Effect of rumen-protected choline on performance, blood metabolites, and hepatic triacylglycerols of periparturient dairy cattle

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ABSTRACT

The effects of a dietary supplement of rumen-protected choline on feed intake, milk yield, milk composition, blood metabolites, and hepatic triacylglycerol were evaluated in periparturient dairy cows. Thirty-eight multiparous cows were blocked into 19 pairs and then randomly allocated to either one of 2 treatments. The treatments were supplementation either with or without (control) rumen-protected choline. Treatments were applied from 3 wk before until 6 wk after calving. Both groups received the same basal diet, being a mixed feed of grass silage, corn silage, straw, and soybean meal, and a concentrate mixture delivered through transponder-controlled feed dispensers. For all cows, the concentrate mixture was gradually increased from 0 kg/day (wk –3) to 0.9 kg of dry matter (DM)/d (day of calving) and up to 8.1 kg of DM/d on d 17 postcalving until the end of the experiment. Additionally, a mixture of 60 g of a rumen-protected choline supplement (providing 14.4 g of choline) and of 540 g of soybean meal or a (isoenergetic) mixture of 18 g of palm oil and 582 g of soybean meal (control) was offered individually in feed dispensers. Individual feed intake, milk yield, and body weight were recorded daily. Milk samples were analyzed weekly for fat, protein, and lactose content. Blood was sampled at wk –3, d 1, d 4, d 7, d 10, wk 2, wk 3, and wk 6 and analyzed for glucose, nonesterified fatty acids, and β -hydroxybutyric acid. Liver biopsies were taken from 8 randomly selected pairs of cows at wk –3, wk 1, wk 4, and wk 6 and analyzed for triacylglycerol concentration. We found that choline supplementation increased DM intake from 14.4 to 16.0 kg/d and, hence, net energy intake from 98.2 to 109.1 MJ/d at the intercept of the lactation curve at 1 day in milk (DIM), but the effect of choline on milk protein yield gradually decreased during the course of the study. Choline supplementation had no effect on milk yield, milk fat yield, or lactose yield. Milk protein yield was increased

from 1.13 to 1.26 kg/d at the intercept of the lactation curve at 1 DIM, but the effect of choline on milk protein yield gradually decreased during the course of the study. Choline supplementation was associated with decreased milk fat concentration at the intercept of the lactation curve at 1 DIM, but the effect of choline on milk fat concentration gradually decreased as lactation progressed. Choline supplementation had no effect on energy-corrected milk yield, energy balance, body weight, body condition score, and measured blood parameters. Choline supplementation decreased the concentration of liver triacylglycerol during the first 4 wk after parturition. Results from this study suggest that hepatic fat export in periparturient dairy cows is improved by choline supplementation during the transition period and this may potentially decrease the risk for metabolic disorders in the periparturient dairy cow. **Key words:** dairy cattle, choline, fatty liver, transition cow

INTRODUCTION

Fat metabolism in dairy cows changes dramatically in the last 10 to 5 d before calving (Drackley and Andersen, 2006). In this period, fat is mobilized by increased lipolysis in fat depots and, consequently, increased amounts of NEFA are released into circulation. The liver plays a central role in coordinating fatty acid metabolism (Drackley and Andersen, 2006). In the liver, NEFA can be oxidized completely or incompletely, the latter resulting in the formation of ketone bodies. The liver also re-esterifies NEFA into triacylglycerol (**TAG**). To release TAG from the liver, TAG is packaged into very-low-density lipoproteins (**VLDL**). Very-low-density lipoproteins are primarily synthesized in the liver and consist of a core of endogenously synthesized TAG surrounded by polar phospholipids and the apolipoproteins B-100, C, and E (Sundaram and Yao, 2010).

Excessive fat mobilization may stimulate fatty acid esterification in the liver. When this increased esterification is not balanced by an increased synthesis and release of VLDL, TAG may accumulate in the liver. Hepatic lipidosis, known as fatty liver disease, is a major

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disorder in transition dairy cows. A field study in the Netherlands involving data from 213 cows from 9 herds indicated that the prevalence of fatty liver disease in dairy cows was 54% (Jorritsma et al., 2001). Hepatic lipidosis is regarded as a contributing or predisposing factor to other periparturient metabolic disorders, infectious diseases, and impaired reproductive performance of dairy cows (Drackley and Anderson, 2006).

Stimulating VLDL synthesis in the liver can increase hepatic TAG release and, consequently, prevent hepatic lipidosis. Choline is an essential component for the synthesis of phosphatidylcholine (PC), being the most important phospholipid component of VLDL (Vance, 2002). Yao and Vance (1988) demonstrated in rats that choline deprivation inhibits hepatic PC biosynthesis and decreases VLDL release. In dairy cows, choline in feedstuffs is extensively degraded in the rumen (Sharma and Erdman, 1989), limiting the intestinal supply of choline. The limited intestinal supply of choline could inhibit PC biosynthesis and, consequently, VLDL synthesis in dairy cows. Protecting choline through lipid encapsulation should decrease ruminal degradation and increase the intestinal supply of choline in dairy cows. Indeed, Cooke et al. (2007) observed a decrease in hepatic TAG concentration when supplementing 15 g/d of rumen-protected choline (RPC) to nonlactating, pregnant dairy cows in which hepatic lipidosis was induced by restricting feed intake to 30% of the energy requirements for maintenance and pregnancy. Two studies have been reported in which the effect of RPC supplementation on hepatic TAG concentration and performance in periparturient dairy cattle were studied (Hartwell et al., 2000; Piepenbrink and Overton, 2003). In both studies, supplementing RPC had no significant effect on hepatic TAG. The lack of significance was attributed either to unbalanced variation in BCS (Hartwell et al., 2000) or in the incidence of abomasal displacement (Piepenbrink and Overton, 2003).

