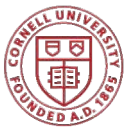
Choline biology in dairy cattle: Contemporary perspectives and future prospects



Joseph W. McFadden, Ph.D.
Associate Professor of Dairy Cattle Biology
Northeast Agribusiness & Feed Alliance Faculty Fellow
Department of Animal Science
McFadden@cornell.edu

Presented as a webinar for the Methyl Donors and 1-C Metabolism mini-symposium by Balchem & Feedstuffs on 4/29/2020

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What we know about rumen-protected choline feeding

- Increased yields of milk and ECM
  - More pronounced in cows fed diets low in metabolizable Met
  - ~50% explained by improved DMI
- Increased milk fat and protein yield
- Increased retention of body condition
- Decreased postpartum disease

Meta-analyses: Sales et al., 2010; Humer et al., 2019; Arshad et al., 2020

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…but this is not your average choline talk:
What's in the research pipeline and why does it matter?

- **Topic #1:** How might fatty acid nutrition optimize choline efficacy?
- **Topic #2:** Should we be concerned about lower gut choline degradation and trimethylamine *N*-oxide?
- **Topic #3:** Why should we consider lysophosphatidylcholine (lysolecithin) as an immunomodulator?

Topic #1

Fatty acid nutrition to optimize methyl donor efficacy

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Dietary choline and fatty liver prevention:

Charles Best & Fredrick Banting

August 1. At this time it was noticed that the dog was unable to bark, otherwise in excellent condition.

October 20. Dog in excellent condition, weight 8.8 kgm. Discontinued "lecithin" and substituted 10 grams suet and liberal doses (adult human) of irradiated ergosterol (oscodal) and yeast extract (marmite). The animal progressively lost weight and showed decline in its general condition until its death.

December 19. The urine became progressively decreased in volume, darker in color and positive for bile. Feces became very dark to begin with, gradually looser with a bloody diarrhea preceding death. Loss of appetite was marked. The animal refused to eat for two or three days during which time it apparently improved somewhat in condition. Any food which was eaten affected it in a most unfavorable manner. The suet, "oscodal" and "marmite" with sugar were given twice daily when the animal failed to eat. When the animal's appetite failed, the insulin dosage was reduced to 4 units twice daily to avoid reactions. The dog became suddenly weak before it died and showed an extreme degree of jaundice. Administration of sugar failed to have any appreciable recuperative effect. It was interesting to note that about five weeks after the commencement of the vitamin administration, the animal recovered some ability to bark. Post-mortem examination showed liver content of 22.0 per cent fat. Iodine number of liver fat 67.

March 28. Sugar excretion 14.8 grams.

March 29. Sugar excretion 18.1 grams. Urine and feces progressively lighter in color. "Lecithin" continued in diet for remainder of experiment. Animal in excellent condition throughout and history uneventful until termination of experiment October 3, 1928. Weight 5.8 kgm. Dog guillotined on this date. Post-mortem examination showed pancreatic tissue "about the size of a split pea," no islet tissue present. Liver fat content 3.75 per cent—Iodine number of liver fat 118. Liver glycogen content 7.90 per cent. Muscle glycogen content 1.40 per cent.

Hershey and Soskin, 1931; Zeisel et al., 2012

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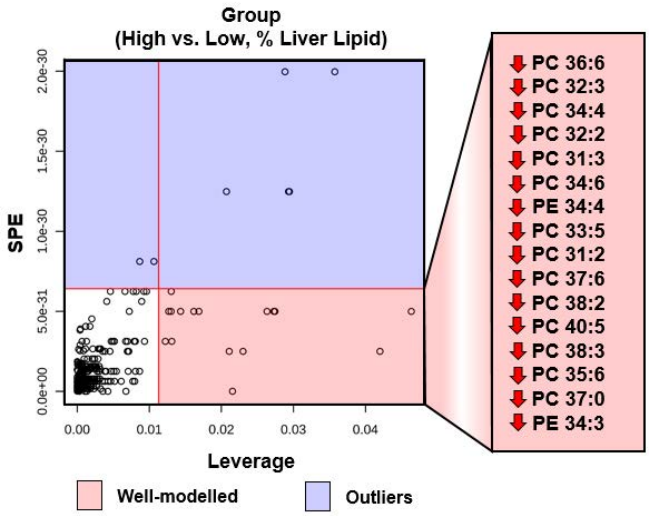
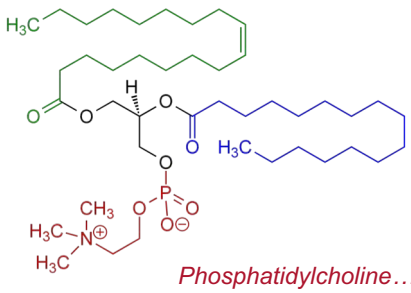
Fatty liver develops in cows with low phosphatidylcholine





