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Extruded Blend of Soybean Meal and Sunflower Seeds for Dairy Cattle in Early Lactation

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EXTRUDED BLEND OF SOYBEAN MEAL AND SUNFLOWER SEEDS FOR DAIRY CATTLE IN EARLY LACTATION

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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DEDICATION

To Janelle, who has shared my dreams; and to the Drackley and Keltgen families, who have encouraged and supported those dreams.

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JKD

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ABSTRACT

An extruded blend of 44% crude protein soybean meal (50%), sunflower seeds (45%), and premix (5%) was evaluated as a protein and energy source for dairy cows in early lactation. Thirty Holstein cows (24 multiparous and 6 primiparous) were assigned to either a corn-oats-soybean meal concentrate (SBM) or a concentrate where soy-sunflower blend replaced all soybean meal and portions of corn and oats (SSF). Dry matter of total mixed diets was 36% corn silage, 21% alfalfa haylage, and 43% concentrate. Yields of milk (33.6 and 33.8 kg/day) and 4% fat-corrected milk (30.9 and 30.5 kg/day) were similar for cows fed SBM or SSF, while percentages of total solids (11.92 and 11.38), fat (3.55 and 3.30), protein (2.91 and 2.74), and solids-not-fat (8.38 and 8.09) were lower in milk from cows fed SSF. Milk from cows fed SSF contained fewer short and medium chain fatty acids, more 18-carbon fatty acids, and was more unsaturated than from cows fed SBM. Intakes of dry matter and changes in body weight were not different between diets. Ruminal fluid pH and molar ratio of acetate to propionate were higher in cows fed SSF, while concentrations of total volatile fatty acids and ammonia were lower in cows fed SSF. Concentrations of essential amino acids in arterial serum and calculated uptakes of amino acids by the mammary gland were similar between diets. Transfer efficiencies of phenylalaine, valine, isoleucine, leucine, and tyrosine were lower in cows fed SSF. In cows fed either diet, methionine appeared to be most limiting, while tryptophan and arginine were least limiting.

INTRODUCTION

High producing dairy cows in early lactation may be limited by nutrient intake (177). These cows rely extensively on mobilization of long-chain fatty acids from adipose tissue to furnish energy and preformed fatty acids for milk fat synthesis (19). Adding fat to diets for cows in early lactation may provide long-chain fatty acids for milk fat and improve the energy economy of the cow (85, 115, 162) while avoiding problems of excessive starch intake from high-grain diets (120). Protein requirements are often higher than can be supplied by rumen microbial synthesis (150). Some protein may be mobilized from body stores for milk protein synthesis; however, no specific storage polypeptide exists and even essential enzymes may be catabolized to support milk protein synthesis (19). Therefore, it would be prudent to minimize the amount of body protein used in early lactation by increasing daily intake of a high-quality rumen-undegradable protein source (30, 35, 150). Since added fat supplies no energy to rumen microbes, a low rumen-degradability protein source is beneficial (115).

Oilseeds are convenient sources of protein and fat (120). Heat-treating these seeds may reduce protein degradation in the rumen (131, 150, 168). Some studies have shown increased milk yields when feeding extruded (5, 104, 156, 160, 184) or roasted soybeans (129, 130, 136, 146). Data is limited on heat treatment of other oilseeds, although extruded sunflower seeds showed no benefits over rolled sunflower seeds in one study (99).

If heat-processed, a combination of soybean meal and whole sunflower seeds might be advantageous for dairy cattle in early lactation. Added fat from sunflower seeds, coupled with the higher fiber of the seed hulls, might enable increased energy intake and efficiency without reducing ration fiber content. Higher fiber might help counteract the milk fat depression often seen with unsaturated fats (33, 47, 120, 176). Heat treatment should increase dietary protein escaping degradation in the rumen (150). The combination of soybean and sunflower proteins should provide a more balanced amino acid mixture, since lysine is first limiting in sunflower meal while methionine is limiting in soybean meal (152).

Therefore, a trial was designed to determine the effects of an extruded blend of soybean meal and sunflower seeds on milk production and composition in dairy cattle during early lactation. The arterio-venous (A-V) difference technique was used to determine if amino acid supply to the mammary gland was altered by the supplement. This technique also allowed estimation of amino acids possibly limiting production, and supplied information concerning the role of amino acid supply in the milk protein depression caused by added dietary fat (120).

LITERATURE REVIEW

Increasing Postruminal Supply of Amino Acids

Ruminal synthesis of microbial protein is insufficient to meet the demands of high-producing dairy cows (147). Researchers have attempted to increase the quantity of dietary protein which escapes undegraded from the rumen. Schingoethe et al. (153) observed greater synthesis of β-casein by bovine mammary tissue in vitro when the quantity of essential amino acids (EAA) in the medium was increased. It was postulated that providing more amino acids to the small intestine would increase the supply of amino acids to the mammary gland and stimulate milk synthesis. The potential for such a response could reasonably be due to several factors (35). 1) An increase in the amino acids which are limiting production. 2) Providing more glucogenic amino acids, thus sparing glucose for lactose synthesis or to furnish energy to the mammary gland. 3) Alteration of hormone levels which control milk synthesis.

Clark (35) reviewed experiments where nutrients were infused postruminally to lactating cows. Postruminal infusion of casein increased milk yield by an average of 5 to 8% and milk protein yield by 10 to 15%. Infusions of various amino acid mixtures generally failed to increase milk yield but elicited small responses in milk protein yield and percentage. Glucose infusion also tended to increase milk and milk protein yields. More recent studies (39,

113) showed responses to casein but not glucose infusion. Rogers et al. (145) obtained increased yields of milk and milk protein from high-producing cows infused postruminally with casein, soybean meal, or cottonseed meal. Abomasal casein infusion stimulated growth hormone release, while abomasal infusion of glucose did not (19).

It is apparent from these studies that increased supply of amino acids postruminally may result in improvements in milk and milk protein yields. In order to design practical methods to exploit this and interpret results, an understanding of amino acid metabolism in the mammary gland is helpful. This topic has been reviewed recently (35, 38, 101).

Amino Acid Metabolism in the Mammary Gland

Free amino acids in blood are the major precursors in milk proteins synthesized within the mammary gland (92). Amino acid uptake is dependent upon three factors (101): 1) arterial concentrations of amino acids, 2) mammary blood flow (MBF), and 3) the extraction process by which carrier systems transfer blood amino acids across basal membranes of the secretory cells. Growth hormone may increase amino acid uptake by increasing MBF (101). Other hormones such as insulin (89) may play a role in regulating the extraction process, but arterial concentration of amino acids is probably the greatest factor in determining uptake (89, 92). According to Mepham (101), once inside the cell, amino acids may

1) undergo RNA-directed polymerization to form proteins, 2) be metabolized to carbon dioxide, urea, polyamines, and nonessential amino acids (NEAA), 3) be retained in the cells in the form of structural proteins or enzymes, or 4) pass unchanged into milk, blood, or lymph. For most amino acids the first two possibilities are quantitatively the most important.

To study uptake of amino acids by the mammary gland, the arterio-venous (A-V) difference technique is widely used. This technique uses concentrations of amino acids in arterial blood entering and venous blood leaving the mammary gland, along with a measurement or estimation of MBF. Calculations of amino acids taken up by the mammary gland can then be compared to output in milk proteins to estimate limiting amino acids. The accuracy of this technique depends on reliable MBF values.

Mammary blood flow has been measured by several different methods as discussed by Linzell (92). Kronfeld et al. (86), using the antipyrine injection method, derived the equation Y=1.0 + .42X, where Y=MBF in liters/min and X=milk yield in kg/day. This equation yields a blood flow to milk yield ratio of approximately 650:1. Reynolds et al. (137) compared the antipyrine method with the continuous thermodilution method and electromagnetic method in goats. Continuous thermodilution and electromagnetic methods gave results similar to each other and to a bleed-out method, while the antipyrine method overestimated MBF by about 30%. Bickerstaffe et al. (18) measured MBF in cows of 457 liters/liter milk produced, using

either continuous thermodilution or urea dilution techniques. Linzell (92) reviewed experimental results and concluded that a 500:1 ratio of blood to milk was a good estimate for cows. Peeters et al. (128) measured a mean value of 507 liters/liter milk using a modified electromagnetic technique in cows. Recently, Waghorn and Baldwin (183) developed a balance model for metabolites in milk synthesis. Using a blood:milk ratio of 500:1, amino acid uptake was underestimated by 75% and had to be adjusted to balance the model.

An important consideration is that blood concentrations must be adjusted for hematocrit or packed cell volume (92), which in cows is normally about 30% (127). Thus, if plasma amino acids are measured, MBF of 500:1 would be adjusted to 350 liters plasma/liter milk.

Amino acid analysis must also be accurate if the estimate of uptake is to be reliable. Tryptophan has not been analyzed in many trials. Analyses of cystine, the dicarboxylic amino acids, and the amide amino acids all may be inaccurate unless careful procedures are followed (133).

Schingoethe et al. (153) found that bovine mammary cells required methionine, lysine, threonine, phenylalanine, leucine, isoleucine, valine, histidine, tryptophan, arginine, and cystine for synthesis of β-casein and β-lactoglobulin. Jorgensen and Larson (81) found that bovine mammary cells could convert a significant amount of phenylalanine to tyrosine but mammary cells of the

rat could not. Verbeke et al. (181) found active phenylalanine hydroxylase activity in goat mammary glands when perfusate phenylalanine concentrations were four times greater than physiological content, but not when physiological phenylalanine concentrations were maintained. Black et al. (22) found that only 10 to 12% of tyrosine in casein was derived from phenylalanine. Thus, tyrosine may also be an EAA in intact cows.

While adequate amounts of carbon and nitrogen are taken up by the gland to account for synthesis of milk proteins, there is an imbalance between amounts of EAA and NEAA taken up (38). Uptake of EAA is generally in excess of milk protein needs, while NEAA are not taken up in sufficient quantities to account for their output in milk (38, 101). This suggests extensive conversion of EAA to NEAA in the mammary gland.

In experiments using A-V difference techniques, uptakes of methionine, phenylalanine, and tyrosine were generally very close to their output in milk protein (18, 36, 39, 49, 128, 163, 180). Uptakes of lysine, histidine, and threonine were only slightly in excess of outputs in milk. Although studies are limited, catabolism of these EAA is probably very low in the mammary gland. Threonine may be converted to glycine via threonine aldolase (181). Reports on metabolism of tryptophan in bovine mammary gland are lacking.

The branched chain amino acids (isoleucine, valine, and leucine) appear to undergo extensive catabolism in the gland.

Wohlt et al. (187), using bovine mammary slices, found substantial transfer of ^{14}C from valine, leucine, and isoleucine to β-hydroxyisobutyrate, isovalerate, and methylmalonate, respectively. These compounds are normal intermediates in catabolism of the branched-chain amino acids in other tissues, leading to citric acid cycle intermediates through acetyl-CoA and succinyl-CoA. Small amounts of label were also recovered (187) in glutamate and aspartate. Roets et al. (143) found that 30% of labelled valine was oxidized to CO_2 and that 10% was reversibly transaminated during one passage through the mammary gland. In another study (142), 24% of labelled leucine was oxidized to CO_2 with 4 to 8% participating in reversible transamination reactions. The branched-chain acids appear to be significant sources of carbon and nitrogen for NEAA synthesis.

Large excesses of arginine are taken up by the mammary gland, as well as substantial amounts of ornithine and citrulline (38, 101) which are not components of milk proteins. Labelled arginine was catabolized extensively by the mammary gland (37), with most of the recovered ^{14}C found in proline, ornithine, and glutamate (37). Ornithine may also donate its terminal amino group via transamination to produce several NEAA, especially glutamate, alanine, and serine (144). In turn, glutamate, alanine, and aspartate are important sources of other NEAA (22). Another important metabolic pathway of ornithine leads to production of the polyamines, spermine and spermidine (110), which may be important regulators of mammary protein synthesis (38). The contribution

of citrulline to synthesis of NEAA is probably low in bovine mammary tissue, since activities of the enzymes arginosuccinate synthetase and arginosuccinase are low (37).

Since methionine apparently cannot be converted to cystine in the bovine mammary gland (153), researchers have sought to explain the deficient uptake of free cystine by the gland, noted in several studies (38, 39, 49, 163). Some cystine may be lost in samples during storage (133). Recently Baumrucker et al. (15) measured a significant uptake of glutathione (a tripeptide of glutamate, cysteine, and glycine) from erythrocytes. Uptake and subsequent hydrolysis of glutathione produced sufficient cystine to account for output in milk proteins. Isotopic labelling studies confirmed that free amino acids from glutathione were indeed incorporated into milk proteins.

Amino Acids Limiting Synthesis of Milk Protein

Studies attempting to determine limiting amino acids in ruminants have been summarized by Clark (35) and Bergen (17). Techniques used include in vitro cell preparations, blood amino acid concentrations, A-V differences to compare uptake to output, and abomasal, intravenous, or intramammary infusions of amino acids. Limiting amino acids in ruminants have been more difficult to determine than in nonruminants, as dietary amino acids are extensively degraded and the profile of absorbed amino acids may be much different than that consumed (17).

Park et al. (125), using culture techniques, indicated that lysine was first-limiting and either methionine, valine, or arginine second-limiting for protein synthesis by rat mammary tissue. Clark et al. (40) found that cystine, threonine, and methionine were first, second, and third-limiting amino acids for synthesis of β-casein and β-lactoglobulin by bovine mammary cells in culture. A later study (41) showed methionine, tryptophan, and cystine to be limiting synthesis of β-casein and β-lactoglobulin by bovine mammary cells in vitro. In the same study (41), NEAA decreased with increased EAA in the medium, suggesting that NEAA might become limiting as EAA supply was increased.

Bergen (17) summarized factors which make blood amino acid profiles difficult to interpret in ruminants. Certain amino acids (e.g. threonine) may be preferentially catabolized in the liver, while the branched-chain amino acids may not be metabolized to any degree by the liver (35). The branched-chain amino acids have been utilized as indicators of increased protein absorption, although increased plasma concentrations may be more a factor of reduced degradation (17). Since amino acids and proteins contribute to the glucose needs of the high-producing cow (111), there may not be a direct relationship between composition of absorbed amino acids, plasma amino acids, and output of amino acids in milk (35). In addition, free amino acids in blood make up only a small portion of the body's total amino acids (17). Blood profiles cannot be used as independent indicators of nutritional status, since the

size of the free amino acid pool is much smaller than the fluxes of amino acids in and out of free tissue pools (17). Foldager et al. (57) found that plasma amino acids were affected by form and amount of dietary nitrogen, milk yield, stage of lactation, and time after feeding. With these qualifications, blood amino acid profiles still may provide insight into nutritional status if coupled with other measures (17).

Halfpenny et al. (67) measured increased NEAA and decreased EAA in plasma and increased milk protein yield in cows fed a high energy diet compared to a low energy diet. The authors concluded that NEAA, particularly glutamate and proline, may limit milk synthesis. However, Mepham and Linzell (102), could find no evidence to support this conclusion. Recent work (29) has shown that proline supplementation will spare arginine uptake and increase the efficiency of protein utilization.

Broderick et al. (25) infused 800 g/day casein into the abomasum and got an 11.6% increase in milk protein production, accompanied by decreased NEAA and increased EAA in plasma. Based on the assumption that a limiting amino acid will only accumulate in plasma after tissue demands are met, it was suggested (25) that increased plasma concentrations of leucine, isoleucine, valine, and phenylalanine might implicate these amino acids as limiting for milk protein synthesis. However, the authors also admitted that increased concentrations might simply reflect reduced degradation capacity. In another study, Broderick et al. (26) fed

formaldehyde-protected casein, and using the same assumptions (25) to evaluate limiting amino acids, proposed that methionine, valine, and lysine were limiting milk production.

In comparing relative abundance of plasma amino acids to their content in milk protein, Foldager et al. (57) estimated methionine, phenylalanine, threonine, and lysine to be the first four limiting amino acids. Using the same method, Rogers et al. (145) found that methionine, phenylalanine, lysine, threonine, and leucine were least abundant in plasma compared to milk protein for cows abomasally infused with casein. The same study showed that limiting amino acids for cows infused with soybean meal may have been methionine, phenylalanine, lysine, leucine, and isoleucine.

A study (36) using A-V differences in cows fed formaldehyde-treated soybean meal suggested that histidine, phenylalanine, methionine, lysine, and threonine were the five most limiting amino acids, based on the smallest uptake to output ratios for these amino acids. From the data of Bickerstaffe et al. (18), the same five EAA were utilized the most completely for milk synthesis. Studies where A-V differences were measured in cows abomasally infused with casein (39, 49, 163) demonstrated increased EAA in plasma and suggested methionine, lysine, phenylalanine, histidine, and threonine were the five most limiting amino acids.