To study the effects of choline supplementation during the periparturient period, an experiment was carried out to study the effects of supplementation of RPC on performance parameters, including feed intake, milk production, blood metabolites, and liver TAG concentration of dairy cows from 3 wk before until 6 wk after calving.

MATERIALS AND METHODS

Animals and Treatments

All experimental protocols and interventions were approved by the Ethics Committee on Animal Experiments of the Animal Sciences Group of Wageningen University and Research Centre, the Netherlands.

The experiment was carried out between January 5 and April 26, 2009 as a complete randomized block designed structure comprising 38 multiparous Holstein-Friesian cows (16 second-parity, 10 third-parity, and 12 older cows). Cows were paired in 19 blocks on the basis of similarity in parity (second-parity, third-parity, and older cows), 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. The cows were dried-off 8 wk before the expected calving date. Four weeks before the expected date of calving, the selected cows were moved to a separate experimental dry cow group. On the day of calving, cows were separated from the experimental dry cow group and housed in a straw-bedded calving pen. After calving, the cows were moved to the lactating cow group. Cows were milked twice daily at 6 a.m. and 5 p.m. in a milking parlor.

Cows within each block were randomly allocated to 1 of 2 treatment groups, choline (CHOL) or control (CON). Cows in treatment group CHOL were supplemented daily with 60 g of a RPC source (Reashure; Balchem Corp., New Hampton, NY) that was mixed with 540 g of soybean meal (SBM). The Reashure product contained 24% choline and, therefore, 14.4 g/d of choline was supplied to each cow in the CHOL treatment group. Cows in treatment group CON did not receive any choline supplementation, but were supplemented with a mixture of SBM and palm oil (582 and 18 g/d, respectively) to obtain equal protein, energy, and crude fat supplementation. The experimental treatments started 3 wk before the expected calving date (wk -3) and lasted until 6 wk postcalving (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 (Table 2). 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 (Table 2). The daily allowance of this concentrate was increased by 0.45 kg of DM/d from 0.9 kg of DM/d on the day of calving up to 8.1 kg of DM/d on d 17 postcalving. The maximum level of concentrate was maintained from d 17 until the end of the experimental period.

Compound concentrates, SBM with RPC, and SBM with palm oil were fed individually using 3 transponder-controlled concentrate dispensers. The total daily allowance of the concentrates was partitioned over 6

Table 1. Ingredients of the feed mixtures and amounts of supplement fed pre- and postcalving¹

Item	Pregalving treatment		Postcalving treatment	
	CON	CHOL	CON	CHOL
Ingredients of feed mixture (% of DM)				
Wilted grass silage	29.8	29.8	52.3	52.3
Corn silage	29.6	29.6	34.7	34.7
Grass seed straw			6.2	6.2
Wheat straw	32.3	32.3		
SBM solvent extract	7.0	7.0	3.1	3.1
SBM formaldehyde treated	—	—	3.0	3.0
Vitamin and mineral premix	0.8 ²	0.8 ²	0.5 ³	0.5 ³
Magnesium oxide	0.5	0.5	—	—
Salt	—	—	0.2	0.2
Concentrate dispensers (kg/cow per day)				
SBM with palm oil	0.6		0.6	
SBM with choline		0.6		0.6
Pre-lactation concentrate	1.0	1.0		
Lactation concentrate			9.0	9.0

¹CON = control group, which did not receive any choline supplementation; CHOL = choline-supplemented group, which received daily supplementation of 60 g of a rumen-protected choline source (Reashure; Balchem Corp., New Hampton, NY) that was mixed with 540 g of soybean meal (SBM). The Reashure product contained 24% choline and, therefore, 14.4 g/d of choline was supplied to each cow in the CHOL treatment group. Cows in treatment group CON were supplemented with a mixture of SBM and palm oil (582 and 18 g/d, respectively) to obtain equal protein, energy, and crude fat supplementation.

²Dry cow mineral and vitamin premix provided 10.8 mg of Cu; 10 mg of Zn; 12 mg of Mn; 0.12 mg of Co; 0.32 mg of I; 0.14 mg of Se; 2,500 IU of vitamin A; 500 IU of vitamin D₃; and 40 IU of vitamin E per kilogram of feed mixture DM.

³Lactating cow mineral and vitamin premix provided 6 mg of Cu; 12.5 mg of Zn; 15 mg of Mn; 0.09 mg of Co; 0.6 mg of I; 0.17 mg of Se; 2,500 IU of vitamin A; 500 IU of vitamin D₃; and 7 IU of vitamin E per kilogram of feed mixture DM.

consecutive time windows of 4 h each. The compound concentrates were dispensed at a rate of 0.3 kg/min. Soybean meal with RPC and SBM with palm oil were dispensed at a rate of 0.1 kg/min. Daily, fresh precalving and postcalving feed mixtures were prepared using

a self-propelled mixer wagon equipped with a cutter loader system and an electronic weighing unit. The fresh weight formulations of the feed mixtures were adjusted twice per week for changes in DM concentration of the roughages in the feed mixtures. The feed mixtures were

Table 2. Ingredient composition of the compound concentrates fed pre- and postcalving

Feed ingredient (%)	Close-up concentrate	Early-lactation concentrate
Corn	36.9	33.3
Wheat	16.4	18.7
Soybean meal (SBM)		10.7
SBM, formaldehyde treated	11.7	6.7
Corn gluten feed		9.7
Palm kernel expeller	10.0	7.3
Cane molasses	2.5	3.0
Palm oil	2.7	3.0
Citrus pulp	7.9	2.2
Canola meal	3.6	2.0
Canola meal, formaldehyde treated	3.9	
Calcium carbonate		1.0
Soy hulls	0.2	
Sodium chloride		0.5
Urea		0.5
Monocalcium phosphate		0.1
Magnesium oxide	0.3	
Ammonium chloride	2.6	
Vitamin and mineral premix	1.3 ¹	1.3 ²

¹Provided 19 mg of Cu; 24 mg of Zn; 12.2 mg of Mn; 0.36 mg of Co; 3.0 mg of I; 0.50 mg of Se; 8,000 IU of vitamin A; 1,840 IU of vitamin D₃; and 200 IU of vitamin E per kilogram of concentrate.

