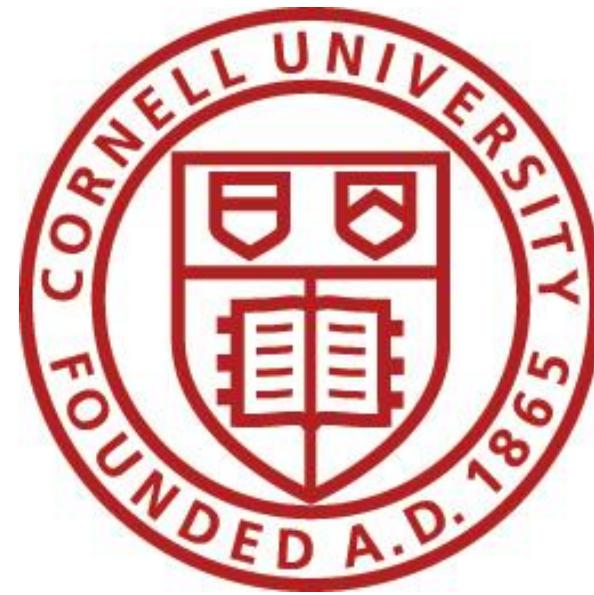


Rethinking Nitrogen Efficiency and Amino Acid Requirements – a More Holistic Approach

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Today's Talk

- Cows are changing, and we need to be conscious of this
- We have updated requirements and estimations of amino acid supplies and considerations for other nutrients and this might require context
- **Protein synthesis** is required for lactose synthesis, fatty acid synthesis and milk protein synthesis
- Thus, the concept of N efficiency is not just related to milk protein output; it is related to energy-corrected milk, as all components require N as part of the synthetic processes

Nitrogen Emissions – Ammonia and Human Health

- Agriculture contributes over 81% of total NH_3 global emissions (Van Damme et al., 2021)
- Currently, NY air emissions are regulated at PM10 and PM2.5 (particulate matter @ 10 microns or 2.5 microns) (<https://dec.ny.gov/environmental-protection/air-quality/monitoring>)
- Higher ammonia emissions result in higher PM2.5 which results in greater health concerns for humans (Chronic Obstructive Pulmonary Disorder, lung cancer, and premature death)
- Eastern US is high in emissions – NY has this on its list of emissions to abate or reduce
- As an industry, we need to be conscious of this and outrun regulations by taking steps to reduce N emissions

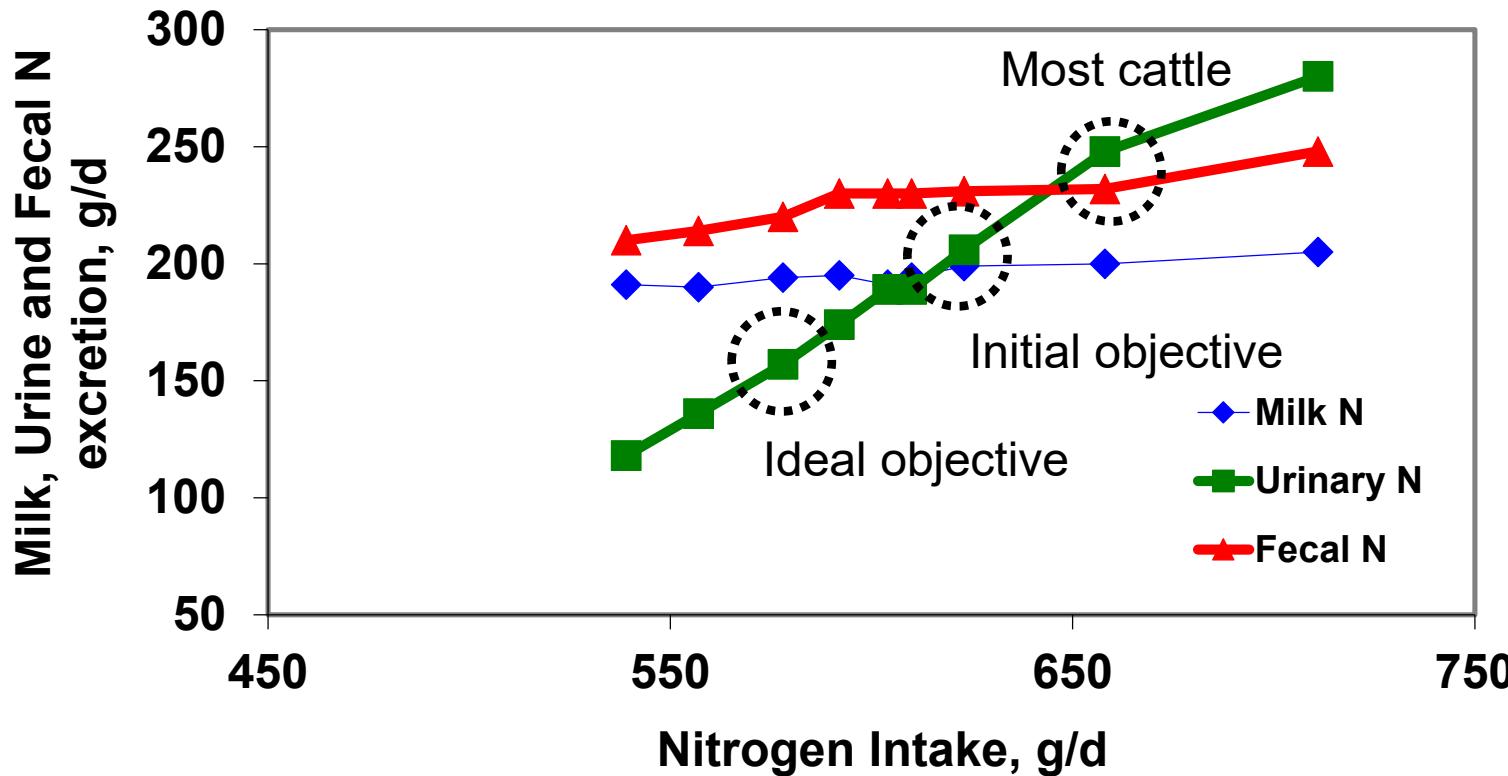
Efficiency of Use of Intake Nitrogen

- This is a tough metric for ruminants since they require non-protein N for rumen function
- When this is described for non-ruminants the N-currency is amino acids
- Higgs and others determined a N efficiency of ~72-73% on a weighted average using just absorbed EAA
- Theoretical efficiency limit 40 to 45% in lactating dairy cattle (Van Vuuren and Meijs, 1987; Hvelplund and Madsen, 1995)
- Practical limit is ~38 to 40% (high cow groups are achieving this)
- Although it is an ambiguous metric, it can be useful if extended to whole body N metabolism

Practical Objectives

- **Improve Feed Efficiency** – provide diets that encourage the mammary gland to utilize as many absorbed nutrients as possible
- This **reduces Carbon intensity** by dilution and is generally financially rewarding
- **Reduce Urinary Nitrogen excretion** – improves feed efficiency, energy efficiency, and income over feed costs and helps mitigate N effects on eutrophication and nitrous oxide emissions

Nitrogen excretion in diets varying in dietary nitrogen



Milk Nitrogen: ~200 g or 1.28 kg (2.81 lb) protein

Urinary N:

Most Cattle: ~250 g
Initial Objective: ~200 g
Ideal Objective : ~150 g

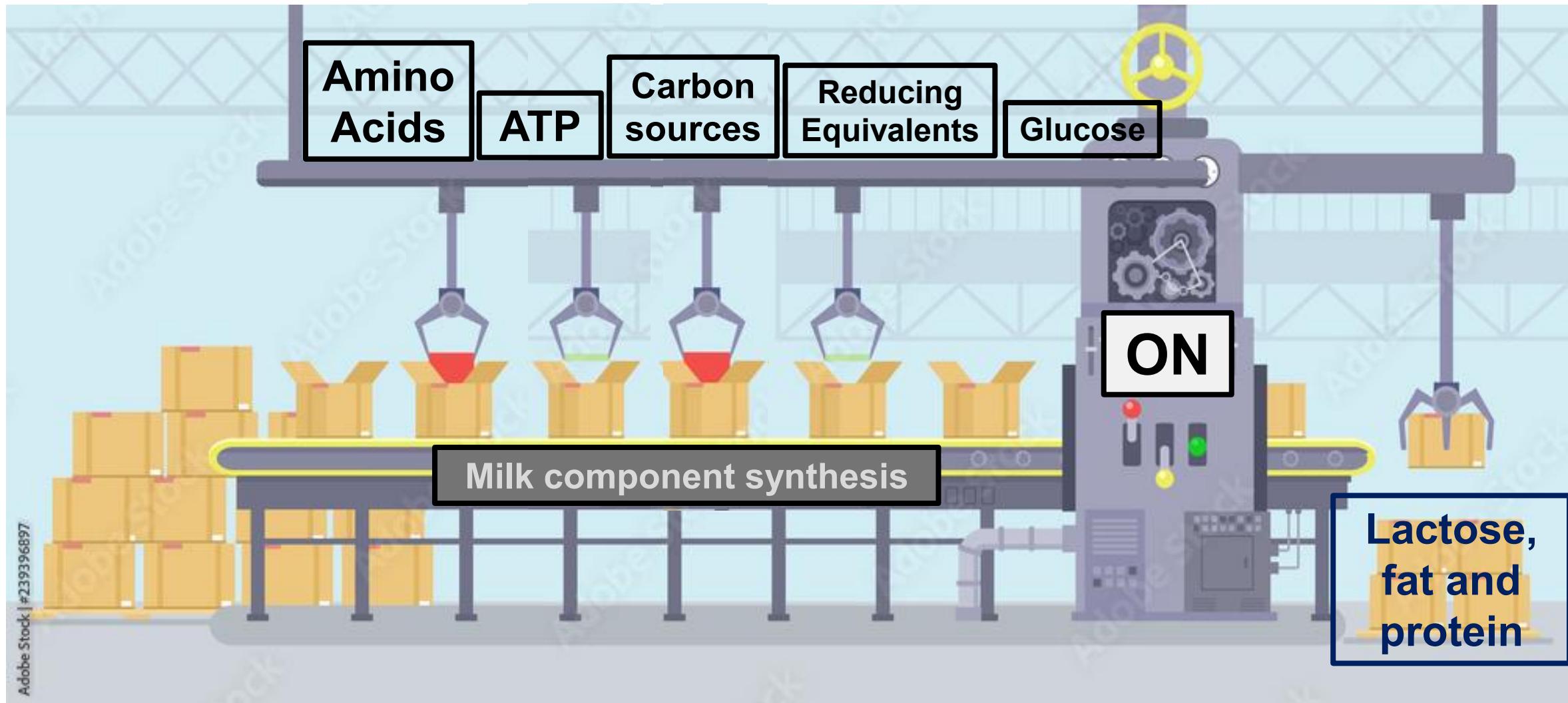
Productive N:Urinary N

Most Cattle: 0.8
Initial Objective: 1.0
Ideal Objective : 1.3

Metrics can be used as a proxy for improvements of Productive N:Urinary N

The Conveyor Belt of Milk Component Production

- Meeting amino acid requirements improves overall nutrient and energy use efficiency for milk and component production



- How should we reconsider the requirements of amino acids in lactating cows and how does this change our

