Evaluating Fish meal as a Protein Source for Lactating Dairy Cows.

by

James Nobles Spain

Dissertation submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of
Doctorate of Philosophy
in
Animal Science

APPROVED:

Carl E. Polan, Chairman	R. Michael Akers
Walter G. Niehaus	Bruce A. Watkins
Kenneth E. Webb, Jr.	William E. Vinson, Department Head

December 19, 1989

Blacksburg, Virginia

Evaluating Fish meal as a Protein Source for Lactating Dairy Cows.

by

James Nobles Spain

Carl E. Polan, Chairman

Animal Science

(ABSTRACT)

A series of experiments were conducted to measure the mode of action by which fishmeal exerts its affect on milk composition of dairy cows. Production Study One measured the effect of increased fishmeal intake on milk production and milk composition in dairy cattle. Milk fat percentage and yield were decreased by increased fishmeal intake. This decrease was not associated with changes in ruminal fermentation patterns. Plasma long-chain n-3 polyunsaturated fatty acids were increased with increased fishmeal intake.

Mammary slices from lactating bovine mammary gland were incubated with eicosapentaenoic acid. Changes in ^{14}C-acetate metabolism were measured. Acetate oxidation and incorporation into milk lipid were not changed by eicosapentaenoic acid. However, tissue from mammary gland of cows milked 6 to 8 h prior to slaughter had 2 fold higher activity than tissue milked 1 h before slaughter.

Disappearance of dry matter, crude protein, and lipid in fish meal from undegradable bags in the rumen was measured. Dry matter and crude protein degradation of fish meal were similar to published values. Lipid escaped rapidly with greater than 70% removed by 8 h. A subsequent fish oil infusion study revealed that intraruminal fish oil treament did not significantly alter ruminal fermentation, or change fatty acid profiles in duodenal digesta, plasma, or milk. Duodenal infusion increased plasma concentrations of n-3 fatty acids but did not affect milk lipid fatty acid composition.

A second production study compared the effects of fish meal versus fish oil on milk production and composition, and changes in fatty acids in plasma and milk. Fish meal significantly increased plasma n-3 fatty acids compared to the fish oil treatment. Residual fatty acids contained in fish meal seemed to be protected from rumen degradation and probably remained intact for digestion and absorption since plasma n-3 fatty acid concentrations increased. No changes in milk yield or composition were due to the experimental treatments. Fat in fish meal and fish oil source differed significantly in their ability to alter milk composition and plasma fatty acid profiles.

Acknowledgements

Many people have made significant contributions towards completing the work described in this dissertation. For their contributions, a sincere thank you is extended to the people listed below.

Dr. Polan has been an adviser, teacher, and trusted friend. For this and the guidance he has provided me through out my post-graduate education I am very grateful.

I appreciate Dr. Webb's training in critical reveiw of published work and for serving on my graduate committee.

For training me in techniques of lipid chemistry and providing stimulating discussion on lipid and vitamin nutrition I thank Dr. Bruce Watkins.

I appreciate the contribution and perspective Dr. Niehaus provided as a committee member and for the stimulating discussion of lipid metabolism.

Dr. Mike Akers for teaching me tissue culture techniques which are a very important part of this dissertation and for giving a little push when a little push was needed.

Dr. William Vinson for the leadership provided as department head and providing a sounding board for graduate student concerns.

Dr. Joseph Herbein who has been supportive and helpful in every way, including critical review of experimental protocols, stimulating discussions on metabolism, and listening to alot of bad jokes.

For their daily support which was critical for the success of this dissertation, I thank , , and the farm crew at the V.P.I. Dairy Cattle Center.

To the faculty of the Department of Dairy Science who were supportive and helped in my professional development during the last five years, I extend my appreciation.

I wish to thank the Pratt Nutrition Foundation for the financial support that has allowed me to obtain the training while providing proper environment for my family.

I thank and the other departmental technicians too numerous to name but who have made unselfish contributions to the work I have done here.

For the outstanding job and help, I thank , , and the other technicians at the Forage Testing Lab.

For running the milk samples, I thank the technicians at DHI lab.

To my fellow graduate students who have been irreplacable and dear friends, I offer my sincere appreciation.

To my family of Faith at First Christian Church, I offer my heartfelt thanks for giving my family your support, friendship, during our stay. You have been like family and we will miss you.

I thank my parents who taught me to dream and to work to make that dream come true.

I thank my girls, my wife and daughter , for the sacrifices they have made for me for they were always there with love, patience and understanding.

To my Heavenly Father who blessed me with all the people listed above who formed an environment full of love, friendship, and support within which I have lived, I thank You. I pray that I might meet Your expectations.

Table of Contents

List of Illustrations

List of Tables

Introduction

Crude protein recomendations for dairy cattle have been revised to include contributions from intake of ruminally undegradable protein. Research suggests ruminants may increase production when bacterial protein flow to the small intestine is supplemented with undegraded dietary protein. Bacterial crude protein is estimated to be 80% digestible but total flow of bacterial protein may be inadequate to meet the daily requirements of high producing dairy cows. Protein sources differ in their amino acid composition and ruminal degradability. While proteins from different sources may be similar in total degradability, rates of degradation may differ greatly. Therefore, rates of release and degradation of specific amino acids may also vary across protein sources. These differences may be important in maintaining proper nitrogen and amino acid balance within the rumen and in meeting the desired amino acid supply to the small intestine. Amino acids supplied by supplemental protein sources must be those limiting for animal production in order to achieve enhanced production responses.

Fish meal has been shown to enhance flow of amino acids to absorptive sites of the small intestine. From a practical standpoint, addition of fish meal to the diet has been shown to increase milk protein yield with concurrent decreases in milk fat yields. Some data suggest that fish meal alters ruminal fermentation patterns similar to those found with high carbohydrate diets. However, other

studies failed to demonstrate changes in ruminal fermentation patterns with fish meal supplemented rations, but decreased milk fat content and milk yields persisted.

Literature Review

Ruminal protein degradation

Dietary protein is significantly altered by proteolytic activity of ruminal microorganisms. Tamminga (69) reported up to 80% of dietary protein may be degraded to ammonia and corresponding keto-acid. NRC (46) discussion of ruminal protein degradation suggested degradation be divided to include proteolysis of dietary protein and degradation of amino acids. As reviewed by Huber and Kung (26), ruminal proteolytic activity is a function of microbial population due to association of proteolytic enzymes with the bacterial cell membrane. As cited by NRC, Russell et al. (59) reported that degradation of peptides to amino acids may limit rate of protein degradation. Nolan and Leng (48) estimated that 30 percent of dietary protein was utilized as amino acids. Similarly, Pilgram et al.(53) measured ammonia incorporation into ruminal bacteria and protozoa and found 60 to 70 % of bacterial protein was derived from ammonia-nitrogen. Rates at which individual amino acids were degraded also differed when individual amino acids were incubated in mixed rumen microbial cultures (13). Rates of in vitro degradation of essential amino acids ranged from .88 mmoles per hour for arginine to .09 mmoles per h for methionine. Schiefinger et al. (62) incubated individual amino acids with pure cultures and reported significant differences in amino acid degradation between species and subspecies. They concluded that ruminal protein degradation

was influenced by interactions between ruminal microorganisms. Cotta and Russell (16) suggested that increased amino acid and peptide availability could increase microbial protein synthesis. In fact, as reviewed by Nocek and Russell (47), peptide uptake and utilization for protein synthesis may be important in ruminal microbial growth.

As summarized by Satter and Roffler (61) microbial protein can provide over 50 percent of metabolizable protein in ruminants. One factor associated with meeting protein requirements of lactating cows is the ability of the diet to efficiently maximize microbial protein synthesis (66). Nocek and Russell (47) observed that calculated maximal microbial protein synthesis was achieved when ruminal available carbohydrate and protein degradation was synchronized. In a series of experiments, Hume and co-workers (27) measured factors that influence microbial protein synthesis. Microbial protein synthesis was increased in sheep fed a protein free diet from 33 to 50 g per d when N intake was increased in from 2 to 9 g per d (27). When urea and volatile fatty acids were replaced with casein, microbial protein increased from 90 to 101 g per day. These data support the conclusion that preformed nutrients may stimulate microbial growth (47).

Sniffen and Robinson (66) reviewed concepts involved in microbial yield. These factors included liquid and solid digesta flow. Increased flow rates increased flow of microorganisms to the small intestine which resulted in increased microbial efficiencies due to decreased maintenance. Acidic ruminal pH has been shown to decrease microbial fermentation and growth. Therefore, physical factors such as flow rates and environmental quality such as pH interact with nutrient availabilty to influence microbial growth and efficiency of use as a nutrient source.

Post-ruminal protein supply

As discussed above, microbial fermentation significantly alters dietary protein. Also, the supply of microbial protein to absorptive sites of the small intestine is an important component which con-

tributes to meeting daily amino acid requirements of ruminants. Post-ruminal protein infusion data suggest microbial protein, while a high quality protein, may be produced in quantities which fail to meet the needs of high producing cows. Clark (15), in an extensive review, reported that post-ruminal infusion of casein resulted in increased milk and milk protein yield. Several recent studies support Clark's findings (38,57,68). Ranawana and Kellaway (57) infused 45 g casein per day post-ruminally and increased daily milk, protein and lactose production compared to control and glucose infusions. Casein infusion also increased N retention. These data were similar to data of Lough et al. (37) and Konig et al. (38) whom reported increased milk production or milk protein yields with casein infusions. Storm and Orskov (68) improved nitrogen retention in cattle with infusion of amino acids in ratios similar to amino acids found in ruminal microorganisms. Therefore, production potential of genetically superior cows may be reached by supplementing amino acids to the small intestine above production of rumen microbial protein.

Satter (60) discussed strategies of enhancing amino acid flow to the small intestine of lactating cows. Inclusion of protein resistant to microbial degradation has been extensively investigated. Protein supplements normally degraded extensively in the rumen may be treated chemically or with heat to increase ruminal escape. Bas et al. (4) used Ca-lignosulfate treatment of soybean meal to decrease in vitro crude protein degradation from 71 to 55% of original protein. Ca-lignosulfate treated soybean meal also increased total non-ammonia-nitrogen flow from continuous culture fermenters. Muller et al. (43) decreased ruminal degradation of whey by formaldehyde treatment but also decreased total digestibility of whey protein. These data demonstrate the importance of measuring treatment effects on ruminal degradability as well as digestibility.

Heat treatment of protein supplements has also been studied as a means of increasing ruminal escape of dietary protein. Roasting soybean meal at temperatures above 144 C was shown to negatively affect total tract digestibility (54). Acid detergent insoluble nitrogen was significantly increased from 6% of total N to 18 and 60% at 159 and 185 C, respectively. Chick growth studies showed decreased gains and feed efficiencies from feeding soybean meal which had been heated at 159 and 185 C. Schingoethe et al. (63) compared heat-treated soybean meal, extruded soybeans,

and untreated soybean meal as protein supplements for lactating cows. Soluble and degradable protein content of heated soybean meal was lower than extruded beans or control meal. Milk yields were greater for cows fed extruded beans compared with cows fed heated soybean meal. Fat-corrected-milk (FCM) was similar for extruded and heated supplements. Control soybean meal diets supported similar levels of FCM production as test diets when supplemented with rumen protected methionine. Heat treatment may increase ruminal escape of protein without adversely affecting N-digestibility, thus enriching or enhancing amino acid flow to small intestine.

Alternatives to chemical or heat treatment of supplements exist in utilization of by-product feeds which are naturally resistant to rumen microbial proteolysis. Satter (60) and the NRC (46) list several by-product protein sources with undegraded protein contents exceeding 50% of the protein content. As discussed with heat and chemical treatment, amino acid supply and digestibility are important in assuring high protein quality as well as quantity. Polan et al. (55) reported that diets supplemented with dried and wet brewers grains supported higher milk and milk protein yields compared to soybean meal supplemented diets. Brewers dried grains have been found to increase post-ruminal flow of valine, methionine, leucine, and phenylalanine in ruminants (40). Several authors as cited by Arambel and Coon (1) suggested that the amino acids most often limiting milk production include methionine, histidine, and phenylalanine.

Animal by-products have also been utilized to provide dietary sources of rumen escape protein. Waltz et al. (75) compared effects of feeding soybean meal, blood meal, or feather meal on amino acid flow and absorption at the small intestine of lactating cows. Feather meal significantly increased flow of non-NH_3-N to the duodenum. However, non-NH_3-N absorbed from the small intestine (g/d) was similar for all diets. An isonitrogenous mixture of feather meal and blood meal increased total (essential and nonessential) amino acid absorption from the small intestine versus SBM diet. Titgemeyer et al. (71) compared protein sources and their potential to alter post-ruminal amino acid flow and absorption in steers. Steers were fed a basal diet with soybean meal, corn gluten meal, blood meal, or fish meal replacing a portion of the corn starch of the basal diet. Addition of any of the supplements increased total N flow to the small intestine. Blood meal, corn

gluten meal, and fish meal increased flow of dietary protein to the small intestine compared to soybean meal. Non-bacterial N

absorbed at the small intestine was highest for the by-product supplements (blood meal,74%, corn gluten meal,80%, and fish meal,74%). Fish meal increased flow of each amino acid except cysteine compared to soybean meal. Blood meal increased Lys, His, Arg, and Val. Corn gluten meal improved Met,Ile, Leu, and Tyr flow. Fish meal did not increase total amino acid flow or amino acid-N absorbed which the authors attributed to lower bacterial protein flows associated with fish meal treatment. McCarthy et al. (39) increased flow of Arg and Glu to small intestine with addition of fish meal to diet. Zerbini et al. (78) fed cannulated cows fish meal or soybean meal supplemented diets and reported higher amino acid intake on the soybean meal diet. Total flow of amino acids to the duodenum was similar between supplements. Addition of fish meal to the diet did result in decreased bacterial protein flow, but rumen escape protein of fish meal supported similar levels of total amino acid flow as noted above. Therefore, fish meal could be used to manipulate amino acid flow to post-ruminal absorptive sites of the small intestine. It should be noted that the data presented above illustrates the importance of defining limitations of production diets from the standpoint of microbial protein synthesis, amino acid composition, concentration of post-ruminal digesta flow, and protein digestibility.

Effect of fish meal on animal performance.

Fish meal has been investigated as a protein source for ruminant diets. Porter (56) classified fish meal research into three areas: fish meal protein used in milk replacers, growing animal diets , and lactation diets. Fish meal, as previously discussed, may be utilized as a ruminally undegradable protein source in diets of ruminating young stock and lactating ruminants. Gill et al. (19) fed ryegrass silage to 3 month old Friesian steers with increments of fish meal (0,50,100, or 150g per

kg silage DM). Fish meal supplementation increased live-weight gain and steers fed 150g fish meal per kg silage had higher live-weight gains and carcass weight at slaughter. Estradiol increased the response to fish meal at the150 g level of supplementation. Buchanan-Smith and Mowat (12) also observed increased weight gains and feed efficiencies in steer and heifer calves fed alfalfa-grass silage (steers) or corn silage (heifers) when supplemented with fish meal. Data of Gill et al. (19) and Buchanan-Smith and Mowat (12) failed, however to address responses which might be expected from any other dietary source of true protein added to silage diets. Zerbini and Polan (79) compared growth rates and feed efficiencies in fifty ruminating Holstein bull calves fed one of four protein sources. Fish meal fed calves had higher rates of gain (.84 kg/d) than corn gluten meal (.75 kg/d), and cottonseed (.75 kg/d) fed animals, but were similar to the soybean meal treatment (.82 kg/d). These data suggest that protein sources with similar rates of ruminal degradability such as fish meal and corn gluten meal may not supply escape amino acids limiting animal performance. Thonney and Hogue (70) fed fish meal or cottonseed meal to 195 kg Holstein steers and recorded similar responses as described by Zerbini and Polan (79).

Milk production responses also have been reported after inclusion of fish meal in diets of lactating cows. Pike et al. (52) found substitution of 2 kg of fish meal for 4 kg of commercial concentrate increased milk yields on two commercial dairy farms. Replacement of soybean meal with fish meal increased milk yield on three commercial farms when fed to cows between weeks 9 and 15 of lactation (41). Graded fish meal addition (0, 40, 80, and 120 g/kg concentrate) to diets composed of ryegrass silage and barley resulted in linear increases in fat-corrected-milk yields for 2 to 15 wk post-partum (49). Milk composition was not different but calculated ME deficit was increased by fish meal.

