Dissecting the Latest Choline Meta-Analysis

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Pictures by Bonnie Mohr http://www.bonniemohr.com/

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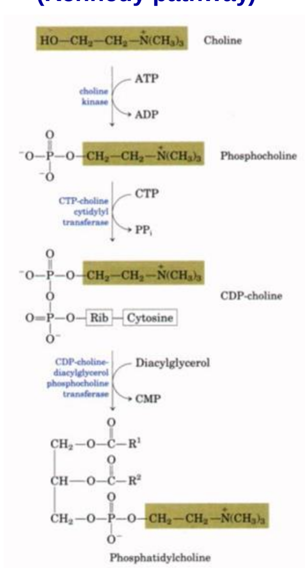
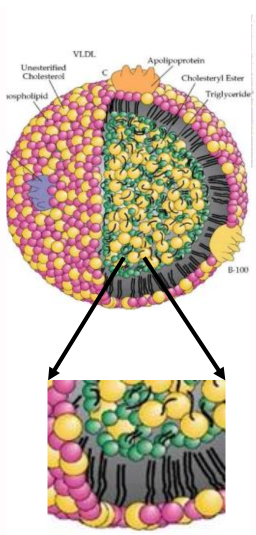
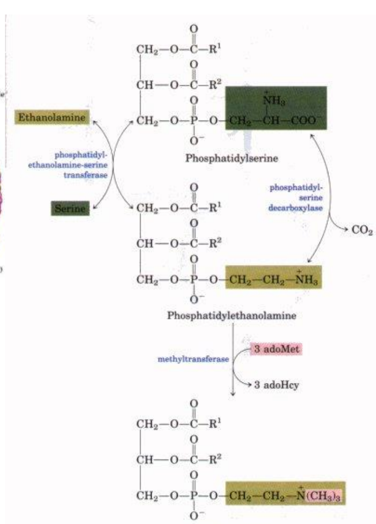
Methyl Donors Play a Role in Phospholipid Synthesis

CDP-Choline Pathway (Kennedy pathway)



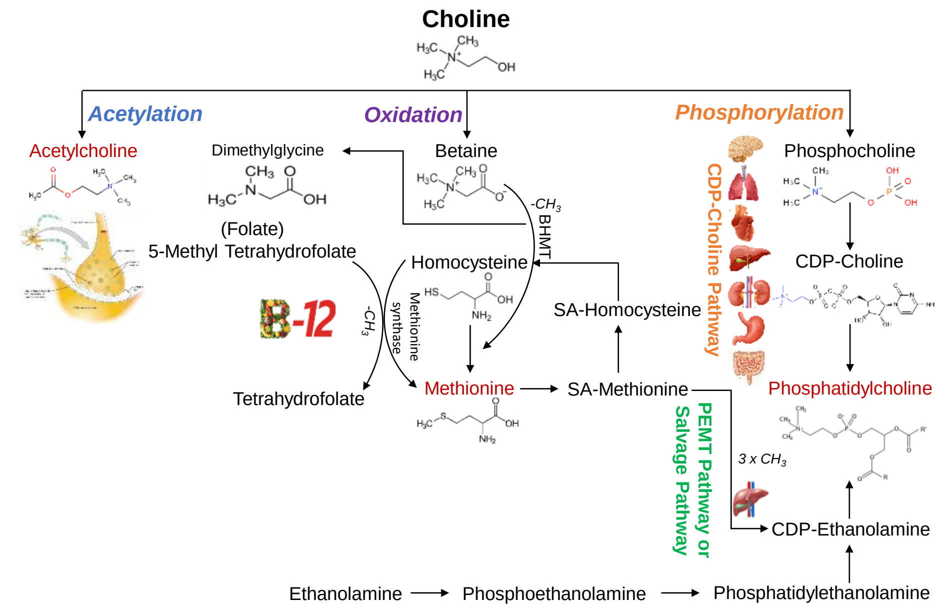


Salvage Pathway



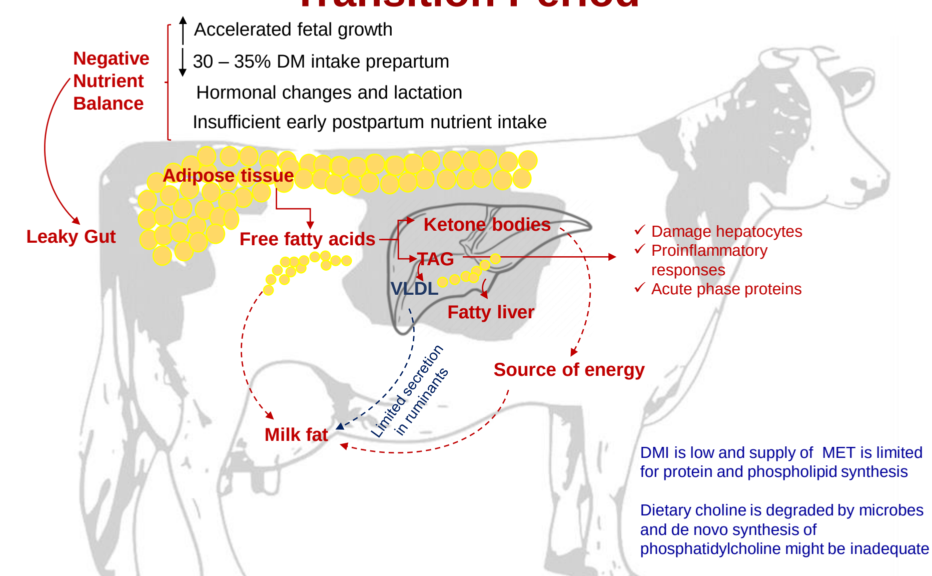
http://www.bioinfo.org.cn/book/biochemistry/chapt20/bio5.htm

