Rumen Microbial Protein: More is **More**!

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What makes microbial protein “more”?

- Let’s start with what happens when “less”
  - Less microbial protein (MicP) = less cells = less microbial enzymes (proteins)
    - Probably not lower starch degradability, SI digestibility better anyway
    - Lower NDF degradabilility (NDFD) in rumen has less compensatory NDFD in the cecum and colon
- Lower DM intake from lower NDFD
  - Less NDFD = less energy for microbial cells
  - Lower DMI = lower everything for MCP supply
  - Lower MCP supply = lower DMI

Adobe Stock

What makes microbial protein (MicP) “more”?

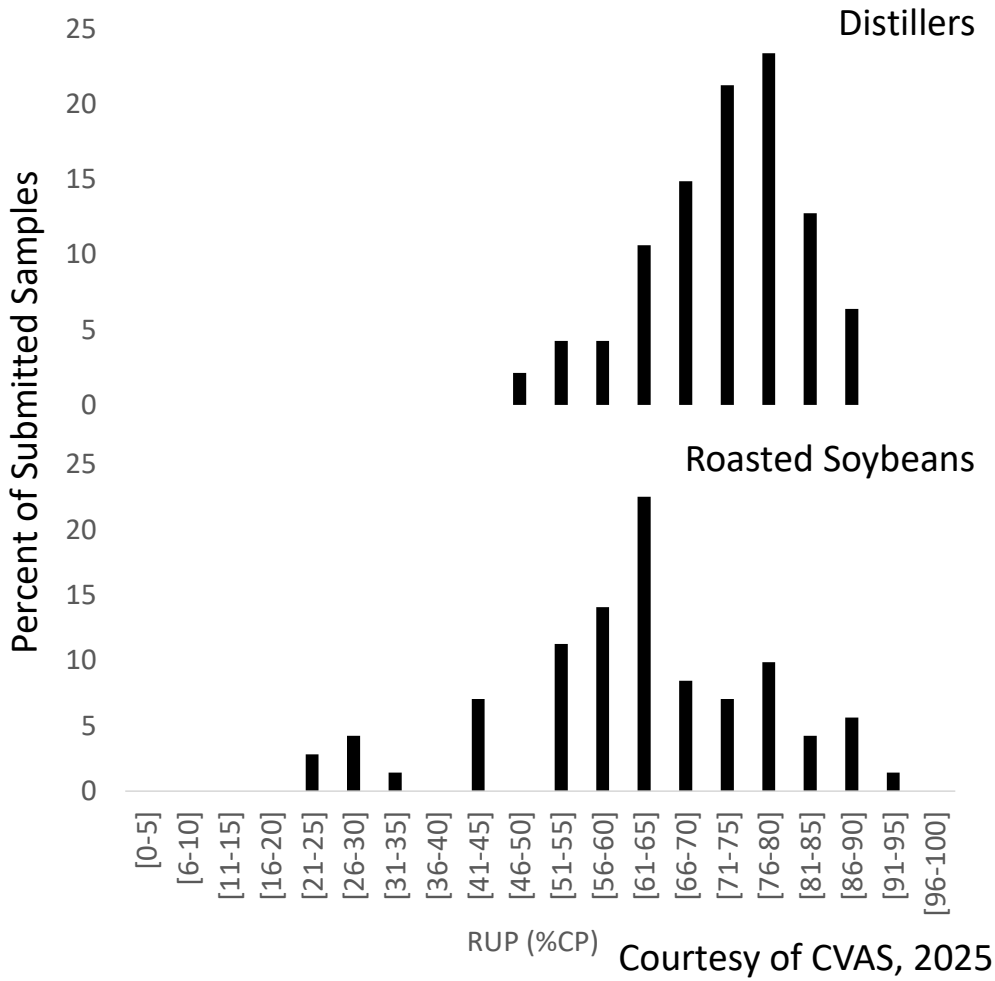
- More MicP is MORE because
  - Increased DMI = increased intake of EVERY nutrient
    - Not just from feed (RUP, FA, etc.)
    - Microbial starch: they are 10 to 15% glycogen ~ starch
    - Microbial lipids: PUFA, c9t11 CLA, bacterial iso and anteiso lipids
  - Cheapest source of protein
    - Even at 80 to 85% conversion efficiency of RDP to Microbial CP
    - Microbial protein is ~ 82.4% true protein x 80% digestible is comparable to feed proteins for metabolizability
  - Convert RDP with lesser quality to higher quality EAA profile
  - Optimizing MicP supply provide opportunity for
    - RP-His (low in microbes) if lower CP diets
    - Probably more consistent responses to RP-Lys, RP-Met

What else makes MicP "more"?

- When microbial protein supply is MORE
  - The rumen is probably more "healthy"
    - Starch and effective fiber must be appropriate to maximize MicP
  - Less N excretion relative to milk protein output
  - Models probably work better
    - More MicP supply
      - Makes cow more responsive to MP
      - RUP or RP-AA responses less likely to be hidden by factors suppressing MicP
    - At least for NASEM, the MP supply system is based on MEASURED MicP flows
      - Directly for measured MicP
      - Indirectly for measured non-ammonia-non-microbial N x 6.25 to adjust kp for RUP to have no mean bias against NANMCP
      - MP = MicP + RUP (partially based on MicP) after adjusting for intestinal digestibilities

Variability of %RUP for Distillers and Roasted Beans

- Distillers is variable and > NASEM's value
- Roasted beans display wider variation than other feedstuffs
  - Different roasters on farms?
- Microbial Protein is constant
  - 82.4% TP of 80% intestinal digestibility
    - Cell walls more resistant
- High RUP feeds risk underheating vs heat damage and low feeding value for the cow
- Extra RDP for MicP could be wasteful but not negative (Roy et al., JDS 109:9409)



Courtesy of CVAS, 2025

Slide adapted from B.A. Wenner

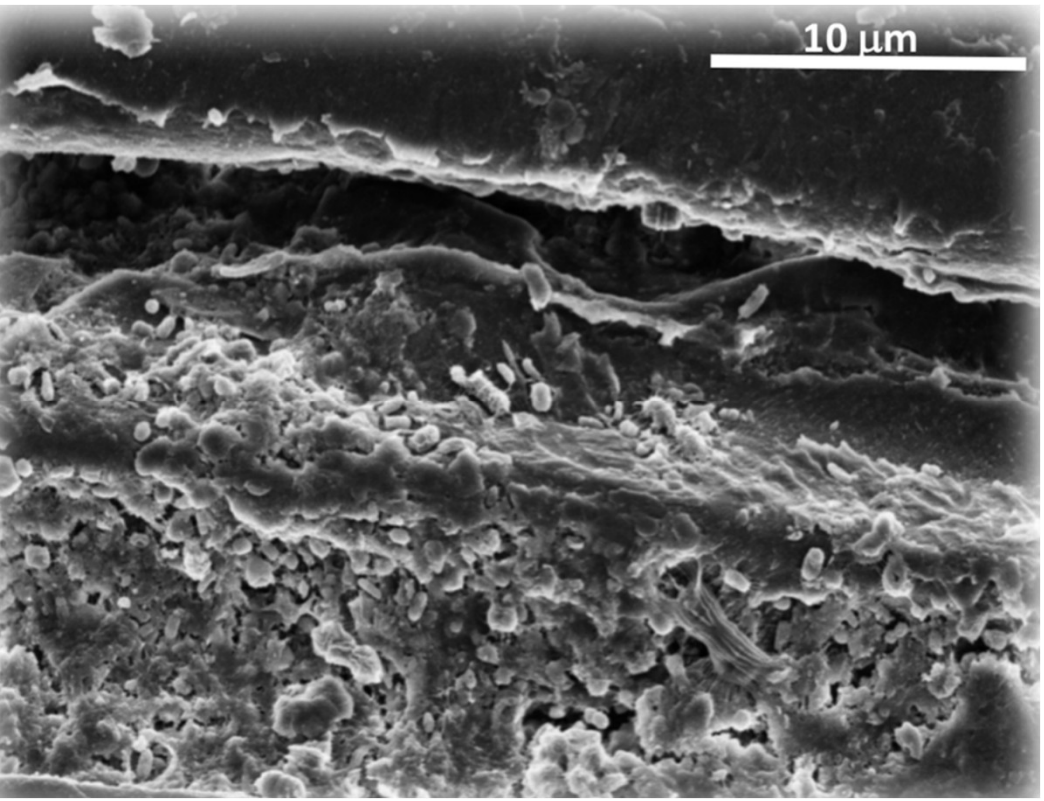
Waterman Dairy Complex: Rumen microbial protein production is complex too

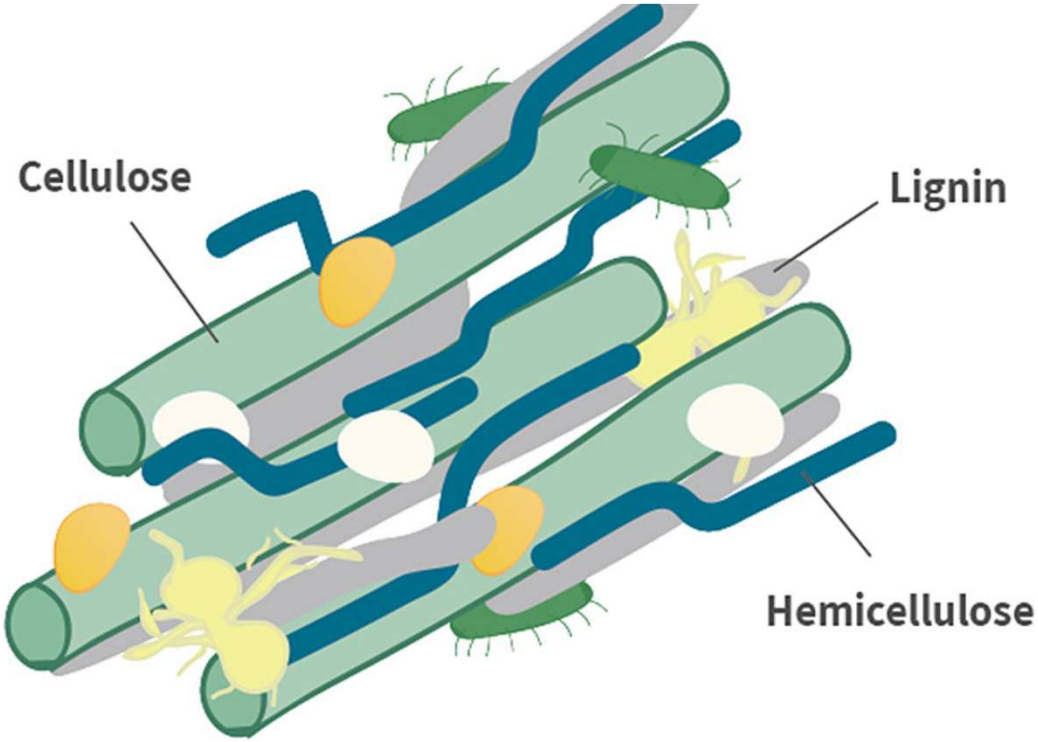
Rumen microbial protein

- Metagenomics only explains part
- Using technology with animal responses

What if too many cows/robot? Cow comfort?
What if robotic feeding still doesn't prevent sorting?
Robot more important than heifer development?
Etc. etc.