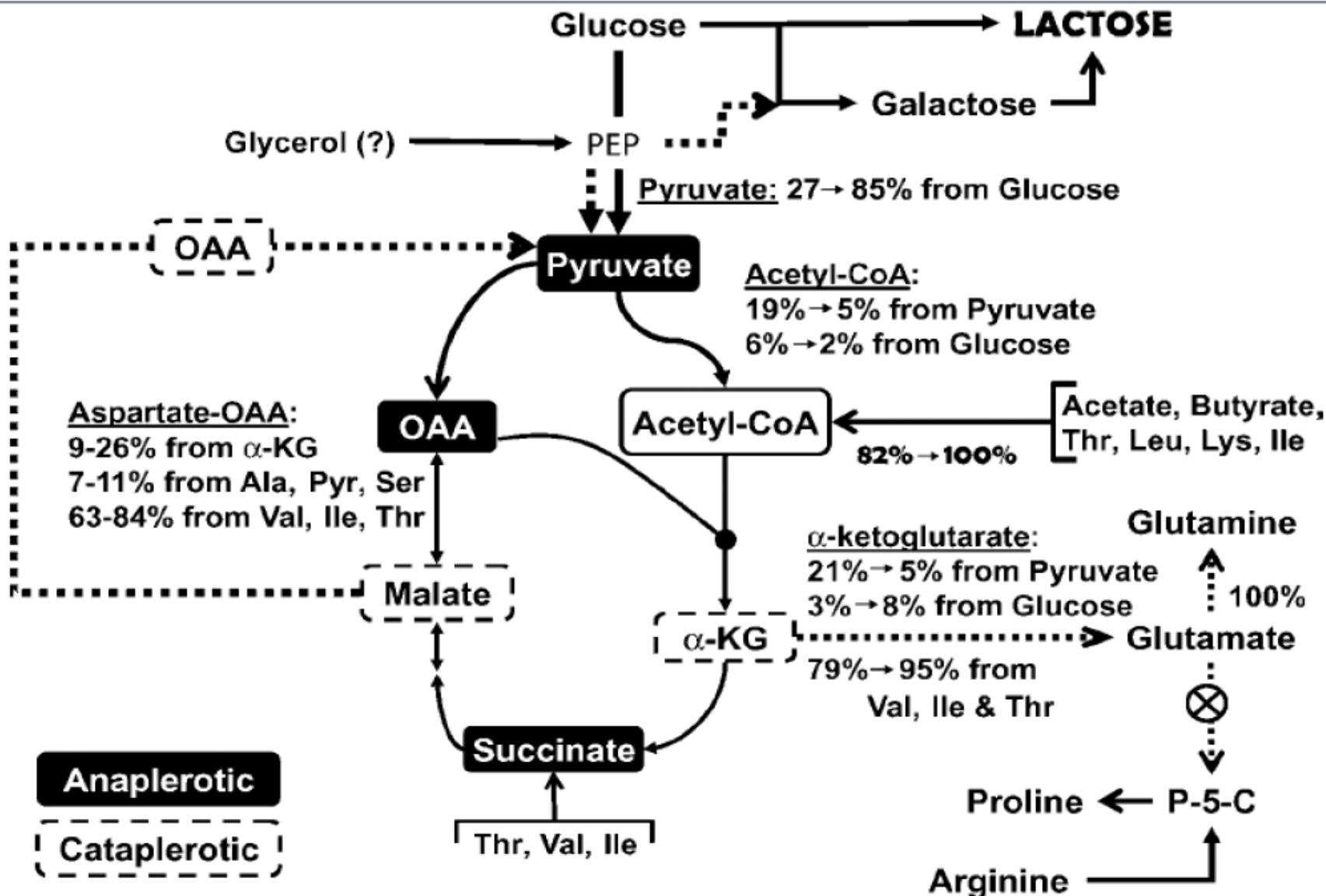


Protein-energy interactions

“Although it has been traditional to consider ‘protein’ and ‘energy’ metabolism as separate entities in mammalian metabolism, most scientists recognize this is an artificial divide. Indeed, they should be considered together as this reflects how nutrients are ingested and utilized as part of normal feeding patterns during evolution.”

Lobley, G. E. 2007. Protein-energy interactions: horizontal aspects. Pages 445-462 in Proc. Energy and protein metabolism and nutrition. Butterworths, Vichy, France.

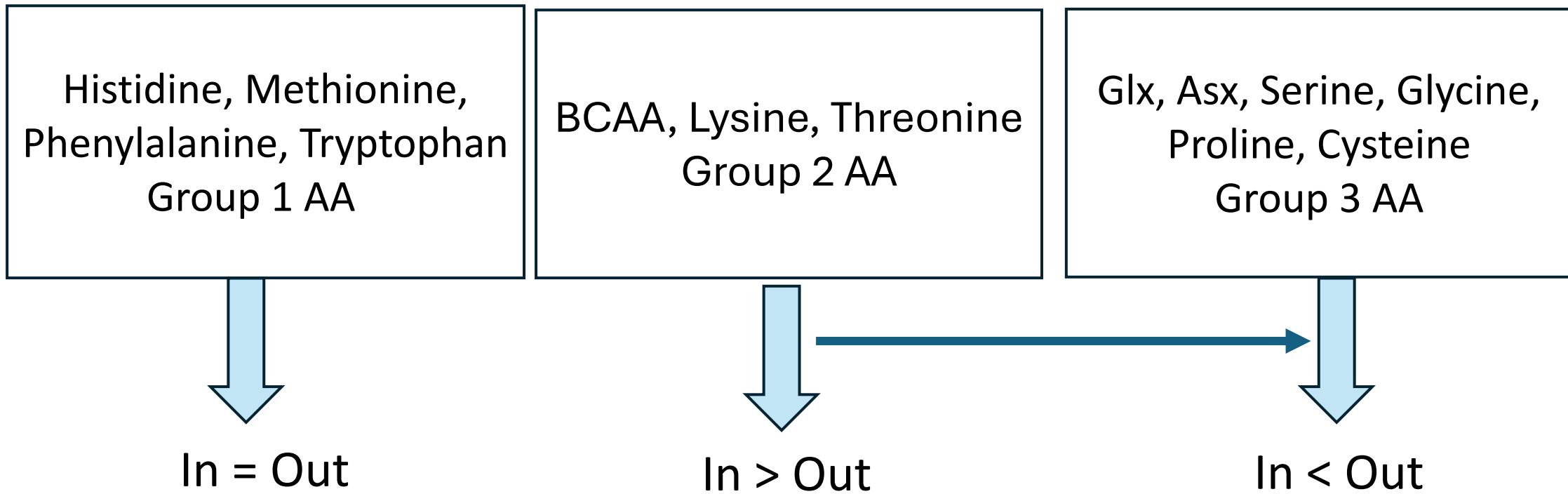
Interconversions in Mammary Gland Explants



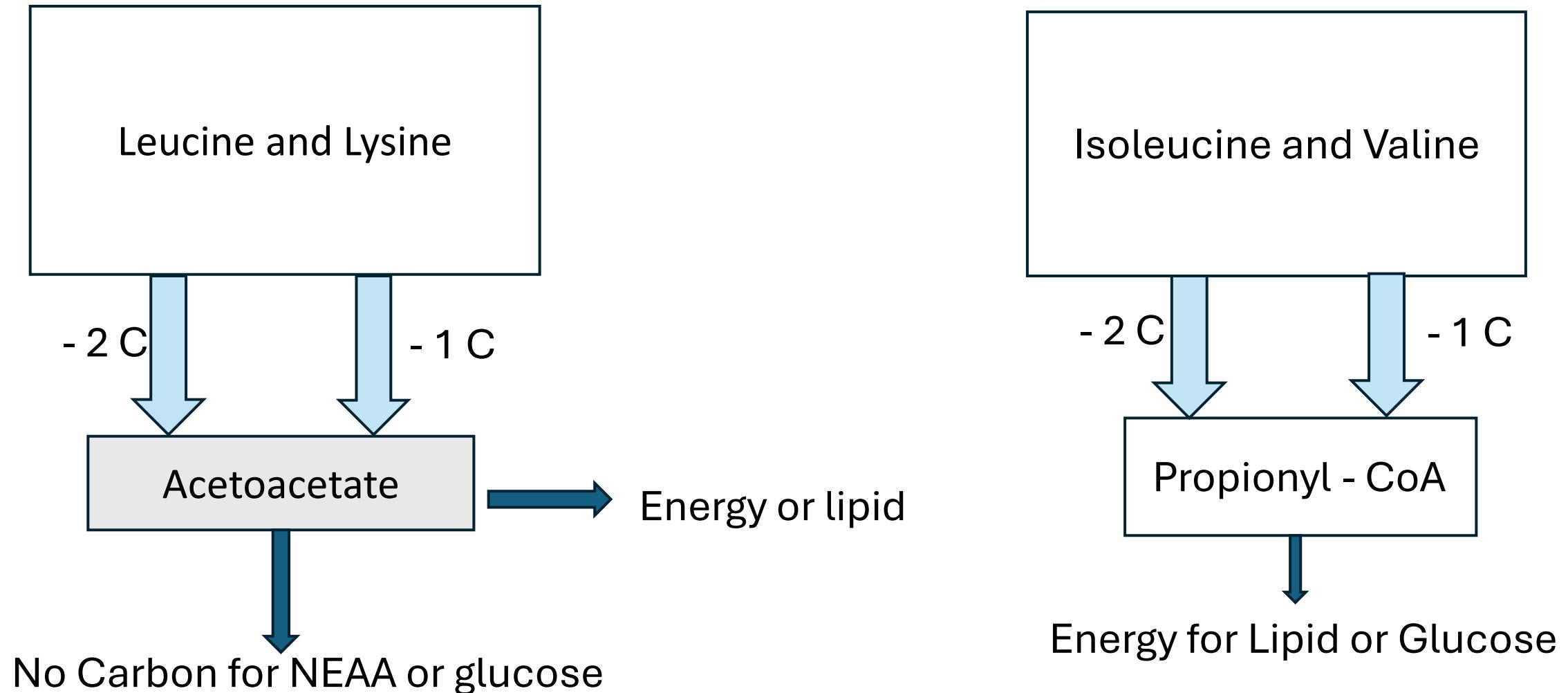
Amino Acid N uptake across the mammary gland – Raggio et al., 2006

mmol/h Nitrogen	Control	Casein	Propionate	Casein + Propionate
Total uptake	163.0	189.5	178.0	212.8
EAA	81.3	100.7	86.4	109.2
NEAA	81.7	88.8	91.7	103.6
Total output	156.1	186.6	165.2	200.9
EAA	68.9	82.6	73.0	88.8
NEAA	87.1	104.3	92.2	112.2
EAA in - out	12.4	18.1	12.4	20.4
NEAA in - out	-5.4	-15.5	-0.5	-8.6

