

Hello To Trans-Fatty Acids and Good-bye To Fatty Liver in Dairy Cows

*Lokenga Badinga, Kurt T. Selberg, and Charles R. Staples
Institute of Food and Agricultural Sciences
Department of Animal Sciences
University of Florida, Gainesville*

Introduction

In dairy cattle, the period of transition from late pregnancy to early lactation is characterized by dramatic changes in nutrient demand that necessitate coordinated changes in body tissue metabolism to meet requirements of energy, glucose and amino acids by the mammary gland (Bauman and Curie, 1980; Bell, 1995; Overton et al., 2001). Failure of the transition cow to appropriately adjust her metabolism to support increased nutrient requirements of early lactation may lead to metabolic disorders, suboptimal milk production and compromised postpartum reproductive efficiency. One of the key metabolic adaptations that occur in support of lactation involves extensive mobilization of body fat to meet the increased demand for energy. However, if the rate of non-esterified fatty acid (NEFA) mobilization from adipose tissue exceeds that of their metabolism in the liver, the syndrome of hepatic lipidosis or fatty liver may result, which can ultimately lead to prolonged recovery from other disorders, increased incidence of health problems, and compromised liver function (Drackley, 1999; Grummer, 1993; Veenhuizen et al., 1991). Therefore, the research missions of our laboratory are 1) to identify dietary components that can reduce periparturient metabolic upsets due to decreased feed intake, 2) improve milk production without negatively affecting its composition, and 3) stimulate ovarian activity to improve postpartum reproductive efficiency in dairy cattle.

Lipid Metabolism During the Transition Period

Dairy cows in early lactation have higher energy requirements than can be supported by dietary intake. As a result, the cow must utilize body fat as a source of energy. However, if the rate of fatty acid (FA) mobilization from adipose tissue exceeds that of their oxidation and export out of the liver, triglyceride accumulates within the hepatocytes, and the acetyl-CoA that is not incorporated into the tricarboxylic acid (TCA) cycle is converted to ketones (Goff and Horst, 1997; Figure 1). Factors controlling the relative flux of NEFA between oxidation and esterification pathways are not fully understood in dairy cows. The translocation of FA into the mitochondria, a step that is required for β -oxidation to occur, is regulated by the enzyme carnitine acyl transferase-1 (CAT-1). In postpartum dairy cows, the activity of CAT-1 was reported to be greater at d 30 of lactation than at d 60, 90 or 180 of lactation (Aiello et al., 1984). Additionally,

CAT-1 activity was shown to be inhibited by malonyl-CoA and methylmalonyl-CoA (Brindle et al., 1985; Knapp and Baldwin, 1990). As suggested by Zammit (1990), inhibition of CAT-1 by methylmalonyl-CoA may be a mechanism to link supply of propionate from ruminal fermentation with the need for NEFA oxidation. Studies with rodents indicated that the sensitivity of CAT-1 to malonyl-CoA inhibition also may be controlled by the physiological state of the animal. During conditions of low circulating insulin or insulin resistance, CAT-1 appears to be less sensitive to inhibition by malonyl-CoA (Zammit, 1996). Thus, in rodents, ketogenic states are characterized by increased expression of CAT-1, decreased intracellular concentration of malonyl-CoA, and decreased sensitivity of CAT-1 to inhibitory effects of malonyl-CoA (Reviewed in Drackley, 1999). The nature and extent to which dietary management of the dairy cow during the transition period affect hepatic CAT-1 activity and/or synthesis remains to be determined.

In addition to mitochondrial oxidation, NEFA can be oxidized in peroxisomes. Unlike the mitochondrial oxidative pathway, the initial oxidative step in peroxisome is catalyzed by an oxidase, which results in the production of hydrogen peroxide rather than reduced NAD (Drackley, 1999). In addition, because peroxisomes do not contain a respiratory chain linked to ATP formation, peroxisomal oxidation is not subject to control by energy demands of the cell (Drackley, 1999). These characteristics make peroxisomal oxidation well suited to partially oxidize FA that are poor substrates for mitochondrial enzymes.

The greatest factor contributing to hepatic lipidosis may be the ruminant's inability to export TG as very low density lipoproteins (VLDL). Kleppe et al. (1988) compared rates of FA esterification and secretion in hepatocytes isolated from rats and goats. After 4 h of incubation in the presence of high FA concentrations, both rat and goat hepatocytes had similar rates of TG synthesis, but rat hepatocytes secreted approximately 25 times more TG than goat hepatocytes. Apolipoprotein B100 (Apo B100) is the major protein of VLDL, and its concentration in the liver is inversely related to hepatic TG concentration. Gruffat et al. (1997) examined stage of lactation-dependent regulation of Apo B100 in high-producing dairy cows during the first 12 wk of lactation. Cows were fattened during gestation and were underfed just after parturition to increase fat mobilization and induce hepatic lipidosis. Concentration of Apo B100 in liver was approximately 25% lower during the first 4 wk of lactation than during late pregnancy. Hepatic Apo B100 concentrations returned to prepartum levels by 12 wk of lactation. These observations implied that periparturient liver lipidosis may be induced, in part, by the inability of transition dairy cows to secrete adequate amounts of Apo B100 during early lactation.