Dairy cow's rumen is packed with feed: 10^{10} to 10^{11} cells per gram, but stratified within rumen





Forage particles that provide effective fiber also are colonized by adherent specialist fibrolytics
Morais and Mizrahi (doi: 10.1093/femsre/fuz007)

Microbes in the rumen (Microbial Protein)

- Protozoa
  - Large entodiniomorphids very active with fiber degradation
  - Small entodiniomorphids more predatory to bacteria, most hardy and abundant
  - Isotrichids quench oxygen and produce lots glycogen but recycle in the rumen
- Bacteria
  - Core (almost all animals have these) bacteria that are mostly functional
  - Generalists that can be very functional or can compete with more functional ones
- Archaea
  - Methanogen patterns in high vs low methane emitters
  - Inhibiting them often yields higher emitted hydrogen (still trying to figure why so much)
- Fungi
  - Probably fewer in dairy fed high quality forage compared with beef
- Viruses
  - Diverse and sometimes specifically targeting certain microbial hosts

Microbes in the rumen

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- Bacteria
  - Core (almost all animals have these) bacteria that are mostly functional
  - Generalists that can be very functional versus those that are redundant
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  - Methanogens divide into groups that are in high vs low methane emitters
  - Inhibiting them often yields higher emitted hydrogen (still trying to figure why so much)
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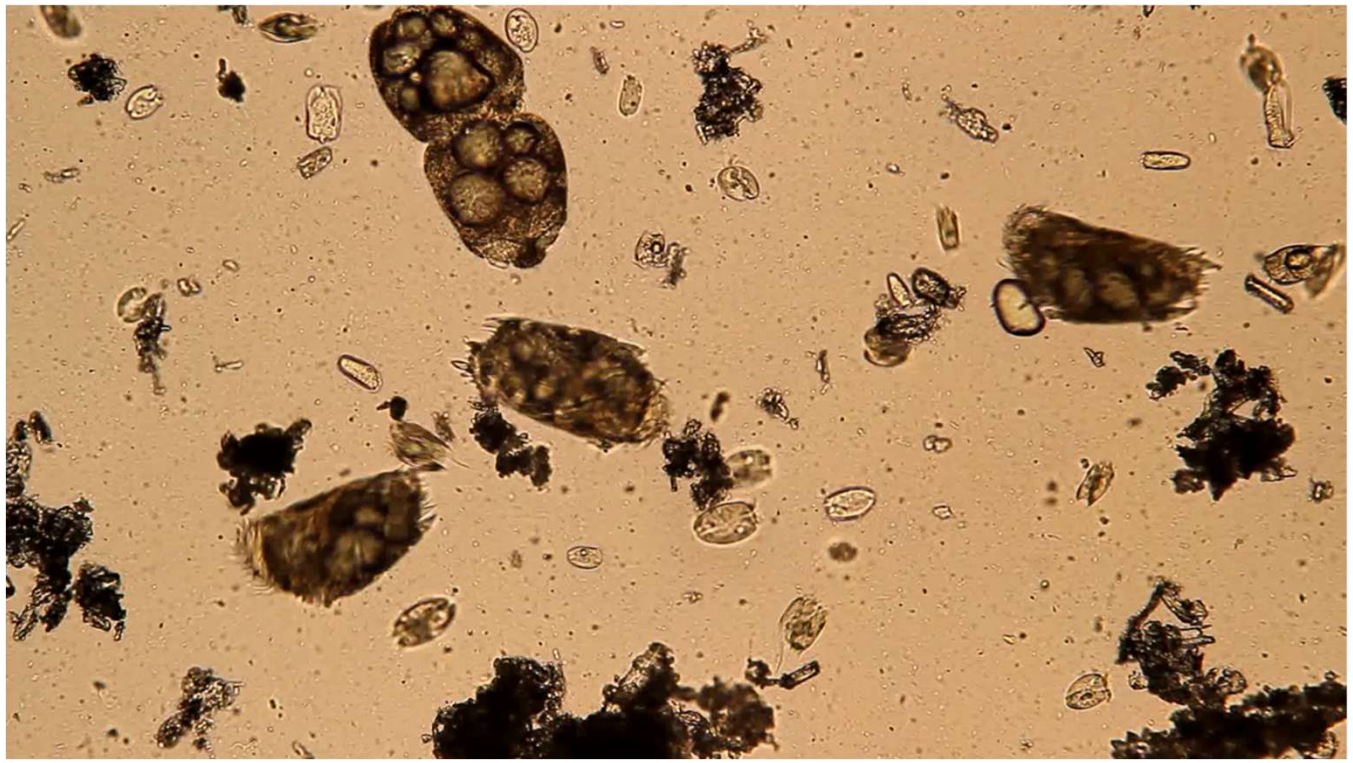
Most efficient rumen microbial consortia compete for substrate but also rely on cross-feeding

- Cellulolytics form a base
  - Very effective at hydrolyzing crystalline cellulose
  - Highly specialized cellulase factories with supportive proteins to gird together enzymes and bind tightly to cellulose
  - Effective at cleaving hemicellulose, even though they make poor use of the resultant sugars for fermentation/ATP synthesis
  - Not proteolytic, many cannot make their own BCAA (need BCVFA from below)
- Hemicellulolytics and pectinolytics
  - Adhere and often interact with cellulolytics
  - Many have proteolytic activity
- Amylolytics also tend to be proteolytic
- If we ↓ RDP, proteolytics do not share AA (etc.) with cellulolytics

Microbes in the rumen

- Protozoan dividing
- Small entodiniomorphids
- Dots are bacteria

- In their “real world”, feed particles are packed



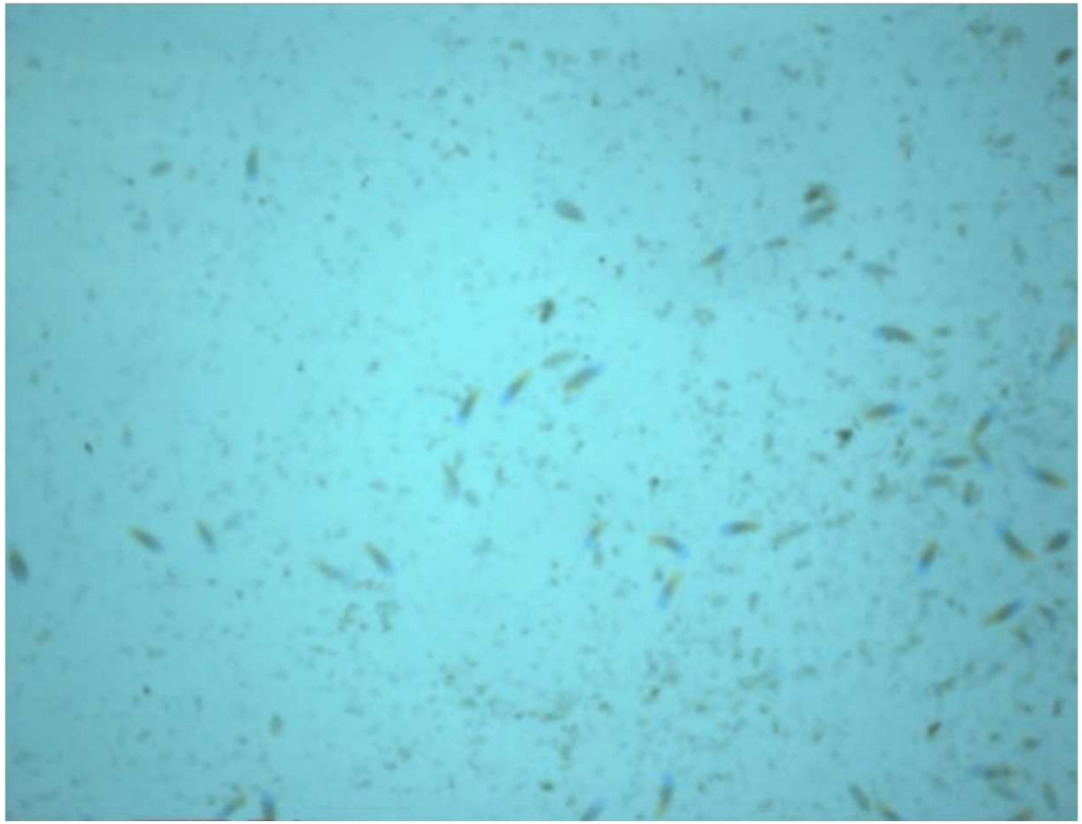
Defaunation: hitting a nail with a sledge hammer

- Dozens of studies with defaunated animals (protozoa completely removed)
  - Up to 30% increase in microbial protein (Newbold et al., 2015 doi: 10.3389/fmicb.2015.01313)
    - But NDFD suppressed, and other drawbacks (Firkins et al., 2020; doi: 10.3389/fmicb.2020.00123)
  - Most defaunation studies with sheep at very low DMI
    - Defaunation less likely increase MicP with dairy cattle at high DMI
  - Small entodiniomorphids are most predatory to bacteria
    - Protozoal abundance and recycling decreases with increasing DMI
    - Protozoa all prefer CHO for energy (small entos use starch), not bacteria
  - Larger entodiniomorphids major fiber degraders (meta-gen/proteomics)
  - If we could selectively target isotrichids, that would be key
    - They partially sequester in the rumen, don't degrade fiber, can use sugars though

Despite dozens/hundreds of studies to kill off protozoa, they don't want to die

Sample with fast-swimming isotrichids

- Inhibitor added to left, diffusing toward center
- Although no barrier, isotrichids sense inhibiting conditions and swim away from that gradient
- Test tubes allow no "away"
- "Away" in the rumen is ventral ↓



Plant bioactives

- Most are in vitro (Patra, Ramon-Morales, Khiaosa-ard, Belanche, etc. reviews)
  - Potential adaptation by protozoa, bacterial degradation of PB's
- Some inhibitors do suppress protozoa in some situations
  - More consistency is needed through research
- Multi-purpose roles by plant bioactives
  - Do they potentially inhibit clostridial AA fermenters? Methanogens?
  - Combined effects of different products on different microbes?
- One size does not fit all: plant bioactives documented the larger role of isotrichids for methanogenesis (Dai et al., 2022; doi: 10.3168/jds.2021-21139)
  - But as of yet, no selective inhibitor against isotrichids
  - Inhibition of entodiniomorphids could ↓ NDFD, which would likely ↓ MicP

Amino Acid of Mixed Microbes

AA, % of total AA	NorFor	Gresner	Sok (NASEM)
Arg	5.1	4.9	4.7
His	2.4	1.9	1.9
Ile	5.9	5.5	5.7
Leu	7.9	7.7	7.9
Lys	7.4	8.0	8.1
Met	2.5	2.3	2.3
Phe	5.7	5.4	5.4
Thr	5.3	5.3	5.3
Trp	1.6	1.5	1.2
Val	5.9	5.6	5.7
Protozoa, % Total	10	15	16.5

Gresner et al. (2021; doi: 10.1111/jpn.13676)

Protozoal N is 16.5% of microbial N in NASEM was derived before these publications

- Ahvenjarvi et al. (2018; doi: 10.3168/jds.2016-12316)
  - 25% of microbial N flow
- Fessenden et al. (2019; doi: 10.3168/jds.2018-15448)
  - 17% of microbial N, 21% of microbial AA
- Dineen et al. (2021; doi: 10.3168/jds.2020-19203)
  - 20-24% of microbial N, 23% of microbial EAA flow
- Assuming no protozoal outflow because of partial sequestration of isotrichids is unrealistic (Firkins et al. 2020; 10.3389/fmicb.2020.00123)

History: Protozoal Outflow and Lysine

- Jouany (1996) using mostly DAP (protozoa by difference) > 16.5% avg
  - DAP overestimates bacterial protein (i.e., could underestimate protozoal N) because of passage of “ghost” cell walls (Broderick, 1992)
  - They discuss protozoa higher in Lys and lower in Ala than bacteria
  - Numerous papers since the 1960’s suggest higher Lys in protozoa, and most of these used gravitation to separate protozoa (heavily contaminated w bacteria)
    - Concentration of bacterial cell walls and bacterial DAP conversion to Lys
    - High Lys proteins in protozoa (e.g., ubiquitin itself high in Lys targeting Lys residues on proteins)?