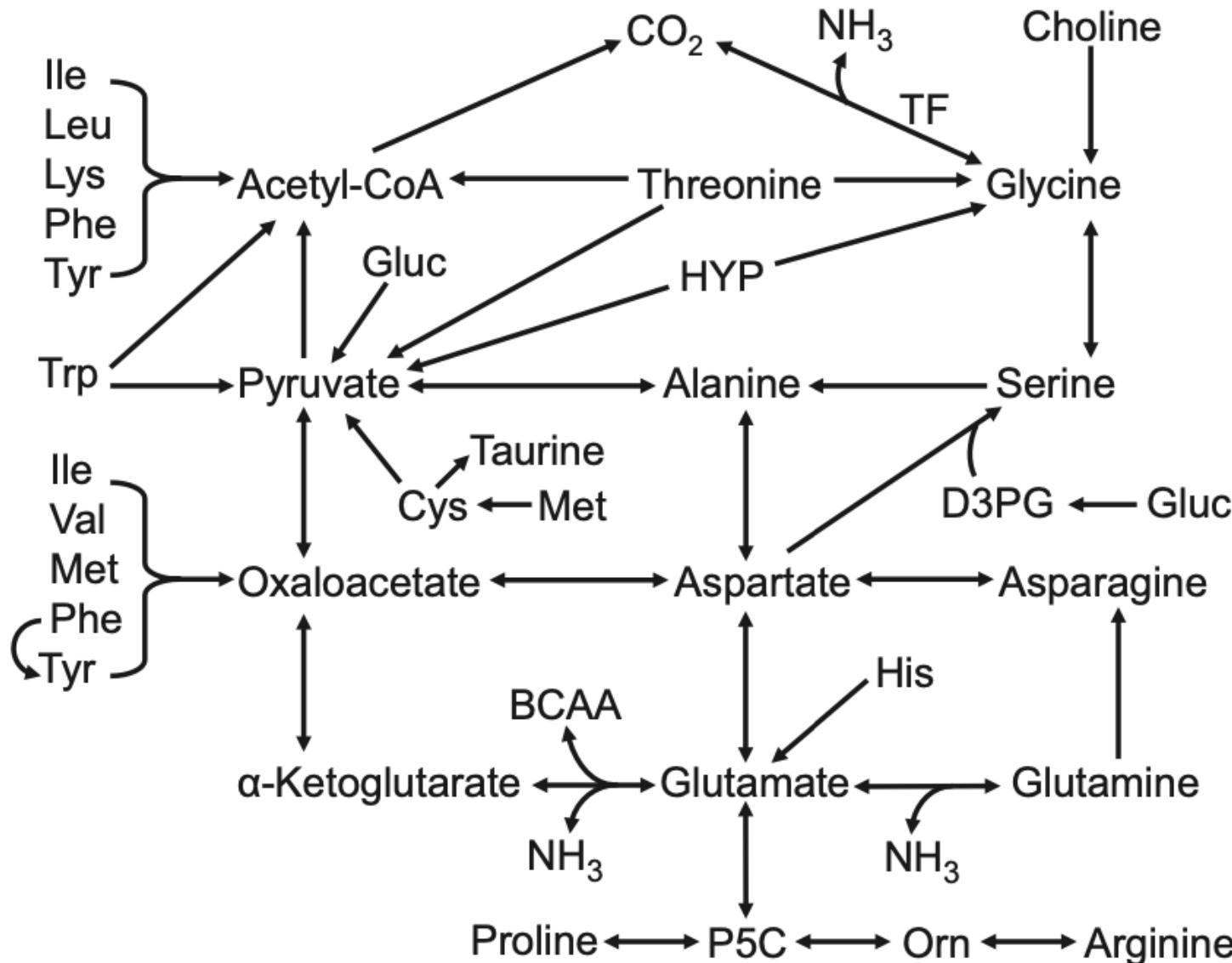
Amino Acid N uptake across the mammary gland – Raggio et al., 2006



Fate of Carbon from Amino Acids – Lobley, 2007



“Non-Essential” but Required Amino Acids



Mammary adaptability in varying nutrient supplies

Shifts in nutrient profile and supply → alterations in their efficient use according to mammary demand.

Extraction of BCAA changes across lactation

- Cellular maintenance and anabolic response (Mepham, 1982)

Lysine undergoes obligate catabolism in mammary (Lapierre, 2009)

- Supplies N for NEAA synthesis
- Level of catabolism can shift in accordance with NEAA supply

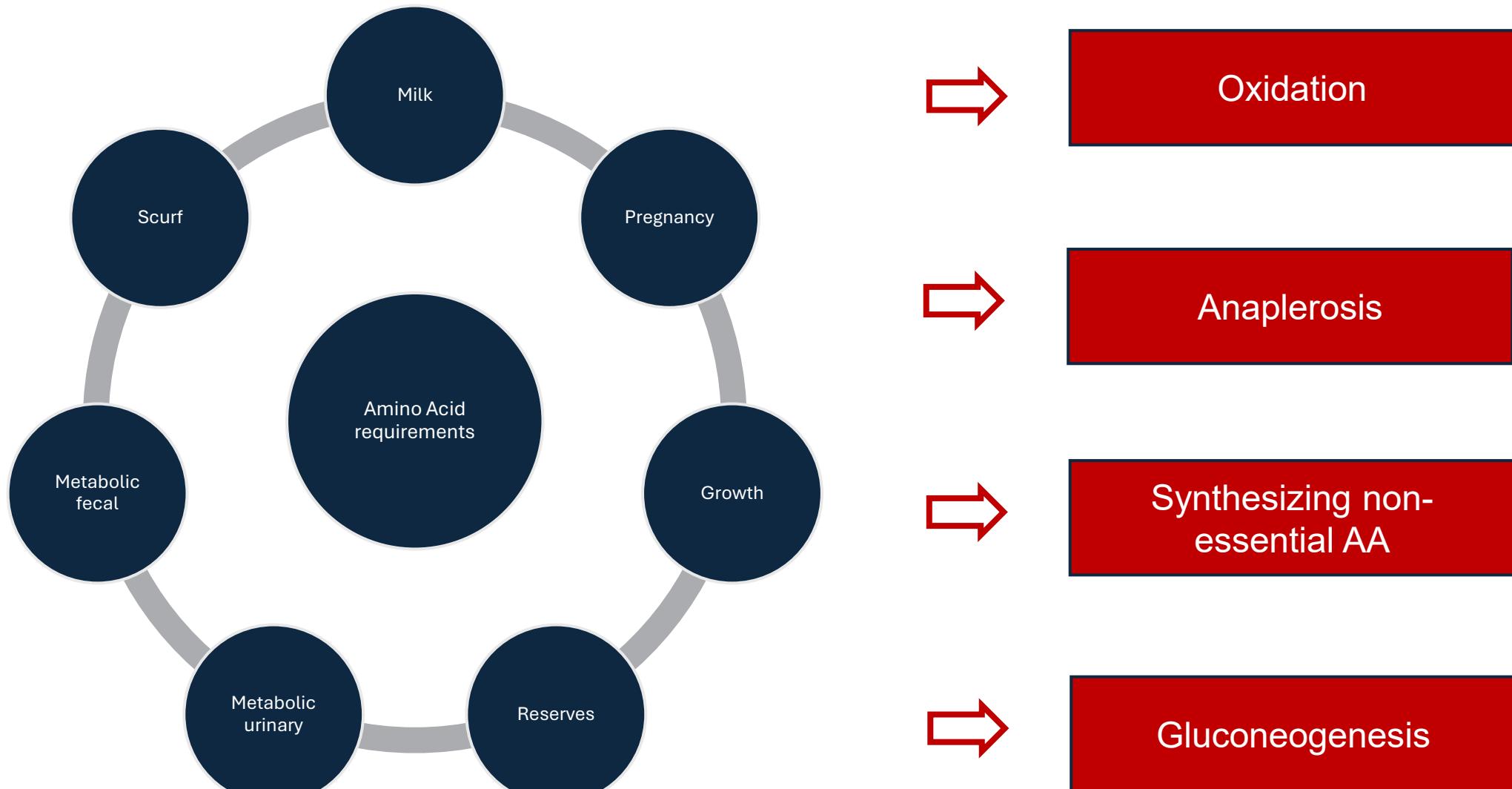
Arginine is taken up in drastic excessive relative to milk protein output (~2.5x)

- Catabolism products include proline, ornithine, and urea (O'Quinn et al., 2002)
- Proline content in milk casein = 10.4% (2nd highest to glutamine)

AA Group (Mepham, 1982)			
	1	2	3
Amino Acid	Histidine	Isoleucine	Alanine
	Phenylalanine	Leucine	Asparagine
	Methionine	Valine	Cysteine
	Tyrosine	Lysine	Glutamine
	Tryptophan	Arginine*	Glycine
		Threonine	Proline
		*	
			Serine
Efficiency (AA -N uptake/AA-N Milk)	1	> 1.15	< 1

* Suggested group according to Lapierre et al. (2012)

‘Efficiency’ Of Essential AA Use (Additional Requirements)



Optimum Supply Of Each EAA Relative To Metabolizable Energy – CNCPS v7.0

Higgs, 2014; Higgs et al., 2023

AA	R ²	Efficiency from our evaluation	Lapierre et al. (2007)	g AA/ Mcal ME	% EAA
Arg	0.81	0.61	0.58	2.04	10.2%
His	0.84	0.77	0.76	0.91	4.5%
Ile	0.74	0.67	0.67	2.16	10.8%
Leu	0.81	0.73	0.61	3.42	17.0%
Lys	0.75	0.67	0.69	3.03	15.1%
Met	0.79	0.57	0.66	1.14	5.7%
Phe	0.75	0.58	0.57	2.15	10.7%
Thr	0.75	0.59	0.66	2.14	10.7%
Trp	0.71	0.65	N/A	0.59	2.9%
Val	0.79	0.68	0.66	2.48	12.4%

Lys and Met requirements 14.9%, 5.1% - Schwab (1996) 2.9:1

Lys and Met requirements 14.7%, 5.3% - Rulquin et al. (1993) 2.77:1

Redescribing AA balancing with more precision for higher milk yields and metabolic demand

- As milk and component yields increase the precision needed for diet formulation also needs to increase
- This is true for amino acids and will be true for fatty acids once we better understand the requirements
- CNCPS v6.55 EAA targets from v7 derivations:
 - Methionine – 1.19 g/Mcal ME
 - Histidine – 1.19 g/Mcal ME
 - Lysine - 3.2 g/Mcal ME

Review of recent experiment evaluating nutrient use efficiency

Dose titration of Rumensin[©]— nothing to do with amino acids, except the diets were formulated using the latest information related to EAA levels – grams per Mcal ME

192 cows were used in a replicated pen study

16 cows per pen, milked 3x per day

Prior to the experiment, the cows were producing 42 kg, 4.1% fat and 3.1% true protein

	DM kg
Corn silage	8.85
Haylage - MML	4.90
Corn ground fine	4.54
SBM	1.72
SoyPass	1.45
Citrus Pulp	1.13
Wheat midds	1.13
Dextrose	0.40
Blood meal	0.25
Bergafat 100	0.15
Energy Booster 100	0.15
Sodium bicarb	0.10
Rumen protected methionine	0.03
Rumen protected lysine	0.03
Levucell SC	0.01
Vitamins and Minerals	0.41
Total	25.27

Rumen modifier study diet chemistry – formulated

DM, %	45.1
CP, %	15.75
Sol CP, %CP	31.5
aNDFom, %	31.6
Sugar, %	4.92
Starch, %	26.33
EE, %	4.4
ME, mcal/kg	2.65
ME, Mcal @25.5 kg DMI	68
Forage, % DMI	54.3
Forage, %BW	0.93
Methionine, g/Mcal ME	1.19
Lysine, g/Mcal ME	3.03
Methionine, g	82
Lysine, g (methionine x 2.7)	222

Diet/Intake related information – Methionine, Histidine and Lysine levels – CNCPS v7 translated to v6.5

Cows consumed approximately **71-72 mcals ME** per day

Methionine @ 1.19g/Mcal ME = $1.19 * 71.5 = 85$ g

Lysine @ 3.2 times Met/Mcal ME = $3.2 * 71.5 = 229$ g

Histidine @ 1.19 g/Mcal ME = $1.19 * 71.5 = 85$ g

Meeting the requirements should improve energetic efficiency and milk component yields

Milk, energy corrected milk, feed efficiency and body weight of cows fed four levels of Rumensin[©]

	Treatment					
Item	0	11g	14.5g	18g	SEM	P-Value
DMI, kg/d	26.9	26.8	26.7	27.7	0.31	0.21
Milk Yield, kg/d	39.1	39.9	39.6	39.6	0.4	0.33
ECM, kg/d,	45.9	46.9	47.1	46.8	0.51	0.11
Feed Efficiency, ECM/feed	1.71	1.74	1.76	1.70	0.02	0.93
BCS	2.9	3.1	3.0	2.9	0.2	0.7
BW, kg	693	690	693	692	2.3	0.96
PUN, mg/dL	9.13	9.23	9.19	8.88	0.16	0.36