Effects of Dietary Fat on Lipid Metabolism In Periparturient Dairy Cows

In rodents, dietary fat induces widespread metabolic changes that include increased peroxisomal and mitochondrial oxidation of FA (Kroetz et al., 1998;

Malewiak et al., 1988), decreased esterification of FA (Malewiak et al., 1988), altered profiles and clearance of plasma lipoproteins (Yang et al., 1993), and altered responsiveness to endocrine signals (Dax et al., 1990). Because many of these changes in lipid metabolism induced by supplemental fat also occur during starvation or negative energy balance in rodents, it has been suggested that they might also be important metabolic adaptations during the transition period in dairy cows (Drackley, 1999).

Grum et al. (1996) examined the effect of prepartum fat supplementation on hepatic composition and lipid metabolism in periparturient Holstein cows. At 1 d after parturition, cows that were fed the high-fat diet prepartum had little TG accumulation in the liver (1.4% of wet weight) compared with those fed isocaloric control (7.3%) or high-grain (5.9%) diets. The lower liver lipid concentrations were associated with less marked increases of plasma NEFA around parturition, reduced capacity of liver slices to esterify palmitate, and increased peroxisomal β -oxidation in liver homogenates (Figure 2). The authors concluded that the high-fat diet fed throughout the dry period likely resulted in a coordinated set of adaptations in lipid metabolism that culminated in less hepatic TG accumulation at parturition. The effect of the high-fat diet appeared to be specific to the periparturient period because a high-fat diet fed from d 21 to 300 of lactation did not result in increased peroxisomal β -oxidation (Grum, 1994).

Because the dry matter intake (DMI) tends to decrease in dairy cows fed supplemental fat (Grum et al., 1996), Douglas et al. (1998) examined periparturient lipid metabolism in dairy cows fed isocaloric diets with or without supplemental fat (4% of dietary DM) either at ad libitum intake (~120% of NRC requirements for NE_L) or restricted intake (~80% of NE_L requirements). Cows fed either diet at restricted intake had less accumulation of lipid in the liver 1 d after calving. The effects of dietary fat were smaller but additive to those of intake, resulting in lower TG concentrations in liver at 1 d postpartum for cows fed the high-fat diet.

Factors responsible for the altered lipid metabolism and decreased lipid accumulation in liver of cows fed the high-fat diet remains to be determined. Data from the University of Illinois (Grum et al., 1996; Douglas et al., 1998) would suggest that hepatic peroxisomal oxidation of FA may be induced by high-fat diets or negative nutrient balances during the transition period. In addition, a high-fat diet fed throughout the dry period does not appear to predispose cows to fatty liver syndrome, at least in cows that are losing body condition during the nonlactating period. Additional research is needed to differentiate the effects of nutrient intake from those of source of supplemental energy on periparturient lipid metabolism and the incidence of fatty liver in dairy cattle.

Production Responses to Supplemental Conjugated Linoleic Acid (CLA) and Monoene *Trans*-Fatty Acids During the Transition Period

The inability of high-producing dairy cows to keep energy intake in balance with energy requirements for maintenance and milk production imposes considerable metabolic stress on the lactating animal. We examined the effects of ruminally-protected CLA and *trans*-fatty acid (*t*FA) isomers on productivity and metabolism of transition dairy cows (Selberg, 2002). Thirty-eight pregnant, nonlactating Holstein cows were assigned randomly to receive a control (n=17), CLA-supplemented (n=10) or *t*FA-supplemented (n=11) diet from approximately 4 wk before calving through 7 wk after parturition. The control group received no fatty acid supplement prepartum or postpartum (Table 1). The CLA treatment group received 231 g/d prepartum and 258 g/d postpartum of a ruminally-protected CLA mixture (Bioproducts, Inc., Kingsburg, CA). The *t*FA treatment group received 214 g/d prepartum and 261 g/d postpartum of a ruminally-protected *trans*-octadecenoic fatty acid mixture (Bioproducts, Inc., Kingsburg, CA). Blood samples were collected weekly and plasma immediately harvested for subsequent metabolite assays. At d 2, 14, and 28 postpartum, liver tissue samples were obtained by biopsy and snap-frozen at -80°C for subsequent determination of total lipid and TG contents.

Prepartum body weight (BW), DMI and BCS did not differ among dietary treatments. When expressed as a percentage of BW, postpartum DMI was consistently lower for cows fed the *t*FA compared with those CLA or the control diet (Figure 3). Postpartum DMI did not differ between control and CLA treatment groups. Milk production patterns were different for cows fed the *t*FA compared with those fed CLA and the control diet (Figure 4). Milk production increased and peaked earlier (within 3 wk of lactation) in *t*FA than in control and CLA groups. With the exception of the first week of lactation, 3.5% fat-corrected milk weights were similar across the three dietary treatments (Figure 4). Unlike *t*FA, which had no effects on milk fat percentage, supplemental CLA greatly suppressed milk fat concentration after 4 wk of lactation (Figure 5). Feeding CLA or *t*FA had no detectable effects on milk protein concentration during the first 7 wk of lactation (Figure 6).

Metabolic Responses to Supplemental Conjugated Linoleic Acid (CLA) and Monoene *Trans*-Fatty Acids During the Transition Period

As a result of reduced DMI, cows fed the *t*FA were in a greater negative energy balance than those receiving CLA or the control diet at 1 wk postpartum (Figure 7). Conversely, after parturition, blood urea nitrogen (BUN) concentrations were greater in cows fed the *t*FA -enriched diet than those fed the control or CLA-supplemented diet (Figure 7). This observation would suggest that postpartum dairy cows fed *t*FA-enriched diets may alter their metabolism to utilize mobile amino acids, rather than body fat, as the primary source of supplemental energy during lactogenesis.

