FACTORS ASSOCIATED WITH MILK FAT SECRETION OF COWS IN RESPONSE TO CONTRASTING AVAILABLE ENERGY CONSUMPTION

by

Demetrio Zanartu

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

DOCTOR OF PHILOSOPHY

in

Animal Sciences

APPROVED

C. E. Polan, Chairman

R. E. Blaser

K. E. Webb

G. M. Jones

M. L. McGilliard

J. M. White

June, 1979

Blacksburg, Virginia

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to the following persons.

Dr. C. E. Polan for his continued assistance and counsel through the graduate program. His suggestions in the preparation of this manuscript are greatly appreciated.

Dr. R. E. Blaser, Dr. K. E. Webb, Dr. M. L. McGilliard, Dr. G. M. Jones, and Dr. J. M. White for their suggestions in the preparation of this manuscript and advice and service as members of the graduate committee.

Mr. C. N. Miller and his staff at the Dairy Research Center for his help during the development of these studies.

Dr. G. W. Niehaus for his help during the third experiment.

Special appreciation is expressed to his wife, , for her sacrifice and continued and invaluable encouragement during his graduate program.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Lipid metabolism with dairy cows has been studied for a number of years, but still there are no clear answers regarding the metabolic alterations involved in the dramatic decline in milk fat production and increase in body weight gains when energy density is increased or physical form of the ration altered.

The importance of keeping a normal fat content is well known. Low fat indicates a poor efficiency of energy utilization for milk production, resulting in more deposition of energy into the adipose tissue than the milk.

Where fluid milk is being priced on milk solids criteria, it is still important to keep a normal fat test from an economical and metabolical standpoint.

A number of theories have been proposed to explain alterations in lipid metabolism. An acetic acid shortage may be caused by the replacement of cell wall digesting bacteria by bacteria that digest cellular content producing relatively more propionic acid than acetic acid. It is known that approximately 40% by weight of fatty acids associated with ruminant milk fat are synthesized in the mammary gland. Also, ruminants utilize acetic and butyric acid as primers in fatty acid synthesis. The importance of this concept has been curtailed by studies showing that exogenous acetic acid contribution showed no important drop during low milk fat production (49).

It has been suggested that antiketogenic properties of propionic acid reduce ketone bodies in peripheral blood, thereby reducing the

primers in fatty acid synthesis. Even though this may occur to a small degree, it can be ignored.

Hormonal shifts that occur due to changes in amounts of glucogenic compounds and glucose have been proposed to explain alterations in lipid metabolism. Insulin, as well as growth hormone, thyroxine and epinephrine, may be involved in the regulation of the balance between lipolysis and lipogenesis. Even though this seems to be an explanation, contradictory results have been obtained when testing this theory. The discrepancy may be explained because concepts of mono-gastric hormonal control of lipid metabolism were applied to ruminants.

Our laboratory pursued the concept that the energy status of the animal was involved in milk fat depression as proposed by Jenny, et al. (41). In this research, cows in early (45 days) lactation were compared with cows in mid lactation (150 days) for responses to milk fat depression, when fed high concentrate diets ad libitum. Milk fat depression was lower for cows in early lactation than for those in mid lactation.

However, the changes in lipid metabolism are not explained adequately by any of the theories; therefore, it appeared desirable to investigate the association of other metabolic alterations with lipid metabolism when feeding milk fat depressing rations. Furthermore, conducting the research at a specific physiological stage would control some of the variables that affect lipid metabolism and improve the validity of the data.

The overall objectives of this research were:

1. To further understand the regulation of lipid metabolism, specifically milk fat production, as influenced by the concentrations and ratios of rumen fermentation products, blood metabolic parameters and ration characteristics.
2. To investigate the role of total fermentable energy on milk fat depression.
3. To monitor the effect and interaction of the variables during different stages of lactation.
4. To measure changes in different enzymes involved in fatty acid synthesis when rations with varying amounts of fermentable energy are fed to dairy cows.

LITERATURE REVIEW

Energy metabolism in the lactating cow

Mammary tissue is one of the most active tissues in the body with regard to lipid synthesis. The principal source of carbon for fatty acid synthesis in nonruminants is glucose. Studies conducted by Folley and French (26, 27) demonstrated marked differences between ruminant and nonruminant mammary tissue with regard to the kind of substrates used for de novo fatty acid synthesis. This was also found to be true for adipose tissue of ruminants compared with liver tissue of nonruminants (8). The inability of ruminants to use glucose as a source of carbon has been demonstrated in liver and adipose tissue slices (31) and perfused mammary gland (32). Cow and sheep liver showed negligible activity for the enzymes ATP citrate lyase (EC. 4.1. 3.8) and NADP malate dehydrogenase (EC. 1.1.1.40), which explains ruminants inability to utilize glucose.

Hardwick (32) explained that glucose is not an important source of carbon for fatty acid synthesis due to a compartmentation of acetyl CoA based on its low diffusion through the mitochondrial membrane. Pyruvate is oxidized to acetyl CoA only inside the mitochondrion and this must be changed into a more diffusible compound in order to exit. Citrate, on the other hand, formed from acetyl CoA is found in considerable quantities in milk, meaning it can leave the mitochondria easily.

Acetate found in the cytosol can be utilized for fatty acid synthesis. It is converted to acetyl CoA via acetyl CoA synthetase

(8, 51). This enzyme, widely distributed in animal tissues, catalyzes the initial reaction in acetate metabolism. The use of acetate as a major substrate for fatty acid synthesis is, according to Ballard et al. (8) a useful adaptation of carbon metabolism in the adult ruminant. The fetal ruminant has the capacity to utilize glucose carbon for lipogenesis, but this ends after birth with the acquisition of the rumen microflora. Bickerstaffe et al. (20) using isotope dilution techniques, estimated that in lactating dairy cows the percentage of fatty acids derived from acetate were C_{10}: 36-87%; C_{12}: 34-57%; C_{14}: 25-57%; C_{16}: 8-35% and no activity in C_{18} or longer.

Even though ruminants have developed the capacity to utilize fibrous feedstuffs and their fermentation end products, when cows are fed different concentrate to grain ratios, the capacity to utilize this product is altered. Annison et al. (2) found that when cows were changed from high roughage to low roughage diets, blood acetate decreased from 7.8 to 4.5 mg/dl; also, the acetate entry rate fell from 3230 to 1630 mg/minutes and the contribution of acetate to total CO_2 from 33 to 24 mg/minutes, and mammary uptake of acetate from 280 to 220 mg/minutes. Based on changes in specific activity of acetate measured across the mammary gland, Annison et al. (2) demonstrated substantial reduction on endogenous acetate production. This is in agreement with former observations by Annison and White (1) and Bergman and Wolf (16).

Glucose utilization in fatty acid synthesis

Even though in ruminants glucose is not the main source of carbon for fatty acid synthesis, glucose incorporation into fatty acids and activities of relevant enzymes can be increased substantially by infusing glucose post-ruminally or intravenously (14). The infusion of glucose into lambs dramatically increased glucose utilization for lipogenesis relative to acetate with forty four and nine fold increase in activity of ATP citrate lyase and NADP malate dehydrogenase.

Glucose plays an essential role in cell metabolism and caloric needs of the body cannot be satisfied by fatty acids alone. According to Bergman (17) adequate blood glucose and tissue glycogen must be maintained for this purpose. The role of glucose in fat synthesis is in the formation of α-glycerol-phosphate which is the specific precursor of glycerol. A second role is furnishing NADPH which is formed via the pentose pathway, required as a reducing agent in fatty acid synthesis. A third role, although not as essential, is as carbon substrate for fatty acid synthesis.

Work done by Smith and Glascock (67) showed that appreciable amounts of glucose were oxidized by sheep udder tissue, especially when acetate was present. Glucose oxidation rate via the tricarboxylic acid (TCA) cycle was equal to or greater than that found in lactating rat mammary slices. They further observed that addition of acetate resulted in increased participation of the pentose phosphate pathway in glucose catabolism. This also increased the activity of the TCA cycle. Although sheep udder tissue incorporates acetate into

fatty acids when it is the only substrate, there was a considerable increase in acetate incorporation when glucose was added to the medium (9).

Role of β-Hydroxybutyric acid (BHBA) in fatty acid synthesis

Results of in vivo experiments by Popjak et al. (59, 60) in lactating goats and cows, established that acetate and BHBA contributed equally to the initial four carbons of fatty acids synthesized in mammary gland. Studies done by Kinsella et al. (43) have shown that bovine fatty acid synthetase (FAS) complex has a marked preference for butyryl CoA compared to acetyl CoA. Although the concentration of butyryl CoA is much lower than acetyl CoA in lactating bovine mammary tissue, Kinsella pointed out that the apparent Km of the FAS is lower for butyryl CoA. Acetate is nevertheless the major source of the remaining carbons in the fatty acid chain (13, 43). While BHBA can be incorporated into fatty acids as the initial four carbon primer unit, any subsequent carbon incorporation depends on metabolism to acetyl CoA by the enzyme β-hydroxybutyrate dehydrogenase (BHBDH). Cellular distribution of BHBDH is not equal (10). The enzyme is located almost exclusively in mitochondria, thus BHBA cannot furnish significant quantities of carbon for fatty acid synthesis beyond the initial four carbon unit because of the absence of citrate cleavage pathway (13).

Source of reducing equivalents for fatty acids synthesis

Lipogenesis or fatty acid synthesis require large amounts of reducing equivalents as NADPH during fatty acid elongation. Glucose,

the normal source of carbon for lipogenesis in nonruminant tissues, also provides the reducing equivalents, NADPH, by oxidation through the hexose monophosphate pathway and by decarboxylation of malate via citrate cleavage pathway (8).

In ruminants, under normal conditions enough glucose may be metabolized via the hexose monophosphate pathway to supply adequate amounts of NADPH. This is supported by the high activities of glucose-6-phosphate dehydrogenase (G6PDH) (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (6PGDH) (EC 1.1.1.44) found in adipose tissue and liver from cows and sheep (31).

The source of reducing equivalents for fatty acid synthesis in ruminant adipose tissue (14) and mammary gland (11) differs from non-ruminants. In ruminants, NADPH is generated in the pentose cycle (via G6PDH and 6PGDH) and the isocitrate cycle (via NADP-isocitrate dehydrogenase (ISOCDH) (EC 1.1.1.42). The activity of cytosolic NADP-ISOCDH has been found to be extremely high in ruminant adipose tissue as compared to nonruminants (14), probably because of its involvement in acetate utilization for NADPH production. Similar species differences may occur in mammary tissue.

Baldwin et al. (7) and Yang and Baldwin (79) indicated that the isocitrate cycle furnished a minimum of one fourth of the NADPH neces sary to support lipogenesis with the remainder arising from the pentose shunt. Malate transhydrogenation cycle, an important source of NADPH in rat adipose tissue, plays according to Baldwin and co-workers, a very minor role in ruminants because of the low activity of NADP malate dehydrogenase and extremely limited carbon flux through this pathway.

Bauman et al. (10) were the first to propose involvement of NADP-isocitrate dehydrogenase in the generation of NADPH for fatty acid synthesis in ruminants. The isocitrate cycle also involves a transhydrogenation system in the mitochondria, employing glutamate dehydrogenase (13). According to these authors, this allows for a recycling of α-ketoglutarate to citrate in the mitochondria. The extent of transhydrogenation would be controlled by NAD^+/NADH ratio in mitochondria. Bauman et al. (11) postulated that the pentose phosphate cycle and isocitrate cycle contributed approximately equal amounts of NADPH in cow and sheep mammary gland tissue. The relative importance of different pathways involved in generation of NADPH could depend on available carbon sources, and the energy (ATP) requirements of the cells (11). Flatt (25) pointed out that in the process of metabolite incorporation into fatty acids there is a net ATP yield which varies according to the pathway employed to generate NADPH. Bauman and Davis (13) suggested that in ruminant mammary gland an ATP deficit occurs rather than a net ATP yield. This is because glucose oxidation occurs primarily in the pentose phosphate cycle, with the triose phosphate formed undergoing extensive recycling. On the other hand, they argue, that utilization of acetate to generate ATP via isocitrate cycle, is an ATP yielding process. With a functional mitochondrial transhydrogenation scheme, Bauman and Davis (13) calculated the isocitrate cycle yields a net of three moles of ATP per mole of acetate incorporated into fatty acids. With the mitochondrial transhydrogenation scheme nonfunctional, carbon returns to the mitochondria as α-ketoglutarate and is cycled in the normal fashion to OAA. In this case, the isocitrate cycle yields a

net of 14 moles of ATP in the process of producing two moles of NADPH needed for a mole of acetate incorporation into fatty acids. Therefore, even though the isocitrate cycle involves acetate instead of glucose in the production of NADPH, its contribution towards generation of NADPH is controlled by the ATP levels in the mammary cell. After ATP requirements have been met, additional reducing equivalents must arise from the pentose phosphate cycle.

Baldwin et al. (7) proposed that in order to sustain higher rates of fatty acid synthesis in adipose tissue of a lactating cow, basal metabolism and simultaneously TCA cycle flux would have to increase to enable additional NADPH formation via isocitrate pathway. Alternatively, to make this possible they stated, glucose availability and pentose cycle flux would have to increase. These concepts help to explain the tendency to increase adipose fat deposition during high energy feeding. At the mammary gland level, where a great amount of glucose is being oxidized and utilized for lactose synthesis, an excessive carbon flux through the TCA cycle could stop the transhydrogenation being carried on via glutamate dehydrogenase. As a result, excessive production of ATP would occur which will finally stop the isocitrate cycle as a source of NADPH, and replace it by the pentose shunt.

Fatty acid synthesis

Observations by Baldwin et al. (7); Opstvedt and Ronning (54); and Yang and Baldwin (79) support the theory that TCA cycle flux as determined, by basal metabolic rate and/or glucose availability,

limits the rate of ruminant adipose and mammary lipogenesis. Fatty acid synthesis from acetate in vitro by ruminant adipocites is highly dependent on glucose availability (4, 79). As previously mentioned, when acetate and glucose were added to sheep mammary tissue culture, glucose oxidation rate via TCA cycle was equal to or greater than that found in lactating rat mammary slices (67). The addition of acetate resulted also in increased participation of the pentose phosphate pathway in glucose metabolism. The authors suggested that probably the factor responsible for this effect is the close relationship that exists in mammary tissue between the pentose phosphate pathway and the systems for the synthesis of fatty acids, these being dependent on the supply of NADPH.

Work by Bickerstaffe (18) has shown that acetate, BHBA and plasma free and esterified fatty acids are the main precursors of milk fatty acids in several species. Isotopic data (20) confirmed that acetate, stearate and palmitate are very important precursors of fat in the cow. They found significant arteriovenous differences for acetate, propionate, BHBA, triglycerides but not for free fatty acids or cholesterol esters. They also reported that in contrast to goats, cows had a significant mammary uptake of triglycerides from low density lipoprotein (LDL).

Work with goats (78) showed that hydrolysis in the capillaries was a prerequisite for triglyceride uptake by the mammary gland. They also found that the mechanism by which plasma triglycerides are taken up and incorporated into milk fat involves hydrolysis and re-esterification within mammary tissue. There was also evidence that lipoprotein

lipase (LPL) was involved in the hydrolysis of triglyceride before uptake.

Ration characteristics seems to alter the pattern of uptake of milk fat constituents. When cows were fed low roughage diets, reduced uptake of plasma triglycerides by the udder were observed by Annison and coworkers (2) even though the circulating levels were not reduced. Similar results were obtained by West and Passey (77) for glucose and palmitate when glucose was infused into the peripheral circulation of sheep. Annison and coworkers (2) suggested that the reduced uptake observed in cows secreting low milk fat could be due to impaired lipoprotein lipase activity due to changes in fatty acid composition of plasma triglycerides.

Annison et al. (2) also found a reduction of biohydrogenation in milk fat depressed animals. Nevertheless, it seems unlikely that unsaturation would be responsible for the reduced susceptibility of triglyceride to lipase action based on the successful results obtained by Bickerstaffe and Annison (19) in treating milk fat depressed goats with duodenal infusions of sunflower oil, which is rich in linoleic acid.

In a more recent study, Storry et al. (69) found that in the low milk fat syndrome, the capacity of the mammary gland to absorb preformed fatty acid was not impaired. They also found a decrease in all milk fatty acids except linoleic (C18:2) when cows were changed from high roughage diet to low roughage diets. To account for the reduced incorporation, they proposed an increased uptake of long chain fatty acids by adipose tissue at the expense of mammary tissue, in agreement with West

and Passey (77). McClymont and Vallance (50) suggested that an increased influx of glucose into the circulation might influence milk fat synthesis by shifting the balance of fatty acid release and esterification. According to West and Passey (77), the infusion of insulin either into peripheral or portal circulation increased the uptake of glucose and decreased the uptake of palmitate by the tissue of sheep. Apparently, the effect of insulin on the metabolism of free fatty acids is mediated through its effect on glucose metabolism. Glucose and insulin seem to suppress fatty acid release by accelerating esterification without altering the rate of lipolysis. The net result is a decrease in the release of free fatty acids into the plasma, producing a caloric shift from fatty acids to glucose. They also observed that when plasma glucose fell below 40 mg/dl, the effect of insulin on fatty acid release was overcome and reversed, probably by a sympathoadrenal response to hypoglycemia. Insulin infusions in goats (64) increased C_{12}-C_{18} fatty acids in milk fat, probably due to a release of adrenaline which promoted mobilization of fat in response to a decrease in peripheral blood glucose.