Milk fat, protein and urea nitrogen of cows fed four levels of Rumensin®

Item	Treatment				SEM	P-Value
	0	11g	14.5g	18g		
Average days in milk	190	168	193	184	7.2	--
DMI, kg/d	26.9	26.8	26.7	27.7	0.31	0.21
Milk Yield, kg/d	39.1	39.9	39.6	39.6	0.4	0.33
ECM, kg/d,	45.9	46.9	47.1	46.8	0.51	0.11
Milk fat, %	4.60	4.67	4.72	4.67	0.05	0.2
Milk fat, kg	1.79	1.83	1.85	1.83	0.02	0.02
Milk true protein, %	3.35	3.38	3.37	3.39	0.01	0.07
Milk protein, kg	1.30	1.33	1.32	1.33	0.01	0.15
MUN, mg/dL	8.92	10.20	9.65	9.56	0.12	<0.01

Fatty acid profile of milk from cows fed four levels of Rumensin[©]

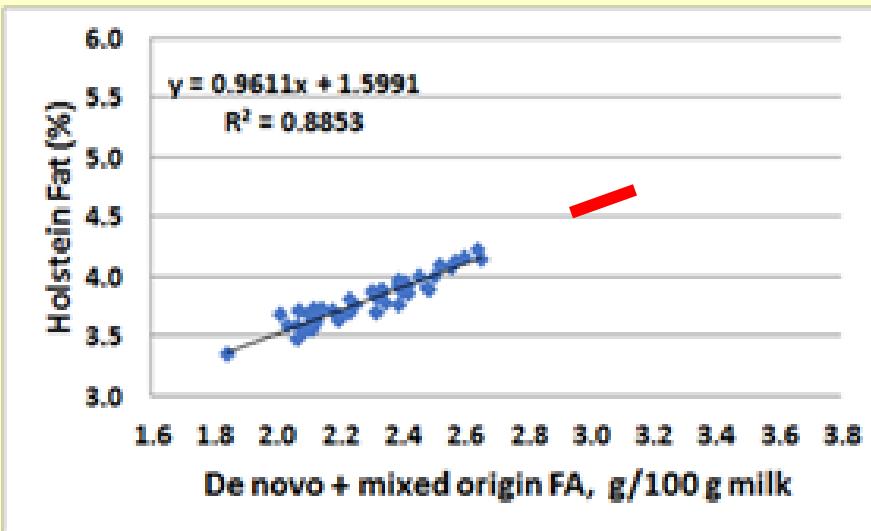
Item	Treatment				SEM	P-Value
	0	11g	14.5g	18g		
De novo fatty acid, g/100g	1.131	1.157	1.168	1.156	0.01	0.03
De novo fatty acid, kg	0.44	0.45	0.46	0.46	0.005	0.32
Mixed fatty acid, g/100g	1.856	1.881	1.918	1.897	0.02	0.02
Mixed fatty acid, kg	0.73	0.74	0.75	0.75	0.009	0.39
Preformed fatty acid, g/100g	1.34	1.33	1.38	1.35	0.02	0.23
Preformed fatty acid, kg	0.52	0.52	0.54	0.53	0.007	0.29
Fatty acid chain length	14.6	14.5	14.5	14.5	0.01	0.83
Double Bonds	0.23	0.23	0.23	0.23	0.002	0.42

Milk de novo and mixed fatty acids in Holsteins compared to Jersey milk components – red lines are from Benoit et al., 2022

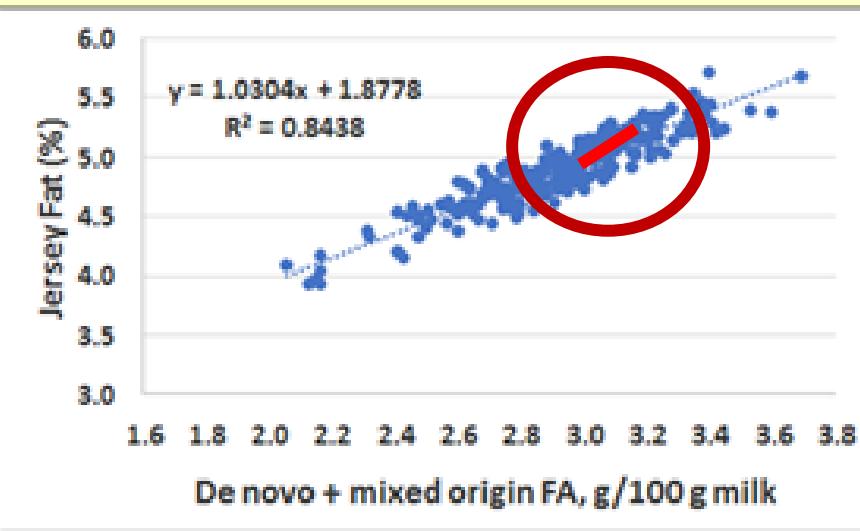
Holstein vs. Jersey Farms 2019

De novo + mixed origin fatty acids and bulk tank milk fat

Holstein



Jersey



Similar slope and high R^2 for the strong relationship between de novo + mixed origin fatty acid concentration and bulk tank milk fat concentration for Jersey and Holstein bulk tank milk. (herd average days in milk 150 to 200 days)

Which mammary fatty acids are involved in increasing milk fat, and where and how are these amino acids responsible for milk fat synthesis and yield?



Methionine prototype dose titration study objectives

- The objective of this study was to evaluate a newly developed rumen-protected Methionine product (RPMet) when Lysine and Histidine were not considered co-limiting.
- A second objective was to further evaluate the AA requirement approach developed for CNCPS v7 (Higgs, 2014; Higgs et al., 2023)

Table 1. Amino acid formulation levels by treatment.

	RP Met g / Mcal ME	Lys g / Mcal ME	Hist g / Mcal ME
Treatment 1	0.86	3.2	1.19
Treatment 2	1.05	3.2	1.19
Treatment 3	1.19	3.2	1.19

Study Design

- 144 Holstein dairy cows assigned to a replicated pen study
 - 16 cows per pen, 3 pens per treatment
 - Diets fed once per day to 5% refusals
 - Sand bedded stalls, one stall per cow, free-choice water
 - Cows milked 3 times per day
- Cows were randomly assigned to treatment based on BW, milk yield, DIM and parity. (12 multiparous and 4 primiparous cattle per pen)
- Covariate period was 14 days, with sampling the last three milkings of the period
- Treatment period was 70 days for 84 total days of the experiment
- Milk sampled every 7 days, 3 contiguous milkings, all analyzed individually
- Pen level DMI measured daily
- BW and BCS measured/observed weekly

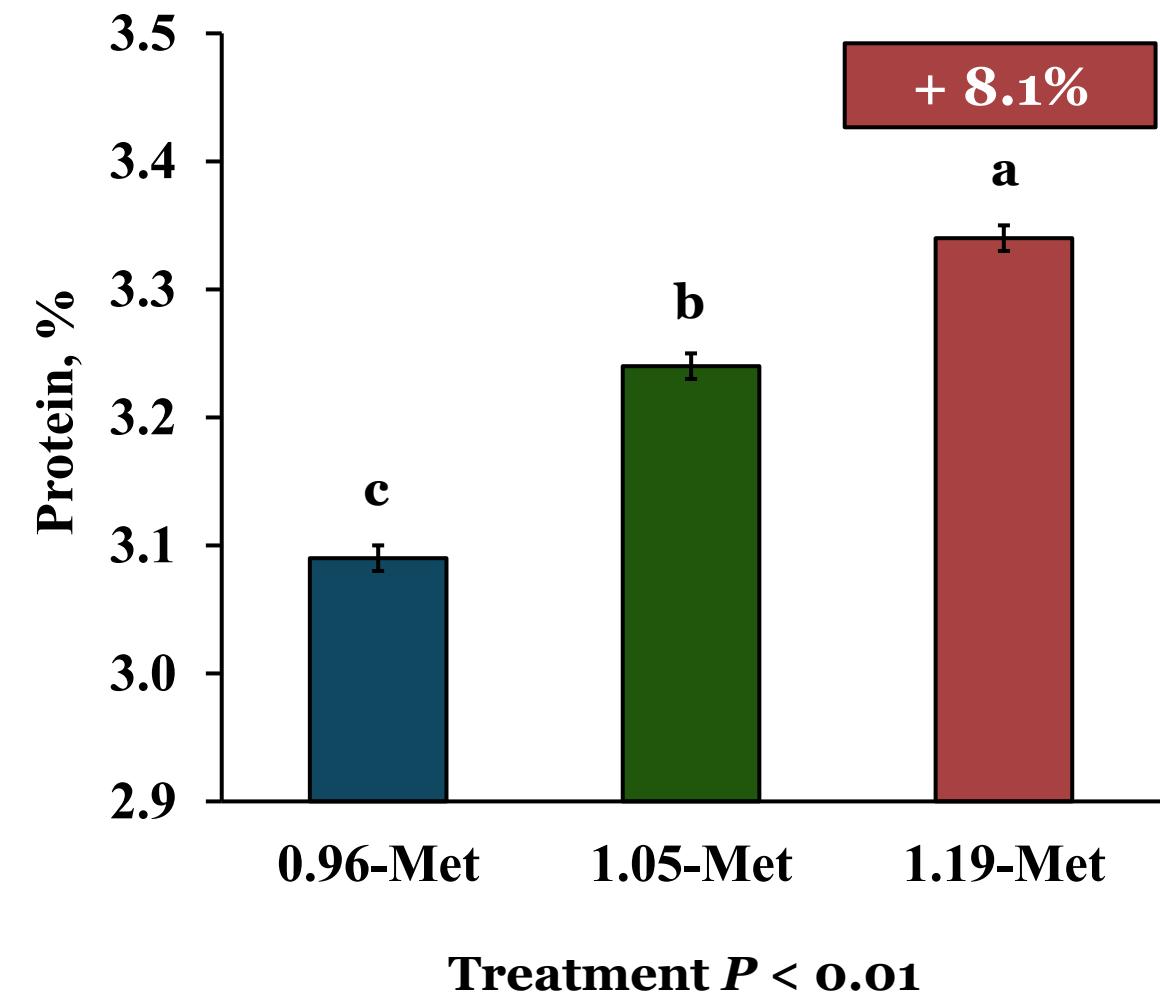
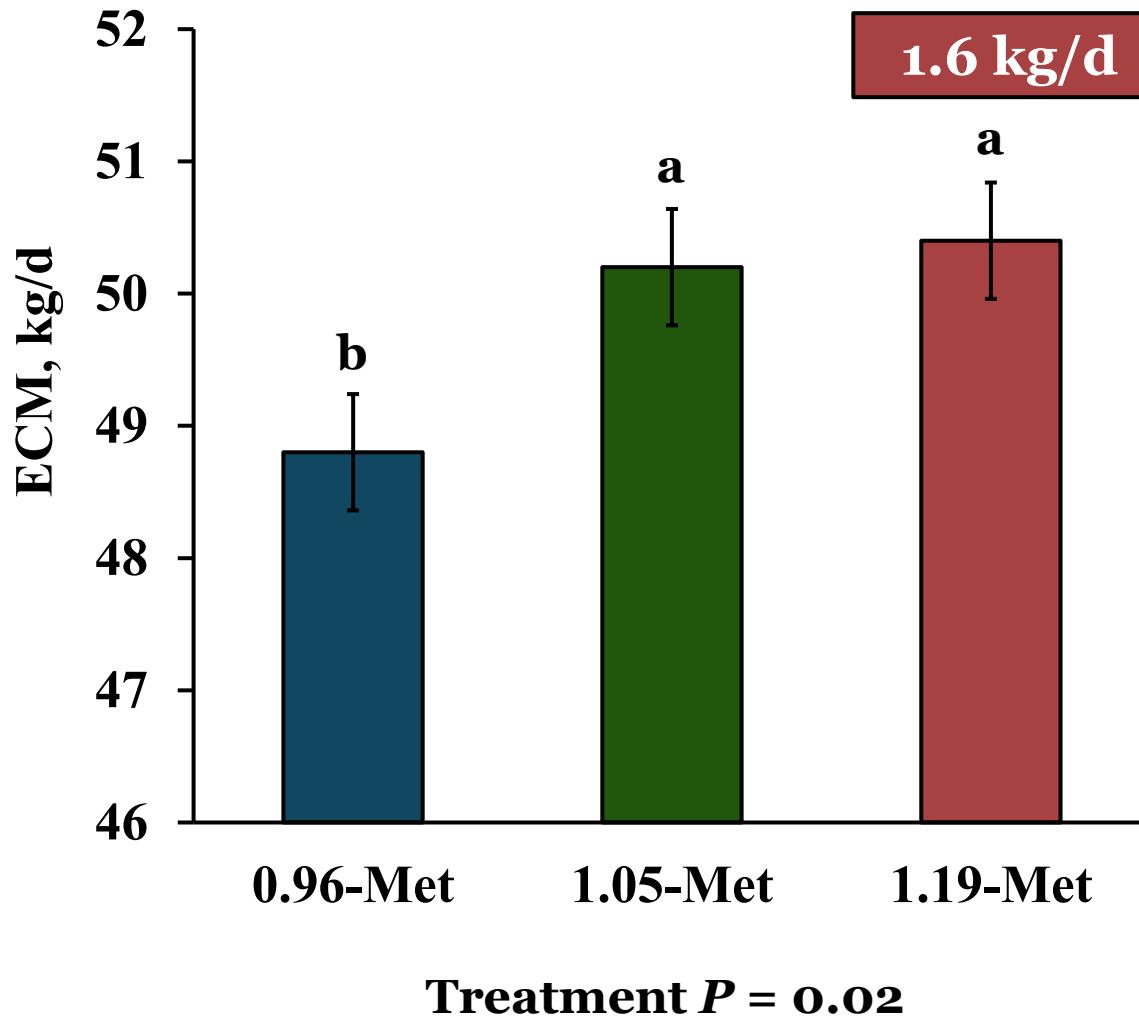
Composition of grain mix for cattle fed varying levels of methionine.