Jouany, doi: 10.1093/jn/126.suppl_4.1335S
Broderick, doi: 10.3168/jds.S0022-0302(92)78024-2

Fessenden et al. (2017; JDS 100:7211)

Table 3. Amino acid profile (% of total AA) of microbial isolates using a 24-h hydrolysis time

	Bacteria AA[1]				Protozoa AA[1]			
Item	A	B	C	D	A	B	C	D
EAA								
Arg	5.4	5.0	5.0	—	5.5	5.4	5.4	4.7
His	2.1	2.1	2.0	—	2.9	2.5	2.3	2.0
Ile	5.0	4.0	4.9	—	4.7	3.8	5.7	5.5
Leu	4.8	5.6	6.8	—	5.5	6.1	4.6	4.2
Lys	4.7	7.5	4.8	—	5.7	8.8	5.3	10.2
Met	3.3	4.5	2.6	—	2.7	3.1	2.1	3.8
Phe	6.6	6.0	6.8	—	7.4	6.5	7.3	7.6
Thr	6.3	5.7	5.4	—	5.4	4.8	6.1	4.7
Trp	5.7	5.5	5.3	—	4.6	4.5	3.1	1.4
Val	6.7	5.9	5.6	—	5.7	4.7	4.7	4.7
Total EAA	50.8	51.9	49.3	—	50.1	50.2	46.5	48.8

Or are the processes to separate these fractions increasing Lys from bacterial cell walls in bacteria and those consumed by protozoa?

Trial D from T. Hackmann:

- Cleanest protozoal prep to date
- Greater contamination with bacteria would dilute Lys differential

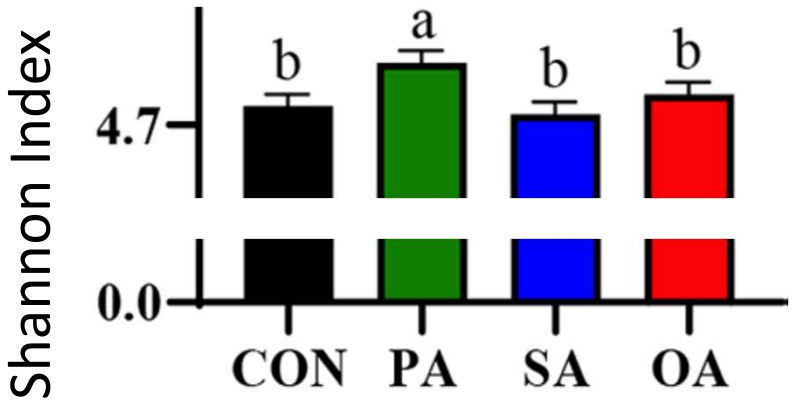
Supplemental fat and microbial protein

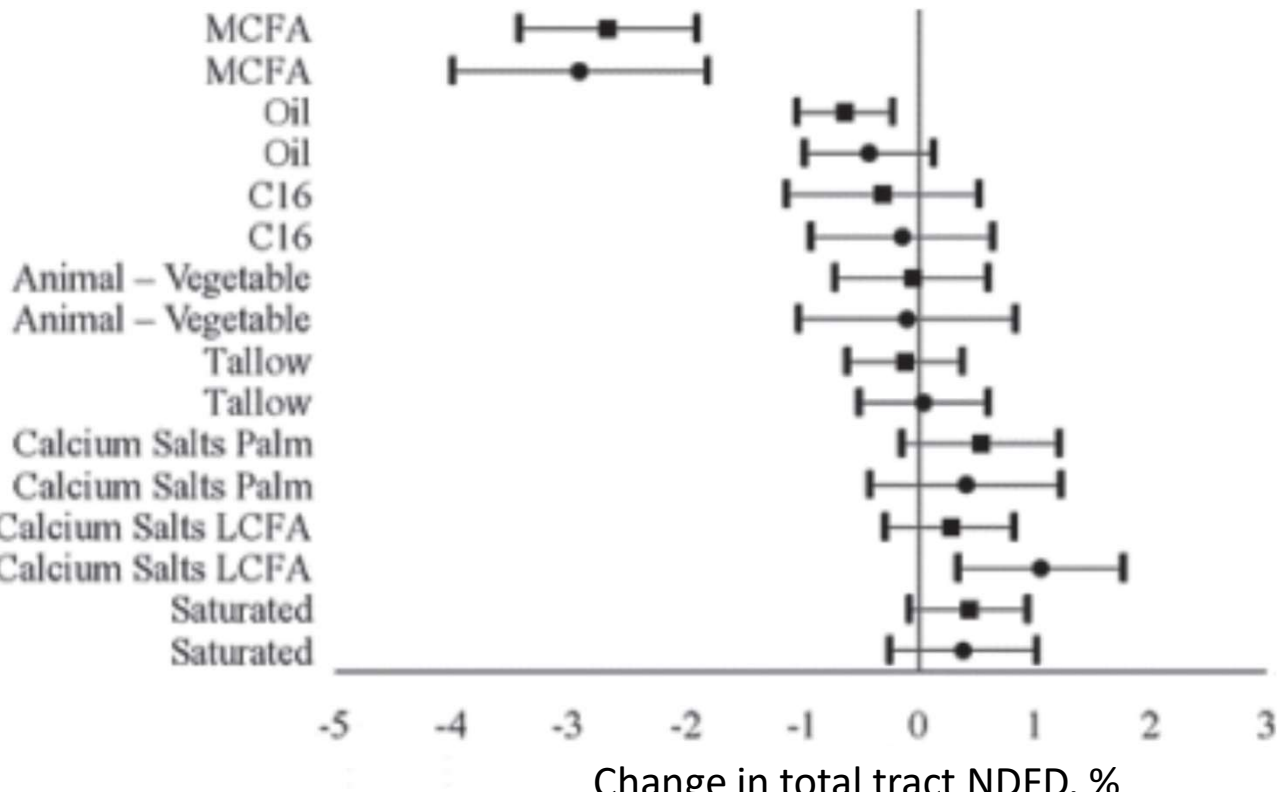
- Some work showing an optimal blend of PUFA inhibits protozoa
  - Objectives more to suppress methanogenesis, uncertain about MicP
  - But overdosing can ↓ DMI (probably more likely than ↓ NDFD)
  - Don't want excess suppression: PUFA → protozoal membranes and pass to SI
- MCFA toxic to protozoa but can ↓NDFD and ↓DMI, don't recommend
- Palmitic (16:0) and oleic (18:1) can actually improve NDFD and MicP
  - Probably not through protozoal inhibition
  - Results 16:0 > 18:1, but probably need both (16:0 poorly digestible wo 18:1)
  - Incorporation into membranes to spare carbon for other cellular purposes
  - ↑ NDFD compensates for less dietary CHO (main energy source for microbes)

1.5% FA and bacterial N outflow
(Sears et al., 2024; doi: 10.3168/jds.2023-23568)

	Control	Palmitic	Stearic	Oleic
NDFD	b	+6% unit[a]	b	-8% unit[c]
Bacterial N, g/d	0.771[b]	0.851[a]	0.758[b]	0.785[b]
Bact N, g/kg NDFD	82.7	80.8	81.2	91.4
RDP, % CP	55.8[b]	60.1[a]	56.8[b]	55.8[b]

- Palmitic increased NDFD and bacterial N and Shannon index (richness and evenness)
- Oleic (probably too high) decreased NDFD but not bacterial N
- **Oleic from soybeans released slowly (not negative)**





Weld and Armentano (2017; doi: 10.3168/jds.2016-11500)…included 16:0 in triglyceride form.

Vegetable oil had 3.7% unit increase in ruminal NDFD (not shown here)

Ca salts are free FA

dos Santos Neto et al. (2021; doi: 10.3168/jds.2021-20699) included only free FA sources.
Palm FA ↑ NDFD by 4.5% unit

Item	Control	MIX	PALM
DMI, kg/d	24.8	24.8	24.9
Nutrient digestibility, %			
DM	64.1[b]	64.9[ab]	65.7[a]
NDF	43.3[b]	44.3[b]	47.8[a]
Total FA[5]	75.9[a]	70.7[b]	73.9[ab]

Does increasing starch increase MCP?

Yes, but probably not as much as you think

Sauvant and Nozière (2016; doi: 10.1017/S1751731115002670)

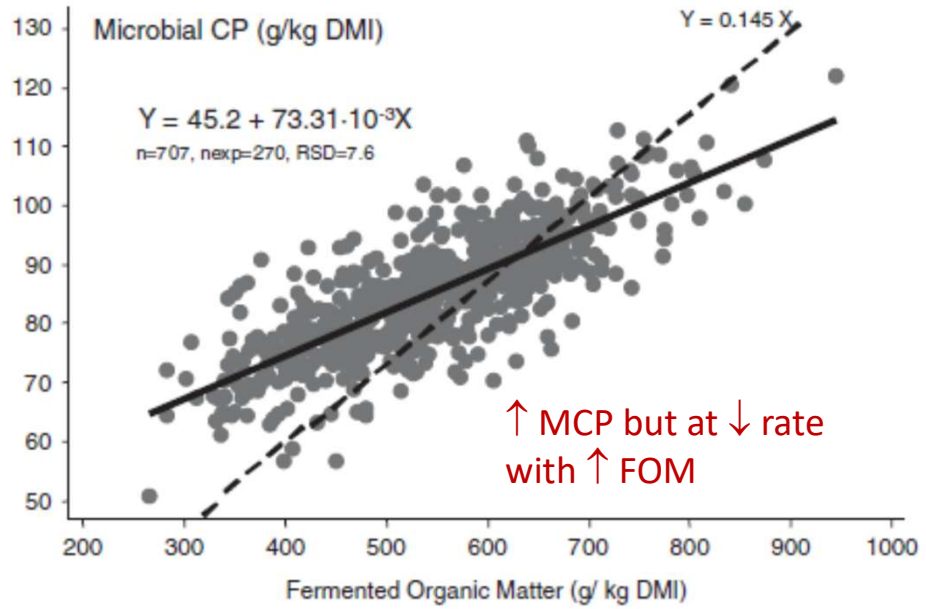


Figure 6 Intra-experiment relationship between microbial CP production and the fermented OM in the rumen.

FCP ~ RDP

resOMtDR ~ NFC truly degraded

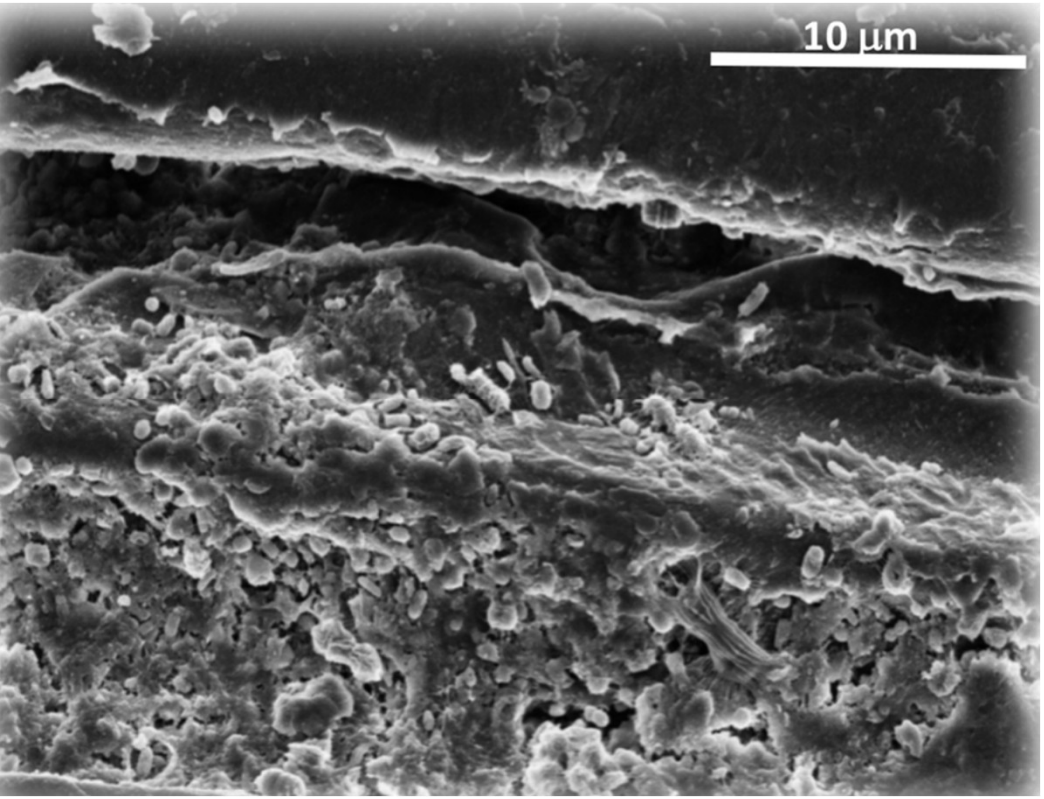
$$\text{MicCP} = 38.7 + 0.23\,\text{FCP} + 55.3 \times 10^{-3}\,\text{resOMtDR} + 42.7 \times 10^{-3}\,\boxed{\text{NDFduo}}$$

$$(n = 446,\ n\,\text{exp} = 174,\ \text{RSD} = 6.5) \qquad (38)$$

This relationship, based on three few related criteria, reflects the respective roles of the dietary components that support microbial growth (FCP and resOMtDR) and those that allow outflow of rumen microbes from the rumen to the duodenum (NDFduo).