Enzyme control of lipid metabolism

A number of enzymes are involved in the synthesis of fatty acids, both at the mammary and adipose tissue levels.

Although the controlling factors of lipogenesis in ruminant animals are not fully understood, results by Bauman (12) indicate that within the cell the regulation is modulated via acetyl CoA carboxylase. Mellenberger et al. (48) showed that in mammary

gland of a multiparous post-partum cow, activity of the cytosolic enzyme acetyl CoA synthetase and acetyl CoA carboxylase closely paralleled changes in lipogenic capacity of mammary tissue slices. They suggested that acetyl CoA carboxylase and perhaps acetyl CoA synthetase represented important regulatory enzymes in fatty acid synthesis. Bauman (14) confirmed similar results for ruminant adipose tissue and mammary tissue. Acetyl CoA carboxylase was the only enzyme whose activity closely paralleled the changes in the rate of fatty acid synthesis. Acetyl CoA synthetase sharply increased after parturition in primiparous and multiparous cows, goats, and rats, followed by a gradual decrease in enzyme activity as lactation progressed (51).

According to Bauman and Davis (13), changes in acetyl CoA carboxylase activity in ruminant mammary and adipose tissue noted during fasting and refeeding, were due to alterations in the synthesis and/or degradation rates of the enzyme. Support for acetyl CoA carboxylase serving as the rate limiting enzyme in fatty acid synthesis is the fact that it exists in an active and inactive form (13). Similar to nonruminants, acetyl CoA carboxylase purified from bovine adipose tissue oscillates between a catalytically inactive protomeric state and a catalytically active polymeric state (45).

Diet composition and physical form on lipid metabolism

Diets in which the roughage is ground or diets with low proportion of roughage and high proportions of concentrate can cause a dramatic fall in the content and yield of fat in milk and significant changes in

its fatty acid composition. Van Soest and Allen (71) stated that the degree of milk fat depression is related to the concentration of rumen propionic acid. They also found a decrease in arterial acetate, and decreased utilization by mammary and peripheral tissues. When isocaloric low roughage and high roughage diets were fed to cows in mid lactation, milk yield was unaffected (23), but a decrease in milk fat percentage was observed in low roughage cows.

Jorgensen and Schultz (39) showed a greater effect of pelleted over conventional rations for developing milk fat depression. They observed a significant decrease in glucose and increase in ketone bodies on pelleted corn rations. They found that type and physical form of the concentrate were more important than the ratio of concentrate to forage. When pelleted corn, pelleted oats and pelleted corn plus thyroprotein were fed to cows in early lactation, a significant decrease in milk fat was observed in pelleted corn. When pelleted corn rations were switched back to normal rations, higher test and lower body gains were obtained. A better response in terms of milk fat secretion was observed when pelleted oats (higher fiber) and pelleted corn plus thyroprotein (catabolic effect) were fed. Addition of soybean oil meal, mechanically extracted, or lard to the pelleted corn did not increase fat secretion (40).

Kinsella et al. (43) confirmed that cows in restricted roughage diets produced less fat throughout lactation. They found a rapid increase in short and medium chain fatty acids after parturition, but decreased palmitic and stearic acid, probably due to preferential utilization by adipose tissue.

Jenny et al. (41) observed a significant increase in α-glycerol phosphate dehydrogenase and LPL of adipose tissue during milk fat depression. They also noted little or no change of these enzymes in mammary gland. No difference was observed between stages of lactation, however, midlactation cows showed a greater change in ruminal VFA, blood glucose and insulin. They attributed the lack of responsiveness of serum insulin and glucose to the metabolic demands for high milk production.

Digesta flow rate and volatile fatty acids

Variation in flow is sometimes correlated with the composition of the mixture of acids in the rumen. Isaacson et al. (38) working with different dilution rates and volatile fatty acid ratios on continuous culture studies observed that dilution rates did not affect total VFA or acetate production, but as the dilution rate increased, propionate tended to increase and butyrate to decrease. Similar results were obtained with sheep by Hodgson and Thomas (33, 34). At 33, 42, 55, and 67 g of dry matter/kg metabolic weight/day respectively, a gradual increase in the molar percentage of propionic acid was observed. When steers were fed a diet of cubed barley at 80% of ad libitum, the proportion of propionic acid in the rumen was low and there was a large population of protozoa (70). When ad libitum feeding was reestablished, the number of protozoa decreased and propionic concentration was higher.

Ration characteristics and efficiency of energy utilization

Calorimetric experiments involving intraruminal infusions of fatty

acids indicated that changes in the mixture of fermentation end products have a pronounced effect on the distribution of energy between the udder and the adipose tissue, but very little on the efficiency of utilization of energy for milk secretion (4, 55). Similar results were obtained varying the diet composition (24) although in some experiments there are indications that the utilization of metabolizable energy for milk secretion may be low for rations consisting solely or mostly of forage (22).

Armstrong et al. (3) showed that for maintenance needs of ruminants, mixtures of different molar proportions of volatile fatty acids were utilized with equal efficiency. Inefficient utilization was only observed when acetic acid was infused alone. The authors explained that being non-glucogenic, acetic acid is dependent on a supply of glucogenic materials to yield OAA for efficient oxidation. Orskov (57) proposed to better understand efficiency of energy utilization, the concept of an optimum proportion of non-glucogenic to glucogenic energy. He reported that dairy cows differed from growing animals in being able to tolerate a higher NGR (non-glucogenic ratio) before efficiency was depressed. He suggested that the inefficient utilization of energy at high NGR values was due to lack of OAA derived from C_3 carbon, and shortage of NADPH for fatty acid synthesis (coming from pentose phosphate cycle). At the adipose tissue level, an excess of ATP will be produced resulting in increased heat production. Nevertheless, Hovell et al. (35) suggested that this excess ATP may be required, and thus efficiently utilized when acetate oxidation occurred in the mammary gland. On the other hand, with a high proportion of propionic acid at

low NGR values, there is evidence from sheep and goats that the capacity of the liver is exceeded (56).

In order to retain an efficient utilization of energy in dairy cows, Orskov (57) suggested that NGR values should be below four. If NGR values are much below three, the partition of energy will begin to suffer insofar as milk fat percentage will be reduced and body fat synthesis increased. Results obtained by Latham et al. (46) and Storry and Rook (68) in dairy cows agreed with the concept of NGR presented by Orskov.

EXPERIMENT I

Objectives

1. To determine the effect of restricted energy intake of high concentrate rations on butterfat production.
2. To determine the effect of VFA alteration on lipid metabolism in lactating cows.
3. To study possible interrelations of ration and metabolic variables with milk fat depression.

Materials and methods

Twenty-one Holstein cows in mid-lactation were stratified according to fat test and milk production, and assigned at random to each of: (1) roughage ration (14% crude protein (CP), 22% crude fiber (CF)) restricted to NRC (53); (2) concentrate(15.7% CP, 11.7% CF) restricted to NRC (53) requirements; (3) concentrate (15.7% CP, 11.7% CF) ad libitum and fed for 30 days. Roughage ration consisted of corn silage, chopped hay, and 36% CP pelleted concentrate in a ratio 70:10:20 on a dry matter (DM) basis. Concentrate rations consisted of long hay, and a 16.8% CP pelleted concentrate in a ratio 15:85 on a DM basis. Composition of the concentrate mixture is shown in Appendix Table 1.

Feed intake and milk production were recorded daily. Blood via jugular puncture and rumen fluid samples via stomach tube were taken about days 15 and 30, 2.5 to 3 hours post-afternoon feeding. Body weight (BW) and 2-day composite milk samples were taken weekly. Milk fat content was determined on a Foss Milko-Tester. Ruminal VFA were

measured by gas liquid chromatography by a procedure similar to that of Baumgardt (15). Blood glucose was determined by a Harleco kit.[1]

Covariate analysis was used to correct for preexperimental values of all parameters measured. A regression model was used to statistically evaluate the influence of the experimental rations on the animal's metabolism. Least square means and standard errors were obtained, and orthogonal contrasts were designed.

The mathematical model used was:

$$Y_{(ij)}{}^{k} = \mu + b_1(IV) + T_i + P_j + TP_{ij} + \varepsilon_{(ij)}{}^{k}$$

where

μ = overall mean

B = regression of y on IV, where IV is initial value for the variable being studied

T_i = effect of the i^{th} Treatment $\quad i = 1, 2, 3$

P_j = effect of the j^{th} Period $\quad j = 1, 2,$ or $1, 2, 3, 4$ depending on the variable

$G_{(ij)}{}^{k}$ = random error

An example of the analysis of variance (ANOVA) breakdown is shown in Table 1, also ANOVA tables for every parameter measured are shown in Appendix Table 2.

---

[1] Harleco, Philadelphia, PA 19143.

Table 1. Analysis of variance for milk fat percentage for Experiment I.

SOURCE	df	SS	MS	F	PROBABILITY
Covariate	1	12.62	12.62	48.58	0.001
Treatment (T)	2	13.12	6.06	25.26	0.001
Period (P)	3	3.91	1.30	5.02	0.001
T x P	6	1.94	.32	1.25	NS
Error	70	18.18	.26		
Total	82	74.49			
R^2		.76			

Table 2. Least square means for intake, body weight, milk and fat production.

PARAMETER	RATIONS						ORTHOGONAL CONTRASTS P < .05	
	CONCENTRATE(1)		RESTRICTED(2) CONCENTRATE		ROUGHAGE(3)		1 vs 2	1 + 2 vs 3
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE		
Dry Matter Intake (kg)	17.7	± .4	12.9	± .6	14.0	± .6	*	*
Body Weight (kg)	573.0	±5.2	546.0	±5.6	564.0	±4.8	*	
Daily Milk (kg)	20.9	± .4	18.8	± .5	18.5	± .4	*	*
Milk Fat (%)	2.5	± .2	3.3	± .1	3.6	± .1	*	*
Energy Intake (Mcal)[1,2]	36.4		26.5		21.3			
Dry Matter Intake kg/100 kg Body Weight	3.1		2.4		2.5			

[1]Calculated based on NRC (53).

[2]Concentrate: 2.05 Mcal/kg DM, Roughage: 1.52 Mcal/kg DM.

Results

Least square means for dry matter intake (DMI) are presented in Table 2. Significant differences ($P < .01$) were found among rations, due to the treatment characteristics. Table 2 also shows least square means for body weight. Significant difference ($P < .01$) was found among rations. Ad libitum concentrate cows were heavier than restricted cows.

Milk production, as shown in Table 2, was significantly different ($P < .01$) among rations. Cows fed concentrate rations (ad libitum and restricted) produced more milk than the roughage ration ($P < .01$). Also, ad libitum concentrate feeding resulted in more milk than restricted ($P < .01$), probably due to high energy intake.

Fat test presented in Table 2, showed significant differences among rations ($P < .01$). Cows on concentrate rations tested lower than those on roughage rations ($P < .01$). Also, milk from ad libitum concentrate fed cows was significantly lower ($P < .01$) than those on restricted concentrate, which showed an intermediate value.

No significant difference ($P < .01$) was found between ad libitum and restricted cows for serum glucose (Table 3). When concentrate rations were compared to roughage fed cows, a significant difference ($P < .01$) was found.

Molar proportion of VFA (Table 3) showed a pattern similar to that reported in the literature for these types of rations (73). Acetate decreased significantly ($P < .01$) and propionate and valerate increased significantly ($P < .01$) for concentrate rations compared to roughage

Table 3. Least square means for serum glucose and rumen VFA.

PARAMETER	RATIONS			ORTHOGONAL CONTRASTS P < .05	
	CONCENTRATE (1) AD LIBITUM	RESTRICTED (2) CONCENTRATE	ROUGHAGE (3)	1 vs 2	1 + 2 vs 3
	$\bar{X}$ SE	$\bar{X}$ SE	$\bar{X}$ SE		
Serum glucose (mg/dl)	46.7±1.4	49.7±2.0	31.5±1.2		*
Ruminal VFA (moles/100 moles)					
Acetate	60.0± .8	64.7± .6	67.3± .1		*
Propionate	32.3±1.3	30.9±1.0	25.9±1.1		*
Butyrate	11.1±1.0	10.4±1.0	10.8±1.1		
Valerate	2:5± .3	1.5± .3	1.0± .3	*	*
Total (µmole/ml)	88.0	84.5	77.0		
NGR[1]	2.4	2.7	3.3		

[1] NGR = Nonglucogenic ratio = $\frac{\text{acetate + 2 butyrate + valerate}}{\text{propionate + valerate}}$, as molar %.

ration, independent of the level of intake. When VFA concentration was considered, cows on ad libitum concentrate showed higher values than cows on the other rations.

NGR values, calculated from VFA values showed marked differences among treatments (Table 3). Roughage and restricted concentrate rations showed values that according to Orskov (57) will allow an efficient utilization of energy. NGR values of ad libitum concentrate cows suggested an inefficient utilization of energy for milk fat production compared with fat deposition.

Discussion

Cows on concentrate rations produced more milk agreeing with other reports (23, 39). This is probably accounted for by higher total intake of energy and crude protein compared with restricted and roughage rations. Even though DMI was lower for restricted fed cows compared to those fed roughage, milk production was similar. The greater caloric density of concentrate in the restricted ration allowed them to produce as much milk as roughage cows.

Fat test was also affected by ration characteristics agreeing with other reports (23, 39), but influenced also by the amount of fermentable energy consumed. Cows in restricted rations had more fat concentration compared to ad libitum fed concentrate, presumably caused by a decrease in the total amount of nonglucogenic VFA absorbed from the rumen. Nevertheless, restricted cows had fat test still lower than roughage cows, suggesting that metabolic alterations are not fully

solved by decreasing the total intake of fermentable energy.

Serum glucose, in contrast to some reports (20, 28, 41), did not show differences among concentrate diets despite the differences observed in fat test and milk production. Due to the increase observed in total VFA concentration as well as NGR values for cows fed ad libitum concentrate, one could suggest an increase in glucose turnover rate to explain the similar values found for glucose among treatments.

When NGR values are compared, cows on restricted concentrate seem to utilize energy for milk fat production more efficiently than to cows fed ad libitum concentrate.

EXPERIMENT II

Objectives

1. To determine the effect of different energy intakes on milk fat secretion, fermentation products and blood metabolic parameters throughout the lactation cycle.
2. To determine interrelations between energy intake, chemical properties of the ration, fermentation products and blood metabolic parameters.

Materials and methods

Twenty-four Holstein cows were assigned to outcome groups of four cows, each based on anticipated day of calving. They were randomly allotted to each one of the four treatments to be specified.

Data on the different parameters mentioned in Experiment I was collected in a similar fashion for a period of 180 days, except blood was obtained from the tail via artery or vein. Extra blood was collected via jugular puncture at 35 and 150 days from every cow for lipoprotein analysis. Ruminal fluid, buffering capacity and pH were determined and recorded immediately after rumen sampling.

Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analysis were performed on feed samples according to Van Soest and Wine (75), Van Soest (72, 74) and Robertson and Van Soest (61).

Four experimental rations were fed: (1) roughage (16.0% CP, 19.8% CF) ad libitum, (2) concentrate (15.7% CP, 10.1% CF) ad libitum, (3) concentrate (15.7% CP, 10.1% CF) restricted to NRC (53) recommendation

and (4) normal (14.7% CP, 13.8% CF) ad libitum.

Cows on high concentrate rations were kept on the roughage ration for 21 days and changed gradually over a 12-day period to the experimental concentrate ration.

The roughage ration consisted of corn silage, ground shelled corn, chopped hay and 38% CP concentrate in a ratio 50:15.8:15:19.2 on a DM basis. Concentrate rations consisted of long alfalfa hay and a 15.7% CP concentrate, in an approximate ratio 13:87 on a dry matter basis. Normal ration consisted of corn silage, 33% CP concentrate, ground shelled corn and hay in a ratio 29.3:18.6:46.4:5.6 on a DM basis. Concentrate ingredients are shown in Appendix Table 3.

