Considerations of Amino Acid Utilization in Dairy Cattle – Focus on Lysine

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Introduction

- Feed efficiency and carbon intensity are directly correlated
  - Any increase in feed efficiency reduces carbon intensity (feed C neutral)
- Essential amino acids are required for protein synthesis, nutrient signaling, and conversion to other metabolites like non-essential amino acids, enzymes and hormones
- The system is constantly running, but it is not always using the energy efficiently – parallels energy spilling in bacteria
- There is an obligate requirement for amino acids in fatty acid synthesis and all of this is integrated in liver and mammary metabolism but is not well discussed

Meta-Analysis of Dietary Methionine and Lysine Impacts on Milk Protein

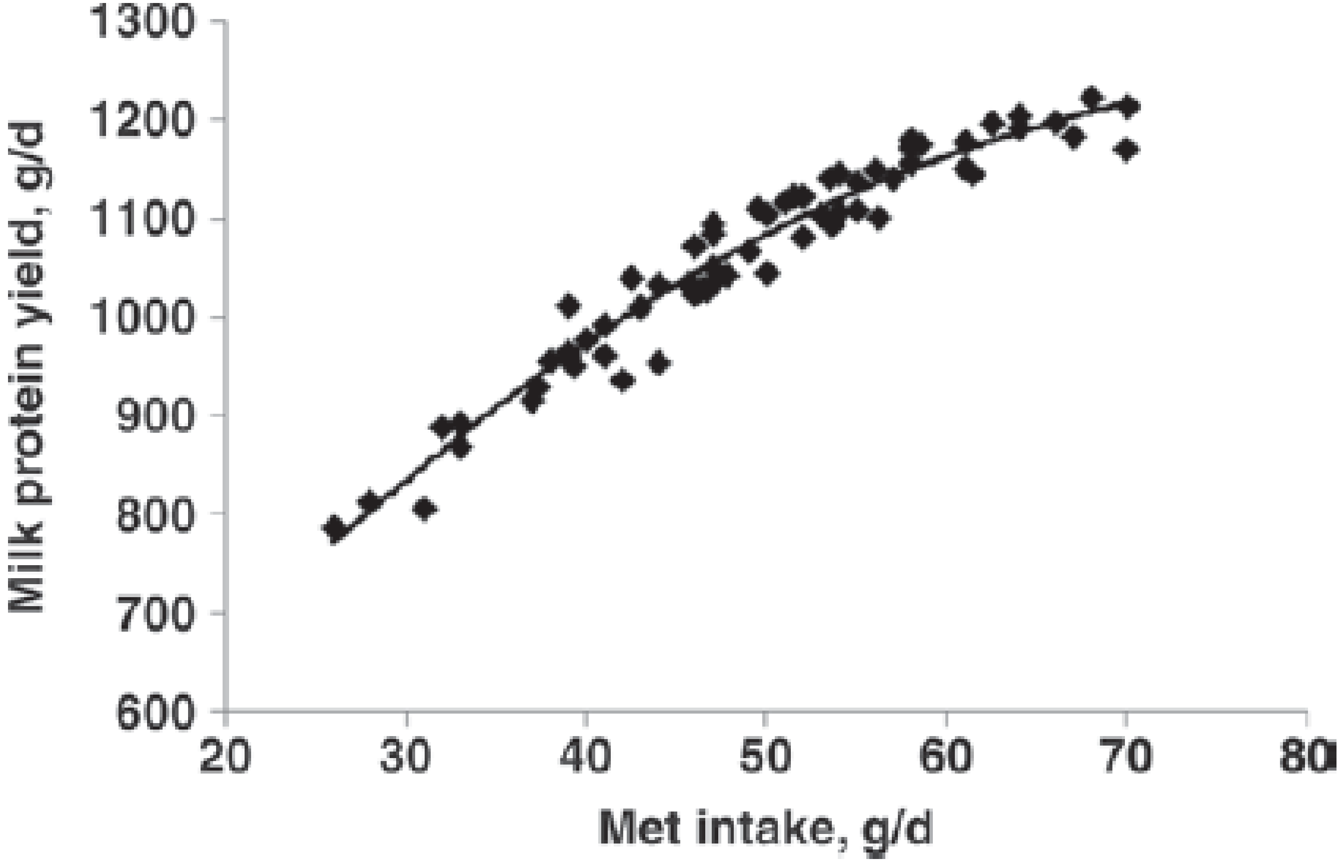

Figure 3. Plot of experiment adjusted milk protein yield (g/d) versus model-predicted milk protein yield (solid line) response to Met intake (g/d).

Vyas and Erdman, 2009

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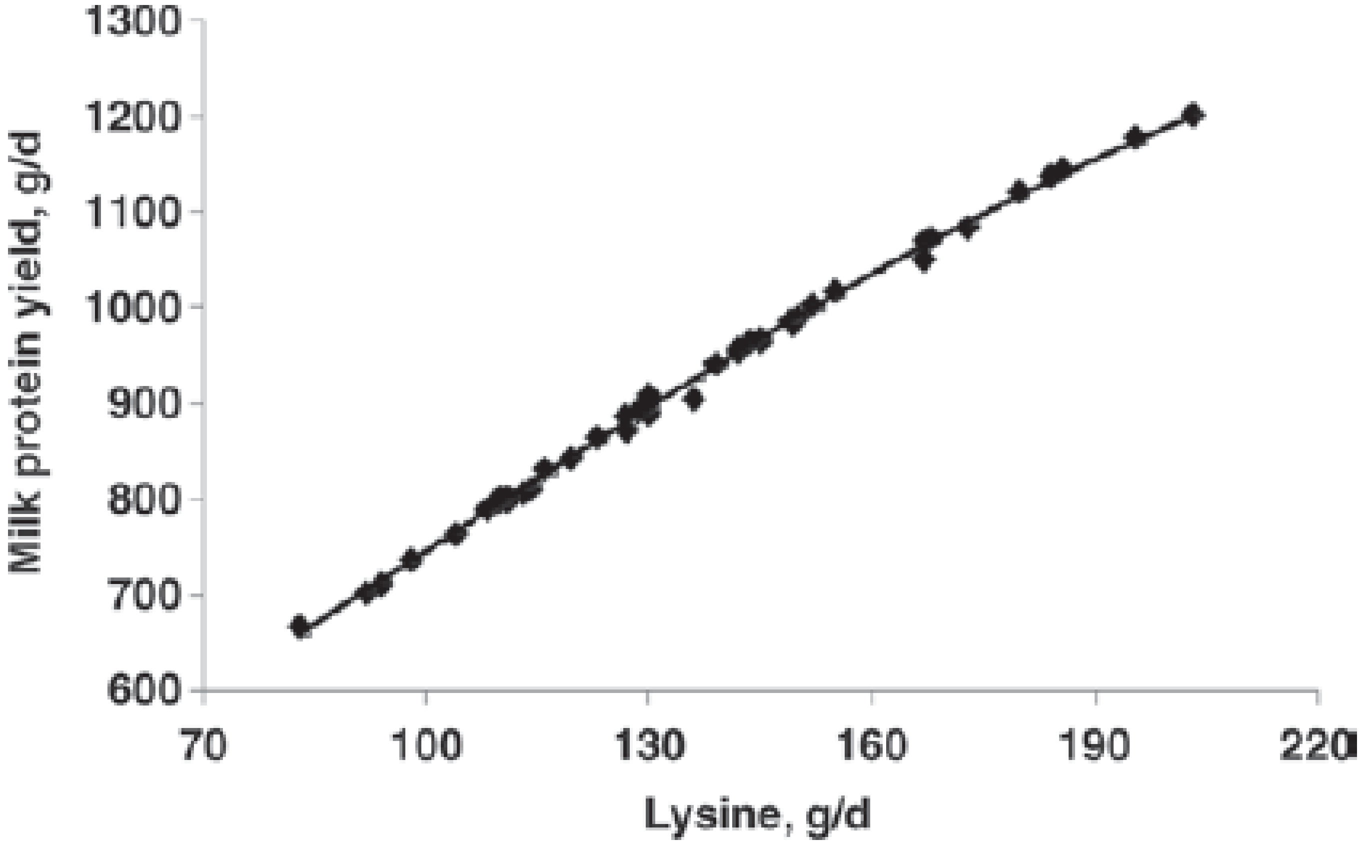
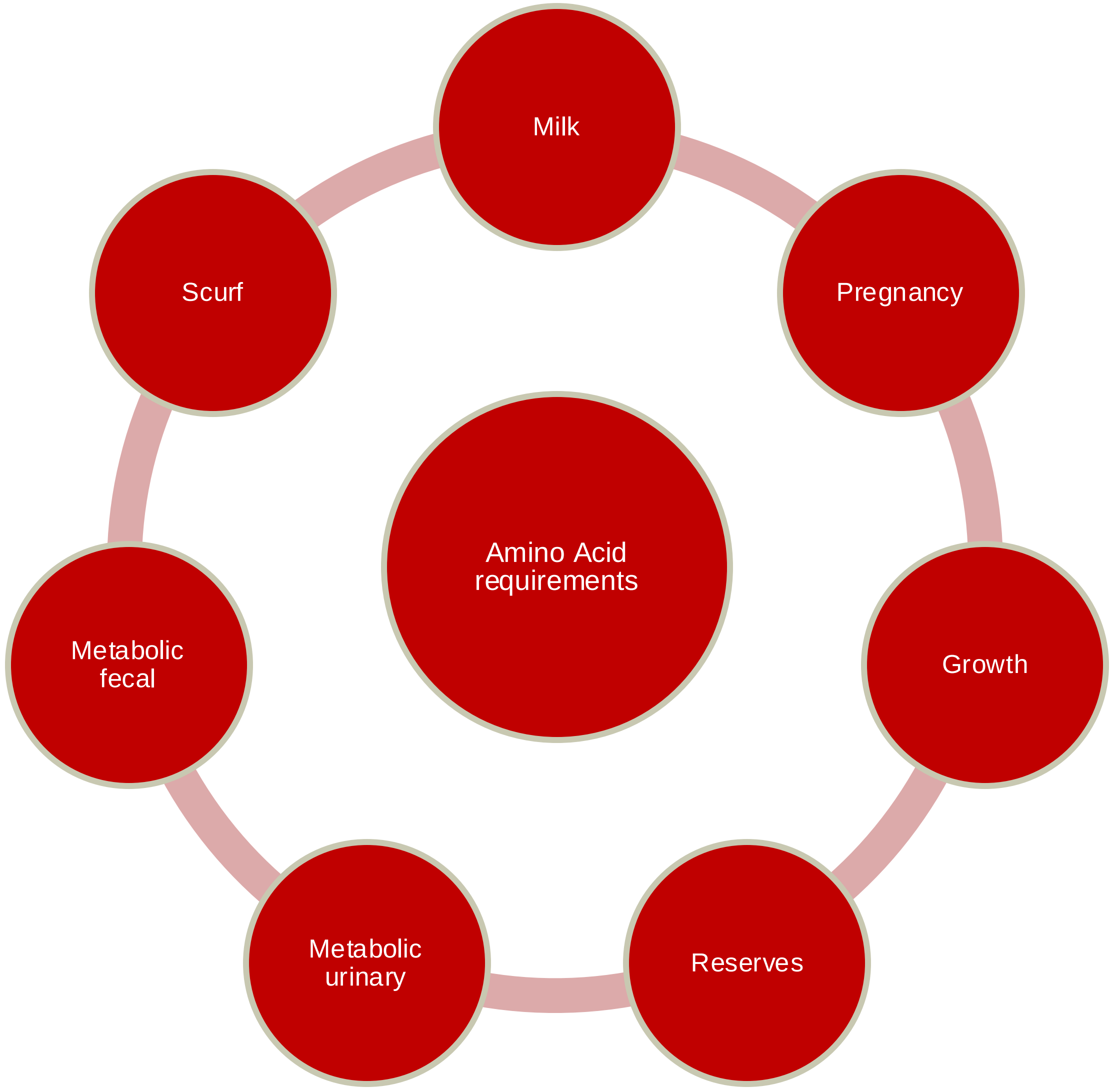


Figure 4. Plot of experiment adjusted milk protein yield (g/d) versus model-predicted milk protein yield (solid line) in response to Lys intake (g/d).

