Challenges with measuring choline bioavailability in the dairy cow

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Topics for discussion

- Bioavailability defined
- Established knowledge about choline
- How gastrointestinal bacteria limit choline bioavailability
  - The role of trimethylamine and trimethylamine *N*-oxide (TMAO)
- Methods and limitations to measure choline bioavailability

What is choline bioavailability?

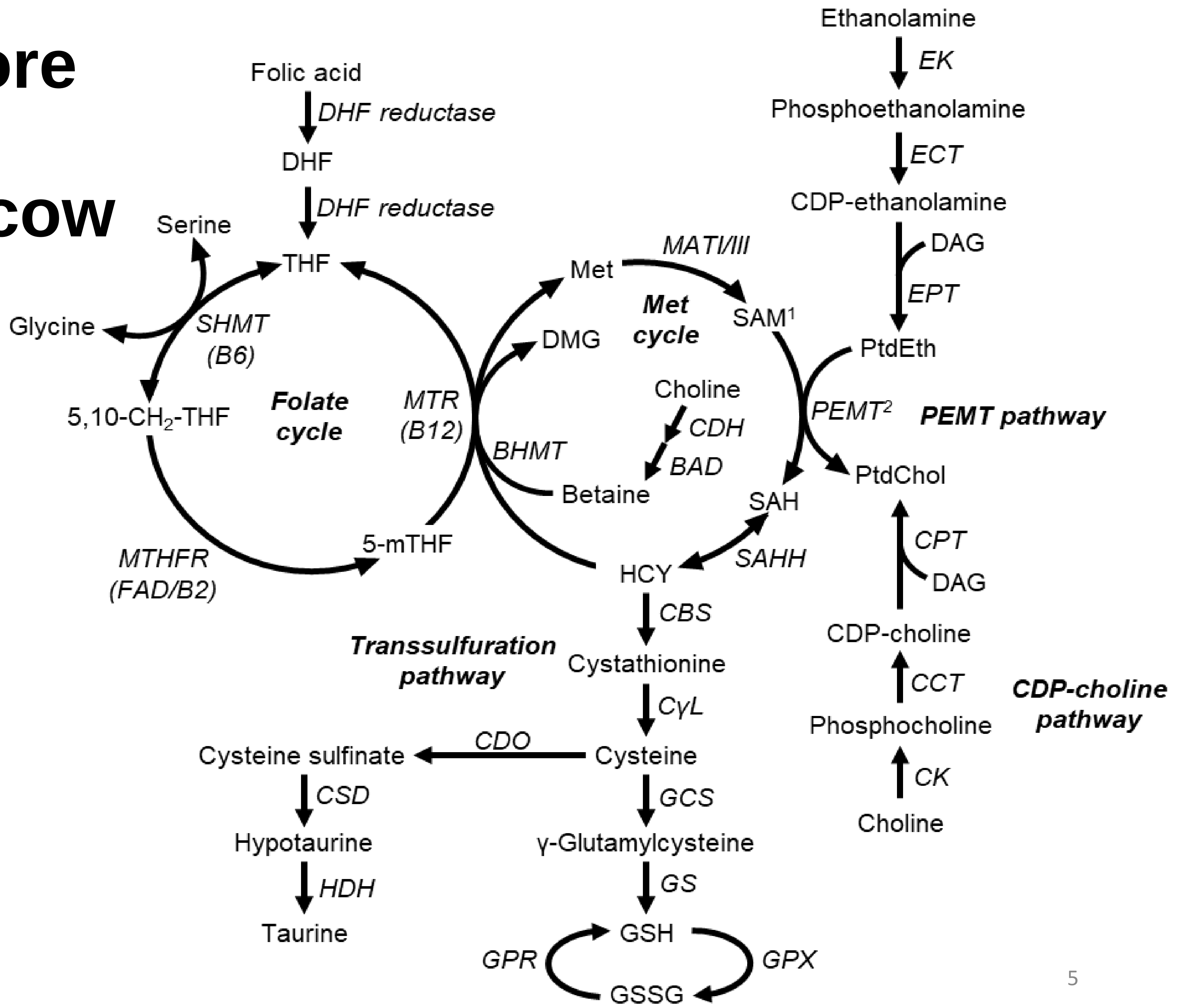
Term	Definition
Abundance	The amount of choline (ion or salt; total or rumen-protected) in the diet
Digestibility	The amount of choline that escapes rumen fermentation and released from encapsulation by the digestive process
Absorbability (metabolizable)	The amount of choline, which was released from encapsulation at the small intestine, that is actually absorbed into the cow
Bioavailability	The amount of choline, which was absorbed, that was actually utilized by the cow for a biological function; influenced by intrinsic and extrinsic factors

Adapted from Godber, 1990

Choline bioavailability is not…

- the amount of choline that escapes ruminal fermentation
- the amount of choline not measured in faeces
- the amount of choline measured at the small intestine
- the amount of choline not used for biological functions

The metabolic core of how choline functions in the cow



Key choline metabolites:
Betaine
Phosphatidylcholine
Lysophosphatidylcholine
Sphingomyelin
Phosphocholine
Glycerophosphocholine
Acetylcholine

McFadden et al., 2020

Choline and choline metabolite concentrations of common feed ingredients

	Feed ingredient							
Metabolite[1]	Soybean hulls	Corn silage	Grass silage	Corn grain	Canola meal	Beet pulp	Megalac[2]	Grass hay
Bet	4.7	19.3	7.7	1.3	3.5	43.5	6.3	11.9
Cho	16.8	37.4	55.7	8.9	72.2	3.1	92.9	38.5
ACho	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.1
GPCho	7.1	0.6	0.1	10.6	24.4	0.5	0.1	1.0
PCho	0.6	0.1	0.2	0.5	0.9	0.9	0.0	0.3
LPC	2.6	0.5	0.4	2.7	42.5	8.8	10.6	0.5
PC	22.3	0.2	0.7	28.9	98.5	102.6	91.2	2.6
SM	0.1	0.0	0.0	0.0	0.0	0.0	1.6	0.0
TCM[3]	23.5	37.9	56.0	17.9	104.3	19.4	108.2	39.6

[1]Betaine (Bet), free choline (Cho), acetylcholine (ACho), glycerophosphocholine (GPCho), phosphocholine (PCho), lysophosphatidylcholine (LPC), phosphatidylcholine (PC), sphingomyelin (SM), total choline moiety (TCM).

[2]Arm & Hammer Animal Nutrition, Princeton, NJ.

[3]Total choline moiety (TCM) is the sum of choline ion originating from Cho, ACho, GPCho, PCho, LPC, PC, and SM.

de Veth et al., 2016; mg/100 g of feed

Ruminal (in vitro) choline disappearance (%)

	Time of incubation, h								
Sample	.25	1	3	6	12	18	24	36	48
Corn silage									
$\overline{X}$	3.6	9.7	17.3	23.9	31.3	36.1	39.7	45.4	50.0
SE	3.62	5.12	9.53	12.64	14.71	14.53	13.77	12.96	13.63
Corn									
$\overline{X}$	41.3	54.8	56.3	54.1	56.4	62.2	57.3	62.2	68.3
SE	12.63	7.40	11.55	25.34	8.58	5.76	4.62	5.32	3.20
Cottonseed meal									
$\overline{X}$	77.8	79.2	83.1	85.8	82.4	83.3	84.9	87.5	89.7
SE	3.15	3.28	2.88	1.70	2.24	2.02	1.59	1.69	1.69
Choline stearate									
$\overline{X}$	92.3	96.3	96.5	99.4	96.8	96.9	96.8	96.9	98.6
SE	4.32	1.46	1.41	2.91	1.40	1.82	1.63	1.50	.28
Choline chloride									
$\overline{X}$	97.7	97.1	97.7	97.5	98.8	99.3	99.8	98.9	99.0
SE	.29	.11	.23	.31	.07	.34	.23	.35	.38