Treatment by week interactions were detected for plasma NEFA and β -hydroxybutyric acid (BHBA) concentrations (Figure 8). Plasma NEFA and BHBA concentrations were higher in cows supplemented with CLA than those fed the control or *t*FA-enriched diet at 1 wk postpartum. Interestingly, feeding *t*FA prevented the rise in plasma NEFA that was detected in the CLA treatment group. Similar to plasma lipid metabolites, postpartum fat accumulation in the liver varied among the dietary treatments (Figure 9). Mean liver lipid concentration in cows fed CLA and control diets rose from d 2 to 14 and subsequently decreased at d 28. In contrast, total hepatic lipid concentration did not change across days in cows fed TRANS (day by diet interaction, $P < 0.001$). Similar results were observed for liver triglyceride percentages. Feeding *t*FA to transition Holstein cows prevented fat accumulation in the liver at week 2 postpartum. At 2 wk after parturition, the frequency distributions of cows with liver TG concentrations greater than 30 mg/g wet tissue were 50, 70, and 40%, for control, CLA, and *t*FA treatment groups, respectively (treatment by week interaction).

Conclusions and Implications

Results provide the first direct evidence that supplemental CLA and *trans*-octadecenoic fatty acids may affect bovine mammary and body metabolism through distinct signaling mechanisms. The observation that dietary *trans* fatty acids prevented fat accumulation in the liver imply that these mono-unsaturated fatty acids may become a producer-friendly nutritional strategy to reduce the incidence of fatty liver syndrome in postpartum dairy cows.

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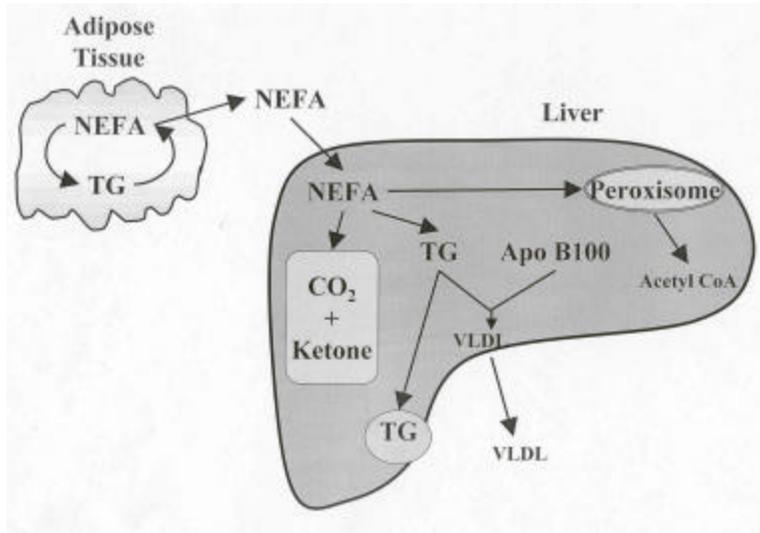


Figure 1. Schematic representation of lipid metabolism in adipose tissue and liver during the transition period.

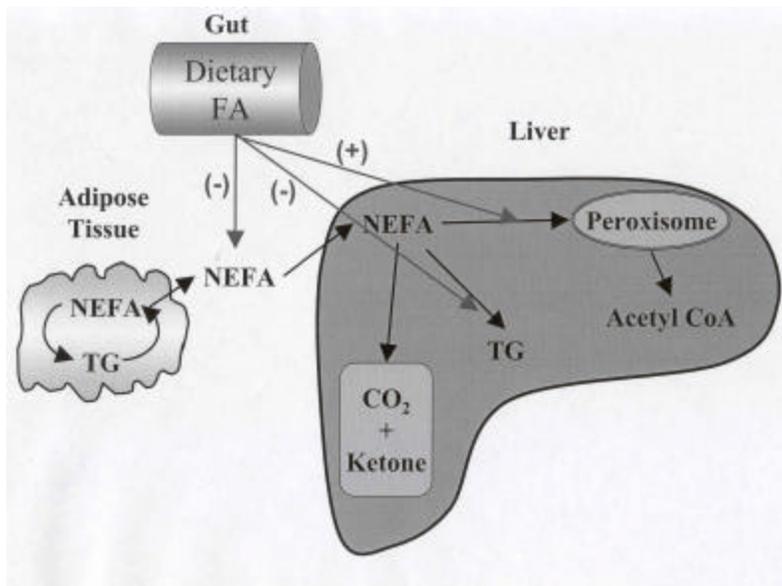


Figure 2. Regulation of NEFA metabolism by dietary fatty acids in periparturient dairy cows (based on Grum et al., 1996).

Table 1. Experimental treatments

Physiological State	Dietary treatments		
	Control n=17	CLA ¹ n=10	tFA ² n=11
	----- g/d -----		
Prepartum	0	231	214
Postpartum	0	258	261

¹CLA mixture contained 3.81% c8,t10; 6.34% c9,t11; 5.37% c8,t10; 8.19% c11,t13; 7.88% t10,c12; and 12.19% other CLA isomers.

