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# Milk production and energy metabolism in ruminants fed 2-ketoisocaproate

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# Milk production and energy metabolism in ruminants fed 2-ketoisocaproate

by

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# INTRODUCTION

Since his origin, man has learned to domesticate animals and utilize them as a source of labor, meat, milk, clothing, and various other supplies. Animal agriculture was important to the people of the Old Testament: Abel was a shepherd; Abraham, Isaac, Jacob, Job, and others had vast herds of sheep, goats, cattle, camels, and donkeys. Domestic animals served a vital role in the development of civilization as we know it today. In Genesis 3, God gave Adam the cultural mandate -- "to develop and preserve His creation," to be a steward of the earth.

This, I believe, is God's mandate for us today also -- to continually learn about creation and explore new ways to develop it while recognizing that all of creation has value in and of itself and that in our developing, we must also "preserve." This is a difficult task to comprehend, let alone carry out, but for animal scientists I think it means that we must explore and develop new ways to use animals to serve the needs of a growing human population while recognizing that animals are not just machines but fellow creatures and must be treated with respect. We must develop more efficient systems for using animals to produce quality human-consumable products (i.e., meat, milk, eggs) from substances that are less favorable for human consumption (i.e., forages, industrial byproducts). To maximize efficiency, inputs of substances that are human-consumable also may be required. Bywater and Baldwin (1980) have calculated efficiency values for the major animal industries (Table 1).

Table 1. Percentage returns on digestible inputs

| | Total Inputs | | Human-Edible Inputs | |
|---|---|---|---|---|
| | Energy | Protein | Energy | Protein |
| Dairy Industry | 23 | 29 | 101 | 181 |
| Beef Industry | 5 | 5 | 57 | 109 |
| Pork Industry | 23 | 38 | 58 | 86 |
| Poultry Industry | 15 | 30 | 31 | 75 |

In my opinion, animal agriculture has and will continue to play a pivotal role in meeting the needs of an ever-increasing world population. About 30% of the land in this country is currently rangeland or pastureland (Wedin et al., 1975), and good soil stewardship requires that much be kept in pasturing systems. Therefore, for the foreseeable future, animals will be needed to utilize this land for human food production.

2-Ketoisocaproate, KIC, has previously been reported to have effects on mammalian protein metabolism. With these reports in mind, we thought that KIC also might have effects on protein metabolism of the mammary gland of a lactating animal and therefore increase the efficiency with which animals use dietary protein to produce milk protein. As the data reported in this thesis indicate, KIC actually seems to increase the production of milk fat to a greater degree than that of milk protein. This was a surprise, and we have yet to understand the mechanism for this effect.

One could argue, in light of the current glut of dairy products in the U.S. and the apparent detrimental role of animal fats in human health, that an increase in milk fat is not needed at this time, and, in

fact, is not good stewardship of resources. Under the current milk pricing system in this country, however, the price a farmer receives for the milk he produces is determined partly by its fat content. Therefore, KIC potentially could increase the dairyman's profit. But in light of God's cultural mandate for man, the only valid reason for continued research on KIC and lactation is that KIC does hold promise for increasing the energetic and protein efficiency of the lactating cow. Additionally, a better understanding of the mechanism of KIC's effects will increase our understanding of the basic physiology of a lactating dairy animal and therefore possibly enable us to increase the efficiency of lactation. This idea that KIC may hold potential for increasing the efficiency of milk production is the basis for the research presented in this dissertation.

The work for this dissertation is divided into two parts. In the first part, studies with lactating goats and cows demonstrated that KIC increases milk fat production. The mechanism of action, however, was not readily apparent from the data collected in these studies. Thus, a study was conducted with growing lambs to 1) determine whether the effect was unique for KIC among the leucine metabolites, 2) determine whether the mechanism involved changes in glucose, acetate, or protein turnover, and 3) determine whether lipid deposition in body stores also was altered. By answering these questions, we hoped to gain insight into the mechanism of action for KIC in lactating ruminants. Results of the protein turnover measurements are reported in a dissertation by Paul J. Flakoll.

# GENERAL REVIEW OF THE LITERATURE

## Metabolism of the Lactating Ruminant

The capacity of the lactating mammary gland to produce milk is determined by the degree of development of the mammary gland prepartum and by the ability of the animal to supply the necessary substrates for milk synthesis. This review will deal primarily with the topic of substrate supply and some basic aspects of milk synthesis. More about the topic of mammary gland development can be found in reviews by DeLouis et al. (1980), Tucker (1979), and by several authors in volumes edited by Larson and Smith (1974).

During her 305-day lactation, a high-producing dairy cow will secrete ~1300 kg of milk solids, about double her body weight. At parturition, she shifts from a physiological state of near maintenance (requiring about 13 Mcal $NE_l$, 1 kg crude protein, 38 g calcium, and 27 g phosphorus per day) to the lactational state in which her requirements are increased to about 38 Mcal $NE_l$, 3.8 kg protein, 130 g calcium, and 90 g phosphorus. The 40 kg of milk she produces per day contains 27 Mcal gross energy, 1.3 kg protein, 50 g calcium, and 40 g phosphorus. As one might expect, this abrupt shift to the lactational state requires major changes in her metabolism - changes that are complex and closely regulated. The mammary gland becomes the focal point of the body, and nutrients are partitioned to it rather than to other body tissues (Figure 1). The term "homeorhesis" has been used to describe this focusing of the various body systems toward establishing and providing for one particular body process, i.e., the synthesis of milk. Fatty acids,

glycerol, and amino acids are mobilized from adipose tissue and muscle, gluconeogenesis is increased, calcium and phosphorus are mobilized from bone, appetite is increased, and blood flow to the mammary gland is increased. The activity of many mammary gland enzymes, such as lipoprotein lipase and acetyl-CoA carboxylase, is also increased. These changes are coordinated by a number of hormonal systems, including growth hormone and insulin.

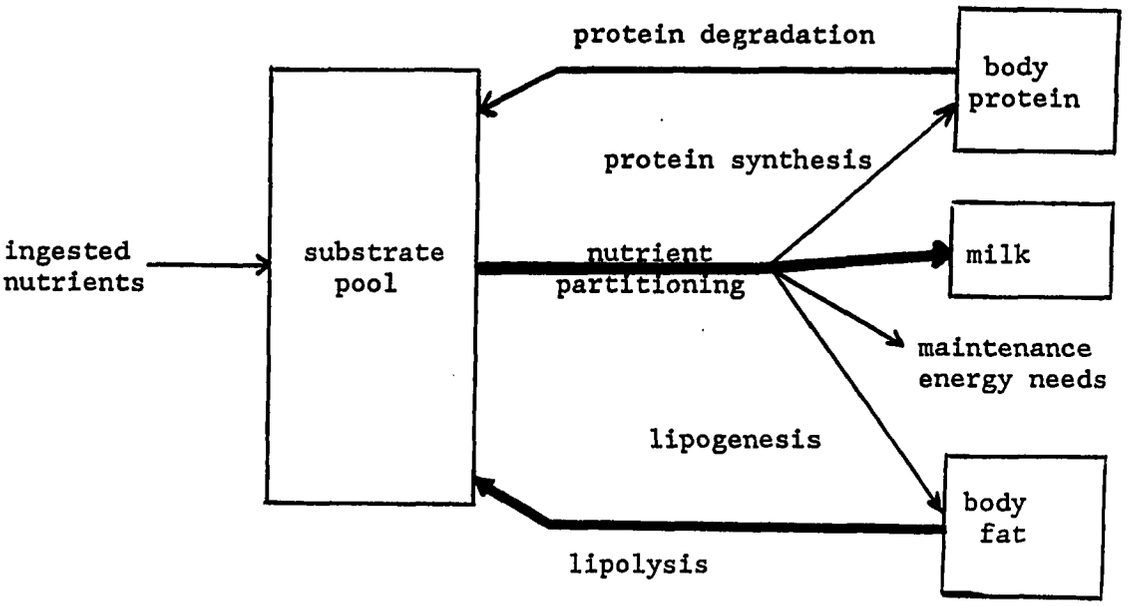


Figure 1. General flow of metabolites during early lactation

Milk synthesis

Concentration of metabolites in arterial blood, blood flow to the mammary gland, and transport of metabolites across the mammary membranes are all important factors in determining substrate supply to the synthetic processes within the mammary gland. Of these three, Davis and Collier (1985) have proposed that blood flow and transport across membranes are the most important. A cow producing 30 kg milk per day secretes daily 1600 g of lactose, 1100 g fat, and 1000 g protein and requires inputs to the mammary gland of about 2200 g glucose, 760 g fat, 950 g acetate, 170 g ketone bodies, and 1000 g of amino acids (Baldwin and Smith, 1979). Although concentrations of many of these metabolites in blood are not elevated during lactation, mammary blood flow is increased, and transport mechanisms for triglyceride-fatty acids, glucose, and amino acids are enhanced.

Fat synthesis Milk fat is 98% triglyceride (Christie, 1981) with fatty acids being derived from two sources. Preformed lipids in the blood account for about 60% by weight or 40 molar % of milk fatty acids, and de novo synthesis of fatty acids within the gland accounts for the rest (Bickerstaffe and Annison, 1974). Nearly all the C4-C14 fatty acids and 60% of C16 are synthesized de novo, whereas the C18 fatty acids and the other 40% of C16 are transported into the gland from blood (Palmquist et al., 1969).

De novo fatty acid synthesis In ruminants, the primary substrates for de novo synthesis are acetate and 3-hydroxybutyrate (BHB). Fatty acid synthesis is a cytosolic process (Davis and Bauman, 1974) in

which successive two-carbon units are added to a "primer" fatty acyl-CoA. Ruminant fatty acid synthetase prefers to use a four-carbon acyl-CoA as its "primer" (Smith and Abraham, 1971), and BHB supplies about 50% of the initial four carbons of de novo fatty acids (Palmquist et al., 1969). Because BHB dehydrogenase is found almost exclusively in the mitochondria, and because the citrate cleavage pathway is not very active in ruminant mammary gland, very little BHB-carbon is incorporated into fatty acids after the initial four carbons (Bauman et al., 1970). BHB contributes no more than 20% of the carbon for de novo synthesis, and more than 80% of the total BHB incorporated into fatty acids is found in the initial four carbons (Smith and McCarthy, 1969). The other 80% of de novo fatty acid-carbon is derived primarily from acetate.

Although glucose is the major source for fatty acid synthesis in nonruminant mammary cells (Bauman et al., 1970), citrate cleavage enzyme activity is low in ruminant mammary tissue, and therefore ruminants use very little glucose for fatty acid synthesis (Bauman et al., 1970; Bauman and Davis, 1975). Low citrate cleavage enzyme activity may not totally explain this lack of glucose incorporated into fatty acids, however, because lactate can be used as a fatty acid precursor (Whitehurst et al., 1978), other evidence suggests that the extensive use of glucose for the synthesis of lactose, glycerol, and reducing equivalents may simply limit its availability for entrance into the TCA cycle and subsequent exit from mitochondria as citrate (Forsberg et al., 1985).

Acetate, on the other hand, is readily available for use in fatty acid synthesis. Acetyl-CoA conversion to malonyl-CoA is considered to be

the first committed step of fatty acid synthesis, and the activity of the enzyme that catalyzes this reaction, acetyl-CoA carboxylase, is enhanced greatly at parturition (Bauman and Davis, 1975). Acetyl-CoA carboxylase activity is regulated both by changes in its rate of synthesis and by interconversion between active and inactive states. High concentrations of cyclic-AMP or long-chain fatty acyl-CoA favor the inactive form (Storry et al., 1973), whereas a high protein concentration favors the active form (Bauman and Davis, 1974). Regulation of fatty acid chain length seems to be a function of the ratio of acetyl-CoA to malonyl-CoA, with increases in this ratio favoring synthesis of shorter fatty acids (Smith and Dils, 1966). Mammary fatty acid synthetase does not synthesize significant amounts of fatty acids longer than 16 carbons.

Use of preformed plasma fatty acids As mentioned earlier, 60% by weight of milk fatty acids are derived from transport of preformed plasma fatty acids into the mammary gland. The major sources of these fatty acids are chylomicrons and very low-density lipoproteins (VLDL); no net mammary uptake of nonesterified fatty acids (NEFA) seems to occur (Bauman and Davis, 1974). Lipoprotein lipase within the capillary wall hydrolyzes the triglyceride fatty acids (TGFA) of chylomicrons and VLDL and thus enables their transport into mammary cells.

Palmitic, stearic, and oleic acids are the principal fatty acids of plasma lipids. After uptake by the mammary gland, they are used almost exclusively for milk fat synthesis and account for almost all the C18 and half of the C16 milk fatty acids (Davis and Bauman, 1974). According to Baldwin and Smith's (1979) model, 10% also are oxidized as an energy

source. High concentrations of long-chain fatty acyl-CoA seem to inhibit de novo fatty acid synthesis within the mammary gland (Storry et al., 1973). Therefore, in early lactation, when much body lipid is being mobilized and is available to the mammary gland, less short- and medium-chain fatty acids (except butyrate) are synthesized.

The blood fatty acids of ruminants are more saturated than those of nonruminants because of microbial hydrogenation within the rumen (Dawson and Kemp, 1970). Fatty acid chain length and degree of saturation are the main determinants of melting point for a triglyceride, and a liquid nature at body temperature generally is considered to be a desirable property of milk fat. Therefore, to compensate for this microbial hydrogenation, a desaturase enzyme is present within the mammary gland that actively converts stearic acid to oleic acid (Bickerstaffe and Annison, 1970). This enzyme is relatively specific for stearic acid, but palmitic acid also is desaturated, and this occurs at a rate equal to 20% that of stearic (Bickerstaffe and Annison, 1968). The fatty acid composition of ruminant milk fat in comparison to dietary, ruminal, plasma, and adipose tissue lipid is shown in Table 2.

In the cow, glucose supplies about 60-70% of the glycerol for fatty acid esterification (Luick and Kleiber, 1961). The other 40% of milk fat glycerol is from the original glycerol hydrolyzed from triglycerides of plasma transport lipoproteins (Bauman and Davis, 1974). The mammary arteriovenous difference for free glycerol is zero, and almost no glycerol is oxidized within the mammary gland; therefore, on a molar basis, the percentage of glycerol from plasma would be expected to equal

the percentage of fatty acids from plasma (40 molar %).

Table 2. Fatty acid composition of lipid from alfalfa hay, rumen contents, bovine plasma, ovine fat depots, and bovine milk

| Fatty acid | Fatty acid composition (weight percentage) | | | | | |
|---|---|---|---|---|---|---|
| | Alfalfa hay[a] | Rumen contents[a] | Blood tri-glycerides[b] | Perirenal adipose tissue[c] | Subcutaneous adipose tissue[c] | Milk fat[b] |
| 4:0 | | | | | | 1[d] |
| 6:0 | | | | | | 2 |
| 8:0 | | | | | | 1 |
| 10:0 | | | | | | 4 |
| 12:0 | | | | | | 4 |
| 14:0 | 1 | 2 | 5 | 3 | 3 | 13 |
| 16:0 | 35 | 33 | 29 | 21 | 20 | 31 |
| 16:1 | 1 | 0 | 4 | 4 | 4 | 3 |
| 18:0 | 4 | 45 | 27 | 30 | 19 | 11 |
| 18:1 | 3 | 8 | 30 | 40 | 48 | 25 |
| 18:2 | 24 | 4 | 5 | 3 | 3 | 4 |
| 18:3 | 32 | 8 | 1 | 1 | 2 | 1 |

[a]Katz and Keeney, 1966. (Bovine rumen contents, 2 h post-feeding).

[b]Bitman et al., 1984. (Holstein cows in mid-lactation).

[c]Scott et al., 1971. (Sheep).

[d]Christie (1981) reports the weight percentage of C4 to be ~3.5.

Lactose synthesis Lactose is the predominant sugar of milk, and its synthesis has been reviewed by Jones (1978), Larson (1985), and several others. Briefly, glucose is converted to UDP-galactose in a series of reactions, and then UDP-galactose reacts with another glucose to form the disaccharide lactose. Lactose production is thought to be the osmotic regulator of total milk production. Of the glucose taken up by the bovine mammary gland, 60-70% is incorporated into lactose (Wood et al., 1965), so that for each 72 g of glucose uptake, 1 kg of milk is

produced (Kronfeld, 1982).

Protein synthesis The major milk proteins are casein, beta-lactoglobulin, and alpha-lactalbumin. Excess essential amino acids and insufficient nonessential amino acids are taken up by the mammary gland (Davis and Bauman, 1974), and some amino acid metabolism must occur before amino acids are activated for protein synthesis. Amino acids are not thought to be used as a net energy source in lactating mammary gland (Baldwin and Smith, 1979); however, studies indicate that the branched-chain amino acids may serve as an energy source (Wohlt et al., 1977).

Protein synthesis in mammary gland is similar to that of other cells and is controlled at the levels of gene transcription rate, mRNA stability, and mRNA translation rate. Although the quantitative role of protein degradation in controlling the net production of casein is unknown, ruminant mammary tissue has the capacity for intracellular degradation of newly-synthesized secretory proteins (Wilde and Knight, 1986). If protein degradation is quantitatively important in controlling net synthetic rate of milk protein, then hormones or factors that inhibit proteolysis might result in increased mammary gland protein production or energetic efficiency. Casein degradation is known to be inhibited in culture by leupeptin (O'Hare et al., 1986).

Energy sources for milk biosynthesis The lactating mammary gland requires ATP as a source of energy for milk synthesis and NADPH as a source of reducing equivalents for de novo fatty acid synthesis. In ruminant mammary gland, ATP is formed primarily by acetyl-CoA oxidation within the mitochondria by the TCA cycle and electron transport chain;

NADPH is produced equally by the pentose phosphate pathway and isocitrate cycle (Bauman and Davis, 1975). Glucose oxidation accounts for 40-50% of the total $CO_2$ output of the gland, but most of this glucose is oxidized in the pentose phosphate pathway for NADPH production (Baldwin and Smith, 1979). Although not as important as it is in nonruminants, glucose is permissive for de novo fatty acid synthesis in ruminant mammary gland (Forsberg et al., 1984). Of total glucose use by mammary cells, about 30% is by the pentose phosphate pathway and less than 10% is by the glycolytic pathway (Wood et al., 1965). Because glycerol is needed for fatty acid esterification, very little glucose is oxidized in the TCA cycle. Acetate is probably the major source of ATP in the mammary gland because it is very available and can be oxidized readily in the TCA cycle (Davis and Bauman, 1974). Acetate accounts for about 35% of total $CO_2$ output by lactating mammary gland, and half of the acetate taken up by the mammary gland is oxidized to $CO_2$ (Baldwin and Smith, 1979; Annison and Linzell, 1964). BHB and long-chain fatty acids are only minor energy sources for the mammary gland (Davis and Bauman, 1974), whereas fatty acids may contribute to as much as 14% of total $CO_2$ output, and ketones supply less than 10% (Baldwin and Smith, 1979).

Efficiency of the lactating mammary gland Whereas the major determinant of milk secretion rate is thought to be glucose uptake by the mammary gland, the major determinant of mammary gland efficiency has been proposed to be long-chain fatty acid uptake (Kronfeld, 1982). The partial efficiency for the conversion of acetate to milk fat is 72%, but the partial efficiency for conversion of long-chain fatty acids to milk

fat is 95% (Baldwin et al., 1985). Therefore, it is more efficient to use long-chain fatty acids to produce milk fat than acetate, and the greater the amount of body lipid being mobilized, the greater the efficiency of the mammary gland. This difference in partial efficiencies for milk fat synthesis provides a basis for optimization of milk production and efficiency through dietary and hormonal manipulation.

Effect of metabolite supply Nutrient supply to the mammary gland directly influences milk synthesis (Moe and Tyrrell, 1986), but it seems quantitatively important only if demands of mammary gland are not already met. In fasted animals, when glucose was in short supply, milk secretion rate was decreased, and, if glucose then was infused into the mammary blood supply, milk yield could be improved dramatically (Linzell, 1967). However, providing extra glucose, amino acids, or acetate to an already well-supplied mammary gland resulted in no changes in milk synthesis (Peel et al., 1982; Clark, 1975; Linzell, 1967). Providing extra long-chain fatty acids also resulted in no change in milk synthesis but did change milk fatty acid composition by decreasing the proportion from de novo fatty acid synthesis (Hansen and Knudsen, 1987; Storry et al., 1973).

Extra-mammary metabolism in support of milk synthesis

A cow producing 9500 kg milk per lactation likely will be in negative energy balance until about the sixteenth week of lactation, when daily milk production is about 80% of peak daily production (Bauman and Currie, 1980). During the first month alone, one third of mammary gland energy requirements are met by mobilization of body reserves (hence the

term, "repartitioning of nutrients"). Adipose tissue lipid is the major mobilized substrate during this period; however, some body protein also may be mobilized to support milk production. Other "support processes" that are altered during lactation are increased appetite, increased volatile fatty acid (VFA) production in the rumen, and enhanced gluconeogenesis. The liver especially is involved in this support metabolism. Support functions of liver include conversion of propionate, lactate, glycerol, and amino acids to glucose, coordination of lipid metabolism by removing NEFA from blood and packaging them into VLDL or oxidizing them to ketone bodies or acetate, detoxification of ammonia from enhanced dietary protein intake and ruminal degradation, and secretion of somatomedin-C, the probable mediator of growth hormone's lactogenic action.

Lipid metabolism Ruminant lipid metabolism is different from that of nonruminants in that the microbial population of the rumen hydrolyzes and hydrogenates most dietary fatty acids (Dawson and Kemp, 1970). The major fatty acids of forages and cereal grains are linolenic acid and linoleic acid, respectively (Christie, 1981). Most of these unsaturated acids are converted to stearic acid in the rumen so that unsaturated fatty acids make up less than 20% of absorbed fatty acids (Katz and Keeney, 1966; see Table 2).

Whole-body lipid turnover The liver synthesizes less than 10% of whole-body fatty acid synthesis in ruminants (Bauman and Davis, 1975); this seems advantageous for the ruminant because the main function of liver is gluconeogenesis. Ruminant liver takes up about 25%

of whole-body non-esterified fatty acid flux (Bergman et al., 1971) and hepatic NEFA uptake is dependent on NEFA concentration in blood (Bell, 1981). Most NEFA (50-90%) taken up by the liver are oxidized to ketones (Katz and Bergman, 1969), with the rest packaged into VLDL or oxidized to acetate, and only a very small fraction is oxidized completely to $CO_2$ (Connelly et al., 1964).

Chylomicrons and VLDL are both important in lipid transport vehicles in the ruminant (Bell, 1981; Bickerstaffe and Annison, 1974; Palmquist et al., 1969), and almost all net fatty acid uptake by mammary gland from blood is triglyceride-fatty acid with VLDL being the primary source of mammary gland preformed fatty acids. VLDL concentration of blood, however, is very low in the ruminant (Palmquist et al., 1969) and is even less in lactating cows than in nonlactating cows (Varman and Schultz, 1968). Whether VLDL concentration is low in ruminants because production by liver is also very low or because ruminant peripheral tissues remove VLDL more rapidly from blood than do nonruminant tissues is not known.

Palmitate flux in lactating cows producing about 22 kg milk per day was measured isotopically to be 260 g/d at four weeks postpartum (Konig et al., 1984) and 500 g/d at a stage of lactation not mentioned (Bickerstaffe and Annison, 1974). Palmitate makes up about one-third of blood fatty acids; therefore, daily NEFA turnover during early lactation is likely about 1-2 kg/d in a high-producing cow. Baldwin and Smith (1979) have estimated NEFA and TGFA turnover in a lactating cow producing 30 kg milk per day to be 1.5 kg/d and 1.0 kg/d, respectively. Most NEFA are derived from lipolysis in adipose tissue and, before being used by

the mammary gland, are taken up by the liver and converted to ketones, TGFA, or acetate. During lactation, at least 75% of TGFA are used by the mammary gland (Baldwin and Smith, 1979), whereas NEFA are used by several nonmammary peripheral tissues. Heart uses some NEFA and TGFA (Bell, 1981), and skeletal muscle also uses NEFA, although muscle probably first converts them to triglycerides, and these triglycerides are then the preferred fuel (Dagenais et al., 1976). In a study by Bell and Thompson, 1979 with fed steers, one leg used 13% of total NEFA flux; there was, however, a net release of NEFA by the leg, but this net release may have been caused by adipose depots within the leg.

Lipid metabolism in adipose tissue Lipid is the major form of stored energy in the animal kingdom, presumably because it is energy-dense and thus decreases body weight and improves mobility. Most of the stored lipid in animals is in the form of triglycerides, with the C16 and C18 fatty acids predominating.

In ruminants, adipose tissue is composed primarily of palmitic, stearic, and oleic acids (Table 2). Acetate, ketones, and some lipogenic amino acids are used to synthesize these fatty acids de novo and, along with preformed fatty acids from blood, are used to form triglycerides. A lactating cow producing 30 kg milk per day probably converts about 20 mol acetate and 2 mol ketones to about 3 mol (800 g) of fatty acids in adipose tissue per day (calculations based on Baldwin and Smith, 1979).

Unlike the very young ruminant, the mature ruminant has a relatively inactive citrate cleavage pathway (Hanson and Ballard, 1968), which may explain partly the relatively low use of glucose carbon for fatty acid

synthesis. Glucose, however, does serve as a precursor for the glycerol portion of triglycerides and is probably the major source of NADPH through it oxidation in the pentose phosphate pathway (Vernon, 1981).

Adipose tissue fatty acid synthesis, unlike that of mammary gland, does produce C18 acids. Similar to mammary gland, adipose tissue has an active desaturase enzyme that converts stearic to oleic acid; this enzyme seems to be more active in subcutaneous than internal fat depots (Payne and Masters, 1971), resulting in a more unsaturated fat profile in subcutaneous depots (Table 2). Other differences among adipose tissue depots are: 1) subcutaneous fat contains a greater fraction of its fatty acids from de novo synthesis than perirenal fat (Christie, 1981), and 2) in young ruminants internal fat lipogenesis is more active than that of subcutaneous fat, but in mature ruminants lipogenesis is much more active in subcutaneous and intramuscular fat depots that in internal depots (Bauman and Davis, 1975).

Early in lactation, the energy demands of milk production are greater than net energy intake. During the first month of lactation, as much as 33% of milk production may be from body reserves, and during the first 5 or 6 weeks, the total amount of body fat used may be as much as 50 kg (Bines and Hart, 1982; Belyea et al., 1978). After peak lactation, however, enery intake matches energy needs so that very little body fat is lost; body stores are replenished as lactation progresses further. Changes in adipose tissue lipid metabolism during lactation are summarized in Table 3.

Table 3. Changes in adipose tissue lipid metabolism during lactation

| Variable | Pregnancy | Lactation early | Lactation mid | Lactation late | Reference[1] |
|---|---|---|---|---|---|
| Plasma NEFA | | very high | | | a |
| Plasma glycerol | | high | | | a |
| Energy balance | + | - | 0 | + | b |
| Adipocyte volume | | low | low | | c |
| Visceral fat | | low | low | | d |
| In vitro lipolysis | low | high | medium | low | e |
| Response to noradrenaline | | high | | | f |
| HSL[2] activity | low | high | medium | low | g |
| LPL[3] activity | medium | low | high | medium | h |
| LPL activity | low | low | high | high | g |
| FA[4] synthesis | | low | high | high | i |
| FAS[5] activity | ---- very little change ---- | | | | j |
| Response to glucose | | low | | | f |
| Glucose oxidation | | low | high | high | i |
| G-6-P DH[6] activity | | low | | | j |
| Acetate oxidation | | low | high | high | i |
| FA re-esterification | medium | low | high | medium | h |
| Glycerol-3-P synthesis | medium | low | high | high | i |
| Glyc-3-P DH[7] activity | ---- very little change ---- | | | | j |

[1]a=Schwalm and Schultz, 1976.
b=Vernon, 1981.
c=Vernon and Taylor, 1986.
d=Smith and Baldwin, 1974.
e=Metz and van den Bergh, 1977.
f=Vernon and Finley, 1985.
g=McNamara et al., 1987.
h=Shirley et al., 1973.
i=Gritchting et al., 1977.
j=Baldwin et al., 1973.

[2]Hormone sensitive lipase.

[3]Lipoprotein lipase.

[4]Fatty acid.

[5]Fatty acid synthetase.

[6]Glucose-6-phosphate dehydrogenase.

[7]Glycerol-3-phosphate dehydrogenase.

The net result of these changes is that lipogenesis is slightly suppressed and lipolysis is greatly enhanced during early lactation. The greater effect on lipolysis than lipogenesis suggests that the fatty acid synthetic activity of mammary gland is supplemented by that of adipose tissue, so that after synthesis, NEFA are exported for eventual use by mammary gland (Moe and Tyrrell, 1986). A contrasting hypothesis is that adipose tissue and mammary gland are in competition with each other, and during lactation the hormonal profile favors mammary gland. This idea is supported by work showing that lipogenic rate in adipose tissue was about 50% lower in heifers of high genetic merit than those of low genetic merit (McNamara and Hillers, 1986).

Much of the NEFA released by adipose tissue during this time is from partial hydrolysis of adipose tissue triglyceride, resulting in a relatively high NEFA to glycerol ratio in blood (Yang and Baldwin, 1973). This ratio has been shown to be greater in high-producing cows during early lactation than in low-producers (Bell, 1981).

Many different factors are considered to be important in the regulation of adipose tissue metabolism in ruminants (Table 4). Lipogenesis is decreased during fasting, partly because of decreased acetate and glucose availability, but during lactation very little change in the concentration of these substrates occurs (Vernon, 1981). Lipogenesis is also a function of insulin secretion rate (Bell, 1981), and membrane transport of glucose likely is decreased during lactation because of decreased insulin concentration; acetate entry into adipose cells, however, would probably be unchanged. Although fatty acid

synthetase activity is not altered, acetyl-CoA carboxylase activity, which is known to be affected by insulin, may be decreased. Decreased NADPH production by the pentose phosphate pathway may also be a major reason for a decrease in lipogenesis during early lactation.

Table 4. Principal factors regulating adipose tissue metabolism in ruminants[a]

| Factor | LPL[b] | lipogenesis | esterification | lipolysis |
|---|---|---|---|---|
| Glucose | +[c] | + | + | |
| Acetate | | + | | |
| Fatty acids | | -[d] | + | - |
| Insulin | + | + | | - |
| Catecholamines | | - | | + |
| Glucagon | | | | + |
| Growth hormone | | - | | + |

[a]Table 13, page 326, in Vernon, 1981.

[b]Lipoprotein lipese.

[c]Stimulating effect.

[d]Inhibitory effect.

