demonstrated slight increases in plasma glucose. But in in vitro studies with steer liver slices (151) and sheep hepatocytes (165) propionate gluconeogenesis was inhibited by ammonium chloride. These results would agree with those in rats since propionate metabolism is similar to pyruvate and malate in that it requires the malate shuttle. If ruminant lactate metabolism follows the same pattern as that in the rat, ammonia should increase gluconeogenesis from lactate.

2.6 SUMMARY

The purpose of this literature review has been to describe ruminant gluconeogenesis and point out the important precursors and the influence of physiological and nutritional factors. Propionate plays a major role in the gluconeogenic process by providing carbon to synthesize more than half of the ruminant's glucose requirement. Lactation and pregnancy affect both glucose requirements and the animal's ability to provide necessary substrates (i.e. decreased feed intake). Ration fiber and nitrogen degradability or ammonia-producing ability are known to be important to animal production which is related to their effects on intermediary metabolism. The interrelationships between these two influences are complex but through in depth study can become useful tools to regulate potential productivity.

Chapter III

EXPERIMENT 1

3.1 INTRODUCTION

In recent years, ruminant protein needs have been defined by metabolizable protein (49), soluble protein (171), bypass protein (51) and limiting amino acids (135,142). All of these methods try to account for the action of the rumen upon the protein. Another protein term, which describes the fraction including nonprotein nitrogen and microbially degraded protein, is rumen degradable nitrogen (RDN). This is determined using the in situ polyester bag technique and estimates rate of protein degradation (122).

Fiber content of rations has been connected with milk fat depression (26,157), fat cow syndrome (126) and altered rumen fermentation (26,157). Increasing grain content or lowering ration fiber has been shown to increase the proportion of propionate in the rumen (26,157). Ration fiber form also influences ruminant metabolism (117). Work by Nocek et al. (122) showed a definite interaction of physical form with nitrogen degradation. Forage-containing rations have a less rapid initial rate of nitrogen degradation regardless of RDN content.

Another aspect which has not been as well studied is the effect of these ration components on intermediary metabolism. The degradability of nitrogenous compounds in the rumen affects blood ammonia concentration, which in turn affects several biochemical pathways (i.e. urea cycle, gluconeogenesis, etc.). Volatile fatty acids (VFA) are the major energy sources for the ruminant. Both lipid and gluose synthesis require VFA as substrates (16,75). Propionate is the major gluconeogenic precursor in the ruminant and can account for up to 60% of the glucose produced (174). Infusion of neither acetate nor butyrate results in net synthesis of carbohydrate (3), while lactate is an efficient glucgenic precursor (4,87,131).

The metabolic fate of gluconeogenic precursors has not been well examined under both varying RDN levels and form of fiber in the ration. The objectives of this study were to determine the effects of (a) ration fiber content and physical form, (b) ration rumen degradable nitrogen and (c) potential interactions between the two on in vitro calf liver propionate and lactate metabolism.

3.2 MATERIALS AND METHODS

3.2.1 Animals and Diets

Thirty-six Holstein bull calves which were fed experimental rations from 8 to 20 weeks of age were used. Animals were assigned to a 3x3 factorial arrangement with physical forms of rations of chopped hay (CH), ground hay (GR) and all concentrate (CONC) and rumen degradable nitrogen (RDN) of 30, 45, and 60%. Ration compositions were described by Nocek et al. (123). All rations were formulated to contain 13% crude protein.

Orchardgrass hay length ranged from .2 to 2 cm in the GR ration and 6 to 10 cm in the CH ration. Ration RDN was estimated from individual feed ingredient RDN determined by Nocek et al. (122). To validate the varying levels of RDN, rumen ammonia nitrogen was determined. Rumen fluid samples were taken 2 hours postprandial at 8, 11, 14, 17 and 20 weeks of age and assayed using the Conway procedure (57). Rumen ammonia nitrogen for 30, 45 and 60% RDN rations were 3.7±.2, 7.0±.3 and 11.5±.4 mg/100 ml respectively.

3.2.2 Samples and Incubation Procedure

At 20 weeks of age animals were slaughtered and liver samples obtained. One sample was frozen immediately in liquid nitrogen for composition analysis. Another sample was place in ice-cold saline (.9% NaCl) for transport to the

laboratory. From a tissue core (14 mm diameter), slices weighing between 75 and 125 mg were cut using a Stadie-Riggs microtome. Slices were kept in cold calcium free Krebs-Ringer bicarbonate buffer until blotted, weighed, and placed in 25 ml side arm incubation flask containing 3 ml of incubation media. Flasks were gassed with oxygen:carbon dioxide (95:5). Triplicate incubations were carried out for one hour at 37C with an oscillation rate of 90 strokes/min.

Incubation media (pH 7.4) consisted of Krebs-Ringer bicarbonate buffer containing; 15 mM sodium acetate, 10 mM sodium propionate, 2 mM sodium pyruvate, 5 mM n-butyric acid, 20 mM L-lactic acid, 2.5 mM D-glucose and 5 mM ammonium chloride. The radioactive tracers (.5microcurie/flask) used were [1-^{14}C]sodium propionate, [2-^{14}C]sodium propionate or [U-^{14}C]sodium lactate.[1]

Scintanalyzed phenethylamine (.2 ml), injected through the top stopper into the center well one minute prior to incubation termination, was used to collect carbon dioxide. Incubations were halted by acidification of the media by injection of 1N HCl (.5 ml) via the side arm. After one hour to allow for carbon dioxide collection, centerwells were placed in scintillation vials with .2 ml of deionized water and 4 ml of RIA FLUOR[2] scintillation fluid.

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[1]New England Nuclear, Boston, MA.

Radioactivity was measured by liquid scintillation counting with a Delta 300 Model 6890.[3] The internal standardization method was used to determine counting efficiency (82%) and to account for quench. Liver slices and media were transferred to separate test tubes and frozen until analyzed.

3.2.3 Assays

Glucose was separated from incubation media (neutralized with .28 ml of 1N KOH) by anion exchange columns (8x30 mm) of Dowex 1 (200-400 x8 mesh; formate form). After application of 1 ml of incubation media to the column, glucose was eluted with 5 ml of deionized water. The column was recharged by rinsing with 15 ml of 1M ammonium formate (pH 7.0). Eluates were lyophilized then dissolved in 1.5 ml deionized water. Glucose was assayed using enzymatic-colorimetric determination with glucose oxidase and peroxidase as described in Sigma Technical Bulletin No. 510 (1973). Labelled glucose was determined, as previously described, by measuring radioactivity in .4 ml of concentrated eluates. Radioactivity in carbon dioxide and glucose derived from [U-^{14}C]lactate was divided by three to account for the three ^{14}C per lactate molecule.

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[2]New England Nuclear, Boston, MA.

[3]Searle and Co., Des Plaines, IL.

Samples frozen (in liquid nitrogen) at slaughter were used to determine liver composition. Dry matter, protein, fat and ash were determined by proximate analysis (8) of homogenized liver tissue (tissue:water, 1:1). Using a modification of the procedure of Lo et al. (107), glycogen content was determined for liver obtained at slaughter and liver slices frozen after incubation.

Analysis of variance was performed using RDN and physical form as main effects. When significant main effects were found, by F test at $P<.05$, differences between means were tested for significance at $P<.05$ using Duncan's New Multiple Range Test (154).

3.3 RESULTS AND DISCUSSION

Growth parameters (Table 1) of the calves, metabolic body weight (MBW,$kg^{\bullet 75}$) and liver weight were not affected by RDN treatments, but were affected by ration physical form. Metabolic body weight and liver weight were greatest in CONC-fed calves. GR-fed calves achieved body weights similar to CONC-fed calves, but had higher blood glucose levels and significantly smaller livers. Calves fed CH rations consumed consistently less feed, had lower daily gains and a feed efficiency of 4.0 kg intake/kg gain compared to 3.8 and 3.1 for GR- and CONC-fed calves, respectively (121). Growth

of the CH-fed calves may have been limited by their ability to consume adequate amounts of the coarse ration. Neither dry matter nor ash content of liver tissue was affected by either physical form or RDN. An inverse relationship between liver protein and liver fat content was found for both physical form and RDN. Within physical form, protein content was high and fat content low in livers of CH-fed calves. Livers of calves fed 30% RDN rations had lowest protein and highest fat content. Liver glycogen was low in GR- and CH-fed calves, but liver protein high.

Propionate and lactate metabolism to carbon dioxide (Table 2) were unaffected by RDN. Physical form affected ($P<.05$) production from [1-^{14}C]propionate and [U-^{14}C]lactate. Agreeing with other workers (102) metabolism of [1-^{14}C]propionate into carbon dioxide was greater than from [2-^{14}C]propionate in liver slices. Labelled carbon in [1-^{14}C]propionate is lost as carbon dioxide with one turn of the TCA cycle but not until the second or later cycling with [2-^{14}C]propionate. When [2-^{14}C]propionate is converted directly to glucose, no labelled carbon dioxide is produced (103,170). Thus, [2-^{14}C]propionate metabolism reflects both TCA cycle activity and gluconeogenic rate. Also, the amount of labelled carbon dioxide derived from [1-^{14}C]propionate versus [2-^{14}C]propionate indicates the predominance of pro-

Table 1. Effect of ration physical form and RDN on metabolizable body weight (MBW), liver weight, blood glucose and liver composition[1].

Ration	MBW	Liver Wt	Blood Glucose	Liver				
				DM	Ash	Protein	Fat	Glycogen
	kg	kg	mg/dl	%		% DM		mg/g DM
Physical Form								
CH	33.3 ± .8^{b}	1.56 ± .07^{c}	72.2 ± 2.3^{b}	26.5 ± .3	5.11 ± .18	65.25 ± .87^{a}	2.44 ± .12^{b}	114.2 ± 10.4^{b}
GR	37.8 ± 1.3^{a}	1.97 ± .10^{b}	83.1 ± 2.4^{a}	27.2 ± .2	5.60 ± .23	67.50 ± 1.19^{a}	3.03 ± .23^{a}	109.6 ± 11.6^{b}
CONC	39.4 ± 1.4^{a}	2.59 ± .14^{a}	82.3 ± 2.6^{a}	26.9 ± .2	5.50 ± .13	58.25 ± 1.56^{b}	2.71 ± .13^{ab}	212.9 ± 14.2^{a}
% RDN								
30	37.5 ± 1.8	2.07 ± .21	81.5 ± 2.7	26.7 ± .2	5.35 ± .19	61.39 ± 2.04^{b}	3.04 ± .21^{a}	149.5 ± 18.9
45	35.9 ± 1.1	2.05 ± .14	78.0 ± 2.7	26.9 ± .3	5.18 ± .14	63.02 ± 1.73^{ab}	2.48 ± .14^{b}	151.7 ± 23.4
60	37.2 ± 1.2	2.00 ± .13	78.1 ± 2.4	27.0 ± .3	5.66 ± .21	65.76 ± 1.30^{a}	2.66 ± .14^{ab}	142.0 ± 16.2

[1]Mean ± SEM of twelve calves.

[abc]Column means with different superscripts within Physical Form or RDN are significantly different ($P < 0.05$).

pionate carbon flow through the gluconeogenic pathway and emphasizes the ability of ruminant liver to remove propionate derived from rumen fermentation (13).

Glucose production from propionate and lactate (Table 3) was greatest for CONC- and 60% RDN-fed calves, although significant ($P<.05$) only from lactate within physical form. One reason for the lack of significance is the interaction between RDN and physical from (Figure 3,4,5). In production from both [1-^{14}C]propionate and [2-^{14}C]propionate (Figure 3 and 4) interaction is shown by the decrease in CH-fed animals at 30% RDN. Interaction in lactate metabolism (Figure 5) was expressed by a large increase in CONC-fed animals at 45 and 60% RDN while CH-and GR-fed calves showed little variation across RDN levels.

The interaction between physical form and RDN could be caused by a variety of factors. Changes in physical form can affect feed intake and rate of passage. Degradability of protein affects rumen nitrogen metabolism and amount of rumen by-pass protein. To formulate specific ration RDN a variety of feedstuffs were used. Therefore, major protein sources and amino acid composition were not the same across rations. When all of these variables are combined many reasons for interactions are possible. The above can result in different amounts of essential and nonessential amino acids

Table 2. Effect of ration physical form and RDN on labelled CO_2 production[1,2].

Ration	Propionate		Lactate
	1-^{14}C	2-^{14}C	U-^{14}C
Physical Form			
CH	527 ± 27^{b}	49 ± 5	31 ± 2^{b}
GR	508 ± 36^{b}	79 ± 18	37 ± 4^{ab}
CONC	641 ± 46^{a}	61 ± 6	49 ± 7^{a}
% RDN			
30	541 ± 39	61 ± 11	38 ± 5
45	581 ± 41	49 ± 4	39 ± 6
60	544 ± 43	77 ± 14	39 ± 4

[1] μmoles substrate metabolized to CO_2/ liver X kg MBW.

[2] Mean ± SEM of 12 calves with 3 samples/calf.

[ab] Column means with different superscripts within Physical Form or RDN are significantly different ($P<0.05$).

Table 3. Effect of ration physical form and RDN on labelled glucose production from propionate and lactate[1,2].

Ration	Propionate		Lactate
	1-^{14}C	2-^{14}C	U-^{14}C
Physical Form			
CH	231 ± 37	383 ± 71	41 ± 5[b]
GR	200 ± 36	322 ± 52	44 ± 5[b]
CONC	265 ± 27	497 ± 45	70 ± 8[a]
% RDN			
30	232 ± 32	418 ± 50	44 ± 5
45	210 ± 32	338 ± 70	50 ± 7
60	253 ± 42	441 ± 60	58 ± 9

[1] μmoles substrate metabolized to glucose/liver X kg MBW.

[2] Mean ± SEM of 12 calves with 3 samples/calf.

[ab] Column means with different superscripts within Physical Form or RDN are significantly different ($P<0.05$).

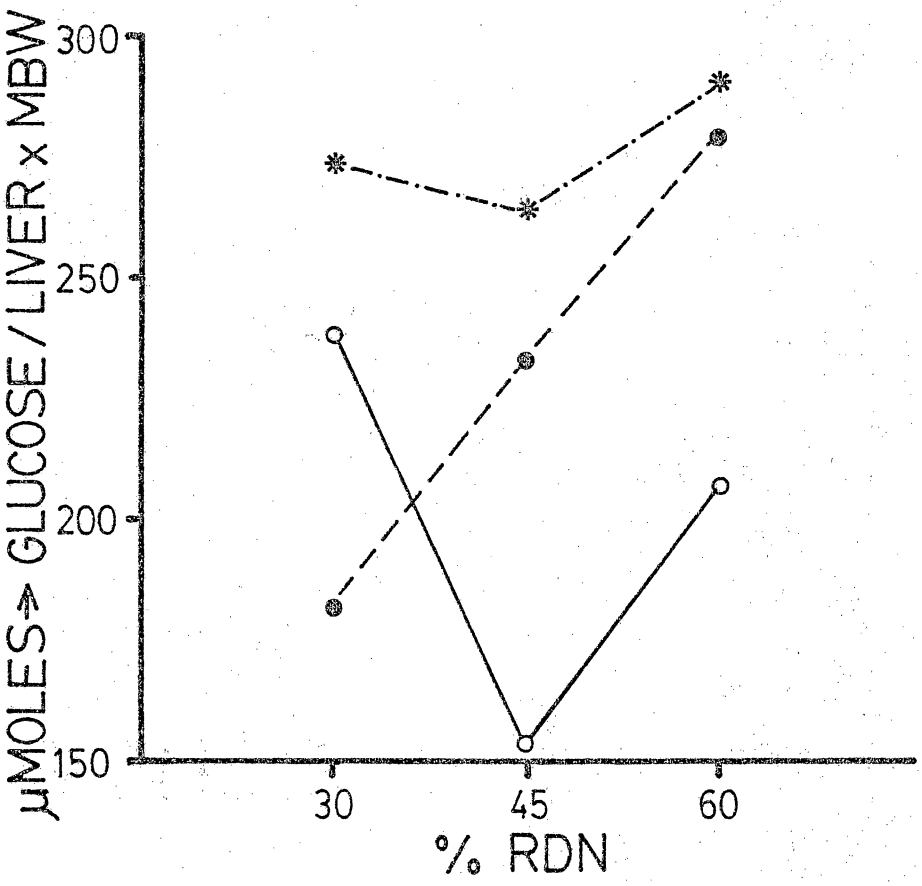

Figure 3. Effect of ration physical form and rumen degradable nitrogen (% RDN) on labelled glucose production from [1-^{14}C] propionate. (ground ——, chopped ---, concentrate —·—)

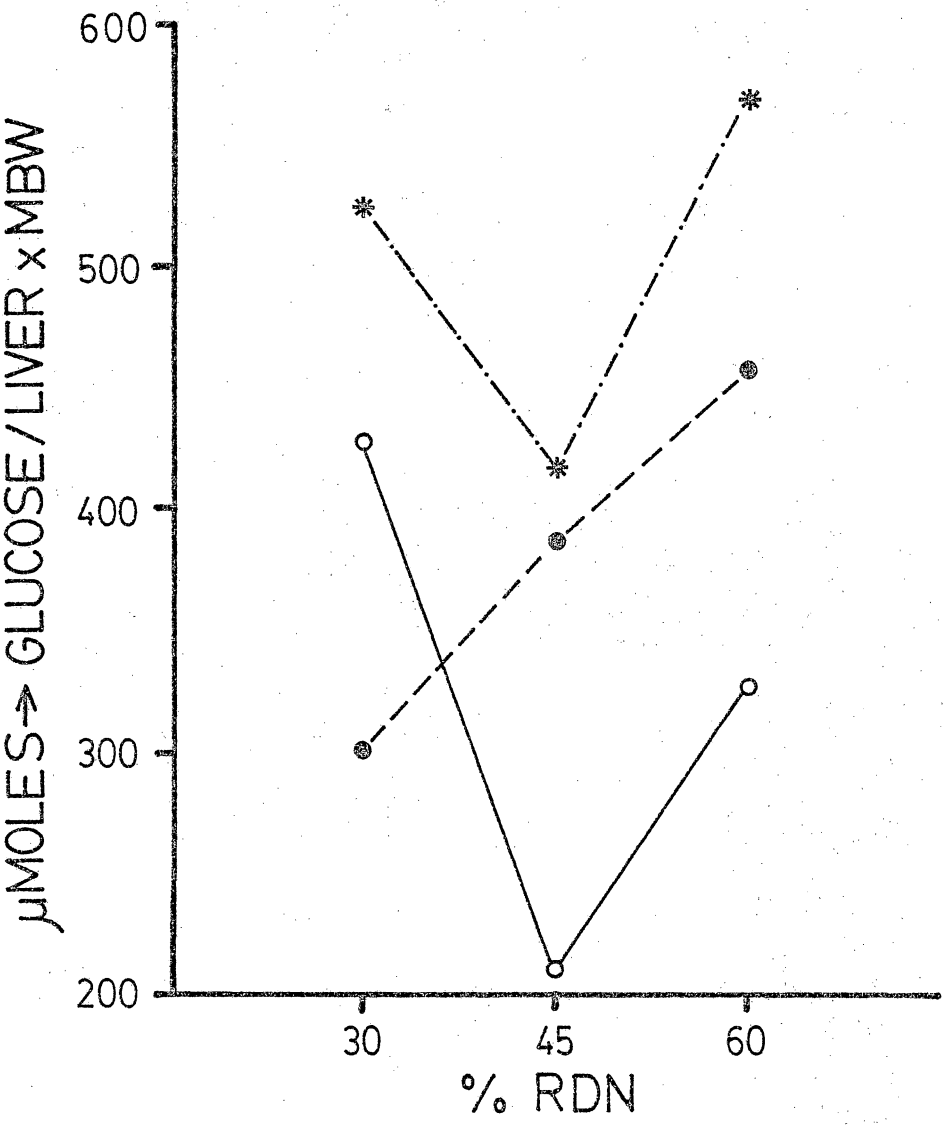

Figure 4. Effect of ration physical form and rumen degradable nitrogen (% RDN) on labelled glucose production from [2-^{14}C] propionate. (ground ——, chopped ---, concentrate —·—)