NDFduo (passed NDF) replaced ruminally degraded NDF

Worth another view



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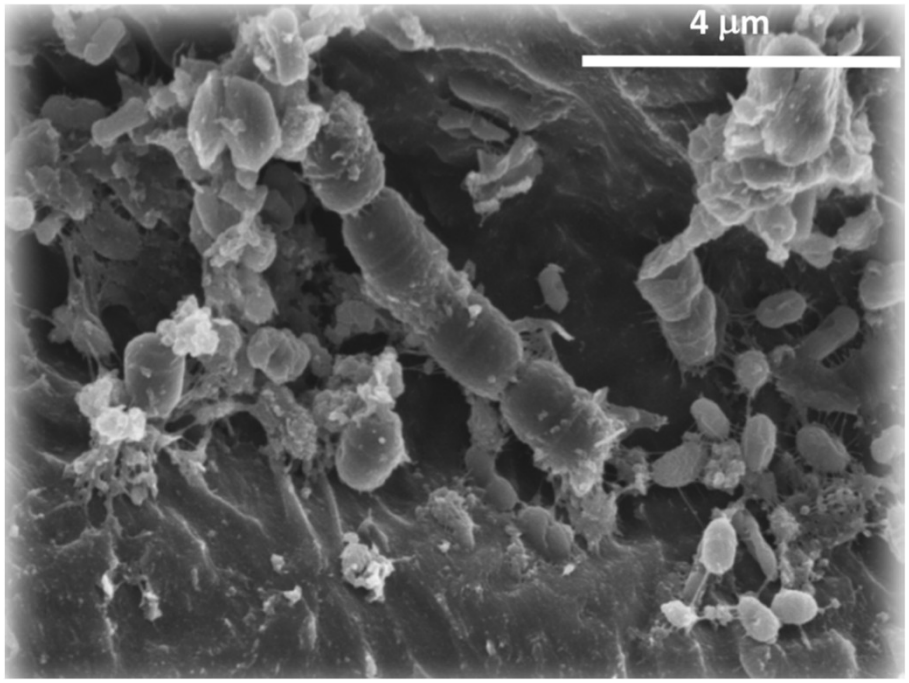


Forage particles that provide effective fiber also are colonized by adherent specialist fibrolytics that pass with the UNDEGRADED particles

Grain processing has modest effect on MicP

Table 8. Least squares means of ruminal digestibilities (% of intake) and duodenal flow of microbial N (g/d) from lactating cows fed different grain sources[a]

	Starch, apparent			NDF			OM, true			Microbial N		
Grain	n[b]	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE
Corn												
Dry, cracked or rolled	6	44.6	4.6	6	48.1	3.3	9	52.3	2.5	10	276	14
Dry, ground	77	52.3	2.1	52	44.9	2.2	69	48.6	1.6	73	257	10
Steam-flaked	9	56.9	4.4	9	41.9	3.1	6	52.8	2.7	4	296	14
High-moisture	12	86.8	8.6	2	47.1	6.2	12	60.1	4.5	12	236	26
Sorghum												
Dry, rolled or ground	4	48.1	6.8	4	49.6	7.1	4	49.2	5.0	4	278	38
Steam-flaked	7	74.0	5.9	7	43.9	7.3	3	56.3	4.7	6	357	34
Barley												
Dry- or steam-rolled	15	71.2	2.8	15	37.6	3.1	15	56.2	2.3	11	299	13
Steam-rolled, hull-less	3	67.9	5.2	3	36.8	3.6	3	56.4	2.9	3	274	17

[a]All least squares means were adjusted for the random effect of experiment and for the mean of all continuous variables remaining in the final model (See Table 9).
[b]Number of treatment means.

Firkins et al. (2001; doi: 10.2527/jas2001.79E-SupplE218x

Increasing ruminal starch degradability

Inconsistent increase in microbial protein synthesis (MPS)
i.e., = were 2x the ↑

Note inconsistent ammonia decrease (↓) with MPS increase (↑)

Hristov and Jouany (2005)
Doi: 10.1017/S0021859608007697

Starch source	Ammonia	MPS
Roasting of barley	N/R[b]	N/R
SRB[c] vs. GSC	↓	=
Tempering of barley	N/R	N/R
Barley vs. maize	=	↑
Expanded vs. cracked maize	↓	=
Ground vs. rolled maize or dry vs. HMC[c]	=	=
SFC vs. TRC[c]	↑	=
Barley vs. maize	N/R	↑
Barley vs. maize	↓	=
Barley vs. maize	=	↑
Processing of barley	↓	=
Barley vs. maize	=	N/R
Barley vs. maize	N/R	N/R
Maize particle size	↓	=
SRC vs. CGC[c]	↓	=
Processing of barley	=	N/R
Processing of barley	=	(↑)[‡]
HMEC vs. CSC[c]	↓	N/R
SFC[c] vs. ground maize	↓	↑
Barley[d] vs. maize	↑	=[d]

Does increasing starch availability increase MicP consistently in dairy studies (e.g., MSU, OSU)?

- Depends on the study, not as consistent as you think
  - If starch degradability (StarchD) > NDFD, why not more starch = more MicP???
    - ↑ StarchD usually ↓ NDFD
    - ↑ StarchD usually ↓ efficiency of microbial protein synthesis (EMPS)
  - Are models covering this ground?
    - Not as well as could, but nor has the research: less can't make more
    - Lab in vitro starch or NDF are doing one at a time…
      and won't capture negative assoc effects
      - Hard to capture these in vitro anyway: less can't make more

Increasing Starch Degradability of Corn Grain (B. Wenner)

Normalized to Base Diet

Increasing starch degradability

- Increases NFC Bacteria
- No effect on FC Bacteria
- Little effect on NDFD unless model predicts low rumen pH
  - Little consideration to "carbohydrate effect"

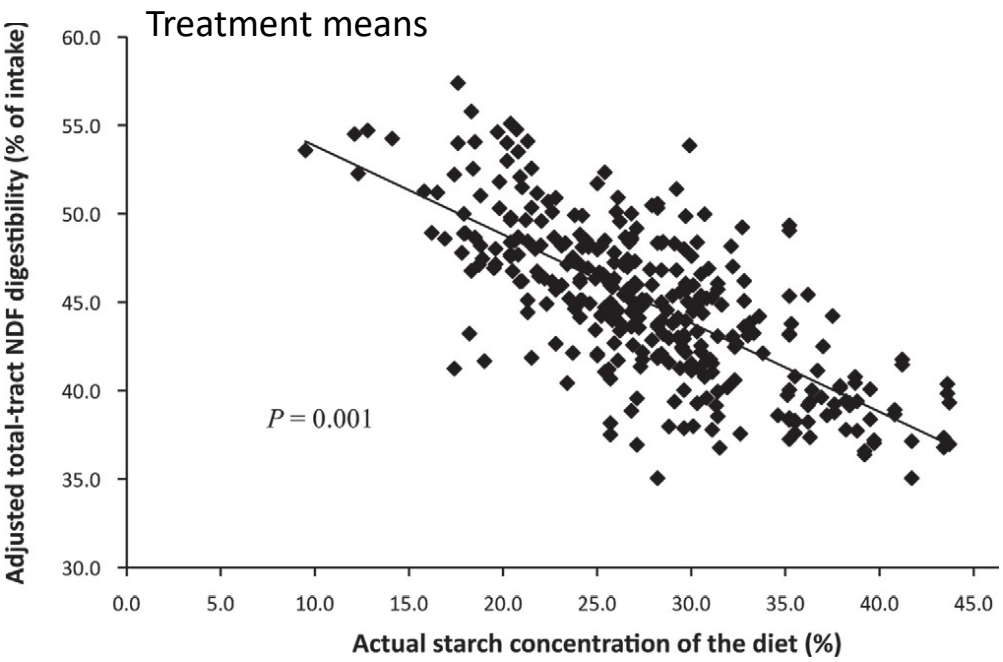
5% improvement in rumen starchD

- Saves 5-8 cents in MP intake

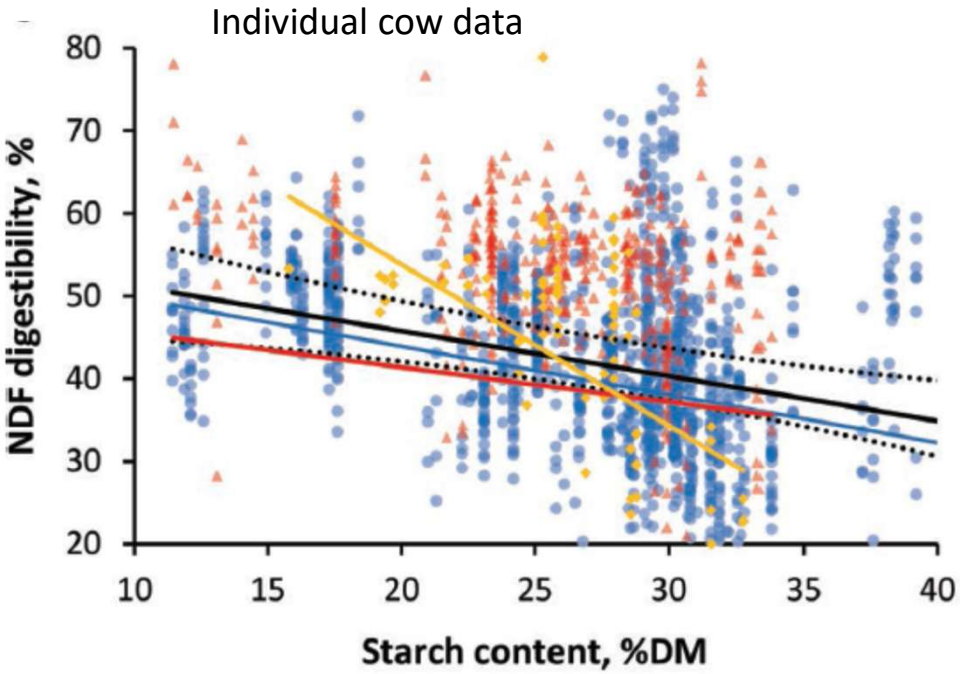
But is it real?