²Provided 35.9 mg of Cu; 61.2 mg of Zn; 52.3 mg of Mn; 0.45 mg of Co; 1.4 mg of I; 0.31 mg of Se; 7,495 IU of vitamin A; 1,970 IU of vitamin D₃; and 103 IU of vitamin E per kilogram of concentrate.

supplied in 24 feed weighing troughs with transponder-controlled access gates (Insentec BV, Marknesse, Netherlands). Each weighing trough was accessible to all experimental cows. The maximum occupation rate was 1.6 cow/weighing trough. Each visit of a cow to a trough yielded a data log including cow identification, time of start and end of the visit, and the fresh weight consumed (difference between weight at start and end of the visit). The troughs were continuously accessible 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.

Feed Sampling and Feed Analysis

Daily, the feed mixture was sampled for DM analysis. Concentrate DM was determined once at delivery of each new batch from the supplier. The DM concentration of each feed mixture and each batch was determined from the weight difference before and after oven drying at 104°C during 36 h. The daily DMI of each cow was calculated by multiplying the daily fresh feed intake with the DM concentration.

Each ingredient of the feed mixtures was sampled twice per week for DM analyses. Weekly, representative samples of the roughages were taken immediately before mixing and stored at -20°C.

Samples of the concentrates were taken weekly. Samples of 5 consecutive weeks were pooled into 1 composite sample, which was used for chemical analysis and determination of the feeding values at the Agricultural Laboratory North Netherlands (ALNN, Ferwert, the Netherlands).

Crude ash content was determined gravimetrically after incineration at 550°C (ISO 5984; ISO, 2002). Crude protein concentration was calculated as $6.25 \times \text{N-Kjeldahl}$ (ISO 5983; ISO, 2005). Concentrations of NDF and ADF were determined according to Van Soest et al. (1991). Crude fat concentration was determined gravimetrically as the ether extract (ISO 6492; ISO, 1999). Sugar concentrations were determined as described by Van Vuuren et al. (1993). Starch concentration was determined as glucose, using the amyloglucosidase method (ISO 15914; ISO, 2004) after releasing the starch by heating in a boiling water bath in the presence of 2 N HCl (Cone, 1991). In vitro OM digestibility was determined according to Tilley and Terry (1963). Digestible OM was calculated as $(1000 - \text{ash}) \times \text{OM digestibility}$. The NE_L , intestinal digestible protein (DVE), and intestinal digestible methionine (DVMet)

were calculated from the chemical composition and OM digestibility according to the prescription of the Central Bureau for Livestock Feeding (CVB, 2008). For each cow, the voluntary DMI was calculated as the fresh weight of the consumed forage mixture multiplied with the DM concentration; DMI was calculated as voluntary DMI plus the consumed concentrate DM. The daily intake of feed ingredients and nutrients was calculated by multiplying the DMI of each feed with the nutrient concentration in feed DM.

Milk Yield and Milk Composition

Milk weights were recorded automatically at each milking. Weekly, milk samples of each cow were taken at 4 consecutive milkings (2 a.m. milkings and 2 p.m. milkings). Both a.m. milk samples were pooled to 1 composite sample; p.m. milk samples were processed likewise. The composite a.m. and p.m. milk samples were analyzed for fat, protein, and lactose concentration and SSC by Qlip (Zutphen, the Netherlands), using a Foss MilkoScan infrared automatic analyzer (Foss Electric, Hillerød, Denmark). Weighed means of concentrations and yields of fat, protein, and lactose of each cow were calculated from the recorded milk weights and the concentrations of fat, protein, and lactose in the composite a.m. and p.m. milk samples.

BW, BCS, and Energy and Protein Balance

The precalving BW were recorded weekly at the same time of day. The postcalving BW were automatically recorded twice per day at entry in the milking parlor. Weekly, cows were scored for body condition from 1 (thin) to 5 (fat) according to Boxem et al. (1998).

Net energy and DVE balances were calculated according to the CVB (2008).

Blood Sampling and Plasma Analysis

Blood samples were taken on Mondays in wk -3, 2, 3, and 6, and on d 1, 4, 7, and 10 postcalving. Blood samples were collected from the jugular vein in lithium-heparin-coated tubes (Vacuette, type 455084; Greiner Bio-One, Frickenhausen, Germany) for analyses of BHBA and in EDTA-coated tubes (Vacuette, type 455036, Greiner Bio-One) for analysis of glucose and NEFA. Immediately after collection, blood samples were placed in ice water. Within 1 h after collection, blood samples were centrifuged at $1,500 \times g$ for 10 min and, subsequently, 1 mL of blood plasma was transferred to a vial and stored at -20°C until further analysis.

Concentrations of plasma metabolites were determined by enzymatic-photometric analysis using a

HumaLyzer 3000 photometric analyzer (Human Diagnostics, Wiesbaden, Germany).

Glucose concentration was determined using the hexokinase/glucose-6-phosphate dehydrogenase method (Barthelma and Czok, 1962) using a test kit (Glucose Liqui UV Mono set; Instruchemie BV, Delfzijl, the Netherlands). The increase in NADH, which is proportional to the glucose concentration, was measured using a photometric analyzer (HumaLyzer 3000; Human Diagnostics).