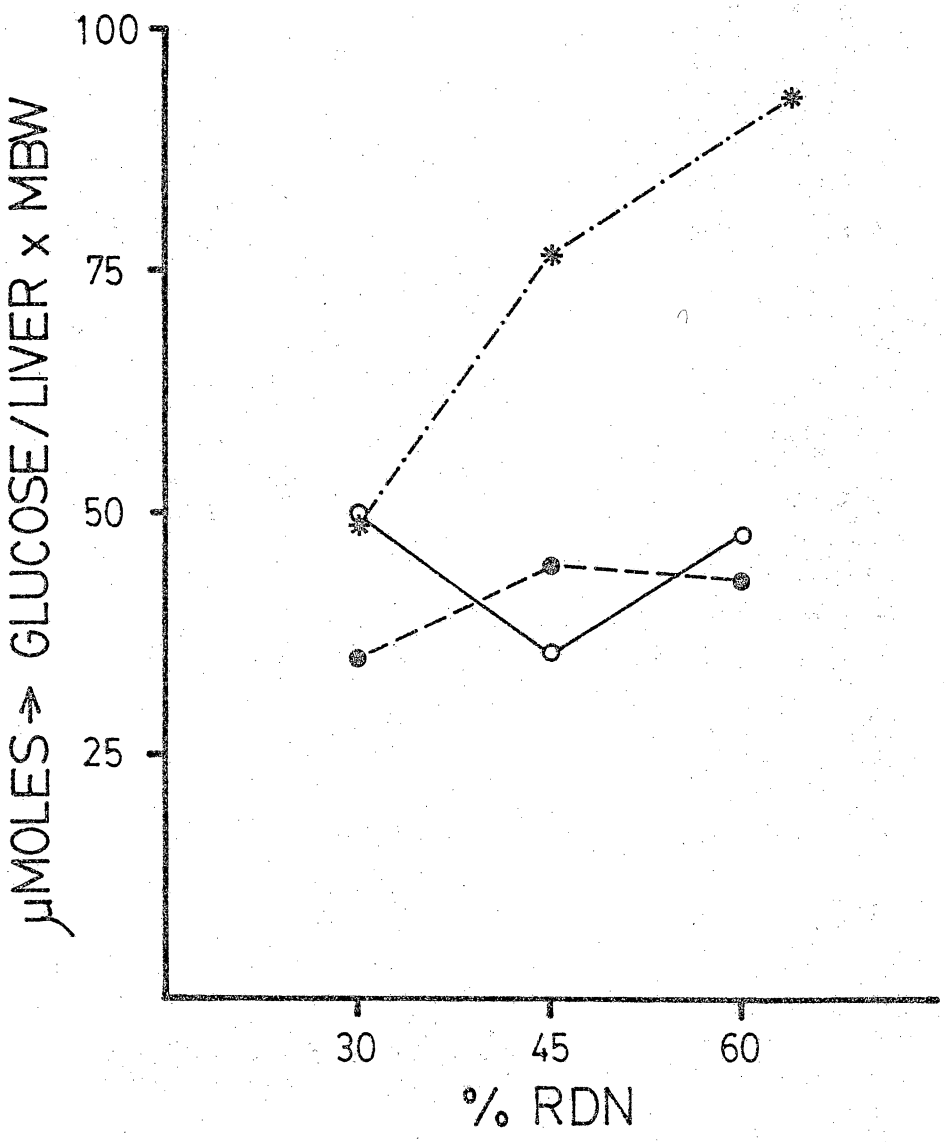

Figure 5. Effect or ration physical form and rumen degradable nitrogen (% RDN) on labelled glucose production from [U-^{14}C] lactate. (ground ——, chopped ---, concentrate —·—)

available for metabolism after digestion. This includes use of amino acids for gluconeogenesis which could influence propionate utilization. Exact causes for the observed interactions cannot at present be determined but need further study.

More labelled glucose was produced from [2-^{14}C]propionate than [1-^{14}C]propionate in agreement with Seto et al. (143). According to Leng et al. (103) when propionate metabolism to glucose follows the pathway: propionate - succinate - PEP - glucose, approximately 50% as much [1-^{14}C]propionate as [2-^{14}C]propionate should appear in glucose. In our study approximately 58% as much [1-^{14}C]propionate as [2-^{14}C]propionate appeared in glucose.

Gluconeogenesis from propionate was greatest ($P<.05$) in calves fed CONC rations and 60% RDN rations. Analyses of rumen epithelial enzymes reported by Nocek et al. (123) showed highest activity of propionyl-CoA synthetase (EC 6.2.1._), which regulates entry of propionate into the TCA cycle and gluconeogenesis, occurring in animals fed these rations. Blood and rumen propionate as well as blood glucose was highest for CONC-fed calves (121). Since 80 to 85% of the the absorbed propionate is taken up by the liver (13), it appears that more propionate is available, more glucose is synthesized and higher blood glucose occurs in

CONC ration calves. It is difficult to examine RDN effects because of its interaction with ration physical form. Animals fed CH ration produced less glucose from propionate than CONC- or GR- fed calves at 30% RDN. The lower intake, longer rumen retention time and lower degradability of the 30% RDN, CH ration as well as the lowest propionyl-CoA synthetase activity may be the causes of the decreased gluconeogenesis from propionate.

Lactate is a gluconeogenic precursor (4,87,134) and also fills an anaplerotic role (13) in ruminant metabolism. Ruminal lactate concentration was lowest in CONC-fed calves (121) but lactate absorption increases with increased concentrate feeding (87) which could provide more available lactate. It appears from work by Reilly and Chandrasena (134) that glucose production from lactate is dependent upon lactate availability and this seems to be reflected by noted increases in gluconeogenesis from lactate in animals fed all concentrate rations in this study.

Calves fed 60% RDN rations had highest glutamate dehydrogenase (EC 1.4.1.3) activity and aspartate aminotransferase (EC 2.6.1.1) activity in ruminal epithelium as well as highest ruminal ammonia nitrogen concentrations and plasma urea nitrogen (121). Infusions of ammonium chloride into ewes caused increases in blood glucose and lactate (70).

The present report indicates increases in % RDN in CONC-fed calves causes increased gluconeogenesis from lactate. Work done in rats (85,172,178) demonstrated that ammonium chloride increases lactate gluconeogenesis by stimulating transamination to aspartate because the aspartate shuttle is needed to produce glucose from lactate. Lactic dehydrogenase produces the needed reducing equivalents to increase glutamate production for transamination. This stimulation could account for the increases in glucose production from lactate in 45 and 60% RDN CONC ration calves.

The enzyme activities in the rumen epithelium (123) indicate that the calves adapted to the ration treatments. Work done by Ash and Baird (7) demonstrated that the activity of propionyl-CoA synthetase was 3 to 4 times greater in liver than in rumen epithelium. If these same enzymes increase in liver during this adaptation, they could account for the changes in gluconeogenic rate. Blood metabolite concentrations of glucose and plasma urea nitrogen also substantiate these results. In 30% RDN-fed calves both lactate and propionate metabolism was similar for GR- and CONC-fed animals. At 45 and 60% RDN rations, CONC- and CH-fed calves had similar gluconeogenic rates from propionate but glucose from lactate was significantly ($P<.05$) greater in livers of CONC-fed calves compared to GR- and CH-fed animals. Control of gluconeogenesis from propionate and lactate appeared dif-

ferent as demonstrated by physical form and RDN effects on glucose production and deserve further investigation.

Chapter IV

EXPERIMENT 2

4.1 INTRODUCTION

Lactation places a great stress on the dairy cow. Baird (10) recently divided metabolism in the dairy cow into three modes using the carbohydrate status of the animal. These were nonlactating animals, animals in early lactation and fasted or ketotic cows. This emphasizes the degree of altered metabolism during lactation. Workers have shown differences between lactating and nonlactating cows in glucose concentration and utilization, insulin concentration and response to exogenous glucose loads (glucose tolerance) (13,10,18,78,108,160). Stage of lactation has also been shown to effect the dairy cow. Early in lactation the demands of the mammary gland take priority over maintenance (11) resulting in a weight loss. During lactation the quantity of milk produced affects metabolism (78,79). Late in lactation the pregnant animal must meet the high requirements of the growing fetus (11,25,82,152). It is therefore necessary to define how and what metabolic changes are occurring.

Ration composition can affect milk constituents (26,29,89,139,157,164) and intermediary metabolism (9,16,50,65,66,88,174). Increasing concentrate in the

ration, which lowers fiber concentration, has been associated with increases in serum glucose and serum insulin (64,65,66,88,90) as well as milk fat depression (26,29,89). The diet fed must meet the needs of the animal to allow maximum productivity. Therefore lactation and ration effects are difficult to separate and their interactions are important to intermediary metabolism and control of metabolic disorders.

Lactation is a complex physiological state which is metabolically important to the cow and economically important to the dairy industry. This importance has long been recognized, but the changes that occur during the different stages of lactation have been less well defined. Another problem is the confounding of the lactational effects by the ration fed. In an effort to examine these problems a study was conducted with the objectives being to determine the effects of: 1) stages of lactation; 2) ration composition; and, 3) interaction between the two on in vitro propionate metabolism in the liver of the dairy cow.

4.2 MATERIALS AND METHODS

4.2.1 Animals and Diets

Ten lactating Holstein cows were fed a control ration (Table 4) for at least two weeks prior to first tissue sam-

pling at 30 days (n=4 of the 10) or 60 days (n=10) postpartum. Five dry cows were also fed the control ration at least two weeks prior to tissue sampling. After 60 days postpartum five of the lactating cows were changed to a high concentrate ration (HI CONC) (Table 4). The control ration was corn silage based with 18% crude fiber. The HI CONC ration was primarily ground shelled corn and ground orchardgrass hay with 10.5% crude fiber. Individual intake of the complete rations were recorded daily by use of a Pinpointer feeder every other week of the experiment when cows were kept in a free stall area. During the week of biopsy cows were housed in a tie stall barn and feed intake recorded on a daily basis. Milk samples were taken weekly and analyzed for fat, protein and total solids.

Two days before biopsy, rumen and blood samples were taken. Blood samples were obtained via right jugular vena puncture. Samples were drawn by syringe at approximately 2 hours postprandial. Tubes contained .03M benzamidine and 1.2 mg sodium EDTA/ml as an anticoagulant and were used for glucose and insulin determinations. Samples were kept on ice until centrifuged at 1000 x g 15 min at 5C. Plasma was decanted and frozen for subsequent analysis. After blood sampling rumen fluid samples were taken via stomach tube (with metal strainer) using a vacuum pump. A 5 ml aliquot

Table 4. Ration composition and proximate analysis[1].

Ingredient	Control	HI CONC
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_L, mcal/kg	1.6	1.7
Ca, %	.826	.837
P, %	.513	.516

[1]Calculated on dry matter basis.

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

was pipetted into a testtube containing 1 ml of 25% (wt/vol) meta-phosphoric acid, frozen at -20C for subsequent VFA analysis.

4.2.2 Samples and Incubation Procedure

All lactating cows were liver biopsied at 60,90,120 and 180 days postpartum. Only four of the cows were biopsied at 30 days. Each dry cow was liver biopsied once after adjustment period to control ration. Cows were initially injected with 2-3 cc (5 mg/ml) Rompun* intramuscularly, then 1-2 cc Rompun intravenously to serve as a mild sedative. Local anesthesis, Lidocaine (20 cc), was injected subcutaneously on the right side in the area of the eleventh and twelfth ribs 10 to 20 cm from spine. Upon desensitization of the tissue, a 5 cm incision was made through skin and muscle layers between the eleventh and twelfth ribs. Intercostal muscle and peritoneum were penetrated using the trocar end of a biopsy tool.

Using the cutting edge of the biopsy tool (1.5 x 50 cm) and the suction generated by the retraction of the trocar, a liver core was cut and withdrawn. The core was rinsed once and transported to laboratory in ice cold saline. The peritoneum was closed using a Ford interlocking suture. Muscle

*Rompun, xylazine (20 mg/ml), Haver-Lockhart, Shawnee, Kansas.

layers were intermittently closed with simple interrupted sutures and the skin by a series of horizontal sutures.

The liver core was cut using a Stadie-Riggs microtome into slices weighing 75 to 125 mg. Slices were kept in cold calcium free Krebs-Ringer bicarbonate buffer until blotted, weighed and placed in 25 ml side arm incubation flasks containing 3 ml incubation media. Flasks were gassed with oxygen : carbon dioxide (95:5). Triplicate incubations were conducted for 1h at 37C with an oscillation rate of 90 strokes/min. The remaining liver core was frozen in a -70C freezer and kept frozen until analyzed for protein.

Incubation media (pH 7.4) consisted of 2 ml calcium free Krebs-Ringer bicarbonate buffer and 1 ml stock substrate solution. The final concentration of substrates was 15 mM sodium acetate, 10 mM sodium propionate, 1 mM sodium pyruvate, 5 mM N-butyric acid, 10 mM L-lactic acid, 2.5 mM D-glucose and 5 mM ammonium chloride. The radioactive tracer used (.5microcurie/flask) was the sodium salt of [2-^{14}C]propionic acid obtained from New England Nuclear, Boston, Mass.

Scintanalyzed phenethylamine (.2 ml), injected through the top stopper into the centerwell one minute prior to incubation termination, was used to collect carbon dioxide. Incubations were halted by acidification of the media by

injection of 5% (vol/vol) perchloric acid (.5 ml) via the side arm. After an hour to allow for carbon dioxide collection centerwells were placed in scintillation vials with deionized water (.2 ml) and Phasar scintillation fluid[5] (4 ml). Radioactivity was measured using a Delta 300 Liquid Scintillation Counter Model 6890.[6] Media was transferred to a testtube, neutralized by .1 ml of 20% KOH and centrifuged at 2960 g for 15 minutes. Supernatant (2.5 ml) was pipetted into a testtube containing .5 ml phosphate buffer (pH 6.8, .5M potassium phosphate monobasic, .5M potassium phosphate dibasic) and frozen until analyzed.

4.2.3 Assays

Glucose was separated from neutralized incubation media (pH 7.0) by anion exchange columns (8 x 35 mm) of Dowex 1 (200-400 x 8 mesh) formate form. After application of 1 ml incubation media to column, glucose was removed by rinsing with 7.5 ml deionized water. Columns were cleaned and regenerated by rinsing with 10 ml 1M ammonium formate (pH 7.0) then 12 ml deionized water. The glucose solution was lyophilized then dissolved in 1 ml deionized water. Labelled glucose was assayed as previously described in

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[5]New England Nuclear, Boston, Mass.

[6]Searle and Co., Des Plaines, Ill.

Experiment 1 by measuring radioactivity in .4 ml final glucose solution.

Rumen fluid samples were thawed and centrifuged at 1000 x g for 15 minutes. A 3 ml aliquot of supernatant was added to .5 ml of 30 mM isocaproic acid (internal standard). A Bendix Model 2600 gas chromatograph was used to analyze rumen fluid for concentrations of acetic, propionic and butyric acids (63). The acids were separated on a 183 cm by 2 mm glass column packed with 10% SP-1200/1% phosphoric acid liquid phase on 80/100 chromosorb WAW packing.[7] The concentrations for each of the VFA were determined by integrator calculated area under the curve.

Blood samples were analyzed for glucose and insulin. Deproteinized blood was assayed for plasma glucose using enzymatic, colorimetric determination with glucose oxidase and peroxidase (described in Sigma Technical Bulletin No. 510, 1973). Insulin was determined using a standard double antibody radioimmunoassay (173) using guinea pig antibody insulin, antiguinea pig gamma globulin (Research Products International Corp., Elk Grove Village, IL) and purified crystalline bovine insulin (Lilly Research Laboratories, Indianapolis, IN).

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[7]Supelco Inc., Bellefonte, Pa.

Frozen liver samples were assayed for protein content. Approximately one gram of liver tissue was homogenized in 10% sucrose, 10 mM glycylglycine (pH 7.4) (10 ml/g tissue) and centrifuged at 800 x g for 5 minutes. Supernatant protein was determined using the method of Lowry et al. (109).

Incubation data from 90 to 180 days postpartum cows and dry cows were analyzed by covariate analysis, 60 day data used as the covariate, with day and ration as main effects. All data were also examined within days and rations for ration and day effects respectively. Significant effects, by F test at .05 level, were tested for differences between least square means at the .05 level using Tukey test (154).

4.3 RESULTS AND DISCUSSION

Milk yield and composition, body weight and feed consumption of the lactating cows is shown in Table 5. Data is divided by stage of lactation and ration fed starting at 60 days postpartum. Cows at 60 days were all fed the control diet but are grouped according to subsequent rations fed. Although randomly assigned the HI CONC cows weighed less, ate more feed and produced more milk. To correct for this the 60 day sample was used as a covariate for incubation data. Cows on the HI CONC ration consistently produced more milk. As found by other workers (26,29,89,157) milk fat

percent was depressed (P<.05) when a high grain low fiber ration, HI CONC, was fed. Neither milk protein nor milk solids-not fat (SNF) percents varied greatly between rations. Body weights were lowest at 60 days coinciding with peak production. Weight gains were slower in HI CONC cows than control cows due most likely to the higher production. As previously mentioned the HI CONC cows consumed more dry matter (DM) which was significant only at peak intake, 90 days postpartum. Due to the higher energy concentration in the HI CONC ration (.35 vs .33 Mcal/kg DM) and higher intake, HI CONC cows reached a positive energy balance before control cows. Energy balance was calculated by substracting NRC requirements from energy intakes.

Rumen fluid, blood and liver analyses are shown in Table 6. Little change occurred in rumen acetate, propionate or butyrate concentration over stage of lactation. The only exception being a large increase in control cows at 120 days. The higher VFA concentrations in HI CONC cows apparent at 60 days is probably due to the higher intake which has been noted by other workers (174). Changing the ration from control to HI CONC after the 60 day sample did not result in the expected increase in the proportion of rumen propionate. The lack of increase could be caused by: 1) rumen fluid samples not taken at proper time, 2)increased

Table 5. Effect of ration and stage of lactation on milk yield, milk composition, body weight and feed intake[1].

