SUBSEQUENT MILK PRODUCTION AND METABOLIC RESPONSE OF FIRST-CALF HEIFERS FED WHOLE RAW SOYBEANS DURING THE LAST TRIMESTER OF GESTATION

by

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(ABSTRACT)

Sixteen pregnant heifers (8 per group) were fed diets containing soybean meal or whole raw soybeans (WSB) to evaluate effects of the supplemental dietary fat provided by WSB during the last trimester of gestation (90 d) on performance in the first 5 wk of the subsequent lactation, during which both groups were fed the same diet. Despite similar DMI by both groups, heifers fed WSB had greater ether extract (EE) intake and tended to weigh more after calving. By 35 DIM, heifers previously fed WSB had greater milk yield with a tendency for greater milk fat yield, although DMI was similar for both groups. Blood obtained by jugular venipuncture four times per day during gestation (-90 d, -69 d, -48 d, and -27 d), on day of calving (0 d), and during lactation (+35 d) was used to evaluate glucose and NEFA response to diet. Glucose concentration in blood plasma at -69 d and 0 d was lower in heifers fed WSB; whereas, plasma NEFA concentration was greater at -69 d, -48 d, and +35 d, but lower at 0 d. Basal

concentrations of glucose and NEFA in plasma and their response to insulin challenge (.26 IU per kg BW) were similar for both dietary groups at -30 d and +35 d. Plasma NEFA concentrations in response to insulin challenge, however, were greater at -30 d than at +35 d. Basal plasma triacylglycerol concentration was greater at -30 d and lower +35 d, due to feeding WSB during gestation. In addition, plasma 16:0 and 18:1 (%, wt per wt) concentrations were lower and 18:2 higher at -30 d in heifers fed WSB. At 35 DIM, heifers previously fed WSB again had lower 16:0 and higher 18:2 in plasma. Dietary treatment during gestation had no influence on long chain fatty acid concentrations in adipose tissue. Supplemental dietary fat provided by WSB for 90 d prior to parturition apparently altered the supply and metabolism of lipids in a manner that improved milk production during early lactation.

DEDICATION

I dedicate this thesis to my parents, Richard and Rebecca Wasserstrom for their love and support.

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INTRODUCTION

The addition of fat to diets of lactating cows increases energy density of the diet and therefore may improve the energy status of the cow, particularly in early lactation. Dairy cows in early lactation are fed diets containing supplemental fat to increase milk and milk fat production, and decrease extent of negative energy balance (Schauff et al., 1992). Diets with added fat increase rate of gain and improve growth efficiency (kg BW gain per kg DMI) of growing ruminants. Supplemental dietary fat apparently improves efficiency of growth by allowing greater dietary energy intake without an alteration in dry matter intake (Albro et al., 1993).

Feeding supplemental fat to ruminants (lactating or growing) increased the quantity of long chain fatty acids (LCFA) flowing to the small intestine for absorption (Sutton, 1985 and Coppock, 1985). Triacylglycerol and non esterified fatty acids (NEFA) in blood plasma increased in response to greater LCFA absorption (Steele, 1983). The metabolic efficiency of triacylglycerol synthesis in adipose tissue and (or) the mammary gland can be improved by feeding fat, because it is more efficient to use dietary LCFA for synthesis of triacylglycerol than to synthesize LCFA de novo. By providing more LCFA via the diet, glucose and acetate are spared (Ekeren et al., 1992) for other functions such as growth, fetal development, or milk production.

Sixteen pregnant heifers were fed diets containing soybean meal or whole raw soybean (WSB) to evaluate the influence of fat provided by WSB fed during the last trimester of gestation on subsequent lactation performance. Heifers (or cows) with greater body condition at parturition produce more milk during early lactation and throughout their entire lactation (Seymour and Polan, 1986; Garnsworthy and Huggett, 1992). Whole raw soybeans were added to the diet as a supplemental fat source to improve body condition prior to calving.

Metabolic parameters including blood glucose, NEFA, triacylglycerol, and long chain fatty acids (LCFA), along with DMI and BW, were measured during gestation to evaluate the direct effects of WSB during the 90 d prior to parturition. Milk, milk fat, milk protein, and milk lactose production were measured weekly to evaluate the indirect effects of gestation dietary treatment on lactation performance. Dry matter intake, BW, and blood metabolites including glucose, NEFA, triacylglycerol, and LCFA also were measured in the 5^{th} wk of lactation to evaluate the influence of dietary fat provided by WSB in the last trimester of gestation.

REVIEW OF LITERATURE

Supplemental Fat in Diets for Growing Ruminants

Providing growing ruminants with high energy diets can improve feed efficiency, average daily gain, as well as alter blood lipids and adipose tissue fatty acid composition. Greater energy density of the diet elevates energy intake at the same DMI. Therefore, animals gain more than animals fed more traditional feed stuffs, resulting in improved feed efficiency (Park et al., 1983). High producing cows rely heavily on tissue reserves in early lactation. Greater body condition at calving decreases body condition loss in early lactation (Garnsworthy and Huggett, 1992). Cows with greater body condition at calving also had greater peak production and total lactation yield when compared with thinner cows (Seymour and Polan, 1986; Garnsworthy and Huggett, 1992). Adding fat to diets fed during the last trimester of gestation may improve body condition prior to parturition, and enhance subsequent lactation performance.

Several studies have evaluated the influence of dietary fat on DMI, BW, growth efficiency, blood metabolites, and adipose tissue of growing ruminants. Garrett et al. (1976 a and b) evaluated the influence of formalin-protected polyunsaturated vegetable oil (PO) and tallow (PT) on growing steers in a comparative slaughter trial. Steele (1983) examined the relationship between intestinal uptake of fatty acids and their concentration in blood plasma of sheep

fed diets containing a high oil concentrate mixture. Soybean oil was 8% of the weight in the concentrate of the high oil diet and replaced starch on an isoenergetic basis. Park et al. (1983) fed growing Holstein heifers sunflower seeds as a source of supplemental fat at 0, 10, 20, and 30% of the diet DM to evaluate the effects of dietary lipid on BW, growth efficiency, and blood metabolite patterns. Smith et al. (1984) evaluated the interrelationships of diet, age, fat deposition, and lipid metabolism in growing steers fed corn concentrate or pelleted alfalfa (roughage) diets. The effect of LCFA added to the diet of growing heifers on productivity, rumen function, and changes in body composition was examined by van Houtert et al. (1990). Heifers were fed various levels of calcium salts of LCFA (0, 100, 200, or 300 g per day) in a 2 x 4 factorial design. Lough et al. (1992) evaluated the influence of canola seed and soy lecithin fed to growing ram lambs on total lipid content, cholesterol content, and fatty acid composition of carcass tissues. The relationship between lipid metabolism and ME intake was examined by Smith et al. (1992) with growing heifers fed the same basal diet with varying levels of pelleted ground corn to achieve .76, 1.12, 1.43, 1.74, and 2.05 times the estimated ME requirement. Albro et al. (1993) fed growing steers diets containing WSB, extruded soybeans (ESB), or soybean meal (62%) and rolled barley mixture (38%) (SBM-B) to evaluate the effects of WSB, ESB and SBM-B supplements on feed intake, nutrient utilization, and animal performance of growing steers.

Dry Matter Intake. When steers were fed diets containing either whole sunflower seeds and whole soybeans (PO) or tallow and soybean meal (PT), less DMI and ME were consumed by steers fed the PT and PO diets (Garrett et al., 1976b). Supplement PO contained 70% whole sunflower seeds plus 30% whole soybeans, and supplement PT contained 40% tallow plus 60% soybean meal. The authors attributed the decrease in intake to the lipid component of the diet. Diets contained 10% fat from the supplements, which is two times the level generally recommended for inclusion in diets for growing ruminants (Garrett et al., 1976b). Park et al. (1983) fed growing heifers diets containing 0, 10, 20, or 30% sunflower seeds and concluded that decreased intake in response to sunflower seed supplementation was due to increased concentration of dietary fat. The ether extract (EE) content of the control and 10% sunflower seed diet, 3.6 and 5.4%, respectively, most closely resembles traditional diets (3 to 6%) fed to growing ruminants. Even at this level of dietary fat, Park et al. (1983) noted a decrease in DMI in response to feeding sunflower seeds. Feed intake in steers fed either corn concentrate or alfalfa (roughage) diets was decreased in steers fed the concentrate diet (Smith et al., 1984). However, ME intake for both groups was similar. Although ME intake was similar, NE_g was greater (9.91 versus 6.00 Mcal per d) in concentrate-fed steers. The authors concluded that steers had decreased DMI in response to the greater energy density of the concentrate diet. Forage DMI and total DMI did not differ for heifers supplemented with 100, 200,

or 300 g per d calcium salts of LCFA when compared to heifers fed a control diet (van Houtert et al., 1990). Albro et al. (1993) fed ruminally cannulated steers WSB, ESB or SBM-B supplements and allowed forage (grass hay) intake to vary. When steers were supplemented with WSB they had greater total DMI (expressed as percent of BW) versus when supplemented with ESB, and intakes were similar when supplemented with SBM-B. In a performance study, Albro et al. (1993) fed forty growing steers the same WSB, ESB and SBM-B supplements and noted no differences in forage DMI, but a slight trend for total DMI to decrease when steers were supplemented with WSB and ESB. The slight decrease in DMI was attributed to the increased energy density from the lipid portion of the WSB and ESB. In studies with growing ruminants it appears, therefore, that the addition of small amounts of fat to the diet does not change DMI or has a tendency to decrease DMI. The decrease in DMI appears to be in response to increased energy density of the diet.

Digestion. Van Houtert et al. (1990) evaluated rumen fermentation characteristics of diets containing calcium salts of LCFA when fed to ruminally cannulated steers. They observed a reduction in VFA and ammonia nitrogen concentrations in rumen fluid when calcium salts of LCFA were added to the diet. They attributed the reduction to a decrease in activity and numbers of cellulolytic bacteria when LCFA were added to the diet. However, ruminal fermentation

apparently is not affected in the same manner when whole soybeans supply dietary lipid. Albro et al. (1993) reported that supplement DM and NDF digestibility in growing steers was not affected by WSB or ESB when compared to diets containing SBM-B. There were also no differences in forage DM or NDF digestibility among supplements, suggesting that overall extent of DM and NDF digestibility was not influenced by WSB or ESB. Because digestibility of NDF was not affected by supplementation, adequate fiber content of the diet may have reduced the negative effects of dietary fat provided by the WSB and ESB on fiber digestibility. Ruminal pH was reduced when steers were supplemented with WSB and ESB, the decrease was attributed to greater total VFA concentrations in the rumen when WSB and ESB supplements were fed. Molar proportions of acetate were decreased but the ratio of acetate to propionate (Ac:Pr) was not influenced when WSB and ESB were fed. Fat supplementation increased ruminal ammonia concentrations across all sampling times, however, release was more uniform when steers were fed WSB versus ESB and SBM-B. More uniform release of ruminal ammonia suggested that whole soybeans have a slower rate of degradability than the SBM-B.