Vyas and Erdman, 2009

‘Efficiency’ Of Essential AA Use (Additional Requirements)



Amino Acid requirements

Milk

Pregnancy

Growth

Reserves

Metabolic urinary

Metabolic fecal

Scurf

⇨ Oxidation

⇨ Anaplerosis

⇨ Synthesizing non-essential AA

⇨ Gluconeogenesis

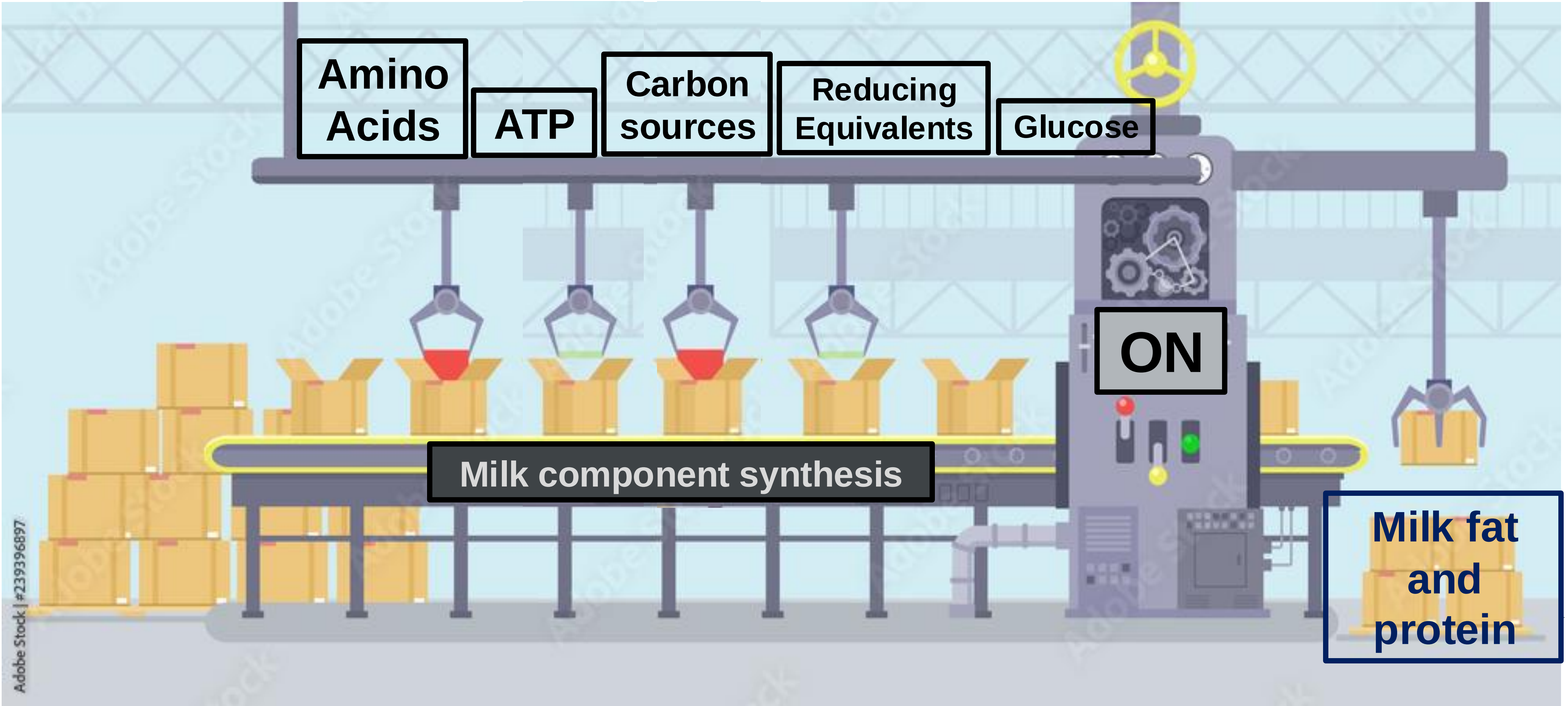
Protein-energy interactions

"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.

The Conveyor Belt of Milk Component Production

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



Nutrient signaling and metabolic flexibility in the mammary gland: Key to improved NUE?

Mammary gland is one of the most adaptable organs in mammals

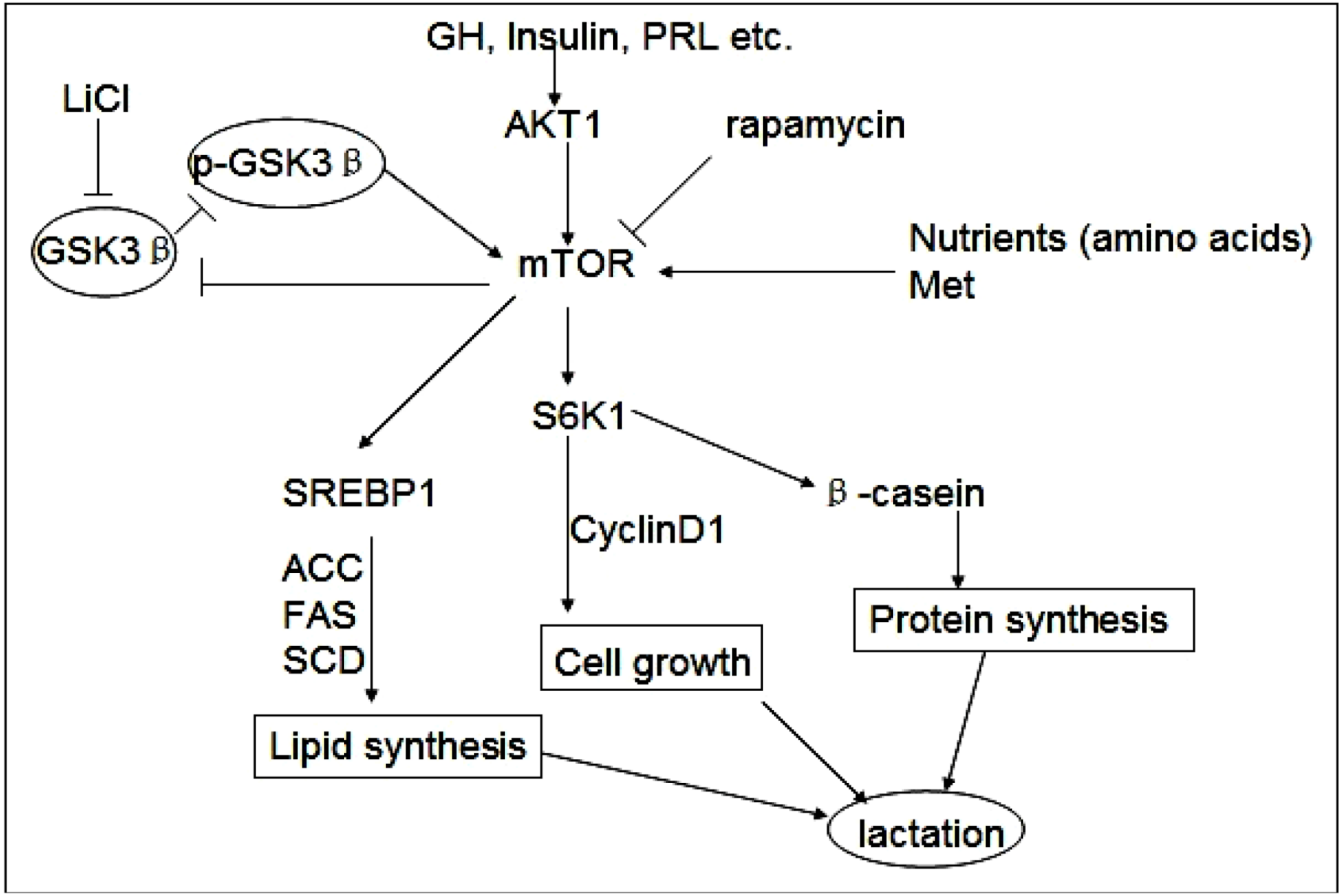
- Main sources of nutrient uptake for intermediary metabolism: acetate, glucose, ketones, and AA
- Ability to manipulate blood flow according to lactation requirements and in recognition of varying nutrient supply
- Uptake to output ratio of AA in mammary gland is not uniform across AA and changes in response to profile and supply of AA observed in circulation → Group 1, 2, and 3 AA

Milk protein synthesis requires activation/repression of key metabolic pathways

- mTORC1 and AMPk pathways
  - Activated through hormone signaling (insulin, IGF-1), intracellular nutrients (AA supply; Leucine), and energy status (ATP:AMP ratio)
- Integrated stress response (ISR) pathway
  - Reduces cellular anabolic load in the presence of intracellular stress
  - Indirectly inhibited by insulin and IGF-1 and ATP status
- Unfolded protein response (UPR) pathway
  - Restores endoplasmic reticulum homeostasis through multiple cellular responses
  - Initiation causes direct phosphorylation of PERK → activation of ISR pathway