	Ration[2]	Stage of Lactation				
		30D	60D	90D	120D	180D
n	C	4	5	5	5	4
	HC		5	5	5	5
Milk, kg/d	C	28.3 ± 1.2	26.0 ± .8	23.5 ± 1.4	22.9 ± 1.3	20.0 ± 1.5
	HC		29.2 ± 2.0	29.7 ± 2.5	25.2 ± 3.2	23.2 ± 2.5
Milk fat, %[3]	C	3.58 ± .26	3.23 ± .13	3.47 ± .08	3.80 ± .26	3.76 ± .07
	HC		3.24 ± .09	3.03 ± .16	3.21 ± .15	3.32 ± .11
Milk Protein, %	C	2.98 ± .07	3.06 ± .11	3.27 ± .11	3.61 ± .17	3.71 ± .07
	HC		2.85 ± .13	3.43 ± .05	3.64 ± .05	3.53 ± .06
Milk SNF, %	C	11.58 ± .45	11.57 ± .20	12.17 ± .16	12.68 ± .41	12.48 ± .10
	HC		11.68 ± .13	11.92 ± .16	12.46 ± .27	12.05 ± .15
Body weight, kg	C	581 ± 47	564 ± 35	567 ± 33	587 ± 31	628 ± 31
	HC		543 ± 22	556 ± 22	559 ± 24	593 ± 31
DM Intake, kg/d[4]	C	16.1 ± .8	16.4 ± .7	17.9 ± .7	17.1 ± .7	18.2 ± .9
	HC		18.8 ± 1.3	23.3 ± 1.6	19.7 ± .8	19.2 ± 1.4
Energy Balance, Mcal[5,6]	C	-3.15 ± 1.51	.90 ± 1.20	3.44 ± .78	1.50 ± 1.11	4.98 ± 1.97
	HC		1.69 ± 2.17	11.31 ± 1.49	8.12 ± 1.30	7.59 ± 1.64

[1]Mean ± SEM.

[2]C = Control Ration, HC = HI CONC Ration.

[3]Significant differences ($P < .05$) between rations at 90 d, 120 d and 180 d.

[4]Significant differences ($P < .05$) between rations at 90 d.

[5]Calculated by subtracting NRC requirements (120) from energy intake.

[6]Significant differences ($P < .05$) between rations at 90 d.

propionate transport out of the rumen and/or 3) an actual nonresponse to the high concentrate diet. Plasma glucose averaged 66.8 mg/100 ml for all cows regardless of diet or lactation stage. No significant differences occurred. Plasma insulin also showed no significant differences but an increase was noted in the HI CONC cows starting at 60 days. The usual trend (78,79,90,163) of increasing level of insulin as lactation progresses was found. Liver protein did not change over stage of lactation. Feeding the HI CONC ration significantly ($P<.05$) increased liver protein, 13.84 and 14.15 mg protein/g liver tissue, control and HI CONC, respectively.

Covariate analysis of incubation data, including labelled carbon dioxide and labelled glucose production, was used to account for differences between the two ration groups of cows, HI CONC and control, that existed at 60 days before the HI CONC ration was fed. Although unadjusted means were significantly different, adjusted means were calculated and increased significance of differences in most instances.

Propionate metabolism into carbon dioxide is shown in Figure 6. Dry cow and 60 day cow data are unadjusted means. At 60 days hepatic carbon dioxide production was found to be different ($P<.05$) between the two ration groups. Stage of lactation affected ($P<.05$) propionate metabolism to carbon dioxide with higher production at 120 days compared to 180

Table 6. Effect of ration and stage of lactation on rumen VFA production, blood glucose and insulin concentration, and liver protein[1].

	Ration[2]	Stage of Lactation			
		30D	60D	90D	120D
Rumen VFA, μmole/ml					
Acetate	C	5.99 ± .27	5.94 ± .85	5.98 ± 1.03	8.09 ± .60
	HC		7.33 ± 1.24	6.16 ± .37	6.62 ± .48
Propionate	C	2.64 ± .15	2.65 ± .35	2.83 ± .55	3.16 ± .25
	HC		3.15 ± .48	2.94 ± .40	3.23 ± .38
Butyrate	C	1.52 ± .05	1.37 ± .23	1.37 ± .36	1.49 ± .40
	HC		1.75 ± .23	1.29 ± .07	1.41 ± .08
Plasma Glucose, mg/100 ml	C	65.0 ± 2.6	65.1 ± 1.5	66.7 ± .9	68.9 ± 1.1
	HC		64.6 ± 1.8	67.8 ± 1.4	69.6 ± 3.4
Plasma Insulin, ng/ml	C	.59 ± .06	.61 ± .06	.73 ± .12	.80 ± .11
	HC		.71 ± .16	.82 ± .06	.94 ± .09
Liver Protein, mg/g	C	13.55 ± 1.44	14.04 ± .08	13.08 ± .75	12.43 ± 1.36
	HC		15.01 ± .87	13.04 ± .73	14.60 ± .72

[1] Unadjusted mean ± SEM.

[2] C = Control Ration, HC = HI CONC Ration.

days. By ration, HI CONC cow livers produced more ($P<.05$) carbon dioxide than control cow livers. The conversion of [2-^{14}C]propionate to ^{14}C-carbon dioxide occurs basically via the TCA cycle. These results show less carbon dioxide production occurring as lactation continues. When carbon dioxide production is calculated on a per mg protein basis (Table 7) the same differences between stages of lactation exist. However, the ration difference, i.e., HI CONC greater than control, is not observed. This is caused by the noted significant increase in liver protein in HI CONC livers. Therefore the carbon dioxide production parallels the protein increase and could be caused in part by increases in enzymes involved in carbon dioxide synthesis.

The capacity to produce glucose from propionate changed (Figure 7) between 60 and 180 days lactation. Feeding the HI CONC ration significantly increased ($P<.05$) labelled glucose production. It is well documented that high concentrate rations increase the proportion of propionate (26,65,170,174) produced in the rumen although not demonstrated by our rumen fluid analysis. Atwal and Sauer (9) found that substrate concentration was the major limiting factor for hepatic gluconeogenesis when using beef liver slices. Although Young et al. (176) did not find selected enzymes involved in carbohydrate metabolism to be easily

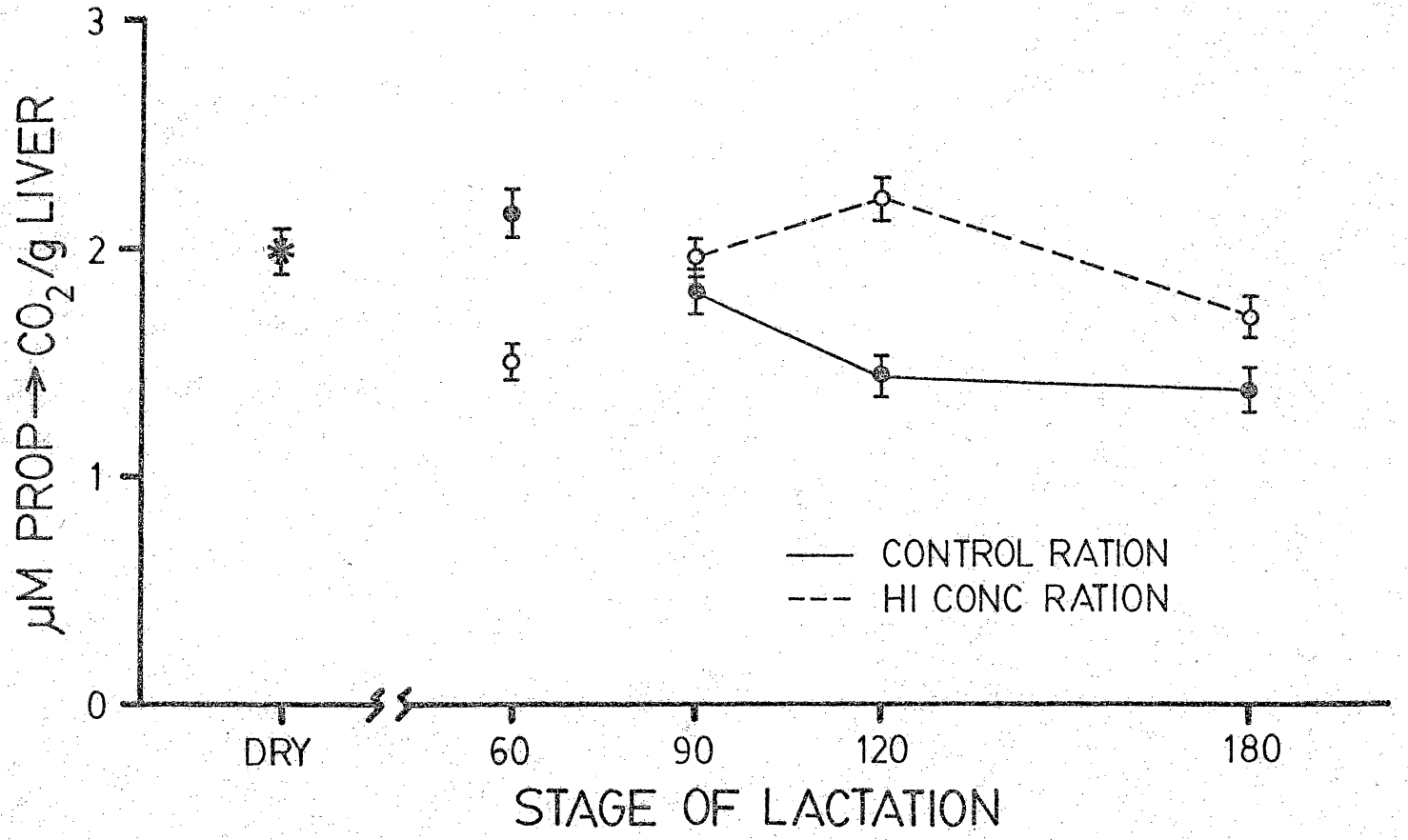

Figure 6. Effect of stage of lactation and ration on labelled CO_2 production from [2-^{14}C] propionate. Mean ± SEM.

Table 7. Effect of ration and stage of lactation on labelled CO_2 and glucose production on a liver protein basis[1,2].

	Ration[3]	Stage of Lactation		
		90D	120D	180D
CO_2	C	.137 ± .007	.122 ± .008	.107 ± .008
	HC	.119 ± .007	.127 ± .007	.090 ± .007
Glucose	C	.092 ± .009	.117 ± .009	.103 ± .010
	HC	.104 ± .009	.116 ± .009	.083 ± .009

[1]μmoles propionate metabolized to CO_2 or glucose/mg protein.

[2]Adjusted mean ± SEM.

[3]C = Control Ration, HC = HI CONC Ration.

influenced by dietary changes, our results tend to indicate that the liver of animals fed a diet capable of producing a high proportion of propionate was able to metabolize more propionate by gluconeogenesis.

Labelled glucose production calculated on a protein basis (Table 7) showed different results from production on a tissue basis. The effect of stage of lactation which was nearly significant on a tissue basis, was significant ($P<.05$) with 120 day cows producing more labelled glucose than 90 and 180 day cows. Ration effects which were noted when expressed on a tissue basis again were eliminated by putting on a protein basis. The cause, as with the carbon dioxide production, is the increased liver protein when HI CONC ration was fed. As mentioned, at least part of the increase in protein could be due to increased amounts of enzymes and would account for increased metabolism of propionate to carbon dioxide and glucose.

Another factor which could be affecting carbon dioxide and glucose production is the size of the liver. Since these animals were not slaughtered liver weights are not known, but increases in liver weight can cause increases in amount of production with no change in rate of metabolism. Very little information is actually known about changes in liver size or composition with ration changes or stage of lactation.

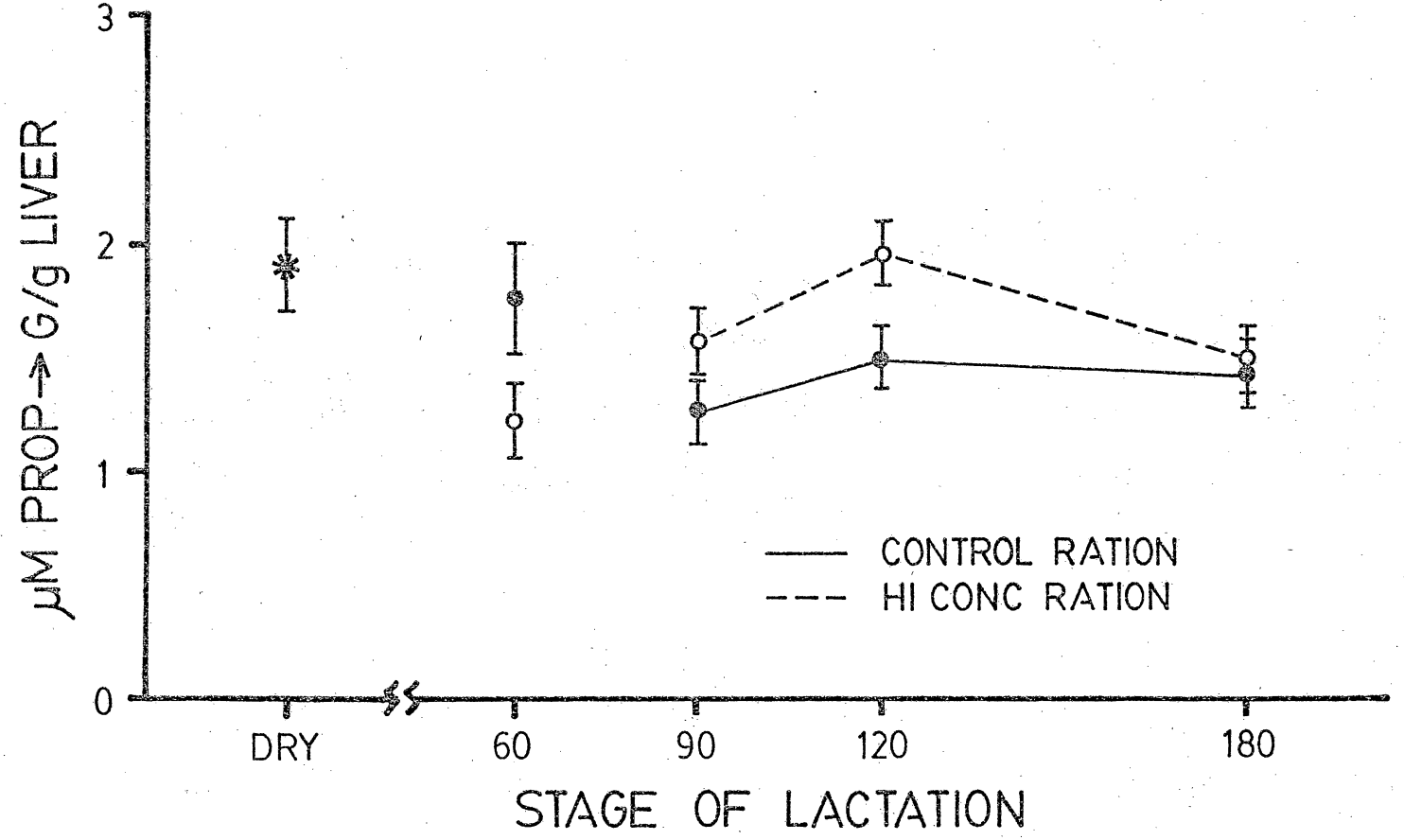

Figure 7. Effect of stage of lactation and ration on labelled glucose production from [2-^{14}C] propionate. Mean ± SEM.

The ratio of labelled carbon dioxide to labelled glucose (Figure 8) can provide an indication of the propionate metabolism occurring. Theoretically, if all propionate is converted to glucose, no labelled carbon dioxide is produced from [2-^{14}C]propionate; only labelled glucose. The carbon dioxide/glucose ratio indicates the relative flow of propionate carbon through the TCA cycle, relative to the flow of propionate carbon to glucose. Neither stage of lactation nor ration appeared to influence this ratio which averaged 1.3 for the adjusted means (Figure 8).

As previously mentioned four of the ten cows were biopsied at 30 days postpartum. Comparison of mean labelled carbon dioxide and labelled glucose production between dry, early lactation (30 days) and later lactation cows is in Table 8. Carbon dioxide production is very similar between all three groups but labelled glucose is significantly higher for 30 day cows than other cows. The ^{14}C-carbon dioxide / ^{14}C-glucose ratio indicates a metabolic adjustment at 30 days. The carbon dioxide/glucose ratio mean of .7 for the 30 day cows shows a predominance of gluconeogenesis. This agrees with work by Bauman and Currie (25) that found dramatic increases in gluconeogenesis during lactogenesis. In goats, Davis et al. (59) found an increase in glucose uptake 2 to .5 days prepartum showing a greater need for

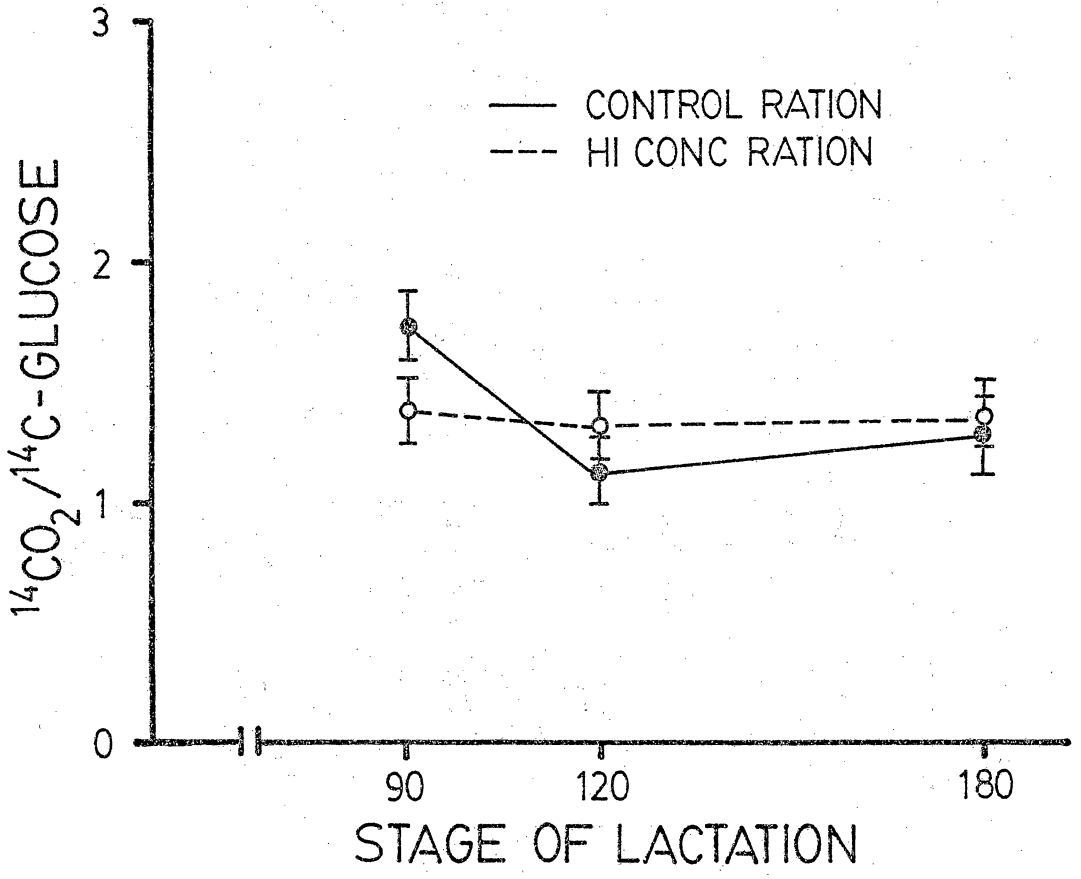

Figure 8. Effect of stage of lactation and ration on labelled CO_2/ labelled glucose ration. Mean ± SEM.

glucose early in lactation. However, Mathias and Elliot (114) found that propionate was not metabolized more rapidly early in lactation. One possible reason for this disagreement is the difference in stages of lactation used, 30 days postpartum versus 41 days, respectively. Rapid metabolic changes may have occurred between these time periods although both are early in lactation.