Phosphatidylcholine…. more than just choline

McFadden Lab (Unpublished); plasma

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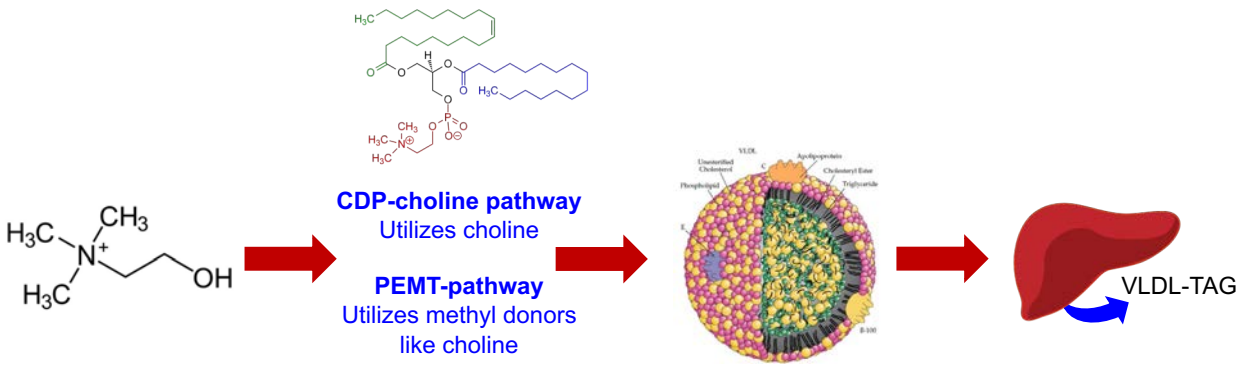
Rumen-protected (RP) choline feeding has been shown to lower liver triglyceride accumulation in fresh cows

- Hartwell et al., 2000
  - 0, 6, 12 g/d choline; -28 to 120 d
  - **Liver triglycerides were decreased** by feeding RPC to high BCS cows
- Elek et al., 2008, 2012
  - 0, vs. 25 with 50 g/d choline pre and postpartum; -21 to 60 d
  - **Decreased liver triglyceride** and circulating BHBA concentrations
- Zom et al., 2011; Goselink et al., 2013
  - 0 or 14.4 g/d choline chloride; -21 to 42 d
  - RPC **decreased liver TAG** but did not affect blood NEFA or BHBA
  - RPC increased expression of genes related to processing of fatty acids and VLDL assembly

Not observed in all studies.

How does RP-choline feeding prevent fatty liver disease?



Choline from diet

Phosphatidylcholine synthesis in liver

Assembly of triglyceride (TAG)-rich very-low-density lipoproteins (VLDL) in liver

VLDL-TAG secretion from liver

In non-ruminants: Yao and Vance, 1990; Verkade et al., 1993

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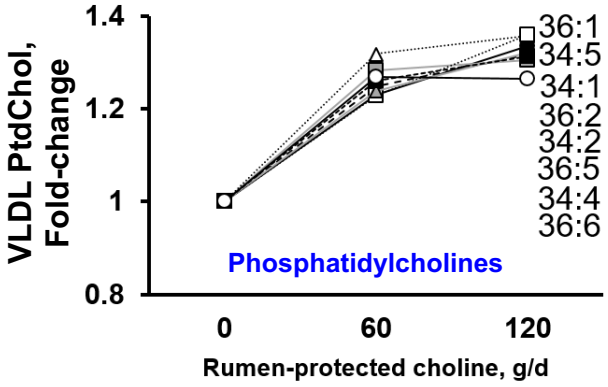
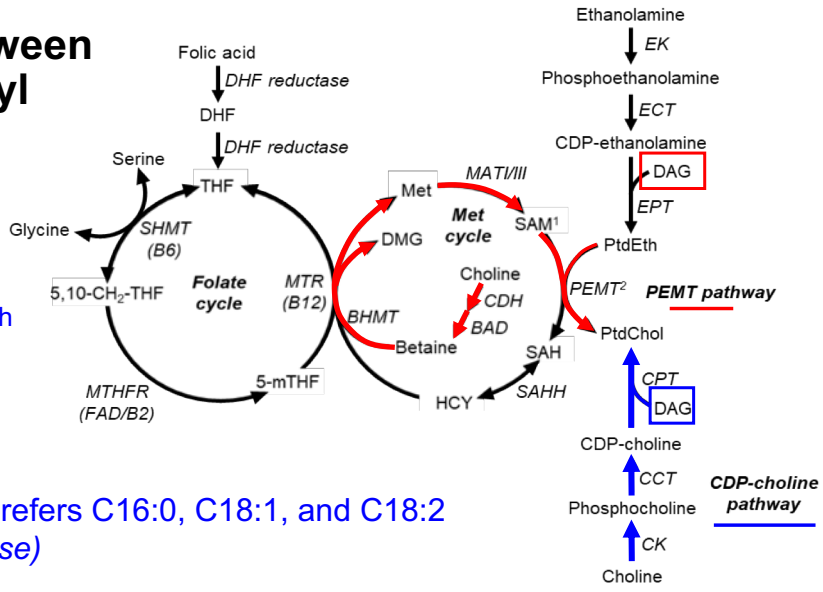
Rumen-protected choline feeding increases VLDL-PtdChol prepartum Holstein dairy cows experiencing negative energy status



- **RP-choline feeding…**
  - Increased hepatic phosphatidylcholine
  - Increased plasma VLDL-TAG
  - Decreased liver TAG

Zenobi et al., 2018; Myers et al., 2019 (ADSA Abstract); ReaShure®; VLDL represents TAG-rich fraction from FPLC