The plasma concentrations of BHBA were determined by enzymatic oxidation with D-3-hydroxybutyrate dehydrogenase (Williamson et al., 1962) using a test kit (β -HBA Reagent Set; Instruchemie BV). The increase in NADH, which is proportional to the BHBA concentration, was measured using a photometric analyzer (HumaLyzer 3000; Human Diagnostics).

Plasma free fatty acid concentrations were determined by an enzymatic-colorimetric method using test kits (Wako NEFA R1 set and Wako R2 set; Instruchemie BV). The extent of colorization, which is proportional to the concentration of free fatty acids, was measured at 545 nm using a photometric analyzer (HumaLyzer 3000; Human Diagnostics).

Liver Biopsy and Triacylglycerol Analysis

Liver biopsies were performed with a core group of 16 cows from 8 randomly selected blocks on Mondays of wk -3, 1, 4, and 6 postcalving. For the biopsy, cows were in a standing position fixed in a headlock feed barrier. The position of the liver was located by percussion and the area was shaved, cleaned with water and a detergent, and disinfected (Sterillium; Bode Chemie, Hamburg, Germany). A 2-cm skin incision was made at the eleventh intercostal space and a biopsy needle (250 mm $\times$ 2 mm; Versteeg Verspaningstechniek BV, Maarsbergen, the Netherlands) was inserted to obtain liver tissue. While inserting, the needle was rotated around the axis to release liver tissue. Then, the needle was drawn back with the outer opening of the needle closed. The liver tissue was pushed out of the needle with an air-filled syringe into cryogenic vials (Cryo.s; Greiner Bio-One). This was repeated until approximately 2 g of liver tissue was collected for analysis. After that, the incision was sutured and protected with a film dressing spray (Opsite; Smith & Nephew BV, Hoofddorp, the Netherlands). The liver tissue samples were frozen immediately in liquid N and stored at -80°C awaiting further analysis.

Before analyses, liver samples were thawed and adhered water was removed using paper tissues. The concentrations of liver TAG were determined using enzymatic hydrolysis of triglycerides with lipase into

glycerol and fatty acids (Jacobs and Vandemark, 1960) using the Triglycerides LiquiColor Mono test kit (Instruchemie BV). Colorization of quinoneimine, which is proportional to the concentration of TAG in the sample, was measured at 550 nm using photometric analysis (HumaLyzer 3000, Human Diagnostics).

Statistical Analysis

For each cow, weekly means of DMI, nutrient intake, milk and milk constituent yield, BW, and BCS, and net energy and DVE balance were calculated from the daily and weekly recorded measurements.

Mixed model analysis was performed using the REML procedure in Genstat twelfth edition (2009). A polynomial curve model with a subject-specific general slope and intercept $y = a + bx + cx^2$ was used to describe the curves of DMI, milk yield, milk constituent yield, and milk composition during the first 6 wk of lactation. Treatment, DIM (both linear and quadratic), and their interactions were included as fixed effects in the model. Repeated measurements within the same cow were considered to be correlated and, therefore, an autoregression term was included in the model. Cow, block, experimental week, and the cow by week interaction were included as random effects in the model.

The model structure was

$$Y_{ijk} = \beta_0 + \beta_1 \text{DIM}_j + \beta_2 \text{DIM}_j^2 + \Delta\beta_0 T_i + \Delta\beta_1 (T \times \text{DIM}_{ij}) + \Delta\beta_2 (T \times \text{DIM}_{ij}^2) + \varepsilon_{ijk},$$

where Y_{ijk} = the response (DMI, milk yield, milk constituent yield, milk composition, BW, BCS); β_0 = the intercept; β_1 = the fixed effect for DIM; β_2 = the fixed quadratic effect of DIM; $\Delta\beta_0$ = the effect of RPC (T) on the intercept as the difference between CHOL and CON; $\Delta\beta_1$ = the fixed effect of the interaction of RPC (T) by DIM as the difference between CHOL and CON; $\Delta\beta_2$ = the fixed effect of the interaction of RPC (T) by DIM^2 as the difference between CHOL and CON; and ε_{ijk} = the residual variance.

Differences in the shape of the curve are indicated by significant treatment effects on parameters $\Delta\beta_0$, $\Delta\beta_1$, and $\Delta\beta_2$.

Liver TAG and plasma NEFA, BHBA, and glucose concentrations were analyzed, using the following model:

$$\log(Y)_{ijk} = \beta_0 + \beta_1 \text{DIM}_i + \beta_2 \text{DIM}_i^2 + \beta_3 \text{TD}_j + \beta_4 \text{TD}_j^2 + \varepsilon_{ijk},$$

where $\log Y$ = the log-transformed concentrations of liver TAG, NEFA, BHBA, or glucose; β_0 = the intercept; β_1 = the fixed effect for DIM; β_2 = the fixed

quadratic effect of DIM; β_3 = the effect of treatment day TD_j (control: $TD = 0$); β_4 = the quadratic effect of treatment day TD_j^2 (control: $TD = 0$); and ε_{ijk} = the residual variance.

Differences were declared significant at $P < 0.05$.

RESULTS

Feed and Feed Intake

The chemical composition and feeding values of the feeds are presented in Table 3 and Table 4.

Weekly means for DMI of both treatments during the experiment are presented in Figure 1. The DMI during the pre- and postcalving treatment period averaged 13.1 and 20.5 kg/d for CON and 13.1 and 21.3 kg/d for CHOL, respectively.

Choline supplementation significantly increased DMI at the start of lactation, as indicated by the significantly higher $\Delta\beta_0$ (Table 5). Because a fixed and similar amount of concentrates was fed to both treatment groups, the higher DMI at the start of lactation for CHOL was attributable to a significantly higher voluntary intake from the feed mixture than for CON (Table 6). Consequently, choline supplementation significantly increased NE_L intake at the start of lactation (Table 6).