Hormone-sensitive lipase activity, on the other hand, is altered at parturition and is known to be regulated indirectly by cAMP concentration (Bauman and Davis, 1975). In nonruminants, catecholamines and glucagon increase and insulin decreases cAMP concentration, so a decrease in insulin concentration would increase cAMP concentration and thus enhance lipolysis. However, insulin is reported to have little effect on cow adipose tissue lipolysis (Yang and Baldwin, 1973), and the response to lipolytic hormones is much less in ruminants than in nonruminants (Bauman and Davis, 1975). Adenosine has been implicated as a potential regulator

of lipolysis in ruminant adipose tissue during lactation, and factors which alter adenosine metabolism may therefore alter lipid metabolism (Vernon and Taylor, 1986). Insulin to growth hormone ratio likely is involved in the regulation of adipose tissue metabolism, and these two hormones will be discussed further in section III.

Ketone metabolism Much of the mobilized lipid from adipose tissue is oxidized by liver as an energy source, but because hepatic oxidation of fatty acids during lactation is incomplete, the ketone bodies, acetoacetate and BHB, are produced and released to the blood. In fed ruminants, ketones also are synthesized from ruminally produced butyrate. Half of this ruminal butyrate is converted to ketones in the rumen epithelium (Weigand et al., 1972), with most of the remaining butyrate converted to ketones in the liver. Studies by Sutton (1985) show that, for lactating cows fed a normal, barley-based diet and eating 10 kg digestible dry matter (DDM) per day, portal absorption of butyrate and BHB was about 500 and 600 g daily, respectively. Whereas the major precursor of liver ketone synthesis is likely butyrate which has escaped metabolism in rumen epithelium (Bell, 1981), NEFA oxidation is undoubtedly an important precursor for ketogenesis in fed lactating cows in negative energy balance.

Baldwin and Smith (1979) estimated that 1100 g ketones per day entered the blood pool. If we assume that 25% of blood NEFA are taken up by liver (Bergman et al., 1971) and that 50-90% of these are oxidized to ketones (Katz and Bergman, 1969), then, based on Baldwin and Smith's model with a NEFA entry rate of 5.5 mol/d, about 30-40% of ketones are

derived from adipose tissue lipid. In a study by Pethick et al. (1983), NEFA contributed about 20% of ketone carbon oxidized to $CO_2$ in fed nonpregnant sheep. Lomax and Baird (1983) reported that portal uptake of butyrate, BHB, and acetoacetate in fed cows (2 lactating and 2 nonlactating) was 150, 360, and 100 g/d, and total splanchnic output was 30, 800, and -25 g/d, respectively. These data indicate that 1) liver removes 80% of portal blood butyrate and all portal blood acetoacetate, and probably converts them along with some NEFA from adipose tissue to BHB, 2) liver produces 40-50% of splanchnic ketone output, 3) portal absorption accounts for 77% of of total splanchnic ketogenesis, and 4) 55% of net liver ketogenesis is from sources other than butyrate, e.g. amino acids and fatty acids. Because liver BHB output is greater in well-fed lactating cows than in nonlactating cows (Baird et al., 1975) and lipid mobilization is high during early lactation, the contribution of splanchnic ketones from NEFA is probably greater in lactating cows than nonlactating cows; thus the 30-40% value calculated from data of Baldwin and Smith (1979) may hold true.

Heitmann et al. (1987) have estimated the net contribution to total splanchnic ketogenesis of portal-drained viscera and liver to be 36 and 11 g/d in fed sheep, -6 and 140 g/d in fasted sheep, and 60 and 40 g/d in lactating sheep. The contribution of liver to splanchnic ketogenesis is thus 23%, 100%, and 40% for fed, fasted, and lactating sheep, respectively.

Regulation of hepatic ketogenesis from endogenous lipid In non-ruminants, the same bihormonal control that operates for

gluconeogenesis seems to hold true for ketogenesis (McGarry and Foster, 1980). Whereas insulin's major role in controlling hepatic ketogenesis seems to be in the regulation of NEFA supply, glucagon seems to have more of an effect on liver itself by enhancing its ketogenic machinery. The glucagon to insulin ratio, however, actually may be more important in regulating hepatic metabolism than the absolute concentration of either hormone alone.

The current working theory of the hepatic regulation of ketogenesis from NEFA has been discussed by McGarry and Foster (1980) and Bell (1981). In nonruminants, the rate-limiting step in the oxidation of long-chain fatty acids (LCFA) in liver cells seems to be entrance into the mitochondria, which is catalyzed by the enzyme carnitine acyltransferase-I (CAT-I). Glucagon seems to increase hepatic carnitine concentrations, which presumably would increase CAT-I synthesis, and therefore LCFA entrance into the mitochondria (McGarry and Foster, 1980). Malonyl-CoA is a potent inhibitor of CAT-I, and its concentration is decreased by a greater glucagon to insulin ratio. LCFA-CoA also decreases the synthesis of malonyl-CoA and thereby indirectly enhances its own oxidation (McGarry and Foster, 1980). Therefore, when fatty acid synthesis is enhanced in animals that synthesize fatty acids in liver, the newly synthesized fatty acids would be routed toward esterification and not oxidation. In the ruminant, however, fatty acids are synthesized primarily in adipose tissue or mammary gland, and malonyl-CoA seemingly would serve no purpose in liver other than CAT-I regulation. Studies by Aiello and coworkers (1984) indicate that CAT-I is the regulatory step of

hepatic ketogenesis in ruminants also. In their studies, they also found that ketone production from both palmitate and buyrate was greatest in liver slices obtained from cows at 30 days into lactation as compared to other stages of lactation. Whereas a high concentrate diet enhanced gluconeogenesis from propionate and decreased ketogenesis from palmitate, it seemed to have no effect on hepatic butyrate metabolism. Thus it seems that CAT-I is a regulatory step for ruminant ketogenesis because CAT-I is not needed for mitochondrial uptake of short- and medium-chain fatty acids but is needed for LCFA.

Once in the mitochondria, fatty acids undergo ß-oxidation to acetyl-CoA. Acetyl-CoA may enter the TCA cycle; however, because ruminant liver is geared toward gluconeogenesis, oxaloacetic acid may be limiting for complete oxidation of most fatty acids (Bell, 1981). This would result in elevated mitochondrial acetyl-CoA concentrations. HMG-CoA synthase, which is the rate-limiting step in conversion of acetyl-CoA to ketones, is found in large quantities only in liver (Williamson et al., 1968), and its activity is dependent on the acetyl-CoA to free CoA ratio (Sauer and Erfle, 1966). For a given input of LCFA to the liver then, ketone output seems dependent on the liver's ability to generate acetyl-CoA.

The exact mechanism controlling the partition of LCFA between the pathways of esterification and oxidation in ruminants is not well understood. The liver's capacity to secrete VLDL seems less than its ability to esterify fatty acids, and apoprotein synthesis may be be limiting in ruminant liver (Bell, 1981). Rapid uptake of LCFA by hepatic mitochondria and limited availability of glucose for hepatic glycerol

production likely also are involved in directing LCFA metabolism in liver of lactating cows in negative energy balance.

Peripheral use of ketones Ketones serve as an alternative fuel source for body tissues when carbohydrates are in short supply or cannot be used efficiently; during lactation, ketones also are used in milk synthesis. The major factor influencing the utilization rate of blood ketones in rats is concentration (Williamson et al., 1971), and ketones are used as an oxidizable substrate in heart, kidney, skeletal muscle, and mammary gland.

Ruminant heart prefers to use ketones as its fuel source (Bell, 1981), and skeletal muscle from fed sheep used ketones for 15% of $CO_2$ production (Pethick et al., 1983). Whereas ketones can replace glucose as fuel source in the brain of nonruminants (Owen et al., 1967), ruminant brain relies almost exclusively on glucose, even during ketonemia (Lindsay and Setchell, 1976).

Bovine mammary gland extracts about 20% of acetoacetate and 45% of BHB from inflowing blood (Laarveld et al., 1985), and calculations based on Baldwin and Smith's (1979) lactating cow model indicate that mammary gland accounts for 16% of total ketone turnover. However, whether entry rate includes conversion of acetoacetate to BHB in liver is not mentioned, so mammary uptake actually may be greater (20-25%) based on net ketone entry rate. In the lactating sheep model of Heitmann et al. (1987), mammary gland, hindquarters, kidney, and liver remove 43%, 38%, 10%, and 9%, respectively, of total ketones entering the blood. However, the authors do not include use by peripheral tissues other than

hindquarter, so this 43% uptake by mammary gland is probably too high. Combining the results of these two studies leads to the conclusion that mammary gland probably uses 20-30% of available ketones.

Ketones are reported to have several effects on metabolism. They may spare glucose from oxidation (Soling and Kleineke, 1976), inhibit muscle proteolysis and thereby decrease gluconeogenesis from amino acids (Sherwin et al., 1975), and decrease adipose tissue lipolysis (Williamson and Hems, 1970). These effects seem related to the reported stimulation of insulin secretion by ketones, but lipolysis also is thought to be decreased directly by ketones (Williamson and Hems, 1970). This effect on lipolysis has led several authors to suggest an "auto-regulatory hypothesis" for ketone metabolism, i.e., that ketones produced from mobilized lipid feed back to adipose tissue, inhibit lipolysis, and thereby indirectly inhibit their own synthesis. Heitmann et al. (1987) proposed that this auto-regulation is of greater importance in the relatively insulin-insensitive ruminant, especially the lactating dairy cow because insulin does not seem needed by the mammary gland.

Acetate metabolism Based on the model of Baldwin and Smith (1979), total acetate entry rate for a cow producing 30 kg milk per day is almost 4000 g/d. Other laboratories have determined total acetate entry rate to be 3700 g/d in cows producing 19 kg milk (Bickerstaffe and Annison, 1974) and 2500 g/d in cows producing 22 kg milk (Konig et al., 1984). Acetate entry rate was 6.5 g/kg/d in lactating ewes (King et al., 1985; Pethick and Lindsay, 1982), almost twice the entry rate of 3.6 g/kg/d in nonlactating sheep (Pethick et al., 1981) and equivalent to

about 2000 g/d for a lactating cow if based on metabolic body size. Because none of the above measurements of acetate flux measured portal acetate specific radioactivity, acetate taken up by the liver likely had a specific radioactivity 50% less than that of arterial blood; therefore, if liver accounts for about 20& of total acetate use, then acetate entry was probably overestimated by about 10% (Pethick et al., 1981). Assuming that acetate concentration is about 1.5 mM (90 mg/l), and that extracellular fluid volume in a lactating cow is about 60 1, then the blood acetate pool is about 5 g and must turnover once every 2-3 min.

Blood acetate in ruminants is derived from two major sources--portal absorption and endogenous production. Most absorbed acetate is derived from microbial fermentation in the rumen, but about 10-15% is from intestinal fermentation (Sutton, 1985).

Portal absorption of acetate peaks about 2 hours after feeding of a silage meal to nonlactating cows (Huntington and Reynolds, 1983), and its concentration in blood peaks at 1-2 mM within several hours after a meal. The concentration then gradually decreases to about .5 mM by 12-20 hours (Bassett, 1974). Lomax and Baird (1983) determined portal absorption of acetate in fed cows (2 lactating and 2 nonlactating) to be 1500 g/d and total splanchnic output to be 2000 g/d. Sutton (1985) reports that portal absorption of acetate in lactating cows is 2900 g/d (290 g/kg digestivel dry matter).

Liver production of acetate is thought to be an alternative to ketogenesis, with the balance favoring acetate when carnitine concentration is relatively high (Bell, 1981). However, Pethick et al.

(1981) found that factors that promote ketogenesis do not proportionally affect acetate release. Hepatic production of acetate instead of ketones should be an advantage to the lactating ruminant because acetate is a more important source of carbon for mammary gland lipogenesis than are ketones (Palmquist et al., 1969).

Pethick et al. (1981) have determined that in fed, insulin-treated, diabetic sheep, the contribution of gut, liver, and muscle to total acetate production was 78, 20, and 2%, respectively; therefore, 22% of total entry was endogenous production of which 90% was from liver. Bergman and Wolff (1971) found that endogenous production accounted for 20% of total entry rate in fed sheep and 80% in fasted sheep. In the fasted sheep, only 20% of endogenous production was from liver. In a fed lactating cow under energy stress, total entry rate of acetate is probably about 4000 g/d, with about 60% from ruminal fermentation, 8% from intestinal fermentation, 16% from liver, and 16% from peripheral tissues.

Use of acetate Conflicting reports exist as to the use of acetate by liver. Cook and Miller (1965) found that absorbed acetate from the gut is not removed by liver; this low hepatic uptake seems consistent with the relatively high activity of hepatic acetyl-CoA hydrolase compared to that of acetyl-CoA synthetase (Knowles et al., 1974). Liver, therefore, would be expected to release acetate from incomplete oxidation of metabolites and to use very little acetate as an energy source, especially in a lactating cow with restricted hepatic TCA cycle activity. Pethick et al. (1981), however, determined acetate use

by liver, gut, and muscle of fed, insulin-treated diabetic sheep to be 17, 25, and 54% of total entry rate, respectively. The discrepancy between these studies is not understood, but Pethick et al. (1981) determined that the reported acetyl-CoA synthetase activity of Knowles et al. (1974) is sufficient to account for hepatic acetate uptake and subsequent metabolic use. Studies of both Pethick et al. (1981) and Bergman and Wolff (1971) indicate that gut tissues directly utilize 30% of absorbed acetate and that most of this acetate is oxidized.

The major factor determining acetate use by peripheral tissues, including mammary gland, is arterial concentration (Pethick et al., 1981; Moore and Christie, 1981). Mammary gland extracts about 60% of arterial acetate in sheep (Pethick and Lindsay, 1982) and accounts for 40% of whole-body acetate use in lactating goats (Davis and Bauman, 1974), 17% in lactating sheep (Pethick and Lindsay, 1982), and 24% in lactating cows (Baldwin and Smith, 1979).

Net muscle uptake accounts for about half of total acetate use. During lactation in sheep, actual muscle uptake decreases slightly and, because of the increased total entry rate, fractional uptake decreases to half (Pethick and Lindsay, 1982). Almost all acetate taken up by muscle is oxidized in the TCA cycle (Holdsworth et al., 1964; Bell, 1981); however, significant amounts can be stored as acetyl-carnitine. In a 50-kg sheep, 6 g of acetate can be stored in this way (Snoswell and Koundakjian, 1972). Some acetate also may be used for intramuscular lipogenesis. Acetate accounts for about 33% of muscle $O_2$ use, and this figure decreases during lactation to 22% (Pethick and Lindsay, 1982).

Heart also uses some acetate, but ruminant brain uses acetate for less than 5% of its energy needs (Lindsay and Setchell, 1976). Acetate is probably the major energy substrate for kidney from a fed ruminant (Bell, 1981).

About 30-40% of whole-body acetate use is oxidized in insulin-treated diabetic sheep (Pethick et al., 1981), and 35-55% is oxidized in lactating cows (Konig et al., 1984; Baldwin and Smith, 1979). Whereas acetate has effects similar to ketones in that it may inhibit lipolysis and spare glucose from oxidation in skeletal muscle (Pethick and Vernau, 1984), it does not stimulate insulin secretion (Trenkle, 1981).

Glucose metabolism A cow producing 40 kg milk needs about 3000 g glucose per day (Hart, 1983). Baldwin and Smith (1979) estimated that glucose entry rate in a cow producing 30 kg is 3300 g/d. Konig et al. (1984) determined non-Cori cycle glucose flux to be 1900 g/d in lactating cows.

Very little glucose is absorbed from the gastrointestinal tract in ruminants. Sutton (1985) estimated that as much as 4-9% of digestible energy intake is from absorbed glucose (which calculates to 400-800 g glucose per day) in lactating cows, depending on diet composition. Huntington and Reynolds (1987) found the net glucose flux across portal tissues in lactating cows to be -250 g/d. Ruminants, then, must rely on gluconeogenesis to meet almost all their glucose needs. This major and continuous function of the liver is evidenced by the constantly high activity of liver gluconeogenic enzymes (Baird et al., 1968). Thus, in contrast to nonruminants, gluconeogenesis in ruminants is greatest

immediately postprandially and is directly proportional to dry matter intake (Steel and Leng, 1973a). A small increase in the activity of some gluconeogenic enzymes is observed during lactation (Mesbah and Baldwin, 1983), when gluconeogenesis is known to be greatly enhanced (Bennink et al., 1972). In the regulation of gluconeogenesis, glucagon seems to be more involved after feeding, whereas the glucocorticoids seem more involved during fasting (Trenkle, 1981). During lactation, both of these hormones may be involved, because both increased feed intake and decreased energy balance are likely responsible for increased gluconeogenesis.

The major precursors for gluconeogenesis are propionate, amino acids, glycerol, and lactate (Hart, 1983). Sutton (1985) reports that daily portal absorption in lactating cows producing 30 kg milk is about 400 g glucose, 1200 g propionate, and 270 g lactate. Lomax and Baird (1983) determined portal uptake of propionate and lactate to be 600 and 360 g/d, respectively, in fed cows (2 lactating and 2 nonlactating). Baldwin and Smith (1979) have estimated total daily entry rates in lactating cows producing 30 kg milk to be 3300 g glucose, 2000 g propionate, 220 g glycerol, and 750 g lactate. Their model shows that 70% of propionate, all glycerol, and all lactate is used for biosynthesis. If we use this model and assume that all biosynthesis with these metabolites is gluconeogenesis, that 5% of glucose entry rate may be absorbed, and that half of lactate entry rate is Cori cycling, then gluconeogenic carbon is 55% propionate, 11% gut lactate, 11% lactate from Cori-cycling, 7% glycerol, and 10% amino acids. These figures agree with

others: 20-60% from propionate in fed ruminants (Steel and Leng, 1973b), 2-15% of whole-body glucose flux in ruminants from Cori cycling (Baird et al., 1983), and 5% of non-Cori cycle gluconeogenesis from glycerol (Bergman et al., 1968).

There is discrepancy, however, over the contribution of amino acids. Bergman and Heitmann (1978) have reported a 30-40% contribution from amino acids, and Lomax and Baird (1983) determined that 9% was from alanine, glycine, serine, and threonine. Oldham and Smith (1982) state that the contribution of amino acid carbon to net glucose synthesis is likely to be less than 5% of glucose requirement in high-yielding cows. If we assume a cow producing 30 kg is in zero protein balance and is absorbing 1600-1800 g amino acids, then about 700 g amino acids would be available for gluconeogenesis daily. Direct oxidation of amino acids exceeds gluconeogenesis by 4:1 in nonlactating ruminants (Lindsay, 1976), but in lactating ruminants the fraction converted to glucose is likely much greater. Potential glucose yield from amino acids is thought to be 550 g per kg of protein (Krebs, 1965). If we consider glucose yield to be as much as 500 g from 1 kg excess amino acids (of which some are oxidized directly), then even for a cow in early lactation with net protein mobilization of 250 g/d (a maximal value), gluconeogenesis from amino acids cannot account for more than 17% of total glucose needs.

Glucose use During lactation, mammary gland in sheep and cows extracts about 30% of arterial glucose (Pethick and Lindsay, 1982). Calculations based on Baldwin and Smith's (1979) model of a lactating cow producing 30 kg milk with whole body glucose flux of 3300 g/d show that

mammary gland daily takes up 2200 g glucose and uses 1520 g (69%) for lactose synthesis, 120 g (5%) for glycerol synthesis, and 560 g (25%) for oxidation. These figures agree with previous measurements (Annison and Linzell, 1964; Annison et al., 1974).

Because of this demand of mammary gland for glucose, a metabolite in short supply, ruminant metabolism is altered so that glucose is spared during lactation. Ruminant liver, adipose tissue, and mammary gland lack an active citrate cleavage pathway (Young et al., 1969; Bauman et al., 1970; Hanson and Ballard, 1968), so glucose is not used for fatty acid or ketone synthesis. Acetate is used instead for fatty acid synthesis, and acetate is used via the isocitrate pathway to supply half the NADPH needed for fatty acid synthesis by mammary gland (Bauman and Davis, 1975). Glucose is used in fat synthesis during lactation to supply the other half of NADPH and to supply about half the glycerol for esterification (Bell, 1981). During early lactation, muscle use of glucose decreases from 57% to 32% of oxygen uptake (Pethick and Lindsay, 1982), and glucose oxidation in adipose tissue also is decreased (Gritchting et al., 1977). Ruminant brain, however, relies almost exclusively on glucose even during ketonemia (Lindsay and Setchell, 1976) and probably also during lactation.

Protein metabolism A lactating cow producing 30 kg milk synthesizes 1 kg of milk protein per day; this is equivalent to a 6 kg gain in body weight per day. The minimal dietary crude protein requirement to support this production is 2360 g crude protein (CP) (Oldham and Smith, 1982).

Huntington (1984), in studies using lactating cows producing 24 kg milk and consuming 2400 g CP per day, has determined portal amino acid (AA) uptake to be 860 g/d or 36% of CP intake. Of this 860 g AA, 720 g or 84% would be secreted into milk. The figure of 36% of CP intake being taken up as AA in the gut is similar to that for sheep--48 g AA uptake with 156 g CP intake (Tagari and Bergman, 1978). By using Iowa State University's metabolizable protein (MP) system, however, a greater value for AA uptake is obtained. In the cow study, calculations reveal an MP uptake of 1300 g/d; therefore, if this predicted MP value is correct, portal uptake of amino acids accounts for only 66% of MP uptake. This figure agrees with measured values from the sheep study in which the authors found that only 30-80% of essential AA disappearing from gut appeared in portal plasma. The most likely reason for this discrepancy between MP intake and portal AA absorption is that di- and tripeptides account for about 25% and up to 70% of amino nitrogen absorbed in the gut (Alpers, 1986; Webb, 1986). For a 600-kg cow producing 30 kg milk, calculated MP and net protein values are 1600 and 1100 g/d, respectively. Of this, 960 g is secreted into milk, and 140 g is net protein for maintenance (endogenous losses). Net protein need for maintenance, however, is actually a result of inefficiency in protein turnover.

Protein turnover Catabolism of amino acids is the result of 1) unbalanced AA intake and 2) the extent to which proteins are "turned over". The components of plasma AA turnover are: inputs (diet and proteolysis) and outputs (AA oxidation and protein synthesis). Body protein turnover is the daily degradation and resynthesis of body

proteins and requires energy input. Whereas some protein turnover probably is required to maintain a dynamic system (adapting to new situations), there is much about protein turnover we do not understand. In drowing or lactating animals, in which protein production is desired, much protein turnover seems inefficient.

One of the primary problems encountered in measuring protein turnover is determining the specific radioactivity (SA) of the precursor pool for protein synthesis. Protein turnover in ruminants is even more difficult to measure than that of nonruminants because of the additional problems of nitrogen recycling through the rumen and determination of amino acid absorption.

If we assume that leucine equals 7% of total protein, work by Pell et al. (1986) indicates that fed 58-kg ewes have a plasma protein turnover of 286 g/d. Protein synthesis, oxidation, degradation, and absorption are 243, 43, 186, and 100 g/d, respectively. After a 3-day fast, plasma AA turnover decreased to 229 g/d, and synthesis, oxidation, and degradation changed to 182, 47, and 229 g/d, respectively. In another study, whole-body protein synthesis in pregnant ewes (60 kg) was 264 g/d (Schaefer and Krishnamurti, 1984), very similar to that of fed nonpregnant ewes.

In 20-kg growing lambs, whole-body protein synthesis was calculated to be 240 g/d by using plasma leucine SA or 610 g/d by using intracellular leucine SA (Davis et al., 1981). Whole-body protein absorption and oxidation were 114 and 95 g/d, respectively; therefore, net protein synthesis was only 19 g/d, a very low value for growing

lambs. Of total protein synthesis, 20% was in skin, 34% gut, 9% liver, and 20% carcass (muscle).

Lobley and coworkers (1980) measured protein turnover in two 250-kg heifers gaining 600 g/d and in a 630-kg dry cow. Estimates for whole-body synthesis were 1.6 times greater when tissue homogenate SA was used instead of plasma SA; if an average SA was used for calculations, then synthesis was 2000 g/d for heifers and 2500 g/d for the cow. The heifers were gaining about 110 g protein per day, but feed intake was not recorded. According to NRC requirements, they were probably consuming ~360 g MP, so synthesis degradation, absorption, and oxidation were 2000, 1890, 360, and 250 g/d. The cow was fed a maintenance diet with 650 g CP/d (about 330 g MP), so degradation must have been equal to synthesis (2500 g/d) and absorption and oxidation were both 330 g/d. Percentage contribution of carcass muscle, gastrointestinal tract, liver, and hide to total protein synthesis was 15, 42, 7, and 17%, respectively. Carcass muscle protein degradation, then, was about 200 g/d for growing heifers and 375 g/d for mature cow. These values are similar to those obtained from a study by Gopinath and Kitts (1984), in which muscle protein degradation in growing 430-kg steers was determined to be 560 g/d by 3-methylhistidine excretion, of which half is likely from splanchnic muscle.

In a lactating cow, whole-body protein turnover is very likely increased over that in a nonlactating cow. AA absorption is at least four times that of the dry cow studied by Lobley et al. (1980), and nonmammary whole body protein synthesis is probably similar. In

lactating rats, protein synthesis in mammary gland doubled from day 3 to 15 with no change in liver protein synthesis (Jansen and Hunsaker, 1986). Calculations from data by Pethick and Lindsay (1982) indicate that muscle uptake of oxygen may decrease 15% with lactation in sheep, and Baldwin et al. (1985) estimates carcass heat production to be decreased 8% in lactating cows; if this is true, it may be associated with a decrease in skeletal muscle protein synthesis.

From nitrogen balance studies, cows in early lactation producing 24 kg milk per day were shown to lose about 40 g protein per day; by week 20, the balance was again positive with a gain of 56 g/d (Huntington, 1984). When fed protein-deficient diets, cows can lose up to 20 kg of body protein during early lactation (Botts et al., 1979). Using potassium-40 counting, Belyea and others (1978) determined body protein loss in dairy cows during the first 6 weeks of lactation to be 10 kg. In comparative slaughter studies, however, Bines and Hart (1982) found that, although adipose tissue weight had declined 40% by peak lactation (week 5), no change in muscle or bone weights had occurred. Another study has shown a 20% reduction in bovine trapezius muscle fiber size during the first eight weeks of lactation, compared with a 50% reduction in subcutaneous fat thickness (Roberts et al., 1979). In summary, then, body protein net loss during early lactation is not as great as fat loss, and likely seldom exceeds 250 g/d.

Baldwin et al. (1985) estimated nonmammary energy expenditure to be increased from 10.6 Mcal/d in nonlactating cows to 11.6 Mcal/d in lactating cows. Digestive tract, liver, and heart account for the

increase, whereas muscle and adipose tissue heat production actually are decreased. Because of the close association between heat production and protein turnover, it seems likely that protein turnover follows the same trend.

From the above discussion, whole-body protein turnover is estimated to be 4600 g/d in a cow producing 30 kg milk per day during early lactation. Inputs into plasma are 1700 g from absorption and 2900 g from proteolysis. Outputs are 900 g for oxidation and 3750 g for synthesis, of which 1000 g is in milk. Based on these estimates, I make the following conclusions and comments:

1) 21% of plasma AA turnover is oxidized in the lactating cow compared to 12% in the dry cow. Liver is more metabolically active during lactation (Baldwin et al. 1985), and, because of increased glucose needs, possibly more amino acids are catabolized by liver. This 850 g of amino acids could provide as much as 500 g glucose (Krebs, 1965). However, a significant portion, especially of the branched-chain amino acids, may be oxidized in other tissues as an energy source because of decreased glucose use by nonmammary tissues. In underfed steers and sheep, nitrogen balance can be maintained during negative energy balance, indicating that ruminants can distinguish between protein and energy-yielding nutrients and do not readily use protein as an energy source (Orskov et al., 1983). Although this seems less true for the lactating cow (compared with the dry cow, 80% of amino acids are recaptured as protein during a time of great energy demand.

2) Nonmammary protein synthesis is decreased slightly in the

lactating cow, but still accounts for 70% of total protein synthesis. Of nonmammary protein synthesis, liver, gastrointestinal tract, and heart probably account for more, and skeletal muscle and hide account for less in a lactating cow than in a nonlactating cow.

3) Net mobilization of protein is 150 g/d. If this were converted directly to milk protein (unlikely), then the net contribution of body protein to milk protein would be 15%. This rate of net mobilization probably would occur until about wk 6 of lactation, after which it would gradually decrease and net body protein synthesis would be positive during mid and late lactation. At these rates, net mobilization and resynthesis of body protein during a lactation cycle would be about 6-10 kg.

Maintenance energy requirement Baldwin and coworkers (1985) have divided whole-body maintenance energy expenditure into three major classes: 40-50% is work functions (liver, heart, kidney, nerve, and lung work), 15-25% is cell component synthesis (primarily protein and membrane lipids), and 25-35% is membrane transport (mostly associated with membrane potential maintenance and $Na^+$, $K^+$-ATPase).

Contribution of ion pumping The above estimates for ion pumping are derived primarily from work by Milligan and coworkers. They have found that, in calf muscle in vitro, ouabain inhibited 40% of respiration and that the energy needed for peptide synthesis was less than 4% of oxygen consumption (Gregg and Milligan, 1982). No apparent relation existed between these two functions. Milligan and McBride (1985) reported that in vitro ruminant tissues used 26-46%, 28-61%, and

18-54% of their total oxygen consumption to support $Na^+,K^+$-transport in skeletal muscle, duodenal epithelium, and hepatocytes, respectively, and that these values were increased in tissues from lactating animals. In 1986, however, Milligan and Summers reported that previous estimates for ion pumping were probably too high, and that, during fasting or when using a complex media for incubations, only 20% of energy expenditure was caused by $Na^+,K^+$-ATPase. They also stated that previous estimates for the contribution of protein synthesis are likely too low because muscle in vitro does not synthesize protein as rapidly as in vivo.

Contribution of protein turnover Estimates for the contribution of protein synthesis to energy requirement vary from 8 to 40%. In 40-kg sheep, Millward and Garlick (1976) calculated it to be 8% based on a value of 3.6 kJ heat per g protein synthesized. Lobley et al. (1980) used a value of 4.5 kJ per g protein (4-5 ATP per peptide bond) and demonstrated that, depending on which AA pool is the precursor pool, 16-28% of heat production was the result of protein synthesis in growing heifers and a dry cow. Reeds et al. (1982) reported a contribution of 20% of heat production from protein synthesis in growing pigs, and Young et al. (1983) summarized the literature to give a minimal estimate of 15-25%. Davis et al. (1981) used intracellular leucine SA to calculate protein turnover in growing lambs and found net protein synthesis to be only 5% of total protein synthesis. They then used the figure of 4.5 kJ/g protein and determined protein synthesis to account for 42% of heat production.