Inter-Relationships Among Pathways



Adapted: Artegoitia et al. (2014) PLoS ONE 9:e103412

Intermediary Metabolism During the Transition Period



Adapted from Drackley (1999) J. Dairy Sci. 82:2259-2273

Prevalence of Fatty Liver in Dairy Cows Reported in the Literature

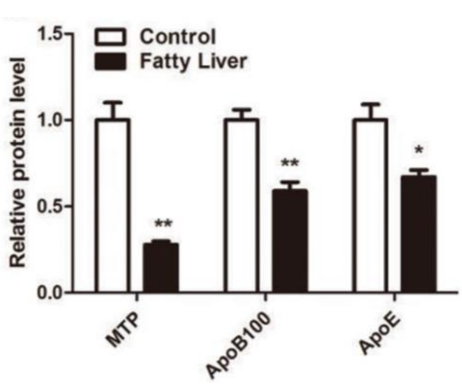
Study	Prevalence of fatty liver, %	
	Moderate (5 to 10% TAG)	Severe (> 10% TAG)
Reid (1980)	48	15
Reid (1980)	33	5
Jorritsma et al. (2000)	45	NR
Jorritsma et al. (2000)	40	14
Gerloff et al (1986)	20	15
Herd (1991)	>24	24
Lima et al. (2013)	28	17

49.5% of the early lactation cows develop at least moderate fatty liver (>5% TAG)

Adapted from Bobe et al. (2004) J. Dairy Sci. 87:3105–3124

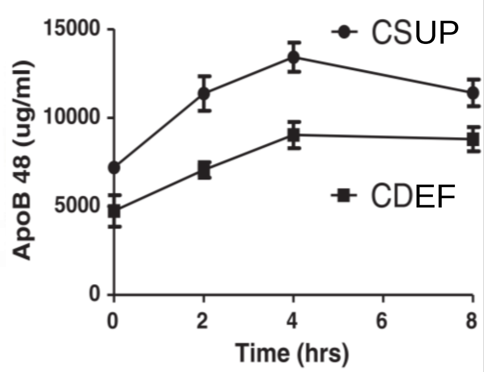
Role of Phosphatidylcholine in Lipoprotein Synthesis

VLDL Assembly



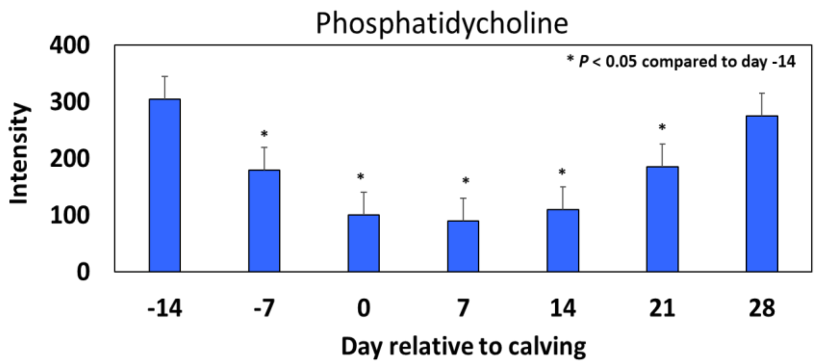
Jia et al. (2019) J. Dairy Sci. 102:833-845

Chylomicron Assembly



Da Silva et al. (2015) J. Nut. Bio. 26:1077-1083

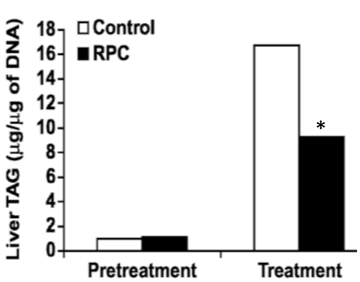
Phosphatidylcholine is Lowest Around Calving



Imhasly et al. (2015) BMC Vet Res. 11: 252

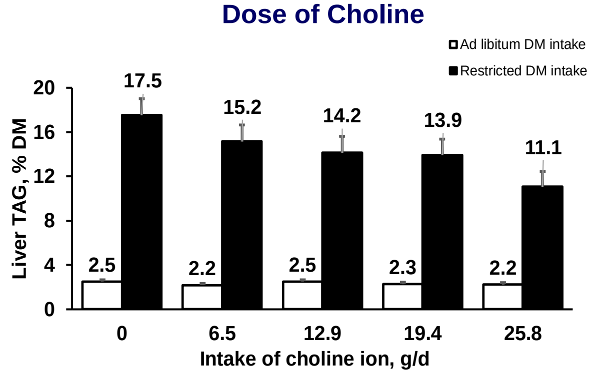
Lipotropic Effects of Choline in Dry Cows

Effect of Choline



Cooke et al. (2007) J. Dairy Sci. 90:2413-2418

Dose of Choline



Zenobi et al. (2018) J. Dairy Sci. 90:5902-5923

Supplemental RPC and Hepatic Triacylglycerol

- ✓110 parous prepartum dry cows
- ✓Weekly cohort of cows were blocked by BCS (4.02 ± 0.5) at 232 d of gestation and assigned randomly (22/treatment) to 1 of 5 treatments:
  - ✓**CON** = 0 g of choline ion
  - ✓**L12.9** = 12.9 g/d of choline ion in a rumen-protected choline form with low concentration (28.8% choline chloride)
  - ✓**L25.8** = 25.8 g/d of choline ion in a rumen-protected choline form with low concentration (28.8% choline chloride)
  - ✓**H12.9** = 12.9 g/d of choline ion in a rumen-protected choline form with high concentration (60% choline chloride)
  - ✓**H25.8** = 25.8 g/d of choline ion in a rumen-protected choline form with high concentration (60% choline chloride)
- ✓5 d of ad libitum intake and 9 d of feed restriction at 50% of the NE_L required for maintenance and pregnancy

Arshad et al. (2020) J. Dairy Sci. (Abstr.)

