Effect of dietary rumen-protected choline on milk production of dairy cows: A meta-analysis

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ABSTRACT

Research studies presented inconsistent results on the effects and action of choline in dairy cow diets. A meta-analysis was conducted to quantify the effects of dietary rumen-protected choline on production characteristics of dairy cows. Dry matter intake (kg/d), milk yield (kg/d), milk fat (% and kg/d), and milk protein (% and kg/d) were evaluated as dependent variables in models. The number of treatment means varied from 20 obtained in 7 studies for milk fat and protein contents to 34 from 11 studies (12 experiments) for milk yield. Accounting for experiment as a random effect, DMI, milk yield, milk protein content, and milk protein yield could adequately be related to levels of dietary rumen-protected choline chloride by a logistic model. Marginal responses in milk yield decreased from 131.5 to 0.037 g of milk/g of dietary rumen-protected choline chloride when supplementation increased from 6 to 50 g/d. From estimated values for the metabolizable Met supplied by diets, it appears that dietary rumen-protected choline chloride functions as a methyl donor to spare Met for milk protein synthesis. However, more accurate input data on Met status of diets are needed to confirm this. Within the range of 6 to 50 g/d of rumen-protected choline chloride, milk fat content decreased linearly at a rate of 0.00339% for a 1 g/d increase in dietary rumen-protected choline chloride. This illustrates that dietary rumen-protected choline chloride has no effect on milk fat content. Numerous physiological and dietary factors probably related to responses obtained with dietary rumen-protected choline supplementation, and the precise mechanism of choline action in the lactating dairy cow warrants further investigation.

Key words: rumen-protected choline, milk production, milk composition, meta-analysis

INTRODUCTION

Choline is a trimethylated quaternary amine involved with transport of fat from the liver. It provides labile methyl groups for transmethylation reactions and is required for the synthesis of phosphatidylcholine found in cell membranes (Hartwell et al., 2000; Zahra et al., 2006). Its ability to increase milk fat synthesis (Erdman et al., 1984) and to treat cows suffering from fatty livers after parturition (Cooke et al., 2007) had stimulated research on dietary supplementation of choline for dairy cows. However, extensive degradation of unprotected dietary choline by rumen bacteria necessitates the use of rumen-protected supplements containing choline chloride, which subsequently allow the release and absorption of choline in the small intestine (Atkins et al., 1988; Deuchler et al., 1998).

The bioavailability of dietary choline in dairy cows is likely to be influenced by factors such as dose, mode of administration (topdress vs. incorporated in compound feed), lactation stage (Baldi and Pinotti, 2006), dietary protein (Hartwell et al., 2000), and Met supply (Brüsemeister and Südekum, 2006; Davidson et al., 2008). These could have contributed to the inconsistency in results obtained with choline supplementation among studies, as summarized in reviews by Pinotti et al. (2002) and Brüsemeister and Südekum (2006).

Narrative literature reviews are based on the results of null hypothesis significance testing presented by individual studies (Sales, 2009). The obvious subjectivity involved in the process limits the relevance of conclusions (Sauvant et al., 2008). With statistical techniques applied to account for variation among different research studies, the current study aimed to quantify the effects of the inclusion of dietary rumen-protected choline (RPC) in dairy cow diets on milk production and characteristics.

MATERIALS AND METHODS

Data Sources

Internet search engines and publishers' Web sites were used to identify studies that have evaluated the influence of dietary choline on production characteristics of

Received January 25, 2010.

Accepted April 5, 2010.

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dairy cows. Selection criteria included 1) dietary choline supplement should be rumen-protected; 2) no treatment was applied that involved restriction or excess of any nutrient in the basal diet; 3) study was presented in a peer-reviewed journal; (4) study has been published in English to extract all relevant information.

Eleven studies (Table 1) were identified that fulfilled in the required criteria. The study of Xu et al. (2006) presented means from 2 independent experiments. Both were found suitable for inclusion in the data set. Inconsistent reporting of independent variables and large differences in variables such as period of choline supplementation and lactation stage of experimental cows were obtained among individual studies. Corresponding authors were contacted when information on the mode of application or unit of dose of RPC (total product or choline chloride) were unclear.