Average Daily Gain and Feed Efficiency. The addition of fat to the diets of growing ruminants does not change DMI or has a tendency to decrease DMI. Body weight gain, however, is increased in animals fed fat, thus improving growth

efficiency. Steers fed diets containing fat supplements of either whole sunflower seeds and whole soybeans (PO) or tallow and soybean meal (PT) gained 10% more weight than control-fed steers despite lower DMI (Garrett et al., 1976b). Garrett et al. (1976b) attributed the gain to greater energy density of the supplemented diets and to an increase in efficiency of ME utilization for maintenance and growth. The authors related the improved efficiency of ME utilization to increased efficiency of triacylglycerol synthesis in adipose tissue depots, thus sparing glucose and acetate for other functions. Smith et al. (1984) noted a greater increase in BW and fat gain in steers fed the concentrate diet compared with steers fed the roughage diet despite similar ME intake. The increase in gain was attributed to increased NE_g. The increase in NE_g reflected the nature of the nutrients absorbed from the gastrointestinal tract in animals fed the concentrate diet. In the study with growing steers fed WSB, ESB, or SBM-B supplements, forage DMI was not affected by treatment, but, a trend for lower total DMI for steers fed ESB and WSB compared to steers fed SBM-B was observed and attributed to the greater energy density of WSB and ESB (Albro et al., 1993). Supplementation with ESB or WSB improved average daily gain (ADG) and feed efficiency (kg DMI per kg BW gain) by nearly twofold compared to steers fed the SBM-B supplement despite a trend for lower DMI.

Park et al. (1983) reported improved growth efficiency (kg BW gain per kg DMI) for heifers fed increasing levels of sunflower seed (0, 10, 20 or 30% of diet

DM). The improved growth efficiency with increasing level of supplementation was inversely related to DMI. As noted earlier the EE of the 0 and 10% sunflower seed diets (3.6 and 5.4%) most closely resemble diets traditionally fed to growing ruminants. When heifers were fed 10% sunflower seed, growth efficiency was increased to 15.2% compared with 12.8% for the 0% sunflower seed diet. The authors attributed the improvement in growth efficiency to improved digestibility of dietary components resulting, in an increase in digestibility of nitrogen, lipid, and energy. Supplementing growing ruminant diets with fat appears to enhance growth despite tendencies for decreased DMI. The increase in growth efficiency appears to be due to an improvement in nutrient utilization.

Blood Metabolites. Blood lipids including, triacylglycerol, total cholesterol and NEFA appear to be altered in response to feeding supplemental dietary fat to ruminants. The most notable alterations in blood lipids are seen as the amount of LCFA flowing from the rumen to the small intestine for absorption increases substantially (Palmquist and Jenkins, 1980; Sutton et al., 1970). For example, Park et al. (1983) reported a linear increase in total blood lipid, total cholesterol, triacylglycerol, and NEFA in heifers fed diets containing increasing levels of sunflower seed, with the greatest concentrations seen in heifers receiving the greatest amount of sunflower seed (20 and 30% of diet DM). The increase in

cholesterol was proportional to the increase in total blood lipid, suggesting that cholesterol accounted for the total lipid response to increasing dietary fat. Total lipid was greater, 512 mg per dL, in heifers fed 10% sunflower seeds when compared to heifers fed 0% (417 mg per dL) sunflower seed. Total cholesterol was 148 mg per dL for heifers fed 10% sunflower seed versus 99 mg per dL in heifers fed 0%. Therefore, even increasing dietary EE from 3.6 to 5.4% increases total blood lipid and cholesterol. The authors reasoned that the increase in plasma lipids with fat supplementation represented a greater transfer of dietary lipid to the blood. The diets containing fat also caused a concurrent reduction of blood glucose concentration. The authors concluded that fatty acids from sunflower seeds were apparently metabolized as either ketogenic or lipogenic substrate, therefore, decreasing availability of gluconeogenic nutrients in heifers fed diets containing fat, thereby, influencing the synthesis of glucose.

Steele (1983) further evaluated the relationship between intestinal uptake of fatty acids and their concentrations in blood lipids in ruminally and duodenally cannulated sheep fed a concentrate mixture containing 8% soybean oil. He found that the major LCFA were approximately 90% digested, indicating dietary fatty acids can be absorbed efficiently by ruminants. There was a linear relationship between uptakes of 16:0, 18:0, and 18:2 from the small intestine and their concentrations in triacylglycerol and triacylglycerol-free lipids (cholesterol and NEFA). There was also a linear relationship between 18:1 and its concentration

in triacylglycerol and triacylglycerol-free lipids, however the slope of the regression line was much greater than the other three fatty acids. Smith attributed the increase in 18:1 to the desaturation of 18:0 to 18:1 in the intestinal wall. The addition of soybean oil to the diet increased the proportion of fatty acids absorbed from the lower gut. He attributed the increase in fatty acid absorption to greater intake of fatty acids provided by the soybean oil. Concentration of fatty acids in plasma triacylglycerol had a much greater dependence on intestinal uptake than other plasma lipids such as NEFA and cholesterol. Steele et al. (1983) concluded that the relative amounts of each fatty acid absorbed by the intestine determines composition of plasma triacylglycerol and hence composition of depot fats of ruminants. Therefore, fatty acid composition of dietary fat can potentially influence adipose tissue composition. The extent of the diet's influence appears to be dependent on the level of supplementation, amount of fatty acids reaching the small intestine, rate of absorption of each fatty acid from the small intestine, and fatty acid composition of plasma triacylglycerol.

Adipose Tissue Metabolism. Studies previously cited (Garrett et al., 1976a and 1976b; Steele, 1983; Lough et al., 1992) also evaluated the influence of dietary fat on amount and fatty acid composition of adipose tissue. Lough et al. (1992) reported an increase in total lipid content and amount of subcutaneous

adipose tissue from lambs fed canola seed, soy lecithin and a combination of the two fat sources. Canola seed tended to increase total cholesterol content of subcutaneous adipose tissue, whereas soy lecithin tended to decrease total cholesterol content. Lough et al. (1993) showed in a later study with growing lambs fed palm oil as a source of dietary fat that increases in adipose tissue were the result of greater efficiency of conversion of dietary fat to body fat in contrast to the conversion of acetate and glucose to body fat. The alteration in adipose tissue composition was attributed to a decrease in 16:0 fatty acids and a concurrent increase in 18:0 and 18:1 fatty acids as the result of feeding canola seed, soy lecithin and combinations of canola seed and soy lecithin.

Fatty acid composition of adipose tissue may more closely reflect dietary fatty acid composition if whole oilseeds are fed. Ekeren et al. (1992) reasoned that in the whole seed form, fatty acids in sunflower seed were less susceptible to ruminal biohydrogenation, and therefore, greater amounts of unsaturated fatty acids should reach the small intestine for absorption. Other forms of unsaturated dietary fat also alter adipose tissue fatty acid concentrations in sheep and cattle (Faichney et al, 1972; Garrett et al., 1976a; Garrett et al., 1976b; Steele et al., 1983; van Houtert et al., 1990; Lough et al., 1992). Specifically, these studies reported higher concentrations of unsaturated fatty acids in adipose tissue in animals fed diets supplemented with partially-protected, unsaturated fat. Results are consistent with studies concerning nonruminants fed diets containing

unsaturated fat (Leveille, 1970). In nonruminants, dietary lipids generally decrease lipogenic activity in adipose tissue by depressing lipogenic enzyme activity and increasing uptake of dietary fatty acids into tissue triacylglycerol (Leveille, 1970). The result is a greater incorporation of dietary fatty acids into adipose tissue. Lough et al. (1992) reported a decrease in total saturated fatty acids in subcutaneous adipose tissue in growing lambs fed canola seed or soy lecithin as unsaturated fatty acid sources. Garrett et al. (1976a) found the major change in fatty acid composition of subcutaneous adipose tissue of lambs fed a commercially-prepared, formalin-protected, unsaturated fat at 40% of diet DM was an increase in 18:2 and decreases in palmitic, palmitoleic and oleic acids. Data from steers fed the same supplement at 33% of diet DM indicated that the amount of 18:2 stored in body fat was positively correlated with consumption and absorption of linoleic acid. Additionally, the greater linoleic acid concentration was associated with lower concentrations of 14:0, 14:1, 16:0, 16:1, and 18:1 in subcutaneous fat. Garrett et al. (1976b) reported an increase in incorporation of dietary unsaturated fatty acids into adipose tissue after feeding steers diets containing whole sunflower seed and WSB (PO) in a concentrate mix. The steers had greater 18:2 content with decreased 16:0, 16:1, and 18:1 of subcutaneous fat compared to control and PT fed steers. The same whole sunflower seed plus WSB and tallow plus soybean meal were added to diets of growing lambs and steers (Yang et al., 1978). Yang et al. (1978) reported a decrease in synthesis of

adipose fatty acids from acetate and an increase in uptake of preformed fatty acids from blood triacylglycerol, as indicated by increased lipoprotein lipase activity. There was also a tendency for increased *in vitro* free fatty acid release from adipose tissue of fat-fed animals. Increased free fatty acid release from adipocytes apparently reflects a decrease in fatty acid esterification in fat-fed animal. Yang et al. (1978) confirmed this by measuring glucose incorporation into triacylglycerol-glyceride as an index of fatty acid esterification and noted a trend for lower glucose incorporation in fat-fed animals. The primary response to dietary fat, however, was decreased *in vitro* rates of incorporation of acetate and lower specific activities of isocitrate dehydrogenase and glucose-6-phosphate dehydrogenase. Isocitrate dehydrogenase and glucose-6-phosphate dehydrogenase provide $NADPH_2$ for fatty acid synthesis. Decreased enzyme activity coupled with decreased rates of acetate incorporation suggests a decrease in de novo synthesis of fatty acids for adipose tissue in response to fatty acids provided by the diet.

The extent of dietary LCFA incorporation into the adipose tissue of ruminants is dependent on age, level of intake, balance of nutrients available to the animal, and physiological state of the animal (van Houtert et al., 1990). The conclusions were based on previous work in which Pothoven et al. (1975) provided ad libitum and restricted access to diets containing corn grain and extruded soybean oil at 9% of the diet DM to growing steers. Steers with ad libitum access had greater amounts of subcutaneous adipose tissue and had greater rates of

acetate incorporation into LCFA up to 505 kg BW, the point at which rates of incorporation began to decline. Pothoven et al. (1975) reasoned that rate of fatty acid synthesis decreased with increasing fatness. Therefore, the addition of fat to the diet may increase the efficiency of adipose tissue fatty acid synthesis by decreasing the need for acetate.

Regulation of Adipose Tissue Metabolism

Adipose tissue metabolism is an important contributor to ruminant energy expenditures (Baldwin et al., 1976). Baldwin et al. (1976) estimated a cow weighing 550 kg, in energy balance, with an ME intake of 43.8 Mcal per d that 4.5 and .5% of a cows daily energy expenditure can be attributed to lipogenesis and lipolysis plus triacylglycerol resynthesis, respectively, in adipose tissue. Maintenance requirement for the same cow was 12.5 Mcal per d and milk energy output was 20.8 Mcal per d. Similarly, in a growing steer gaining .42 kg of fat per d, adipose energy expenditures equal 9.1% of total heat production (19.1 Mcal). The estimated contributions of maintenance, lipogenesis and lipolysis plus triacylglycerol resynthesis are 2.4, 5.5 and 1.2% respectively. The contribution of adipose tissue to whole animal lipogenesis for the lactating cow and the growing steer are 54 and 85%, respectively. The amount of fat in the diets of ruminants can significantly affect adipose energy expenditures. The efficiency of conversion to triacylglycerol for absorbed dietary fat and for acetate are 96% and 72%

respectively. Considering fermentation losses from the conversion of dietary carbohydrates to acetate and the efficiency of converting acetate to fatty acid, the estimate of efficiency of converting dietary carbohydrate to fatty acid decreases to about 50%. Therefore, the efficiency of converting dietary fat to body fat is much greater than converting dietary carbohydrate to fat. Garrett et al. (1976b) reported that efficiencies of conversion of ME available for gain were 43.8% for control diets and 59.1% for a diet containing 25% of commercially-protected (formalin) tallow supplement. Therefore, the addition of fat to the diet can improve efficiency of lipid deposition in growing and lactating animals (Baldwin et al., 1976). Baldwin et al. (1976) based their conclusions on results from Leveille (1970), which indicated a depression in adipose tissue lipogenesis in fat-fed rats. Lipogenic activity, measured as glucose-6-phosphate dehydrogenase activity was inversely related to fat content of the diet.