The interaction between fatty acid and methyl donor metabolism



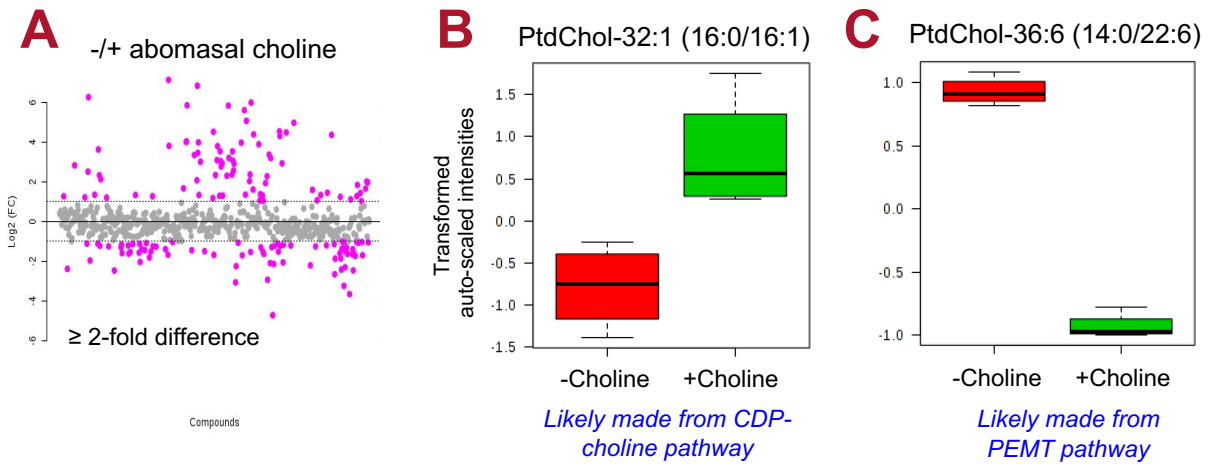
In transition cow liver,
C16:0, C18:1, and C18:2 are high
C18:3, C20:4, C22:6 are low

The CDP-choline pathway prefers C16:0, C18:1, and C18:2 *(likely utilizes FA from adipose)*

The PEMT pathway prefers C20:4, C22:5, C22:6

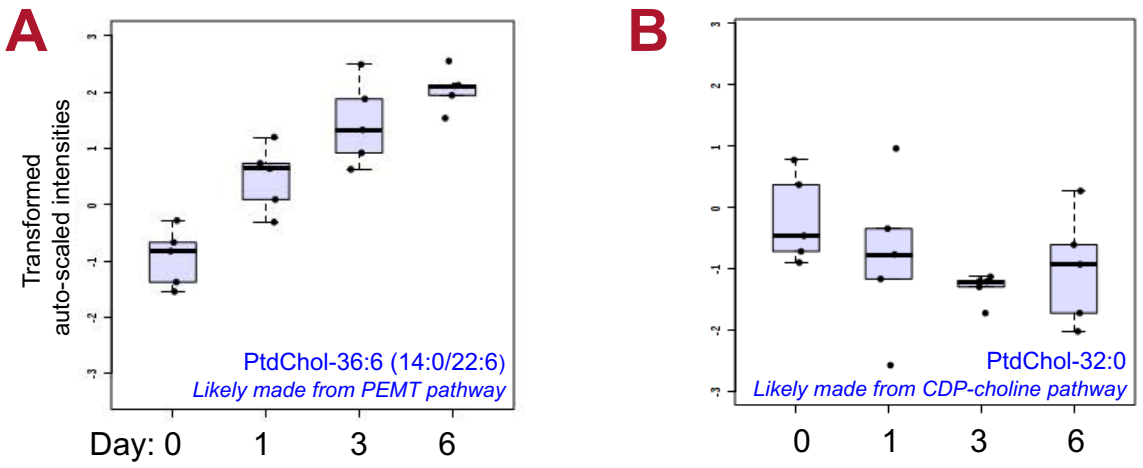
DeLong et al., 1999; Based on non-ruminant findings

Abomasal choline infusion differentially modifies hepatic PtdChol supply in cows



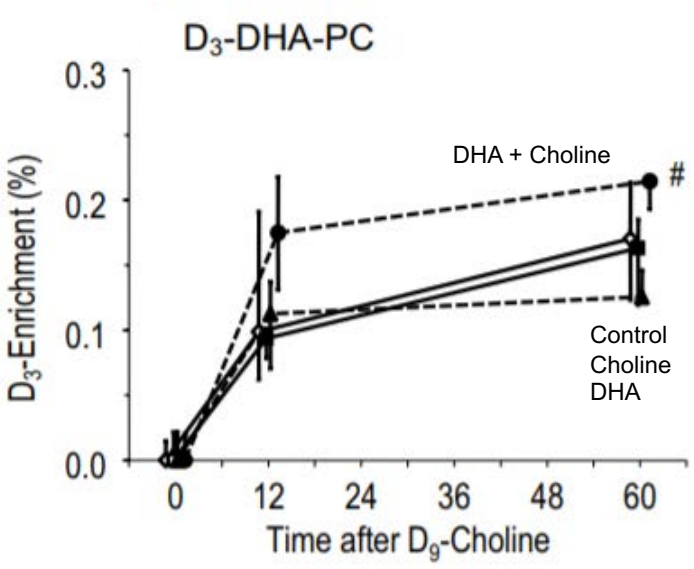
Myers et al., 2019; unpublished

Abomasal omega-3 FA (DHA) infusion increases plasma PtdChol-36:6 in cows



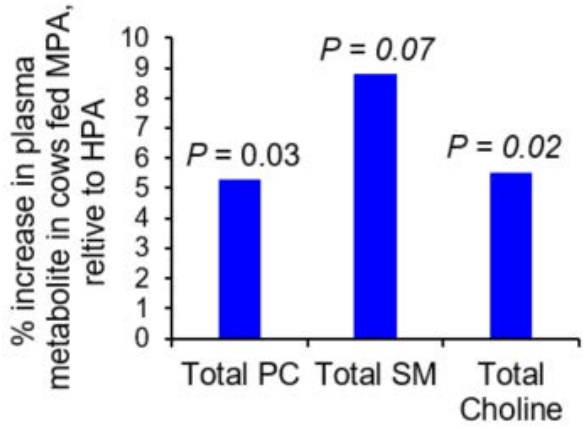
Myers et al., 2019; unpublished

In humans, DHA + choline promotes PEMT pathway activation in humans



Bernhard et al., 2019

Feeding prilled palm fatty acids containing less C16:0/more C18:1 increased plasma total choline in lactating dairy cows