²tFA mixture contained 20.62% C18:1 t6-8; 10.47% C18:1 t9; 10.62% C18:1 t10; 7.05% C18:1 t11; and 8.73% C18:1 t12.

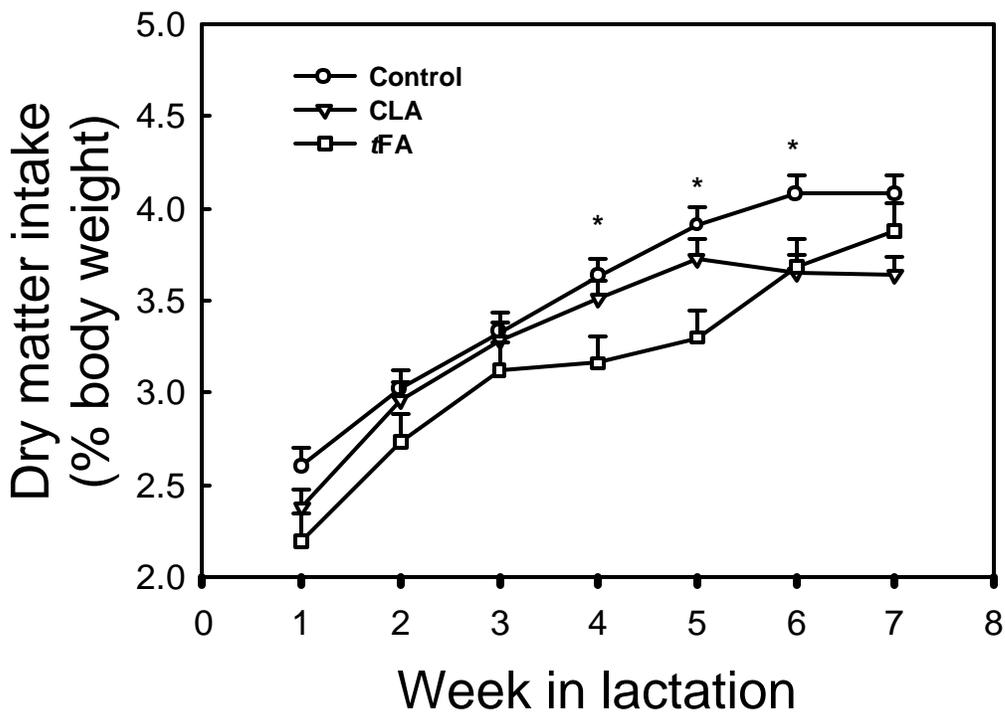


Figure 3. Postpartum DMI in Holstein cows fed a control, CLA-supplemented, or tFA-based diet.

