This is to certify that the

dissertation entitled

EFFECTS OF ISOACIDS ON NUTRIENT-ENDOCRINE INTERACTIONS IN LACTATING HOLSTEIN CATTLE

presented by

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has been accepted towards fulfillment
of the requirements for

Ph.D degree in Animal Science

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Date October 28, 1988

EFFECT OF ISOACIDS ON NUTRIENT-ENDOCRINE INTERACTIONS IN LACTATING HOLSTEIN CATTLE

by

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A THESIS

Submitted to Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Animal Science

1988

ABSTRACT

EFFECT OF ISOACIDS ON NUTRIENT-ENDOCRINE INTERACTIONS IN LACTATING HOLSTEIN CATTLE

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The purpose of this work was to determine if the production response in lactating cows fed isoacids (a blend of isobutyric acid, isovaleric acid, 2-methylbutyric acid and valeric acid) involves endocrine and metabolic interactions. A dose-titration study was conducted to obtain an effective dosage. Forty eight cows were distributed into five groups receiving isoacids over a complete lactation. The diets did not contain urea. The dosages were 0.0, 0.4, 0.8, 1.2, and 1.6 % of the concentrate dry matter. The results showed that the highest dosage increased milk yield by 3.0 kg ($p < .1$) during the first third of lactation, and by 2.29 kg ($p < .1$) over a complete lactation. Body weight loss during the first third of lactation was enhanced by isoacids ($p < .05$).

A second trial was conducted to determine the endocrine and metabolic milieu in cows fed isoacids. Eight multiparous cows were distributed into a control and a isoacid group. The results show that feeding isoacids at 1.6% of the concentrate dry matter increased milk yield ($p.14$) by 2.95 kg/d. Feed efficiency was improved ($p<.05$). Plasma levels of growth hormone, insulin and cortisol were

not affected. Isoacids increased plasma urea nitrogen ($p<.05$) in the post milking period. Daily mean plasma glucose was decreased ($p<.05$) by isoacids. Ruminal concentrations of total volatile fatty acids (VFA), acetate, isovalerate and the acetate:propionate ratio were increased in treated animals. In a third experiment, supplementation of 40 g/d of either isovalerate, 2-methylbutyrate or phenylacetate did not affect milk yield, feed efficiency, ruminal VFA or plasma concentrations of growth hormone, glucose and urea nitrogen. However, plasma insulin levels were reduced ($p<.05$) in all treatments.

The higher acetate levels found in the second trial represent an energy substrate that does not trigger an insulin response. This non-insulin stimulating energy is especially well suited for the physiology of the lactating cow.

ACKNOWLEDGEMENTS

I would like to express my appreciation and gratitude to the members of my commitee during my Ph D. program.

To my academic advisor, Dr. Robert Cook, for his guidance, support and advice throughout the years of my Ph D program.

To Dr. Werner G. Bergen for his continuing interest in my academic development through two graduate programs.

To Dr. Jack L. Kostyo for his friendship, support and guidance. His interest in developing my research abilities is deeply appreciated.

To Dr. Melvin T. Yokoyama for his objectivity and sound counsel throughout my graduate studies.

Additionally, deep appreciation is also expressed to Dr. Maynard Hogberg for his interest and support during my Ph D program.

Last but not least, I would like to thank my fellow students and the personnel of the dairy barn. Their friendship, help and support made this work possible.

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INTRODUCTION

The basic research of the late 1940's and early 1950's in the fields of metabolism and nutrient requirements of the anaerobic rumen bacteria led to the application of the new knowledge in productive situations with farm animals. The information gained from these early trials, in turn generated the interest that resulted in the development of products that optimize or modify the fermentative processes of tbe ruminal ecosystem. This has been the case of products like the antibiotics monensin and lasalocid, and growth factors like isoacids.

In the case of Isoacids, the ruminal effect is not the suppression of microbial populations, but the stimulation of cellulolytic bacteria. The first in vitro experiments were carried out to elucidate the role of branched-chain volatile fatty acids and valeric acid as growth factors for cellulolytic rumen bacteria. Their first application to field stuations was aimed at the stimulation of microbial growth and improvement of the digestion of high-fiber feedstuffs. The ensuing interest led to their inclusion in other types of diets that, as in the case of rations for high-producing cows at the beginning of lactation, contain moderate amounts of fiber and high levels of protein. The fact that in these conditions, isoacids have been shown to increase milk production with little or no increases in feed intake, has

led to the search for their mechanism of action in post-absorptive effects that can repartition nutrients towards milk synthesis.

The development of the commercial product containing isoacids (Eastman. IsoPlus, Nutritional Supplement), a mixture of isovaleric, 2-methyl butyric, isobutyric and valeric acids, has occurred at a time when knowledge in the areas of endocrinology and metabolism has grown exponentially. A number of studies on possible endocrine and metabolic effects of Isoacids have been carried out in lactating and non-lactating ruminants. The results, although far from conclusive, stress the need to integrate digestive and metabolic events, especially in the areas of nutrient-endocrine interactions in lactating ruminants.

The aim of the present work is to investigate some of these interactions in the lactating cow. The studies will cover productive responses in milk yield with different dosages and at different stages of lactation. The digestive, endocrine end metabolic effects of isoacids fed as mixture and as individual acids will also be reported.

CHAPTER ONE

LITERATURE REVIEW

Early Studies.-

The uniqueness of the ruminal ecosystem to synthesize protein from non protein compounds and to produce volatile fatty acids (VFA) through the fermentation of protein and carbohydrates has long been recognized. Towards the end of the last century, Zuntz (1891) postulated that ruminal bacteria could synthesize protein from non-protein nitrogenous compounds. Decades later, Loosli et al (1949), investigated the ability of ruminants to synthesize amino acids. In their work, they measured the amino acid content and composition of the protein synthesized in the rumen of sheep and goats fed protein-free diets in which urea was the only source of nitrogen. Their results showed that all essential amino acids could be synthesized in the rumen, from non-protein compounds. The metabolism of protein in the rumen was further studied by El-Shazly (1952). His work showed that microorganisms present in rumen fluid from sheep fed a casein-concentrate-hay diet posessed the ability to produce volatile fatty acids (VFA) of branched and straight chain during the degradation of protein. His results also suggested that the synthesis of these VFA occurred through the fermentation of the branched chain amino acids leucine,

isoleucine and valine into the respective branched chain isovaleric, 2-methylbutyric and isobutyric acids (Isoacids). Additionally, he postulated that the isoacids were produced in coupled Stickland-type reactions in which an easily reduced amino acid, such as proline, was reduced to aminovaleric acid. El-Shazly (1952) also suggested that the isoacids could be subsequently utilized in the synthesis of branched-chain higher fatty acids of 15 and 17 carbons found in ruminant fat.

Eldsen and Lewis (1953) later confirmed in vitro several of the previous results. They found that Gram negative cocci, isolated from the rumen of sheep produced isoacids and valeric acid. Other research (Annison, 1954) showed that the results of El-Shazly (1952) could be essentially reproduced in sheep fed a variety of diets. This established that the ruminal production of isoacids from amino acids was a constant phenomenon of the digestive physiology of ruminants.

In vitro studies.-

Biosynthesis of Isoacids and Valeric Acid.-

Research on the properties of compounds present in rumen fluid indicated that the isoacids and valeric acid enhanced cellulose digestion. Bentley et al (1954) showed that the cellulolytic activity of rumen microbes in a defined, protein-free medium was augmented two fold by isobutyric and isovaleric acids, and nearly three fold by n-valeric acid. The amino acid precursors also stimulated

cellulolytic activity. The addition of valine increased cellulose digestion two fold and the combined addition of valine and proline enhanced cellulolysis by nearly three fold. This supports the theory of El-Shazly (1952) that isoacids arise from the degradation of branched-chain amino acids and easily reduced amino acids such as proline in coupled Stickland-type reactions. Bentley and coworkers (1955) also found that the addition of valeric acids and isoacids to a purified, protein free-medium enhanced the growth of rumen microbes.

The ruminal production of isoacids and valeric acid from protein largely depends on proteolytic activity, although it should be noted that valeric acid can also be synthesized from carbohydrates (Bryant, 1973). As described before, the resulting amino acids can enhance the growth and activity of cellulolytic bacteria. This process illustrates the interdependence between cellulolytic and non-cellulolytic species in the rumen ecosystem. These bacterial interactions have been reviewed by Allison (1969), Bryant (1973) and Hoover (1986). Isoacids are largely produced by non-cellulolytic species. The isoacids in turn stimulate the growth of and activity of cellulolytic species that degrade cellulose to soluble sugars that can then be used by sugar-utilizing, non cellulolytic species. Moreover, the lysis of bacteria in the rumen can provide an additional source of the branched-chain amino acid precursors of the isoacids (Bryant, 1973; Miura et al, 1980).

As described earlier, El-Shazly (1952) postulated that the production of isoacids in the rumen occurs through Stickland-type reactions in which some compounds are oxidized and others are reduced. Additional evidence appeared in the research of Dehority et al (1957), who showed that the production of isoacids from aminoacids by rumen microbes has a branched-chain component and a straight-chain component. Their work measured the effect of branched-chain amino acids and proline on cellulose degradation. Their results showed that the combination of proline and a branched chain amino acid had an additive effect. It also was shown that the different branched-chain amino acids could substitute for each other but not for proline. These results were consistent with a system in which the enhancement of cellulolytic activity is due to the VFA's produced from their precursor amino acids in a coupled, Stickland-type reaction. In this case, one of the branched-chain amino acids valine, leucine or isoleucine would provide the oxidized component and proline, which is easily reduced, would provide the second, necessary component of a Stickland-type reaction. Further research by Dehority and coworkers (1958) showed that this was the case. Their work on the metabolism of C-14 labelled valine and proline by rumen microorganisms established that the conversion of branched-chain amino acid to isoacid occurs in two steps. First, an oxidative deamination yields an intermediate alpha-ketoacid and then a decarboxylation that produces the corresponding isoacid. In the case of valine, the amino acid is first deaminated to alpha-

ketoisovaleric acid and then decarboxylated to isobutyrate. In the case of leucine, the steps are the deamination to alpha-ketoisocaproate and the decarboxylation to isoleucine. With isoleucine, the deamination results in alpha-keto, beta-methyl-n-valeric acid, and the final decarboxylation produces 2-methylbutyrate.

With respect to C-14 proline and valeric acid, the results showed that the process is different but, as proline can provide the second, reduced component for a Stickland reaction, it is closely related to the oxidative deamination and decarboxylation of the branched-chain amino acids described above. The results indicated that C-14 proline first undergoes the reductive cleavage of its carbon ring to form alpha-aminovaleric acid and then the deamination that yields valerate.

Specific requirements for isoacids.-

In regard to the specific requirements of the major types of cellulolytic bacteria for isoacids and valeric acid, Bryant and Doetsch (1955) investigated the growth requirements of Bacteroides succinogenes in a purified, protein-free medium, and showed that it requires a straight-chain acid such as valerate and a branched-chain acid, such as isobutyrate or isovalerate. In a later study, Allison and coworkers (1958) studied the effect of isoacids and short, straight-chain VFA's on the growth of cellulolytic bacteria

in a purified, protein-free medium. In their experiment, isobutyrate, 2-methylbutyrate and isovalerate were added at 0.0128 millimoles/acid/100 ml. Their experiments included three strains of Ruminococcus flavefaciens and two strains of Ruminococcus albus isolated from bovine rumen fluid from three different animals fed four different rations in four locations, in order to insure a representative sample of rumen microbial populations. Their data showed that all the strains tested were stimulated by the VFA's. The authors indicated that the stimulation of growth of B. succinogenes (Bryant and Doetsch, 1955) and the differen strains of R. albus and R. flavefaciens (Allison and collaborators, 1958) had major physiological significance since those species are among the most numerous cellulolytic microorganisms in the rumen.

The body of research on the effects of isoacids and valeric acid on the stimulation of growth and activity of rumen cellulolytic bacteria has been reviewed extensively (Dehority et al, 1967; Allison, 1969; Bryant, 1973; Hoover, 1987). As research expanded, experiments including non-cellulolytic species have demonstrated that the growth of some ruminal non-cellulolytic microbes is also stimulated by isoacids. An early report of Wegner (1962) indicated that a strain of Borrelia and a gram-positive coccus required straight and branched-chain VFA's. Bryant and Robinson (1961) found that a non-cellulolytic strain of Ruminococcus albus also required isoacids. Allison et al

(1961) quoted unpublished observations that indicate that several strains of Eubacterium ruminantium also require VFA for as growth factors, and more recently, Stanton and Canale-Panola (1980) reported that Treponema bryantii, a sugar-utilizing rumen spirochete that grows in coculture with Bacteroides succinogenes requires isobutyrate for optimal growth. This latter microorganism is the same as the Borrellia first reported in the research of Wegner (1962).

Amino acid synthesis from isoacids.-

Ammonia can be used for protein synthesis by over 80% of rumen bacteria. It is also an obligatory requirement for over 20% of rumen bacteria (Allison, 1969). This emphasizes the importance of amino acid synthesis from non protein nitrogen sources and diverse carbon chains in the rumen ecosystem. In the case of branched-chain amino acids, cellulolytic bacteria require branched chain volatile fatty acids for synthesis of the corresponding amino acids (Allison, 1969; Bryant, 1973; Cook, 1985). In 1962, Allison and collaborators showed that C-14 labelled isovalerate was incorporated into the protein and lipid fractions of the cellulolytic microorganism Bacteroides succinogenes. Analysis of the microbial protein showed that all the labelled isovalerate in the protein was in the form of leucine. These findings were similar to those of Wegner (1962) which showed that R. flavefaciens converted isobutyrate into bacterial

lipids and valine. The results of Allison and coworkers (1962) also indicated that it was the intact isovalerate molecule that was incorporated as leucine. This suggested that the synthesis occurred through the direct carboxylation of the isoacid into the corresponding alpha-ketoacid and subsequent amination into the corresponding branched-chain aminoacid. This was shown to be the case by Allison and Bryant (1963), who cultured Ruminococcus flavefaciens in the presence of labelled CO_2 and isovalerate, 2-methylbutyrate and isobutyrate. Their results showed that the labelled carbon dioxide was used for the carboxylation step in the respective synthesis of isoleucine, leucine and valine. During the synthesis of amino acids, rumen bacteria are unique in that they form the alpha ketoacid precursor by carboxylating the carboxyl group of the respective volatile fatty acid. In the case of the straight-chain valeric acid, it is mostly utilized for the synthesis of higher fatty acids and aldehydes by the cellulolytic bacteria in the rumen (Bryant, 1973).