In support of the higher value, Lobley (1986) measured protein

synthesis (based on blood leucine irreversible loss), heat production, and fat and protein deposition in beef steers fed at either maintenance or 1.6 times maintenance. By using heat production values of 4.5 kJ/g protein synthesized and 320 kJ/MJ fat deposited, he calculated an expected increase in heat production, which was only 56% the observed increase. Whereas his value for the cost of fat deposition may have been underestimated, the cost of protein synthesis was quite likely underestimated. Using intracellular leucine SA to calculate protein synthesis would have doubled the rate of synthesis, and the value of 4.5 kJ/g protein synthesized (4-5 ATP per peptide bond) also may be an underestimate (see discussions by Reeds et al., 1985; Milligan and Summers, 1986). In studies with adult man, 50% of energy expenditure is related statistically to protein turnover, which implies that indirect costs of protein turnover may be significant.

Contribution of different tissues Organ size is an indicator of heat production by that organ. Koong et al. (1985) has shown that in the gastrointestinal tract, liver, kidneys, and pancreas of well-fed pigs make up a greater share of total body weight and that fasting heat production is greater than in undernourished pigs. These enlarged organs are metabolically more active than other body tissues and, therefore, are thought to cause the increased whole-body heat production. Thus, based on data from Smith and Baldwin (1974) (Table 5), we would expect a lactating cow to have a greater basal heat production than a nonlactating cow of equal body weight. This is evidently true, as Moe (1981) has reported that ME for maintenance is 20% greater in

lactating than in nonlactating cows.

Table 5. Effect of lactation on organ weights[a]

| | Nonlactating | Lactating |
|---|---|---|
| | kg tissue | |
| Digestive tract | 26.4 | 33.9 |
| Liver | 9.1 | 11.4 |
| Heart | 2.6 | 3.2 |
| Visceral fat | 51.1 | 42.0 |
| Carcass | 406.3 | 375.2 |
| Skin | 33.0 | 31.3 |
| Kidney and brain | 2.6 | 2.6 |
| Mammary | 13.1 | 22.7 |
| Other (head, hooves, etc.) | 105.8 | 127.7 |
| Total | 650.0 | 650.0 |

[a]From Table 5, p. 1059, in Smith and Baldwin (1974).

In studies with 60-kg ewes, Oddy et al. (1984) determined that heat production was 8.5 MJ/d in dry ewes and 12.9 MJ/d in ewes producing 708 g milk per day. Skeletal muscle accounted for 28% of total heat production in dry ewes but only 14% in lactating ewes. Milk energy output was 3.1 MJ/d (23% of ME intake) and mammary gland energy use was only .29 MJ/d less than 3% of total heat production or metabolizable energy (ME) intake. Pethick and Lindsay (1982), however, have found that the mammary gland in lactating sheep uses 10-14% of total oxygen consumption. Calculations from the model of Baldwin and Smith (1979) reveal that if amino acids are not considered as an energy source, then 20% of heat production from oxidation of metabolites postprandially occurs in the mammary gland of a lactating cow producing 30 kg milk.

Splanchnic tissues are metabolically very active tissues. In a study by Reynolds et al. (1986), lactating cows producing 27 kg milk per

day consumed daily 18 kg dry matter with 49 Mcal ME. Energy expended as heat was 32 Mcal/d and total oxygen consumption was 6600 l/d. Portal-drained viscera and liver accounted for 20 and 25% of oxygen consumption, respectively. Webster (1980) measured heat production in sheep fed at maintenance and found that the gut contributed 20%; when fed at twice maintenance, this contribution increased to 23%. Liver accounted for 18% of oxygen consumption in another study with fed sheep (Thompson and Bell, 1976). Webster (1981) has summarized literature to show that ruminant splanchnic tissues account for about 50% of total heat production.

In summary, for a cow in early lactation, the contribution of skeletal muscle, mammary gland, gastrointestinal tract, and liver to body heat production is approximately 15%, 15%, 20%, and 25%, respectively. Considerable animal variation in maintenance energy requirement is thought to exist and could be used to increase the gross efficiency of production (Baldwin et al., 1985). By optimizing nutrient balance for a lactating cow, we may be able to decrease the work load of liver and thus decrease liver hypertrophy during lactation and decrease maintenance requirement. If maintenance requirement was decreased from 130 to 90 kcal/kg$\cdot^{75}$ and feed intake remained constant at three times maintenance requirement, then there would be a 15% increase in nutrients available to mammary gland for increased milk synthesis (Baldwin et al., 1985).

Overview of support metabolism A lactating cow producing 30 kg milk requires about 60 Mcal digestible energy (DE) per day. If the diet fed is 40% hay with a corn-based concentrate, then, from estimates of Sutton (1985), the digestion products are 12.6 Mcal acetate, 7.8 Mcal

propionate, 6.0 Mcal butyrate, 2.4 Mcal higher VFA, and 14 Mcal heat plus methane in the rumen; 12.6 Mcal AA, 6 Mcal LCFA, 4 Mcal glucose, and 2 Mcal VFA in the small intestine; and 5 Mcal VFA in the large intestine. Thus, VFA account for 55% of DE and probably 64% of absorbed energy.

Nutrients, however, are metabolized by intestinal tract mucosa and liver and also are produced from endogenous sources of ruminal VFA production, about 15% of propionate is probably converted to lactate and over half of butyrate is absorbed as ketone bodies (Baird et al., 1975). The direct contribution of major metabolites to respiratory $CO_2$ in fed sheep is: acetate, 32%; propionate, 19%; glucose, 9% (half from propionate); butyrate, 9%; NEFA, 2%; ketones, 10% (80% from butyrate); and other, 19% (e.g., amino acids, TGFA,). During early lactation, the contribution from glucose likely is decreased, and the contribution from NEFA and ketones increased.

The previous discussion has demonstrated that whole-body metabolism in a lactating cow is focused on the production of milk. In adipose tissue, fatty acid synthesis is decreased and lipolysis is increased, so that increased lipid is available to mammary gland for triglyceride synthesis and to other tissues for energy needs. In muscle, the rate of protein synthesis decreases below the rate of degradation because of the rapid use of plasma amino acids by mammary gland and liver, so that there is net mobilization of protein from muscle. And in liver, nutrients likely are spared from complete oxidation and used for increased gluconeogenesis, ketogenesis, acetogenesis, and export of lipoprotein lipid; availability of oxaloacetic acid may be important in the

partitioning between these pathways (Bell, 1981). Nonmammary peripheral tissues use less glucose and more lipid and ketones as an energy source, and mammary gland rapidly draws nutrients from blood. These changes in metabolism closely are regulated by the animal's endocrine system.

Hormonal control of lactation

Insulin, glucocorticoids, and prolactin are required for mammary development in most mammals, including ruminants. These three hormones also are required for the maintenance of lactation in most mammals; maintenance of lactation in ruminants, however, does not seem to require prolactin or insulin, but instead requires growth hormone (Vernon and Flint, 1983).

Although changes in the blood hormonal profile occur through the lactation cycle, these changes are relatively small compared with the differences observed between high- and low-producing cows. (Hart et al., 1978). Possibly, the relative ratios of blood hormones (namely, growth hormone to insulin) are more important than the individual concentrations themselves. Changes in sensitivity, however, likely are also involved.

Insulin Insulin concentration is reported to reach a minimum during early lactation (Koprowski and Tucker, 1973), but this decrease may not always be very large (de Boer et al., 1985). Insulin is also lower in high-producing cows and is thought to reflect energy balance (Vernon, 1981).

Propionate and butyrate, but not acetate, stimulate acute release of insulin in cattle. Normally after feeding, therefore, insulin concentrations are high, and storage of nutrients as triglyceride and

protein is favored. Insulin secretion, however, seems less responsive to insulinotropic agents during lactation (Lomax et al., 1979). In a high-producing cow, no large increase in insulin concentration occurs after feeding normal diets, so the flow of energy and amino acids to mammary gland is favored over other peripheral tissues because mammary gland does not seem to require insulin for nutrient utilization (Trenkle, 1981). Insulin concentration, however, is increased after feeding high-grain diets with a subsequent decrease in mammary competitiveness (Bell, 1981). Insulin concentration is decreased in energy-restricted, ketonemic cows (de Boer et al., 1985) and decreases after the post-feeding period (Bassett, 1974).

Studies with rats and with ruminants suggest that adipose tissue is somewhat resistant to insulin during early lactation (Flint et al., 1979; Vernon, 1981). Whereas placental lactogen may be the antagonist in rats, it does not seem to be the insulin-antagonist in ruminants, and growth hormone is likely the inhibitor (Tyson and Felig, 1971).

As mentioned, insulin does not seem needed for glucose uptake and lactose synthesis in bovine mammary gland (Hove, 1978). Insulin receptors have been identified in bovine mammary tissue and are relatively abundant. During lactation, however, they are less abundant and seemed to have no effect on lactose synthesis (Baumrucker, 1986a,b). If insulin is not needed by mammary gland, then it would be beneficial to the cow to minimize insulin concentration during lactation. Insulin inhibits the gluconeogenic activity of glucagon and catecholamines at the liver (Hart, 1983), and, in fed sheep, liver removes 40% of insulin

turnover (Heitmann et al., 1986). Insulin is a storage hormone for peripheral tissues and thus decreases the supply of NEFA, glycerol, and amino acids for liver processing and mammary uptake (Hart, 1983). Insulin, therefore, decreases milk fat yield and partitions nutrients toward body stores (Kronfeld et al., 1963).

Growth hormone In contrast to insulin, growth hormone partitions nutrients from deposition in adipose tissue toward milk synthesis and preserves body protein relative to adipose tissue during energy deficit (Hart, 1983). In other words, it favors mammary gland at the expense of adipose tissue. Another viewpoint is that growth hormone coordinates adipose tissue and mammary gland to act synergistically rather than antagonistically; thus, adipose tissue uses acetate during lactation to synthesize fatty acids that then are exported for use by mammary gland (Kronfeld, 1982). This effectively increases the lipogenic capacity of the lactating mammary gland.

In ruminants, growth hormone seems to take the place of prolactin, and its concentration in blood is elevated during early lactation (Hart et al., 1978; Koprowski and Tucker, 1973), although only slightly in some studies (de Boer et al., 1985). Growth hormone concentration is greater in high-producing cows than in low-producers at peak lactation, and changes in its concentration positively are correlated with changes in milk yield (Hart, 1983; Hart et al., 1980). Its concentration is also greater in underfed lactating cows than in those fed adequately (Hove and Blom, 1973) and is elevated in energy-restricted ketonemic cows (de Boer et al., 1985). Growth hormone concentration seems to reflect both energy

balance and stage of pregnancy and lactation (Vernon, 1981).

Growth hormone's importance in lactation is demonstrated by numerous studies showing that exogenous growth hormone dramatically increases milk yield in lactating cows (Tyrrell et al., 1982; Bauman et al., 1985). Acutely, growth hormone increases milk yield through mobilization of body tissues (Hart, 1983); however, in longer studies, appetite is eventually increased so that nutrient intake matches the increased demands of the mammary gland (Bauman et al., 1985).

Exogenous growth hormone, therefore, increases the fraction of absorbed nutrients put into milk and decreases the fraction used by nonmammary tissues for maintenance and deposition. This results in increased gross efficiency of milk production (milk energy / feed energy) up to 20% (Bauman et al., 1985), with no change in the partial efficiency of milk synthesis (milk energy / ME intake above maintenance) (Tyrrell et al., 1982; Kronfeld, 1982). There is, however, an increase in mammary efficiency (milk energy / ME uptake by mammary gland) caused by the increased availability of preformed fatty acids for milk fat synthesis (Kronfeld, 1982).

The mechanism of growth hormone's action seems to be chiefly an increase in nutrient supply to the mammary gland through stimulation of lipolysis and indirectly appetite (Hart, 1983). Support for this "push" hypothesis comes from a study by Bines et al. (1980) in which exogenous growth hormone resulted in the same percentage response in both low- and high-producing dairy cows. In the low-producers, blood glucose concentration also was increased, indicating that the mammary gland of

these cows lacked the ability to use all of the increased nutrient supply.

Other studies, however, support a galactopoietic effect of growth hormone ("pull" hypothesis). In goats, growth hormone increased mammary acetyl-CoA synthetase actively (Marinez et al., 1976). When growth hormone was infused into only one side of the mammary gland, however, no direct galactopoietic effect was observed (Hart, 1983). Additionally, although bovine growth hormone specifically binds to liver, binding was not observed in mammary tissue (Tucker and Merkel, 1987). These studies suggest that if growth hormone is galactopoietic, liver-secreted factor may be its mediator. Currently, the mediator of growth hormone's action on mammary gland is thought to be insulin-like growth factor-I (IGF-I). Baumrucker (1986a,b) has reported the presence of IGF-I receptors in bovine mammary tissue and that addition of IGF-I to mammary slices in vitro stimulated lactose synthesis. Another possibility, however, is a recently discovered liver factor that seems to mediate prolactin's lactogenic action in mice (Hoeffler and Frawley, 1987).

In summary, growth hormone seems very important in lactating ruminants, and its action probably involves both a "push" of milk synthesis through increased supply and a "pull" of nutrients to the mammary gland through endocrine stimulation of milk synthesis. Whether the stimulation of appetite is caused by growth hormone, by a mediator of growth hormone, or indirectly by effects on energy balance is not known.

Glucagon Little is known about glucagon's role in ruminant lactation. Liver seems to be the primary target organ for glucagon, and,

in fed sheep, liver accounts for 30% of glucagon removal in the body (Heitmann et al., 1986). At the liver, glucagon stimulates gluconeogenesis (Hart, 1983) and probably ketogenesis (McGarry and Foster, 1980) directly and also indirectly through enhanced substrate supply. In ruminants, however, the lipolytic response to glucagon is much less than it is in nonruminants (Bauman and Davis, 1975). Hart (1983) reports that glucagon concentration is slightly less in high-producing cows during peak lactation than in low producers, and that its concentration increases as lactation progresses to the later stages. De Boer et al. (1985), however, observed a slightly greater glucagon concentration during early lactation than during the dry period. Whereas these two studies seem irreconcilable, the period after feeding may be important and, also, the relative concentrations of glucagon and insulin may be more important than absolute amounts.

Glucocorticoids The glucocorticoids exert a permissive effect on the action of gluconeogenic hormones in the liver and the lipolytic action of hormones at adipose tissue (Hart, 1983). Although they likely do not directly stimulate the enzymes of gluconeogenesis, exogenous glucocorticoids enhance gluconeogenesis from amino acids in the liver (Reilly and Ford, 1974). In adrenalectomized sheep, this effect on gluconeogenesis was observed within 3 h after infusion of cortisol at a dose which gave a ten-fold rise in cortisol concentration (Reilly and Black, 1973). Cortisol also increases amino acid mobilization (Hart, 1983), and this effect also seems to occur after only hours of infusion (Reilly and Black, 1973).

The glucocorticoids also seem to play a role in fat distribution. Hyperactive adrenal glands in human patients often cause muscle-wasting and sometimes produce uneven fat distribution, with depletions of fat in extremities and excessive fat in the neck and back area ("humpback"). Whether cortisol also affects fat distribution in ruminants is uncertain. Glucocorticoids bind and are taken up by mammary tissue, suggesting that they are associated with lactational events (Tucker, 1979). When exogenous glucocorticoids are given to lactating cows, however, the effects have not been consistent. Whereas Braun et al. (1970) observed a decrease in milk yield, Swanson and Lind (1976) reported increased milk yield and milk fat yield. In two studies using injections of ACTH, milk yield was decreased and milk fat content was increased, with little change in milk fat yield (Flux et al., 1954; Varner and Johnson, 1983). In the study of Varner and Johnson (1983), blood glucose concentration was increased two-fold, and this increase was thought to be at least partly caused by inhibition of glucose uptake by peripheral tissues, including mammary gland. In summary, whereas glucocorticoids may promote milk fat synthesis, their effects on lactose production through effects on glucose metabolism may counteract any net increase in milk fat production.

Other hormones Several other hormones likely are involved to some extent in ruminant lactation. The catecholamines promote gluconeogenesis in liver and lipolysis in adipose tissue (Hart, 1983). Prolactin is required to initiate differentiation of mammary tissue and synthesis of milk at parturition but not after lactation has been

established (Tucker and Merkel, 1987). The thyroid hormones are permissive for many aspects of metabolism and increase basal metabolic rate. When given exogenously, they increase milk yield in-short term studies but decrease milk yield in long-term studies. The sex steroids are important for mammary gland development, and, during lactation, they may be involved with growth hormone and glucocorticoid secretion (Tucker and Merkel, 1987).

Effects of dietary supply on lactation

Several dietary-related methods have been used to try to increase milk production. Of these, only those factors which alter milk fat production will be discussed.

Dietary fat Although feeding dietary fat does not generally alter milk fat production in cows fed normal diets (Palmquist, 1976), it can change milk fat composition and, if unsaturated fats are used, even depress milk fat production. Addition of 10% oleate in the diet alters rumen function, thereby decreasing acetate production, fatty acid synthesis, and milk fat yield (Steele and Moore, 1968). Addition of 10% tallow (mainly stearate and oleate), however, resulted in no change in rumen VFA production or milk fat yield but increased the percentage of C18:0 and C18:1 and decreased the percentage of C6 to C16 fatty acids in milk fat (Storry et al., 1973). Using rumen-protected fats in lactating cow diets can increase the fraction of C18:2 up to 35% (Banks et al., 1983). The reason for a decreased percentage of C6 to C16 fatty acids is likely that high concentrations of LCFA-CoA from plasma fats depress de novo fatty acid synthesis.

High-grain diet High-grain, low-forage diets can depress milk fat yield by as much as 60% (Christie, 1981). Bell (1981) summarizes changes that occur during milk fat depression from high-grain diets. One theory for this effect is the "glucogenic theory." This theory holds that high concentrations of dietary starch enhance propionate production (Annison et al. 1974), which in turn increases glucose production and insulin secretion (Walker and Elliot, 1973). Elevated insulin concentration then increases adipose tissue lipoprotein lipase activity and lipogenesis (Benson et al., 1972), depresses adipose lipolysis (Yang and Baldwin, 1973), and thereby decreases hepatic fatty acid availability, hepatic VLDL release, and plasma lipid uptake by mammary gland.

The effects of high-grain diets on ruminal acetate production are not definitive. Annison et al. (1974) observed no change in ruminal acetate concentration and attributed decreased plasma concentration to depressed endogenous production by body tissues. Sutton (1985) also reported decreased acetate and BHB concentrations in blood, but he measured a decrease in net ruminal acetate production. Davis and Brown (1970) measured a decrease in acetate to propionate concentration ratio but no change in rumen acetate production. He then summarized literature to show that supplementation of high-grain diets with acetate has yielded variable results.

Although the exact mechanism of milk fat depression is not understood, the net effect of high-grain diets is to increase nutrient use by nonmammary tissues and decrease use by mammary gland (Moe and

Tyrrell, 1986).

Feeding frequency Increased feeding frequency reduces the milk fat depression associated with high-grain diets and also has been shown to decrease plasma concentrations of insulin, glucagon, and free fatty acids (Sutton et al., 1986). In a study by Hart (1983), feeding a 90% grain diet six instead of two times per day halved blood insulin concentration and almost doubled milk fat content. The reason for these effects is likely that blood insulin is elevated for several hours after feeding and that increased grain in the diet and increased meal size both promote this increase in insulin (Hart, 1983). Therefore, by increasing feeding frequency, meal size is decreased, insulin is decreased, and milk fat depression is less severe. No effect of feeding frequency on prolactin, growth hormone, or thyroxine concentrations were observed.

Ruminal volatile fatty acids Effects of VFA infusions into the rumen are variable. Lough et al. (1983) observed that ruminal acetate infusion increased milk fat content and yield relative to propionate infusion; however, Armstrong and Blaxter (1965) have shown that propionate infusions depress milk synthesis and increase body fat (as we would expect based on the discussion of high-grain diets). In studies by Rook and Storry, infusing about 1000 g of acetate per day into the rumen resulted in a 20% (100 g) increase and a 15% (70 g) increase in milk fat yield (Rook and Balch, 1961; Rook et al., 1965). They also measured an 11% (60 g) increase when 1500 g were infused daily (Storry and Rook, 1965). Orskov et al. (1969) fed lactating cows a fat-depressing diet and then supplemented it with acetic acid at 15% of ME intake; this reduced

(but not completely) the observed milk fat depression and also increased concentrations of C12, C14, and C16 fatty acids in milk.

Ruminal butyrate infusions of about 900 g per day increased milk fat yield 10% (65 g) in cows studied by Rook and Balch (1961) and Rook et al. (1965). And in another study, 750 g per day increased milk fat yield 5% (40 g); the fraction of milk fat that was C4 to C14, C16, and C18 fatty acids was increased 24%, increased 13%, and decreased 25%, respectively (Storry and Rook, 1965).

In related studies, ruminal infusion of 500 g isovaleric acid increased milk fat yield 6% (35 g) (Rook et al., 1965), and supplementing fat-depressing diets with 5% butanediol either increased milk fat yield by 150 g (Hess and Young, 1972) or had no significant effect (Bonner et al., 1976).

## Effects of 2-Ketoisocaproate on Metabolism

### Metabolism of leucine and 2-ketoisocaproate

Leucine, like the other branched-chain amino acids (BCAA), is metabolized differently than most indispensable amino acids (Harper et al., 1984). Whereas most amino acids are catabolized almost completely by liver, leucine is oxidized extensively by peripheral tissues, and studies in rats indicate that liver has less capacity than peripheral tissues to degrade BCAA. The initial catabolic step for BCAA is catalyzed by a unique transaminase, BCAA aminotransferase, which is widely distributed among tissues and results in production of 2-ketoisocaproate (KIC).

In sheep, 40% of absorbed leucine is removed by liver, where some is

oxidized but most is used for synthesis of export proteins; the other 60% is taken up by peripheral tissues and either used for protein synthesis or transaminated to KIC (Bergman, 1986). Peripherally produced KIC undergoes further oxidation or is released to blood, eventually removed by liver, and then either converted back to leucine or oxidized (Bergman, 1986). The metabolic fate of orally administered KIC in ruminants is not known; in dogs, however, 70% of an orally administered KIC load is metabolized by gastrointestinal mucosa and liver, with liver catabolism of KIC resulting in ketone bodies (70%) and leucine (30%) (Abumrad et al., 1982b). Although metabolism of KIC in ruminants is thought to differ from that of nonruminants, greater than half of blood KIC is removed by splanchnic tissues (Pell et al., 1986); therefore, in ruminants, most orally administered KIC likely is metabolized also in splanchnic tissues.

Classically, leucine catabolism is as follows (Rodwell, 1979): leucine is transaminated to KIC either in cytosol or mitochondria; in mitochondria, KIC is oxidatively decarboxylated to isovaleryl-CoA, which is catabolized eventually to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) and then acetoacetic acid and acetyl-CoA. Mitochondrial HMG-CoA is not transported into cytosol, and therefore cholesterol biosynthesis from leucine carbon in adipose tissue (Rosenthal et al., 1974) must be through resynthesis of HMG-CoA from the ketone body endproducts of mitochondrial leucine catabolism.

An alternative pathway of leucine catabolism that has been discovered recently in rat and human liver is oxidation of cytosolic KIC

to 3-hydroxyisovalerate (Sabourin and Bieber, 1983). Because 3-Hydroxyisovalerate is structurally similar to HMG-CoA, it may be an inhibitor of HMG-CoA reductase, the rate-limiting step of cholesterol synthesis. Changes in cholesterol synthesis in specific organs could potentially alter steroid synthesis and may explain some of the unique metabolic effects of leucine and KIC.

Effects of 2-ketoisocaproate on metabolism

Leucine and KIC have effects on metabolism that are unique among the amino and keto acids. Leucine and KIC both inhibit proteolysis and stimulate protein synthesis in vitro (Tischler et al., 1982; Kirsch et al., 1976; Buse and Reid, 1975; Chua et al., 1979) and reduce nitrogen excretion in vivo during times of extreme protein catabolism (Ell et al., 1978; Mitch et al., 1981; Sapir et al., 1983; Sax et al., 1986). Isovaleryl-CoA is a further catabolite of leucine and there is evidence that isovalerate also decreases proteolysis in vitro (Chua et al., 1979), suggesting that a further step in leucine catabolism may be responsible for the effects of leucine and KIC.

Protein turnover likely accounts for a minimum of 15-25% of maintenance energy requirement (Young et al., 1983; Lobley et al., 1980). Thus, if nutrient absorption is constant, decreased protein turnover should increase the energy available for lipogenesis and (or) increase net protein synthesis. Additionally, mechanisms controlling protein metabolism and those controlling lipid and carbohydrate metabolism are not distinct. Therefore, effects of compounds within the leucine metabolic pathway on protein metabolism indicate that they also may alter

energy metabolism. Previous observations are that: 1) leucine increases insulin concentration and decreases glucose uptake and oxidation by human forearm (Abumrad et al., 1982a); 2) KIC decreases pyruvate oxidation in muscle from fasted rats (Chang and Goldberg, 1978), decreases gluconeogenesis in perfused kidney (Stumpf et al., 1978), decreases glucagon secretion, and increases insulin secretion in perfused pancreas (Leclercq-Meyer et al., 1979; Holze and Panten, 1979); 3) isovalerate does not stimulate insulin secretion from perfused pancreas (Vague et al., 1975); and 4) leucine must be metabolized, presumably to KIC, to elicit increased insulin secretion from perfused pancreas and decreased pyruvate oxidation from rat muscle (Vague et al., 1975; Chang and Goldberg, 1978). Additionally, patients with postsurgical trauma exhibited elevated plasma ketone body concentration in response to KIC infusion (Sapir et al., 1983).

## REFERENCES CITED

Abumrad, N. N., R. P. Robinson, B. R. Gooch, and W. W. Lacy. 1982a. The effect of leucine infusion on substrate flux across the human forearm. J. Surg. Res. 32:453-463.

Abumrad, N. N., K. L. Wise, P. E. Williams, N. A. Abumrad, and W. W. Lacy. 1982b. Disposal of 2-ketoisocaproate: roles of liver, gut, and kidneys. Am. J. Phyiol. 243:E123-E131.

Aiello, R. J., T. M. Kenna, and J. H. Herbein. 1984. Hepatic gluconeogenic and ketogenic interrelationships in the lactating cow. J. Dairy Sci. 67:1707-1715.

Alpers, D. H. 1986. Uptake and fate of absorbed amino acids and peptides in the mammalian intestine. Fed. Proc. 45:2261-2267.

Annison, E. F., and J. L. Linzell. 1964. The oxidation and utilization of glucose and acetate by the mammary gland of the goat in relation to their over-all metabolism and to milk formation. J. Physiol. 175:372-385.

Annison, E. F., R. Bickerstaffe, and J. L. Linzell. 1974. Glucose and fatty acid metabolism in cows producing milk of low fat content. J. Agric. Sci., Camb. 82:87-95.

Armstrong, D. G., and K. L. Blaxter. 1965. Effects of acetic and propionic acids on energy retention and milk secretion in goats. Pages 59-70 in K. L. Blaxter, ed. Energy Metabolism: Proceedings of the 3rd symposium. Euro. Assoc. Animal Prod. Publ. 11. Academic Press, New York.

Baird, G. D., K. G. Hibbitt, G. D. Hunter, P. Lund, M. Stubbs, and H. A. Krebs. 1968. Biochemical aspects of bovine ketosis. Biochem. J. 107:683-689.

Baird, G. D., H. W. Symonds, and R. Ash. 1975. Some observations on metabolite production and utilization in vivo by the gut and liver of adult dairy cows. J. Agric. Sci., Camb. 85:281-296.

Baird, G. D., J. G. Van der Walt, and E. N. Bergman. 1983. Whole-body metabolism of glucose and lactate in productive sheep and cows. Br. J. Nutr. 80:249-265.

Baldwin, R. L. and N. E. Smith. 1979. Regulation of energy metabolism in ruminants. Pages 1-27 in H.H. Draper, ed. Advances in Nutritional Research. Volume 2. Plenum Press, New York.

Baldwin, R. L., J. R. Reichl, S. Louis, N. E. Smith, Y. T. Yang, and E. Osborne. 1973. Effects of age, pregnancy, and lactation on rat, guinea pig, and cow adipose enzyme activities and cow adipose metabolism. J. Dairy Sci. 56:340-349.

Baldwin, R. L., N. E. Forsberg, and C. Y. Hu. 1985. Potential for altering energy partition in the lactating cow. J. Dairy Sci. 68:3394-3402.

Banks, W., J. L. Clapperton, and W. Steele. 1983. Dietary manipulation of the content and fatty acid composition of milk fat. Proc. Nutr. Soc. 42:399-406.

Bassett, J. M. 1974. Diurnal patterns of plasma insulin, growth hormone, corticosteroid and metabolite concentrations in fed and fasted sheep. Aust. J. Biol. Sci. 27:167-181.

Bauman, D. E., and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514-1529.

Bauman, D. E., and C. L. Davis. 1974. Biosynthesis of milk fat. Chapter 2, pages 31-75 in B. L. Larson and V. R. Smith, eds. Lactation. A comprehensive treatise. Volume II. Academic Press, New York.

Bauman, D. E., and C. L. Davis. 1975. Regulation of lipid metabolism. Pages 496-509 in I. W. McDonald and A. C. I. Warner, eds. Digestion and metabolism in the ruminant. Univ. N. E. Publishing Unit, Armidale, Australia.

Bauman, D. E., R. E. Brown, and C. L. Davis. 1970. Pathways of fatty acid synthesis and reducing equivalent generation in mammary gland of rat, sow, and cow. Arch. Biochem. Biophys. 140:237-244.

Bauman, D. E., P. J. Eppard, M. J. DeGeeter, and G. M. Lanza. 1985. Responses of high-producing dairy cows to long-term treatment with pituitary somatotropin and recombinant somatotropin. J. Dairy Sci. 68:1352-1362.

Baumrucker, C. R. 1986a. Insulin like growth factor 1 (IGF-1) and insulin stimulates lactating bovine mammary tissue DNA synthesis and milk production in vitro. J. Dairy Sci. 69 (Suppl. 1):120.

Baumrucker, C. R. 1986b. Insulin-like growth factor 1 (IGF-1) and insulin stimulates DNA synthesis in bovine mammary tissue explants obtained from pregnant cows. J. Dairy Sci. 69 (Suppl. 1):120.

Bell, A. W. 1981. Lipid metabolism in liver and selected tissues and in the whole body of ruminant animals. Pages 363-410 in W. W. Christie, ed. Lipid metabolism in ruminant animals. Pergamon Press, Elmsford, NY.

Bell, A. W., and G. E. Thompson. 1979. Free fatty acid oxidation in bovine muscle in vivo: effects of cold exposure and feeding. Am. J. Physiol. 237(4):E309-E315.