The glucose metabolism in the ruminant is of major importance due to its almost totally endogenous source. Lactation increased the glucose requirement (13,31) but this is not caused by a change in nonmammary utilization (48). With certain diets it is possible that glucose may limit milk yield (127) and that the cow will conserve glucose for lactational purposes (55).

Enzymes involved in gluconeogenesis are also affected by diet and lactation. Dry matter intake has been found to be positively correlated with PEPCK (160) but other workers (176) found little influence of diets on enzyme activity. Although not demonstrated yet in ruminants, lactation affects partitioning of nutrients in monogastrics. In rats, protein and amino acids are preferentially used for enzyme synthesis during lactation (129). Glucose oxidation enzymes are saturated in dry cows but not in lactating cows (18) demonstrating another lactational effect on enzymes.

Table 8. Effect of stage of lactation on labelled CO_2 production, labelled glucose production and labelled CO_2/labelled glucose ratio[1].

	Stage of Lactation		
	Dry	30D	60-180D
n (cows)	5	4	36
$^{14}CO_2$, µmole/g	2.04 ± .04	1.98 ± .19	1.78 ± .03
^{14}C-Glucose, µmole/g	1.40 ± .07	3.29 ± .64	1.47 ± .04
$^{14}CO_2/^{14}$C-Glucose	1.66 ± .11	.70 ± .17	1.38 ± .04

[1]Unadjusted mean ± SEM.

One calculation which involves both lactational stage and ration is energy balance. Early in lactation cows are in negative energy balance as found in this study. The stress of lactation increases the energy requirement even though the cow is unable to consume enough energy to meet these needs. Metabolic control (hormone mediation) has been found to be associated with intake and energy balance (147). Jenny et al. (90) suggested the lack or response to a high grain ration early in lactation to be caused by the high energy demands. The increase in gluconeogenesis found early in lactation in this study may also be caused by the negative energy balance. Our results show that the liver has the potential to increase gluconeogenesis in response to feeding a low fiber high concentrate ration, but the magnitude of the response is lower than this increase noted earlier in lactation (30 days) when cows are in negative energy balance.

Chapter V

SUMMARY AND CONCLUSIONS

5.1 CALF AND COW COMPARISONS

Two experiments were conducted to examine ruminant gluconeogenesis. In Experiment 1, 20 week old calves were used to study labelled carbon dioxide and glucose production from propionate, both [1-^{14}C]propionate and [2-^{14}C]propionate, and lactate. In Experiment 2, gluconeogenesis from propionate, [2-^{14}C]propionate, was examined using Holstein cows at various stages of lactation. Both studies used in vitro liver slice incubations in similar incubation media. Comparing the results from these experiments, similarities and differences between growing and lactating ruminants can be found.

In Experiment 2 labelled carbon dioxide/glucose ratio was found to vary with stage of lactation (Table 8). Dry cows and lactating cows 60 to 180 days postpartum had similar ratios that were more than twice as large as the ratio of 30 day cows. When this ratio is calculated for Experiment 1 differences between the two can be seen (Table 9). When [1-^{14}C]propionate tracer is used the ratio (overall average=2.2) is large as expected due to the large loss of ^{14}C as ^{14}C-carbon dioxide. The ratio when using

[2-^{14}C]propionate is much smaller (overall average=.15) and the lactate ratio (overall average=.74) is between the two.

Comparing the [2-^{14}C]propionate results in the two experiments, adult ruminants have a ratio almost eight times larger than growing calves. Lactating cows (>60 days postpartum) had an average ratio of 1.4 versus the calf ratio average of .15. As mentioned a low ratio indicates a preference for glucose production over carbon dioxide production. Therefore it appears that calves produce more glucose while cows, except early in lactation, produce more carbon dioxide. It is necessary to compare the absolute values from each experiment to determine where the real differences occur.

Table 10 repeats the labelled carbon dioxide production from both experiments. Production is slightly larger from [2-^{14}C]propionate for Experiment 2 than Experiment 1 but not greatly different. This small difference would not account for the large ratio differences. When examining the labelled glucose production (Table 11) the reason for the ratio differences is apparent. Glucose production from the calf liver is approximately 4.5 times greater than cow liver production on a per gram of liver tissue basis. This large increase in glucose production as well as the slightly lower carbon dioxide production accounts for the ratio differences found in Table 9.

Table 9. CO_2/Glucose ratios for Experiment 1 and Experiment 2[1].

	[1-^{14}C] Prop	[2-^{14}C] Prop	[U-^{14}C] Lactate
Experiment 1. Calves			
Ration			
CH	2.14	.12	.72
GR	2.34	.22	.80
CONC	2.42	.13	.70
30%	2.33	.15	.84
45%	2.41	.12	.70
60%	2.12	.18	.69

Experiment 2. Cows		
Stage	C	HC
30D	.70	
60D	2.47	
90D	1.71	1.37
120D	1.28	1.69
180D	1.24	1.34
DRY	1.66	

[1] C = Control Ration, HC = HI CONC Ration.

Table 10. Labelled CO_2 production for Experiment 1 and Experiment 2[1,2].

	[1-^{14}C] Prop	[2-^{14}C] Prop	[U-^{14}C] Lactate
Experiment 1. Calves			
Ration			
CH	11.23	1.06	1.97
GR	9.82	1.50	2.16
CONC	9.83	.95	2.24
30%	10.07	1.14	2.09
45%	10.35	.87	2.08
60%	10.47	1.48	2.20

Experiment 2. Cows		
Stage	C	HC
30D	1.98	
60D	1.78	
90D	1.80	1.94
120D	1.42	2.20
180D	1.37	1.68
DRY	2.04	

[1] µmoles metabolized to CO_2/gram liver.

[2] C = Control Ration, HC = HI CONC Ration.

Table 11. Labelled glucose production for Experiment 1 and Experiment 2[1,2].

	[1-^{14}C] Prop	[2-^{14}C] Prop	[U-^{14}C] Lactate
Experiment 1. Calves			
Ration			
CH	5.24	8.69	.91
GR	4.20	6.89	.90
CONC	4.07	7.56	1.07
30%	4.33	7.73	.83
45%	4.30	6.99	.99
60%	4.95	8.35	1.07

Experiment 2. Cows

Stage	C	HC
30D	3.28	
60D	1.46	
90D	1.25	1.57
120D	1.48	1.94
180D	1.42	1.48
DRY	1.40	

[1] μmoles metabolized to glucose/gram liver.

[2] C = Control Ration, HC = HI CONC Ration.

High glucose production in growing calves would be expected. All rapidly growing animals have high requirements for glucose. Young ruminants are no exception. But their carbohydrate metabolism changes as they mature, from being similar to monogastrics to a true ruminant metabolism (18). This means that these young ruminants also have a higher blood glucose level to maintain than a mature ruminant. Growth and a high blood glucose level cause an increased need for glucose which is accompanied by the demonstrated increase in rate of glucose production. Lactating cows as previously mentioned have a high need for glucose for lactation but mature ruminants have lower blood glucose levels to maintain and their carbohydrate metabolism is dependent upon acetate and not glucose for energy. These differences can account for the physiological adaptation of an increased rate of glucose production in calves.

Using data from other ruminant liver slice experiments the labelled carbon dioxide/glucose ratio can be calculated. Using fed sheep liver slices Leng and Annison (102) studied propionate metabolism with both [1-^{14}C]propionate and [2-^{14}C]propionate. The ratios calculated ranged from 4.5 to 5.4 and .22 for [1-^{14}C]propionate and [2-^{14}C]propionate, respectively. Seto et al. (143) also used both radioactive isotopes but with cow liver slices. In this study the rat-

ios were 4.8 to 5.0 and .20 to .34 for [1-^{14}C]propionate and [2-^{14}C] propionate, respectively. In both these experiments the ratios are very similar to that of the calves in Experiment 1. To try to understand why these ratios differ from the cows' ratio in Experiment 2 it is important to examine each study. First the animals used vary; sheep, cows and growing calves. In the study by Seto et al. (143) no indication is given as to the physiological status of the cows used (age, lactating, or pregnant). Another important factor is the method of tissue handling. In all the studies except for Experiment 2 the liver tissue was obtained from slaughtered animals. Due to the time and variation which might occur between slaughters it is possible that this could cause differences from the biopsy technique used in Experiment 2. Thirdly the incubation media used could cause variation. In the studies by Leng and Annison (102) and Seto et al. (143) the media consisted only of the precursor, propionate. This would not explain the similarity between the calf study and these studies or why Experiment 1 and Experiment 2, which had similar media consisting of several precursors, had different values but might help explain the differences between the cow experiments. The above are several possibilities that could help explain at least part of the variation between the labelled carbon dioxide/glucose ratios.

As previously stated calf liver had a greater labelled glucose production than cow liver when expressed on a per gram of liver tissue basis. But for a true comparison glucose production on a total animal basis should be made. With the calf study this is easy because at slaughter the entire liver weight was recorded. Thus multiplying this weight by micromoles of propionate metabolized to glucose per gram the micromoles of propionate metabolized per animal can be calculated. This value is then multiplied by the molecular weight of propionate to obtain the weight of glucose produced. The overall average daily production is 1.11 kg of glucose produced per animal. With the cow study the calculations are more involved due to using the biopsy technique. To calculate total animal production it is necessary to know liver weight which cannot be obtained directly, but using the weight of the liver as a percent of body weight it can be estimated. Little work has been done to determine the liver weight as a percent of body weight but using years of recorded data from Beltsville's slaughter lab[8] (unpublished) it has been possible to make estimates. Due to the completeness of the records available it was also possible to study the effect of stage of lactation on liver weight. From this data the estimates by stage of lactation are: dry,

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[8]U.S.D.A., SEA-AR, Ruminant Laboratory, BARC-East, Beltsville, MD, 20705.

1.2; 1-36 days, 1.3; 37-65 days, 1.7; 66-95 days, 1.2; 95-125 days, 1.4; 126-155 days, 1.3; and 156-185 days, 1.3% of body weight as liver. Comparing the calf results the liver weight ranged from 1.3 to 2.2% of the body weight with an average of 1.6%. It is interesting to note with the cows that the liver is larger early in lactation as others have suggested (13). Using the previous estimates and the body weight an estimated liver weight can be obtained and the glucose production can be calculated as with the calves. The average total animal glucose productions are: 30 days, 1.83 kg; 60 days, 1.04 kg; 90 days, .70 kg; 120 days, 1.01 kg; and 180 days, .85 kg glucose. These values must not be compared to the animals' requirements remembering that these glucose values are only from propionate which may supply up to 60% of the total glucose requirement (174). For growing calves the production of 1.11 kg glucose would adequately meet the requirement of .65 kg (177).

With lactating cows the requirements can be estimated from milk production. Young (175) calculated requirements using milk production, a lactose content of 4.9%, estimates of 60% total glucose for lactose synthesis and 90% of glucose from gluconeogenesis, and the molecular weight of glucose. Table 12 lists calculated glucose production and requirements for Experiment 1 and Experiment 2. The produc-

tion in Experiment 2 meets between 36 to 88% of the calculated requirements. Using these calculations, glucose production in both experiments is in the range of expected values. It is important to remember that all these calculations include use of estimates as well as combining in vivo and in vitro data.

5.2 SUBSTRATE CONCENTRATION EFFECTS IN INCUBATION MEDIA

The effect of substrate concentration in the incubation media was also studied with both calf and lactating cow liver. The methods and materials for these studies are in the appendix which also includes regression equations and graphs. Three compounds important in ruminant hepatic gluconeogenesis were examined. They include propionate, the major substrate, glucose, the endproduct, and ammonium chloride, a compound which effects the urea cycle as well as gluconeogenesis. A brief discussion of the results from these studies will be included here.

Increasing propionate concentration in the media increased both labelled carbon dioxide and glucose production (expressed as percent of production with standardly used media concentrations) from calf liver. Carbon dioxide production from cow liver responded in the same manner but labelled glucose production showed little increase with

Table 12. Glucose production and requirements for calves and lactating cows[1,2].

	Production	Requirement
Experiment 1. Calves		
Ration		
CH	.96	.65
GR	.90	
CONC	1.47	
30%	1.20	
45%	.90	
60%	1.22	
Experiment 2. Cows		
Stage		
30D	1.83	2.08
60D	1.04	2.03
90D	.70	1.96
120D	1.01	1.76
180D	.85	1.58

[1] kg/animal.

[2] Calculations explained in text. Requires from (175) and (177).

higher concentrations of propionate. The increases noted are in agreement with the results of Atwal and Sauer (9) that hepatic gluconeogenesis is limited by substrate concentration. When 30 day cows alone are examined glucose production was low and tended to decrease with higher propionate concentrations. This would demonstrate the importance of utilization of other substrates early in lactation and that the liver is working to capacity and further increases are not beneficial.

Increasing glucose concentration in the media stimulated carbon dioxide production up to 2.5 mM but then had an inhibitory effect in calf liver. In cow liver no change in carbon dioxide production was noted. Labelled glucose production was inhibited by increasing glucose concentration in calf liver similar to results by Seto et al. (143). In cow liver little change occurred but a trend of decreased glucose production with increasing concentration was noted. Cows early in lactation (30 days postpartum) were more sensitive to glucose concentration and exhibited a greater depresssion in glucose production at higher glucose concentrations. The inhibition of glucose production by liver demonstrates a feedback inhibition which has been shown by others working with ruminants (18,55,108,143,160).

The third compound, ammonium chloride, caused a decreased carbon dioxide and glucose production from propionate in calf liver as concentration in the media increased. This has been suggested by others (151,172) to be due to the removal of TCA cycle intermediates. With cow liver, ammonium chloride concentration increases caused no change in carbon dioxide production. Labelled glucose production in cow liver, unlike that in calf liver, was increased by increasing ammonium chloride concentrations. These findings are in disagreement with most previous work. Two studies (17,70) infusing sheep with ammonium chloride did find increases in blood glucose but most in vitro studies with propionate (151,165) find depressed glucose production. In Experiment 2, a depressing effect was noted above 7.5 mM ammonium chloride. Garwacki et al. (70) suggested interference with glucose production would occur only at high blood ammonia levels.

5.3 SUMMARY

Two experiments were conducted to examine nutritional and physiological effects on ruminant hepatic gluconeogenesis. The factors studied were ration rumen degradable nitrogen and ration physical form with growing calf liver and stage of lactation and ration fiber with Holstein cows.

In Experiment 1, 36 Holstein bull calves were assigned to a 3x3 factorial design with rumen degradable nitrogens of 30, 45 and 60% and ration physical forms of chopped hay, ground hay and all concentrate. Liver samples obtained at slaughter were sliced and incubated in a media containing: 15 mM acetate, 10 mM propionate, 5 mM butyrate, 20 mM lactate, 2 mM pyruvate, 2.5 mM glucose and 5 mM ammonium chloride. Metabolism was traced using [1-^{14}C]propionate, [2-^{14}C]propionate and [U-^{14}C]lactate. Collected labelled carbon dioxide and isolated labelled glucose were measured. Carbon dioxide production was not affected by RDN but production from [1-^{14}C]propionate and [U-^{14}C]lactate was greatest for CONC-fed calves. Labelled glucose production was greatest for CONC-fed calves and 60% RDN-fed calves although significant ($P<.05$) only for lactate metabolism in CONC-fed calves. Ration RDN did not have a consistent effect across all physical forms. This interaction was demonstrated by calves on the 30% RDN, CH ration causing less glucose production from propionate than other 30% RDN-fed calves. Calves fed 45 and 60% RDN, CONC rations produced more glucose than calves fed either hay containing ration.

From Experiment 1, it was concluded that: 1) concentrate-fed calves produced more glucose than calves fed either hay ration, with calves fed ground hay rations being most simi-

lar to calves fed the all concentrate ration, 2) increasing available ammonia via increased ration RDN stimulated lactate metabolism to glucose and 3) propionate and lactate metabolism were affected by ration RDN and physical form as well as the interaction between the two.

In Experiment 2, 10 lactating and 5 dry Holstein cows were used to study stage of lactation and ration fiber effects. Liver biopsies were conducted at 30, 60, 90, 120, and 180 days postpartum and once on dry cows. All cows were fed a control ration (18% CF) until after 60 day sampling when half were changed to a high concentrate ration (10.5% CF). Feed intake and milk yield and composition were recorded. Liver samples obtained by biopsy were sliced and incubated in a media containing: 15 mM acetate, 10 mM propionate, 5 mM butyrate, 10 mM lactate, 1 mM pyruvate, 2.5 mM glucose and 5 mM ammonium chloride. Metabolism was traced using [2-^{14}C]propionate. Collected labelled carbon dioxide and isolated labelled glucose were measured. Due to differences between ration groups, the 60 day sampling was used as a covariate.

Milk fat percent was depressed by feeding the high concentrate ration but milk protein and solids-not-fat were not affected. Energy balance was more positive for high concentrate cows due to greater intake and higher energy concen-

tration of the ration. Carbon dioxide production was greater at 120 days than 180 days. Cows fed high concentrate produced more carbon dioxide than control cows. Labelled glucose production was significantly increased by feeding the high concentration ration. Carbon dioxide/glucose ratio did not vary greatly during lactation after 60 days or during the dry period. Early in lactation (30 days) glucose production was greatly increased compared to all other cows.

From Experiment 2, it was concluded that: 1) carbon dioxide production was significantly affected by ration fiber and stage of lactation, 2) gluconeogenic capacity of the liver was increased by feeding a low fiber, high concentrate ration and 3) the liver can respond to the physiological stress of lactation, early in lactation when in negative energy balance.

Chapter VI

EPILOGUE

The study described in this dissertation has addressed itself to several of the unexplained areas in ruminant nutrition. It has attempted to begin to answer some of the questions which will expand our understanding of ruminant intermediary metabolism. Research in ruminant metabolism is expanding but at present is far behind the extensive nonruminant work that has been conducted. It is important to remember that the ruminant is in many ways a specialized animal but also that techniques and knowledge gained from nonruminant research can and should be applied to the ruminant to further our understanding.