Oldham et al. (49) recorded increased milk and milk protein production when fish meal replaced urea in diets of lactating Fresian cows. Positive production responses were present during early and mid-lactation. Milk fat yield was increased during early lactation at the high fish meal level, but was significantly decreased by the fish meal diet during mid-lactation. Several other studies have reported similar decreases in milk fat content and yields when fish meal was fed to lactating dairy

cows. Wohlt et al. (76) fed corn silage based diets supplemented with soybean meal, corn gluten meal or fish meal to contain 16% CP. Diets were fed during the first 18 wk of lactation. Fish meal diet had higher DM digestibility and tended to support higher milk production than corn gluten meal diet. Milk protein was similar for all diets. Cows fed fish meal had significantly lower milk fat content with a trend towards lowered milk fat yield (g/d). Spain et al. (67) fed corn silage based total mixed rations similar to (76) containing soybean meal, corn gluten meal and fish meal. They also fed two combination diets in which 50% of supplemental crude protein was supplied from soybean and fish meal or soybean meal and corn gluten meal. Cows fed diets containing fish meal or 50% fish meal had significantly lower milk fat content and daily yield. Zerbini et al. (78) also reported significantly lower milk fat content and yield with inclusion of fish meal in diets of lactating dairy cows. Therefore, while fish meal supports high milk production it has had a negative effect on milk fat content and yields.

Dietary and Metabolic Factors that Influence Milk Fat Production

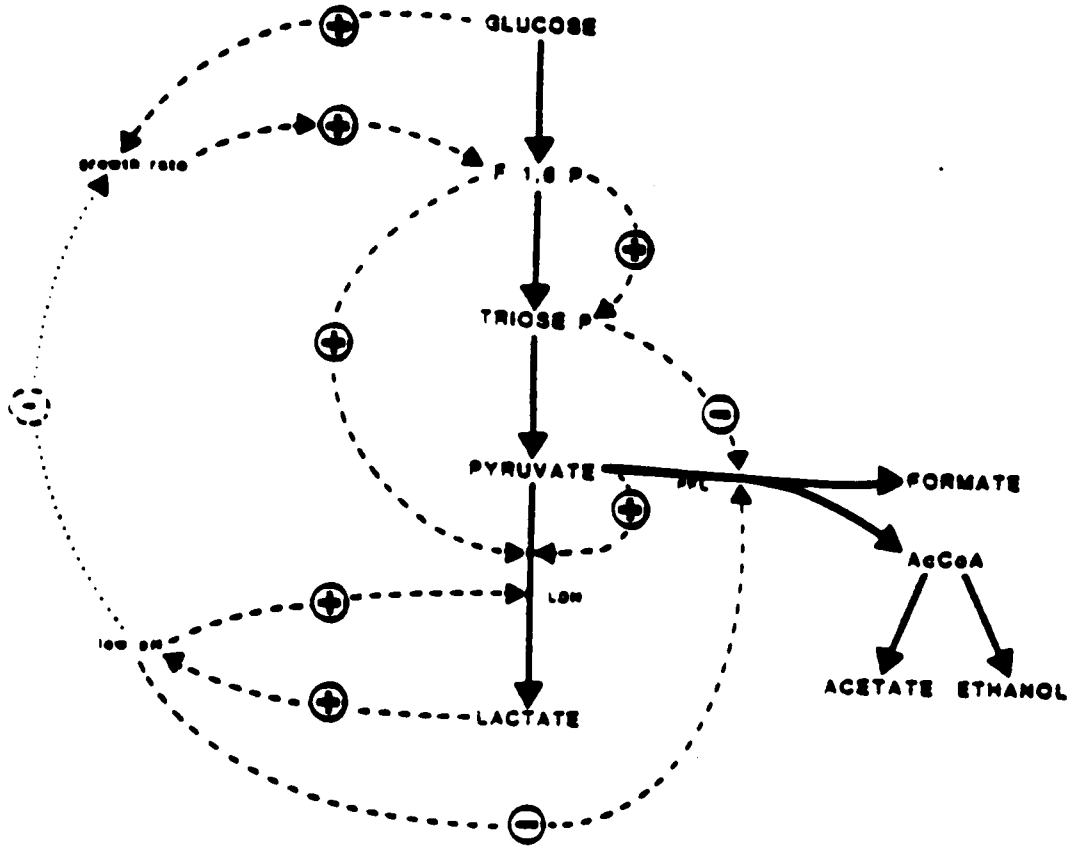
Milk synthesis by mammary secretory epithelial cells involves a milieu of reactions and interactions, within the mammary cell, the mammary gland, and the whole animal. Ruminants, in contrast to monogastrics, are unique given the pre-gastric fermentation of the diet by ruminal microorganisms. Microbial fermentation significantly alters dietary components prior to absorption and utilization by the animal. In fact, pre-gastric fermentation has resulted in the evolution of metabolic and biosynthetic relationships unique to ruminants.

Milk fat synthesis may be affected at any number of points in the complex chain of events. Alteration of ruminal volatile fatty acid proportions by dietary manipulation and its effects on milk composition have been thoroughly documented (17,33,77). Feeding large amounts of soluble and rapidly fermentable carbohydrates with limited quantities of fiber has been shown to alter ruminal

volatile fatty acid patterns and subsequent milk composition and component yield (8,17). Increased grain results in decreased rumination and saliva flow associated with chewing activity (65,73). Reduced saliva flow limits flow of salivary buffers to the rumen. Concurrently, rapid lactic acid production from the soluble carbohydrates in grain contribute to lower rumen pH (58). The resulting drop in pH limits activity of cellulolytic microorganisms which may limit acetate production (65). These relationships are summarized in Figure 1.

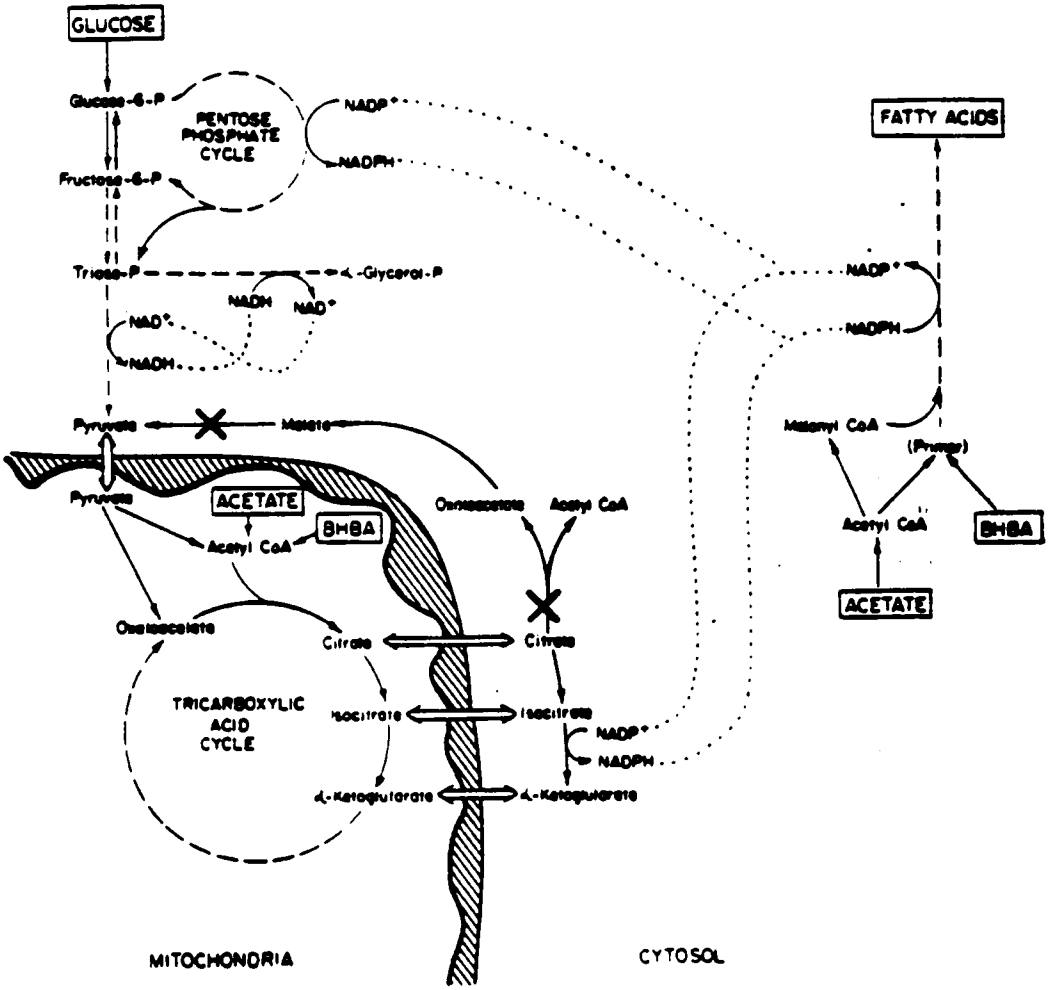
Changes in ruminal fermentation alters quantities and proportions of ruminal VFA. Davis (17) found no change in ruminal acetate production between control (1223 mmoles/h) and high grain diets (1171 mmoles/h). Bauman et al. (8) fed diets similar to those of Davis (17) which increased propionate production from 13 moles/d (control diet) to 31 moles/d (high grain diet). Changes in ruminal VFA production and proportions result in metabolic shifts within the animal as well. Increased ruminal propionate production has been associated with increased plasma glucose and insulin concentrations of lactating dairy cows fed high grain diets (85% of diet dry matter supplied by concentrate) (31). Jenny et al. (32) found increased levels of 16:0, 18:1, and 18:2 with decreased levels of 10:0, 12:0, 14:0 and 14:1 in milk fat of cows fed high grain diets. These workers suggested that high grain feeding resulted in repartitioning of milk triglyceride precursors from mammary secretory cells to adipose tissue.

De novo synthesis of fatty acids from acetate can provide up to 50% of the fatty acids secreted in milk triglycerides. Two studies by Balmain et al. (2,3) substantiated early theories concerning the importance and role of acetate for milk fat synthesis in mammary tissue of ruminants. Numerous laboratories have used in vitro culture of lactating mammary slices or isolated secretory cells to measure mammary activity and metabolic regulation (24,35,36). Bauman et al. (6) identified enzymatic differences between mammary gland tissue of ruminants and monogastrics as illustrated in Figure 2 which notes species' differences and pathways involved. These differences included lower glucose-6-phosphate dehydrogenase, ATP-citrate lyase, and NADP-malate dehydrogenase activity in cow versus rat mammary tissue. In contrast, ruminant mammary tissue had almost 20-fold higher NADP-isocitrate dehydrogenase activity than rat and sow mammary tissue. These



Russell and Hino (58)

Figure 1. Proposed mechanisms involved in altered volatile fatty acid in the rumen of cows fed high energy diets.



Bauman et al. (6)

Figure 2. Enzymes and pathways involved in milk fat synthesis in mammary gland of ruminants.

enzyme differences resulted in marked differences in substrate utilization. Acetate-1-^{14}C incorporation into fatty acids was signifcantly higher in ruminant than monogastric mammary tissue slices. Glucose utilization for fatty acid synthesis was significantly higher for monogastrics. Fatty acid profiles of mammary tissue incubated with radiolabelled precursors indicated that tissue incubated in vitro produced fatty acids in similar proportions as reported for in vivo studies (6). The remaining 50% of fatty acids incorporated into milk triglyceride are absorbed from the blood (7,18). These fatty acids are primarily long chain fatty acids from adipose lipolysis or directly from the diet. Emery (18) noted a significant increase in mammary lipoprotein lipase (LPL) activity with initiation of lactation. Mammary LPL increased 24 h prior to parturition in guinea pigs, reaching maximum activity 2 h after parturition. In fact, chylomicron uptake was highly correlated with mammary LPL activity (42). Dietary levels of forage and grain have been shown to influence mammary LPL activity with high energy (grain) diets decreasing LPL activity in mammary gland (18).

Long chain fatty acid availability has been shown to affect mammary triglyceride synthesis (24,34). Kinsella and Gross (34) reported palmitate to be the preferred fatty acid for initiation of acylation in bovine mammary microsomes. An increased activity in dispersed goat mammary cells was achieved when palmitate was added to culture media (24). Hansen and Knudsen (23) incubated dispersed goat mammary cells with laurate, palmitate, or oleate. Palmitate increased de novo fatty acid synthesis and subsequent incorporation of butyrate. Oleate inhibited synthesis of fatty acids measured except butyrate which was not changed. Palmitate also increased triglyceride synthesis when added to in vitro mammary cultures (22). Stearate and linoleate inhibited triglyceride synthesis compared with control incubations (23).

Diets of lactating dairy cows typically contain lipid at five percent or less of the diet dry matter. As discussed in previous sections, pregastric fermentation by rumen microorganisms significantly alter ingested dietary carbohydrate and protein. Rumen microorganisms also significantly alters dietary fatty acids. Palmquist and Jenkins (50) summarized dietary fatty acid compositions of typical dairy diets which indicated a high proportion of polyunsaturated fatty acids. In contrast, the

majority of milk fatty acids and tissue fatty acids consist of saturated fatty acids (25). Alteration of dietary fatty acid through biohydrogenation results in this dramatic difference between dietary and tissue lipid composition.

Biohydrogenation has been reported to occur as outlined in Figure 3 (25). Fatty acids must be in free form before biohydrogenation can occur (50). Bickerstaffe et al. (11) reported that 90 percent of 18:1, 18:2, and 18:3 were biohydrogenated by rumen microorganisms. Addition of long chain fatty acids to diets of cannulated cows increased duodenal flow of polyunsaturated fatty acids. These data indicated dietary supplements may provide fatty acids that exceed microbial capacity for biohydrogenation. Stereochemistry of the fatty acid may also influence the ability of microorganisms to hydrogenate with respect to positional isomers of long-chain fatty acids. Figure 4 depicts spatial arrangement of microbial biohydrogenation. The ability of rumen microorganisms to hydrogenate long-chain polyunsaturated fatty acids that are not present in normal diets may also escape microbial biohydrogenation and reach the small intestine unaltered.

Dietary fat supplementation may be utilized to supplement energy, but may also alter ruminal fermentation. Addition of fat to in vitro fermentors decreased acetic acid and increased propionic acid production (29). This shift was associated with decreased fiber digestibility. In contrast, calcium salts of long-chain fatty acids did not change ruminal VFA or fiber digestibility (20). Cows fed diets supplemented with unsaturated fatty acids (yellow grease) had lower acetate:propionate ratio as well as decreased milk fat percentage and yield (30). Palmquist and Jenkins (50) listed possible effects of dietary lipid on fiber digestibility including a physical barrier inhibiting attachment of particle associated bacteria. Although the inhibitory effect has not been defined, altered ruminal fiber digestibility due to lipid supplementation has resulted in decreased milk fat content and yield.

Addition of fat to the diet has been investigated as a means of providing fatty acids for milk fat synthesis. Dietary fats are used to increase energy density of the diet dry matter. Dietary fats also have been used to alter the fatty acid composition of milk and adipose tissue of ruminants (72,21).

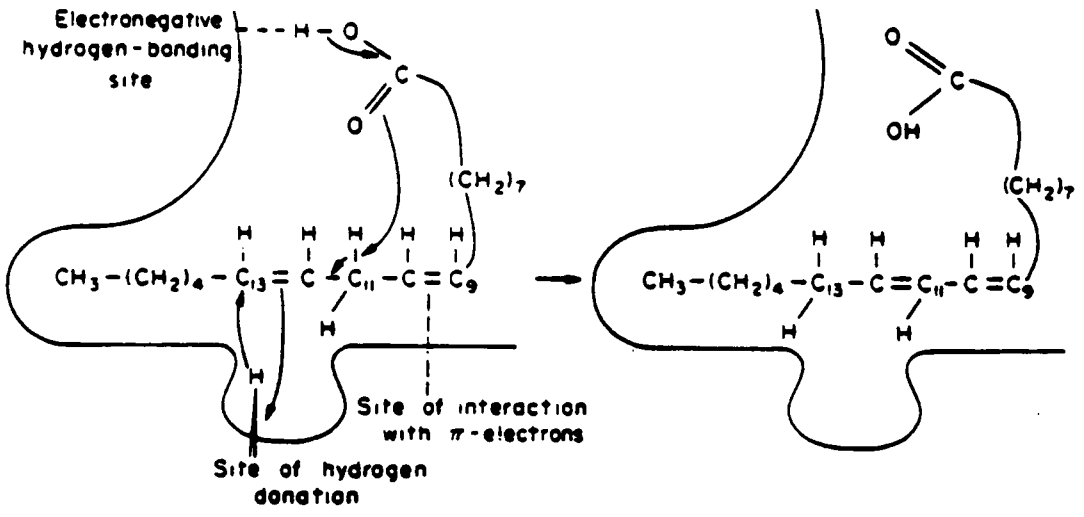
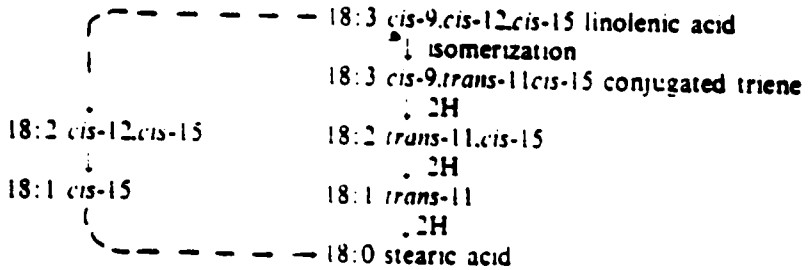


Figure 3. Enzymatic biohydrogenation of polyunsaturated fatty acids by ruminal microorganisms.