- ↑ Starch should penalize NDFD (therefore FC bacteria production)
- ↓ microbial efficiency (pay more attention to peptides)

Each 1% unit increase in starch decreases <u>total tract</u> NDFD by



<u>0.48% unit</u> (Ferraretto et al., 2013; doi: 10.3168/jds.2012-5932)



<u>0.59% unit</u>; NASEM discount only 30% of NRC (2001) (de Souza et al., 2018; doi: 10.3168/jds.2017-13344)

Meta-Data from Studies with Dairy Cattle

- Rumen-degraded NDF (g/d) was **75%** of rumen-degraded starch (g/d) (Roman-Garcia et al., 2016)
  - Degraded and undegraded NDF important for microbial protein

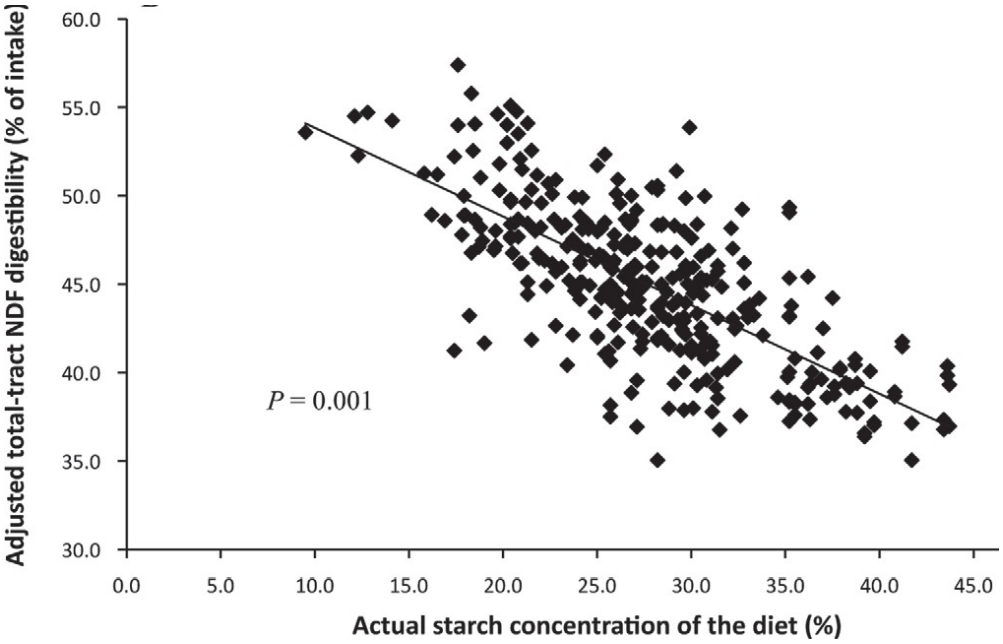
- Total tract digestibility of NDF vs starch (de Souza et al., 2018)
  - 40, 49, 44 % NDFD in MI, OH, GA (**44.3%** avg across 3, not weighted n)
  - 94, 92, 90 % starchD in MI, OH, GA (**92.0%** avg, not weighted n)

  - 30, 34, 35% NDF in diet (avg. **33.0%** unweighted)
  - 27, 26, 26% starch in diet (avg **26.3%** unweighted)

$$\frac{0.443 \times 33.0}{0.920 \times 26.3} = 60.4\%$$

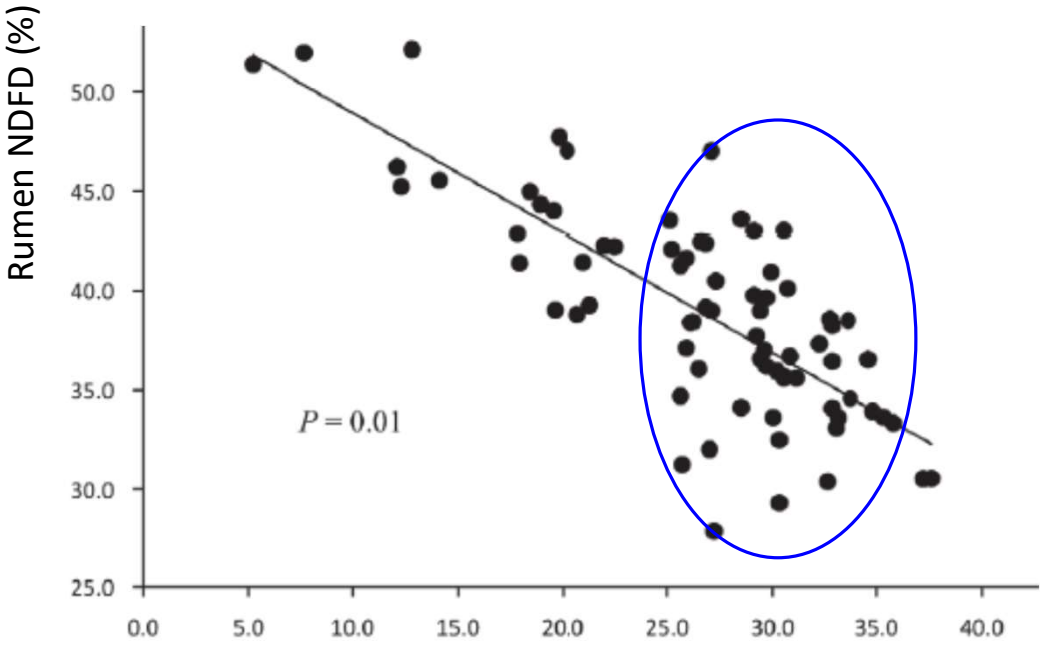
- **Summary: 60.4% of digested CHO from NDF**
  - **Every 1% unit ↑ in starch discounted 0.25% unit from ↓ NDFD**

Each 1% unit increase in starch decreases ruminal NDFD > total NDFD, and more dispersion



0.48% unit for total tract

(Ferraretto et al., 2013; doi: 10.3168/jds.2012-5932)



0.605% unit for ruminal

(Ferraretto et al., 2013; doi: 10.3168/jds.2012-5932)

Why does starch decrease microbial efficiency?

- Unlike cellulolytics (marathoners), amylolytics are sprinters
  - Fibrous particles have longer retention (mat dynamics) and slower kd, but soluble carbohydrates would have faster passage and faster kd
    - ↑ efficiency for cellulolytics vs ↑ growth rate for amylolytics
  - Cellulolytics compete for surface area, but amylolytics compete for substrate
    - Wasting ATP (↑ maintenance) from starch degradation isn't "waste" if they did the wasting and got more substrate vs competition
  - Excellent paper from Lingga et al. (2026; doi: 10.3168/jds.2025-27284)
    - Glucose vs cellulose as substrates (one or other) had similar EMPS
  - Instead, combined diets with higher starch are the reason for ↓ EMPS
  - Higher starch ↑ competition for common resources, more "selfish" consortia (Firkins et al., 2025; doi: 10.3168/jds.2024-25863)

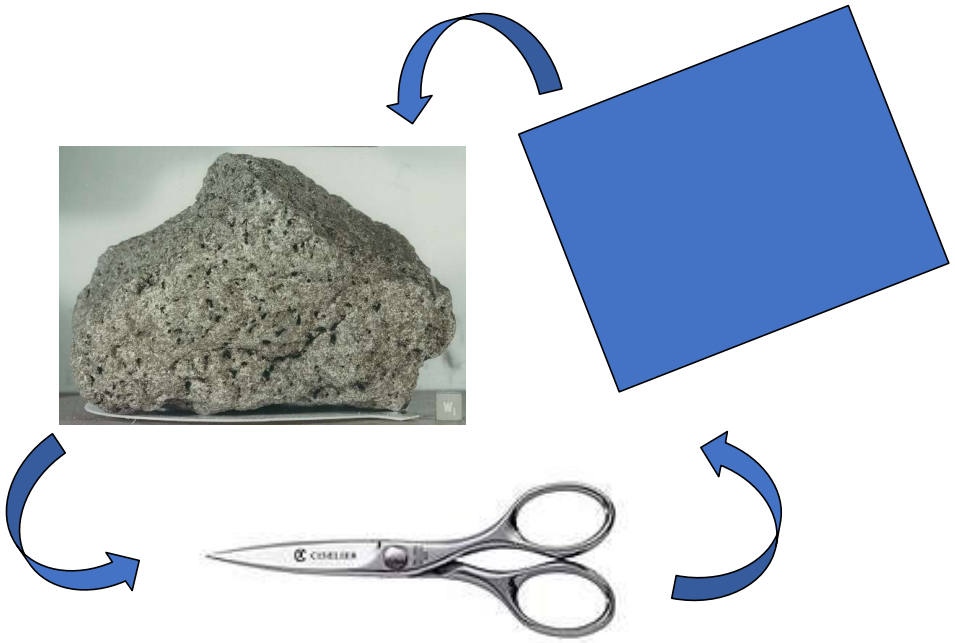
Reasons for Negative Associative Effects

(Firkins et al. 2025; doi: 10.3168/jds.2024-25863)

- Byproduct fiber to dilute starch lessens the negative effects of starch
  - But byproduct NDF has greater extent of NDFD and more risk for depressed degradation!
- Many papers on pH and NDFD
  - Cellulolytic cultures less able to adjust to intracellular pH
  - Li et al. (2021; doi: 10.1186/s42523-021-00092-6) supported negative role after directly adding acid into the rumen
  - Management can improve low pH response (buffers, forage particle size, additives, etc.)
- "Carbohydrate effect" independent of pH probably the same or greater magnitude of problem as low pH (unless SARA)
  - Challenge for management to improve what we don't know as much
  - Competition among cellulolytics and amylolytics for growth factors

Rock, Paper, Scissors for Fibrolytic Base

Moraïs and Mizrahi (2019; doi: 0.1093/femsre/fuz007)



Research: pH not too low (lactate not accumulate), forage quality, adequate RDP and other shared nutrient resources

"Rock, Paper, Chop!" Cartoon Daffy always wins

Analogous to acidosis challenge studies disrupting diversity

Reducing Corn Silage and adding BCVFA

Redoy et al., 2025; doi: 10.3168/jds.2024-25358

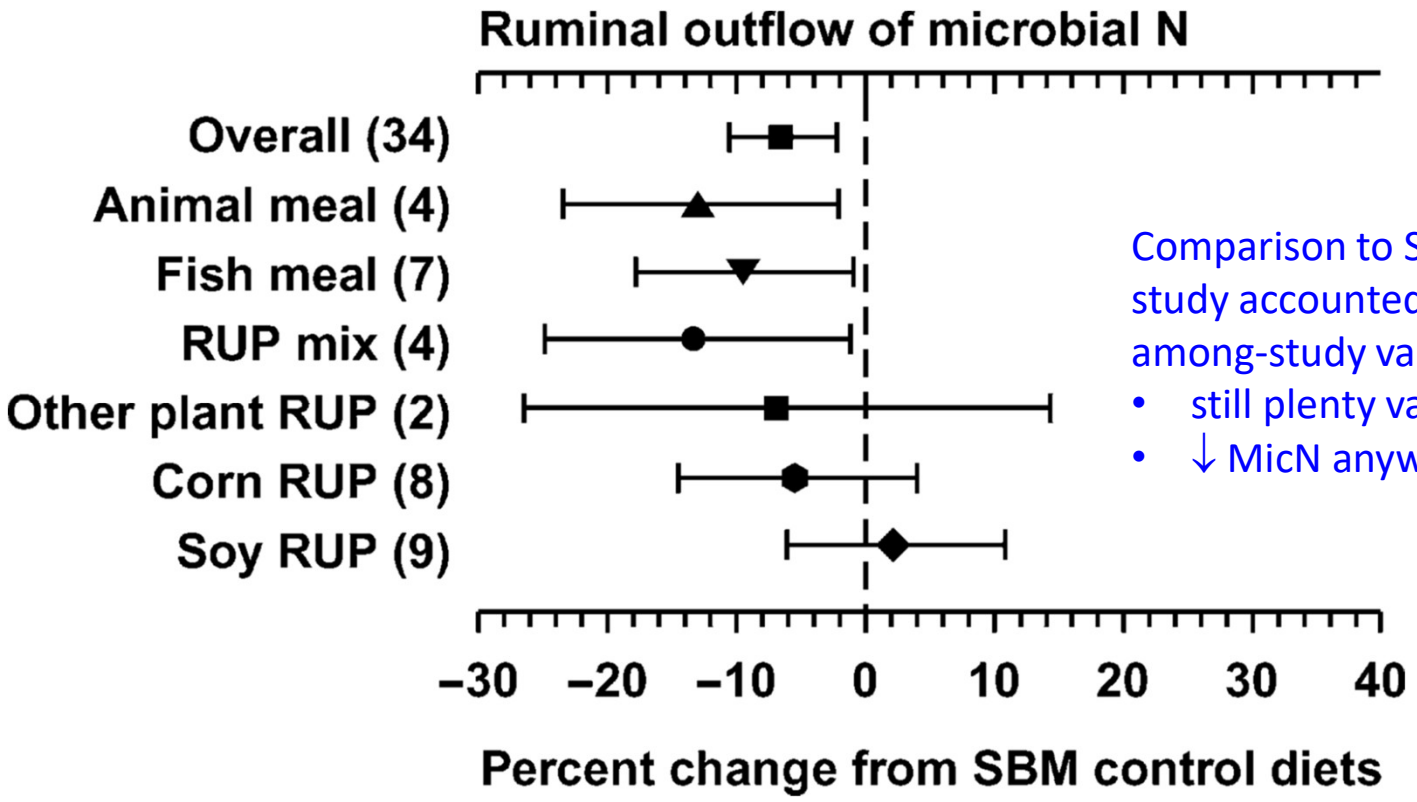
	Hi Forage		Low Forage	
DM basis	– BCVFA	+ BCVFA	– BCVFA	+ BCVFA
fNDF (excl WCS), %	20.7		16.6	
nfNDF, %	5.7		16.6	
Starch, %	32.4		30.7	
RDP, % (urea 0.5% CP)	10.3		10.5	
NEL, Mcal/kg	1.58		1.58	
DMI, kg/d[F]	25.7	26.2	27.5	28.1
ADG, kg/d[F, B, Inter]	0.64	0.69	0.93	1.33
ECM, kg/d[F, B, Inter]	39.0	41.9	42.8	42.2
ECM/DMI[F, Inter]	1.58	1.66	1.60	1.53
NDFD, %[F, B, Inter]	51.9	64.4	63.4	68.3