Ingredients, % DM	Gram of Metab. Met/Mcal ME		
	0.86	1.05	1.19
Grain Mix	4.96	4.99	5.02
Wheat middlings		2.08	
Sugar (sucrose)		0.79	
Soybean hulls		0.42	
Blood meal blend		0.42	
SoyPlus		0.28	
Rumen protected lysine		0.08	
Energy Booster 100		0.07	
Urea		0.07	
Palmit 80		0.05	
Levucell SC		0.01	
Rumensin, 90 g/lb		0.002	
Minerals and vitamins		0.69	
Rumen protected methionine prototype	0.02		0.04

Results – 144 cows, replicated pen study, 48 cows/trt

Parameter	Diet, g Metabolizable Met/Mcal ME			SEM	P value
	0.86	1.05	1.19		
Body Weight, kg	698	705	701	3.3	0.30
Delta BW, kg	16.4	23.9	9.8	6.8	0.35
Dry Matter Intake, kg	26.4	26.5	26.1	0.3	0.59
Milk Yield, kg	44.6	45.3	44.8	0.38	0.38
ECM, kg	48.8^a	50.2^b	50.4^b	0.44	0.02
ECM to DMI	1.87	1.88	1.92	0.017	0.21
Milk True Protein, g/100g	3.09^a	3.24^b	3.34^c	0.010	< 0.01
Milk True Protein, kg	1.38^a	1.46^b	1.49^b	0.011	< 0.01
Milk Fat, g/100g Milk	4.21^a	4.25^a	4.36^b	0.026	< 0.01
Milk Fat, kg	1.88	1.92	1.94	0.023	0.16
MUN, mg/dL	11.20	11.44	11.09	0.120	0.12

Effect of Increasing Methionine on ECM and Protein

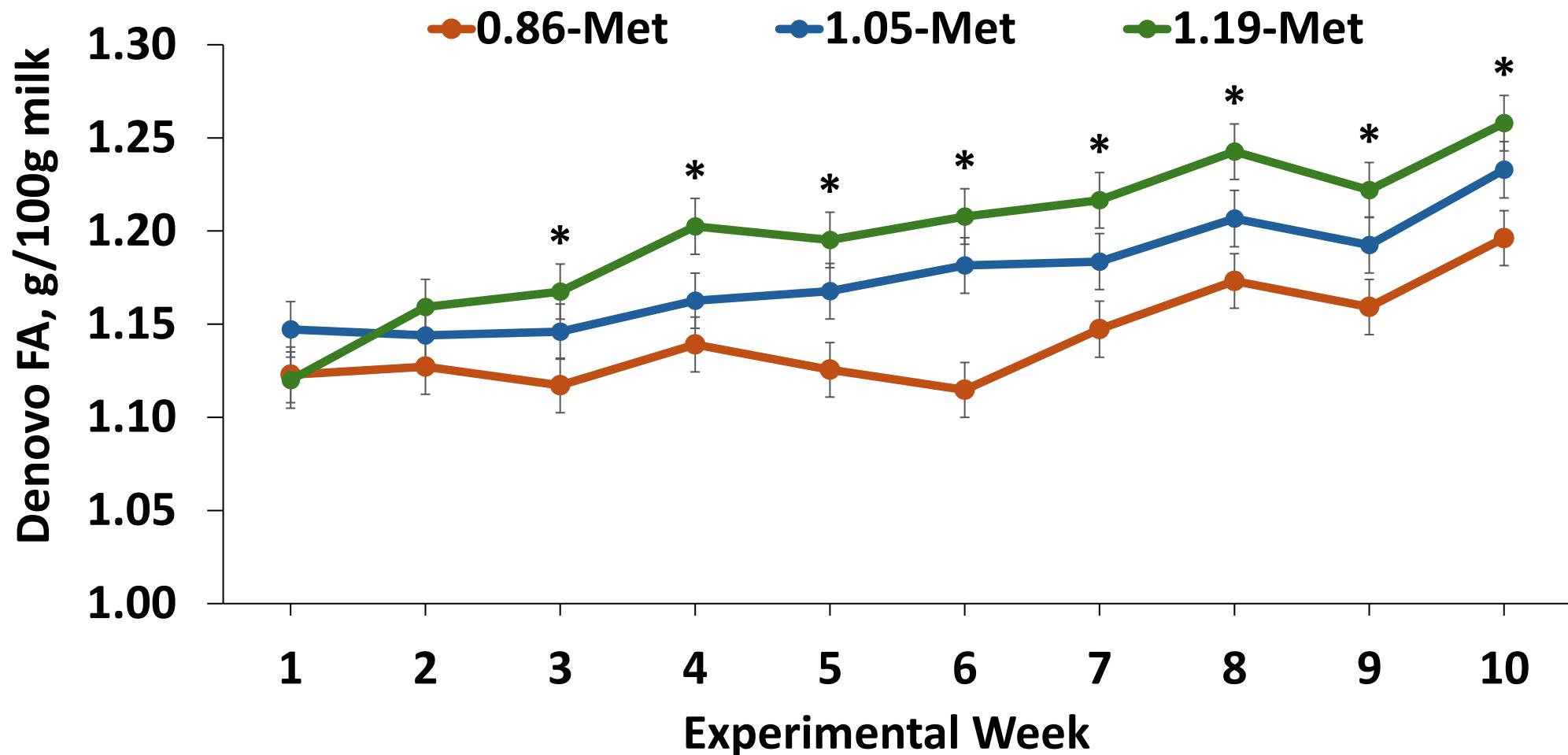


Results

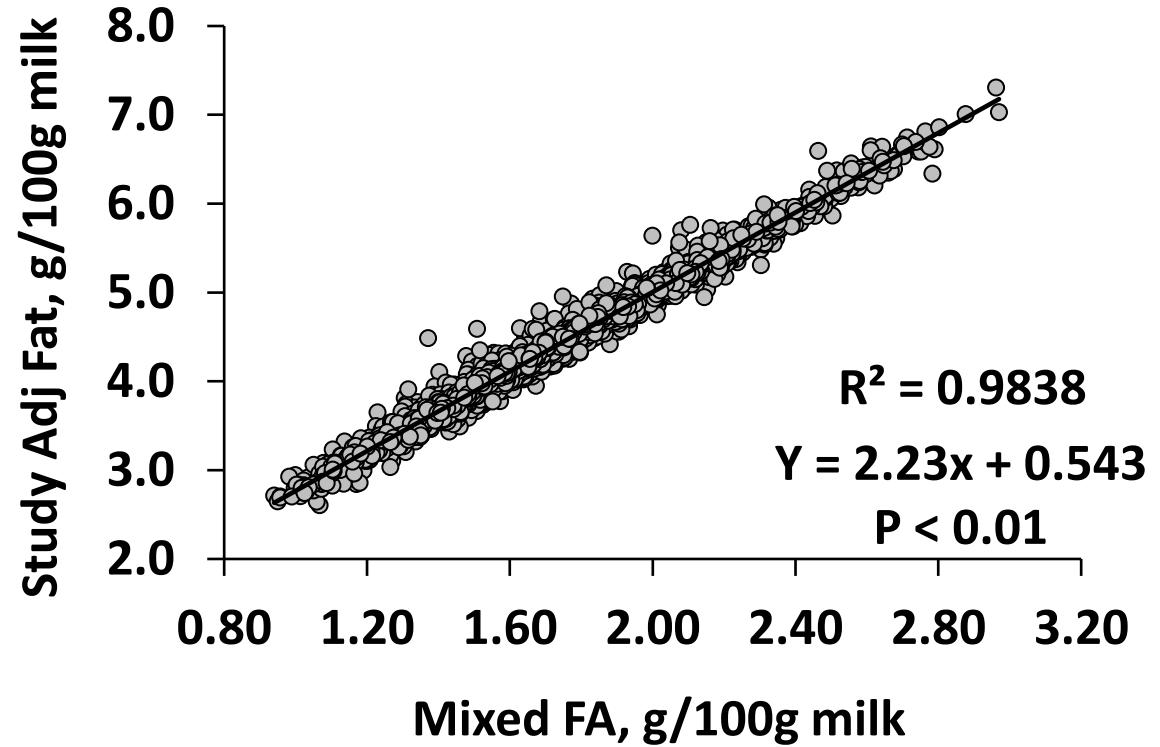
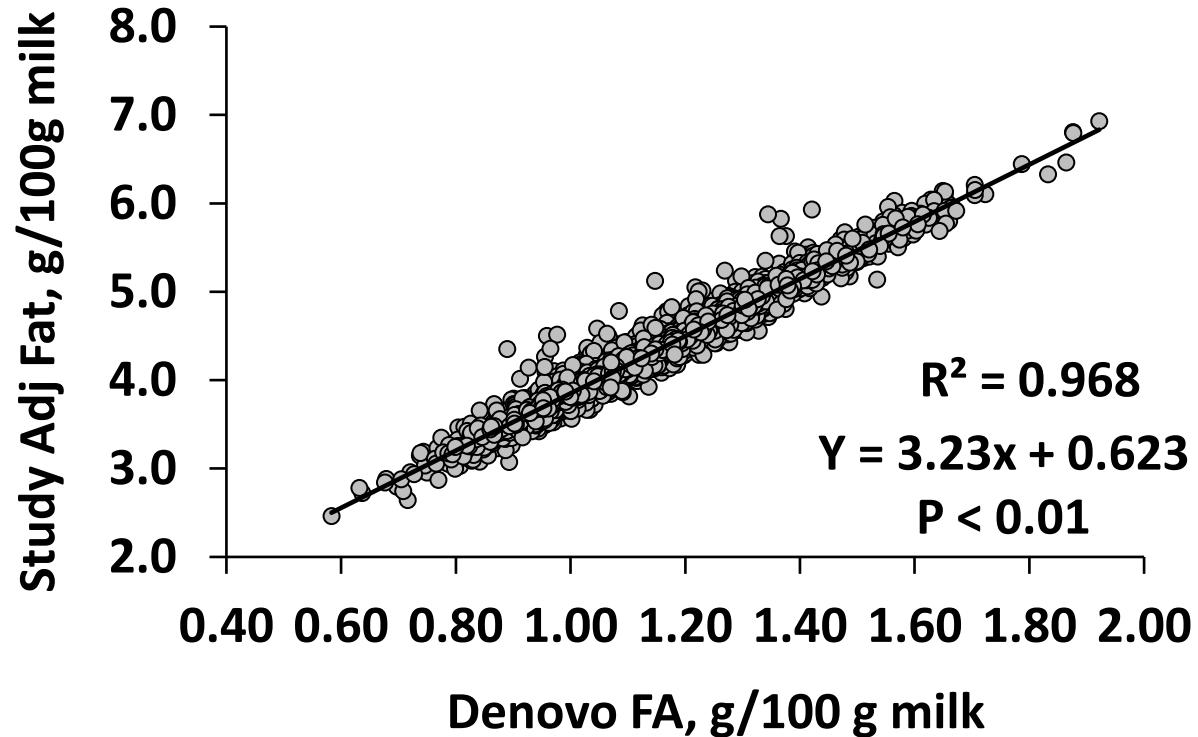
Diet, g Metabolizable Met/Mcal ME