Optimal supply of AA with improved energy status → Maximized anabolic output

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



https://doi.org/10.3390/molecules19079435

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

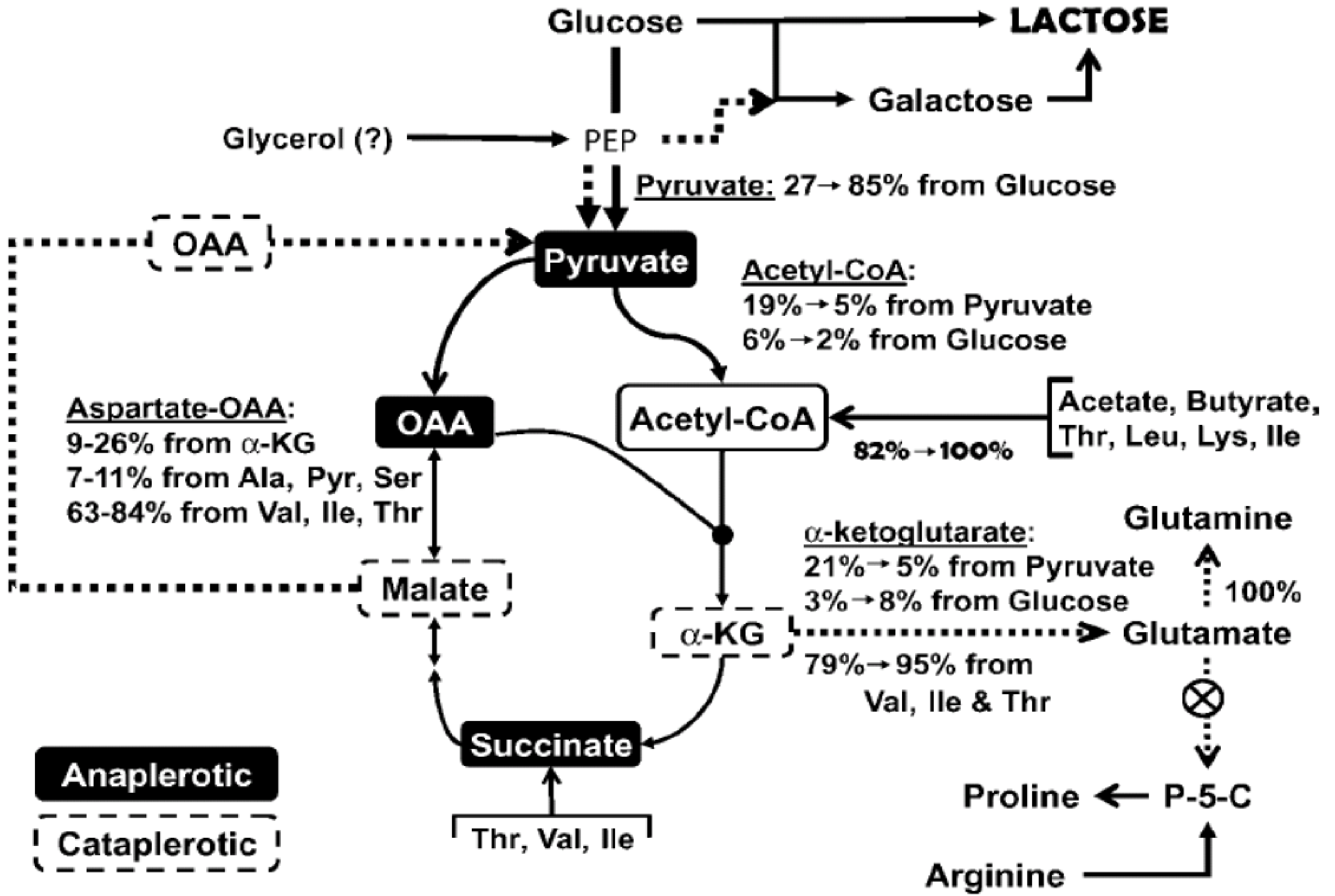
Arginine is taken up in drastic excessive relative to milk protein output (~2.5x)

- 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)

Interconversions in Mammary Gland Explants

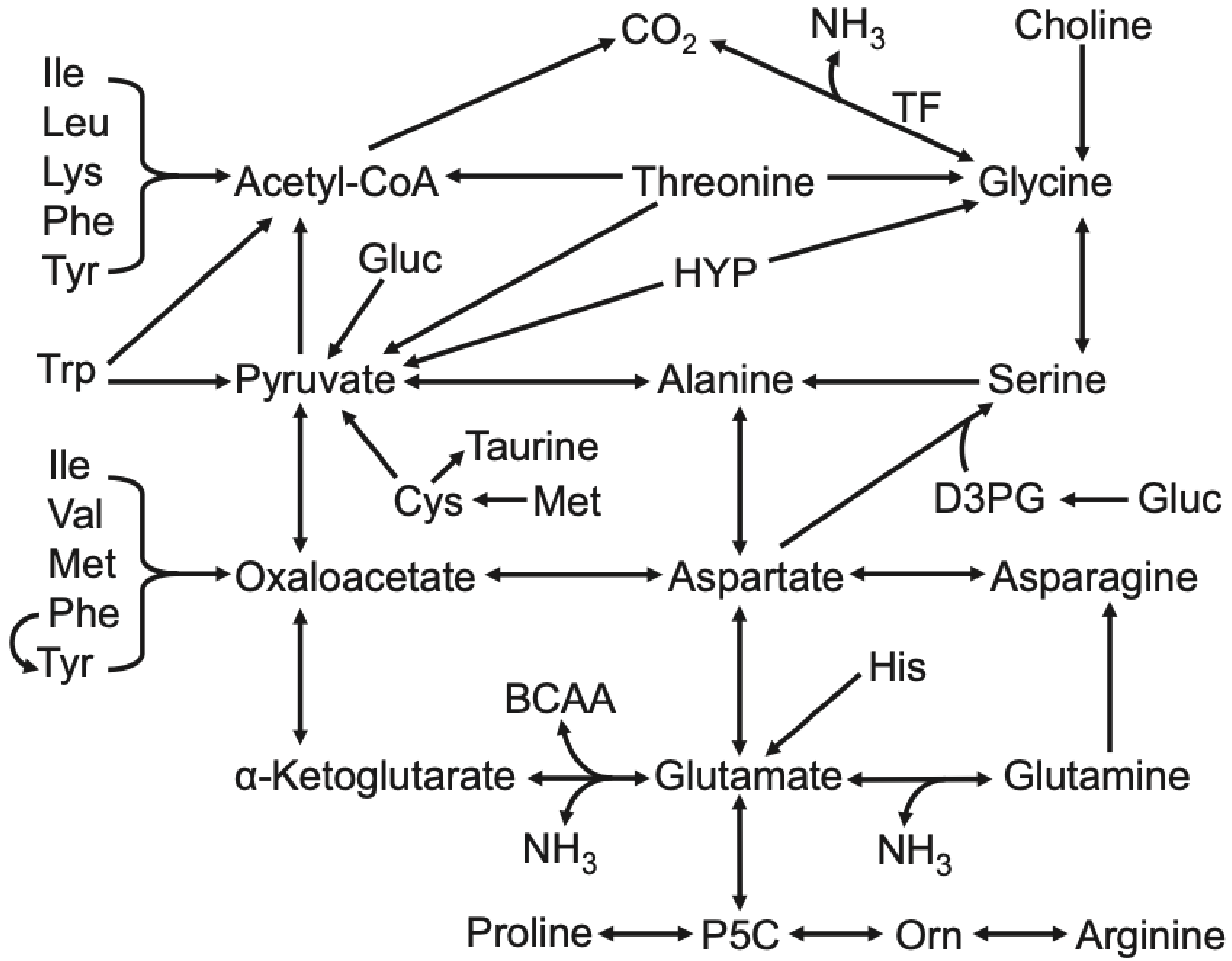


Bequette et al., 2006

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

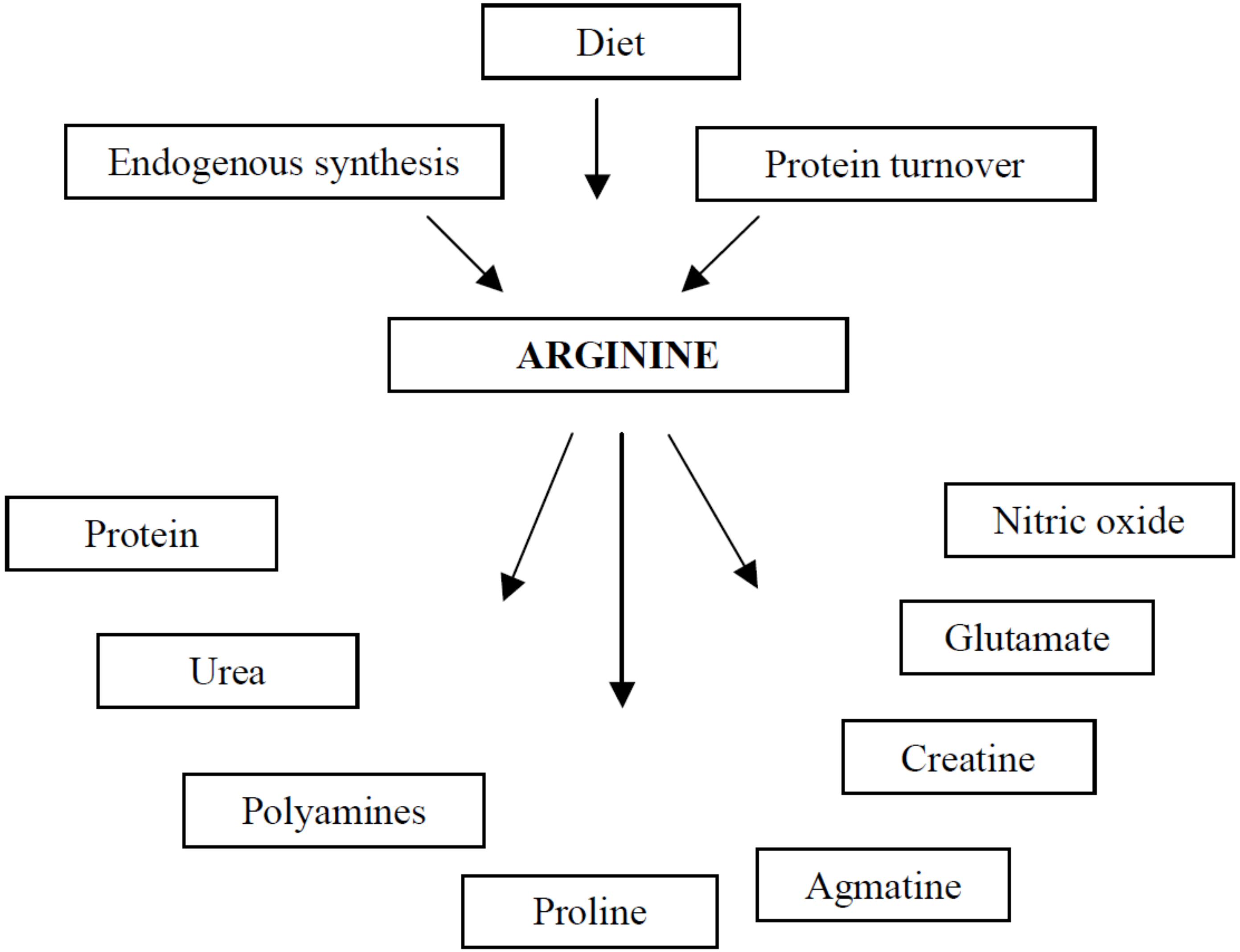
mmol/h Nitrogen	Control	Casein	Propionate	Casein + Propionate
Total uptake	163.0	189.5	178.0	212.8
EAA	81.3	100.7	86.4	109.2
NEAA	81.7	88.8	91.7	103.6
Total output	156.1	186.6	165.2	200.9
EAA	68.9	82.6	73.0	88.8
NEAA	87.1	104.3	92.2	112.2
EAA in - out	12.4	18.1	12.4	20.4
NEAA in - out	-5.4	-15.5	-0.5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

“Non-Essential” Amino Acids



Wu 2022

Sources and metabolic products of arginine. Adapted from (Morris, 2006).