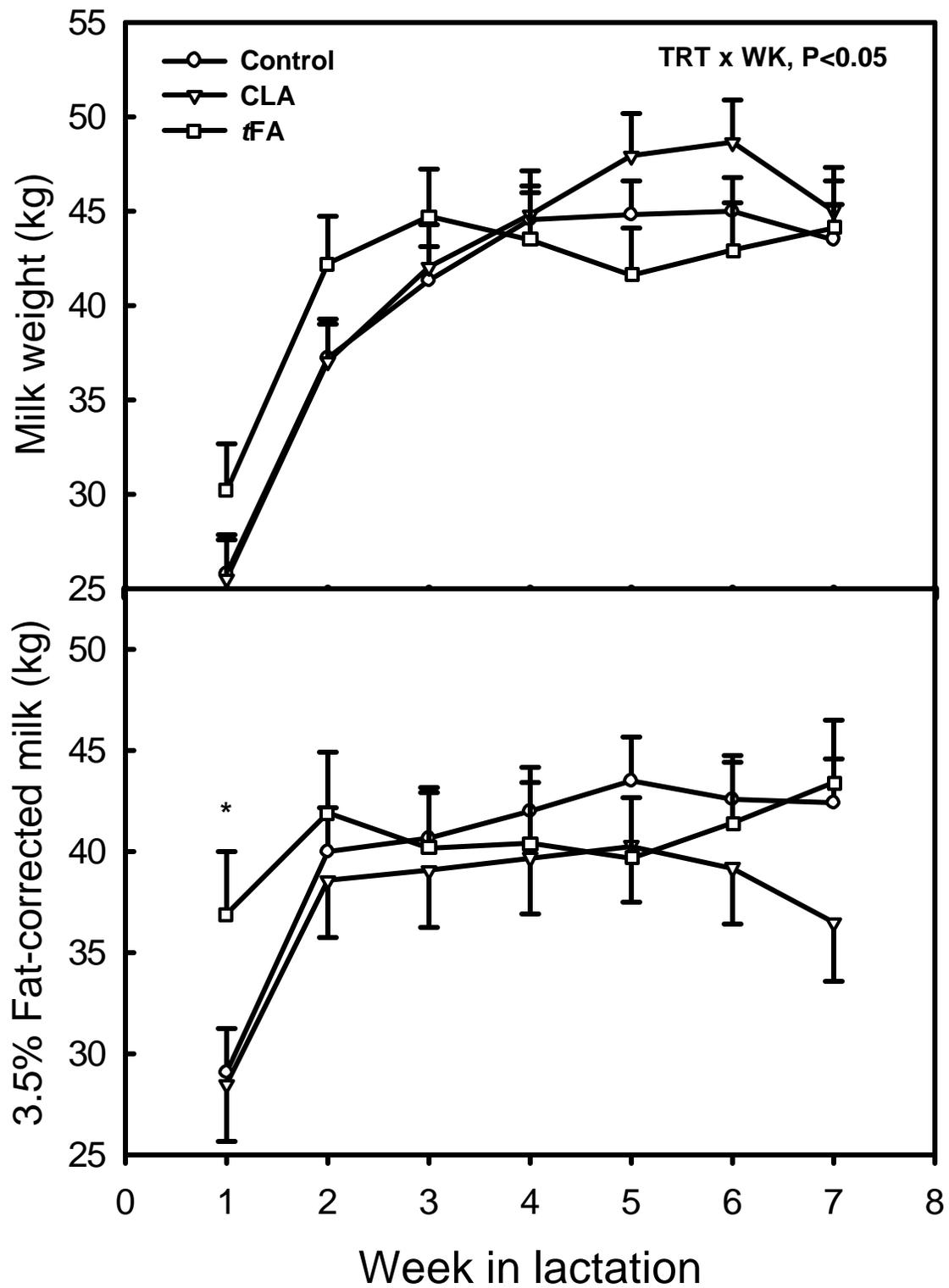


Figure 4. Effects of dietary CLA and *tFA* on milk production (top) and 3.5% fat-corrected milk (bottom) in postpartum Holstein cows.

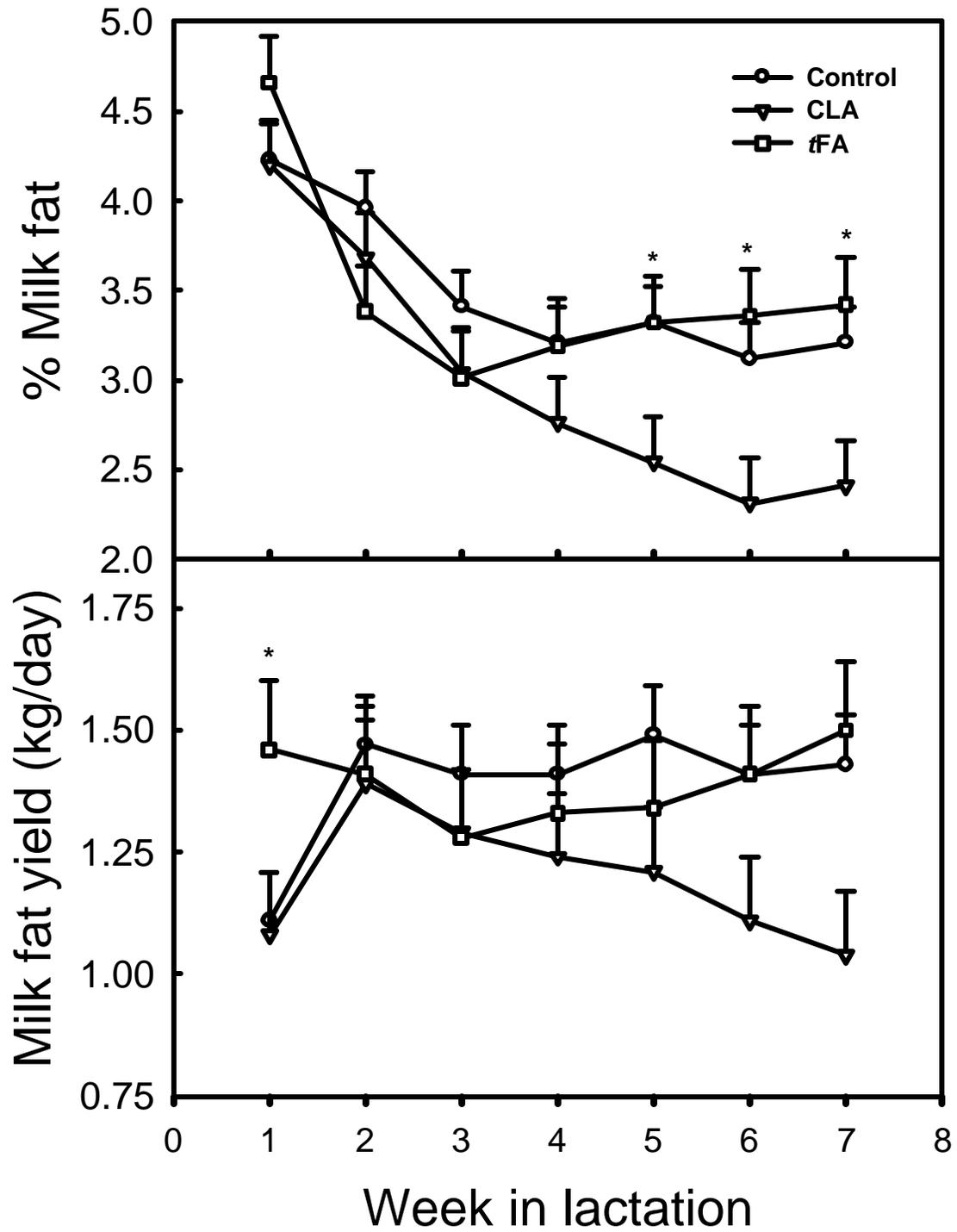


Figure 5. Effects of dietary CLA and *tFA* on milk fat percentage (top) and yield (bottom) in postpartum Holstein cows.