Sharma and Erdman, 1989

An increase in post-ruminal choline increases milk choline yield

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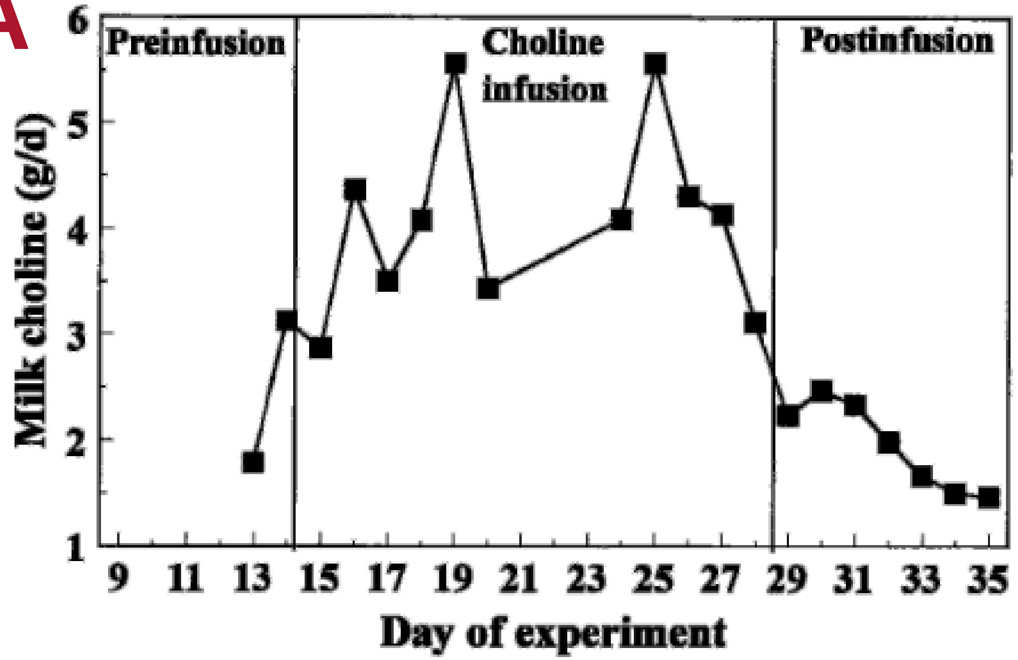

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Item	Choline 0 g/d	25 g/d	50 g/d	75 g/d	SEM	Linear[1]
Milk choline						
mg/L	86	116	118	120	7.9	0.024
g/d	2.56	3.62	3.72	3.82	0.16	0.035

Sharma and Erdman, 1988

If choline from RPC is bioavailable, what milk or plasma metabolites are responsive?

Study	Stage of lactation	Treatment	Sample type	Choline	Betaine	Phosphocholine	DMG	PC	LPC	SM	Total
Deuchler et al., 1998	Early lactation	Abomasal CC	Milk	++							
Engel et al., 2006	Lactating	Fed RPC	Milk/Plasma	NC/NC							
Elek et al., 2008	Early lactation	Fed RPC	Milk	++							
de Veth et al., 2016	Late lactation	Abomasal CC	Plasma	++	++	++		NC	NC	NC	NC
de Veth et al., 2016	Late lactation	Fed RPC	Plasma	+	++	NC		NC	NC	NC	NC
Zenobi et al., 2018	Dry	Fed RPC	Plasma	NC	NC	-		++	++	++	++
Myers et al., 2019	Dry	Fed RPC	Isolated VLDL/LDL					++			
Potts et al., 2019	Late lactation	Fed RPC	Milk/Plasma	NC/NC	NC/NC	NC/NC		NC/NC	NC/NC	+/NC	NC/NC
	Late lactation	Abomasl CC	Milk/Plasma	++/++	++/++	++/NC		NC/NC	NC/NC	NC/NC	NC/NC
	Mid lactation	Fed RPC	Milk/Plasma	NC/-	NC/NC						
	Transition	Fed RPC	Milk	NC	- or +			NC	NC	NC	NC
Myers et al., 2019	Late lactation	Abomasal CC	Plasma	++	++	++	++	NC	NC	NC	NC
Piantoni et al., 2021	Late lactation	Fed RPC	Milk	NC	++	NC					
France et al., unpublished	Early lactation	RPC bolus	Milk/Plasma	++/++	++/++	++/++		NC/-	NC/ND	NC/NC	NC/NC
France et al., unpublished	Late lactation	RPC bolus	Milk/Plasma	NC/++	++/NC	ND/NC		NC/NC	ND/-	+/NC	NC/NC

NC = no change; ND = not detected

How gastrointestinal bacteria limit choline bioavailability: The role of trimethylamine *N*-oxide

The role of gut bacteria and TMAO

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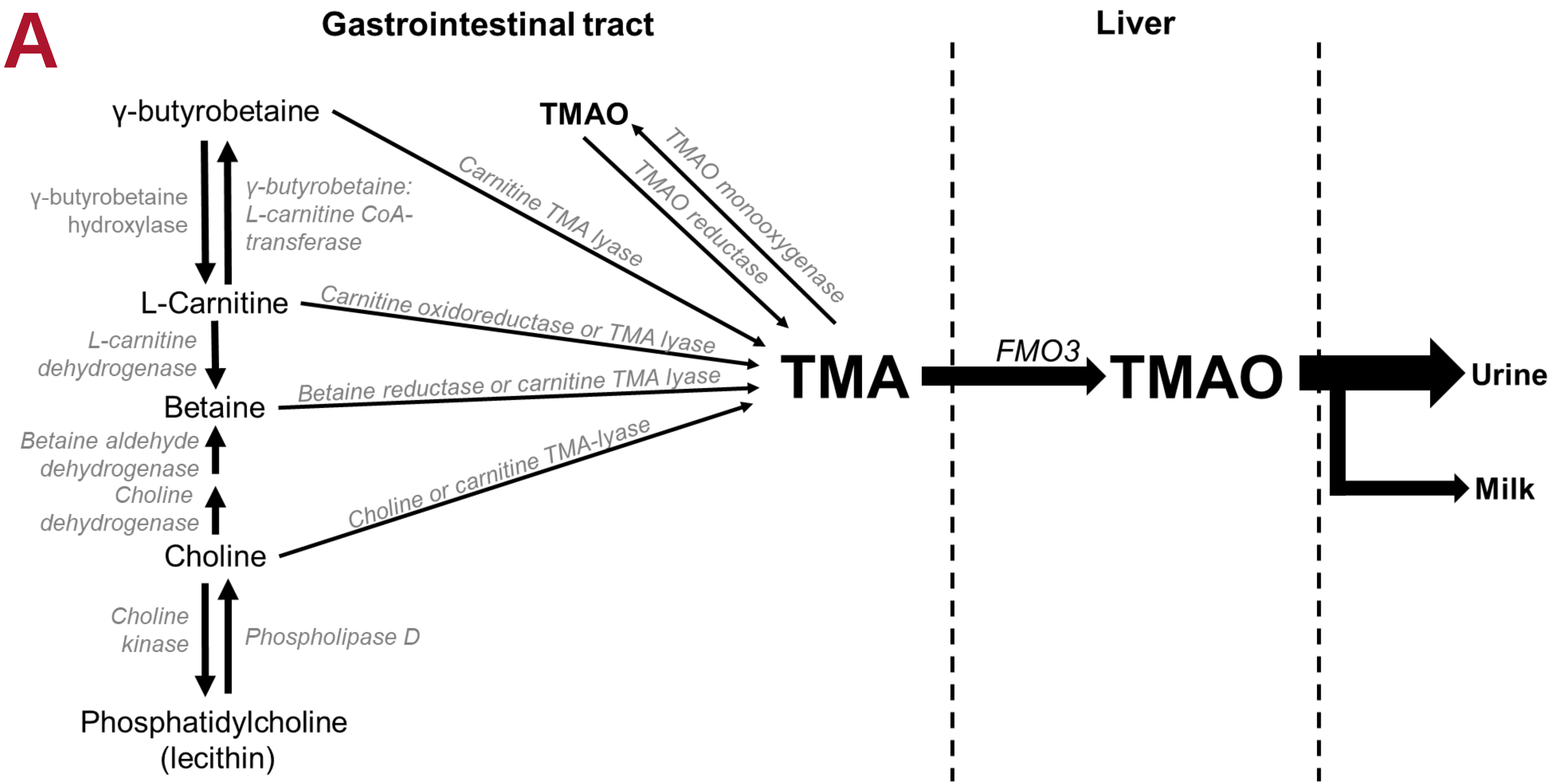