Effects on in vitro digestibility of feeds.-

As described earlier, most of the pioneering in vitro work on the cellulolytic activity of isoacids was conducted in systems with defined, purified media with either only non protein nitrogen or defined amounts of casein hydrolyzate. As the interest in the practical application of those early findings increased, it became necessary to investigate the

effects of isoacids on the degradation of the fibrous components of diverse feedstuffs in systems that included diverse types of protein. These systems would not only resemble more closely the actual biochemical environment of the rumen but would also help determine if the addition of isoacids would stimulate cellulolytic activity even though feed proteins present could provide isoacids. The earlier in vitro studies of Bryant and Robinson (1961) and Dehority and coworkers (1967) included casein hydrolyzate in the growth media. Their results indicated that isoacids stimulated growth and activity of major cellulolytic bacteria even when there was protein present. The protein utilized in the media was casein hydrolyzate, which is a high quality protein, and therefore high in essential amino acids such as the branched-chain amino acids. These data supported the belief that isoacids could enhance microbial growth and cellulolytic activity in the presence of protein. However, it should be noted that in many experiments the largest responses in cellulolytic activity do occur when protein levels are lower and non-protein nitrogen is included (Quispe, (1982); Brondani, (1985); Kone and coworkers (1986).

Russell and Sniffen (1984) studied the effect of the addition of isoacids and valeric acid to mixed rumen bacteria in terms of microbial protein synthesis. In their study, they utilized inocula from dairy cattle fed either timothy hay or a 60% concentrate, 40% mixed grass hay diet. With the inoculum from the cow fed timothy hay, microbial protein

synthesis was increased 11% by isovalerate, 16% by isobutyrate and 18% by the combined addition of all four isoacids and valeric acid. But with the inoculum from the cow fed concentrate-hay, there were no differences between control and isoacid treated incubations. It must be noted however that a maximal single isoacid level of 2mM was utilized, and subsequent research has shown that more useful data can be obtained with a wider range of concentrations. Gorosito et al (1985)measured the in vitro effect of isoacid and valeric acid supplementation on the digestion of plant cell wall components of different feedstuffs and filter paper, used as a cellulose control. In their study, they included a range of concentrations fom .15 to 5mM. Their data shows that isoacids added at 1.76 mM improved the digestion of filter paper, and cell walls of alfalfa hay, orchard grass and corn silage, but not of timothy hay or cannary grass. When the dose-response to isoacids was measured in incubations with wheat straw in which isoacids were included at up to 5 mM, the responses in digestion were dose-related in all cell wall components. Their results, together with those of Russell and Sniffen (1984) emphasize the importance of both dosage and type of feed in properly determining digestive responses to isoacid addition. Additional data has confirmed the previous observations. In the study of Cummings and Papas (1985), a mixture of 22.8% each, of isobutyrate, isovalerate and 2-methylbutyrate, and 31.5% valerate was added to incubations of mixed rumen

bacteria with diets of diverse composition. The isoacids were added at proportions ranging from .5 to 1.5% of the dry matter added to the incubation. The results indicated that the addition of isoacids improved dry matter digestibility and microbial growth even when protein levels as high as 16% were included in the incubations. However, their results also show that not all the responses were consistent at different levels of dietary protein and concentrations of isoacids. Although additional basic and applied research is needed to fully explain the inconsistencies in responses, some reports suggest that the degradability of the protein may be a major factor, since it would determine the availability of branched chain amino acid precursors of the isoacids for the bacterial populations in the system. This has been shown by Varga and coworkers (1988), who added isoacids to in vitro incubations of rumen bacteria and either formaldehyde-treated or untreated soybean meal. Their results show that when the degradability of soybean meal had been reduced by treatment with formaldehyde, the addition of isoacids improved the digestibility of cell wall components. This substantiates the theory that the response to isoacid supplementation is more evident when the dietary protein is not degraded easily. In this case the release of branched-chain amino acid precursors of the isoacids is low and possibly limits growth and activity of cellulolytic microorganisms unless isoacids are supplemented.

In vivo studies.-

The increases in cellulose digestion and production of microbial protein reported in the early studies had obvious potential for the improvement of the productivity of farm ruminants. This stimulated research on the effects of isoacids in farm ruminants. Lassiter and coworkers (1958a), fed valeric acid and a combination of valeric and isovaleric acids to lactating cows, and reported that daily intakes of 8.64 g of valeric acid stimulated the digestibility of dry matter, organic matter, crude fiber and nitrogen-free extract. Daily intakes of the same amount of valeric acid or a combination of 5.22 g of valeric acid and 2.13 g of isovaleric acid reduced urinary nitrogen and improved nitrogen retention, although milk yield and milk fat were not affected by the supplementation of the acids. The same authors report in another study (Lassiter et al, 1958b) that daily intakes of 3.6 g valeric acid and 1.0 g isovalerate increased growth rates in dairy heifers. In a later study with lambs (Cline et al, 1966), fed a mixture of 4.18 g isobutyrate, 5.9 g isovalerate and 1.18 g n-valeric acid per day, in purified diets containing 39 and 59% cellulose. Their results show that in the 39% cellulose diet, the isoacids increased cellulose and dry matter digestibilities. Their effects on nitrogen metabolism indicate that the acids lowered rumen ammonia levels and increased the digestibility and retention of dietary nitrogen. With the 59% cellulose, however, the addition of isoacids did not affect cellulose

digestibility or the measurements of nitrogen metabolism. The authors suggest that the inconsistency may have been due to the fact that urea was used as nitrogen source and may have been hydrolyzed too quickly and the production of ammonia was probably uncoupled from the slower fermentation of the other dietary components. This would in turn have meant that there actually was a nitrogen deficiency at the time where cellulolytic activity was more intense. This again underscores the need to have a coupled fermentative process in which all factors required for microbial cell synthesis and activity, such as ammonia, volatile fatty acids (VFA), sulfur, and carbon chains are available within the same time frame (Bergen and Yokoyama, 1978). This requirement is evident again in the report of Van Gylswyk (1970), who supplemented 2.0% isoacids to a low protein diet fed to sheep. The results indicate that the isoacids enhanced the digestion of hemicellulose and bacterial number after the low protein hay had been supplemented with urea as a nitogen source. More recent reports (Quispe, 1982; Brondani, 1985) also indicate that in high fiber, low protein rations, sulfur and nitrogen requirements have to be met before isoacids can elicit increases in microbial growth and cellulose digestion.

Oltjen and coworkers (1971) supplemented branched-chain VFA's and phenylacetic acid to steers fed diets containing urea or isolated soy protein. Phenylacetic acid was included since, like isoacids, it can be utilized by rumen bacteria to synthesize amino acids, in this case

phenylalanine (Allison, 1969; Bryant, 1973). Additionally, a recent report confirmed that phenylacetic acid can stimulate cellulose digestion by rumen cellulolytic bacteria (Stack et al, 1983). The results of Oltjen and coworkers (1971), showed that steers fed the urea diet had better nitrogen retention and lower urinary nitrogen when supplemented with a combination of isoacids and phenylacetic acid at 1.03% of the diet. The analysis of major VFA produced in the rumen showed that the addition of the acids increased the molar proportions of acetate in the urea diet.

Felix et al (1980a) added different mixtures of isoacids to growing heifers and lactating cows fed urea-supplemented rations. In their study, they measured the growth rate of the heifers and the digestive effect of isoacids in the lactating cows. The dairy heifers received daily 10 g of each of the five acids, and in the digestion trial with lactating cows, the isoacids were added at 80 g/day of either of two mixtures of isoacids and valeric acid. The first mixture consisted of 28% isobutyrate and 24% of each of the 5 carbon acids. The second mixture contained 36% isobutyrate, 17% isovalerate, 17% 2-methylbutyrate and 30% n-valerate. Their results showed that isoacids improved the growth rate of heifers and that the effect was higher in the younger animals. With respect to the digestive effects in lactating cows, the supplementation of either mixture of isoacids improved nitrogen retention and lowered urinary nitrogen, although there were no differences in the overall

digestibility of dry matter.

In another study, Felix et al (1980b) supplemented the same two mixtures described previously, to dairy cows during early lactation. In this study, the animals received concentrate-forage diets supplemented with either urea or soy protein. The results show that addition of either mixture of isoacids described above, to the urea-supplemented diets enhanced milk production and improved persistency of lactation. The determination of ruminal volatile fatty acids showed that isoacids increased the ruminal concentration of acetate while leaving propionate and butyrate unaffected. Their results showing increases in acetate concur with those of Oltjen et al (1971) in steers. Later reports on the rate of acetate production (Quispe, 1982; Brondani; 1986) also show a stimulatory effect of isoacids, although it should be noted that Brondani (1986) found a decrease in propionate concentrations in sheep supplemented isoacids intraruminally and fed an all hay ration. With regard to the effects on nitrogen metabolism Felix et al (1980b) showed that either of the mixtures of isoacids added to urea-supplemented diets lowered rumen ammonia nitrogen levels as well as plasma urea nitrogen concentrations. The effects on rumen ammonia nitrogen are in agreement with the results of Cline et al in urea-fed lambs and with the data of Oltjen and collaborators (1971) in steers fed the urea diets. With regard to the effects on plasma urea nitrogen, the results agree with the data of Oltjen et al (1971) in the urea-supplemented diets

but not in steers fed the soybean-supplemented ration, in which there were no changes when isoacids were added. The overall effects on nitrogen economy and ruminal nitrogen metabolism are also congruent with the overall model of nitrogen metabolism in ruminants. Owens and Bergen (1983) have reviewed the matabolism of nitrogen in the ruminant. During ruminal fermentation, ammonia is released from protein and non protein nitrogen sources by rumen microbes. The excess ammonia that is not utilized by the rumen microbiota is absorbed through the rumen wall and converted to urea in the liver. The urea thus formed can in turn be lost through urinary excretion or recycled to the rumen via salivary flow and diffusion into the rumen epithelium. In the case of isoacid supplementation, as isoacids increase the growth of cellulolytic rumen bacteria and synthesis of microbial protein, they also increase the fixation of ammonia into bacterial protein, thereby reducing rumen ammonia concentrations. This in turn attenuates the flow of nitrogen to the circulation and the fraction of this flow that is lost through the urine. Additionally, after ruminal fermentative events have subsided, they increase the ability of the rumen to utilize the fraction of urea nitrogen that is recycled to the rumen. The increased capacity to utilize nitrogen is especially important if diets high in non-protein nitrogen are fed at the beginning of lactation to high-producing cows. During that period, cows fed 30% or more of non protein nitrogen can be in negative nitrogen balance. It is in this period that the improvements in nitrogen retention found in

isoacid-fed ruminants would be more beneficial.

Lactation responses.-

In their study with dairy cows in early lactation, Felix et al (1980b) reported that feeding 80 g of either of the two isoacid mixtures described in the previous section improved milk yield by up to 11% over the production of animals fed urea-supplemented diets. Their data also show that in cows fed urea-supplemented diets, the isoacid-treated animals usually lost less weight during the experiment. However, body weight changes were not significantly different between controls fed soy protein and isoacid-supplemented cows.

Papas et al (1984) studied the production responses of lactating cows to different mixtures of isoacids in urea-supplemented diets. The study encompassed a complete lactation and measured responses in milk production, milk composition and body weight changes in cows fed six different amounts of combinations of ammonium salts of the 5 carbon acids and isobutyric acid. Their results show that computer modeling indicated an optimal blend of 89 g/day of isoacids containing 61 g of the ammonium salts of the 5 carbon acids and 28 g of ammonium isobutyrate. This blend has been the basis of the commercial product as either ammonium or calcium salts. When supplemented as aqueous blend, the mixture is equivalent to 120 g/day with 74% solids that contain 89 g of anhydrous ammonium salts. In terms of the individual free

acid composition, the mixture contains 23.6 g isobutyric acid, 19.2 g 2-methylbutyric acid, 14.8 g isovaleric acid and 18.6 g valeric acid. The results of the study are based on a similar mixture, that was added at 94 g/day of ammonium salts. This similar mixture contained 63.45 g of ammonium salts of 5 carbon acids and 30.55 g ammonium isobutyrate. Their report indicated that supplementation of this mixture increased milk yield over the entire lactation, although the increase was more evident during early lactation, when isoacids increased milk production up to 11 % or 6.6 lb/day. Milk fat percentage was not affected by isoacids. Feed intake data showed that isoacid-supplemented cows ate slightly more than the unsupplemented animals, although the increases in feed intake were proportionally smaller than the increases in milk yield, suggesting a more efficient utilization of the feed in the isoacid treatment. Body weight changes in this study showed that cows fed isoacids lost more weight than unsupplemented cows, particularly during early lactation.

In a subsequent whole lactation study, Pierce-Sandner et al (1985) utilized the blend defined by computer model in the study of Papas and coworkers (1984) in lactating cows fed diets containing either corn gluten meal and urea, or soybean meal or cottonseed meal as the primary protein supplement. In these trials, Isoacids increased milk production by 7% and milk fat percentage was unchanged. Feed efficiency was higher in the isoacid-fed cows and body weight records indicated

that isoacid-fed cows tended to lose more weight than control cows, suggesting an improved capacity to mobilize body reserves of energy in the isoacid- treated animals. The report of Pierce-Sandner et al (1985) agree with the majority of the data produced in the work of Papas et al (1984). However it should be noted that in the study of Pierce-Sandner and coworkers (1985), the largest response in milk production in isoacid-treated animals was seen at the begining of the peak of lactation.

The stimulatory effect of isoacids on milk production has also been evaluated in commercial farm trials. Rogers and Cook (1986) determined the response to the suplementation of the commercial blend of isoacids defined in the work of Papas et al (1984). The data of Rogers and Cook (1986) shows that heifers and cows fed isoacids produced 2.3 kg more milk than controls and that the response was stronger in animals in early lactation, when the increases were 2.7 kg. Their results also showed that milk fat percentage was not affected. A noteworthy point of this report is that even though the rations consisted of mixed forage-grain without urea supplementation, there was a production response to isoacids. In another commercial trial, Rogers et al (1986) reported that supplementation of 100 g/ day of calcium salts of isoacids to a variety of rations increased milk yield by 1.8 kg/day. although the majority of the response occurred in multiparous cows. In another commercial farm trial, Newman et al (1986) reported that cows

supplemented 82 g/ day of Ca salts of isoacids in a variety of concentrate-forage rations, produced more milk than the controls. In this study again, the productive response was larger in the mature cows than in heifers and the percentage of milk fat was unchanged by isoacid treatment.

The relationship between productive response and stage of lactation was investigated by Blumer and coworkers (1986), who measured the response to the addition of 86 g of Ca salts of isoacids to diets that contained no urea. In their work, isoacids were introduced in the diet of animals at different stages of lactation. The results show that the responses were higher when isoacids were introduced before the peak of lactation, intermediate when the addition began after the peak of lactation and lower when the cows were exposed to isoacids in the later parts of lactation. In this respect, their results agree with those of Papas et al (1984).