Parameter	0.86	1.05	1.19	SEM	Treatment
Milk fat, g/100g milk					
Denovo	1.14^a	1.17^b	1.20^b	0.010	< 0.01
Mixed	1.65^x	1.67^{xy}	1.70^y	0.015	0.07
Preformed	1.16	1.15	1.19	0.013	0.20
Milk fat, % Total fat					
Denovo	28.79^a	29.33^b	29.34^b	0.09	< 0.01
Mixed	41.83	41.61	41.56	0.15	0.40
Preformed	29.33	29.08	29.07	0.17	0.43

Effect of increasing Met on De novo FA Synthesis



Relationship between De novo and Mixed FA and Milk Fat when balanced for AA



- **5,116 Individual Cow Observations from 3 studies**
- Study 1: Met and His 1.19g/Mcal ME and Lys 3.20g/Mcal ME; Study 2: Met increased from 0.86g/Mcal ME to 1.19g/Mcal ME (3 treatments) and His and Lys set at 100%, Study 3: Lys increased from 2.98g/Mcal ME to 3.18g/Mcal ME and Met and His set at 100%

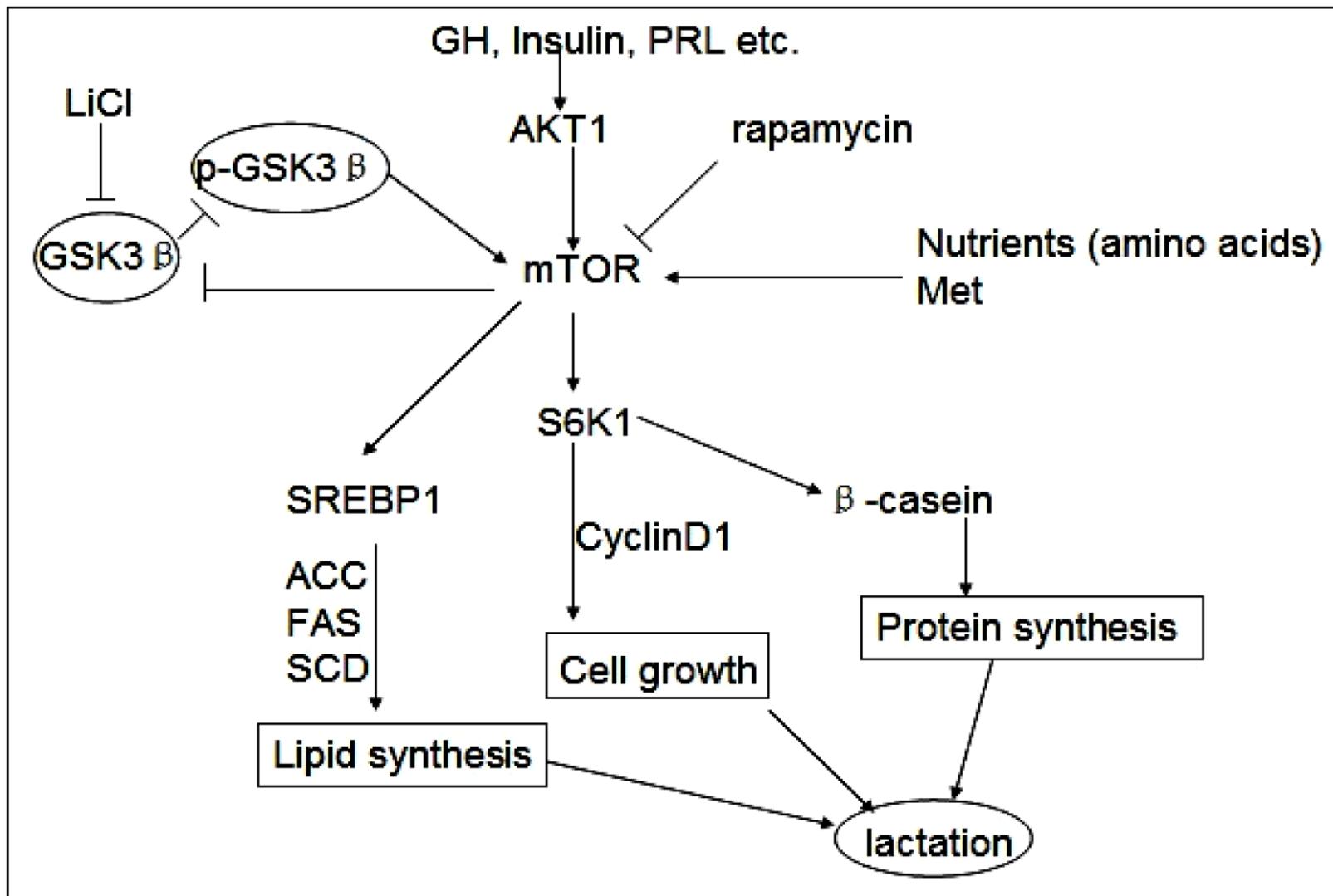
CNCPS Version 7 Predicted AA Supply for Two Diets

CNCPS Version 7 Predicted AA Supply											Diet #1 Analyzed Milk Data	
Diet #1	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Ave Trial DIM	193 ± 50
AA Supply, g/d	188	83.5	175	294	255	92.5	183	175	49.8	202	DMI, kg	27.0
AA Req, g/d (ME basis)	156	82.2	181	272	240	94	177	156	50.3	192	Milk, kg	39.5
AA Balance, g/d (ME basis)	+33	+1.3	-5.5	+22	+15	-1.5	5.5	+18	-0.6	+10	ECM, kg	45.2
AA Balance, % (ME basis)	121%	102%	97%	108%	106%	98%	103%	112%	99%	105%	Fat, %	4.53
Diet #2	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Fat, kg	1.79
AA Supply, g/d	177	77.0	171	278	272	93.4	174	167	47.9	191	Protein, %	3.37
AA Req, g/d (ME basis)	160	84.7	187	281	247	97.0	183	161	51.9	198	Protein, kg	1.33
AA Balance, g/d (ME basis)	+17	-7.7	-16	-3.0	+25	-3.6	-8.4	+6.6	-4.0	-6.7	Diet #2 Analyzed Milk Data	
AA Balance, % (ME basis)	110%	91%	91%	99%	110%	96%	95%	104%	92%	97%	Ave Trial DIM	157 ± 20
											DMI, kg	27.6
											Milk, kg	46.7
											ECM, kg	49.4
											Fat, %	3.98
											Fat, kg	1.86
											Protein, %	3.14
											Protein, kg	1.47

- How do we interpret the metabolic interaction between amino acids and milk components, particularly fat?

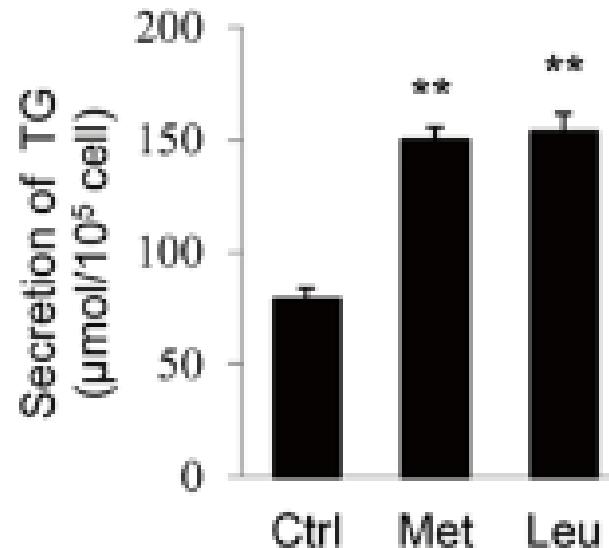


Pathways and Regulatory Signals for Regulation of Protein Synthesis in the Mammary Gland

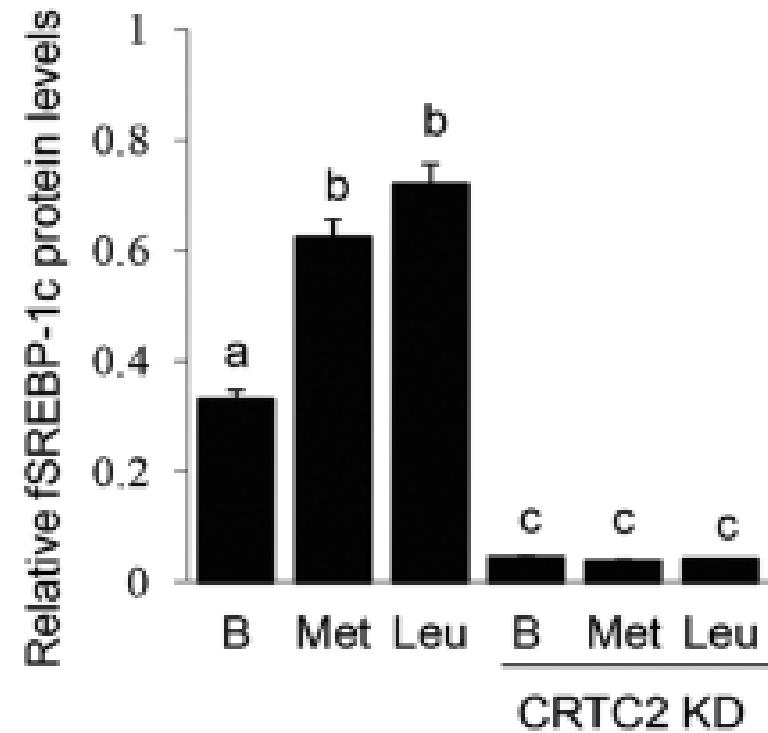
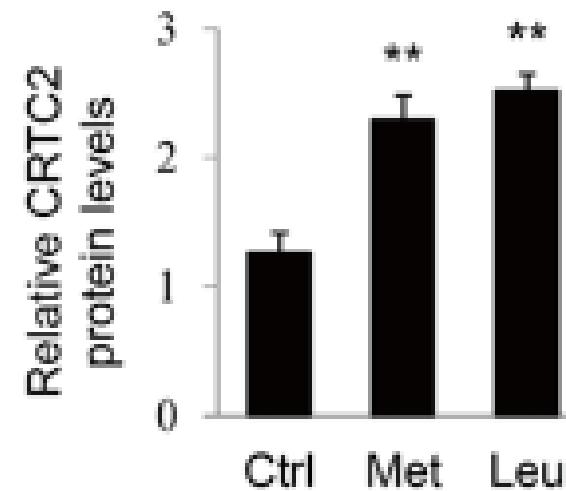