Flatt et al. (1970) proposed that the maximum rate of glucose converted to fatty acid in nonruminants is limited by generation of sufficient ATP and $NADPH_2$ from glucose for fatty acid synthesis. Therefore, Flatt et al. (1970) reasoned that rates of ATP and $NADPH_2$ utilization for maintenance functions of adipose tissue might be determined by ADP and NADP availability for fatty acid synthesis. The pentose phosphate pathway, isocitrate dehydrogenase and the malic enzyme system generate 64, 32 and 4%, respectively, of the $NADPH_2$ required for fatty acid synthesis in the ruminant (Smith and Crouse, 1984). To

sustain higher rates of fatty acid synthesis, basal metabolism and tricarboxylic acid cycle activity would have to increase to provide additional ATP and $NADPH_2$. Flatt et al. (1970) suggested that tricarboxylic acid cycle activity, as determined in part by basal metabolic rate and (or) glucose availability, limits the rate of ruminant adipose lipogenesis, because they reported that ruminant adipocytes were highly dependent on glucose availability when synthesizing fatty acids from acetate *in vitro*.

Age and Physiological State. Adipose tissue undergoes metabolic adaptations during pregnancy and lactation. These adaptations include anabolism during mid pregnancy followed by a shift to catabolism in late pregnancy and a dramatic catabolic shift in early lactation. Blood lipid patterns and adipose tissue lipogenesis, lipolysis and enzyme activities change to accommodate pregnancy, lactation, or growth. The changes are due in part to changes in blood hormone patterns that accompany each physiological state. Very early pregnancy is characterized by lipid deposition, whereas lipid mobilization is typical prior to parturition. A possible explanation for increased lipogenesis in early pregnancy is increased insulin secretion in response to increased feed intake. Lipolysis in late pregnancy is more difficult to explain but may be due to increased concentrations of pituitary hormones and glucocorticoids in maternal blood (Baldwin et al., 1976).

Baldwin et al. (1976) further explained that early and peak lactation represent a period of lipid mobilization along with depletion of blood triacylglycerol in adipose cells, elevated blood NEFA, and elevated total blood lipid. At peak lactation (3 to 6 wk post calving), blood NEFA concentrations decrease due to the increased removal of lipids from the blood by the mammary gland. Lipogenic activity in adipose tissue increases to accompany the onset of lactation as indicated by rates of incorporation of acetate into lipids. Lipolytic activity in adipose tissue increases in response to the onset of lactation as indicated by rates of incorporation of acetate into lipids, but lipolytic capacity also is elevated as indicated by rates of glycerol and fatty acid release from adipocytes incubated with and without epinephrine (Yang and Baldwin, 1973). The changes in adipose tissue, increased lipogenesis and lipolysis with an emphasis on lipid mobilization, are in support of lactation and energy deficit during early and peak lactation.

In an *in vitro* study with adipose tissue slices from adult (2 to 4 years of age), pregnant (7^{th} to 8^{th} mo of first pregnancy at about 2 years of age), virgin (12 to 16 months of age), and lactating (mid-lactation and 2 to 4 years of age) nonpregnant cows, Baldwin et al. (1973) characterized adipose tissue metabolism as related to age and physiological state by evaluating enzyme activity. Activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were slightly lower in pregnant and lactating animals and lower in adult versus virgin animals. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate

dehydrogenase are responsible for providing $NADPH_2$ for fatty acid synthesis through the pentose phosphate pathway. Enzyme activities tend to decrease with age as exhibited by consistently lower activities in adult animal adipose tissue compared to samples from virgin animals (Baldwin et al., 1973 and Smith et al., 1984). Pregnancy and lactation apparently retard this trend. Decreased enzyme activity in adipose tissue from pregnant and lactating animals is most likely attributed to age rather than pregnancy or lactation because enzyme activities in the adult nonpregnant, nonlactating adipose tissue are similar to those in the pregnant and lactating animals. Enzyme activities cannot be attributed either to feed intake or energy status because the pattern of activity is not consistent with these characteristics. Adult nonlactating and lactating groups likely represent the two extremes of energy excess and energy deficit, yet have similar patterns of enzyme activity.

Further evaluation of adipose tissue metabolism from lactating and nonlactating cows characterized rates and patterns of substrate oxidation (Baldwin et al., 1973). Lactating cows fed a diet containing 40% concentrate and 60% alfalfa were compared to cows fed the same diet but injected with 750 to 1,000 units of insulin daily for 4 wk and cows fed a 100% concentrate diet. Rates of acetate-1-^{14}C and glucose-1-, -2-, -6-, and -U-^{14}C oxidation were lower in nonlactating than lactating cows. Rates of glucose-1- and -2-^{14}C oxidation were not affected by dietary treatment of lactating cows but there was an apparent

increase in glucose-6-^{14}C oxidation in cows fed the 100% concentrate diet. Relative rates of glucose oxidation were 1.0, .5 and .25 for glucose-1-,-2-, and -6-^{14}C, respectively. Baldwin et al. (1973) concluded that these rates of oxidation indicated that the major route of glucose oxidation in cow adipose tissue is the pentose phosphate pathway in which considerable recycling occurs. Acetate oxidation, however, was increased by insulin treatment.

Rates of utilization of glucose and acetate for lipid synthesis in adipose tissue slices also were evaluated. When considering radioactivity incorporated into triacylglycerol 95% of the glucose carbon was incorporated into glyceride-glycerol; whereas, 100% of the acetate carbon was incorporated into fatty acids. Therefore, Baldwin et al. (1973) concluded that glucose incorporation into lipids is an index of fatty acid esterification and acetate incorporation is an index of fatty acid synthesis. Triacylglycerol and fatty acid synthesis was twofold greater in lactating tissue compared to nonlactating adipose tissue.

Hormonal Regulation. The primary acute effectors of adipose tissue function include insulin, glucagon, and catecholamines. Acute responses occur within minutes of hormonal stimulation and are short in duration. Catecholamines bind to receptors closely associated with adenylate cyclase on the cell membrane. The number of receptor sites per cell varies with diet and physiological state. Binding of glucagon and catecholamines to the cell receptor increases adenylate cyclase activity and, thus, the formation cAMP (adenosine-3',

5' cyclic monophosphate). The increase of cAMP activates protein kinase, which in turn activates triacylglycerol sensitive lipase to increase triacylglycerol hydrolysis. Insulin, acting acutely, decreases tissue cAMP either by inhibiting adenylate cyclase, increasing cAMP phosphodiesterase activity, or both. These acute actions of insulin may be responsible for its antilipolytic effect and partially responsible for its lipogenic effect (Baldwin et al., 1976). Insulin as a chronic effector of adipose tissue decreases lipolytic activity and increases lipogenic capacity. Chronic responses are evident over a period of time, and induce changes in basal metabolism of a tissue or response to acute hormonal stimuli. Insulin's primary lipogenic actions include increased capacity for incorporation of glucose and acetate into triacylglycerol, increased glycerol-3-phosphate dehydrogenase activity, and increased acetyl-CoA carboxylase activity.

Etherton et al. (1986) further investigated the role of insulin in lipogenesis with bovine adipose tissue *in vitro*. The authors found that synthesis of triacylglycerol from acetate and glucose was increased by insulin in the short-term (1 to 3 h). Lipogenic capacity decreased markedly when adipose tissue was cultured *in vitro* for 48 h without insulin, but addition of insulin maintained lipogenic capacity. Hydrocortisone did not affect lipogenesis in short-term incubations, however, it greatly affected lipogenesis in combination with insulin after 48 h. Hydrocortisone alone decreased the lipogenic activity of adipose tissue, but hydrocortisone plus insulin increased the ability of insulin to maintain

lipogenic activity compared to insulin alone. The lipogenic response to hydrocortisone and insulin was related linearly to the concentration of insulin, and indicates the importance of insulin in chronic regulation of adipose tissue lipogenesis. Hydrocortisone and insulin act synergistically to maintain the lipogenic enzyme mass, because in the absence of hydrocortisone and insulin enzyme mass decreases. The potentiated lipogenic ability of insulin in the presence of hydrocortisone, also may have been due to an increase in the number of insulin receptors.

High fat diets apparently decrease insulin sensitivity and responsiveness, resulting in insulin resistance *in vivo* and *in vitro* (Watarai et al., 1988). *In vivo* studies (Maegawa et al., 1986) showed a decrease in glucose clearance during continuous infusion of glucose and insulin in rats fed a high fat diet. High fat diets caused increased plasma insulin concentration, no difference in glucose concentration, and decreased insulin binding as compared to control fed rats. However, insulin receptor numbers for both groups were comparable (Watarai et al., 1988). Decreased insulin binding appears to be due to decreased affinity of insulin to the receptor for insulin. Watarai et al. (1988) also reported decreased responsiveness to insulin stimulation as measured by glucose uptake and concluded that high fat diets induced insulin resistance manifested by hyperinsulinemia. Watarai et al. (1988), however, hypothesized that high fat diets induced a decrease in insulin binding to adipocytes due to an alteration in lipid

composition and viscosity of the plasma membrane rather than a decrease in insulin receptor affinity.

Whole Raw Soybeans for Lactation Diets

Although, WSB was not added to the lactating cow diet in the present study, data from lactating cows fed WSB is useful in evaluating the influence of fat provided by WSB on DMI, digestion, rumen environment, blood plasma metabolites and milk production. Whole raw soybeans are added to the diet of lactating cows as a source of fat and protein. Increased intake of LCFA in response to added dietary fat improved metabolic efficiency of energy utilization for milk production (Schauff et al., 1992). Long chain fatty acids provided by the diet can be incorporated directly into milk fat, which decreases energy expended to synthesize fatty acids, sparing glucose and acetate for other functions of the mammary gland (Schauff et al., 1992).

Previous studies with lactating cows have evaluated the influence of WSB on DMI, digestion, ruminal fermentation, and milk production. Mohamed et al. (1988) fed early-and mid-lactation cows diets containing soybean or cottonseed meal; soybean meal and soybean oil or cottonseed meal and cottonseed oil; whole soybeans or cottonseeds; roasted soybeans or roasted cottonseed. The CP content of all diets was 16.5%. Bernard (1990) fed 24 multiparous Holstein cows diets

containing soybean meal (control), whole raw soybeans, or roasted soybeans to determine the influence of raw or roasted soybeans on lactation performance. The effects of WSB and tallow on ruminal fermentation, apparent total tract nutrient digestibilities, energy and N utilization, milk production, and milk composition were evaluated by Schauff et al. (1992). Five mid- to -late lactation cows were fed either control, WSB, whole roasted soybeans, cracked roasted soybeans, or ground roasted soybeans in a digestion trial to determine the effects of roasting WSB and effects of particle size of roasted soybeans on ruminal fermentation, nutrient digestibility, and cow performance (Tice et al., 1993)

Dry Matter Intake. In digestion trials with lactating cows, Mohamed et al. (1988), Bernard (1990), Schauff et al. (1992), and Tice et al. (1993) found conflicting results concerning the effect of WSB on DMI when added to the diet as a fat supplement. Mohamed et al.(1988) reported a decrease in total DMI when diet DM contained 20% WSB or 16.5% whole cotton seed, as compared with cows fed diets containing 20% roasted soybeans or 16.5% roasted cottonseed. However, due to the higher energy density of the WSB diet, calculated net energy intake was not decreased. In contrast, Bernard (1990) fed lactating cows isocaloric, isonitrogenous diets containing 21% soybean meal in diet DM, 9.4% WSB, or 9.4% roasted soybeans and found no differences in DMI due to dietary treatment. Ether extract and ADF intakes, however, were greater in cows fed

WSB due to greater EE and ADF in WSB. Schauff et al. (1992) fed diets containing 2.5% tallow, 10% WSB or combinations of the two fat sources (10% WSB plus 2.5% tallow or 10% WSB plus 4% tallow). Schauff et al. (1992) reported that DMI was not affected by source or amount of dietary fat. Tice et al. (1993) fed diets containing 19.7% of either WSB, roasted soybean, cracked soybeans, or ground soybean. Dry matter intake did not differ for cows fed control versus soybean diets, WSB, or roasted soybeans, thus confirming Bernard's (1990) observations.