*Cows fed **MPA** (72% C16:0, 12% C18:1) vs. **HPA** (99% C16:0)*

Wang et al., In Review; *P*-values based on concentration data (not presented)

Conclusion #1

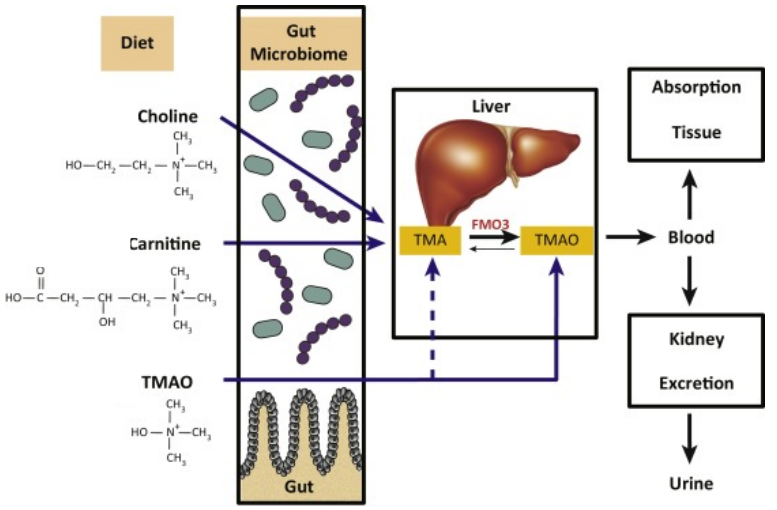
- Low PtdChol supply is a key feature of fatty liver in dairy cows.
  - Likely due to low PUFA and choline
- Delivery of post-ruminal PUFA may support PtdChol synthesis in cows.
  - Should consider interactions with dietary fatty acid digestibility
  - Improved insulin sensitivity, and reduced lipolysis and inflammation are added benefits
  - Fresh cow considerations
    - Keep total dietary fat 4-6.5%
    - Consider commercial fats with protected UFA
    - SFA:UFA ratio < 0.75:1
    - n-6:n-3 FA ratio < 5:1 in fresh cows
    - C18:3 (flax) likely has similar benefit as DHA (fish oil) but need to feed more
    - Feed 12.5 to 20 g of RP-choline ion*
    - Feed Met at 2.4% of MP; Lys:Met ratio ~2.8:1

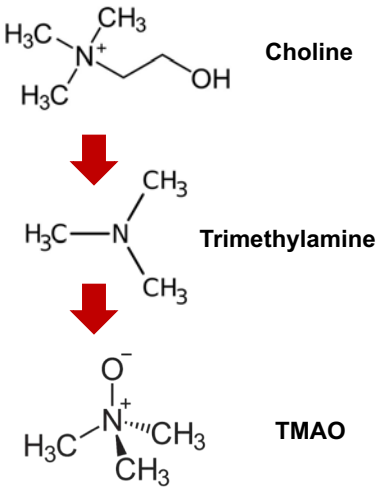
*Understand bioavailability

Topic #2

Gastrointestinal choline degradation and trimethylamine *N*-oxide

Choline is degraded to trimethylamine (by microbes), which is converted to trimethylamine *N*-oxide (TMAO) in liver





Cho and Caudill, 2017

Unprotected choline is degraded to trimethylamine in rumen

A

Item	Choline, g/kg ration DM 0	10	20	SEM
DM Intake, kg/d	18.3	16.9	16.8	.43
Duodenal DM flow, kg/d	10.50	9.92	9.65	.48
Dietary	8.68	8.08	7.86	.46
Bacterial	1.82	1.85	1.78	.05
Duodenal CP flow, kg/d	2.44	2.52	2.36	.14
Nonbacterial (dietary plus NH_3 N)	1.59	1.68	1.53	.09
Bacterial	.84	.86	.83	.05
Choline intake, g/d	23.5	176.7	325.9	6.57[a]
Duodenal choline flow, g/d	1.17	1.34	2.48	.30[b]

B

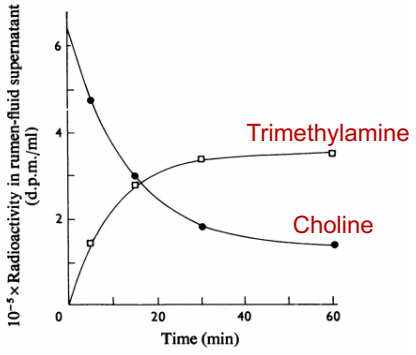
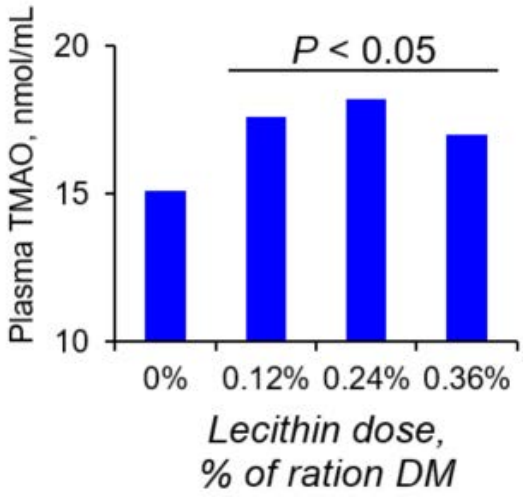