Belyea, R. L., G. R. Frost, F. A. Martz, J. L. Clark, and L. G. Forkner. 1978. Body composition of dairy cattle by potassium-40 liquid scintillation detection. J. Dairy Sci. 61:206-211.

Bennink, M. R., R. W. Mellenberger, R. A. Frobish, and D. E. Bauman. 1972. Glucose oxidation and entry rate as affected by the initiation of lactation. J. Dairy Sci. 55:712-713.

Benson, J. D., E. W. Askew, R. S. Emery, and J. W. Thomas. 1972. Metabolism of fatty acids by adipose tissue and liver of cows fed normal, restricted roughage, or MgO supplemented rations. J. Dairy Sci. 55:83-92.

Bergman, E. N. 1986. Splanchnic and peripheral uptake of amino acids in relation to the gut. Fed. Proc. 45:2277-2282.

Bergman, E. N., and R. N. Heitmann. 1978. Metabolism of amino acids by the gut, liver, kidneys, and peripheral tissues. Fed. Proc. 37:1228-1232.

Bergman, E. N., and J. E. Wolff. 1971. Metabolism of volatile fatty acids by liver and portal-drained viscera in sheep. Am. J. Physiol. 221:586-592.

Bergman, E. N., D. J. Starr, and S. S. Reulein. 1968. Glycerol metabolism and gluconeogenesis in the normal and hypoglycemic ketotic sheep. Am. J. Physiol. 215:874-880.

Bergman, E. N., R. J. Havel, B. M. Wolfe, and T. Bohmer. 1971. Quantitative studies of the metabolism of chylomicron triglycerides and cholesterol by liver and extrahepatic tissues of sheep and dogs. J. Clin. Invest. 50:1831-1839.

Bickerstaffe, R., and E. F. Annison. 1968. The desaturation of stearic acid by mammary-gland tissue of the lactating goat and sow. Biochem. J. 108:47P-48P.

Bickerstaffe, R., and E. F. Annison. 1970. The desaturase activity of goat and sow mammary tissue. Comp. Biochem. Physiol. 35:653-665.

Bickerstaffe, R., and E. F. Annison. 1974. The metabolism of glucose, acetate, lipids and amino acids in lactating dairy cows. J. Agric. Sci., Camb. 82:71-85.

Bines, J. A., and I. C. Hart. 1982. Metabolic limits to milk production, especially roles of growth hormone and insulin. J. Dairy Sci. 65:1375-1389.

Bines, J. A., I. C. Hart, and S. V. Morant. 1980. Endocrine control of energy metabolism in the cow: the effect on milk yield and levels of some blood constituents of injecting growth hormone and growth hormone fragments. Br. J. Nutr. 43:179-188.

Bitman, J., D. L. Wood, H. F. Tyrell, D. E. Bauman, C. J. Peel, A. C. G. Brown, and P. J. Reynolds. 1984. Blood and milk lipid responses induced by growth hormone administration in lactating cows. J. Dairy Sci. 67:2873-2880.

Bonner, J. M., J. W. Young, and P. J. Berger. 1976. Effects of 1,3-butanediol in alleviating and preventing milk fat depression in cows. J. Dairy Sci. 59:431-438.

Botts, R. L., R. W. Hemken, and L. S. Bull. 1979. Protein reserves in the lactating dairy cow. J. Dairy Sci. 62:433-440.

Braun, R. K., E. N. Bergman, and T. F. Albert. 1970. Effects of various synthetic glucocorticoids on milk production and blood glucose and ketone body concentrations in normal and ketotic cows. J. Am. Vet. Med. Assoc. 157:941-946.

Buse, M. G., and S. S. Reid. 1975. Leucine: a possible regulator of protein turnover in muscle. J. Clin. Invest. 56:1250-1261.

Bywater, A. C., and R. L. Baldwin. 1980. Alternative strategies in food-animal production. Pages 1-30 in R. L. Baldwin, ed. Animals, feed, food, and people. An analysis of the role of animals in food production. Westview Press, Boulder, CO.

Chang, T. W., and A. L. Goldberg. 1978. Leucine inhibits oxidation of glucose and pyruvate in skeletal muscles during fasting. J. Biol. Chem. 253:3696-3701.

Christie, W. W. 1981. The effects of diet and other factors on the lipid composition of ruminant tissues and milk. Pages 193-226 in W. W. Christie, ed. Lipid metabolism in ruminant animals. Pergamon Press, Elmsford, NY.

Chua, B., D. L. Siehl, and H. E. Morgan. 1979. Effect of leucine and metabolites of branched chain amino acids on protein turnover in heart. J. Biol. Chem. 254:8358-8362.

Clark, J. H. 1975. Lactational responses to post-ruminal administration of proteins and amino acids. J. Dairy Sci. 58:1178-1197.

Connelly, J. D., H. H. Head, and W. F. Williams. 1964. Metabolism of albumin-bound palmitate-1-$C^{14}$ by the isolated perfused goat liver. J. Dairy Sci. 47:386-390.

Cook, R. M., and L. D. Miller. 1965. Utilization of volatile fatty acids in ruminants. I. Removal of them from portal blood by the liver. J. Dairy Sci. 48:1339-1345.

Dagenais, G. R., R. G. Tancredi, and K. L. Zierler. 1976. Free fatty acid oxidation by forearm muscle at rest, and evidence for an intramuscular lipid pool in the human forearm. J. Clin. Invest. 58:421-431.

Davis, C. L. 1967. Acetate production in the rumen of cows fed either control or low-fiber, high-grain diets. J. Dairy Sci. 50:1621-1625.

Davis, C. L., and D. E. Bauman. 1974. General metabolism associated with the synthesis of milk. Chapter 1, pages 3-30 in B. L. Larson and V. R. Smith, eds. Lactation. A comprehensive treatise. Volume II. Academic Press, New York.

Davis, C. L., and R. E. Brown. 1970. Pages 545-565 in A. T. Phillipson, ed. Physiology of digestion and metabolism in the ruminant. Oriel Press, Newcastle upon Tyne, England.

Davis, S. R., T. N. Barry, and G. A. Hughson. 1981. Protein synthesis in tissues of growing lambs. Br. J. Nutr. 46:409-419.

Davis, S. R., and R. J. Collier. 1985. Mammary blood flow and regulation of substrate supply. J. Dairy Sci. 68:1041-1058.

Dawson, R. M. C., and P. Kemp. 1970. Biohydrogenation of dietary fats in ruminants. Pages 504-518 in A.T. Phillipson, ed. Physiology of digestion and metabolism in the ruminant. Oriel Press, Newcastle upon Tyne, England.

de Boer, G., A. Trenkle, and J. W. Young. 1985. Glucagon, insulin, growth hormone, and some blood metabolites durng energy restriction ketonemia of lactating cows. J. Dairy Sci. 68:326-337.

DeLouis, C., J. Djiane, L. M. Houdebine, and M. Terqui. 1980. Relation between hormones and mammary gland function. J. Dairy Sci. 63:1492-1513.

Ell, S., M. Fynn, P. Richards, and D. Halliday. 1978. Metabolic studies with keto acid diets. Am. J. Clin. Nutr. 31:1776-1783.

Flint, D. J., P. A. Sinnett-Smith, R. A. Clegg, an R. G. Vernon. 1979. Role of insulin receptors in the changing metabolism of adipose tissue during pregnancy and lactation in the rat. Biochem. J. 182:421-427.

Flux, D. S., S. J. Folley, and S. J. Rowland. 1954. The effect of adrenocorticotrophic hormone on the yield and composition of the milk of the cow. J. Endocrinol. 10:333-339.

Forsberg, N. E., R. L. Baldwin, and N. E. Smith. 1984. Roles of acetate and its interactions with glucose and lactate in cow mammary tissue. J. Dairy Sci. 67:2247-2254.

Forsberg, N. E., R. L. Baldwin, and N. E. Smith. 1985. Roles of glucose and its interactions with acetate in maintenance and biosynthesis in bovine mammary tissue. J. Dairy Sci. 68:2544-2549.

Gooden, J. M., and A. K. Lascelles. 1973. Effect of feeding protected lipid on the uptake of precursors of milk fat by the bovine mammary gland. Aust. J. Biol. Sci. 26:1201-1210.

Gopinath, R., and W. D. Kitts. 1984. Growth, N-methylhistidine excretion and muscle protein degradation in growing beef steers. J. Anim. Sci. 59:1262-1269.

Gregg, V. A., and L. P. Milligan. 1982. In vitro energy costs of NA+, K+ATPase activity and protein synthesis in muscle from claves differing in age and breed. Br. J. Nutr. 48:65-71.

Gritchting, G., R. L. Baldwin, and N. E. Smith. 1977. Effect of stage of lactation and fasting on cellularity and lipogenesis in cow adipose tissue. J. Dairy Sci. 60 (Suppl. 1):120. (Abstract)

Hansen, H. O., and J. Knudsen. 1987. Effect of exogenous long-chain fatty acids on individual fatty acid synthesis by dispersed ruminant mammary gland cells. J. Dairy Sci. 70:1350-1354.

Hanson, R. W., and F. J. Ballard. 1968. The metabolic fate of the products of citrate cleavage. Biochem. J. 108:705-713.

Harper, A. E., R. H. Miller, and K. P. Block. 1984. Branched-chain amino acid metabolism. Ann. Rev. Nutr. 4:409-454.

Hart, I. C. 1983. Endocrine control of nutrient partition in lactating ruminants. Proc. Nutr. Soc. 42:181-194.

Hart, I. C., J. A. Bines, S. V. Morant, and J. L. Ridley. 1978. Endocrine control of energy metabolism in the cow: comparison of the levels of hormones (prolactin, growth hormone, insulin and thyroxine) and metabolites in the plasma of high- and low-yielding cattle at various stages of lacation. J. Endocrinol. 77:333-345.

Hart, I. C., S. E. Lawrence, and T. B. Mepham. 1980. Effect of exogenous growth hormone on mammary blood flow and milk yield in lactating goats. J. Physiol. 308:46P.

Heitmann, R. N., S. C. Sensenig, C. K. Reynolds, J. M. Fernandez, and D. J. Dawes. 1986. Changes in energy metabolite and regulatory hormone concentrations and net fluxes across splanchnic and peripheral tissues in fed and progressively fasted ewes. J. Nutr. 116:2516-2524.

Heitmann, R. N., D. J. Dawes, and S. C. Sensenig. 1987. Hepatic ketogenesis and peripheral ketone body utilization in the ruminant. J. Nutr. 117:1174-1180.

Hess, G. S., and J. W. Young. 1972. Preventing and alleviating milk fat depression by feeding 1,3-butanediol to cows. J. Dairy Sci. 55:1097-1105.

Hoeffler, J. P., and L. S. Frawley. 1987. Liver tissue produces a potent lactogen that partially mimics the actions of prolactin. Endocrinology 120:1679-1681.

Holdsworth, E. S., E. Neville, C. Nader, I. G. Jarrett, and O. H. Filsell. 1964. The metabolism of acetate in the sheep. Biochim. Biophys. Acta 86:240-249.

Holze, S., and U. Panten. 1979. Studies on the role of ß-cell metabolism in the insulinotropic effect of 2-ketoisocaproic acid. Biochim. Biophys. Acta 588:211-218.

Hove, K. 1978. Maintenance of lactose secretion during acute insulin deficiency in lactating goats. Acta Physiol. Scand. 103:173-179.

Hove, K., and A. K. Blom. 1973. Plasma insulin and growth hormone in dairy cows; diurnal variation and relation to food intake and plasma sugar and acetoacetate levels. Acta Endocrinol. 73:289-303.

Huntington, G. B. 1984. Net absorption of glucose and nitrogenous compounds by lactating Holstein cows. J. Dairy Sci. 67:1919-1927.

Huntington, G. B., and P. J. Reynolds. 1983. Net volatile fatty acid absorption in nonlactating Holstein cows. J. Dairy Sci. 66:86-92.

Huntington, G. B., and C. K. Reynolds. 1987. Oxygen consumption and metabolite flux of bovine portal-drained viscera and liver. J. Nutr. 117:1167-1173.

Jansen, G. R., and H. Hunsaker. 1986. Effect of dietary protein and energy on protein synthesis during lactation in rats. J. Nutr. 116:957-968.

Jones, E. A. 1978. Lactose biosynthesis. Pages 371-385 in B. L. Larson, ed. Lactation. A comprehensive treatise. Volume IV. Academic Press, New York.

Jurgens, M. H. 1978. Animal feeding and nutrition. 4th ed. Kendall/Hunt Publishing Company, Dubuque, Iowa. 427 pp.

Katz, M. L., and E. N. Bergman. 1969. Hepatic and portal metabolism of glucose, free fatty acids, and ketone bodies in the sheep. Am. J. Physiol. 216:953-960.

Katz, I., and M. Keeney. 1966. Occurrence of ketostearic acids in the rumen. J. Dairy Sci. 49:967-970.

King, K. R., J. M. Gooden, and E. F. Annison. 1985. Acetate metabolism in the mammary gland of the lactating ewe. Aust. J. Biol. Sci. 38:23-31.

Kirsch, R. E., L. O'C. Frith, and S. J. Saunders. 1976. Stimulation of albumin synthesis by keto analogues of amino acids. Biochim. Biophys. Acta 442:437-441.

Knowles, S. E., I. G. Jarrett, O. H. Filsell, and F. J. Ballard. 1974. Production and utilization of acetate in mammals. Biochem. J. 142:401-411.

Konig, B. A., J. D. Oldham, and D. S. Parker. 1984. The effect of abomasal infusion of casein on acetate, palmitate and glucose kinetics in cows durng early lactation. Br. J. Nutr. 52:319-328.

Koong, L. J., C. L. Ferrell, and J. A. Nienaber. 1985. Assessment of interrelationships among levels of intake and production, organ size and fasting heat production in growing animals. J. Nutr. 115:1383-1390.

Koprowski, J. A., and H. A. Tucker. 1973. Bovine serum growth hormone, corticoids and insulin during lactation. Endocrinology 93:645-651.

Krebs, H. A. 1965. Inaugural lecture: some aspects of gluconeogenesis (the relations between gluconeogenesis and ketogenesis). Pages 1-9 in K. L. Blaxter, ed. Energy Metabolism: Proceedings of the 3rd symposium. Euro. Assoc. Anim. Prod. Publ. 11. Academic Press, New York.

Kronfeld, D. S. 1982. Major metabolic determinants of milk volume, mammary efficiency, and spontaneous ketosis in dairy cows. J. Dairy Sci. 65:2204-2212.

Kronfeld, D. S., G. P. Mayer, J. M. Robertson, and F. Raggi. 1963. Depression of milk secretion during insulin administration. J. Dairy Sci. 46:559-563.

Laarveld, B., R. K. Chaplin, and R. P. Brockman. 1985. Effects of insulin on the metabolism of acetate, ß-hydroxybutyrate, and triglycerides by the bovine mammary gland. Comp. Biochem. Physiol. 82B:265-267.

Larson, B. L. 1985. Biosynthesis and cellular secretion of milk. Pages 129-163 in B. L. Larson, ed. Lactation. Iowa State University Press, Ames, Iowa.

Larson, B. L., and V. R. Smith. 1974. Lactation. A comprehensive treatise. Academic Press, New York.

Leclercq-Meyer, V., J. Marchand, R. Leclercq, and W. J. Malaisse. 1979. Interactions of 2-ketoisocaproate, glucose and arginine in the secretion of glucagon and insulin from the perfused rat pancreas. Diabetologia 15:121-126.

Lindsay, D. S. 1970. Pages 438-451 in A. T. Phillipson, ed. Physiology of digestion and metabolism in the ruminant. Oriel Press, Newcastle upon Tyne, England.

Lindsay, D. B. 1976. Amino acids as sources of energy. Pages 183-195 in D. J. A. Cole, K. N. Boorman, P. J. Buttery, J. Lewis, R. J. Neale, and J. Swan, eds. Protein metabolism and nutrition. Euro. Assoc. Anim. Prod. Publ. 16. Butterworths, London.

Lindsay, D. B., and B. P. Setchell. 1976. The oxidation of glucose, ketone bodies and acetate by the brain of normal and ketonaemic sheep. J. Physiol., Lond. 259:801-823.

Linzell, J. L. 1967. The effect of infusions of glucose, acetate and amino acids on hourly milk yield in fed, fasted and insulin-treated goats. J. Physiol. 190:347-357.

Lobley, G. E. 1986. The physiological bases of nutrient responses; growth and fattening. Proc. Nutr. Soc. 45:203-214.

Lobley, G. E., V. Milne, J. M. Lovie, P. J. Reeds, and K. Pennie. 1980. Whole body and tissue protein synthesis in cattle. Br. J. Nutr. 43:491-502.

Lomax, M. A., and G. D. Baird. 1983. Blood flow and nutrient exchange across the liver and gut of the dairy cow. Br. J. Nutr. 49:481-496.

Lomax, M. A., G. D. Baird, C. B. Mallinson, and H. W. Symonds. 1979. Differences between lactating and non-lactating dairy cows in concentration and secretion rate of insulin. Biochem. J. 180:281-289.

Lough, D. S., E. C. Prigge, W. H. Hoover, and G. A. Varga. 1983. Utilization of ruminally infused acetate or propionate and abomasally infused casein by lactating goats. J. Dairy Sci. 66:756-762.

Luick, J. R., and M. Kleiber. 1961. Quantitative importance of plasma glucose for synthesis of milk fat glycerol. Am. J. Physiol. 200:1327-1329.

Marinez, D. I., C. A. Ricks, and R. M. Cook. 1976. Utilization of volatile fatty acids in ruminants. 8. Acetate activation in mammary tissue. J. Agric. Food Chem. 24:927-935.

McDowell, G. 1983. Hormonal control of glucose homoeostasis in ruminants. Proc. Nutr. Soc. 42:149-167.

McGarry, J. D., and D. W. Foster. 1980. Regulation of hepatic fatty acid oxidation and ketone body production. Ann. Rev. Biochem. 49:396-420.

McNamara, J. P., and J. K. Hillers. 1986. Regulation of bovine adipose tissue metabolism during lactation. 1. Lipid synthesis in response to increased milk production and decreased energy intake. J. Dairy Sci. 69:3032-3041.

McNamara, J. P., D. C. McFarland, and S. Bai. 1987. Regulation of bovine adipose tissue metabolism during lactation. 3. Adaptations of hormone-sensitive and lipoprotein lipases. J. Dairy Sci. 70:1377-1384.

Mesbah, M. M., and R. L. Baldwin. 1983. Effects of diet, pregnancy, and lactation on enzyme activities and gluconeogenesis in ruminant liver. J. Dairy Sci. 66:783-788.

Metz, S. H. M., and S. G. van den Bergh. 1977. Regulation of fat mobilization in adipose tissue of dairy cows in the period around parturition. Neth. J. Agric. Sci. 25:198-211.

Milligan, L. P., and B. W. McBride. 1985. Shifts in animal energy requirements across physiological and alimentational states. J. Nutr. 115:1374-1382.

Milligan, L. P., and M. Summers. 1986. The biological basis of maintenance and its relevance to assessing responses to nutrients. Proc. Nutr. Soc. 45:185-193.

Millward, D. J., and P. J. Garlick. 1976. The energy cost of growth. Proc. Nutr. Soc. 35:339.

Mitch, W. E., M. Walser, and D. G. Sapir. 1981. Nitrogen sparing induced by leucine compared with that induced by its keto analog, 2-ketoisocaproate, in fasting obese man. J. Clin. Invest. 67:553-562.

Moe, P. W. 1981. Energy metabolism of dairy cattle. J. Dairy Sci. 64:1120-1139.

Moe, P. W., and H. F. Tyrrell. 1986. The physiological bases of nutrient responses during lactation. Proc. Nutr. Soc. 45:215-220.

Moore, J. H., and W. W. Christie. 1981. Lipid metabolism in the mammary gland of ruminant animals. Pages 227-277 in W. W. Christie, ed. Lipid metabolism in ruminant animals. Pergamon Press, Elmsford, NY.

Noble, R. C. 1981. Digestion, absorption and transport of lipids in ruminant animals. Pages 57-88 in W. W. Christie, ed. Lipid metabolism in ruminant animals. Pergamon Press, Elmsford, NY.

Oddy, V. H., J. M. Gooden, and E. F. Annison. 1984. Partitioning of Nutrients in Merino Eves I. Contribution of skeletal muscle, the pregnant uterus and the lactating mammary gland to total energy expenditure. Aust. J. Biol. Sci. 37:375-388.

O'Hare, M., P. Kirvin, H. Razooki-Hasan, C. Wilde, D. A. White, and R. J. Mayer. 1986. Secretion-coupled protein degradation: studies on mammary casein. Biochimica et Biophysica Acta 889:49-58.

Oldham, J. D., and T. Smith. 1982. Protein-energy interrelationships for growing and for lactating cattle. Pages 103-130 in E. L. Miller and I. H. Pike, eds. Protein contribution of feedstuffs for ruminants: application to feed formulation. Butterworth Scientific, Boston, Mass.

Orskov, E. R., W. P. Flatt, P. W. Moe, and A. W. Munson. 1969. The influence of ruminal infusion of volatile fatty acids on milk yield and composition and on energy utilization by lactating cows. Br. J. Nutr. 23:443-453.

Orskov, E. R., N. A. MacLeod, S. T. M. Fahmy, L. Istasse, and F. D. DeB. Hovell. 1983. Investigation of nitrogen balance in dairy cows and steers nourished by intragastric infusion. Effects of submaintenance energy input with or without protein. Br. J. Nutr. 50:99-107.

Owen, O. E., A. P. Morgan, H. G. Kemp, J. M. Sullivan, M. G. Herrera, and G. F. Cahill, Jr. 1967. Brain metabolism during fasting. J. Clin. Invest. 46:1589-1595.

Palmquist, D. L. 1976. A kinetic concept of lipid transport in ruminants. A review. J. Dairy Sci. 59:355-363.

Palmquist, D. L., C. L. Davis, R. E. Brown, and D. S. Sachan. 1969. Availability and metabolism of various substrates in ruminants. V. Entry rate into the body and incorporation into milk fat of D(-)ß-hydroxybutyrate. J. Dairy Sci. 52:633-638.

Payne, E., and C. J. Masters. 1971. The in vitro incorporation of $^{14}C$-labelled fatty acids into ovine liver and omental adipose tissue. Int. J. Biochem. 2:623-643.

Peel, C. J. T. J. Fronk, D. E. Bauman, and R. C. Gorewit. 1982. Lactational response to exogenous growth hormone and abomasal infusion of a glucose-sodium caseinate mixture in high yielding dairy cows. J. Nutr. 112:1770-1778.

Pell, J. M., E. M. Caldarone, and E. N. Bergman. 1986. Leucine and 2-ketoisocaproate metabolism and interconversions in fed and fasted sheep. Metabolism 35:1005-1016.

Pethick, D. W., and D. B. Lindsay. 1982. Acetate metabolism in lactating sheep. Br. J. Nutr. 48:319-328.

Pethick, D. W., and B. Vernau. 1984. The effect of acetate on the utilization of glucose by skeletal muscle on the sheep hindlimb. Can. J. Anim. Sci. 64 (Suppl. 1):291-292.

Pethick, D. W., D. B. Lindsay, P. J. Barker, and A. J. Northrop. 1981. Acetate supply and utilization by the tissues of sheep in vivo. Br. J. Nutr. 46:97-110.

Pethick, D. W., D. B. Lindsay, P. J. Barker, and A. J. Northrop. 1983. The metabolism of circulating non-esterified fatty acids by the whole animal, hind-limb muscle and uterus of pregnant ewes. Brit. J. Nutr. 49:129-143.

Reeds, P. J., K. W. J. Wahle, and P. Haggarty. 1982. Energy costs of protein and fatty acid synthesis. Proc. Nutr. Soc. 41:155.

Reeds, P. J., M. F. Fuller, and B. A. Nicholson. 1985. Metabolic basis of energy expenditure with particular reference to protein. Pages 46-57 in J. S. Garrow and D. Halliday, eds. Substrate and energy metabolism. John Libbey, London.

Reilly, P. E. B., and A. L. Black. 1973. Early effects of cortisol on glucose and alanine metabolism in adrenalectomized sheep. Am. J. Physiol. 225:689-695.

Reilly, P. E. B., and E. J. H. Ford. 1974. The effect of betamethasone on gluscose production and on gluconeogenesis from amino acids in sheep. J. Endocrinol. 60:455-461.

Reynolds, C. K., G. B. Huntington, H. F. Tyrrell, and P. J. Reynolds. 1986. Splanchnic tissue and whole animal oxygen consumption by lactating Holstein cows. J. Dairy Sci. 69 (Suppl. 1):193.

Roberts, C. J., I. M. Reid, B. V. Pike, and B. R. Turfrey. 1979. Tissue mobilization in dairy cows in early lactation. Proc. Nutr. Soc. 38:68A.

Rodwell, V. W. 1979. Catabolism of the carbon skeletons of amino acids. Pages 406-429 in H. A. Harper, V. W. Rodwell, and P. A. Mayes, eds. Review of physiological chemistry. 17th edition. Lange Medical Publications, Los Altos, CA.

Rook, J. A. F., and C. C. Balch. 1961. The effects of intraruminal infusions of acetic, protpionic and butyric acids on the yield and composition of the milk of the cow. Br. J. Nutr. 15:361-368.

Rook, J. A. F., C. C. Balch, and V. W. Johnson. 1965. Further observations on the effects of intraruminal infusions of volatile fatty acids and of lactic acid on the yield and composition of the milk of the cow. Br. J. Nutr. 19:93-99

Rosenthal, J., A. Angel, and J. Farkas. 1974. Metabolic fate of leucine: a significant sterol precursor in adipose tissue and muscle. Am. J. Physiol. 226:411-418.

Sabourin, P. J., and L. L. Bieber. 1983. Formation of 3-hydroxyisovalerate by a 2-ketoisocaproate oxygenase in human liver. Metabolism 32:160-164.

Sapir, D. G., P, M. Stewart, M. Walser, C. Moreadith, E. D. Moyer, A. L. Imbembo, M. B. Rosenshein, and S. Munoz. 1983. Effects of 2-ketoisocaproate and of leucine on nitrogen metabolism in postoperative patients. Lancet 1:1010-1014.

Sauer, F., and J. D. Erfle. 1966. On the mechanism of acetoacetate synthesis by guinea pig liver fractions. J. Biol. Chem. 241:30-37.

Sax, H. C., M. A. Talamini, and J. E. Fischer. 1986. Clinical use of branched-chain amino acids in liver disease, sepsis, trauma, and burns. Arch. Surg. 121:358-366.

Schaefer, A. L., and C. R. Krishnamurti. 1984. Nutrient deprivation and protein synthesis in the pregnant ewe, fetus, and placenta. Can. J. Anim. Sci. 64 (Suppl.):289-290.

Schwalm, J. W., and L. H. Schultz. 1976. Relationship of insulin concentration to blood metabolites in the dairy cow. J. Dairy Sci. 59:255-261.

Scott, T. W., L. J. Cook, and S. C. Mills. 1971. Protection of dietary polyunsaturated fatty acids against microbial hydrogenation in ruminants. J. Am. Oil Chem. Soc. 48:358-364.

Sherwin, R. S., R. G. Hendler, and P. Felig. 1975. Effect of ketone infusions on amino acid and nitrogen metabolism in man. J. Clin. Invest. 55:1382-1390.

Shirley, J. E., D. Morrow, and R. S. Emery. 1973. Mammary and adipose lipoprotein lipase and glyceride synthesis as regulators of blood fat uptake. J. Nutr. 103:XXII. (Abstract)

Smith, G. J., and S. McCarthy. 1969. Synthesis of milk fat from ß-hydroxybutyrate and acetate in mammary tissue. Biochim. Biophys. Acta 176:664-666.

Smith, N. E., and R. L. Baldwin. 1974. Effects of breed, pregnancy, and lactation on weight of organs and tissues in dairy cattle. J. Dairy Sci. 57:1055-1060.

Smith, S., and S. Abraham. 1971. Fatty acid synthetase from lactating rat mammary gland. J. Biol. Chem. 246:2537-2542.

Smith, S., and R. Dils. 1966. Factors affecting the chain length of fatty acids synthesised by lactating-rabbit mammary glands. Biochim. Biophys. Acta 116:23-40.

Snoswell, A. M., and P. P. Koundakjian. 1972. Relationships between carnitine and coenzyme a esters in tissues of normal and alloxan-diabetic sheep. Biochem. J. 127:133-141.

Soling, H. D., and J. Kleineke. 1976. Species dependent regulation of hepatic gluconeogensis in higher animals. Pages 369-462 in R. W. Hanson and M. A. Mehlman, eds. Gluconeogenesis: Its regulation in mammalian species. Wiley, New York.

Steel, J. W., and R. A. Leng. 1973a. Effects of plane of nutrition and pregnancy on gluconeogenesis in sheep. 1. The kinetics of glucose metabolism. Br. J. Nutr. 30:451-473.

Steel, J. W., and R. A. Leng. 1973b. Effects of plane of nutrition and pregnancy on gluconeogensis in sheep. 2. Synthesis of glucose from ruminal propionate. Br. J. Nutr. 30:475-489.

Steele, W., and Moore, J. H. 1968. The effects of mono-unsaturated and saturated fatty acids in the diet on milk-fat secretion in the cow. J. Dairy Res. 35:353-360.

Storry, J. E., and J. A. F. Rook. 1965. Effect in the cow of intraruminal infusions of volatile fatty acids and of lactic acid on the secretion of the component fatty acids of the milk fat and on the composition of blood. Biochem. J. 96:210-217.

Storry, J. E., A. J. Hall, and V. W. Johnson. 1973. The effects of increasing amounts of dietary tallow on milk secretion in the cow. J. Dairy Res. 40:293-299.

Stumpf, B., H. Kraus, R. Kasten, and G. Ahrens. 1978. Inhibition of gluconeogenesis in isolated rat kidneys by branched chain 2-ketoacids. Pediatr. Res. 12:1039-1044.

Sutton, J. D. 1985. Digestion and absorption of energy substrates in the lactating cow. J. Dairy Sci. 68:3376-3393.

Sutton, J. D., I. C. Hart, W. H. Broster, R. J. Elliott, and E. Schuller. 1986. Feeding frequency for lactating cows: effects on rumen fermentation and blood metabolites and hormnes. Br. J. Nutr. 56:181-192.

Swanson, L. V., and R. E. Lind. 1976. Lactational response of dairy cows to oral administration of a synthetic glucocorticoid. J. Dairy Sci. 59:614-619.

Tagari, H., and E. N. Bergman. 1978. Intestinal disappearance and portal blood appearance of amino acids in sheep. J. Nutr. 108:790-803.