A wide range of experimental techniques are employed to study metabolism, including: injections, infusions, perfusions, tissue homogenates, tissue slices and isolated cells. Injections (27,38,93,111,155,162) and infusions (20,47,81,84,91,141), two in vivo techniques, have been used to study the effects of different compounds, their concentration, pool size and clearance rates. Both of these have been widely used in the ruminant as well as the nonruminant. Perfusions of organs are commonly used in studies with small monogastrics such as the rat (94,118,124). Although perfu-

sions are used in ruminant research, in work with the liver they are not widely employed due to the size of the liver and the expense of large amounts of compounds. One experiment which did use liver perfusions was conducted by Gill et al. (72) to study the effect of glucagon on hepatic propionate metabolism in sheep.

The use of tissues versus whole animal or organ studies involves the problem of collection and preparation of the tissue. In the two experiments of this study tissue was collected at slaughter (Experiment 1) or by biopsy (Experiment 2). Obtaining tissue at slaughter has the advantages of not requiring surgery and providing additional information such as organ weights. Biopsy, on the other hand, has the prime advantage of allowing repeated samples on the same animal at various times but has an ever present risk of endangering future work with the animal. Biopsies of small sample size are routine in human medical treatment. Larger samples as used in this study are not as common but are in use (50). The technique of choice in future work is the biopsy with improvements in procedures to provide adequate tissue at minimum risk to experimental animals.

Once obtained the preparation of the tissue is important to be able to determine metabolic function. Slices of tissues have been used to study gluconeogenesis in rats both

with hepatic (75,76) and renal (94,110) tissue. In this study as well as others (9,75,76,143) liver slices have been employed to examine ruminant gluconeogenesis. A more recent technique is the use of isolated hapatocytes. The isolation of cells has been well examined (28,37) and is continually being modified to improve viability. At present most gluconeogenic research using hepatocytes has been done with rats (6,41,52,56,71,73,83,94,145,149,150,161) but also has been used with rabbits (179) and chickens (125). Ruminant researchers have suceeded in using this technique with sheep (73,136) but present usage is limited until modifications are perfected.

The use of isolated hepatocytes provides several distinct advantages over slices. Viability of the cells is easily determined compared to a slice which may be partially damaged during preparation. Uniformity of tissue and membrane is more easily assured. Isolated cells can be used for a longer period of time and this increases the number of experiments possible. Two lost advantages when changing from slices to cells are cell to cell contact in a physiological structure and ease of preparation. Future work in ruminant gluconeogenesis will benefit from use of isolated hepatocytes from tissue obtained by biopsy.

To continue the work begun in this study isolated cells and liver slices should be used. Hepatocyte work is definitely important for advancement of knowledge but also important is the knowledge of how results from cells and slices compare. In this way information gathered from past work can be incorporated into future work. This involves repetition of some work but will be beneficial to assure consistency of cell work. New work should involve exploration of more substrates especially amino acids as well as their concentrations.

In this study two of the major factors influencing the lactating cow were examined, lactation stage and ration composition. These two should be further studied in connection with other effects such as hormones and enzymes. Lactation stage needs to be closely examined to determine as much as possible about its interaction with the animal's metabolism. Ration effects have long been examined by in vivo experiments. Through feeding trials extensive knowledge has been gained about composition effects on health, gain, gestation and lactation. Less work has been done in vitro but by combining the two (as in Experiment 2) the effect of ration on in vitro metabolism can be examined. In vitro it is also possible to study how incubation media changes or substrate availability affects metabolism. As pointed out earlier

ration and lactation effects are hard to separate due to the close relationship between the two. Another factor which effects both of these and also is affected by both is the hormonal balance in the animal. Research combining these three is the most important to making determinations about ruminant gluconeogenesis.

Hormone research in the ruminant is an area which demands further investigation. Table 13 summarizes some of the research that has been conducted. Important points to note include: most research has been done in vivo with very few in vitro studies, work has been done both with sheep and cows and insulin is the most commonly studied hormone. Horino et al. (84) examined insulin response in rats, rabbits, pigs, sheep and cows. This kind of comparison work is valuable in pointing out differences and similarities between different species. Lactation as mentioned earlier is closely related to the animal's hormone pattern. Milking was found to cause release of insulin and corticoids but not growth hormone (98). Growth hormone concentration decreased as lactation advanced, but increased as gestation progressed (98). Feeding and hormones have also been closely associated. Both propionate and butyrate stimulate insulin secretion but not via hyperglycimia (112). In one study Stern et al. (156) found no insulin increase from propionate or buty-

rate intraruminally administered but did if these substrates were injected intravenously. Other workers (162) have shown that feeding grain increases insulin secretion and works through increases in the proportions of propionate and butyrate ruminally produced.

Insulin is one of the most studied hormones but how it causes its documented effects is unknown. One suggestion is that it regulates a primary satiation in the ruminant by increasing hepatic propionate uptake from the portal circulation immediately after feeding (39). Glucagon is another important hormone to gluconeogenesis in the monogastric but research in the ruminant is just starting. The insulin/glucagon ratio in the ruminant seems to be low (23). Growth hormone has been shown to increase milk production (27). Two theories that attempt to account for this are: 1) growth hormone affects the partitioning of nutrients to the mammary gland and/or 2) growth hormone increases the ability to synthesize lactose and milk fat. Correlations between these hormones and feed intake demonstrate the relationship. These significant correlations with intake of digestible nutrients suggest that energy balance is important during lactation especially early in lactation (24,147). This brief summary indicates the gaps in hormone work and that more in vitro work needs to be conducted.

Table 13. List of ruminant hormone studies.

Hormone(s) Studied	Animal	Technique	Reference
Insulin	Lactating & Dry Cows	in vivo - infusions	Baird et al., 1980 (13)
Insulin	Lactating Cows	in vivo - infusions	Baldwin et al., 1972 (14)
Insulin, Glucagon, Growth Hormone	Wethers	in vivo - infusions	Bassett, 1971 (20)
Insulin, Growth Hormone	Lambs, Sheep	in vivo	Bassett, 1974 (21)
Insulin, Growth Hormone, Corticosteroids	Wethers	in vivo	Bassett, 1974 (22)
Insulin, Growth Hormone	Sheep	in vivo	Bassett et al., 1971 (24)
Insulin	Ewes	in vivo - injections	Berzins, 1979 (38)
Insulin	Wethers	in vivo - infusions	Bhattacharya and Alulu, 1975 (39)
Insulin, Glucagon, Somatostatin	Ewes	in vivo - infusions	Brockman, 1979 (46)
Insulin, Glucagon	Ewes	in vivo - infusions	Brockman et al., 1975 (47)
Insulin	Rams, Lactating Cows	in vivo	Evans et al., 1975 (65)
Glucagon	Ewes	in vitro - perfusions	Gill et al., 1981 (72)
Insulin, Growth Hormone, Prolactin	Dairy & Beef Cows	in vivo	Hart et al., 1980 (78)

Table 13. Cont'd

Hormone(s) Studied	Animal	Technique	Reference
Insulin	Lactating Cows	in vivo	Hart et al., 1978 (79)
Insulin	Sheep, Lambs	in vivo - infusions	Hertelendy et al., 1969 (81)
Insulin	Lactating Cows	in vivo	Jenny et al., 1974 (90)
Insulin	Lactating Cows	in vivo	Jenny et al., 1975 (88)
Insulin	Calves	in vivo - infusions	Johnson et al., 1981 (91)
Insulin	Lambs	in vivo - injections	Kamalu and Trenkle, 1978 (93)
Insulin, Growth Hormone, Corticoids	Lactating Cows	in vivo	Koprowski and Tucker, 1973 (98)
Insulin	Wethers	in vivo - infusions	Manns and Boda, 1967 (111)
Insulin	Sheep	in vivo - infusions	Manns et al., 1967 (112)
Insulin	Goats	in vivo - injections	Stern et al., 1970 (156)
Insulin	Lactating Cows	in vivo	Smith et al., 1976 (147)
Insulin	Sheep	in vivo - injections	Trenkle, 1970 (162)
Insulin, Growth Hormone	Lactating Cows	in vivo	Vasilatos and Wangsness, 1979 (163)
Insulin	Lactating Cows	in vivo	Walker and Elliot, 1973 (164)

In conjunction with hormone research characterization of hormone receptors is important. In rats, rates of association and dissociation, and insulin and receptor degradation have been measured (69,158). Ruminant work has been started in the area of binding and receptor work with sheep (73) and cows (140). These must be examined to help understand hormone actions.

To get a complete metabolic picture of the animal it is necessary to study enzymes, their presence, absence, concentrations and activities. In this study enzyme knowledge was limited. Enzyme information can help explain results found in experiments. Reviews (113,169) of enzymes dealing with glucose metabolism tend to deal mostly with monogastric data due to the restricted ruminant knowledge. Enzymes which workers have dealt with include: ATP-citrate lyase (75,76,176), NADP-malate dehydrogenase (75,76), acetyl-CoA synthetase (75), glucose-6-phosphate dehydrogenase (75,176), 6-phosphate-gluconate dehydrogenase (75), propionyl-CoA synthetase (7), phosphoenolpyruvate carboxykinase (50,176), malic enzyme (176), aspartate aminotransferase (76), glucokinase (15) and hexokinase (15). This research will provide a good background to understand intermediary metabolism but it must be continued to include how enzymes change during lactation and with different rations.

The purpose of this section was to briefly cover some of the future areas in ruminant metabolism. The present work was a beginning in this direction which needs to be continued to acquire an understanding of the differences and similarities between nonruminants and ruminants.

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Table 14. Labelled CO_2 production from [1-^{14}C] propionate - Experiment 1[1].

Ground	Chopped	Concentrate
30% RDN		
(77) 11.332	(495) 10.647	(500) 11.514
(103) 5.482	(522) 10.808	(510) 11.642
(126) 10.830	(98) 11.697	(99) 7.813
(589) 10.365	(125) 8.783	(131) 9.938
$\bar{X}$ ± SEM 9.502 ± 1.355	10.484 ± .612	10.222 ± .891
45% RDN		
(492) 10.736	(514) 12.260	(59) 5.887
(513) 9.111	(74) 12.982	(75) 10.038
(109) 9.614	(104) 10.962	(91) 9.190
(117) 7.460	(553) 13.276	(582) 12.713
$\bar{X}$ ± SEM 9.230 ± .681	12.370 ± .516	9.457 ± 1.407
60% RDN		
(76) 11.219	(84) 11.635	(61) 13.642
(93) 13.200	(537) 12.736	(515) 9.389
(120) 6.291	(111) 9.083	(100) 10.650
(586) 12.258	(143) 9.942	(116) 5.554
$\bar{X}$ ± SEM 10.742 ± 1.538	10.849 ± .823	9.808 ± 1.675

[1] µmoles substrate metabolized to CO_2/gram liver.

Table 15. Labelled CO_2 production from [2-^{14}C] propionate - Experiment 1[1].

Ground	Chopped	Concentrate
30% RDN		
(77) 1.388	(495) 1.254	(500) 1.514
(103) .552	(522) .529	(510) 1.222
(126) -	(98) .678	(99) .718
(589) 2.527	(125) 1.014	(131) -
$\bar{X}$ ± SEM 1.489 ± .572	.869 ± .164	1.151 ± .232
45% RDN		
(492) .778	(514) .886	(59) .978
(513) .811	(74) .944	(75) .915
(109) .706	(104) 1.034	(91) .662
(117) .782	(553) 1.079	(582) -
$\bar{X}$ ± SEM .769 ± .022	.986 ± .044	.852 ± .097
60% RDN		
(76) 1.066	(84) 1.172	(61) .988
(93) 2.950	(537) .988	(515) .917
(120) 1.161	(111) 1.031	(100) 1.048
(586) 3.762	(143) 2.121	(116) .508
$\bar{X}$ ± SEM 2.235 ± .669	1.328 ± .267	.865 ± .122

[1] µmoles substrate metabolized to CO_2/gram.

Table 16. Labelled CO_2 production from [U-^{14}C] lactate - Experiment 1[1].

Ground		Chopped		Concentrate	
30% RDN					
(77)	.820	(495)	.664	(500)	.775
(103)	.363	(522)	.411	(510)	.434
(126)	.584	(98)	.655	(99)	.705
(589)	1.087	(125)	.787	(131)	1.060
$\bar{X}$ ± SEM	.714 ± .156		.629 ± .079		.744 ± .129
45% RDN					
(492)	.701	(514)	.611	(59)	-
(513)	.605	(74)	.463	(75)	.476
(109)	.495	(104)	.672	(91)	.806
(117)	.620	(553)	.727	(582)	1.443
$\bar{X}$ ± SEM	.605 ± .042		.618 ± .057		.908 ± .284
60% RDN					
(76)	.820	(84)	.719	(61)	.631
(93)	.933	(537)	.803	(515)	.866
(120)	.585	(111)	.417	(100)	.714
(586)	1.040	(143)	.971	(116)	.318
$\bar{X}$ ± SEM	.844 ± .097		.728 ± .116		.632 ± .115

[1] µmoles substrate metabolized to CO_2/gram liver.

Table 17. Labelled glucose production from [1-^{14}C] propionate - Experiment 1[1].

Ground	Chopped	Concentrate
30% RDN		
(77) 9.078	(495) 4.222	(500) 3.074
(103) 3.975	(522) 5.970	(510) 5.886
(126) 4.940	(98) 4.394	(99) 5.106
(589) 1.482	(125) 1.299	(131) 2.556
$\bar{X}$ ± SEM 4.869 ± 1.581	3.971 ± .974	4.156 ± .797
45% RDN		
(492) 6.685	(514) 8.708	(59) 1.526
(513) 2.595	(74) 3.657	(75) 4.065
(109) 1.497	(104) 5.081	(91) 5.657
(117) 1.566	(553) 1.616	(582) 3.456
$\bar{X}$ ± SEM 3.086 ± 1.226	4.766 ± 1.494	3.676 ± .854
60% RDN		
(76) 4.277	(84) 10.582	(61) 4.240
(93) 7.676	(537) 6.424	(515) --
(120) 1.363	(111) 5.141	(100) 5.097
(586) 2.615	(143) 2.123	(116) --
$\bar{X}$ ± SEM 3.983 ± 1.368	6.068 ± 1.754	4.668 ± .428

[1] µmoles substrate metabolized to glucose/gram liver.

Table 18. Labelled glucose production from [2-^{14}C] propionate - Experiment 1[1].

Ground		Chopped		Concentrate	
30% RDN					
(77)	15.777	(495)	4.665	(500)	7.194
(103)	5.548	(522)	10.786	(510)	9.098
(126)	8.333	(98)	7.226	(99)	7.002
(589)	4.946	(125)	3.845	(131)	8.358
$\bar{X}$ ± SEM	8.651 ± 2.487		6.630 ± 1.561		7.913 ± .496
45% RDN					
(492)	9.444	(514)	18.192	(59)	3.352
(513)	3.227	(74)	4.229	(75)	4.648
(109)	1.046	(104)	6.796	(91)	6.666
(117)	3.174	(553)	2.387	(582)	10.140
$\bar{X}$ ± SEM	4.223 ± 1.813		7.901 ± 3.547		6.202 ± 1.479
60% RDN					
(76)	6.145	(84)	18.042	(61)	7.270
(93)	9.540	(537)	8.900	(515)	10.476
(120)	3.561	(111)	7.611	(100)	8.954
(586)	6.128	(143)	5.269	(116)	--
$\bar{X}$ ± SEM	6.344 ± 1.226		9.956 ± 2.798		8.900 ± .926

[1] μmoles substrate metabolized to glucose/gram liver.

Table 19. Labelled glucose production from [U-^{14}C] lactate - Experiment 1[1].

Ground		Chopped		Concentrate	
30% RDN					
(77)	1.351	(495)	.533	(500)	.727
(103)	.536	(522)	.864	(510)	.873
(126)	1.117	(98)	1.179	(99)	.853
(589)	.994	(125)	.441	(131)	.484
$\bar{X}$ ± SEM	1.000 ± .171		.754 ± .168		.734 ± .089
45% RDN					
(492)	.846	(514)	1.653	(59)	--
(513)	.856	(74)	.810	(75)	.987
(109)	.489	(104)	.832	(91)	1.353
(117)	.468	(553)	.330	(582)	1.122
$\bar{X}$ ± SEM	.665 ± .108		.906 ± .275		1.154 ± .107
60% RDN					
(76)	.726	(84)	1.248	(61)	1.157
(93)	1.467	(537)	1.066	(515)	1.991
(120)	.775	(111)	.670	(100)	1.180
(586)	.741	(143)	.747	(116)	--
$\bar{X}$ ± SEM	.927 ± .180		.933 ± .136		1.443 ± .274

[1] µmoles substrate metabolized to glucose/gram liver.

Table 20. Total glucose - Experiment 1[1].

Ground	Chopped	Concentrate
30% RDN		
(77) 3.865	(495) 3.402	(500) 6.199
(103) 4.872	(522) 3.130	(510) 6.898
(126) 6.448	(98) 5.648	(99) 7.887
(589) 7.772	(125) 5.075	(131) 8.592
$\bar{X}$ ± SEM 5.739 ± .861	4.314 ± .619	7.394 ± .529
45% RDN		
(492) 6.403	(514) 6.387	(59) 6.130
(513) 3.338	(74) 4.706	(75) 7.623
(109) 5.007	(104) 5.060	(91) 6.074
(117) 3.962	(553) 6.233	(582) 6.828
$\bar{X}$ ± SEM 4.678 ± .670	5.596 ± .419	6.664 ± .363
60% RDN		
(76) 4.460	(84) 7.984	(61) 7.216
(93) 6.903	(537) 5.923	(515) 4.041
(120) 6.871	(111) 5.474	(100) 7.488
(586) 6.199	(143) 7.113	(116) 4.642
$\bar{X}$ ± SEM 6.108 ± .573	6.374 ± .811	5.842 ± .881

[1]mg/gram liver.

Table 21. Calf body weights - Experiment 1[1].

Ground	Chopped	Concentrate
30% RDN		
(77) 125.2	(495) 104.3	(500) 93.0
(103) 154.2	(522) 115.7	(510) 149.7
(126) 117.5	(98) 106.6	(99) 143.8
(589) 133.4	(125) 87.5	(131) 181.4
$\bar{X}$ ± SEM 132.6 ± 7.9	103.5 ± 5.9	142.0 ± 18.3
45% RDN		
(492) 98.4	(514) 111.1	(59) 123.4
(513) 102.1	(74) 111.1	(75) 121.1
(109) 120.2	(104) 108.0	(91) 136.1
(117) 158.8	(553) 100.7	(582) 131.5
$\bar{X}$ ± SEM 119.9 ± 13.8	107.7 ± 2.5	128.0 ± 3.5
60% RDN		
(76) 116.6	(84) 108.9	(61) 124.3
(93) 152.0	(537) 98.4	(515) 123.4
(120) 130.6	(111) 137.4	(100) 154.2
(586) 117.9	(143) 96.2	(116) 133.4
$\bar{X}$ ± SEM 129.3 ± 8.2	110.2 ± 9.5	133.8 ± 7.2

[1] kg.

Table 22. Calf liver weights - Experiment 1[1].

