TAKE HOME MESSAGES

- Fatty liver in dairy cattle represents a large problem for dairy producers in Illinois as well as across the United States in terms of animal health and costs to the farm.

- Supplemental lipid in the form of long-chain fatty acids has shown potential as a nutritional strategy to prevent or minimize excessive lipid deposition in liver after calving.

- Saturated and unsaturated fatty acids both increase expression of fatty acid oxidation genes. However, they can have different effects on important physiological functions such as gluconeogenesis and the liver response to harmful pathogens (e.g. inflammation).

- Palmitic acid and stearic acid may yield some benefits for the transition cow, but we need to be aware that these may prove to be pro-inflammatory.

- EPA (20:5n-3), one of the main omega-3 fatty acids in fish meal and fish oil, may prove to be a major fatty acid for increasing expression of key genes instrumental for fatty acid oxidation in the liver.

INTRODUCTION

From three weeks prepartum to three weeks postpartum, the dairy cow undergoes major metabolic challenges and physiological adaptations. This period, termed as the “transition period”, is characterized by a reduction in voluntary feed intake as well as increasing energy demands. This leads to a period of negative energy balance (NEB) in which the cow must mobilize fatty acids (FA) from body reserves. This results in dramatic increases of non-esterified fatty acids (NEFA) in blood, which are taken up by liver and used in part for oxidation to help generate energy during this deficit period. In comparison to other animals, however, the dairy cow has an impaired ability to secrete triglycerides (TG) to the bloodstream, and hepatic lipidosis, or fatty liver, may occur when excessive amounts of NEFA are re-esterified to TG instead of being oxidized. Fatty liver as well as ketosis, another disease affecting transition cows, represent a major concern for dairy producers in Illinois as well as across the USA.

One of the main goals over the past 15 years in transition cow research has been to prevent TG deposition in the liver by maximizing fatty acid oxidation. Supplementation of the fresh cow diet with lipid sources may be a means of doing this. Several companies in the industry today market supplemental lipid that can be added to diets (e.g. Virtus Nutrition - EnerG II, StrataG w/ EPA/DHA Omega-3 fatty acids, Prequel 21 w/ Omega-6 Technology; Arm & Hammer Nutrition – Megalac®, Megalac Plus™, Megalac® R). These lipid sources are stated to do such things as...
“improving milk components and milk production efficiency” to “reducing NEFA levels” and “improving reproductive status”. More importantly from a nutritional stand-point, these lipids contain differing fatty acid profiles. Specific fatty acids could play a role in the activation of certain regulatory genes in the liver that could in turn activate expression of other genes involved in the regulation of tissue metabolism. Both dietary saturated (e.g. palmitic acid [16:0]) and unsaturated fatty acids (e.g. oleic acid [18:1 cis9]) can have distinct effects on the expression of these genes, but unsaturated fatty acids can be extensively biohydrogenated in the rumen, leading to higher amounts of saturated fatty acids (e.g. stearic acid [18:0]) leaving the rumen. Although many commercially available sources of lipids for dairy cattle offer some degree of protection against rumen hydrogenation, the biological effects that are associated with specific fatty acids, both saturated and unsaturated, are still not well defined. Studying the biological events brought on by fatty acids at the gene level might prove to be useful to limit fatty liver by maintaining or increasing fatty acid oxidation, as well as other important physiological functions such as gluconeogenesis and the liver response to harmful stimuli (e.g. inflammation) like pathogens.

As an initial step to develop feeding strategies to optimize liver function during the transition period, we have used a bovine cell culture system to evaluate the potential effects of saturated and unsaturated fatty acids on liver gene expression. Our goal is to use this data to determine the viability of feeding certain fatty acids and, potentially, commercial lipid supplements to minimize hepatic lipidosis and optimize liver function/health in transition dairy cows.