Chandler and Polan (31) calculated transfer efficiencies (amino acid secreted in milk protein as a percentage of the amino acid in plasma entering the mammary gland) for cows at peak

lactation and found the five most limiting (highest transfer efficiencies) amino acids were methionine, lysine, phenylalanine, tyrosine, and threonine. Using the same method, Vik-Mo et al. (182) calculated phenylalanine and lysine to be limiting in cows abomasally infused with casein. Methionine, threonine, leucine, histidine, and tyrosine also were in rather short supply. Casein infusion consistently increased EAA and decreased NEAA in plasma.

Schwab et al. (157) infused different combinations of amino acids into the abomasum and concluded that methionine and lysine were first and second-limiting. Fisher (56) infused methionine, lysine, or histidine intravenously to cows fed a corn-corn silage-urea diet. Results were interpreted to mean methionine was first-limiting, with valine, leucine, isoleucine, or tyrosine possibly being second-limiting. Histidine infusion caused decreased dry matter (DM) intakes and milk protein yield, as did the highest amount of methionine. Very few experiments are similar to (56) in which possible effects of amino acid imbalances and interactions are examined, similar to work with nonruminants. Oldham (111) stated that work with nonruminants needs to be better applied to amino acid utilization in ruminants.

Heat Treatment of Proteins to Increase Ruminal Bypass

Recent reviews (30, 35, 93, 112, 114, 147, 150) have summarized research to find practical methods of reproducing the positive results of abomasal protein infusion. Formaldehyde treatment

(36, 134, 188) has received the most interest, but results have been variable. Sidhu and Ashes (159) demonstrated a nearly complete loss of tyrosine and reduced availability of other EAA in protein meals treated with 3% formaldehyde. Rae and Ingalls (134) found that tyrosine supplementation improved milk production in cows fed formaldehyde-treated canola meal. Over-protection of protein is a major problem with formaldehyde treatment (150).

Another promising method of increasing ruminal protein escape is heat treatment (147, 150). Heat is routinely applied in preparation of many protein sources (147), but additional heat processing may further improve utilization of the protein in ruminants.

Heating proteins causes formation of amide bonds between free amino groups (e.g. lysine) and carboxyl groups in proteins, similar to the Maillard reaction (20). More importantly, the free amino groups of lysine may form amide linkages with the amide groups of asparagine and glutamine, with loss of ammonia (21). These reactions make proteins less soluble in the rumen (24, 59, 66, 152, 168, 170) and may also protect the protein from microbial degradation in the rumen by blocking reactive sites of proteolytic enzymes (24). Many of these condensation reactions are reversible with acid hydrolysis (21), allowing digestion in the abomasum. Heating soybeans at 140^{o}C for 4 h reduced ruminal protein degradation by 84% but reduced digestibility only 5% (188). Schingoethe and Ahrar (152) found no decrease in biological value or growth

rates in mice fed heated sunflower or soybean meals compared to the unheated meals. However, heating may destroy cystine, and some linkages may not be acid-reversible (21). Abomasally digestible protein and indigestible heat-damaged protein can be estimated by the acid-pepsin insoluble nitrogen and acid-detergent insoluble nitrogen tests, respectively (60). Van Soest and Sniffen (178) stated that even heat-damaged protein may increase total protein supply to the animal by providing particles for microbial attachment, leading to increased microbial wash-out and so stimulating greater microbial growth.

Early studies with sheep (59, 170) showed reduced rumen ammonia concentrations and improved nitrogen retention and growth when fed heated soybean meals. Ahrar and Schingoethe (2) observed slightly greater milk production in the early part of a trial where extruded soybean meal replaced regular soybean meal in diets fed to cows past peak lactation. Soybean flakes subjected to additional heating during processing (109) supported increased milk yields compared to cows fed conventionally-processed soy flakes. Grummer and Clark (66) found no advantages for heated soybean flakes over regular soybean meal in a trial using a limited number of animals. Kung and Huber (87) and Sahlu et al. (148) reported significantly higher milk yields when high-producing cows in early lactation were fed heat-treated soybean meals than when fed regular soybean meals. In all these trials (2, 66, 87, 109, 148) there were no differences in milk composition due to heat

treatment.

Milk yields were higher in some trials (5, 129, 130, 136, 156, 160, 184) where extruded or heat-processed soybeans were fed, but not in other trials (23, 50, 82, 104, 168). Extruded whole cottonseed supported higher milk yields than raw cottonseed (72), but extruding sunflower seeds had no effect on milk yields (99).

McMeniman and Armstrong (100) measured no increase in amino acid nitrogen reaching the small intestine in cows fed heated field beans (Vicia fabia) compared to unheated beans. Kung et al. (88) observed increased non-ammonia nitrogen (NAN) reaching the duodenum in cows fed heated soybean meal. Heat-treating whole cottonseed (131) resulted in lower rumen ammonia concentrations, more NAN reaching the duodenum, increased efficiency of microbial protein synthesis, and increased absorption of EAA and NEAA than in raw cottonseed. Compared to raw soybeans, roasted soybeans led to lower rumen ammonia concentrations, less branched-chain volatile fatty acids (VFA) in rumen fluid, and more EAA in plasma (130). In another study (129), feeding heated soybean meal rather than regular soybean meal led to greater concentrations of EAA in plasma. Heated soybeans also increased plasma EAA over soybean meal (129), and feeding heated soybean meal or heated soybeans increased plasma concentrations of the branched-chain amino acids. Stern et al. (168) found that feeding diets containing extruded whole soybeans increased availability and tended to increase absorption of EAA in the small intestine compared with diets containing soybean meal or

raw whole soybeans. Results of these studies demonstrate that heat-treating proteins increases the quantity of absorbable EAA available to the animal, which may account for the increased milk production often seen with these diets.

When feeding low rumen-degradable protein sources, sufficient soluble nitrogen must be fed to provide optimum microbial growth (149) or milk production may not be improved. Quality of the bypassed protein is also important (111, 150), as increased quantities of a protein with poor amino acid balance will do little to increase milk production.

Sunflowers as a Source of Protein and Energy

Increasing quantities of oil-type sunflowers have been grown in the upper midwest states in recent years (98, 99, 135, 154). Sunflower meal, available as a by-product of seed-crushing operations, was shown to be an acceptable protein supplement for dairy cattle (151, 154), supporting milk yields equivalent to soybean meal (154). Nitrogen in sunflower meal is more soluble in buffer systems than soybean (151, 152) and cottonseed meals (151), but less soluble than nitrogen in linseed, peanut, or rapeseed meals (151). Lysine is the first-limiting amino acid in sunflower meal relative to milk protein, while methionine is first-limiting in soybean meal (152). Biological value of sunflower meal was similar to that of a casein reference diet (1). Whole sunflower seeds (5, 53, 55, 99, 135) and whole-plant sunflower silage (98,

172) have high fat contents and thus are good sources of both protein and energy.

Addition of Fats to Dairy Cattle Rations

Use of fats in rations for dairy cattle in early lactation has received much research interest in recent years. There are several possible advantages to addition of fat, summarized by Palmquist (115). First, fat may increase total energy intake and milk production in early lactation. Second, fat may replace portions of starchy ingredients in high-grain diets, allowing increased use of digestible fiber without reducing total energy intake. High-grain diets may lead to overfattening, decreased milk fat percent, and acidosis. Experimental proof of the advantages in this approach has been offered by Palmquist and Conrad (118, 119). Finally, fat may improve metabolic efficiency of lactation, as demonstrated by Kronfeld et al. (85). This increased efficiency is due to two factors: 1) Less energy is expended incorporating dietary fatty acids into milk fat than in de novo synthesis of fatty acids. 2) Oxidation of fatty acids is about 10% more efficient than oxidation of acetate from other sources. However, if excessive fat is fed, rumen fermentation may be adversely affected so that overall efficiency is not improved.

Many types of fat have been evaluated for use in ruminant diets, including tallow and other saturated greases, hydrolyzed fats, vegetable oils, and whole oilseeds. This discussion will be

concerned mainly with feeding oilseeds and their unsaturated oils to dairy cattle. Use of other fats in dairy rations was reviewed recently (120).

Effects of Unsaturated Fats on Rumen Function

Recent reviews have discussed lipid metabolism in the rumen (68, 120, 122). Early studies with unsaturated fats such as corn oil showed reduced digestibility of cellulose in sheep (27, 48, 64, 185, 186) and steers (185). Another study (140) showed no effect on fiber digestibility from feeding sunflower or rapeseed oils to steers. Galbraith et al. (58) and Henderson (71) demonstrated that unsaturated fatty acids were inhibitory to various cellulolytic and methanogenic bacteria of the rumen. Linseed oil or its unsaturated fatty acids reduced methane production in sheep (44). Fatty acids may also coat feed particles and prevent or reduce microbial attack (120). Addition of calcium has effectively reversed depressions in fiber digestibility when feeding unsaturated fats to growing ruminants (27, 48, 53, 64, 121, 185, 186) which may be due to formation of insoluble fatty acid salts or soaps (120). Depressions in fiber digestibility with added dietary fat have not usually been noted in lactating cows (5, 34, 45, 55, 74, 96, 118, 119, 161, 164, 171), probably because of high intakes, which reduce digestibility (177). Palmquist (117) observed that any reduction in fiber digestion from added fats was a reduction in rate and not extent of fiber digestion.

Reports of the effects of unsaturated fats on ruminal VFA concentrations have been variable. Some workers have noted increases in the relative abundance of propionate (23, 53, 75, 76, 84, 96, 118, 158, 166, 179) while others noted no change (3, 8, 28, 55, 75, 90, 94, 99, 104, 132, 138, 155). Increased propionate production could be expected if methanogenesis were inhibited. Van Maanen et al. (175) used isotope dilution to measure propionate production in steers fed monesin, and found that slight increases in rumen propionate concentrations greatly underestimated the true increase in propionate production. This suggests a similar problem might exist with using VFA concentrations as indicators of production rates in animals fed unsaturated fats.

Addition of fat decreased ruminal ammonia concentrations in some studies (76, 84, 171), which was attributed to reduction of protozoal numbers. However, Macleod et al. (95) observed no reduction in protozoa with added fats. Reduction in protozoal populations resulted (76, 171) in increased microbial protein reaching the small intestine, due to less recycling of bacteria through protozoa. Efficiency of bacterial protein synthesis was decreased slightly in (76). Thus, net effects of unsaturated fats on microbial protein supply may be difficult to predict.

Effects of Unsaturated Fats on Feed Intake and Milk Production

Consumption of DM was not different from control rations when cows were fed rations containing whole cottonseed (3, 4, 45,

161, 173), cottonseed oil (61, 165), sunflower seeds (34, 99, 135), sunflower oil (34), sunflower silage (172), whole or ground soybeans (70, 75, 104, 132, 166), extruded or heated soybeans (23, 50, 82, 104, 146), soybean oil (61, 94, 116, 132, 155, 166), or safflower oil (179). However, in other cases feed intake was reduced when feeding sunflower seeds (5, 55), sunflower silage (98), soybeans (118, 184), soybean oil (65), canola seed (83), or groundnut (peanut) oil (164). No consistent pattern with ration or fat type is apparent. As stated by Smith et al. (162), DM intake may decline when feeding added fat so that intake of energy remains unchanged.

Responses in milk yield to addition of unsaturated fats were observed in some studies (3, 10, 52, 65, 70, 73, 74, 96, 99, 135, 146, 156, 160, 166). In general, responses were greatest in cows in early lactation, with fats added at less than 6% of total ration DM. Source or form of fat did not consistently affect milk yield. Other studies showed no change (4, 45, 55, 61, 75, 82, 90, 94, 104, 106, 118, 132, 138, 155, 160, 161, 164, 165, 172, 173, 179) or decreased milk yields (5, 8, 28, 75, 83, 92, 126, 184) when various unsaturated fats were fed.

Effect of Unsaturated Fats on Milk Composition

Fat. A major problem with feeding unsaturated fats to lactating cows is the reduction of milk fat percentage (5, 8, 10, 23, 28, 34, 50, 55, 65, 69, 70, 72, 73, 74, 75, 82, 83, 90, 94, 106,

116, 118, 124, 126, 138, 155, 156, 158, 164, 165, 166, 172, 179) unless the fats are protected from ruminal hydrogenation (8, 34, 61, 83, 96, 123, 124, 134). The problem has been discussed in review articles (16, 33, 47, 107, 120, 176). The occurrence and extent of the fat depression seem to be related to the amount and form of the oil, type of ration fed, and length of feeding period.

Feeding free cottonseed oil (28, 69, 106, 165), safflower oil (126, 138, 179) or sunflower oil (34) depressed milk fat percent. However, feeding whole cottonseed had no effect on fat percent (3, 4, 5, 45, 106) or even increased milk fat percent if fed in high-grain rations which normally depress fat percent (52, 160, 161, 173). Increased fat percent with these high-grain diets may have been a factor of slower release of fat into the rumen from the whole seed and the increased amount of highly digestible fiber of the whole seed. Feeding smaller amounts of free cottonseed oil (165) or the same amount but in a high roughage diet (28, 106) did not depress fat percent. On the other hand, Steele (164) fed only ryegrass silage supplemented with groundnut oil (5.7% of DM) and observed depressed fat percent.

Free soybean oil fed at 2% of ration DM did not affect milk fat percent, but reduced fat percent when fed at 4 or 6% of DM (65). Similarly, free soy oil depressed fat percent (69, 73, 74, 90, 94, 132, 166) while the same amounts of oil fed as whole or ground soybeans had no effect (75, 90, 96, 118, 132, 166, 184). Steele (164) found milk fat to be reduced from cows fed free

groundnut oil but not those fed crushed groundnut seeds. Extruded whole soybeans fed at 1.5 kg (160) or 2.0 kg (104) per day did not depress fat percent, but did when fed at 2.3 kg per day or more (23, 50, 82, 156). Soybeans heat-treated by other methods (146, 184) did not depress milk fat content even when fed in excess of 2.5 kg per day. It is apparent that many complex and as yet not understood factors interact to determine occurrence and severity of milk fat depression when feeding unsaturated fats.

Increasing the amount of calcium in the diet may help prevent negative effects of added fats (119), possible by increasing formation of insoluble soaps in the rumen (120). Finn et al. (55) observed no fat depression in cows fed rolled whole sunflower seeds in a diet containing 1% calcium, but fat was depressed where calcium level was .5%. Rafalowski and Park (135) fed the same amount of whole sunflower seed with .96% calcium and did not observe reduction of milk fat percent, although slower release of fat from the whole seed may also have been a factor.

The mechanism by which unsaturated fats depress milk fat secretion is not understood. The syndrome is in many ways similar to that observed with high-grain, reduced-roughage diets (47). Molar percents of ruminal acetate may be reduced due to increased proportions of propionate (see above), but Davis (46) found no reduction in acetate production in rumens of cows fed fat-depressing diets. If ruminal propionate production is increased as discussed earlier, it could trigger the fat depression syndrome

similar to high-grain diets. Insulin release from increased propionate may suppress release of fatty acids from adipose tissue and increase uptake of acetate by adipose tissue (16), creating a deficiency of milk fat precursors at the mammary gland. Uptake of triglycerides and free fatty acids was reduced in some studies with unsaturated fats (90, 138, 179) where milk fat was depressed.

The fat-depressing effects of unsaturated oils are mediated through the rumen. When oils which depress milk fat when fed are instead infused intravenously (169) or postruminally (13) or are fed in a form protected from ruminal action (8, 61, 96, 124), milk fat percentages are unchanged or increased. The trans-isomers of fatty acids formed in the rumen as normal intermediates of biohydrogenation (68) have been implicated as causative factors in milk fat depression by some workers (8, 158). Protected soybean oil increased milk fat percent, while protected hydrogenated soybean oil depressed fat percent (8). Selner and Schultz (158) fed trans-fatty acids and observed milk fat depression. These trans-isomers may not be as suitable for esterification as cis-substrates (6), and a non-ideal array of fatty acids at the mammary gland may inhibit triglyceride formation (97).