18:2 *cis*-9,*cis*-12 (Linoleic acid)
↓ isomerization
18:2 *cis*-9,*trans*-11 conjugated diene
↓ 2H
18:1 *trans*-11 monoenoic acid
↓ 2H
18:0 stearic acid

Probable sequence of reactions in the biohydrogenation of linoleic acid.



Probable metabolic pathways in the biohydrogenation of linolenic acid.

Harfoot (25)

Figure 4. Illustration of proposed spatial arrangement of enzyme and polyunsaturated fatty acid.

Addition of oleic acid (500 ml per d) caused decreased ruminal acetate and increased ruminal propionate concentrations (64). This shift in ruminal fermentation did not alter milk fat content or yields. However, addition of hydrogenated oils (Crisco) or trans fatty acids decreased milk fat content and yields without altering ruminal fermentation patterns as measured by ruminal VFA concentrations (64). Addition of long-chain polyunsaturated fatty acids to in vitro rumen cultures decreased acetate and increased propionate production compared to control cultures (14). Therefore, dietary lipid may alter ruminal fermentation causing changes in milk fat production, or, added dietary lipid could alter post-ruminal lipid metabolism as discussed above.

Post-ruminal dietary factors other than rumen VFA have also been shown to affect milk fat production. Murphy and Morgan (44) increased milk fat yield by feeding protected tallow to lactating dairy cows. Inclusion of unsaturated fatty acids in diets of lactating cows decreased ruminal acetate (molar proportion) and milk fat percentage (74). However, the authors noted that differences in ruminal VFA patterns were small compared to the large decrease in milk fat content. They indicated potential for polyunsaturated fatty acids to escape biohydrogenation and affect post-absorptive lipid metabolism. A similar response was noted when cod-liver oil (CLO) was infused into the rumen or abomasum of lactating cows (51). Intraruminal CLO infusion decreased milk fat percentage with no change in ruminal pH or VFA patterns. Milk fat percentage also was decreased by intra-abomasal CLO infusion. Content of long-chain polyunsaturated fatty acids in plasma and milk was increased by both treatments. These data support the post-absorptive affect of fish lipid polyunsaturated fatty acids on lipid metabolism proposed by Varman et al. (74) which includes decreased uptake of plasma fatty acids by the mammary gland.

References

1. Arambel, M.J. and C.N. Coon. 1981. Effect of dietary protein on amino acids and microbial protein of duodenal digesta. J. Dairy Sci. 64:2201.

2. Balmain, J.H., S.J. Folley, and R.F. Glascock. 1952. Effects of insulin and of glycerol in vitro on the incorporation of [carboxy- &sup.1.^{4}C]acetate into the fatty acids of lactating mammary gland slices with special reference o species differences. Biochem. J. 522:301.

3. Balmain, J.H., S.J. Folley, and R.F. Glascock. 1954. Relative utilization of glucose and acetate carbon for lipogenesis by mammary-gland slices, studied with tritium, ^{13}C and ^{14}C. Biochem. J. 56:234.

4. Bas, F.J., M.D. Stern, and N.R. Merchen. 1989. Influence of protein supplementation of alkaline hydrogen peroxide-treated wheat straw on ruminal microbial fermentation. J. Dairy Sci. 72:1217.

5. Bauchart, D., M. Doreau, and A. Kindler. 1987. Effect of fat and lactose supplementation on digestion in dairy cows fed long chain fatty acids. J. Dairy Sci. 70:71.

6. 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 Biophys. 140(1):237.

7. Bauman, D.E. and C.L. Davis. 1974. Biosynthesis of milk fat. in Lactation: A Comprehensive Treatise. Volume II. Biosynthesis of Milk/Diseases. Academic Press, N.Y., N.Y.

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

9. Bauman, D.E., D.L. Ingle, R.W. Mellenberger, and C.L. Davis. 1973. Factor affecting in vitro lipogenesis by bovine mammary tissue slices. J. Dairy Sci. 56:1520.

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

11. Bickerstaff, R., D.E. Noakes, and E.F. Annison. 1972. Quantitative aspects of fatty acid biohydrogenation, absorption, and transfer into into milk fat in the lactating goat, with special reference to the cis- and trans-isomers of octadecenoate and linoleate. Biochem. J. 130:607.

12. Buchanan-Smith, J.G. and D.N. Mowat. 1987. Supplementation of silage with fish meal for growing calves. Can J. Anim. Sci. 67:1186 (abst.)

13. Chalupa, W. 1976. Degradation of amino acids by the mixed rumen microbial population. J. Anim. Sci. 43:828.

14. Chalupa, William, Bonnie Rickabaugh, D.S. Kronfeld, and David Skan. 1984. Rumen fermentation in vitro as influenced by long-chain fatty acids. J. Dairy Sci. 67:1439.

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

16. Cotta, M.A. and J.B. Russell. 1982. Effect of peptides and amino acids on efficiency of rumen bacterial protein synthesis in continuous culture. J. Dairy Sci. 65:226.

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

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

19. Gill, M., D.E. Beever, P.J. Buttery, and R.D. Baker. 1985. The effect of estradiol implantation on the response of fish meal in calves offered silage. Proc Nutr. Soc. 44:115A.

20. Grummer, R.R. 1988. Influence of prilled fat and calcium salt of palm oil fatty acids on ruminal fermentation and nutrient digestibility. J. Dairy Sci. 71:117.

21. Gurr, M.I. 1987. Factors affecting the composition of cow's milk - implications for its nutritive value. Nutr. Bulletin. 10:139.

22. Hansen, Hans Otto and Jens Knudsen. 1987. Effect of exogenous long-chain fatty acids on lipid biosynthesis in dispersed ruminant mammary gland epithelial cells and esterification of long-chain exogenous fatty acids. J. Dairy Sci. 70:1344.

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

24. Hansen, H.O., Ditte Tornehave, and Jens Knudsen. 1986. Synthesis of milk specific fatty acids and proteins by dispersed goat mammary epithelial cells. Biochem. J. 238:167.

25. Harfoot, C.G. 1978. Lipid metabolism in the rumen. Prog. Lipid Res. 17:21.

26. Huber, J.T. and L. Kung, Jr. 1981. Protein and nonprotein nitrogen utilization in dairy cattle. J. Dairy Sci. 64:1170.

27. Hume, I.D., R.J. Moir, and M. Somers. 1970. Synthesis of microbial protein in the rumen. I. Influence of the level of nitrogen intake. Aust. J. Agric. Res. 21:283.

28. Hume, I.D. and P.R. Bind. 1970. Synthesis of microbial protein in the rumen. IV. The influence of the level and form of dietary sulfur. Aust. J. Agric. Res. 21:315.

29. Jenkins, T.C. 1987. Effect of fats and fatty acid composition on ruminal fermentation in semi-continuous in vitro cultures. J. Anim. Sci. 64:1526.

30. Jenkins, T.C. and B.F. Jenny. 1989. Effect of hydrogenated fat on feed intake, nutrient digestion, and lactation performance of dairy cows. J. Dairy Sci 72:2316.

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

32. Jenny, B.F., C.E. Polan, and P.T. Chandler. 1972. Acetate and stearate utilization by lactating cows fed high grain, restricted roughage rations. J. Dairy Sci. 55:1481.

33. Jenny, Bruce F., Carl E. Polan, and Forrest 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.

34. Kinsella, J.E. and M. Gross. 1973. Palmitic acid and initiation of mammary glyceride synthesis via phosphatidic acid. Biochim. Biophys. Acta. 316:109.

35. Kinsella, J.E. and R.D. McCarthy. 1968. Biosynthesis of secretory lipids from [2-^{14}C] acetate by bovine mammary cells in vitro. Biochim. Biopyhs. Acta. 164:518.

36. Kinsella, J.E. and R.D. McCarthy. 1968. Lipid composition and secretory activity of bovine mammary cells in vitro. Biochim. Biophys. Acta. 164:530.

37. Konig, Barbara A., J.D. Oldham, and D.S. Parker. 1984. The effect of infusion of casein on acetate, palmitate, and glucose kinetics in cows during early lactation. Br. J. Nutr. 52:319.

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

39. McCarthy, Jr, R.D., T.H. Klusmeyer, J.L. Vicini, J.H. Clark, and D.R. Nelson. 1989. Effects of source of protein and carbohydrate on ruminal fermentation and passage of nutrients to the small intestine of lactating cows. J. Dairy Sci. 72:2002.

40. Merchen, Neal, Tom Hanson, and Terry Klopfenstein. 1979. Ruminal by-pass of brewers dried grains protein. J. Anim. Sci. 49:192.

41. Miller, E.L., N.W. Gawley, I.H. Pike, and G. Newman. 1983. Effect of replacing soya-bean meal by fish meal on milk production by Fresian cows on commercial farms. Nutr. Soc. Proc. 42:62A (abstract)

42. Moore, J.H. and W.W. Christie. 1980. Lipid metabolism in the mammary gland of ruminants. Prog. Lipids Res. 17:347.

43. Muller, L.D., D. Rodriguez, and D.J. Schingoethe. 1975. Formaldehyde treated whey protein concentrate for lactating dairy cattle. J. Dairy Sci. 58:1847.

44. Murphy, J.J. and D.J. Morgan. 1978. Effect of inclusion of protected and unprotected tallow in dairy rations on cow performance. Anim. Prod. 32(3):368.

45. National Research Council. 1989. Nutrient Requirements of Dairy Cattle, 6th Rev Ed. National Academy Press, Washington, D.C.

46. National Research Council. 1985. Ruminant Nitrogen Usage. National Academy Press. Washington, D.C.

47. Nocek, J.E. and J.B. Russell. 1988. Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. J. Dairy Sci. 71:2070.

48. Nolan, J.V. and R.A. Leng. 1972. Dynamic aspects of ammonia and urea metabolism in sheep. Br. J. Nutr. 27:177.

49. Orskov, E.R., G.W. Reid, and C.A.G. Tait. 1987. Effect of fish meal on the mobilization of body stores in dairy cows. Anim. Prod. 45:345.

50. Palmquist, D.L. and T.C. Jenkins. 1980. Fat in lactation rations: A review. J. Dairy Sci 63:1.

51. Pennington J.A. and C.L. Davis. 1975. Effects of intraruminal and intra-abomasal additions of cod-liver oil on milk fat production in the cow. J. Dairy Sci. 58:49.

52. Pike, I.H., E.L. Miller, and M. Clark. 1984. Use of fish meal to reduce compound food usage in high-yielding dairy herds. Anim. Prod. 38:520 (abstract).

53. Pilgrim, A.F., F.V. Gray, R.A. Weller, and C.B. Belling. 1970. Synthesis of microbial protein from ammonia in the sheep's rumen and the proportion of dietary nitrogen converted into microbial nitrogen. Br. J. Nutr. 24:589.

54. Plegge, S.D., L.L. Berger, and G.C. Fahey, Jr. 1982. Effect of roasting on utilization of soybean meal by ruminants. J. Anim. Sci. 55:395.

55. Polan, C.E., T.A. Herrington, W.A. Wark, and L.E. Armentano. 1985. Milk production response to diets supplemented with dried brewers grains, wet brewers grains, or soybean meal. J. Dairy Sci. 68:2016.

56. Porter, Paul A. 1988. Fish meal as an undegradable protein source for ruminants. Southwest Nutrition Conference.

57. Ranwana, S.S.E. and R.C. Kellaway. 1977. Responses to post-ruminal infusions of glucose and casein in lactating goats. Br. J. Nutr. 37:395.

58. Russell, J.B. and T. Hino. 1985. Regulation of lactate production in *Streptococcus bovis*: a spiraling effect that contributes to rumen acidosis. J. Dairy Sci. 68:1712

59. Russell, J.B., C.J. Sniffen, and P.J. Van Soest. 1983. Effect of carbohydrate limitation on degradation and utilization of casein by mixed rumen bacteria. J. Dairy Sci. 66:763.

60. Satter, Larry D. 1986. Protein supply from undegraded dietary protein. J. Dairy Sci. 69:2734.

61. Satter, L.D. and R.E. Roffler. 1975. Nitrogen requirement and utilization in dairy cattle. J. Dairy Sci. 58:1219.

62. Scheifinger, C., N. Russell, and W. Chalupa. 1976. Degradation of amino acids by pure culttures of rumen bacteria. J. Anim. Sci. 43:821.

63. Schingoethe, D.J., D.P. Casper, C. Yang, D.J. Illg, J.L. Sommerfeldt, and C.R. Mueller. 1988. Lactational response to soybean meal, heated soybean meal and extruded soybeans with ruminally protected methionine. J. Dairy Sci. 71:173.

64. Selner, D.R. and L.H. Schultz. 1980. Effects of feeding oleic acid or hydrogenated vegetable oils to lactating cows. J. Dairy Sci. 63:1235.

65. Slyter, L.L. 1976. Influence of acidosis on rumen function. J. Anim. Sci. 43:910.

66. Sniffen, C.J. and P.H. Robinson. 1987. Microbial growth and flow as influenced by dietary manipulation. J. Dairy Sci. 70:425.

67. Spain, J.N., M.D. Alvarado, Carl E. Polan, C.N. Miller, and M.D. McGilliard. 1990. Effect of protein source and energy level on milk composition in mid-lactation dairy cows. J. Dairy Sci. (in press)

68. Storm, E. and E.R. Orskov. 1984. The nutritive value of rumen micro-organisms in ruminants. IV. The limiting amino acids of microbial protein in growing sheep determined by a new approach. Br. J. Nutr. 52:613.

69. Tamminga, S. 1979. Protein degradation in the forestomachs of ruminants. J. Anim. Sci. 49(6):1615.

70. Thonney, M.L. and D.E. Hogue. 1986. Fish meal or cottonseed meal as supplemental protein for growing Holstein steers. J. Dairy Sci. 69:1648.

71. Titgemeyer, E.C., N.R. Merchen, and L.L. Berger. 1989. Evaluation of soybean meal, corn gluten meal, blood meal, and fish meal as sources of nitrogen and amino acids disappearing from the small intestine of steers. J. Anim. Sci. 67:262.

72. Tove, S.B. and G. Matrone. 1962. Effect of purified diets on the fatty acid composition of sheep tallow. J. Nutr. 62:271.

73. Van Soest, P.J. 1964. Ruminant fat metabolism with particular reference to factors affecting low milk fat and feed efficiency: a review. J. Anim. Sci. 24:204.

74. Varman, P.N., L.H. Schultz, and R.E. Nichols. 1968. Effect of unsaturated oils on rumen fermentation, blood components, and milk composition. J. Dairy Sci. 51:1956.

75. Waltz, D.M., M.D. Stern, and D.J. Illg. 1989. Effect of ruminal protein degradation of blood meal and feather meal on the intestinal amino acid supply to lactating cows. J. Dairy Sci. 72:1509.

76. Wohlt, J.E., S.L. Chimel, and J.L. Evans. 1987. Protein level and quality from soybean meal, fish meal and corn gluten meal for dry matter intake and milk production in early lactation by Holstein cows. J. Dairy Sci. 70(Suppl. 1):116 (abst)

77. Zanartu, D., C.E. Polan, L.E. Ferreri, and M.L. McGilliard. 1983. Effect of stage of lactation and varying available energy intake on milk production, milk composition, and subsequent tissue enzymatic activity. J. Dairy Sci. 66:1644.

