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Effect of rumen-protected choline supplementation on liver and adipose gene expression during the transition period in dairy cattle

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ABSTRACT

We previously reported that supplementation of rumen-protected choline (RPC) reduces the hepatic triacylglycerol concentration in periparturient dairy cows during early lactation. Here, we investigated the effect of RPC on the transcript levels of lipid metabolism-related genes in liver and adipose tissue biopsies, taken at wk −3, 1, 3, and 6 after calving, to elucidate the mechanisms underlying this RPC-induced reduction of hepatic lipidosis. Sixteen multiparous cows were blocked into 8 pairs and randomly allocated to either 1 of 2 treatments, with or without RPC. Treatments were applied from 3 wk before to 6 wk after calving. Both groups received a basal diet and concentrate mixture. One group received RPC supplementation, resulting in an intake of 14.4 g of choline per day, whereas controls received an isoenergetic mixture of palm oil and additional soybean meal. Liver and adipose tissue biopsies were taken at wk −3, 1, 3, and 6 to determine the mRNA abundance of 18 key genes involved in liver and adipose tissue lipid and energy metabolism. Milk samples were collected in wk 1, 2, 3, and 6 postpartum for analysis of milk fatty acid (FA) composition. The RPC-induced reduction in hepatic lipidosis could not be attributed to altered lipolysis in adipose tissue, as no treatment effect was observed on the expression of peroxisome proliferator-activated receptor γ, lipoprotein lipase, or FA synthase in adipose tissue, or on the milk FA composition. Rumen-protected choline supplementation increased the expression of FA transport protein 5 and carnitine transporter SLC22A5 in the liver, suggesting an increase in the capacity of FA uptake and intracellular transport, but no treatment effect was observed on carnitine palmitoyl transferase 1A, transporting long-chain FA into mitochondria. In the same organ, RPC appeared to promote apolipoprotein B-containing lipoprotein assembly, as shown by elevated microsomal triglyceride transfer protein expression and apolipoprotein B100 expression. Cows supplemented with RPC displayed elevated levels of glucose transporter 2 mRNA and a reduced peak in pyruvate carboxylase mRNA immediately after calving, showing that supplementation also resulted in improved carbohydrate metabolism. The results from this study suggest that RPC supplementation reduces liver triacylglycerol by improved FA processing and very-low-density lipoprotein synthesis, and RPC also benefits hepatic carbohydrate metabolism.
Key words: dairy cow, choline, fatty liver, gene expression

INTRODUCTION

The transition to lactation is supported by hormone-induced adaptations in fat metabolism in all mammals, including dairy cows (Friggens et al., 2004). These homeorhetic processes are accompanied by an increased release of FA from adipose tissue, elevating blood levels of NEFA. Furthermore, lipolysis is sustained during early lactation as long as energy intake cannot compensate for the increased energy demand of lactation (McNamara, 1991; Grummer, 2008). Aside from being utilized by the mammary gland, part of the circulatory NEFA are taken up by the liver, where they can be metabolized through 1 of 3 major pathways: (1) direct production of energy via oxidation of NEFA in mitochondria or peroxisomes, (2) production of ketone bodies (i.e., acetoacetate, acetone, and BHBA) through partial oxidation, or (3) reesterification into triacylglycerol (**TAG**), which can then either be sequestered in internal stores or be released into the circulation as TAG-rich, very-low-density lipoproteins (**VLDL**; Drackley et al., 2006; Grummer, 2008). In ruminants, however, VLDL secretion is relatively low, which predisposes the animals to hepatic lipidosis and ketosis (Kleppe et al., 1988).