Addition of fats to diets results in milk fat that contains fewer short and medium chain fatty acids (those synthesized within the mammary gland) and more 18-carbon acids (transferred from diet) (11, 28, 33, 34, 42, 54, 55, 61, 75, 83, 95, 96, 98, 99, 104, 107, 126, 132, 155, 158, 160, 161, 162, 164, 165, 167, 169). De novo

synthesis is apparently inhibited by the increased supply of preformed fatty acids, probably at the point of acetyl-CoA carboxylase (120). Dimemma and Emery (51) noted that the supply of medium chain fatty acids may limit the final step of triglyceride formation, which may indicate a problem if synthesis of the medium chain acids is depressed too greatly by unsaturated fats.

Feeding unprotected unsaturated fats increases 18:0 and 18:1 fatty acids in milk fat, with little increase in 18:2 or 18:3 acids, while protected unsaturated fats result in large increases in the polyunsaturated acids of milk fat (33). When feeding unprotected fats, polyunsaturated fatty acids are mostly hydrogenated to 18:1 and 18:0 acids, but those which escape hydrogenation and are absorbed are preferentially esterified to phospholipids and cholesterol esters. These lipids are not sources of fatty acids for milk fat synthesis, but conserve polyunsaturated fatty acids for incorporation into membranes and other essential functions (33). When protected fats are fed, large quantities of polyunsaturates are absorbed and the normal system becomes "swamped", so that some polyunsaturated acids are incorporated into the triglycerides which serve as milk fat precursors (33). It is not clear from the literature why the large quantities of 18:0 and 18:1 fatty acids resulting from hydrogenation of unprotected fats cannot compensate for the reduction in de novo synthesis. These acids would be incorporated into lipid fractions rich in triglycerides which can donate fatty acids for milk fat synthesis (33). Saturated fats do not

cause milk fat depression, but reduce de novo synthesis and may interfere with ruminal digestion (120). Thus, it appears that products of hydrogenation in the rumen may play a post-absorbative role in the fat depression caused by unsaturated fats.

Protein. Some studies with added unsaturated fats have noted decreased milk protein percentage (3, 5, 10, 23, 45, 52, 55, 74, 94, 96, 98, 99, 104, 123, 124, 138, 160, 161, 165, 166, 184). Others have noted no change (4, 50, 52, 61, 65, 70, 73, 75, 83, 90, 106, 118, 119, 132, 135, 146, 155, 164, 170, 179) or an increase in milk protein (8, 34, 75, 116) with added unsaturated fats. Block et al. (23) noted decreased milk protein from cows fed extruded soybeans compared to raw soybeans. Saturated fats and protected unsaturated fats may also depress milk protein percent (120).

What causes the reduction in milk protein to occur in some cases and not in others and the mechanism by which it occurs are unknown. Dunkley et al. (54) noted that the reduction was specifically in the casein fraction of milk protein. Some have suggested (162) that replacement of carbohydrates with fats may result in a shortage of glucose precursors or reduce microbial protein production. However, as noted earlier, added fats may increase microbial protein supply to the small intestine (76, 171). Palmquist and Moser (123) measured decreased glucose clearance rates in cows fed added fat, and milk protein percent was reduced. In that experiment (123) dietary protein was in excess of requirements, which

discredits an absolute protein shortage as the cause of reduced milk protein.

Insulin may be essential for mammary protein synthesis (123). Some workers (62) observed increased milk protein synthesis in lactating goats intravenously infused with insulin. Others (89) found that extraction of leucine, isoleucine, valine, tyrosine, and aspartic acid from plasma was increased when circulating insulin was increased in lactating cows. Palmquist and Moser (123) observed decreased plasma insulin and increased tissue insulin resistance in trials where protected fats caused reduced milk protein. It is conceivable that reduced insulin action might limit uptake of amino acids or synthesis of proteins in the mammary gland. However, this theory is inconsistent with possible stimulation of insulin secretion if unsaturated fats increase ruminal propionate production, which may be a factor in the milk fat depression observed with these fat sources (see above). More research is needed to determine relationships between unsaturated fats and their effects on ruminal VFA production, hormone release, and milk fat and protein synthesis.

MATERIALS AND METHODS

An extruded blend of 44% crude protein-soybean meal (50%), whole, oil-type sunflower seed (45%), and premix (5%) was evaluated as a protein and energy supplement for dairy cows in early lactation. The soy-sunflower blend[1] was prepared by processing soybean meal, sunflower seeds, and premix (sodium bentonite, lignin sulfonate, and ammoniated lignin sulfonate) through an extruder at 140°C.

Thirty Holstein cows (24 multiparous and 6 primiparous) were paired based on lactation number and previous production. Multiparous cows were yielding at least 27 kg and primiparous cows at least 23 kg daily by the end of the 3rd wk postpartum. One cow from each pair was assigned randomly to a diet with either a control concentrate mix (SBM) containing corn, oats, and soybean meal or a concentrate mix where the soy-sunflower blend replaced all of the soybean meal and a portion of the corn and oats (SSF) (Table 1).

Total mixed diets consisted of 36% dry matter (DM) as corn silage, 21% alfalfa haylage, and 43% of the respective concentrate mix (Table 1). Diets were formulated to be isonitrogenous at 100% of protein requirements of 600 kg cows producing 35 kg milk daily (108), with diet SSF hypercaloric to increase energy intake (108) from added fat. Cows were individually fed once daily for ad libitum intake the respective experimental diets from wk 4 through

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[1]Triple "F" Feeds, Des Moines, IA.

TABLE 1. Ingredient content of concentrate mixes containing soybean meal (SBM) or soy-sunflower blend (SSF).[1]

Ingredient	Diet	
	SBM	SSF
	(% of DM)	
Ground corn	36.5	24.8
Ground oats	36.5	24.8
Soybean meal, 44% CP	24.3	...
Soy-sunflower blend	...	47.7
Dicalcium phosphate	1.6	1.6
Trace mineral salt	1.1	1.1

[1]Mixes contained 8,800 IU added vitamin A and 2,200 IU added vitamin D/kg.

wk 15 postpartum, with amounts fed and refusals recorded daily. Body weights were recorded 3 consecutive days at beginning and end of the trial and once during the 4th and 8th wk of trial.

Cows were milked twice daily with milk weights recorded at each milking. Two 24 h (p.m. plus a.m.) milk samples were collected from each cow during wk 3 postpartum (pretrial) and one 24 h sample was taken every 2 wk throughout the trial. Samples were analyzed for total solids by the Mojonnier method (9), fat by Milk-O-Tester MK-II[2], and protein by Kjeldahl (7). Remaining amounts of milk samples during wk 4 and 12 of the trial were lyophilized and the fat extracted by the Roese-Gottleib procedure (9). Butyl esters of fatty acids from the ten highest producing cows on each treatment were prepared by methods of Jones and Davidson (79) and separated by gas-liquid chromatography using 10% EGSS-X on 100/120 Gas Chrom P[3] in a .32 x 305 cm stainless steel column. Column temperature was programmed at 6°C/min from 75 to 200°C. Six times during the trial, additional p.m.-a.m. samples were taken from two cows chosen at random from each treatment. Raw samples were composited by treatment, and duplicate samples (identified only by number) were evaluated for flavor by a trained taste panel using the official American Dairy Science Association-Dairy and Food Industry Supply Association scorecard approximately 8 h after a.m.

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[2]N. Foss Electric, Hillerod, Denmark.

[3]Applied Science Laboratories, State College, PA.

milking.

Feeds were sampled weekly and composited monthly for analyses. Samples were dried at 57°C for 48 h in a forced-air oven for determination of DM. Dried samples were ground and analyzed for crude protein, ether extract, and ash (7), acid detergent fiber (ADF) (60), and neutral detergent fiber (NDF) (141). Samples of soybean meal, soy-sunflower blend, and composites of all monthly samples of feeds were analyzed for calcium by atomic absorption spectrophotometry (174). Samples of mixed diets were prepared from monthly feed composites. Samples of the mixed diets, soybean meal and soy-sunflower blend were analyzed for soluble nitrogen (N) in 10% Burroughs' mineral buffer (43), soluble and degradable N by the ficin protease procedure described by Sahlu et al. (148), acid detergent insoluble N (ADIN) (60), and acid pepsin insoluble N (APIN) (60).

To determine amino acid content of feeds, .2 to .5 g of soybean meal, soy-sunflower blend, and composites of monthly feed samples were hydrolyzed in 6 N HCl in sealed tubes containing N_2 gas for 4 h at 145°C. Hydrolysates were evaporated to dryness, diluted with sodium citrate buffer (pH 2.2), filtered, and analyzed on an amino acid analyzer[4]. Separation of amino acids was on ion-exchange columns with sodium citrate buffers ranging in pH from 3.49 to 6.40. Tryptophan was measured in separate .2 to .3 g

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[4]Spinco 120 Automated Amino Acid Analyzer, Beckman Instruments, Inc., Palo Alto, CA.

samples by a modification of the methods of Jones et al. (80). Samples were mixed with 50 mg maltodextrin and 5 ml of 6 N NaOH. Tubes were vortexed, evacuated, filled with N_2 gas, and autoclaved for 18 h at 120°C and 1.05 kg/cm^2. The hydrolysate was transferred to a 50 ml volumetric flask, 5 ml of 6 N HCl was added while shaking the flask, and the mixture brought to volume with pH 6.4 sodium citrate buffer. After centrifuging for 30 min at 30,000 x g, 250 µl was applied to an amino acid analyzer[5]. Tryptophan was eluted at 72°C with pH 6.4 sodium citrate buffer.

Once monthly samples of ruminal contents were obtained 2 to 3 h after feeding by applying vacuum to an esophageal tube fitted with suction strainer into 300 ml bottles containing .5 ml saturated mercuric chloride. Samples were analyzed for pH and then filtered through four layers of cheese cloth. A 10-ml sample of ruminal fluid was centrifuged, the supernatant acidified with .5 ml of .1 N HCl, and frozen until analyzed for ammonia-N (32). Another 10-ml sample was acidified with 2 ml 25% metaphosphoric acid and centrifuged. The supernatant was frozen until analyzed for volatile fatty acids (VFA) on a neopentylglycol succinate column in a gas chromatograph (14). Blood was drawn from the jugular vein at time of rumen sampling and serum analyzed for urea (32). At the first rumen sampling (5 to 7 wk postpartum) blood samples were obtained from the coccygeal artery or vein and the subcutaneous abdominal

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[5]Beckman 118 BL Automated Amino Acid Analyzer, Beckman Instruments, Inc., Palo Alto, CA.

vein. Serum from the ten highest-producing multiparous cows on each treatment was deproteinized by the method of Mondino et al. (105). Amino acids were eluted on a single-column amino acid analyzer[6] by ion exchange with lithium citrate buffers of pH 2.83, 3.70, and 3.75 with 6% 2-propanol added (91). Uptake of serum amino acids by the mammary gland was calculated as in (39, 49), using an estimate of blood flow derived from several sources (18, 86, 92, 128, 183) as 575 liters/kg milk produced, corrected for an average packed cell volume of 30.5% (127). This yielded a serum flow to milk yield ratio of 400:1. Comparisons of uptake and arterial serum concentrations of amino acids to amino acids in milk protein (39, 49) used the amino acid composition of milk protein given by Jacobson et al. (77). Transfer efficiencies were calculated as in (182).

Variance of data was analyzed using the General Linear Models procedure (63). Milk yield and composition data were adjusted by analysis of covariance using yield and composition during wk 3 postpartum as covariates. Differences due to treatment, time, and treatment by time interaction were considered. Amino acid data only was analyzed by the ANOVA procedure (63) for analysis of variance with differences due to treatment being considered.

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[6]Beckman 118 BL Automated Amino Acid Analyzer, Beckman Instruments, Inc., Palo Alto, CA.

RESULTS AND DISCUSSION

Chemical composition of feeds and total diets is in Table 2. Total diets contained similar dry matter (DM) and crude protein, with diet soy-sunflower blend (SSF) higher in ether extract, neutral detergent fiber (NDF) and acid detergent fiber (ADF) because of addition of the whole sunflower seed in the soy-sunflower blend. Diets were formulated to contain .65% calcium, slightly higher than National Research Council (NRC) recommendations (.60%) since research showed that additional calcium should be added when feeding added fat (119). The soy-sunflower blend that was fed contained more calcium than original samples, resulting in slightly higher calcium in diet SSF.

The extruded soy-sunflower blend contained less nitrogen (N) soluble in either 10% Burroughs' solution or in ficin protease buffer (Table 3) than soybean meal. This resulted in less soluble N in diet SSF than SBM. Sunflower protein was more soluble than soybean protein (151, 152), but heat treatment reduced solubility of dietary proteins (1, 23, 24, 59, 66, 87, 104, 109, 148, 152). Rumen degradability of N in SSF was higher than SBM (Table 3) as estimated by the ficin protease assay. Undegraded N was higher for soy-sunflower blend than soybean meal (25.6 vs 13.7%), but diets SBM and SSF did not differ in undegraded N. Sahlu et al. (148) stated that samples of mixed diets may give better indications of N solubility and degradability than individual feeds. Heated soybean meals contained less ficin-degradable N than regular soybean

TABLE 2. Chemical composition of supplements, concentrate mixes, forages, and total diets containing soybean meal (SBM) or soy-sunflower blend (SSF).

Component	Supplements only		Concentrate mixes		Alfalfa haylage	Corn silage	Total diets[1]	
	SBM	SSF	SBM	SSF			SBM	SSF
Dry matter (DM), %	93.3	95.1	90.1	93.0	56.9	43.8	58.2	58.4
	(% of DM)							
Crude protein	48.8	29.5	19.7	19.4	20.2	8.0	15.4	15.4
Ether extract	1.7	18.3	2.9	10.4	1.9	2.5	2.6	6.0
Neutral detergent fiber	19.1	42.0	25.0	29.6	47.0	47.2	37.3	39.4
Acid detergent fiber	12.2	19.4	10.4	13.6	38.4	26.0	21.9	23.1
Ash	6.2	9.1	5.7	8.3	9.2	5.0	6.3	7.3
Calcium	.28	.36	.51	.70	1.52	.26	.66	.71

[1]Computed.

TABLE 3. Solubility and degradability estimates for nitrogen (N) in soybean meal (SBM), soy-sunflower blend (SSF), and respective total diet samples.[1]

	Supplements		Total diets	
	SBM	SSF	SBM	SSF
	(% of N)			
Solubility in 10% Burroughs' solution	18.0	10.5	40.3	33.6
Ficin protease assay				
Solubility	42.2	13.6	45.1	37.1
Degradability	44.1	60.8	32.1	40.8
Undegraded	13.7	25.6	22.8	22.1
Acid detergent insoluble-N (ADIN)	3.6	5.7	5.4	5.9
Acid pepsin insoluble-N (APIN)	6.7	13.7	15.0	17.0

[1]36:21:43 (corn silage:alfalfa haylage:concentrate, dry matter basis).

meal (148), and in vivo studies (88, 168) showed that less N was degraded in rumens of cows fed heated soybean meal or heated soybeans. Differences in degradable N in this study may be due to different proteins and degree of heat treatment.

The amount of heat-damaged protein, as estimated by acid detergent insoluble-N (ADIN), was increased slightly in the soy-sunflower blend (Table 3). The amount of unavailable protein estimated by acid pepsin insoluble-N (APIN) was more than doubled in the soyflower blend and was higher in diet SSF. The extrusion process may have over-protected the protein in the soy-sunflower blend. Extruded soybean meal was only slightly higher in ADIN and APIN than regular soybean meal (148), but heating sunflower meal at 138°C or 149°C for 4 h resulted in decreased digestibilities of DM and crude protein, reduced N retention, and lower weight gains in rats (1).

Milk yield (Table 4) was similar from cows fed SBM or SSF. As shown in Figure 1, cows fed SBM reached a higher peak yield (35.6 vs 34.6 kg/day) and peaked earlier in lactation (6 vs 10 wk postpartum) than cows fed SSF. However, cows fed SSF tended to be more persistent, yielding more milk from week 10 through week 15 postpartum. This resulted in the similar overall means for milk yield. Other trials in which sunflower seeds were fed in amounts similar to this study showed milk yields were unchanged (55, 99, 135).

Yield of 4% fat-corrected milk was similar for both diets,

TABLE 4. Milk yield, composition, and flavor score from cows fed diets containing soybean meal (SBM) or soy-sunflower blend (SSF).