78. Zerbini, E., Carl E. Polan, and J.H. Herbein. 1988. Effect of dietary soybean meal and fish meal on protein digesta flow in Holstein cows during early and midlactation. J. Dairy Sci. 71:1248.

79. Zerbini, E. and Carl E. Polan. 1985. Protein sources evaluated for ruminating Holstein calves. J. Dairy Sci. 68:1416.

Goals

The goal of this research work was to re-evaluate the use of fish meal as a protein source for lactating dairy cattle. Inclusion of fish meal has been shown to result in decreased milk fat content. In all the studies described, a primary goal was to identify the mechanisms or site of action by which fish meal has its negative affect on milk fat production.

Production studies involving small groups of animals were conducted. Blood and rumen parameters that have been shown to influence or correlate with milk fat content were monitored. Mammary tissue fatty acid synthesis was measured using in vitro culture techniques to determine if a tissue level effect was involved in decreased milk fat content. More specific objectives for individual experiments are stated with the introduction of the respective sections.

Effect of Addition of Fish Meal to the Diet of Lactating Holstein Cows.

Introduction

Inclusion of protein resistant to degradation by rumen microorganisms in diets of lactating cows is currently recommended (14). Several protein sources have been reported to enhance amino acid flow to absorptive sites of the small intestine above that provided by microbial amino acid nitrogen (23,24,25). Fish meal is a by-product protein supplement which has been utilized to provide ruminal escape protein. Studies with cattle which had been surgically altered to allow measurement of post-ruminal amino acid flow have shown addition of fish meal to the diet increases dietary amino acid flow to the small intestine (11,23,25). Data from production trials have indicated milk fat yields may be reduced with addition of fish meal to the diet (16, 19,20). However, others have reported no difference in milk fat yields (3,11). The effect of fish meal on milk fat concentrations has been suggested to be associated with altering ruminal fermentation due to residual oil content of fish meal and the high content of polyunsaturated fatty acids in the oil. Several studies have failed to show significant changes in ruminal VFA patterns or dry matter digestibilities in cows fed fish meal (16,20).

Therefore, this study was undertaken to reexamine this theory with the goal of determining the appropriate level of fish meal in diets of lactating dairy cows. The effect of fish meal intake on milk yield and composition, concentrations of ruminal VFA and ammonia, plasma urea, glucose, and long-chain fatty acids were measured in order to identify a metabolic factor or factors affected by fish meal which may be responsible for decreased milk fat production.

Materials and Methods

Eight Holstein cows were assigned to two replicates of a 4x4 Latin square which included four cows per replicate receiving four different diets over four consecutive 20 d periods. Cows averaged 131 ± 14 d in milk and 27 ± 2 kg milk per d at the start of the study. Diets were corn silage based, total mixed rations (Table 1) and were fed twice daily (0600 and 1500 h) in amounts adequate to insure 10% refusal. Fish meal was substituted for corn gluten meal on a isonitrogenous basis. Cows were adjusted to diets for 7 d followed by a 13 d collection period. Milk production and DM intake was recorded daily. Milk samples were collected at consecutive milkings on d 7 and 13 of the collection period and placed in sealed plastic bags containing potassium dichromate (150 mg per sample) as preservative. Samples were stored at room temperature for 24 h before being analyzed with a four channel spectrophotometer (Multispec Mark I, Foss Food Technology, Eden Plaines, MN.) for milk fat, protein, and lactose.

Approximately 150 ml rumen fluid were sampled by aspiration 2 to 3 h after the afternoon feeding on day 12 of the experimental period. A 5 ml aliquot was placed in a plastic tube containing four drops of concentrated sulfuric acid for ammonia analysis. Another 10 ml aliquot was stored frozen in a separate plastic tube for VFA analysis. Also on day 12 of the experimental period, 30 ml of

Table 1. Diet components, amounts and composition of diets with increments of fish meal substituted for corn gluten meal.

Ingredient	Diets[1]			
	C[2]	LF	MF	HF
Corn silage	62.3	62.9	62.0	61.9
High moisture corn	20.3	20.5	20.2	20.2
Soybean meal	10.1	10.3	10.1	10.1
Corn gluten meal	7.3	3.7	2.5	---
Fish meal	--	2.6	5.2	7.8
Composition				
DM	42.0	44.0	44.0	42.0
ADF	25.2	25.1	24.7	24.4
CP	17.2	17.5	17.3	17.2

[1]Reported as percent of dry matter.
[2]C = Control,LF = Low fish meal,MF = Medium fish meal,HF = High fish meal.

blood were sampled by jugular puncture. Ten ml subsamples were placed in plastic tubes containing 100 units of heparin each. All samples were stored on ice for transport to laboratory. Blood samples were centrifuged at 3,220 x g for 20 min with temperature maintained at 5 C. Plasma was decanted and stored at -20 C until analyzed. Plasma used for fatty acid analysis was stored at -80 C. Rumen fluid was stored at -20 C until analyzed.

Diet components and total mixed ration samples were collected weekly. DM was determined by drying samples at 100 C in a forced air oven for 24 h. Dried samples were ground to pass through a 2 mm screen. Kjeldahl nitrogen (1) and ADF (5) was measured on ground sub-samples.

Plasma samples were thawed to room temperature and analyzed for glucose using a colorimetric glucose assay (Sigma, St. Louis, MO.). Plasma urea was quantified by the method of Coluombe and Favreau (4). Plasma fatty acid composition was measured using the following procedure. A 1.5 ml aliquot was transferred to an acid washed extraction tube. Seven ml methanol was added and the sample was homogenized for 20 sec. Fourteen ml chloroform were then added with the sample rehomogenized for 20 sec. The sample was centrifuged for 3 min at 800 x g. Solvent was filtered through Whatman filter paper (No.40). The precipitate was resuspended in 12 ml 2:1 chloroform:methanol (v:v) and homogenized for 20 sec. The sample was then centrifuged and filtered as previously described into the tube containing original filtrate. To the filtrate, 8.5 ml 0.88% aqueous KCl was added, sample capped and mixed vigorously for 5 min using a benchtop test tube shaker. Following shaking samples were then allowed to separate into aqueous and solvent phases. The aqueous layer was removed and the solvent phase concentrated under N_2 to approximately 2 ml. Concentrated solvent was transferred to a 15 ml screw top tube. Decaheptenoic acid (615.6 μg/sample) was added and used as an internal standard. Samples were then concentrated under N_2 to near dryness. Extracted lipid was saponified with 400 μl 0.5 N NaOH for 5 min at 100 C. Tubes were cooled under cool water, 400 μl boron triflouride were added and they were incubated as for saponification. Tubes were cooled to room temperature, 0.9 ml iso-octane and 4.0 ml deionized water were added, and they were then shaken for 5 min. Phases were separated by centrifugation

for 10 min at 2000 rpm iso-octane was transferred to 1.5 ml vials containing sodium sulfate. Fatty acids were then determined by gas chromatography as previously described (18).

Acidified rumen fluid samples were centrifuged at 3,220 x g for 20 min and analyzed for ammonia (12). Ruminal VFA concentrations were determined by the method previously documented (20).

Data were analyzed statistically using the GLM procedure of SAS (21). The statistical model used was $Y = \mu + C_i + Trt_j + Pd_k + (TrtPd)_{jk}$ + residual, where μ = mean, C_i is ith cow, Trt_j is jth treatment(diet), Pd_k is kth period, and $(TrtPd)_{jk}$ is the interaction between jth treatment and kth period. Treatment means were tested by error means. Means difference were evaluated by Tukey's procedure (13). Linear and quadratic response relationships were measured by orthogonal contrasts (10).

Results

DM was consumed in a quadratic response ($P<.05$) to addition of fish meal in the diet (Table 2). DM intake of cows fed low fish meal (21.2kg/d) was higher than cows fed high fish meal (19.5 kg/d) with control and medium fish meal diets intermediate. Milk production was not affected by diet. Milk fat percentage was decreased in a linear response to increasing intake of FM. Milk protein and lactose percentage were not different among diets (Table 2).

Total concentrations of VFA in rumen fluid ranged from 145.3 to 165.6 mmol/dl but were not different due to diet (Table 3). Individual VFA concentrations also were not different due to diets. Molar proportions of ruminal VFA were not different with the exception of butyrate. Molar proportion of butyrate was higher in cows fed medium fish meal (13.5%) than high fish meal (12.5%) ($P<.05$). The acetate to propionate ratio ranged from 2.6 to 2.8 but was not different due to dietary treatment.

Table 2. Effect of fish meal on DM intake, milk production, and milk composition.

Item		Diets[1]				
		C[2]	LF	MF	HF	SEM
DM *intake*[B]	(kg/d)	20.6[a,b]	21.2[b]	20.1[a,b]	19.5[b]	.1
Milk	(kg/d)	25.3	26.2	25.5	25.6	.4
Fat[A]	(%)	3.5[a]	3.2[a,b]	3.1[b]	3.0[b]	.04
Protein	(%)	3.4	3.4	3.4	3.3	.01
Lactose	(%)	4.7	4.7	4.7	4.7	.01

[1] For diet composition see Table 1.
[2] C = control, LF = low fish meal, MF = medium fish meal, HF = high fish meal.
[a,b] Values in same row with different superscript differ $P < .05$.
[A,B] Denotes significant linear (A) or quadratic (B) response to dietary treatment, respectively.

Plasma glucose concentration was highest for cows fed high fish meal (73.62 mg/dl) but was not different from means for other treatments (Table 4). Addition of fish meal tended to decrease plasma urea concentrations but treatment means were not different (Table 4). Ruminal ammonia appeared quadratic in response ($p<.15$) similar in pattern to DM intake ($p<.15$).

Plasma long-chain fatty acid content was altered by increased fish meal intake (Table 5). Unsaturated fatty acids 16:1n7, 18:3n3, 20:4n6, 20:5n3 and 22:6n3 increased linearly with increased fish meal intake. In contrast, 18:3n6 and 20:3n6 decreased with increased fish meal intake. Saturated fatty acids, 18:1n9, and 18:2n6 were not altered by diet.

Discussion

Diet effect on DM intake, while different, was not of a sufficient magnitude to alter milk production between diets (Table 2). Differences in DM intake may reflect some benefit of different dietary nitrogen components with respect to ruminal fermentation (15). While milk production failed to respond to diet, milk fat content was significantly decreased with increasing FM intake. Milk fat decreased linearly from 3.5% on control to 3.0% on high fish meal. No differenes in ruminal VFA concentrations were observed. Ruminal VFA concentrations and ratios have been shown to directly influence milk fat yields in lactating ruminants (9,21). Plasma glucose has also been reported to reflect changes in ruminal fermentation end products (8). While feeding high fish meal resulted in the highest plasma glucose measurement, plasma glucose concentrations across all diets were not representative of fermentation changes associated with decreased milk fat production. So in summary, cows fed linear increments of fish meal exhibited a concurrent linear decrease in milk fat content in the absence of significant changes in milk volume, ruminal VFA patterns, or plasma

Table 3. Effect of fish meal on ruminal volatile fatty acids.

Item	Diets[1]				
	C[2]	LF	MF	HF	SEM(n = 31)
(mmol/dl)					
Total	165.1	154.5	165.6	145.3	5.3
Acetate	99.8	92.1	98.5	88.3	3.2
Propionate	36.8	35.8	37.9	32.8	1.4
Isobutyrate	1.6	1.5	1.6	1.5	.04
Butyrate	21.4	20.2	22.4	18.2	.8
Isovalerate	2.9	2.6	2.7	2.5	.1
Valerate	2.6	2.3	2.4	1.9	.1
(mol/100mol)					
Acetate	60.6	59.5	59.7	60.9	.4
Propionate	22.2	23.3	22.7	22.5	.4
Isobutyrate	1.0	1.0	1.0	1.0	.02
Butyrate	12.9	13.0	13.5[a]	12.5[b]	.08
Isovalerate	1.8	1.7	1.6	1.7	.07
Valerate	1.6	1.5	1.4	1.3	.05
Acetate:propionate	2.8	2.6	2.7	2.8	.06

[1] For diet composition see Table 1.
[2] C = control, LF = low fish meal, MF = medium fish meal, HF = high fish meal.
ab Values in same row with different superscript differ, $p < .05$.

Table 4. Effect of fish meal on plasma glucose, urea and ruminal ammonia.

Item	Diets[1]				
	C[2]	LF	MF	HF	SEM(n = 32)
			(mg/dl)		
Plasma glucose	68.1	68.0	69.5	73.6	2.3
Plasma urea-N	21.4	21.3	21.1	20.1	.7
Ruminal ammonia-N	4.8	6.6	5.5	4.8	.4

[1] For diet composition see Table 1.
[2] C = control, LF = low fish meal, MF = medium fish meal, HF = high fish meal.

Table 5. Effect of different amounts of fish meal on plasma long-chain fatty acid levels (μg/ml).

Fatty acid	Diets[1]				
	C[2]	LF	MF	HF	SEM(n = 32)
16:0	239	198	187	237	38
16:1[A]	12[a]	12[a]	14[a]	19[b]	1
17:0	18	16	15	19	2
18:0	384	318	292	359	189
18:1n9	126	121	117	167	18
t18:2n6	5	5	5	6	1
c18:2n6	708	690	713	622	21
18:3n6[A]	16	14	11	10	1
18:3*n*3[B]	22[a]	23[a]	27[a,b]	31[b]	1
20:0	2	trace	2	3	1
20:3*n*6[A]	43[a]	40[a,b]	35[a,b]	31[b]	1
20:4n6[A]	38	41	47	51	3
20:5*n*3[C]	9[a]	19[a,b]	30[b,c]	42[c]	2
22:0	1	trace	1	2	1
22:4n6	5	5	5	4	1
22:6n3[B]	9	13	17	22	2

[1] For diet composition see Table 1.
[2] C = control, LF = low fish meal, MF = medium fish meal, HF = high fish meal.
[a,b,c] Values in same row with different letters differ $p < .05$.
[A,B,C] Significant linear to diet at $p < .05$, $p < .005$, $p < .0005$, respectively.

glucose. In addition, dietary fiber was fed at a level which should be conducive to higher milk fat production than measured in the three diets containing fish meal.

Changes in milk fat yields may reflect some change in post-absorptive lipid metabolism. Considerable effort has been directed towards defining effects of n-3 fatty acids on lipid metabolism in monogastric animals. Reports indicate inclusion of n-3 fatty acids in experimental diets of monogastrics result in a decreased activity in several key lipogenic enzymes (6,7). Acetyl CoA carboxylase and fatty acid synthetase activity were greatly reduced in mice fed diets high in eicosapentaenoic acid (20:5n3). This n-3 fatty acid is also found in higher concentrations in plasma of mice fed marine oil with high 20:5n3 levels (22). Fish meal contains up to 30% of its fatty acids in n-3 form.

Inclusion of fish meal in increasing concentrations altered plasma polyunsaturated fatty acid profiles. Plasma 20:5n3 and 22:6n3 were increased in a linear response which coincided with increased fish meal intake as well as a linear decrease in milk fat. It is important to note the ability of these highly unsaturated fatty acids to survive ruminal biohydrogenation thus allowing for the absorption of these metabolically active long-chain polyunsaturates. In addition to altered plasma n-3 fatty acids, 20:3n6 was decreased and 20:4n6 was increased in cows fed fish meal. This may indicate lipid metabolism involving long-chain polyunsaturated fatty acids is indeed changed by the higher levels of circulating n3 fatty acids.

While previously published data indicate different effects on long-chain polyunsaturated fatty acids in blood, this work was not done with ruminants. Therefore, a number of events may be contributing to decreased milk fat yield including decreased de novo fat synthesis at the mammary gland, impaired long-chain uptake or incorporation into milk fat triglycerides. Thus, metabolically active fatty acids may have the potential to alter or repartition nutrients from one metabolic system to another.

References

1. Association of Official Analytical Chemists. 1975. Official method of analysis, 12th ed. Assoc. of Anal. Chem. Washington, D.C.

2. Bauman, D.E., C.L. Davis, and H.F. Bucholtz. 1971. Propionate production in the rumen of cows fed either a control or high-grain, low-fiber diet. J. Dairy Sci. 54:1287.

3. Broderick, G.A. 1989. Fish meal versus soybean meal for lactating cows fed alfalfa as sole forage. J. Dairy Sci. 72(Suppl. 1):299. (abst.)

4. Coloumbe, J.J. and L. Favreau. 1963. A new simple method for colorimetric determination of urea. Clin. Chem. 9:102.

5. Goering, H.K. and P.J. Van Soest. 1970. Forage fiber analysis: apparatus, reagents, procedures, and some applications. U.S. Dept. of Agric. Handbook No. 379.