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Phylum	Genus or Species
TMA *N*-oxide	
Bacteroidetes	*Flavobacterium*
Proteobacteria	*Achromobacter*
	Aeromonas
	Alteromonas
	Photobacterium
	Rhodopseudomonas
	Salmonella
	Shewanella[a]
	Shigella
	Vibrio
Betaine	
Firmicutes	*Haloanaerobacter*
Proteobacteria	*Desulfuromonas*
Carnitine	
Proteobacteria	*Achromobacter*
	Serratia
	Shigella
Choline	
Proteobacteria	*Achromobacter*
	Shigella
Ergothioneine	
Proteobacteria	*Burkholderia*
	Campylobacter
	Stigmatella
	Sinorhizobium[b]

Myers and McFadden, 2020; Fennema et al., 2016

Ruminal and post-ruminal choline degradation increases trimethylamine (TMA)/TMAO

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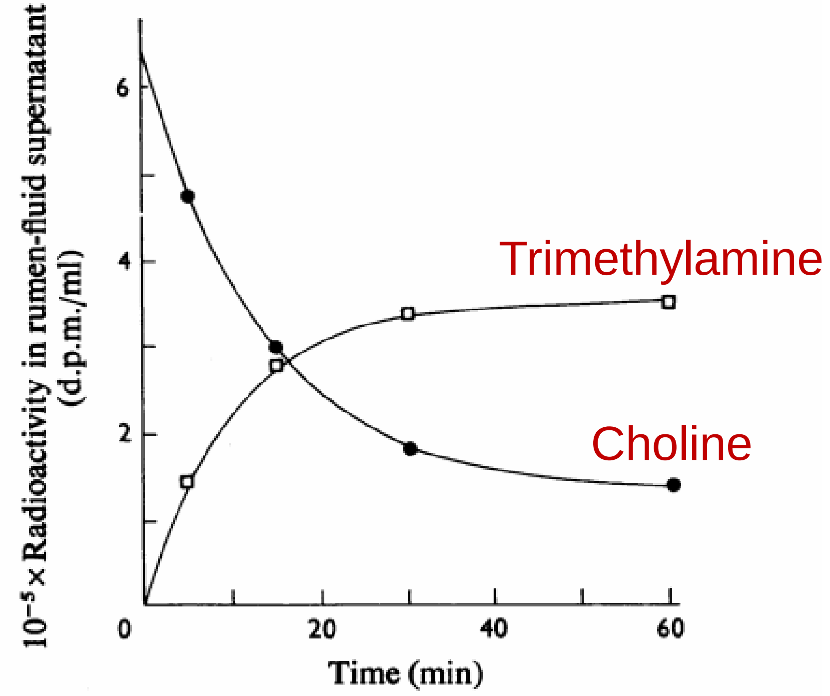

Fig. 3. *Metabolism of choline chloride to trimethylamine in rumen fluid in vitro*

Ruminal choline degradation

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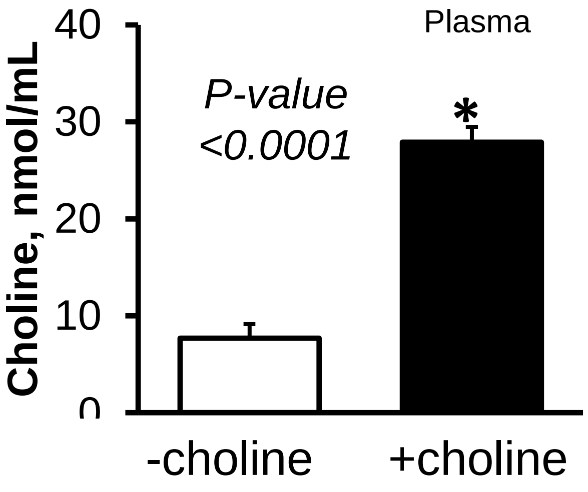

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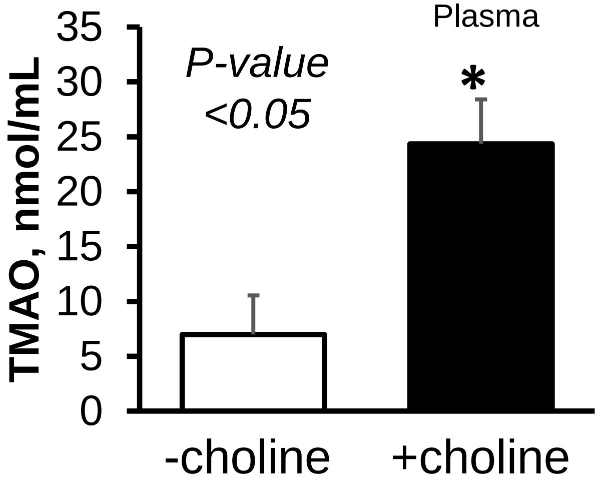
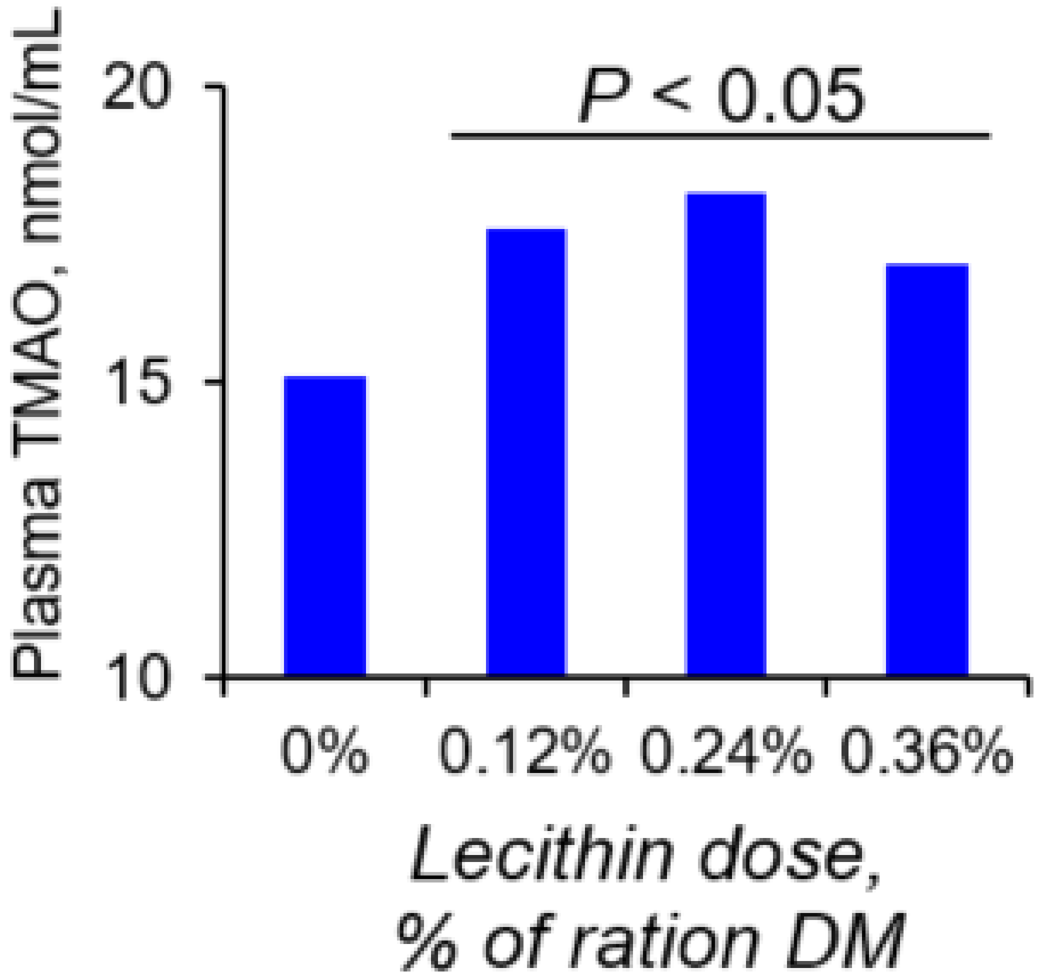