Ground	Chopped	Concentrate
30% RDN		
(77) 1.77	(495) 1.41	(500) 1.77
(103) 2.45	(522) 1.59	(510) 2.63
(126) 1.59	(98) 1.72	(99) 2.95
(589) 2.27	(125) 1.09	(131) 3.62
$\bar{X}$ ± SEM 2.02 ± .20	1.45 ± .14	2.74 ± .38
45% RDN		
(492) 1.32	(514) 1.68	(59) 2.61
(513) 2.09	(74) 1.72	(75) 2.72
(109) 1.90	(104) 1.59	(91) 2.27
(117) 2.45	(553) 1.59	(582) 2.72
$\bar{X}$ ± SEM 1.94 ± .24	1.64 ± .03	2.58 ± .11
60% RDN		
(76) 1.81	(84) 1.54	(61) 2.13
(93) 2.31	(537) 1.32	(515) 2.45
(120) 1.81	(111) 2.04	(100) 2.90
(586) 1.86	(143) 1.45	(116) 2.31
$\bar{X}$ ± SEM 1.95 ± .12	1.59 ± .16	2.45 ± .16

[1] kg.

Table 23. Calf liver analysis: Dry matter - Experiment 1[1].

Ground	Chopped	Concentrate
30% RDN		
(77) 26.32	(495) 25.21	(500) 25.92
(103) 27.61	(522) 26.48	(510) 27.45
(126) 26.81	(98) 27.49	(99) 26.85
(589 25.89	(125) 26.68	(131) 27.91
$\bar{X}$ ± SEM 26.66 ± .37	26.46 ± .47	27.03 ± .43
45% RDN		
(492) 27.85	(514) 27.82	(59) 26.23
(513) 26.67	(74) 26.89	(75) 27.19
(109) 27.81	(104) 27.04	(91) 26.53
(117) 26.23	(553) 24.80	(582) 27.93
$\bar{X}$ ± SEM 27.14 ± .41	26.64 ± .65	26.97 ± .38
60% RDN		
(76) 27.59	(84) 24.97	(61) 25.79
(93) 27.59	(537) 26.07	(515) 26.98
(120) 28.02	(111) 27.79	(100) 27.15
(586) 27.79	(143) 26.72	(116) 27.06
X ± SEM 27.75 ± .10	26.39 ± .59	26.74 ± .32

[1]%.

Table 24. Calf liver analysis: Protein - Experiment 1[1].

Ground		Chopped		Concentrate	
30% RDN					
(77)	67.45	(495)	69.72	(500)	59.39
(103)	63.65	(522)	68.87	(510)	55.05
(126)	--	(98)	60.83	(99)	55.32
(589)	63.30	(125)	--	(131)	50.32
$\bar{X}$ ± SEM	64.80 ± 1.33		66.47 ± 2.83		55.02 ± 1.85
45% RDN					
(492)	65.04	(514)	64.21	(59)	58.55
(513)	70.82	(74)	65.00	(75)	55.05
(109)	61.33	(104)	62.75	(91)	63.42
(117)	73.60	(553)	64.54	(582)	51.98
$\bar{X}$ ± SEM	67.70 ± 2.77		64.12 ± .49		57.25 ± 2.46
60% RDN					
(76)	69.43	(84)	65.41	(61)	67.23
(93)	66.64	(537)	67.23	(515)	56.19
(120)	68.96	(111)	61.69	(100)	59.45
(586)	72.31	(143)	67.54	(116)	67.03
$\bar{X}$ ± SEM	69.34 ± 1.16		65.47 ± 1.34		62.48 ± 2.77

[1]% on a dry matter basis.

Table 25. Calf liver analysis: Glycogen - Experiment 1[1].

Ground		Chopped		Concentrate	
30% RDN					
(77)	80.77	(495)	165.33	(500)	184.99
(103)	151.03	(522)	103.25	(510)	241.60
(126)	--	(98)	52.58	(99)	224.80
(589)	135.34	(125)	19.50	(131)	154.46
$\bar{X}$ ± SEM	122.38 ± 21.29		85.16 ± 31.79		201.46 ± 19.66
45% RDN					
(492)	132.28	(514)	146.40	(59)	246.32
(513)	50.76	(74)	98.74	(75)	292.35
(109)	86.01	(104)	96.00	(91)	244.40
(117)	25.92	(553)	110.93	(582)	164.41
$\bar{X}$ ± SEM	73.74 ± 23.08		113.02 ± 11.59		236.87 ± 26.57
60% RDN					
(76)	91.99	(84)	134.60	(61)	136.25
(93)	149.15	(537)	148.14	(515)	234.99
(120)	108.64	(111)	68.94	(100)	216.94
(586)	23.75	(143)	130.69	(116)	37.80
$\bar{X}$ ± SEM	93.38 ± 26.13		120.59 ± 17.62		156.50 ± 45.01

[1]mg/gram dry matter.

Table 26. Calf liver analysis: Ether extract - Experiment 1[1].

Ground	Chopped	Concentrate
30% RDN		
(77) 3.72	(495) 3.04	(500) 2.98
(103) 4.77	(522) 2.56	(510) 2.15
(126) --	(98) 3.05	(99) 2.72
(589) 2.41	(125) 3.10	(131) 2.90
$\bar{X}$ ± SEM 3.63 ± .68	2.94 ± .13	2.69 ± .19
45% RDN		
(492) 2.50	(514) 2.07	(59) 2.65
(513) 2.74	(74) 1.98	(75) 2.28
(109) 2.21	(104) 2.42	(91) 2.01
(117) 3.60	(553) 2.34	(582) 3.01
$\bar{X}$ ± SEM 2.76 ± .30	2.20 ± .11	2.49 ± .22
60% RDN		
(76) 2.74	(84) 2.39	(61) 3.42
(93) 2.44	(537) 2.26	(515) 2.57
(120) 2.95	(111) 2.02	(100) 2.48
(586) 3.29	(143) 2.10	(116) 3.30
$\bar{X}$ ± SEM 2.86 ± .18	2.19 ± .08	2.94 ± .24

[1]% on a dry matter basis.

Table 27. Calf liver analysis: Ash - Experiment 1[1].

Ground	Chopped	Concentrate
30% RDN		
(77) 5.62	(495) 5.85	(500) 5.41
(103) 4.36	(522) 5.59	(510) 5.00
(126) --	(98) 4.35	(99) 4.93
(589) 6.20	(125) 5.99	(131) 5.60
$\bar{X}$ ± SEM 5.39 ± .54	5.44 ± .37	5.24 ± .16
45% RDN		
(492) 5.24	(514) 4.62	(59) 5.38
(513) 4.84	(74) 4.88	(75) 5.64
(109) 4.68	(104) 4.83	(91) 5.30
(117) 5.84	(553) 4.80	(582) 6.11
$\bar{X}$ ± SEM 5.15 ± .26	4.78 ± .06	5.61 ± .18
60% RDN		
(76) 6.77	(84) 4.00	(61) 5.62
(93) 6.05	(537) 5.60	(515) 5.01
(120) 6.37	(111) 5.33	(100) 5.53
(586) 5.59	(143) 5.52	(116) 6.53
$\bar{X}$ ± SEM 6.20 ± .25	5.11 ± .38	5.67 ± .32

[1]% on a dry matter basis.

Table 28. Effect of rumen degradable nitrogen on rumen and blood parameters, VFA transport and selected enzymes - Experiment 1[1,2].

Parameter	RDN 30%	RDN 45%	RDN 60%
Ruminal[3], moles/100 mole of acid			
Acetate	57.8 ± 1.1	56.7 ± 1.1	56.6 ± .9
Propionate	29.4 ± 1.3	30.5 ± 1.2	30.3 ± 1.1
Butyrate	8.7 ± .4	8.9 ± .5	8.7 ± .4
Lactate, µg/100 ml	367 ± 63$_{c}$	449 ± 71$_{b}$	282 ± 54$_{a}$
NH_3-N, mg/100 ml	3.7 ± .2^{c}	7.0 ± .3^{b}	11.5 ± 4^{a}
pH	6.3 ± .1	6.3 ± .1	6.3 ± .1
Blood[4]			
Acetate, mg/100 ml	21.9 ± 1.1	21.6 ± 1.1	20.5 ± 1.3
Propionate, mg/100 ml	3.1 ± .2	3.2 ± .3	3.0 ± .2
Butyrate, mg/100 ml	1.4 ± .2	1.3 ± .2	1.2 ± .2
Glucose, mg/100 ml	81.5 ± 2.7$_{b}$	78.0 ± 2.7$_{b}$	78.1 ± 2.4$_{a}$
PUN, mg/100 ml	11.6 ± .7^{b}	12.6 ± .8^{b}	16.3 ± 1.6^{a}
Lactate Production[5,6]	36.4 ± 5	35.5 ± 6	33.8 ± 5
Transport[5,6]			
Acetate	739 ± 129^{b}	1066 ± 176^{ab}	1192 ± 260^{a}
Propionate	174 ± 33^{c}	219 ± 35^{b}	260 ± 71^{a}
Acetate/Propionate	4.6 ± .7	6.2 ± 1.6	6.8 ± 1.4
Enzymes[6,7]			
Lactate Dehydrogenase	4.8 ± 1.1	3.1 ± .6	3.0 ± .2
NADP-Malate Dehydrogenase	1.0 ± .1	1.4 ± .2	1.1 ± .1
Propionyl-CoA Synthetase	12.5 ± 3.5$_{ab}$	14.3 ± 4.7$_{b}$	17.5 ± 1.5$_{a}$
Glutamate Dehydrogenase	.41 ± .04^{ab}	.38 ± .03^{b}	.43 ± .02^{a}
Ala Aminotransferase	1.0 ± .1$_{ab}$	.9 ± .1$_{b}$	1.2 ± .2$_{a}$
Asp Aminotransferase	3.8 ± .3^{ab}	2.6 ± .4^{b}	4.1 ± .6^{a}

[1] Dissertation, J. E. Nocek, 1980, VPI&SU.

[2] Row means with different superscripts are significantly different ($P < .05$) by Duncans.

[3] Mean ± SEM of 18 calves with 5 sample periods per calf.

[4] Mean ± SEM of 18 calves with 2 sample periods per calf.

[5] µg/min/gm dry tissue.

[6] Mean ± SEM of 6 20-week old calves with duplicate values per calf.

[7] µmole/min/gm tissue.

Table 29. Effect of ration physical form on rumen and blood parameters, VFA transport and selected enzymes - Experiment 1[1,2].

Parameter	Physical Form		
	GR	CH	CONC
Ruminal[3], moles/100 mole of acid			
Acetate	65.4 ± .4[a]	60.0 ± .7[b]	45.6 ± .5[c]
Propionate	21.4 ± .5[c]	25.8 ± .8[b]	43.0 ± .7[a]
Butyrate	9.4 ± .2[a]	9.9 ± .3[a]	7.0 ± .5[b]
Lactate, µg/100 ml	403 ± 69[ab]	465 ± 74[a]	230 ± 40[b]
NH_3-N, mg/100 ml	7.5 ± .5[a]	7.5 ± .5[a]	7.3 ± .5[b]
pH	6.7 ± .1[a]	6.6 ± .1[a]	5.6 ± .1[b]
Blood[4]			
Acetate, mg/100 ml	24.3 ± 1.6[a]	22.4 ± 1.2[a]	17.3 ± .8[b]
Propionate, mg/100 ml	3.1 ± .2[ab]	2.6 ± .2[b]	3.6 ± .3[a]
Butyrate, mg/100 ml	1.4 ± .2	1.4 ± .3	1.2 ± .2
Glucose, mg/100 ml	83.1 ± 2.4[a]	72.2 ± 2.3[b]	82.3 ± 2.6[a]
PUN, mg/100 ml	13.9 ± .7	12.5 ± .8	14.1 ± 1.7
Lactate Production[5,6]	42.8 ± 5	26.4 ± 5	36.5 ± 5
Transport[5,6]			
Acetate	1487 ± 209[a]	1097 ± 157[a]	413 ± 55[b]
Propionate	424 ± 38[a]	132 ± 14[b]	97 ± 13[b]
Acetate/Propionate	3.4 ± .3[b]	9.7 ± 1.6[a]	4.5 ± .5[b]
Enzymes[6,7]			
Lactate Dehydrogenase	4.1 ± 1.1	3.0 ± .7	3.7 ± .4
NADP-Malate Dehydrogenase	1.1 ± .2	1.0 ± .1	1.1 ± .1
Propionyl-CoA Synthetase	14.8 ± 2.9[b]	10.5 ± 2.2[b]	19.1 ± 4.3[a]
Glutamate Dehydrogenase	.41 ± .03	.38 ± .03	.42 ± .02
Ala Aminotransferase	1.2 ± .2	1.0 ± .1	.9 ± .1
Asp Aminotransferase	3.9 ± .6	3.1 ± .3	3.5 ± .6

[1] Dissertation, J. E. Nocek, 1980, VPI&SU.

[2] Row means with different superscripts are significantly different ($P < .05$) by Duncans.

[3] Mean ± SEM of 18 calves with 5 sample periods per calf.

[4] Mean ± SEM of 18 calves with 2 sample periods per calf.

[5] µg/min/gm dry tissue.

[6] Mean ± SEM of 6 20-week old calves with duplicate values per calf.

[7] µmole/min/gm tissue.

Table 30. Ration formulations and specifications using ruminal nitrogen degradabilities obtained by the *in situ* polyester bag technique[1,2].

Ingredients	30% RDN			45% RDN			60% RDN		
	Ground	Chopped	Concentrate	Ground	Chopped	Concentrate	Ground	Chopped	Concentrate
Orchardgrass	39.6	40	--	38	38	--	37	37	--
Corn	43	44.8	81	26.1	26.1	74	18	20	60
Wheat Bran	--	--	--	--	--	--	25	28.5	10
Soybean Meal	--	--	8.8	.5	.5	--	--	--	--
Corn Gluten Meal	5.2	5	--	--	--	--	.5	--	--
Cottonsead Meal	--	--	--	7.5	7.5	4	--	--	--
Dehy. Alfalfa	--	--	--	7.0	7.5	4	--	--	--
Corn Starch	--	--	--	9.2	9.2	6.8	--	3.9	17
Urea	--	--	--	--	--	1.0	.4	.4	1.8
Molasses (liq.)	7	5	5	6.5	6.5	5.0	10	5	5.0
Water	3	3	3	3	3	3	3	3	3
TM Salt	1	1	1	1	1	1	1	1	1
$CaCO_3$	1	1	1	1	1	1	1	1	1
Vit.[3] Mix[3]	.2	.2	.2	.2	.2	.2	.2	.2	.2
Specifications									
Cr. Prot.	13.1	13.06	13.01	13.05	13.05	13.09	12.97	13.02	13.053
ADF (%)[5]	15.51	15.69	5.49	16.43	16.43	6.02	14.08	15.41	4.48
Energy (Mcal/d)[4]	10.90	10.92	12.08	10.04	10.04	11.86	9.56	9.49	11.50
RDN (%)	30.64	29.8	29.64	45.57	45.57	45.15	59.40	60.45	60.19
Soluble (%)[6]	17.31	16.29	18.03	28.55	28.55	38.65	30.03	29.33	50.37
CA (g/d)[4]	19.55	19.47	14.50	22.16	22.16	15.7	20.26	20.08	21.73
P (g/d)[4]	10.3	10.53	10.51	10.78	10.78	10.08	17.15	18.72	16.77

[1] From (121).

[2] Calculated on dry matter basis.

[3] Vit. A = 19008 IU/kg; Vit. D = 21648 IU/kg.

[4] Assumed 3.2 kg/d intake.

[5] % ADF, Energy Ca and P taken from NRC (120).

[6] % Soluble N value obtained from Dairy Ration Formulation.

Table 31. Labelled CO_2 production - Experiment 2[1].

Cow	Stage of Lactation				
	30D	60D	90D	120D	180D
Control Ration					
1211		1.939	1.453	1.798	1.378
1187	1.496	2.098	2.144	1.597	-
1239	2.007	2.237	2.560	1.774	-
1230		2.664	1.866	1.876	1.108
1231		1.533	1.292	1.494	1.351
HI CONC Ration					
1106		1.177	2.129	1.966	2.105
1178	2.448	1.302	1.554	2.592	1.439
1229	1.984	2.178	2.210	1.510	1.241
1191		1.422	1.319	2.066	1.359
1222		.930	1.411	1.796	1.382

[1] μmoles [2-^{14}C] propionate metabolized/gram liver.

Table 32. Labelled glucose production - Experiment 2[1].

Cow	Stage of Lactation				
	30D	60D	90D	120D	180D
Control Ration					
1211		4.181	2.616	1.568	.849
1187	5.092	1.450	1.550	1.760	2.537
1239	2.689	1.445	.676	2.308	-
1230		3.830	1.235	1.372	.340
1231		2.096	1.358	.914	1.394
HI CONC Ration					
1106		2.095	1.697	2.173	.602
1178	2.182	1.232	1.726	3.969	.801
1229	3.188	.260	3.409	-	1.893
1191		2.234	1.687	2.311	2.115
1222		2.801	1.656	3.725	1.382

[1] μmoles [2-^{14}C] propionate metabolized/gram liver.

Table 33. Total glucose - Experiment 2[1].

Cow	Stage of Lactation				
	30D	60D	90D	120D	180D
Control Ration					
1211		-28.00	-10.77	-3.35	2.08
1187	-5.72	-12.41	-6.30	5.63	11.89
1239	-18.09	-14.23	5.52	-9.10	-
1230		-14.05	-.57	-7.66	.49
1231		-9.03	-8.96	2.63	5.91
HI CONC Ration					
1106		-.11	-9.31	-12.65	-1.24
1178	-14.04	-12.59	-11.07	-12.81	6.66
1229	-8.71	-8.31	-6.46	-	9.19
1191		6.34	-5.68	-11.26	3.39
1222		-2.49	-5.73	-4.87	6.04

[1]mg/gram liver.

Table 34. Labelled CO_2 production, labelled glucose production and total glucose for dry cows - Experiment 2.

Cows	$^{14}CO_2$[1]	^{14}C-Glucose[1]	Total Glucose[2]
1112	1.870	1.471	-7.51
1057	2.135	2.267	-4.69
953	1.795	1.566	-5.72
1101	1.802	1.991	-6.90
1084	2.310	2.130	-9.06

[1] µmoles [2-^{14}C] propionate metabolized/gram liver.

[2] mg/gram liver.

Table 35. Basal blood glucose concentrations - Experiment 2[1].

Cows	Stage of Lactation			
	30D	60D	90D	120D
Control Ration				
1211		63.3	64.8	70.7
1187	58.7	63.3	68.2	65.7
1239	71.6	64.1	65.5	70.2
1230		69.7	68.2	66.7
1231		--	--	71.4
HI CONC Ration				
1106		70.0	72.0	83.2
1178	65.4	65.9	65.0	68.1
1229	64.4	64.5	66.6	65.2
1191		63.6	65.3	65.3
1222		58.9	70.0	66.1

[1]mg/100 ml.

Table 36. Basal blood insulin concentrations - Experiment 2[1].