6. Herzberg, G.R. and M. Rogerson. 1988. Hepatic fatty acids synthesis and triglyceride secretion in rats fed fructose or glucose-based diets containing corn oil, tallow or marine oil. J. Nutr. 118:1061.

7. Iritani, N., K. Inoguchi, M. Endo, E. Fukude, and M. Morita. 1980. Identification of shellfish fatty acids and their effects on lipogenic enzymes. Bioch. et Biophys. Acta. 618:378.

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

9. 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.

10. Lentner, M. and T. Bishop. 1986. Experimental Design and Analysis. Valley Book Company, Blacksburg, VA.

11. McCarthy, R.D., T.H. Klusmeyer, J.L. Vicini, J.H. Clark, and D.R. Nelson. 1989. Effects of source of protein and carbohydrate on ruminal fermentation and passage of nutrients to the small intestine of lactating cows. J. Dairy Sci. 72:2002.

12. McCullough, H. 1967. The determination of ammonia in whole blood by direct colorometric method. Clin. Chem. Acta. 17:297.

13. Mosteller, F. and J.W. Tukey. 1977. Data Analysis and Regression. Addison-Wiley, N.Y.

14. NRC. 1989. Nutrient Requirements of Dairy Cattle. National Academy Press, Washington, D.C.

15. Nocek, J.E. and J.B. Russell. 1988. Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. J. Dairy Sci. 71:2070.

16. Oldham, J.D., D.J. Napper, T. Smith, and R.J. Fulford. 1985. Performance of dairy cows offered isonitrogenous diets containing urea or fish meal in early and mid-lactation. Br. J. Nutr. 53:337.

17. Orskov, E.R., G.W. Reid, and C.A.G. Tait. 1987. Effect of fish meal on the mobilization of body energy in dairy cows. Anim. Prod. 45:345.

18. Phetteplace, H.W. and B.A. Watkins. 1989. Effects of various n-3 lipid sources on fatty acid composition in chicken tissues. J. Food Composit. Analysis (In press).

19. Pike, I.H. and E.L. Miller. 1984. Use of fish meal to reduce compound food usage in high - yielding dairy herds. Anim. Prod. 38:520 (abst)

20. Spain, J.N., M.D. Alvarado, C.E. Polan, C.N. Miller, and M.D. McGilliard. 1990. Effect of protein source and energy level on milk composition in lactating dairy cows. J. Dairy Sci. (In Press).

21. Statistical Analysis System Institute Inc. 1985. SAS User's Guide. SAS Institute, Cary, N.C.

22. Takeuchi, H., A. Ito, T. Murose, M. Nagai, and K. Murenatsu. 1984. Changes in plasma fatty acids profiles of plasma and tissues in rats fed fish oil. J. Agric. Biol. Chem. 48:2125.

23. Titgemeyer, E.C., N.R. Merchen and L.L. Berger. 1989. Evaluation of soybean meal, corn gluten meal, blood meal, and fish meal as sources of nitrogen and amino acids disappearing from the small intestine of steers. J. Anim Sci. 67:262.

24. Windschilt, P.M. and M.D. Stern. 1988. Evaluation of calcium lignosulfonate - treated soybean meal as a source of rumen protected protein for dairy cattle. J. Dairy Sci. 71:3310.

25. Zerbini, E., Carl E. Polan, and J.H. Herbein. 1988. Effect of dietary soybean meal and fish meal on protein digesta flow in Holstein cows during early and mid-lactation. J. Dairy Sci. 71:1248.

Effect of Eicosapentaenoic Acid and Time Post Milking on In Vitro Mammary Tissue Metabolism.

Introduction

In vitro measurement of fatty acid synthesis in mammary secretory epithelial cells has been utilized to quantify factors influencing lipid synthesis. Ruminants generate approximately 50% of milk fatty acids from acetate via de novo synthesis. Therefore, factors which influence acetate incorporation into fatty acids may influence milk fat synthesis and yield. Bauman et al. (1) reported several parameters could affect in vitro synthesis of milk lipid. Time post slaughter and temperature of pre - incubation media did not alter ^{14}C-acetate incorporation by mammary slices. However, culture media acetate and glucose concentrations were found to decrease incorporation at levels below 10 and 25 mM, respectively. Bauman et al. (2) reported similar findings in that addition of glucose (10mM final concentration) increased acetate utilization for fatty acid synthesis four times over control which contained the same media except for glucose.

Several reports have indicated linear increases in acetate incorporation and CO_2 production during incubation intervals up to 3 h (1,5). King et al (8) measured u-^{14}C-acetate uptake in lactating ewes and reported 60% uptake from arterial blood. Hansen et al. (5) reported addition of 500 mM palmitic acid to dispersed goat mammary cells increased the percentage of 4:0, and 6:0 fatty acids with a concurrent decrease in 8:0, 10:0, 12:0, 14:0, and 16:0. Frozen-thawed mammary cells had similar changes which were attributed to presence of long-chain fatty acids released from cells damaged by freeze-thaw process. Kinsella and McCarthy (9) reported freshly prepared mammary cells incorporated newly synthesized fatty acids into triglycerides. Hansen and Knudsen (4) found addition of palmitic acid stimulated synthesis and incorporation of butyric acid from acetate. These same workers in a companion paper reported addition of palmitate stimulated incorporation of fatty acids into triacylglycerols by dispersed mammary cells. In contrast, stearic and oleic acid strongly inhibited triacylglyceride synthesis (5). Therefore, long-chain fatty acids may influence lipid synthesis in ruminant mammary cells.

Feeding of whole oil seeds which act to protect lipid from microbial degradation in the rumen have been shown to alter both plasma and milk fatty acid composition (10,11). Fish meal has been shown to alter plasma fatty acid composition by increasing n-3 fatty acid levels with a tendency to decrease n-6 fatty acids (12). This change may be important given the ability of n-3 fatty acids to inhibit lipid synthesis in monogastrics (6,14,17). With respect to milk fat synthesis in the ruminant and contribution of de novo fatty acid synthesis to milk lipid, decreased milk fat yields which often occur with inclusion of fish meal in diets of lactating dairy cows may be associated with changes in long-chain fatty acids available at the mammary gland.

This study was conducted to determine the effect of eicosapentaenoic acid (20:5n3) on de novo fat synthesis from acetate. Influence of time post milking on tissue activity was also measured. In addition to these two aspects, the affect of acidification of culture with or without the tissue in the culture flask also was evaluated.

Materials and Methods

Five lactating cows were sacrificed by standard slaughter procedures at the Virginia Polytechnic Institute and State University Meats Laboratory. Before transport to slaughter, animals were given oxytocin and milked at the dairy center. Cows 3,4, and 5 were milked only on the right side. Therefore, the left side milk was removed at the routine time which was 5 to 7 h prior to slaughter. Following exsanguination, the mammary gland was removed. Tissue samples were taken from the right rear quarter (milked) in all cows and from the left rear quarter (unmilked) on the last three cows. Tissue sampling site was approximately 5 to 8 cm below the fat pad attachment. Tissue was placed in cold media 199 (M199, Gibco Laboratories, Life Technologies,Inc., Grand Island, N.Y.) with milked and unmilked tissue placed in separate containers, placed in ice, and transported to the laboratory.

The tissue culture technique used was as described by Bauman et al (1) with the following protocol. Tissue slices weighing approximately 130 to 150 mg wet weight were obtained using a hand held microtone. After weighing, slices were placed in individual flasks containing fresh M199 at ambient temperature. Culture vessels were flushed with a stream of oxygen (95%):carbon dioxide (5%) and capped with reaction flask caps fitted with center wells. Each center well contained a single slice of filter paper. Treatments included control media or control media containing .05 mg eicosapentaenoic acid per ml. All flasks contained 3 ml total volume and 1.5 μCi ^{14}C-acetate.

Tissue slices were incubated in a shaking water bath at 37 C and 80 oscillations per min. Incubations were stopped at 1, 2, and 3 h after being placed in water bath by injecting 200μl concentrated HCl. Prior to acidification, 200μl phenethylamine were injected through the cap into center well to trap CO_2. After acidification, vessels were returned to water bath and shaken for 1 h as described above. With cow 2, at 2 and 3 h, three control and three treated vessels were acidified with the tissue in the vessel with remaining tubes acidified after removal of tissue from the tube. After removal from water bath, reaction caps were removed and center wells placed in scintillation

vials. Deionized water (200μl) and 4 ml Phasar (Amersham, Arlington Hieghts, IL) were added to scintillation vials. Tissue slices were removed, rinsed with physiological saline, and placed in glass saponification tubes (25 x250) containing 5 ml 5 N NaOH.

Tissue were refluxed for 5 h at 90 C in 25 x 250 mm tubes capped with a glass marble. Sufficient concentrated HCl was added to the saponification vessel to turn congo red indicator paper to blue. Tubes were allowed to air cool followed by addition of 5 ml deionized water. Ten ml petroleum ether were added to each tube and mixed thoroughly by vortexing. Solvent-aqueous layers were allowed to separate. The petroleum ether layer was aspirated to a screw top glass tube and capped with a teflon lined cap. The extraction was repeated and the petroleum ether fractions were combined. One ml extract was transferred to a plastic scintillation vial with 15 ml toluene based commercial counting cocktail (Scintiverse II, Fisher Scientific Company, Fair Lawn, N.J.). Ether extracts and CO_2 traps were counted using a Beckman liquid scintillation counter for 10 min per sample.

Data were analyzed using the General Linear Procedure of SAS (13). An example analysis of variance is shown in Table 6. All cows were included in measuring affects of EPA treatment on tissue utilization of acetate. Only cows 3,4, and 5 were utilized for the effects and interactions with milked or unmilked tissue. Means squares were tested by interactions of main effects that contained cow. Variables containing cow were tested by mean square error value.

Results

Acetate incorporation into the petroleum ether extract increased over time (Table 7). Acetate oxidation to CO_2 was significantly influenced by time x cow x eicosapentaenoic acid interaction. Results of culture acidification with tissue remaining or removed prior are reported in Table 8.

Table 6. Analysis of variance table with corresponding degrees of freedom for milked and unmilked tissue incubations.

Source	df
COW	2
TISSUE	1
COWxTISSUE	2
EPA[B]	1
COWxEPA	2
EPAxTISSUE	1
COWxTISSUExEPA	2
TIME	2
TIMExCOW	4
TIMExTISSUE	2
TIMExTISSUExCOW	4
TIMExEPA	2
TIMExCOWxEPA	4
TIMExTISSUExEPA	2
TIMExTISSUExEPAxCOW	4

[A] Source of variation was tested by interaction that included cow.
[B] EPA = Eicosapentaenoic acid

Carbon dioxide production and fatty synthesis increased linearly as described above. However, if tissue remained in the culture vessel during acidification, CO_2 captured as estimated by ^{14}C was higher than in flasks from which tissue was removed prior to acidificaton. Fatty acid synthesis measured as labelled product recovery was not different due to presence or absence of tissue in the culture system during acidification.

In the last three cows, effect of time post-milking was designated as Tissue effect (Table 9). Again, CO_2 and fatty acid synthesis were significantly affected by time, cow and the two way interaction. As reported in Tables 9 and 10 respectively, unmilked tissue (6 to 8 h post-milking) had a higher acetate utilization for fatty acid synthesis ($p<.10$) and increased CO_2 production ($p<.12$). There was also an interaction between cow and tissue as well as an interaction involving time and tissue for both fat and CO_2 production.

Discussion

Numerous factors have been previously reported to affect mammary cell function. Given the importance of de novo fatty acid synthesis in milk fat production, factors influencing this anabolic process may cause shifts in vivo. EPA has been shown to significantly inhibit activity of hepatic lipogenic enzymes (6,7,14). As shown in Table 7, addition of EPA (.05mg/ml) did not alter incorporation of acetate into petroleum ether extract or oxidation to CO_2. Previous tissue culture work with n-3 fatty acids in monogastrics has involved a feeding period prior to animal sacrifice, tissue culture, and enzyme analysis (6,7,14). Our culture procedure was only conducted over 3 h, indicating short term exposure to EPA does not alter acetate utilization by lactating mammary tissue.

Table 7. Effect of eicosapentaenoic acid addition to culture media on acetate conversion to fatty acids and carbon dioxide.

Treatment	Time(h)	Fatty acid[1]	SEM	CO_2[2]	SEM
Control	1	17	2	117	66
	2	30	5	233	61
	3	42	5	38	61
EPA[3]	1	18	5	112	60
	2	33	5	267	61
	3	40	5	367	62

[1]Nanomoles acetate ($x10^2$) converted to fatty acids per 100mg wet tissue.
[2]Nanomoles acetate ($x10^2$) converted to CO_2 per 100mg wet tissue.
[3]EPA = eicosapentaenoic acid (20:5n3)

Table 8. Effect of eicosapentaenoic acid and acidification on label recovery in fatty acids and carbon dioxide.

Treatment	Acidification	Time(h)	Ether extract	CO_2[a]
			(cpm x 10^2/100mg)	
Control	Before[1]	2	17	76
		3	29	137
	After	2	17	52
		3	28	110
EPA	Before	2	18	69
		3	33	156
	After	2	18	70
		3	33	126

[1] Before = acidified before removing tissue.
After = acidified after removing tissue.

[a] Significant effect of acidification ($p < .01$).

Table 9. Effect of tissue, time and eicosapentaenoic acid on acetate incorporation into fatty acids.

Treatment	Time	Tissue type		
		Milked	Unmilked	SEM
	(h)	(nmol acetate incorporated x 10^2)		
Control	1	14	29	7
	2	24	53	7
	3	34	70	7
EPA	1	17	31	7
	2	26	53	7
	3	27	68	7

Table 10. Effect of tissue, time and eicosapentaenoic acid on acetate conversion to carbon dioxide reported as nmoles acetate ($x10^2$) converted to carbon dioxide per 100mg tissue.

Treatment	Time (h)	Tissue type		
		Milked	Unmilked	SE (n = 40)
Control	1	148	240	72
	2	262	480	72
	3	458	683	72
EPA	1	149	257	73
	2	323	494	73
	3	387	649	73

Acidification of culture with tissue present yielded higher CO_2 recovery than if tissue was removed before acidification. This may reflect the removal of tissue CO_2 before lowering culture pH. There may also be some tissue activity after acidification which could also contribute to this difference. Our data do not allow a conclusion to be drawn on this point.

Time of slaughter with respect to time post-milking must also be considered and controlled. Bauman et al. (1) studied effect of time post-slaughter and found reasonable delays of up to 1 h did not affect tissue function. Our data set is limited with respect to numbers ($n = 3$). However, the trend seemed to be strong given the level of significance with limited cow numbers. Tissue type significantly altered levels of acetate conversion to fatty acids and CO_2. In fact, tissue slices from the unmilked portion of the mammary gland had two times more acetate incorporated into extract than did milked tissue. This may reflect the presence of some limiting metabolite presence in unmilked tissue that was removed by milking. For example, Hansen et al. (5) reported palmitate stimulated milk triglyceride synthesis over control mammary gland tissue incubation. With respect to previous in vitro mammary cultures, response to added fatty acids may be different as shown by this data with time post-milking or suckling.

Conclusions

Eicosapentaenoic acid did not alter mammary utilization of acetate during short-term in vitro incubation. This may reflect a time response required for EPA to affect mammary function. Time of slaughter relative to last milking did affect acetate utilization. This difference in tissue activity may involve a large number of mechanisms including systemic, tissue and cellular changes in the animal and mammary gland after milking. Handling of tissue during culture may also influence quantitative values although qualitative responses seemed to be similar. Future investigations uti-

lizing in vitro culture of mammary tissue from lactating animals must consider and should report time post-milking prior to mammary gland removal.

References

1. Bauman, D.E., D.L. Ingle, R.W. Mellenberger, and C.L. Davis. 1973. Factors affecting in vitro lipogenesis by bovine mammary tissue slices. J. Dairy Sci. 56:1520.

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

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

4. Hansen, Hans Otto and Jens Knudsen. 1987. Effect of exogenous fatty acids on lipid biosynthesis in dispersed ruminant mammary gland epithelial cells: Esterification of long-chain exogenous fatty acids. J.Dairy Sci. 70:1344.

5. Hansen, H.O., D. Tornehave, and J. Knudsen. 1986. Synthesis of milk specific fatty acids and proteins by dispersed goat mammary-gland epithelial cells. Biochem. J. 238:167.