METHODS

For our study, we utilized a bovine kidney cell culture system which is metabolically similar to bovine liver cells. In comparison to liver cells, these kidney cells are easier to grow and keep alive. Treatments included a broad range of saturated and unsaturated fatty acids important to ruminant nutrition and metabolism including palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1 cis9), trans10-octadecenoic acid (18:1 trans10), trans-vaccenic acid (18:1 trans11), linoleic acid (18:2n-6), rumenic acid (18:2 cis9trans11 CLA), trans10,cis12-CLA (18:2 trans10cis12 CLA), linolenic acid (18:3n-3), arachidic acid (20:0), EPA (20:5n-3), and DHA (22:6n-3), as well as the compound Wy-14,643 a known peroxisome-proliferator activated receptor (PPAR) agonist. Bovine cells were incubated with each treatment for 6 hours. Over 30 genes were selected for investigation because they play crucial roles in the overall process of fatty acid oxidation (e.g. ACOX1, CPT1A), triglyceride synthesis (e.g. SCD, SREBF1), inflammation (e.g. HP, IL6), and gluconeogenesis (e.g. PCK1, PC) in liver. For this report, we shall focus on fatty acids 16:0, 18:0 and 20:5n-3. Genes we will discuss here are those important for long-chain fatty acid oxidation (e.g. ACOX1, CPT1A), triglyceride synthesis (e.g. SCD, SREBF1), inflammation (e.g. HP, IL6), and gluconeogenesis (e.g. PCK1, PC) in liver. For this report, we shall focus on fatty acids 16:0, 18:0 and 20:5n-3. Genes we will discuss here are those important for long-chain fatty acid oxidation, specifically CPT1A, ACOX1, PPAR and PPARG1A; gluconeogenesis, particularly PCK1; triglyceride synthesis/lipogenesis specifically SREBF1 and SCD; and inflammation or “disease”-related genes including HP, SAA1 and IL6.

RESULTS AND DISCUSSION

Long-chain fatty acid metabolism - Carnitine palmitoyltransferase 1A (CPT1A), a rate limiting enzyme for fatty acid oxidation in the liver, increased in expression with all LCFA treatments (Figure 1). Of those, relative to control, 16:0 resulted in >400% change, 18:0 in ~300%, and 20:5n-3 ~150% change. On the other hand, peroxisomal oxidation, assessed by ACOX1, had
little change over control, with no treatments being significant. PPAR and its co-activators (e.g. PPARGC1A) are a group of nuclear receptors closely involved with cellular lipid metabolism in various tissues including liver. Interestingly, no significant changes in expression of PPARA over control were seen with any of the treatments. PPARGC1A, however, had a ~200% change relative to control with 16:0 and 18:0 and ~150% with 20:5n-3 (Figure 1).

_Gluconeogenesis_ – Almost all LCFA treatments caused significant changes relative to control when PCK1 expression was examined (Figure 1). 16:0 and 18:0 resulted in up-regulation of ~200%, while 20:5n-3 resulted in ~800% down-regulation.

_TG synthesis/Lipogenesis/Cholesterol synthesis_ – 16:0 and 18:0 induced up-regulation of SREBF1 by 300 and 200% relative to control (Figure 1). SCD also was up-regulated by ~600, 400 and 150% with 16:0, 18:0 and 20:5n-3 relative to control.

_Inflammation/disease_ – HP was up-regulated with 16:0, 18:0 and 20:5n-3, being 130, 50 and 90% higher relative to control. The saturated fatty acids caused ~75% up-regulation, while 20:5n-3 caused >150% down-regulation of IL6 expression. An almost 900 and 1000% up-regulation was seen for SAA1 when 16:0 and 18:0 were used.

Results from palmitic acid and stearic acid proved to be very interesting when discussing the topic of the transition cow. These saturated fatty acids were the most potent and are highly abundant in bovine blood. On the one hand, they appear to stimulate an inflammatory response but they also stimulated desirable effects such as fatty acid oxidation, gluconeogenesis, and synthesis of cholesterol (essential for lipid export from liver). EPA proved to have very interesting and encouraging results in terms of transition and fatty liver. LCFA oxidation was increased and inflammation was decreased, while lipogenesis was not affected. Gluconeogenesis, however, was dramatically decreased when using EPA, something that may not be as desirable.

![Figure 1. Metabolic and disease-related gene expression in bovine cells in response to palmitic acid, stearic acid, and EPA.](image-url)
Overall, these in vitro results suggest that supplementing transition dairy cattle rations with lipid sources might be desirable to stimulate fatty acid oxidation in liver. However, our data also show that different metabolic and physiological responses might occur depending on the nature of the fatty acids fed. For example, lipid supplements that are high in the fatty acid EPA (20:5n-3) might be the most beneficial in terms of prevention of fatty liver (e.g. reduced triglyceride synthesis, increased oxidation). Palmitic acid and stearic acid may yield some benefits for the transition cow, but we need to be aware that these may prove to be pro-inflammatory.

Our data are the first to examine comprehensively the effects of specific fatty acids on the behavior of several key genes which are important for optimal liver function and health. Results from this initial in vitro study provide support for follow-up studies with transition cows. Those studies are required to test biological effects and determine optimal feeding doses of dietary lipid supplements rich in, for example, palmitic acid, stearic acid, oleic acid, linoleic acid, and/or EPA. Several lipid sources for dairy cattle are commercially available (see above) but the molecular mechanisms of how they work have not been examined. This information is essential for dairy farmers in terms of minimizing costs and optimizing transition success.