Thompson, G. E., and A. W. Bell. 1976. The energy metabolism of the liver measured in vivo during cold exposure of sheep. Pages 37-40 in Energy metabolism of farm animals, Proceedings of the 7th symposium. Eur. Assoc. Anim. Prod. Publ. 19.

Tischler, M. E., M. Desautels, and A. L. Goldberg. 1982. Does leucine, leucyl-tRNA, or some metabolite of leucine regulate protein synthesis and degradation in skeletal and cardiac muscle? J. Biol. Chem. 257:1613-1621.

Trenkle, A. 1981. Endocrine regulation of energy metabolism in ruminants. Fed. Proc. 40:2536-2541.

Tucker, H. A. 1979. Endocrinology of lactation. Sem. Perinatol. 3:199-223.

Tucker, H. A., and R. A. Merkel. 1987. Applications of hormones in the metabolic regulation of growth and lactation in ruminants. Fed. Proc. 46:300-306.

Tyrrell, H. F., A. C. G. Brown, P. J. Reynolds, G. L. Haaland, C. J. Peel, D. E. Bauman, and W. D. Steinhour. 1982. Administration of bovine growth hormone to high yielding Holstein cows. I. Influence on in vivo energy metabolism. J. Dairy Sci. 65 (Suppl. 1):120. (Abstract)

Tyson, J. E., and P. Felig. 1971. Medical aspects of diabetes in pregnancy and the diabetogenic effects of oral contraceptives. Med. Clin. N. Am. 55:947-959.

Vague, P., G. Ramahandridona, C. Di Campo-Rouge, and F. Mahmoud. 1975. Kinetics of the early insulin response of the perfused rat pancreas to various metabolites and tolbutamide. Diabete & Metab. 1:185-189.

Varman, P. N., and L. H. Schultz. 1968. Blood lipids of cows at different stages of lactation. J. Dairy Sci. 51:1971-1974.

Varner, M. A., and B. H. Johnson. 1983. Influence of adrenocorticotropin upon milk production, milk constituents, and endocrine measures of dairy cows. J. Dairy Sci. 66:458-465.

Vernon, R. G. 1981. Lipid metabolism in the adipose tissue of ruminant animals. Pages 279-362 in W. W. Christie. Lipid metabolism in ruminant animals. Pergamon Press, Elmsford, NY.

Vernon, R. G., and E. Finley. 1985. Regulation of lipolysis durng pregnancy and lactation in sheep. Response to noradrenaline and adenosine. Biochem. J. 230:651-656.

Vernon, R. G., and D. J. Flint. 1983. Control of fatty acid synthesis in lactation. Proc. Nutr. Soc. 42:315-331.

Vernon, R. G., and E. Taylor. 1986. Enzymes of adenosine metabolism of sheep adipose tissue: changes in activity with season, pregnancy and lactation. Horm. Metab. Res. 18:369-373.

Walker, C. K., and J. M. Elliot. 1973. Effect of roughage restriction on serum insulin in the dairy cow. J. Dairy Sci. 56:375-377.

Webb, K. E., Jr. 1986. Amino acid and peptide absorption from the gastrointestinal tract. Fed. Proc. 45:2268-2271.

Webster, A. J. F. 1980. Energy costs of digestion and metabolism in the gut. Pages 469-484 in Y. Ruckebusch an P. Thivends, eds. Digestive physiology and metabolism in ruminants. AVI Publishing, Westport, CT.

Webster, A. J. F. 1981. The energetic efficiency of metabolism. Proc. Nutr. Soc. 40:121.

Wedin, W. F., H. J. Hodgson, and N. L. Jacobson. 1975. Utilizing plant and animal resources in producing human food. J. Anim. Sci. 41:667-685.

Weigand, E., J. W. Young, and A. D. McGilliard. 1972. Extent of butyrate metabolism by bovine ruminoreticulum epithelium and the relationship to absorption rate. J. Dairy Sci. 55:589-597.

Whitehurst, G. B., D. C. Beitz, M. A. Pothoven, W. R. Ellison, and M. H. Crump. 1978. Lactate as a precursor of fatty acids in bovine adipose tissue. J. Nutr. 108:1806-1811.

Wilde, C. J., and C. H. Knight. 1986. Degradation of newly-synthesised casein in mammary explants from pregnant and lactating goats. Comp. Biochem. Physiol. 84B:197-201.

Williamson, D. H., and R. Hems. 1970. Metabolism and function of ketone bodies. Pages 257-281 in W. Bartley, H. L. Kornberg, and J. R. Quayle, eds. Essays in cell metabolism. Wiley-Interscience, London.

Williamson, D. H., M. W. Bates, and H. A. Krebs. 1968. Activity and intracellular distribution of enzymes of ketone-body metabolism in rat liver. Biochem. J. 108:353-361.

Williamson, D. H., M. W. Bates, M. A. Page, and H. A. Krebs. 1971. Activities of enzymes involved in acetoacetate utilization in adult mammalian tissues. Biochem. J. 121:41-47.

Wohlt, J. E., J. H. Clark, R. G. Derrig, and C. L. Davis. 1977. Valine, leucine, and isoleucine metabolism by lactating bovine mammary tissue. J. Dairy Sci. 60:1875-1882.

Wood, H. G., G. J. Peeters, R. Verbeke, M. Lauryssens, and B. Jacobson. 1965. Estimation of the pentose cycle in the perfused cow's udder. Biochem. J. 96:607-615.

Yang, Y. T., and R. L. Baldwin. 1973. Lipolysis in isolated cow adipose cells. J. Dairy Sci. 56:366-374.

Young, J. W., S. L. Thorp, and H. Z. De Lumen. 1969. Activity of selected gluconeogenic and lipogenic enzymes in bovine rumen mucosa, liver and adipose tissue. Biochem. J. 114:83-88.

Young, V. R., H. N. Munro, D. R. Matthews, and D. M. Bier. 1983. Relationship of Energy Metabolism to Protein Metabolism. Pages 43-73 in G. Kleinberger and E. Deutsch, eds. New Aspects of Clinical Nutrition. S. Karger, Basel, Switzerland.

# EXPLANATION OF DISSERTATION FORMAT

This dissertation has been prepared according to the alternate dissertation format as described in the Iowa State University Graduate College Thesis Manual. Use of this format involves the presentation of two or more sections, each of which is in a form suitable for publication in a scientific journal. Two independent papers have been prepared from research performed in partial fulfillment of requirements for the Ph.D. degree. A general summary and conclusions follow these papers.

# SECTION I. MILK PRODUCTION AND COMPOSITION IN COWS AND GOATS FED 2-KETOISOCAPROATE

# Milk production and composition in cows and goats fed 2-ketoisocaproate

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## ABSTRACT

To examine whether 2-ketoisocaproate supplementation affects milk production, 10 goats were fed either 0 or 1.1% calcium-2-ketoisocaproate for 2 wk and 12 cows were fed either 0 or .75% sodium-2-ketoisocaproate for 3 wk. Supplementation with 2-ketoisocaproate significantly increased milk fat content, milk fat yield, and 4% fat-corrected milk yield in cows by an average of 5, 10, and 8%, respectively, for the treatment period. In cows, response of milk fat yield to 2-ketoisocaproate was 14% during wk 1 of treatment but diminished to 7% by wk 3. Milk yield and milk protein yield tended to be greater in cows fed 2-ketoisocaproate, but milk production efficiency and body weight were unaltered in either species. Supplementation with 2-ketoisocaproate had no significant effect on fatty acid composition of milk but tended to increase the percentage of $C_{10}$ and $C_{12}$ fatty acids. In goats, supplementation with 2-ketoisocaproate had no significant effects on milk production or composition, but trends toward increased milk fat and protein content were observed. Results indicate that 2-ketoisocaproate acutely stimulates milk fat production in lactating cows, and that this effect seems to diminish with time.

# INTRODUCTION

2-Ketoisocaproate (KIC) is the first metabolite in the degradative pathway of leucine and, like leucine, has been reported to reduce muscle proteolysis (Tischler et al., 1982) and stimulate protein synthesis (Kirsch et al., 1976). Clinical studies have demonstrated that KIC reduces nitrogen excretion by humans during renal failure (Ell at al., 1978), fasting (Mitch et al., 1981), and postsurgical trauma (Sapir et al., 1983). Patients with postsurgical trauma also exhibited elevated plasma ketone body concentrations in response to KIC infusion (Sapir et al., 1983). In addition, KIC stimulated insulin secretion and inhibited glucagon secretion from perfused rat pancreas (Leclercq-Meyer et al., 1979).

If dietary KIC also affects hormone secretion or the metabolism of protein, fat, or glucose in lactating animals, then KIC also might alter the production of milk protein, fat, or lactose. To date, however, there are no reports on the effects of dietary KIC during lactation. Therefore, studies were conducted to determine if dietary KIC alters milk yield, milk composition, or milk production efficiency of goats and cows.

# MATERIALS AND METHODS

## Experimental Design

### Animals

For trial 1, ten lactating, nonpregnant Saanen goats (about 130 d postpartum) were assigned randomly to either a control or treatment group. Goats were milked twice daily (0700 and 1700 h). In trial 2, eight Holstein and four Brown Swiss cows were assigned to either a control or treatment group in a randomized block design, according to parity number and breed. Four Holstein cows and two Brown Swiss cows were primiparous; all others were multiparous. All cows were 151 to 300 d postpartum and estimated to be 66 to 148 d pregnant. Cows were milked twice daily (0230 and 1400 h).

### Basal diets

Goats and cows were fed their respective diets ad libitum twice daily (0730 and 1730 h). Feed refusals were collected before the morning feeding. Composition of diets with calculated values for net energy of lactation (NE), crude protein, and calcium concentration is shown in Table 6. In both trials, animals consumed protein and calcium in excess of their requirements. Leucine, as a percentage of total amino acids in the ration, was calculated to be 7.4% in trial 1 and 8.1% in trial 2.

### Protocol and treatments

Goats in trial 1 were adjusted gradually to the basal ration and then fed the basal ration for 2 wk prior to a 2 wk treatment period. During this treatment period, control goats were fed the basal ration, whereas KIC-treated goats were fed the basal ration supplemented with

1.1% calcium-$(KIC)_2$ (DM basis; Table 6).

In trial 2, cows were adjusted gradually to the basal ration and then fed the basal ration for 2 wk before treatments began. Dietary treatments were imposed for the next 3 wk as a treatment supplement added to each cow's ration at both the morning and evening feedings. KIC-treated cows received sodium-KIC equivalent to .75% of the diet dry matter. To partly protect KIC from degradation by rumen bacteria, KIC in trial 2 was fed as extruded pellets of a mixture of sodium-KIC, corn gluten meal, hydrogenated soy oil, and paraffin in a weight ratio of 20:10:9:1 (Smith and Boling, 1984). Additionally, .5 mg d-alpha-tocopherol (Sigma Chemical Company, St. Louis, MO) was added per gram of soy oil. Pellets were stored at -20°C to minimize oxidation of KIC in the fat mixture. Control pellets contained no KIC and were fed equivalent to .375% of the diet dry matter. To ensure consumption of the treatment supplements, 2% of the concentrate in the total ration was combined with the treatment supplement for each cow for each day, and this mixture was added to the respective cow's ration during feeding.

Measurements

Milk yield, feed consumption, and body weight were measured daily for each goat in trial 1. Milk samples collected from four milkings at the end of each week were analyzed for fat and protein concentrations; milk fat, milk protein, and 4% fat-corrected milk yields were calculated. Blood samples were taken twice per week into heparinized tubes, and plasma was collected and stored at -20°C.

In trial 2, daily milk yield and feed consumption were measured for

Table 6. Composition of control and 2-ketoisocaproate (KIC)-supplemented diets fed to lactating goats and cows

| Ingredient | Goats | | Cows | |
|---|---|---|---|---|
| | Control | KIC | Control | KIC |
| | ----- % of diet dry matter----- | | | |
| Alfalfa hay, 17% CP | 35.8 | 35.4 | 41.3 | 41.0 |
| Corn silage | -- | -- | 18.0 | |
| Ground corn | 15.1 | 15.0 | 11.7 | 11.6 |
| Ground oats | 18.8 | 18.6 | 17.2 | 17.1 |
| Whole cottonseed | 5.1 | 5.0 | -- | -- |
| Soybean meal, solvent-extracted | 15.6 | 15.4 | -- | -- |
| Soybean meal, expeller-extracted[a] | -- | -- | 7.5 | 7.4 |
| Molasses, sugarcane | 7.2 | 7.1 | 2.1 | 2.1 |
| Dicalcium phosphate | .49 | .48 | 1.0 | 1.0 |
| Limestone | .73 | .72 | -- | -- |
| Buffers[b] | .59 | .58 | -- | -- |
| Trace mineralized salt | .49 | .48 | .45 | .45 |
| Trace minerals[c] | .06 | .06 | -- | -- |
| Vitamins[d] | .06 | .06 | .05 | .05 |
| Extruded KIC pellets[e] | -- | -- | -- | 1.46 |
| Extruded control pellets[f] | -- | -- | .71 | -- |
| Calcium-$(KIC)_2$[g] | -- | 1.09 | -- | -- |
| $NE_l$, Mcal/kg[h] | 1.65 | 1.66 | 1.56 | 1.57 |
| Crude protein, % of DM[h] | 20.7 | 20.5 | 16.2 | 16.0 |
| Leucine, % of DM[h] | 1.54 | 1.52 | 1.31 | 1.30 |
| KIC, % of DM | .85 | | .64 | |
| Calcium, % of DM[h] | 1.02 | 1.15 | .91 | .90 |

[a]Soy-Plus, West Central Cooperative, Ralston, IA.

[b]83% sodium bicarbonate, 17% magnesium oxide.

[c]Trace Minerals Premix 60, Land O'Lakes, Inc., Fort Dodge, IA.

[d]ADEK, Protein Blenders, Inc., Iowa City, IA.

[e]KIC pellets contained 23.3% corn gluten meal (Clinton Corn Products, Clinton, IA), 25.3% hydrogenated soy oil (Hunt-Wesson Foods, Inc., Fullerton, CA), and 51.4% sodium-KIC (SOBAC, Paris, France) on a DM basis.

[f]Control pellets contained 47.9% corn gluten meal and 52.1% hydrogenated soy oil on a DM basis.

[g]SOBAC, Paris, France.

[h]Net energy of lactation calculated from National Research Council values (National Research Council, 1978; 1981; 1982).

each cow for 2 wk before treatments began and for 2 wk after treatments ended. Milk samples were collected from both milkings on d 2, 3, 5, and 6 of each week; samples from d 2 and 3 and from d 5 and 6 were pooled for each cow. These samples were analyzed for protein and fat contents, and milk protein, milk fat, and 4% fat-corrected milk yields were calculated for 2 times per wk. Milk samples that had been pooled weekly were analyzed for KIC concentration of individual fatty acids and fatty acid composition, and yields were calculated. Body weights were measured three times per week. Blood was collected into heparinized tubes at 0900 h on d 3 and 6 of each week, and sodium fluoride was added immediately. Samples were put on ice and, after centrifugation, plasma was collected and stored at -20°C.

In both trials, gross energetic efficiency of milk production was calculated by dividing the estimated gross energy of milk output by the estimated NE intake. To estimate NE intake, both sodium and calcium salts of KIC were assigned a NE value of 2.3 Mcal/kg dry salt. This value was calculated by assuming 35 moles ATP produced per mole of KIC and comparing this conversion with those for fat and carbohydrate.

## Methods of Analysis

Milk samples of trial 1 were analyzed by an infrared milk analyzer (Foss Milkoscan 104) by Mid-America Dairymen, Inc., Newton, IA. Milk samples from trial 2 also were analyzed by infrared spectroscopy with a Foss Milkoscan 203 at the Swiss Valley Farms Laboratory, Waterloo, IA. Milk and plasma KIC were quantified by adding 20 nmol 2-ketocaproate (KC) as an internal standard to .5 g lyophilized milk or 1 ml plasma and

extracting with methylene chloride (Nissen et al., 1982). The extract then was dried under nitrogen gas and derivatized with 100 ul N-methyl-N-(t-butyl-dimethylsilyl)tri-fluoroacetamide containing 1% t-butyl-dimethylchlorosilane (Regis Chemical Company, Morton Grove, IL). Both KIC and KC were monitored at 301 AMU by using a gas chromatography-mass spectrometry system (Hewlett-Packard 5970). KIC concentration of milk or plasma was calculated by comparing the KIC to KC ratio of samples with that of standards. Relative concentrations of $C_{10}$ to $C_{18}$ fatty acids in milk were determined by gas chromatography (Varian 3700G) of fatty acid methyl esters (Arrendale et al., 1983). Plasma glucose was quantified by a glucose oxidase assay kit (Worthington Diagnostic Systems, Inc., Freehold, NJ). 3-Hydroxybutyrate content in deproteinized plasma was measured by enzymatic assay (Williamson and Mellanby, 1974). Plasma was analyzed for concentration of cortisol by radioimmunoassay kit (Travenol-Genentech Diagnostics, Cambridge, MA) and for insulin concentration by radioimmunoassay using bovine insulin antibody (Trenkle, 1970). Plasma glucagon concentration was determined by double-antibody radioimmunoassay using rabbit anti-porcine glucagon antiserum (BioTek Research, Inc., Lenexa, KS). The concentration of insulin-like growth factor I (IGF-I) in plasma was measured by T. Elsasser (USDA-ARS, Beltsville, MD) by radioimmunoassay using human IGF-I antibody (Underwood et al., 1982) after dissociation of IGF-I from its binding protein with acetic acid. Nitrogen content of feed was determined by Kjeldahl method (AOAC).

Statistical analysis

Means for each cow were calculated for the 2-wk pretreatment period and 3-wk treatment period. Pretreatment means by cow were used as covariables for adjustment of 3-wk treatment means in analysis of covariance, and these covariate-adjusted means are reported as values for control and KIC-fed animals during the treatment period. Effect of KIC treatment was considered to be significant if the probability of a greater F value by chance was less than .05 ($P<.05$) in the analysis of covariance.

Additionally, for the cow study, period effects on milk fat yield were analyzed statistically by using the split plot design of Table 7; results are shown in Table 9. Contrasts for treatment by day interaction of 1) the average of 2-wk pretreatment and 2-wk posttreatment periods vs. 3-wk treatment period, 2) wk 1 vs. the average of wk 2 and 3 of treatment period, and 3) the average of 2-wk pretreatment period vs. 2-wk posttreatment period were analyzed. Effects on milk fat content and yield of milk and fat-corrected milk also were analyzed by using this design (Appendix A). Differences were considered to be significant if $P<.05$ and to be trends if $P<.25$ and $P>.05$.

Table 7. Analysis of variance (ANOVA) model for treatment effect on milk fat yield by week (two observations per week) for the cow study

| Variable | df | SS | MS | F | Pr>F | conserv.[a] Pr>F |
|---|---|---|---|---|---|---|
| Trt[b] | 1 | 1281704 | 1281704 | 9.11 | .019 | |
| Block | 3 | 6971726 | 6971726 | 16.5 | .002 | |
| Trt*Block | (3) | (216827) | | | | |
| Cow(Trt*Block) | (4) | (768230) | | | | |
| Error(A)[c] | 7 | 985057 | 140722 | | | |
| Day | 13 | 157281 | 12099 | 3.77 | .0001 | .081 |
| Trt*Day | 3 | 112679 | 8668 | 2.70 | .0022 | .13 |
| Day*Block | (39) | (153467) | | | | |
| Trt*Day*Block | (39) | (101317) | | | | |
| Day*Cow(Trt*Block) | (52) | (162962) | | | | |
| Error(B)[d] | 130 | 417745 | 3213 | (Root MSE = 56.7) | | |
| Total | 167 | 9926192 | | | | |
| Contrasts by period | | | | | | |
| Day | | | | | | |
| Mean trt vs. pooled pre- + post-trt | 1 | 45667 | 45667 | 14.2 | .0002 | .004 |
| Wk 1 vs. wk 2 + 3 | 1 | 46836 | 46836 | 14.6 | .0002 | .004 |
| Pre- vs. post-trt | 1 | 6683 | 6683 | 2.08 | .15 | .18 |
| Trt*Day | | | | | | |
| Mean trt vs. pooled pre- + post-trt | 1 | 71084 | 71084 | 22.1 | .0001 | .0009 |
| Wk 1 vs. wk 2 + 3 | 1 | 14500 | 14500 | 4.51 | .036 | .060 |
| Pre- vs. post-trt | 1 | 413 | 413 | .13 | .72 | .75 |

[a]Probability of a greater F calculated with conservative degrees of freedom (df) for period effects. Conservative df are calculated by dividing both terms of F test by df for day; thus, for trt*day effect, probability of a greater F is determined using 1 and 10 df, not 13 and 130 df.

[b]Treatment.

[c]Error for nonrepeated effects = Trt*Block + Cow(Trt*Block).

[d]Error for period effects = Day*Block + Trt*Day*Block + Day*Cow(Trt*Block).

## RESULTS

Milk production and composition results from both trials are presented in Table 8. Goats fed control diets produced an average of 3.0 kg milk per d with 3.2% fat and 2.7% protein. Milk, milk fat, and milk protein yields were not altered significantly by KIC supplementation. Whereas KIC increased milk fat content by 11% and milk protein content by 5% in goats, these increases were only trends.

Table 8. Covariate-adjusted yield and composition of milk from goats and cows fed control and 2-ketoisocaproate (KIC)-supplemented diets (average of treatment period)

| Variable | Goats | | | Cows | | |
|---|---|---|---|---|---|---|
| | Control | KIC | Pr>F | Control | KIC | Pr>F |
| Milk yield, kg/d | 2.96 | 2.97 | .9 | 21.4 | 22.5 | .19 |
| Milk fat content, % | 3.17 | 3.52 | .10 | 4.00 | 4.19 | .03 |
| Milk protein content, % | 2.68 | 2.82 | .13 | 3.51 | 3.49 | .35 |
| Milk fat yield, g/d | 96.3 | 105.6 | .4 | 845 | 930 | .017 |
| Milk protein yield, g/d | 79.7 | 83.6 | .4 | 731 | 767 | .17 |
| 4% FCM yield[a], kg/d | 2.63 | 2.77 | .5 | 21.2 | 22.9 | .04 |
| Lactose content[b], % | 4.41 | 4.34 | .4 | 4.81 | 4.82 | .9 |

[a]4% Fat-corrected milk yield, calculated as (.4 x milk yield + 15 x milk fat yield).

[b]%Lactose of milk from cows determined by (%total solids - %fat - %protein - .7% for minerals).

In the cow trial, control cows produced about 21 kg milk per d with 4.0% fat and 3.5% protein. When compared with controls, cows fed KIC produced more milk (5%), and this milk had a 5% greater fat content. The calculated milk fat and 4% fat-corrected milk yields were greater by 10% and 8%, respectively, in cows fed KIC. Protein content was not different between groups, but protein yield tended to be greater in cows fed KIC.

The milk fat yield response occurred by d 3 of treatment, which was the initial measurement of milk composition (Figure 2). During the entire first wk of treatment, cows fed KIC produced 14% more milk fat than did controls (Table 9). By wk 2 and 3, however, this response had diminished from wk 1 ($P<.06$) to only 7% above that of controls. Increased milk fat yield in cows fed KIC during wk 1 and the decrease during wk 2 and 3 resulted from changes in both milk yield and milk fat content. No difference in milk fat production between groups was evident during the 2 wk after treatments had ended (Table 7).

Neither the energetic efficiency of milk production calculated as milk energy output / NE intake nor the efficiency of feed protein conversion to milk protein were significantly greater in goats and cows fed KIC than in controls (Table 10). The partial energetic efficiency of milk production [milk energy output /(NE intake minus maintenance NE requirement)] also was not significantly altered by treatment. Partial energetic efficiency was calculated to be 70% for control cows and 73% for those fed KIC. Body weight gain also was not altered by KIC supplementation.

Fatty acid composition of milk was not significantly altered by feeding KIC; however, the percentage of $C_{10}$ and $C_{12}$ fatty acids tended to increase in cows fed KIC (Table 11). Milk KIC concentrations were not different between KIC-treated and control cows (3.43 M and 3.40 M, respectively).

Plasma KIC concentration of goats fed KIC was not different from that of control goats (14.5 uM vs. 13.2 uM, respectively). Cows fed KIC

tended to have a greater concentration of 3-hydroxybutyrate in plasma, but glucose concentration was not different (Table 12). Plasma cortisol concentration was not altered by dietary treatment. Glucagon and insulin concentrations in plasma tended to be less in cows fed KIC, and insulin to glucagon ratio also tended to be less in cows fed KIC. Plasma IGF-I concentration in cows fed KIC tended to be less than those of controls.

Table 9. Covariate-adjusted yield of milk, fat, and fat-corrected milk by week in cows fed control and 2-ketoisocaproate (KIC)-supplemented diets

| Variable | Week of study[a] | | | | | | | Pr>F | |
|---|---|---|---|---|---|---|---|---|---|
| | -2 | -1 | 1 | 2 | 3 | 4 | 5 | Trt[b] | Wk[c] |
| Milk yield, kg/d (Av SEM[d] = .6) | | | | | | | | | |
| Control | 21.9 | 21.1 | 22.0 | 21.6 | 20.6 | 21.5 | 21.0 | | |
| KIC | 21.7 | 21.3 | 23.2 | 22.5 | 21.1 | 20.2 | 21.1 | .02 | .5 |
| Milk fat content, % (Av SEM = .08) | | | | | | | | | |
| Control | 3.97 | 3.98 | 3.94 | 4.03 | 4.05 | 4.14 | 4.16 | | |
| KIC | 3.97 | 3.99 | 4.24 | 4.16 | 4.15 | 4.19 | 4.11 | .014 | .09 |
| Milk fat yield, g/d (Av SEM = 23) | | | | | | | | | |
| Control | 855 | 832 | 862 | 861 | 814 | 876 | 853 | | |
| KIC | 848 | 839 | 981 | 925 | 868 | 848 | 864 | .001 | .06 |
| 4%-Fat corrected milk yield, kg/d (Av SEM = .5) | | | | | | | | | |
| Control | 21.8 | 20.9 | 21.7 | 21.5 | 20.4 | 21.7 | 21.2 | | |
| KIC | 21.2 | 21.1 | 24.0 | 22.9 | 21.5 | 20.8 | 21.4 | .002 | .14 |

[a]Wk -2 and -1 are pretreatment period, wk 1, 2, and 3 are treatment period, and wk 4 and 5 are posttreatment period.

[b]Probability of a greater F for the contrast "wk 1, 2, and 3 vs wk -2, -1, 4, and 5" of treatment x day interaction by using conservative df in the ANOVA model of Table 7.

[c]Probability of a greater F for the contrast "wk 1 vs. wk 2 and 3" of treatment x day interaction by using conservative df.

[d]Average standard error of a mean calculated from the root mean square error of the ANOVA model of Table 7.

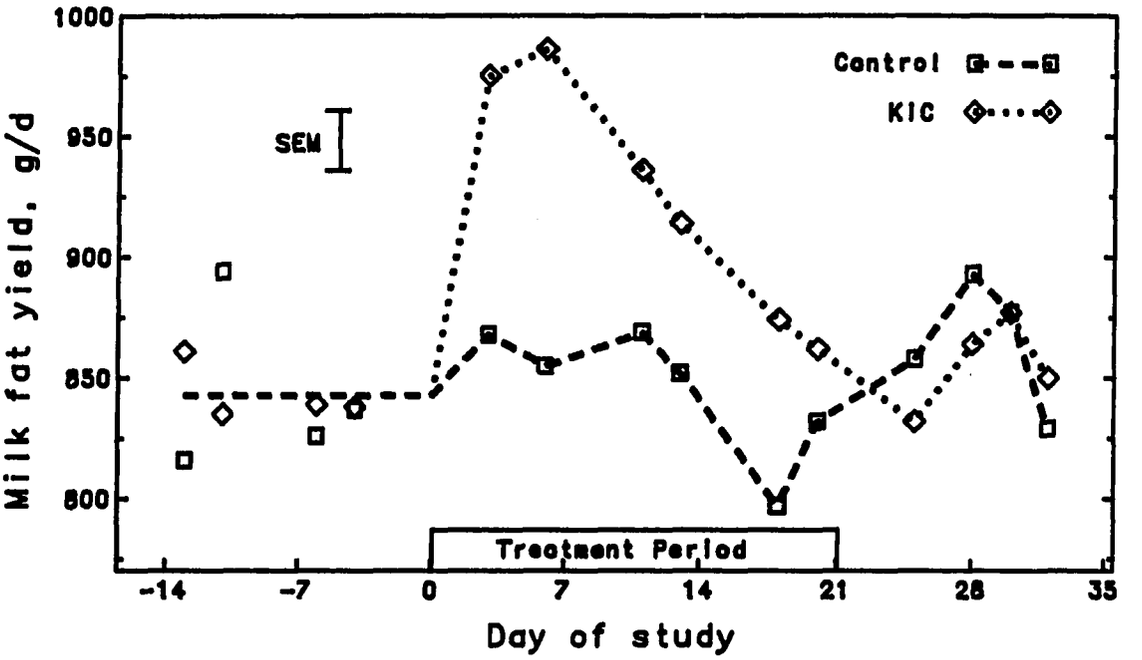


Figure 2. Covariate-adjusted milk fat yield of cows fed control and 2-ketoisocaproate (KIC)-supplemented diets. Milk fat yield was calculated for two times per week. The average of four pretreatment values was used as the covariate so that pretreatment means (heavy dashed line) were equal; all symbols represent covariate-adjusted yield. Average standard error of a mean (SEM) was calculated to be 23 g/d by using the root mean square error from an ANOVA model including treatment, day, treatment x day, block, treatment x block, and cow-within-treatment x block.

Table 10. Covariate-adjusted feed intake, efficiency of milk production, and body weight of goats and cows fed control and 2-ketoisocaproate (KIC)-supplemented diets (average of treatment period)

| Variable | Goats | | | Cows | | |
|---|---|---|---|---|---|---|
| | Control | KIC | Pr>F | Control | KIC | Pr>F |
| Dry matter intake, kg/d | 2.52 | 2.62 | .6 | 20.7 | 21.2 | .3 |
| Milk energy output[a], Mcal/d | 1.83 | 1.94 | .5 | 16.0 | 17.1 | .06 |
| Net energy intake, Mcal/d | 4.16 | 4.33 | .6 | 32.4 | 33.3 | .3 |
| Gross energy efficiency[b], % | 43.9 | 44.8 | .7 | 49.2 | 51.5 | .6 |
| Milk protein output, g/d | 79.7 | 83.6 | .4 | 731 | 767 | .17 |
| Crude protein intake, g/d | 521 | 535 | .7 | 3349 | 3401 | .5 |
| Protein efficiency[c], % | 15.1 | 15.7 | .7 | 21.8 | 22.3 | .7 |
| Body weight, kg | 62.5 | 61.9 | .5 | 598 | 602 | .3 |

[a]Calculated as in Tyrrell and Reid (1965) by [milk yield x (.0921 x %fat + .0490 x %solids-not-fat - .0563)]. Solids-not-fat content of goat milk calculated by (%lactose + %protein + .7% for minerals).