Diet

Endogenous synthesis

Protein turnover

ARGININE

Protein

Urea

Polyamines

Proline

Nitric oxide

Glutamate

Creatine

Agmatine

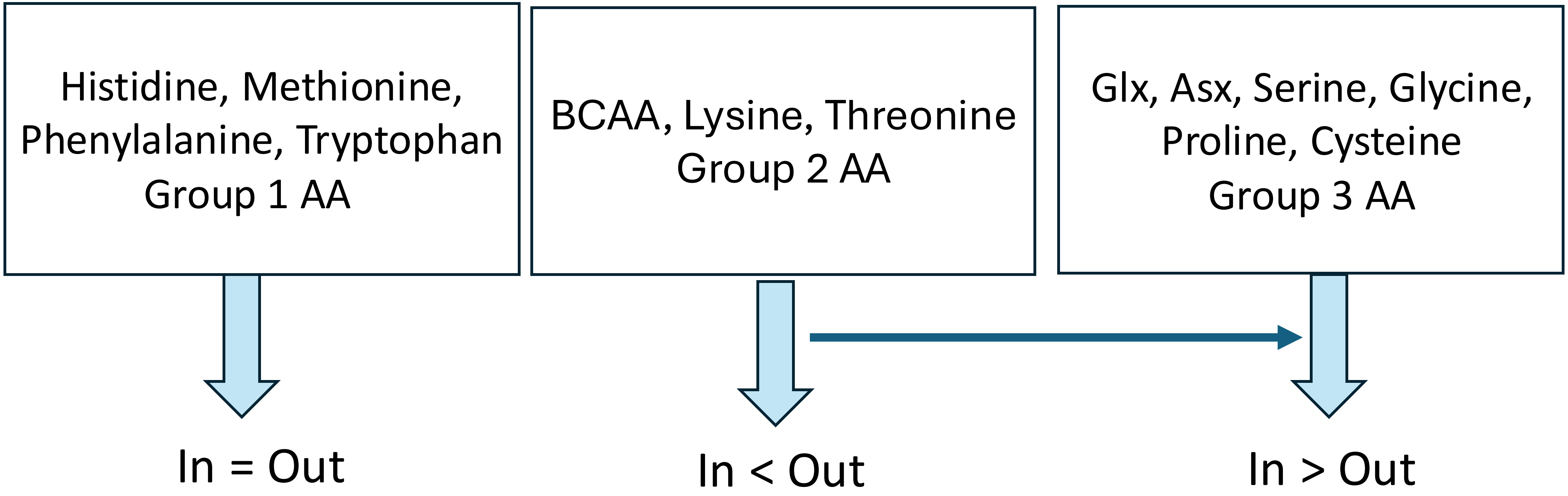
Lapierre et al. 2012

Mammary Arg uptake to output 2:45:1

Range 0.88 to 4.18

47 observations

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



Histidine, Methionine, Phenylalanine, Tryptophan
Group 1 AA

BCAA, Lysine, Threonine
Group 2 AA

Glx, Asx, Serine, Glycine, Proline, Cysteine
Group 3 AA

In = Out

In < Out

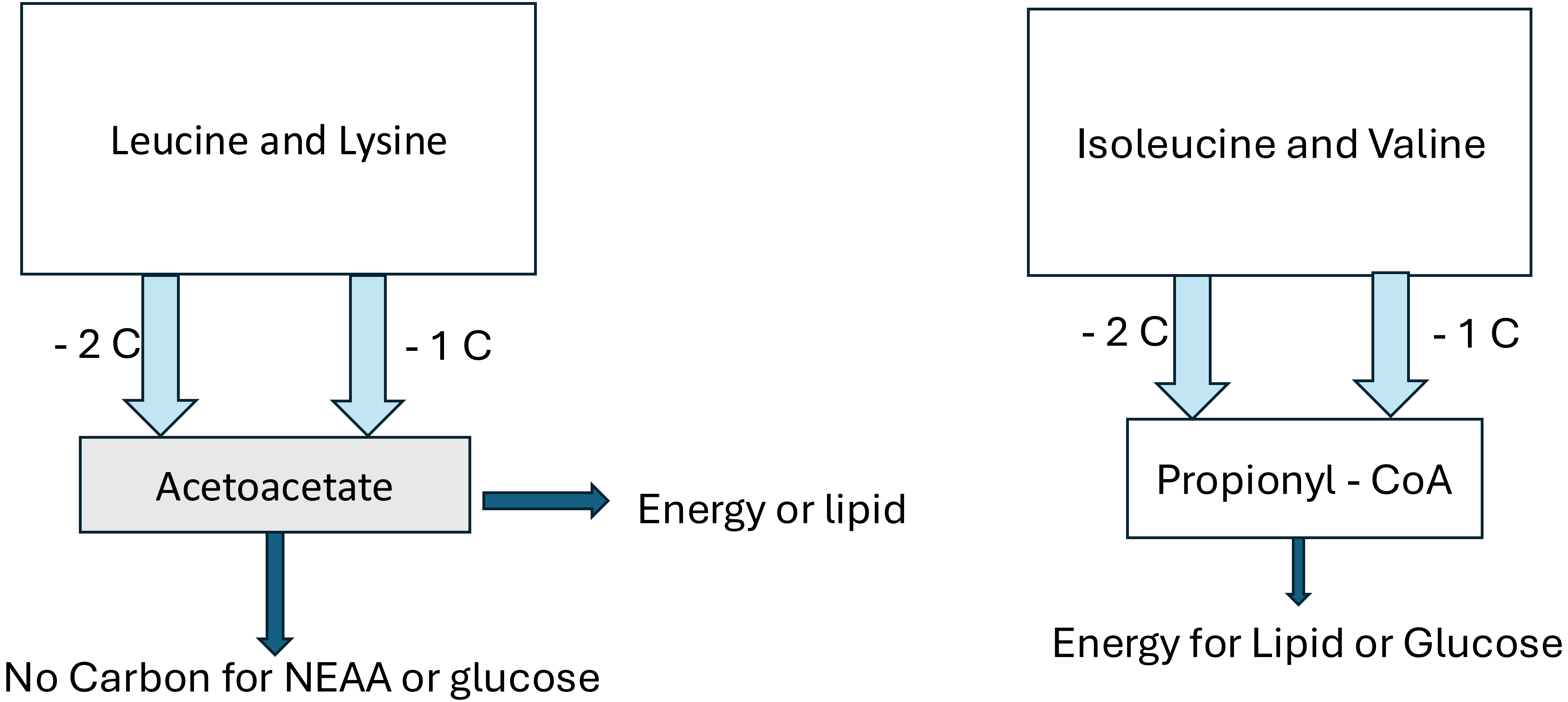
In > Out

Amino Acid Carbon Balance – Half Mammary Gland (grams)

	Group 1 AA	Group 2 AA	Group 3 AA	Total
Intake	134	275	212	621
Output	133	246	259	638
Difference	1	29	-47	-17