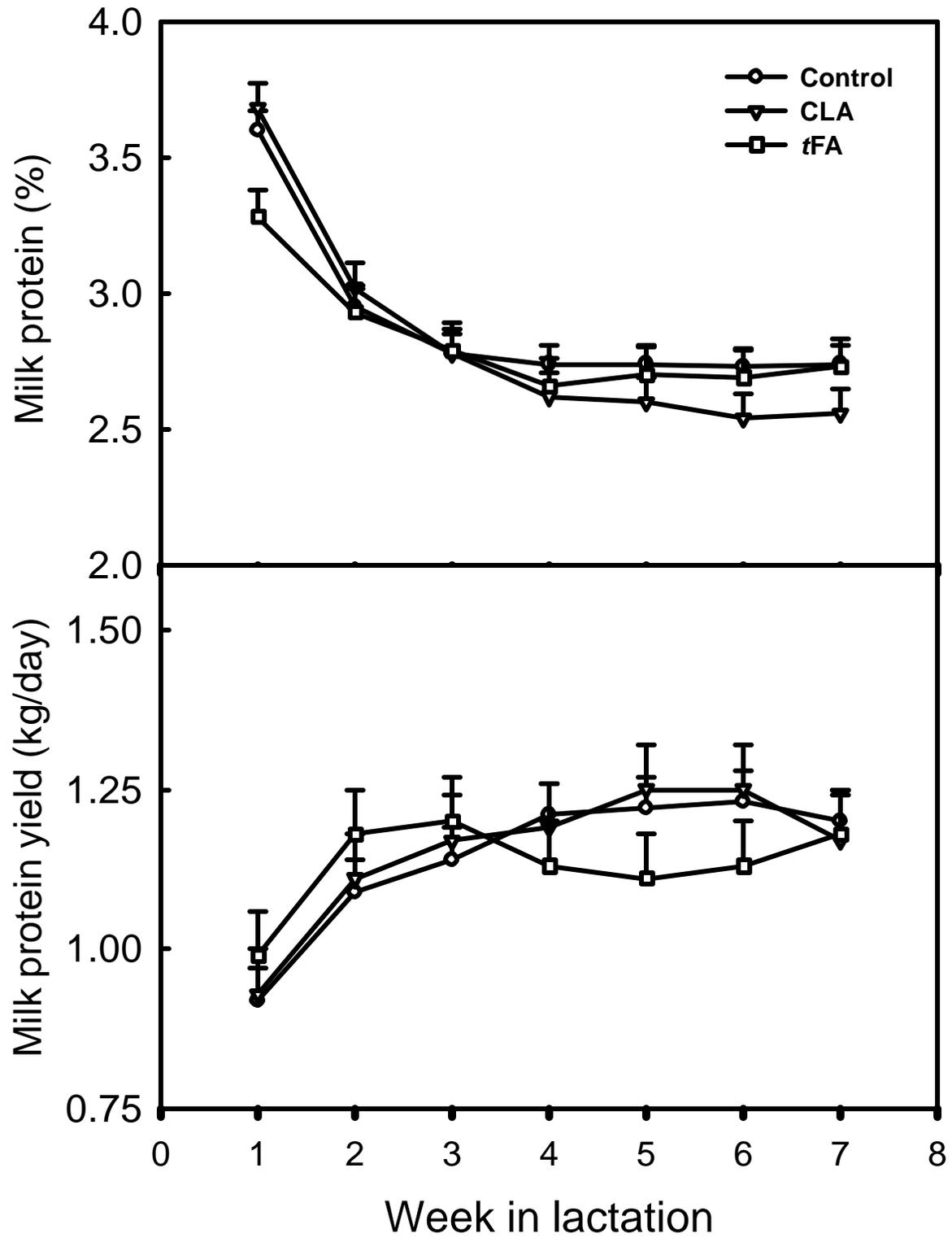


Figure 6. Effects of dietary CLA and *tFA* on milk protein percentage (top) and yield (bottom) in postpartum Holstein cows.