Cows	Stage of Lactation			
	30D	60D	90D	120D
Control Ration				
1211		.711	.502	.802
1187	.774	.716	1.050	.919
1239	.528	.487	.724	1.136
1230		.541	.649	.548
1231		--	--	.605
HI CONC Ration				
1106		.636	.895	1.208
1178	.554	.537	.646	1.084
1229	.505	.542	.796	.885
1191		1.340	.747	.728
1222		.507	1.011	.794

[1]ng/ml.

Table 37. Liver protein - Experiment 2[1].

Cows	Stage of Lactation				
	30D	60D	90D	120D	180D
Control Ration					
1211		--	12.71	11.97	14.46
1187	10.85	14.13	--	14.47	14.30
1239		--	11.80	14.51	--
1230		--	15.24	8.77	10.52
1231		13.96	12.56	--	15.34
HI CONC Ration					
1106		12.46	13.43	--	--
1178	14.07	14.87	14.77	15.03	--
1229	15.74	17.19	11.08	12.57	--
1191		13.88	14.31	14.82	--
1222		16.64	11.61	15.98	17.94

[1]mg/gram liver.

Appendix B

APPENDIX 2 - SURFACE RESPONSE STUDIES

B.1 METHODS AND MATERIALS

COWS

Animals and procedures were the same as Experiment 2 with the following changes. Incubation media (pH 7.4) consisted of 2 ml calcium free Krebs-Ringer bicarbonate buffer and 1 ml substrates; sodium salts of acetate (15 mM) and pyruvate (1 mM), n-butyric acid (5 mM), and L-lactic acid (10 mM). Five concentrations of each of the following were used in a response surface design: sodium salt of propionate (5,7.5,10,12.5, and 15 mM), D-glucose (0,1.25,2.5,3.75, and 5 mM), and ammonium chloride (0,2.5,5,7.5 and 10 mM). Concentrations are expressed in terms of final concentration in the incubation media. Propionate metabolism was traced using the radioactively labelled isotope [2-^{14}C]propionate, .5microcurie/flask.

CALVES

Animals and procedures were the same as Experiment 1 with the following changes. Incubation media was the same as mentioned with the following substrate changes; acetate (20 mM), pyruvate (2 mM) and L-lactic acid (20 mM). The tracers used were [2-^{14}C]propionate and [U-^{14}C]lactate at .5microcurie/flask.

B.1.1 Assays

Glucose was assayed as stated in Experiments 1 and 2. Data was analyzed by multiple regression. Regression equations ($P<.05$) were obtained by backward elimination on the initial variables of propionate concentration (PROP), $PROP^2$, glucose concentration (G), G^2, ammonium chloride concentration (NH), NH^2, PROP x G, PROP x NH, G x NH, and PROP x G x NH.

B.2 RESULTS

Figure 9 shows a graphical representation of the experimental design. Regression equations for calf (Table 38) amnd cow (Table 39) experiments are given with R^2 values and statistical probabilities. Graphs (Figures 10-15) show effects of substrate concentrations on carbon dioxide and glucose production.

B.3 DISCUSSION

At present the usefulness of this kind of experiment is debatable in this research. Central composite designs allow the use of a wide range of concentrations. This is beneficial to obtain results but the value of the observations is questionable. Some of the concentrations used in these experiments represent extremes and are not necessarily phy-

siological. The additional problem of varying three substrate concentrations simultaneously leads to interpretation problems.

The data given here is interesting but not fully understandable. Until more knowledge is gained about enzymes and hormones in ruminant liver metabolism this approach would be best avoided.

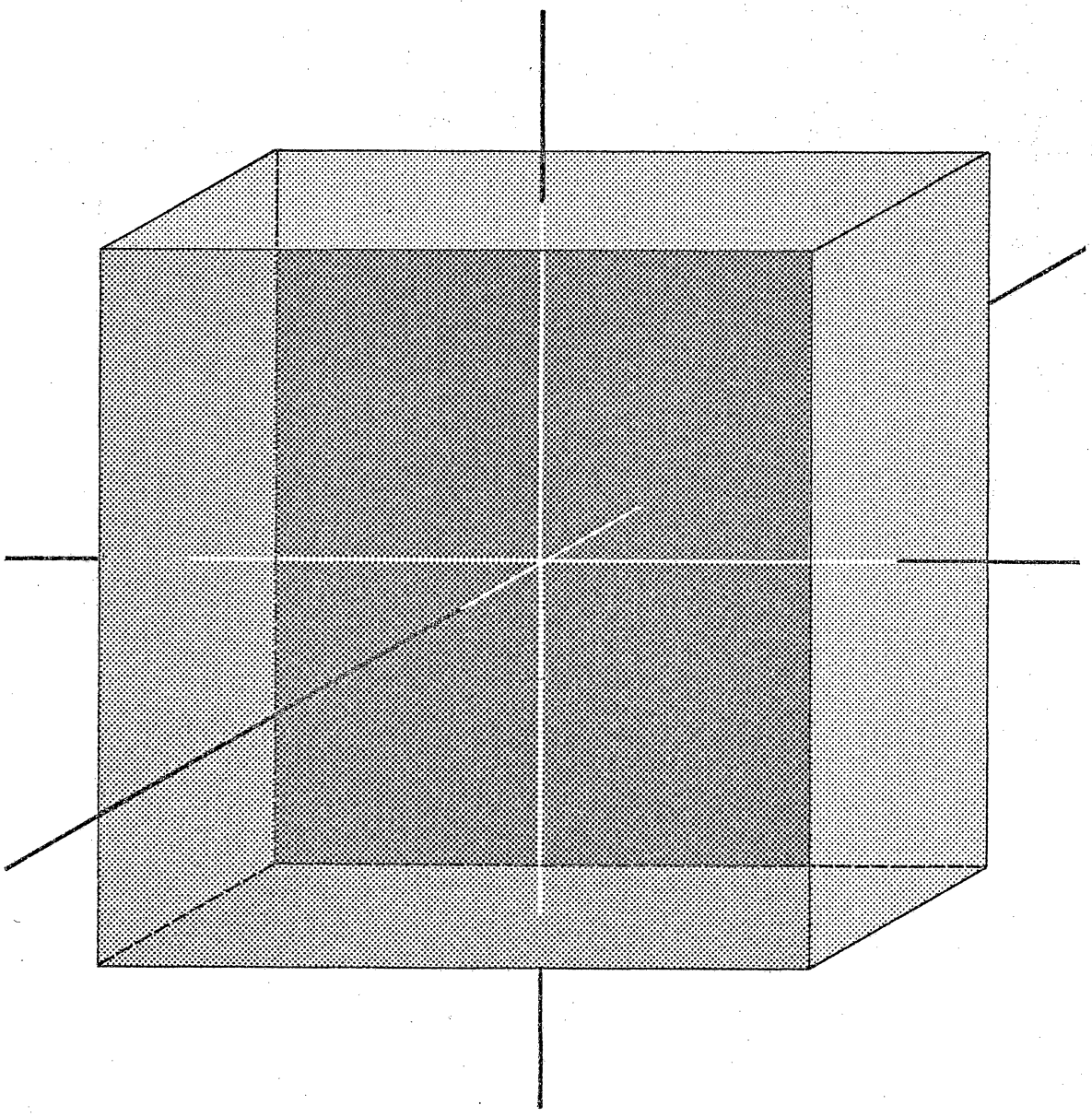

Figure 9. Central composite design for three factors.

Table 38. Multiple regression equations from central composite experiments with bull calves[1,2].

Equation	R^2	P<
[2-^{14}C] Propionate		
$^{14}CO_2$ = -131.64 + 29.64 (P) + 24.64 (G) + 8.01 (NH) - .87 (P^2) - 5.06 (G^2) - NH^2	.41	.0001
^{14}C-Glucose = -70.19 + 40.01 (P) - 18.43 (G) + 9.27 (NH) - 1.13 (P^2) + .90 (NH^2) - 2.72 (PNH) + .18 (PGNH)	.13	.049
Total Glucose = 270.45 - 11.50 (P) - 28.78 (G) -14.03 (NH) + .26 (P^2) + 1.01 (NH^2) + 1.69 (PG)	.15	.01
[U-^{14}C] Lactate		
$^{14}CO_2$ = 254.83 - 26.86 (P) + 2.44 (NH) + 1.48 (P^2) + 3.35 (G^2) - 1.67 (PG)	.09	.155
^{14}C-Glucose = 287.92 - 31.96 (P) + 1.73 (P^2) + 5.37 (G^2) - 2.72 (PG) + .36 (PNH)	.08	.184
Total Glucose = 79.16 + 16.30 (NH) + .21 (P^2) -1.24 (G^2) - .71 (NH^2) - .75 (PG) - -.82 (PNH)	.13	.049
[2-^{14}C] Propionate		
$^{14}CO_2$ = 195.36 - 11.57 (P) - 37.33 (G) - 4.17 (L) + 1.06 (P^2) + .03 (L^2) + 1.83 (PG) + 1.04 (GL)	.48	.0001
^{14}C-Glucose = -61.15 + 21.20 (P) + 15.51 (G) -.43 (P^2) + 2.85 (G^2) - 2.88 (PG)	.22	.0004
Total Glucose = 5.36 + 7.75 (P) + 16.37 (G) + 5.14 (L) + .21 (P^2) - .06 (L^2) - 2.64 (PG) - .31 (PL)	.22	.003

[1]Expressed as a % of response when centerpoint concentrations are used.

[2]Concentration variables in mM where: P = propionate; G = glucose; NH = NH_4Cl; L = lactate; P^2 = propionate squared; G^2 = glucose squared; NH^2 = NH_4Cl squared; L^2 = lactate squared; PG, PNH, PL, GNH, GL, PGNH, PGL = interaction terms.

Table 39. Multiple regression equations from central composite experiments with dairy cows[1,2].

Equation	R^2	P<
[2-^{14}C] Propionate		
All Cows		
$^{14}CO_2$ = 96.43 - 31.93 (G) - 16.40 (NH) + 3.53 (PG) + 1.74 (PNH) + 6.55 (GNH) -.70 (PGNH)	.35	.0001
^{14}C-Glucose = 46.60 + 4.45 (P) + 10.39 (NH) - .25 (P^2) - .71 (NH^2) - 1.74 (GNH) + .15 (PGNH)	.05	.0003
Total Glucose = -150.77 + 168.90 (G) + 1.03 (P^2) + 1.53 (NH^2) - 8.08 (PG) - 5.79 (GNH)	.04	.001
30 day Cows		
$^{14}CO_2$ = 3.36 + 13.98 (P) - 10.67 (G) - .47 (P^2) + 1.19 (PG) - .50 (PNH) + .21 (PGNH)	.41	.0001
^{14}C-Glucose = 114.35 - 17.38 (G) - .41 (P^2) - 3.24 (G^2) + 2.33 (PG) + 2.22 (GNH)	.33	.0007
Total Glucose = 2.42 - 37.57 (G) + 5.66 (G^2) + 7.11 (PG) + 14.41 (GNH) - 1.36 (PGNH)	.46	.0001

[1]Expressed as a % of response when centerpoint concentrations are used.

[2]Concentration variables in mM where: P = propionate; G = glucose; NH = NH_4Cl; P^2 = propionate squared; G^2 = glucose squared; NH^2 = NH_4Cl squared; PG, PNH, GNH, PGNH = interaction terms.

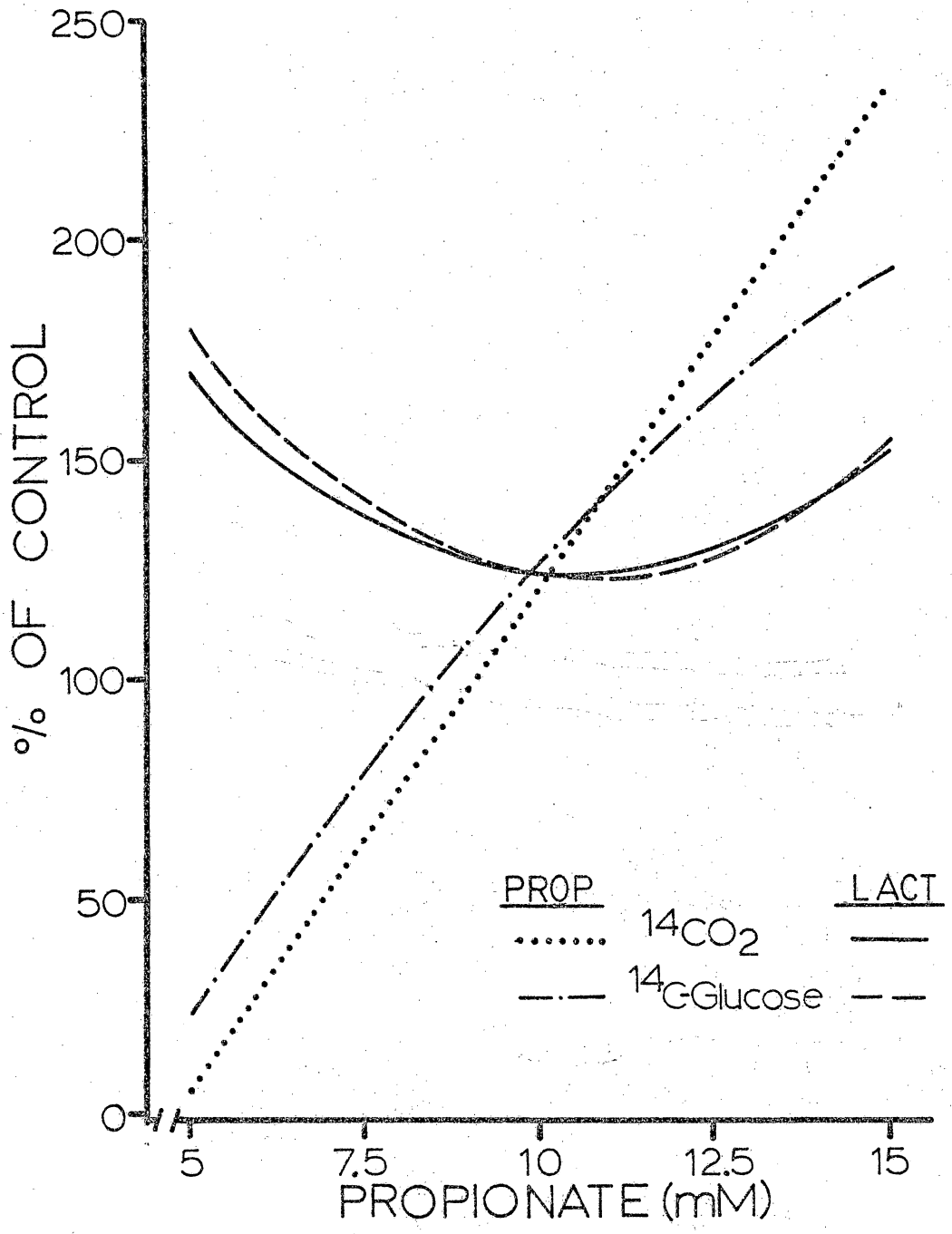

Figure 10. Effect of propionate concentration on labelled CO_2 and labelled glucose production from [2-^{14}C] propionate and [U-^{14}C] lactate in bull calves. (Control = Centerpoint concentrations)

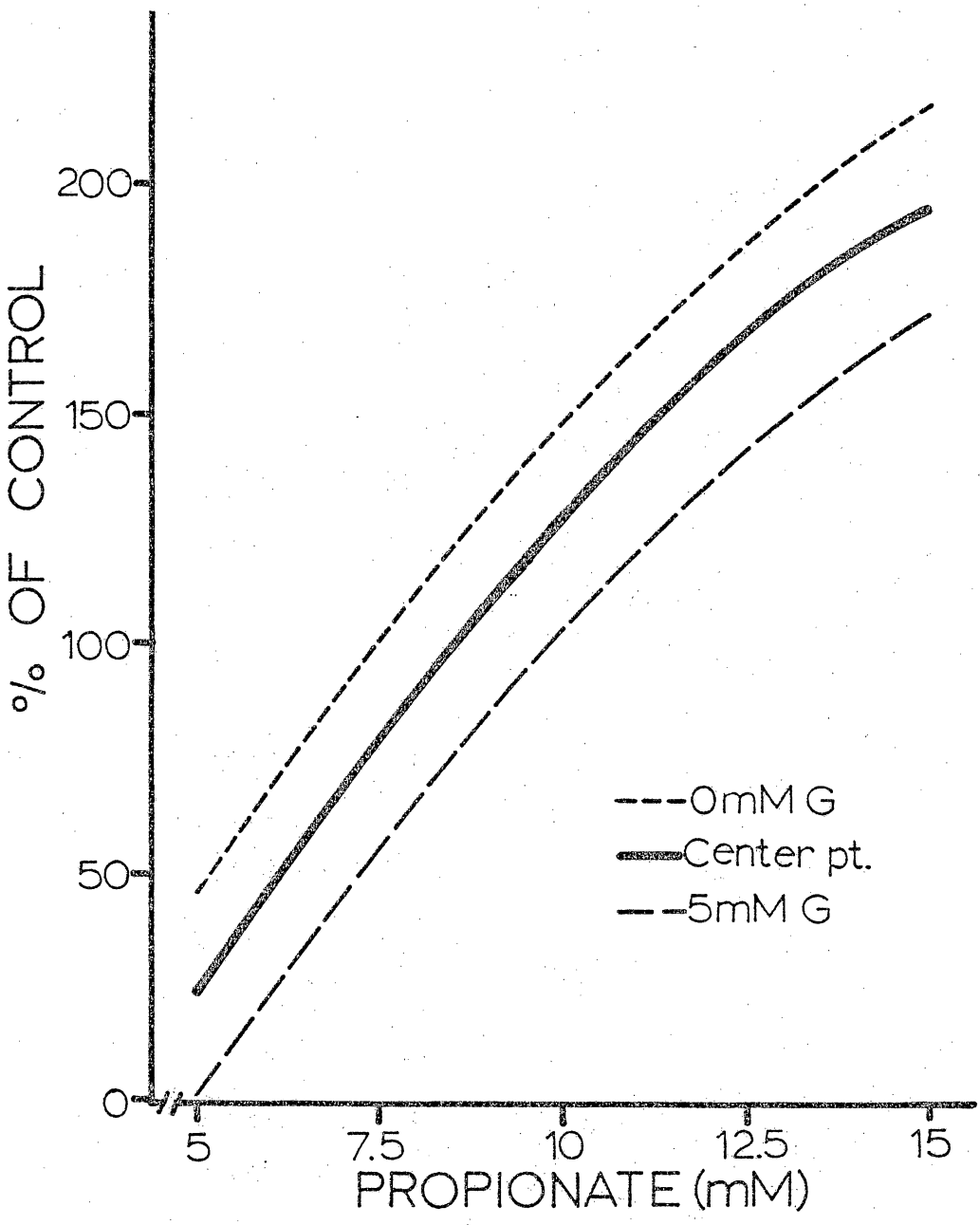

Figure 11. Effect of glucose concentration on labelled glucose production from [2-^{14}C] propionate in bull calves. (Control = Centerpoint concentrations)