Fig. 3. *Metabolism of choline chloride to trimethylamine in rumen fluid in vitro*

Choline → trimethylamine

Hayward and Stadtman, 1959; Neill et al., 1978; Sharma and Erdman, 1988;
Reaction controlled by TMA Lyase

Feeding phosphatidylcholine (i.e., lecithin) increases plasma [TMAO] in lactating cows

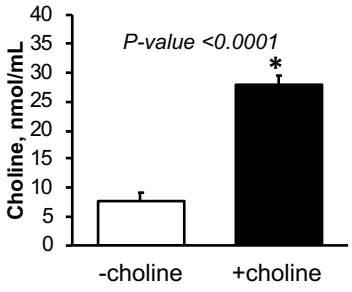


Wang et al., In Review

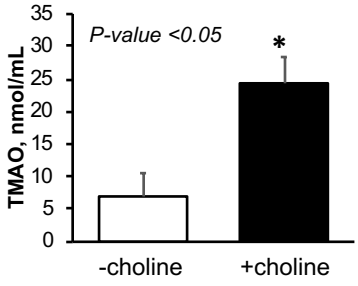
Abomasal infusion of choline chloride increases plasma [TMAO] in lactating cows

A

B



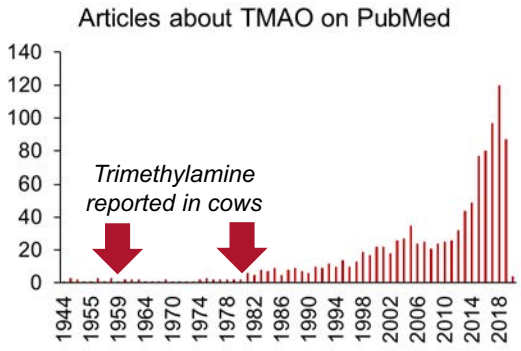
C



Myers et al., 2019

TMAO accumulation is related to poor health

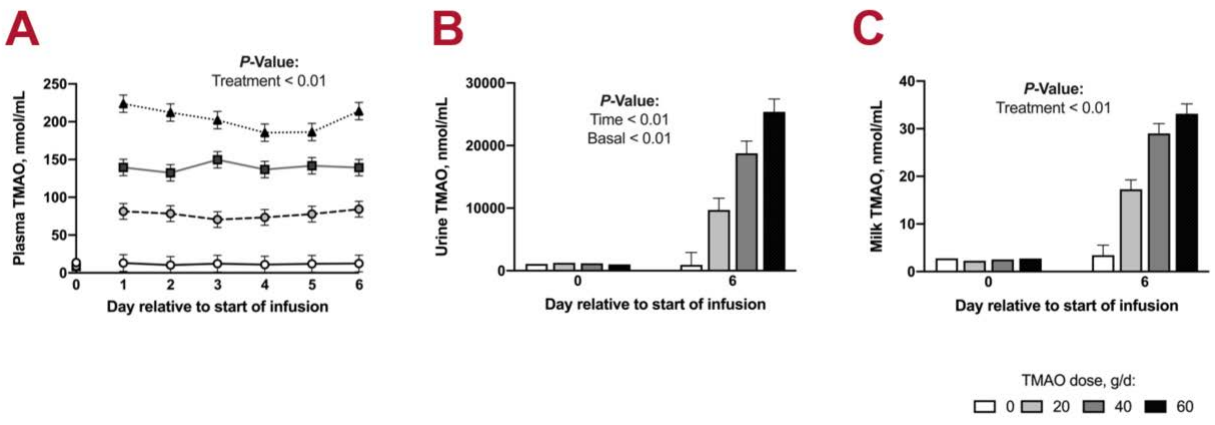
- Non-alcoholic fatty liver disease
- Inflammation
- Insulin resistance (type-2 diabetes)
- Obesity
- Oxidative stress
- Cardiovascular disease
- Cancer
- CKD (Chronic kidney disease)



Plasma [TMAO] has been shown to be associated with fatty liver in cows.

Xu et al. 2015; Chen et al., 2016; Tan et al., 2019

So we studied the effects of intravenous TMAO infusion in early lactation Holstein cows



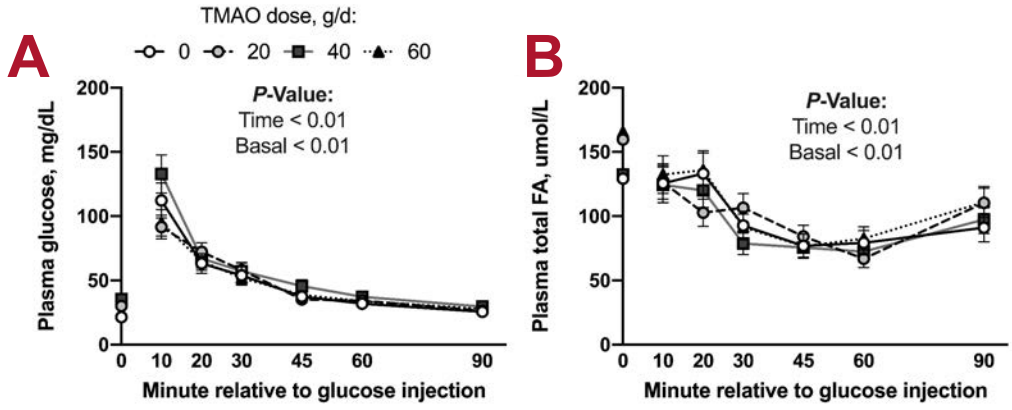
Replicated 4 × 4 Latin square; 8 cows; continuous i.v. infusion

