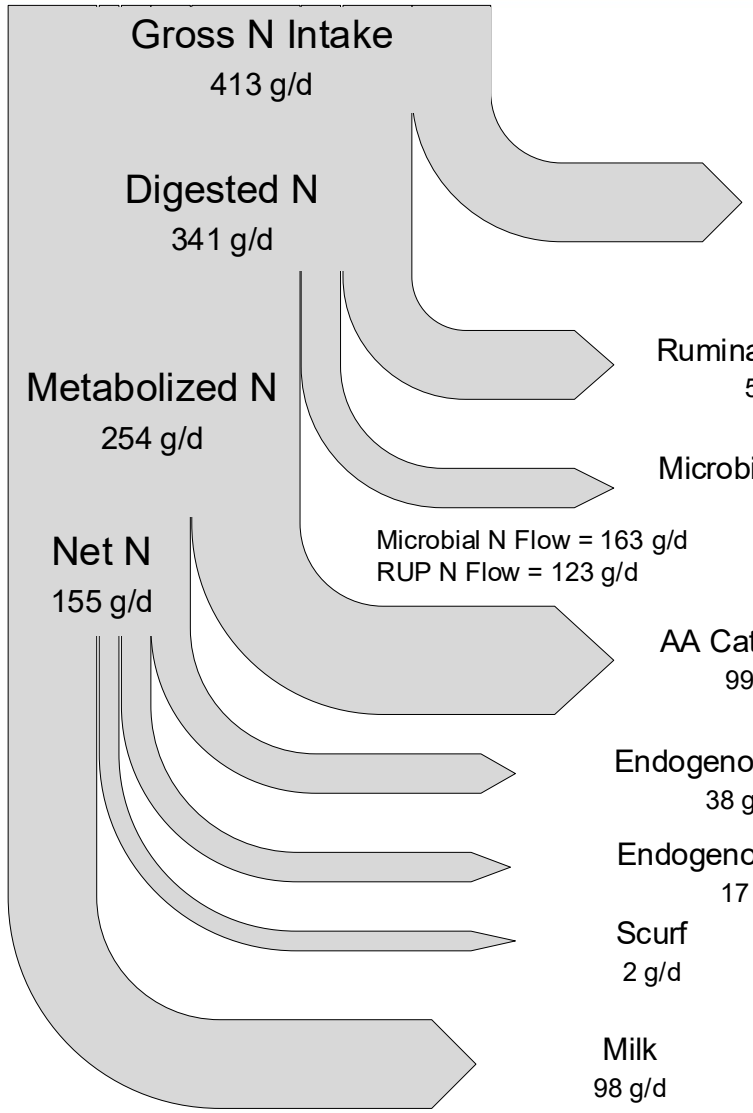
Protein and Amino Acid Requirement System

Mark D. Hanigan, Virginia Tech
Jeff Firkins, Ohio State
Helene Lapierre, Ag Canada