6. Herzberg, G.R. and M. Rogerson. 1988. Hepatic fatty acid synthesis and triglyceride secretion in rats fed fructose- or glucose-based diets containing corn oil, tallow or marine oil. J. Nutr. 118:1061.

7. Iritani, N., K. Inoguchi, M. Endo, E. Fukude, and M. Morita. 1980. Identification of shellfish fatty acids and their effects on lipogenic enzymes. Biochim. et Biophys. Acta 618:378.

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

9. Kinsella, J.E. and R.D. McCarthy. 1968. Biosynthesis of secretory lipids from [2-^{14}C] acetate by bovine mammary cells in vitro. Biochim. Biophys. Acta. 164:518.

10. Mohamed, O.E., L.D. Satter, R.R. Grummer, and F.R. Efle. 1988. Influence of dietary cottonseed and soybean on milk production and composition. J. Dairy Sci. 71:2677.

11. Rofalowski, W. and C.S. Park. 1982. Whole sunflower seed as a fat supplement for lactating cows. J. Dairy Sci. 65:1484.

12. Spain, J.N., Carl E. Polan, and B.A. Watkins. 1989. Effect of fish meal on milk yield, composition, ruminal volatile fatty acids, and plasma fatty acid profiles. J. Dairy Sci. 72(Suppl.1):507.

13. Statistical Analysis System Institute Inc. 1985. SAS User's Guide. SAS Institute. Cary, N.C.

14. Yang,Y.T. and M.A. Williams. 1978. Comparison of C_{18}-, C_{20}-, C_{22}-unsaturated fatty acids in reducing fatty acid synthesis in isolated rat hepatocytes. Biochim. et Biophys. Acta. 531:133.

In situ Measurement of Fish Meal Degradability.

Introduction

Other workers have reported decreased milk fat content with infusion of polyunsaturated marine oil into the rumen of lactating dairy cows (4,8). Addition of fish meal to diets of lactating cows has resulted in similar decreases in milk fat content (6). Fish meal does contain a substantial quantity of residual lipid compared to other protein meal supplements (5). Dry matter and crude protein degradability of fish meal has been described (3,9). However, availability of residual oil from fish meal has not been reported. Therefore, this study was conducted to determine the ruminal availability or release of residual oil in fish meal as well as to further characterize ruminal degradability of DM and CP.

Materials and Methods

Three ruminally fistulated cows were housed in a tie-stall barn and were individually fed a total mixed ration (Table 11) twice daily. Approximately 10g fish meal was placed in dacron polyester bags (143 cm^2) with a pore size of 57 microns. Bags were fastened to metal chains attached to nylon cords with 3 bags per incubation time period per cow. Ruminal resident times for the bags were 2,4,8,12,24,48,72 and 120 h. At the beginning of incubation, bags were placed on the ventral floor of the rumen. They were allowed to mix and be moved within the rumen to mimic normal digesta mixing and movement in the rumen. Bags were placed in the rumen in reverse order over time to allow removal of all bags simultaneously.

At removal, bags were individually rinsed to remove particles from the bag exterior. Bags were then placed in a continuous tap water rinse system for 24 h. During the continuous rinse, the system was emptied twice and refilled with fresh water. After rinsing, bags were suspended in a forced air oven and dried for 48 h at 65 C. Dried residues were composited within cow by residence time period. Composites were ground through a .5 mm screen. Each composite was analyzed for DM, CP (I) and lipid by refluxing residue with petroleum ether for 1 h (Soxtec System HT, Itecator).

Results and Discussion

Nutrient composition of pre-incubated fish meal is reported in Table 11. These values are similar to those previously reported for Menhaden fish meal (9). Average DM, CP, and ether extract disappearances are reported in Table 12. By 24 h, 37% of original DM was removed from the bag. CP disappearance was similar to DM at 30.6% after 24 h resident time. Ether extract was removed very rapidly with 75% removed after only 2 h and 92% of original lipid material removed after 24

Table 11. **Diet composition and nutrient analysis of fish meal prior to in situ incubation.**

Diet Components	Percent	
Barley Silage	77.9	
Dry Corn	8.2	
Soybean meal	12.2	
Herd mineral mix	1.7	
Item	**Diet**	**Fish meal**
Dry matter	48.9	94.3
Crude protein	17.4	61.8
Acid detergent fiber	24.2	4.2
Ether extract		9.4

Table 12. In situ nutrient disappearance of DM, CP, and ether extract during incubation in the rumen.

Time (h)	% Disappearance		
	Dry matter	Crude protein	Ether extract
2	28.9	17.6	75.2
4	29.8	17.9	84.2
8	31.4	22.5	83.9
12	32.5	22.0	84.4
24	37.1	30.6	92.1
48	50.2	49.7	92.1
72	59.8	64.4	88.9
120	71.8	82.8	92.9

h. These values have not been corrected for microbial contamination and appreciable amounts of residual rumen microbes could make this value higher for protein.

These data suggest that while fish meal DM and CP are resistant to microbial degradation in the rumen, residual lipid is readily removed from the feedstuff. DM and CP disappearance rates are similar to those of Zerbini and Polan (9). With respect to residual lipid, it is obvious this material is available to react within the rumen environment. Ready availability of dietary lipid, especially in large quantity, may affect rumen microbial metabolism (4,5). These data do not address the availability of specific fatty acids which may differ in their release and uptake. Individual fatty acid residues may not significantly alter total flow to post-ruminal absorptive sites. However, given the potential of n-3 fatty acids to alter lipid metabolism (2), post-ruminal flow of these particular fatty acids common to fish meal may be important to identify in future work of this nature.

References

1. Association of Official Analytical Chemists. 1980. Official methods of analysis. 13th ed. Assoc. of Official Analytical Chemists. Washington, D.C.

2. Herzberg, G.R. and M. Rogerson. 1988. Hepatic fatty acid synthesis and triglyceride secretion in rats fed fructose- or glucose-base diets containing corn oil, tallow, or marine oil. J. Nutr. 118:1061.

3. Mehrez, A.Z., E.R. Orskov, and J. Opstvedt. 1980. Processing factors affecting degradability of fish meal in the rumen. J. Anim. Sci. 50:737.

4. Nicholson, J.W.G. and J.D. Sutton. 1971. Some effects of unsaturated oils given to dairy cows with rations of different roughage content. J. Dairy Res. 38:363.

5. National Research Council. 1989. Nutrient requirements of dairy cattle. 6th Rev. ed. National Academy of Science, Washington, D.C.

6. Oldham, J.D., D.J. Napper, T. Smith, and R.J. Fulford. 1985. Performance of dairy cows offered isonitrogenous diets containing urea or fish meal in early and mid-lactation. Brit. J. Nutr. 53:337.

7. Palmquist, D.L. and T.C. Jenkins. 1980. Fat in lactation rations:Review. J. Dairy Sci. 63:1.

8. Pennington, J.A. and C.L. Davis. 1975. Effects of intraruminal and intra-abomasal additions of cod-liver-oil on milk fat production in the cow. J. Dairy Sci. 58:49.

9. Zerbini, E. and Carl E. Polan. 1985. Protein sources evaluated for ruminating Holstein calves. J. Dairy Sci. 68:1416.

Effects of Ruminal or Duodenal Marine Fish Oil Infusion on Milk Production, Milk Composition, Ruminal VFA, Duodenal Digesta Flow, and Plasma Fatty Acid Composition.

Introduction

Fish meal has been utilized to increase and alter amino acid flow to the small intestine of ruminants (17,21). Addition of fish meal to rations of lactating dairy cow has resulted in decreased milk fat (9,21). Decreased milk fat content has also been reported for cows fed marine oil (11,18). Site of action of marine oil on milk fat production has not been determined. Nicholson and Sutton (8) reported fish oil decreased ruminal acetate concentrations which corresponded with decreased milk fat. Pennington and Davis (11) found no difference in ruminal acetate with fish oil infusion. Both ruminal and abomasal fish oil infusions resulted in decreased milk fat content (11).

Fish meal has been shown to alter ruminal VFA concentrations (21). Other studies have shown decreased milk fat with no changes in ruminal VFA levels (13). Plasma levels of n-3 fatty acids were increased when cows were fed fish meal (14). Fish meal or fish oil feeding may affect milk fat production by altering ruminal fermentation as well as altering post-ruminal lipid metabolism (3). This study was conducted to measure effects of fish oil addition to the rumen versus duodenal infusion on performance and ruminal, duodenal, and plasma fatty acids.

Materials and Methods

Six lactating Holsteins (130 ± 9 d post-partum) were assigned to two replicates of a 3 x 3 Latin square. The design included three 10 d periods during which cows received one of three treatments. Cows had been surgically fitted with rumen fistulae and duodenal T-type cannulae. At the initiation of experiment, average milk production was 34.0 ± 3.9 kg milk per d. Cows were housed in a tie-stall barn. Treatments were corn gluten meal addition to rumen, corn gluten meal-fish oil addition to rumen, and corn gluten meal added to the rumen and fish oil infusion into duodenum. All cows were fed the same basal diet twice daily for 7 d prior to initiation of and throughout the 30 d experiment (Table 13). Cows were fed 30% of daily ration at 0600 with the remaining 70% fed at 1500. Orts were removed daily and sampled for individual cows every three days of each period. Fish oil was a crude Menhaden oil and its fatty acid composition is listed in Table 14. Cows were dosed twice daily 2 h after each feeding. Cows on duodenal treatment recieved one half of dose with remainder given 1 h later.

Cows were milked twice daily with production recorded. Milk samples were collected at consecutive am-pm milkings on day 4,7, and 10 of each experimental period. Samples were placed in plastic bags containing potassium dichromate, stored for 24 h at room temperature, and analyzed by 4-channel spectrophotometer (Multispec Mark I, Foss Food Technology, Eden Plaine, MN).

Table 13. Diet components and level of feeding.

Item	% Dry Matter
Corn silage	51.9
Alfalfa haylage	16.4
Concentrate[1]	28.8
Corn gluten meal	2.9
% CP	17.48
% ADF	19.35

[1]Concentrate (% as fed):dry corn,57.5;soybean meal,27.0;dried brewer's grains,12.6;minerals,3.0.

Table 14. Fatty acid composition of crude Menhaden fish oil as measured

Fatty acid	μg/mg
12:0	trace
14:0	85
15:0	5
16:0	175
16:1	100
17:0	12
17:1	7
18:0	30
18:1	64
18:2	48
18:3n6	2
18:3n3	7
20:0	3
20:1	8
20:2	trace
20:3n6	10
20:4n6	trace
20:3n3	trace
20:5n3	112
22:1n9	trace
22:6n3	59

On day 10, a liter sample was collected at the pm milking and transported to lab at ambient temperature. A 225 ml aliquot was centrifuged at 9,500 x g for 1 h at 10 C. Lower aqueous layer was aspirated by suction and fat layer removed. Separated milk fat was placed in individual plastic bags, which were sealed in larger plastic bags, and stored at -70 C until analyzed for fatty acid composition (S).

Rumen fluid and blood samples were collected 2 h post-dosing on d 10. Rumen fluid was collected by straining rumen digesta from various regions of the rumen through four layers of cheese cloth. Rumen pH was measured by pH meter calibrated with standards after collection at the dairy center. Rumen fluid was mixed and two 5 ml aliquots were transferred to plastic tubes and placed on ice. One tube contained four drops concentrated sulfuric acid and was used for rumen ammonia determination (4). Samples were stored at -20 C until analyzed. Rumen fluid was also analyzed for volatile fatty acids (13).

Blood samples were collected by puncture of the jugular vein and transferred to plastic tubes containing 200 units heparin. Blood was centrifuged at 3,220 x g for 20 min at 10 C. Plasma was decanted and stored in plastic tubes at -20 C until analyzed. Plasma samples used for fatty acid analysis were stored at - 70 C. Plasma samples (1.5 ml) were extracted and analyzed for fatty acids by gas chromatagraphy as described by Phetteplace and Watkins (12).

One liter of total flow duodenal digesta samples were collected over the last three days of each period to allow compositing of samples collected at 0100, 0500, 0900, 1300, 1700, and 2100. Duodenal digesta pH was measured at each sample time as described for rumen fluid. Duodenal samples were stored in sealed plastic cups at -20 C. For analysis, duodenal samples were thawed to room temperature and composited by cow for each period. Dry matter content was determined as the loss of weight following freeze-drying 120 h. Dried samples were ground to pass through a .5 mm screen. Acid detergent fiber (ADF) was measured by the method of Goering and Van Soest

(1). Acid insoluble ash was determined by ashing the ADF residue at 500 C for 4 h. Duodenal DM fatty acids were extracted by the method of Outen et al. (10) and analyzed by gas chromatography as described for plasma fatty acid analysis. Duodenal cytosine was determined as described by Zerbini and Polan (20).

Diet component samples were collected weekly, All feed and ort samples were dried at 100 C for 24 h to measure DM. ADF and acid detergent insoluble ash were measured as described for duodenal samples. Kjeldahl-N was measured and reported as CP.

Statistical analysis was by the General Linear Model of SAS (16). Experimental model was x = μ + replicate + cow(replicate) + period + treatment. Treatments were tested by the error mean square. Means were compared by Tukey's procedure (7).

Results

Dry matter intake averaged 19.7 kg per d for all three treatments and was not different (Table 15). Average daily milk yields also were similar for treatments ranging from 31.3 to 32.0 kg per day. No significant differences in milk composition or component yields were measured (Table 15).

Ruminal volatile fatty acid concentrations tended to be lower when cows recieved ruminal oil versus control or duodenal oil treatments (Table 16). There were no differences due to treatment in concentrations, proportions, or ratios of ruminal VFA (Table 16). Rumen pH was not changed by treatment (Table 16).

Table 15. Effect of ruminal or duodenal fish oil infusion on intake, milk production, composition, and component yields.

Item		Control	Ruminal	Duodenal	SEM (n = 18)
DM intake	(kg/d)	19.7	20.2	19.2	.2
Milk	(kg/d)	31.3	31.5	32.0	.2
Fat	(%)	3.2	3.2	3.1	.04
Protein	(%)	3.1	3.1	3.1	.01
Lactose	(%)	4.7	4.8	4.7	.01
SNF	(%)	8.6	8.6	8.6	.01
Fat	(kg/d)	1.0	1.0	1.0	.01
Protein	(kg/d)	1.0	1.0	1.0	.01
Lactose	(kg/d)	1.5	1.5	1.5	.01
SNF	(kg/d)	2.7	2.7	2.7	.02

Duodenal digesta DM content and total DM flow were not significantly different among treatments (Table 17). Duodenal digesta pH was also unaffected by treatment. Duodenal cytosine was not changed due to treatment. Duodenal fatty acid profiles were altered. The ruminal oil treatment increased digesta content of 20:0 (Table 18). Butyric acid was highest (p,.05) in duodenal digesta of cows receiving control treatment (Table 18).

Plasma fatty acid concentrations were altered by fish oil infusion and response differed among individual fatty acids (Table 19). Saturated fatty acids were not altered by treatment. Palmitoleic (16:1) was increased by duodenal fish oil infusion ($p<.09$) but 18:1 was not different for all three treatments. Polyunsaturated fatty acids were markedly changed by duodenal fish oil infusion. Gamma linolenic (18:3n6) tended to be decreased by duodenal infusion to 22 versus 30 μg per ml for control treatment (Table 19). Arachidonate (20:4n6) was increased by duodenal treatment over control and ruminal dosing. All n-3 fatty acids were significantly increased by duodenal fish oil versus the other treatments (Table 19). Although circulating fatty acid profiles were altered, milk fatty acid composition was not different (Table 20).

Discussion

Conflicting reports exist with respect to the means by which fish oil or fishmeal exerts its affect on milk fat production. Milk fat production in this study was low but was not different due to treatment (Table 15). Pennington and Davis (11) decreased milk fat content by infusing 225 g destearinated cod-liver oil into the rumen or abomasum of lactating cows. Both treatments decreased milk fat content with no differences in ruminal VFA patterns. Varman et al (18) reported decreased ruminal acetate but not of the magnitude associated with large decreases in milk fat content resulting from fish oil supplementation. Varman and co-workers also measured decreased arteriovenous differences in mammary triglyceride uptake suggesting a possible post-absorptive ef-

Table 16. Effect of fish oil addition to rumen or duodenum on ruminal volatile fatty acid concentrations and ratios.