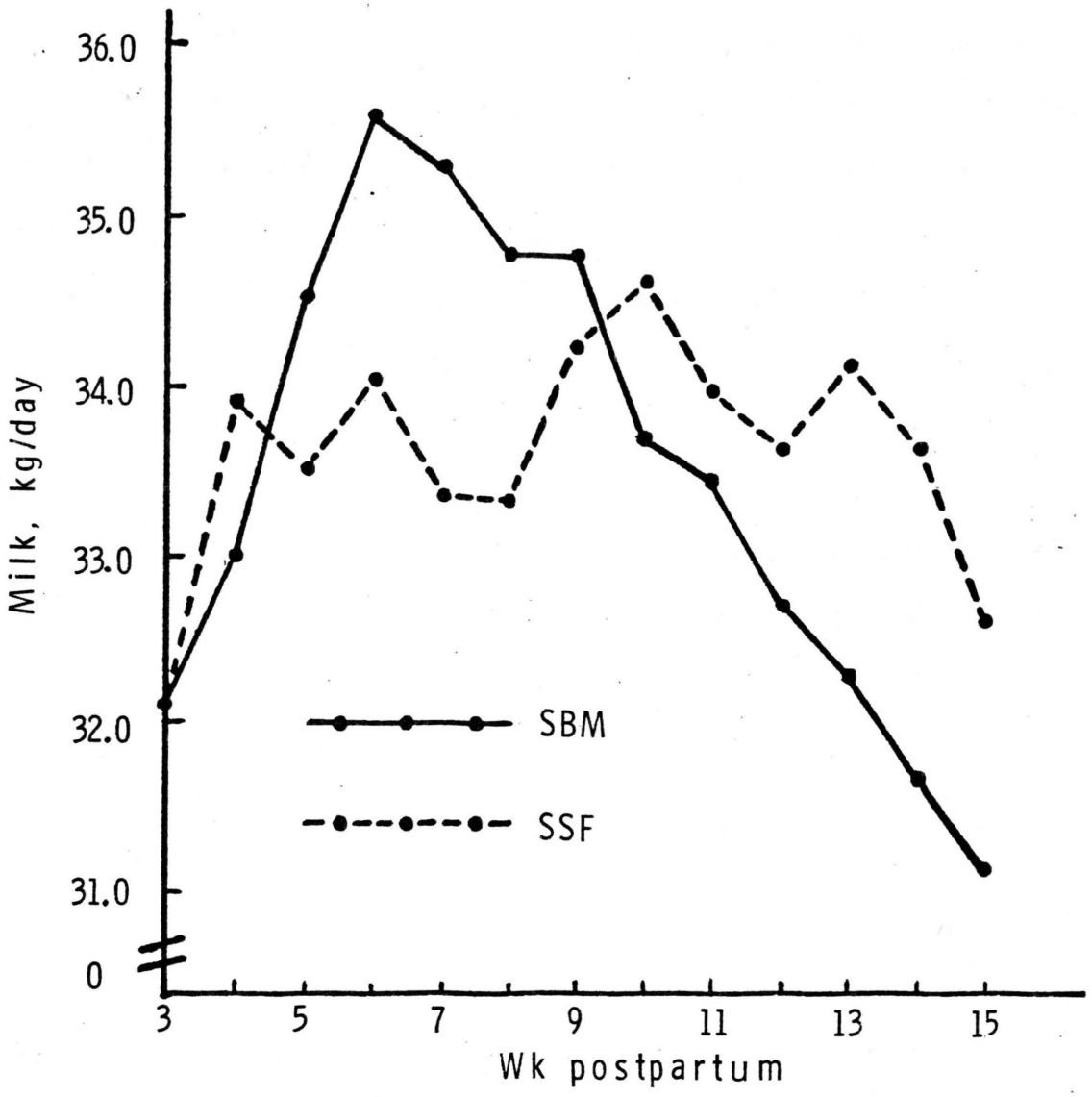
Measurement	Diet		SE
	SBM	SSF	
Milk, kg/day	33.6	33.8	.29
4% Fat-corrected milk, kg/day	30.9	30.5	.31
Solids-corrected milk, kg/day	30.2	29.4*	.28
Fat, %	3.55	3.30**	.06
Fat, kg/day	1.17	1.13	.02
Solids-not-fat, %	8.38	8.09**	.04
Solids-not-fat, kg/day	2.78	2.76	.03
Protein, %	2.91	2.74**	.02
Protein, kg/day	.96	.94	.01
Total solids, %	11.92	11.38**	.07
Total solids, kg/day	3.95	3.87	.03
Milk flavor score[1]	8.4	8.7*	.08

*P<.05.

**P<.01.

[1]Scale of 1 to 10 with 10 best.

Figure 1. Milk yield of cows fed diets containing soybean meal (SBM) or soybean meal-sunflower seed blend (SSF).


36.0
35.0
34.0
33.0
32.0
31.0
0
Milk, kg/day
SBM
SSF
3
5
7
9
11
13
15
Wk postpartum

while yield of solids-corrected milk was lower for cows fed SSF due to depressed total solids content (Table 4). Percentages of fat, solids-not-fat, and protein were lower in milk from cows fed SSF. Depressions in fat percent (5, 8, 10, 23, 28, 34, 55, 65, 69, 70, 72, 73, 74, 75, 82, 90, 94, 106, 116, 118, 124, 126, 138, 155, 156, 158, 164, 165, 166, 172, 179) and protein percent (3, 5, 10, 23, 45, 52, 55, 74, 94, 96, 98, 99, 104, 123, 124, 138, 160, 161, 165, 166, 184) are common when feeding unsaturated fats. Figure 2 shows that the milk fat depression did not become apparent until week 6 of the trial (9 wk postpartum), similar to results of Finn et al. (55). Selner and Schultz (158) found that fat depression took 2 to 3 wk to develop in cows fed hydrogenated vegetable oils, while the fat depression reported by Block et al. (23) was present within 1 wk after cows were fed heated soybeans.

Protein percent (Figure 3) was depressed by about 3 wk of experiment (6 wk postpartum). Percent of lactose-plus-ash (solids-not-fat minus protein) also was lower (5.47 vs 5.35%) in cows fed SSF, and was consistently lower from week 2 of experiment (5 wk postpartum) through the end of the trial (data not shown). This may indicate reduced lactose content of milk. Others (124, 135, 162) reported reduced lactose-plus-ash content in milk from cows fed added fat. Since lactose is the major osmotic constituent determining fluid volume secreted (13), it is unclear how milk yield could remain unchanged even though content of lactose, protein, and fat was decreased. Dunkley et al. (54) noted that the

Figure 2. Milk fat percent of cows fed diets containing soybean meal (SBM) or soybean meal-sunflower seed blend (SSF).

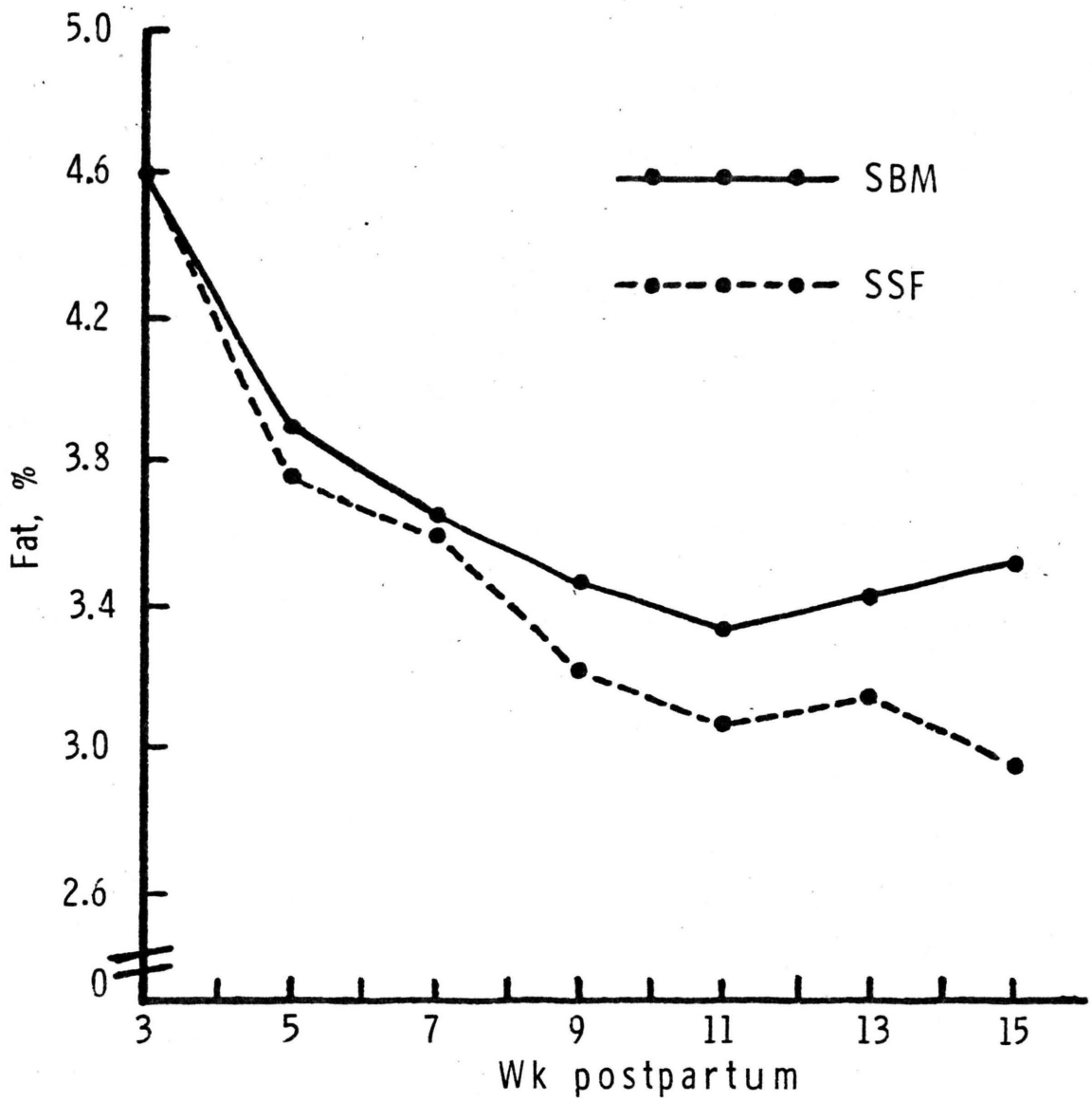
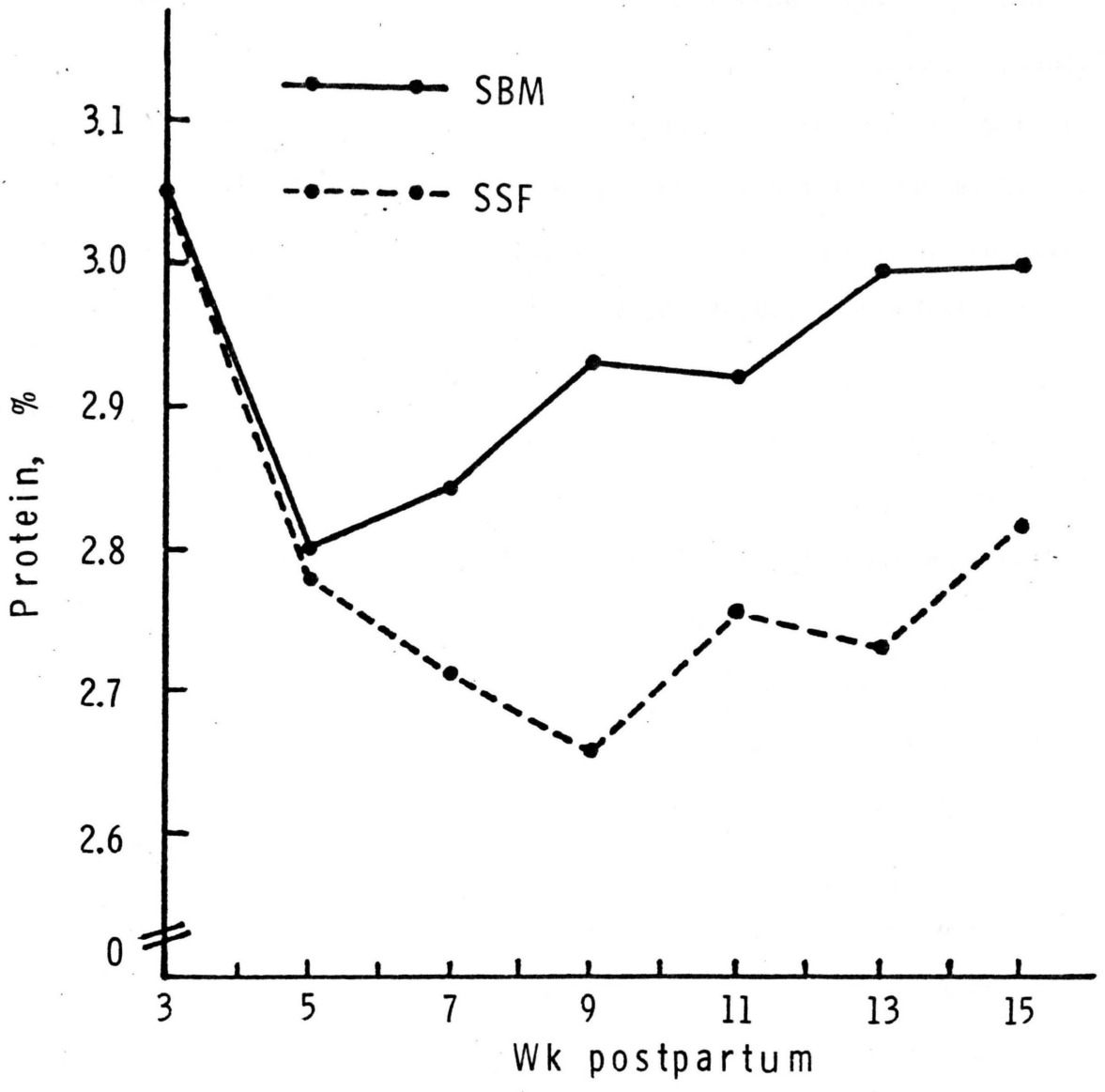

5.0
4.6
4.2
3.8
3.4
3.0
2.6
0
Fat, %
SBM
SSF
3
5
7
9
11
13
15
Wk postpartum

Figure 3. Milk protein percent of cows fed diets containing soybean meal (SBM) or soybean meal-sunflower seed blend (SSF).


SBM
SSF
3.1
3.0
2.9
2.8
2.7
2.6
0
Protein, %
3
5
7
9
11
13
15
Wk postpartum

reduced milk protein from added fat was specifically due to reduced casein, and that reduced microbial synthesis from added fat could decrease the supply of amino acids and glucose to the mammary gland. Smith et al. (162) speculated that decreases in protein and lactose content of milk were due to altered glucose metabolism caused by addition of fat to diets. As a result of these reduced percentages (Table 4), yields of fat ($P<.08$), protein ($P<.07$), and total solids ($P<.07$) tended to be reduced in cows fed SSF.

Milk flavor score (Table 4) was slightly higher from cows fed SSF. Flavor defects were similar between diets. No oxidized flavor was detected, which may occur if milk fat is made more unsaturated. However, milk was tasted within 24 h and was not "challenged" with oxidizing agents such as light or metals.

Milk fat from cows fed SSF contained more unsaturated fatty acids (24.1 vs 41.1 g/100 g total fatty acids) than those fed SBM (Table 5), indicating that some of the unsaturated acids of sunflower oil escaped total hydrogenation in the rumen. Since there were no significant diet by time interactions ($P>.10$), data from samples taken before and after fat depression occurred with SSF were pooled within diet to give means shown in Table 5. Synthesis of fatty acids from 6:0 to 16:0 was decreased, while transfer of 18-carbon fatty acids from the blood into milk fat was increased in agreement with (11, 28, 33, 34, 42, 54, 55, 61, 75, 83, 95, 96, 98, 99, 104, 107, 126, 132, 155, 158, 160, 161, 162, 164, 165, 167, 169). De novo synthesis is apparently inhibited by reduced activity

TABLE 5. Fatty acid composition of milk fat from cows fed diets containing soybean meal (SBM) or soy-sunflower blend (SSF).[1]

Fatty acid[2]	Diet		SE
	SBM	SSF	
	— (g/100 g) —		
4:0	4.0	4.2	.17
6:0	2.9	1.8**	.10
8:0	1.7	.8**	.07
10:0	3.5	1.5**	.07
12:0	3.8	1.9**	.12
14:0	12.1	6.9**	.27
16:0	29.0	18.9**	.70
16:1	2.6	2.8	.10
18:0	10.8	16.1**	.41
18:1	19.4	33.5**	.53
18:2	1.6	4.0**	.13
18:3	.5	.8**	.06
Other	8.1	6.8	.51

**P<.01.

[1]Means of samples taken week 7 and week 15 postpartum from the ten highest-producing cows on each treatment.