[b]Calculated as milk energy output x 100 / net energy intake.

[c]Calculated as milk protein output x 100 / crude protein intake.

Table 11. Covariate-adjusted composition of fatty acids in milk fat of cows fed control and 2-ketoisocaproate (KIC)-supplemented diets (average of treatment period)

| Fatty acid | Control | KIC | change | Pr>F |
|---|---|---|---|---|
| | ------ weight % [a] ------- | | | |
| $C_{10:0}$ | 2.70 | 3.14 | .44 | .09 |
| $C_{12:0}$ | 3.94 | 4.24 | .30 | .08 |
| $C_{14:0}$ | 12.6 | 12.6 | .01 | .9 |
| $C_{16:0}$ | 34.0 | 33.8 | -.24 | .6 |
| $C_{16:1}$ | 1.83 | 1.87 | .04 | .8 |
| $C_{18:0}$ | 13.6 | 13.3 | -.29 | .5 |
| $C_{18:1}$ | 26.7 | 26.8 | .04 | .9 |
| $C_{18:2}$ | 3.73 | 3.59 | -.13 | .4 |
| $C_{18:3}$ | .88 | .71 | -.16 | .07 |
| Total $C_{10-14}$ | 19.3 | 20.0 | .75 | .2 |
| Total $C_{16}$ | 35.8 | 35.6 | -.20 | .7 |
| Total $C_{18}$ | 44.9 | 44.4 | -.55 | .3 |

[a] Weight percentage of fatty acids measured.

Table 12. Covariate-adjusted concentration of metabolites and hormones in plasma of cows fed control and 2-ketoisocaproate (KIC)-supplemented diets (average of treatment period)

| Variable | Control | KIC | SEM | Pr>F |
|---|---|---|---|---|
| Glucose, mg/dl | 61.6 | 62.0 | 1.0 | .8 |
| 3-hydroxybutyrate, mg/dl | 5.06 | 5.67 | .24 | .14 |
| Insulin, ng/ml | 1.34 | 1.15 | .10 | .23 |
| Glucagon, pg/ml | 283 | 267 | 21 | .21 |
| Insulin : glucagon | 4.91 | 4.29 | .32 | .21 |
| Cortisol, ng/ml | 3.7 | 4.3 | .5 | .5 |
| IGF-I, ng/ml | 64 | 46 | 7 | .13 |

# DISCUSSION

The major finding of this study is that KIC stimulated milk fat content and total milk fat yield in cows. This fat yield response was 10% in both cows and goats, but statistically significant ($P<.02$) only in cows. The milk fat percentage response also was significant ($P<.03$) only in cows, but a trend toward increased milk fat content also was observed in goats fed KIC.

The response of milk fat production to dietary change also has been demonstrated to be similar for goats and cows in other studies. For example, low dietary fiber rations decreased the fat content of milk in both species, although goats were less susceptible (Askew et al., 1971; Calderon et al., 1984). In the present study, changes in milk fat content seemed greater in goats than in cows. A difference in response might have been expected because goats were fed a diet containing less fiber than were cows. Additionally, goats were fed the calcium salt of KIC, whereas cows were fed the sodium salt. Both salts of KIC are completely soluble in rumen fluid (data not shown), but the sodium salt is more readily soluble. Lipid-coating of sodium-KIC for the cow study presumably blocked this rapid solubility, however, so that some KIC likely bypassed the rumen and reached the lower tract.

The milk fat yield response of cows to KIC supplementation was acute and was greatest during the first wk of treatment (14% increase); by day 20, yield had diminished to 7% over controls. Whether this diminished response to KIC was caused by adaptation of the rumen microflora population or of the animal's metabolism is unclear. Possibly, the trend

toward a decrease in IGF-I concentration in plasma of cows fed KIC may have been involved in this diminished response.

The structural and metabolic similarity of KIC and isovaleric acid suggests that KIC, a branched-chain ketoacid, might elicit a production response similar to that of the branched-chain fatty acids. The milk fat response to dietary KIC, however, contrasts with the production response observed in recent work in dairy cows supplemented with mixtures of the branched-chain fatty acids (Papas et al., 1985); although branched-chain fatty acid salts (90 g/d) increased milk yield (13%), they did not increase milk fat content. In an earlier study (Rook et al., 1965), however, intraruminal administration of 500 ml isovaleric acid (about 460 g) increased milk fat content (8%) and milk fat production (6%). Because the major effect of dietary KIC also seemed to be an increase in milk fat content and milk fat yield, the possibility exists that leucine-specific metabolites (i.e., KIC and isovaleric acid) may produce different metabolic effects than other branched-chain acids. This specificity also has been observed in studies in which leucine altered protein degradation and synthesis much more than either isoleucine or valine (Baldwin et al., 1985; Tischler et al., 1982), and KIC decreased protein degradation to a much greater degree than did 2-ketoisovalerate (Chua et al., 1979).

In this study, cows were fed about 130 g KIC per d, and the increase in milk fat production was 140 g/d during wk 1 and averaged about 85 g/d for the 3 wk trial. In previous experiments, daily infusions of 1000-1500 g acetic acid or approximately 900 g of butyric acid into the rumen of lactating dairy cows resulted in increased milk fat yields of 60-100

and approximately 65 g, respectively (Rook and Balch, 1961; Rook et al., 1965). Additionally, 1,3-butanediol fed at 5% of the diet to cows with milk fat-depression increased milk fat yield 90-140 g/d (Hess and Young, 1972). In consideration of these low conversion efficiences, it seems unlikely that KIC carbon could contribute all of the average 85 g/d increase in milk fat yield, unless conversion of KIC to fatty acids was much more efficient than that of short-chain fatty acids or ketone body precursors.

No significant difference was observed in milk fatty acid composition of the dietary treatments. Whereas the $C_{10}$ to $C_{14}$ fatty acids primarily are synthesized de novo in the mammary gland, the $C_{18}$ fatty acids are derived largely from preformed plasma fatty acids (Palmquist et al., 1969). Therefore, no fatty acid composition difference indicates that increased milk fat synthesis in response to KIC supplementation was the result of both an increase in de novo fatty acid synthesis and an increase in transport of blood lipids into the mammary gland. The effect of KIC on de novo synthesis, however, seems more pronounced. Although the percentage of $C_{14}$ in milk fat was not increased, the percentage of $C_{10}$ and $C_{12}$ tended to be increased in cows fed KIC. This increase in percentage of $C_{10}$ and $C_{12}$ was observed during all three weeks of the treatment period, and similar changes were observed in the goat trial (data not shown). Increased percentage of C10 and C12 in milk fat may be related to the observation that cows fed KIC tended to have an increased (12%) concentration in plasma of 3-hydroxybutyrate, which serves as the primer for about half of de novo

milk fatty acids (Palmquist et al., 1969). De novo fatty acid chain length is thought to be a function of the ratio of primer-CoA to malonyl-CoA at the site of synthesis (Smith and Dils, 1966), so an increase in the proportion of shorter-chain fatty acids might be expected with increased 3-hydroxybutyrate concentration. A rise in the concentration in plasma of ketone bodies, which are products of KIC catabolism, has been observed previously in postoperative patients treated with KIC (Sapir et al., 1983).

A mechanism involving direct action of KIC on peripheral tissues is unlikely because 1) elevated concentrations of KIC in peripheral blood plasma and milk were not detected in goats and cows fed KIC in our studies, 2) a significant amount of the KIC fed in these studies likely did not bypass the rumen and therefore the amount of KIC available to body tissues was less than the amount fed, and 3) 85% of an orally administered KIC load given to dogs is metabolized by gastrointestinal mucosa and liver to leucine and ketone bodies (Abumrad et al., 1982). Although metabolism of KIC by ruminants is thought to differ from that of nonruminants, greater than half of blood KIC is removed by splanchnic tissues (Pell et al., 1986). Therefore, most orally administered KIC would not be expected to reach peripheral tissues, so the stimulation of milk fat production by KIC likely was an indirect action on the mammary gland related to splanchnic metabolism of KIC.

Splanchnic tissues account for about 50% of total protein turnover in nonlactating cows (Lobley et al., 1980) and 45% of heat production in lactating cows (Reynolds et al., 1986). Moreover, protein turnover

likely accounts for 16-28% of total body heat production nonlactating cows (Lobley et al., 1980). Therefore, if KIC decreases protein turnover, as indicated by in vitro studies with non-ruminant tissues and studies with whole animals (Chua et al., 1979; Ell et al., 1978; Sapir et al., 1983; Tischler et al., 1982), then the ATP requirement of splanchnic tissues and also the maintenance energy requirement of a lactating animal would be decreased. A decreased maintenance energy requirement might allow more lipogenic substrates to be used by the mammary gland for milk fat synthesis. In support of this concept, Baldwin et al. (1985) have proposed that if maintenance energy requirement was decreased from 130 to 90 kcal/kg$\cdot^{75}$, with feed intake constant at three times maintenance, then nutrient availability to the mammary gland would be increased 15%.

Another possibility is that KIC might act indirectly on the mammary gland or other peripheral tissues by way of hormones or KIC-specific metabolites. The trend toward a lesser insulin to glucagon ratio in cows fed KIC may be partly responsible for increased milk fat yield in cows fed KIC. Concentrations of cortisol, insulin, glucagon, and IGF-I in plasma, however, were not altered significantly by KIC supplementation in our study. Possibly, other hormones are involved in the response, or secretion rate is being altered to a greater degree than concentration.

In summary, milk fat content tended to increase in lactating goats fed a 1.1% KIC diet and was increased significantly in lactating dairy cows fed .75% KIC in the diet dry matter. Milk fat production was increased significantly only in cows. Whereas the mechanism for these effects seems to be postruminal, a ruminal mechanism cannot be

discounted. Further work is needed to determine the optimal KIC dosage for maximal stimulation of milk synthesis, to determine if KIC can prevent the milk fat depression associated with low fiber diets, and to elucidate the mechanism by which KIC stimulates milk fat production.

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## REFERENCES

Abumrad, N. N., K. L. Wise, P. E. Williams, N. A. Abumrad, and W. W. Lacy. 1982. Disposal of 2-ketoisocaproate: roles of liver, gut, and kidneys. Am. J. Physiol. 243:E123.

Arrendale, R. F., G. W. Chapman, and O. T. Chortyk. 1983. Gas chromatographic analyses of fatty acids on laboratory-prepared fused silica silar 10C capillary columns. J. Agric. Food Chem. 31:1334.

Askew, E. W., J. D. Benson, J. W. Thomas, and R. S. Emery. 1971. Metabolism of fatty acids by mammary glands of cows fed normal, restricted roughage, or magnesium oxide supplemented rations. J. Dairy Sci. 54:854.

Baldwin, R. L., N. E. Forsberg, and C. Y. Hu. 1985. Potential for altering energy partition in the lactating cow. J. Dairy Sci. 68:3394.

Buse, M. G., and S. S. Reid. 1975. Leucine: a possible regulator of protein turnover in muscle. J. Clin. Invest. 56:1250.

Calderon, J., E. J. De Peters, N. E. Smith, and A. A. Franke. 1984. Composition of goat's milk: changes within milking and effects of a high concentrate diet. J. Dairy Sci. 67:1905.

Chua, B., D. L. Siehl, and H. E. Morgan. 1979. Effect of leucine and metabolites of branched chain amino acids on protein turnover in heart. J. Biol. Chem. 254:8358.

Ell, S., M. Fynn, P. Richards, and D. Halliday. 1978. Metabolic studies with keto acid diets. Am. J. Clin. Nutr. 31:1776.

Hess, G. S., and J. W. Young. 1972. Preventing and alleviating milk fat depression by feeding 1,3-butanediol to cows. J. Dairy Sci. 55:1097.

Kirsch, R. E., L. O'C. Frith, and S. J. Saunders. 1976. Stimulation of albumin synthesis by keto analogues of amino acids. Biochim. Biophys. Acta 442:437.

Leclercq-Meyer, V., J. Marchand, R. Leclercq, and W. J. Malaisse. 1979. Interactions of 2-ketoisocaproate, glucose and arginine in the secretion of glucagon and insulin from the perfused rat pancreas. Diabetologia 15:121.

Lobley, G. E., V. Milne, J. M. Lovie, P. J. Reeds, and K. Pennie. 1980. Whole body and tissue protein synthesis in cattle. Br. J. Nutr. 43:491.

Mitch, W. E., M. Walser, and D. G. Sapir. 1981. Nitrogen sparing induced by leucine compared with that induced by its keto analog, 2-ketoisocaproate, in fasting obese man. J. Clin. Invest. 67:553.

National Research Council. 1978. Nutrient requirements of dairy cattle. 5th ed. Natl. Acad. Sci., Washington, DC.

National Research Council. 1981. Nutrient requirements of goats: Angora, dairy, and meat goats in temperate and tropical countries. Natl. Acad. Sci., Washington, DC.

National Research Council. 1982. United States - Canadian tables of feed composition. 3rd ed. Natl. Acad. Sci., Washington, DC.

Nissen, S. L., C. Van Huysen, and M. W. Haymond. 1982. Measurement of branched chain amino acids and branched chain 2-ketoacids in plasma by high-performance liquid chromatography. J. Chromatog. 232:170.

Palmquist, D. L., C. L. Davis, R. E. Brown, and D. S. Sachan. 1969. Availability and metabolism of various substrates in ruminants. V. Entry rate into the body and incorporation into milk fat of D(-)-3-hydroxybutyrate. J. Dairy Sci. 52:633.

Papas, A. M., S. R. Ames, R. M. Cook, C. J. Sniffen, C. E. Polan, and L. Chase. 1985. Production responses of dairy cows fed diets supplemented with ammonium salts of iso C-4 and C-5 acids. J. Dairy Sci. 67:276.

Pell, J. M., E. M. Caldarone, and E. N. Bergman. 1986. Leucine and 2-ketoisocaproate metabolism and interconversions in fed and fasted sheep. Metabolism 35:1005.

Reynolds, C. K., G. B. Huntington, H. F. Tyrrell, and P. J. Reynolds. 1986. Splanchnic tissue and whole animal oxygen consumption by lactating Holstein cows. J. Dairy Sci. 69(suppl. 1):193.

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

Rook, J. A. F., C. C. Balch, and V. W. Johnson. 1965. Further observations on the effects of intraruminal infusions of volatile fatty acids and of lactic acid on the yield and composition of the milk of the cow. Br. J. Nutr. 19:93.

Sapir, D. G., P, M. Stewart, M. Walser, C. Moreadith, E. D. Moyer, A. L. Imbembo, M. B. Rosenshein, and S. Munoz. 1983. Effects of 2-ketoisocaproate and of leucine on nitrogen metabolism in postoperative patients. Lancet 1:1010.

Smith, S. I., and J. A. Boling. 1984. Lipid coating as a mode of protecting free methionine from ruminal degradation. J. Anim. Sci. 58:187.

Smith, S., and R. Dils. 1966. Factors affecting the chain length of fatty acids synthesized by lactating-rabbit mammary glands. Biochim. Biophys. Acta 116:23.

Tischler, M. E., M. Desautels, and A. L. Goldberg. 1982. Does leucine, leucyl-tRNA, or some metabolite of leucine regulate protein synthesis and degradation in skeletal and cardiac muscle? J. Biol. Chem. 257:1613.

Trenkle, A. 1970. Effects of short-chain fatty acids, feeding, fasting and type of diet on plasma insulin levels in sheep. J. Nutr. 100:13.

Tyrrell, H. F., and J. T. Reid. 1965. Prediction of the energy value of cow's milk. J. Dairy Sci. 48:1215.

Underwood, L. E., A. J. D'Ercole, K. C. Copeland, J. J. Van Wyk, T. Hurley, and S. Handwerger. 1982. Development of a heterologous radioimmunoassay for somatomedin C in sheep blood. J. Endocrinol. 93:31.

Williamson, D. H., and J. Mellanby. 1974. D-(-)-3-hydroxybutyrate. Page 1836 in H. U. Bergmeyer, ed. Methods in enzymatic analysis. Vol. 3. Academic Press, London.

# SECTION II. EFFECT OF FEEDING LEUCINE, 2-KETOISOCAPROATE, OR ISOVALERATE TO GROWING LAMBS ON LIPID DEPOSITION AND WHOLE-BODY TURNOVER OF ACETATE AND GLUCOSE

# Effect of feeding leucine, 2-ketoisocaproate, or isovalerate to growing lambs on lipid deposition and whole-body turnover of acetate and glucose

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## ABSTRACT

To determine if leucine or its metabolites alter energy metabolism in growing lambs, 30 mixed-breed lambs were fed a control diet or ruminally-protected L-leucine, 2-ketoisocaproate (KIC), or isovalerate (IVA) at a molar equivalent of .05% leucine in the diet. Lambs were fed diets for ~10 wk and slaughtered upon reaching 50 kg body weight. During wk 8, plasma glucose and acetate turnover rates were measured. Perirenal fat deposition was decreased ($P<.01$) in lambs fed KIC by 45% and tended to be decreased in lambs fed leucine by 29%. KIC also tended to decrease ($P<.09$) backfat thickness by 25%. Lambs fed leucine tended to have a slower growth rate and less efficient feed conversion when compared with lambs fed KIC. Plasma glucagon concentration was significantly less in lambs fed leucine, KIC, and IVA than in controls during wk 4 and tended to be less in lambs fed KIC and IVA during wk 8. Dietary treatment did not alter significantly glucose flux or concentration or acetate flux, concentration, or oxidation rate; however, trends toward increased acetate flux were observed in lambs fed leucine and KIC diets. In summary, KIC decreased lipid deposition and seemed to decrease glucagon concentration in plasma of growing lambs; effects of KIC on lipid deposition were unique among the leucine metabolites studied.

# INTRODUCTION

The branched-chain amino acid leucine and its first catabolite, 2-ketoisocaproate (KIC), have effects on metabolism that are unique among the amino and keto acids. Leucine and KIC both inhibit proteolysis and stimulate protein synthesis in vitro (Tischler et al., 1982; Kirsch et al., 1976; Buse and Reid, 1975; Chua et al., 1979) and reduce nitrogen excretion in vivo during times of extreme protein catabolism (Ell et al., 1978; Mitch et al., 1981; Sapir et al., 1983; Sax et al., 1986). Isovaleryl-CoA is a further catabolite of leucine, and there is evidence that isovalerate also decreases proteolysis in vitro (Chua et al., 1979), suggesting that a further step in leucine catabolism may be responsible for the effects of leucine and KIC.

Protein turnover likely accounts for a minimum of 15-25% of maintenance energy requirement (Young et al., 1983; Lobley et al., 1980). Thus, if nutrient absorption is constant, decreased protein turnover should increase the energy available for lipogenesis and (or) increase net protein synthesis. Additionally, mechanisms controlling protein metabolism and those controlling lipid and carbohydrate metabolism are not distinct. Therefore, effects of compounds within the leucine metabolic pathway on protein metabolism indicate that they also may alter energy metabolism. Previous observations are that: 1) leucine increases insulin concentration and decreases glucose uptake and oxidation by human forearm (Abumrad et al., 1982a); 2) KIC decreases pyruvate oxidation in muscle from fasted rats (Chang and Goldberg, 1978), decreases gluconeogenesis in perfused kidney (Stumpf et al., 1978), decreases

glucagon secretion, and increases insulin secretion in perfused pancreas (Leclercq-Meyer et al., 1979; Holze and Panten, 1979); 3) isovalerate does not stimulate insulin secretion in perfused pancreas (Vague et al., 1975); and 4) leucine must be metabolized, presumably to KIC, to elicit increased insulin secretion and decreased pyruvate oxidation (Vague et al., 1975; Chang and Goldberg, 1978).

In recent studies by our research group, intraperitoneal injection of 3 g KIC per day and dietary supplementation with 15 g KIC per day increased growth rate in lambs by 10% (Flakoll et al., 1986; Flakoll and Nissen, 1986). Supplementation of lactating cow diets with .75% KIC increased milk yield and milk fat yield by 5% and 10%, respectively (VandeHaar et al., 1986). Whether these effects are specific to KIC or are general effects of leucine and its catabolites is not known. Therefore, studies were conducted in growing lambs to determine if dietary supplementation with ruminally-protected leucine, KIC, or isovalerate would alter growth rate, fat deposition, concentrations of insulin and glucagon in plasma, or whole-body glucose or acetate turnover.

# METHODS

## Animals and Diets

Thirty mixed-breed ram lambs (21 kg initial body weight) were allocated randomly to one of four dietary treatment groups. Animals were fed ad libitum a corn-soy basal diet (Table 13) supplemented with 5 g ammonium chloride per day, were caged individually, and had free access to water.

Table 13. Composition of basal diet

| Ingredient | % of total mix (as fed basis) |
|---|---|
| Ground corn | 60 |
| Expeller soybean meal[a] | 21 |
| Dehydrated alfalfa meal | 10 |
| Liquid molasses | 7.5 |
| Corn oil | .38 |
| Calcium carbonate | .50 |
| Salt | .50 |
| Vitamin A premix[b] | .10 |
| Trace mineral premix[c] | .02 |
| TOTAL | 100.00 |
| Dry matter[d], % | 88 |
| Crude protein[d], % | 17 |
| Net energy for maintenance[d], Mcal/kg | 1.9 |
| Net energy for gain[d], Mcal/kg | 1.3 |
| Calcium[d], % | .5 |
| Phosphorus[d], % | .4 |
| Leucine[d], % of total protein | 10.4 |

[a]West Central Cooperative, Ralston, IA.

[b]$5.2 \times 10^6$ IU/kg.

[c]13.5% calcium, 12% zinc, 8% manganese, 10% iron, 1.5% copper, .2% iodine, and .1% cobalt.

[d]Calculated from National Research Council values (1982).

Dietary treatments were 1) leucine (n=7), 2) 2-ketoisocaproate (KIC; n=8), 3) isovalerate (IVA; n=7), or 4) limestone (control; n=8) and were supplemented to the ration at feeding (2x/d) as zein-coated pellets (Table 14; Appendix B; VandeHaar et al., 1987). Pellets were made by thoroughly mixing zein and 88% ethanol and adding limestone, bentonite, and either calcium-$(KIC)_2$, calcium-$(IVA)_2$, leucine, or more limestone. This mixture was extruded through a 3 mm die directly into liquid nitrogen for freeze-fracturing. Frozen pellets were allowed to dry slowly at room temperature. After drying, pellets were shaken under forced air and sprayed with a solution of 20% zein in 88% ethanol (100 ml/kg pellets). Average pellet size was 2 x 8 mm, and density was 1.1 - 1.2 g/ml as determined by buoyancy in salt solutions. From rumen bag studies, ruminal bypass of zein-protected compounds was estimated to be about 50%. Calcium-$(IVA)_2$ was made by combining calcium hydroxide and isovaleric acid, and the salt produced was calculated to be 73% calcium-$(IVA)_2$ and 27% calcium hydroxide.

Lambs were fed their respective pellets at a rate of 1 g/d for the first 6 wk, 1.5 g/d for wk 6-10, and 2 g/d for wk 10 to time of slaughter. These doses are equivalent to about 1 g pellets per kg diet and 2.5 mmol leucine, KIC, or IVA per kg diet (molar equivalent of .05% leucine added to the diet). Two control lambs, one lamb fed leucine, and one fed IVA developed urinary calculi or other complications and were removed from the study.

Lambs were weighed weekly, and total feed consumption for each 2 wk period was recorded. Jugular blood samples were collected at 4 wk into

sodium-EDTA-coated tubes and immediately centrifuged, and plasma was stored at -70° C for hormone analysis. Lambs were killed by electrical shock and exsanguination at about 50 kg body weight after an 18-h fast. Hide, carcass, and perirenal adipose tissue were separated and weighed. Carcasses were severed between the tenth and eleventh ribs for measurement of backfat thickness.

Table 14. Composition of treatment supplements[a]

| | Leucine | KIC | IVA | Control |
|---|---|---|---|---|
| | ---------- g/kg ---------- | | | |
| Zein[b] | 450 | 450 | 450 | 450 |
| Ethanol | 278 | 278 | 278 | 278 |
| Water | 37 | 37 | 37 | 37 |
| Limestone[c] | 160 | 100 | 80 | 450 |
| Sodium Bentonite[d] | 50 | 50 | 50 | 100 |
| L-Leucine[e] | 340 | | | |
| Calcium-$(KIC)_2 \cdot H_2O$[f] | | 400 | | |
| Calcium-$(IVA)_2 \cdot H_2O$[f] | | | 420 | |

[a]Dried treatment pellets contained approximately 2.5 mol leucine, KIC, or IVA, and 64, 91, 97, or 180 g calcium per kg leucine, KIC, IVA, or control pellets, respectively.

[b]Freeman Industries, Tuckahoe, NY.

[c]325 mesh; Iowa Limestone Company, Des Moines, IA.

[d]200 mesh; American Colloid Company, West Des Moines, IA.

[e]Sigma Chemical Company, St. Louis, MO.

[f]SOBAC, Paris, France.

## Isotope Infusion Study

During wk 8, when average body weight was 40 kg, whole-body flux of glucose and acetate was studied in four lambs per day. On the day

before each study, lambs were removed from feed at 2000 h and placed in metabolism stalls. At 0730 h on the day of study, lambs were fed 50 g of feed with .75 g of their respective treatment pellets. Right and left jugular veins were catheterized, and, at about 1000 h, a primed dose-continuous infusion of [1-$^{14}$C] acetate (ICN Radiobiochemicals, Irvine, CA) and [6-$^{3}$H] glucose (ICN Radiobiochemicals) was begun into the right jugular vein at .71 and .28 uCi/min, respectively. Priming doses of labelled acetate and glucose and [$^{14}$C] bicarbonate were 28 and 10 uCi, respectively. Additionally, 50 uCi of [$^{14}$C] bicarbonate (Calatomic, Los Angeles, CA) was given to prime the blood $CO_2$ pool.

Blood from left jugular vein and expired air were sampled every 15 min from 120 min to 180 min after start of infusion. Blood was collected into sodium-EDTA-coated tubes, stored on ice, and centrifuged, and plasma was stored at -70° for metabolite flux and hormone analysis. Expired air was collected for 1-2 min into meteorological balloons by using a nose-cone face mask connected to a respiratory valve. $^{14}CO_2$ was trapped by slowly bubbling the air sample through 250 ml 1 M ethanolamine, 2 ml aliquots of this solution then were transferred to scintillation vials, and radioactivity was counted for determination of breath $^{14}CO_2$ output per min.

## Methods of Analysis

Plasma was analyzed for concentration of cortisol by radioimmunoassay kit (Travenol-Genentech Diagnostics, Cambridge, MA) and for insulin concentration in plasma of wk 8 samples by radioimmunoassay using bovine insulin antibody (Trenkle, 1970). Plasma glucagon

concentration was determined by radioimmunoassay using porcine glucagon antibody (Harris et al., 1979) for wk 4 samples and double-antibody radioimmunoassay using rabbit anti-porcine glucagon (BioTek Research, Inc., Lenexa, KS) for wk 8 samples. The concentration of insulin-like growth factor I (IGF-I) in plasma was measured by T. Elsasser (USDA-ARS, Beltsville, MD) by radioimmunoassay using human IGF-I antibody (Underwood et al., 1982) after dissociation of IGF-I from its binding protein with acetic acid. Plasma leucine and KIC concentration were determined by gas chromatography-mass spectrometry after protein precipitation, separation of amino and keto acids by ion-exchange chromatography, extraction of ketoacids with methylene chloride (Nissen et al., 1982), and derivatization with N-methyl-N-(t-butyl-dimethylsilyl)tri-fluoroacetamide containing 1% t-butyl-dimethylchlorosilane (Regis Chemical Company, Morton Grove, IL).

Plasma glucose concentration and specific radioactivity (SRA) were determined by modification of Schmidt et al. (1975). Briefly, perchloric acid was used to precipitate protein in 1 ml plasma; after centrifugation, supernatant was neutralized with potassium hydroxide and poured through a cation-exchange column and anion-exchange column in series and eluted with $H_2O$. Filtrate was dried at 25° and resuspended in 1 ml water, of which 50 ul was used to determine glucose concentration by glucose oxidase assay kit (Worthington Diagnostic Systems, Inc., Freehold, NJ). The remaining 950 ul was diluted with scintillation fluid, and $^3H$ counts were measured. Whole-body glucose flux was calculated as [$^3H$] glucose infusion rate / SRA of plasma glucose.

Plasma acetate concentration and SRA were determined by using high-performance liquid chromatography for separation of acetate. Protein in 2 ml plasma with 3 umol malonic acid added as internal standard was precipitated by addition of 4 ml .5 N perchloric acid, and, after centrifugation, supernatant was poured through a disposable column filled with 2 ml 50% cation-exchange resin (Dowex-50W, Sigma Chemical Co., St. Louis, MO) and eluted with 2 ml .01 N hydrochloric acid. Eluent was heated to 90° for 10 min to remove acetoacetate, and then organic acids were extracted twice from sample with diethyl ether (purified for fat extraction; Mallinckrodt, Paris, KY) and back-extracted with 1 ml .05 N sodium hydroxide. This back-extract was dried under nitrogen gas, resuspended with 125 ul HPLC-grade water and 55 ul of suspended cation-exchange resin (Dowex-50W), and 115 ul was chromatographed by HPLC using cation-exchange guard and analytical columns (Cation $H^+$ and Aminex HPX-87H; Bio-Rad Laboratories, Richmond, CA) with .02 M phosphoric acid at .6 ml/min as the eluent. At 1.1 min after injection, a column-switching valve (Rheodyne, Cotati, CA) was activated so that solvent flowed first through the analytical column and then the guard column and detector; thus, very late eluting compounds (later than 25 min) eluted within the first 8 min (Nazareth et al., 1984). Acetate eluted at about 17 min, was detected by absorbance of UV light at 214 nm, and was collected for determination of radioactivity by scintillation counting. Peak height was used to measure amount of acetate and compared to the peak height of internal standard (malonic acid) for calculation of concentration in plasma. Recovery of acetic and malonic acids was 44% and 18%,

respectively. Acetate SRA was used to estimate whole-body acetate flux and conversion to $CO_2$ by the equations 1) acetate flux = infusion rate of [$^{14}C$] acetate / acetate $^{14}C$ SRA, and 2) acetate oxidation = $^{14}CO_2$ output in expired air / acetate $^{14}C$ SRA.

Determination of significant treatment effects was by analysis of variance with the ANOVA table including treatment and error; contrasts within the treatment component were made between control and treatment groups. Treatment effects were considered significant if the probability of greater F was less than .05 ($P<.05$). Effects were considered to be trends if $P<.25$ and $P>.05$.