Digestion. Substituting unsaturated fat for part of the concentrate in a diet increases energy density of the diet but can have adverse affects on fiber digestion. Rumen microbes can be partially protected from the adverse affects, however, by feeding whole oilseeds. In the previously cited study by Mohamed et al. (1988), rumen pH and ammonia were not affected by fat source when WSB and whole cottonseed were compared to roasted soybean and roasted cottonseed. The EE content of the WSB diet was 11.6 % compared with 2.1% in the soybean meal diet. The EE content was 10.8, 12.6, and 12.7% for whole cottonseed, roasted cottonseed, and roasted soybean diets, respectively. Acetate and propionate, as a proportion of total ruminal VFA, were not affected by diet; however, concentrations of butyrate were reduced. Ruminal Ac:Pr also was decreased due to the addition of oilseeds to the diet. The reduction in Ac:Pr with

fat supplementation was attributed to a reduction in fiber digestion and decreased numbers of methanogenic bacteria. Bernard (1990) fed cows diets containing soybean meal, WSB, or roasted soybean to evaluate the influence of processing procedures on apparent nutrient digestibilities, milk production and milk composition. The EE content of the soybean meal diet was 2.8% and 3.7% for the WSB diet. Apparent digestibility of EE, ADF, and NDF were lower ($P <$ .01) and DM and CP tended ($P < .10$) to be lower in cows fed soybean meal versus soybeans. Dry matter, ADF and CP digestibilities tended to be greater in cows fed WSB versus cows fed roasted soybean. Addition of fat to the diets of lactating cows may be a means of reducing the negative effects of high starch diets on fiber digestion, because the increased fiber digestibility of diets containing soybeans was apparently due to greater fiber and lower starch content compared with the SBM diet. Increased apparent EE digestibility for diets containing WSB was due to greater EE intake for cows fed WSB versus soybean meal.

Schauff et al. (1992) found that lactating cows consuming diets containing WSB, tallow, or combinations of tallow and WSB had lower concentrations of total VFA in ruminal fluid. Additionally, WSB in the diet tended to decrease proportions of acetate and increase propionate, resulting in a decreased Ac:Pr. Total tract digestibilities of DM, OM, cellulose, and CP decreased when WSB was fed; whereas, digestibilities of ADF, NDF, and hemicellulose were not altered. The apparent digestibilities of total C18 fatty acids and total LCFA were

decreased by supplemental fat. The authors attributed the decrease in fatty acid digestibilities to the degree of biohydrogenation in the rumen and concentration of fat in the diet. The gross energy for the WSB diet was 4.72 Mcal per kg, 4.87 Mcal per kg for the WSB plus 2.5% tallow, 4.92 Mcal per kg for the WSB plus 4.0% tallow compared with 4.58 Mcal per kg for the control diet. They reasoned that biohydrogenation of unsaturated fatty acids in the rumen apparently provided greater flow of 18:0 to the small intestine, which in turn decreased the overall digestibility of the fatty acids in the gastrointestinal tract.

Tice et al. (1993) fed diets containing 19.7% WSB, whole roasted soybean, cracked soybean, or ground soybean. Overall, diets contained 15%CP, 35% NDF, 19% ADF, and 1.74 NE_L Mcal per kg. A control diet was used for comparison and contained 15.7 % CP, 30.6% NDF, 16.8% ADF, and 1.78 NE_L Mcal per kg. There was no effect due to roasting soybeans on ruminal pH. However, cows had greater ruminal pH 9 h after feeding WSB than after feeding whole roasted soybeans. Ruminal NH_3 N tended (P = .06) to be greater at 9 h after feeding and was greater at 12 h post-feeding when cows were fed WSB versus whole roasted soybean. The higher NH_3 N was attributed to greater protein degradability (lower UIP) of the WSB. When fed the control diet, cows had a lower molar percentage of acetate and higher propionate than when fed WSB diets. The increase in propionate was attributed to the greater starch content of the control diet. Intestinal and total tract digestibilities of OM were similar

among treatments. Intake and total tract apparent digestibility of NDF was lower when the control diet was fed compared to when cows were fed the WSB diets. The authors hypothesized that the soy hulls associated with the WSB may have been more digestible than the fiber sources they replaced in the control diet, which may partially account for the decrease in total tract digestibility of the control diet.

Blood Metabolites. Adding partially protected fat to the diets of ruminants increases the amount of LCFA and triacylglycerol flowing to the small intestine for absorption (Sutton et al., 1970). In studies with lactating cows fed WSB, blood serum concentrations of glucose and insulin were not affected; however, percent unsaturated LCFA (% 18:1 plus % 18:2 plus 18:3) in triacylglycerol was increased (Mohamed, et al. 1988). Plasma amino acid concentrations also were reduced by feeding WSB. The decrease was attributed to a decrease in microbial protein synthesis due to inhibition of microbial growth by 18:1 fatty acids or their precursors (Mohamed, et al. 1988). Schauff et al. (1992) reported NEFA concentrations in blood plasma increased with increasing amount of dietary fat. Glucose concentration, however, was reduced when cows were fed diets containing WSB, tallow, or combinations of the two fat sources (10% WSB plus 2.5% tallow or 10% WSB plus 4% tallow). Mohamed et al. (1988), Bernard

(1990), and Schauff et al.(1992) concluded that blood lipids increased due to the increased absorption of fat from the gastrointestinal tract.

Milk, Milk Fat and Milk Protein Production. Dietary fat supplementation improves efficiency of milk production by providing preformed fatty acids for milk triacylglycerol synthesis and sparing glucose and gluconeogenic precursors for other metabolic functions. Milk fat and milk protein depression are concerns when feeding fat, particularly with unsaturated fat sources such as WSB.

Milk yield and milk fat percentage were not effected by the addition of WSB to the diet, however milk protein percentage was reduced (Mohamed et al., 1988). The authors attributed the decrease in protein percentage to a reduction in milk casein fraction. In the case of WSB and roasted soybean, whey proteins were also reduced possibly as a result of decreased plasma amino acids. Additionally, Bernard (1990) observed no differences in milk yield, percentages of milk fat or milk protein when raw and roasted soybeans were fed, indicating there were no benefits to roasting soybeans prior to their addition to the diets. When Schauff et al. (1992) compared the effects of WSB, tallow and combinations of the two fat sources on milk and milk protein production, there were no changes due to the addition of fat to the diet. Milk fat percentage and milk fat yield, however, tended (P = .06) to increase when WSB was fed.

Studies with lactating cows fed WSB help evaluate the influence of WSB on DMI, digestion, rumen fermentation and efficiency of either milk production or growth. When evaluating lactating cow data, differences in intake, rumen environment, and metabolic demand need to be kept in mind. Lactating cow DMI is greater than pregnant nonlactating cow DMI. The metabolic demands of milk production on lactating cows is also much greater than the demands of growth and pregnancy. In spite of the differences in DMI and physiological state, WSB appears to improve efficiency of triacylglycerol synthesis by providing preformed LCFA. Improvements in triacylglycerol synthesis efficiency spares glucose and acetate for other metabolic functions such as growth or milk production.

MATERIALS AND METHODS

Animals

Sixteen pregnant Holstein heifers with expected calving dates within a 100 d range were paired according to DHIA estimated average transmitting ability for milk, milk fat, and milk protein production. One heifer from each pair was assigned to the control diet and the other assigned to the WSB diet. Heifers were housed in a free-stall barn with access to a Pinpointer feeder (4000B, AIS Corp., Cookeville, TN) in order to monitor daily intake for 2 wk prior to the start of the study. At this time all heifers were fed the control diet. Heifers then were assigned according to diet to one of two pens, each with access to a Pinpointer feeder, in a counter-slope facility at 90 d prior to parturition. At 30 d prepartum, heifers were moved into one of two larger pens in which they continued to receive their assigned diet, but feed intake was not monitored. At 10 d prepartum, heifers were moved to a maternity lot where they were adjusted to a lactation diet. Approximately 2 d prior to parturition heifers were moved to individual box stalls and remained there until 24 h after calving. All heifers were housed, fed, and milked in a single group until 35 DIM. Dry matter intake during lactation was measured from 21 to 35 DIM in a free-stall barn with a Pinpointer feeder.

Diets

Control and WSB diets contained (DM basis) 23.7% corn silage, 61% orchardgrass hay, and 15.3% concentrate mix (ground corn, soybean meal, and trace-mineralized salt). Whole raw soybeans were substituted for all soybean meal and a small amount of corn grain to obtain a protein content in the WSB diet similar to the control diet. Water containing 10% liquid molasses was added prior to feeding to increase palatability. Heifers were fed 50% of their daily allotment at 0600 and 1300. Ingredient and chemical composition of the diets are given in Table 1. All diets were formulated to meet or exceed NRC (1989) requirements for heifers gaining .7 kg per d.

The lactation diet (Table 2) was formulated for a 590 kg cow producing 36 kg milk per d with 3.5% butterfat. Undegraded intake protein content of the diet was 40%, 5% above the NRC (1989) recommendations due to the protein sources provided. Supplemental fat was not added to the lactation diet in order to evaluate the influence of gestation diet on lactation performance.

Measurements and Sampling

Forages and concentrate mixtures (control and WSB) were sampled every 3 wk throughout the gestation feeding period. Forages and high-moisture corn in the lactation diet were sampled at 3 wk intervals during the period when the lactation diet was fed. Concentrates for the lactation diet also were sampled

Table 1. Ingredient and chemical compositon of diets fed prepartum[a]

	Control	WSB
Ingredient		
Corn silage	23.7[b]	23.7
Orchardgrass hay	61.0	61.0
Ground corn	5.4	4.0
Soybean meal, 44% CP	9.4	---
Whole raw soybeans	---	10.8
Molasses	.4	.4
Trace-mineralized salt[c]	.1	.1
Chemical Composition[b,d]		
DM, %	70.5	70.6
CP	12.6	12.2
ADF	33.2	33.9
NDF	58.8	59.5
Ether extract	3.1	4.7
DE, Mcal/kg of DM[e]	2.75	2.78

[a]Heifers were fed experimental diets beginning 90 d prepartum.

[b]Percent of diet DM, unless otherwise indicated.

[c]Trace-mineralized salt contained: NaCl, 97.3%; Zn, .35%; Fe, .34%; Mn, .20%; Cu, .033%; I, .007%; Co, .005%.

[d]Based on averages of triplicate analyses of forages and concentrate. Ten samples of each were composited.

[e]Estimated from NRC.

Table 2. Ingredient and chemical composition of lactation diet.