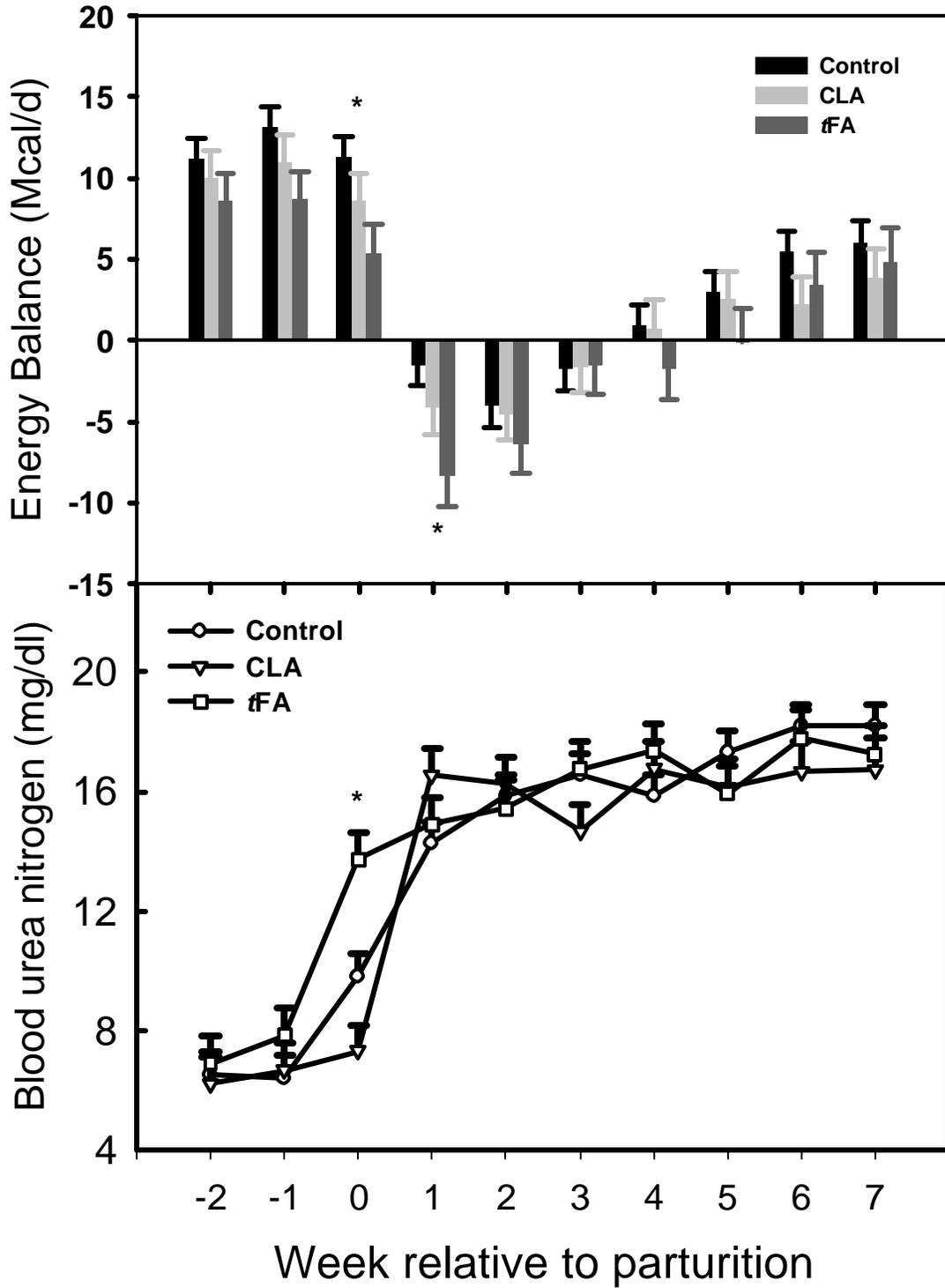


Figure 7. Effects of dietary CLA and *tFA* on energy balance (top) and blood urea nitrogen (bottom) in periparturient Holstein cows.