Feed intake and milk production were recorded daily during the 180-day period. One-day composite milk samples were taken once a week and analyzed for fat in a Milko-Tester instrument. Milk protein was determined by the dye binding method in a Foss-Pro-Milk instrument. Milk solids were determined by a gravimetric procedure based on an oven drying method. Ruminal VFA were measured as in Experiment I. Buffering capacity was measured by titrating 50 ml of filtered rumen fluid up to pH 5.0 from pH 6 with 0.1 N HCl and expressed as meq of acid per 50 ml of rumen fluid.

Serum glucose was determined as in Experiment I. Serum VFA were determined by a procedure outlined by Supelco Inc.[2] Serum ketone

---

[2] Chromatography Lipids, Vol. IX No. 2, 1975. Supelco, Inc., Bellefonte, PA 16823.

bodies were measured according to Williamson and Mellanby (75). Serum lipoprotein were separated by an adaptation of the procedure used by Rudel et al. (65) and quantitated according to Lowry et al. (47) by a modification done by Schacterle and Pollack (66).

Data was arranged in four periods as follows: Period 1 = 1-6 weeks; Period 2 = 7-14 weeks; Period 3 = 15-21 weeks; Period 4 = 22-26 weeks.

A general linear model was used to statistically evaluate the influence of the experimental rations. Least square means and standard errors were obtained. Orthogonal contrasts were set up.

The mathematical model used was:

$$Y_{ijkm} = \mu + P_i + R_j + PR_{ij} + C_{k(j)} + PC_{ik(j)} + \varepsilon_{m(ijk)}$$

where

μ = overall mean

P_i = effect of the i^{th} Period $\quad i = 1, 2, 3, 4$

R_j = effect of the j^{th} Ration $\quad j = 1, 2, 3, 4$

$C_{k(j)}$ = effect of the k^{th} cow within the j^{th} Ration

$k = 1, 2, 3, 4, 5, 6$

$\varepsilon_{m(ijk)}$ = random error

An example of ANOVA breakdown is presented in Table 4. Also ANOVA for all parameters are shown in Appendix Tables 4, 5, and 6.

Results

Dry matter intake (DMI) values presented in Table 5 showed a significant difference among rations. Cows on concentrate rations

Table 4. Analysis of variance for milk fat percentage in Experiment II.

SOURCE	df	SS	MS	F	PROBABILITY
Ration (R)	3	50.08	16.69	6.87	0.002
Cow (Ration)	20	48.61	2.43	8.53	0.001
Period (P)	3	43.49	14.49	50.85	0.001
R x P	9	16.65	1.85	6.49	0.001
Error	535	152.52	.28		
Total	570	318.73			
R^2		.52			

(restricted and ad libitum) consumed significantly less ($P < .05$) dry matter compared to cows in control rations (Roughage and Normal). Cows on restricted concentrate consumed the least amount, as expected. Period as well as ration by period interaction were also significant ($P < .10$) and the responses are shown in Figure 1. As time progressed, all animals except restricted animals increased DMI, reaching peak intake by period two (7-14 weeks). Cows on the roughage ration continued to increase DMI up to period 3, slightly decreasing thereafter as the other cows.

Dry matter intake for restricted cows was constantly decreased because restriction was based on NRC (53) requirements which decreased as lactation progressed due to decreased milk production.

Intake of fiber expressed as neutral detergent fiber (NDF) and acid detergent fiber (ADF) is shown in Table 5. Significant differences ($P < .01$) were observed among rations. Intake of NDF and ADF were affected by the level of DMI and the type of rations. Cows on concentrate rations consumed less than cows on control rations ($P < .01$). Cows on restricted concentrate did not consume significantly less than cows ad libitum. Significant differences ($P < .01$) were also found among periods and also for ration by period interaction, as shown in Figures 2 and 3. Intake of NDF and ADF increased rapidly with time for cows on roughage and normal rations, as expected. Cows on ad libitum concentrate rations showed a slight increase up to the second period, decreasing thereafter. Intake in restricted cows started higher than ad libitum and constantly declined throughout the experiment.

Table 5. Least square means for body weight, intake, milk and fat production.

PARAMETER	RATIONS				ORTHOGONAL CONTRASTS P < .05		
	ROUGHAGE (1)	CONCENTRATE (2) AD LIBITUM	RESTRICTED (3) CONCENTRATE	NORMAL (4)	1 + 4 vs 2 + 3	2 vs 3	1 vs 4
	$\bar{X}$ SE	$\bar{X}$ SE	$\bar{X}$ SE	$\bar{X}$ SE			
Body Weight (kg)	578.3±2.20	547.1±2.20	517.1±2.20	574.4±2.20			
Intake (kg/day)							
Dry Matter	18.2± .20	16.3± .20	13.3± .20	20.8± .20			
Neutral Detergent Fiber	6.3± .09	3.4± .08	2.8± .09	5.8± .08	*		
Acid Detergent Fiber	4.0± .05	1.7± .04	1.4± .05	2.9± .04	*		
Crude Protein	2.9± .06	2.6± .05	2.1± .05	3.1± .05	*		
Daily Milk (kg)	28.4± .28	30.3± .28	29.1± .28	29.1± .28			
Fat Test (%)	3.5± .05	2.8± .05	2.9± .05	3.4± .05			
Milk Solids (%)	11.7± .09	10.8± .08	10.9± .08	11.9± .10	*		
Milk Protein (%)	3.4± .03	3.3± .03	3.1± .03	3.5± .04	*	*	
FCM (kg)	26.6± .29	25.0± .28	24.5± .29	27.1± .30			

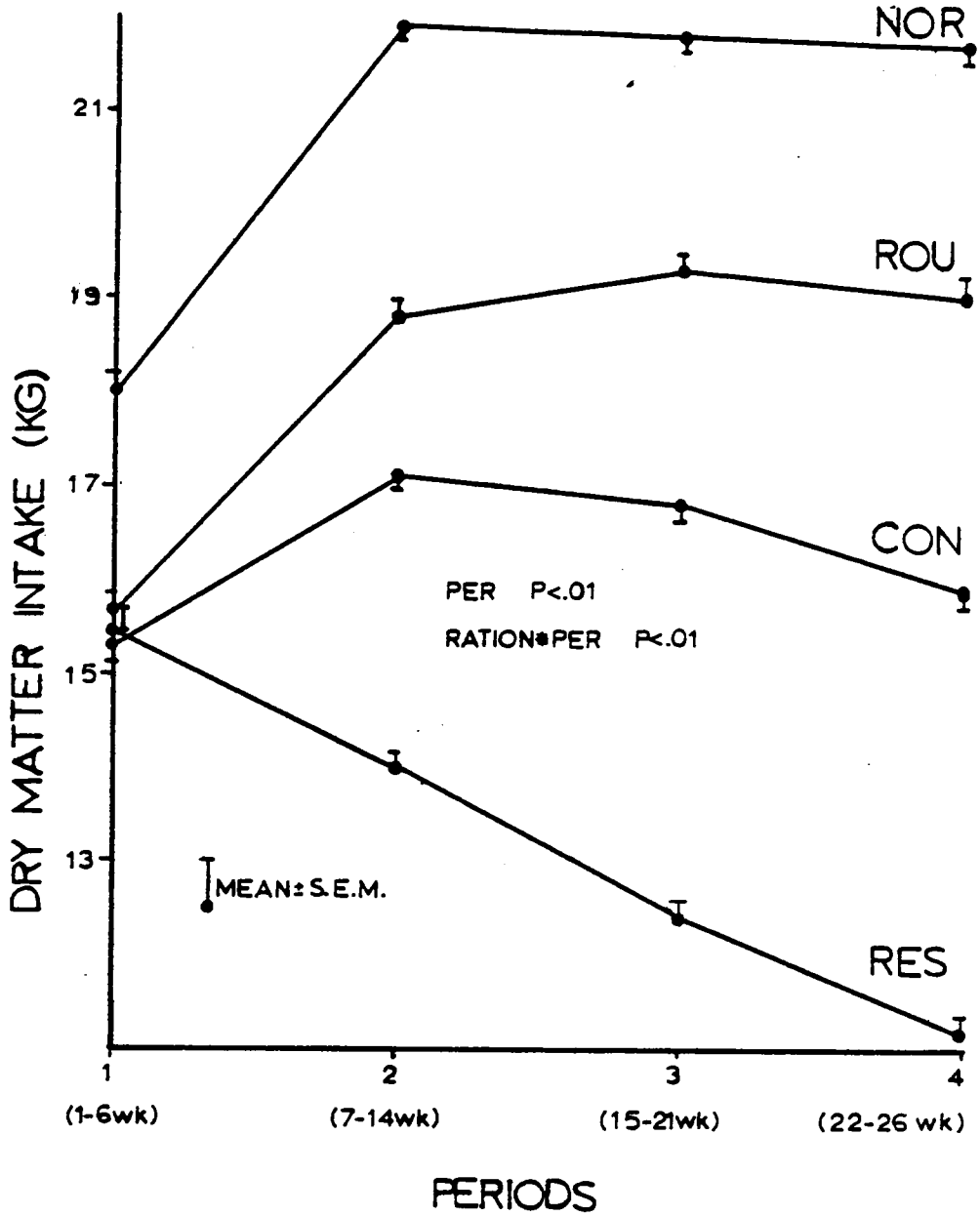

Figure 1. Changes in dry matter intake with time for Experiment II.

NOR = Normal ration; ROU = Roughage ration; CON = Concentrate ration; RES = Restricted ration.

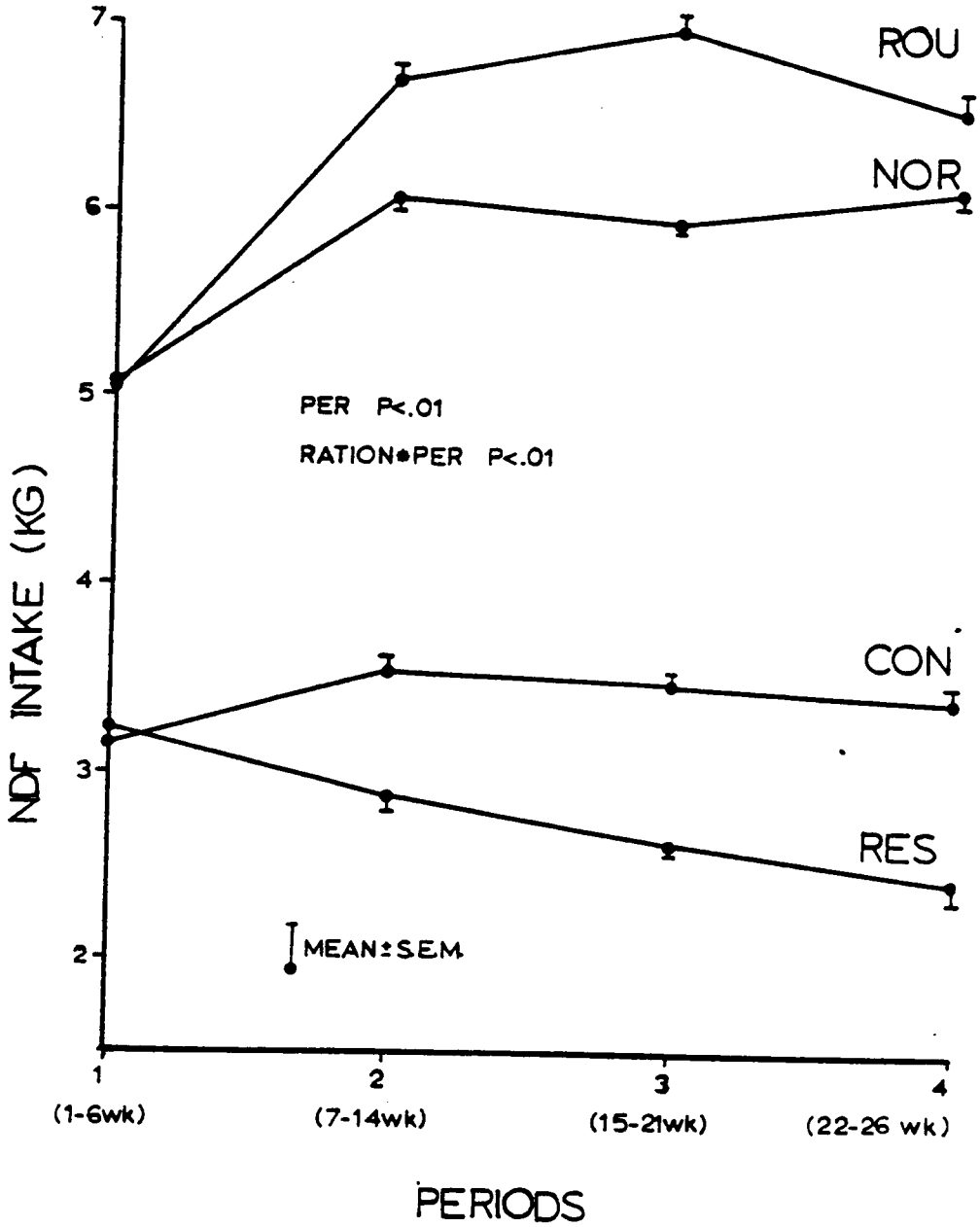

Figure 2. Changes in neutral detergent fiber (NDF) intake with time for Experiment II.

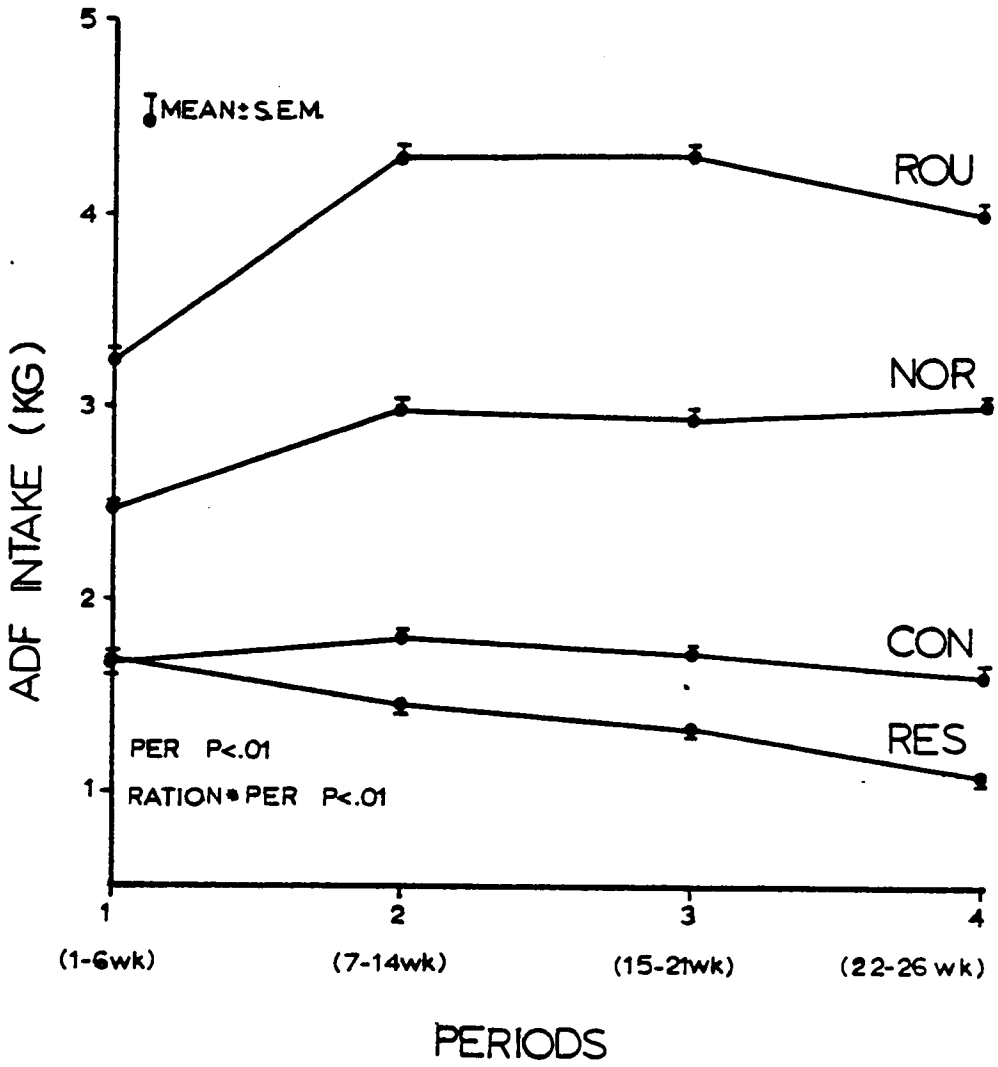

Figure 3. Changes in acid detergent fiber (ADF) intake with time for Experiment II.

Crude protein (CP) intake was significantly different ($P < .01$) among rations, as shown in Table 5. Concentrate ration showed lower ($P < .01$) intake of CP compared with control rations. Also, restricted cows consumed less ($P < .10$) CP than ad libitum fed cows. Significant difference among periods ($P < .01$) and for ration by period interaction ($P < .01$) are shown in Figure 4. Maximum intake of CP was reached around the second period except for roughage ration where intake continued up to the third period. Crude protein intake constantly decreased in cows fed restricted concentrate.