# RESULTS

Effects of leucine, KIC, and IVA on feed intake, growth rate, feed efficiency, perirenal lipid deposition, and backfat thickness are reported in Table 15. Leucine, KIC, and IVA had no significant effect on feed intake. Growth rate and feed efficiency were decreased in lambs fed leucine when compared to those fed KIC, and feed efficiency tended to be greater in lambs fed KIC than controls ($P<.2$). Perirenal fat deposition at time of slaughter was decreased in lambs fed KIC by 45% ($P<.01$) and tended to be decreased in lambs fed leucine ($P<.08$) when compared with controls. KIC also tended to decrease backfat thickness (25%, $P<.09$).

Leucine, KIC, and IVA significantly decreased plasma glucagon concentration during wk 4 by 18% ($P<.03$), 23% ($P<.01$), and 31% ($P<.01$), respectively, but had no significant effect on glucagon concentration during wk 8 (Table 16). Treatment also had no significant effect on plasma insulin concentration during wk 8, but insulin to glucagon ratio tended to be greater in lambs fed KIC than in lambs fed leucine ($P<.06$). Additionally, no significant effects were observed on plasma IGF-I or cortisol concentration.

Steady state SRA for plasma glucose was achieved by 135 min post-infusion (Figure 3), and average SRA of the four measurements between 135 min and 180 min was used to evaluate effect of treatments. Leucine and its metabolites had no significant effect on plasma glucose concentration or turnover (Table 17). Plasma glucose concentration averaged 66 mg/dl, and mean glucose turnover was 16 umol/kg·min. Steady state also was achieved for acetate SRA by 120 min (Figure 3). Acetate flux was

approximately 36 umol/kg·min, and oxidation to $CO_2$ accounted for 37% of total acetate flux. Dietary treatment had no significant effect on acetate flux or oxidation, but acetate flux in lambs fed KIC and leucine tended to be greater than that of lambs fed control and IVA diets. The flux of acetate to compounds other than $CO_2$ also tended to be greater in lambs fed KIC and leucine. Acetate concentration in plasma was about 230 nmol/ml for all treatments.

Table 15. Effect of leucine, KIC, and IVA on feed intake, growth rate, and fat deposition in lambs fed to 50 kg body weight

| Variable | Control | Leucine | KIC | IVA | SEM[a] | Pr>F[b] |
|---|---|---|---|---|---|---|
| Number of animals | 6 | 8 | 6 | 6 | | |
| Initial weight, kg | 21.6 | 20.9 | 21.5 | 21.0 | 1.3 | .9 |
| Growth rate, g/d | 323 | 287 | 334 | 315 | 20 | .2[c] |
| Feed intake, g/d | 1430 | 1339 | 1394 | 1386 | 50 | .5 |
| Gain to feed ratio | .224 | .215 | .239 | .226 | .020 | .2[d] |
| Days to slaughter | 92 | 102 | 87 | 93 | 15 | .3[e] |
| Carcass weight, kg | 26.8 | 26.4 | 26.3 | 26.6 | .1 | .3 |
| Perirenal fat, g | 792 | 566 | 435 | 668 | 89 | .03[f] |
| Backfat thickness, cm | .95 | .86 | .72 | .73 | | .23[g] |

[a]Pooled standard error of a mean, calculated as pooled standard deviation from the ANOVA model / square root of the harmonic mean.

[b]Probability of a greater F for effect of treatment component in ANOVA model.

[c]KIC vs. leucine (P<.04).

[d]KIC vs. leucine (P<.03), vs. control (P<.2).

[e]KIC vs. leucine (P<.08).

[f]KIC vs. control (P<.006), vs. leucine (P<.24), vs. IVA (P<.05); leucine vs. control (P<.07); IVA vs. control (P<.3).

[g]KIC vs. control (P<.08).

Table 16. Effect of leucine, KIC, and IVA on concentrations of plasma hormones of lambs[a]

| Variable | Control | Leucine | KIC | IVA | SEM[b] | Pr>F[c] |
|---|---|---|---|---|---|---|
| Number of animals | 6 | 8 | 6 | 6 | | |
| Plasma hormone concentrations | | | | | | |
| cortisol, ng/ml, wk 8 | 1.8 | 1.4 | 1.9 | 2.3 | .4 | .4 |
| insulin, ng/ml, wk 8 | .87 | .73 | .99 | .98 | .3 | .4[d] |
| glucagon, pg/ml, wk 4 | 365 | 300 | 282 | 252 | 8 | .003[e] |
| wk 8 | 326 | 321 | 295 | 295 | 16 | .4[f] |
| IGF-I, ng/ml, wk 4 | 198 | 212 | 220 | 201 | 15 | .6 |
| insulin to glucagon ratio, wk 8 | 2.68 | 2.26 | 3.43 | 3.33 | .4 | .2[g] |
| Plasma leucine concentration, uM, wk 8 | 242 | 254 | 215 | 247 | 31 | .6 |
| Plasma KIC concentration, uM, wk 8 | 15.3 | 13.3 | 11.0 | 14.0 | 1.5 | .3[h] |

[a]Blood samples collected during wk 4 or 8 after overnight fast

[b]Pooled standard error of a mean, calculated as pooled deviation from the ANOVA model / square root of the harmonic mean.

[c]Probability of a greater F for the effect of treatment component in ANOVA model.

[d]KIC vs. leucine (P<.14).

[e]Control vs. treatments (P<.0009), vs. leucine (P<.02), vs. KIC (P<.005), vs. IVA (P<.001); leucine vs. IVA and KIC (P<.2); IVA vs. KIC (P<.3).

[f]KIC vs. control (P<.2), vs. leucine (P<.3).

[g]KIC vs. control (P<.22), vs. leucine (P<.06).

[h]KIC vs. control (P<.06).

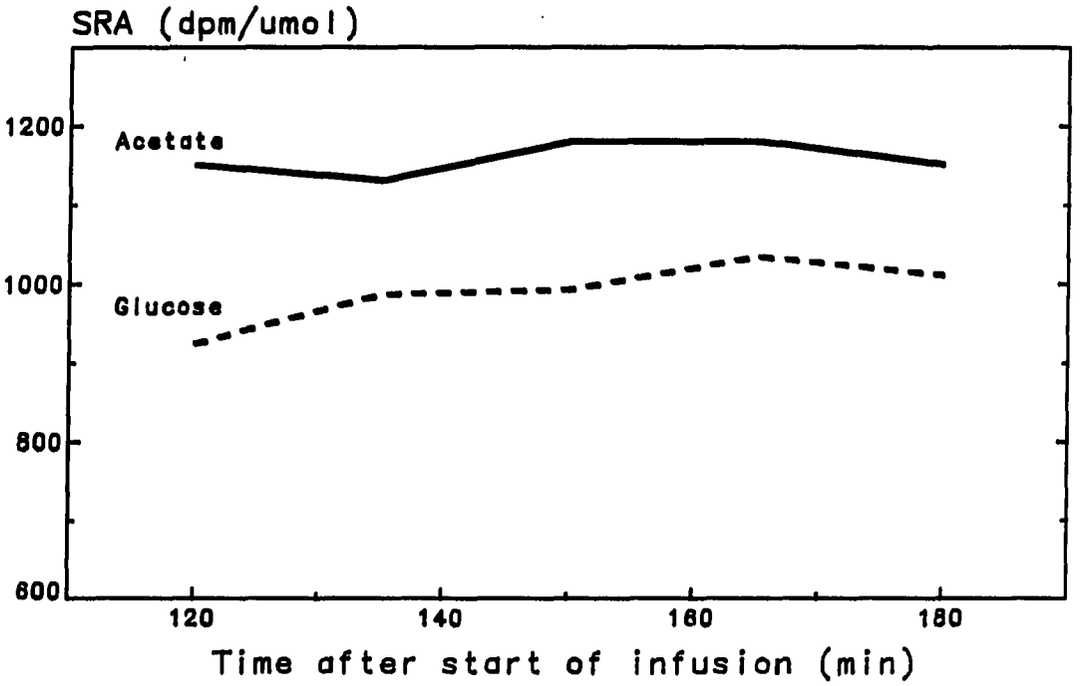


Figure 3. Average specific radioactivity (SRA) of glucose and acetate in plasma of all lambs at 15 min intervals during the sample collection period of infusion studies. Isotopic steady state was reached for glucose and acetate by 135 and 120 min, respectively, after start of infusion.

Table 17. Effect of leucine, KIC, and IVA on plasma glucose and acetate metabolism of lambs during wk 8 after overnight fast

| Variable | Control | Leucine | KIC | IVA | SEM[a] | Pr>F[b] |
|---|---|---|---|---|---|---|
| Number of animals[c] | 6 | 6 | 7 | 5 | | |
| Body weight | 41 | 38 | 41 | 40 | | |
| Plasma glucose | | | | | | |
| Concentration, mg/dl | 68.6 | 64.0 | 64.6 | 63.2 | 3.0 | .6 |
| Flux, umol/kg·min | 16.0 | 17.3 | 15.9 | 15.4 | 1.0 | .6 |
| Plasma acetate | | | | | | |
| Concentration, nmol/ml | 263 | 221 | 226 | 209 | 40 | .8 |
| Flux, umol/kg·min | 34.2 | 38.7 | 40.9 | 30.2 | 3.5 | .2[d] |
| % Oxidized | 38.1 | 34.7 | 36.7 | 37.8 | .9 | .7 |
| Non-oxidized flux, umol/kg·min | 20.8 | 25.4 | 25.8 | 18.8 | 2.2 | .1[e] |
| Glucose plus acetate flux, umol/kg·min | 50.2 | 55.8 | 56.8 | 45.1 | 3.7 | .2[d] |
| Acetate to glucose flux ratio | 2.17 | 2.22 | 2.66 | 2.04 | .26 | .4[f] |

[a]Pooled standard error of a mean, calculated as pooled deviation from the ANOVA model / square root of the harmonic mean.

[b]Probability of a greater F for the effect of treatment component in ANOVA model.

[c]Infusion studies were not performed successfully in all lambs.

[d]KIC vs. control (P<.2), vs. IVA (P<.05).

[e]KIC vs. control (P<.12), vs. IVA (P<.04).

[f]KIC vs. control (P<.2), vs. IVA (P<.11), vs. leucine (P<.24).

# DISCUSSION

The major finding of this study is that KIC significantly decreased perirenal fat deposition and tended to decrease subcutaneous lipid deposition and increase growth rate in growing lambs. This effect seemed unique to KIC, although trends were observed toward the same responses in lambs fed leucine. Other effects observed in this study, which are reported by Flakoll et al. (1987b), were significant increases in the weights of selected muscles and a trend for decreased days to slaughter for lambs fed KIC. Leucine and IVA seemed to have no effect on growth. Also observed were enhanced immune function in response to KIC and suppressed immune function in response to leucine (Kuhlman et al., 1987).

Although leucine metabolites did not significantly increase growth rate in the present study, growth rate tended to be greater in lambs fed KIC when compared with lambs fed leucine. This difference between diets does not seem related to a branched-chain amino acid antagonism, because no significant difference in feed intake was observed. This is consistent with previous studies in lambs infused intraperitoneally with 3 g KIC per day or fed 1% KIC and cattle fed .05% zein-protected KIC (Flakoll et al., 1986; Flakoll and Nissen, 1986; Flakoll et al., 1987a).

A trend toward decreased subcutaneous fat deposition also is supported by previous studies with growing lambs (Flakoll et al., 1986; Flakoll and Nissen, 1986). Additionally, effects on fat deposition in lambs are supported by studies in lactating animals, in which KIC increased fat output into milk (VandeHaar et al., 1986). Because fat deposition in body tissues during lactation seems to compete with fat

output into milk, increased milk fat production may reflect decreased net lipid deposition in body tissues.

Glucose flux and concentration in this study are consistent with previous reports of total entry rate of glucose (including recycling) in sheep (White et al., 1969); sheep in the present study were fasted 14 h, so glucose production likely was about 80 - 90% of that in the fed state (Armentano et al., 1984). Although glucose use by tissues and gluconeogenesis previously have been shown to be decreased by KIC (Stumpf et al., 1978; Stumpf and Kraus 1978), no effect on glucose turnover was observed in the present study, possibly because animals were fasted overnight.

Measures of acetate concentration in plasma and whole-body acetate flux and oxidation are also consistent with previous studies (Pethick and Lindsay, 1978; Pethick et al., 1981). Effects of KIC on acetate metabolism have not been reported; however, KIC administration does increase ketone production in postoperative humans (Sapir et al., 1983) and, thus, might have been expected to also increase acetate production. The maximal increase in acetate production rate from catabolism of dietary leucine, KIC, or IVA supplements would be about .7 umol/kg·min. However, KIC and leucine tended to increase acetate flux rate (relative to control and IVA treatments) almost 7 umol/kg·min. Whether this trend is related to the changes also noted in lipid deposition is not understood. Because lambs were studied after an overnight fast, this increase indicates that endogenous acetate production was likely enhanced. Despite this possible change in total flux, however, no change

was observed in the percentage of plasma acetate oxidized to $CO_2$. The rate of acetate conversion to compounds other than $CO_2$ was increased in lambs fed KIC and leucine diets. This increased non-oxidative flux indicates that the recycling of acetate to lipid and back to acetate was greater in these lambs, and that their adipose tissue may have been metabolically more active.

In the present study, lambs were supplemented with only 2.5 mmol leucine, KIC, or IVA. Leucine absorption from the diet in sheep is considered to be approximately 50 mmol/d (Tagari and Bergman, 1978); therefore, the maximal increase in leucine absorption expected from leucine supplementation would be only 5%. KIC and IVA absorption in unsupplemented animals, however, are much less than that of leucine, so equimolar amounts of supplemental KIC or IVA represent a much greater relative change in KIC and IVA absorption. Of KIC reaching the small intestine, 85% is used by gut and liver in dogs (Abumrad et al., 1982a), and, in lambs, gut and liver removed more than half of blood KIC (Pell et al., 1986). Therefore, KIC is metabolized primarily by splanchnic tissues and very little of an oral dose would be expected to reach peripheral tissues. This indicates that effects of KIC probably are mediated by metabolism in gut tissues and liver and may be caused by changes in hormone secretion rates.

Plasma glucagon concentration was decreased in lambs fed all leucine metabolites during wk 4, and, during wk 8, lambs fed KIC tended to have a greater insulin to glucagon ratio in plasma. This is consistent with previous studies showing decreased glucagon secretion and increased

insulin secretion in response to KIC (Leclercq-Meyer et al., 1979; Holze and Panten, 1979). Increased insulin to glucagon ratio in lambs fed KIC, however, likely would have increased rather than decreased lipid deposition in adipose depots unless other hormones or metabolites also were altered.

Effects of KIC on cortisol metabolism have not been reported, although leucine may increase cortisol concentration (Munro et al., 1963). Whereas no effect on plasma total cortisol concentration was observed in the present study, we previously have observed significant decreases in total cortisol concentration in cattle fed KIC (data not reported), and effects on free cortisol concentration are not known. IGF-I secretion, which is responsive to plane of nutrition (Kenison et al., 1987), seems a likely candidate for mediating KIC action because it is involved in controlling growth and is secreted by liver, a tissue that rapidly metabolizes KIC. Although no change was observed in total IGF-I concentration, effects of KIC on secretion rate or bound to free ratio are not known.

In summary, decreased perirenal and subcutaneous lipid deposition and increased growth rate in lambs fed KIC seems to be a unique effect of this metabolite within the leucine catabolic pathway. The mechanism for this effect is not understood but seems to be post-ruminal and may be related to changes in glucagon and insulin secretion.

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## REFERENCES CITED

Abumrad, N. N., R. P. Robinson, B. R. Gooch, and W. W. Lacy. 1982a. The effect of leucine infusion on substrate flux across the human forearm. J. Surg. Res. 32:453-463.

Abumrad, N. N., K. L. Wise, P. E. Williams, N. A. Abumrad, and W. W. Lacy. 1982b. Disposal of 2-ketoisocaproate: roles of liver, gut, and kidneys. Am. J. Physiol. 243:E123.

Armentano, L. E., S. E. Mills, G. deBoer, and J. W. Young. 1984. Effects of feeding frequency on glucose concentration, glucose turnover, and insulin concentration in steers. J. Dairy Sci. 67:1445.

Buse, M. G., and S. S. Reid. 1975. Leucine: a possible regulator of protein turnover in muscle. J. Clin. Invest. 56:1250.

Chang, T. W., and A. L. Goldberg. 1978. Leucine inhibits oxidation of glucose and pyruvate in skeletal muscles during fasting. J. Biol. Chem. 253:3696-3701.

Chua, B., D. L. Siehl, and H. E. Morgan. 1979. Effect of leucine and metabolites of branched chain amino acids on protein turnover in heart. J. Biol. Chem. 254:8358.

Ell, S., M. Fynn, P. Richards, and D. Halliday. 1978. Metabolic studies with keto acid diets. Am. J. Clin. Nutr. 31:1776.

Flakoll, P. J., and S. L. Nissen. 1986. Evaluation of lamb performance in response to oral admistration of 2-ketoisocaproate. J. Anim. Sci. 63(Suppl. 1):452.

Flakoll, P. J., P. Ostaszewski, and S. L. Nissen. 1986. Evaluation of lamb performance in response to intravenous or intraperitoneal admistration of 2-ketoisocaproate. J. Anim. Sci. 63(Suppl. 1):452.

Flakoll, P. J., M. J. VandeHaar, G. Kuhlman, J. A. Roth, and S. L. Nissen. 1987a. Evaluation of steer growth, immune function, and carcass composition in response to oral administration of ruminally protected 2-ketoisocaproate. J. Anim. Sci. 65(Suppl. 1):476.

Flakoll, P. J., M. J. VandeHaar, and S. Nissen. 1987b. Muscle growth and whole-body leucine metabolism of growing lambs fed leucine, 2-ketoisocaproate, and isovalerate. Fed. Proc. 46:1178.

Harris, V., G. R. Faloona, and R. H. Unger. 1979. Glucagon. Page 643 in B. M. Jaffe and H. R. Behrman, eds. Methods of hormone radioimmunoassay. 2nd ed. Academic Press, Inc., New York.

Holze, S., and U. Panten. 1979. Studies on the role of beta-cell metabolism in the insulinotropic effect of 2-ketoisocaproic acid. Biochim. Biophys. Acta 588:211.

Kenison, D. C., C. C. Chase, Jr., G. T. Schelling, F. M. Byers, and L. W. Greene. 1987. Somatomedin C levels in steers immunized against somatostatin and fed two levels of nutrition. Fed. Proc. 46:1178.

Kirsch, R. E., L. O'C. Frith, and S. J. Saunders. 1976. Stimulation of albumin synthesis by keto analogues of amino acids. Biochim. Biophys. Acta 442:437.

Kuhlman, G., J. A. Roth, P. J. Flakoll, M. J. VandeHaar, and S. Nissen. 1987. The effect of supplemental dietary leucine and leucine metabolites on immune function in growing lambs. J. Anim. Sci. 65(Suppl. 1):475.

Leclercq-Meyer, V., J. Marchand, R. Leclercq, and W. J. Malaisse. 1979. Interactions of 2-ketoisocaproate, glucose and arginine in the secretion of glucagon and insulin from the perfused rat pancreas. Diabetologia 15:121.

Lobley, G. E., V. Milne, J. M. Lovie, P. J. Reeds, and K. Pennie. 1980. Whole body and tissue protein synthesis in cattle. Br. J. Nutr. 43:491.

Mitch, W. E., M. Walser, and D. G. Sapir. 1981. Nitrogen sparing induced by leucine compared with that induced by its keto analog, 2-ketoisocaproate, in fasting obese man. J. Clin. Invest. 67:553.

Munro, H. N., M. H. Steele, and W. C. Hutchison. 1963. Blood coricosterone-levels in the rat after administration of amino-acids. Nature 199:5000.

National Research Council. 1982. United States - Canadian tables of feed composition. 3rd ed. Natl. Acad. Sci., Washington, DC.

Nazareth, A., L. Jaramillo, B. L. Karger, and R. W. Giese. 1984. Automated analysis of antiepileptic drugs in serum by column-switching high-performance liquid chromatography. J. Chromatogr. 309:357.

Nissen, S. L., C. Van Huysen, and M. W. Haymond. 1982. Measurement of branched chain amino acids and branched chain 2-ketoacids in plasma by high-performance liquid chromatography. J. Chromatogr. 232:170.

Pell, J. M., E. M. Caldarone, and E. N. Bergman. 1986. Leucine and 2-ketoisocaproate metabolism and interconversions in fed and fasted sheep. Metabolism 35:1005-1016.

Pethick, D. W., and D. B. Lindsay. 1978. Acetate production by sheep hind-limb muscle. Proc. Nutr. Soc. 37:101A.

Pethick, D. W., D. B. Lindsay, P. J. Barker, and A. J. Northrop. 1981. Acetate supply and utilization by the tissues of sheep in vivo. Br. J. Nutr. 46:97.

Reynolds, C. K., G. B. Huntington, H. F. Tyrrell, and P. J. Reynolds. 1986. Splanchnic tissue and whole animal oxygen consumption by lactating Holstein cows. J. Dairy Sci. 69(Suppl. 1):193.

Sapir, D. G., P, M. Stewart, M. Walser, C. Moreadith, E. D. Moyer, A. L. Imbembo, M. B. Rosenshein, and S. Munoz. 1983. Effects of 2-ketoisocaproate and of leucine on nitrogen metabolism in postoperative patients. Lancet 1:1010.

Sax, H. C., M. A. Talamini, and J. E. Fischer. 1986. Clinical use of branched-chain amino acids in liver disease, sepsis, trauma, and burns. Arch. Surg. 121:358.

Schmidt, S. P., J. A. Smith, and J. W. Young. 1975. Rapid determination of [carbon-14] glucose specific radioactivity for in vivo glucose kinetics. J. Dairy Sci. 58:952.

Stumpf, B., H. Kraus, R. Kasten, and G. Ahrens. 1978. Inhibition of gluconeogenesis in isolated rat kidneys by branched chain 2-ketoacids. Pediatr. Res. 12:1039-1044.

Tagari, H., and E. N. Bergman. 1978. Intestinal disappearance and portal blood appearance of amino acids in sheep. J. Nutr. 108:790.

Tischler, M. E., M. Desautels, and A. L. Goldberg. 1982. Does leucine, leucyl-tRNA, or some metabolite of leucine regulate protein synthesis and degradation in skeletal and cardiac muscle? J. Biol. Chem. 257:1613.

Trenkle, A. 1970. Effects of short-chain fatty acids, feeding, fasting and type of diet on plasma insulin levels in sheep. J. Nutr. 100:13-23.

Underwood, L. E., A. J. D'Ercole, K. C. Copeland, J. J. Van Wyk, T. Hurley, and S. Handwerger. 1982. Development of a heterologous radioimmunoassay for somatomedin C in sheep blood. J. Endocrinol. 93:31.

VandeHaar, M. J., P. J. Flakoll, D. C. Beitz, and S. Nissen. 1986. Effect of feeding 2-ketoisocaproate to dairy cows on milk production. J. Dairy Sci. 69(suppl. 1):111.

VandeHaar, M. J., P. J. Flakoll, D. C. Beitz, and S. Nissen. 1987. A new method to protect compounds from ruminal degradation using the corn protein zein. J. Anim. Sci. 65(suppl. 1):501.

Vague, P., G. Ramahandridona, C. Di Campo-Rouge, and F. Mahmoud. 1975. Kinetics of the early insulin response of the perfused rat pancreas to various metabolites and tolbutamide. Diabete & Metab. 1:185-189.

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

# GENERAL SUMMARY AND CONCLUSIONS

The major finding of these studies is that KIC alters lipid metabolism in ruminants. KIC increased milk fat production in lactating cows and goats and decreased perirenal and subcutaneous lipid deposition in growing lambs. These results might seem inconsistent unless one considers that the mechanisms controlling milk fat production and those controlling body lipid deposition are quite different. For example, insulin increases net body lipid deposition but decreases milk fat production; growth hormone increases milk fat production but decreases net body lipid deposition. During lactation, lipid deposition in body tissues seems to compete with lipid output into milk. Increased milk fat production, therefore, may reflect decreased net deposition of lipid in body tissues of a lactating animal.

Some possible mechanisms for the effects of KIC on lipid metabolism observed in these studies are:

1. KIC may directly alter lipogenesis and lipolysis in adipose tissue and lipogenesis in mammary gland. Adipose tissue is a major site for metabolism of leucine (Rosenthal et al., 1974; Wijayasinghe et al., 1983) and probably of KIC also; however, if KIC does alter lipid metabolism, it likely does so in a manner similar to that of ketone bodies, which seem to inhibit lipolysis. Bovine mammary gland removes leucine from plasma in excess of its output into milk and uses some of this excess as an energy and carbon source (Wohlt et al., 1977).

2. KIC may be converted to ketone bodies in liver and thereby provide increased lipogenic substrate for milk fat synthesis when fed at

a dose similar to that of the lactation studies (.75%). The dose of leucine metabolites in the lamb study, however, was likely too low (.05%) to significantly increase the availability of lipogenic precursor for adipose tissue lipogenesis.

3. KIC may indirectly alter lipid metabolism through effects on skeletal muscle protein breakdown. If feed intake is constant and muscle proteolysis is decreased during lactation, then protein synthesis might be decreased similarily; thus, energy use by muscle would be decreased, and increased energy (acetate, ketones, fatty acids) would be available for milk fat synthesis. If proteolysis is decreased in a growing animal, however, little change in synthesis might occur; therefore, amino acid deposition as muscle protein might be increased, and amino acid availability as an energy and carbon source for adipose tissue lipogenesis would be decreased.

4. Because the primary sites for metabolism of orally-administered KIC are likely gut and liver tissues, metabolic effects of KIC may be greatest in these tissues. Possible effects at the gut and liver level are: 1) decreased splanchnic tissue energy use because of decreased protein turnover, 2) increased whole-body glucose availability because of decreased glucose use by gut tissues and increased gluconeogenesis, 3) decreased cortisol secretion because of decreased cholesterol synthesis through inhibition of HMG-CoA reductase, and 4) altered secretion of IGF-I or other gut hormones. Any of these changes might alter whole-body lipid metabolism in growing or lactating ruminants.

5. A decreased insulin to glucagon ratio or increased growth

hormone secretion rate in response to KIC would have resulted in effects similar to that observed. These hormonal changes would increase milk fat synthesis with little change in milk fatty acid composition in a lactating cow and decrease body fat deposition in a growing lamb. However, no effect or a decrease was observed in the concentration of IGF-I, the probable mediator of growth hormone action, and, although insulin to glucagon ratio tended to be less in cows fed KIC, it tended to be greater in lambs fed KIC and IVA. Increased insulin to glucagon ratio in response to KIC is supported by previous studies in nonruminants and would be expected to have effects opposite those observed.

6. Recent studies suggest that KIC alters immune function in ruminants. If KIC enhances the immune function of lactating mammary gland, then a decrease in mastitis incidence and a subsequent increase in milk and milk fat production might be expected. A possible mechanism for the immune system to decrease adipose tissue lipid deposition is based on the recent finding that lymphocytes can secrete ACTH and endorphins (Blalock and Smith, 1985). If KIC significantly alters the secretion of ACTH by lymphocytes, then changes would be expected in the secretion of cortisol, a hormone known to alter lipid deposition.

Based on available evidence from these and other studies, the most likely mechanism for the effect of KIC on lipid metabolism is through decreased protein turnover of splanchnic tissues and skeletal muscle. This would be consistent with the apparent partitioning of nutrients toward milk and muscle and away from body lipid stores. Lowered muscle maintenance needs in lactating cows might increase the nutrient supply to

mammary gland. Because muscle in a lactating cow uses mostly acetate, ketones, and fatty acids for its energy needs, lowered energy requirement with no change in feed intake would increase the flow of these nutrients to mammary gland and might increase milk fat production without a large change in milk fatty acid composition. In growing animals, lowered skeletal muscle proteolysis with no change in protein synthesis and feed intake should increase net protein deposition and decrease the energy available for lipogenesis.

In a fasted animal, as in the lamb infusion study, muscle likely uses fatty acids as its primary energy source and releases some acetate from incomplete oxidation of fatty acids. (Although muscle acetate uptake may be greater than release, peripheral release of acetate accounts for 80% of endogenous acetate production in fasted sheep - Bergman and Wolff, 1981.) If KIC decreased protein turnover during fasting, then increased release of acetate might occur. Increased peripheral production of acetate could explain the increase in milk fat production observed in the cow and goat studies of section I.

The increase in non-oxidative acetate flux observed in lambs fed KIC indicates that lipogenesis was greater in these lambs; however, lipid deposition was significantly less in these lambs. Possibly, adipose tissue was metabolically more active in lambs fed KIC. If adipose tissue of lactating cows was synthesizing and releasing fatty acids more actively, then an increase in milk fat production might be expected because adipose tissue would be adding to the lipogenic capacity of the mammary gland.

The role of the major partitioning hormones (i.e., growth hormone, IGF-I, insulin) in the mechanism of KIC action is not known. No significant difference between control and KIC-fed animals was observed for concentrations of IGF-I or insulin. A trend toward decreased IGF-I concentration in lactating cows fed KIC was observed; however, decreased IGF-I concentration likely would not result in increased milk fat production. When compared with controls, insulin to glucagon ratio tended to be less in cows fed KIC but greater in lambs fed KIC. Decreased insulin to glucagon ratio would be expected to have effects on lipid metabolism consistent with those observed in the lactation studies. The differential response to KIC of insulin to glucagon ratio in lactating cows and growing lambs may be related to the reported decreased responsiveness of insulin secretion to insulinotropic agents during lactation (Lomax et al., 1979).

Although cortisol concentration was not altered in the present studies, decreases in cortisol concentration of lambs fed KIC have been observed in previous studies. Cortisol alters lipid metabolism and therefore could be involved in this partitioning effect, but the role of cortisol in milk production is not understood.

The greatest effect of KIC on milk fat production in the lactating cow study was within the first week of KIC supplementation. The greatest effect of KIC on growth was during the first month of supplementation during the lamb study (data not shown). The ratio of internal to subcutaneous lipogenesis decreases with age in ruminants (Bauman and Davis, 1975); thus, a greater effect of KIC during the first month of

treatment would account for the observation that perirenal lipid deposition was decreased to a greater extent than subcutaneous lipid deposition.

In conclusion, KIC increased milk fat production in lactating cows and goats and decreased lipid deposition in adipose tissue of growing lambs. The mechanism of these effects is not known but seems to be postruminal and may involve changes in protein turnover. Further work is needed to better define the optimal dosage of KIC and its mechanism of action.