Acute intravenous TMAO infusion does not modify milk production in early lactation cows

Item	TMAO, g/d				SEM	*P*-value
	0	20	40	60		
DMI	23.7	24.0	23.0	24.1	1.16	0.89
Milk yield, kg/d	48.0	47.9	47.0	47.7	1.89	0.98
Milk solids, kg/d						
Fat	1.80	1.89	1.79	1.77	0.07	0.55
Protein	2.33	2.30	2.28	2.36	0.09	0.93
Lactose	1.28	1.27	1.25	1.27	0.05	0.97
Milk composition, %						
Fat	3.68	3.74	3.83	3.78	0.12	0.25
Protein	2.70	2.67	2.69	2.68	0.04	0.94
Lactose	4.89	4.83	4.88	4.95	0.04	0.11
3.5% FCM, kg/d	49.8	51.2	49.3	49.1	0.65	0.72
ECM, kg/d	47.7	48.7	47.2	47.1	1.55	0.80
Efficiency (milk/DMI)	2.01	2.17	2.05	1.96	0.13	0.54
MUN	7.55	8.02	7.72	7.71	0.32	0.60

Replicated 4 × 4 Latin square; 8 cows; continuous i.v. infusion

Acute intravenous TMAO infusion does not modify glucose tolerance in early lactation cows



No change in basal glucose, triglyceride, or total FA with treatment.
No change in serum liver enzymes.
No overt changes in metabolome.

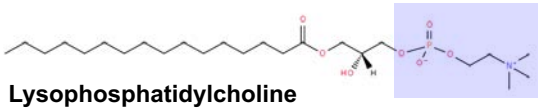
Replicated 4 × 4 Latin square; 8 cows; continuous i.v. infusion

Conclusion #2

- Trimethylamine *N*-oxide does not appear to influence energy metabolism or challenge health in early lactation cows.
- Choline is subject to ruminal and lower-gut degradation to trimethylamine.
  - The influence on choline bioavailability needs to be defined.
- Data in non-ruminants suggests that unsaturated fatty acid feeding is means to shift the gut microbiota and slow trimethylamine formation.

Topic #3

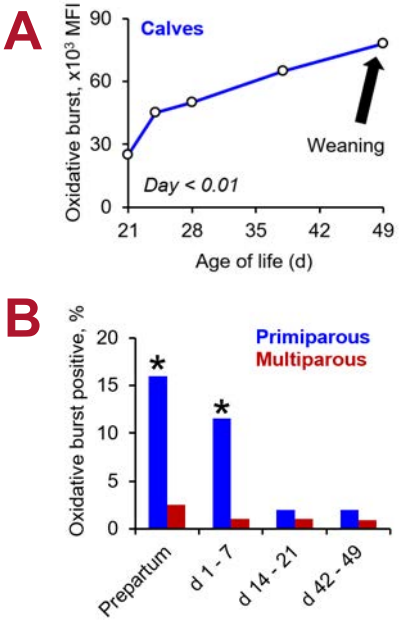
Lysophosphatidylcholine and Immunomodulation



Lysophosphatidylcholine

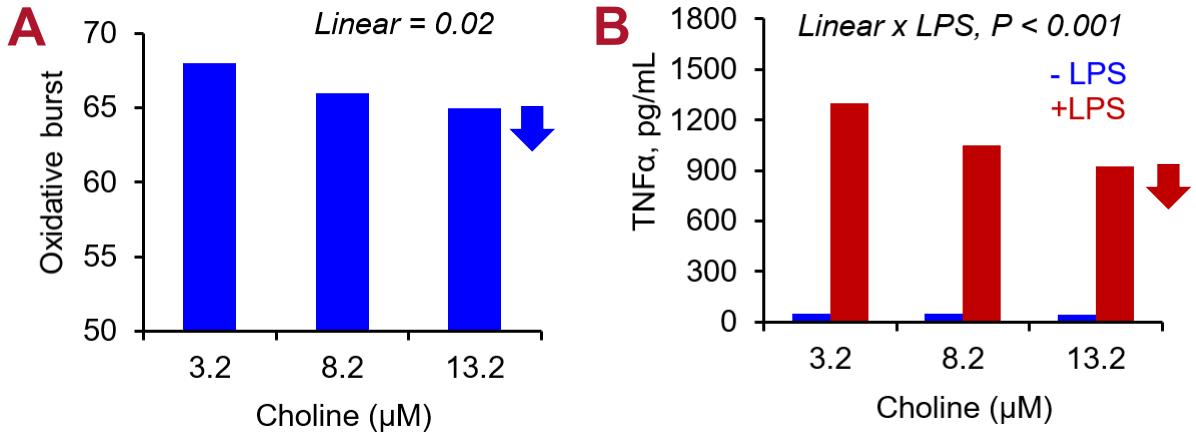
Neutrophils and immunity

- Neutrophils are cells of the innate immune system (first line of defense).
- Help fight infection by ingesting microorganisms and releasing enzymes to kill invaders.
- Involves the production of oxygen free radicals (i.e., oxidative burst).
- Pre-weaned calves and transition cows are susceptible to pathogen infection.