Post-ruminal choline degradation;
Plasma choline and TMAO increase in parallel

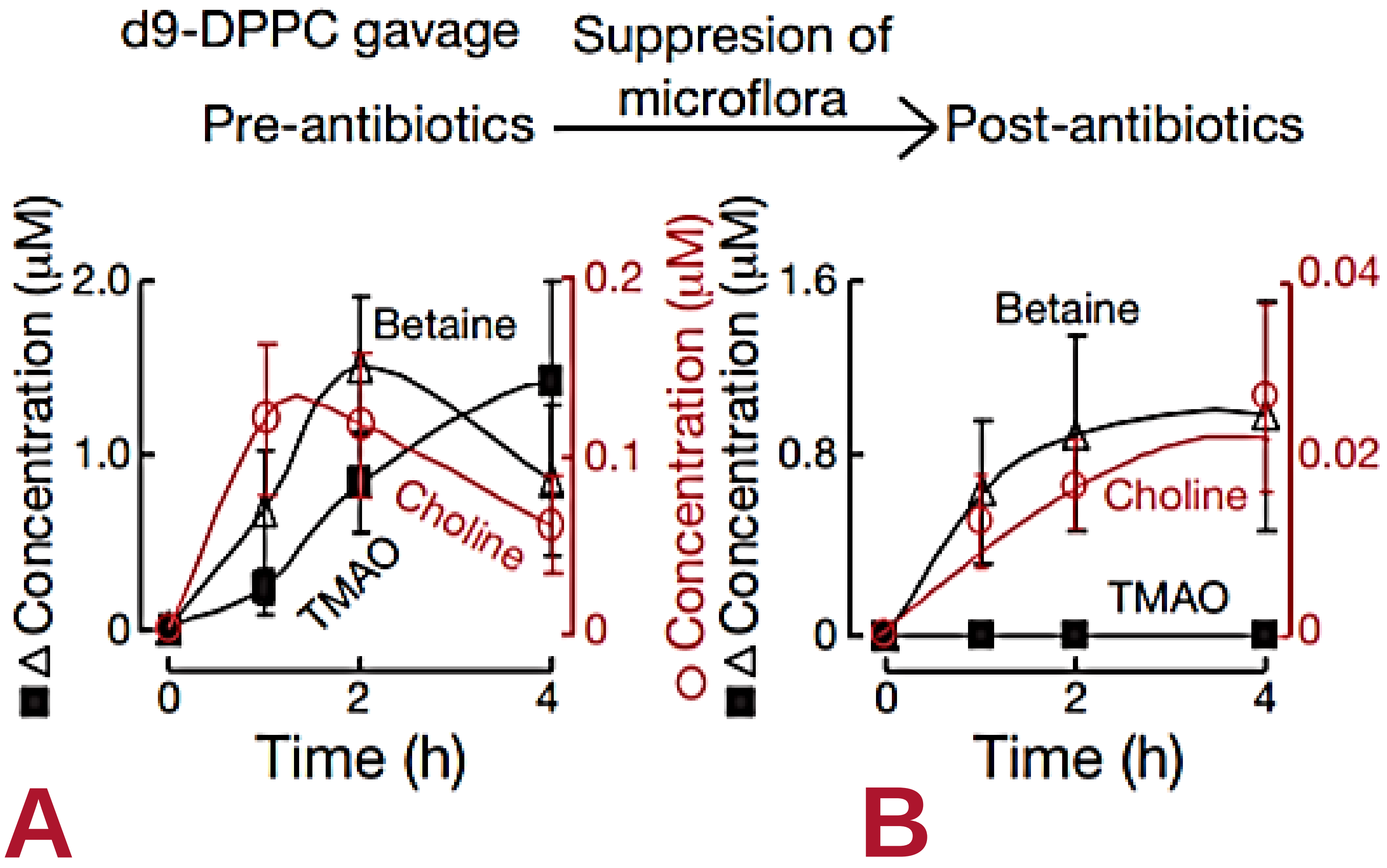
Hayward and Stadtman, 1959; Neill et al., 1978; Sharma and Erdman, 1988; Myers et al., 2019
Reaction controlled by TMA Lyase