In all studies animals received TMR. Studies other than Deuchler et al. (1998), Xu et al. (2006), and Toghdory et al. (2007) indicated that recommendations by NRC (2001) or earlier versions were used for diet formulation. Studies by Eastridge et al. (2006) and Janovick Guretzky et al. (2006) included a breed (Jersey) other than Holstein. Except for Xu et al. (2006), where treatments means included primiparous cows, multiparous cows were used in all studies that reported parity number. Hartwell et al. (2000), Piepenbrink and Overton (2003), Janovick Guretzky et al. (2006), and Elek et al. (2008) stated that cows were in a >2 parity stage. Several studies included a prepartum treatment with dietary RPC (Table 1).

Commercial products were used as dietary choline supplements. This necessitated a description of products as presented in Table 2. Deuchler et al. (1998) and Xu et al. (2006) did not mention trade names of products used. Rumen stability of 85% for Reashure (Balchem Corp., Slate Hill, NY), determined by *in vitro* analysis, is in agreement with a value of 79% after 8 h of incubation in rumen-cannulated adult ewes reported by Elek and Husveth (2007).

Attempts to adjust RPC for rumen stability were complicated by differences in evaluation methods applied for different products (Table 2) and uncertainty on the adequacy of some methods. Consequently, dose of choline were standardized to rumen-protected choline chloride (**RPCC**) equivalents.

Variables

With a minimum of 6 experiments for any outcome arbitrarily set as a requirement for evaluating the effect of treatment, DMI (kg/d), milk yield (**MY**; kg/d), milk fat content (**MFC**; %), milk fat yield (**MFY**; kg/d), milk protein content (**MPC**; %), and milk protein yield

(**MPY**; kg/d) were the only dependent variables that could be considered for analyses. Relevant descriptive statistics of milk production responses are summarized in Table 3.

Mode of application was unsuitable as a discrete (qualitative) independent variable in models. This was justified by uncertainty on the definition of top dress versus blended. In some studies in which mode of application was stated as topdress, dietary RPC was blended with a diet component or part of the TMR before it was applied (Table 1). Furthermore, the mode of application described as blended was represented by a limited number of studies (3) and values, resulting in unbalanced data. The inclusion of a prepartum treatment period in several experiments, and Latin square designs with short time periods per treatment per cow in some experiments, rendered the use of trial length as a continuous independent variable unrealistic. Stage of lactation as a possible discrete independent variable was hampered by an unequal distribution of categories and by periods of sampling that would overlap for some stages (Table 1).

The Cornell Net Carbohydrate and Protein System (**CNCPS**; version 6.1.30.0, Cornell University, Ithaca, NY) software was used to estimate dietary metabolizable Met and Met balance. However, with inadequate feed ingredient descriptions and animal characteristics ill defined by individual studies, model inputs had to be based on default values and numerous assumptions. Although estimates are presented in Table 1 to be used in the discussion, these values were found to be too unstable to be included as a continuous independent variable. Furthermore, studies were selected for the current evaluation based on suggested optimal diet formulation. This was done to avoid any possible suboptimal nutrient intakes. However, this has eliminated data from studies in which diet manipulation was applied to create possible limitations, which would be needed to investigate the interaction between RPC supplementation and metabolizable Met supply. The presence or absence of a prepartum treatment with dietary RPC could be added as a discrete fixed variable, whereas dose (g/d of RPCC) presented a continuous variable.

Model Development

Exponential (Ørskov and McDonald, 1979), Gompertz (Emmans, 1989), logistic (Doepel et al., 2004), and Richards (Norris et al., 2007) models were evaluated to describe the relationship between dose and dependent variables. The NLMIXED procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC) was applied to include the random effects of experiment in models, as described by Vyas and Erdman (2009).