Lobley, 2007; Lemosquet et al., 2009

Fate of Carbon from Amino Acids – Lobley, 2007



Leucine and Lysine

- 2 C

- 1 C

Acetoacetate

Energy or lipid

No Carbon for NEAA or glucose

Isoleucine and Valine

- 2 C

- 1 C

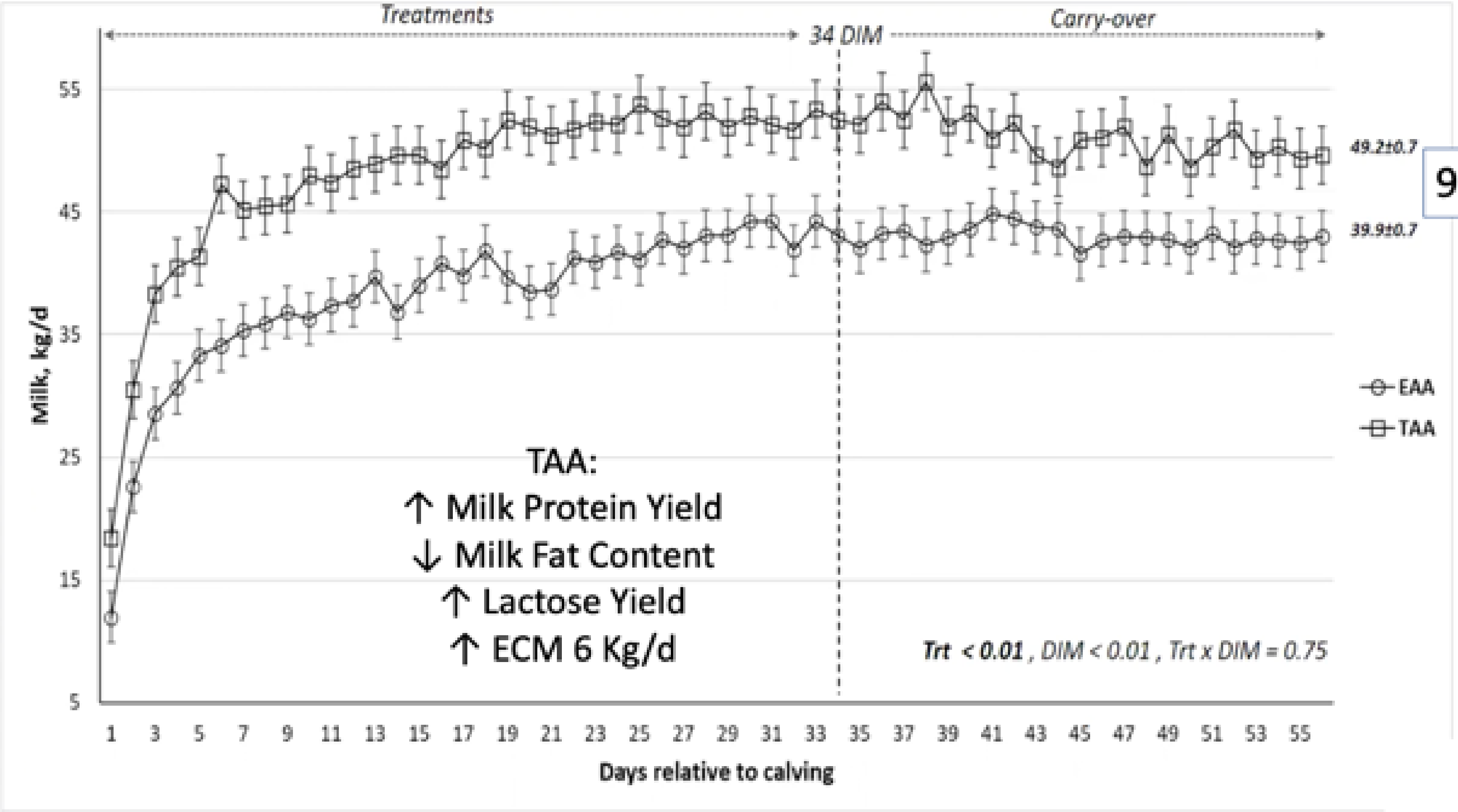
Propionyl - CoA

Energy for Lipid or Glucose

Non-Essential AA Infusions in Fresh Cows

Bahloul et al., 2021

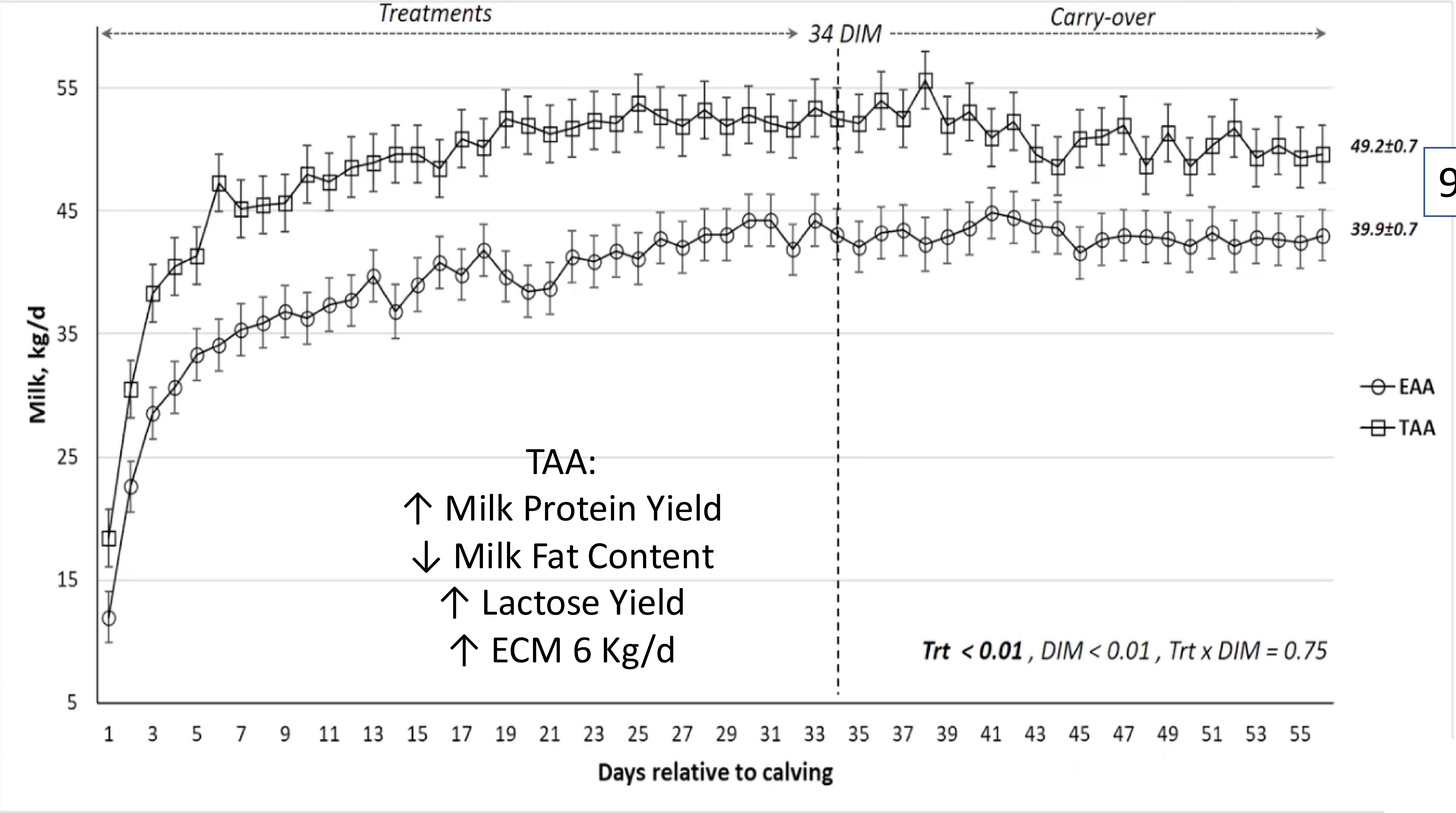
- 9 Holstein Cows, Calving to 50 DIM
- 2 Trts: TAA or EAA, Casein AA Profile
- Abomasal infusions



TAA:
↑ Milk Protein Yield
↓ Milk Fat Content
↑ Lactose Yield
↑ ECM 6 Kg/d

9 kg

Bahloul et al., 2021



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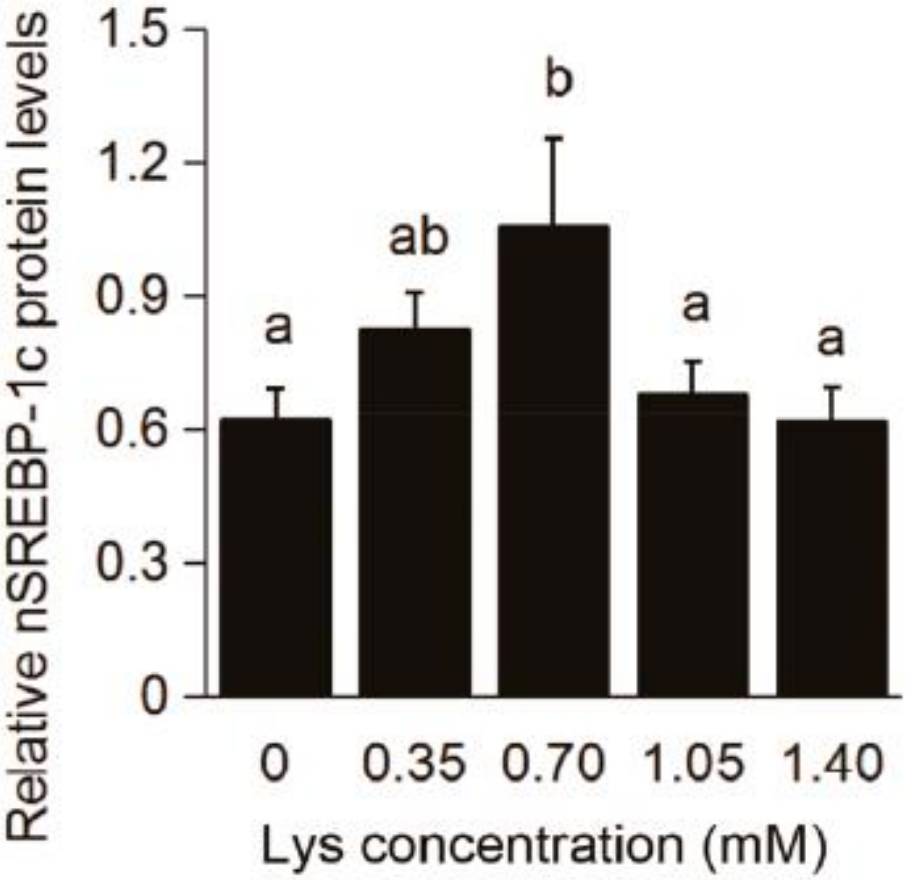
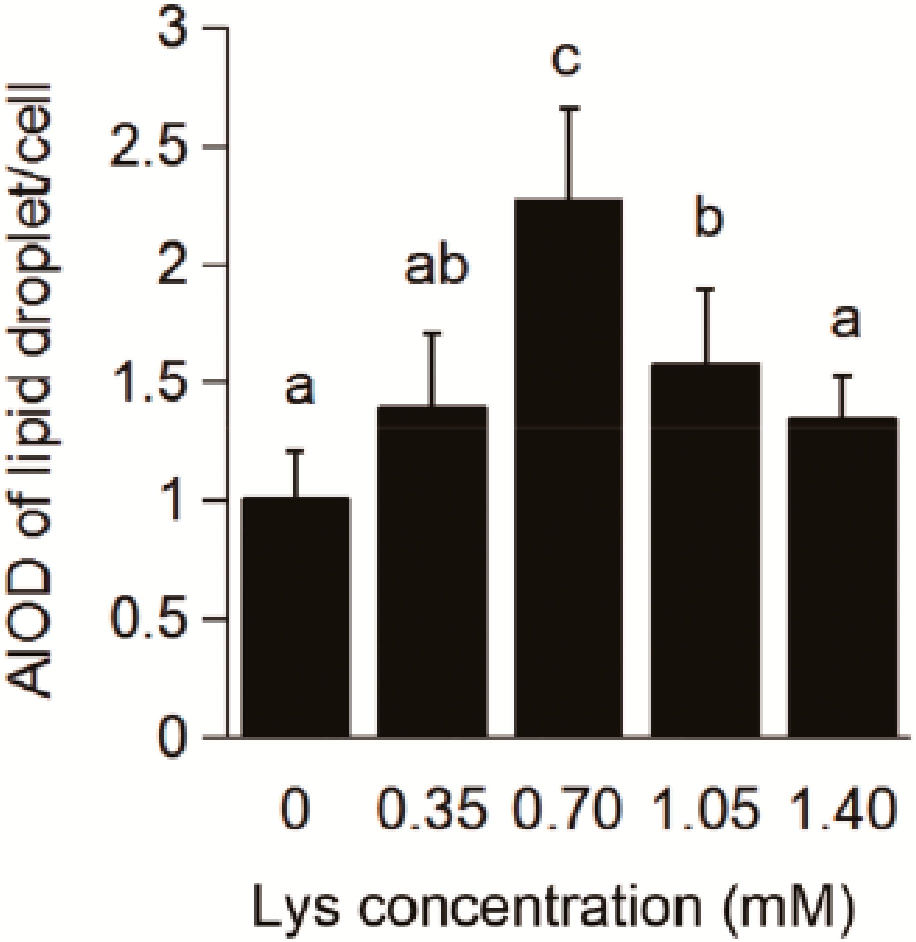
Bahloul et al., 2021