Other experiments however, have failed to demonstrate a positive rsponse to isoacids. Fieo and coworkers (1984), in a short-term study with a Latin square design, did not see a response in milk production to the addition of 120 g of an aqueous blend of isoacids to diets supplemented with corn-gluten meal and urea. Klusmeyer et al (1987) added a similar amount of isoacids to a forage-concentrate diet that did not include urea. Their results showed no significant response in milk yield or milk fat percentage. It must be noted that their trial started at an average of 77

days into lactation and if the data of Papas et al (1984), and Blumer et al (1986) hold true, the cows in this trial may have been less responsive to isoacids. On the other hand, if the results of Pierce-Sandner et al (1985) apply, the animals in this post-peak experiment should have responded to isoacid treatment. In another study, Moore and collaborators (1987) did not find a response to the addition of similar amounts of isoacids to forage-concentrate diets, either with cows at the begining or mid-lactation. The reasons for the inconsistencies are not clear. A valid explanation requires a more complete knowledge of dose responses and the mechanism of action of isoacids in lactating cattle. The body of research indicates that isoacids have clear effects on nitrogen metabolism in ruminants fed high fiber, high non-protein nitrogen diets but in rations rich in preformed protein such as those fed to high producing dairy cattle, it is unlikely that effects on ammonia utilization and production of microbial protein can account for increases of up to 11% in milk yield (Papas et al 1984). The body of research suggests that particularly during early lactation, it is the supply of energy and mobilization of endogenous reserves of energy that are critical in the high producing cow (Bauman and Currie, 1980; Bines and Hart, 1982; Hart 1983). The ability of isoacids to stimulate cellulolytic activity in the rumen, has clear advantages in the extraction of dietary energy during early lactation and merits attention especially in terms of its effects on the endocrine-metabolic

milieu of the cow. Additionally, the fact that in the whole-lactation trials of Papas et al (1984) and Pierce-Sandner and collaborators (1985) isoacid-fed animals lost more weight during lactation is suggestive of an enhanced ability to mobilize endogenous reserves of nutrients. This underscores the need to investigate further the mechanism of action of isoacids in high-producing lactating cattle.

Dose-responsiveness.-

First, it is important to reevaluate the dose-response to isoacid treatment. The previous section shows that a more reliable dosage is clearly desirable. The experiments in vitro (Cummins and Papas, 1984; Gorosito, et al, 1985) with a wide range of dosages, indicate that dosage is critical in determining the response to isoacids. The evidence in vivo also emphasizes the importance of dosage. When very low levels of isoacids were used as in the study of Lassiter et al (1958a) with lactating cattle, there was no response in milk yield. The lactation studies described before show responses to isoacids added at around 90 g of ammonium or Ca salts /cow/day and clearly show that at this level, isoacids can elicit an increase in milk yield. It is also important to note that the level and blend utilized in most of these studies was defined in the computer model of Papas et al (1984), which also clearly shows dose-responsiveness in cows fed rations that did include urea. Although a great deal was learned from the model, it should

be noted that the results in the report were based on the response to a blend that although similar, was not the same as the one defined in their computer model. Also, the amount actually supplemented was somewhat higher than the one defined by the model. When Pierce-Sandner et al (1985) utilized the optimal blend, their results did show an enhancement in milk production, however, the increases in milk yield occurred mostly during the second and last thirds of lactation, and not in early lactation, when most of the controversial results have been reported. It is possible that within the specific conditions of the experiment, the computer model indicated an effective but vulnerable level of supplementation whose effects are susceptible to variations in the characteristics of the feeds. That the interactions between feed degradability and isoacids are critical, is evident in the in vitro work of Varga and collaborators (1988) with soybean meals of different degradability. Additionally, little is known about the behavior of isoacid salts in the rumen concerning their distribution and time of residence. Ration composition and feed processing may also be important. It is again possible that changes in the dilution rate and dietary interactions between fibrous and starchy concentrate components (where isoacids are added) affect the dosage required for a reliable productive response. In this regard, it is critical to reevaluate the dose response of isoacids and investigate the mechanism of action at levels of supplementation that can reliably induce a positive response

in milk yield. Moreover, the investigation of the effects of other blends also merits further research. As can be seen in the *in vitro* and *in vivo* experimental evidence reviewed, diverse combinations of isoacids have been effective. This is underscored by a recent report of Hlalo *et al* (1987) which shows that supplementation of isobutyric acid alone, elicited increases in milk production comparable to those of whole blends. Although due to the diversity of the specific requirements of the ruminal bacterial population it is safer to utilize combinations of isoacids, it is also clear that the investigation of effects of individual isoacids merits attention, since it could yield a product that is more cost-effective in commercial situations.

Mechanisms of action.-

Apart from the well documented effects on nitrogen-utilization and cellulose digestion, relatively little is known about other biological effects of isoacids. As described before. The improvements in milk yield without significant increases in feed intake found by Papas *et al*, (1984) and Pierce-Sandner *et al*, (1985) in isoacid-fed, lactating cattle, suggest a post-absorptive effect that redirects nutrients towards the mammary gland. Additionally, in the same two whole lactation studies, isoacid-supplemented cows lost more weight during lactation than control-fed animals. This is an indication of an improved capacity to mobilize endogenous reserves of nutrients and is

also suggestive of a post absorptive effect on tissue mobilization. It is unlikely that isoacids can directly exert an effect on the mammary gland of ruminants, since volatile fatty acids of three and more carbons are removed by the liver (Ricks and Cook, 1981). In the search for a post-absorbtive effect of isoacids, research has focused on the endocrine milieu. Hart and coworkers (1980), have described that higher-producing cattle have higher levels of circulating growth hormone and lower levels of plasma insulin than low producers. Research on the metabolic effects of hormones has established that the enhancement of milk production in dairy cattle treated with growth hormone (GH) is associated with a better feed efficiency and an increased capacity to mobilize endogenous reserves of energy (Hart, 1983; Bauman and Mc Cutcheon, 1986). Isoacid supplementation has also resulted in improvements in feed efficiency and body weight changes, this suggests an enhanced capacity for tissue mobilization, the possibility that isoacids increase plasma GH has been explored in several studies. Fieo et al (1984) found higher GH levels in plasma of isoacid-treated cows, but the fact that milk yield was not affected makes it difficult to integrate the endocrine and productive aspects of isoacid supplementation. More recently, Klusmeyer et al (1987) and Kik (1987) reported that GH levels were not affected by isoacid treatment in lactating dairy cattle. However, here again it is difficult to draw definite conclusions since the responses in milk yield were not

significantly higher (Klusmeyer et al, 1987) or were small (Kik, 1987).

With regard to insulin, a reduction in insulin levels is desirable in lactating cattle. The body of research on the endocrine regulation of lactation has been extensively reviewed (Bauman and Currie, 1980; Bines and Hart, 1982; Hart, 1983) and shows that in ruminants, insulin stimulates the deposition of nutrients into peripheral tissues and away from the insulin-independent lactating mammary gland. As mentioned before, Hart and collaborators (1980) found that low circulating insulin levels are associated with higher milk yields. Moreover, different investigators (Kronfeld et al, 1963; Schmidt, 1966; Laarveld, 1981) have shown that the administration of insulin to lactating cattle has deleterious effects on milk synthesis. Research on the effects of isoacids on plasma insulin has shown lower concentrations in hay-fed sheep (Brondani, 1986) and essentially unchanged levels in lactating cows fed forage-concentrate rations (Towns and Cook, 1984; Kik, 1987; Klusmeyer, 1987). Isoacids can enhance the digestibility of cell wall components (Gorosito et al, 1985), as well as the rate of production (Quispe, 1982) and concentrations (Oltjen et al, 1971; Felix et al, 1980b) of acetate in the rumen. This indicates a capacity to enhance the extraction of dietary energy from the fibrous components of the diet. The fact that insulin levels are unchanged in isoacid-suplemented lactating cattle has physiological significance for lactation since, if insulin

remains unchanged, extra energy would be more available for milk synthesis.

The above observations are also supported by research on the effects of VFA's on hormones. Bines and Hart (1984) have shown that propionate is the only VFA secretagogue of insulin. Their results essentially confirm the earlier obsebrvations of Horino et al, (1968) with intravenous infusions of supraphysiologic concentrations of VFA's. The results of Bines and Hart (1984) were obtained with intraruminal infusions of physiological concentrations of VFA. This makes their study more relevant in explaining the effects of compounds that, like isoacids, affect VFA production in the rumen. In the case of isoacids. propionate levels are not increased (Oltjen et al, 1971; Felix et al 1980b; Brondani, 1986; Kone et al, 1986; Klusmeyer et al 1987) and the increases in VFA's occur mostly through acetate (Oltjen et al, 1971; Felix et al 1980b; Quispe, 1982; Brondani, 1986; Kone et al, 1986).

With regard to plasma metabolites, the data has not shown a clear effect in isoacid fed cattle. Brondani (1986) did not find an effect on plasma glucose in non-lactating sheep. In research with lactating cattle, (Kik, 1987; Klusmeyer, 1987), plasma glucose levels have not been affected in isoacid-fed cows. plasma urea nitrogen (PUN) levels in isoacid-supplemented ruminants have been lower in lactating cattle fed urea-supplemented diets (Felix et al, 1980). In non lactating ruminants, Cline et al (1966) found

unchanged PUN levels with lambs, Oltjen et al (1971), working with steers, found levels of PUN that were lower with urea-supplemented diets and unchanged with soybean meal-supplemented diets. The above results suggest that the type of dietary nitrogen is important in determinig the response of PUN levels to isoacid supplementation. Here again, in the case of the lactating cow it is important to evaluate the response in conditions where the animals are showing a productive response. Also it is desirable to test it in diets that contain little or no urea. In rations containing less degradable nitrogen sources, the levels of PUN would be affected less by ruminal events and would be a closer reflection of changes in the mobilization of endogenous protein during lactation.

CHAPTER TWO

DOSE-TITRATION TRIAL

Introduction.-

As described in the previous section, research has established that the dietary addition of different blends of isoacids and valeric acid can increase milk yield in lactating cattle. However, the inconsistencies in some of the experiments described before, underscore the need for further research. Questions remain unanswered, regarding the mechanism of action of isoacids, particularly in terms of their effects on the endocrine-metabolic milieu. Additionally, it is necessary to reevaluate the optimal dosage in order to obtain more consistent responses. The aim of the present work is to determine whether the increases in milk production in cows fed isoacids, involve digestive, endocrine or metabolic effects. This overall aim is addressed in the specific objectives of the three experiments described in the following sections.

Specific Objective.-

To determine the dose response to isoacid supplementation in terms of milk yield, feed intake, feed efficiency and body weight changes. This objective has clear importance for the determination of the useful dosages at

which to supplement isoacids in practical situations. To accomplish this, it is also necessary that the research be conducted under conditions that resemble the feeding and management practices found in commercial operations. In a complete lactation experiment, the report of Papas et al (1984), showed a positive response to 94g/day of isoacids. They also identified the first third of lactation as the most responsive period. Their results are supported by the findings of Blumer and coworkers (1986) both in terms of dosage range and stage of lactation. The results of Pierce-Sandner et al (1985) with similar dosages also show increases in milk yield beginning during the first period and lasting throughout the whole lactation. However, the data of Fieo et al 1984), Klusmeyer et al (1987) and Moore et al (1987) with cows fed similar dosages during the first third of lactation did not show responses in milk yield. It is necessary to reassess the optimal dosage ranges at different stages of lactation in order to obtain a reliable response to isoacids. Most importantly, a reliable dosage is necessary in order to investigate the mechanism of action in animals that exhibit a productive response.

Experimental Work.-

The purpose of this experiment was to address the questions of the above specific objective. The study investigated over a complete lactation, the dosage required for a reliable response. The criteria to evaluate the response were milk production, feed intake, feed efficiency

and body weight changes. Body weight changes were included as one of the criteria since they are an indication of the ability of the animal to mobilize endogenous nutrients to support milk synthesis.

Materials and Methods.-

Fifty five lactating multiparous Holstein cows from the Michigan State University Dairy Research and Teaching Center were selected for this study. The cows were randomly assigned to either one of five experimental groups of 11 cows each. The assignment was determined three weeks before the expected parturition date. Each group received a different dosage of the optimal blend of isoacids as defined in the study of Papas et al (1984).

The isoacid dosages, expressed as a percentage of the concentrate dry matter fed were 0.0%, 0.4%, 0.8%, 1.2% and 1.6%. During the experiment, 7 cows had to be eliminated due to diverse clinical and reproductive problems. This left 48 cows that completed 200 days or more in lactation. The data of those 48 cows were evaluated. Table 1 contains a description of the experimental groups. The index of productive capacity utilized in this experiment was the Dairy Herd Improvement Association (DHIA) computation of Estimated Relative Productive Ability (ERPA).

TABLE 1. Mean age and ERPA of experimental groups.-

	Treatment Groups					
	0.0%	.4%	.8%	1.2%	1.6%	SEM*
No. Cows	10	10	9	10	9	
Age (mo)[a]	48.2	49.9	50.6	52.1	51.2	4.65
ERPA[a]	-164	-112	28	-168	-204	240

* Standard error of the mean for each treatment group

[a]Means were not different between treatment groups (p .10)

Ration composition and management.-

Before parturition, the cows were gradually adapted to the isoacid blend over a period that began three weeks before the expected calving date. During this period the cows were fed corn silage and alfalfa hay ad libitum. This was supplemented with a grain mixture containing, in the case of treated cows, 87.5% corn, 9.9% Soybean meal, 3.2 % isoacids and 2.0% of the Trace Mineral Salt (TMS) described for the lactation diet . In the case of control cows, the prepartum supplement was 89.1% Corn, 9.9% Soybean Meal and 2.0% TMS. The amount of the above grain mixtures supplemented was 1 kg during the third week prepartum, 2 kg during the second week prepartum and 4 kg during the week before the expected parturition date. During lactation, the animals were fed a total mixed ration divided in equal amounts between morning (3;00 am) and afternoon (1:00pm) feedings. The diet consisted of corn silage, alfalfa hay, high moisture corn and a protein supplement that also

contained vitamins, mineral salts and the isoacid blend.

The concentrate portion of the diet consisted of the high moisture corn and the protein supplement. The isoacids were included as part of the protein supplement. The same ingredients were utilized for the whole experiment. However, in order to adjust to the changes in nutritional needs, the ration formulation was modified for three periods. For period 1, during the first 112 days of lactation, the diet included on a dry matter basis, 50% forage (Corn Silage and Alfalfa Hay) and 50% grain concentrate (High Moisture Corn and Protein Supplement) For period 2, during days 113-224, the proportion of Forage:Concentrate was changed to 60%:40% and for period 3, between days 225-305, the proportion was again changed to 70% forage and 30% concentrate.

The ration was formulated to meet the nutrient requirements of lactating cattle for mature cows of 600 kg body weight and producing over 29 kg of fat corrected milk (FCM)/day during period 1; 21kg FCM/d during period 2 and 14 kg FCM/d during period 3. (National Research Council, 1978). The ration formulation, protein supplement composition and total nutrient composition of the diet appear in tables 2 and 3 respectively.