Modified from Belli et al., 2018 and Llamas Moya et al., 2008 (see pubs for data)

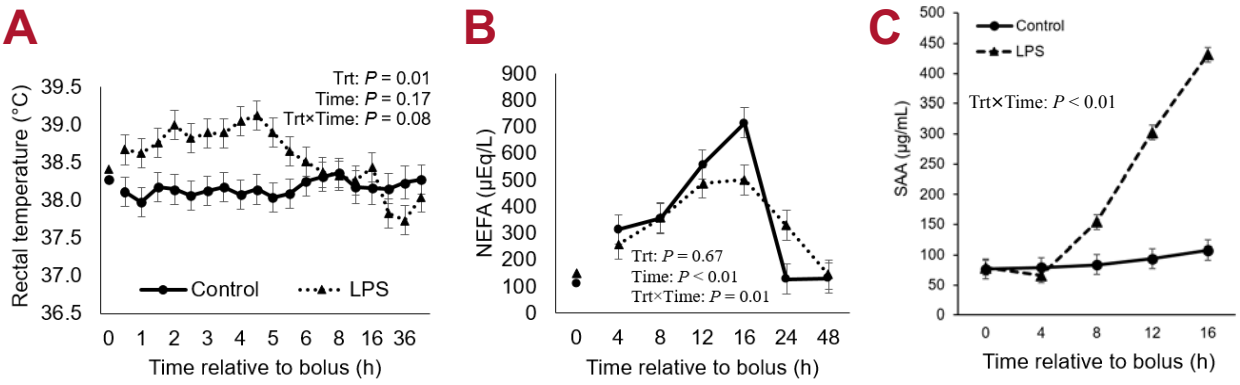
Choline modulates neutrophil function



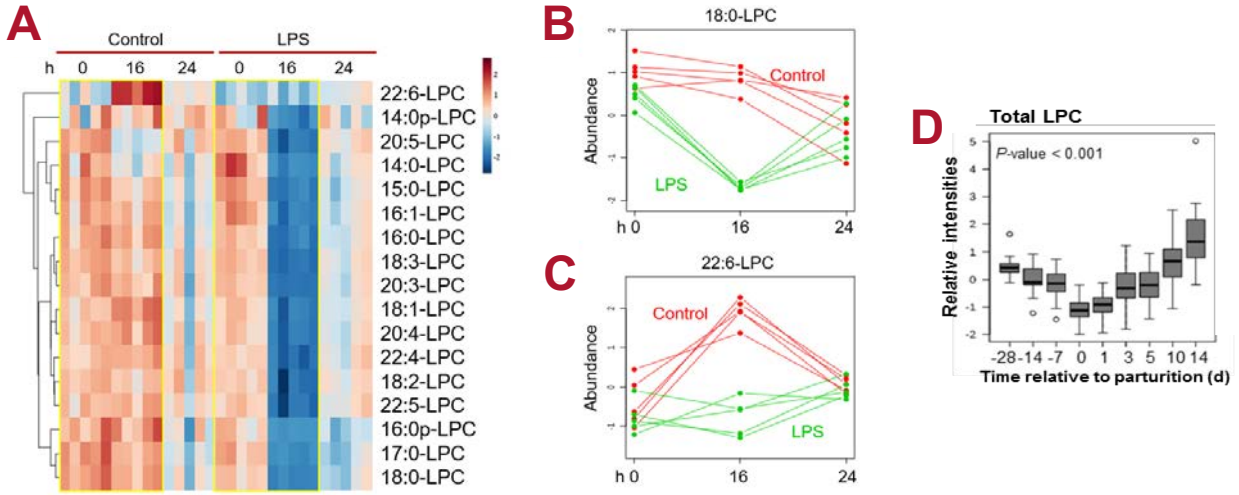
Choline modulated expression of genes involved in choline metabolism to reflect increased utilization.

Modified from Garcia et al., 2018 (see pub for data)

The endotoxin response in lactating cows



Endotoxin decreases circulating lysophosphatidylcholines in dairy cows



Plasma ceramide and lysophosphatidylcholine inversely correlate with mortality in sepsis patients

Wolfgang Drobnik,* Gerhard Liebisch,* Franz-Xaver Audebert,§ Dieter Fröhlich,† Thomas Glück,§ Peter Vogel, Gregor Rothe,* and Gerd Schmitz[1,*]**

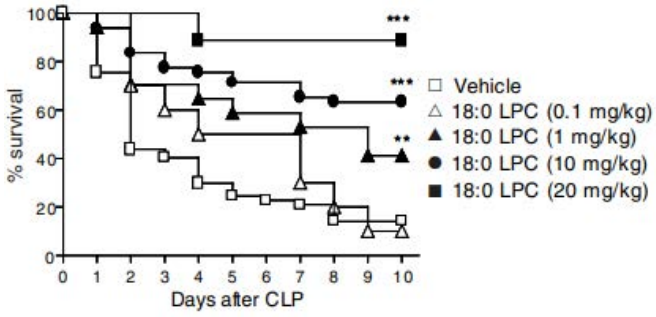
Institute for Clinical Chemistry* and Laboratory Medicine, Department of Anesthesiology,† Department of Internal Medicine I,§ and Department of Surgery,** University of Regensburg, Germany

Therapeutic effects of lysophosphatidylcholine in experimental sepsis

Ji-Jing Yan[1,4], Jun-Sub Jung[1,4], Jung-Eun Lee[1,4], Jongho Lee[1,4], Sung-Oh Huh[1,3,4], Hee-Sung Kim[1,3], Kyeong Cheon Jung[2], Jae-Young Cho[1], Ju-Suk Nam[1], Hong-Won Suh[1], Yung-Hi Kim[1] & Dong-Keun Song[1,3]