Milk Yield and Milk Composition

Choline supplementation significantly increased milk protein yield at the start of lactation (Figure 2), but had no significant effect on milk yield and yields of milk fat and lactose during the first 42 d postcalving (Table 5). Choline supplementation significantly decreased milk fat concentration at the start of lactation, as indicated by the significantly lower $\Delta\beta_0$. However, a tendency existed ($P = 0.09$) for a treatment $\times$ DIM interaction on milk fat concentration, resulting in a less severe decrease of milk fat concentration for CHOL than for CON. Choline supplementation had no significant effect on concentrations of milk protein and lactose.

BW, BCS, and Energy and DVE Balance

At calving, the average BW was 669 and 684 kg and the average BCS was 3.2 and 3.3 for CON and CHOL, respectively. Choline supplementation had no significant effect on BW and BCS. Also, no differences between treatments were observed for NE_L and DVE balance (Table 6).

Blood Metabolites and Liver Triacylglycerols

Choline supplementation had no significant effect on the plasma concentrations of NEFA, BHBA, and glucose (Table 7).

Table 3. Chemical composition, in vitro digestibility, and feeding values of the feeds

Parameter	SBM ¹					Concentrate		
	Corn silage	Grass silage	Grass straw	Wheat straw	Untreated	Formaldehyde treated ²	SBM and palm oil	SBM and choline
DM (%)	31.8	37.1	83.3	84.6	86.8	86.8	87.7	87.8
OM (%) of DM	95.4	89.6	92.5	91.4	93.1	93.1	93.2	93.0
CP (%) of DM	6.9	17.2	4.9	3.1	47.6	53.7	50.2	51.5
NH ₃ -N (%) of total N	—	6	4	3	—	—	—	—
Crude fat (%) of DM	3.3	4.1	1.0	1.0	3.1	2.4	8.9	9.2
NDF (%) of DM	38.6	44.1	68.4	79.0	14.4	18.6	11.0	12.2
ADF (%) of DM	21.9	26.6	40.1	51.7	9.4	8.7	5.1	6.0
Sugars (%) of DM	1.0	9.3	11.7	<0.5	10.8	11.1	10.0	9.6
Starch (%) of DM	36.1	—	—	—	1.0	2.4	0.5	0.4
IVDOM ³ (%)	77.2	80.4	54.2	42.7	90.8	91.2	86.7	85.4
NE _L (MJ/kg of DM)	6.8	6.6	4.0	2.9	8.0	7.9	8.6	8.6
DVE ⁴ (%) of DM	5.1	6.2	2.0	-0.9	26.7	42.4	24.4	24.5
DVMet ⁴ (%) of DVE)	2.5	2.2	—	—	1.7	1.6	2.1	2.1

¹Soybean meal.

²MervoBest (Pre-Mervo BV, Utrecht, the Netherlands).

³In vitro digestible OM.

⁴Intestinal digestible protein (DVE) and intestinal digestible methionine (DVMet) calculated according to CVB (2008).

Figure 1. Numerical daily mean DMI of 2 groups of dairy cattle receiving either no supplemental choline (control treatment; dotted line) or supplemental choline (solid line).

The stage of lactation had a significant linear and quadratic effect on liver TAG, with the greatest concentration of TAG at wk 1 after calving (Figure 3). Choline supplementation significantly decreased liver TAG concentration in wk 1 and 4 but not in wk 6, as indicated by the significantly lower $\Delta\beta_3$ and the significantly higher $\Delta\beta_4$ for this parameter (Table 7).

DISCUSSION

Feed Intake

In our study, RPC was fed as a mixture with SBM using transponder-controlled concentrate dispensers, which allowed control and monitoring of the individual intake of RPC. All cows consumed their daily allowance of 60 g of RPC independent of the voluntary intake of the feed mixture. This meant that directly after calving, when voluntary intake was lowest, the relative proportion of RPC in the overall diet was highest, with the proportion of RPC decreasing as voluntary intake increased. A choline requirement for dairy cows has not been determined, but Pinotti et al. (2005) suggested that supplementation with 12 to 20 g/d is probably adequate for periparturient dairy cows. The assumed supply of 14.4 g/d of choline from 60 g of Reashure is within the range suggested by Pinotti et al. (2005).

Effects of choline supplementation on DMI, as reported in the literature, have indicated either no effect on DMI in periparturient cows (Hartwell et al., 2000; Pinotti et al., 2003; Janovick Guretzky et al., 2006; Elek et al., 2008) or a tendency for increased DMI postcalving (Lima et al., 2007). In our study, the initial level of feed intake at the start of lactation for CHOL was increased compared with CON.

Table 4. Chemical composition and nutritive value of the feed mixtures

Item	Precalving diet	Postcalving diet
DM (%)	44.2	38.4
OM (% of DM)	91.2	91.4
CP (% of DM)	11.6	14.7
Crude fat (% of DM)	2.8	3.4
NDF (% of DM)	51.1	41.8
ADF (% of DM)	31.8	24.6
Sugars (% of DM)	4.0	6.6
Starch (% of DM)	10.8	12.6
NE _L (MJ/kg of DM)	5.5	6.5
DVE ¹ (% of DM)	5.0	7.2
DVMet ¹ (% of DVE)	2.1	2.1

¹Intestinal digestible protein (DVE) and intestinal digestible methionine (DVMet) calculated according to CVB (2008).

The significantly greater NE_L intake for CHOL at 1 DIM was because of the higher voluntary DMI of the feed mixture on the CHOL treatment compared with CON.

Milk Yield, Milk Composition, BW, and BCS

Choline supplementation in our study resulted in an increased milk protein yield at the start of lactation, whereas other performance parameters were not significantly affected by choline supplementation. Reported effects of choline supplementation on milk yield, milk constituent yields, and milk composition vary from no effect to a significant increase. Some authors reported that milk yield did not change with choline supplementation (Hartwell et al., 2000; Janovick Guretzky et al., 2006) whereas others observed a tendency for higher milk yields (Piepenbrink and Overton, 2003; Lima et al., 2007) or significantly increased milk yield (Erdman and Sharma, 1991; Pinotti et al., 2003; Zahra et al., 2006; Elek et al., 2008).