[2]Expressed as number of carbons:number of double bonds.

of acetyl-CoA carboxylase resulting from increased supply of preformed fatty acids (120). While positional isomers were not determined in the present study, increased 18:1 in milk fat from cows fed SSF is likely due in part to trans-isomers as in other studies where polyunsaturated fats were fed (11, 34, 158, 167). These trans-18:1 isomers are normal intermediates in the biohydrogenation of linoleic (18:2) and linolenic (18:3) acids occurring in the rumen (68). As in (11, 28, 96, 99, 104, 118, 155, 161, 162, 165, 167), butyrate was not decreased in milk fat from cows fed SSF. Butyrate may be synthesized by routes other than the malonyl-CoA pathway (130).

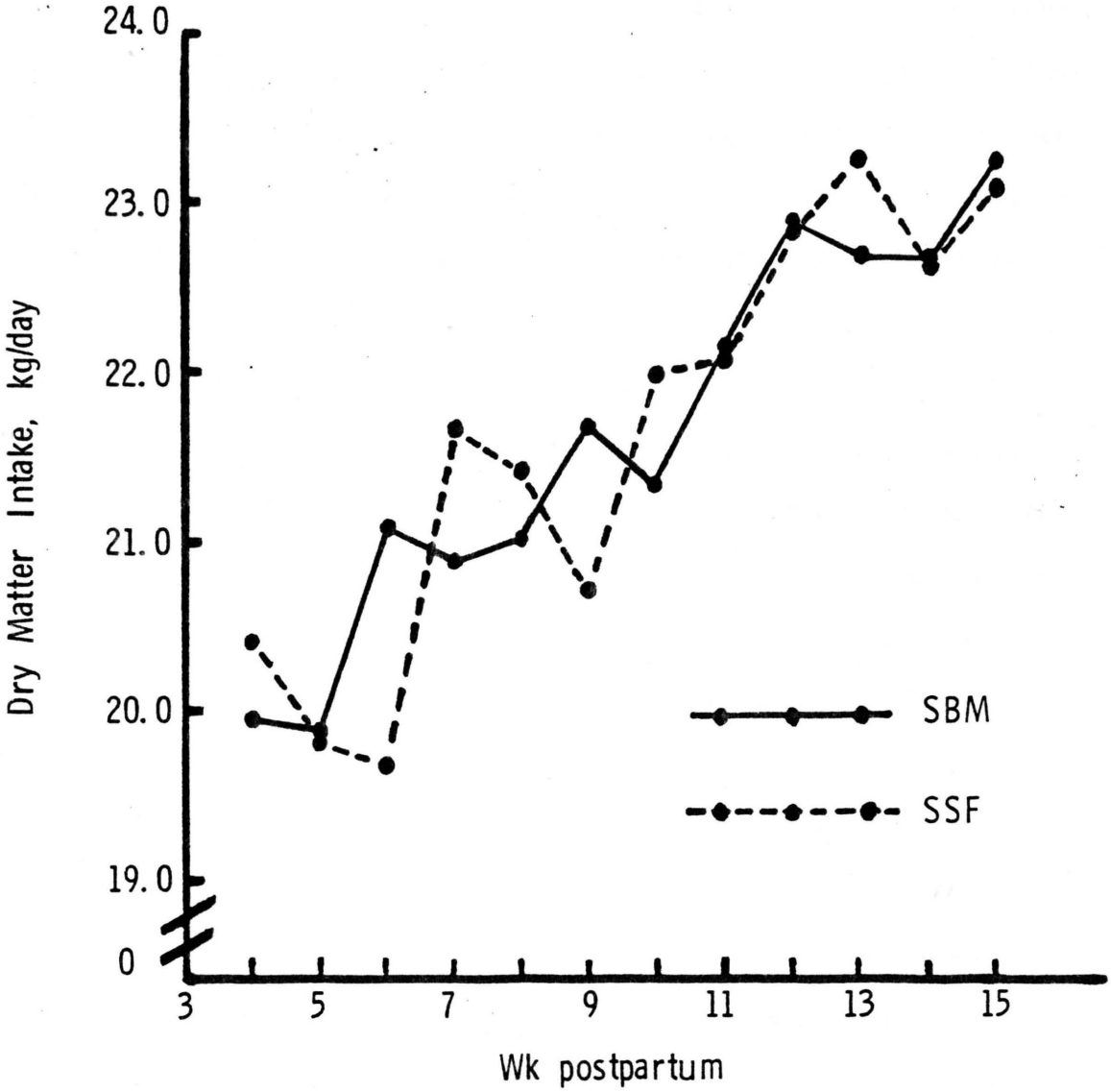
Consumption of DM was not different between diets (Table 6), in agreement with other studies with added fat (3, 4, 23, 34, 45, 61, 70, 75, 82, 94, 99, 104, 116, 132, 135, 146, 155, 161, 165, 166, 172, 173, 179). Decreases in DM intake noted in some studies (5, 55, 65, 83, 98, 118, 164, 184) may be due to negative effects of fat on ruminal digestion (120) or to altered hormonal status in response to lipid metabolites which results in no increase in energy intake (162). Supplying additional protein or amino acids postruminally may increase energy utilization and DM intake (35), possibly from increases in growth hormone release (19). As in Figure 4, there were no differences in DM intake between diets during the experiment. Intake of crude protein was similar between diets (Table 6), but ether extract intake was increased over two-fold in cows fed SSF.

TABLE 6. Nutrient intakes and body weight (BW) changes for cows fed diets containing soybean meal (SBM) or soy-sunflower blend (SSF) during weeks 4 to 15 postpartum.

Item	Diet		SE
	SBM	SSF	
Dry matter intake, kg/day	21.7	21.7	.28
Dry matter intake, kg/100 kg BW	3.56	3.63	.11
Crude protein intake, kg/day	3.34	3.34	.04
Ether extract intake, kg/day	.56	1.28**	.02
Beginning BW, kg	605	599	13.5
Ending BW, kg	612	595	14.8
BW change weeks 4 to 7, kg/day	-.42	-.35	.27
BW change weeks 8 to 11, kg/day	.48	.22	.20
BW change weeks 12 to 15, kg/day	.19	-.07	.20
BW change weeks 4 to 15, kg/day	.09	-.05	.09

**P<.01.

Figure 4. Dry matter intake of cows fed diets containing soybean meal (SBM) or soybean meal-sunflower seed blend (SSF).


24.0
23.0
22.0
21.0
20.0
19.0
0
Dry Matter Intake, kg/day
3
5
7
9
11
13
15
Wk postpartum
SBM
SSF

There were no differences in body weight change (Table 6) between diets over the 12-wk experiment or by 4-wk periods. Palmquist and Conrad (118) suggested that addition of fat to high-fiber diets might limit weight gain in early to midlactation. Since output of milk energy (Table 4) and body weight gain (Table 6) were not increased, and methane loss (44, 71, 73) and heat increment for incorporation of dietary fat into milk fat (162) are usually reduced with added fat, SSF must have contained less digestible energy than SBM even though gross energy was higher in SSF. This may be a consequence of negative effects of fat on rumen fermentation. As indicated by increased APIN in SSF (Table 3), N in SSF may have been less digestible, possibly resulting in insufficient amino acids for optimum utilization of additional dietary fatty acids (111) and mobilized fatty acids of adipose tissue (19).

Proportions of the VFA in the rumen are shown in Table 7. Molar percent of acetate increased while propionate decreased in cows fed SSF. Butyrate percent was unchanged. The increased acetate to propionate ratio in cows fed SSF might be a result of additional dietary fiber in SSF (Table 2). Many trials with added fats have shown increased proportions of propionate in rumen fluid (23, 53, 75, 76, 84, 96, 118, 158, 166, 179). Reduced methanogenesis causes a shift in hydrogen utilization from methane to propionate (177). Increased percentages of isobutyrate and valerate in cows fed SSF probably reflect differences in dietary protein composition and ruminal degradation between protein sources (96).

TABLE 7. Volatile fatty acids (VFA), ammonia, and pH in ruminal fluid, and urea in serum in cows fed diets containing soybean meal (SBM) or soy-sunflower blend (SSF).

Variable	Diet		SE
	SBM	SSF	
VFA			
Acetate, moles/100 moles	51.3	54.5**	.56
Propionate, moles/100 moles	26.6	23.4**	.75
Isobutyrate, moles/100 moles	.8	1.0*	.04
Butyrate, moles/100 moles	16.7	16.8	.36
Isovalerate, moles/100 moles	2.2	2.3	.10
Valerate, moles/100 moles	2.4	2.1**	.07
Acetate/Propionate	2.0	2.4**	.07
Total,µmol/ml	92.0	76.4**	1.92
Ruminal pH	6.26	6.52**	.04
Ruminal ammonia, mg/dl	16.5	13.0**	.79
Serum urea, mg/dl	16.0	15.2	.49

*P<.05.

**P<.01.

Total VFA concentrations were decreased in ruminal contents from cows fed SSF (Table 7), which may be a result of fatty acid-induced toxicity in ruminal cellulolytic and methanogenic bacteria (58, 71) and replacement of readily fermentable carbohydrates with poorly-digested sunflower hulls (Table 1). Reduced concentrations of acetate and butyrate might indicate reduced production rates and subsequent shortage of fatty acid precursors. However, Davis (46) found no reduction in production of acetate even though concentrations were lower in rumens of cows fed a fat-depressing diet.

Ruminal pH was higher in cows fed SSF, reflecting lower VFA concentrations. A significant ($P<.01$) diet x time interaction showed that mean pH in cows fed SSF was lower at the third sampling time relative to the first two samples (6.31 vs 6.63 and 6.62), while in cows fed SBM, pH was relatively constant over all three sampling times. Decreased pH at time 3 in cows fed SSF was associated with non-significant increases in total VFA and propionate.

Concentration of ammonia in ruminal fluid was lower in cows fed SSF (Table 7), consistent with other studies with heat-treated proteins (23, 87, 88, 104, 130, 131). Some workers (76, 84, 171) observed decreased ruminal ammonia when feeding added fats which was attributed to reduction of protozoal numbers and less bacterial recycling. Lower ammonia concentrations in the present study might also be a result of reduced microbial activity as indicated in lower total VFA concentrations. Ammonia concentrations with both diets appeared adequate for optimal microbial growth (149). Serum

urea concentrations were similar between diets.

Amino acid content of feeds and total diets is shown in Table 8. Diet SSF contained slightly more methionine, phenylalanine, valine, and arginine but less lysine than SBM, corresponding to differences in amino acid composition of soybean meal and sunflower meal (152). Diet SSF contained more EAA than SBM, with the exception of lysine. Amino acid balance may have been improved somewhat with the soy-sunflower blend.

Concentrations of amino acids in arterial and venous serum, and A-V differences are given in Table 9. In general, concentrations did not vary greatly between diets, although total EAA tended to be higher and total NEAA lower in arterial serum from SSF fed cows. Some workers (23, 129, 130) noted increased concentrations of EAA in plasma of cows fed heated protein sources, while others (2, 82) did not. Halfpenny et al. (67) observed increased NEAA and decreased EAA in plasma of cows fed a high energy diet. Palmquist and Conrad (118) found no effect of dietary fat on plasma EAA concentrations. It was suggested (118) that ratios of EAA to NEAA below .6 indicate a protein deficiency. Ratios in this study were .77 for SBM and .94 for SSF, indicating adequate protein supply.

Increased concentrations of the branched-chain amino acids (valine, isoleucine, and leucine) in plasma or serum are considered indicators of increased amino acid absorption from the small intestine (130), since these amino acids undergo negligible catabolism by the liver (35). In this trial, amino acid absorption was not

TABLE 8. Amino acid composition of supplements, concentrate mixes, forages, and total diets containing soybean meal (SBM) or soy-sunflower blend (SSF).

Amino acid	Supplement only		Concentrate mix		Alfalfa haylage	Corn silage	Total diet[1]	
	SBM	SSF	SBM	SSF			SBM	SSF
	(% of dry matter)							
Methionine	.70	.52	.29	.34	.23	.14	.22	.25
Lysine	2.67	1.27	.89	.81	.65	.20	.59	.56
Phenylalanine	2.22	1.35	.87	.98	.68	.31	.63	.68
Threonine	1.85	1.15	.77	.76	.65	.26	.56	.56
Valine	2.18	1.25	.78	.89	.96	.39	.68	.73
Isoleucine	2.09	1.25	.78	.84	.69	.28	.58	.61
Leucine	3.62	2.19	1.55	1.61	1.07	.72	1.15	1.18
Histidine	1.13	.71	.47	.45	.26	.14	.31	.31
Arginine	3.05	1.99	1.18	1.32	.35	.23	.66	.73
Tryptophan	.53	.31	.21	.19	.13	.04	.13	.12
Tyrosine	1.65	.86	.60	.62	.43	.24	.43	.45
Half-cystine	.37	.16	.13	.12	<.01	<.01	.06	.05
Proline	2.49	1.41	1.09	1.27	1.20	.43	.88	.96
Aspartic acid	5.36	2.57	2.15	1.66	2.48	.52	1.63	1.42
Glutamic acid	7.41	4.52	3.25	2.14	1.14	.98	1.98	1.52
Serine	2.35	1.37	1.01	.86	.71	.30	.69	.63
Glycine	1.81	1.19	.83	.86	.70	.29	.61	.62
Alanine	1.68	1.22	.88	.94	.83	.56	.74	.78
Total amino nitrogen	6.45	3.85	2.66	2.60	2.01	.93	1.90	1.88

[1]Computed.

TABLE 9. Concentration of amino acids in arterial and venous serum, and arterio-venous (A-V) difference in cows fed diets containing soybean meal (SBM) or soy-sunflower blend (SSF).

Amino acids	Arterial serum			Venous serum			A-V difference		
	SBM	SSF	SE	SBM	SSF	SE	SBM	SSF	SE
	(μmoles/dl)								
Methionine	1.9	1.9	.12	1.1	.8+	.09	.9	1.1	.14
Lysine	7.3	8.1	.43	2.6	3.3*	.24	4.7	4.8	.37
Phenylalanine	4.2	4.9	.33	2.1	2.8+	.27	2.1	2.1	.21
Threonine	7.6	7.7	.51	4.9	4.3	.43	2.6	3.4	.47
Valine	26.0	30.2	2.92	19.0	24.4	2.64	7.0	5.7	1.19
Isoleucine	12.8	17.5	2.03	7.3	12.8*	1.51	5.5	4.7	.86
Leucine	14.6	17.8	1.72	8.2	11.8	1.55	6.4	6.0	.77
Histidine	5.0	5.3	.22	3.5	3.8	.23	1.5	1.5	.16
Arginine	14.5	15.8	.73	10.1	10.1	.67	4.4	5.7	.69
Tryptophan	3.6	4.0	.34	2.7	3.0	.25	.9	1.0	.28
Total essential amino acids	97.4	113.1	7.70	61.4	77.1	6.39	36.0	36.0	4.11
Tyrosine	3.8	4.8*	.33	1.7	2.7*	.27	2.1	2.1	.17
Half-cystine	.4	.4	.03	.3	.4*	.02	.1	<.1	.04
Proline	7.8	8.5	.53	5.7	6.4	.64	2.1	2.1	.47
Aspartic acid	2.3	2.4	.14	1.7	1.8	.15	.6	.6	.19
Glutamic acid	7.0	7.3	.28	3.4	3.8	.20	3.6	3.6	.30
Serine	9.4	9.4	.70	7.2	6.0	.96	2.2	3.4	.62
Glycine	44.4	38.5	3.89	43.7	36.8	4.03	.7	1.7	1.65
Alanine	22.7	22.5	1.86	17.4	16.2	1.69	5.3	6.2	.98
Asparagine	4.6	4.8	.33	2.2	3.8*	.43	2.4	1.0*	.46
Glutamine	23.6	22.3	1.35	22.7	18.2*	1.13	.9	4.1	1.59
Ornithine[1]	4.5	4.8	.30	2.0	2.1	.16	2.5	2.7	.25
Citrulline[1]	9.6	11.6	1.07	9.0	10.3	1.07	.7	1.3	.48
Taurine[1]	5.8	5.4	.41	5.4	4.7	.39	.4	.6	.46
Total nonessential amino acids	126.1	120.9	5.90	106.1	96.0	6.15	20.0	25.0	4.49

+P<.10.
*P<.05.
[1]Not included in total nonessential amino acids.

increased greatly by diet SSF as concentrations of the branched-chain amino acids were not significantly higher than with diet SBM. Differences for isoleucine and leucine approached statistical significance ($P<.12$ and .19).