Lysophosphatidylcholine therapy prevents sepsis-induced mortality

A



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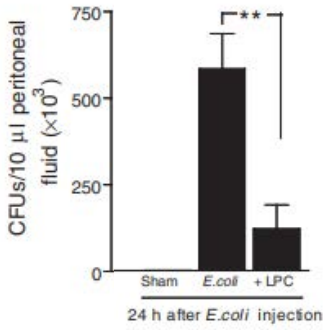
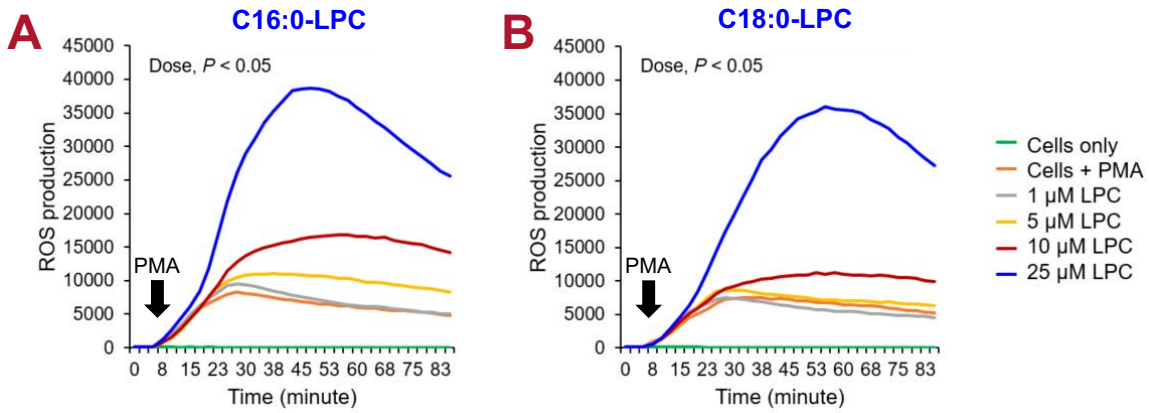


LPC activates innate and adaptive immunity.

Yan et al., 2004

Saturated LPC enhances oxidative burst in neutrophils isolated from pre-weaned calves

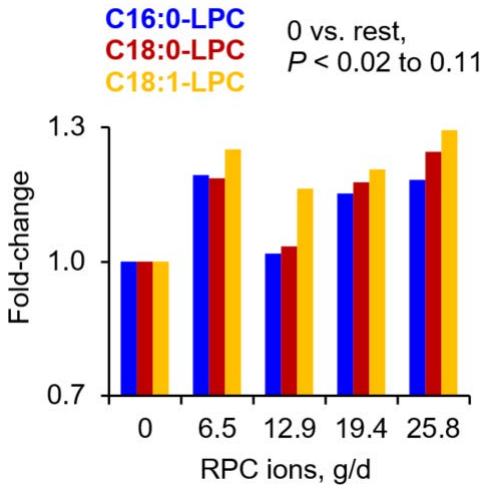


PMA = phorbol myristate acetate (promotes oxidative burst); pilot data;
ROS production = chemiluminescence

Rumen-protected choline feeding increases circulating LPC

RP-choline feeding may not increase circulating free choline.

Need to study choline metabolites.



Modified from Zenobi et al., 2018.
Significance based on plasma concentrations as described in publication.

Conclusion #3

- Immunosuppression and endotoxemia are characterized by low circulating lysophosphatidylcholine concentrations in dairy cows.
- In vitro data suggest that saturated LPC activate neutrophils.
- Rumen-protected choline feeding increases circulating LPC.
  - Future research is likely to define an immunomodulatory role for choline.
- We should consider effects of lecithin and lysolecithin on immunity.

One last thing…

J. Dairy Sci. 103
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Symposium review: **One-carbon metabolism and methyl donor nutrition in the dairy cow***

J. W. McFadden,[1]† C. L. Girard,[2] S. Tao,[3] Z. Zhou,[4] J. K. Bernard,[3] M. Duplessis,[2] and H. M. White[5]
[1]Department of Animal Science, Cornell University, Ithaca, NY 14853
[2]Agriculture and Agri-Food Canada, Sherbrooke Research and Development Centre, Sherbrooke, QC, Canada J1M 0C8
[3]Department of Animal and Dairy Science, University of Georgia, Tifton 31793
[4]Department of Animal Science, Michigan State University, East Lansing 48824
[5]Department of Dairy Science, University of Wisconsin, Madison 53706

The lab

- Dr. Eduardo Rico (Post-doc)
- Philip Wang (visiting postdoc)
- Amanda Davis (PhD student; NSF Fellow)
- Ananda Fontoura (PhD student; FFAR Fellow)
- Brianna Tate (PhD student)
- William Myers (PhD student)
- Tanya France (PhD student)
- Awais Javid (PhD student)
- Feiran Wang (visiting PhD student)
- Alumni: Alice Mathews, Yu Zang, Sina Saed Samii, Zach Phipps

Collaborators

- Dr. Charles Staples
- Dr. Lance Baumgard
- Dr. Norman Haughey

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Choline biology in dairy cattle: Contemporary perspectives and future prospects

Questions?

Joseph W. McFadden, Ph.D.
Associate Professor of Dairy Cattle Biology
Northeast Agribusiness & Feed Alliance Faculty Fellow
Department of Animal Science
McFadden@cornell.edu

Presented as a webinar for the Methyl Donors and 1-C Metabolism mini-symposium by Balchem & Feedstuffs on 4/29/2020