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***ReaShure*[®]-XC**

Precision Release Choline

Balchem Research Summary

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

A study conducted by R. M. A. Goselink, J. van Baal, H. C. A. Widjaja, R. A. Deckker, R. L. G. Zom, M. J. de Veth, and A. M. van Vuuren. Wageningen UR Livestock Research, Netherlands. Published in the February 2013 issue of the Journal of Dairy Science (Volume 96, pages 1-15).

Background

Balchem now manufactures a high-quality, ruminally protected choline product called ReaShure®-XC *Rumen Protected Choline*, the next generation of ReaShure which provides bioavailable choline in a more concentrated product.

During the transition period, hormonal changes and negative energy balance cause dramatic increases in fatty acid mobilization from adipose tissue, blood nonesterified fatty acid (NEFA) concentration, and uptake of NEFA by the liver. Ideally, NEFA are either completely oxidized to provide energy to the liver or are re-esterified to triglyceride (TG) and exported as a constituent of very low density lipoproteins (VLDL). The capacity of these metabolic pathways is exceeded during the transition period; consequently TG accumulates in the liver and ketone production from NEFA increases leading to fatty liver and subclinical or clinical ketosis. Research over the past decade (Cooke et al., 2007; Zom et al., 2011; Lima et al., 2012) has indicated that feeding ReaShure® rumen-protected choline can reduce TG accumulation in the liver and incidence of ketosis. This probably occurs by alleviating a choline deficiency because unprotected choline in feeds is extensively degraded by microorganisms in the rumen. Fatty liver is a classic symptom of choline deficiency in nonruminant animals. In nonruminants, extensive research shows that choline is required for the synthesis of phosphatidylcholine (PC), which in turn is required for VLDL assembly and secretion from the liver. The pathophysiology of fatty liver development during choline deficiency is presumed to be similar in nonruminants and ruminants such as the transition dairy cow; however, it has not been studied. Goselink et al. (2013) recently examined gene expression in liver and adipose tissue of control or ReaShure supplemented transition cows to provide insight into the mechanisms for reduction of liver TG.

Trial Design

Sixteen multiparous Holstein Friesian dairy cows in good body condition (mean = 3.25) and not experiencing subclinical ketosis were fed 12,9 gr/day/cow of choline ion in a rumen protected form or a control diet from 3 weeks prior to calving until 6 weeks postcalving. This dose of ReaShure provides 15 g/day of choline in a form that is protected from degradation in the rumen. Cows were paired according to parity and expected calving date, and cows within each pair were randomly assigned to treatment. Cows were housed in groups and transponder-controlled feeders were used to dispense 582 g soybean meal and 18 g palm oil per day to control cows and 540 g soybean meal and 12,9 gr/day/cow of choline ion in a rumen protected form per day to treatment cows. In addition to these treatments, a dry cow

feed mixture (grass silage/corn silage/wheat straw/soybean meal/premix) was consumed ad libitum and cows were gradually increased up to 0,9 kg of additional concentrate per day prior to calving. After calving, treatments continued and cows consumed a lactating cow feed mixture (grass silage/corn silage/grass seed straw/soybean meal/premix) ad libitum and were gradually increased from 0,9 to 8,1 kg of additional concentrate per day. Liver and adipose tissue were sampled at 3 weeks prior to calving and at 1, 3, and 6 weeks postpartum. Gene expression of key enzymes involved in fatty acid and energy metabolism were measured.