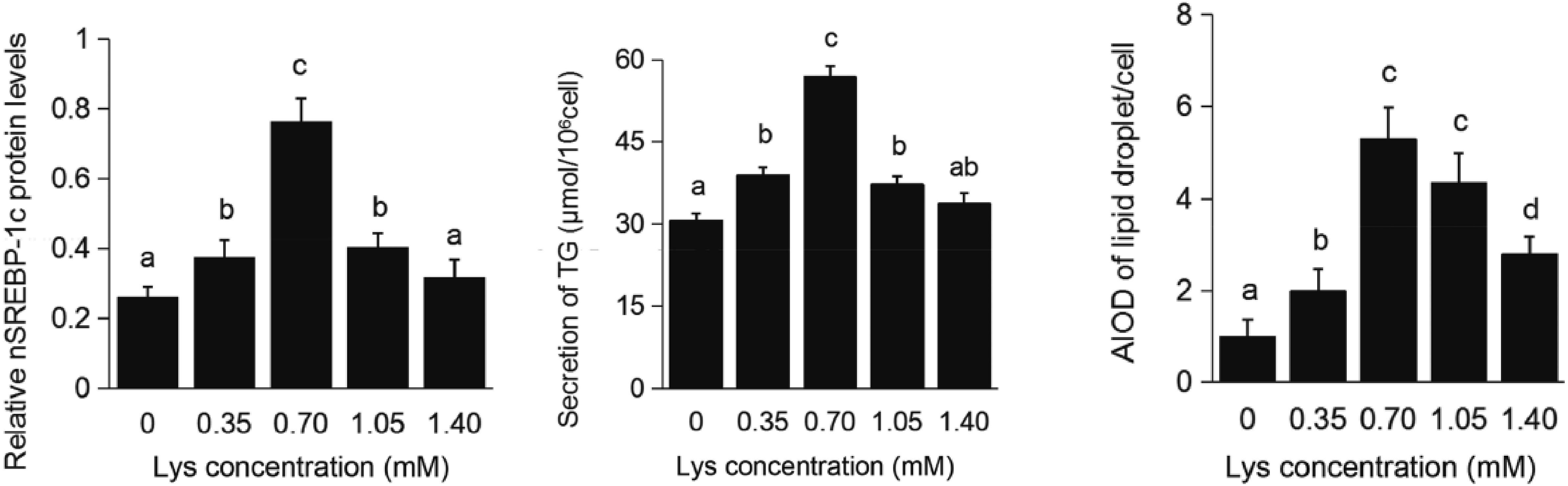
Effects of Lys on milk fat synthesis in the absence of Fatty Acids.





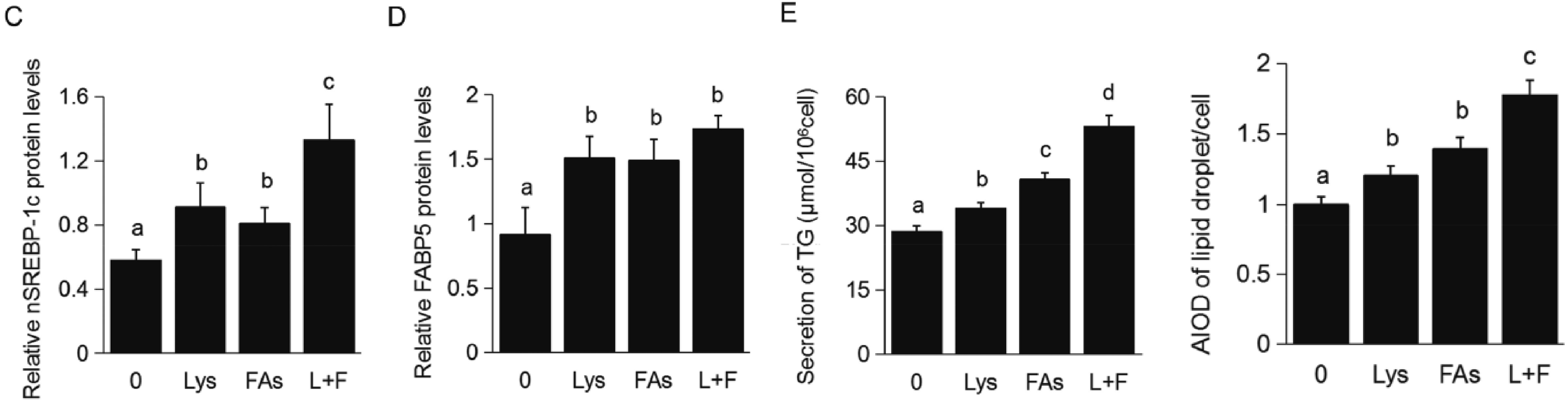
Li et al., 2019

Effects of Lysine on Milk Fat Synthesis in the Presence of Fatty Acids



Li et al., 2019

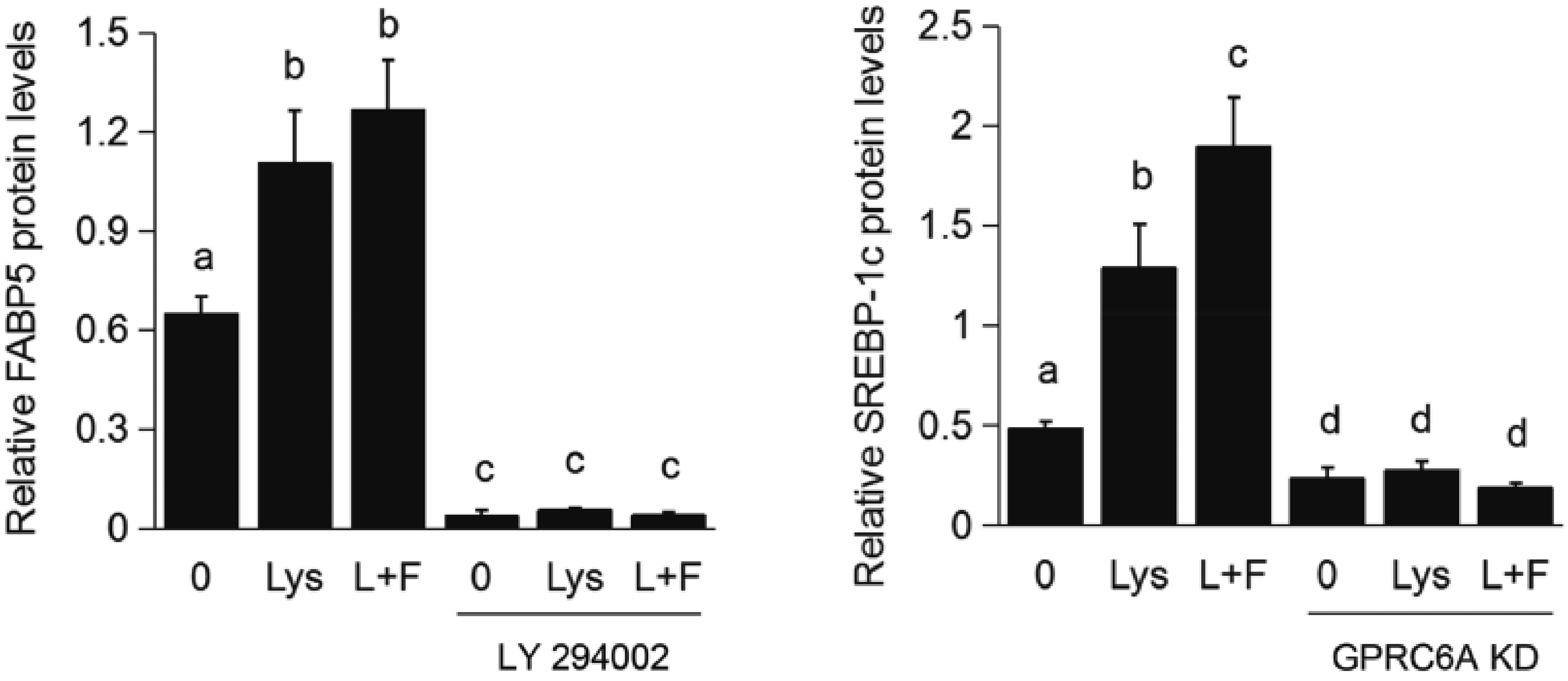
Effects of Lysine together with Fatty Acids on Milk Fat Synthesis



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

Li et al., 2019

Effects of PI3K Inhibition on Lysine Stimulated FABP5 Expression and SREBP-1c Expression and Maturation



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 theGPRC6A- the G protein-coupled receptor class 6A – which induces the PI3K/AkT (phosphatidy linositol 3-kinase) pathway

- FABP5 – Fatty acid binding protein 5 which regulates lipid metabolism

Effects of feeding rumen-protected lysine during the postpartum period on performance and amino acid profile in dairy cows: A meta-analysis

	Lysine % MP			
	6.5	**8.5**	**SEM**	**P**
Milk, kg	32.1	34.0	1.3	0.02
ECM, kg	33.4	35.8	1.6	0.03
Milk fat, %	3.68	3.73	0.12	0.07
Milk fat, kg	1.17	1.27	0.06	0.05
Milk protein, %	3.09	3.18	0.03	0.04
Milk protein, kg	0.99	1.06	0.05	0.07
Lactose, %	4.81	4.72	0.07	0.14