Least square means for body weight (BW) are presented in Table 5. No significant difference was observed due to rations. Differences among periods ($P < .01$) and ration by period interaction ($P < .01$) are shown in Figure 5. As expected, weight change was significantly different for periods. In general, cows maintained weight (control rations) or lost weight (concentrate rations) up to week seven, constantly gaining weight thereafter. Cows on the restricted ration paralleled the weight increase of ad libitum cows. Cows on control rations did gain more weight during the whole experiment compared to concentrate fed cows.

No significant differences were observed for milk production among the different rations (Table 5). Significant differences ($P < .01$) were observed between periods, probably attributed to stage of lactation. Even though milk least square means for ad libitum concentrate were larger than for restricted concentrate, the differences due to ration were not significant.

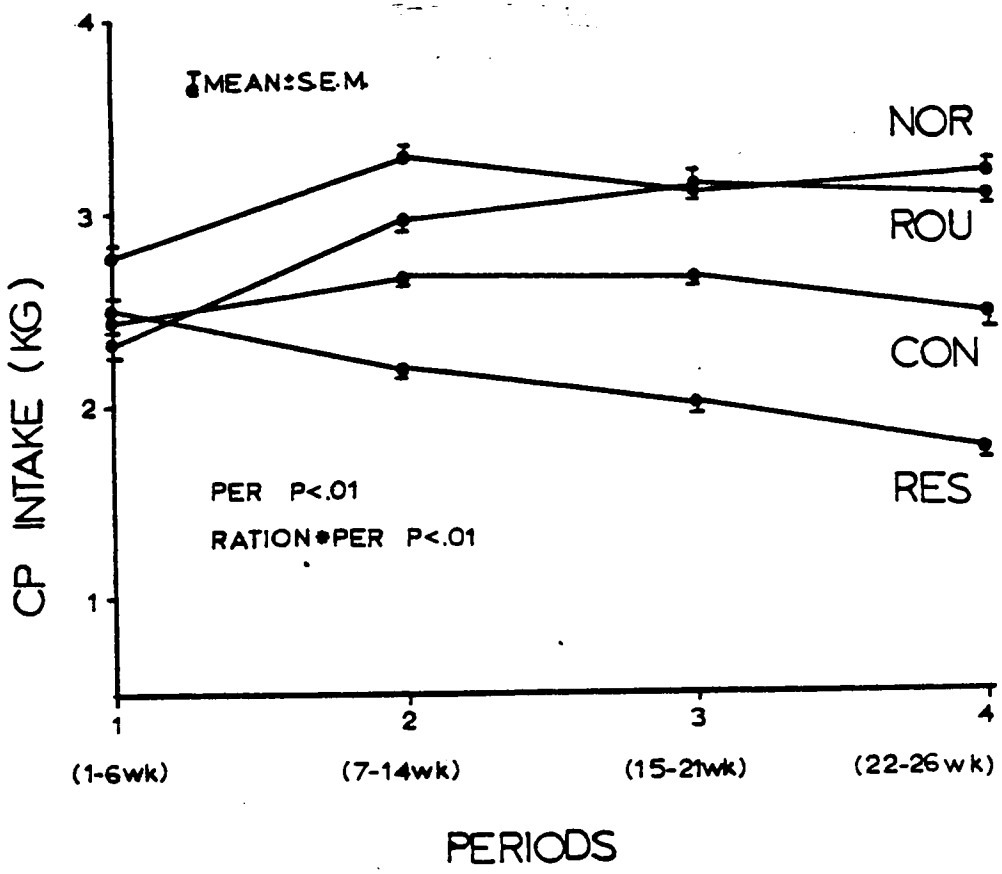

Figure 4. Changes in crude protein (CP) intake with time for Experiment II.

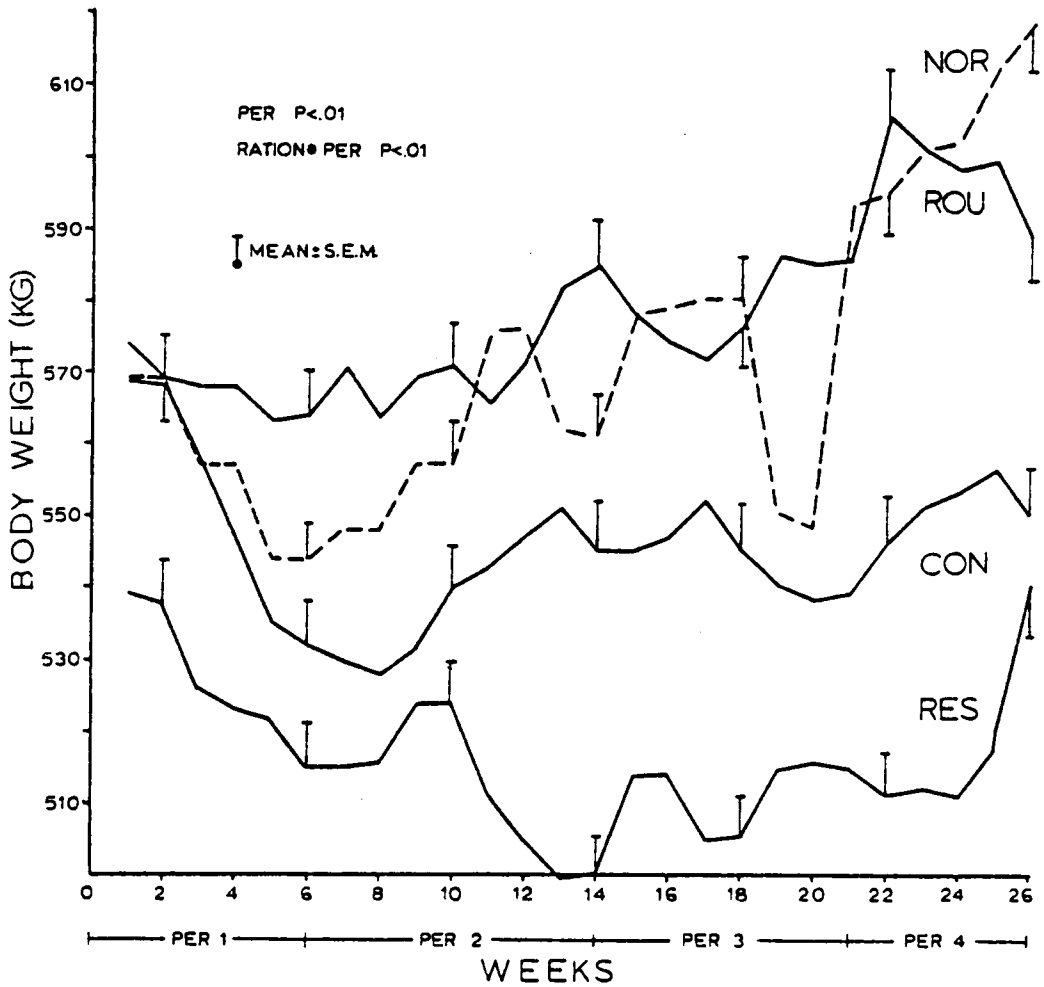

Figure 5. Changes in body weight with time for Experiment II.

Milk components, as shown in Table 5, were affected by the different rations. Milk solids showed significant differences ($P < .01$) between control and concentrate rations. Concentrate rations had similar values even though intake differed; also milk protein showed significant differences due to rations ($P < .01$). Cows in control rations had higher milk protein compared to cows fed concentrate ($P < .01$). Also, restricted concentrate cows showed significantly lower values ($P < .05$) compared to ad libitum concentrate.

Figure 6 shows milk protein plotted by periods ($P < .01$). The ration by period interaction was significant ($P < .03$). In general, milk protein tended to increase as lactation progressed, except for restricted cows that showed no change. Cows on roughage rations had a marked decrease during the second period, increasing thereafter in a similar fashion to cows on normal and ad libitum concentrate rations. The decrease can not be explained by similar differences in feed intake.

As shown in Table 5, fat test was significantly different among rations ($P < .01$). Cows fed concentrate rations had lower values compared with cows fed control rations. Figure 7 shows weekly variation in milk fat production for the different rations. Periods and rations by period interaction were significant ($P < .01$). A decrease in fat test from the time of parturition to week 8 or 9 was observed for every ration, being more pronounced for cows in concentrate rations.

Restricted concentrate feeding maintained milk fat secretion compared to ad libitum concentrate, the change being noticeable after the eighth week. Maximum improvement was reached by week 13, and maintained

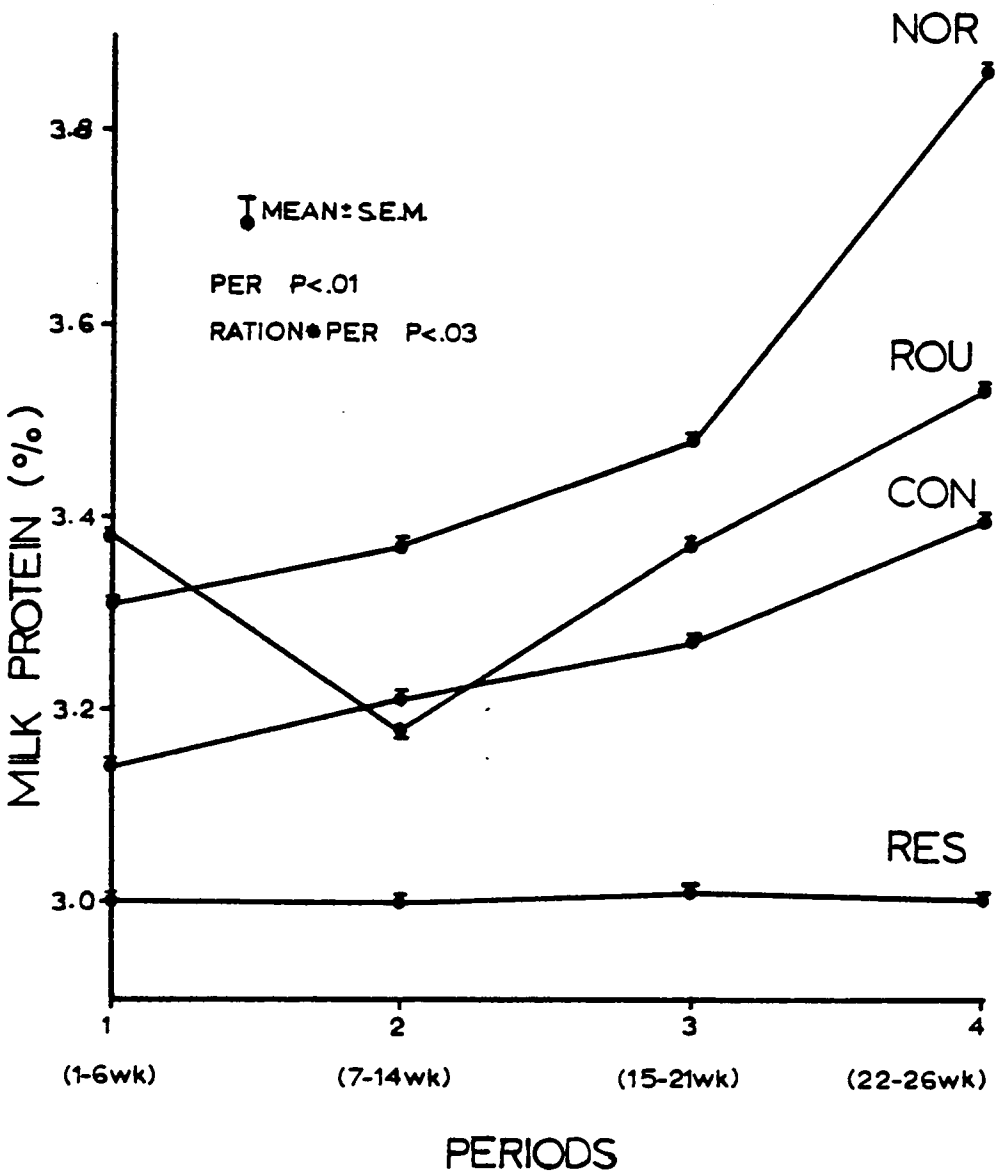

Figure 6. Changes in milk protein percentage with time for Experiment II.

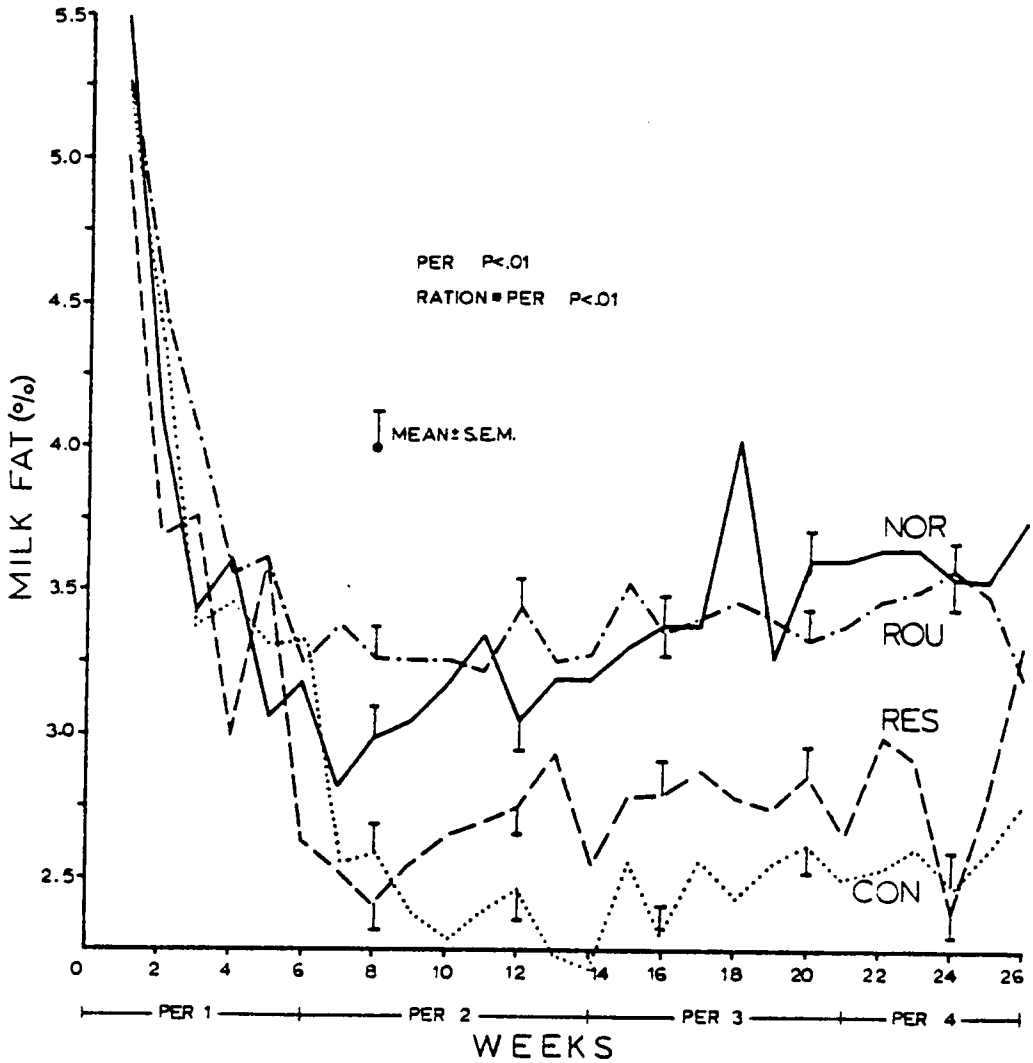

Figure 7. Weekly changes in milk fat percentage for Experiment II.

throughout the experiment. Nevertheless, no significant difference was observed between restricted and ad libitum rations when averages for the whole experiment are considered.

Fat corrected milk (FCM) presented in Table 5 showed a pattern very similar to fat percentage, largely due to the minimal difference in milk production among rations.

Molar proportion of ruminal VFA and total VFA concentration of rumen fluid differed among rations (Table 6). Significant differences among concentrate and control rations were found for acetate ($P < .12$), propionate ($P < .01$) and butyrate ($P < .02$). No difference was observed between restricted and ad libitum concentrate rations, presumably due to the same composition of both diets. Significant difference among periods ($P < .04$) and significant ration by period interaction ($P < .10$) were found for propionate (Figure 8). All the rations except roughage had an increase in propionate molar proportion up the second period leveling off or decreasing later to values similar to the ones observed in period one. Restricted cows maintained a narrow range of fluctuations. Roughage cows were the only ones that ended with higher values than in period one, but yet remained lower than the other diets.