Synthesis of VLDL requires TAG, phospholipids, cholesterol esters, microsomal triglyceride transfer protein (**MTTP**), and apolipoproteins such as apolipoprotein B100 (Bernabucci et al., 2004). Choline, a quasi-

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vitamin with various functions, is incorporated into phosphatidylcholine, the major phospholipid of VLDL. When phosphatidylcholine is limiting, choline supplementation may improve the rate of VLDL synthesis and thereby prevent excessive TAG accumulation in the liver (Grummer, 2008). In contrast to humans and rodents, choline availability in ruminants is hampered by the loss of dietary choline by extensive microbial degradation (Sharma and Erdman, 1989), which means that supplements should be industrially protected against ruminal degradation. Indeed, we and others have previously demonstrated that rumen-protected choline (**RPC**) supplementation to dairy cattle reduces fat accumulation in the liver (Cooke et al., 2007; Zom et al., 2011), increases milk production (Elek et al., 2008) and milk protein production (Zom et al., 2011). Yet, the underlying molecular mechanisms for the beneficial effects of RPC in periparturient dairy cattle are not fully understood.

In the present study, we propose a model for the action of RPC on FA processing by the bovine liver during early lactation, based on the temporal gene-expression profiles of 18 key energy metabolism-related enzymes in liver and adipose tissue, assessed by real-time quantitative PCR (**qPCR**) and the FA composition of milk to assess adipose mobilization. This model may be helpful in defining new strategies of RPC supplementation to reduce the incidence of hepatic lipidosis and ketosis in dairy cattle.

MATERIALS AND METHODS

Animals and Treatments

All experimental protocols and interventions were approved by the Ethical Committee on Animal Experiments of the Animal Sciences Group of Wageningen University and Research Centre (Lelystad, the Netherlands). The experiment was carried out between January 5, and April 26, 2009, as a complete randomized block designed structure comprising 16 Holstein-Friesian cows (7 second-parity, 5 third-parity, and 4 older cows), within a larger performance trial described by Zom et al. (2011). Cows were paired in 8 blocks on the basis of similarity in parity, expected date of calving and milk performance in the previous lactation (in order of priority).

Cows were housed in a cubicle shed and were kept in separate dry cow and lactating cow groups. Four weeks before the expected date of calving, cows were moved to the precalving group. On the day of calving, cows were separated from the dry cow group and housed in a straw-bedded calving pen. After calving, the cows were moved to the postcalving group. Cows were milked twice daily at 0600 and 1700 h in a milking parlor.

Cows within each block were randomly allocated to either the control (**CON**) or choline (**CHOL**) treatment group. Cows in the CHOL group received daily 60 g of an RPC source (ReaShure; Balchem Corp., New Hampton, NY) that was mixed with 540 g of soybean meal. As ReaShure contained 24% choline, each CHOL cow received 14.4 g of choline per day. Cows in group CON did not receive any choline supplementation, but were supplemented with a mixture of soybean meal and palm oil (582 and 18 g/d, respectively) to supply equal protein, energy, and crude fat levels. The experimental treatments started 3 wk before the expected calving date (wk −3) and lasted until 6 wk after calving (wk 6).

Diets and Feeding Management

From 4 wk before calving until calving, cows received ad libitum the precalving feed mixture (Table 1) supplemented with a close-up compound concentrate. The daily concentrate allowance was increased gradually from zero at d 21 to 0.9 kg of DM on the expected day of calving.

After calving, cows received ad libitum the postcalving feed mixture (Table 1), supplemented with an early-lactation compound concentrate. The daily allowance of this concentrate was increased with 0.45 kg of DM/d from 0.9 kg of DM/d on d 0 (i.e., calving) up to 8.1 kg of DM/d on d17. The maximum level of concentrate was maintained from d 17 until the end of the experimental period at d 43. Concentrate ingredients and chemical composition of all feeds are described by Zom et al. (2011).

The compound concentrates as well as the CHOL or CON supplement were fed individually using 3 transponder-controlled concentrate dispensers. The feed mixtures were supplied in feed weighing troughs with transponder-controlled access gates (Insentec BV, Marknesse, the Netherlands) which were continuously accessible for each cow, except during milking and when refusals were removed and fresh feed was supplied. Daily, between 1030 and 1100 h, feed refusals were removed from the troughs and a fresh feed mixture was supplied. To ensure ad libitum intake of the feed mixture, the refusal weight was at least 10% of the fresh weight at offer. The cows had unrestricted access to fresh drinking water.

Tissue Sampling

Liver and adipose tissue biopsies were taken on Mondays of wk −3, just before the experimental treatment