TABLE 2.-Ration formulation.-

	% of Dry Matter		
Feed Ingredient	Period 1	Period 2	Period 3
Corn Silage	37.5	45.0	52.5
Alfalfa Hay	12.5	15.0	17.5
High Moisture Corn	30.0	24.0	18.0
Protein Supplement	20.0	16.0	12.0

The above rations provided 16.0% Crude Protein for Period 1; 14.8% Crude Protein for period 2 and 13.7% Crude protein for Period 3. Net Energy for Lactation was 1.72 Mcal/kg dry matter for period 1; 1.62 Mcal/kg dry matter for period 2 and 1.52 Mcal/kg dry matter for period 3. Acid Detergent Fiber was at least 21% throughout the experiment. The ration also contained the following nutrients .70% Ca; .42% P; .30% Mg; .80% K .46% NaCl; .22% S; .10ppm Se; 3,200IU/kg Vit. A and 300IU/kg Vit. D.

The nutrient composition of the diets was monitored throughout the whole trial from weekly composite samples. No major corrections were necessary during the trial. In order to insure an adequate consumption, individual intakes and weigh back amounts were monitored daily. Based on individual consumption the amount offered was calculated to provide at least a 10% weighback.

TABLE 3.-Percentage composition of protein supplements.-

	Treatments				
Ingredient *	0.0%	0.4%	0.8%	1.2%	1.6%
Soybean meal	86.22	85.26	84.31	83.36	82.41
Corn	4.53	4.48	4.43	4.38	4.33
Sodium Bicarb.	1.25	1.25	1.25	1.25	1.25
Dical-P	.25	.25	.25	.25	.25
Trace Minerals	.25	.25	.25	.25	.25
Vitamins & Se	.25	.25	.25	.25	.25
Mg O	.05	.05	.05	.05	.05
AS-Isoacids **	-----	1.00	2.00	3.00	4.00

* Supplement was fed as 40% of the grain-concentrate

** When mixed with the grain in the proportions described in Table 1, the respective isoacid levels are 0.0%; .4%; .8%; 1.2% and 1.6% of the concentrate.

Isoacid composition and dosage.-

Isoacids were added as a percentage of the grain-protein concentrate mixture. For the first third of lactation, the proportion of the concentrate was 50% of the total dry matter intake. Total dry matter intake was estimated as 20 kg/cow day. This is in agreement with the NRC (1978) requirements for multiparous cows weighing 600 kg and producing over 30 kg of 4% Fat Corrected Milk (FCM). The expected amount of isoacids consumed daily was calculated based on an expected dry matter intake of 10 kg concentrate/cow/day. The isoacid blend utilized was the same defined in the computer model of Papas et al (1984). This is an aqueous blend of ammonium salts of the acids. It contains 26% water and 74% solids. In terms of the free acids, the blend is a combination of 31% isobutyric acid, 25.2% 2-methyl butyric acid, 19.4% isovaleric acid and 24.4%

valeric acid.

Quality control.-

Feed analysis of samples collected as described before, was conducted by standard proximate analysis procedures at two different laboratories. Crude Protein was determined according to the principle of Kjeldahl (N x 6.25). The determination of Acid Detergent Fiber was performed as described by Goering and Van Soest (1970). Mineral Analysis was conducted by atomic absorption spectophotometry in a Perkin-Elmer atomic absorption spectophotometer model 5000 (Perkin-Elmer Corporation., Norwalk, Conn.06856). The composition of the mixture of isoacids was verified by gas chromatography in a Hewlett-Packard gas chromatograph model 5030-A, equipped with a 7671-A automatic sampler and a 3880A integrator-recorder (Hewlett-Packard Co., Avondale, Penn.) The columns were all-glass, packed with 10% SP-1200/1% H3PO4 on 80/100 Chromosorb W AW (Supelco Inc., Bellefonte, Penn.) The samples were run according to the procedures of Ottenstein and Bartley (1971a, 1971b).

Variables Measured.-

Feed Intake.-

Feed intake was calculated daily by subtracting the weigh back refused from the amount of feed offered. The determination of dry matter was conducted from composite samples on a weekly basis. The percentage of dry matter was obtained from the differences in weight between as fed and

oven-dried feed samples.

Milk Production.-

The cows were milked twice daily, at 0400 and 1500 h The milk yields were recorded from each milking as indicated by digital electronic scales connected to Boumatic milking machines. Milk yields were not corrected for milk fat content since isoacids do not affect milk fat percentage (Papas and coworkers, 1984; Pierce-Sandner and coworkers, 1985).

Body Weight.-

Cows were weighed after parturition and then every two weeks throughout lactation, the cows were weighed at the same time of the day in order to minimize variability due to feed intake or loss due to milking.

Statistical Analysis.-

The data were evaluated by the least squares analysis of the Statistical Analysis System. The dose-responses and differences in milk yields were determined by analysis of covariance. In the analysis, the blocking criterion was the calving dates. The Estimated Relative Productive Ability (ERPA) was utilized as a covariate to account for differences in productive potential among the experimental groups. Feed Intake, Feed Efficiency and Body weight changes were also analyzed by the least squares model of the

Statistical Analysis System. The model utilized in the evaluation of the production responses can be represented as follows.-

$$Y = U + Ti + Rij + B(\bar{X}ij - \bar{X}) + Eij$$

Where

Y is the variable measured.

U is the overall mean

Ti is the effect due to the i treatment

Rij is the effect of the j block of the i treatment

B is the coefficient of the covariate

$\bar{X}ij$ is the observed value of the covariate

$\bar{X}$ is the general mean of the covariate

Eij is the random error

Results and discussion.-

Production data.-

The effects of the different dosages of isoacids on milk yield, dry matter intake and feed efficiency appear on Tables 4, 5 and 6. The dosages appear in the tables as % ammonium salts of isoacids (% AS-Isoacids). The dosage values indicate the percentage of the concentrate dry matter at which the AS-Isoacids were added. Milk yield is reported both in actual values and in values adjusted for the Estimated Relative Productive Ability (ERPA) covariate as described in the statistical analysis. Feed efficiency is expressed as kg of milk yield per kg of dry matter intake. The data in Table 4 illustrates the productive response of the cows receiving the different dosages of isoacids over the first third (weeks 1 - 15) of lactation. The statistical analysis model described in the previous section did not indicate a dose-related response for the different isoacid dosages. However, the response is significant for individual, independent pair-wise comparisons. The increases in milk yield, relative to control, of the group receiving isoacids at 1.6% of the concentrate were significant (p.07). The response in this group was 3 kg/ day or 8.28% over the control group. The means of the dry matter intake show a small, numerical, dose related response that is not statistically significant. Feed efficiency was not statistically significant, and the numerical values do not suggest an effect except in the group where isoacids were supplemented at 1.6 % of the concentrate. In this group,

feed efficiency was 4.5% better than in the control cows.

TABLE 4.-Means for actual milk yield, adjusted milk yield, feed intake and feed efficiency during the first 15 weeks of lactation

	% AS-Isoacids *					
	0.0	0.4	0.8	1.2	1.6	SEM[a]
Milk Yield (Actual) kg/d	36.1	36.9	37.1	36.5	39.0[b]	1.54
Milk Yield (Adjusted) kg/d	36.2	36.8	36.7	36.6	39.2[b]	1.31
Dry Matter Intake kg/d	20.5	21.0	21.1	21.2	21.3	1.31
Feed Eff. (MY/DMI)	1.75	1.74	1.75	1.73	1.83	.059

* Dosages as percentage of the concentrate dry matter
a Standard error of treatment means
b Treatment means with different superscript differ ($p < .1$)

Table 5 contains the productive responses to the different isoacid dosages during the second third of lactation (weeks 16 - 30). During this period, there were no differences among treatments ($p < .1$). It should be noted however, that the individual, pair-wise comparison between the feed efficiency of the controls and the 0.4% treatment were significant (p .05). The reasons for this single improvement in feed efficiency at the lowest level of isoacid supplementation (0.4% of the concentrate) are not clear. It is interesting though, that the improvement came

as a result of a small numerical increase in milk yield and a reduction in feed intake.

TABLE 5.-Means for actual milk yield, adjusted milk yield, feed intake and feed efficiency between weeks 16 - 30.

	% AS-Isoacids *, **					a
	0.0	0.4	0.8	1.2	1.6	SEM
Milk Yield Actual (Kg)	29.2	30.8	31.7	28.3	30.6	1.17
Milk Yield Adjusted (Kg)	29.3	30.8	31.5	28.3	30.6	1.17
Feed Int. (Kg D.M.)	21.8	20.8	22.2	21.5	22.1	1.64
Feed Eff. (MY/FI)	1.35	1.50	1.40	1.32	1.39	.056

* Dosages as percentages of the concentrate dry matter
** Means among treatments did not differ ($p < .05$)
a Standard error of treatment means

It is also noteworthy that the first change in dietary regime (Table 2) ocurred during this period. The change represented a shift in forage : concentrate ratios from 50:50 to 60:40. The design of the trial was to keep the isoacid dosage constant as a percentage of the concentrate dry matter. This represented, in absolute quantities, a reduction in the grams of the isoacid blend consumed daily. The cows in the 0.4% dosage showed a small increase in milk production in spite of the changes in the forage-concentrate ratio. These changes could have conceivably induced the reduction in feed intake in those animals. The improved feed efficiency may be a reflection of an enhanced extraction of

dietary energy. Table 6 contains the actual amounts of the isoacid blend consumed during the three different dietary regimes.

TABLE 6.-Calculated daily intakes of the isoacid blend during the different dietary regimes.

	% AS-Isoacids *, **					a
	0.0	0.4	0.8	1.2	1.6	SEM
Days 1 - 112 (g/d)	0.0	42.3	84.8	128.2	171.3	3.8
Days 113-224 (g/d)	0.0	32.9	71.1	102.2	141.5	3.3
Days 225-305 (g/d)	0.0	22.4	48.1	71.7	95.4	2.2

* Dosages as percentage of the concentrate dry matter.
** Means among treatments differ ($p < .01$)
a Standard error of treatment means

The productive responses over the complete lactation appear in Table 7. The complete lactation was standardized to 305 days according to the DHIA table of Mc Daniel and collaborators (1965). The statistical analysis model described in the previous section did not indicate that the differences among treatments were dose related or significant. However, independent, pair-wise comparisons between individual treatments and the control group show results similar to those of the first third of lactation. In this case, the increases in milk yield of the group that received the isoacid blend at 1.6% of the concentrate

were significant (p .06). The increases represent 8.09% over control yields. In absolute amounts, this is 701 kg of milk over the 305 day lactation, which is equivalent to 2.29 kg milk / day.

TABLE 7.-Means for actual milk yield, adjusted milk yield, feed intake and feed efficiency during the complete 305 day lactation.

	% AS-Isoacids *					
	0.0	0.4	0.8	1.2	1.6	SEM[a]
Milk Yield Actual (Kg)	8,665	9,028	9,318	8,556	9,366[b]	332
Milk Yield Adjusted (Kg)	8,682	9,021	9,252	8,547	9,392[b]	266
Feed Int. (Kg D.M.)	6,058	5,745	6,362	5,932	6,180	237
Feed Eff. (MY/FI)	1.44	1.58	1.47	1.46	1.53	.056

* Dosages as percentages of the concentrate dry matter
a Standard error of treatment means
b Treatment means with different superscripts differ (p <.1)

Discussion.-

The data from the above tables show that isoacids can enhance milk production by 8.28 % or 3.0 kg/day in the first third of lactation and that the response over the total lactation is 8.09% or 2.29 kg/ day. The magnitude of the responses is consistent with the enhancements reported in a complete lactation trial by Papas et al (1984). In their study, the increases were 3.0 kg/d for the first third of lactation and 2.6 kg/d for the 305 day lactation. Here it

should be noted that the responses of first third of lactation in the present study were obtained with intakes over 171.3 g/d of the aqueous blend of isoacids. This is equivalent to 126.7 g of anhydrous ammonium salts of the acids (AS-VFA). Additionally, in this study the diet contained no urea. In the study of Papas et al (1984), with cows supplemented urea, the responses were seen with the addition of 94 g/ day of ammonium salts of isoacids. The study of Pierce-Sandner et al (1985) with urea-supplemented diets showed that the addition of 120 g of aqueous blend (or 89 g anhydrous AS-VFA) enhanced milk yield by an average of 1.8 kg/ day over a 305 day lactation. The data from the present trial suggest that in diets containing no urea, the amount of isoacids required is higher than in urea-supplemented diets. The results of the present work also confirm previous reports (Papas et al, 1984, Blumer and coworkers, 1986) that lactating cattle are more responsive to isoacid treatment during the first third of lactation. In the present work, no significant differences were observed in milk yield during the second third. The data also suggests that the absolute amount of isoacids added must be kept constant. In the present trial, the dosages were kept constant as a percentage of the concentrate. However, due to shifts in the forage-concentrate ratio in the second third of lactation, the daily intake of aqueous blend in the 1.6% group was reduced to 141.5 g/d. Although this is still higher than the 120 g/d recommended by Papas et al (1984)

and used by Pierce-Sandner et al (1985), no response was seen during this period. In this respect, the data also concurs with reports (Rogers and Newman, 1986) that isoacids have a minimal carryover effect.

With respect to the productive responses of the 305 day-lactation, the data shows a response that in pair-wise comparisons was significant (p .06). The data is in agreement with the report of Papas et al (1984), that shows increases in milk yield over a standardized 305-day lactation. However, the respose of this study over the same period is .31 kg/d lower. This reduced response is conceivably due to the lower absolute amounts fed after changes in dietary regimes during the second and third parts of lactation. Again, this underscores the importance of maintaining a high dosage in absolute amounts over the complete lactation.

The present study suggests a need for higher amounts of isoacid in rations that contain preformed protein and no urea. The lack of response at lower dosages merits attention. Klusmeyer et al (1987) did not find a productive response to the supplementation of 120 g/d of aqueous blend in lactating cattle. They concluded that the preformed dietary protein already provided enough branched-carbon chains for maximal fermentation and milk production. However, their explanation would not explain why the present study showed a response with higher levels of isoacid supplementation. If the dietary protein were a sufficient source of branched-chain fatty acids, additions of even

larger amounts of isoacids should not have produced a response. Although additional research is needed for a complete explanation, it is possible that the higher requirement shown here is affected by varying fermentation patterns in the rumen. With urea-supplemented diets, the release of ammonia would be rapid (Chalupa, 1968). This would couple an enhanced availability of ammonia to the presence of the isoacids in the rumen before they can be absorbed or catabolized. In the case of preformed protein-rations, the isoacids may not remain in meaningful amounts during the time the maximal rate of ammonia release occurs. Interestingly enough, the ruminal VFA data reported by Klusmeyer et al (1987), does not show increases in any of the isoacid components that were supplemented to the ration. This may help explain the absence of productive responses in their study. In preformed-protein diets, if the dietary protein is degraded more slowly, the amount of isoacids needed should be higher. This would increase the probability that the ruminal concentrations of isoacids are still high during the period of optimal ammonia release. Additionally, this theory helps explain why diets with preformed protein show irregular responses. In these diets, differences in degradability and rate of passage would affect the pattern of ammonia release. The need to couple ammonia release with the presence of the acids has been postulated by Cline and coworkers (1966). This also underscores the overall requirement for the coordinated presence of all the required

factors for microbial growth (Bergen and Yokoyama, 1978).