Table 1. Summary of studies used to evaluate the effect of dietary rumen-protected choline supplementation on DMI, milk yield (MY), milk fat content (MFC), milk fat yield (MFY), milk protein content (MPC), and milk protein yield (MPY) in lactating dairy cows

Study	Country	Dose, g/d of choline chloride	Mode of application	Cows/treatment, n	Period postpartum, d	Met, ¹ % of MP	Met balance, ² g/d	Outcomes used
Chung et al. (2009)	United States	0, 12.5, 25	Topdress	6 ³	41–51	1.84	+1.1	DMI, MY
Deuchler et al. (1998)	United States	0, 50	Blended	5	— ⁴	1.80	+4.9	DMI, MY, MFC, MPC
Eastridge et al. (2006)	United States	0, 15, 30	Blended	12	0–90	— ⁵	— ⁵	DMI, MY, MFC, MPC
Elek et al. (2008)	Hungary	0, 50	Topdress ⁶	16	0–60 ⁷	1.83 ⁸	+14.4	DMI, MY, MFC, MFY, MPC, MPY
Erdman and Sharma (1991) ⁹	United States	0, 15, 30, 45	Blended	8, 7, 7, 7 ³	155–176	1.94	+10.0	DMI, MY, MFC, MFY, MPC, MPY
Hartwell et al. (2000) ¹⁰	United States	0, 6, 12	Topdress ⁶	7	0–120 ¹¹	1.89	+17.1	DMI, MY, MFC, MFY, MPC, MPY
Janovick Guretzky et al. (2006) ¹²	United States	0, 15	Topdress	24	0–21 ¹¹	1.85 ⁸	–0.6	DMI, MY, MFC, MFY, MPC, MPY
Piepenbrink and Overton (2003)	United States	0, 11.3, 15, 18.8	Topdress	12	0–63 ¹¹	2.14 ⁸	+3.5	DMI, MY, MFC, MFY, MPC, MPY
Pinotti et al. (2003)	Italy	0, 20	Topdress ¹³	13	0–30 ¹¹	1.85	+1.0	MY, MFC, MFY, MPC, MPY
Toghdory et al. (2007)	Iran	0, 6, 12.5	— ⁶	8 ³	— ¹⁴	1.91 ⁸	— ⁵	MY, MFC, MFY, MPC, MPY
Xu et al. (2006) ¹⁵	China	0, 7.5	Topdress	7	0–21 ¹¹	1.93	–2.4	DMI, MY, MFC, MPC
		0, 11.3, 22.5, 33.8	Topdress	9	0–15 ¹¹	1.92	–4.0	DMI, MY, MFC, MFY, MPC, MPY

¹Indicated for basal diets.²Lowest value identified among different treatments within study.³Latin square design.⁴Midlactation, trial duration 14 d.⁵Inadequate data for calculation.⁶Blended with grain.⁷Included a prepartum treatment of 25 g/d of dietary rumen-protected choline chloride.⁸Value presented by author.⁹Means for 16.5% CP treatment.¹⁰Means for 4.0% prepartum RUP treatment.¹¹Included a prepartum treatment of dietary rumen-protected choline chloride at similar levels as applied postpartum.¹²Values for outcomes combined for Holsteins and Jerseys.¹³Blended with 0.5 kg of TMR.¹⁴21-d periods, stage of lactation not indicated.¹⁵Two independent experiments.

Table 2. Description of commercial rumen-protected choline products used in the different studies

Product	Manufacturer	Study	Choline chloride, % (wt/wt)	Rumen stability, %
Capshure choline	Balchem Corp., Slate Hill, NY	Hartwell et al. (2000)	25	85 ¹
Norcol-25	Nordos Italy, Bussolengo, Italy	Elek et al. (2008)	25	20.4 ²
Overcholine 45% Coated	Ascor Chimici, Forli, Italy	Pinotti et al. (2003)	45	—
Pro-Choline 50	—	Chung et al. (2009)	50	50 ³
Reashure	Balchem Corp., Slate Hill, NY	Piepenbrink and Overton (2003); Eastridge et al. (2006); Janovick Guretzky et al. (2006); Toghdory et al. (2007)	25	85 ¹
Showa Denko	KK, Yokyo, Japan	Erdman and Sharma (1991)	—	>87 ⁴

¹In vitro analysis (Deuchler et al., 1998).