Arterio-venous differences were similar for the two diets (Table 9). Barry (12) concluded that in order to state that a compound is absorbed, the A-V difference should be greater than 20% of the arterial concentration in lactating animals. Using this criterion, SBM cows showed no net uptake of glycine, glutamine, citrulline, or taurine. Cows fed SSF showed no net uptake of half-cystine, glycine, glutamine, citrulline, or taurine by the mammary gland. In cows fed SSF, A-V difference for valine was only 18.9% of arterial concentration, but this was within one standard deviation of 20% and thus considered to be taken up. As a group, total NEAA did not exceed the 20% rule for either diet.

Table 10 shows calculated uptake (A-V difference times daily blood flow) and output of amino acids in milk protein by the mammary gland. Uptakes were not different between diets. Most EAA were taken up in adequate or excessive amounts relative to outputs in milk protein as indicated by uptake to output ratios greater than 1.0. Mean ratios for methionine were less than 1.0 for both diets but within one standard deviation, and could vary with differences in actual blood flow. Uptakes of methionine, phenylalanine, and tyrosine were very close to output in milk protein, and uptakes of lysine, leucine, and histidine only slightly in excess of output

TABLE 10. Uptake and output of amino acids by the mammary gland, and ratio of uptake to output in cows fed diets containing soybean meal (SBM) or soy-sunflower blend (SSF).

Amino acids	Uptake[1]			Output			Uptake/Output		
	SBM	SSF	SE	SBM	SSF	SE	SBM	SSF	SE
	(g/day)								
Methionine	19.0	24.4	3.1	24.8	24.2	.9	.78 (1)[2]	.97 (1)[2]	.12
Lysine	100.5	105.5	8.7	77.3	75.3	2.9	1.31 (4)	1.40 (6)	.10
Phenylalanine	50.0	51.3	5.6	46.8	45.6	1.8	1.08 (3)	1.11 (2)	.11
Threonine	45.3	60.6	8.4	43.9	42.8	1.6	1.04 (2)	1.39 (5)	.18
Valine	119.6	101.8	21.3	62.9	61.3	2.4	1.89 (7)	1.60 (7)	.31
Isoleucine	106.3	92.5	17.7	56.2	54.8	2.1	1.90 (8)	1.63 (8)	.28
Leucine	122.2	118.0	15.9	92.5	90.1	3.5	1.32 (5)	1.29 (3)	.15
Histidine	34.9	33.8	3.9	25.7	25.1	1.0	1.37 (6)	1.35 (4)	.15
Arginine	111.4	146.8	17.3	33.3	32.5	1.2	3.37 (10)	4.58 (10)	.54
Tryptophan	26.8	30.4	8.2	13.3	13.0	.5	2.01 (9)	2.44 (9)	.62
Total essential amino acids	736.0	765.2	89.4	474.9	469.8	17.8	1.55	1.63	.16
Tyrosine	55.7	57.9	4.8	48.6	47.4	1.8	1.16	1.21	.09
Half-cystine	1.9	1.1	.7	8.1	7.9	.3	.24	.15	.09
Proline	35.8	36.9	8.0	94.4	92.0	3.5	.38	.37	.09
Aspartic acid	11.5	11.5	3.6	75.3	73.4	2.8	.15	.16	.05
Glutamic acid	76.4	77.5	6.8	208.8	203.5	7.8	.54	.64	.08
Serine	32.4	52.6	9.5	53.4	52.0	2.0	.63	1.03	.18
Glycine	6.0	16.6	17.1	19.1	18.6	.7	.35	1.08	.95
Alanine	67.7	84.0	12.7	32.4	31.6	1.2	2.12	2.62	.39
Total nonessential amino acids	287.4	338.1	40.0	533.0	523.8	20.0	.54	.64	.08
Ornithine	48.2	52.2	4.4						

[1]Calculated as A-V difference multiplied by estimated blood flow per day.

[2]Numbers in parentheses indicate apparent sequence of limiting amino acids.

in milk protein, similar to (18, 36, 39, 49, 128, 163, 180). Threonine uptake was equivalent to output for cows fed SBM and slightly in excess for cows fed SSF. Use of these amino acids for oxidation or synthesis of NEAA is probably limited (35, 38, 101) although leucine contributes carbon and nitrogen for NEAA synthesis and may be oxidized as an energy source (142).

Uptakes of valine, isoleucine, and tryptophan were substantially greater than outputs in milk protein. Valine and isoleucine may be oxidized or converted to NEAA (143, 187). Metabolism of tryptophan in bovine mammary gland has received very limited study, probably due to difficulties in analysis. Tryptophan is transferred almost quantitatively to milk protein in the guinea pig mammary gland (103), subject to no appreciable metabolism in the gland. From excessive uptake in the present study it appears that tryptophan may be metabolized in the bovine mammary gland.

As in previous studies (18, 36, 39, 49, 128, 163) uptake of arginine was greatly in excess of output in milk protein for cows fed SBM or SSF. Ornithine was also taken up by the mammary gland in significant amounts by cows on both diets. Arginine and ornithine are important sources of NEAA in milk proteins, especially proline and glutamate (37). Ornithine is also a precursor of spermidine, a polyamine which may be an important regulator of mammary protein synthesis (110).

A ranking based on uptake to output ratios (Table 10) would give methionine, threonine, phenylalanine, lysine, and leucine as

the first five amino acids limiting production in cows fed SBM. In SSF fed cows, methionine, phenylalanine, leucine, histidine, and threonine would be most limiting. If tyrosine is considered an EAA, it would be fourth-limiting in SBM and third-limiting in SSF. These amino acids have frequently been cited as limiting milk production, although the relative order varies widely (18, 25, 26, 31, 36, 39, 49, 56, 57, 145, 157, 163, 182). A problem with this method of determining limiting amino acids is that it ranks amino acids from least to most used in pathways other than direct incorporation into milk protein, which may not be an accurate prediction of amino acids truly limiting milk protein synthesis (38).

Uptakes of NEAA were less than output in milk protein (Table 10), with the exception of tyrosine (which may be an EAA) and alanine. The NEAA are synthesized in large amounts from EAA in the mammary gland (35, 101). Uptake of half-cystine was extremely low relative to output in milk protein, as seen in (38, 39, 49, 163). Cysteine may be essential for protein synthesis in bovine mammary tissue (153) but can be supplied from glutathione in erythrocytes (15).

Extraction percentages (Table 11) were similar to those reported by others (39, 49, 163). Extractions of valine, leucine, isoleucine, and tyrosine were higher in cows fed SBM than SSF, which may be due to the tendency for higher arterial concentrations of these amino acids in SSF cows (Table 9).

Transfer efficiencies, or the percentage of arterial amino

TABLE 11. Extraction percentages and transfer efficiencies of serum amino acids in cows fed diets containing soybean meal (SBM) or soy-sunflower blend (SSF).

Amino acid	Extraction[1]			Transfer efficiency[2]				
	SBM	SSF	SE	SBM		SSF		SE
	(%)							
Methionine	44.1	56.0	6.1	59.4	(1)[3]	61.4	(1)[3]	3.8
Lysine	64.5	59.3	2.6	49.9	(2)	44.4	(2)	2.5
Phenylalanine	50.2	42.7	3.9	46.9	(3)	40.2^{+}	(3)	2.4
Threonine	34.9	42.8	4.4	33.6	(5)	33.9	(4)	2.3
Valine	28.8	18.9^{+}	4.0	15.6	(8)	12.4^{+}	(8)	1.2
Isoleucine	45.8	26.6**	3.7	25.5	(6)	18.0*	(7)	2.2
Leucine	48.1	34.3^{+}	4.8	35.6	(4)	27.9*	(5)	2.6
Histidine	31.2	27.7	3.2	22.8	(7)	21.0	(6)	.8
Arginine	30.4	35.6	3.7	9.0	(10)	8.3	(10)	.5
Tryptophan	24.5	23.5	5.7	12.9	(9)	11.6	(9)	.9
Tyrosine	56.6	45.4*	3.4	49.4		39.3*		3.4
Half-cystine	20.8	8.5	9.1	106.1		103.1		8.3
Proline	27.4	24.3	5.6	71.8		67.9		4.7
Aspartic acid	23.1	23.5	7.2	172.6		160.4		10.3
Glutamic acid	50.6	47.9	3.0	138.6		130.4		6.3
Serine	23.6	38.4	6.7	38.7		37.3		2.7
Glycine	1.5	4.6	4.3	4.2		4.5		.3
Alanine	23.3	28.6	3.8	11.5		11.4		.9

$^{+}$P<.10.

*P<.05.

**P<.01.

[1] A-V difference/arterial concentration x 100.

[2] $\frac{\text{Amino acid output in milk (g/day)}}{\text{Arterial serum amino acid (g/liter) x serum flow (liter/day)}} \times 100.$

[3] Numbers in parenthesis indicate apparent limiting order.

acid incorporated into milk protein, are also shown in Table 11. Values are similar to those in (31, 182). If it is assumed that amino acids utilized most efficiently are those limiting production, then methionine, lysine, phenylalanine, leucine, and threonine were the first five limiting amino acids in cows fed SBM. Methionine, lysine, phenylalanine, threonine, and leucine were most limiting in SSF cows. Except for lysine replacing histidine in cows fed SSF, limiting amino acids by this method were the same for both diets as those in Table 10, although the relative order after methionine varied. Tyrosine would be third-limiting in SBM and fourth-limiting in SSF if considered an EAA. Tryptophan and arginine appeared to be least limiting by both methods for cows fed either diet.

Transfer efficiencies for phenylalanine, valine, isoleucine, leucine, and tyrosine were lower in cows fed SSF (Table 11). One explanation is that extraction was reduced to a level compatible with some other more limiting amino acid. Also, the combination of soybean meal and sunflower proteins may have provided a more balanced amino acid supply, in which case reduced transfer efficiencies may indicate improved supply of those amino acids. Another possibility is that the added fat of SSF exerted a direct negative effect on utilization of these amino acids in protein synthesis, possibly mediated through hormones such as insulin. Insulin stimulated protein synthesis in goats (62) and caused increased uptake of leucine, isoleucine, valine, tyrosine, and aspartate in cows (89).

GENERAL DISCUSSION

Cows fed SSF produced similar quantities of milk as cows fed SBM, but milk solids content was decreased. Reasons for the depression in solids in this and other studies are not known. Cows fed SSF were calculated to enter positive energy balance at about 7 wk postpartum, immediately preceeding appearance of the low-fat milk syndrome at 8 to 9 wk postpartum. Three cows fed SSF became ketotic prior to this, one at 7 wk postpartum and two at 6 wk postpartum. One of these was removed from experiment, and the data was discarded. The other two cows recovered quickly after treatment and their data was used in analysis of the trial.

High-grain, restricted roughage diets depress milk fat through increased ruminal propionate production, which causes release of insulin (16, 33, 47, 176). Insulin causes adipose tissue to compete with the mammary gland for lipogenic substrates by suppressing release of fatty acids from and increasing uptake of acetate by adipose tissue (19). As in this study, propionate concentrations have not always been higher in cases of low-fat milk caused by feeding unsaturated fats (8, 28, 55, 75, 90, 94, 138, 155). However, due to the extremely high demand for glucose in the early lactation cow, propionate may not accumulate in the rumen to the same degree as in midlactation (78). Propionate concentrations and molar percentages may not be indicative of actual production rates (175). As the cow enters positive energy balance, propionate may stimulate increased insulin concentrations which may cause depressed milk fat.

Palmquist and Moser (123) measured increased insulin concentrations in cows fed a blended animal-vegetable fat, which suggests insulin may have been increased in the present trial.

Although mean molar percents of ruminal propionate were lower in cows fed SSF (Table 7), cows showing the most severe milk fat depression on SSF tended to have increased propionate and total VFA concentrations at the third rumen sampling time compared to the first. This may be due to adaptation of the rumen microbes over time to the toxicity of unsaturated fat, and to increased DM intake (Figure 4) which increases rate of passage and favors propionate production (177). The significant diet x time interaction showing lower rumen pH after occurrence of milk fat depression in cows fed SSF also indicates increased total VFA. Increased propionate at the time of transition to positive energy balance might trigger insulin release. Also, reduced acetate concentration in cows fed SSF might indicate some reduction in acetate production relative to SBM. An alteration of ruminal VFA as the cause for the milk fat depression is consistent with observations of delayed onset in early lactation (55), more rapid development in cows in positive energy balance (8, 23, 118, 138, 155, 158), and lack of depression with additional limestone (55) and whole seeds (3, 4, 5, 45, 106, 132, 164) which provide a slower release of oil into the rumen.

Insulin-mediated suppression of fatty acid release from adipose tissue reduces a major source of fatty acids to the early lactation cow for synthesis of very-low-density lipoproteins (VLDL),

the major triglyceride carrier for milk fat synthesis (16). The trans-isomers of oleic acid produced during ruminal biohydrogenation of polyunsaturated fat (68) may not be ideal substrates and may inhibit synthesis of VLDL, of which the preferred fatty acids are stearic, palmitic, and oleic acids (68). Altered VLDL carrying these trans-fatty acids may not be taken up (97) and the fatty acids incorporated into milk fat (6) by the mammary gland as well as other "normal" triglycerides. Thus, without fatty acids from adipose tissue, these ruminally altered fatty acids may contribute to lower milk fat formation. Regardless of the exact mechanism, milk fat depression caused by unsaturated fats involves a ruminal effect. When fats which depress milk fat when fed are instead infused into the abomasum (139) or blood (169), or are fed in a form "protected" from the rumen (8, 61, 96, 124) milk fat is usually increased rather than depressed.

Reduced solids-not-fat and protein in cows fed SSF may be a problem of glucose metabolism, as proposed by Smith et al. (162). Lower total VFA may indicate reduced propionate production relative to SBM cows, possibly creating a glucose insufficiency. Lactose-plus-ash percentages appeared to be lower almost immediately after initiation of the trial, and protein percentages were depressed by 4 wk into the trial. Ketosis in three cows during this time period suggests that glucose needs were not being met (16). The specific reduction in casein (54) may be related to the fact that casein is primarily an export protein (13). Synthesis of casein might be

reduced during glucose shortage before other milk proteins which serve other more vital functions in the gland. Milk protein began to increase in SSF cows between weeks 9 and 11 postpartum (Figure 3), soon after cows entered positive energy balance at about 7 wk postpartum.

Palmquist and Moser (123) indicated that feeding fat induced tissue insulin resistance and that amino acid uptake or protein synthesis might be inhibited. Although amino acid uptakes were not different in this trial, blood samples from SSF cows were taken (mean 45 days postpartum) just as protein percent started to decrease. In light of previous work (62, 89), lower transfer efficiencies for the branched chain amino acids and tyrosine in this trial could indicate a direct effect of insulin or other hormones on protein synthesis caused by added fat. Exact mechanisms for depression of milk solids in this trial can not be determined in the absence of more detailed metabolite and hormone data.

REFERENCES

1 Abondano, E. A., D. O. Erickson, R. L. Harrold, and W. D. Slanger. 1985. Quality of sunflower meal protein as affected by protein protection methods. J. Anim. Sci. 61(Suppl. 1):132. (Abstr.)

2 Ahrar, M., and D. J. Schingoethe. 1979. Heat-treated soybean meal as a protein supplement for lactating cows. J. Dairy Sci. 62:932.

3 Anderson, M. J., D. C. Adams, R. C. Lamb, and J. L. Walters. 1979. Feeding whole cottonseed to lactating dairy cows. J. Dairy Sci. 62:1098.

4 Anderson, M. J., R. C. Lamb, and J. L. Walters. 1980. Comparison of four levels of whole cottonseed for lactating dairy cows. J. Dairy Sci. 63(Suppl. 1):154. (Abstr.)

5 Anderson, M. J., Y. E. M. Obadiah, R. L. Boman, and J. L. Walters. 1984. Comparison of whole cottonseed, extruded soybeans, or whole sunflower seeds for lactating dairy cows. J. Dairy Sci. 67:569.

6 Askew, E. W., J. D. Benson, J. W. Thomas, and R. S. Emery. 1970. Metabolism of fatty acids by cows fed restricted roughage rations. Fed. Proc. 29:691. (Abstr.)

7 Association of Official Analytical Chemists. 1980. Official methods of analysis. 13th ed. AOAC, Washington, DC.

8 Astrup, H. N., L. Vik-Mo, A. Ekern, and F. Bakke. 1976. Feeding protected and unprotected oils to dairy cows. J. Dairy Sci. 59:426.