In terms of recommendations of adequate dosages of isoacids, this report emphasizes the need to explore the effects of diverse dosages. In the literature, the recommended amounts are usually expressed as g of isoacids/cow/day. It is difficult to express a recommended dosage of isoacids on a body weight basis since the cow changes weight throughout lactation (Bauman and Currie, 1980). However it is also desirable to standardize the dosages taking into account the weight of the animal. The results reported here suggest a required dosage of 171.3 g/cow/day during the first third of lactation. In a mature 600 kg cow, this is equivalent to 0.285 g/kg body weight. On a metabolic body weight basis ($BW^{.75}$) the same 600 kg represent a metabolic weight of 121.23 and a suggested dosage of 1.413 g isoacids/unit of metabolic body weight.

The data presented in this study suggests that the dosage identified by Papas et al (1984) was an effective but vulnerable range. The results of the present experiment indicate that only the highest treatment level elicited a production response. This also suggest that this treatment level may represent the low end of the effective dosage range for diets that do not include non protein nitrogen. It is necessary to investigate the production response to higher levels of isoacid supplementation in lactating cows fed varying nitrogen sources. However, it must also be emphasized that the practical problems involved in

conducting this type of whole lactation experiments are enormous. The present study required 48 cows and lasted over 18 months. The massive requirements of labor, availability of animals and data reduction are likely to tax the resources of most research institutions. However it is also clear that if isoacids are to be used in practical situations, additional research is needed to determine the adequate dosage ranges.

Body weight changes.-

The changes in body weight reported in this section represent the maximal percent body weight loss and the average daily weight change. The maximal percent weight loss is the maximal amount of weight that the cow lost in early lactation expressed as a percent of the initial postpartum body weight. This is a reflection of the ability of the cow to mobilize endogenous reserves of nutrients to meet lactation demands. The average daily weight change is the daily weight change over the number of days in lactation. This is an indicator of the ability of the cow to maintain and enhance endogenous reserves of nutrients over the whole lactation. Table 8 describes the dose-response of these variables in the different treatments.

TABLE 8.-Percentage maximal weight loss during the first third of lactation and daily body weight change over the complete lactation.

	% AS-Isoacids *					a
	0.0	0.4	0.8	1.2	1.6	SEM
Max. Wt. Loss (% of B. Wt)	4.39	4.14	4.13	5.71 b	6.75 b	.965
Wt. Change (g/d)	367	258 c	272 c	222 c	199 c	43.7

* Dosages as percentages of the concentrate dry matter.
a Standard error of treatment means
b Means with different superscript differ ($p < .05$)
c Means with different superscript differ ($p < .01$)

The results indicate that maximal percent weight loss during the first third of lactation was dose related and significant. The differences were significant ($p < .05$) in the groups that received isoacids at 1.2 and 1.6% of the concentrate. The daily weight change over the complete lactation shows that addition of isoacids leads to a reduction in body weight gain in lactating cows fed diets unsupplemented with urea. This response was dose-related and the differences were significant ($p < .01$) in all isoacid-supplemented groups.

Discussion.-

The data support a role for isoacids in enhancing the mobilization of endogenous nutrients to support lactation. The results also agree with the reports of Papas et al (1984) and Pierce-Sandner et al (1985) that indicate that cows fed isoacids had lower rates of weight gain throughout

lactation. Overall this suggests an attenuated capacity to store nutrients. This implies a redistribution of energy and nutrients away from peripheral tissue accretion. In the lactating cow this means an increased availability of nutrients for the mammary gland. More importantly, the results of the present study show that cows supplemented with isoacids lost a higher percentage of body weight during the first third of lactation. This increased maximal weight loss during the first third of lactation indicates an improved capacity to mobilize nutrients.

During early lactation, the increases in feed consumption cannot fulfill the requirements of lactation and most cows go into negative energy balance. In turn this makes lactation heavily dependent on the mobilization of endogenous nutrients (Belyea and coworkers, 1978; Bauman and Currie, 1980; Bines and Hart, 1982). During this period, the cow may draw up to 33% of the energy required to maintain lactation from endogenous reserves (Bauman and Currie, 1980). The enhanced ability of isoacid-treated cows to mobilize endogenous reserves during the first third of lactation is clearly beneficial during this critical period. This also helps explain why the largest production effects in isoacid-fed cows are seen during the first third of lactation. Additionally, the data suggest that isoacids help redirect nutrients towards lactation. This implies a repartitioning effect that will be studied in the following chapters.

CHAPTER THREE

ENDOCRINE-METABOLIC EFFECTS OF COMBINED ISOACIDS

Specific objective.-

To determine whether or not the productive responses to the supplementation of isoacid blends involves endocrine and metabolic interactions.

This objective requires that the determination of digestive, metabolic and endocrine effects be performed with animals that are showing a productive response. Therefore, this objective depends on the determination of optimal dosages and stages of lactation addressed in chapter 2. The results of Fieo, et al, (1984) and Klusmeyer et al, (1987) on the endocrine-metabolic effects of isoacids cannot provide a definitive answer since the animals they utilized did not exhibit a productive response. Brondani (1985) found that isoacids decreased insulin in non-lactating sheep. This experiment will determine whether or not there is the same effect in lactating cows.

Experimental work.-

The study encompassed the digestive-endocrine-metabolic effects of isoacids in lactating cattle. The measurements encompassed the concentration of volatile fatty

acids (VFA) in rumen fluid as an indication of digestive events. The endocrine variables analyzed were plasma insulin, growth hormone (GH) and cortisol. insulin and GH were analyzed because of their regulatory effects on nutrient partitioning and milk synthesis in ruminants (Bauman and Currie, 1980; Bines and Hart, 1982; Hart, 1983). Cortisol was measured because it affects the mobilization of endogenous protein (Spencer, 1985). Since aminoacids are gluconeogenic precursors (Young, 1977), an enhancement in the availability of aminoacids for gluconeogenic purposes would be beneficial for lactation.

Glucose was measured since it is an indicator of overall carbohydrate metabolism. In the lactating cow, glucose is also a major determinant of the rate of milk secretion (Bines and Hart 1982). PUN was measured since it would provide an indication of the status of nitrogen metabolism in the ruminant. Also, PUN levels have been shown to be affected by isoacid feeding (Felix et al, 1980b). As indicated in the specific objective, the variables will be measured in animals that exhibit a productive response to isoacid supplementation. The dosage was obtained from the analysis of productive effects in Experiment 1 and consists of 1.60% of the concentrate dry matter. In terms of the absolute amount, the expected intake , as described in chapter 2 , is a minimum of 160 g/d of the aqueous blend.

Distribution of experimental animals.-

For this study, multiparous cows in early lactation were assigned to two experimental groups of 4 cows each. Days into lactation and the average milk production of the 14 previous days was used as a criterion to distribute the animals into groups of similar stage of lactation and milk production. Table 9 shows the pretreatment means of milk yield, dry matter intake and feed efficiency. In the table, feed efficiency is calculated as kg milk produced/kg dry matter consumed.

TABLE 9.-Pretreatment means of milk yield, dry matter intake and feed efficiency * , **

	Control	Isoacids	SEM[a]
Milk Yield (kg/d)	35.67	35.52	3.40
Dry matter Intr' (kg/d)	22.14	23.23	1.66
Feed Efficiency	1.61	1.52	0.07

* Means (n = 4) of 14 days before treatment began
** Treatment means do not differ ($p < .05$)

Ration composition and isoacid dosage.-

The cows in this experiment were fed the same feed ingredients described in Chapter 2. The ration composition also was the same described in Chapter 2 for the first third of lactation. In this study, the control cows received the

control (0.0%) diet and the treated cows received the 1.6% isoacid diet. In this experiment, however, a different blend of isoacids was utilized.

In this case, the blend was an equal weight mixture supplemented as an aqueous blend (74% solids) of 25 % of the ammonium salts of each of the different acids. In terms of the molar proportions, the blend contains 28 % isobutyrate and 24 % of each of isovalerate, 2-methyl butyrate and valerate. This blend is the same that was used in the studies of Felix et al (1980a and 1980b). In those studies, the blend was shown to be efficacious in eliciting a productive response in lactating cattle. As described in Chapter 2 the level of supplementation of 1.6% of the concentrate dry matter should produce a minimum daily intake of 160 g of the isoacid blend.

Feeding and milking schedules.-

The protocol for feeding and milking was the same as the one described in Chapter 2. The diets in this experiment were fed for 31 days. For the purpose of analysis of production responses, the data of the first fourteen days of treatment was not included in the analysis since this period was allowed for adaptation to the diets. The productive responses included were calculated from averages of the 14 days following the adaptation period.

Plasma Samples.-

After the 28 th day of treatment, a catheter was placed in the jugular vein of the cows. The collection of plasma samples represented a stressful procedure that could affect the endocrine configuration of the cows. To minimize stress, the cows were sham-sampled the following day. The samples collected for analysis were taken 36 hours after inserting the cannulas. Serial samples were collected every 30 minutes for 9,5 hours. The plasma samples were taken beginning at 1030 h. of day 30. This sampling protocol included three sampling periods. Period 1 included 7 samples before feeding. Period 2 included 6 samples between feeding and milking and Period 3 included 6 samples taken after milking.

Approximately 15 ml of blood was collected in 20 ml heparinized tubes. Plasma was then obtained by centrifugation and immediately frozen in 2.5 ml aliquots. The samples were then kept frozen at -20 C until they were analyzed for hormones and metabolites.

Rumen fluid samples.-

Rumen fluid samples were collected with an esophageal cannula on day 31. The time of collection was 4 hours after feeding. This schedule was selected because fermentative events in the rumen are more intense during this period (Church, 1979). Approximately 250 ml of rumen fluid were collected. The fluid was then strained, acidified with 50%

formic acid, and kept frozen at -20 C until analysis of VFA. The determination of VFA concentrations in ruminal fluid was performed by gas chromatography, following the procedures of Ottenstein and Bartley (1971a) and Ottenstein and Bartley (1971b).

Analysis of hormones and blood metabolites.-

Hormone concentrations were analyzed by radioimmunoassay procedures validated for cattle. Growth hormone was measured using the GH kit kindly supplied by the National Hormone and Pituitary Program of the National Institute of Health. Plasma insulin was measured by a commercial solid-phase assay kit (Micromedic Systems Inc. Horsham, Pa) and Plasma cortisol was also measured with a commercial solid phase antibody kit (Micromedic Systems Inc. Horsham, Pa).

Plasma glucose was measured using a commercial kit for the coupled glucose oxidase and peroxidase method (Sigma Chemical Co. St. Louis Mo.). The analysis of Plasma Urea Nitrogen was performed with a commercial kit for the urease procedure of Berthelot (Sigma Chemical Co. ST. Louis, MO.) In all cases the values of hormones and plasma metabolites were confirmed by other laboratories in randomly selected samples.

Statistical analysis.-

The statistical analysis of the data was performed by

repeated measurement procedures of analysis of variance in the analysis of hormones and metabolites. For the analysis of ruminal volatile fatty acid values, comparisons between means were conducted by Student's T tests. In the analysis of milk yield, dry matter intake and feed efficiencies, the values were analyzed by analysis of covariance where the pre-trial values of milk yield, dry matter intake and feed efficiency were used as covariates. The model for the analysis was as follows.-

$$Y = U + Ti + B (Xij - \bar{X}) + Eij$$

Where

Y is the measured variable

U is the overall mean

Ti is the effect of the i treatment

B is the coefficient of the covariate

Xij is the observed value of the covariate for the i treatment

$\bar{X}$ is the overall mean of the covariate

Eij is the random error

Results and discussion.-

Production responses.-

The production response to the supplementation of the isoacid blend appears in Table 10. In the statistical model described in the previous section, the production data of the trial was adjusted using pretrial values as a covariate. The data shown in Table 9 consists of the adjusted means of kg of milk yield, kg of dry matter intake and feed efficiency. Feed efficiency is defined as kg milk/kg dry matter intake.

TABLE 10.-Effect of isoacids on milk production, dry matter intake and feed efficiency. *

	Control	Isoacids	SEM[a]
Milk Yield (kg/d)	33.72	36.67[d]	1.26
Dry matter intake (kg/d)	26.70	22.68[c]	1.03
Feed efficiency[b]	1.25	1.62[c]	0.07

* Means (n = 4 per group) of 14 days after adaptation period
a Standard error of treatment means
b Milk yield/Dry matter intake
c Treatment means differ ($p < .05$)
d Treatment means differ ($p .14$)

The results show that the addition of isoacids at 1.6% of the concentrate dry matter enhanced milk production by 2.95 kg/d. This represented a 8.7% increase over the control cows. The results are almost identical to the data for the same dosage in Chapter 2. However in this trial the

results were significant at p .14. In comparing results, it must be borne in mind that the number of animals per treatment was 4 in this study. This small number makes it more difficult to achieve significance at p <.1 than in the titration trial of the previous section. The response in feed intake shows that control cows consumed 4.02 kg/d more (p <.05) than isoacid-fed cows. In turn, the differences in milk yield and dry matter intake resulted in an improvement in feed efficiency of 22% in the isoacid group (p <.05). The effects in this trial are also a reflection of the ability of the animals to adapt to the experimental diets.

In the present experiment, the diet also contained on a dry basis, 50 % concentrate. From the data of table 10, this is equivalent to 11.43 kg of concentrate dry matter consumed daily. With a supplementation level of 1.6% of the concentrate dry matter, the absolute amount of isoacids consumed was 181 g of aqueous blend/day. This in turn represents 134 g of anhydrous ammonium salts of volatile fatty acids . Compared with the recommended amount of 89 g/d of AS-VFA (Papas et al , 1984), the levels suggested by the experiments in the present studies are at least 40 % higher. The results of the present chapter also support the suggestion that in diets that do not include urea, the amount of isoacids required for a response is higher than in urea-supplemented diets.