²After 8 h in situ incubation in rumen-cannulated adult ewes (Elek and Husveth, 2007).

³After 48 h in situ incubation in rumen-cannulated cows (Chung et al., 2009).

⁴In vitro analysis (Erdman and Sharma, 1991).

From the 4 models, only the logistic model did not present problems to converge to a solution. This model predicts the dependent variable (Y) as follows:

$$Y = \frac{A}{1 + Be^{-(\text{dose}/k)}},$$

where A = plateau of the response, B = amplitude of the curve, k = steepness of the curve, and dose = dose (g/d) of RPCC.

The first derivative of the model is used to calculate the marginal rate of production (Doepel et al., 2004):

$$\frac{dy}{dx} = \frac{ABe^{-(\text{dose}/k)}}{k \left[1 + Be^{-(\text{dose}/k)} \right]^2}.$$

Mean and linear model biases, with predicted values for the x-axis, were tested according to St-Pierre (2003). To calculate model root mean square errors, responses (Y-values) were adjusted according to St-Pierre (2001) to take into account the random effect of experiment.

The high number of parameters that had to be estimated for this model prevented separate analyses for cows that received a prepartum treatment with dietary RPCC and those that did not. Furthermore, the model

did not fit values presented for MFC and MFY. Visual evaluation of MFC (Figure 1) and MFY (Figure 2) responses as influenced by RPCC supplementation levels in individual studies did not expose any consistent intra-and interstudy relationships. These data were analyzed by the MIXED procedure of SAS (SAS Institute Inc.) according to methodology described in detail by St-Pierre (2001) and Sauvant et al. (2008). Dose was included as a fixed effect, and the effect of experiment included as random. In contrast with the NLMIXED procedure, the MIXED procedure includes a WEIGHT statement. Treatment means were weighted by the reciprocal of their variance (n/SEM^2) scaled to 1 to ensure homogeneity of variance for models. Dose was considered significant at $P < 0.05$, with a tendency for significance at $P < 0.10$. Prediction bias was evaluated as described above.

RESULTS AND DISCUSSION

DMI, Milk Yield, and Milk Protein

Parameter estimates obtained with the logistic model for DMI, MY, MPC, and MPY are shown in Table 4. Large standard errors for the steepness of curves (k) indicated that the sum of the squared deviations around the predicted values was insensitive to changes in this parameter (Hill and Lewicki, 2007). However, no mean

Table 3. Summary statistics for dependent variables

Variable	Studies, n	Experiments, n	Values	Mean	Median	Minimum	Maximum	SD
DMI, kg/d	9	10	29	20.39	20.40	14.20	28.48	4.160
Milk yield, kg/d	11	12	34	34.87	34.35	24.40	48.93	6.392
Milk fat content, %	10	11	31	4.084	4.000	3.210	5.770	0.651
Milk fat yield, kg/d	7	7	20	1.364	1.405	0.880	1.780	0.245
Milk protein content, %	10	11	31	3.191	3.120	2.880	3.740	0.218
Milk protein yield, kg/d	7	7	20	1.134	1.154	0.868	1.310	0.107

Figure 1. Responses in milk fat content on rumen-protected choline chloride supplementation obtained in individual studies. Treatment means that were statistically different are connected by dashed lines: $\circ$ = Toghdory et al. (2007). Treatment means that were not statistically different are connected by solid lines: $\blacktriangle$ = Erdman and Sharma (1991); $*$ = Deuchler et al. (1998); $\blacksquare$ = Hartwell et al. (2000); $\diamond$ = Piepenbrink and Overton (2003); $\blacklozenge$ = Pinotti et al. (2003); $\square$ = Eastridge et al. (2006); $\bullet$ = Janovick Guretzky et al. (2006); $\times$ = Xu et al. (2006) experiment 1; Δ = Xu et al. (2006) experiment 2; $+$ = Elek et al. (2008). Color version available in the online PDF.

and linear prediction biases ($P > 0.05$) were detected for any dependent variable, as illustrated for MY in Figure 3. Furthermore, small root mean square errors relative to the ranges of dependent variables (Table 3) demonstrated that data were adequately described by the models.