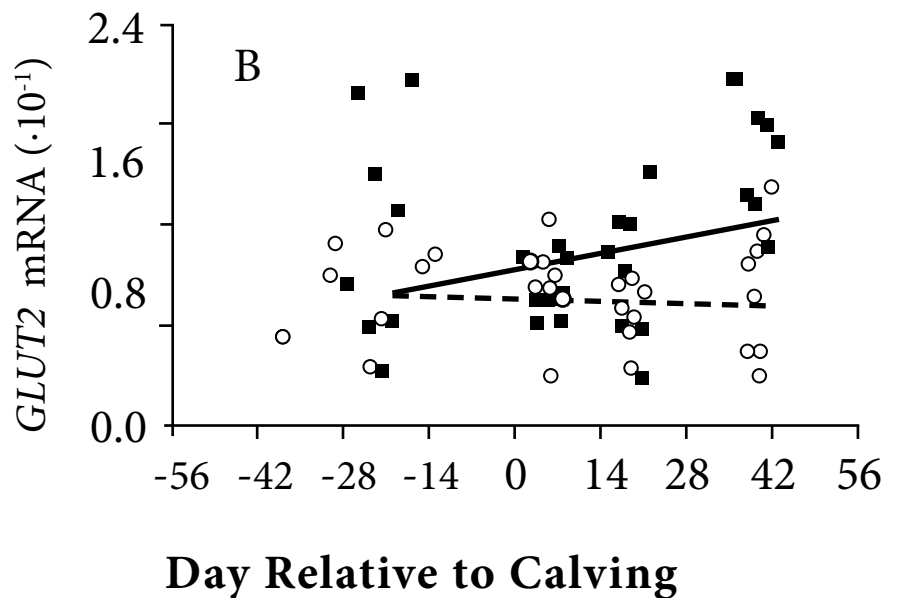
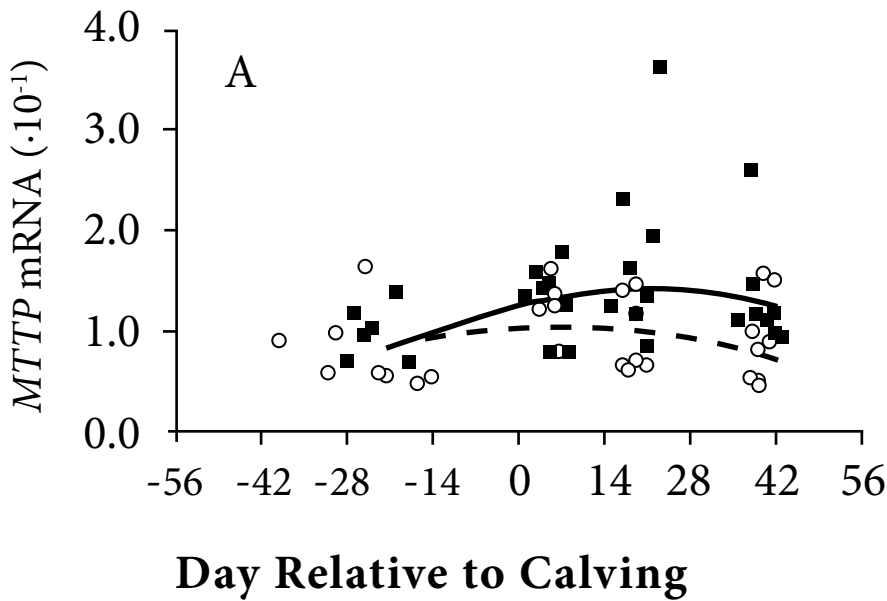
Results and Discussion

Effects of feeding ReaShure on feed intake, milk production and composition, blood metabolites, and liver TG from this trial were previously published (Zom et al., 2011). ReaShure significantly reduced liver TG at 1 and 4 weeks postpartum.

Feeding ReaShure did not affect adipose tissue gene expression of enzymes controlling lipolysis (Goselink et al., 2013) which suggests that choline does not influence liver TG levels by reducing fatty acid mobilization from adipose tissue and subsequent NEFA uptake by the liver. Cows fed ReaShure-XC showed greater gene expression of liver microsomal TG transfer protein (MTTP; Figure 1). MTTP functions at the endoplasmic reticulum of the liver cell and is required for packaging TG into a VLDL and secreting it from the liver. The elevated MTTP suggests that supplementing choline facilitates TG export out of the liver and helps the transition cow cope with elevated NEFA uptake by the liver. Choline supplementation increased liver gene expression of apolipoprotein B100, a constituent of VLDL that is also required for VLDL secretion. This also suggests that VLDL TG export is enhanced when improving the choline status of transition cows. Although expression of genes involved in PC synthesis was not measured in this study, increased PC synthesis possibly could have been involved in triggering the expression of genes coding for MTTP and apolipoprotein B100 synthesis.

Interestingly, expression of genes (e.g. GLUT2; Figure 1) related to glucose production was also affected by feeding ReaShure. Previous research indicated ReaShure increases glucose storage as glycogen in the liver (Piepenbrink and Overton, 2003) and liver cells with lower TG content have higher rates of glucose synthesis (Strang et al., 1998). Ruminants absorb very little glucose from the gastro-intestinal tract and are highly dependent on the liver to synthesize glucose. Glucose is the precursor for lactose synthesis by the mammary gland. Lactose production is the primary determinant for volume of milk produced.

Figure 1. ReaShure supplementation (solid line=RPC; dotted line=control) enhances expression of *MTTP*, a gene involved in VLDL assembly and secretion (panel A) and *GLUT2*, a gene involved in carbohydrate metabolism (Panel B).



Conclusions

Substantial evidence indicates that feeding ReaShure affects transition cows at the “whole animal” level, i.e., milk production, health, and reproduction (Oelrichs et al., 2004; Grummer, 2012; Lima et al., 2012). Additionally, previous research indicates that feeding ReaShure affects transition cows at the “organ” level, i.e., reduces liver fat during the transition period and negative energy balance (Cooke et al., 2007; Zom et al., 2011). The current study from Wageningen UR Livestock Research provides the first evidence that feeding ReaShure affects transition cows at the “molecular level”. The effects observed at the molecular level are very consistent with previous observations at the organ or whole animal level. Choline-enhanced expression of genes involved in VLDL assembly and secretion correlates with lower liver TG content; alteration of genes involved with carbohydrate metabolism correlates with higher liver glycogen content and enhanced milk production.

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