Plasma hormones and metabolites.-

Hormones.-

The plasma concentrations of growth hormone, insulin and cortisol appear respectively in Tables 11, 12 and 13. The tables include the comparisons between control and isoacid-supplemented cows during the prefeeding period (Period 1), the period between feeding and milking (Period 2) and the post milking period (Period 3). With regard to the number of plasma samples included in each period, Period 1 represents the mean of 7 serial plasma samples. Period 2 indicates the mean of 6 serial plasma samples, and period 3 represents 6 serial plasma samples. Included as the total is the overall mean of all samples for the treatment group in question.

In the case of growth hormone (GH), the results are described in Table 11.

TABLE 11.-Effect of isoacids on plasma levels of growth hormone (GH) *

	Control	Isoacids	SEM[a]
	ng / ml		
Period 1	4.89	4.79	.155
Period 2	5.05	4.71[b]	.062
Period 3	3.70	3.71	.143
Total	4.55	4.41	.051

* Means of samples obtained on day 29
a Standard error of treatment means
b Treatment means differ $p < .05$

The results show that with the exception of Period 2, isoacid supplementation had no effect on plasma GH levels. The reduced values in period two are .34 ng/ ml lower than the control values. This reduction represents less than 10% of the control mean and is not likely to be biologically relevant.

In animals treated with exogenous GH, the effects on milk production are associated with two or three fold increases in the daily mean GH concentration (Kik, 1987). It must also be noted here that the significance in this small difference arose partly because of a reduced standard error. This suggests that the significance may be an artifact of numerical manipulations. In regard to the other data shown, the prefeeding (Period 1), postmilking (Period 3) and total means were not different between treatments.

The physiological relevance of the pulsatile secretion of GH is well documented (Bauman and Mc Cutcheon, 1986), however, there were no significant differences in the pattern of secretion accross the serial plasma samples taken in this study.

The results of plasma insulin levels in control and isoacid-fed cows appear in Table 12.

TABLE 12 .- Effects of isoacids on plasma levels of insulin *,**

	Control	Isoacids	SEM[a]
	------ uU / ml ------		
Period 1	9.31	8.72	.36
Period 2	23.38	24.11	2.32
Period 3	21.82	25.85	3.29
Total	18.17	19.56	1.73

* Means of samples obtained on day 29
** Treatment means do not differ ($p < .05$)
a Standard error of treatment means.

The results indicate that mean plasma insulin levels did not differ between contol and isoacid-supplemented animals in any of the periods described. In both groups, the increases in the post feeding (Period 2) and post milking (Period 3) values are consistent with an insulin response to the uptake of nutrients in the postprandial period.

The results of plasma cortisol concentrations in control and isoacid-fed cows appear in Table 13.

TABLE 13.-Effect of isoacids on plasma levels of cortisol *

	Control	Isoacids	SEM[a]
	ng / ml		
Period 1	1.52	1.59	.076
Period 2	1.81	1.67	.027[b]
Period 3	1.34	1.35	.051
Total	1.56	1.54	.037

* Means of samples obtained on day 29
a Standard error of treatment means
b Means differ ($p < .05$)

The results indicate that overall, plasma cortisol was largely unchanged by isoacid supplementation. The small difference seen in Period two is .14 ng/ml and represents 10% of the mean values for the control cows in this period. It is unlikely that such a small difference over this period is physiologically relevant. As was the case with the differences seen in table 11, the significance seen here is partly the result of the smaller standard error of the mean. The values for the prefeeding (Period 1) and postmilking (Period 3) means do not differ. Likewise, the overall total mean is not different between treatments.

Plasma metabolites.-

With regard to the effect of isoacid-feeding on plasma glucose, the results appear in Table 14.

TABLE 14.-Effect of isoacids on plasma levels of glucose *

	Control	Isoacids	SEM[a]
	mg / ml		
Period 1	50.94	47.25[b]	.912
Period 2	46.91	40.23[c]	2.01
Period 3	46.30	40.10	2.79
Total	48.05	42.53[c]	1.32

* Means of samples obtained after 29 days of treatment
a Standard error of treatment means
b Difference between means was significant (p =.06)
c Treatment means differ (p <.05)

The results indicate a reduction in plasma glucose in the isoacid group. The reduced glucose levels were consistent over the three periods measured. The differences were significant (p <.05) in the post-feeding periods and in the total mean. In the prefeeding period (Period 1), the decreases were significant (p .06). The overall decreases in the isoacid group were 5.5 mg / dl and are equivalent to 15 % of the control group.

The effects of isoacid supplementation on plasma urea nitrogen (PUN) in control and isoacid-supplemented cows appear in Table 15.

TABLE 15.-Effect of isoacids on plasma levels of plasma urea nitrogen (PUN) *

	Control	Isoacids	SEM[a]
	------- mg / ml ------		
Period 1	12.22	12.71	1.21
Period 2	10.43	11.39	0.87
Period 3	12.15	14.82[b]	0.73
Total	11.60	12.97	0.84

* Means of samples obtained on day 29
a Standard error of treatment means
b Treatment means differ ($p<.05$)

Volatile fatty acids (VFA) analysis.-

The effects of isoacids on ruminal concentrations of VFA in control and isoacid treated cows appear in table 16.

TABLE 16.-Effect of isoacids on ruminal concentrations of volatile fatty acids (VFA) *

	Control	Isoacids	SEM[a]
	----- mmoles / dl ------		
Acetate	5.01	6.57[b]	0.19
Propionate	2.28	2.21	0.14
Isobutyrate	0.12	0.18	0.02
Butyrate	0.76	0.92	0.07
2-M. Butyrate	0.10	0.14	0.02
Isovalerate	0.13	0.20[c]	0.02
Valerate	0.08	0.12	0.02
Total	8.51	10.36[b]	0.29
Acetate/Propion.	2.21	2.97[b]	0.11

* Means (n = 4) of rumen fluid sampled 4 h. after feeding
a Standard error of treatment means
b Treatment means differ ($p < .01$)
c Treatment means differ ($p < .05$)

The results show that supplementation of the acids increased acetate concentrations ($p < .01$) by 1.56 mmoles/dl.This represents an enhancement of 31 % over controls. Isoacid addition also resulted in significant increases in the concentrations of isobutyrate ($p < .05$) and isovalerate ($p < .05$). The concentrations of butyrate and 2-methylbutyrate in isoacid-treated cows also showed numerical increases that approached significance. Total ruminal VFA were 21.7% higher ($p < .01$) in the treated cows.

Since the concentrations of propionate were not affected, the ratio of acetate to propionate was increased.

The effect of isoacids on ruminal VFA molar percentages in control and isoacid-supplemented cows are shown in Table 17.

TABLE 17.-Effect of isoacids on molar percentages of ruminal volatile fatty acids *

	Control	Isoacids	SEM[a]
Acetate	58.95	63.37[b]	0.70
Propionate	26.76	21.33[b]	1.02
Isobutyrate	1.42	1.73	0.17
Butyrate	9.09	8.96	0.93
2-M. Butyrate	1.20	1.42	0.20
Isovalerate	1.55	1.96	0.25
Valerate	1.02	1.22	0.18

* Means (n = 4) of rumen fluid sampled 4 h. after feeding
a Standard error of treatment means
b Treatment means differ p (<.01)

The results indicate that isoacids increased the molar percentages of acetate ($p < .01$) and reduced the molar percentages of propionate ($p < .01$). These changes came as a result of higher molar concentrations of acetate and total VFA in the isoacid group while propionate concentrations remained unchanged. It is important to underscore that the lower molar percentages of propionate represent a proportion of the total VFA. In terms of actual absolute amounts, as indicated in table 16, propionate remained unaffected by isoacid treatment.

Discussion.-

As indicated in the specific objective of this chapter, the goal of this experiment was to integrate production responses with digestive, hormonal and metabolic events. This objective requires an integrated discussion of the results.

The production responses with isoacids at 1.6% of the concentrate dry matter, essentially reproduced the results of the titration experiment. It is unlikely that the productive responses are due to the energy supplied by the isoacids per se. An equal weight mixture of isoacids contains approximately 6.5 kcal / g of gross energy (CRC, 1978). According to the tables for nutrient requirements of dairy cattle (National Research Council, 1978), even if this caloric density were entirely utilized as dietary energy, the amount of energy in 180 g of isoacids could not account for the production responses. The increased milk yield in this experiment allowed for the measurement of digestive and metabolic variables in animals that exhibited a reproducible production response within the same dietary conditions as those described in the previous chapter.

The results of the endocrine measurements indicate that the supplementation of isoacids did not change the concentrations of GH, insulin or cortisol. As explained previously, the small differences in GH and cortisol during Period 2 were partly due to the reductions in standard error in those means. The body of research in lactating cows indicates that the magnitude and time-frame of the

differences in period 2 are too small to account for a biological response (Hart and coworkers, 1980; Bauman and Mc Cutcheon, 1985; Kik, 1987). Moreover, the lower levels were found in the isoacid-fed group. The fact that milk yield was higher in this same group argues against a biological relevance for the small reduction in GH. group in Period 2. Overall, the total mean for plasma GH levels did not show a response to isoacid supplementation. In terms of GH responses, the present results do not support the report of Fieo et al (1984) who found increased GH levels in the plasma of isoacid treated cows. With regard to the data of Klusmeyer et al (1987), and Kik (1987) the present results support their reports that GH levels do not change in cows treated with isoacids. However, their results were obtained from cows that did not exhibit a significant productive response. The present data, on the other hand, was obtained in cows where isoacids did increase milk production and feed efficiency. This provides a more conclusive evidence that the increases seen in milk production in isoacid-fed cows are not related to plasma concentrations of growth hormone.

With regard to cortisol, the small differences of Period 2 in the isoacid group are not consistent with the lack of response in the other periods. Cortisol enhances the mobilization of endogenous reserves of protein (Hart, 1983; Spencer, 1985). The fact that plasma urea nitrogen (PUN) were not affected during this period argues against a physiological meaning for the differences in cortisol

levels.

The lack of effect on insulin concentration merits attention. Research has shown that plasma insulin levels in isoacid-fed cows are not increased (Kik, 1987; Klusmeyer et al, 1987). This is clearly beneficial for the lactating cow, as insulin draws nutrients away from the mammary gland of ruminants (Bines and Hart, 1982; Hart, 1983). The results of the present study support the previous reports of Kik (1987) and Klusmeyer et al (1987) with isoacid-fed cows. However, here again, as was the case with GH, the previous results were obtained in cows that did not show a clear productive response. In contrast, the results of the present study were obtained with cows that exhibited a productive response. This, as in the case of GH, provides more conclusive evidence that plasma concentrations of insulin are not increased in cows fed isoacids.

The lower levels of glucose in all periods studied are probably related to milk synthesis. Glucose is needed for lactose synthesis and is also a major regulator of the rate of milk secretion (Bines and Hart, 1982). The higher milk production found in the treated cows should have increased the glucose drain through the mammary gland. The lower glucose concentrations in the isoacid group are probably a reflection of the enhancement in milk yield.

The data on PUN levels show that isoacid-fed cows had higher PUN concentrations. The increases in PUN support the effect of isoacids on the enhanced mobilization of endogenous nutrient reserves seen in Chapter 1. Belyea et al

(1978) have shown that the lactating cow mobilizes endogenous protein reserves in the first third of lactation. The mobilized protein can be a source of aminoacids for gluconeogenic purposes (Young, 1977) or casein for milk synthesis (Hart, 1983). Although the data of Chapter 1 does not include body composition studies, it does support the suggestion that protein was being mobilized during the first third of lactation. In turn, this enhanced mobilization and turnover of endogenous protein should increase the concentration of urea in the blood. The increased PUN levels of the present experiment support the theory that isoacids enhance the mobilization of endogenous reserves of nutrients during the first third of lactation.

The effects on ruminal VFA concentrations also merit consideration in the overall metabolic effects of isoacids. The data from this chapter shows increases in ruminal acetate in the isoacid group. This confirms previous reports that isoacids increase acetate concentrations in lactating cattle (Felix et al, 1980; Quispe, 1982; Brondani, 1986). The increases in ruminal acetate and total VFA found in the present study suggest that isoacids increased cellulolytic activity and enhanced ruminal fermentation. The extra energy represented by the enhanced acetate levels has the advantage that acetate does not stimulate insulin secretion (Horino and coworkers, 1968; Bines and Hart, 1984). The fact that insulin is not increased means a higher availability of the extra energy for the lactating mammary gland. Additionally,

the lack of stimulation of insulin favors the enhanced ability of isoacid-fed cows to mobilize endogenous nutrients.

The lack of effect of isoacids on propionate concentrations also deserves attention. Propionate has been shown to increase insulin concentrations (Horino et al, 1968; Bines and Hart, 1982; Emmanuel and Kennelly, 1984). Additionally, intraruminal infusion of propionic acid has been shown to restrict feed intake in cattle (Elliot and collaborators, 1984). Therefore, in the lactating cow in early lactation it seems desirable to limit propionate production. Nevertheless, it must also be considered that propionate is a major gluconeogenic source (Young, 1977; Hart, 1983) This implies that a reduction in propionate might negatively affect the supply of glucose to the mammary gland. In view of the previous considerations, the unchanged propionate levels in the isoacid group are a convenient physiological compromise. In isoacid fed-cows, the enhanced acetate concentrations provide non-insulin-stimulating energy while the stable propionate levels insure a constant cluconeogenic supply.

Overall, the above effects provide an example of the interactions between digestive and metabolic effects. Additionally, the results underscore the importance that the nutrients have on the post absorptive distribution of energy.

CHAPTER FOUR

EFFECTS OF INDIVIDUAL ACIDS

Specific objective.-

To determine the productive and physiological responses to individual acids fed at the levels in which they are present in isoacid blends. Different blends of isoacids have been shown to produce similar increases in milk yield (Felix and collaborators, 1980b; Papas et al, 1984; Pierce-Sandner et al, 1985). This suggests that it is the total amount of isoacids and not the specific individual proportions that determine the biological effects. In support of this, Hlalo et al (1987) showed that when isobutyrate alone was fed at dosages comparable to whole blends, the magnitude of the productive responses were similar. However, there is a paucity of data on the effects of the other individual acids. It is necessary to ascertain the effect of supplementing individual acids at levels similar to those at which they are present in the blends.

Experimental design and dosages.-

In this study, multiparous lactating cows were fed either 0.0 or 40 g/d of individual acids. The acids studied

were isovaleric acid, 2-methylbutyric acid and phenylacetic acid. The isoacids were added as the hydrated ammonium salts (74%) solids. This represents 29.6 g of the anhydrous ammonium salts. Phenylacetic acid was added as 40 g of the free acid/day. As indicated before, the amount of isoacids represents the range in which they are present in the higher dosages of isoacid blends in chapters 2 and 3.

Experimental protocol.-

For this experiment, 4 cows were assigned to each treatment. The pretrial means of milk production, dry matter intake and feed efficiency were computed from the values of the 14 previous days. This pretrial data was used to assign the cows to treatments and reduce pretrial variation among treatments. The data was also used as a covariate to adjust the productive responses observed in the trial.