Few research studies have found any significant effect of dietary RPC on DMI of dairy cows. de Ondarza et al. (2007) obtained a decrease ($P < 0.05$) in DMI in a commercial herd when feeding as little as 3.1 g/d of RPCC over a 4-wk period. In a study by Zahra et al. (2006), DMI was increased during the period 3 wk prepartum through 4 wk postpartum with 14 g/d of dietary RPCC supplementation in fat (BCS ≥ 4 , 3 wk before calving) cows, with no response found in nonfat cows. Intake of 282 g/d of unprotected choline chloride decreased DMI, with no effect found at an intake level of 159 g/d (Sharma and Erdman, 1988). Abomasal infusion of choline up to 90 g/d tended to be quadratically related to DMI, with the highest intake at 30 g/d of infusion (Sharma and Erdman, 1989).

Dose-response research studies on MY as influenced by dietary RPCC supplementation have predominantly used ANOVA as the only statistical test to separate treatments. This complicated comparison with current results. Both Piepenbrink and Overton (2003) and Xu et al. (2006) found maximum tendencies at 11 g/d of

RPCC with cows evaluated during the transition period. However, upper limits of dietary RPCC values evaluated in those studies were restricted to 18.8 and 33.8 g/d, respectively.

Several studies have illustrated the influence of different dietary and physiological factors on the effect that dietary RPC has on MY. A nonsignificant tendency for the highest MY at 36 g/d of RPCC intake (range: 0–51 g/d) was reported in early-lactation cows fed a diet that contained 148 g/kg of CP (Erdman and Sharma, 1991). In contrast, Erdman and Sharma (1991) obtained no maximum in MY up to 59 g/d of RPCC intake with midlactation cows despite different (130 vs. 165 g/kg of DM) dietary CP treatments. When feeding a low (40 g/kg of DM) RUP diet prepartum to cows, doses of 0, 6, and 12 g/d of RPCC increased MY during the first 56 d of lactation, whereas responses when feeding 62 g/kg of DM RUP prepartum were nonsignificant. At 120 d of lactation, responses were nonsignificant for both of the above 2 treatments (Hartwell et al., 2000).

Several mechanisms have been suggested (Pinotti et al., 2002; Brüsemeister and Südekum, 2006) for the positive effect of dietary RPC on milk protein synthesis: 1) a sparing of methyl groups (and thus Met as the methyl group donor) needed for choline synthesis; 2) RPC may serve as a methyl group donor for remethylation of homocysteine via its metabolite betaine; and 3)

Figure 2. Responses in milk fat yield on rumen-protected choline chloride supplementation obtained in individual studies. Treatment means that were statistically different are connected by dashed lines: $\blacklozenge$ = Pinotti et al. (2003); $+$ = Elek et al. (2008). Treatment means that were not statistically different are connected by solid lines: $\blacktriangle$ = Erdman and Sharma (1991); $\blacksquare$ = Hartwell et al. (2000); $\diamond$ = Piepenbrink and Overton (2003); $\bullet$ = Janovick Guretzky et al. (2006); $\circ$ = Toghdory et al. (2007). Color version available in the online PDF.