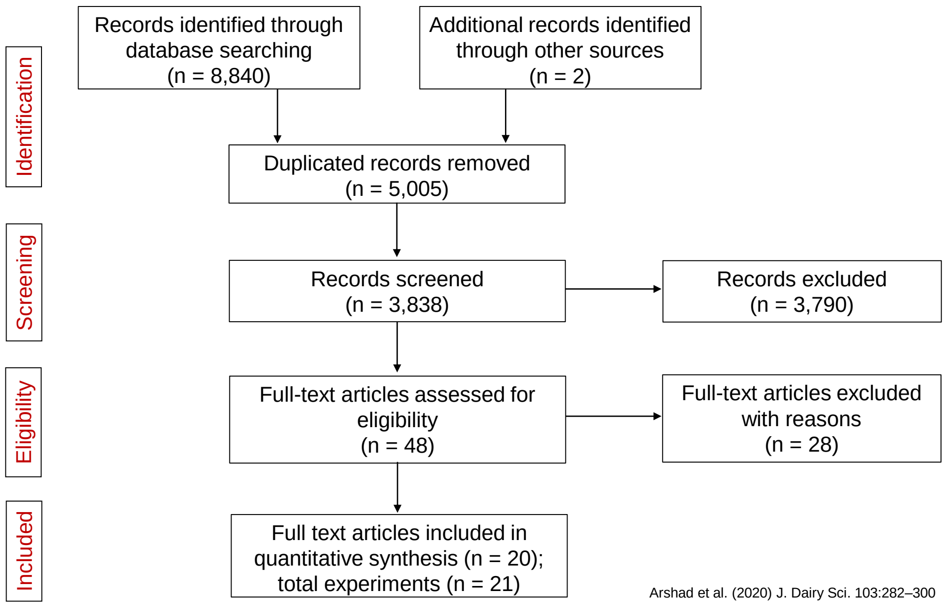
Response to Treatments During Feed Restriction

	Treatment					
Item	CON	L12.9	L25.8	H12.9	H25.8	SE
NE intake, Mcal/d	7.05	7.04	6.81	7.29	7.09	0.20
Plasma						
NEFA, m*M*	0.837	0.854	0.835	0.848	0.777	0.062
BHB, m*M*	0.801	0.779	0.758	0.769	0.760	0.061
Liver TAG, %						
As is*†	9.32	6.59	5.05	6.61	6.00	0.55
DM*†	26.33	18.59	14.55	18.29	16.75	1.65
Liver glycogen, %						
As is*‡†	1.83	2.59	3.55	3.13	4.07	0.18
DM*‡†	5.01	7.26	10.32	8.63	11.58	0.57
Ratio TAG to glycogen						
As is*†	5.19	2.39	1.29	2.02	1.35	0.31
DM*†	5.20	2.40	1.29	2.03	1.36	0.31

Effect $P < 0.05$: *choline; ‡concentration; †amount; §interaction

Arshad et al. (2020) J. Dairy Sci. (Abstr.)

Meta-Analysis of Rumen-Protected Choline During Transition



Arshad et al. (2020) J. Dairy Sci. 103:282–300

Meta-Analysis of Published Literature

- ✓ 20 manuscripts with 21 randomized experiments
  - ✓ 66 treatment means and 1,313 parous cows

Data collected

- ✓ **Predictors**
  - ✓ Individual experiment (random effect)
  - ✓ Amount of choline ion fed
  - ✓ Ingredient and nutrient composition of pre- and postpartum diets
  - ✓ Diets entered into NRC (2001) to calculate pre- and postpartum intakes of:
    - ✓ NE
    - ✓ MP
    - ✓ Metabolizable methionine
- ✓ **Responses of interest:** LSM and SEM/SD and frequency of events
  - ✓ DM intake
  - ✓ BW and BCS
  - ✓ Productive performance
  - ✓ Concentrations of metabolites in blood
  - ✓ Hepatic composition
  - ✓ Incidence of diseases

Arshad et al. (2020) J. Dairy Sci. 103:282–300

Mathematical Approaches

- ✓ Classic mixed effects meta-analysis and meta-regression to determine the effect of supplementing choline ion prepartum
  - ✓ METAN and METAREG procedures of STATA release 14
  - ✓ Weighted according to SD and number of experimental units to account for the precision of each experiment
- ✓ Mixed effects meta-analysis to determine the optimum amount of choline ion supplemented
  - ✓ MIXED and GLIMMIX procedures of SAS ver. 9.4
  - ✓ Weighted by the inverse of SEM squared ($1/SEM^2$) to account for the precision of each experiment
- ✓ **Choline as a continuous variable:**
  - ✓ **Fixed effects:** Choline, Choline x Choline, Dietary NE_L content prepartum (**NE_LPr**), Metabolizable methionine as percentage of MP pre- (**METMPPr**) and postpartum (**METMPPo**), Choline x NE_LPr, Choline x METMPPr, and Choline x METMPPo
- ✓ **Choline as a categorical variable:**
  - ✓ **Fixed effects:** Choline (not supplemented vs. supplemented), NE_LPr, and METMPPr
- ✓ All models included the random effect of experiment

Arshad et al. (2020) J. Dairy Sci. 103:282–300

Descriptive statistics of nutrient intake and estimated duodenal flow based on NRC (2001)[1]