Total VFA concentration showed significant differences among rations ($P < .06$). As shown in Table 6, cows on restricted concentrate showed values similar to cows in control rations, but were less than cows fed ad libitum concentrate.

Non glucogenic ratio (NGR), as defined by Orskov (57) [(acetate + 2 butyrate + valerate) ÷ (propionate + valerate) in molar %], was

Table 6. Least square means for buffer capacity, pH and ruminal volatile fatty acids.

PARAMETER	RATIONS								ORTHOGONAL CONTRASTS P < .05		
	ROUGHAGE (1)		CONCENTRATE (2) AD LIBITUM		RESTRICTED (3) CONCENTRATE		NORMAL (4)		1 + 4 vs 2 + 3	2 vs 3	1 vs 4
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE			
Buffer capacity (meq HCL/50 ml rumen fluid)	1.92	± .06	2.03	± .06	1.99	± .06	1.81	± .06			
pH	6.73	± .05	6.31	± .06	6.52	± .06	6.65	± .06	*	*	
Ruminal VFA (moles/100 moles)											
Acetate	60.62	± .70	57.04	± .70	57.81	± .70	58.02	± .70			
Propionate	23.11	± .60	27.73	± .60	27.10	± .60	26.30	± .60	*		*
Butyrate	12.59	± .30	11.15	± .30	11.32	± .40	12.14	± .30	*		
i-Valerate	1.82	± .10	1.99	± .10	1.80	± .10	1.65	± .10			
Valerate	1.56	± .10	1.92	± .10	1.70	± .10	1.85	± .10			
Total	85.92	±1.78	93.87	±1.76	89.24	±1.89	86.65	±1.79	*		
NGR[1]	3.64	± .10	2.84	± .10	3.00	± .10	3.09	± .10	*		*

[1] NGR = Nonglucogenic ratio = $\left(\frac{\text{acetate + 2 butyrate + valerate}}{\text{propionate + valerate}}\right.$, as molar %).

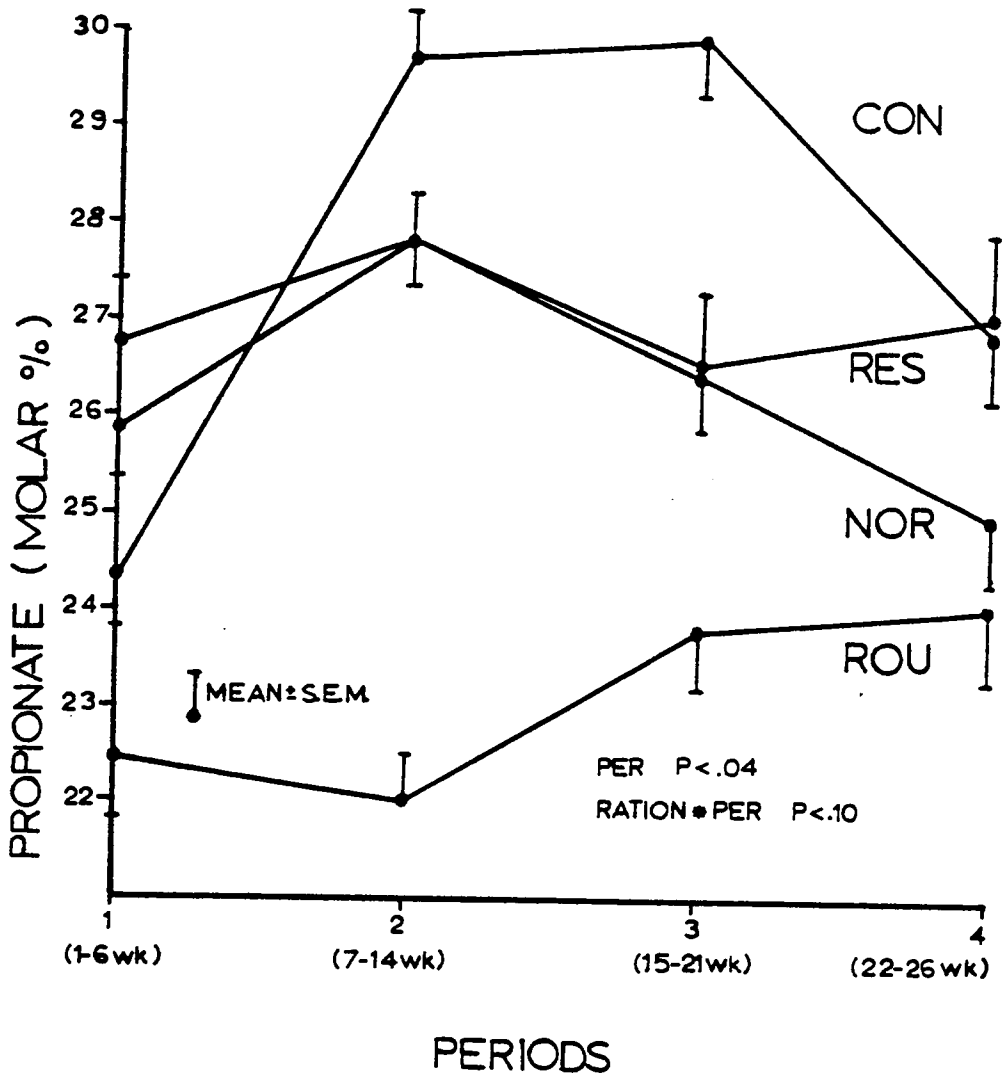

Figure 8. Changes in molar proportion propionate with time for Experiment II.

significant between rations ($P < .01$). As shown in Table 6, restricted concentrate rations did improve NGR values compared to ad libitum concentrate and approached NGR values for control diets. Differences were observed in NGR values as lactation progressed, probably due to the changes in glucogenic VFA molar ratio mentioned above.

As expected, due to the different characteristics of the rations, significant differences ($P < .01$) were observed for ruminal pH. As shown in Table 6, cows on ad libitum concentrate feeding, developed a lower pH compared to the other diets. Restricted concentrate feeding seems to aleviate the pH drop to a certain degree. No significant differences were observed for buffering capacity of the rumen, even though, a slight trend for a reduction in buffering capacity can be observed in concentrate diets (Table 6). It seems that cows in concentrate diets overcame satisfactorily the dietary challenge and maintained throughout the experiment pH and buffering capacity similar to that of control cows. These findings are also supported by the fact that none of the cows in concentrate rations developed any metabolic disorder during the experimental period.

Least square means for serum glucose are presented in Table 7. No significant difference was observed among rations, even though in concentrate rations, glucose was slightly higher.

Serum VFA least square means are presented in Table 7. Only acetate showed a significant difference between concentrate and control rations ($P < .07$). Smaller values were observed for concentrate rations. This agrees with a report by Annison et al. (2) that shows a decrease in endogenous production of acetate where high concentrate diets are

Table 7. Least square means for serum glucose, serum volatile fatty acids, beta-hydroxybutyrate and lipoproteins.

PARAMETER	RATION			
	ROUGHAGE (1)	CONCENTRATE (2) AD LIBITUM	RESTRICTED (3) CONCENTRATE	NORMAL (4)
	$\bar{X}$ SE	$\bar{X}$ SE	$\bar{X}$ SE	$\bar{X}$ SE
Serum Glucose (mg/dl)	49.76± .93	52.86± .94	51.75± .90	51.44± .98
Serum (μ mole/ml)				
Acetate	2.26± .14	1.46± .14	1.50± .14	1.68± .14[1]
Propionate	.10± .02	.10± .02	.10± .02	.14± .02
Butyrate	.05± .01	.04± .01	.05± .01	.06± .01
BHBA (μ mole/10 ml)	3.80± .59	2.23± .49	2.89± .42	
Lipoproteins (mg/dl)[2]				
VLDL	2.24± .78	3.06±1.94	1.25± .53	
LDL	7.76±1.62	9.01±1.51	9.61±3.16	
HDL	99.31±7.41	116.08±16.71	83.43±15.10	

[1]Difference among treatments for acetate $P < .07$.

[2]VLDL = Very low density lipoprotein; LDL = Low density lipoprotein; HDL = High density lipoprotein.

fed.

Least square means for ketone bodies expressed as beta-hydroxybutyric acid (BHBA) are presented in Table 7. No significant difference was observed among rations. Nevertheless, concentrate rations showed smaller values compared with control rations, results that agree with reports found in the literature (71).

Least square means for the different lipoproteins are also found in Table 7. Lipoprotein profiles and actual quantification of the amount of lipoprotein were done for a number of animals in every ration. Even though changes in the total amount of lipoprotein was observed among cows, no trend was found in terms of diet effect on the different lipoproteins. A great variation between cows was observed.

Discussion

Dry matter intake (DMI) followed a normal pattern as lactation progressed for every ration, except restricted concentrate. As expected, cows in concentrate rations consumed less dry matter, ADF and NDF than cows in control rations. Cows in control rations did consume more crude protein (CP) compared to concentrate diets. Even though rations were formulated to contain equal amounts of CP, the energy-protein ratio was larger for concentrate fed cows, resulting in a lesser intake of total ration and consequently of ration components. This difference in intake and the energy-protein ratio partially explain the similarity obtained in milk production for different rations.

Examining ration intake and milk production provides some explanation of changes observed in body weight throughout the lactation

cycle. All cows maintained or lost weight up to the eighth week (Figure 5), increasing their energy deposition thereafter. Although not expected, cows on ad libitum concentrate did not gain the most weight, but closely paralleled body weight gains of cows in roughage ration. According to Jahn et al. (42), cows on high concentrate rations are expected to have higher energy deposition than cows on normal rations. One possible explanation could be the appetite control mechanism. Due to the high energy density of concentrate rations, it is possible to suggest a chemostatic regulation of feed intake (52).

Body fill could also be affecting BW changes. According to Jahn et al (42), animals consuming rations containing approximately 10% ADF - 15% CP will have an approximate body fill of 10% conpared to 14% for cows receiving rations containing 20% ADF - 15% CP. When BW values from Table 5 are corrected for body fill, true body weight differences are minimal. Nevertheless, even though this correction seems applicable to BW averages for the experiment, when BW corrections from the latter part of the experiment are computed, cows in normal rations showed substantially higher BW than cows in concentrate rations.

Milk solids for concentrate fed cows were lower compared to control cows partially due to lower values for butterfat and protein components. These results disagree with findings by Huber and Bowman (36). They reported that protein content of milk was significantly higher for high concentrate fed cows (hay: conc.15:85) compared to low concentrate (hay: conc.75:25). In another experiment, Huber and Bowman (37), working with grazing cows and different levels of grain supplementation reported increases in solids-not-fat with increasing levels of

supplementation reported increases in solids-not-fat with increasing levels of supplementation. Cows had available high quality pasture, high in protein. This was not true for our experiment where CP was similar for all rations independent of energy concentration. Results reported by Boman (21) tend to agree with our results, on the basis that the level of fiber in the ration could affect milk protein. He found that cows fed grain ad libitum plus 0.2 kg hay/day, had milk protein content no higher than cows receiving hay, corn silage and normal levels of grain, probably due to depressed intake and digestive disturbances observed in ad libitum concentrate fed cows. Also our data shows than when ad libitum and restricted concentrate fed cows are compared, restricted cows showed lower milk protein values due to lower CP intake.

Fat test, as reported in several places in the literature was lower for cows fed concentrate rations (Figure 7); nevertheless, when the overall average of the experiment is considered, no differences existed between ad libitum and restricted concentrate fed cows. All cows showed a decrease in fat test up to week eight or nine, which coincide with a decrease in BW and gradual increase in milk production. By week eight, cows have normally reached their maximum production and their milk production decline begins.

Dry matter intake (DMI) increased during this time so that usually just a few days beyond peak milk production, the animal energy status changes from a negative one in which fat and protein is drawn out of tissue to support production, to a positive one in which energy accumulates. During the period prior to this change, milk fat percentage

probably does not decline due to ration effect, because of the support given by the animals tissue. Once this change is established, as it can be seen in Figures 5 and 7, concentrate fed cows showed an inverse relationship between BW changes and fat test. These observations are nevertheless not applicable to control cows, where independent of BW gains, milk fat percentage was maintained throughout the lactation. These findings tend to support observations by Jenny et al. (41) with respect to the lesser susceptibility to milk fat depression of concentrate fed cows in early lactation compared with mid lactation. Nevertheless, due to the different behavior of control cows, it is suggested that other factors such as change in fermentation products and blood metabolic parameters might be also involved in this metabolic alteration. This will be in agreement with some of the theories that have been proposed to explain milk fat depression (50, 73).

When rumen fermentation products are considered, a ration effect on total VFA concentration as well as molar proportion was observed. Total concentration of VFA was found to be higher for ad libitum concentrate compared to the other rations, including restricted concentrate. This suggests that the amount of VFA absorbed from the rumen could be greater for concentrate diets increasing the amount of glucogenic and nonglucogenic precursors available for milk and fat synthesis. However, negative results had been reported in the literature (62, 63) when attempts were made to induce milk fat depression and increase milk production by infusing propionate and other VFA ruminally and post-ruminally.

Molar proportion of VFA was also altered and the evaluation is simplified by looking at NGR values. NGR was significantly different for different rations. Control showed higher values, suggesting a better ratio between glucogenic and nonglucogenic VFA according to the Orskov concept (57). This indicates energy is utilized in a more efficient way in control rations compared to concentrate rations. This largely coincides with production response as shown by FCM (Table 5). Nevertheless, NGR values for concentrate rations are still considered reasonable in terms of efficiency by Orskov but based on body weights gains and milk fat production by these cows, efficiency was much lower.

No significant difference was found for serum glucose among rations. It is reasonable to suggest that cows in ad libitum concentrate would be expected to produce more glucose with lower NGR values and more propionate production. However, because it was not shown for concentrate cows, the turn over rate of glucose must be greater.

Work found in the literature for glucose (41, 71) generally disagreed with the present findings. The difference can be explained perhaps due to different sampling time, physiological stage of the animal, level of production and duration of the experiment. Figure 9 shows serum glucose levels for the different rations throughout the experimental period. Even though there was no significant difference among rations, significant difference among periods can be seen, which would then agree with certain literature (41, 73).

If period 3 is considered, ad libitum concentrate showed the highest values for blood glucose, with restricted cows being located in

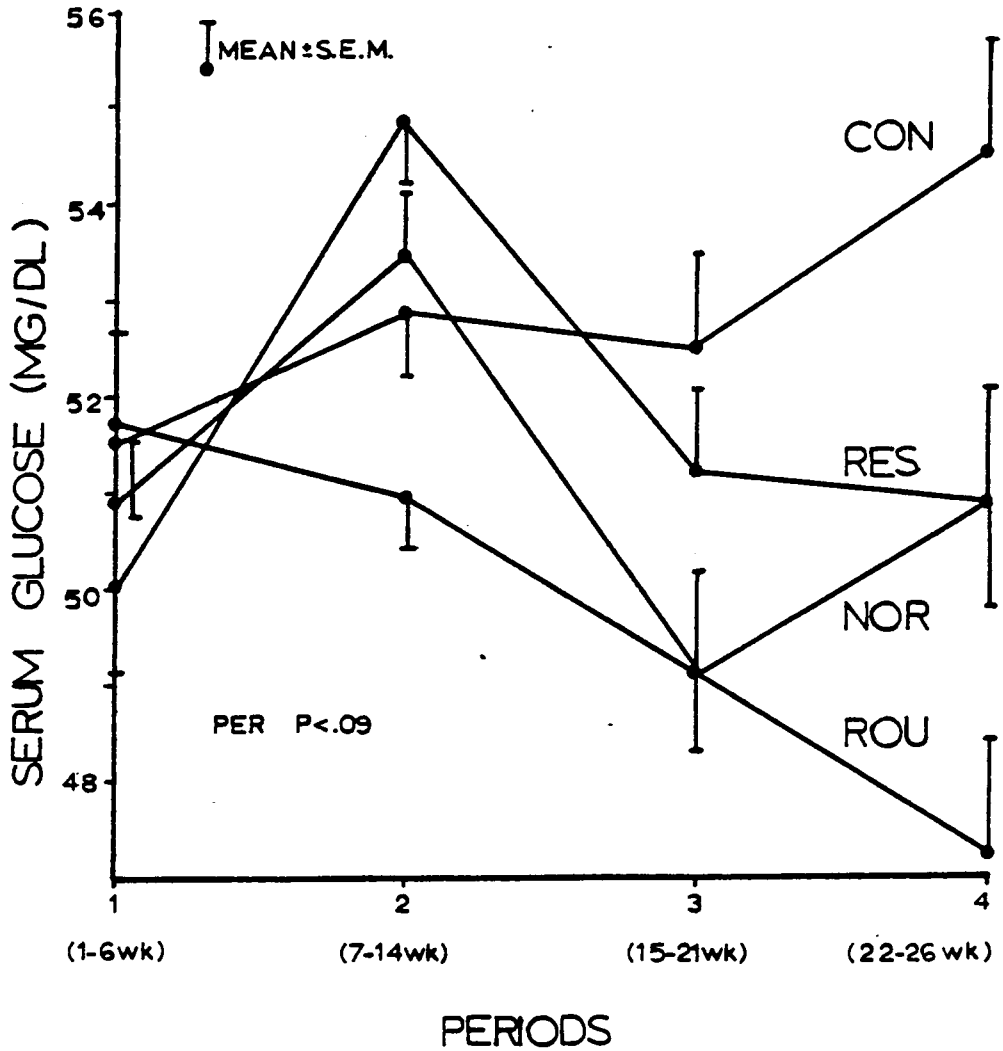

Figure 9. Changes in serum glucose with time for Experiment II.

between control and ad libitum cows. This trend was also found although not significant in Experiment I, where cows in mid-lactation (150 days) were followed for 30 days.