Table 4. Logistic model parameter estimates ($\pm$ SE) and root mean square error (RMSE, adjusted for the random effect of experiment) for rumen-protected choline chloride supplementation on dependent variables

Parameter ¹	DMI, kg/d	Milk yield, kg/d	Milk protein content, %	Milk protein yield, kg/d
A	20.53 $\pm$ 1.318	35.40 $\pm$ 1.794	3.242 $\pm$ 0.241	1.455 $\pm$ 0.566
B	0.0230 $\pm$ 0.0145	0.0635 $\pm$ 0.0206	0.0234 $\pm$ 0.0773	0.3456 $\pm$ 0.5202
k	7.970 $\pm$ 11.61	5.347 $\pm$ 6.129	84.07 $\pm$ 249.7	84.20 $\pm$ 118.1
Experiment variance	16.90 $\pm$ 7.612	35.31 $\pm$ 14.65	0.0318 $\pm$ 0.0146	0.00843 $\pm$ 0.00478
Model variance	0.3056 $\pm$ 0.0992	1.975 $\pm$ 0.595	0.00658 $\pm$ 0.00207	0.00119 $\pm$ 0.00047
RMSE	0.448	1.137	0.067	0.028

¹A = plateau of the response; B = amplitude of the curve; k = steepness of the curve.

betaine may also spare Met by responding as methyl group donor in some metabolic processes. This would leave a larger supply of Met for milk protein synthesis and would be especially effective in cows fed diets with a low Met passage to the small intestine (Janovick Guretzky et al., 2006).

As estimated by the CNCPS software, deficiencies in metabolizable Met supply in studies used in the current data set were limited (Table 1) to Janovick Guretzky et al. (2006) (1 treatment) and Xu et al. (2006) (6 treatments). An average between Holsteins and Jerseys was used for animal characteristic inputs in the software for the study of Janovick Guretzky et al. (2006), seeing that milk production responses to dietary RPCC supplementation could not be separated for breeds in the article. According to the ideal protein approach, however, diets in all studies presented Met contents lower than the 2.4% of MP suggested by NRC (2001) to maximize milk and milk protein production. It should be stressed that

the CNCPS uses a factorial approach to determine daily requirements for and supply of absorbed AA compared with results obtained with dose-response trials used for the ideal protein concept (Brüsemaster and Südekum, 2006). In the current data set, statistically significant differences in MPC responses to dietary RPCC supplementation were obtained in the studies by Hartwell et al. (2000), where it decreased when RPCC increased, and Elek et al. (2008), where it increased. However, differences in individual studies were evaluated by tests that lacked statistical power. Although it seems that increases in MPC with dietary RPCC supplementation in this quantitative review could be attributed to the Met sparing effect of RPCC, this needs confirmation from further research on the interaction between dietary Met and RPCC in dairy cows.

Responses in MPY with increasing dietary levels of RPCC could be attributed to corresponding responses in both MY and MPC (Table 4). Studies by Erdman and Sharma (1991) with midlactation cows and Elek et al. (2008) with cows during the periparturient period associated a higher MPY in response to dietary RPCC supplementation with an increase in MY. On the contrary, Pinotti et al. (2003) and Toghdory et al. (2007) did not find any significant increase in MPY, notwithstanding a higher MY and constant MPC. When feeding 40 g/d of choline (derived from a mix of choline chloride and phosphatidylcholine; S. Davidson, Department of Animal Science, North Carolina State University, Raleigh; personal communication) with a Met-limiting diet to multiparous cows from 28 to 91 DIM, a significant increase in MPY was related to an increase in MY. However, no differences were detected with primiparous cows (Davidson et al., 2008).

The effects of dietary RPCC levels on marginal rates of MY and MPY are presented in Figure 4, with model-predicted yields included. When RPCC supplementation increased from 6 to 50 g/d, marginal MY responses decreased from 131.5 to 0.037 g of milk/g of RPCC (Figure 4a). This drastic decrease was not reflected in MPY, where the corresponding decrease was from 3.18 to 2.33 g of milk protein/g of RPCC (Figure 4b). Based

Figure 3. Plot of residuals against predicted milk yield in response to rumen-protected choline chloride supplementation. Mean prediction bias: $P = 0.583$; linear prediction bias: $P = 0.577$.