	Lactation diet
Ingredient	
Corn silage	20.0[a]
Alfalfa silage	41.6
High-moisture corn	28.5
Concentrate	
Blood meal	3.9
Soybean meal, 44% CP	3.3
2:1 mineral[b]	2.6
Chemical Composition[c]	
DM, %	57.4
CP	17.7
ADF	19.3
NDF	29.9
Ether Extract	3.4
NE_L, Mcal/kg of DM[d]	1.58

[a]Percent of diet DM, unless otherwise indicated.
[b]2:1 mineral contained: Ca, 19%; P, 10%; Mg, 5%; S, 2%; K, .90%; Fe, .56 %; Cu, .14% ; Mn, .46%; Zn, .6%; Co, .014%; Se, .001%.
[c]Based on averages of triplicate analyses of concentrates, for which five samples were composited for analysis, and separate (five of each) analyses of forages and high-moisture corn.

every 3 wk, but samples were composited for analysis. Dry matter content was determined by oven drying at 60°C for 48 h. Dry samples were ground through a 1 mm screen (Thomas Wiley Laboratory Mill). Equal (10 g) aliquots of feed samples were composited. Samples were stored in sealed containers until determination of chemical composition. All feed samples, with the exception of the lactation diet forages, were analyzed in triplicate. Lactation diet forages were analyzed by Virginia Tech forage testing lab in duplicate.

Although, feed intake was measured daily by a Pinpointer feeder, daily intakes for the last 5 d of each week were used to calculate weekly averages. Individual intake determined prior to the start of the experimental period was used as a covariate in the statistical analysis to evaluate treatment effects on DMI between 90 and 30 d prior to calving and 21 to 35 DIM.

Heifers were weighed every week prior to the 1300 feeding. Body weights from each of the 2 wk prior to starting the experimental period were averaged to determine an initial weight. After moving the heifers to the maternity lot, body weights were recorded for three consecutive days and averaged to determine a final gestation weight. On the day of parturition, weights of each heifer (after expulsion of the placenta) and calf (prior to first feeding) were recorded.

Blood samples were obtained by jugular venipuncture at 0600, 1200, 1800 and 2400 (4x per d) starting 90 d prepartum, then every 3 wk during gestation, on the day of calving, and at 35 DIM. Blood (12 mL) was transferred

from syringe to tubes containing 572 IU heparin in .1 mL and centrifuged at 3,200 x g for 10 min. Plasma was removed and stored at -70°C until glucose and NEFA analyses.

Insulin challenges were given via indwelling jugular catheter at 90 d prior to calving (-90 d), 30 d prior to calving (-30 d) and 35 DIM (+35 d). Heifers were catheterized on the day of challenge and allowed to rest for 2 h prior to challenge. Four basal blood samples (-20, -15, -10, and -5 min) were taken prior to injection of insulin (.26 IU per kg BW). Following insulin injection, blood samples were taken every 5 min for the first 45 min, then every 15 min until 150 min. Blood was transferred from syringe to tubes containing 572 IU heparin and centrifuged at 3,200 x g for 10 min. Plasma was removed and frozen at -70°C until glucose, NEFA, triacylglycerol, and fatty acid analysis.

Body condition was evaluated using ultrasound prior to insulin challenge at -90 d and -30 d, then once a week between 7 and 35 DIM. Measurements were at the interface between the 12^{th} and 13^{th} rib over the longissimus muscle and on the caudal point of the buttock according to Miles et al. (1983). Measurements were taken on alternating sides of each animal.

Subcutaneous adipose tissue biopsies were obtained on the day following an insulin challenge during gestation (-90 and -30 d). Tissue samples were taken from the rump region (5 to 15 cm from the tail head and 6 to 12 cm from the dorsal midline). The heifers were given .05 mL xylazine (20 mg per mL) via

jugular venipuncture and 6 mL of Lidocaine hydrochloride (2%) administered in an inverted "L" shape prior to incision. Adipose tissue samples were washed with sterile saline (.09% NaCl) and frozen at -70°C until fatty acid analysis.

Chemical Analyses

Acid detergent fiber and NDF were determined using the method of VanSoest et al. (1991). To avoid overestimating NDF concentration in concentrates, .5 g samples were treated with 50 mL of 8 M urea plus 50 μL of amylase (A3306 dietary fiber kit; Sigma) for 5 min at 80 to 90°C. Samples then were incubated for 4 h at room temperature, diluted with 50 mL of NDF solution, boiled for 1 h and filtered through tared glass crucibles. Nitrogen was determined using the Kjeldahl procedure (AOAC, 1990).

Ether extract was determined (AOAC, 1990) using .5 g of composited samples of forages and concentrate mixtures. Samples were diluted with petroleum ether (50 mL), placed on EE apparatus (Soxtec System HT 1043 Extraction unit, Tecator Inc., Switzerland), allowed to boil during extraction for 30 min, and rinsed for 1 h. Ether was evaporated for 15 min before placing thimbles into a drying oven for 2 to 3 min at 105°C. Samples were allowed to cool in a desiccator, then weighed.

Glucose and NEFA concentrations in blood plasma samples taken 4x per d were determined by Sigma glucose-oxidase diagnostic kit (number 510-A) and

Biochem Diagnostics NEFA-C-AR kit (number 999-75401). All basal plasma samples (-20 to -5 min) from the insulin challenges were assayed individually for determination of glucose concentration. Equal aliquots (1 mL) of the basal plasma were pooled to determine basal NEFA concentrations. All remaining samples (+5 through +150 min) were assayed to determine glucose response to insulin. Only samples taken every 15 min after insulin injection were used to determine NEFA response to insulin. Basal concentrations of free glycerol, true triglyceride and total triacylglycerol concentrations in pooled blood plasma (as noted for NEFA analyses above) were determined using a Sigma Triglycerides Diagnostic kit (number 337-B). Concentrations of glycerol and true triacylglycerol in response to insulin were not determined due to cost of the kits.

Fatty acids in adipose tissue (100 to 200 mg), pooled basal samples (2 mL) from the insulin challenges, and feed concentrate mixtures (500 mg) were extracted according to Bligh et al. (1959). Samples were mixed with 50, 10, or 30 μL of 17:1 (10-heptadecenoic methyl-code U-42-M; 40 μg per μL) internal standard for adipose tissue, plasma, and concentrate, respectively, prior to methylation. Extracted fatty acids were methylated according to Al-Athari and Watkins (1988). Five, 5, or 2 μL of extract from plasma, adipose tissue and concentrates, respectively, were injected by auto-sampler into a Hewlett Packard 5890A gas chromatograph (Hewlett Packard Co., Sunnyvale, CA) equipped with a flame ionization detector and Norton Analytical Data System. Identification of

peaks was based on retention times for fatty acid standards (Nu-Chek-Prep Inc., Elysian, MN). A calibration table was constructed using known concentrations of 16:0, 16:1*n*7, 17:0, 18:0, 18:1*n*9, trans 18:2, 18:2*n*6, 18:3*n*3, 18:3*n*6, 20:0, 20:1*n*9, 20:3*n*6, 20:4*n*6, 22:4*n*6, and 22:6*n*3 fatty acids. The internal standard was used to correct sample peaks before quantifying fatty acids with a HP 3393A integrator (Hewlett Packard Co., Sunnyvale, CA).

Statistical Analysis

The general linear model (SAS, 1990) with repeated measures was used to test differences due to dietary treatment. Means were accepted as different at $P < .10$ and for the purpose of discussion trends are indicated when $P = .10$ to .15. Measurements of BW, DMI, blood plasma metabolites and fatty acids in adipose tissue prior to the start (-90 d) of the experimental period were used as covariates in the analyses. Estimated average transmitting ability was used as a covariate for analysis of milk, milk fat, and milk protein production.

Model:

$$Y_{ik} = a + T_i + \beta(x_i) + D_k + TD_{ik} + E_{ik}$$

Where:

Y = dependent variable

a = constant

T = diet (i = 1, 2)

β = regression coefficient

x = covariate measurement

D = days relative to calving (k = 1, 2...5, or 12)[a]

TD = T x D interaction

E = residual error

[a]The values for k represent the range of number of repeated measures, which varied according to dependent variable. For example: k = 1, 2 for insulin challenge parameters (including basal concentrations of plasma triacylglycerol and individual fatty acids), k = 1...5 for 4 x per d plasma parameters, milk production parameters, and DMI, and k = 1...12 for body weight. An example of an analysis of variance table is presented in Appendix Table 6, in which k = 1, 2 and days relative to calving are -30 for k = 1 and +35 for k=2. Data from adipose tissue biopsies were the only data for which there were no repeated measures. In this case, D and TD were removed from the model. A sample analysis of variance table for adipose tissue fatty acid content is given in Appendix Table 7.

RESULTS AND DISCUSSION

Gestation Responses

Dry Matter Intake. Average DMI between 90 and 30 d prior to parturition did not differ due to diet (Table 3). In addition to SBM, corn grain was removed from the control diet to accommodate the whole soybeans, but the change in DE due to WSB (4.01 Mcal DE/kg) in exchange for SBM (3.70 Mcal) and corn (3.75 Mcal) did not have a major influence on total DE content (Table 1). Due to the small differences in the DE content of the diets, DE intake also was not significantly affected by diet. Dietary EE content, however, was influenced by the exchange and resulted in higher EE intake by heifers fed WSB. Lack of an effect of WSB on DMI agrees with other studies with growing ruminants fed diets with supplemental fat (van Houtert et al., 1990; Albro et al., 1993). The increase in EE intake by heifers fed WSB agrees with studies in which larger quantities of fat were included in the diet (Garrett et al., 1976 a and b; Park et al., 1983; van Houtert et al., 1990; Albro et al., 1993; Lough et al., 1993).

Body Weight. There was no effect of diet on BW prior to parturition. However, the tendency for a difference in body weight after calving was evident after removal of variation associated with calf, placenta, and fluid weights

Table 3. Daily dry matter, digestible energy, and ether extract intake of Holstein heifers fed control or whole raw soybean (WSB) diets[a]

	Control	WSB	SE	$P <$
DMI, kg/d[b]	9.9	10.2	.3	.50
DMI, % BW	1.79	1.81	.04	.75
DE, Mcal/d[c]	27.2	28.4	.9	.19
DE, Mcal/100 kg	4.92	5.02	.12	.57
EE intake, kg/d	.30	.47	.01	.01

[a]Heifers were fed experimental diets beginning 90 d prepartum.
[b]Averages of average weekly intake from 90 d to 30 d prepartum.
[c]Estimated from NRC (1989).

Table 4. Prepartum body weight of Holstein heifers in response to feeding control or whole raw soybean (WSB) diets for 90 d prior to calving

	Diet			
Days[a]	Control	WSB	SE	$P <$
-90[b]	531			
-76	545	545	4	.98
-62	560	559	6	.85
-48	564	569	6	.58
-34	570	573	6	.73
-20	568	575	7	.51
-10	570	583	7	.30

[a]Days prior to expected calving date.

[b]All heifers were fed the control diet for 2 wk.

Table 5. Body weights of Holstein heifers at calving and postpartum in response to feeding control or whole raw soybean (WSB) diets 90 d prior to parturition

	Diet fed prepartum			
Item	Control	WSB	SE	$P <$
Calving				
	----------kg----------			
Cow	512	531	9	.14
Calf	38.9	38.4	1.8	.84
Other[a]	19.2	14.0	5.8	.55
Total [b]	570	583	7	.30
Postpartum				
7	511	526	7	.14
14	510	517	7	.57
21	499	506	6	.44
28	494	496	6	.76
35	491	501	6	.20

[a]Weight of placenta and associated fluids.

[b]Cow weight before calving.