EXPERIMENT III

Objectives

1. To determine the effect of different energy intakes on cow lipid metabolism.
2. To monitor the changes in enzyme concentrations or activities in mammary and adipose tissue when cows are fed restricted amounts of fermentable energy.

Materials and Methods

Twelve cows in mid lactation (150 days) were used in this experiment. Outcome groups of three cows were made according to milk production and fat test and randomly assigned to the different rations. The experimental rations were as in Experiment II, roughage, concentrate ad libitum and concentrate restricted to NRC (53) requirements. Cows on concentrate rations had an adjustment period of 12 days.

Weekly one day composite milk samples were taken and analyzed for fat content in a Foss-Milko-Tester. When fat depression was established, daily composite milk samples were taken for three to four days before the animal was slaughtered. A similar procedure was followed for roughage fed cows.

Cows in restricted concentrate feeding were first fed concentrate ad libitum and monitored as cows in ad libitum concentrate until milk fat depression occurred. From the onset of milk fat depression, daily composite milk samples were taken for a period of four to five days

and analyzed for fat content. Consequently, intake of concentrate was restricted to NRC (53) recommendations for their level of milk production, fat test and body weight. Milk fat changes for restricted cows were monitored daily thereafter until fat test came up to predepressed levels. When predepressed levels were established, cows were slaughtered.

In every case, mammary tissue from healthy quarters and renal fat tissue samples were placed in cold isotonic saline and brought into the laboratory. Accurate weight and recording were done, and later homogenized (1:2 vol) in a Polytron instrument[3] for 5-10 seconds. The homogenization solution contained 0.28 M Sucrose, 0.1 M Sodium EDTA, 0.1 M reduced glutathione and 0.1 M Potassium Phosphate buffer, pH 7.8. Samples were centrifuged at 800 g for 10 min. at 4°C, and the resulting supernatant centrifuged at 102,000 g for 60 min. at 4°C. Samples were stored at -90°C until analysis.

Fatty acid synthetase activity was determined using the procedure outlined by Goodridge (30). Isocitrate dehydrogenase was assayed according to the procedure outlined by Plaut (58) and glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase according to Clock and McLean (29). A Gilford spectrophotometer (kinetic model) with the temperature of chamber kept at 35°C was used to measure responses.

A general linear model was used to statistically evaluate the effect of the different treatments. Least square means and standard

---

[3]Brinkman Instruments, Westbury NY 11590.

error were also obtained.

The mathematical model used was:

$$Y_{ijkm} = \mu + T_i + R_j + TR_{ij} + C_{k(j)} + TC_{ik(j)} + \varepsilon_{m(ijk)}$$

where

μ = overall mean

T_i = effect of the ith Tissue $\quad i = 1, 2$

R_j = effect of the jth Ration $\quad j = 1, 2, 3$

$C_{k(j)}$ = effect of the kth Cow within each Ration

$k = 1, 2, 3, 4$

$\varepsilon_{m(ijk)}$ = random error

An example of the analysis of variance (NOVA) breakdown is presented in Table 8. Also ANOVA tables for all enzymes studied are shown in Table 7 of the Appendix.

Results

As shown in Table 9, 6 phosphogluconate dehydrogenase (6 PGDH) activity in mammary and adipose tissue did not show significant differences among rations. A significant difference was observed for tissues ($P < .01$).

Mammary tissue showed from 5 to 6 times higher activity per gram of wet tissue compared to adipose tissue depending on the ration. Cows in restricted concentrate showed a trend for higher enzymatic activity in mammary tissue compared to ad libitum concentrate and roughage diet. In fat tissue, higher enzymatic activity was present in cows on concentrate rations and lower for roughage fed cows.

Table 8. Analysis of variance for fatty acid synthetase activity in Experiment III.

SOURCE	df	SS	MS	F	PROBABILITY
Ration (R)	2	0.071	0.036	0.34	NS
Cow (Ration)	9	1.043	0.104	1.06	NS
Tissue (T)	1	3.159	3.159	32.14	0.001
R x T	2	0.028	0.014	0.14	NS
Error	9	0.786	0.098		
Total	23	5.648			
R^2		.860			

Table 9. Least square means for activity of different enzymes, tissue protein and milk production.

PARAMETER	MAMMARY RATIONS				ADIPOSE RATIONS			
	RESTRICTED CONCENTRATE	CONCENTRATE AD LIBITUM	ROUGHAGE	SIGNIFICANCE	RESTRICTED CONCENTRATE	CONCENTRATE AD LIBITUM	ROUGHAGE	SIGNIFICANCE
Enzyme activity (μ mole/min/g wet tissue)	$\bar{X}$ SE	$\bar{X}$ SE	$\bar{X}$ SE		$\bar{X}$ SE	$\bar{X}$ SE	$\bar{X}$ SE	
6-P-gluconate dehydrogenase	1.94± .27	1.67± .27	1.62± .27	NS	.31± .27	.36± .27	.27± .27	NS
Glucose 6-P dehydrogenase	.83± .45	.83± .45	.99± .45	NS	.86+ .47	1.98± .45	1.03± .45	NS
Isocitrate dehydrogenase	21.64±2.77	22.32±2.77	21.52±2.77	NS	.91±2.77	.80±2.77	1.01±2.77	NS
Fatty acid synthetase	.76± .15	.94± .15	.96± .15	NS	.08± .15	.09± .15	.13± .15	NS
Protein (mg/g wet tissue)	27.91	35.20	34.15		3.93	3.23	4.42	
Daily Milk (kg)	20.30	20.90	20.20		20.30	20.90	20.20	

Least square means for enzyme glucose-6-phosphate dehydrogenase (G6PDH) are presented in Table 9. No significant differences were observed for rations or tissues. A slightly higher activity was observed for the adipose tissue.

No real differences were observed for isocitrate dehydrogenase activity in adipose and mammary tissue due to rations (Table 9). Significant differences were found for tissue. Mammary tissue was 21 (roughage) to 28 times (restricted) more active than adipose tissue per gram of wet tissue.

No significant differences were found among rations for fatty acid synthetase complex (FAS) activity (Table 9). Significant differences ($P < .01$) were observed between tissues. In concentrate fed cows, mammary tissue averaged 10 times more activity than adipose tissue. In roughage fed cows, mammary tissue activity was only seven times more active than adipose tissue.

Discussion

Results on enzymatic activity for 6-phosphogluconate dehydrogenase (6PGDH) and glucose-6-PO_4 dehydrogenase (G6PDH) agree in trend, but not in magnitude, with values reported in the literature for adipose tissue (6). Even though values presented in Table 9 were approximately 10 times higher than those reported by Baldwin et al. (6) for adipose tissue, three to six times higher activity was found in G6PDH compared to 6PGDH. Although not significant, rations seemed to have an effect on the activity of these two enzymes. Ad libitum concentrate resulted in the highest activity for G6PDH, restricted and roughage being

similar and lower in value. Enzymatic activity for isocitrate dehydrogenase (ISOCDH) in adipose tissue was found similar to values reported by the same authors.

Baldwin et al. (6) suggested that age of the animal and not physiological stage was responsible for differences in activity. Our results indicate that different periods within a lactation cycle could also account for changes in activity. Based on different parameters measured and overall condition of the animal throughout the lactation cycle, Experiment II suggests that changes in enzymatic activity due to a treatment could be obtained around 45-98 day compared to 150 days.

According to Experiment II, after 45 to 98 days, cows appear to be less susceptible to milk fat depression and to possible changes in activity of enzymes involved in lipid metabolism, independent of diet characteristics.

Opstvedt et al. (54) found that the enzymatic capacity for fat synthesis in adipose tissue was higher in cows fed all concentrate rations than cows fed all hay rations. Activities of many of the enzymes involved in fatty acid synthesis tended to be slightly lower in mammary tissue from cows fed concentrate than from cows fed hay. They found fatty acid synthetase (FAS) activity to be one half for concentrate cows. Even though enzymatic activity was higher for mammary tissue in our study, no difference existed between ad libitum concentrate and roughage rations.

Mellenberger et al. (48) working with cows that ranged in milk production from 18 to 40 kg/day at peak lactation found values similar to ours. Glucose-6-PO_4 dehydrogenase and 6 PGDH activity for mammary

tissue at 40 days post partum agree with ours, suggesting that independent of ration effects, the level of enzymatic activity seems to be maintained prior to peak lactation continuing up to 150 days.

Fatty acid synthetase activity found in mammary tissue was similar to those reported by Mellenberger et al. (48) at 40 days post partum, suggesting again a constant activity of the enzyme throughout the lactation cycle.

Isocitrate dehydrogenase activity in mammary tissue was found to be similar to values reported by Mellenberger et al. (48) and was found to be more active than in adipose tissue. According to Mellenberger et al. (48), ISOCDH is an important enzyme for providing a large percentage of NADPH for fatty acid synthesis in ruminants. Pentose shunt enzymes according to the authors, do play a role compared to nonruminants.

Our results indicate that, independent of the amount of glucogenic precursors available, cows in mid lactation obtained a large portion of the reducing equivalents through the isocitrate cycle.

The minimal effect of ration on enzyme activity could also be interpreted as not directly related to response between tissue metabolism and enzyme activity (5). Ad libitum or restricted intake of fermentable energy could affect metabolite concentration and mammary and adipose tissue metabolism without noticeable change in enzyme activity. This could explain the partial increase obtained for fat test where energy intake was restricted compared to ad libitum intake.

GENERAL DISCUSSION

Even though Experiment I indicated that milk fat depression was alleviated by controlling the amount of available energy consumed, results were confounded by the fact that intake for the roughage group was restricted to NRC (53) requirements. Also, 150 days post partum may not be the most appropriate time for a good comparison, based on observations from Experiment II. In Experiment I, cows on ad libitum concentrate showed higher milk production presumably due to higher total ration and energy intake. Cows on restricted and roughage rations were restricted in energy and crude protein creating the potential for milk production favorable for ad libitum concentrate cows.

Due to the level of restriction and the higher fiber content of the ration, cows on roughage ration showed higher fat test. Cows restricted in concentrate showed intermediate values compared to ad libitum and roughage cows.

Molar proportion of VFA changed, mainly due to propionate increase when concentrate was fed. Non-glucogenic ratio (NGR) showed a more favorable value for restricted cows compared to ad libitum due to a slight increase in acetate (57). That could increase fat synthesis at mammary tissue level by providing a balanced proportion of metabolites.

Similar results were obtained for Experiment II at 150 days when compared to Experiment I. Cows fed ad libitum concentrate did produce more milk than cows fed restricted concentrate or control rations. Also, molar percent VFA changed for cows on concentrate rations due to

an increase in propionate production. When fat test was considered, restricted concentrate cows showed intermediate values between ad libitum concentrate and control rations. Dry matter intake, nevertheless, was higher for cows fed normal rations compared to concentrate rations. When dry matter intake is considered for the whole experiment, concentrate cows consumed less than normal cows, suggesting a chemostatic regulation of intake due to the higher energy density of the ration. If crude protein intake in considered, concentrate cows could be considered to be borderline according to NRC (53) recommendations for protein consumption. Based on Table 5 and NRC (53), cows in concentrate should receive 2.81 kg of protein per day, but only consumed 2.6 kg. Roughage cows required 2.81 kg and consumed 2.9 kg per day.

Limited intake of crude protein and fiber probably affected milk components. Milk solids were lower for concentrate fed cows largely due to a decrease in milk protein and milk fat. When lactose-mineral content was calculated from Table 1, no apparent difference was observed for the different rations.

When fat test was followed on a weekly basis (Figure 7), a marked decrease was observed for every animal independent of the experimental ration up to week eight or nine. This decrease coincides with an increase in milk production and decrease in body weight. After this period, fat test stabilized at a given level depending on the energy concentration of the ration consumed. Concentrate fed cows showed lower fat test values compared to control cows and fat changes were inversely related to body weight gains. When restricted cows are

compared with ad libitum concentrate cows for milk fat test differences were observed after peak of lactation. When values for week 10 to 24 are compared, restricted cows had 3.17% milk fat as compared to 2.88% for ad libium concentrate cows, suggesting an adverse effect of metabolite concentration on milk fat secretion.

According to others (6, 57), cows in control groups were not expected to gain weight because of the type of ration fed. However, when comparing cows on control and concentrate rations, the former gained more weight even after correction for body fill. No inverse relationship occurred for control cows between body weight changes and fat test (Figures 5 and 7). This suggests that in addition to crude fiber content of the ration, amount of available energy consumed and energy status of the animal, other factors must be involved in controlling milk fat secretion in ruminants.

Therefore, it seemed important to look at several key enzymes involved in fatty acid synthesis under different intakes of available energy both in mammary and adipose tissue (Figure 10). There were no significant differences due to rations for G6PDH(1), 6PGDH(2), ISOCDH (3) and FAS complex (4) activity in either adipose or mammary tissue. When 6-P-GDH activity for adipose tissue was plotted against mammary tissue for every cow in the different rations, a negative relation was found. Increases in adipose tissue activity were accompanied by decreases in mammary enzymatic activity but were not consistent enough to allow statistical significance. Even though some small differences can be seen (Table 4), results agree with the literature

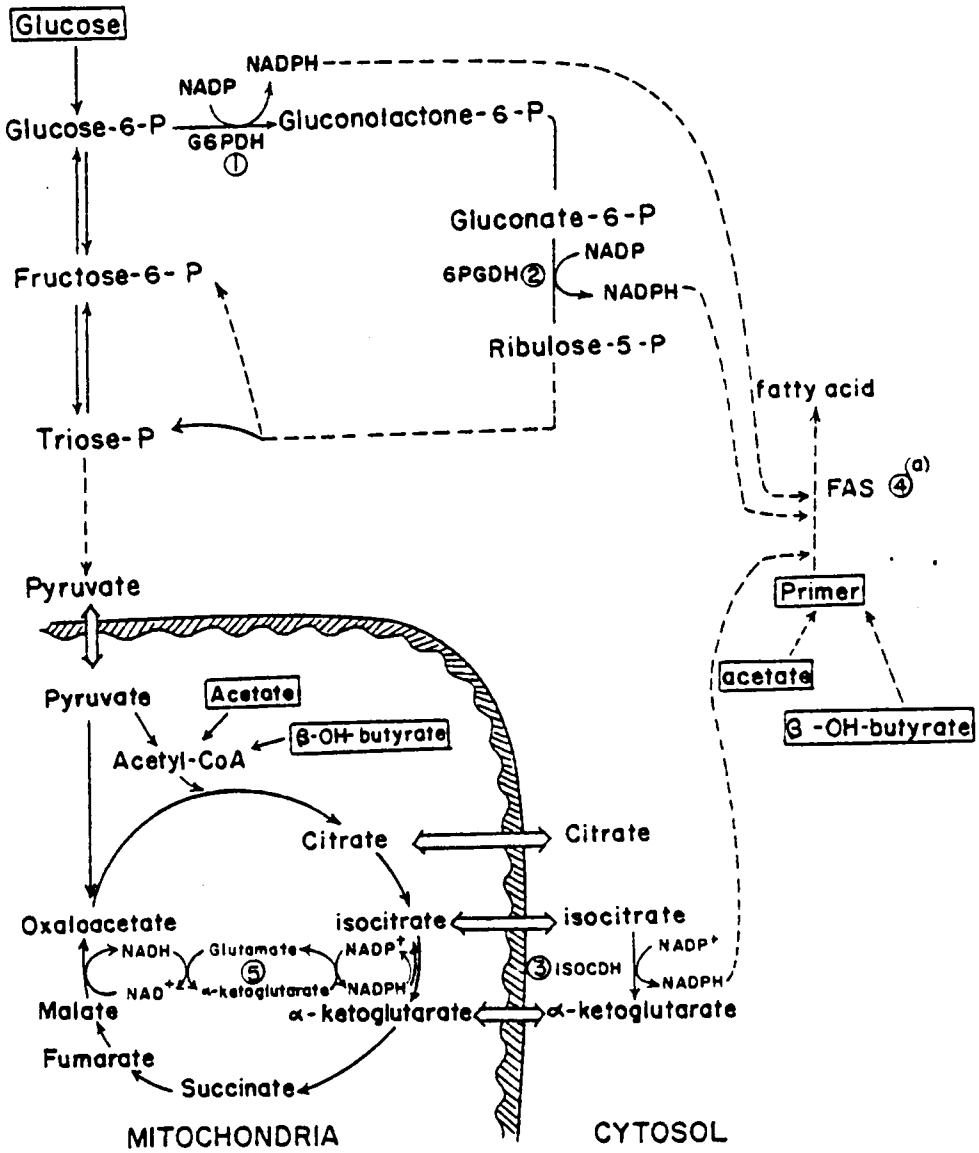

Figure 10. Schematic representation of NADPH generation for fatty acid synthesis in ruminants. Adapted from (13).