9 Atherton, H. V., and J. A. Newlander. 1977. Chemistry and testing of dairy products. 4th ed. AVI Publ. Co., Inc., Westport, CT.

10 Banks, W., J. L. Clapperton, M. E. Ferrie, and A. G. Wilson. 1976. Effect of feeding fat to dairy cows receiving a fat-deficient diet. I. Milk yield and composition. J. Dairy Res. 43:213.

11 Banks, W., J. L. Clapperton, and M. E. Ferrie. 1976. Effect of feeding fat to dairy cows receiving a fat-deficient diet. II. Fatty acid composition of the milk fat. J. Dairy Res. 43:219.

12 Barry, J. M. 1964. A quantitative balance between substrates and metabolic products of the mammary gland. Biol. Revs. 39:194.

13 Bath, D. L., F. N. Dickinson, H. A. Tucker, and R. D. Appleman. 1978. Dairy cattle: Principles, practices, problems, profits. 2nd ed. Lea & Febiger, Philadelphia, PA.

14 Baumgardt, B. R. 1964. Practical observations on the analysis of free volatile fatty acids (VFA) in aqueous solutions by gas-liquid chromatography. Bull. 1, Dep. of Dairy Sci., Univ. Wisconsin, Madison.

15 Baumrucker, C. R., P. A. Pocius, and T. L. Riss. 1981. Glutathione utilization by lactating bovine mammary secretory tissue in vitro. Biochem. J. 198:243.

16 Bell, A. W. 1980. Lipid metabolism in the liver and selected tissues in the whole body of ruminant animals. Prog. Lipid Res. 18:117.

17 Bergen, W. G. 1979. Free amino acids in blood of ruminants-physiological and nutritional regulation. J. Anim. Sci. 49:1577.

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

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

20 Bjarnason, J., and K. J. Carpenter. 1969. Mechanism of heat damage in protein. I. Models with acylated lysine units. Br. J. Nutr. 23:859.

21 Bjarnason, J., and K. J. Carpenter. 1970. Mechanism of heat damage in protein. II. Chemical changes in pure proteins. Br. J. Nutr. 24:313.

22 Black, A. L., E. Yeh, and T. Chapman. 1972. Interconversion of amino acids in lactating dairy cows. Fed. Proc. 31:681. (Abstr.)

23 Block, E., L. D. Muller, L. C. Griel, Jr., and D. L. Garwood. 1981. Brown midrib-3 corn silage and heat extruded soybeans for early lactation cows. J. Dairy Sci. 64:1813.

24 Broderick, G. A., and W. M. Craig. 1980. Effect of heat treatment on ruminal degradation and escape, and intestinal digestibility of cottonseed meal protein. J. Nutr. 110:2381.

25 Broderick, G. A., T. Kowalczyk, and L. D. Satter. 1970. Milk production response to supplementation with encapsulated methionine per os or casein per abomasum. J. Dairy Sci. 53:1714.

26 Broderick, G. A., L. D. Satter, and A. E. Harper. 1974. Use of plasma amino acid concentration to identify limiting amino acids for milk production. J. Dairy Sci. 57:1015.

27 Brooks, C. C., C. B. Garner, C. W. Gehrke, M. E. Muhrer, and W. H. Pfander. 1954. The effect of added fat on the digestion of cellulose and protein by ovine rumen microorganisms. J. Anim. Sci. 13:758.

28 Brown, W. H., J. W. Stull, and G. H. Stott. 1962. Fatty acid composition of milk. I. Effect of roughage and dietary fat. J. Dairy Sci. 45:191.

29 Bruckental, I., and E. Alumot. 1984. Proline supply for efficient nitrogen utilization by lactating ruminants. Can. J. Anim. Sci. 64(Suppl.):285.

30 Chalupa, W. 1975. Rumen bypass and protection of proteins and amino acids. J. Dairy Sci. 58:1198.

31 Chandler, P. T., and C. E. Polan. 1972. Considerations in interpretation of serum amino acids in lactating cows. J. Dairy Sci. 55:709. (Abstr.)

32 Chaney, A. L., and E. P. Marbach. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130.

33 Christie, W. W. 1979. The effects of diet and other factors on the lipid composition of ruminant tissues and milk. Prog. Lipid Res. 17:245.

34 Clapperton, J. L. 1982. The effect of sunflower oil on the fatty acid composition of the milk of cows fed either a fat-depressing diet or grass silage. J. Sci. Food Agric. 33:741.

35 Clark, J. H. 1975. Lactational responses to postruminal administration of proteins and amino acids. J. Dairy Sci. 58:1178.

36 Clark, J. H., C. L. Davis, and E. E. Hatfield. 1974. Effects of formaldehyde treated soybean meal on nutrient use, milk yield and composition, and free amino acids in the lactating bovine. J. Dairy Sci. 57:1031.

37 Clark, J. H., R. G. Derrig, C. L. Davis, and H. R. Spires. 1975. Metabolism of arginine and ornithine in the cow and rabbit mammary tissue. J. Dairy Sci. 58:1808.

38 Clark, J. H., H. R. Spires, and C. L. Davis. 1978. Uptake and metabolism of nitrogenous components by the lactating mammary gland. Fed. Proc. 37:1233.

39 Clark, J. H., H. R. Spires, R. G. Derrig, and M. R. Bennink. 1977. Milk production, nitrogen utilization and glucose synthesis in lactating cows infused postruminally with sodium caseinate and glucose. J. Nutr. 107:631.

40 Clark, R. M., P. T. Chandler, and G. S. Park. 1978. Limiting amino acids for milk protein synthesis by bovine mammary cells in culture. J. Dairy Sci. 61:408.

41 Clark, R. M., P. T. Chandler, C. S. Park. and A. W. Norman. 1980. Extracellular amino acid effects on milk protein synthesis and intracellular amino acid pools with bovine mammary cells in culture. J. Dairy Sci. 63:1230.

42 Cook, L. J., T. W. Scott, and Y. S. Pan. 1972. Formaldehyde-treated casein-safflower oil supplement for dairy cows. II. Effect on the fatty-acid composition of plasma and milk lipids. J. Dairy Res. 39:211.

43 Crooker, B. A., C. J. Sniffen, W. H. Hoover, and L. L. Johnson. 1978. Solvents for soluble nitrogen measurements in feedstuffs. J. Dairy Sci. 61:437.

44 Czerkawski, J. W., K. L. Blaxter, and F. W. Wainman. 1966. The effect of linseed oil and linseed oil fatty acids incorporated into the diet on metabolism of sheep. Br. J. Nutr. 20:485.

45 Dale, R. L., R. E. Roffler, and V. M. Thomas. 1984. Dairy cattle fed rations containing 0, 10, or 20% whole cottonseed. J. Dairy Sci. 67(Suppl. 1):114. (Abstr.)

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

47 Davis, C. L., and R. E. Brown. 1970. Low-fat milk syndrome. Page 545 in Physiology of digestion and metabolism in the ruminant. A. T. Phillipson ed. Oriel Press Limited, Newcastle, England.

48 Davison, K. L., and W. Woods. 1963. Effect of calcium and magnesium upon digestibility of a ration containing corn oil by lambs. J. Anim. Sci. 22:27.

49 Derrig, R. G., J. H. Clark, and C. L. Davis. 1974. Effect of abomasal infusion of sodium caseinate on milk yield, nitrogen utilization and amino acid nutrition of the dairy cow. J. Nutr. 104:151.

50 Dijk, H. J. Van, G. D. O'dell, P. R. Perry, and L. W. Grimes. 1983. Extruded versus raw ground soybeans for dairy cows in early lactation. J. Dairy Sci. 66:2521.

51 Dimenna, G. P., and R. S. Emery. 1981. Palmitate and octanoate metabolism in bovine mammary tissue. J. Dairy Sci. 64:132.

52 Dowlen, H. H., M. J. Montgomery, B. J. Bearden, and W. W. McNeil. 1984. Utilization of cottonseed in dairy rations. J. Dairy Sci. 67(Suppl. 1):234. (Abstr.)

53 Drackley, J. K., A. K. Clark, and T. Sahlu. 1985. Ration digestibilities and ruminal characteristics in steers fed sunflower seeds with additional calcium. J. Dairy Sci. 68:356.

54 Dunkley, W. L., N. E. Smith, and A. A. Franke. 1977. Effects of feeding protected tallow on composition of milk and milk fat. J. Dairy Sci. 60:1863.

55 Finn, A. M., A. K. Clark, J. K. Drackley, D. J. Schingoethe, and T. Sahlu. 1985. Whole rolled sunflower seeds with or without additional limestone in lactating dairy cattle rations. J. Dairy Sci. 68:903.

56 Fisher, L. J. 1972. Response of lactating cows to the intravenous infusion of amino acids. Can. J. Anim. Sci. 52:377.

57 Foldager, J., J. T. Huber, and W. G. Bergen. 1980. Factors affecting amino acids in blood of dairy cows. J. Dairy Sci. 63:396.

58 Galbraith, H., T. B. Miller, A. M. Paton, and J. K. Thompson. 1971. Antibacterial activity of long chain fatty acids and the reversal with calcium, magnesium, ergocalciferol, and cholesterol. J. Appl. Bacteriol. 34:803.

59 Glimp, H. A., M. R. Karr, C. O. Little, P. G. Woolfolk, G. E. Mitchell, Jr., and L. W. Hudson. 1967. Effect of reducing soybean protein solubility by dry heat on the protein utilization of young lambs. J. Anim. Sci. 26:858.

60 Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analysis (apparatus, reagents, procedures, and some application). US Dep. Agric., Agric. Handbook 379.

61 Goering, H. K., T. R. Wrenn, L. F. Edmondson, J. R. Weyant, D. L. Wood, and J. Bitman. 1977. Feeding polyunsaturated vegetable oils to lactating cows. J. Dairy Sci. 60:739.

62 Golovan', V. T., and V. N. Shpotakovskii. 1978. Effect of insulin on secretion of milk proteins (translated title). Page 35 in Fiziologicheski i biokhimicheskie osnovy povysheniya produktivnosti sel'skokhozyaistvennykh zhivnotnykh. M. V. Frunze, ed. Kishinevskii Sel'skokhozyaistvennyi Institut, Kishinev, USSR. Dairy Science Abstr. 42:932.

63 Goodnight, J. H. 1979. ANOVA procedure, page 121 and GLM procedure, page 245 in SAS User's Guide. J. T. Helwig and K. A. Council, ed. SAS Inst., Inc., Cary, NC.

64 Grainger, R. B., M. C. Bell, J. W. Stroud, and F. H. Baker. 1961. Effect of various cations and corn oil on crude cellulose digestibility by sheep. J. Anim. Sci. 20:319.

65 Gramza, G. J., H. H. Olson, G. C. McCoy, and D. D. Lee. 1979. A comparison of feeding 0, 2, and 4% soybean oil rations to lactating cows. J. Dairy Sci. 62(Suppl. 1):138. (Abstr.)

66 Grummer, R. R., and J. H. Clark. 1982. Effect of dietary nitrogen solubility on lactation performance and protein and dry matter degradation in situ. J. Dairy Sci. 65:1432.

67 Halfpenny, A. F., J. A. F. Rook, and G. H. Smith. 1969. Variations with energy nutrition in the concentrations of amino acids in the blood plasma of the dairy cow. Brit. J. Nutr. 23:547.

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

69 Hawkins, G. E. 1980. Cottonseed and soybean oil in rations of dairy cattle. J. Dairy Sci. 63(Suppl. 1):164. (Abstr.)

70 Hawkins, E. E., J. E. Meader, F. G. Owen, and S. R. Lowry. 1984. Optimizing soybean utilization in dairy rations. J. Dairy Sci. 67(Suppl. 1):125. (Abstr.)

71 Henderson, C. 1973. The effects of fatty acids on pure cultures of rumen bacteria. J. Agric. Sci. 81:107.

72 Higginbotham, G. E., N. P. Johnston, D. Andrus, and J. T. Huber. 1984. Effect of nonprotein nitrogen supplementation of highly insoluble dairy rations on the performance of high producing dairy cows. J. Dairy Sci. 67(Suppl. 1):120. (Abstr.)

73 Honing, Y. van der. 1980. The utilization by high-yielding dairy cows of energy from animal tallow or soya bean oil added to a diet rich in concentrates. Page 315 in European Assoc. for Anim. Prod. Pub. 26.

74 Honing, Y. van der, B. J. Wieman, A. Steg, and B. van Donselaar. 1981. The effect of fat supplementation of concentrations on digestion and utilization of energy by productive dairy cows. Neth. J. Agric. Sci. 29:79.

75 Hutjens, M. F., and L. H. Schultz. 1971. Addition of soybeans or methionine analog to high-concentrate rations for dairy cows. J. Dairy Sci. 54:1637.

76 Ikwuegbu, O. A., and J. D. Sutton. 1982. The effect of varying the amount of linseed oil supplementation on rumen metabolism in sheep. Brit. J. Nutr. 48:365.

77 Jacobson, D. R., H. H. Van Horn, and C. J. Sniffen. 1970. Lactating ruminants. Fed. Proc. 29:35.

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

79 Jones, E. P., and V. L. Davidson. 1965. Quantitative determination of double bond positions in unsaturated fatty acids after oxidative cleavage. J. Am. Oil Chem. Soc. 42:121.

80 Jones, A. D., C. H. S. Hitchcock, and G. H. Jones. 1981. Determination of tryptophan in feeds and feed ingredients by high-performance liquid chromatography. Analyst 106:968.

81 Jorgensen, G. N., and B. L. Larson. 1968. Conversion of phenylalanine to tyrosine in the bovine mammary secretory cell. Biochem. Biophys. Acta 165:121.

82 Kenna, T. M., and C. G. Schwab. 1979. Extruded heat-processed soybeans and N-hydroxy-methionine-Ca for cows in early lactation. J. Dairy Sci. 62(Suppl. 1):74. (Abstr.)

83 Kennelly, J. J. 1983. Whole canola seed for lactating dairy cows. Page 80 in Agriculture and Forestry Bull., Dep. Anim. Sci., Univ. Alberta, Edmonton.

84 Kowalczyk, J., E. R. Ørskov, J. J. Robinson, and C. S. Stewart. 1977. Effect of fat supplementation on voluntary food intake and rumen metabolism in sheep. Brit. J. Nutr. 37:251.

85 Kronfeld, D. S., S. Donoghue, J. M. Naylor, K. Johnson, and C. A. Bradley. 1980. Metabolic effects of feeding protected tallow to dairy cows. J. Dairy Sci. 63:545.

86 Kronfeld, D. S., F. Raggi, and C. F. Ramberg. 1968. Mammary blood flow and ketone body metabolism in normal, fasted, and ketotic cows. Amer. J. Physiol. 215:218.

87 Kung, L., Jr., and J. T. Huber. 1983. Performance of high producing cows in early lactation fed proteins of varying amounts, sources, and degradability. J. Dairy Sci. 66:227.

88 Kung, L., Jr., J. T. Huber, and L. D. Satter. 1983. Influence of nonprotein nitrogen and protein of low rumen degradability on nitrogen flow and utilization in lactating dairy cows. J. Dairy Sci. 66:1863.

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

90 Larson, S. A., and L. H. Schultz. 1970. Effects of soybeans compared to soybean oil and meal in the ration of dairy cows. J. Dairy Sci. 53:1233.

91 Lee, P., and R. Slocum. 1982. Beckman 118/119 BL application notes, January. Spinco Division of Beckman Instruments, Inc., Palo Alto, CA.

92 Linzell, J. L. 1974. Mammary blood flow and methods of identifying and measuring precursors of milk. Page 143 in Lactation. B. L. Larson and V. R. Smith, ed. Academic Press, London.

93 Macleod, G. K., and D. G. Grieve. 1983. Protein nutrition of dairy cattle. Page 14 in Proc. Cornell Nutr. Conf., Ithaca, NY.

94 Macleod, G. K., and A. S. Wood. 1972. Influence of amount and degree of saturation of dietary fat on yield and quality of milk. J. Dairy Sci. 55:439.

95 Macleod, G. K., A. S. Wood, and Y. T. Yao. 1972. Influence of dietary fat on rumen fatty acids, plasma lipid and milk fat composition in the cow. J. Dairy Sci. 55:446.

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

97 McCarthy, R. D., P. S. Dimick, and S. Patton. 1966. Field observations on the lipids of cows with depressed milk fat test. J. Dairy Sci. 49:205.

98 McGuffey, R. K., and D. J. Schingoethe. 1980. Feeding value of a high oil variety of sunflowers as silage to lactating dairy cows. J. Dairy Sci. 63:1109.