Leu and Met increase SREBP1 synthesis through transcription cofactors in BMEC

D

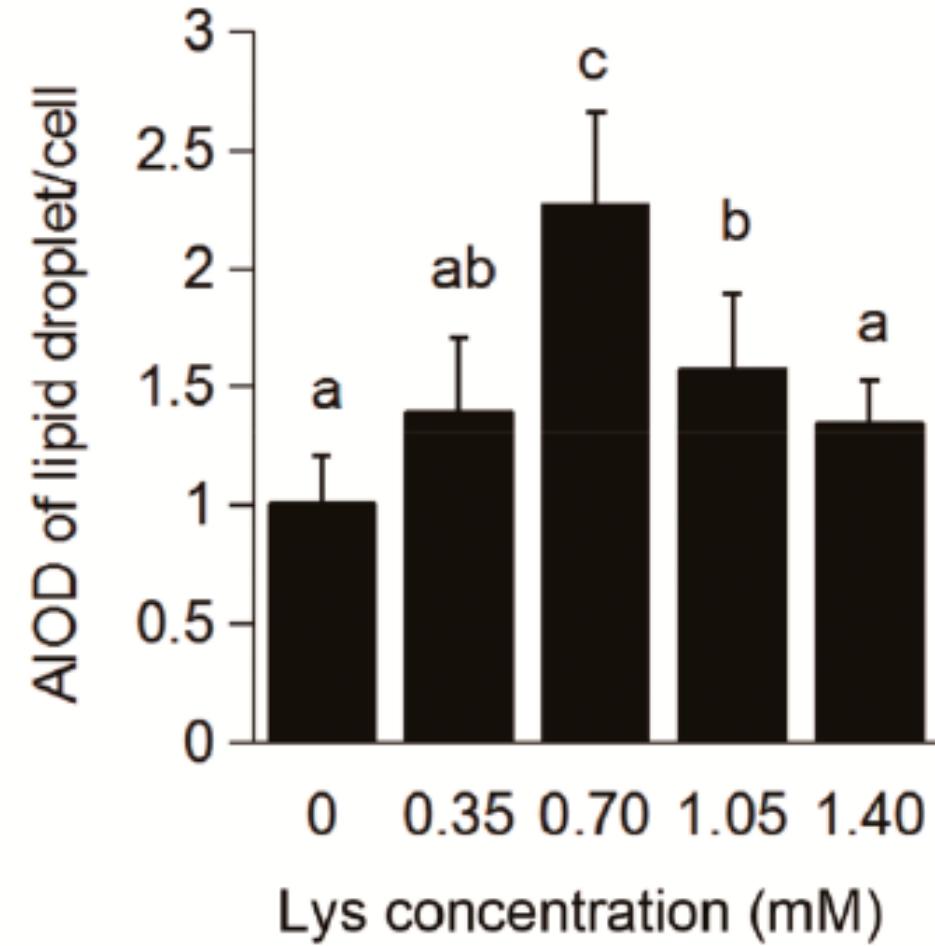
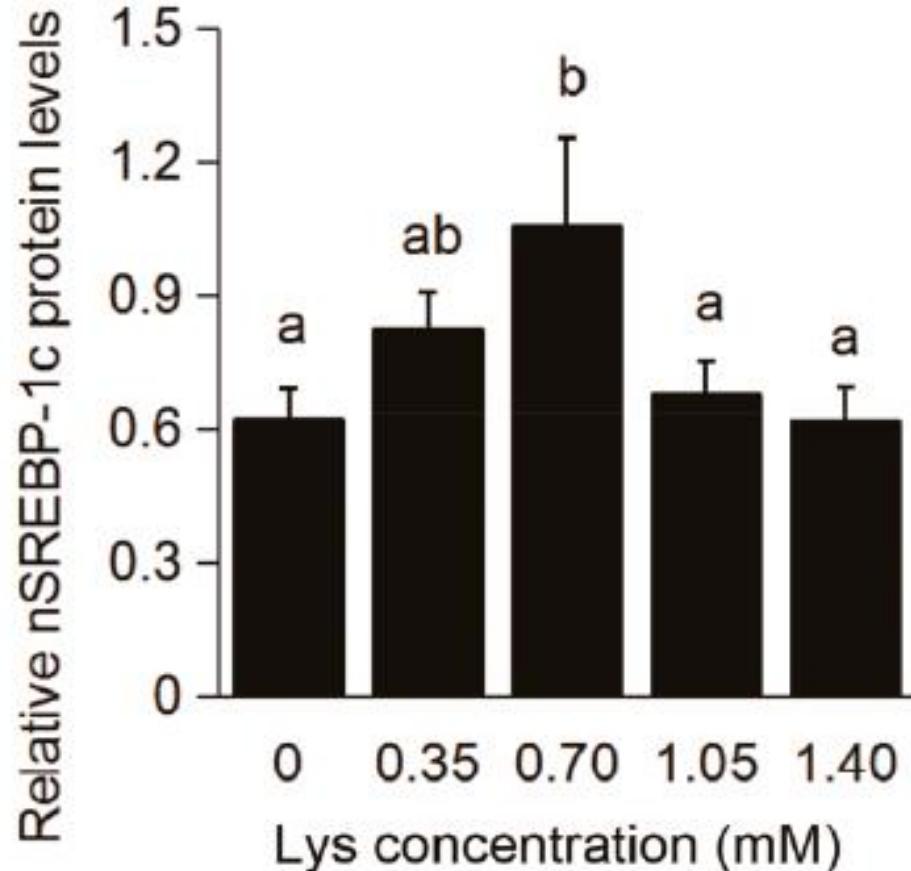


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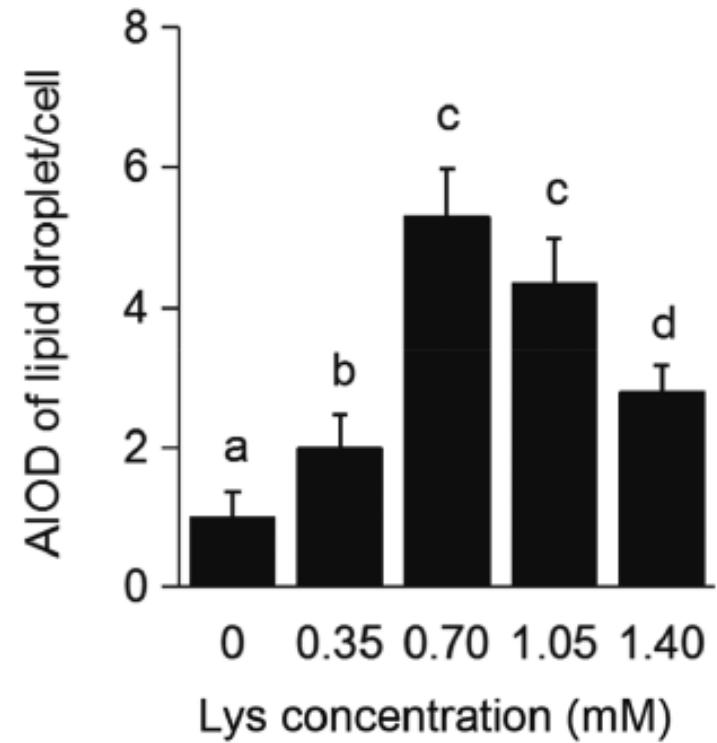
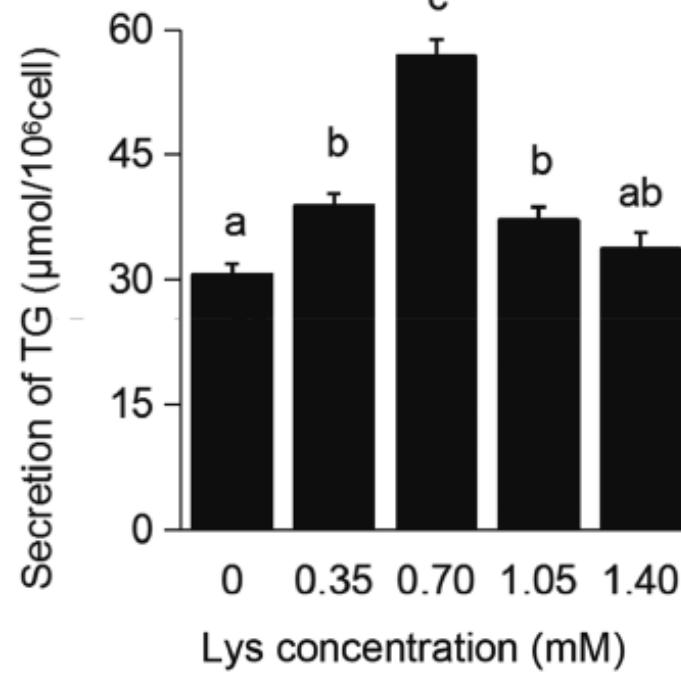
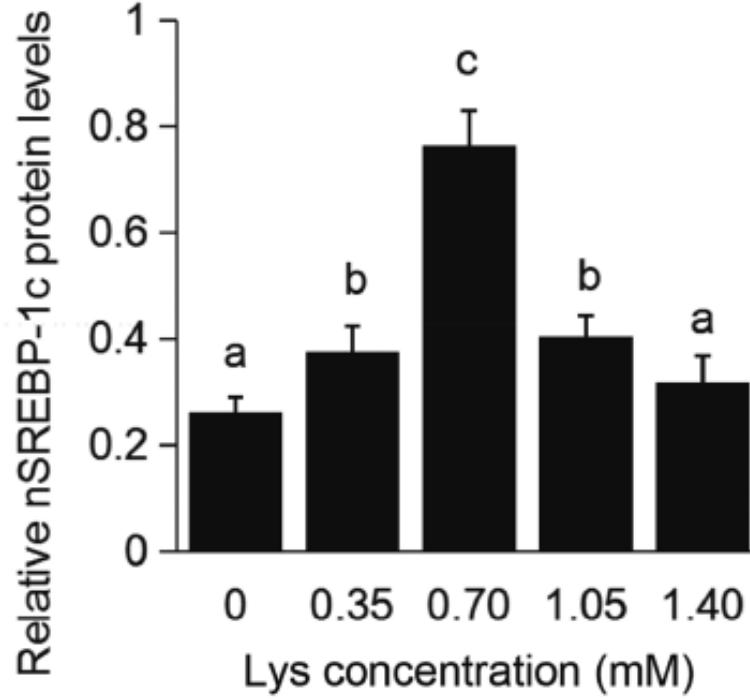


- Met and Leu increase secretion of TG in BMEC (D) through increasing the expression of CRTC2 (E), a c-AMP regulated transcription activator and CRTC2 mediated the effect of Leu and Met on SREBP1 expression
- CRTC2 involved in glucose metabolism and may be involved in lactose synthesis, cross-interactions between nutrients and milk component synthesis

Effects of Lys in Bovine Mammary Cells on Milk Fat Synthesis in the Absence of Fatty Acids