(Table 5). Since WSB heifers averaged .17 kg more fat intake per d for 80 d (-90 to -10 d), then they consumed 13.6 kg more fat during the prepartum period. Assuming 85% digestibility (Holter et al., 1992) of ingested fat, 11.6 kg of 19 kg increase in body weight may have been due to fat in WSB. The remainder of the weight difference may have been due to greater efficiency of utilization of other nutrients, as indicated by previous studies with growing ruminants fed supplemental fat (van Houtert et al., 1990; Smith et al., 1992; Lough et al., 1993 and Albro et al., 1993). Lough et al. (1993) found that growth efficiency was improved when growing ruminants were fed diets containing fat, in spite of DMI similar to that of animals fed diets containing no supplemental fat. Providing dietary fat to growing ruminants apparently decreases de novo synthesis of fatty acids from acetate thus sparing available acetate and glucose for other metabolic functions (van Houtert et al., 1990 and Ekeren et al., 1992). Baldwin et al. (1976) explained this as an increase in efficiency, as it is more efficient to use absorbed dietary fatty acids in triacylglycerol synthesis for adipose tissue than to synthesize fatty acids from glucose and acetate provided by dietary carbohydrate. Postpartum body weights will be discussed later in this chapter.

Ultrasound and Adipose Tissue. Subcutaneous adipose tissue thickness did not differ due to dietary treatment during gestation or due to physiological state. Adipose tissue thickness of heifers fed WSB was 2.2 mm 30 d prior to parturition

compared to 2.3 mm (SE =.10) in control-fed heifers. By 35 DIM subcutaneous adipose tissue thickness measured 1.9 mm (SE = .11) in both groups. The lack of differences in adipose tissue thickness may be due to error associated with ultrasound, such as interpretation and level of technical experience (Brethour, 1992 and Perkins et al., 1992). A major adipose depot in cattle is omental fat and was not measured in the current study (Miles et al., 1983). Therefore, subcutaneous adipose tissue apparently was not the primary site of dietary fat deposition, as indicated by ultrasound within the 80 d feeding period.

Fatty acid composition of adipose tissue (Table 6) did not differ due to dietary treatment during the last trimester of gestation. The lack of difference in adipose tissue fatty acid composition also may be attributed to length of the feeding period. Studies (Garrett et al., 1976 a and b; Yang et al., 1978; Ekeren et al., 1992; Lough et al., 1993) that have reported differences in adipose tissue content had longer feeding periods. Dietary EE content also was not as high as previous studies that have reported differences in adipose tissue fatty acid composition (Garrett et al., 1976 a and b; Yang et al., 1978; Ekeren et al., 1992; Lough et al., 1993). Differences in dietary EE content may have also contributed to the lack of difference in adipose tissue fatty acid composition. As reported earlier, the control and WSB diets contained 3.1 and 4.7% EE, respectively.

Table 6. Fatty acid composition of adipose tissue from Holstein heifers after feeding control or whole raw soybean (WSB) diets for 60 d during gestation

Fatty Acids	Control	WSB	SE	$P <$
	-----% (wt/wt)[a]-----			
16:0	23.0	22.9	1.7	.95
16:1 n7	6.6	6.1	.5	.48
18:0	9.1	8.9	.8	.88
18:1 n9	52.9	51.2	3.6	.75
18:2 n6	1.9	1.8	.1	.78
18:3 n3	.27	.27	.02	.86
Total, $\mu g/mg$[b]	175	185	17	.71

[a]Weight percent of total (16:0 to 20:3 n6). Each of the other fatty acids (17:0, trans 18:2, 20:0, 20:1 n9, and 20:3 n6) accounted for less than one percent.
[b]Adipose tissue wet weight basis.

Blood Metabolites during Gestation. Heifers fed WSB had lower plasma glucose concentration at -69 d compared to heifers fed the control diet (Table 7). However, the difference does not appear to be a response to feeding WSB, but rather to an elevated glucose concentration in control-fed heifers. Greater plasma glucose concentration cannot be attributed to stress, as all animals were treated the same, therefore, all animals were stressed equally. Calving and postpartum glucose concentrations will be discussed later in the chapter. Heifers fed WSB had greater concentrations of NEFA in blood plasma at -69 d and -48 d (Table 7). There were no differences in plasma NEFA concentration at -27 d, which may be due to moving the heifers to a larger group housing facility 3 d prior to sampling. Increased NEFA concentration may be due to increased lipoprotein lipase activity in response to supplemental fat intake (Park et al., 1983). Park et al.(1983) noted an increase in lipoprotein lipase activity in the adipose tissue of heifers fed increasing levels of sunflower seeds (0, 10, 20, and 30 % of diet DM). Calving and postpartum NEFA concentrations will be discussed later in the chapter.

Triacylglycerol concentration in blood plasma and total plasma fatty acid concentration (Table 8) were increased at -30 d in response to feeding WSB. Increases in plasma triacylglycerol concentration can be attributed to increased fat intake and absorption of dietary LCFA provided by the WSB diet. Steele (1983) found that 90% of LCFA provided by the diet were digested. He also found a

Table 7. Prepartum and postpartum glucose and NEFA concentrations in blood plasma of Holstein heifers fed control or whole raw soybean (WSB) diets during gestation[a]

Item	Control	WSB	SE	$P <$
Glucose, mg/dL				
-90 d	67.7[b]			
-69 d	68.8	66.1	.8	.04
-48 d	66.4	66.4	.8	.96
-27 d	66.4	64.5	1.2	.31
0 d[c]	77.7	73.1	1.4	.03
+35 d	64.1	62.5	1.9	.56
NEFA, uEq/L				
-90 d	123[b]			
-69 d	98	168	7	.01
-48 d	128	173	6	.01
-27 d	179	174	10	.89
0 d[c]	323	261	22	.07
+35 d	148	181	12	.07

[a]Heifers were fed experimental diets beginning 90 d prepartum.
[b]Average concentration in blood samples taken at 0600, 1200, 1800, and 2400.
[c]Blood sampling began 6 to 12 h after calving.

Table 8. Prepartum triacylglycerol and fatty acid concentrations in blood plasma from Holstein heifers fed control and whole raw soybean (WSB) diets[a, b]

Item	Control	WSB	SE	$P <$
Triacylglycerol, mg/dL	20.9	25.4	1.8	.10
Fatty Acids, % (wt/wt)[c]				
16:0	17.2	15.8	.3	.01
16:1 n7	1.9	1.7	.2	.54
18:0	23.8	25.0	1.2	.50
18:1 n9	15.6	13.5	.6	.04
18:2 n6	31.8	35.0	.6	.01
18:3 n3	3.9	3.5	.3	.40
18:3 n6	1.1	1.5	.2	.18
20:3 n6	2.5	2.3	.2	.45
20:4 n6	4.1	3.8	.4	.60
Total, μg/mL	771	1158	43	.01

[a]Blood plasma from pooled basal insulin challenge samples taken 30 d prepartum.
[b]Heifers were fed experimental diets beginning 90 d prepartum.
[c]Weight percent of total (16:0 to 22:6 n3) fatty acids. Each of the other fatty acids (17:0, trans 18:2, 22:6 n3) accounted for less than one percent.

linear relationship between absorption of fatty acids and concentrations of triacylglycerol and other plasma lipids such as cholesterol and free fatty acids (triacylglycerol-free lipids). Relative to the amount absorbed, greater proportions of 18:2, 18:1, 16:0 and 18:0, respectively, were incorporated into triacylglycerol-free plasma lipids; whereas, a lower proportion of 18:1 was incorporated into triacylglycerol. The difference in incorporation of 18:1 was attributed to desaturation of 18:0 to 18:1 by intestinal mucosa (Steele, 1983). Absorption of LCFA is almost exclusively in the small intestine, and absorptive efficiency in the small intestine is high (85%) (Sutton, 1985 and Holter et al., 1992). After fatty acids reach the small intestine, they are emulsified by taurocholic acid, phosphatidyl choline, and phosphatidyl ethanolamine, dispersed into micelles, and absorbed by intestinal mucosa (Palmquist and Jenkins, 1980). Triacylglycerols synthesized in the mucosa are transported via the lymph to the heart for distribution to other tissues such as mammary gland or adipose tissue (Palmquist and Jenkins, 1980 and Palmquist and Conrad, 1978).

In the current study, heifers fed WSB during gestation had a greater total fatty acid concentration in blood plasma at -30 d (Table 8) compared with heifers fed the control diet. Plasma 16:0 and 18:1 concentrations were lower, and 18:2 concentration was greater in heifers fed WSB. The alteration in plasma lipids is apparently due to increased LCFA intake and absorption. The WSB concentrate mixture contained 40% (as a percent of total fatty acids) 18:2 and 15% 16:0;

whereas, the control concentrate contained 32% 18:2 and 17% 16:0 (Table 9). Soybean oil contains of 54.2% 18:2 (Macleod and Buchanan-Smith, 1972) which accounts for greater 18:2 in the WSB concentrate. The greater proportion of 18:2 combined with greater total fat intake appears to be responsible for the increase in plasma 18:2 in heifers fed WSB. Increased digestibility of unsaturated fatty acids in the small intestine may also contribute to the increase in plasma 18:2 concentration (Andrews and Lewis, 1970). Andrews and Lewis (1970) concluded that increased absorption of 18:2 and other unsaturated fatty acids is due to the ease with which unsaturated fatty acids form micelles. Decreased plasma 16:0 concentration may be related to decreased digestibility of saturated fatty acids in diets containing supplemental fat (Andrews and Lewis, 1970; Macleod and Buchanan-Smith, 1972).

Blood Metabolites at Calving. Heifers fed WSB during the last trimester of gestation had a lower glucose concentration in plasma at calving (0 d) (Table 7) compared with heifers fed the control diet. Cummins and Sartin (1987) reported a decrease in plasma glucose pool in response to increased dietary fat possibly as result of a reduction in plasma glucagon concentration. Thus, the ratio of insulin to glucagon in blood plasma is elevated, suggesting a decrease in hepatic gluconeogenesis (Cummins and Sartin, 1987).

Nonesterified fatty acid concentration in plasma (Table 7) of all heifers

Table 9. Fatty acid content of control and whole raw soybean (WSB) concentrates

	Diet	
Fatty acids	Control	WSB
	----- % (wt/wt)[a]-----	
16:0	16.8	14.9
16:1 n7	2.8	1.7
18:0	23.1	18.0
18:1 n9	16.4	16.2
18:2 n6	31.7	40.3
18:3 n3	4.9	3.0
20:3 n6	1.6	1.3
20:4 n6	4.6	4.0
Total, μg/mg[b]	173	182

[a]Weight percent of total (16:0 to 22:6 n3) fatty acids. Each of the other fatty acids (17:0, 20:0, 20:1 n9, and 22:6n3) accounted for less than one percent.

[b]Concentrate mixture, dry weight basis.

was greater at calving than during gestation, which may be a response to increased secretions of pituitary hormones such as growth hormone and glucocorticoids such as cortisol in late pregnancy and on the day of calving (Baldwin et al., 1976). However, heifers fed WSB had lower plasma NEFA at calving compared with control-fed animals. Bertics et al. (1992) reported a lower concentration of NEFA in plasma prepartum and at parturition when cows were force-fed, as compared to cows that voluntarily decreased intake prior to parturition. The authors attributed the decrease in NEFA to lower liver triacylglycerol content. Apparently, improved energy intake prior to calving prevents lipid deposition in liver, which is a typical response to negative energy balance. Garnsworthy and Huggett (1992) observed that cows in better body condition in early lactation had greater DMI prepartum and at parturition than thinner animals.