Figure 4. Predicted marginal yield (g; solid lines) and model-predicted yield (kg; dashed lines) of a) milk and b) milk protein per gram of rumen-protected choline chloride supplemented.

on results of choline secretion into mammalian milk, Pinotti et al. (2002) concluded that choline is a limiting metabolite in lactating mammary tissue. High activity of the associated enzymes showed that it is avidly used when available. However, a lack of information on their saturation kinetics, together with the uncertainty on the underlying mechanisms of improved milk production following dietary RPC supplementation in dairy cows (Baldi and Pinotti, 2006), do not allow for intensive speculation on the above phenomenon. A similar analysis as presented in this paper for milk components other than protein and fat, as influenced by dietary RPCC levels, might be helpful. Currently, this is hampered by a lack of an adequate number of values.

Milk Fat

Responses in MFC and MFY were linearly related to dietary RPCC levels:

$$\text{MFC, \%} = 4.07 (\pm 0.178) - 0.00339 (\pm 0.00152) \times \text{RPCC, g/d (n = 31; } P = 0.038), \text{ and}$$

$$\text{MFY, kg/d} = 1.32 (\pm 0.093) + 0.00216 (\pm 0.00080) \times \text{RPCC, g/d (n = 20; } P = 0.019).$$

However, mean and linear prediction biases have occurred for the prediction of MFC (Figure 5). These were attributed to the 2 treatment means from the first experiment in the study of Xu et al. (2006), in which MFC was underestimated. When this experiment was removed from the data set, the effect of dose was still significant ($P = 0.042$). This is in accordance to the well-known negative correlation between MY and MFC, where a decrease in MFC as MY increases could probably be related to a dilution effect (Emanuele, 2003). It indicated that dietary RPCC supplementation had no influence on MFC.

As illustrated by Figure 1, most research studies did not find any significant effect of dietary RPCC supplementation on MFC. Increases in MFY with increasing dietary RPCC levels were related to increases in MY (Pinotti et al., 2003; Elek et al., 2008), MFC (de Ondarza et al., 2007), and MY and MFC (Toghdory et al., 2007). Notwithstanding no response in MFC and a quadratic tendency ($P = 0.15$) for MY, MFY tended ($P = 0.16$) to increase linearly in the study of Piepenbrink and Overton (2003). Milk fat yield did not respond significantly to RPCC supplementation in the study of Erdman and Sharma (1991) with midlactation cows, although MY presented a positive linear response. In results presented by Hartwell et al. (2000), a tendency of a negative linear relation between MFC and dietary RPCC had no influence on MFY. Janovick Guretzky et al. (2006) did not find any effect of dietary RPCC on MFY, attributed to nonsignificant effects obtained for MY and MFC.

Choline (as phosphatidylcholine) contributes to fatty acid transport in blood (Piepenbrink and Overton, 2003) and is also incorporated in phospholipid membranes around fat globules (Elek et al., 2008). Theoretically, more lipoprotein triglycerides might be available to the mammary gland for incorporation into milk fat with an increase in choline supplementation (Erdman et al., 1984). Most of the potential application of RPC in dairy diets has concentrated on its role in lipid metabolism (Overton and Waldron, 2004). However,

Figure 5. Plot of residuals against predicted milk fat content in response to rumen-protected choline chloride supplementation. Residual = $-0.795 (\pm 0.234; P = 0.002) + 0.208 (\pm 0.057, P = 0.011) \times$ predicted milk fat content ($R^2 = 0.31$). Color version available in the online PDF.

the current meta-analysis did not reveal any effect of dietary RPCC supplementation on MFC.

CONCLUSIONS

This meta-analysis conclusively shows that MFC in dairy cows is not influenced by dietary RPCC supplementation. Evidence is presented that dietary RPCC improves MPC by acting as a methyl donor to spare Met; however, this warrants further investigation. In addition, the study provides models to calculate changes in MY and MPY in dairy cows caused by different dietary RPCC levels, which could be applied in practice. Discrepancies among results caused by numerous physiological and dietary factors are accentuated in the article. At this stage, evaluation of these factors is hampered by a lack of suitable values.

ACKNOWLEDGMENTS

The project was supported by the Ministry of Agriculture of the Czech Republic (Prague, Czech Republic, MZE0002701404). The authors thank 2 anonymous reviewers for providing many helpful suggestions on manuscript improvement.

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