(a) FAS multienzyme complex: (1) Acyltransferase, (2) Malonyltransacylase, (3) Dehydratase, (4) Enoylreductase, (5) Acylcarrier protein (ACP), (6) B-ketoacyl ACP Synthetase, (7) B-ketoacyl ACP reductase.

(6, 54) in suggesting a minor involvement of enzymes in explaining the changes in milk fat secretion observed when high concentrate rations are fed. Changes in metabolism could occur due to factors other than changes in enzymatic activity.

Our data supports Baldwin's concept (6) related with the implications of metabolite concentrations in the control of milk fat secretion in the dairy cow.

The lack of response in enzymatic activity due to rations may be explained perhaps by the reduced intake of ration components, particularly crude protein by concentrate cows. Cows in concentrate ration consumed less crude protein than required as mentioned earlier in this discussion, due to a possible chemostatic regulation of intake.

Experiment II also suggests that extreme depression in milk fat secretions was not achieved in ad libitum concentrate cows. These observations are supported by the fact that ad libitum concentrate cows did not gain weight rapidly as shown by other (7, 54).

It has been assumed that cows in high concentrate rations produce more milk compared to normal rations due to a higher availability of milk precursors at the mammary gland level. Nevertheless, our experiment showed that crude protein could be limiting because of the restricted intake of total dry matter due to the high energy density of the ration. Milk production will not be expressed at full potential, unless especial consideration is given to the reduction in dry matter intake when formulating for protein requirements.

Theoretically, ad libitum consumption of concentrate with a proper energy-protein ratio should allow maximum intake of crude protein and available energy for glucose synthesis and provide optimal conditions to express maximum milk production, depressed milk fat secretion, and increase in body weight gains.

Under these conditions, a number of metabolic alterations could occur. Reports in the literature (4, 67, 79) have shown that fatty acid synthesis from acetate in vitro in ruminants is greatly increased in the presence of glucose. Under this theoretical situation, delineated before, glucose availability will be increased resulting in greater amounts of glucose being channelled through the TCA cycle for use in lactose synthesis and for oxidation purposes (7).

Isocitrate cycle provides NADPH for fatty acid synthesis by utilizing acetate and is the main source of reducing equivalents with an operational transhydrogeneration scheme (See item 5, Figure 10). This activity will diminish due to a disruption of the transhydrogenation via glutamate dehydrogenase, with consecutive increase in pentose phosphate cycle activity (See items 1 and 2, Figure 10) when glucose concentration increases (7).

It was shown by Flatt (25) and Bauman and Davis (13) that the individual pathway used by the cell in the generation of reducing equivalents is related to the energy (ATP) needs of the cell. Isocitrate cycle is an ATP generating process and pentose phosphate cycle an ATP deficient process. According to Bauman and Davis (13), isocitrate cycle yields three moles of ATP per mole of acetate incorporated into fatty acids. When transhydrogeneration is not operational,

the isocitrate cycle yields 14 moles of ATP.

Results agree with the literature (48) with respect to a greater involvement of isocitrate cycle (See item 3, Figure 10) compared with the pentose shunt (See items 1 and 2, Figure 10) in providing reducing equivalents for fatty acid synthesis at the mammary gland level under the present conditions (Table 9).

Equal participation of the two pathways is observed for adipose tissue (Table 9), results that agree with the literature (13). Adipose tissue, being a less active tissue compared to mammary gland, does not require the large amount of ATP that will be produced if isocitrate cycle is the predominant souce of reducing equivalents.

Despite the agreement with the literature, overall changes in enzyme activity did not show great response to rations. No appreciable decrease was observed for ISOCDH (Item 3, Figure 10) or increase for 6PGDH (Item 2, Figure 10) and G6PDH (Item 1, Figure 10) in mammary tissue of ad libitum concentrate cows when compared to restricted concentrate. This was expected for high fat depressed cows.

An increase, although not significant, was observed for G6PDH and 6PGDH in adipose tissue for ad libitum concentrate compared to restricted fed cows. This could possibly explain the differences in fat test observed in Figure 7 for these two groups. Increased metabolite concentration could have altered fat synthesizing processes at the mammary gland level in ad libitum concentrate fed cows.

Apparently, as mentioned before, mammary gland enzymes were not responsive to ration effects (5, 54) and other factors like metabolite concentrations or hormonal regulation of these metabolites might be

involved in the control of the metabolic alterations conducive to milk fat depression (54).

Our results support published reports (5, 6, 54) suggesting the possibility that mammary enzyme activity is not the reason for the differences observed in fat test when concentrates are fed. A variety of ration extremes have been used to test the involvement of enzyme activity, but little if any enzyme activity changes have been obtained.

According to our results, mid lactation (150 days), which has been used in most of the reports, is probably not the appropriate time to make enzyme comparisions. If any difference in enzyme activity is to be expressed, comparisons should be done just beyond peak of lactation. It is during this period where drastic changes in the cow metabolism occur because of the heavy demands for synthesis of milk components.

SUMMARY

Three experiments were conducted to better understand the regulation of milk fat secretion by looking at concentration and ratio of rumen fermentation products, blood metabolic parameters and ration characteristics as well as the activities of some enzymes involved in fatty acid synthesis.

In Experiment I, 21 cows in mid lactation (150 days) placed in milk production strata were randomized to (1) roughage ration restricted to NRC (53); (2) concentrate ration ad libitum; or (3) concentrate ration restricted to NRC (53).

Cows fed concentrate rations (ad libitum and restricted) produced more milk than those fed the roughage ration. Ad libitum concentrate feeding resulted in more milk than restricted, probably due to higher energy intake.

Fat content of milk was lower for concentrate as compared to roughage rations; also values for ad libitum were lower than restricted concentrates.

Serum glucose did not differ for cows fed ad libitum and restricted concentrates; however, serum glucose was higher in cows fed concentrate as compared to roughage rations.

Molar proportion VFA values were similar to those obtained by others for similar types rations. Acetate decreased and propionate and valerate were higher for the concentrate than the roughage ration, independent of the level of intake. Total VFA concentration was higher for ad libitum concentrate compared to other rations.

Non-glucogenic ratio (NGR) [defined as (acetate + 2 butyrate + valerate) ÷ (propionate + valerate)] showed marked differences among rations. Restricted concentrate showed a more appropriate balance of glucogenic and nonglucogenic VFA. Cows fed concentrate ad libitum had NGR values that suggest inefficient utilization of energy for milk fat production.

In Experiment II, 24 cows were used from parturition until 180 days in their lactation. The rations used were (1) roughage ad libitum; (2) concentrate ad libitum; (3) concentrate restricted to NRC (53); and (4) normal ration ad libitum.

Cows on concentrate rations (ad libitum and restricted) consumed less dry matter than control cows (roughage and normal). Periods as well as ration by period interaction were also significant. Animals reached peak intake around 45-98 days except for restricted concentrate cows that constantly decreased.

Intake of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were significantly different among rations. Cows in concentrate rations consumed less than control rations. Concentrate rations consumed less CP compared to control rations. A chemostatic mechanism controlling intake of concentrate cows could explain the lower intake.

Significant difference among periods and for ration by period interaction was found for body weight. In general, cows maintained weight (control rations) or lost weight (concentrate rations) up to week seven, constantly gaining weight thereafter. Cows in control rations did gain more weight during the whole experiment compared to concentrate fed cows.

No significant differences were observed for milk production among the different rations. However, milk solids showed significant differences between control and concentrate rations. Concentrate rations had similar values independent of the level of intake. Milk protein, also, showed significant differences among the different rations. Cows in control rations had higher protein values compared to cows in concentrate rations. Restricted concentrate showed lower values than ad libitum concentrate cows.

Concentrate cows had lower fat test than cows in control rations. A decrease in fat test from the time of parturition to week eight or nine was observed for every ration, being more pronounced for cows in concentrate rations. Energy restrictions improved milk fat secretion compared to ad libitum intake after the eighth week.

Molar proportion VFA and total VFA concentration differed among rations. Molar proportion acetate decreased and propionate and valerate increased for concentrate compared to control rations. Restricted concentrate rations did not differ from ad libitum concentrate rations. Molar proportion propionate showed significant variations during the experiment. All rations except roughage showed increasing propionate molar percentage up to period 2 (45-98 days), leveling off later in the experiment.

Total VFA concentration showed differences between rations. Restricted concentrate cows showed values similar to control fed cows but less than ad libitum concentrate. Also, NGR was more favorable for restricted compared to ad libitum concentrate cows.

Even though changes in pH were seen among the rations, no significant differences were found for rumen buffer capacity.

When blood parameters are considered, concentrate cows showed slightly higher glucose values and smaller blood acetate values compared to control cows.

Twelve cows in mid lactation fed the rations of Experiment II excluding normal ration were used to determine the effect of available energy intake on activity of key enzymes involved in fatty acid synthesis.

Activity for 6-phosphogluconate dehydrogenase was not different among rations. Mammary tissue showed from 5 to 6 times higher activity than adipose tissue depending on the diet. Cows on restricted concentrate tended to have higher activity in mammary tissue than ad libitum or roughage cows. Fat tissue activity was higher for concentrate rations and lower for roughage.

No significant differences were observed for rations or tissue for glucose 6-phosphate dehydrogenase.

Isocitrate dehydrogenase did not differ due to rations. Nevertheless, when tissues are considered, mammary was 21 to 28 times more active per gram of wet weight than adipose tissue.

Fatty acid synthetase did not show a difference in activity due to ration. Significant differences were observed between tissues. Mammary averaged 10 times more activity per gram of tissue than adipose tissue. Mammary tissue activity of roughage fed cows averaged seven times more than adipose.

From these experiments, the following has been concluded.

Conclusions

1. Milk fat depression is partially alleviated by limiting the amount of intake of high concentrate rations.
2. Stage of lactation relative to energy status of the animal, influences susceptibility to milk fat depression when high grain rations are fed.
3. Enzymatic activity does not seem to be the main factor involved in controlling milk fat secretion when measured during mid lactation.
4. Excess grain feeding was inefficient for obtaining maximum milk production.

REFERENCES

1. Annison, E. F., and R. R. White. 1961. Glucose utilization in sheep. Biochem, J., 80:162.

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

3. Armstrong, D. G., K. L. Blaxter, and N. McC. Graham. 1957. The heat increment of mixtures of steam volatile fatty acids in sheep. Brit. J. Nutr., 11:392.

4. Armstrong, D. G., and K. L. Blaxter. 1965. Effects of acetic and propionic acids on energy retention and milk secretion in goats. In Energy Metabolism. p. 59. K. L. Blaxler, ed. Adademic Press, New York.

5. Baldwin, R. L., H. J. Lin, W. Cheng, R. Cabrera, and M. Ronning. 1969. Enzyme and metabolite levels in mammary and abdominal adipose tissue of lactating dairy cows. J. Dairy Sci., 52:183.

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

7. Baldwin, R. L., Y. T. Yang, K. Crist, and G. Grichting. 1976. Theoretical model of ruminant adipose tissue metabolism in relation to the whole animal. Fed. Proc. 35:2314.

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

9. Balmain, J. H., S. J. Folley, and R. F. Glascock. 1952. Effects of insulin and of glycerol *in vitro* on the incorporation of (carboxy-^{14}C) acetate into the fatty acids of lactating mammary gland slices with special reference to species differences. Biochem. J., 52:301.

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

11. Bauman, D. E., R. W. Mellenberger, and R. G. Derrig. 1973. Fatty acid synthesis in sheep mammary tissue. J. Dairy Sci., 56:1312.

12. Bauman, D. E. 1974. Fat metabolism in ruminants. Cornell Nutr. Conf. p. 69.

13. Bauman, D. E., and C. L. Davis. 1975. Regulation of lipid metabolism. In Digestion and Metabolism in the ruminant (Proc. IV Symposium on Rum. Physiology). I. W. McDonald A.C.I. Warner (Ed) p. 496.

14. Bauman, D. E. 1976. Intermediary metabolism of adipose tissue. Fed. Proc., 35:2308.

15. Baumgardt, B. R. 1964. Practical Observations on the Quantitative Analysis of Free Volatile Fatty Acids (VFA) in Aqueous Solutions by Gas-Liquid Chromatography. Departmental Bulletin 1. Department of Dairy Science, University of Wisconsin, Madison, WI 53706.

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

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

18. Bickerstaffe, R. 1971. Uptake and metabolism of fat in the lactating mammary gland. In Lactation (ed I. R. Falconer) p. 317. London:Butterworth.

19. Bickerstaffe, R., E. F. Annison. 1971. Triglyceride synthesis in goat and sow mammary tissue. Int. J. Biochem., 2:153.

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

21. Boman, R. L. 1965. The effects of various nutritional factors on the composition of milk. M.S. Thesis, Virginia Polytechnic Institute & State University, Blacksburg, VA.

22. Coppock, E. E., W. P. Flatt, L. A. Moore, and W. E. Stewart. 1964. Relationship between end products of rumen fermentation and utilization of metabolizable energy for milk production. J. Dairy Sci., 47:1359.

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

24. Flatt, W. P., P. W. Moe, L. A. Moore, N. W. Hooven, R. P. Lehmann, E. R. Orskov, and R. W. Hemken. 1969. In K. L. Blaxter, G. Thorbek and J. Kielanowski (Ed.) Energy Metabolism of Farm Animals. p. 221. Oriel Press, Newcastle-upon-Tyne.

25. Flatt, J. P. 1970. Conversion of carbohydrate to fat in adipose tissue: an energy-yielding and, therefore, self-limiting process. J. of Lipid Res., 11:131.

26. Folley, S. J. and T. H. French. 1949. The intermediary metabolism of the mammary gland. 1. Respiration of lactating mammary gland slices in presence of carbohydrates. Biochem. J. 45:117.

27. Folley, S. J., and T. H. French. 1950. The intermediary metabolism of the mammary gland. 3. Acetate metabolism of lactating mammary gland slices with special reference to milk fat synthesis. Biochem. J., 46:465.

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

29. Glock, G. E., and P. McLean. 1953. Further studies on the properties and assay of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of rat liver. Biochem. J., 55:400.

30. Goodridge, A. G. 1972. Regulation of the activity of acetyl coenzyme A carboxylase by palmitoyl coenzyme A and citrate. J. Biol. Chemistry, 247:6946.

31. Hanson, R. W., and F. J. Ballard. 1967. The relative significance of acetate and glucose as precursors for lipid synthesis in liver and adipose tissue from ruminants. Biochem. J., 105: 529.

32. Hardwick, D. C. 1966. The fate of acetyl groups derived from glucose in the isolated perfosed goat udder. Biochem. J., 99:228.

33. Hodgson, J. C., and P. C. Thomas. 1972. The chemical composition and dilution rate of rumen fluid in sheep receiving a diet of barley, hay and flaked maize. Proc. Nutr. Soc. 31:57A.

34. Hodgson, J. C., and P. C. Thomas. 1973. The influnce of the level of feeding on the digestion of a diet consisting of barley, hay and flaked maize. Proc. Nutr. Soc. 32:91A.

35. Hovell, F. D. De B., J. F. D. Greenhalgh, and F. W. Wainman. 1976. The utilization of diets containing acetate salts by growing lambs as measured by comparative slaughter and respiration calorimetry together with rumen fermentation. Brit. J. Nutr. 35:343.

36. Huber, J. T., and R. L. Boman. 1966. Nutritional factors affecting the solids-not-fat content of milk. J. Dairy Sci., 49:816.

37. Huber, J. T., and R. L. Boman. 1966. Effect of grain level and protein content of the grain for grazing cows on milk composition and yield, and certain blood and rumen constituents. J. Dairy Sci. 49:395.

38. Isaacson, H. R. F. C. Hinds, M. P. Bryant, and F. N. Owens. 1975. Efficiency of energy utilization by mixed rumen bacteria in continuous culture. J. Dairy Sci., 58:1645.

39. Jorgensen, N. A., and L. H. Schultz. 1965. Ration effects on rumen acids, ketogenesis, and milk composition. II. Restricted roughage feeding. J. Dairy Sci., 48:1040.