48 multiparous, 16 primiparous; 119 DIM; 2 wk covariate + 8 wk trial

Switching protein sources limited MicN

Ipharraguerre and Clark (2005)

Similar findings in other reviews by Stern et al. (1994) and Santos et al. (1998), who said high-RUP protein sources decreased MicP **76%** of the time



Comparison to SBM within study accounted for among-study variation

- still plenty variation
- ↓ MicN anyway

Clearly, MicP depends on adequate RDP, but what is adequate, and what components of RDP?

J. Dairy Sci. 100:10094–10112
https://doi.org/10.3168/jds.2017-13320

A 100-Year Review: **Protein and amino acid nutrition in dairy cows**[1]

Charles G. Schwab[*2,3] **and Glen A. Broderick**†
*Schwab Consulting LLC, 105 Doc Mac Drive, Boscobel, WI 53805
†Broderick Nutrition & Research LLC, 221 Glen Hollow Road, Madison, WI 53705

"Clearly, optimizing N efficiency in dairy production requires that sufficient RDP be provided to support microbial formation in the rumen."

"Clearly, adequate amounts of rumen-available N are needed to optimize rumen function and maximize synthesis of microbial protein."

Normalized (% of control w/o AA) by cellulolytic pure cultures has same or lower N derived from ammonia when given cellulose

	F. succinogenes		*R. flavefaciens*		*R. albus*	
	Microbial N	N from NH_3	Microbial N	N from NH_3	Microbial N	N from NH_3
Cellulose	214	44.4	281	50.6	103	38.6
Cellobiose	524	63.0	762	50.0	224	65.7

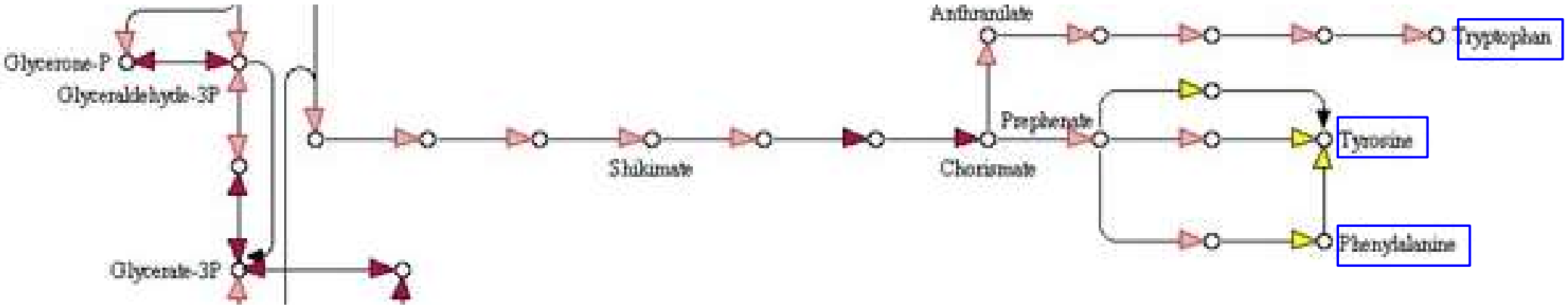
Cellulose was Avicel, which is crystalline and yields ~slow degradation (and growth) rate.

Phe (maybe Pro) synthesis limited growth on cellulose, especially *F. succinogenes*, but they added BCVFA

"While some of these conclusions might at first appear to conflict with accepted dogma, they are in fact fairly consistent with most published data and may help us to resolve some apparent contradictions which appear in the literature."

Atasoglu et al. (2001; 10.1128/AEM.67.6.2819–2822.2001)

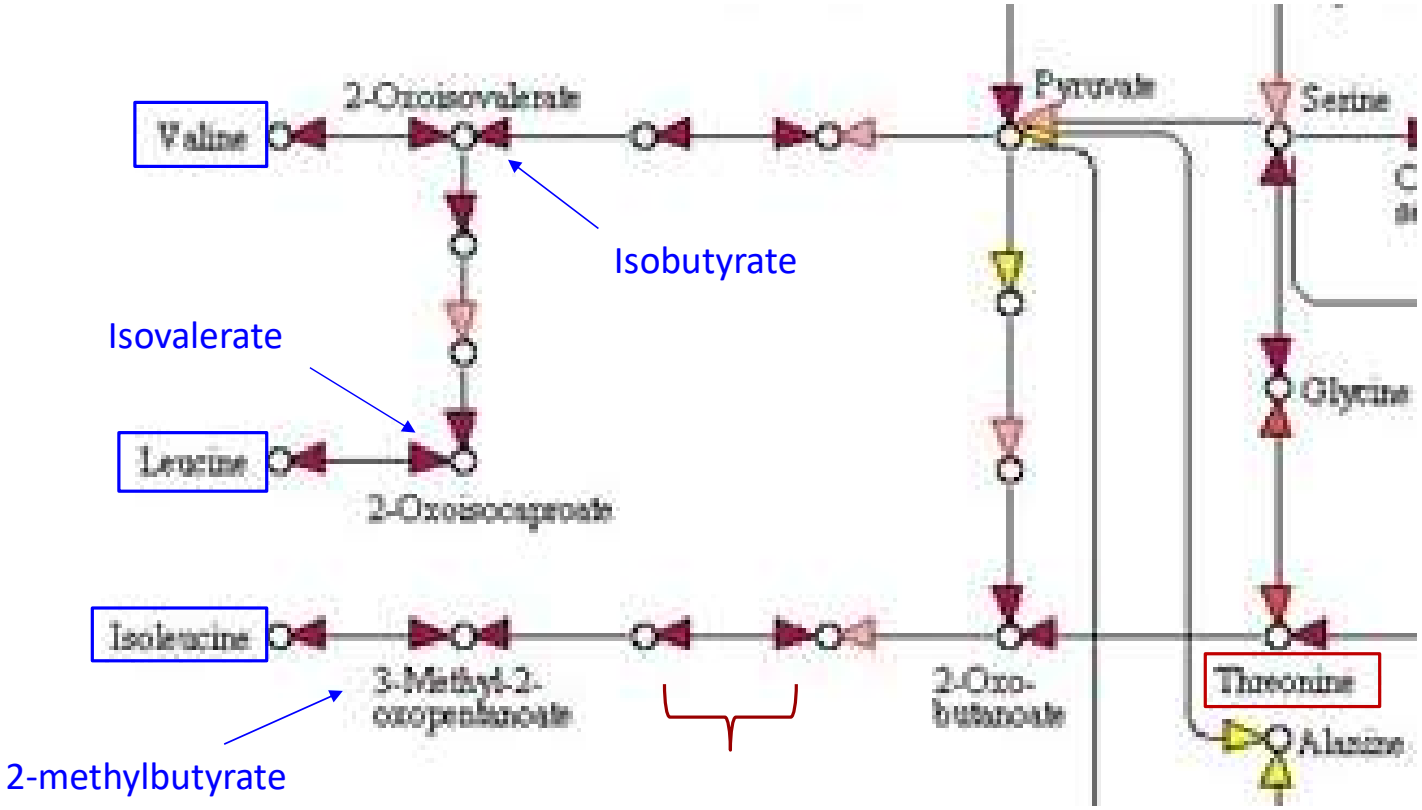
Aromatic AA might limit efficiency of microbial protein synthesis when CHO supply would support rapid growth



AA biosynthesis

- Relatively more complicated pathway than other AA
- Phe critical growth factor for *Ruminococcus*
  - Perhaps others?
- Each aromatic AA has allosteric feedback on its synthesis, but synthesis can limit growth unless preformed AA used

BCAA are growth factors for cellulolytics but perhaps also stimulate growth of many bacteria

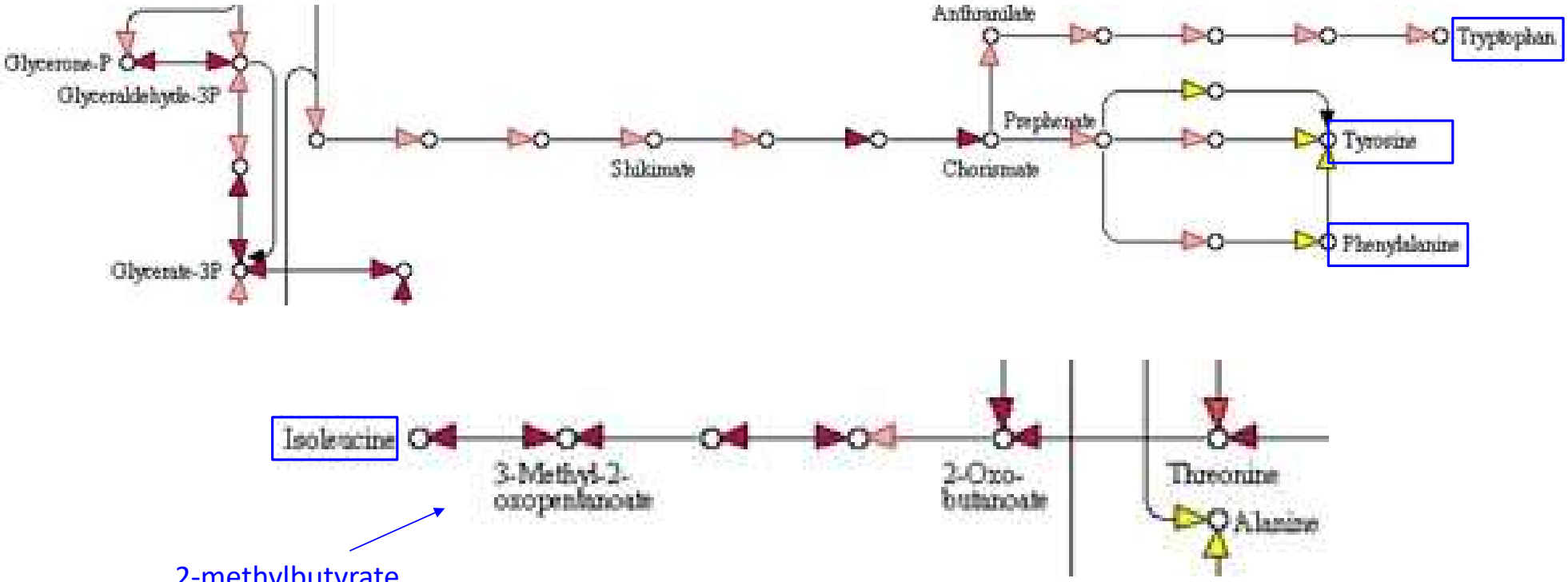


Fibrobacter and *Ruminococcus* often don't express 1 or 2 genes and therefore require BCVFA