Item	Treatment			
	Control	Ruminal	Duodenal	SEM(n = 18)
	mmol/dl			
Total	150.9	140.1	150.7	2.1
Acetate	82.0	80.0	86.8	1.6
Propionate	40.2	33.5	34.7	2.0
Isobutyrate	1.6	1.6	1.7	.03
Butyrate	20.9	18.7	20.5	.1
Isovalerate	3.1	3.1	3.5	.1
Valerate	3.2	3.4	3.6	.1
	mol/100 mol			
Acetate	54.5	57.2	58.3	.8
Propionate	26.3	23.8	22.6	1.1
Isobutyrate	1.1	1.1	1.2	.03
Butyrate	13.9	13.3	13.5	.4
Isovalerate	2.1	2.2	2.3	.1
Valerate	2.1	2.4	2.3	.1
Acetate:propionate	2.2	2.5	2.7	.2
Acet. + Butyrate:Propionate	2.8	3.1	3.3	.2
Rumen pH	5.9	5.8	6.0	.02

Table 17. Effect of fish oil dose into the rumen or duodenum on duodenal digesta dry matter, pH, duodenal digesta flow rates. and cytosine concentrations.

Item		Treatment			
		Control	Ruminal	Duodenal	SEM(n = 18)
Duodenal DM	(%)	6.1	7.0	6.2	.2
Duodenal pH		2.8	2.7	2.8	.03
DM digesta flow	(kg/d)	11.1	18.2	14.3	1.7
Cytosine	μM	46.5	48.6	37.8	1.2

Table 18. Effect of site of fish oil infusion on duodenal digesta fatty acid composition (area percent).

Fatty acid	Treatment			
	Control	Ruminal	Duodenal	SEM(n = 18)
4:0	.8[a]	.4[b]	.5[a]	.05
6:0	.2	.2	.2	.01
8:0	.4	.4	.4	.02
10:0	.2	.2	.2	.02
12:0	.4	.4	.4	.01
13:0	.1	.2	.2	.02
14:0	1.5	1.7	1.4	.04
14:1	.2	.2	.2	.02
15:0	1.0	1.0	1.0	.01
15:1	.5	.4	.5	.05
16:0	18.3	18.9	17.8	.2
16:1	.3	.3	.2	.03
17:0	1.1	1.2	1.1	.02
18:0	51.8	48.7	45.5	1.0
18:1	10.8	14.4	11.3	1.0
c18:2	7.3	7.7	10.4	.5
18:3n6	trace	trace	trace	
18:3n3	.7	.8	.8	.02
20:0	.8[a]	1.0[b]	.8[a]	.02

[a,b]Value in same row with different superscripts differ, $p < .05$.

Table 19. Effect of site of fish oil infusion on plasma long chain fatty acid composition (μg/ml).

Item	Treatment			
	Control	Ruminal	Duodenal	SEM(n = 18)
16:0	281	258	294	20
16:1	14	15	24	2
17:0	24	24	25	2
18:0	443	409	440	30
18:1	181	193	174	11
t18:2	6	14	8	2
c18:2	1100	1163	1126	18
18:3n6	30	25	22	1
20:3n6	66	69	65	2
20:4n6	69[a]	70[a]	82[b]	2
20:5n3	17[a]	28[a]	64[b]	1
22:4n6	8	9	10	1
22:5n3	20	19	28	2
22:6n3	trace	2	12	1

[a,b]Values in same row with different superscripts differ $p < .05$.

Table 20. Effect of site of administration of fish oil on milk fat fatty acid composition (μg/mg).

Item	Treatment			
	Control	Ruminal	Duodenal	SEM(n = 18)
4:0	11.9	19.3	20.3	.8
6:0	15.7	17.5	17.0	.1
8:0	9.5	10.0	9.8	.1
10:0	18.1	23.0	19.5	1.7
12:0	21.0	20.6	22.5	.2
14:0	72.1	76.1	75.9	.9
14:1	5.1	5.1	5.1	.02
15:0	6.9	6.7	6.2	.1
16:0	164.9	166.9	166.2	1.4
16:1	6.1	6.2	7.1	.1
17:0	2.8	2.7	2.2	.03
18:0	51.1	48.4	43.2	.4
18:1	94.8	93.2	86.5	.6
18:2	16.0	15.8	14.4	.1
18:3n6	.3	.0	.0	.02
18:3n3	1.9	1.9	1.7	.02

fect of fish oil fatty acids. In contrast, Storry et al. (15) reported decreased ruminal acetate in cows fed 300 g unprotected cod-liver oil which resulted in decreased milk fat production.

Total ruminal VFA concentrations tended to be lower in cows recieving ruminal doses, but was not significant (Table 16). Concentrations of individual VFA and their ratios were not different. Rumen fluid pH also tended to be lower in ruminally treated cows and might have caused the slight differences in total VFA concentrations. Ruminal pH may reflect the lack of dietary buffers and fiber level of the diet (Table 13).

Flow rates of digesta DM to the duodenum were not changed by treatment (Table 17). Duodenal pH was not altered by treatment (Table 17). Although rumen pH and total ruminal VFA concentrations were slightly lower for ruminal dose, there were no differences in cytosine concentrations in th duodenal digesta. Ruminal fish oil dose did alter concentrations of several fatty acids slightly (Table 18). These differences are physiologically small. Ruminal microorganisms significantly altered polyunsaturated fatty acids of marine oil. In addition to microbial action, the dose of oil was smaller than those used in previous fish oil studies involving lactating dairy cattle (11,15,18).

Plasma fatty acid composition was significantly altered by duodenal infusion of fish oil (Table 19). Arachadonate (20:4n6), eicosapentaenoate (20:5n3), and docosahexaenoate (22:6n3) were all increased by duodenal fish oil infusion (Table 19). Compared to plasma values for ruminally dosed cows treated cows, fish oil fatty acids were extensively altered by ruminal microorganisms. Polyunsaturated fatty acids common to fish oil have been shown to have a negative effect on lipogenesis in monogastrics fed marine oil supplemented diets (19). Published data has measured increased plasma n-3 fatty acids when dairy cows were fed fish meal. However, due to ruminal metabolism, ruminal fish oil dose failed to cause changes in plasma fatty acid profiles. These differences in effect by source suggest differences in ruminal metabolism from fish meal versus fish oil.

Unlike plasma fatty acids, milk fatty acids were not altered significantly (Table 20). Studies where whole oil seeds or protected lipids have been fed have shown significantly altered milk fatty acid

composition (3,5). Pennington and Davis (11) increased unsaturated fatty acid composition of milk with fish oil infusion. Storry et al. (15) increased milk fat content of polyunsaturated fatty acids in cows fed protected fish oil. The lack of a response in milk fatty acid composition may again be due to inadequate levels of circulating n-3 fatty acids to alter milk triglyceride synthesis and composition. This lack of response may also result from the preferential use of saturated and monoenoic fatty acids for milk triglyceride synthesis (11).

Therefore, milk production and composition were not altered by treatment. Dose level was significantly lower than that previously reported, but was calculated to reflect normal lipid intake from a fishmeal supplemented diet. Polyunsaturated fatty acids infused into the rumen were not detected in duodenal digesta indicating modification of free oil fatty acids within the rumen. Duodenal infusion of fish oil did significantly alter plasma fatty acids but did not affect milk fatty acid composition. Length of experimental period might have been inadequate to allow equilibration of fatty acids and maximum response to changes in plasma fatty acid concentrations. Given the biologically active nature of n-3 fatty acids, these fatty acids may be involved in decreased milk fat production in cows fed fish oil.

References

1. Goering, H.K. and P.J. Van Soest. 1970. Forage Fiber Analysis. Agric. Handbook No. 379 ARS.-USDA, Beltsville, MD.

2. Mathers, J.C. and E.L. Miller. 1983. Some effects of fish lipids on rumen metabolism and digestion in sheep. Nutr. Soc. Proc. 42:63A.

3. Mattos, Wilson and D.L. Palmquist. 1974. Increased polyunsaturated fatty acid yields in milk of cows fed protected fats. J. Dairy Sci. 57:1050.

4. McCullough, H. 1967. The determination of ammonia in whole blood by direct colorimetric method. Clin. Chem. Acta. 17:297

5. Mohamed, O.E., L.D. Satter, R.R. Grummer, and F.R. Ehle. 1988. Influence of dietary cottonseed and soybean meal on milk production and composition. J. Dairy Sci. 71:2677.

6. Moore, J.H. and W.W. Christie. 1978. Lipid metabolism in the mammary gland of ruminant animals. Prog. Lipid Res. 17:347.

7. Mosteller, F. and J.W. Tukey. 1977. Data Analysis and Regression. Addison-Wiley, N.Y.

8. Nicholson, J.W.G., and J.D. Sutton. 1971. Some effects of unsaturated oils given to dairy cows with rations of different roughage content. J. Dairy Res. 38:363.

9. Oldham, J.D., D.J. Napper, T. Smith, and Rosemary J. Fulford. 1985. Performance of lactating dairy cows offered isonitrogenous diets containing urea or fishmeal in early and mid-lactation. Br. J. Nutr. 53:337.

10. Outen, G.E., D.E. Beever, and J.S. Fenlon. 1976. Direct methylation of long-chain fatty acids in feeds, digesta, and faeces without prior extraction. J. Sci. Food. Agric. 27:419.

11. Pennington, J.A. and C.L. Davis. 1975. Effects of intraruminal and intra-abomasal additions of cod-liver oil on milk fat production in the cow. J. Dairy Sci. 58:49.

12. Phetteplace, H.W. and B.A. Watkins. 1989. Effects of various n-3 fatty acid sources on fatty acid composition in chicken tissues. J. Food Compos. and Analysis 2: (in press).

13. Spain, J.N., M.D. Alvarado, Carl E. Polan, C.N. Miller, and M.D. McGilliard. 1990. Effect of protein source and energy level on milk composition in mid-lactation dairy cows. J. Dairy Sci (in press).

14. Spain, J.N., Carl E. Polan, and B.A. Watkins. 1989. Effect of fishmeal on milk yield, composition, ruminal volatile fatty acids, and plasma fatty acids profiles. J. Dairy Sci 72(suppl.1):507 (abst.).

15. Storry, J.E., P.E. Brumby, A.J. Hall, and B. Tuckley. 1974. Effects of free and protected forms of codliver oil on milk fat secretion in the dairy cow. J. Dairy Sci. 57:1046.

16. Statistical Analysis System. 1985. SAS User's Guide. SAS Institute. Cary, N.C.

17. Titgemeyer, E.C., N.R. Merchen, and L.L. Berger. 1989. Evaluation of soybean meal, corn gluten meal, blood meal, and fishmeal as sources of nitrogen and amino acids disappearing from the small intestine of steers. J. Anim. Sci. 67:262.

18. Varman, P.N., L.H. Schultz, and R.E. Nichols. 1968. Effects of unsaturated oils on rumen fermentation, blood components, and milk composition. J. Dairy Sci. 51:1956.

19. Yang, Y.T. and M.A. Williams. 1978. Comparison of C_{18}-, C_{20}, and C_{22}- fatty acids on reducing fatty acid synthesis in isolated rat hepatocytes. Biochim. et Biophys. Acta. 531:133.

20. Zerbini, E. and Carl E. Polan. 1985. Protein sources evaluated for ruminating Holstein calves. J. Dairy Sci. 68:1416.

21. Zerbini, E., Carl E. Polan and J.H. Herbein. 1988. Effect of dietary soybean meal and fish meal on protein digesta flow in Holstein cows during early and midlactation. J. Dairy Sci. 71:1248.

Comparison of Fish Meal or Fish Oil Addition to Diets of Lactating Holsteins on Milk Production and Composition,Ruminal Volatile Fatty Acids and Plasma Fatty Acids.

Introduction

By-product feedstuffs are being widely used to provide high quality economical diets for dairy cattle (2). Fish meal is a by-product of fish oil removal from raw fish by a mechanical extraction process. Menhaden fish meal contains 60% crude protein on average with approximately 65% of this protein escaping ruminal degradation (8,19). Fish meal can be used to meet recommended levels of undegradable intake protein. Studies have shown increased amino acid flow to small intestine with fish meal feeding (5,18). Fish meal also contains 8 to 10% residual ether extract (8). As described by Opstvedt (14), a major portion of this lipid material is long-chain polyunsaturated fatty acids. These fatty acids have been implicated as being involved in decreased milk fat production by cows

fed fish meal. Effect on milk fat production may be due to ruminal effects of residual polyunsaturates on fermentation by ruminal bacteria, post-ruminal changes in lipid metabolism, or both (9,12). Decreased cellulytic bacteria activity could decrease ruminal acetate production which has been shown to reduce milk fat production (12). Increasing fish meal intake was associated with linear increases in plasma n-3 fatty acid concentrations of lactating dairy cows with a concurrent linear decrease in milk fat production (16). These data suggest n-3 fatty acids of fish meal escape microbial metabolism within the rumen.

Therefore, this study was conducted to determine effects of fish oil on milk production and composition. This included investigating two sources of fish oil, oil associated with meal or a separate fish oil mixed with the concentrate portion of ration. Effects of fish oil supplementation on rumen function and plasma long-chain fatty acids were also measured.

Materials and Methods

Twelve lactating Holstein cows (3.1 average lactation number) were assigned to one of four replicates of a 3x3 latin square design. Average pre-trial milk production, milk fat production, and milk fat percentage are listed by replicate in Table 21. Cows were housed in a tie-stall barn and fed one of three diets over three 18 d periods. Diets were corn silage - alfalfa haylage based total mixed rations (Table 22). Fish oil was mixed with corn gluten meal-limestone premix, placed in plastic bags and stored refrigerated. Weekly supplies were moved to the barn and stored in bags inside a rubber barrel. Ethoxyquin (500 ppm) was added at time of pre-mixing as an antioxidant. Cows were fed twice daily at 0600 and 1500 in amounts to insure 10% refusal. Orts were recorded 4 d per wk. Cows were milked twice daily and production recorded at each milking.

Table 21. Pre - experiment production and milk composition of cows shown by replicates of a 4x4 experiment.

Item[1]		Replicate 1	2	3	4
Milk production	(kg/d)	27.3	35.0	39.7	43.3
Milk fat	(%)	4.0	2.6	3.4	2.9
Milk fat	(kg/d)	1.1	0.9	1.4	1.3

[1] Item represents average records of the cows from the last DHI test prior to start of study.

Table 22. Diet composition reported as percentage of diet dry matter.

Item	Diet		
	Control	Fish meal	Fish oil
Corn silage	37.5	37.6	37.3
Alfalfa haylage	35.8	36.1	35.8
High moisture corn	14.9	15.0	14.9
Soybean meal	7.5	7.5	7.5
Corn gluten meal	3.7	-	3.9[1]
Fish meal	-	3.8	-
Limestone	0.6	-	0.6

[1] Includes 0.2 kg crude Menhaden fish oil.

Milk samples were collected at four consecutive milkings on d 16,17, and 18. Milk samples were placed in plastic bags containing potassium dichromate, stored for 24 h at room temperature, and analyzed by regional DHI lab using 4-channel spectrophotometer (Multispec Mark I, Foss Food Technology, Eden Plaines, MN). Blood and rumen samples were taken on d 18 following the morning feeding by 2 h. Blood was sampled via jugular venipuncture. Ten ml was transferred to plastic tubes containing 200 units of heparin per tube and stored on ice. Rumen fluid was sampled by esophageal tube, transferred to sealed plastic cups and placed on ice. Plasma and rumen fluid were centrifuged for 20 min at 3,220 x g at 4 C. Supernatants were decanted into plastic tubes, capped and stored at -20 C until analyzed. Plasma samples used for fatty acid analysis were stored at -70 C.

Plasma was analyzed for urea-N (3) and rumen ammonia was measured by a phenol-based colorimetric procedure (6). Plasma samples (1.5 ml) were extracted and analyzed by gas chromatography as decribed by (14). Rumen fluid samples were thawed at room temperature, vortexed, filtered using syringe fitted microfilters (.45 micron) and analyzed for ruminal VFA using the procedure described by Spain et al. (15).

Statistical analysis was by the General Linear Models procedures of SAS (17). Experimental model was defined as $Y = \mu + \text{replicate} + \text{cow(replicate)} + \text{period} + \text{treatment} + \text{residual}$ with treatment means square tested by residual means square term. Means were separated by Tukey's procedure. (7).

Results

Milk production and composition are reported in Table 23. Milk production was not affected by diet. DM intake was similar for cows fed all diets (Table 23). Milk fat percentage tended to be

decreased slightly by fish meal and fish oil with total fat produced (kg/d) not different. Protein, lactose, and total solids-not-fat were not different across diets.