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

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

42. Jahn, E., P. T. Chandler, and R. F. Kelly, 1976. Nutrient accumulation and prediction of body composition of 20-week-old calves fed varying percentages of protein and fiber. J. Am. Sci. 42:736.

43. Kinsella, J. E., D. Bruns, and J. P. Infante. 1975. Fatty acid synthetase of bovine mammary: properties and products. Lipids 10:227.

44. Kinsella, J. E., and G. Houghton. 1975. Phospholipids and fat secretion by cows on normal and low fiber diets: Lactational trends. J. Diary Sci., 58:1288.

45. Kleinschmidt. A. K., J. Moss, M. D. Lane. 1969. Acetyle coenzyme A carboxylase: filamentous nature of the animal enzymes. Science 166:1276.

46. Latham, M. J., J. D Sutton, and M. E. Sharpe. 1974. Fermentation and microorganisms in the rumen and the content of fat in the milk of cows given low roughage rations. J. Dairy Sci., 57:803.

47. Lowry, O. H., N. O. Rosebrough, A. L. Fan, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265.

48. Mellenberger, R. W., D. E. Bauman, and D. R. Nelson. 1973. Metabolic adaptations during lactogenesis. Fatty acid and lactose synthesis in cow mammary tissue. Biochem. J., 136:741.

49. McClymont, G. L. 1951. Volatile fatty acid metabolism of ruminants with particular reference to the lactating bovine mammary gland and the composition of milk fat. Australian J. Agr. Res. 2:158.

50. McClymont, G. L., and S. Vallance. 1962. Depression of blood glycerides and milk-fat synthesis by glucose infusion. Proc. Nutrition Soc., 21: XII.

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

52. Montgomery, M. J., and B. R. Baumgardt. 1965. Regulation of food intake in ruminants. 1. Pelleted rations varying in energy concentrations. J. Dairy Sci. 48:569.

53. Nutrient requirement of dairy cattle. 1971. National Academy of Sciences, National Research Council. Publ. ISBN 0-309-01916-8.

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

55. Orskov, E. R., F. D. Hovell, and D. M. Allen. 1966. Utilization of salts of volatile fatty acids by growing sheep. 2. Effect of stage of maturity and hormone implantation on the utilization of violatile fatty acid salts as a source of energy for growth and fattening. Brit. J. Nutr., 20:307.

56. Orskov, E. R., and C. Fraser, 1974a. The effect of processing of cereals on digestibility, type of rumen fermentation and firmness of subcutaneous fat in sheep. Brit. J. Nutr. 32:71.

57. Orskov, E. R. 1977. Capacity for digestion and effects of composition of absorbed nutrients on animal metabolism. J. Animal Sci., 46:600.

58. Plaut, G. W. E. 1962. Isocitric dehydrogenase (TPN-linked) from pig heart (revised procedure) In Methods in Enzymology vol. 5:645.

59. Popjak, G., T. H. French, and S. J. Folley. 1951. Utilization of acetate for milk-fat synthesis in the lactating goat. Biochem. J., 48:411.

60. Popjak, G., T. H. French, G. D. Hunter, and A. J. P. Martin. 1951. Mode of formation of milk fatty acids from acetate in the goat. Biochem. J., 48:612.

61. Robertson, J. B., and P. J. Van Soest. 1977. Dietary fiber estimation in concentrate feedstuffs. Paper no. 636, presented at 69th meeting ASAS, Univ. Wisconsin, Madison.

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

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

64. Rook, J. A. F., J. B. Hopwood. 1970. The effects of intravenous infusions of insulin and of sodium succinate on milk secretion in the goat. J. Dairy Res., 37:193.

65. Rudel, L. L., A. L. Jason, D. M. Manford and M. S. James. 1974. Characterization of plasms lipoprotein separated and purified by agarose column chromatography. Bioch. J., 139:89.

66. Schacterle, G. R., and R. L. Pollack. 1973. A simplified method for the quantitative assay of small amounts of protein in biological material. Anal. Biochem., 51:654.

67. Smith, R. W. and R. F. Glascock. 1969. The effects of acetate and of pyruvate on the pathways of glucose catabolism in lactating mammary tissue. II. Sheep tissue. J. Dairy Res., 36:469.

68. Storrv, J. E. and J. A. F. Rook. 1965. Effects of intravenous infusions of acetate, beta-hydroxy-butyrate, triglyceride and other metabolite on the composition of the milk fat and blood in cows. Biochem. J. 97:879.

69. Storry, J. E., D. E. Brumby, A. J. Hall, and V. W. Johnson. 1974. Responses in rumen fermentation and milk-fat secretion in cows receiving low-roughage diets supplemented with protected tallow. J. Dairy Res. 41:165.

70. Thomas, P. C. 1975. Diet and milk secretion in the ruminant. World Review of An. Pord., X1:33.

71. Van Soest, P. J., and N. N. Allen. 1959. Studies on the relationship between rumen acids and fat metabolism of ruminants fed on restricted roughage diets. J. Dairy Sci., 42:1977.

72. Van Soest, P. J. 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. J. Assn. O. A. Chem. 46:829.

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

74. Van Soest, P. J. 1967. Development of a comprehensive system of feed analysis and its application to forages. J. Animal Sci., 26:219.

75. Van Soest, P. J., and R. H. Wine. 1967. Use of detergents in the analysis of fibrous feeds. IV. The determination of plant cell wall constituents. J. Assn. O. A. Chem. 50:50.

76. Williamson, D. H., and J. Mellanby. 1965. D-(-)- Hydroxybutyrate In Methods of Enzymatic analysis. 2nd ed. Bergermeyer, H. V. Ed.

77. West, C. E., and R. F. Passey. 1967. Effect of glucose load and of insulin on the metabolism of glucose and of palmitate in sheep. Biochem. J., 102:58.

78. West, C. E., R. Bickerstaffe, E. F. Annison and J. L. Linzell. 1972. Studies on the mode of uptake of blood triglycerides by the mammary gland of the lactating goat. Biochem. J., 126:477.

79. Yang. Y. T., and R. L. Baldwin. 1973. Preparation and metabolism of isolated cells from bovine adipose tissue. J. Dairy Sci., 56:350.

APPENDIX

Appendix Table 1. Concentrate mixtures for rations used in Experiment I.

INGREDIENTS	CONCENTRATE	ROUGHAGE
TM Salt	1.00	1.00
Ground Limestone	1.75	2.75
Dicalcium PO_4	.25	2.75
MgO	.25	.25
Na_2SO_4	.60	.60
Vit mix (12×10^6 U/g vit A, 12×10^6 U/g vit D)	.25	.25
Dried Molasses	1.25	1.25
Wet Molasses	3.00	3.00
Distillers Grain	2.50	2.50
Wheat	5.75	5.75
Pellet Binder	2.00	2.00
Ground Shelled Corn	63.35	77.10
Soybean Meal	18.05	.80
TOTAL	100.00	100.00

Appendix Table 2. Analysis of variance for different parameters in Experiment I.

	DMI[1]		BW		MILK		FAT		FCM	
	df	ss	df	ss	df	ss	df	ss	df	ss
Covariate	1	46.62(a)		229413.64(a)		515.00(a)		48.57(a)		198.81(a)
Treatment (T)	2	86.96(a)		9364.43(a)		98.87(a)		45.26(a)		25.44(c)
Period (P)	3	4.99		1795.93		86.69(a)		5.02(a)		23.62
T x P	6	41.17(f)		1812.85		20.63		1.25		15.28
Residual	71	280.32	70	23318.34	71	325.54	70	18.18	70	367.22
R^2		.79		.93		.68		.76		.48

	BUTYRATE		PROPIONATE		VALERATE		i-VALERATE		GLUCOSE		ACETATE	
	df	ss	df	ss	df	ss	df	ss	df	ss	df	ss
Covariate	1	42.49(e)		10.01(a)		.79		.03		9.49		2.32
Treatment (T)	2	2.08		9.17(a)		9.73(a)		.35		901.46(a)		186.28(d)
Period (P)	1	1.34		2.41		1.05		.26		752.87(a)		4.00
T x P	2	22.50		1.78		.86		.21		281.63		21.50
Residual	20	176.73	20	166.32	20	.15	20	3.49	34	189.18	20	421.75
R^2		.27		.75		.54		.19		.38		.39

1. Units for each parameter can be found on Table 2.

2. a = P < .01; b = P < .05; c = P < .10; d = P < .02; e = P < .04; f = P < .12.

Appendix Table 3. Concentrate mixtures for rations used in Experiments II and III.

INGREDIENTS	ROUGHAGE	AD LIBITUM & RESTRICTED CONCENTRATE	NORMAL
TM Salt	1.20	.24	1.43
Ground Limestone	3.00	.84	5.00
Dicalcium PO_4	4.20	.60	3.60
MgO	1.20	.24	1.43
Na_2SO_4	.50	.10	1.55
Vit Mix ($6x10^5$I.U./g vit A; $4x10^5$I.U./g vit D)	.22	.44	.53
Molasses	4.00	.80	4.00
H_2O	4.00	.80	4.00
SBM	77.00	12.30	74.33
Ground Corn	5.00	84.10	4.20
TOTAL	100.00	100.00	100.00

Appendix Table 4. Analysis of variance for different parameters in Experiment II.

SOURCE	MILK SOLIDS[1]		MILK		BHBA		SERUM BUTYRATE		SERUM PROPIONATE	
	df	ss	df	ss	df	ss	df	ss	df	ss
Ration ←┐	3	43.89(a)[2]		294.04		11.13		.01		.04
Cow(Ration)┘	20	29.06(a)		9770.44(a)		45.71		.02		.31
Period	3	18.86(a)		2186.21(a)		20.63(b)		.01(c)		.11(d)
Ration×Period	9	4.83		66.66		8.38		.01		.09
Residual	203	79.40	586	7112.53	25	45.52	57	.03	153	2.10
R^2		.54		.63		.70		.46		.21

	SERUM ACETATE		RUMEN VALERATE		RUMEN i-VALERATE		RUMEN BUTYRATE	
	df	ss	df	ss	df	ss	df	ss
Ration ←┐	3	17.63(e)		3.48		2.71		63.39(b)
Cow(Ration)┘	20	42.67(a)		32.62(a)		15.41		102.14
Period	3	12.67(a)		4.65(e)		3.77(f)		13.58
Ration×Period	9	10.50		4.07		12.74(a)		54.41
Residual	165	147.31	179	93.07	179	99.43	180	966.49
R^2		.37		.34		.26		.22

1. Units for each parameter can be found in Table 5, 6, 7.

2. a = P < .01; b = P < .02; c = P < .06; d = P < .04; e = P < .03, f = P < .08; g = P < .10; h = P < .12; i = P < .09.

Appendix Table 5. Analysis of variance for different parameters in Experiment II.

SOURCE	RUMEN PROPIONATE[1]		RUMEN ACETATE		GLUCOSE		MILK PROTEIN		BODY WEIGHT	
	df	ss	df	ss	df	ss	df	ss	df	ss
Ration ←┐	3	586.97(a)[2]		339.12(h)		248.29		4.90(a)		352323.89
Cow(Ration)┘	20	684.16(a)		1042.50(a)		3928.56(a)		4.04(a)		1429340.11(a)
Period	3	141.48(d)		78.25		282.23(i)		1.97(a)		30741.80(a)
Ration×Period	9	254.70(g)		194.17		299.92		1.20(e)		34841.61(a)
Residual	180	3057.23	180	3852.86	196	8526.80	211	13.67	569	401770.24
R^2		.40		.32		.36		.46		.82

	BUFFER CAPACITY		pH		FAT TEST		DRY MATTER INTAKE		TOTAL VFA	
	df	ss	df	ss	df	ss	df	ss	df	ss
Ration ←┐		1.27		4.56(a)		50.08(a)		4464.75(a)		1831.28(c)
Cow(Ration)┘		6.28(b)		3.06		48.61(a)		2231.47(a)		4306.88(g)
Period		1.10(g)		1.82(a)		43.48(a)		313.27(a)		1964.51(a)
Ration×Period		1.55		2.29(f)		16.65(a)		827.83(a)		493.45
Residual	178	31.23	180	26.36	535	152.52	572	3192.76	180	26751.20
R^2		.26		.33		.52		.72		.25

1. Units for each parameter can be found in Table 5, 6, 7.
2. a = P < .01; b = P < .02; c = P < .06; d = P < .04; e = P < .03; f = P < .08; g = P < .10; h = P < .12; i = P < .09.

Appendix Table 6. Analysis of variance for different parameters in Experiment II.

SOURCE	FCM[1]		ADF INTAKE		CP INTAKE		NDF INTAKE		NGR	
	df	ss	df	ss	df	ss	df	ss	df	ss
Ration		593.85		220.46(a)		33.73(a)		535.05(a)		16.84(a)
Cow(Ration)		7703.10(a)		25.76(a)		26.20(a)		81.54(a)		21.34(a)
Period		1853.09(a)		5.01(a)		2.80(a)		17.02(a)		3.24(f)
Ration×Period		293.29(a)		9.61(a)		7.60(a)		27.22(a)		4.73
Residual	535	6056.22	251	34.66	251	48.77	251	108.91	179	85.32
R^2		.64		.90		.62		.88		.39

1. Units for each parameter can be found in Table 5, 6, 7.
2. a = P < .01; b = P < .02; c = P < .06; d = P < .04; e = P < .03; f = P < .08; g = P < .10; h = P < .12; i = P < .09.

Appendix Table 7. Analysis of variance for enzymes in Experiment III.

	df	6-P-GLUCONATE DEHYDROGENASE[1]	GLUCOSE 6-P DEHYDROGENASE	ISOCITRATE DEHYDROGENASE	FATTY ACID SYNTHETASE
		sum of squares			
Ration ←┐	2	.08	1.51	.49	.07
Cow(Ration)←┘	9	2.79	9.09	300.87	1.04
Tissue	1	9.56	1.19	2342.44	3.16
Ration x Tissue	2	.30	1.40	1.50	.03
Residual	9	2.13	7.02	275.85	.79
R^2		.87	.65	.91	.86

1. Units for each parameter can be found in Table 9.

The vita has been removed from the scanned document

FACTORS ASSOCIATED WITH MILK FAT SECRETION OF COWS
IN RESPONSE TO CONTRASTING AVAILABLE ENERGY CONSUMPTION

by

Demetrio Zanartu

(ABSTRACT)

Three experiments were conducted to determine the relationship of milk fat secretion to concentration and ratio of rumen fermentation products, blood metabolic parameters, ration characteristics and certain enzymic activity involved in fatty acid synthesis.

In Experiment I, 21 cows in mid lactation were randomized according to milk production to (1) roughage ration (∿22% CF) restricted to NRC; (2) concentrate ration (∿12% CF) ad libitum; or (3) concentrate ration (∿12% CF) restricted to NRC and fed for 30 days.

Ad libitum concentrate showed higher dry matter intake (DMI), body weight (BW), milk production than restricted concentrate. The opposite was true for fat test. Concentrate rations (ad libitum and restricted) when compared to roughage showed higher values for DMI, BW, milk poduction, serum glucose, molar proportion propionate and valerate and lower values for fat test and molar proportion acetate.

In Experiment II, 24 cows 180 d. post parturition, were assigned to four rations based on anticipated parturition. Rations were: (1) roughage ad libitum (∿20% CF); (2) concentrate ad libitum (∿10% CF); (3) concentrate restricted (∿10% CF) to NRC; and (4) normal ration (∿14% CF) ad libitum. Cows on ad libitum and restricted concentrate consumed less dry matter, acid detergent fiber, neutral detergent

fiber and crude protein than control cows (roughage and normal). Cows fed control rations gained more weight than concentrate fed cows. No difference was found for milk production. Milk protein was higher for control cows. Concentrate cows had lower fat test than control cows. Cows on all rations decreased fat test up to week three or four but by week eight or nine, concentrate fed cows had decreased to their lowest fat test. Energy restriction improved milk fat secretion compared to ad libitum intake after the eighth week. Molar proportion VFA favored propionate for concentrate cows and was similar for restricted and ad libitum concentrate. Total ruminal VFA concentration was higher for ad libitum concentrate cows and those cows showed slightly higher glucose and smaller blood acetate compared to control.

Twelve cows in mid lactation were fed the rations of Experiment II to determine the effect of available energy intake on activity of key enzymes of fatty acid synthesis. Activity of 6-phosphogluconate dehydrogenase was not different among rations. Mammary tissue showed from five to six times higher activity than adipose tissue. Fat tissue activity tended to be higher for concentrate rations compared to roughage. No differences among rations were found for glucose 6-phosphate dehydrogenase nor isocitrate dehydrogenase. Mammary tissue was 21 to 28 times more active than adipose tissue for latter enzyme. Fatty acid synthetase showed no difference in acitvity due to rations, but mammary tissue was seven to ten times more active than adipose tissue.