Choline is also a precursor of acetylcholine and a source of methyl groups for resynthesis of methionine from homocysteine (Zeisel et al., 1991). Supplementing choline may, thus, have a sparing effect on the methyl group donor methionine, which for the lactating dairy cow is considered one of the first limiting AA for milk protein synthesis. Choline capacity to spare methionine, through donating methyl groups, is one potential mechanism how choline supplementation may increase milk protein yield. The calculated level of DVMet in the diets as proportion of DVE was 2.1%, which is above the recommended level of 1.9% of DVE as proposed by Subnel (1997) and comparable to the recommendations by Rulquin et al. (2001) and Schwab et al. (2001). Davidson et al. (2008) showed that RPC but not rumen-protected methionine increased milk yield

Table 5. The effect of choline supplementation on DMI, milk yield, and yield and concentration of milk fat, milk protein, and lactose using the statistical model $Y_{ijk} = \beta_0 + \beta_1 \text{DIM}_j + \beta_2 \text{DIM}_j^2 + \Delta\beta_0 T_i + \Delta\beta_1 (T \times \text{DIM}_{ij}) + \Delta\beta_2 (T \times \text{DIM}_{ij}^2) + \varepsilon_{ijk}$ ¹

Item	Parameter ²	Factor			Contrast CHOL vs. CON ³		
		Constant	DIM	DIM ²	Treatment (T)	T × DIM	T × DIM ²
		β_0	β_1	β_2	$\Delta\beta_0$	$\Delta\beta_1$	$\Delta\beta_2$
DMI ⁴ (kg/d)	Estimate	14.41	0.456	−0.00621	1.630	−0.067	0.00089
	SED	0.541	0.04767	0.00102	0.76	0.0671	0.00144
	P-value		<0.001	<0.001	0.03	0.31	0.53
Voluntary DMI ⁵ (kg/d)	Estimate	12.11	0.0250	0.00093	1.573	−0.046	0.00042
	SED	0.536	0.00470	0.00102	0.74	0.0662	0.001418
	P-value		<0.001	0.113	0.03	0.49	0.78
Milk yield (kg/d)	Estimate	27.17	1.0475	−0.01583	1.884	−0.0465	0.00039
	SED	1.476	0.0838	0.01766	1.779	0.1174	0.00247
	P-value		<0.001	<0.001	0.29	0.69	0.88
Milk fat yield (g/d)	Estimate	1,677	16.20	−0.346	1.942	0.9869	0.02331
	SED	87.0	6.662	0.14446	105.8	9.297	0.2013
	P-value		0.172	0.001	0.99	0.92	0.91
Milk protein yield (g/d)	Estimate	1,125	17.706	−0.3054	136.38	−7.775	0.13864
	SED	52.9	4.347	0.09516	62.98	6.061	0.1326
	P-value		<0.001	<0.001	0.03	0.20	0.30
Lactose yield (g/d)	Estimate	1,031	67.272	−1.0553	117.55	−4.782	0.09928
	SED	78.6	6.173	0.13338	102.3	8.617	0.1859
	P-value		<0.001	<0.001	0.25	0.58	0.59
Milk fat (%)	Estimate	6.5007	−0.1457	0.002165	−0.5757	0.04064	−0.000635
	SED	0.1829	0.01708	0.0003741	0.2454	0.02382	0.0005216
	P-value		<0.001	<0.001	0.02	0.09	0.22
Milk protein (%)	Estimate	4.401	−0.08386	0.001297	0.131	−0.0100	0.00017
	SED	0.0729	0.007237	0.000159	0.01022	0.001009	0.0002217
	P-value		<0.001	<0.001	0.20	0.32	0.45
Lactose (%)	Estimate	4.266	0.03150	−0.000534	0.0134	0.000999	−0.00003417
	SED	0.0469	0.004091	0.0008876	0.06419	0.005709	0.0001238
	P-value		<0.001	<0.001	0.85	0.86	0.78

¹ Y_{ijk} = the response (DMI, milk yield, milk constituent yield, milk composition, BW, BCS); β_0 = the intercept; β_1 = the fixed effect for DIM; β_2 = the fixed quadratic effect of DIM; $\Delta\beta_0$ = the effect of rumen-protected choline (T) on the intercept as the difference between choline supplementation (CHOL) and control (CON); $\Delta\beta_1$ = the fixed effect of the interaction of rumen-protected choline (T) by DIM as the difference between CHOL and CON; $\Delta\beta_2$ = the fixed effect of the interaction of rumen-protected choline (T) by DIM² as the difference between CHOL and CON; and ε_{ijk} = the residual variance.

²SED = standard error of difference.

³The CON group did not receive any choline supplementation; the CHOL group received daily supplementation of 60 g of a rumen-protected choline source (Reashure; Balchem Corp., New Hampton, NY) that was mixed with 540 g of soybean meal. The Reashure product contained 24% choline and, therefore, 14.4 g/d of choline was supplied to each cow in the CHOL treatment group. Cows in treatment group CON were supplemented with a mixture of soybean meal and palm oil (582 and 18 g/d, respectively) to obtain equal protein, energy, and crude fat supplementation.

⁴Total DMI from the ad libitum-fed roughage mixture plus fixed amounts of concentrate supplied by dispensers.

⁵DMI intake from the ad libitum-fed roughage mixture.