Insulin Challenge

Blood Metabolites. Exogenous insulin (.26 IU per kg BW) was used to challenge heifers during gestation (-90 and -30 d) and lactation (+35 d) to evaluate the acute effect of insulin on plasma glucose and its secondary effect on adipose tissue metabolism (Baldwin et al., 1976). The initial responses of adipose tissue to insulin are inhibition of free fatty acid release and enhanced lipogenesis. Glucose uptake and utilization via the pentose phosphate pathway also are increased in support of lipogenesis and triacylglycerol synthesis (Murray et al.,

1993). Insulin's primary action, however, may be inhibition of triacylglycerol sensitive lipase, which is responsible for hydrolysis of triacylglycerol to free fatty acids (Baldwin et al., 1976).

The insulin dose used to challenge heifers was similar to the dose used by Michel et al. (1991). Plasma glucose response to insulin challenge at -30 d or +35 DIM in this study did not differ due to prepartum diet (Figure 1). Glucose response to insulin challenge also was not influenced by physiological state (gestation versus lactation).

Insulin appeared to initially inhibit triacylglycerol sensitive lipase, and therefore decreased plasma NEFA concentration (Figure 2). Plasma NEFA response to insulin challenge did not differ due to diet at -30 d or +35 d, but the NEFA response was greater during gestation (-90 and -30 d) than during lactation (+35 d). The decreased response during early lactation may indicate a decrease in adipose sensitivity to insulin. McNamara and Hiller (1986) characterized a decrease responsiveness to insulin *in vitro*, which they attributed to changes in receptor number and sensitization due to changes in physiological state. Decreased dietary energy intake results in a decrease in net energy balance and a decrease in adipose tissue de novo lipogenesis per gram of adipose tissue and per adipocyte. When coupled with the decrease in number and sensitivity of insulin receptors, a decrease in responsiveness to insulin during early lactation apparently results (McNamara and Hiller, 1986). In early lactation there is a concurrent

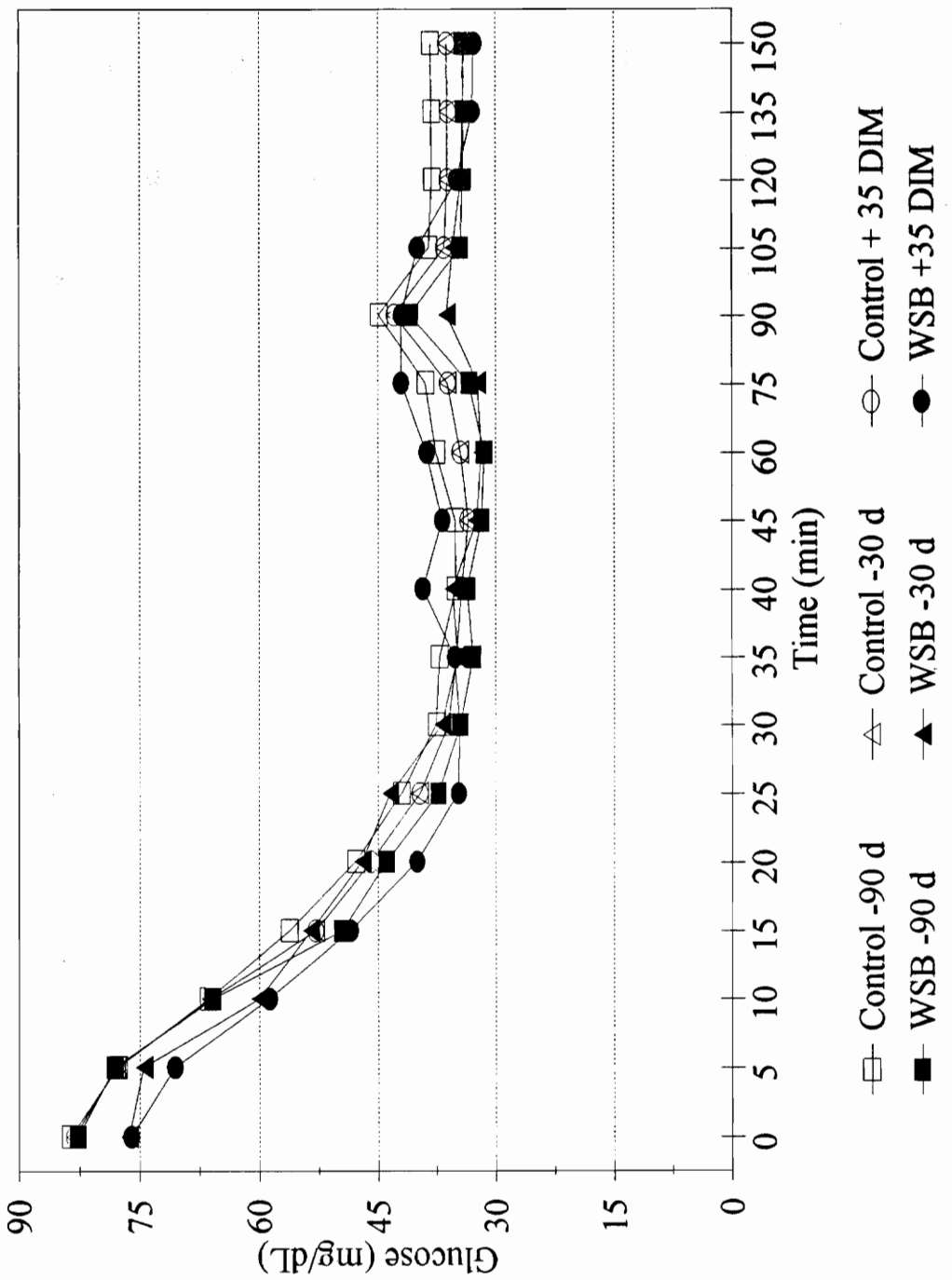

Figure 1. Glucose response to insulin challenge

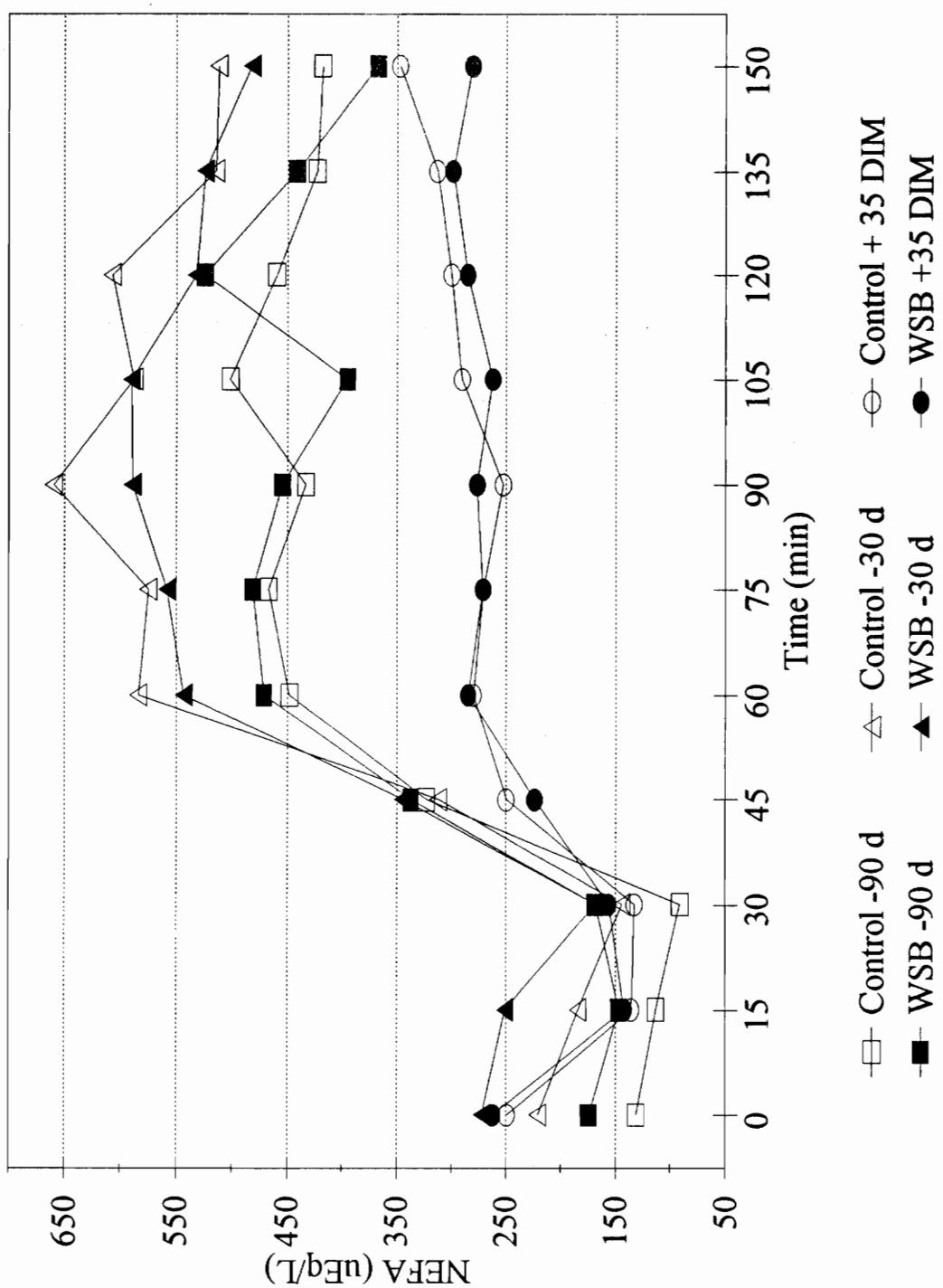

Figure 2. NEFA response to insulin challenge

increase in sensitivity to catecholamines and an increase in β-adrenergic receptors, which also may decrease responsiveness to insulin challenge (McNamara and Hiller, 1986)

Lactation Responses

Dry Matter Intake and Milk Production. Dry matter intake (Table 10), during lactation when both groups were fed the same diet, was not different. Despite similar DMI the heifers previously fed WSB produced 17% more milk during the fifth week of lactation (Table 10). Milk fat and milk lactose yield also tended to be greater for heifers previously fed WSB. The trend for increased milk fat and milk lactose yield appears to be a function of the difference in milk volume, because fat and lactose content (%) was similar for both groups.

Body Weight. Heifers previously fed the WSB diet tended to weigh more in the first week of lactation than control-fed heifers (Table 5). By 28 DIM both groups weighed the same, indicating heifers previously fed WSB lost more body weight (13 kg) in the first four weeks of lactation. Differences in plasma lipid concentrations at 35 DIM suggest that the weight loss was from adipose tissue. The additional body reserves apparently were mobilized to support a higher rate of milk synthesis.

Table 10. Milk and milk component production during the fifth week of lactation of Holstein heifers previously fed control or whole raw soybean (WSB) diets

	Diets fed prepartum			
Item	Control	WSB	SE	$P <$
DMI, kg/d[a]	17.9	19.3	.80	.23
Yield, kg/d				
Milk[b]	28.5	33.4	1.7	.09
Fat	.97	1.11	.06	.13
Protein	.77	.89	.04	.26
Lactose	.69	.79	.05	.15
Milk Composition, %				
Fat	3.45	3.31	.13	.47
Protein	2.70	2.69	.06	.95
Lactose	4.90	4.89	.06	.91

[a]Average dry matter intake from 30 to 35 DIM.
[b]Average milk production from 30 to 35 DIM.