# REFERENCES CITED

Bauman, D. E., and C. L. Davis. 1975. Regulation of lipid metabolism. Pages 496-509 in I. W. McDonald and A. C. I. Warner, eds. Digestion and metabolism in the ruminant. Univ. N. E. Publishing Unit, Armidale, Australia.

Bergman, E. N., and J. E. Wolff. 1971. Metabolism of volatile fatty acids by liver and portal-drained visera in sheep. Am. J. Physiol. 221:586-592.

Blalock, J. E., and E. M. Smith. 1985. A complete regulatory loop between the immune and neuroendocrine systems. Fed. Proc. 44:108-111.

Lomax, M. A., G. D. Baird, C. B. Mallinson, and H. W. Symonds. 1979. Differences between lactating and non-lactating dairy cows in concentration and secretion rate of insulin. Biochem. J. 180:281-289.

Rosenthal, J., A. Angel, and J. Farkas. 1974. Metabolic fate of leucine: a significant sterol presursor in adipose tissue and muscle. Am. J. Physiol. 226(2):411-418.

Wijayasinghe, M. S., L. P. Milligan, and J. R. Thompson. 1983. In vitro degradation of leucine in muscle, adipose tissue, liver and kidney of fed and starved sheep. Biosci. Rep. 3:1133-1140.

Wohlt, J. E., J. H. Clark, R. G. Derrig, and C. L. Davis. 1977. Valine, leucine, and isoleucine metabolism by lactating bovine mammary tissue. J. Dairy Sci. 60:1875-1882.

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# APPENDIX A. STUDIES AND RESULTS RELATED TO SECTION I

## Effect of Intravenous Infusion of KIC in Lactating Goats

Eight lactating goats were used in a study to determine the effect of intravenous infusion of 0, 1, 3, or 6 g of sodium-KIC per day. Treatments of KIC in saline were infused into the jugular vein via indwelling catheters for 1 wk. Daily milk yield was recorded during the treatment period and the 2 previous wk. Composition of milk was measured for 2 d before, 2 d at the start, and 2 d at the end of the treatment period. Results indicated no effect of treatment (Table A1).

Table A1. Effect of intravenous infusion of KIC on milk production in goats

| | Dose | | | |
|---|---|---|---|---|
| | 0 g/d | 1 g/d | 3 g/d | 6 g/d |
| Milk yield, lb/d | | | | |
| Pretrial | 6.9 | 6.5 | 8.7 | 7.45 |
| Trial effect | -.2 | -.4 | -.8 | -.2 |
| Difference from control | | -.4 | -.6 | .0 |
| Milk fat content, % | | | | |
| Pretrial | 3.29 | 3.56 | 3.81 | 3.56 |
| Trial effect | .14 | .28 | .10 | .08 |
| Difference from control | | .14 | -.03 | -.06 |
| Milk protein content, % | | | | |
| Pretrial | 2.85 | 3.00 | 3.27 | 2.85 |
| Trial effect | 2.78 | 3.02 | 3.08 | 2.78 |
| Difference from control | | .09 | -.13 | .00 |

## Blood Response to Oral Doses of KIC Salts

Tests were conducted in goats to determine if the calcium salt of KIC, which is less water soluble, would bypass the rumen to a greater degree than the sodium salt. The results (Table A2) indicated that the calcium salt may have been less degraded in rumen.

Table A2. Blood KIC concentration (uM) response to oral administration of calcium and sodium salts of KIC in goats

| Dose (g) | Time after dose (hours) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 4 | 8 | 20 |
| Calcium-KIC | | | | | | |
| 0 | 17 | -- | ND | ND | ND | 28 |
| 20 | 17 | -- | 43 | 36 | 24 | 24 |
| 40 | 15 | -- | 58 | 48 | 27 | -- |
| Sodium-KIC | | | | | | |
| 0 | 40 | -- | 36 | -- | 40 | 26 |
| 10 | 28 | 34 | 43 | -- | 30 | 38 |
| 20 | 32 | 36 | 26 | -- | 37 | 39 |
| 30 | 39 | 72 | 92 | -- | 53 | 26 |

## Efficacy of Lipid Coating for KIC Bypass

This method is based on work by Smith and Boling (1984; see section I herein) in which plasma methionine concentration was elevated in lambs fed lipid-coated methionine (composed of 40% finely ground corn, 30% coconut oil, 20% D,L-methionine, and 10% zein). The lipid-coated KIC in the cow trial was made by mixing 2 kg sodium-KIC with 1 kg finely ground corn gluten meal and then adding 1 kg of the hot fat mix. The fat mix contained 10% paraffin, 90% hydrogenated soy oil, and .05% d-alpha-tocopherol (490 IU per kg fat). A 50% reduction in 16 h water solubility (37°) of KIC was observed when sodium-KIC was treated by this method.

Extruded pellets also were added to rumen fluid in vitro, but, because of difficulties in quantitating KIC from rumen fluid, disappearance could not be determined. Visual observations indicated that some pellets remained in the tubes after 20 h of incubation. No tests were performed using rumen bags to estimate degradability of lipid-coated KIC.

## Animals Used for Section I Studies

Table A3. Animals used in trials 1 and 2 of lactation studies

| Animal | Breed | Lactation | Days postpartum | Days pregnant | Treatment | Initial milk yield |
|---|---|---|---|---|---|---|
| **Goat trial** | | | | | | |
| 578 | Saanen | 3 | 136 | 0 | KIC | 1.88 |
| 594 | Saanen | 3 | 30 | 0 | KIC | 5.56 |
| 614 | Saanen | 2 | 137 | 0 | control | 2.65 |
| 615 | Saanen | 2 | 135 | 0 | KIC | 2.10 |
| 616 | Saanen | 2 | 137 | 0 | KIC | 4.61 |
| 628 | Saanen | 2 | 140 | 0 | KIC | 2.58 |
| 639 | Saanen | 2 | 136 | 0 | control | 3.07 |
| 660 | Saanen | 1 | 64 | 0 | control | 4.02 |
| 682 | Saanen | 1 or 2 | 140 | 0 | control | 2.54 |
| 698 | Saanen | 3 | 141 | 0 | control | 2.02 |
| Average control | | | 124 ± 15 | | | 2.9 ±.4 |
| Average KIC | | | 116 ± 21 | | | 3.3 ±.7 |
| **Cow trial** | | | | | | |
| 8121 | Holstein | 3 | 314 | 145 | control | 24.9 |
| 8316 | Holstein | 2 | 222 | 132 | control | 28.1 |
| 8488 | Holstein | 2 | 202 | 132 | KIC | 29.1 |
| 8560 | Holstein | 2 | 165 | 80 | KIC | 30.5 |
| 8659 | Holstein | 1 | 243 | 109 | KIC | 24.5 |
| 8704 | Holstein | 1 | 251 | 162 | KIC | 22.6 |
| 8720 | Holstein | 1 | 219 | 91 | control | 22.9 |
| 8752 | Holstein | 1 | 172 | 109 | control | 19.7 |
| 8209 | Brown Swiss | 3 | 215 | 135 | KIC | 15.1 |
| 8223 | Brown Swiss | 3 | 250 | 148 | control | 10.4 |
| 8645 | Brown Swiss | 1 | 211 | 139 | control | 14.0 |
| 8742 | Brown Swiss | 1 | 211 | 112 | KIC | 16.0 |
| Average control | | | 231 ± 19 | 127 ± 9 | | 20 ± 3 |
| Average KIC | | | 215 ± 13 | 122 ± 11 | | 23 ± 3 |

## Analysis of Variance for Effect of KIC on Milk Production

Table A4. Analysis of variance for the effect of KIC on milk yield

| Variable | df | SS | MS | F | Pr>F | conserv.[a] Pr>F |
|---|---|---|---|---|---|---|
| Trt[b] | 1 | 421 | | 9.4 | .018* | |
| Block | 3 | 5631 | 1877 | 41.8 | .0001 | |
| Trt*Block | (3) | (119) | | | | |
| Cow(Trt*Block) | (4) | (195) | | | | |
| Error(A)[c] | 7 | 315 | 44.9 | | | |
| Day | 13 | 96.7 | 7.44 | 3.8 | .0001 | .080 |
| Trt*Day | 13 | 33.2 | 2.55 | 1.3 | .23 | .28 |
| Day*Block | (39) | (91.7) | | | | |
| Trt*Day*Block | (39) | (64.4) | | | | |
| Day*Cow(Trt*Block) | (52) | (101.5) | | | | |
| Error(B)[d] | 130 | 257.6 | (Root MSE = 1.41) | | | |
| Total | 167 | 6754 | | | | |
| Contrasts by period | | | | | | |
| Day | | | | | | |
| Mean trt vs. pooled pre- + post-trt | 1 | 15.2 | 15.2 | 7.68 | .0064 | .020 |
| Wk 1 vs. wk 2 + 3 | 1 | 21.2 | 21.2 | 10.7 | .0014 | .0084 |
| Pre- vs. post-trt | 1 | 7.1 | 7.1 | 3.6 | .060 | .087 |
| Trt*Day | | | | | | |
| Mean trt vs. pooled pre- + post-trt | 1 | 15.6 | 15.6 | 7.88 | .006 | .019 |
| Wk 1 vs. wk 2 + 3 | 1 | .80 | .80 | .40 | .53 | .54 |
| Pre- vs. post-trt | 1 | 2.3 | 2.3 | 1.15 | .29 | .31 |

[a]Probability of a greater F calculated with conservative df for period effects. Conservative df are calculated by dividing both terms of F test by df for day; thus, for trt*day effect, Pr>F is determined by using 1 and 10 df, not 13 and 130 df.

[b]Treatment.

[c]Error for nonrepeated effects = Trt*Block + Cow(Trt*Block).

[d]Error for period effects = Day*Block + Trt*Day*Block + Day*Cow(Trt*Block).

Table A6. Analysis of variance for the effect of KIC on milk fat content

| Variable | df | SS | MS | F | conserv.[a] Pr>F | Pr>F |
|---|---|---|---|---|---|---|
| Trt[b] | 1 | 2.55 | 2.55 | 1.4 | .28 | |
| Block | 3 | 9.17 | 3.06 | 1.7 | .25 | |
| Trt*Block | (3) | (3.93) | | | | |
| Cow(Trt*Block) | (4) | (8.59) | | | | |
| Error(A)[c] | 7 | 12.52 | 1.79 | | | |
| Day | 13 | 1.58 | .122 | 3.33 | .0002 | .098 |
| Trt*Day | 13 | .852 | .0655 | 1.79 | .051 | .21 |
| Day*Block | (39) | (2.09) | | | | |
| Trt*Day*Block | (39) | (1.56) | | | | |
| Day*Cow(Trt*Block) | (52) | (1.10) | | | | |
| Error(B)[d] | 130 | 4.75 | .0365 | (Root MSE = .191) | | |
| Total | 167 | 31.42 | | | | |
| Contrasts by period | | | | | | |
| Day | | | | | | |
| Mean trt vs. pooled pre- + post-trt | 1 | .047 | .047 | 1.30 | .26 | .28 |
| Wk 1 vs. wk 2 + 3 | 1 | .001 | .001 | .03 | .86 | .87 |
| Pre- vs. post-trt | 1 | .707 | .707 | 19.4 | .0001 | .002 |
| Trt*Day | | | | | | |
| Mean trt vs. pooled pre- + post-trt | 1 | .321 | .321 | 8.79 | .0036 | .014 |
| Wk 1 vs. wk 2 + 3 | 1 | .133 | .133 | 3.65 | .058 | .085 |
| Pre- vs. post-trt | 1 | .000 | .000 | .00 | .97 | .98 |

[a]Probability of a greater F calculated with conservative df for period effects. Conservative df are calculated by dividing both terms of F test by df for day; thus, for trt*day effect, Pr>F is determined by using 1 and 10 df, not 13 and 130 df.

[b]Treatment.

[c]Error for nonrepeated effects = Trt*Block + Cow(Trt*Block).

[d]Error for period effects = Day*Block + Trt*Day*Block + Day*Cow(Trt*Block).

Table A7. Analysis of variance for the effect of KIC on 4% fat-corrected milk yield

| Variable | df | SS | MS | F | Pr>F | conserv.[a] Pr>F |
|---|---|---|---|---|---|---|
| Trt[b] | 1 | 634 | 634 | 12.1 | .010 | |
| Block | 3 | 4825 | 1608 | 30.6 | .0003 | |
| Trt*Block | (3) | (93) | | | | |
| Cow(Trt*Block) | (4) | (275) | | | | |
| Error(A)[c] | 7 | 368 | 52.6 | | | |
| Day | 13 | 85.1 | 6.55 | 3.68 | .0001 | .084 |
| Trt*Day | 13 | 50.8 | 3.90 | 2.20 | .013 | .17 |
| Day*Block | (39) | (83.5) | | | | |
| Trt*Day*Block | (39) | (57.4) | | | | |
| Day*Cow(Trt*Block) | (52) | (90.1) | | | | |
| Error(B)[d] | 130 | 231.0 | 1.78 | (Root MSE = 1.33) | | |
| Total | 167 | 6194 | | | | |
| Contrasts by period | | | | | | |
| Day | | | | | | |
| Mean trt vs. pooled pre- + post-trt | 1 | 22.7 | 22.7 | 12.8 | .0005 | .0050 |
| Wk 1 vs. wk 2 + 3 | 1 | 25.9 | 25.9 | 14.6 | .0002 | .0034 |
| Pre- vs. post-trt | 1 | .03 | .03 | .01 | .90 | .92 |
| Trt*Day | | | | | | |
| Mean trt vs. pooled pre- + post-trt | 1 | 31.1 | 31.1 | 17.5 | .0001 | .0019 |
| Wk 1 vs. wk 2 + 3 | 1 | 4.7 | 4.7 | 2.6 | .107 | .14 |
| Pre- vs. post-trt | 1 | .8 | .8 | .46 | .50 | .51 |

[a]Probability of a greater F calculated with conservative df for period effects. Conservative df are calculated by dividing both terms of F test by df for day; thus, for trt*day effect, Pr>F is determined by using 1 and 10 df, not 13 and 130 df.

[b]Treatment.

[c]Error for non-repeated effects = Trt*Block + Cow(Trt*Block).

[d]Error for period effects = Day*Block + Trt*Day*Block + Day*Cow(Trt*Block).

## Effect of KIC on Milk Fat Yield of Individual Cows

Table A8. Effect of KIC on milk fat yield of individual cows

| Cow | Treatment | Block | Average Pretrial | Average Trial | Change | Wk -1 | Wk 1 | Change |
|---|---|---|---|---|---|---|---|---|
| 8316 | Control | H2[a] | 1234 | 1173 | -61 | 1225 | 1266 | 41 |
| 8121 | Control | H2 | 844 | 902 | 58 | 877 | 927 | 50 |
| 8752 | Control | H1[b] | 760 | 762 | 2 | 696 | 745 | 49 |
| 8720 | Control | H1 | 756 | 800 | 44 | 756 | 828 | 72 |
| 8223 | Control | B2[c] | 418 | 344 | -74 | 368 | 313 | -55 |
| 8645 | Control | B1[d] | 619 | 663 | 44 | 638 | 661 | 23 |
| Average Control | | | 772 | 774 | 2 | 760 | 790 | 30 |
| 8448 | KIC | H2 | 1145 | 1238 | 93 | 1153 | 1357 | 204 |
| 8560 | KIC | H2 | 1079 | 1162 | 83 | 1058 | 1218 | 160 |
| 8659 | KIC | H1 | 975 | 1069 | 94 | 978 | 1163 | 185 |
| 8794 | KIC | H1 | 910 | 975 | 65 | 876 | 970 | 94 |
| 8209 | KIC | B2 | 642 | 700 | 58 | 642 | 751 | 109 |
| 8742 | KIC | B1 | 739 | 833 | 94 | 753 | 855 | 102 |
| Average KIC | | | 915 | 996 | 81 | 910 | 1052 | 142 |

[a]Multiparous Holstein.

[b]Primiparous Holstein.

[c]Multiparous Brown Swiss.

[d]Primiparous Brown Swiss.

Effect of KIC on Nitrogen Balance of Lactating Cows

Foley catheters were inserted into the urinary bladder of cows in trial 2 at the end of the pretreatment period and at the end of the treatment period. Urine was collected into plastic oil collectors containing 30 mls sulfuric acid and emptied twice per d. Total urine weight and feces weight were recorded for 48 h, and representative samples of each were taken for later analysis of nitrogen (N) content. Results were analyzed statistically for treatment differences by analysis of covariance using the pretreatment measurement as the covariate (Table A8). No significant differences were observed, although there were slight trends for decreased N output into urine and increased N output into milk.

Table A8. Covariate-adjusted N balance measurements of cows fed control and KIC-supplemented diets

| N component[a] | Control | KIC | Pr>F[b] |
|---|---|---|---|
| | ------ g/d ------ | | |
| Feed N intake | 551 | 544 | .6 |
| Urine N output | 198 | 184 | .4 |
| Feces N output | 179 | 176 | .7 |
| Milk N output | 126 | 132 | .3 |
| Body N retention[c] | 46 | 55 | .6 |

[a]Feed N intake and milk N output calculated from feed intake, milk yield, and milk protein content during the final weeks of pretreatment and treatment periods.

[b]Probability of a greater F for effect of KIC by using analysis of covariance.

[c]Body N retention calculated as
[feed N - (urine N + feces N + milk N).

## Pooled Statistics for Goat and Cow Trials

When both the goat and cow studies were combined for statistical analysis by blocking for species, the pooled percentage increases in milk fat content (6.0%), milk fat yield (9.6%), and 4% fat-corrected milk (7.3%) were significant, $P<.011$, $P<.014$, and $P<.036$, respectively. Pooling the data may be unjustified, however, because the underlying assumption of equal variance may not be true with different species. Whether or not the pooling is justified, the response in the two species was very similar. This similar response in both trials indicates that KIC does have the potential to alter milk production.

## Health Records of Cows Used in Trial 1

Four cows developed clinical signs of kidney infection within one year of the present study; time of infection was likely during the nitrogen balance determinations (11/14/84 to 11/16/84 and 12/4/84 to 12/7/84). Foley urinary catheters were sterilized with ethylene oxide and inserted by the following procedure. 1. The pubic area of each cow was washed with green soap and water, then with zepharin solution. 2. Hands and catheter were lubricated with sterile K-Y jelly. 3. Catheter was inserted without touching tip to external pubic area, and its balloon was filled sterile saline. 4. Catheter was connected to drainage tubing, taped to base of tail, and covered with Furacin cream. No bacteriocide was flushed into the urinary bladder. The pubic area of each cow was washed twice daily with green soap and water and treated with Furacin cream. Catheters were disconnected for milking.

Table A9. Arrangement of cows in stalls during trial

| Stall from north door | West side | | East side | |
|---|---|---|---|---|
| | Cow | Treatment | Cow | Treatment |
| 1 | 8209[a,b] | KIC | 8316[c] | control |
| 2 | 8223 | control | 8121[b,c,d] | control |
| 3 | 8645 | control | 8704[b,d] | KIC |
| 4 | 8742 | KIC | 8659[b,c,e] | KIC |
| 5 | 8488[c] | KIC | 8752 | control |
| 6 | 8560 | KIC | 8720 | control |

[a]Excessive urine output and water consumption (almost double) during second N balance determination.

[b]Developed pyelonephritis after trial period.

[c]Feces very loose during the second N balance determination.

[d]Urinary catheter replaced at least once.

[e]Excessive straining against urinary catheter.

Table A10. Health records of individual cows

| Cow | Due date | Following lactation: Calving date | Days | Milk yield | Health records: date | comments |
|---|---|---|---|---|---|---|
| CONTROL COWS | | | | | | |
| 8121 | 3/31/85 | 3/30/85 | 120 | 10060 | 4/25/85 | bloody urine |
| | | | | | 7/6/85 | diagnosis: kidney infection |
| | | | | | 7/27/85 | sold: low production |
| 8223 | 4/8/85 | 4/4/85 | 309 | 12240 | 3/19/86 | sold: research purposes |
| 8316 | 4/13/85 | 4/12/85 | no record | | 4/12/85 | sold: pendulous udder |
| 8645 | 4/17/85 | 4/14/85 | 308 | 12090 | 10/25/85 | mastitis |
| | | | | | 12/7/85 | sold: low production |
| 8720 | 5/24/85 | 5/26/85 | 298 | 19790 | 9/1/87 | still in herd |
| 8752 | 5/6/85 | 5/10/85 | 293 | 16750 | 1/26/87 | sold: low production |
| COWS FED KIC | | | | | | |
| 8209 | 4/21/85 | 1/3/85 | no record | | 12/15/84 | not eating, taken off trial |
| | | | | | 12/18/84 | diagnosis: kidney infection |
| | | | | | 1/3/85 | aborted |
| | | | | | 2/7/85 | euthanized and necropsied: chronic pyelonephritis |
| 8488 | 4/13/85 | 4/18/85 | 423 | 27570 | 4/20/85 | mastitis |
| | | | | | 9/9/85 | mastitis |
| | | | | | 6/15/55 | sold: reproductive problems |
| 8560 | 6/4/85 | 6/2/85 | 305 | 18910 | 9/1/87 | still in herd |
| 8659 | 5/6/85 | 3/21/85 | no record | | 2/5/85 | not eating |
| | | | | | 2/7/85 | diagnosis: kidney infection |
| | | | | | 2/8/85 | quit milking |
| | | | | | 3/21/85 | aborted |
| | | | | | 4/10/85 | sold as nurse cow |
| 8704 | 3/14/85 | 3/15/85 | 22 | 970 | 4/3/85 | low production, not eating |
| | | | | | 4/4/85 | bloody urine |
| | | | | | 4/5/85 | diagnosis: kidney infection |
| | | | | | 4/6/85 | euthanized and necropsied: pyelonephitis |
| | | | | | daughter | (#9301) is in 1st lactation |
| 8742 | 5/14/85 | 5/11/85 | 331 | 11320 | 9/1/87 | still in herd |

# APPENDIX B. ZEIN-COATING AS A BYPASS METHOD

## Introduction

Several methods have been studied and proposed to protect compounds from ruminal degradation. Among these are: 1) lipid encapsulation (Smith and Boling, 1984); 2) coating with blood (Mir et al., 1984); 3) coating with fish hydrolysate (Mir et al., 1984); and 4) encapsulation with a styrene and 2-methyl-5-pyridine copolymer (Dannelly and Ardell, 1980a, b; Papas et al., 1984). None of these methods meet all of the criteria of Table B1. Therefore, a method using the corn protein zein was developed to protect compounds from ruminal degradation. Zein is highly resistant to microbial degradation and is insoluble in aqueous solutions. Zein dissolves in 80-90% alcohol and shrinks to form a resilient polymer coat when dried. It is a "natural compound" and has previously been used in the pharmaceutical industry to make time-release tablets (Klippel, 1971; Westall and Pimbley, 1972).

## Methods

### Production of zein-protected compounds

The method of production of zein-coated pellets has been described in section II of this document and will be discussed only briefly. Zein-coated pellets of KIC, methionine, and limestone were made by mixing zein, 88% ethanol, limestone, bentonite, and either Ca-$(KIC)_2$, D,L-methionine, or more limestone (Table B2). After extrusion into liquid nitrogen for freeze-fracturing, pellets were slowly dried on nontacky vinyl tarps spread over a concrete floor. After drying, 1 kg pellets

were placed on a mechanical shaker and sprayed with 100 mls 20% zein in 88% ethanol while shaking with air blowing over them.

Table B1.Desired properties for a laboratory-scale method of protecting compounds from ruminal degradation

| |
|---|
| Properties of pellet |
| 1. Size = ~2-3 $mm^3$ for optimal passage time through rumen |
| 2. Density = 1.1-1.2 g/ml for optimal passage time through rumen[a] |
| 3. Resistant to microbial degradation |
| 4. Digestible in lower gastrointestianl tract |
| 5. Stable matrix at room temperature |
| 6. Inert so that protected compound is not chemically altered |
| 7. Physically stable during feed mixing and mastication |
| 8. Acceptable composition to use in feeding animals for human-consumption |
| Properties of method |
| 1. Low capital inputs |
| 2. Readily available ingredients |
| 3. Consistent results easily verified by rumen bag technique |

[a]desBordes and Welch, 1984.

Table B2. Pellet ingredients for efficacy studies of zein-protection

| Ingredients | Pellet type | | |
|---|---|---|---|
| | KIC | Methionine | Control |
| Zein, g | 450 | 450 | 450 |
| 88% Ethanol, g | 315 | 315 | 315 |
| Limestone, g | 100 | 160 | 450 |
| Na-bentonite, g | 50 | 50 | 100 |
| Ca-$(KIC)_2$, g | 400 | | |
| D,L-Methionine | | 340 | |

Determination of bypass value

For determination of resistance to microbial degradation, the rumen bag procedure was employed. Pellets (1.5 g) were placed into 8 x 10 cm

dacron bags, sealed, and weighed. Bags were anchored and placed in the rumen of a fistulated steer (fed an alfalfa hay diet) for varying lengths of time in triplicate. Upon removal, bags were thoroughly rinsed with cold tap water until rinses were visibly clean and then dried at 60° for 24 h. Percentage of KIC or methionine remaining in bags was determined. Pellet bypass value was determined by the cumulative degradation equation (Orskov, 1982, p. 55):

$$P = a + [bc / (c + k)]$$

where P = fraction degraded (bypass fraction = 1 - P)
a = rapidly soluble fraction
b = fraction subject to degradation
c = constant rate for disapperance of b
k = fractional outflow rate (inverse of turnover time)

and a, b, and c are determined from the degradation equation:

$$p = a + b(1 - e^{-ct})$$

where p is the amount of compound lost at time t in an in situ rumen bag study.

To evaluate the digestibility of zein-coated pellets, adult rats were fed diets containing almost 50% KIC pellets. Additionally, feces of lambs and cattle fed KIC and methionine pellets were visually inspected for undigested pellets and analyzed for traces of KIC.

Methods for analysis of KIC and methionine

Dry pellets or feces were weighed and ground, and 100 mg was dissolved in 2 ml 88% ethanol in a 50 ml culture tube. The solution was brought up to 25 ml with 1 N HCl (which caused the zein to come out of solution), vortexed well, heated to 40° for 20 min, and further diluted to 50 ml. For KIC pellets, a 500 ul aliquot was added to a 50 ml culture

tube with 200 nmol norleucine as an internal standard, extracted into methylene chloride, back-extracted into 500 ul .1 M phosphate (pH 7), and analyzed for KIC concentration by reverse-phase HPLC. For methionine pellets, the procedure was identical except that L-leucine was used as the internal standard and amino acids were converted to their respective ketoacids before the extraction (Nissen et al., 1982). Briefly, the initial aliquot was poured through a cation-exchange column, and amino acids were eluted with 25% $NH_4OH$ into a 20 ml vial, dried under $N_2$ gas, and incubated with an L-amino acid oxidase and catalase solution under $O_2$ gas. After acidification, ketoacids were separated as above.

## Results and Discussion

Results are shown in Table B3. Based on the assumption that protected compounds eventually would be completely degraded in rumen bags, estimates of the rapidly soluble fraction (a), the fraction subject to degradation (b), and the constant rate of disappearance of b (c) were 15%, 85%, and 2.9% for KIC pellets and 2%, 98%, and 3.8% for methionine pellets. If turnover time in the rumen is assumed to be 15 h, then fractional outflow rate (k) would be 6.7% and fraction of protected compound bypassing the rumen would be 59% for KIC pellets and 63% for methionine pellets. For turnover times ranging from 12 to 24 h, values for fraction of methionine bypassed range from about 50-70%. Results of the digestibility study with rats and observations from studies feeding protected KIC and methionine to sheep and cattle indicate that zein-protected compounds are completely digestible.

In summary, zein encapsulation seems to meet all of the criteria for

Table B3. Ruminal degradation of protected compounds

| Pellet type | Time in rumen h | Pellet loss % | Compound loss % |
|---|---|---|---|
| KIC (study 1, average pellet = 1.8 x 8 mm) | | | |
| | 7 | 15 | 40 |
| | 12 | 18 | 53 |
| | 24 | 23 | 69 |
| KIC (study 2, average pellet = 2.1 x 8 mm) | | | |
| | 0 | 7 | 9 |
| | 6 | 15 | 22 |
| | 24 | 23 | 55 |
| Limestone (average pellet = 2.1 x 8 mm) | | | |
| | 12 | 1 | |
| Methionine (average pellet = 2.1 x 8 mm, double-coated) | | | |
| | 0 | 0 | 0 |
| | 2 | 4 | 13 |
| | 6 | 8 | 18 |
| | 12 | 13 | 45 |
| | 24 | 20 | 58 |

a laboratory-scale method of protecting compounds from ruminal degradation. Pellet density was ~1.1 g/ml and size was ~2 x 8 mm. Both of these characteristics can easily be altered to maximize outflow rate for species and diet differences. Compounds were protected from ruminal degradation so that ~40% remained in rumen bags after 24 h; if pellet turnover time in the rumen is 15 h, then 60% of the compound will bypass the rumen. Zein pellets seemed to be completely digested.

In conclusion, zein-encapsulation is a relatively simple, laboratory-scale method for protecting compounds from ruminal degradation and may be useful for determining amino acid requirements and specific nutrient effects in ruminants.

## References cited

Dannelly, C. C., and R. E. Ardell. 1980a. Rumen-stable pellets. U.S. Patent 4,196,187.

Dannelly, C. C., and R. E. Ardell. 1980b. Rumen-stable pellets. U.S. Patent 4,181,710.

desBordes, C. K., and J. G. Welch. 1984. Influence of specific gravity on rumination and passage of indigestible particles. J. Anim. Sci. 59:470.

Klippel, K. R. 1971. Sustained release pharmaceutical compositions. U.S. Patent 3,558,768.

Mir, Z., G. K. MacLeod, J. G. Buchanan-Smith, D. G. Grieve, and W. L. Grovum. 1984. Effect of feeding soybean meal protected with sodium hydroxide, fresh blood, or fish hydrolysate to growing calves and lactating dairy cows. Can. J. Anim. Sci. 64:845.

Nissen, S. L., C. Van Huysen, and M. W. Haymond. 1982. Measurement of branched chain amino acids and branched chain alpha-ketoacids in plasma by high-performance liquid chromatography. J. Chromatogr. 232:170.

Orskov, E. R. 1982. Protein nutrition in ruminants. Academic Press, New York.

Papas, A. M., C. J. Sniffen, and T. V. Muscato. 1984. Effectiveness of rumen-protected methionine for delivering methionine postruminally in dairy cows. J. Dairy Sci. 67:545-552.

Smith, S. I., and J. A. Boling. 1984. Lipid coating as a mode of protecting free methionine from ruminal degradation. J. Anim. Sci. 58:187.

Westall, E. B., and K. C. Pimbley. 1972. Color concentrated base dispersion used in tablet film coating. U.S. Patent 3,802,896.