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



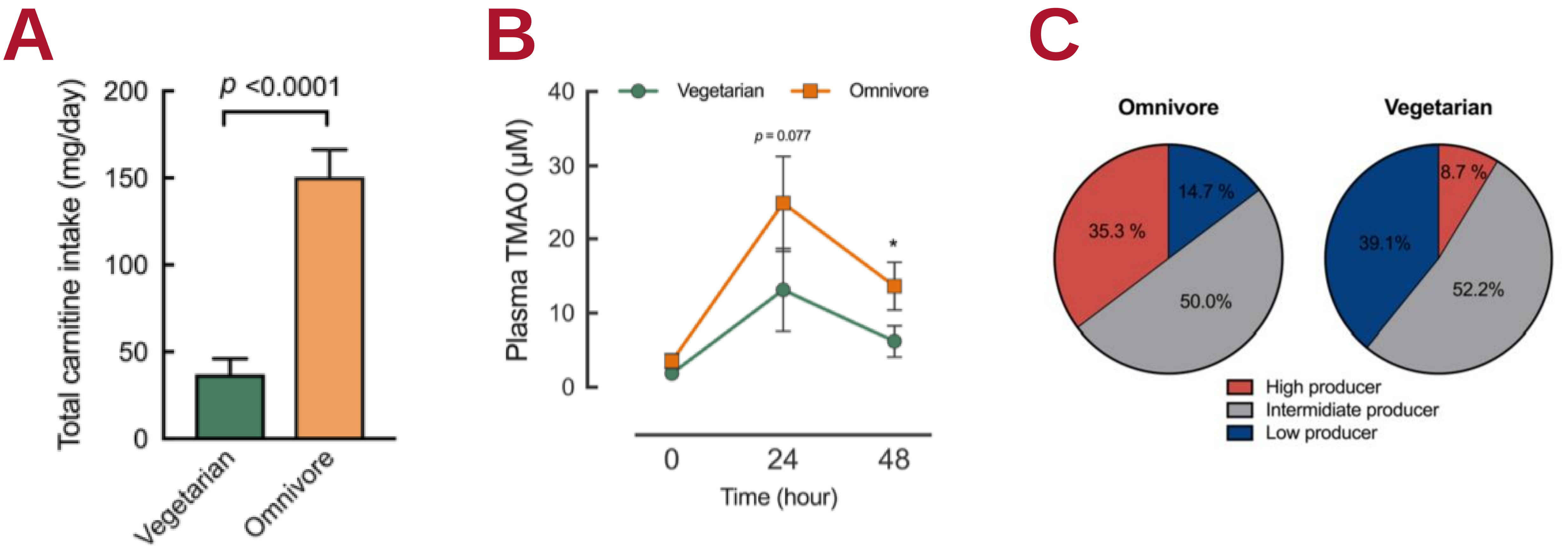
Wang et al., 2021

Obligatory role of gut flora in generation of TMAO



Wang et al., 2011

Diet and microbiome influences TMAO response



Wu et al., 2019; humans

The TMAO response varies by individual

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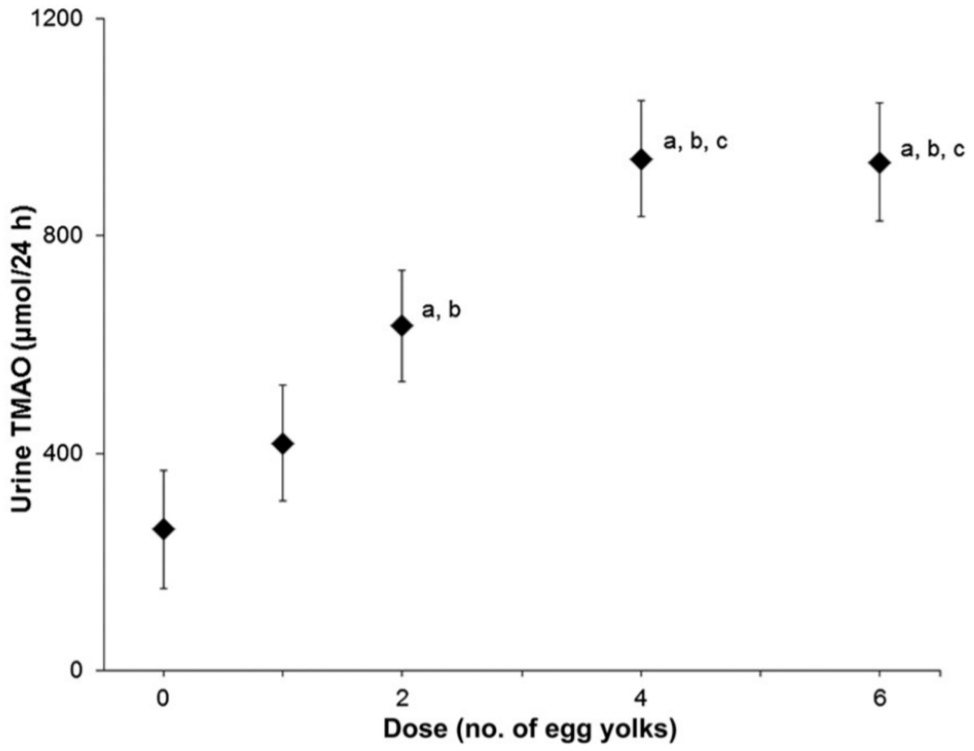

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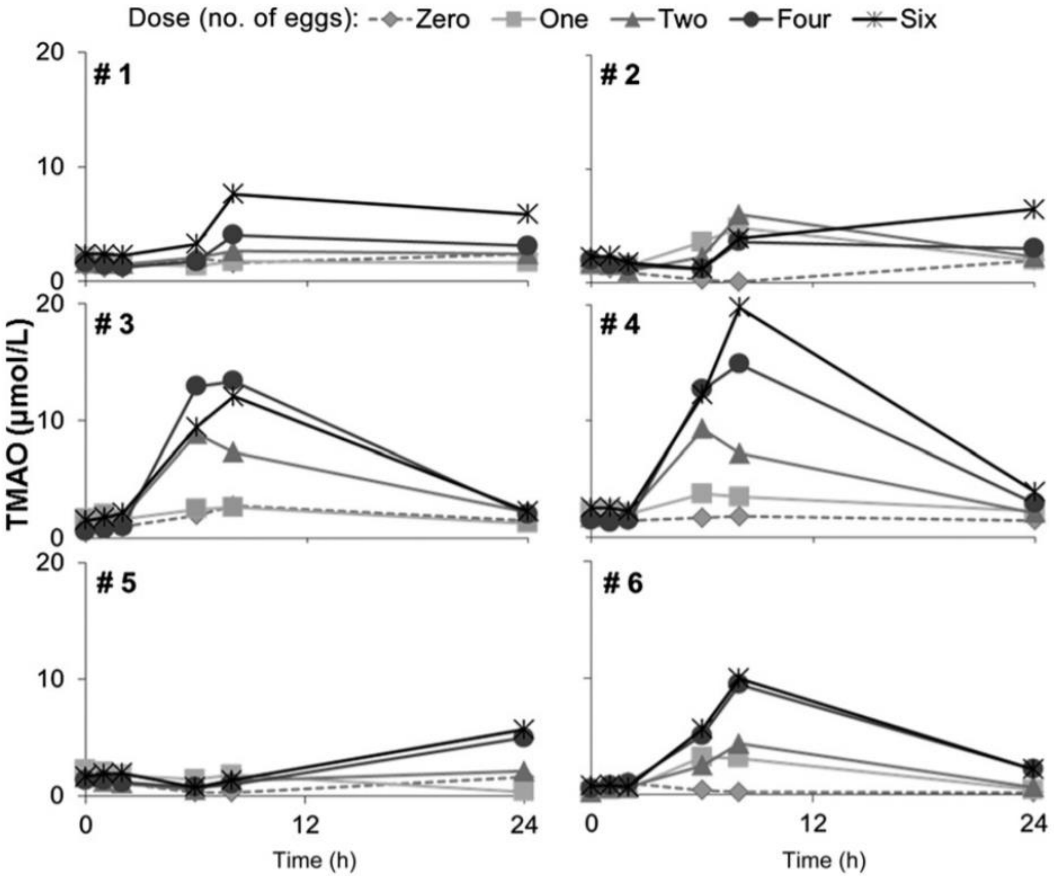

Miller et al., 2014

Bacterial composition and host genotype modulates hepatic FMO3 activity, serum TMAO, and likely choline bioavailability

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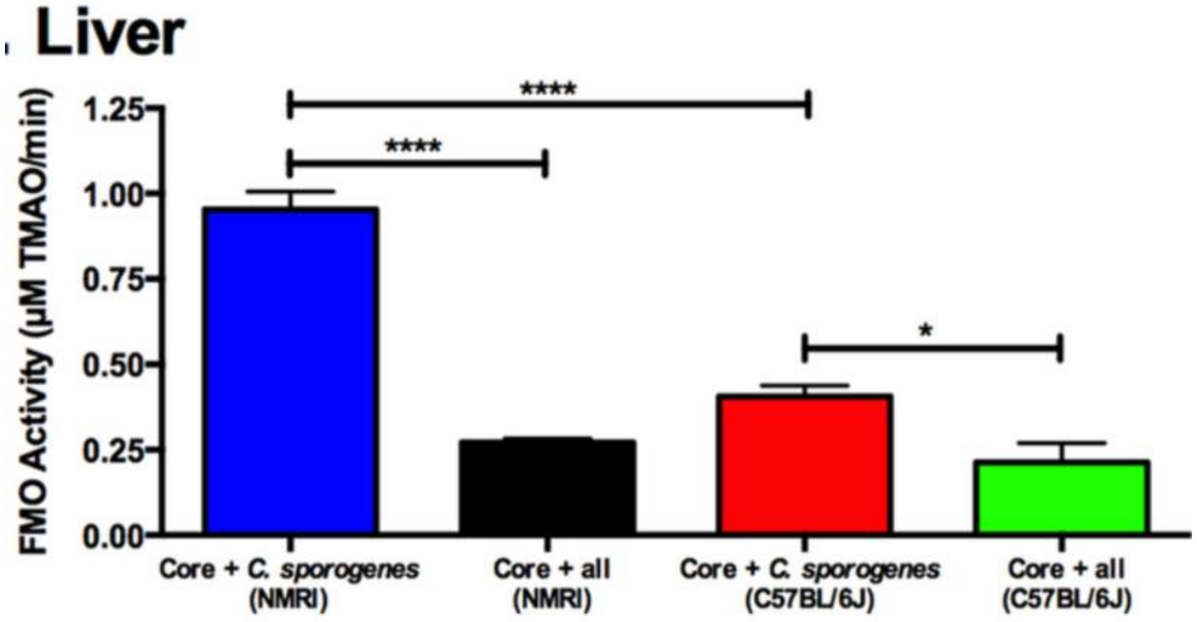