99 McGuffey, R. K., and D. J. Schingoethe. 1982. Whole sunflower seeds for high producing dairy cows. J. Dairy Sci. 65:1479.

100 McMeniman, N. P., and D. G. Armstrong. 1979. The flow of amino acids into the small intestine of cattle when fed heated and unheated beans (Vicia fabia). J. Agric. Sci., Camb. 93:181.

101 Mepham, T. B. 1982. Amino acid utilization by lactating mammary gland. J. Dairy Sci. 65:287.

102 Mepham, T. B., and J. L. Linzell. 1974. Effects of intramammary infusion of nonessential amino acids and glucose in the lactating goat. J. Dairy Res. 41:111.

103 Mepham, T. B., A. R. Peters, and S. R. Davis. 1976. Uptake and metabolism of tryptophan by the perfused guinea-pig mammary gland. Biochem. J. 158:659.

104 Mielke, C. D., and D. J. Schingoethe. 1981. Heat-treated soybeans for lactating cows. J. Dairy Sci. 64:1579.

105 Mondino, A., G. Bongiovanni, S. Pumero, and L. Rossi. 1972. An improved method of plasma deproteination with sulphosalicylic acid for determining amino acids and related compounds. J. Chromatogr. 74:255.

106 Moody, E. G. 1978. Cottonseed and oil in dairy rations at two roughage levels. Feedstuffs 50(41):20.

107 Moore, J. H., and W. Steele. 1968. Dietary fat and milk fat secretion in the cow. Proc. Nutr. Soc. 27:66.

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

109 Netemeyer, D. T., L. J. Bush, J. W. Ward, and S. A. Jafri. 1982. Effect of heating soybean meal for dairy cows. J. Dairy Sci. 65:235.

110 Oka, T., and J. W. Perry. 1974. Arginase affects lactogenesis through its influence on the biosynthesis of spermidine. Nature 250:660.

111 Oldham, J. D. 1984. Amino acid metabolism in ruminants. Page 137 in Proc. Cornell Nutr. Conf., Ithaca, NY.

112 Oldham, J. D., and S. Tamminga. 1980. Amino acid utilization by dairy cows. I. Methods of varying amino acid supply. Livestock Prod. Sci. 7:437.

113 Ørskov, E. R., D. A. Grubb, and R. N. B. Kay. 1977. Effect of postruminal glucose or protein supplementation on milk yield and composition in Friesan cows in early lactation and negative energy balance. Brit. J. Nutr. 38:397.

114 Otterby, D. E., R. G. Lundquist, M. D. Stern, and J. G. Linn. 1983. Slowly degraded proteins and amino acids in diets for dairy cows. Page 1 in Proc. 44th Minnesota Nutr. Conf., Univ. Minnesota, St. Paul.

115 Palmquist, D. L. 1983. Feeding fat to lactating dairy cows. Page 28 in Proc. 44th Minnesota Nutr. Conf., Univ. Minnesota, St. Paul.

116 Palmquist, D. L. 1984. Calcium soaps of fatty acids with varying unsaturation as fat supplements for lactating cows. Can. J. Anim. Sci. 64(Suppl.):240.

117 Palmquist, D. L. 1984. Effects of supplemental fat in various forms on rumen digestibility in sacco. J. Dairy Sci. 67(Suppl. 1):127. (Abstr.)

118 Palmquist, D. L., and H. R. Conrad. 1978. High fat rations for dairy cows. Effects on feed intake, milk and fat production, and plasma metabolites. J. Dairy Sci. 61:890.

119 Palmquist, D. L., and H. R. Conrad. 1980. High fat rations for dairy cows. Tallow and hydrolyzed blended fat at two intakes. J. Dairy Sci. 63:391.

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

121 Palmquist, D. L., and T. C. Jenkins. 1982. Calcium soaps as a fat supplement in dairy cattle feeding. Page 477 in Proc. XII World Congr. Dis. Cattle, Vol. 1. Amsterdam, Netherlands.

122 Palmquist, D. L., and T. C. Jenkins. 1983. Metabolism of fats in the rumen. Paper presented at XVII Conf. on Rumen Function, Chicago, IL.

123 Palmquist, D. L., and E. A. Moser. 1981. Dietary fat effects on blood insulin, glucose utilization, and milk protein content of lactating cows. J. Dairy Sci. 64:1664.

124 Pan, Y. S., L. J. Cook, and T. W. Scott. 1972. Formaldehyde-treated casein-safflower oil supplement for dairy cows. I. Effect on milk composition. J. Dairy Res. 39:203.

125 Park, C. S., P. T. Chandler, and A. W. Norman. 1976. Limiting amino acids for protein synthesis with mammary cells in tissue culture. J. Dairy Sci. 59:868.

126 Parry, R. M., Jr., J. Sampugna, and R. G. Jensen. 1964. Effect of feeding safflower oil on the fatty acid composition of milk. J. Dairy Sci. 47:37.

127 Payne, J. M., G. J. Rowlands, R. Manston, and S. M. Den. 1973. A statistical appraisal of the results of metabolic profile tests on 75 dairy herds. Brit. Vet. J. 129:370.

128 Peeters, G., A. Houvenaghel, E. Roets, A. M. Massart-Leën, R. Verbeke, G. Dhondt, and F. Verschooten. 1979. Electromagnetic blood flow recording and balance of nutrients in the udder of lactating cows. J. Anim. Sci. 48:1143.

129 Pena, F., and L. D. Satter. 1984. Effect of feeding heated soybean meal and roasted soybeans on milk production in Holstein cows. J. Dairy Sci. 67(Suppl. 1):123. (Abstr.)

130 Pena, F., L. D. Satter, and G. A. Broderick. 1984. Effect of feeding roasted soybeans on ruminal and blood plasma measurements in lactating Holstein cows. J. Dairy Sci. 67(Suppl. 1):123. (Abstr.)

131 Pena, F., H. Tagari, and L. D. Satter. 1983. Effect of heat treating whole cottonseed on rumen degradation and flow of protein to the small intestine in Holstein cows. J. Dairy Sci. 66(Suppl. 1):200. (Abstr.)

132 Perry, F. G., and G. K. Macleod. 1968. Effects of feeding raw soybeans on rumen metabolism and milk composition of dairy cows. J. Dairy Sci. 51:1233.

133 Perry, T. L., and S. Hansen. 1969. Technical pitfalls leading to errors in the quantitation of plasma amino acids. Clin. Chem. Acta 25:53.

134 Rae, R. C., and J. R. Ingalls. 1984. Lactational response of dairy cows to oral administration of L-tyrosine. J. Dairy Sci. 67:1430.

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

136 Rakes, A. H., D. G. Davenport, and G. R. Marshall. 1972. Feeding value of roasted soybeans for dairy cows. J. Dairy Sci. 55:529.

137 Reynolds, M., J. L. Linzell, and F. Rasmussen. 1968. Comparison of four methods for measuring mammary blood flow in conscious goats. Amer. J. Physiol. 214:1415.

138 Rindsig, R. B., and L. H. Schultz. 1974. Effect of amount and frequency of feeding safflower oil on related milk, blood, and rumen components. J. Dairy Sci. 57:1037.

139 Rindsig, R. B., and L. H. Schultz. 1974. Effects of abomasal infusions of safflower oil or elaidic acid on blood lipids and milk fat of dairy cows. J. Dairy Sci. 57:1459.

140 Roberts, W. K., and J. A. McKirdy. 1964. Weight gains, carcass fat characteristics, and ration digestibility in steers as affected by dietary rapeseed oil, sunflower seed oil, and animal tallow. J. Anim. Sci. 23:682.

141 Robertson, J. B., and P. J. Van Soest. 1981. The detergent system of analysis and its application to human foods. Page 123 in The analysis of dietary fiber. W. P. T. James and O. Theander, ed. Marcell Dekker, New York, NY.

142 Roets, E., A. M. Massart-Leën, G. Peeters, and R. Verbeke. 1983. Metabolism of leucine by the isolated perfused goat udder. J. Dairy Res. 50:413.

143 Roets, E., A. M. Massart-Leën, R. Verbeke, and G. Peeters. 1979. Metabolism of [U-^{14}C;2,3-^{3}H] - L-valine by the isolated perfused goat udder. J. Dairy Res. 46:47.

144 Roets, E., R. Verbeke, G. Peeters, H. Axmann, and G. Proksch. 1979. Metabolism of ornithine in perfused goat udder. J. Dairy Sci. 62:259.

145 Rogers, J. A., J. H. Clark, T. R. Drendel, and G. C. Fahey, Jr. 1984. Milk production and nitrogen utilization by dairy cows infused postruminally with sodium caseinate, soybean meal, or cottonseed meal. J. Dairy Sci. 67:1928.

146 Ruegsegger, G. J., L. H. Schultz, and D. Sommer. 1983. Response of dairy cows in early lactation to the feeding of heat-treated whole soybeans. J. Dairy Sci. 66(Suppl. 1):168. (Abstr.)

147 Sahlu, T. 1983. Lactational and chemical evaluation of soybean meals heat-treated by two methods. PhD dissertation, South Dakota State Univ., Brookings.

148 Sahlu, T., D. J. Schingoethe, and A. K. Clark. 1984. Lactational and chemical evaluation of soybean meals heat-treated by two methods. J. Dairy Sci. 67:1725.

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

150 Schingoethe, D. J. 1984. Application of rumen bypass proteins in ruminant diets. Anim. Nutr. Res. Highlights, April, Amer. Soybean Assn., St. Louis, MO.

151 Schingoethe, D. J. 1984. Sunflower products in cattle rations. The Sunflower 10(4):24.

152 Schingoethe, D. J., and M. Ahrar. 1979. Protein solubility, amino acid composition, and biological value of regular and heat-treated soybean and sunflower meals. J. Dairy Sci. 62:925.

153 Schingoethe, D. J., E. C. Hageman, and B. L. Larson. 1967. Essential amino acids for milk protein synthesis in the in vitro secretory cell and stimulation by elevated levels. Biochim. Biophys. Acta 148:469.

154 Schingoethe, D. J., J. A. Rook, and F. C. Ludens. 1977. Evaluation of sunflower meal as a protein supplement for lactating cows. J. Dairy Sci. 60:591.

155 Schingoethe, D. J., G. D. Stewart, and F. C. Ludens. 1983. Dried whey-fat blend product as a feed for lactating dairy cows. J. Dairy Sci. 66:2515.

156 Schwab, C. G., S. J. Muise, and J. B. Holter. 1980. The effect of extruded heat-processed soybeans and methionine on performance, nitrogen and energy metabolism, and blood parameters in early lactation cows. J. Dairy Sci. 63(Suppl. 1):140. (Abstr.)

157 Schwab, C. G., L. D. Satter, and A. B. Clay. 1976. Response of lactating dairy cows to abomasal infusion of amino acids. J. Dairy Sci. 59:1254.

158 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.

159 Sidhu, G. S., and J. R. Ashes. 1977. Amino acid availability from protected protein - a critical factor for response in milk production. Proc. Nutr. Soc. Austr. 2:81.

160 Smith, N. E., L. S. Collar, D. L. Bath, W. L. Dunkley, and A. A. Franke. 1980. Whole cottonseed and extruded soybean for cows in early lactation. J. Dairy Sci. 63(Suppl. 1):153. (Abstr.)

161 Smith, N. E., L. S. Collar, D. L. Bath, W. L. Dunkley, and A. A. Franke. 1981. Digestibilities and effects of whole cottonseed fed to lactating cows. J. Dairy Sci. 64:2209.

162 Smith, N. E., W. L. Dunkley, and A. A. Franke. 1978. Effects of feeding protected tallow to dairy cows in early lactation. J. Dairy Sci. 61:747.

163 Spires, H. R., J. H. Clark, R. G. Derrig, and C. L. Davis. 1975. Milk production and nitrogen utilization in response to postruminal infusion of sodium caseinate in lactating cows. J. Nutr. 105:1111.

164 Steele, W. 1984. Lipid supplementation of dairy cow diets. J. Dairy Sci. 67:1716.

165 Steele, W., and J. H. Moore. 1968. The effects of dietary tallow and cottonseed oil on milk fat secretion in the cow. J. Dairy Res. 35:223.

166 Steele, W., R. C. Noble, and J. H. Moore. 1971. The effects of 2 methods of incorporating soybean oil into the diet on milk yield and composition in the cow. J. Dairy Res. 38:43.

167 Steele, W., R. C. Noble, and J. H. Moore. 1971. The effects of dietary soybean oil on milk-fat composition in the cow. J. Dairy Res. 38:49.

168 Stern, M. D., K. A. Santos, and L. D. Satter. 1985. Protein degradation in rumen and amino acid absorption in small intestine of lactating dairy cattle fed heat-treated whole soybeans. J. Dairy Sci. 68:45.

169 Storry, J. E., A. J. Hall, B. Tuckley, and D. Millard. 1969. The effects of intravenous infusions of cod-liver and soya-bean oils on the secretion of milk fat in the cow. Brit. J. Nutr. 23:173.

170 Tagari, H., I. Ascarelli, and A. Bondi. 1962. The influence of heating on the nutritive value of soya-bean meal for ruminants. Brit. J. Nutr. 16:237.

171 Tamminga, S., A. W. van Vuuren, C. J. van der Koelen, H. M. Khattab, and L. G. M. van Gils. 1983. Further studies on the effect of fat supplementation of concentrates fed to lactating dairy cows. 3. Effect on rumen fermentation and site of digestion of dietary components. Neth. J. Agric. Sci. 31:249.

172 Thomas, V. M., G. A. Murray, D. L. Thacker, and D. N. Snedden. 1982. Sunflower silage in rations for lactating Holstein cows. J. Dairy Sci. 65:267.

173 Tomlinson, J. E., W. H. McGee, E. W. Custer, J. W. Fuquay, W. E. Poe, and R. L. Dooley. 1981. Effect of graded levels of whole cottonseed in the diet of dairy cattle upon milk production and composition. J. Dairy Sci. 64(Suppl. 1):141. (Abstr.)

174 Trudeau, D. L., and E. F. Freier. 1967. Determination of calcium in urine and serum by atomic absorption spectrophotometry (AAS). Clin. Chem. 13:101.

175 Van Maanen, R. W., J. H. Herbein, A. D. McGilliard, and J. W. Young. 1978. Effects of monensin on in vivo rumen propionate production and blood glucose kinetics in cattle. J. Nutr. 108:1002.

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

177 Van Soest, P. J. 1982. Nutritional Ecology of the Ruminant. O & B Books, Inc., Corvallis, OR.

178 Van Soest, P. J., and C. J. Sniffen. 1984. Nitrogen fractions in NDF and ADF. Page 73 in Proc. Distillers Feed Res. Council Conf., Cincinnati, OH.

179 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.

180 Verbeke, R., and G. Peeters. 1965. Uptake of free plasma amino acids by the lactating cow's udder and amino acid composition of udder lymph. Biochem. J. 94:183.

181 Verbeke, R., E. Roets, A. M. Massart-Leen, and G. Peeters. 1972. Metabolism of [U-^{14}C]-L threonine and [U-^{14}C]-L phenylalanine by the isolated perfused udder. J. Dairy Res. 39:239.

182 Vik-Mo, L., J. T. Huber, W. G. Bergen, R. E. Lichtenwalner, and R. S. Emery. 1974. Blood metabolites in cows abomasally infused with casein or glucose. J. Dairy Sci. 57:1024.

183 Waghorn, G. C., and R. L. Baldwin. 1984. Model of metabolite flux within mammary gland of the lactating cow. J. Dairy Sci. 67:531.

184 Ward, G. M., J. L. Morrill, M. B. Morrill, and A. D. Dayton. 1984. Evaluation of raw and processed full-fat soybeans for high-producing dairy cows. Page 21 in Kansas State Univ. Agric. Exp. Sta. Rep. 460, Manhattan.

185 Ward, J. K., C. W. Tefft, R. J. Sirny, H. N. Edwards, and A. D. Tillman. 1957. Further studies concerning the effect of alfalfa ash upon the utilization of low quality roughages by ruminant animals. J. Anim. Sci. 16:633.

186 White, T. W., R. B. Grainger, F. H. Baker, and J. W. Stroud. 1958. Effect of supplemental fat on digestion and calcium requirement of sheep. J. Anim. Sci. 17:797.

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

188 Yu, Y. 1978. Effect of treating full-fat whole soybeans with dry heat or formaldehyde on digestibilities of nitrogen and polyenoic acids. J. Dairy Sci. 61:128.