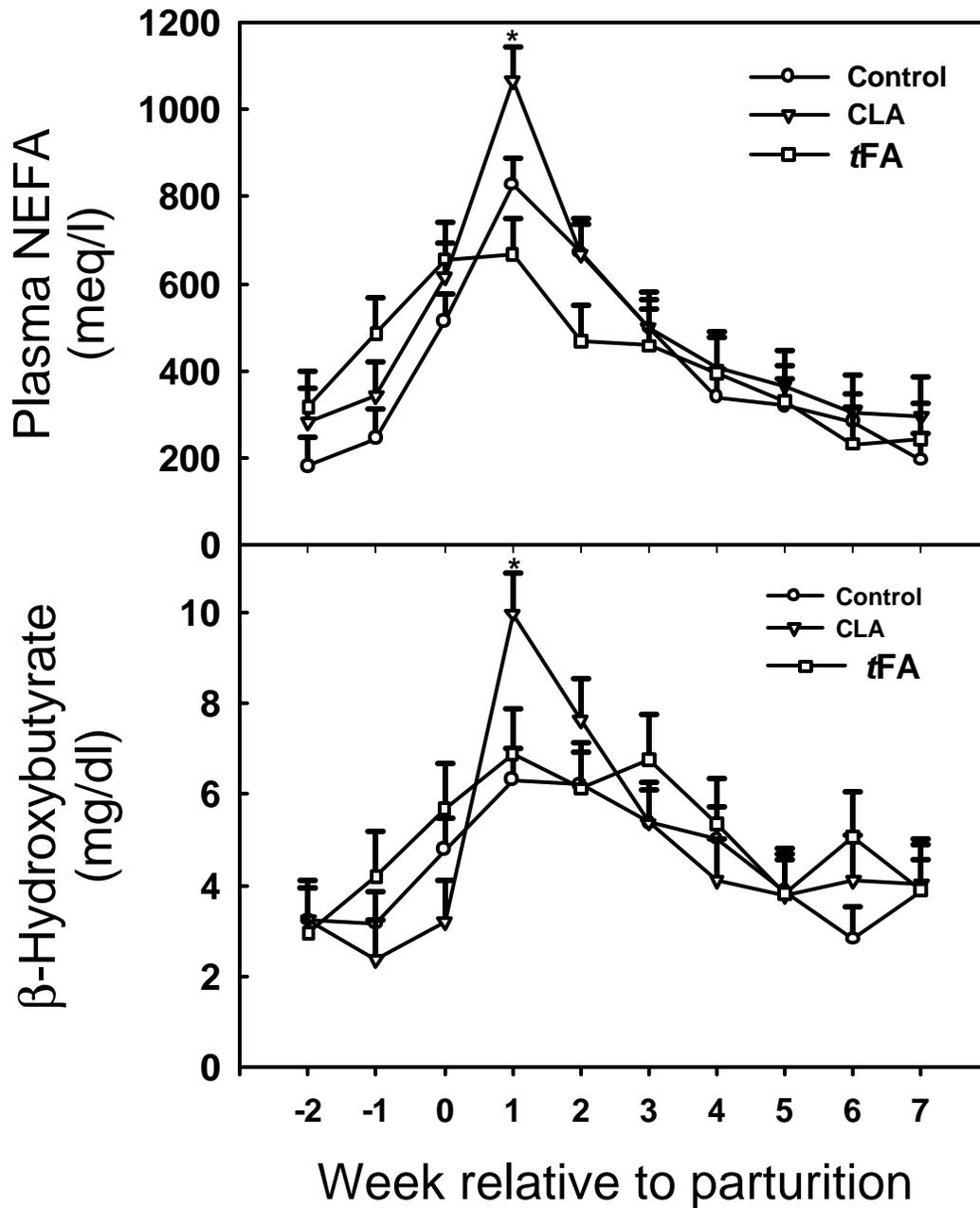


Figure 8. Effects of dietary CLA and *t*FA on plasma NEFA (top) and BHBA (bottom) concentrations in periparturient Holstein cows.

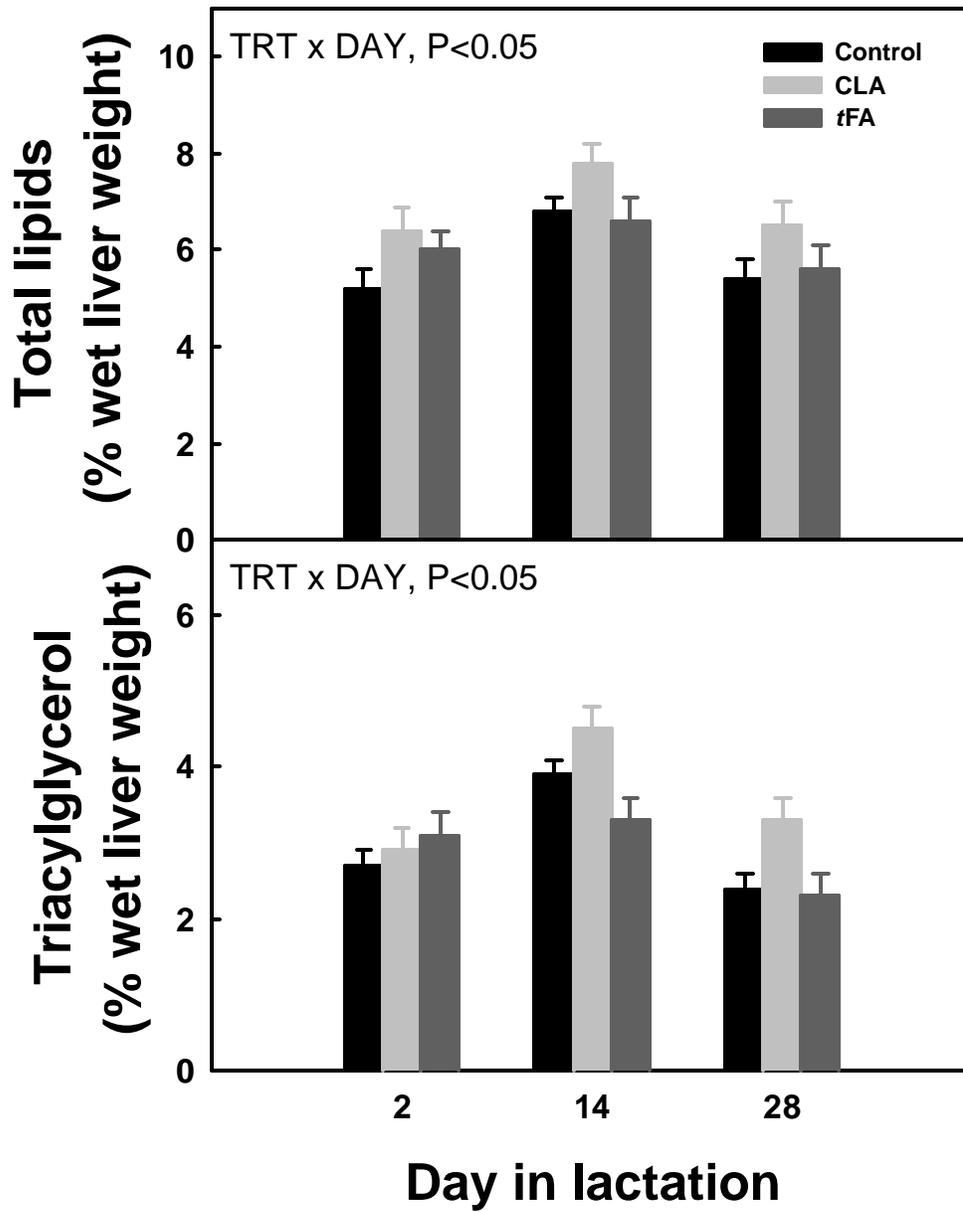


Figure 9. Effects of dietary CLA and *tFA* on total lipid (top) and triglyceride (bottom) contents in liver of postpartum Holstein cows.