Table 6. The effect of choline supplementation on BW, BCS, and energy and protein balance of periparturient dairy cattle using the statistical model $Y_{ijk} = \beta_0 + \beta_1 \text{DIM}_j + \beta_2 \text{DIM}_j^2 + \Delta\beta_0 T_i + \Delta\beta_1 (T \times \text{DIM}_{ij}) + \Delta\beta_2 (T \times \text{DIM}_{ij}^2) + \varepsilon_{ijk}$ ¹

Item	Parameter ²	Factor			Contrast CHOL vs. CON ³		
		Constant	DIM	DIM ²	Treatment (T)	T × DIM	T × DIM ²
		β_0	β_1	β_2	$\Delta\beta_0$	$\Delta\beta_1$	$\Delta\beta_2$
BW (kg)	Estimate	669.0	−1.233	0.022375	14.586	−0.313	0.002735
	SED	13.98	0.3168	0.006508	16.52	0.04458	0.00918
	P-value		<0.001	<0.001	0.38	0.48	0.77
BCS	Estimate	3.23	−0.01344	0.000068	0.09797	−0.004403	0.000016213
	SED	0.1477	0.012485	0.0027	0.1864	0.01753	0.0003806
	P-value		<0.001	0.699	0.60	0.80	0.97
FPCM ⁴ (kg/d)	Estimate	37.92	0.4428	−0.00767	1.041	0.008188	−0.0004
	SED	1.545	0.0985	0.00212	1.771	0.1375	0.000403
	P-value		<0.001	<0.001	0.56	0.95	0.89
Net energy intake (MJ/d)	Estimate	98.203	3.7569	−0.05312	10.897	−0.4899	0.00697
	SED	3.72	0.3356	0.007193	5.188	0.4724	0.0105
	P-value		<0.001	<0.001	0.04	0.30	0.49
Net energy balance (MJ/d)	Estimate	−63.222	2.3315	−0.02864	7.452	−0.5055	0.007298
	SED	5.442	0.4455	0.009682	6.900	0.6217	0.01349
	P-value		<0.001	<0.001	0.28	0.42	0.59
Protein balance (g of DVE/d) ⁵	Estimate	−284	22.491	−0.32564	−148.16	8.719	0.00697
	SED	3.72	7.149	0.1591	93.91	9.941	0.2216
	P-value		<0.001	<0.001	0.12	0.38	0.48

¹ Y_{ijk} = the response (DMI, milk yield, milk constituent yield, milk composition, BW, BCS); β_0 = the intercept; β_1 = the fixed effect for DIM; β_2 = the fixed quadratic effect of DIM; $\Delta\beta_0$ = the effect of rumen-protected choline (T) on the intercept as the difference between choline supplementation (CHOL) and control (CON); $\Delta\beta_1$ = the fixed effect of the interaction of rumen-protected choline (T) by DIM as the difference between CHOL and CON; $\Delta\beta_2$ = the fixed effect of the interaction of rumen-protected choline (T) by DIM² as the difference between CHOL and CON; and ε_{ijk} = the residual variance.

² SED = standard error of difference.

³ The CON group did not receive any choline supplementation; the CHOL group received daily supplementation of 60 g of a rumen-protected choline source (Reashure; Balchem Corp., New Hampton, NY) that was mixed with 540 g of soybean meal. The Reashure product contained 24% choline and, therefore, 14.4 g/d of choline was supplied to each cow in the CHOL treatment group. Cows in treatment group CON were supplemented with a mixture of soybean meal and palm oil (582 and 18 g/d, respectively) to obtain equal protein, energy, and crude fat supplementation.

⁴ Fat (4.0%)- and protein (3.3%)-corrected milk.

⁵ DVE = intestinal digestible protein.

Figure 2. The modeled effect of choline supplementation on milk protein yield in periparturient dairy cattle. Dotted line = control treatment; solid line = choline treatment. Control: milk protein yield (g/d) = 1,125 (SED: 52.9) + 17.71 (SED: 4.35) $\times$ DIM – 0.305 (SED: 0.0952) $\times$ DIM². Treatment effect: 136.3 (SED: 63.0; $P = 0.03$) – 7.78 (SED: 6.06; $P = 0.20$) $\times$ DIM + 0.139 (SED: 0.133; $P = 0.30$) $\times$ DIM²; SED = standard error of difference.

of cows fed a methionine-limited diet. The supply of DVMet above suggested requirements in the current study may explain the absence of an effect of choline supplementation on milk yield.

Choline supplementation increased DMI and, consequently, the energy and nutrient supply at the start of lactation. The increased energy and nutrient supply is another explanation for the increased milk protein yield (DePeters and Cant, 1992).

Figure 3. The modeled effect of choline supplementation on concentrations of triacylglycerols (TAG) in the liver of periparturient dairy cattle. Solid bars = control treatment; open bars = choline treatment. Control: TAG log (g/g of wet tissue) = 4.24 (SED: 0.14) + 0.0230 (SED: 0.0031) $\times$ DIM – 0.00114 (SED: 0.000124) $\times$ DIM². Treatment effect: –0.0330 (SED = 0.00969; $P = 0.001$) $\times$ DIM + 0.000627 (SED: 0.00175; $P < 0.001$) $\times$ DIM²; SED = standard error of difference.