	Control (n = 30)			Choline[2] (n = 36)		
Item	Mean ± SD	Median	Range	Mean ± SD	Median	Range
Content, DM basis						
NE_L, Mcal/kg	1.59 ± 0.08	1.60	1.41 to 1.72	1.61 ± 0.08	1.62	1.41 to 1.72
CP, %	14.3 ± 1.6	14.6	10.9 to 16.5	14.2 ± 1.8	14.6	10.9 to 16.5
MP,[3] %	9.3 ± 1.0	9.3	7.1 to 11.0	9.2 ± 1.1	9.3	7.1 to 11.1
Intake						
DMI, kg/d	12.2 ± 1.9	11.9	8.8 to 16.2	12.3 ± 1.9	12.5	8.5 to 16.8
NE_L, Mcal/d	19.3 ± 2.9	19.3	13.3 to 25.6	19.7 ± 2.9	19.9	13.5 to 26.2
CP, g/d	1,737 ± 334	1,801	1,068 to 2,225	1,752 ± 369	1,823	1,046 to 2,300
Choline ion, g/d	0	0	---	12.8 ± 3.8	12.9	5.6 to 25.2
Metabolizable, g/d						
Protein, g/d	1,124 ± 209	1,180	699 to 1,477	1,138 ± 236	1,214	685 to 1,558
Methionine	23.0 ± 5.0	23.0	15.0 to 35.0	23.1 ± 5.2	23.5	14.0 to 36.0
Lysine	74.3 ± 14.1	75.5	48.0 to 98.0	74.7 ± 15.3	76.0	46.0 to 102.0
Essential AA	507 ± 91	520	326 to 655	512 ± 101	530	320 to 688
Methionine,[4] % MP	2.05 ± 0.22	2.00	1.80 to 2.71	2.04 ± 0.21	2.02	1.80 to 2.77

[1] The concentration of nutrients in diets offered and the intake of nutrients were calculated using the ingredient composition of each diet with NRC (2001) and adjusted to the observed prepartum DMI of control and choline-supplemented cows.
[2] Intake of choline ion was calculated based on the 74.6% molar proportion of choline ion in choline chloride. The amount of rumen-escape choline and its intestinal bioavailability for the different products supplemented were not considered for statistical analysis.
[3] Metabolizable protein was predicted with NRC (2001) and it is expressed as the percentage of the observed DMI.
[4] Metabolizable methionine was predicted using NRC (2001) and it is expressed as the proportion of the MP.

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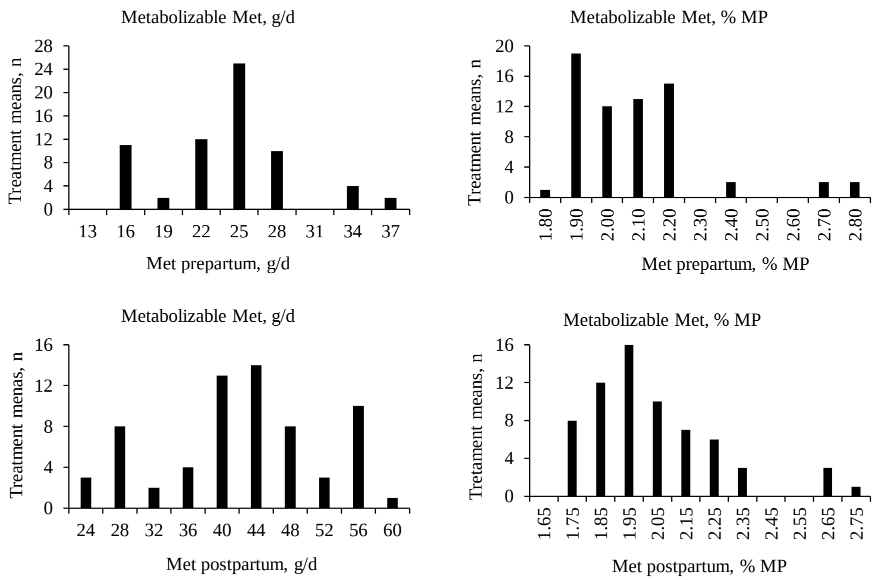
Metabolizable MET Pre- and Postpartum



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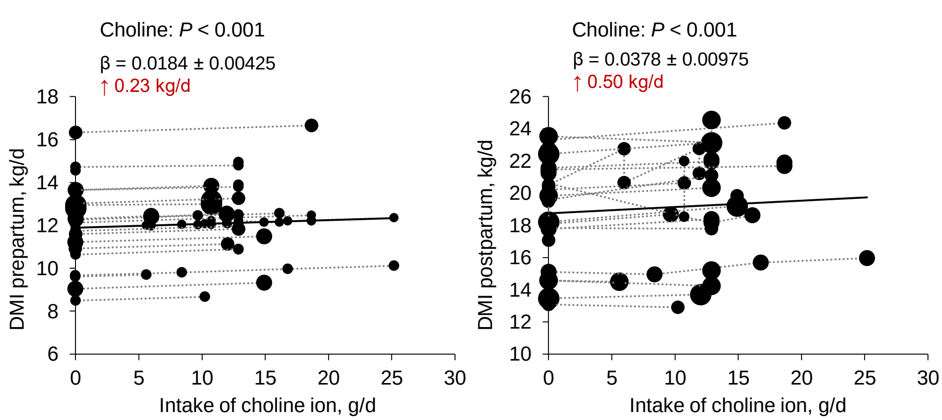
DM Intake Pre- and Postpartum