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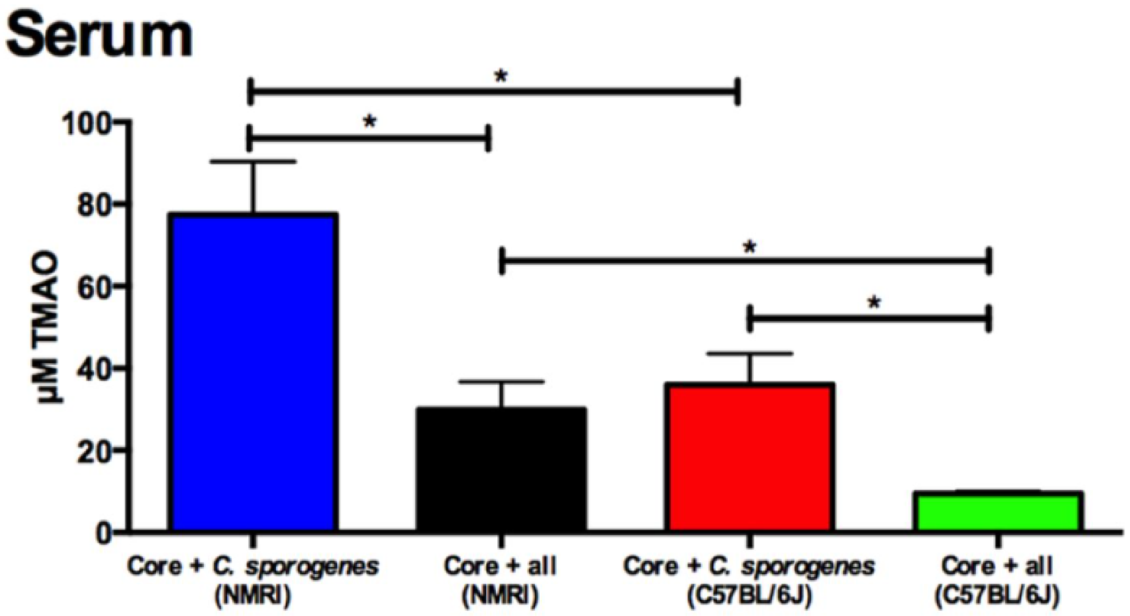
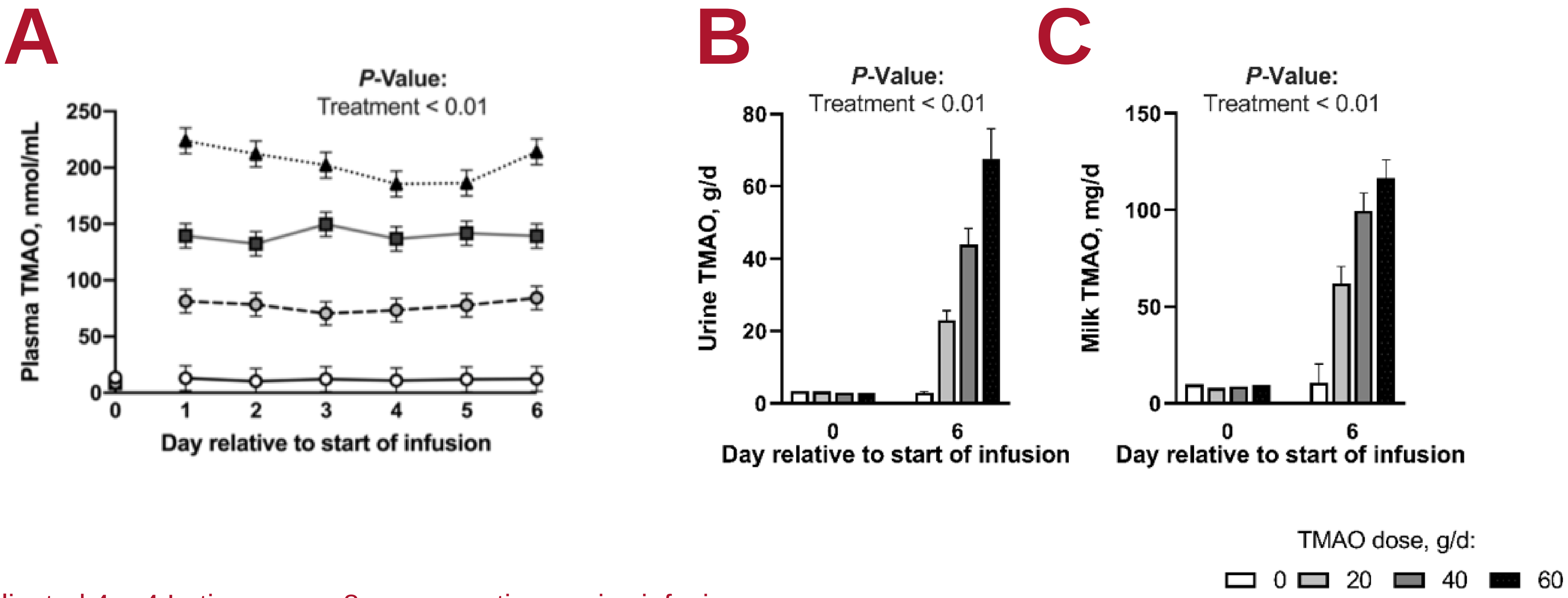


Romano et al., 2015; mice

Intravenous TMAO infusion increases urinary and milk TMAO in early lactation Holstein cows



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

Myers et al., 2021

Methods and limitations to measure choline bioavailability

Area-under-the curve technique/temporal response

- Evaluates temporal changes in plasma and milk choline and choline metabolites in response to increasing doses of RPC in the cow
- TMAO response can be observed
- Supraphysiological bolus dose; does not mimic feeding behavior
- Accounts for handling and allows for mastication by the cow
- Qualitative; not quantitative
- Biological outcomes may be hard to detect
- Good approach for product comparison

A rumen-protected choline bolus increases plasma choline and betaine in a dose-dependent manner

Unpublished

A rumen-protected choline bolus increases milk choline yield in early lactation cows

Unpublished

If choline from RPC is bioavailable, what milk or plasma metabolites are responsive?