BCVFA maybe better than BCAA, anyway, if bacteria don't express BCAA transporters

Thr is an intermediate for several other AA, critical for de novo Ile synthesis

Along with proline (from Glu but another ring), aromatics appear to work with 2MB/Ile (April White thesis)



BCVFA and Rumen-Degraded AA (not RDP)

- Our work with isoacids in continuous cultures
  - The BCAA, especially Ile, are critical
    - 2-methylbutyrate (2MB) is a precursor for Ile
    - Risk for BCAA imbalance if supplemented Ile instead of 2MB
  - Phe and aromatic AA seem to work with BCVFA
  - Pro seems limiting especially if RDP supply of other RD-AA are limiting
- $\geq$ 12% RDP provides enough natural BCVFA
- Need ~ 10% RDP but higher is less efficient
- < 9% RDP limits MicP and benefits from supp BCVFA
- Sweet spot 9 to 11% and depends on degradable starch and NDF
  - More RDP with more RDCHO, perhaps helps limit negative associative effects
  - As RDP, probably can use urea (0.2-0.3%) especially with corn silage-based forage

Preformed AA can improve EMPS

- Analogy: Many of you *could* make your own vitamin and mineral premixes
  - But you would take *space* and *time* to do it
  - More efficient (space, labor, time) to purchase premixes
- Most bacteria can make all AA but still transport preformed ones
  - Probably aromatics (Phe, Tyr, His) and proline take longer (closing rings)
  - BCAA, perhaps Met, synthesis de novo intersects other AA synth pathways
  - With ↑ CHO substrate, preformed AA allows faster and more consistent protein synthesis at ribosomes for faster growth
    - When growth is prioritized in bacteria, dilution of maintenance energy
    - Just like more energy to milk components dilutes maintenance for cows

RDP (85% of INRA) and qPCR

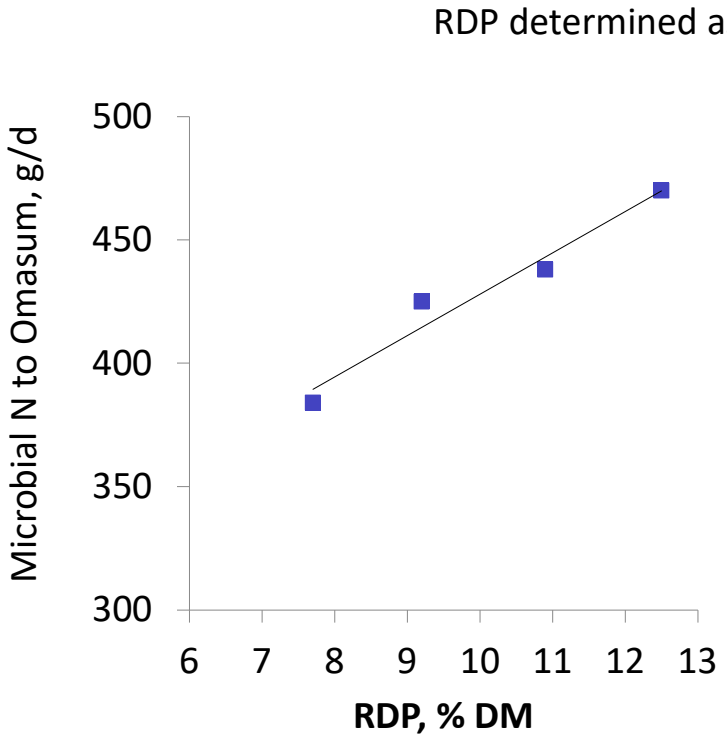
(Belanche et al., 2012; J. Nutr. 142:1684)

Limiting RDP:

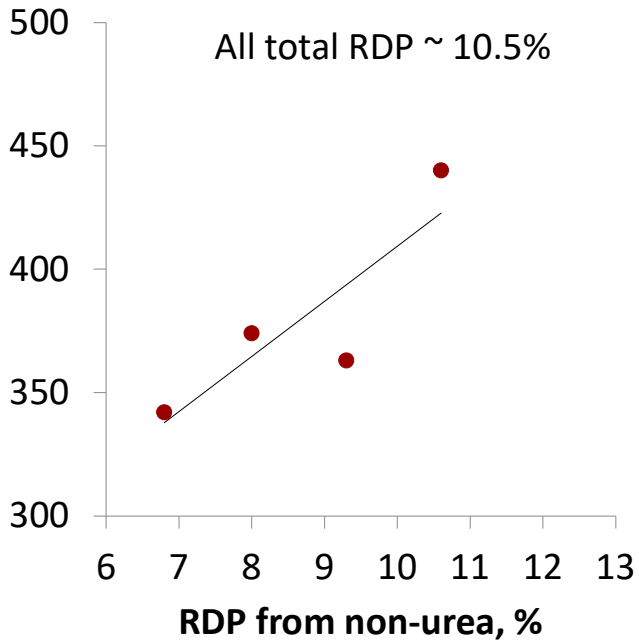
- Bacteria: -13%
- Archaea: -29%
- Protozoa: -19%
- Fungi: -28%
- Decreased bacterial richness especially when low RDP combined with higher starch: COMPREHENSIVE effect

We need more dairy studies on how RDP influences microbial structure, especially networks

Amino-N for Microbes, Not Just Ammonia (It's the carbon skeletons!)



Reynal & Broderick (2005; JDS 88:405)



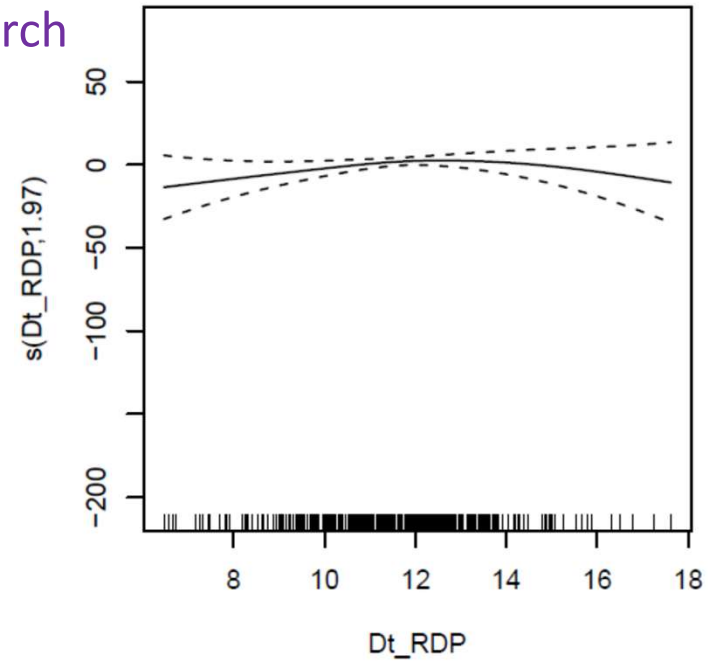
Broderick & Reynal (2009; JDS 82:2822)

Microbial N equation (Moraes et al., 2018)

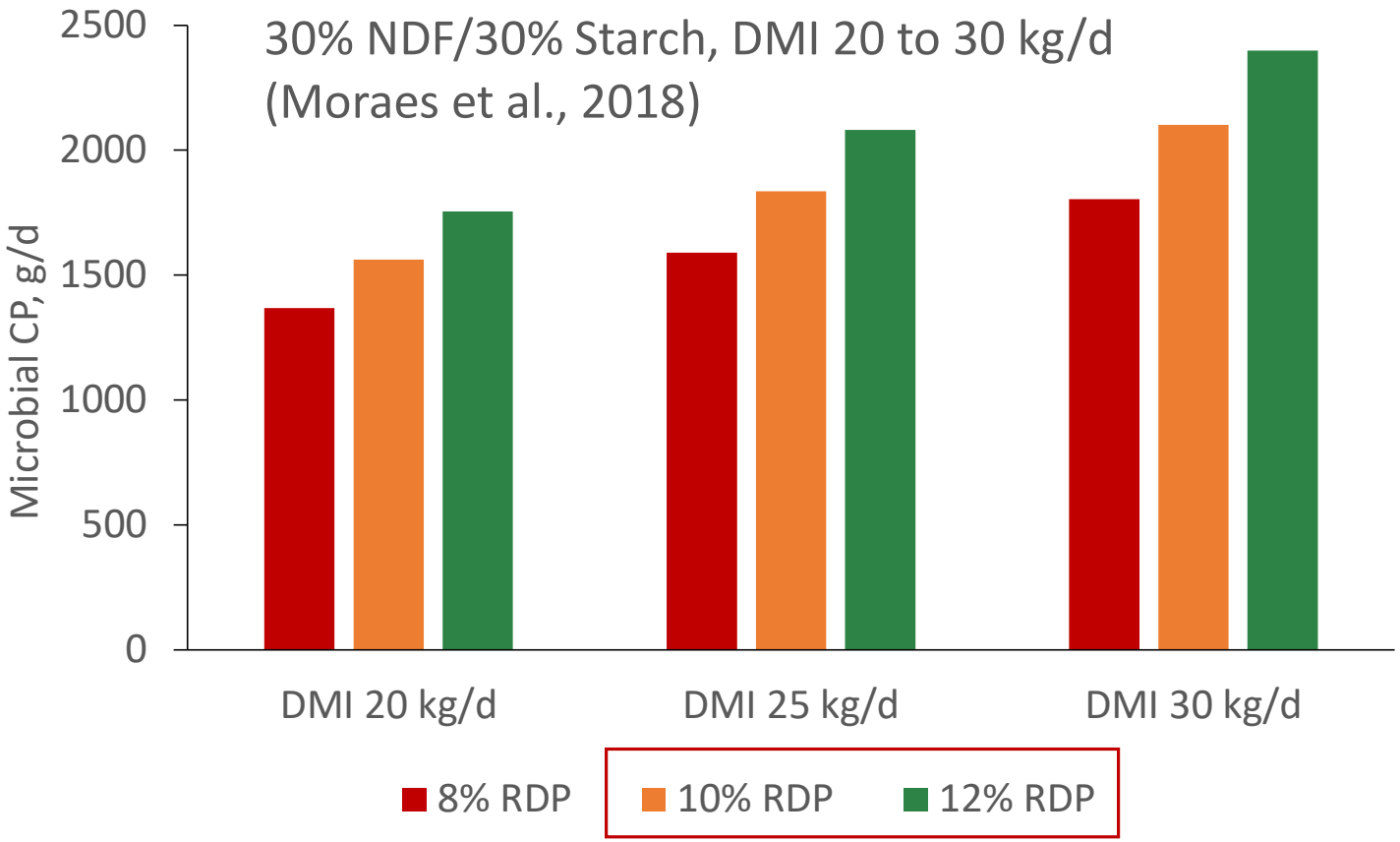
- Microbial N (g/d) = $[ß_0 + (ß_1 \times RDP)]/[(1 + ß_2/RDNDF) + (1 + ß_3/RDS)]$
  - Integrated Michaelis Menten equation (originally by Robin White)
  - Not lesser of RDP and CHO
  - ~ 18% omasal treatment means

- Denominator: rumen-degraded NDF + rumen-degraded starch
  - RDNDF negative slope for dietary starch percentage (neg. assoc. effects, White et al., 2016)
  - Scales for DMI: RDNDF and RDS counter each other
  - Truncate CP > 21.4% (Firkins and Lapierre, 2023)
    doi: 10.19103/AS.2022.0117.02 on many same concepts

- Numerator: intercept and coefficient for RDP intake
- Model is sensitive to RDP, capped at 12%



Providing enough but not too much RDP



Could not find a way to make RDP asymptotic

- Cap the response to 12%
- Recommend 10% RDP above which likely lessen efficiency of conversion of RDP to MicN