* Estimated response when cows are supplemented with 12.9 g of choline ion

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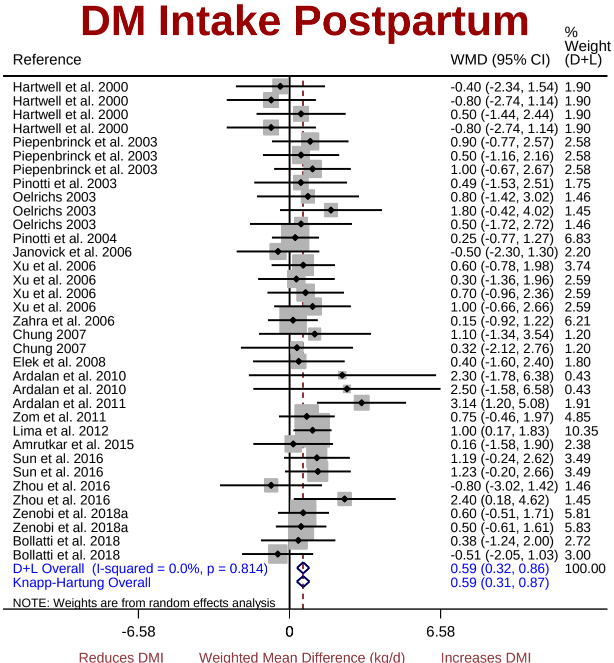
DM Intake Postpartum



WMD = 0 z = 4.34 P < 0.0001

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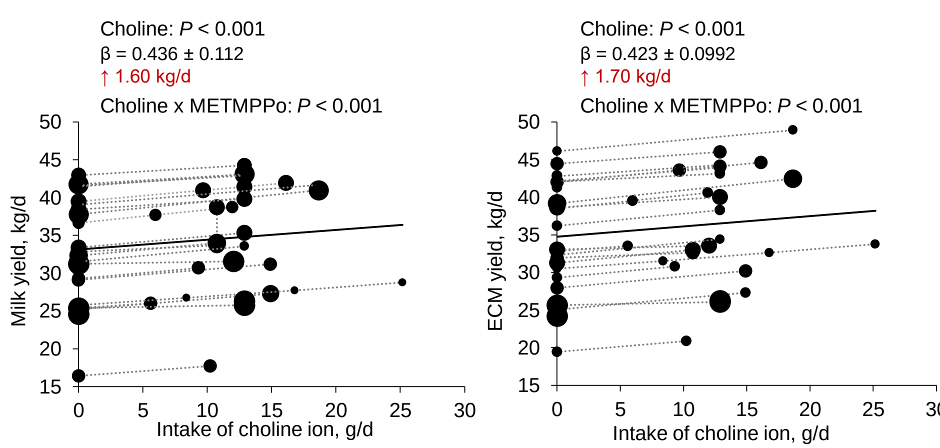
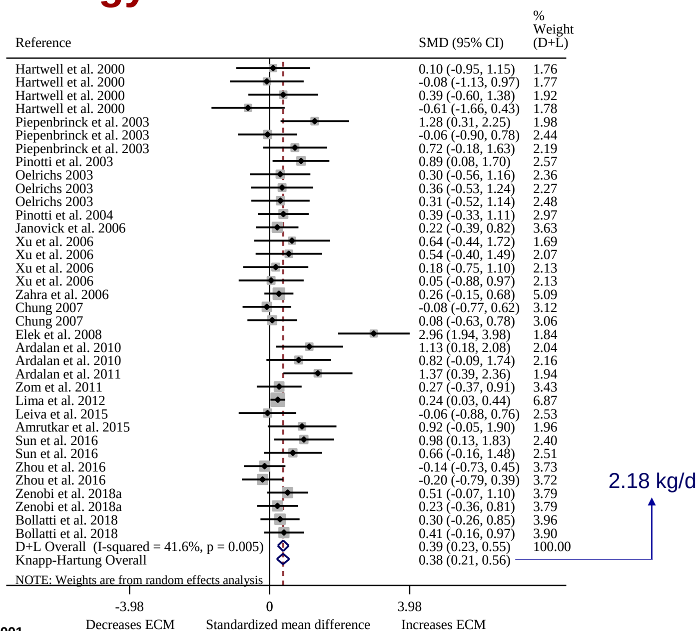
Yields of Milk and Energy-Corrected Milk



* Estimated response when cows are supplemented with 12.9 g of choline ion

Arshad et al. (2020) J. Dairy Sci. 103:282–300

Energy-Corrected Milk Yield



SMD = 0 z = 4.18 P < 0.0001

Arshad et al. (2020) J. Dairy Sci. 103:282–300

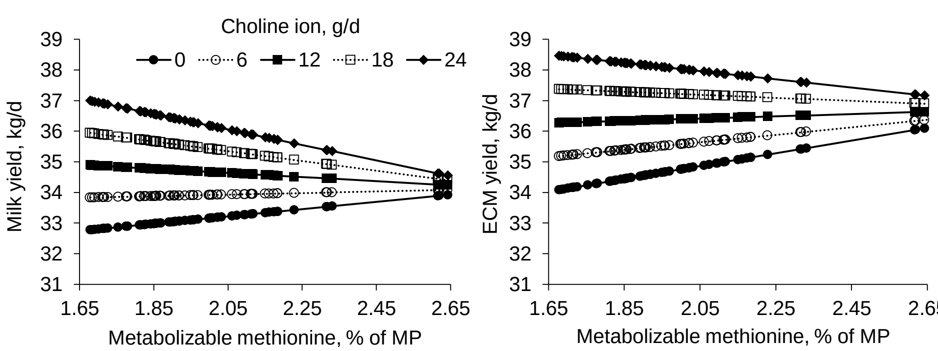
Interactions Between Supplemental Choline and Postpartum Dietary Methionine