Arshad et al., 2024

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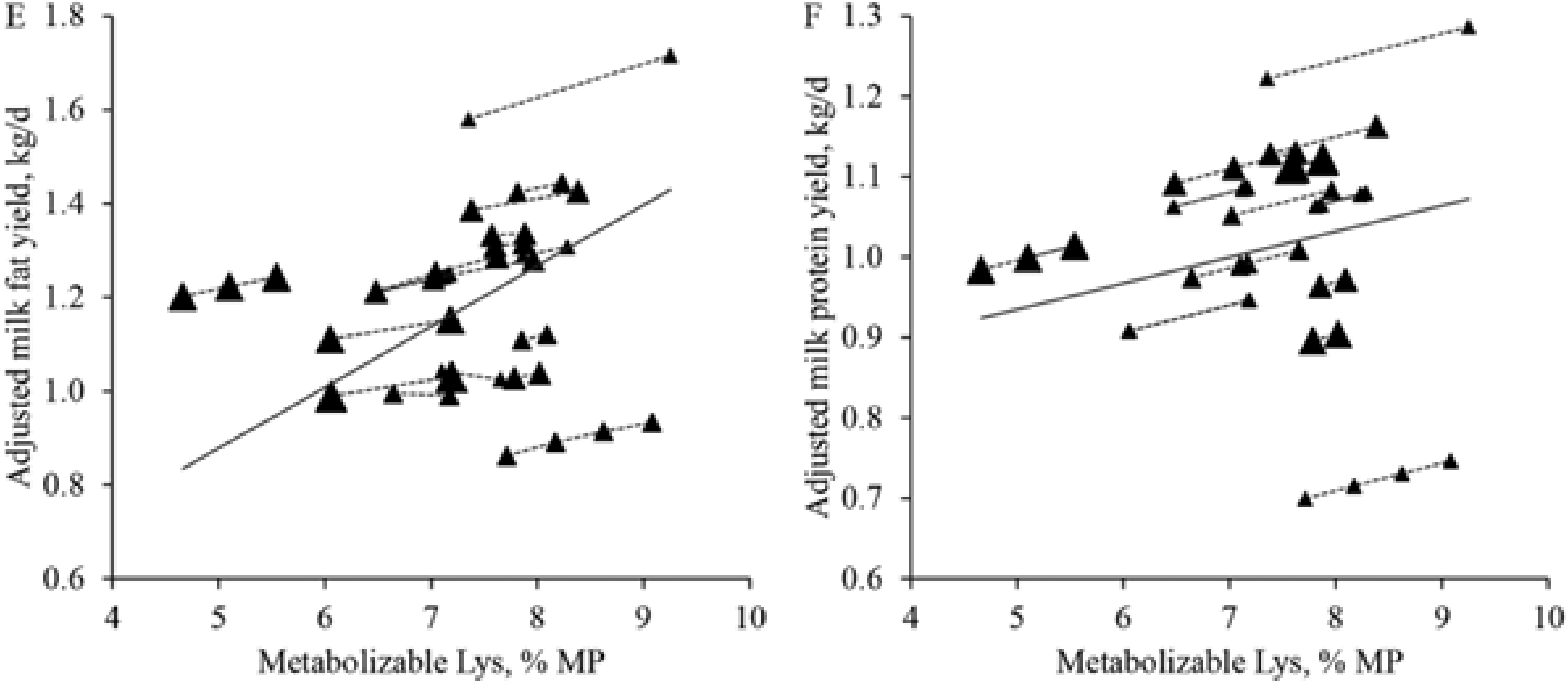


Arshad et al., 2024

Amino Acids and De Novo FA Synthesis

- Lys increased enzymes related to de novo FA synthesis (ACS, ACC, FAS) through upregulation of FABP and SREBP1 (Li et al., 2019)
  - Further increased when supplemented with palmitic acid and oleic acid

- Additionally, Met and Leu increase expression of SREBP1– important regulator of enzymes for milk FA synthesis (Li et al., 2019).

- Arg increased de novo and mixed FA synthesis and expression of ACC, SCD, DGAT1 (Ding et al., 2022)

Fatty Acid Synthetase (FAS)

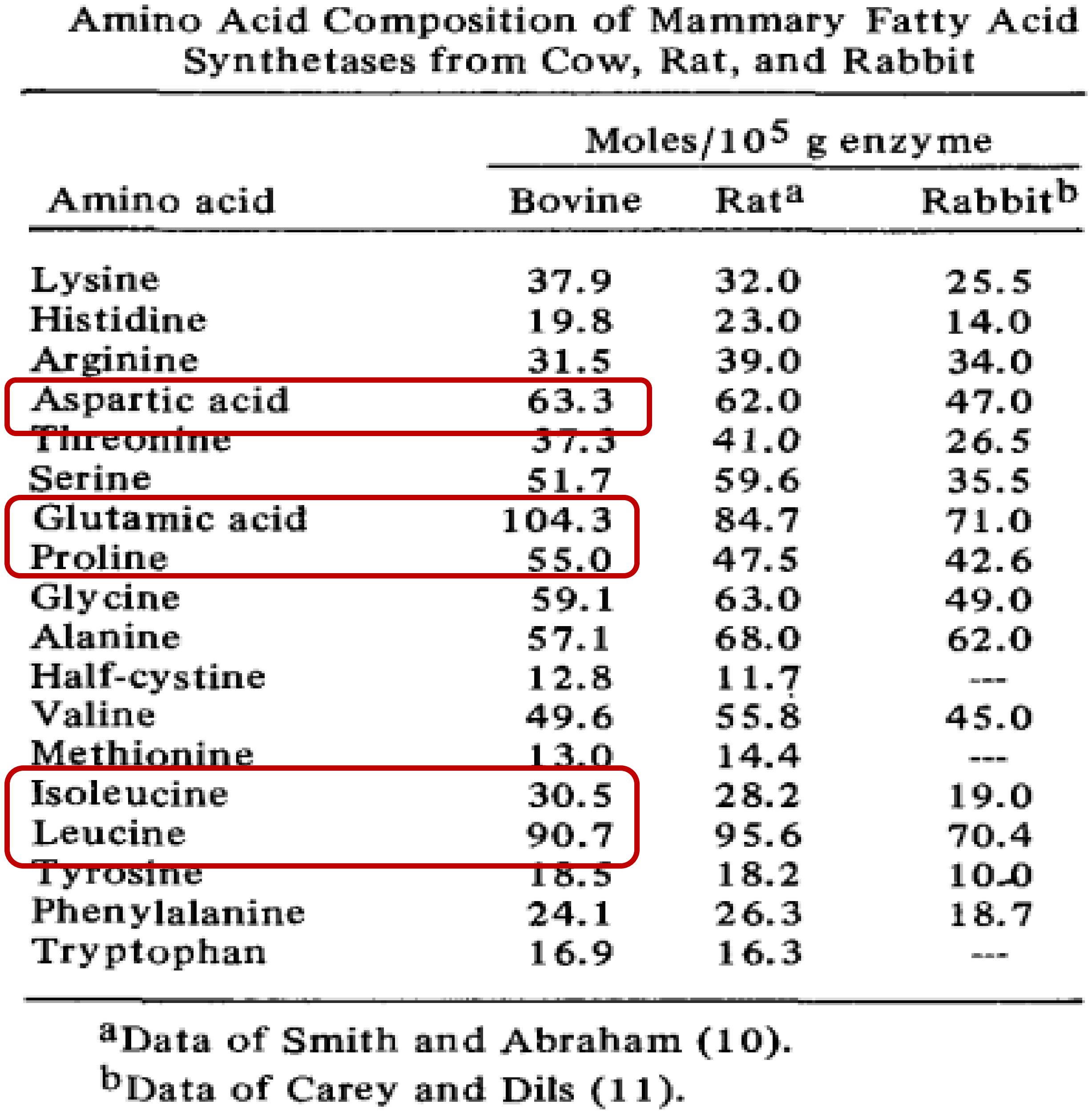
- FAS synthesizes de novo FA by elongating FA carbon chain

- 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

Amino Acid Composition of Bovine Mammary FAS



Amino Acid Composition of Mammary Fatty Acid Synthetases from Cow, Rat, and Rabbit

	Moles/10^5 g enzyme		
Amino acid	Bovine	Rat[a]	Rabbit[b]
Lysine	37.9	32.0	25.5
Histidine	19.8	23.0	14.0
Arginine	31.5	39.0	34.0
Aspartic acid	63.3	62.0	47.0
Threonine	37.3	41.0	26.5
Serine	51.7	59.6	35.5
Glutamic acid	104.3	84.7	71.0
Proline	55.0	47.5	42.6
Glycine	59.1	63.0	49.0
Alanine	57.1	68.0	62.0
Half-cystine	12.8	11.7	---
Valine	49.6	55.8	45.0
Methionine	13.0	14.4	---
Isoleucine	30.5	28.2	19.0
Leucine	90.7	95.6	70.4
Tyrosine	18.5	18.2	10.0
Phenylalanine	24.1	26.3	18.7
Tryptophan	16.9	16.3	---

[a] Data of Smith and Abraham (10).

[b] Data of Carey and Dils (11).

Kinsella et al., 1975

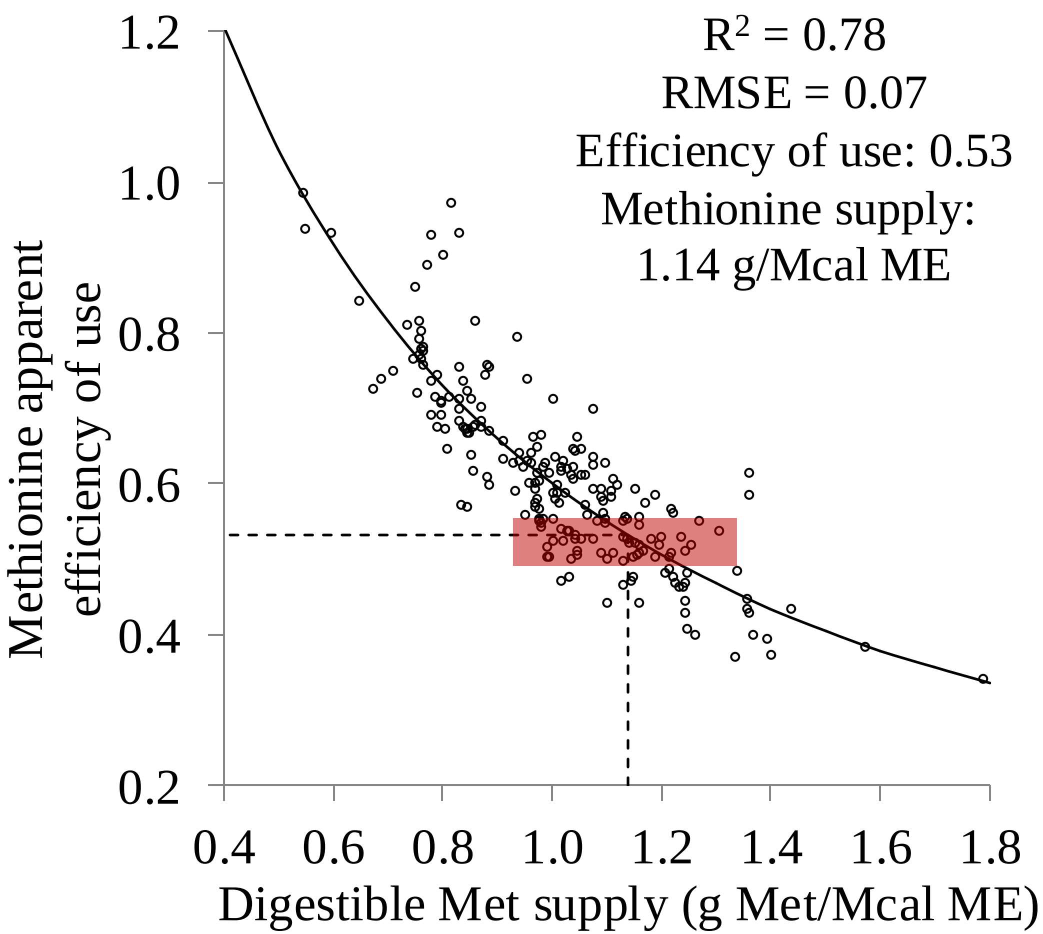
Optimum Supply Of Each EAA Relative To Metabolizable Energy – CNCPS v7.0