The pretrial means of days in lactation, milk yield, dry matter intake and feed efficiency of the cows in the control (C), isovalerate (IV), 2-methylbutyrate (2-MB) and phenylacetate (PA) groups are shown in Table 18. Feed efficiency was calculated as kg milk / kg dry matter intake.

TABLE 18.-Pretrial average days in lactation, milk yield, dry matter intake and feed efficiency of the cows assigned to the groups receiving the control (C), isovalerate (IV), 2-methylbutyrate (2-MB) and phenylacetate (PA) treatments *

	Treatments **				a
	C	IV	2-MB	PA	SEM
Cows/group	4	4	4	4	
Days in lact.	42.5	39.0	43.7	44.7	5.35
Milk Yield (kg/d)	36.4	35.4	35.4	34.9	2.35
Dry matter Intake (kg/d)	21.6	20.3	21.8	20.4	0.99
Feed Efficiency b	1.67	1.73	1.62	1.76	0.47

* Mean values (n = 4) of 14 days before supplementation of the acids
** Means among treatments are not different ($p < .05$)
a Standard error of treatment means
b Kg Milk/kg Dry matter intake

Ration composition and feeding schedule.-

The diets utilized were the same that were described in chapter 2 for the control (0.0% isoacids) and the lower treatment (0.4% isoacids). The only modification made was that in this trial, the acids were added as top dressing in the supplement. The change was done to monitor the consumption of the isoacids and insure that the complete dosage would be consumed. Control cows received the same amount of protein supplement as top dressing.

Daily feeding schedule and management was the same as that described for the titration experiment in chapter 2. The experimental rations were fed for 30 days. As in the case of chapter 2. A two week period was allowed for adjustment to the diets. The data included in the evaluation

of the production responses was collected for 13 days after this adjustment period.

Analysis of plasma hormones and metabolites.-

The hormones measured in this experiment were growth hormone and insulin. The plasma metabolites analyzed were glucose and plasma urea nitrogen. The sampling protocol was similar to the one used in the previous experiment. However, changes were made in that plasma samples were collected on day 29. The plasma samples were collected serially every 30 minutes for 7.5 hours. The 15 samples thus collected included 4 prefeeding samples (Period 1), 6 samples collected between feeding and milking (Period 2) and 5 post milking samples (Period 3).

Ruminal volatile fatty acids.-

Ruminal fluid samples were collected on day 30, as described in chapter 2.

Statistical Analysis.-

The data from this trial was analyzed according to the analysis of variance procedures described in chapter 2. In cases where there were significant differences among means, the comparisons among means were conducted according to Dunett's test for differences among means.

Results.-

The productive responses of this experiment appear on Table 19. The data shows the adjusted means of milk yield, dry matter intake and feed efficiency in the four treatment groups

TABLE 19.-Effect of isovaleric acid (IV), 2-methyl butyric acid (2MB) and phenylacetic acid (PA) on milk yield, feed intake and feed efficiency *

	Treatments **				a
	C	IV	2-MB	PA	SEM
Milk Yield (kg/d)	34.0	34.4	33.0	32.7	0.95
Dry Matter Intake (kg/d)	22.1	22.4	22.5	23.3	0.54
Feed Efficiency b	1.55	1.52	1.48	1.37	.045

* Mean (n = 4) of 13 days after 2 weeks of adaptation to the acids.
** Means among treatments are not different ($p < .05$)
a Standard error of treatment means
b Kg Milk/kg dry matter intake

The data indicates that the addition of the acids did not affect any of the variables measured. This lack of effect shows that the effects on milk yield seen in the previous chapters are not due to individual acids. This data also implies that it is the total amount of isoacids that determines the enhancement in milk yield seen in chapters 2 and 3 with dosages of 1.6% of the concentrate dry matter.

Plasma Hormones and metabolites.-

The effects of the acids on plasma concentrations of hormones and metabolites appear in Tables 20 through 23. Table 20 shows the responses of growth hormone (GH) in the different treatments.

TABLE 20.-Effect of isovaleric acid (IV), 2-methylbutyric acid (2-MB) and phenylacetic acid (PA) on plasma concentrations of growth hormone (GH) *

	Treatments **				a
	C	IV	2-MB	PA	SEM
	ng / ml				
Period 1	5.03	4.16	4.21	5.68	.758
Period 2	4.30	4.21	4.03	4.72	.397
Period 3	3.51	4.06	4.12	3.99	.408
Total	4.28	4.14	4.12	4.80	.414

* Means of plasma samples obtained on day 28
** Means among treatments did not differ ($p < .05$)
a Standard error of treatment means.

The results indicate that plasma concentrations of GH were not affected by treatment with any of the acids. In the case of isovaleric acid and 2-methylbutyric acid, the data is in agreement with the results of the previous chapter. In the case of phenylacetic acid, the data can only indicate that there is no response at the present dosage. The possibility remains that higher dosages may elicit different responses

The effects of treatment on the plasma levels of insulin appear on Table 21.

TABLE 21.-Effect of isovaleric acid (IV), 2-methylbutyric acid (2-MB) and phenylacetic acid (PA) on plasma concentrations of insulin *

	Treatments				
	C	IV	2-MB	PA	SEM a
	uU / ml				
Period 1	10.00	9.78 b	9.42 b	11.90 b	1.29
Period 2	26.88	14.75 c	14.48 c	15.52 c	1.52
Period 3	20.93	14.65 b	13.06 b	13.77 b	1.67
Total	19.27	13.06	12.32	13.73	2.31

* Means of plasma samples obtained on day 28
a Standard error of treatment means
b Treatment means with different superscript differ p <.01
c Treatment means with different superscript differ p <.05

The data shows that plasma concentrations of insulin were reduced by all treatments in the postprandial periods. This suggests that the uptake of nutrients in the treatment groups did not stimulate insulin secretion as much as in the control. This effect in turn is consistent with the role of isoacids in stimulating the release of non-insulin stimulating energy from the diet. In the case of phenylacetic acid, the effects can only be interpreted within the limit of this dosage and type of diet.

Plasma metabolites.-

The effects of treatment on plasma concentrations of glucose appear on Table 22.

TABLE 22.-Effect of isovaleric acid (IV), 2-methylbutyric acid (2-MB) and phenylacetic acid (PA) on plasma concentrations of glucose *

	Treatments **				a
	C	IV	2-MB	PA	SEM
	mg / dl				
Period 1	51.35	50.14	51.41	51.60	3.00
Period 2	54.65	54.15	55.79	51.42	2.96
Period 3	56.69	61.71	62.62	58.14	3.21
Total	54.23	55.33	56.60	53.72	2.89

* Means of plasma samples obtained on day 28
** Treatment means do not differ p <.05
a Standard error of treatment means

The data indicates that plasma glucose was not influenced by treatment with any of the acids. This lack of response was consistent at all periods and in the total mean. The data of chapter 3 did show a reduction in plasma glucose in cows fed higher amounts of isoacids. The data of chapter 3 also indicated an enhancement of milk production in the isoacid-fed cows. The results of this experiment support the observation that plasma glucose levels are not affected if milk production is not changed by isoacids. This implies that plasma glucose levels are a reflection of the production response elicited by isoacids.

The effects of treatment on plasma urea nitrogen concentrations are shown in Table 23.

TABLE 23.-Effect of isovaleric acid (IV), 2-methylbutyric acid (2-MB) and phenylacetic acid (PA) on plasma concentrations of plasma urea nitrogen (PUN) *

	Treatments **				a
	C	IV	2-MB	PA	SEM
	mg / dl				
Period 1	7.35	7.96	8.39	7.00	.64
Period 2	7.49	8.36	8.72	7.09	.62
Period 3	7.13	7.89	8.86	7.08	.78
Total	7.32	8.07	8.66	7.05	.66

* Means of samples obtained on day 28.
** Treatment means do not differ ($p < .05$)
a Standars error of treatment means.

The data shows that PUN concentrations were not influenced by treatment with any of the acids. Here again, the lack of response must be interpreted considering that there was not a production response. The glucose and PUN data suggest that changes in these variables are affected by the productive response to isoacid supplementation. This in turn reduces the likelihood that these plasma metabolites are affected if milk production remains unchanged.

Ruminal volatile fatty acids.-

The effects of the different treatments on ruminal concentrations of volatile fatty acids (VFA) are shown in Table 24. The percentage molar proportions of ruminal VFA appear on table 25.

TABLE 24.-Effect of isovaleric acid (IV), 2-methylbutyric acid (2-MB) and phenylacetic acid (PA) on concentrations of ruminal volatile fatty acids *

	Treatments **				a
	C	IV	2-MB	PA	SEM
	mmoles / dl				
Acetate	6.45	6.92	6.68	6.50	.49
Propionate	2.31	2.47	2.51	2.22	.18
Isobutyrate	0.16	0.15	0.17	0.18	.02
Butyrate	1.02	1.15	1.14	1.20	.15
2-M. Butyrate	0.10	0.12	0.13	0.10	.02
Isovalerate	0.11	0.14	0.12	0.12	.02
Valerate	0.12	0.11	0.12	0.15	.02
Total	10.29	11.08	10.89	10.50	.49
Acetate/propion.	2.85	2.89	2.72	2.91	.29

* Means (n = 4) of rumen fluid sampled 4 h after feeding
** Means among treatment groups do not differ ($p < .05$)
a Standard error of treatment means.

TABLE 25.-Effect of isovaleric acid (IV), 2-methylbutyric acid (2MB), and phenylacetic acid (PA) on molar percentages of ruminal volatile fatty acids *

	Treatments **				a
	C	IV	2-MB	PA	SEM
Acetate	62.7	62.3	61.1	61.5	2.50
Propionate	22.2	22.4	23.1	21.1	1.76
Isobutyrate	1.66	1.38	1.63	1.72	0.27
Butyrate	9.9	10.4	10.5	11.8	1.69
2-M. Butyrate	1.00	1.09	1.24	1.02	0.22
Isovalerate	1.17	1.33	1.10	1.21	0.21
Valerate	1.18	1.06	1.08	1.48	0.24

* Means (n = 4) of rumen fluid sampled 4 h after feeding
** Treatment means do not differ ($p < .05$)
a Standard error of treatment means.

The data show that neither the ruminal concentrations nor the molar proportions of VFA were changed by treatment with any of the acids. The lack of response in VFA in this trial suggests that the amount of acids supplemented was not enough to influence fermentative events in the rumen. This also helps explain why production variables were not affected by the addition of 40 grams of the acids in this experiment.

Discussion.-

The addition of 40 grams of acids did not increase the production of the treated animals. similar results were seen in the titration experiment (chapter 1) when the blend

of isoacids was added at similar amounts. On the other hand, when higher dosages were supplemented in the experiments of chapters 2 and 3, the production response was clear. The amount of acids added in this experiment was the equivalent of the individual isoacids in the dosages that elicited the production responses of the previous trials. However, in this experiment there were no increases in milk yield. This suggests that it is not an individual acid, but the total amount in the blends that determined the production response. This data also supports the findings of Hlalo et al (1987) that isobutyrate alone can elicit a productive response if it is fed at levels comparable to the higher dosages of the blends. Their data also shows that isobutyrate did not affect milk yield when it was added at lower dosages. In this regard, further research is needed to determine the dose responses of the isoacids utilized in the present study. This could lead to the development of less expensive blends if production responses are found at higher levels of individual acid supplementation.

The endocrine responses also merit discussion. The unchanged levels of plasma GH are consistent with the data of chapter 3, and with a number of reports in the literature (Brondani, 1986; Kik, 1987; Klusmeyer et al, 1987). The insulin results however, do not agree with the data of chapter 2. Additionally, while some reports indicate that insulin is not affected by isoacids (Kik, 1987; Klusmeyer et al, 1987) other data (Brondani, 1986) show reductions in plasma insulin in isoacid-supplemented

ruminants. Brondani (1986) explained the lower insulin levels as a result of reduced propionic acid concentrations in the rumen. In this experiment that was not the case, since propionic acid concentrations were not reduced by the acids. Brondani (1986) suggested that insulin secretion may be regulated by neural paths that are influenced by isoacids. The data of the present study lend preliminary and peripheral support to that proposition. The enhancements in acetate production described in the previous chapter, are a form of non-insulin stimulating energy. However, that does not rule out a ruminal or hepatic effect of isoacids on pancreatic function. The ability of propionate to stimulate insulin secretion is well documented (Horino, 1968; Bines and Hart, 1982; Elliot and coworkers, 1984). This effect is regulated in all likelihood, by ruminal or hepatic pathways, as propionic acid is effectively removed from the circulation by the ruminant liver (Ricks and Cook, 1980). This suggests the possibility that there may be similar pathways for the inhibition of insulin secretion by isoacids Clearly this is an area of physiological relevance that needs further research.

With regard to the plasma levels of glucose, the lack of response to the acids ocurred in animals where milk production was not increased. This suggests that the lower glucose levels seen in the isoacid group in the previous chapter may have reflected the increased utilization of glucose by the mammary gland. In the present study, milk

production was not affected by the acids and therefore, the utilization of plasma glucose should have been similar.

A similar case may be made for the PUN results. In the present study, PUN levels were not affected. The addition of acids in this experiment did not increase milk yields. The similar rates of protein turnover were probably reflected in the similar PUN levels found in the different groups.

Volatile fatty acid concentrations were not affected by the acids. This suggests the dosage was too small for a ruminal effect. Here again, it is interesting to note that there was not a productive response. Overall the data suggests that the production responses and endocrine-metabolic effects are largely determined by the ruminal effect. This is also evident in the report of Klusmeyer et al (1987). Their results are mostly negative in terms of production responses and endocrine and metabolic variables. However, it is interesting to mention that in their study, the supplementation of isoacids did not affect ruminal acetate or increased ruminal isoacid levels. Overall , the present results emphasize the importance of feeding the adequate dosage in the production of physiological effects.

CONCLUSIONS

The results of the dose-titration trial show that, in diets unsupplemented with urea, the production response to isoacids requires over 40% more isoacids than was previously indicated in studies in which urea was used. The discrepancy in dosages underscores the importance of diet composition in obtaining a production response to the addition of isoacids. The higher amount required also underscores the need for additional research. Further work is necessary, particularly on the relationship between isoacid utilization by rumen microbes and the pattern of ammonia release with different sources of nitrogen.

The dose-titration responses also showed that milk yield was enhanced only with the higher isoacid dosage (1.6% of the concentrate dry matter). This suggests that the results may have only showed the lower end of the production response. Additional dose-response research is needed to determine if the increases in milk yield can still be further augmented by higher dosages of isoacids. The results indicated that the response was higher during the first third of lactation, and that high isoacid intakes must be maintained throughout lactation. In these observations, the data of the present work is in agreement with the body of previous research.

The effects of isoacids on the enhancement of body

weight loss during the first third of lactation suggest that isoacids enhance the ability of the animal to mobilize endogenous nutrients. This implies an effect on the post-absorptive distribution of energy.