METMPPo = 1.80 % then, ↑ 2.0 kg/d

METMPPo = 2.30 % then, ↑ 1.0 kg/d

METMPPo = 1.80 % then, ↑ 2.1 kg/d

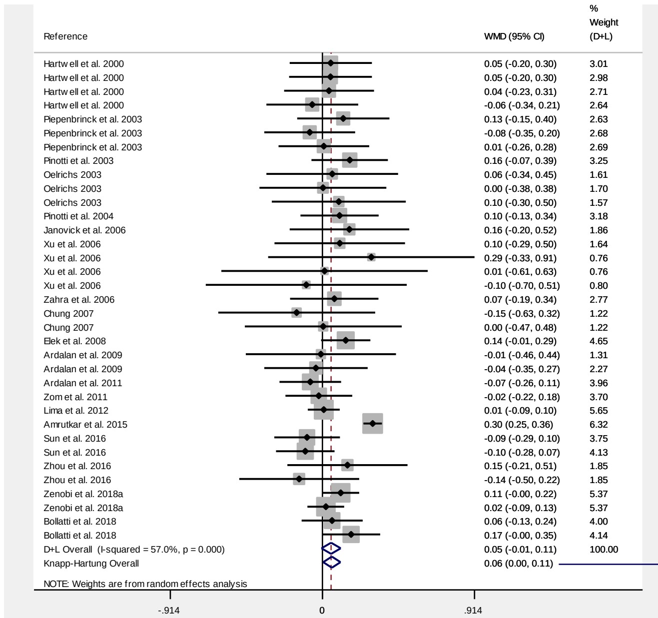
METMPPo = 2.30 % then, ↑ 1.2 kg/d



* Estimated response when cows are supplemented with 12.9 g of choline ion

Arshad et al. (2020) J. Dairy Sci. 103:282–300

Feed Efficiency – ECM/DMI



60 g/d more ECM/kg DMI

WMD=0 : z= 1.75 *P* = 0.08

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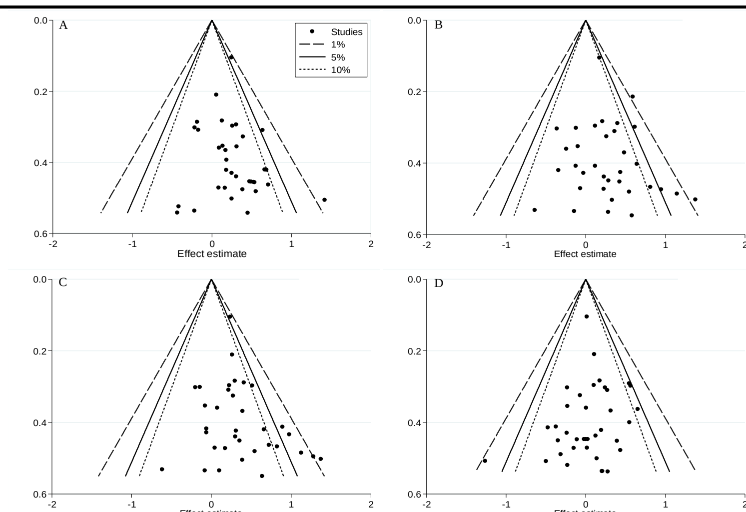
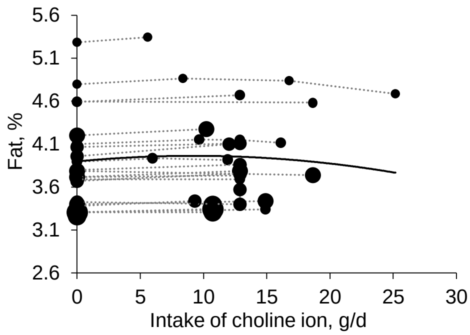


Contour-enhanced funnel plot for postpartum DM intake (A), milk yield (B), energy-corrected milk yield (C), and feed efficiency (ECM/DMI; D) in response to supplementation of choline ion. The lines represent the 90, 95, and 99% CI for treatment comparisons. Standard errors are inversely proportional to the weight of the comparisons in the analysis (small SE represent comparisons with more weight, i.e. larger and more precise experiments). Effect estimates represent the change in the response analyzed resulting from supplementation of choline (values greater than 0 indicate an increase in response resulting from choline supplementation, whereas a value smaller than 0 represents a decrease in response resulting from choline supplementation). Point estimates within the dotted lines represent comparisons with effect of choline with $P > 0.10$; point estimates between the solid and dotted lines represent treatment comparisons with effect of choline with $0.05 < P < 0.10$; point estimates between the solid and dashed lines represent treatment comparisons with effect of choline with $0.01 < P < 0.05$.

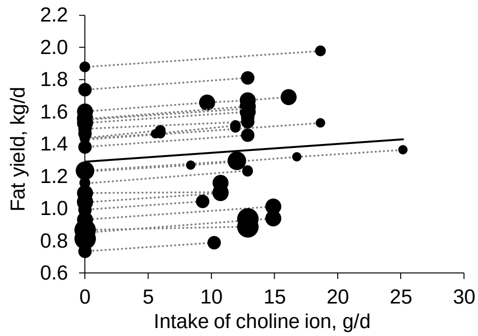
Arshad et al. (2020) J. Dairy Sci. 103:282–300