Total concentrations of ruminal VFA tended to be lower for cows fed fish meal than other dietary treatments (Table 24). Percent propionate and valerate were higher for fish oil than other diets with but concentrations were not different. No other differences in ruminal VFA concentrations were observed. Acetate:propionate as well as acetate + butyrate:propionate ratios were not affected by addition of fish meal or fish oil to total mixed rations.

Plasma fatty acids data are presented in Table 25. Cows fed fish meal had higher 16:1 than either CG or fish oil. Stearic acid and oleic acid were not changed by dietary treatment. Trans gamma-linoleic tended to be increased with addition of fish meal to the diet with cis 18:2n6, 18:3n6, and 20:3n6 tended to be lower with inclusion of fish meal in the diet. In contrast, 18:3n3 and 20:4n6 exhibited a trend to be increased by fish meal. Eicosapentaenoic acid and 22:6n3 were significantly increased by fish meal, with fish oil increasing eicosapentaenoic acid. No other fatty acid concentations were altered significantly by dietary treatment.

Discussion.

Milk production and DM intake were not different across treatments (Table 23). These results agreed with previous work in our lab which showed no adverse affects on production when fish meal was substituted for corn gluten meal. Use of total mixed rations and providing a proper balance of nutrients will support optimal intake and performance. Milk fat content tended to decrease with addition of fish meal or fish oil to diets but was not significantly different. Pennington and Davis (13) reported that cod-liver-oil infusion into the rumen or abomasum of lactating cows de-

Table 23. Effect of dietary treatment on milk production, DM intake, milk composition and component yields.

Item		Diet[1]			
		Control	Fish meal	Fish oil	SEM(n = 32)
Milk	(kg/d)	30.1	30.3	30.1	.2
DM intake	(kg/d)	21.6	21.2	21.4	.3
Milkfat	(%)	3.8	3.7	3.7	.03
	(kg/d)	1.1	1.1	1.1	.01
Milk protein	(%)	3.1	3.1	3.1	.01
	(kg/d)	0.9	0.9	0.9	.01
Milk lactose	(%)	4.8	4.8	4.8	.01
	(kg/d)	1.4	1.4	1.4	.01
Milk SNF	(%)	8.6	8.6	8.6	.02
	(kg/d)	2.6	2.6	2.6	.02

[1] Diets are defined in Table 22.

Table 24. Effect of diet on ruminal volatile fatty acid concentrations and ratios.

Item		Diet[1]			
		Control	Fish meal	Fish oil	SEM(n = 32)
Total	(mMol)	98.4	92.4	98.4	3.8
Acetate	(mMol)	61.9	58.0	62.3	2.4
Propionate	(mMol)	19.0	17.8	18.1	.7
Isobutyrate	(mMol)	1.4	1.4	1.5	.1
Butyrate	(mMol)	11.9	11.3	11.9	.5
Isovalerate	(mMol)	2.7	2.5	2.6	.1
Valerate	(mMol)	1.7	1.7	2.0	.1
Acetate:Propionate		3.4	3.3	3.5	.03
Acet. + Butyr.:Prop.[2]		4.0	3.9	4.1	.04

[1] Diets are defined in Table 2.
[2] (Acetate + Butyrate):Propionate
[a,b] Values with different superscripts differ, $p < .05$.

Table 25. Effect of dietary fish meal or fish oil in lactating cows on plasma fatty acid concentrations (μg/ml).

Item	Diet[1]			
	Control	Fish oil	Fish meal	SEM(n = 32)
16:0	268	288	286	8
16:1	19^{a}	20^{a}	26^{b}	.7
17:0	23	24	24	.7
18:0	397	419	391	12
18:1	172	175	182	5
18:2 (trans)	5	5	7	1
18:2 (cis)	1331	1334	1195	42
18:3n6	31	29	23	1
18:3n3	142	146	167	4
20:3n6	74	74	65	2
20:4n6	75^{a,b}	72^{a}	87^{b}	2
20:5n3	26^{a}	32^{b}	60^{b}	2
22:4n6	9	11	12	.7
22:5n3	22	22	27	1
22:6n3	4^{a}	4^{a}	18^{b}	1

[1] Diets are defined in Table 2.
^{a,b}Values in same row with different a superscript differ ($p < .05$).

creased milk fat percentage but not yields. Fish meal has been reported to decrease milk fat content and yield when fed to lactating dairy cows (10,20) Milk fat yields were not different across diets. Protein, lactose, and SNF were similar for all diets. Oldham et al (10) substituted fish meal for urea in lactating cow diets with milk protein yields were significantly increased with no change in lactose. Fish meal substitution for soybean meal did not alter protein or lactose yields in early and mid-lactation dairy cows (20).

With exception of the molar proportions of propionate and valerate, ruminal concentrations or ratios of volatile fatty acids were not affected by diet. These data differ from those of Pennington and Davis (13) who reported no change in ruminal VFA concentrations with infusion of cod liver oil into the rumen or abomasum. Nicholson and Sutton (9) found the addition of cod liver oil to the grain portion of the diet resulted in decreased ruminal acetate and butyrate concentrations with propionate concentrations increased. However, when low levels of cod liver oil (125ml/d), which was slightly lower than that used in our study (180ml/d), resulted in only modest changes in ruminal VFA concentrations in a 50:50 concentrate:forage diet. Fiber level influenced VFA changes reported by Nicholson and Sutton (9). Ruminal VFA were changed more on the low roughage diet than medium or high roughage diets. Fiber source may also influence response to fish meal or fish oil. Broderick reported fish meal addition to alfalfa silage based diets had no effect on milk composition (1). Differences due to forages may be caused by differences in rapidly fermented soluble carbohydrates which may result in hormonal changes such as increased insulin. This may augment the effects of marine fatty acids inhibitory effect on lipid synthesis.

. Some plasma fatty acids were significantly altered by fish oil and fish meal addition with the effect more pronounced with fish meal. Fish meal supplementation increased plasma 16:1 over levels on control and fish oil (Table 25). Nicholson and Sutton (9) reported linear increases in 16:1 in milk fat with increased fish oil dose per day. Pennington and Davis (13) reported five unidentified long-chain fatty acids were changed by cod liver oil infusion. The unidentified fatty acids may correspond with the 20 and 22 carbon fatty acids found changed by fish meal and fish oil in this

study. As reported in Table 25, fish meal and fish oil increased plasma 20:5n3 concentrations versus plasma concentrations of cows fed control diet. There was also an increase in plasma 22:6n3 when cows were fed fish meal compared to diets control and fish oil. These responses agree with previous work done at our lab with similar levels of fish meal. However, in previous work, fish meal addition to corn silage-based diets was accompanied by decreased milk fat content. In the present study, milk fat was only slightly decreased with a similar rise in plasma n-3 fatty acid concentrations. Diets fed in the present study utilized total mixed rations but 50 percent of the forage was supplied by alfalfa haylage in contrast to corn silage alone. Potential for forage source to influence production responses should be considered and included in future work.

References

1. Broderick, G.A. 1989. Fish meal versus soybean meal for lactating cows fed alfalfa silage as the sole forage. J. Dairy Sci 72(Suppl 1):299.

2. By-Product Feed Handbook. 1988. R.L. Belyea ed. Univ. Missouri, Columbia.

3. Coulombe, J.J. and L. Favrea. 1963. A new simple method for colorimetric determination of urea. Clin. Chem. 9:102.

4. 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.

5. McCarthy,Jr.,R.D., T.H. Klusmeyer,J.L.Vicini, J.H. Clark, and D.R.Nelson.1989. Effects of source of protein and carbohydrate on ruminal fermentation and passage of nutrients to the small intestine of lactating cows. J. Dairy Sci. 72:2002.

6. McCullough, H. 1967. The determination in whole blood by direct colorimetric method. Clin. Chem. Acta. 17:297.

7. Mosteller, F. and J.W. Tukey.1977. Data Analysis and Regression. Addison-Wiley. N.Y.

8. National Research Council. 1989. Nutrient requirements for dairy cattle. 6th Rev. ed. National Academy Press, Washington, D.C.

9. Nicholson, J.W.G. and J.D. Sutton. 1971. Some effects of unsaturated oils given to dairy cows with rations of different roughage to concentrate. J. Dairy Res. 38:363.

10. Oldham, J.D., D.J. Napper, T. Smith, and R.J. Fulford. 1985. Performance of dairy cows offered isonitrogenous diets containing urea or fish meal in early and mid-lactation. Br. J. Nutr. 53:337.

11. Opstvedt, J. 1985. Fish lipids in animal nutrition. International Association of Fish Meal Manufacturers Technical Bulletin.

12. Palmquist, D.L. and T.C. Jenkins. 1980. Fat in lactation rations: Review. J. Dairy Sci. 63:1.

13. Pennington, J.A. and C.L. Davis. 1975. Effects of intraruminal and intra-abomasal additions of cod-liver oil on milk fat production in the dairy cow. J. Dairy Sci. 58:49.

14. Phetteplace, H.W. and B.A. Watkins. 1989. Effects of various n-3 sources on fatty acid composition in chicken tissues. J. Food Comp. Analysis. 2: (in press).

15. Spain, J.N., M.D. Alvarado, Carl E. Polan, C.N. Miller, and M.D. McGilliard. 1989. Effect of protein source and energy level on milk composition in lactating dairy cows. J. Dairy Sci. (in press).

16. Spain, J.N., Carl E. Polan, and B.A. Watkins. 1989 Effect of fish meal on milk yield, composition, ruminal volatile fatty acids, and plasma fatty acid profiles. J. Dairy Sci. 72(Suppl.1):507 (abst).

17. Statistical Analysis System Institute, Inc. 1985. SAS User's Guide. SAS Institute, Cary, N.C.

18. Titgemeyer, E.C., N.R. Merchen, and L.L. Berger. 1989. Evaluation of soybean meal, corn gluten meal, blood meal, and fish meal as sources of nitrogen and amino acids disappearing from the small intestine of steers. J. Anim. Sci. 67:262.

19. Zerbini, E. and C.E. Polan. 1985. Protein sources evaluated for ruminating Holstein calves. J. Dairy Sci. 68:1416.

20. Zerbini, E., Carl E. Polan, and J.H. Herbein. 1988. Effect of dietary soybean meal or fish meal on protein digesta flow in Holstein cows during early and mid-lactation. J. Dairy Sci. 71:1248.

Epilogue

Utilization of fish meal to supplement post-ruminal amino acid flow above bacterial protein could improve milk production. In some studies, cows fed fish meal had decreased milk fat percentage and yields. There have been cases in which ruminal VFA were altered while others reported no changes in ruminal VFA patterns. Therefore, to better utilize fish meal, the way by which fish meal affects milk composition must be better understood.

Milk composition changes reported for cows fed fish meal are similar to those of cows fed or dosed with fish oil. Diet lipid content has been shown to negatively affect milk fat production by decreasing ruminal acetate production due to lower fiber digestibility. Marine fish oil has been reported to decrease ruminal acetate concentration. However, several studies have decreased milk fat by feeding fish oil with no change in ruminal fermentation patterns. Several authors have implicated a post-absorptive effect of long-chain polyunsaturated fatty acids on lipid metabolism. Experiments described in this dissertation were directed towards defining how fish meal exerts its affect on milk composition.

Results of the first production study showed inclusion of fish meal decreased milk fat from 3.5 to 3.0 percent without altering ruminal VFA patterns. In addition, plasma long chain fatty acid profiles were significantly altered with increased fish meal intake increasing plasma n-3 fatty acid con-

centrations to 40 μg/ml. These data indicated n-3 fatty acids escaped microbial biohydrogenation and could potentially alter lipid metabolism. Monogastric data has indicated n-3 fatty acids inhibited or decreased hepatic acetyl CoA carboxylase activity. Acetyl CoA carboxylase has been described as the rate limiting enzyme in de novo fatty acid synthesis in ruminant mammary gland. Given the contribution of de novo fatty acids to milk triglyceride synthesis (50%), increased plasma n-3 fatty acids could inhibit milk fat synthesis.

Mammary tissue incubations were conducted to measure affects of presence of eicosapentaenoic acid on acetate incorporation. Published data showed mammary gland in vitro de novo fatty acid synthesis and subsequent triglyceride synthesis was decreased by polyunsaturated fatty acids. Addition of EPA to cultures did not affect acetate incorporation. These incubations were short term and tissue donors had not been previously fed fish meal or fish oil prior to slaughter. Influence of carbohydrate level and fermentability was not considered and pre-slaughter diets were not controlled.

Although eicosapentaenoic acid did not alter acetate utilization, there was a significant affect of time post-milking on tissue activity. Mammary tissue that was milked 6 to 8 h prior to slaughter had twice the activity than tissue milked 1 h prior to slaughter. These data could reflect differences in cellular metabolites required for maximum activity. Several published in vitro studies illustrated the stimulatory effect of palmitate on de novo lipid synthesis in mammary cells.

Ruminal degradability of fish meal protein has been well documented. Disappearance of residual lipid from fish meal had not been measured. Given possible ruminal effects of residual lipid contained in fish meal, in situ disappearance of lipid from fish meal was measured. Lipid was removed quickly and could possibly affect ruminal fermentation if excessive fish meal was fed. Data from the first production study indicated n-3 fatty acids in fish meal escaped microbial biohydrogenation. Further in situ lipid disappearance work should include residual fatty acid composition. Average literature values for fish meal show a high content of phospholipids. Average published fatty acid composition values show higher concentration of 22:6n3 and total n-3 fatty acids in phospholipids

than neutral lipids. Therefore, fatty acids associated with DM residue may influence post-ruminal fatty acid flow in ruminants fed fish meal.

The fish oil infusion study was conducted to determine the magnitude of ruminal and post-ruminal mode of action of fish lipid on milk composition. In situ results indicated fish oil of fish meal was readily available and that fish oil could be used to maintain similar fatty acid composition for ruminal and post-ruminal treatments. However, reported differences in lipid fractions of fish meal versus fish oil may influence ruminal and post-ruminal metabolism. Level of fish oil dose was selected to reflect levels which would be contained in practical feeding levels of fish meal. In comparison to previously published feeding trials with fish oil, the dosage used was one-half the low levels reported by others. Future fish oil infusion studies should included graded levels of oil and fish meal for a comparison between the two lipid sources.

Comparison of fish meal with fish oil in the second production study indicated distinct differences in plasma fatty acid composition. Cows fed fish meal had increased plasma n-3 fatty acids compared to fish oil fed cows indicating potential differences in ruminal and post-ruminal digestion. Ruminal differences may include rates and extent of lipolysis and biohydrogenation of polyunsaturated fatty acids. Post-ruminal differences could be associated with different digestibilities of phospholipids versus neutral lipids.

Numerous factors described below could possibly interact with fish meal to result in decreased milk fat production. First, decreased ruminal acetate production concurrently with increased propionate due to release of residual lipid could contribute to decreased milk fat production. Similar ruminal VFA shifts in cows fed high grain diets have caused increased plasma insulin. Increased concentrations of plasma insulin may also decrease acetate availability by stimulating de novo lipogenesis in adipose tissue. Decreased lipolysis at adipose tissue associated with higher circulating insulin could also decrease release of long chain fatty acids from adipose tissue and their availability for mammary gland utilization. Therefore, a shift in ruminal fermentation due to fish meal lipid could cause a repartitioning of triglyceride precursors from mammary to adipose tissue.

In addition to ruminal effects, post-ruminal effects could also contribute to decreased milk fat synthesis. Intake of n-3 fatty acids eicosapentaenoic acid and docosahexanoic acid have been associated with decreased lipogenesis and plasma lipid content. Animals fed n-3 enriched diets had decreased hepatic de novo synthesis of fatty acids. Marine oils have also decreased lipoprotein lipase activity. Cows fed fish oil had decreased uptake of chylomicron triglycerides by the mammary gland. Hypolipidemia has also been caused by diets containing n-3 fatty acids and could further decrease pre-formed long chain fatty acid availability for mammary gland utilization for fat synthesis. Thus, shifts in post-ruminal lipid metabolism due to presence of n-3 fatty acids could affect milk fat production.

In conclusion, many dietary factors can combine to influence milk production and compostition. Fishmeal may cause small changes in a number of these factors at once that when measured individually are not dramatically different. However, the total additive affect could be decreased milk fat production. Future research needs to identify changes in several factors described above that can contribute further to the understanding of lipid metabolism and how it is affected by dietary ingredients.

The two page vita has been removed from the scanned document. Page 1 of 2

The two page vita has been removed from the scanned document. Page 2 of 2