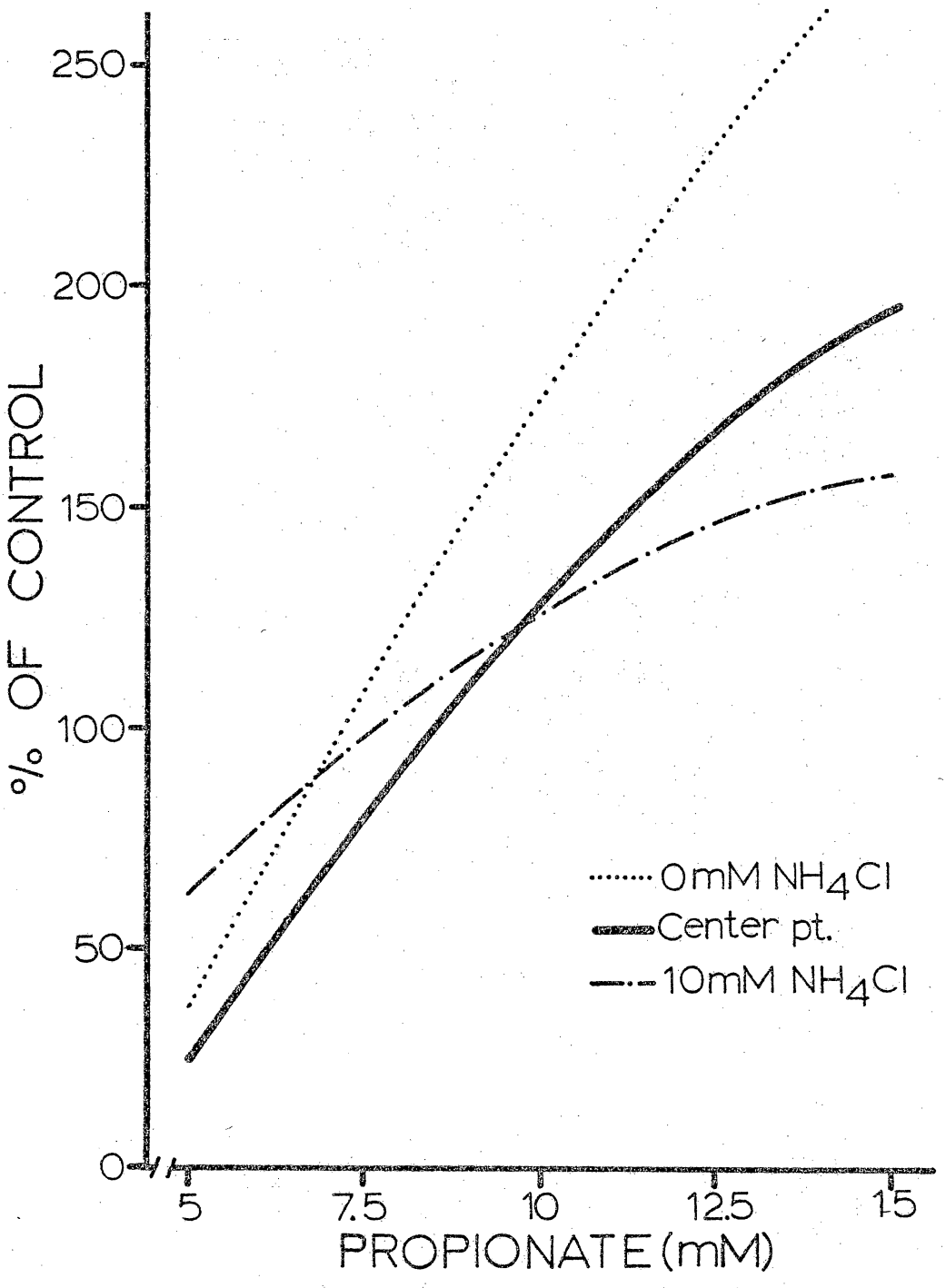

Figure 12. Effect of NH_4Cl concentration on labelled glucose production from [2-^{14}C] propionate in bull calves. (Control = Centerpoint concentrations)

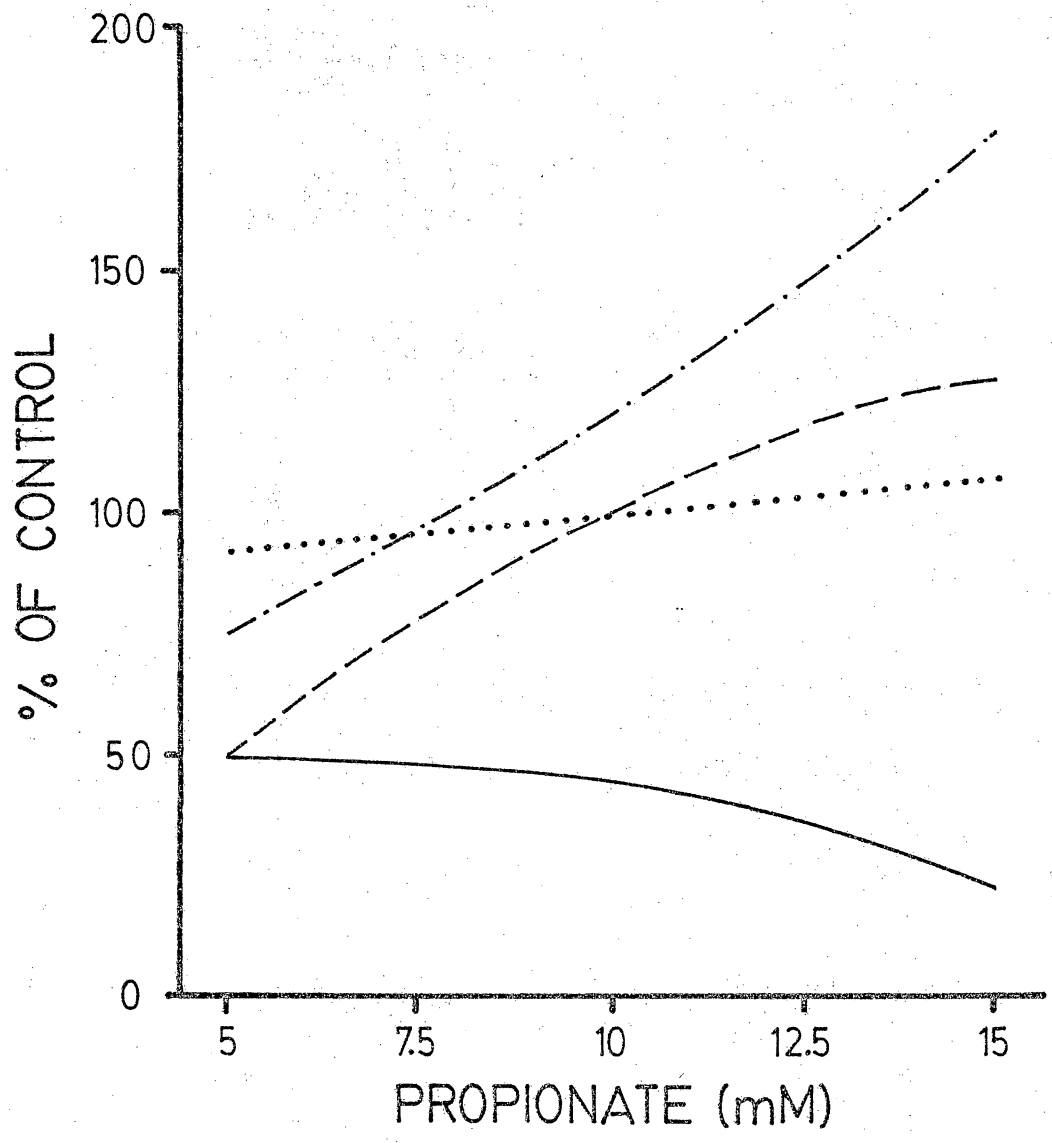

Figure 13. Effect of propionate concentration on labelled CO_2 and labelled glucose production from [2-^{14}C] propionate in dairy cows. (Control = centerpoint concentrations; All cows, CO_2 —·—; 30 day cows, CO_2 ---; All cows, glucose ···; 30 day cows, glucose ——)

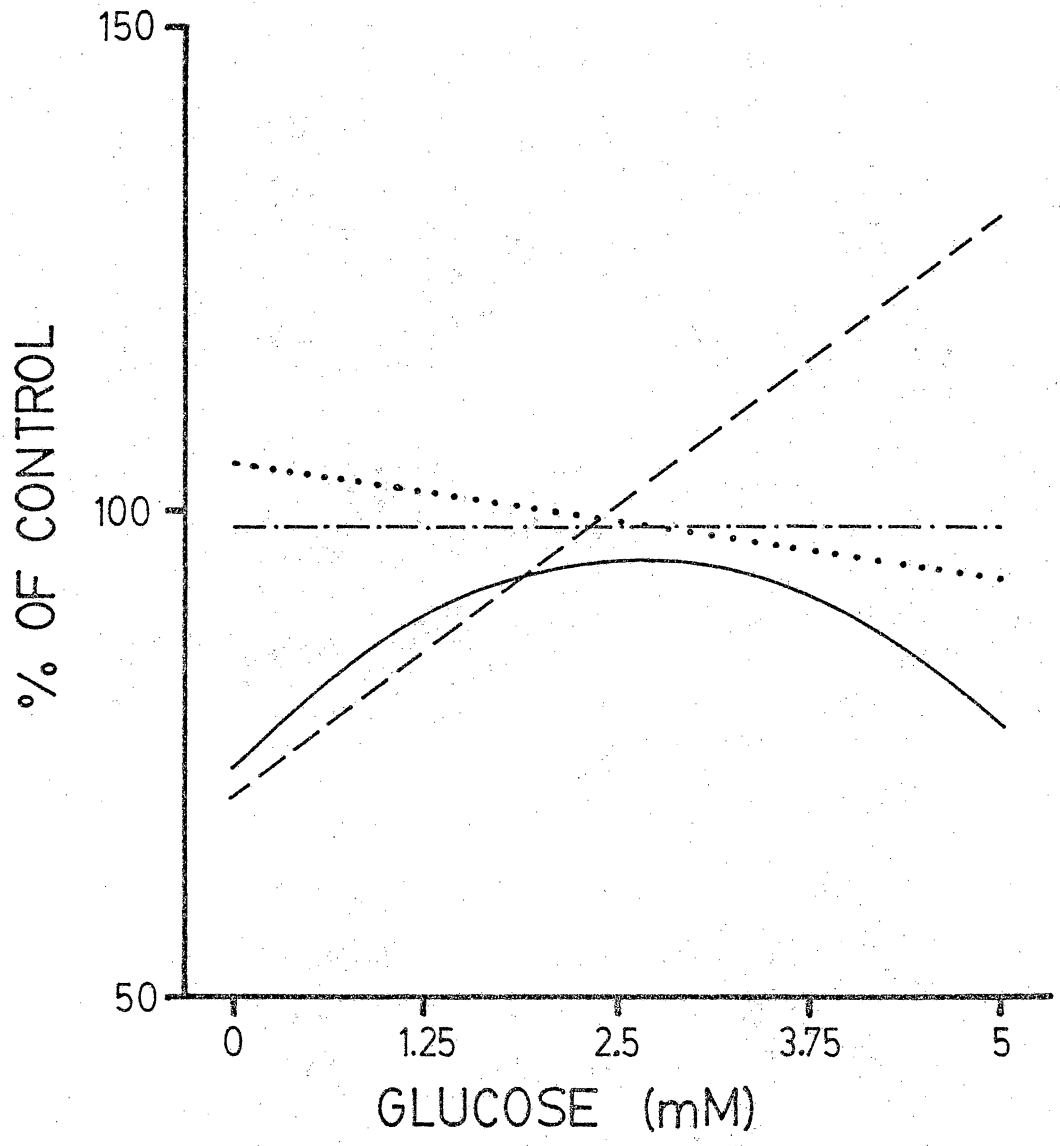

Figure 14. Effect of glucose concentration on labelled CO_2 and glucose production from [2-^{14}C] propionate in dairy cows. (Control = centerpoint concentrations; All cows, CO_2 —·—; 30 day cows, CO_2 ---; All cows, glucose ···; 30 day cows, glucose ——)

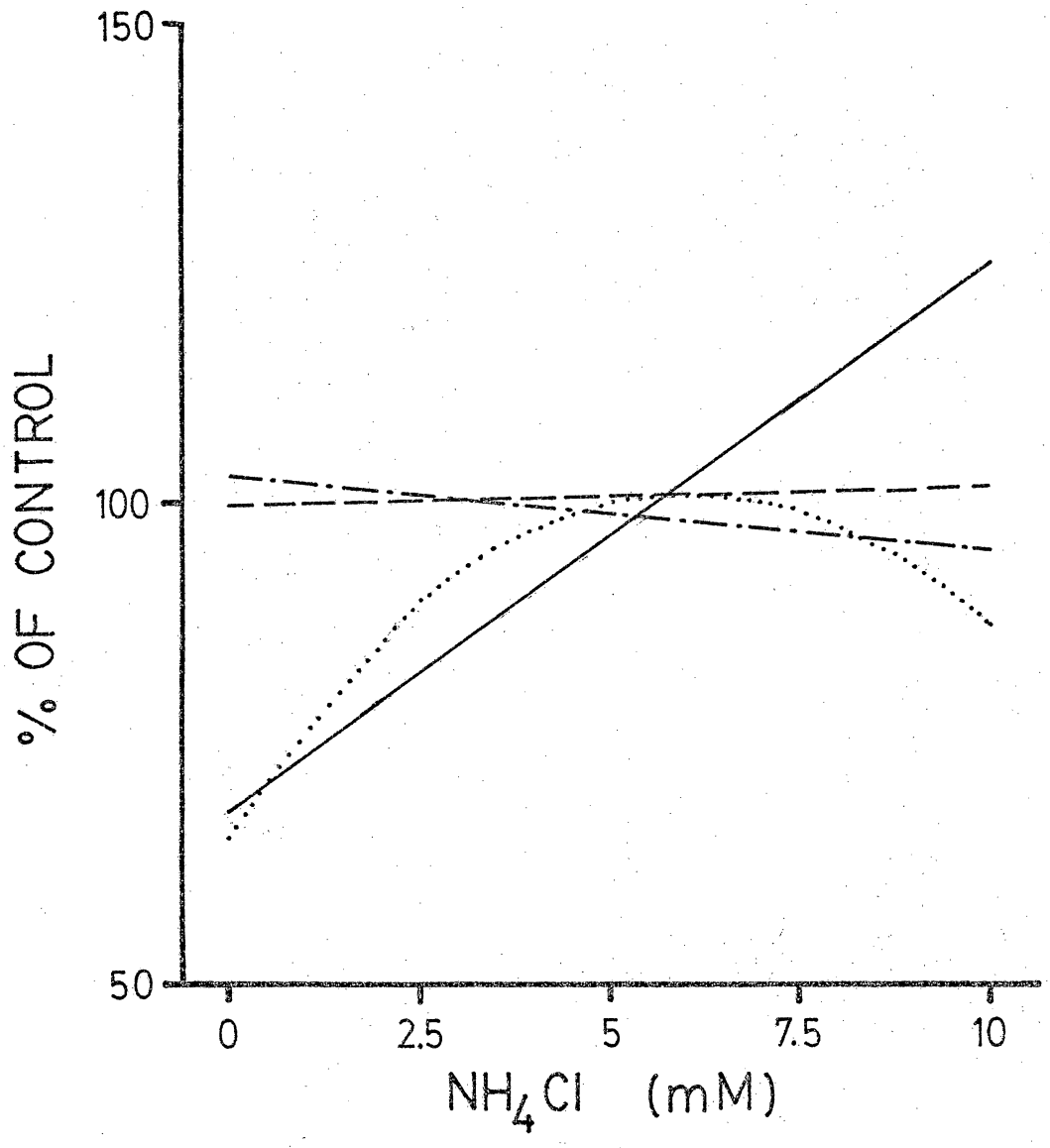

Figure 15. Effect of NH_4Cl concentration on labelled CO_2 and labelled glucose production from [2-^{14}C] propionate in dairy cows. (Control = centerpoint concentrations; All cows, CO_2 —·—; 30 day cows, CO_2 ---; All cows, glucose ···; 30 day cows, glucose ——)

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Gluconeogenesis in the liver of growing and lactating ruminants: The influence of ration composition and stage of lactation.

by

Twila M. Kenna

(ABSTRACT)

Thirty six Holstein bull calves were assigned to a 3X3 factorial design with ration physical forms of chopped hay (CH), ground hay (GR) and all concentrate (CONC) and rumen degradable nitrogen (RDN) of 30, 45 and 60%. Metabolic body weight, liver weight and blood glucose were greatest in CONC-fed calves. Neither dry matter nor ash content of liver tissue was affected by either physical form or RDN. Within physical form, protein content was highest and fat lowest in livers of CH-fed calves. Within RDN, livers of calves fed 30% RDN rations had lowest protein and highest fat content. RDN percent had no effect on carbon dioxide production. Livers from CONC-fed calves produced significantly ($P<.05$) more carbon dioxide from [1-^{14}C]propionate and [U-^{14}C]lactate. Glucose production from propionate and lactate was greatest for CONC-fed and 60% RDN-fed calves, although significant only from lactate within physical form. Interaction between ration physical form and RDN makes interpretation of propionate data

difficult. In 30% RDN-fed calves both lactate and propionate metabolism was similar for GR- and CONC-fed animals. At 45 and 60% RDN rations, CONC- and CH-fed calves had similar gluconeogenic rates from propionate but glucose from lactate was significantly greater in livers of CONC-fed calves compared to GR- and CH-fed animals.

Ten lactating and five dry Holstein cows were liver biopsied at various stages of lactation (30,60,90,120,180 days postpartum and dry) and when fed two rations, control (18% CP) or high concentrate (HI CONC, 10.5% CP). Lactating cows at 30 and 60 days and dry cows were fed the control ration. After 60 days half the cows remained on the control ration and half the lactating cows were changed to the HI CONC ration. Milk fat percent was depressed in cows fed HI CONC ration. HI CONC cows consumed more dry matter and due to the higher energy content reached a positive energy balance before control cows. Rumen fluid volatile fatty acid production was not affected by stage of lactation or ration fed. Both plasma glucose and plasma insulin nonsignificantly increased as lactation progressed. Liver tissue protein did not change over lactation but increased when HI CONC ration was fed. Stage of lactation affected ($P<.0002$) propionate metabolism to carbon dioxide with higher production at 120 days compared to 180 days. By ration, HI CONC cows' livers produced more carbon dioxide than control cows' livers. Feeding the HI CONC ration significantly increased labelled glucose production.

Increased production of both labelled carbon dioxide and glucose by liver from cows fed HI CONC ration was nonsignificant when based on liver protein content. Carbon dioxide/glucose ratio was similar for lactating (>60 days) and dry cows. Cows early in lactation (30 days) had significantly lower carbon dioxide/glucose ratios, indicating a higher glucose production.

Ration and stage of lactation are important factors in ruminant metabolism. Gluconeogenesis, a pathway especially important in ruminants, is affected by both factors. In calves and cows, concentrate rations increased glucose production. Ration RDN, studied in calves, affected lactate metabolism to glucose. The liver of lactating cows showed a greater response (increase in glucose production) to stage of lactation than ration fed demonstrating the importance of energy balance on ruminant metabolism.