Table 7. The effect of choline supplementation on plasma NEFA, BHBA, glucose, and liver triacylglycerol (TAG) concentration in periparturient dairy cattle using the statistical model $\log(Y)_{ijk} = \beta_0 + \beta_1 \text{DIM}_i + \beta_2 \text{DIM}_i^2 + \beta_3 \text{TD}_j + \beta_4 \text{TD}_j^2 + \epsilon_{ijk}$

Item	Parameter ²	Factor			Effect of choline treatment day		
		Constant	DIM	DIM ²	TD _j	TD _j ²	TD _j ²
NEFA [log(mmol/L)]	Estimate SED P-value	β_0 –1.219 0.0669	β_1 0.02450 0.003582 <0.001	β_2 –0.001084 0.0001372 <0.001	β_3 –0.01166 0.08831 0.191	β_4 –0.313 0.00016925 0.111	β_4 –0.313 0.00016925 0.111
BHBA [log(mmol/L)]	Estimate SED P-value	–0.6214 0.003524	0.005395 0.0018608 <0.001	0.00007072 <0.001	0.005215 0.004210 0.219	–0.0000666 0.000085269 0.436	–0.0000666 0.000085269 0.436
Glucose [log(mmol/L)]	Estimate SED P-value	1.260 0.0126	–0.003261 0.0005622 <0.001	–0.0001107 0.00001995 <0.001	0.0001095 0.00118022 0.926	–0.000009793 0.000002363 0.679	–0.000009793 0.000002363 0.679
TAG [log(g/g of wet tissue)]	Estimate SED P-value	4.244 0.1354	0.02297 0.003130 <0.001	–0.001143 0.0001242 <0.001	–0.0330 0.009688 0.001	0.0006271 0.00017501 <0.001	0.0006271 0.00017501 <0.001

¹log Y = the log-transformed concentrations of liver TAG, NEFA, BHBA, or glucose; β_0 = the intercept; β_1 = the fixed effect for DIM; β_2 = the fixed quadratic effect of DIM; β_3 = the effect of treatment day TD_j (control: TD = 0); β_4 = the quadratic effect of treatment day TD_j² (control: TD = 0); and ϵ_{ijk} = the residual variance.

²SED = standard error of difference.

The lack of a significant treatment effect on BW or BCS corresponds to the lack of a significant treatment effect on energy balance. NE_L intake at the start of lactation was higher for CHOL than for CON, but also net energy output as fat-and-protein-corrected milk was higher for CHOL than for CON, although not significantly.

Blood Metabolites and Liver Triacylglycerols

The lack of an effect of RPC on plasma glucose concentration is in agreement with other studies (Janovick Guretzky et al., 2006; Cooke et al., 2007).

The lack of an effect of RPC supplementation on plasma NEFA concentration is in agreement with Hartwell et al. (2000), Piepenbrink and Overton (2003), Janovick Guretzky et al. (2006), and Davidson et al. (2008). However, some studies have reported decreased concentrations of plasma NEFA in response to RPC supplementation (Pinotti et al., 2003; Cooke et al., 2007).

The lack of an effect of RPC supplementation on plasma BHBA concentration is in agreement with observations elsewhere (Piepenbrink and Overton, 2003; Pinotti et al., 2003; Janovick Guretzky et al., 2006; Cooke et al., 2007; Davidson et al., 2008). However, one study reported a tendency ($P = 0.11$) for decreased plasma concentrations of BHBA at 20 DIM when RPC was supplemented from -14 to 30 DIM (Pinotti et al., 2004).

Concentrations of BHBA and NEFA in plasma are often used as indicators for the energy status of dairy cows and to detect subclinical ketosis. For plasma BHBA, different cutoff levels may be used to diagnose subclinical ketosis (Carrier et al., 2004; Hiss et al., 2009). In the Netherlands, subclinical ketosis is diagnosed at plasma BHBA concentration >0.9 mmol/L per mL (Animal Health Services, Deventer, the Netherlands). For NEFA concentration, the threshold level indicating a negative energy balance is 0.6 mmol/L (Hiss et al., 2009). Based on these cutoff levels, we conclude that, on average, the treatment groups did not suffer from subclinical ketosis or a severe negative energy balance.

Although plasma levels of NEFA and BHBA were below the critical values that indicate subclinical ketosis, the hepatic concentrations of TAG in wk 1 and 3 were between 51 and 100 mg/g of wet tissue, indicating moderate lipidosis (Gaal et al., 1983; Jorritsma et al., 2001; Bobe et al., 2004). This indicates that RPC supplementation may also benefit cows at a BCS that is considered adequate at calving and apparently not suffering from subclinical ketosis. Fatty liver is considered a major metabolic disorder in dairy cows and its incidence is strongly associated with other metabolic

disorders (see review by Bobe et al., 2004). In addition, the incidence of infectious diseases such as mastitis and metritis are also associated with fatty liver and are likely related to immune response suppression (Bobe et al., 2004). Therefore, the decrease in liver TAG observed in the current study suggests that choline has the potential to decrease the incidence of metabolic disorders in the periparturient dairy cow beyond fatty liver itself.

The decreased liver TAG concentration with choline supplementation, as observed in our study, agrees with Cooke et al. (2007), who used a feed-restricted dry cow model to induce fatty liver. To our knowledge, Hartwell et al. (2000) and Piepenbrink and Overton (2003) are the only prior published studies to have reported the effects of choline supplementation on liver TAG concentrations in the transition cow. These authors did not report a significant difference in liver TAG concentration after feeding RPC to transition cows, which is in contrast to that reported by Cooke et al. (2007) and what was found in the present study. Less variation in liver TAG concentration, due to greater animal numbers and more frequent sampling, may be the basis for detecting a significant decrease in the present study compared with that of Hartwell et al. (2000) and Piepenbrink and Overton (2003).

In our study, the lack of a significant difference in plasma NEFA and BHBA concentration and in BCS between both treatments suggests that choline supplementation did not alter adipose mobilization or BHBA production in the liver. Therefore, the decreased liver TAG in the first weeks postcalving with choline supplementation is expected to result from increased synthesis of VLDL and a subsequent increase in the release of TAG from the liver.

CONCLUSIONS

Based on the decrease in liver TAG concentration and the absence of a change in plasma NEFA concentration observed in this study, the results suggest that hepatic fat export in periparturient dairy cows was improved by choline supplementation during the transition period and this may potentially decrease the risk for metabolic disorders in the periparturient dairy cow.

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