Blood Metabolites. Plasma triacylglycerol concentration was lower and total plasma LCFA (Table 11) concentration was greater in heifers previously fed WSB. Triacylglycerol in plasma is derived primarily from the diet, and can be used directly for milk production and concurrent requirement for fatty acids. The greater concentration of total LCFA was likely due to greater NEFA content (Table 7) of plasma, indicating mobilization of body reserves to support milk production. Specifically, plasma 16:0 concentration was lower and 18:2 concentration greater (Table 11). Milk production in dairy cattle normally peaks between 3 and 6 weeks postpartum; however, DMI does not peak until several weeks later, resulting in an energy deficit (Dunshea and Bell, 1989). Lipid mobilization in response to negative energy balance in early lactation (parturition to peak lactation) is accompanied by a decrease in adipocyte triacylglycerol, elevated plasma NEFA, and elevated blood lipids. At peak lactation there is an increase in rate of release of glycerol, decreased plasma NEFA, and decreased triacylglycerol concentrations (Baldwin et al.,1976; Bines et al., 1983; Coppock and Wilks, 1991; McNamara and Hiller, 1986). Baldwin et al. (1976), Coppock (1985) and Coppock and Wilks (1991) suggested that the decreasing concentrations of NEFA and triacylglycerol reflect increased mammary gland removal of lipid from plasma to support milk production from early to peak lactation when DMI is not sufficient to meet requirements. It appears that at 35 DIM heifers previously fed the control diet were at peak lactation (Figure 4), but heifers previously fed WSB

Table 11. Postpartum triacylglycerol and fatty acid concentrations in blood plasma of Holstein heifers previously fed control or whole raw soybean (WSB) diets[a, b]

	Diets fed prepartum			
Item	Control	WSB	SE	$P <$
Triacylglycerol, mg/dL	12.2	6.6	1.3	.01
Fatty acid, % (wt/wt)[c]				
16:0	14.2	12.3	1.0	.07
16:1 n7	2.5	2.0	.5	.52
18:0	18.6	16.5	1.2	.25
18:1 n9	13.4	12.2	1.4	.55
18:2 n6	42.9	48.7	3.5	.09
18:3 n3	3.2	3.1	.2	.36
18:3 n6	1.0	1.0	.1	.26
20:3 n6	2.2	2.2	.3	.81
20:4 n6	3.1	2.8	.3	.56
Total, μg/mL	989	1225	165	.34

[a]Blood plasma from pooled basal insulin challenge samples taken 35 d postpartum.
[b]Heifers were fed experimental diets beginning 90 d prepartum.
[c]Weight percent of total (16:0 to 22:6 n3) fatty acids. Each of the other fatty acids (17:0, trans 18:2, 20:0, 20:1 n9, 22:6 n3) accounted for less than one percent.

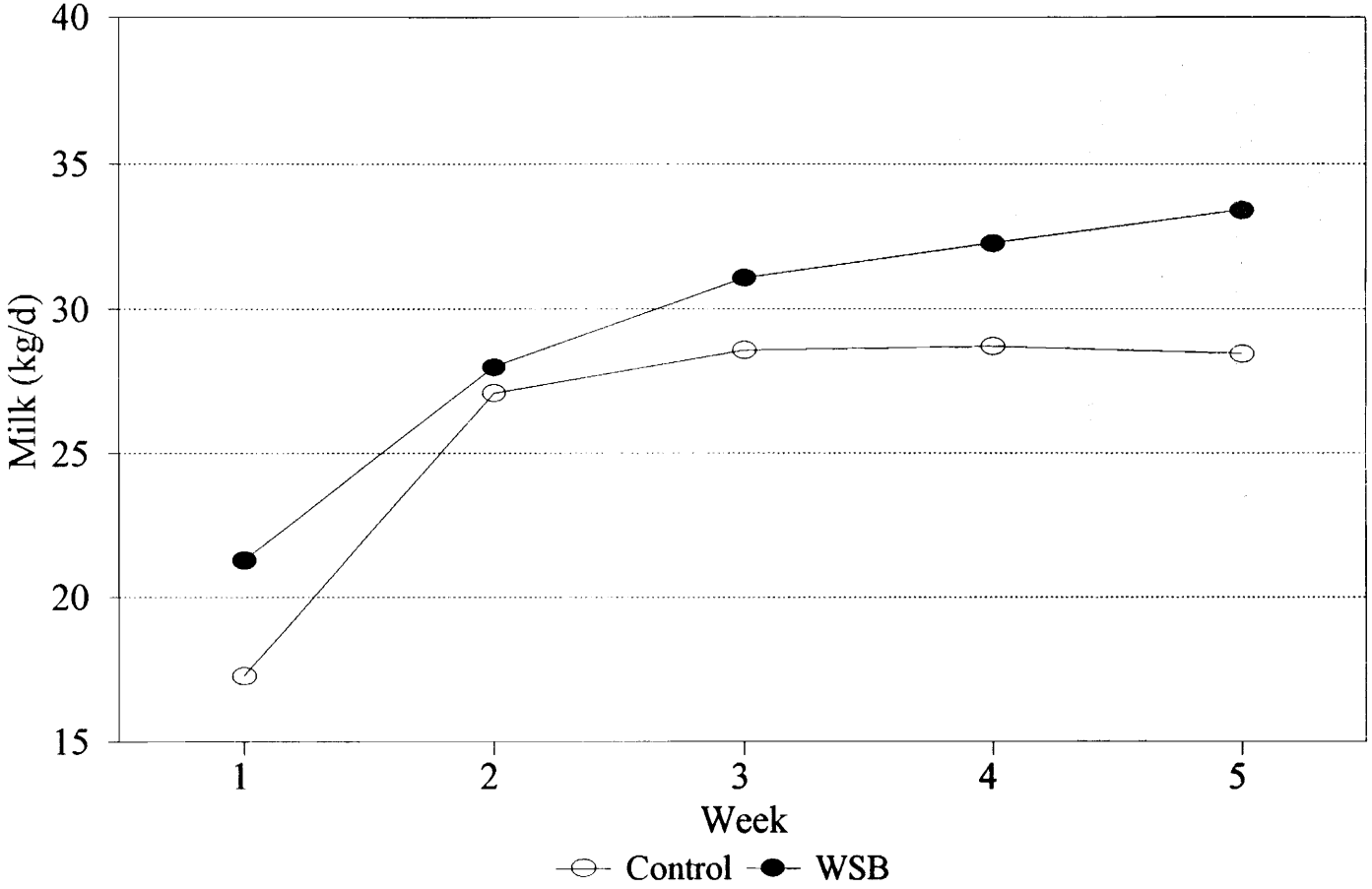

Figure 4. Average weekly milk production by Holstein heifers previously fed control or whole raw soybean (WSB) diets during gestation

had not reached peak. Energy balance calculated from DMI and BW in the fifth week of lactation revealed no differences due to gestation dietary treatment. The greater demand for nutrients to support milk production may account for the greater plasma NEFA and lower triacylglycerol concentrations in heifers previously fed WSB. Availability and use of preformed fatty acids from adipose tissue appears to increase efficiency of milk production and spare glucose and acetate for other metabolic functions, such as milk production (Dunshea and Bell, 1989; Schauff et al., 1992).

IMPLICATIONS

Supplemental fat in the diet of first-calf heifers apparently improves efficiencies of growth and triacylglycerol synthesis, resulting in greater deposition of body reserves prior to parturition. Improved body condition at parturition may decrease the extent of negative energy balance during early lactation, increase peak milk production, and total lactation yield. Body reserves in early lactation are particularly important for the first-calf heifer, because the heifer requires nutrients for growth as well as for milk production. Substitution of WSB for soybean meal in the diet was shown to be an effective method for increasing LCFA intake without affecting DMI. The favorable results obtained from this study may serve as the basis for future work dealing with the evaluation of various types and (or) amounts of whole oilseeds in the diet of heifers or cows prior to parturition.

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APPENDIX

Table 1. Dry matter intake[a] by Holstein heifers fed control or whole raw soybeans (WSB) diets for 90 d prior to parturition

Days[b]	Control	WSB	SE	$P <$
	------kg/d------			
-90[c]	10.2	9.6		
-76	10.2	10.4	.4	.51
-62	10.1	10.2	.4	.81
-48	9.7	10.1	.4	.42
-34	9.8	9.9	.3	.66

[a]Average of 2 wk of daily intake.

[b]Days prior to expected calving date.

[c]All heifers were fed the control diet for 2 wk.

Table 2. Milk production[a] during the first 35 d of lactation of Holstein heifers previously fed control or whole raw soybean (WSB) diets

	Diets fed prepartum			
Days in milk	Control	WSB	SE	$P <$
	-----kg/d-----			
7	17.4	21.3	1.5	.65
14	27.1	28.0	1.5	.67
21	28.6	31.1	1.8	.36
28	28.7	32.3	2.0	.25
35	28.5	33.4	1.7	.08

[a]Average weekly milk production.

Table 3. Milk fat production and milk fat percent[a] during the first 35 d of lactation of Holstein heifers previously fed control or whole raw soybean (WSB) diets

	Diets fed prepartum			
	Control	WSB	SE	$P <$
Fat, kg/d				
14	1.00	.98	.08	.89
21	.92	1.05	.09	.32
28	.97	1.07	.13	.59
35	.97	1.11	.06	.13
Fat, %				
14	3.69	3.46	.15	.31
21	3.25	3.31	.18	.82
28	3.35	3.27	.29	.84
35	3.45	3.31	.47	.67

[a]Average weekly milk fat production.

Table 4. Milk protein production and milk protein percent[a] during the first 35 d of lactation of Holstein heifers previously fed control or whole raw soybean (WSB) diets

	Diets fed prepartum			
	Control	WSB	SE	$P <$
Protein, kg/d				
14	.79	.81	.04	.69
21	.80	.85	.04	.41
28	.79	.86	.05	.31
35	.77	.89	.04	.26
Protein, %				
14	2.91	2.92	.09	.87
21	2.80	2.75	.06	.50
28	2.75	2.66	.06	.27
35	2.70	2.69	.06	.95

[a]Average weekly milk protein production.

Table 5. Milk lactose production and milk lactose percent[a] during the first 35 d of lactation of Holstein heifers previously fed control or whole raw soybean (WSB) diets

	Diets fed prepartum			
	Control	WSB	SE	$P <$
Lactose, kg/d				
14	.81	.85	.08	.31
21	.73	.70	.06	.29
28	.68	.76	.03	.20
35	.69	.79	.05	.15
Lactose, %				
14	4.80	4.82	.08	.87
21	4.93	4.95	.05	.90
28	4.96	4.94	.07	.81
35	4.90	4.89	.06	.91

[a]Average weekly milk lactose production.

Table 6. Analysis of variance table for plasma 16:0 concentration at 30 d prior to parturition[a]

Source	DF	Type III Sum Squares	Mean Square	F Value	P < F
Treatment	1	19.89	19.89	4.15	.06
Covariate	1	28.05	28.05	5.85	.03
Day	1	1.06	1.06	.34	.58
Treatment x Day	1	.35	.35	.11	.75
Error	11	62.31	4.79		

[a]Data obtained from plasma samples -90 d was used as the covariate for analysis of data from -30 d (shown here) and +35 d.

Table 7. Analysis of variance tables for adipose tissue 16:0 concentration 30 d prior to parturition

Source	DF	Sum of Squares	Mean Square	F Value	Pr < F
Model	2	58.3	29.2	1.4	.28
Error	13	269.7	20.8		
Total	15	328.0			

Source	DF	Type III SS	Mean Square	F Value	Pr < F
Diet	1	.09	.09	.00	.95
Covariate	1	52.2	52.2	2.5	.14

VITA

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