Concentration and Yield of Fat in Milk

Choline: $P = 0.59$
Choline x Choline: $P = 0.09$



Choline: $P < 0.001$
β = 0.00555 ± 0.000793
↑ 0.07 kg/d



* Estimated response when cows are supplemented with 12.9 g of choline ion

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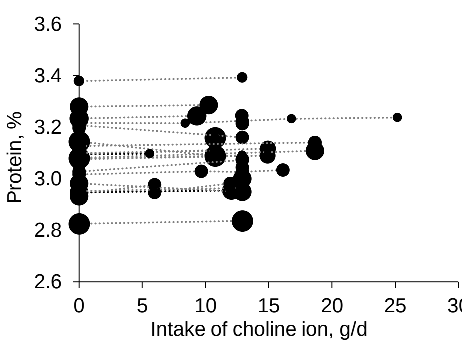
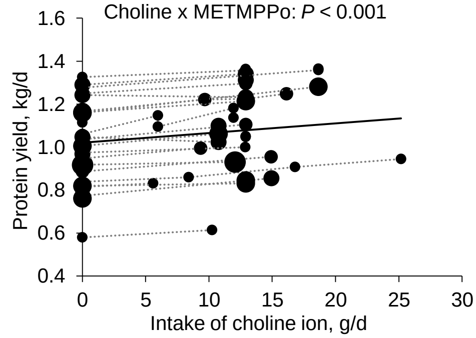
Concentration and Yield of Protein in Milk

Choline: $P = 0.31$



Choline: $P < 0.001$
β = 0.00102 ± 0.000991
↑ 0.05 kg/d
Choline x METMPPo: $P < 0.001$



* Estimated response when cows are supplemented with 12.9 g of choline ion

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Effect of Choline on Health and Liver Composition

		Treatment[1]		
Item	Means (Exp.),[2] n	Control	Choline	*P*-value
Retained placenta	38 (11)	10.6 ± 2.9	7.5 ± 2.2	0.06
Metritis	28 (09)	11.7 ± 2.2	8.7 ± 1.8	0.19
Mastitis	34 (11)	14.8 ± 3.0	11.7 ± 2.5	0.09
Milk fever	38 (11)	2.5 ± 1.5	1.5 ± 0.9	0.23
Displaced abomasum	38 (11)	6.0 ± 1.7	5.2 ± 1.5	0.67
Ketosis	36 (10)	12.0 ± 3.0	12.1 ± 3.0	0.96
Disease cases/cow	40 (12)	0.55 ± 0.1	0.48 ± 0.1	0.23
Liver, % wet basis				
Triacylglycerol	22 (8)	8.79 ± 1.60	8.96 ± 1.60	0.75
Glycogen	10 (4)	1.24 ± 0.06	1.03 ± 0.06	0.01

[1] Treatment as a categorical variable in the statistical models (not supplemented vs. supplemented) because the majority of experiments that reported diseases and hepatic composition supplemented choline ion at 12.9 g/d.

The mean (± SD) amounts of supplemental choline for experiments reporting data on health and hepatic composition were 13.3 ± 2.6 and 13.3 ± 2.1 g/d.

[2] Number of treatment means (experiments) that contributed data for statistical analyses.

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Conclusions

- ✓ Supplementing choline ion as RPC during transition
  - ✓ Increased DM intake and yields of milk, ECM, fat and protein
  - ✓ Tended to reduce the incidence of retained placenta and mastitis
  - ✓ Responses to RPC did not depend on prepartum diet; however, the improvements in lactation performance were influenced by postpartum metabolizable methionine
- ✓ The optimum amount of supplemental choline ion to transition dairy cows was not identified because production responses were linear up to 25 g/d
- ✓ Mechanisms that justify improved productive performance
  - ✓ Increased nutrient intake
  - ✓ Other non-classical mechanisms of choline might be involved beyond effects on hepatic TAG content

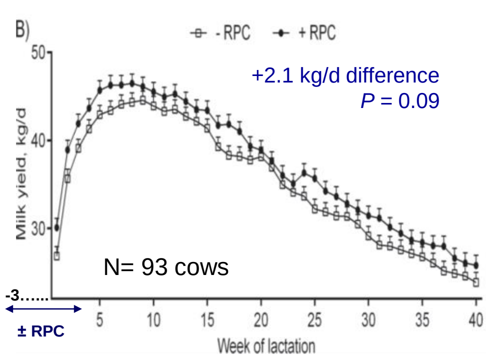
Arshad et al. (2020) J. Dairy Sci. 103:282–300

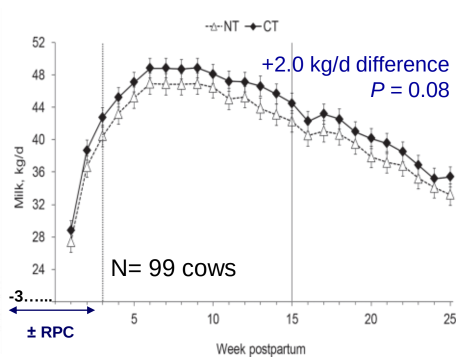
Limitations

- ✓ Data analyzed included only parous cows
  - ✓ Nulliparous represent 30 to 35% of the transition cows on a farm
  - ✓ We have limited knowledge about the response of nulliparous to supplemental choline
- ✓ Not all responses available for all experiments
  - ✓ Different outcomes analyzed with variable database (e.g. disease only contained data from half of the experiments)
- ✓ Investigators **REMOVE** cows from experiments and oftentimes do not report what happened to them, although the treatment was implemented before removal
  - ✓ It is possible that we did not estimate the true effect on health events
- ✓ A large dose-titration experiment is needed to understand the optimum intake of choline ion in transition cows
  - ✓ Parous and nulliparous cows