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France et al., unpublished	Late lactation	RPC bolus	Milk/Plasma	NC/++	++/NC	ND/NC		NC/NC	ND/-	+/NC	NC/NC

NC = no change; ND = not detected

RPC dose and type influences plasma [TMAO]

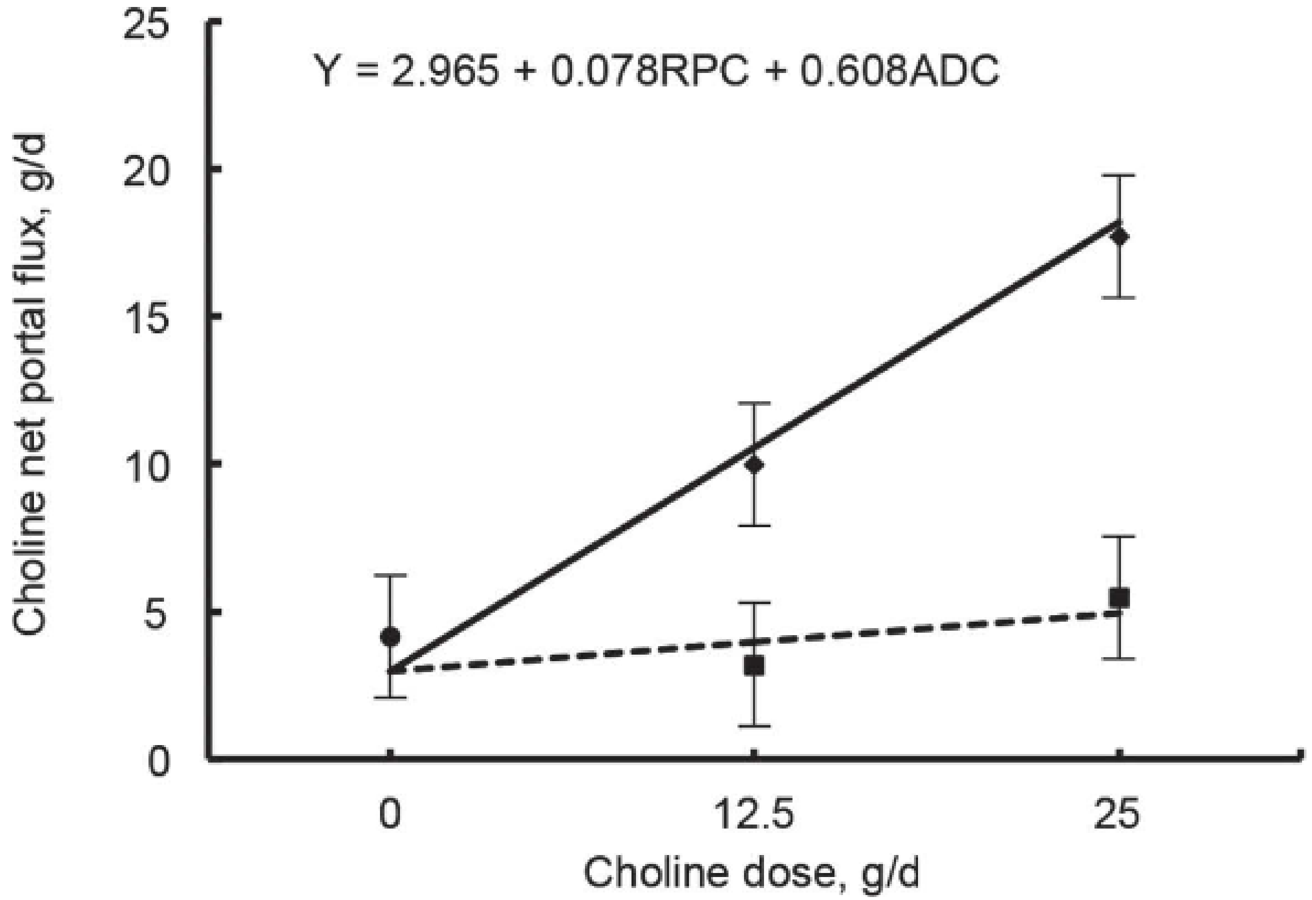
RPC dose and type influences milk [TMAO]

Unpublished; variable choline chloride content

TMAO-response varies to choline challenge in cows

Unpublished; lowest and highest quartiles (n = 3/quartile)

Evaluation of net portal flux: abomasal infusion of choline chloride vs dietary RPC topdress



Limitations:

- Requires intensive approach
- Does not account for intestinal use
- Does not account for intestinal conversion of choline to TMA
- Influenced by stage of lactation and feed intake (rate of passage)
- Rate of flow can vary

de Veth et al., 2016

Three-step in vitro procedure or Ross method

- In situ or in vitro rumen incubation time is fixed
- Particle size can be difficult to control
- Digestive [enzyme] are unknown or based on sheep studies
- Use of bags prolongs lag phase of digestion
- Demonstrated loss of highly soluble components of feeds
- Does not account for ruminal or post-ruminal conversion of choline to TMA; could be highly influenced by host microbiome
  - Inadequate replicates
- Does not account for effect of product handling or mastication by cow
- Does not measure bioavailability of choline or choline metabolites

Calsamiglia and Stern, 1995; Ross et al., 2010

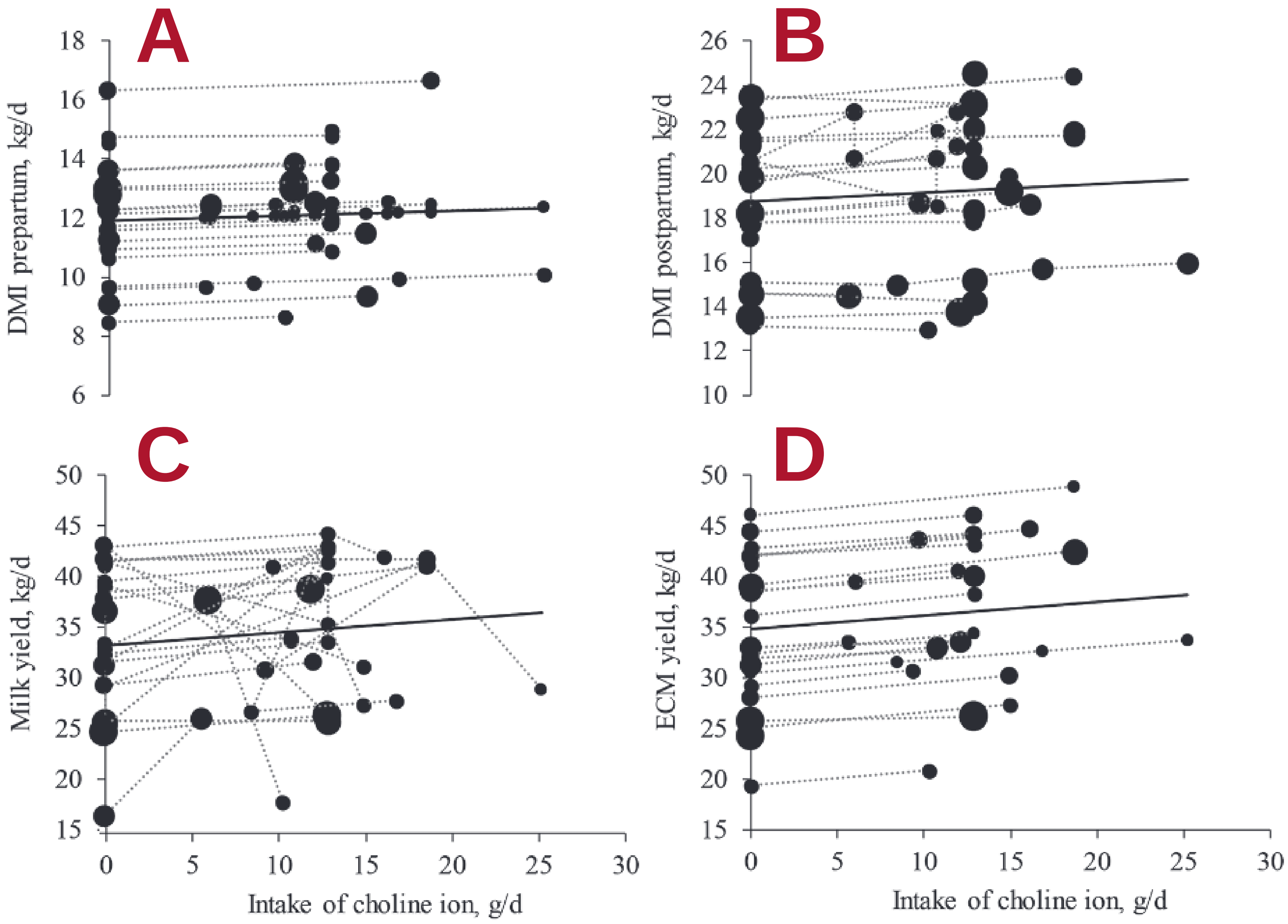
Rumen bypass followed by duodenal insertion