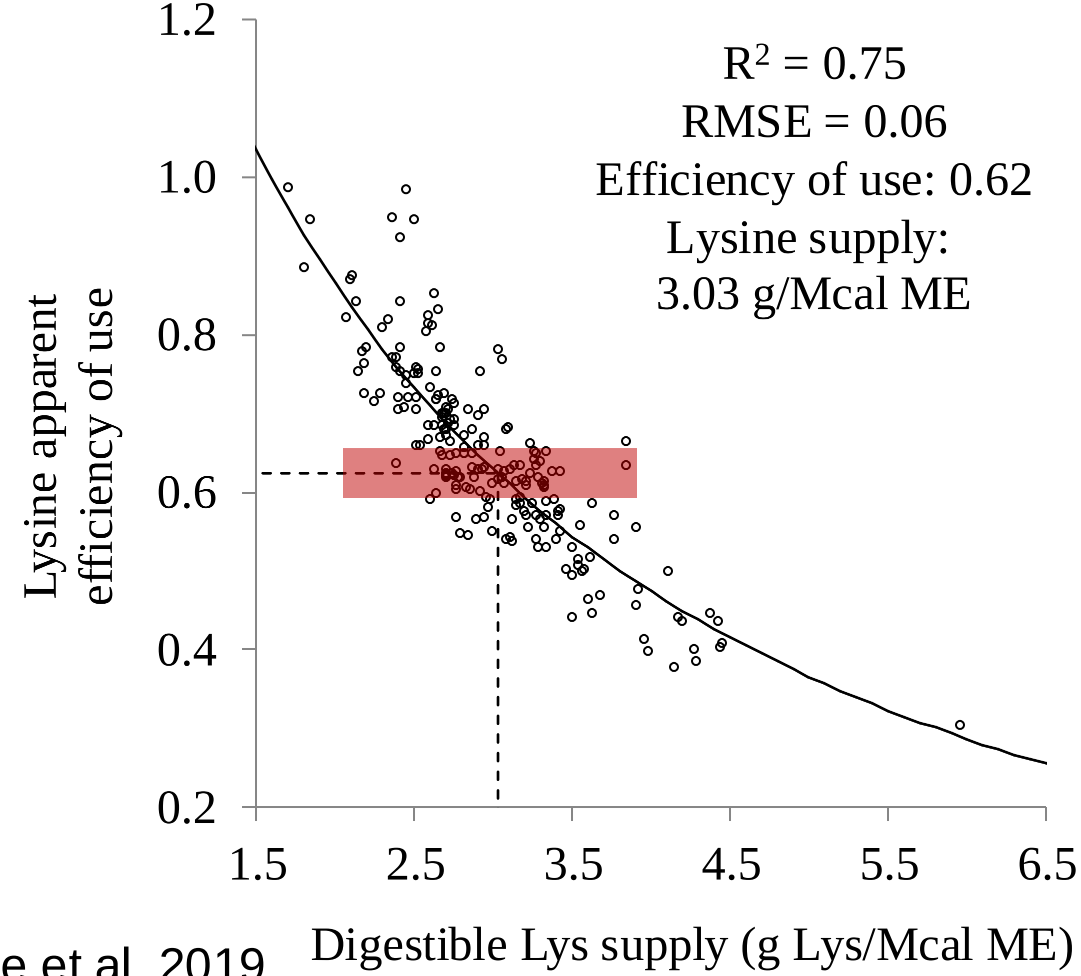
AA	R^2	Efficiency from our evaluation	Lapierre et al. (2007)	**g AA/ Mcal ME**	% EAA
Arg	0.81	0.61	0.58	**2.04**	10.2%
His	0.84	0.77	0.76	**0.91**	4.5%
Ile	0.74	0.67	0.67	**2.16**	10.8%
Leu	0.81	0.73	0.61	**3.42**	17.0%
Lys	0.75	0.67	0.69	**3.03**	15.1%
Met	0.79	0.57	0.66	**1.14**	5.7%
Phe	0.75	0.58	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

Variation exists when contextualizing efficiency of use with amino acid and energy supply





LaPierre et al, 2019

Experimental design to test amino acid balancing

14-week longitudinal feeding trial

144 cows balanced in 9, <u>16-cow pens</u>

3 Diets formulated using CNCPS v.7:

1. Optimum g EAA/Mcal ME (14.8% CP) → **'Control'**
2. -1 Std Dev g EAA/Mcal ME (14.0% CP) → **'Negative'**
3. +1 Std Dev g EAA/Mcal ME (16.3% CP) → **'Positive'**

All diets formulated to be iso-caloric and in ME excess

- ☐ Nitrogen intestinal digestibility tested (Gutierrez-Botaro et al., 2022)
- ☐ Feed AA profile updated to refine supply (Van Amburgh et al., 2017)

LaPierre et al, 2019

Dietary Ingredients, % DM	Negative	Control	Positive
Corn silage	51.5	51.5	50.4
High moisture ear corn	9.4	9.5	9.9
Canola	1.8	9.2	6.3
Triticale	7.3	7.3	8.0
Corn grain	6.4	6.4	6.0
Soybean meal	8.2	5.6	2.7
Soyhulls	9.3	3.8	2.8
Bloodmeal	0.0	0.0	3.1
Dextrose	1.6	1.6	2.2
SoyPlus	0.00	0.91	3.6
Energy booster	0.73	0.73	0.91
Urea	0.62	0.51	0.51
Smartamine M	0.00	0.04	0.05
Smartamine ML	0.00	0.00	0.07
Minerals and vitamins	3.3	2.9	3.2

LaPierre et al, 2019

Chemical Component, % DM	Negative	Control	Positive
Dry Matter, %	44.7	44.5	44.2
Crude Protein	14.0	14.7	16.0
ADICP, % CP	5.70	5.90	5.50
NDICP, % CP	15.0	15.5	18.7
aNDFom	32.4	31.0	31.4
Lignin	2.61	3.00	2.70
Sugar	3.95	4.10	3.90
Starch	29.8	29.3	29.3
Fat	3.50	3.60	3.80
Ash	6.60	6.90	6.60
Ammonia	0.80	0.90	0.80
RDP, % DM	9.50	9.65	9.50
ME, Mcal/kg	2.58	2.60	2.61

LaPierre et al, 2019

					P
Metabolizable supply, g·d^{-1}	Negative	Control	Positive	SEM	Diet
Arginine	141.1^{a}	153.2^{b}	154.1^{b}	1.6	< 0.01
Histidine	60.6^{a}	66.1^{b}	87.1^{c}	0.7	< 0.01
Isoleucine	146.0^{a}	155.2^{b}	146.9^{a}	1.7	0.02
Leucine	223.9^{a}	239.2^{b}	285.5^{c}	2.6	< 0.01
Lysine	201.5^{a}	214.0^{b}	248.1^{c}	2.3	< 0.01
Methionine	69.5^{a}	74.1^{b}	88.3^{c}	0.8	< 0.01
Phenylalanine	148.4^{a}	155.3^{b}	178.3^{c}	1.7	< 0.01
Threonine	142.6^{a}	154.6^{b}	166.8^{c}	1.6	< 0.01
Tryptophan	45.1^{ax}	47.0^{ay}	42.2^{b}	0.5	< 0.01
Valine	157.9^{a}	170.6^{b}	196.3^{c}	1.8	< 0.01
Lys:Met	2.90^{ax}	2.89^{ay}	2.81^{b}	0.003	< 0.01

LaPierre et al, 2019

^{ab} Within a row, means without a common superscript differ (*P* < 0.05) ^{xy} Within a row, means without a common superscript differ (*P* < 0.10)

	Diet				*P*	
Parameters	**Negative**	**Control**	**Positive**	SEM	Enroll	Diet
Intake and lactation performance, kg/d						
Dry matter intake	25.9	26.4	26.4	0.27	0.41	0.37
Milk yield	37.6^{a}	40.5^{b}	41.6^{b}	0.40	0.37	< 0.01
Energy corrected milk yield	40.3^{a}	43.3^{b}	44.2^{b}	0.51	0.01	< 0.01
3.5% fat corrected milk	41.0^{a}	43.7^{b}	44.6^{b}	0.55	0.01	< 0.01
True protein yield	1.14^{a}	1.27^{b}	1.29^{b}	0.02	0.23	< 0.01
Fat yield	1.54^{x}	1.61^{y}	1.65^{y}	0.07	0.05	0.07
Lactose yield	1.79^{a}	1.93^{b}	1.97^{b}	0.04	< 0.01	< 0.01
Milk urea nitrogen, mg/dL	10.5^{a}	11.2^{b}	13.6^{c}	0.14	< 0.01	< 0.01
Body weight and condition						
Body weight change, kg·wk^{-1}	1.73	2.39	2.14	0.35	< 0.01	0.43
Final BCS, 1-5 scale	2.89	2.90	2.91	-	-	0.71
Feed and N efficiency						
Milk Yield:DMI	1.47^{a}	1.57^{b}	1.59^{b}	0.02	0.71	< 0.01
ECM:DMI	1.58^{a}	1.68^{b}	1.69^{b}	0.02	0.26	< 0.01
Milk N:Feed N	0.328^{a}	0.343^{b}	0.321^{a}	0.004	< 0.01	< 0.01

LaPierre et al, 2019

Two herds in Southern PA – both between 100 and 150 cows with diets formulated using similar dietary metrics as the previous study – these values represent the whole herd - these are Holstein cattle. Milk fat in both herds was about 4.2% before dietary interventions. Milk protein was approximately 3.1% prior to diet change.

Herd 1	
Milk yield, lb	90
Milk fat, %	4.64
Milk true protein, %	3.48
Milk fat yield, lb	4.12
Milk protein yield, lb	3.13

Herd 2	
Milk yield, lb	91
Milk fat, %	4.76
Milk true protein, %	3.46
Milk fat yield, lb	4.3
Milk protein yield, lb	3.13

Take home messages

- Insulin is involved in protein synthesis in the mammary gland – for both milk protein and fat
- Amino acids have other roles that involve signaling and supporting the metabolism of other products, such as milk fat and lactose
- Fatty acid enzymes are inducible and sense supplies of nutrients
- Amino acids, such as Lysine, can induce enzymes and signal pathways related to fatty acid synthesis and are required for optimum milk fatty acid yield
- To improve feed efficiency, formulating the correct amount of metabolizable essential amino acids relative to metabolizable energy is necessary

Thank you for your attention

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