Remember bacteria can derive 0.5 to 1 ATP/AA fermented

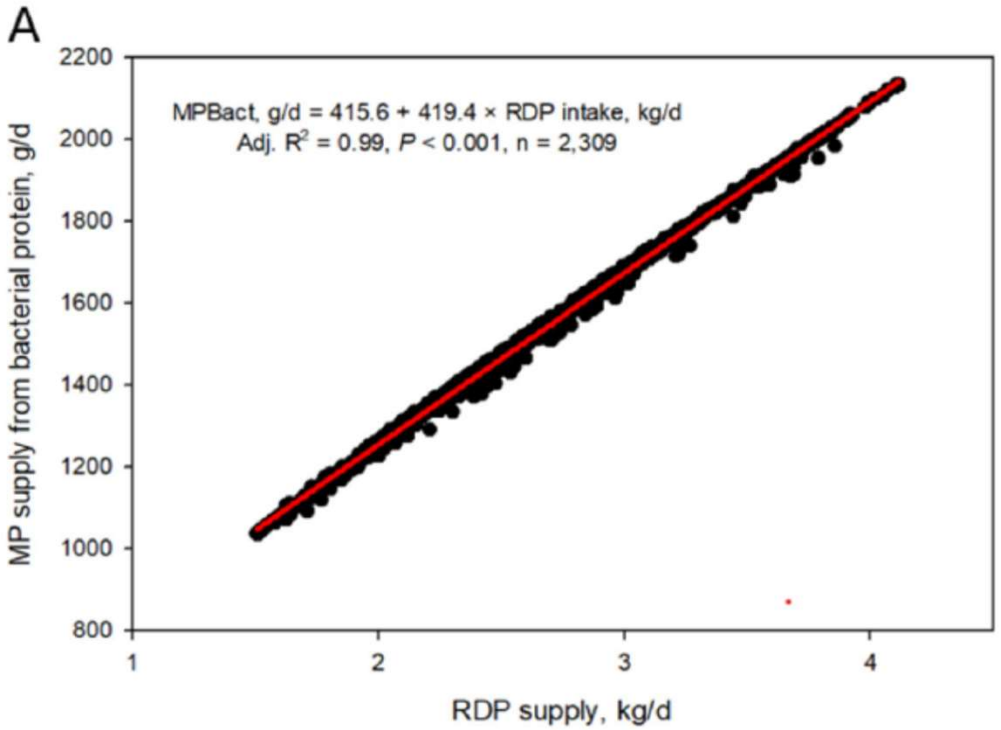
Several strains of proteolytic bacteria could get energy to maintain cell density, just not grow (Wallace et al., 1997)

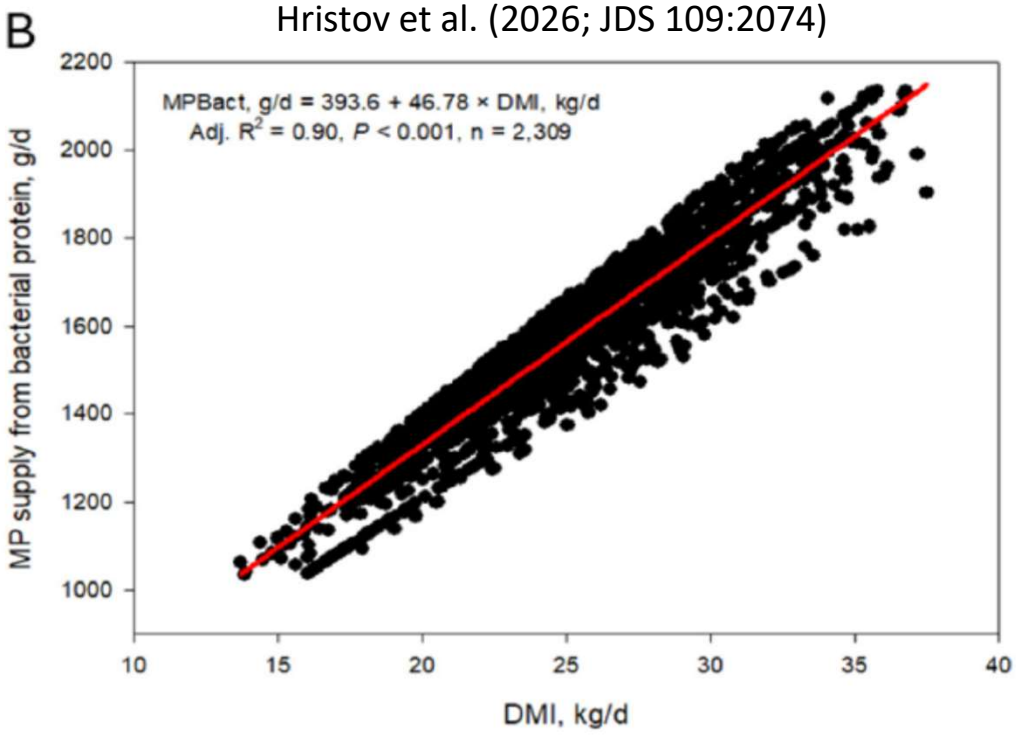
Does RDP intake “drive” MCP prediction?

- Remember 10% RDP in NASEM would be < 10% RDP in NRC
- If drop below 10% RDP with just replacing protein sources, you are just doing Ipharraguerre 2.0
- Equation truncates at 12% RDP
- To increase from 10 to 12% RDP, you would increase SBM ~ 5% units
  - Decrease 5% most likely from grain or nonforage fiber source
  - Decreasing the denominator of the equation

Does RDP intake "drive" MCP prediction?

Hristov et al. (2026; JDS 109:2074)





NASEM model "overdependent" on RDP

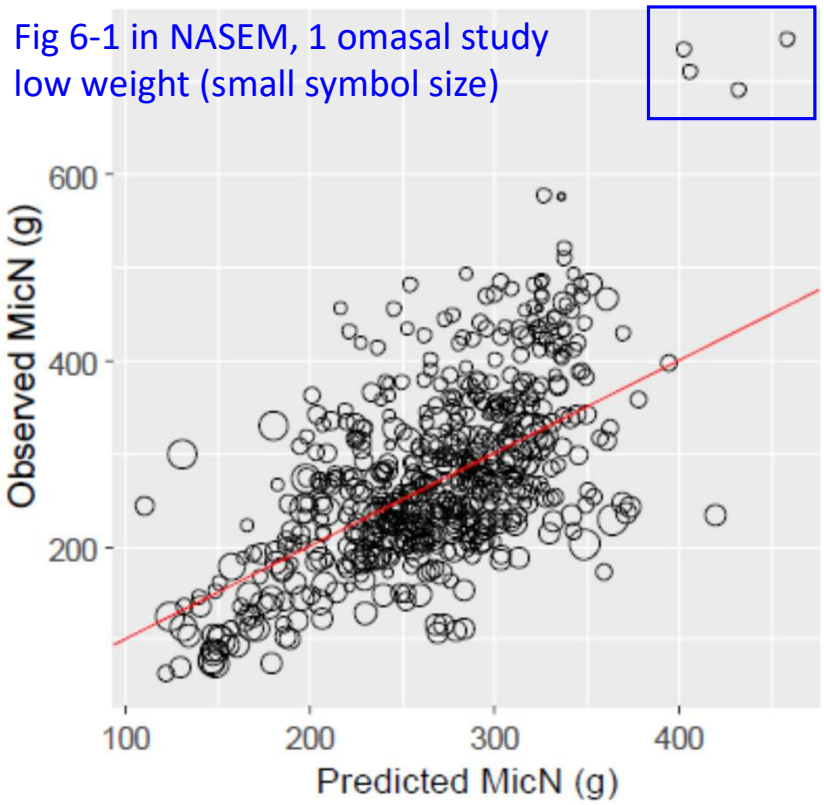
- Assuming Y is predicted MicP, RDPI (A) explains more variability (less dispersion) than DMI alone (B), but…
- Studies only varied 9 to 12% RDP, and RDP was probably same within a study, need to decompose the model error

NASEM model "confounded" with DMI

- Yes… DMI is in RDPI but also in RDNDFI and RDstarchI (starchD inhibits NDFD, which is why more static)
- DMI driving variable in every meta (as panel B)!

Martineau et al. (2023; doi: 10.3168/jds.2022-23215)
Note that JLF is a co-author.

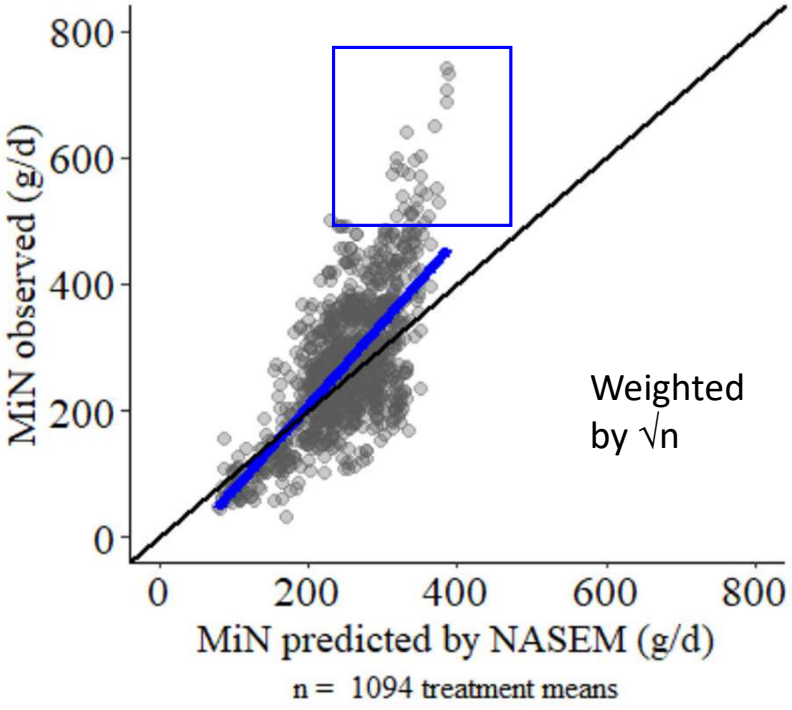
Fig 6-1 in NASEM, 1 omasal study low weight (small symbol size)



Larger circles = ↑weighting (1/SEM)

NASEM: N = 580 treatment means

More omasal studies with high DMI, MCP



Martineau: numerous n added back after NASEM screening, others new

Hanigan et al. (2021; doi: 10.3168/jds.2020-19672) noted importance of RDP using mostly same database

- Significant linear RDP (not significant quadratic RDP^2) on Mic N
- Negative interaction RDP and starch
  - NDFD already penalized for negative effects of starch in White et al. (2017)
  - Increasing starch depresses efficiency of microbial protein synthesis (EMPS), and probably RDP helps to recover that depression

"The significant linear RDP effect and the lack of significance of a quadratic RDP effect herein and in prior work (Bach et al., 2005; Galyean and Tedeschi, 2014)"

"Responses to fermentable carbohydrate and RDP occur simultaneously throughout the substrate range… Given such responses, the relative cost of the ruminally degradable carbohydrate and protein must be weighed against the value of the synthesized microbial protein to determine an optimum feeding level of each."

Conclusions: get MORE!

- MicP is cheapest and best overall source of metabolizable AA
- When you increase MicP, you get other benefits
  - Less likely to depress NDFD, DMI
  - Microbial CHO, lipids, etc.
  - Probably better effectiveness of nutrition models for your farm
- Dietary conditions that improve NDFD, MicP
  - Better balance of rumen-degraded starch and fiber effectiveness, moderate 16:0 (18:1)
  - Moderate RDP—insufficient RDP risks limiting MicP and NDFD
  - Isotope labeling studies (Atasoglu, White, etc.) support need for rumen-degraded AA and not just ammonia, even for fibrolytics
- Many studies to manipulate protozoa and other community members
  - Some promise for sure, but beware “too much of a good thing”
  - Look for consistency

THANKS to Balchem for sponsoring

- All my mentors (UIUC mentors; Drs. Dehority, Palmquist et al. at OSU; members of multi-state projects)
- Students, postdocs who taught me
- Funders of prior projects and symposia
- Family for tolerating an absent-minded prof
- You for listening