Arshad et al. (2020) J. Dairy Sci. 103:282–300

Effects From RPC Continued After Supplementation Ceased

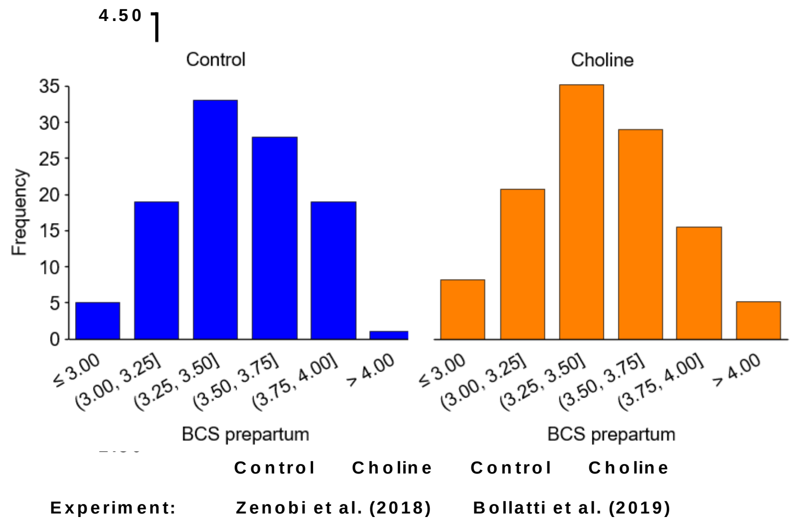




Zenobi et al. (2018) J. Dairy Sci. 101:1088–1110

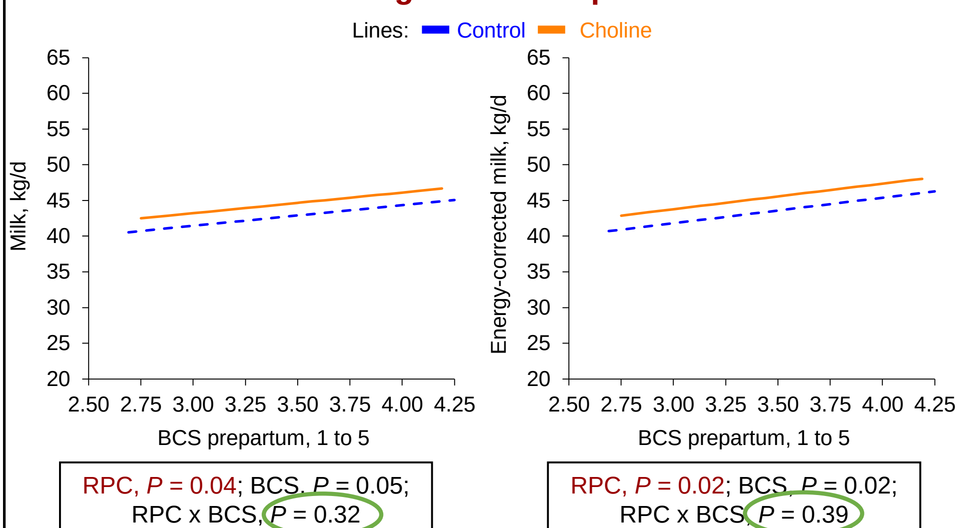
Bollatti et al. (2020) J. Dairy Sci. 103:4174–4191

Distribution of Prepartum BCS According to Treatments



Bollatti et al. (2020) J. Dairy Sci. 103:2272–2286

Effect of RPC During Transition on Yields of Milk and ECM According to BCS Prepartum

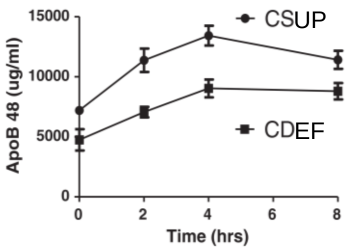


Bollatti et al. (2020) J. Dairy Sci. 103:2272–2286

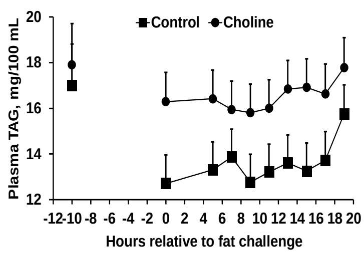
Hypotheses

Fatty acid absorption and transport

Chylomicron Assembly

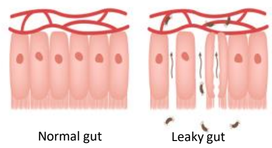


Da Silva et al. (2015) J. Nut. Bio. 26:1077-1083



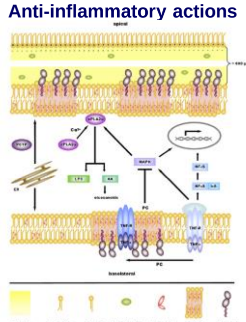
Zenobi et al. (2018) J. Dairy Sci. 101:1–22

Gastrointestinal lining integrity



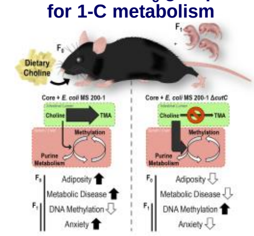
Phosphatidylcholine is critical for gastrointestinal epithelial integrity

Anti-inflammatory actions



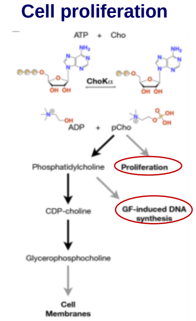
Schneider et al. (2010) Int. J. Mol. Sci. 11:4149-4164

Source of CH_3 groups for 1-C metabolism



Romano et al. (2017) Cell Host & Microbe 22:279–290

Cell proliferation



Kall et al. (2019) Sci. Rep. 9:17121

Thank you

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