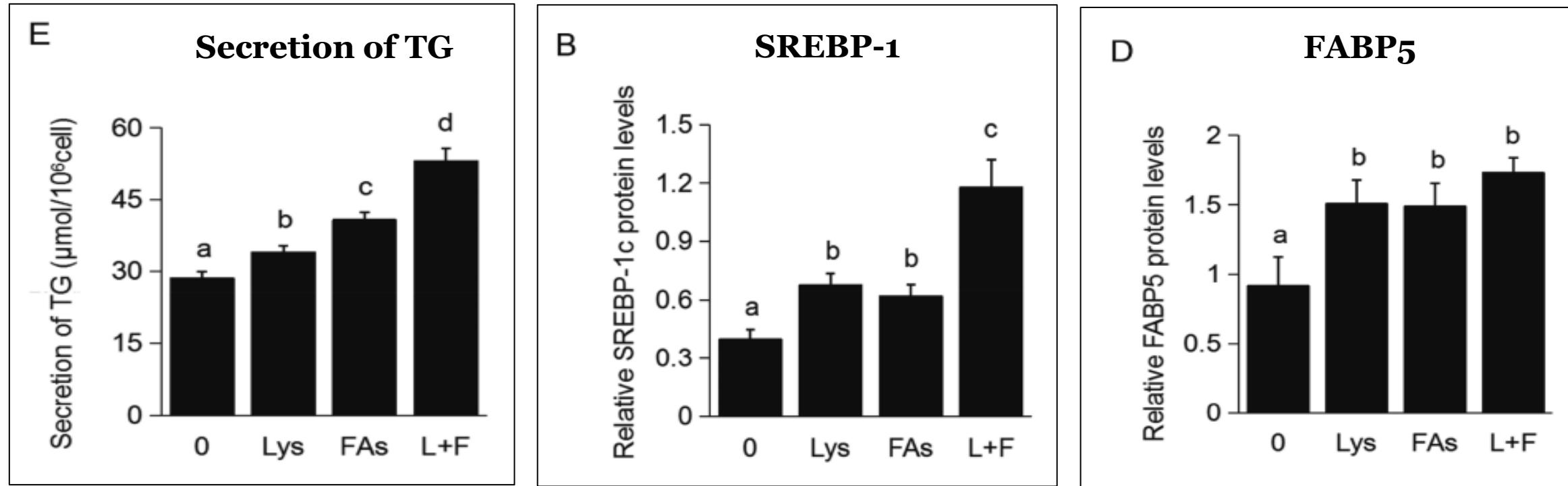


Effects of Lys in Bovine Mammary Cells on Milk Fat Synthesis in the Presence of Fatty Acids (50 μ M Palmitic Acid and 50 μ M Oleic Acid)



Lysine and FA in Bovine Mammary Cell Culture

- In BMEC, Lys increased relative protein levels of FABP and SREBP-1—a key regulator of milk FA synthesis
 - Further increased when supplemented with palmitic acid (PA) and oleic acid (OA)



0 = no treatment, Lys = 0.70 mM lysine, FAs = 50 μ M PA and 50 μ M OA, L+F = Lys and FAs

Li et al., 2019

Lysine and Milk Fat

- In this study , using bovine mammary epithelial cells, Lysine-induced fatty acid-dependent SREBP-1c expression and maturation was used. SREBP-1c
- SREPB-1 is a key regulator of fatty acid synthesis in the mammary gland (Li et al., 2014) and is also sensitive to insulin
- This was done through regulation of the GPRC6A- the G protein-coupled receptor class 6A – which induces the PI3K/AkT (phosphatidylinositol 3-kinase) pathway
- FABP5 – Fatty acid binding protein 5 which regulates lipid metabolism

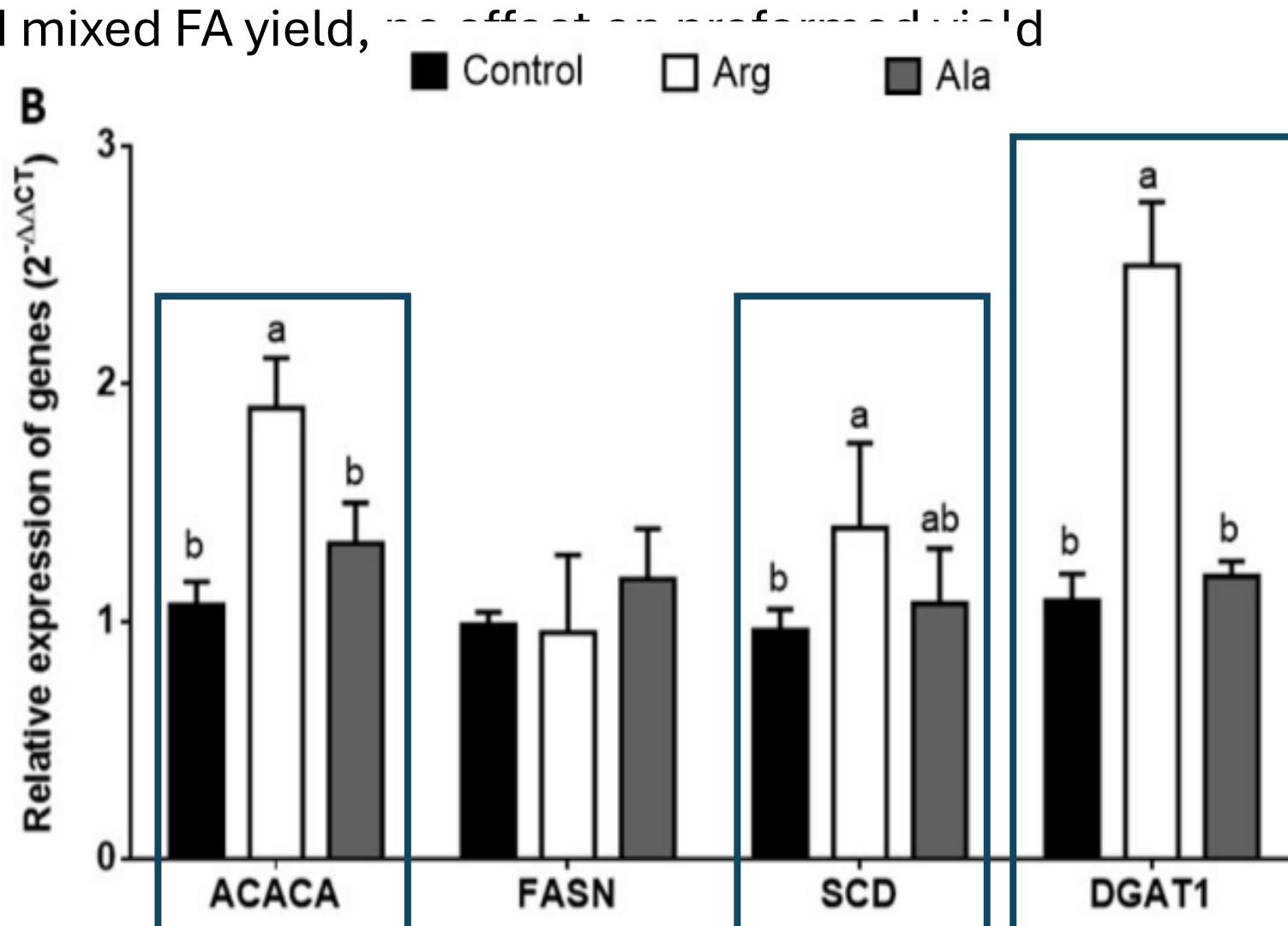
Fatty Acid Synthetase (FAS)

- FAS synthesizes de novo FA by elongating FA carbon chain
- Active sites with AA essential for function and transfer of intermediates during elongation of de novo FA
 - His, Lys, Ser, Cys (Smith et al., 2003; Wettstein-Knowles et al., 2005)
- FAS expression decreased in His- and Lys-deficient human liver cell medium (Dudek and Semenkovich, 1995)
 - This was reversible when His and Lys were reintroduced
- Expression of FAS increased by adding both NEAA and EAA compared each treatment individually (Fukuda and Iritani, 1986)
 - FAS complex likely has requirement for both types of AA

Effect of Arginine Infusion on Milk Fat

- Arg increased milk de novo and mixed FA yield,
- Arg increased the expression of genes involved in FAS

Fatty acid ^{1,2}	Control	Arg
De novo	281.3 ^b	347.7 ^a
Mixed	336.5 ^b	371.4 ^a
Preformed	428.0	404.6



Least squares means for dry matter intake, feed efficiency (FE), milk yield, and milk composition of cows fed a common diet and infused abomasally with water (C), histidine (H), proline (P), or a combination of both AA (H+P).

Variable	Treatment				SE	<i>P</i> ¹
	C	H	H+P	P		
DMI, kg/d	26.6 ^a	26.3 ^{ab}	25.1 ^{bc}	24.8 ^c	0.5	0.04
FE, kg 3.5% FCM/ kg DM	1.95 ^b	1.92 ^b	1.95 ^b	2.11 ^a	0.08	0.07
Yield						
Milk, kg/d	50.2	49.6	48.0	48.7	1.7	0.44
3.5% FCM, kg/d	51.8	50.6	49.0	52.4	2.5	0.34
Fat, g/d	1871.7 ^{†‡}	1804.6 ^{†‡}	1736.9 [†]	1929.7 [‡]	116.1	0.29
Lactose, g/d	2433.9	2427.5	2324.3	2423.9	94.2	0.36
Protein, g/d	1471.8 [†]	1473.6 [†]	1369.8 [‡]	1409.7 ^{†‡}	74.2	0.25
Milk composition, %						
Fat	3.70	3.60	3.63	3.95	0.15	0.29
Lactose	4.85 ^b	4.89 ^b	4.83 ^b	4.97 ^a	0.03	0.01
Protein	2.93	2.96	2.85	2.89	0.06	0.33



How does this work on well managed commercial dairies?

~4,000 Cow Herd in Northern NY – Three bulk tanks - Holsteins and Jerseys (Easter Week 2025 Test) – still holding over 4.2% in June

April		Butterfat			Protein	
Total	Average	4.933913			3.546196	
	Pounds	156,888.37			112,761.81	
Tank	2025	45371-1	45371-2	45371-3	45371-1	45371-2
	Average	4.891320	4.866001	5.425409	3.530502	3.535603
	Pounds	70,962.74	66,794.92	19,331.87	51,220.13	48,532.73
		Holstein	Holstein	Jersey	Holstein	Jersey

~250 genomic Holstein heifers in the same herd on a similar diet –
40+ kg milk, >5.2% fat, >3.8% protein (fat up to 6.5% and protein over 4%)

1,700 Cow Holstein Dairy in Central NY

15,426 kg herd average- Bulk tank fat 4.9-5.0% and true protein 3.5%-
3.6%

Energy-corrected milk (ECM) in the high group >72 kg

ECM feed efficiency of the high group ~2.1

Contextualizing EAA Supplies and Requirements- CNCPS v7 translated to v6.55 and 6.56

- Methionine – 1.19 grams per Mcal ME intake
- Histidine – 1.19 grams per Mcal ME intake
- Lysine – 3.2 grams per Mcal ME intake

Let's assume 59 kg ECM, so that's about 87 Mcals ME

Methionine: $87 * 1.19 = 103$ grams

Histidine: $87 * 1.19 = 103$ grams

Lysine: $87 * 1.19 = 278$ grams

Summary Statements

- Under current selection conditions, cows are an innovation, and we likely have a genotype we don't fully understand
- Data demonstrate that meeting the amino acid requirements enhances energetic efficiency equal to nitrogen efficiency and directly impacts milk fat synthesis
- Holstein cattle can produce milk fat similar Jersey cattle when fed an appropriate diet – meeting the requirements
- We are moving closer to being able to understand how to modify milk composition a proactive manner and this will require more integrated research with amino acids, fatty acids and certain carbohydrates

Thank you for your attention,
for everyone who helped
develop this work, and for
the sponsors who keep it
going