The data showing that isoacids increase ruminal acetate concentrations and total volatile fatty acid levels are in agreement with the effects of isoacids on the growth and activity of cellulolytic bacteria.

The results on the concentrations of insulin suggest that the increases in acetate represent a form of energy that does not trigger an insulinogenic response. A direct effect of isoacids in reducing insulin concentrations cannot be ruled out by the data of the last two chapters. However, it is clear that the ability of isoacids to increase non-insulin-stimulating energy in the form of acetate is a major part of the mechanism of action of isoacids.

The results obtained with individual acids supplemented at 40 g/d can only be interpreted within the limitations of that low dosage. The dosage represents the amounts in which the individual acids are present in isoacid blends. The results suggest that the production responses seen with the isoacid blends are not due to the individual amounts of isovalerate or 2-methylbutyrate. However, it is also clear that additional research is needed with higher dosages of individual acids.

Overall, the data indicates that the ruminal effects of isoacids have endocrine and metabolic implications. The

body of research in lactating cattle shows that it is advantageous to have low insulin concentrations. During early lactation, when energy intake and the mobilization of endogenous nutrients are critical, it is important to avoid stimulating insulin secretion. The fact that acetate does not stimulate insulin secretion is clearly advantageous. In enhancing the concentrations of acetate, isoacids increase the supply of energy in a form that is uniquely well suited for the metabolic requirements of the lactating cow.

LIST OF REFERENCES

Allison, M.J. 1969. Biosynthesis of amino acids by ruminal microorganisms. J. Anim. Sci. 29:797.

Allison, M.J. and M.P. Bryant. 1963. Biosynthesis of branched-chain fatty acids by rumen bacteria. Arch. Biochem. Biophys. 101:269.

Allison, M.J., M.P. Bryant and R.N. Doetsch. 1958. Volatile fatty acid growth factor for cellulolytic cocci of bovine rumen. Science. 128:474

Allison, M.J., M.P. Bryant and R.N. Doetsch. 1962.Studies on the metabolic function of branched-chain VFA growth factors for Ruminococci. I. Incorporation of isovalerate into leucine. J. Bacteriol. 85:523.

Annison, E.F. 1954. Some observations on the volatile fatty acids in the sheep rumen. Biochem. J. 57:400.

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.

Bauman, D.E. and S.N. McCutcheon. 1986. The effects of growth hormone and prolactin on metabolism. In: Control of Digestion and Metabolism in Ruminants L.P. Milligan, W.L. Grovum and A. Dobson (eds). Prentice-Hall, Englewood Cliffs. , NJ, USA, p. 436.

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.

Bentley, O.G., L. Alfred, R.R. Johnson, T.V. Herschberger A.L. Moxon. 1954. The cellulolytic factor activity of certain short-chain fatty acids. Am. Chem. Soc. J. 76:5000.

Bentley, O.G., R.R. Johnson, T.V. Herschberger, J.H. Cline and A.L. Moxon. 1955. Cellulolytic-factor activity of certain short-chain fatty acids for rumen microorganisms in vitro. J. Nutr. 57:389.

Bergen, W.G. and M.T. Yokoyama. 1978. Productive limits to rumen fermentation. J. Anim. Sci. 46:573.

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.

Bines, J.A. and I.C. Hart. 1984. The response of plasma insulin and other hormones to the intraruminal infusion of VFA mixtures in cattle. Can. J. Anim. Sci. 64:304 (Suppl).

Blumer, V.E.,C.H. Dougherty Jr., E.L. Herring and A.K. Clark. 1986. Milk production response to calcium salts of volatile fatty acids in dairy cattle rations when introduced at various stages of lactation. 64 th annual meeting of the Southern Branch, American Dairy Science Association. Nashville, Tenn.

Brondani, A.V. 1986. A Study on the action of teichomycin A2, avoparcin and isoacids in sheep. Ph.D. Thesis. Michigan State University. E. Lansing, Mich.

Bryant, M.P. 1973. Nutritional requirements of the predominant rumen cellulolytic bacteria. Fed. Proc. 32:1809.

Bryant, M.P. and I.M. Robinson. 1961. Some nutritional requirements of the genus Ruminococcus. Appl. Microbiol. 9:91.

Bryant, M.P. and R.N. Doetsch. 1955. Factors necessary for the growth of Bacteroides succinogenes in the volatile fatty acid fraction of rumen fluid. J. Dairy Sci. 38:340

CRC Handbook of Chemistry and Physics. 1978. Weast, R.C. (ed). 58 th edition. CRC Press Inc. Cleveland Oh.

Church, D.C. 1979. Ruminal fermentation of feedstuffs. In: Digestive Physiology and Nutrition of Ruminants. Vol. I. 2nd edition. p. 266. O & B Books. Corvallis, Ore.

Chalupa, W. 1968. Problems in feeding urea to ruminants. J. Anim. Sci. 27:207.

Cline, T.R., U.S. Garrigus and E.E. Hatfield. 1966. Addition of branched-chain and straight-chain volatile fatty acids to purified lamb diets and effects on utilization of certain dietary components. J. Anim. Sci. 25:734.

Cook, R.M. 1985. Isoacids, a new feed additive for lactating dairy cows. In: Proceedings 1985 Maryland Nutrition Conference for Feed Manufacturers. J.A. Doer. (ed)

Cummins, K.A. and A.H. Papas. 1985. Effect of isocarbon-4 and isocarbon-5 volatile fatty acids on microbial protein synthesis and dry matter digestibility in vitro. J. Dairy Sci. 68:2588.

Dehority, B.A., O.G. Bentley, R.R. Johnson and A.L. Moxon. 1957. Isolation and identification of compounds from autolyzed yeast, alfalfa meal and casein hydrolyzatewith cellulolytic factor activity for rumen microorganisms in vitro . J. Anim. Sci. 16:502.

Dehority, B.A., R.R. Johnson, O.G. Bentley and A.L. Moxon. 1958 Studies on the metabolism of valine, proline, leucine and isoleucine by rumen microorganisms in vitro. Arch. Biochem. and Biophys. 78:15.

Dehority, B.A., H.W. Scott and P. Kowaluk. 1967. Volatile fatty acid requirements of cellulolytic rumen bacteria. J. Bacteriol. 94:537.

El-Shazly, K. 1952. Degradation of protein in the rumen of sheep. 2. The action of rumen micro-organisms on amino acids. Biochem. J. 51:647.

Eldsen, S.R. and D.Lewis. 1953. The production of fatty acids by a gram negative coccus. Biochem. J. 55:183.

Elliot, J.M., H.W. Symonds and B. Pike. 1984. Effect on feed intake of infusing sodium propionate or sodium acetate into a mesenteric vein of cattle. J. Dairy Sci. 68:1165

Emmanuel, B. and J.J. Kennelly. 1984. Effect of intraruminal infusion of propionic acid on plasma metabolites in goats. Can. J. Anim. Sci. 64:295 (Suppl).

Felix, A., R.M. Cook and J.T. Huber. 1980a. Effect of feeding isoacids with urea on growth and nutrient utilization by lactating cows. J. Dairy Sci. 63:1943.

Felix, A., R.M. Cook and J.T. Huber. 1980b. Isoacids and urea as a protein supplement for lactating cows fed corn silage. J. Dairy Sci. 63:1103.

Fieo, A.G., T.F. Sweeney, R.S. Kensinger and L.D. Miller. 1984. Metabolic and digestion effects of the addition of the ammonium salts of volatile fatty acids to the diets of cows in early lactation. J. Dairy Sci. 67 (Suppl.1):117 (abs).

Goering, H.K. and P.J. Van Soest. 1970. Forage fiber analyses. US Dep. Agric., Agric. Handbook 379, Washington, DC.

Gorosito, A.R., J.B. Russell and J.P. van Soest. 1985. Effect of carbon-4 and carbon-5 volatile fatty acids on digestion of plant cell wall in vitro. J. Dairy Sci. 68:840.

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

Hart, I.C., J.A. Bines and S. Morant. 1980. The secretion and metabolic clearance rates of growth hormone, insulin and prolactin in high- and low- yielding cattle at four stages of lactation. Life Sci. 27:1839.

Hoover, W.H. Chemical factors involved in ruminal fiber digestion. J. Dairy Sci. 69:2755.

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

Hlalo, D., P. Machado and R.M. Cook. 1987. Effects of individual isoacid (isobutyrate) on lactating Holsteins. J. Dairy Sci. 70 (Suppl 1):216 (Abs).

Kik, N.A. 1987. The effect of bovine somatotropin, iso-plus and a combination of the two in early lactating Holsteins. M.S. Thesis. Michigan State University. E. Lansing, Mich

Klusmeyer, T.H., J.H. Clark, J.L. Vicini, M.R. Murphy and G.C. Fahey Jr. 1987. Effects of feeding or infusing ammonium salts of volatile fatty acids on ruminal fermentation, plasma characteristics and milk production of cows. J. Dairy Sci. 70:50.

Kone, P., P. Machado and R.M. Cook. 1986. Effect of monensin and isoacids on in vitro rumen fermentation. J. Dairy Sci. 69 (Suppl. 1):156 (Abs).

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

Laarveld, B., D.A. Christensen abd R.P. Brockman. 1981. The effect of insulin on net metabolism of glucose and amino acids by the bovine mammary gland. Endocrinology. 108:2217.

Lassiter, C.A., R.S. Emery and C.W. Duncan. 1958a. Effect of alfalfa ash and valeric acid on growyh of dairy heifers. J. Dairy Sci. 41:552.

Lassiter, C.A., R.S. Emery and C.W. Duncan. 1958b. The effect of valeric acid and a combination of valeric and iso-valeric acids on the performance of milking cows. J. Anim. Sci. 17:358.

Loosli, J.K., H.H. Williams, W.E. Thomas, F.H. Ferris and L.A. Maynard. 1949. Synthesis of amino acids in the rumen. Science 110:114.

McDaniel, B.T., R.H. Miller and E.L. Corey. 1965. DHIA factors for projecting incomplete records to 305 days. Dairy Herd Improvement Letter. ARS-44-164. August 1965.

Miura, H., M. Horiguchi and T. Matsumoto. 1980.Nutritional interdependence among rumen bacteria Bacteroides amylophylus, Megasphera eldsenii and Ruminococcus albus. Appl. Environ. Microbiol. 40:294.

Moore, C.L., R.A. Mohr and K.S. Kreier. 1987. Effects of IsoPlus on mid lactation and early lactation Holstein cows. J. Dairy Sci. 70 (Suppl. 1):216 (Abs).

National Research Council. 1978. Nutrient requirements of domestic animals. No. 3. Nutrient requirements of dairy cattle. 5 th rev. ed. Natl. Acad. Sci., Washington, DC.

Newman, D., J.A. Rogers, A.K. Clark and A.C. Spearow. 1986. Milk production response of dairy cows fed calcium salts of volatile fatty acids on 34 commercial dairy farms. J. Dairy Sci. 69 (Suppl 1):158 (Abs).

Oltjen, R.R., L.L. Slyter, E.E. Williams and D.L. Kern. 1971. Influence of branched-chain volatile fatty acids and phenylacetate on ruminal microorganisms and nitrogen utilization by steers fed urea or isolated soy proteins. J. Nutr. 102:479.

Ottenstein, D.M., and D.A. Bartley. 1971a. Separation of free acids C2-C5 in dilute aqueous solution column technology. J. Chromatog. Sci. 9:673.

Ottenstein, D.M., and D.A. Bartley. 1971b. Improved gas chromatography separation of free acids C2-C5 in dilute solutions. Anal. Chem. 43:952.

Owens, F.N. and W.G. Bergen. 1983. Nitrogen metabolism of the ruminant animal. J. Anim. Sci. 57 (Suppl 2):498.

Papas, A.M., S.R. Ames, R.M. Cook, C.J. Sniffen, C.E. Polan and L. Chase. 1984. Production responses of dairy cows fed diets supplemented with ammonium salts of isoC-4 and C-5 acids. J. Dairy Sci. 67:276.

Pierce-Sandner, S.B., A.M. Papas, J.A. Rogers, T.F. Sweeney, K.A. Cummins, H.R. Conrad and L.D. Muller. 1985. Supplementation of dairy cow diets with ammonium salts of volatile fatty acids. J. Dairy Sci. 69:2895.

Quispe-Salas, M.E. 1982. A study of the effects of isoacids, urea and sulfur on the rate of fermentatioin in the rumen. M.S. Thesis. Michigan State University. E. Lansing, Mich.

Ricks, C.A. and R.M. Cook. 1981. Regulation of volatile fatty acid uptake by mitochondrial acyl CoA synthetase. J. Dairy Sci. 64:2236.

Rogers, J.A. and R.M. Cook. 1986. Effect of ammonium salts of VFA on milk production of dairy cows. I. Commercial farm trials-1982. J. Dairy Sci. 69 (Suppl. 1):157 (Abs).

Rogers, J.A., D. Newman. and R.M. Cook. 1986. Effect of calcium salts of VFA on milk production of dairy cows. II. Commercial dairy trials-1982. J. Dairy Sci. 69 (Suppl. 1):157 (Abs).

Russell, J.B. and C.J. Sniffen. 1984. Effect of carbon-4 and carbon-5 volatile fatty acids on growth of mixed rumen bacteria in vitro. J. Dairy Sci. 67:987.

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

Spencer, G.S.G. 1985. Hormonal systems regulating growth. A review. Livestock Production Science. 12:31.

Stack, R.J., R.E. Hungate and W.P. Opsahl. 1983. Phenylacetic acid stimulation of cellulose digestion by Ruminococcus albus 8. Appl. Environ. Microbiol. 46:539.

Stanton, T.B. and E. Canale-Parola. 1980. Treponema bryantii sp. a rumen spirochete that interacts with cellulolytic bacteria. Arch. Microbiol. 127:145.

Towns, R. and R.M. Cook. 1984. Isoacids, a new growth hormone releasing factor. AAAS Annual Meeting, New York, NY. (Abs. No 347).

Varga, G.A., W.H. Hoover, L.L. Junkins and B.J. Shriver. 1988. Effects of urea and isoacids on in vitro fermentation of diets containing formaldehyde-treated or untreated soybean meal. J. Dairy Sci. 71:737.

Van Gylswik, N.O. 1970. The effect of supplementing a low-protein hay on the cellulolytic bacteria in the rumen of sheep and on the digestibility of cellulose and hemicellulose. J. Agric. Sci. Camb. 74:169.

Wegner, E. 1962. The metabolic fate of fatty acids required by certain rumen bacteria. Ph. D. Thesis. University of Wisconsin. Madison, Wis.

Young, J.W. 1977. Gluconeogenesis in cattle: significance and methodology. J. Dairy Sci. 60:1.

Zuntz, N. 1891. Pflug. Arch. F. Physiol. 49:477.