- In situ rumen incubation time is fixed
- Particle size can be difficult to control
- Use of bags prolongs lag phase of digestion
- Demonstrated loss of highly soluble components of feeds
- Does not account for ruminal or post-ruminal conversion of choline to TMA; could be highly influenced by host microbiome
  - Inadequate replicates
- Does not account for effect of product handling or mastication by cow
- Does not measure bioavailability of choline or choline metabolites
- <u>Intestinal insertion likely does not align with pancreatic secretions</u>
- <u>Falsely assumes that choline release in large intestine is bioavailable</u>

Other

- RPC feeding trials
- Plasma free *amino acid* dose-response technique
- Use of stable-labeled choline

Greater milk yield *means* greater choline bioavailability



"…the observed increased in DMI of 0.5 kg/d would likely support at least 1.1 kg of ECM, or 50% of the observed response with supplemental choline ion in the present study."

Arshad et al., 2020

Greater milk yield in diets low in metabolizable Met *means* greater choline bioavailability

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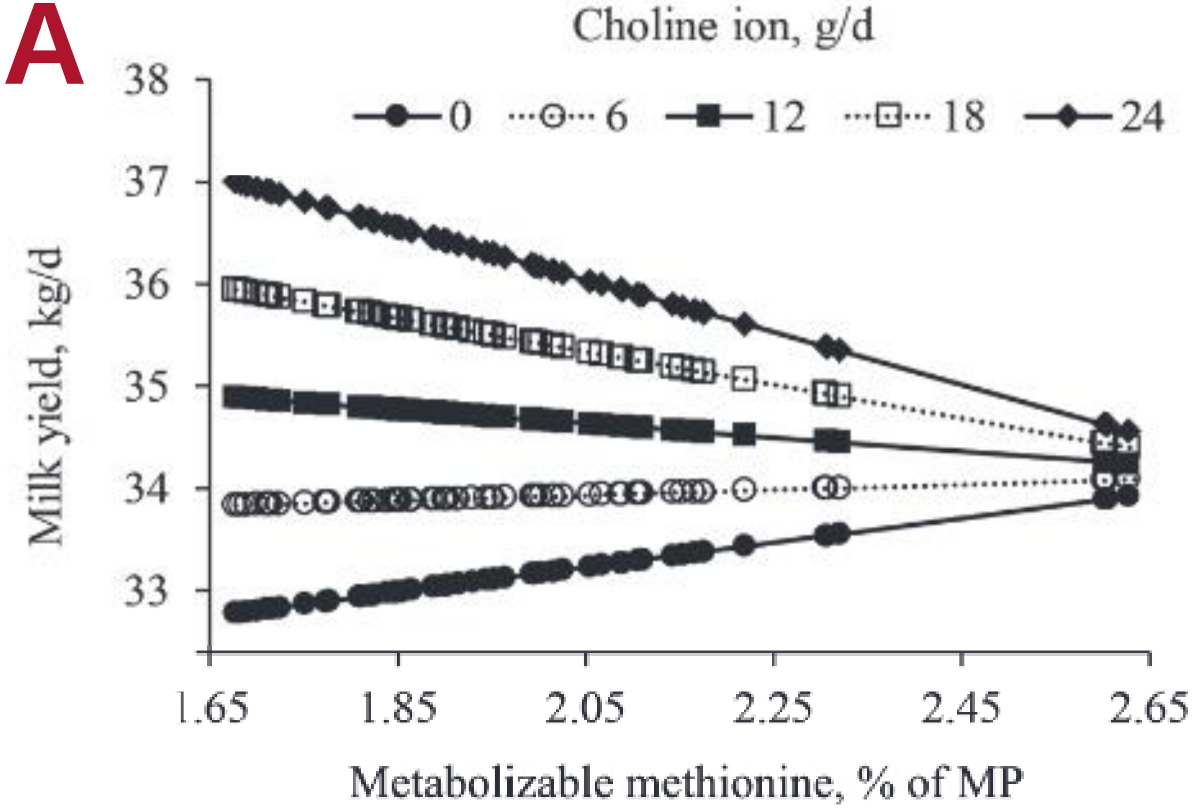

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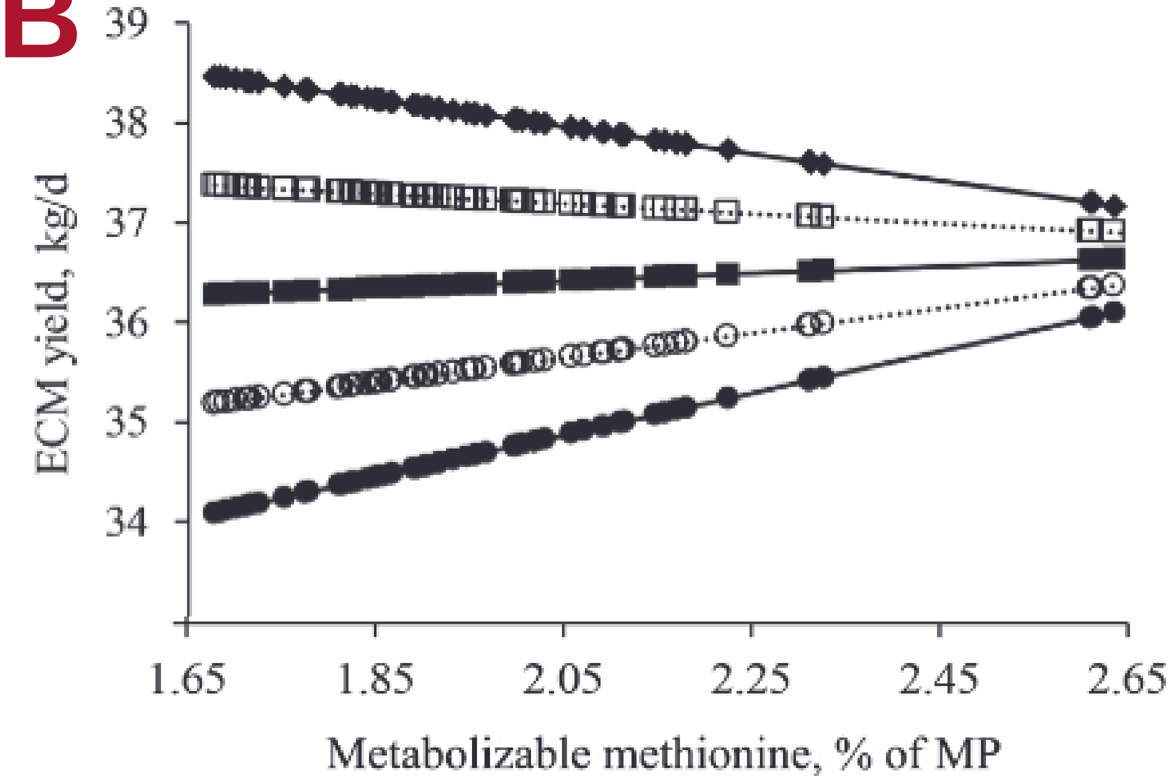

Arshad et al., 2020

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