N Partitioning in the Lactating Ruminant

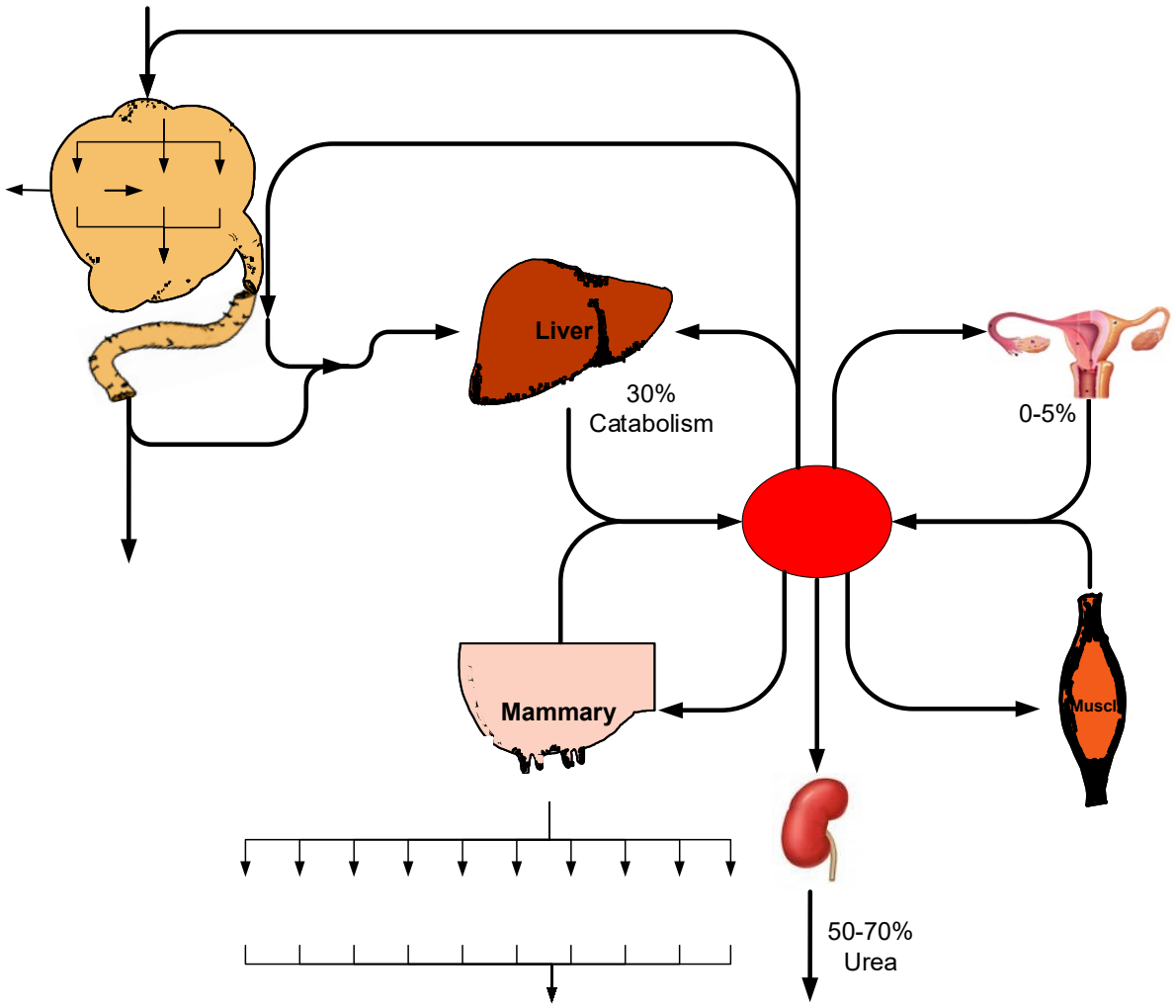
Typical 1990's Diet



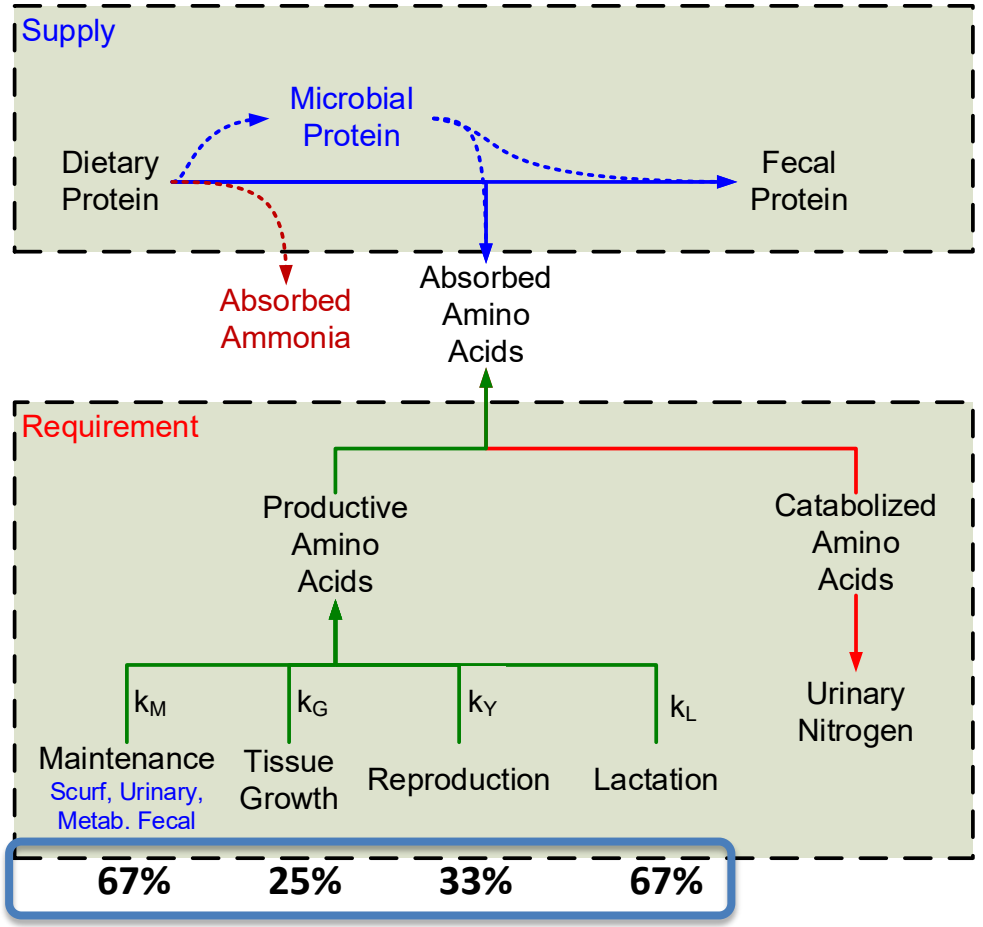
	% of Intake	% of MP
Undigested 72 g/d	17%	
Ruminal NH_3 Loss 55 g/d	13%	
Microbial Nucleic Acids 32 g/d	8%	
Microbial N Flow = 163 g/d RUP N Flow = 123 g/d		
AA Catabolism 99 g/d	24%	39%
Endogenous Fecal 38 g/d	9%	15%
Endogenous Urinary 17 g/d	4%	7%
Scurf 2 g/d	1%	1%
Milk 98 g/d	24%	39%

Adapted from Arrola et al., 2014

Representing the System – 2001

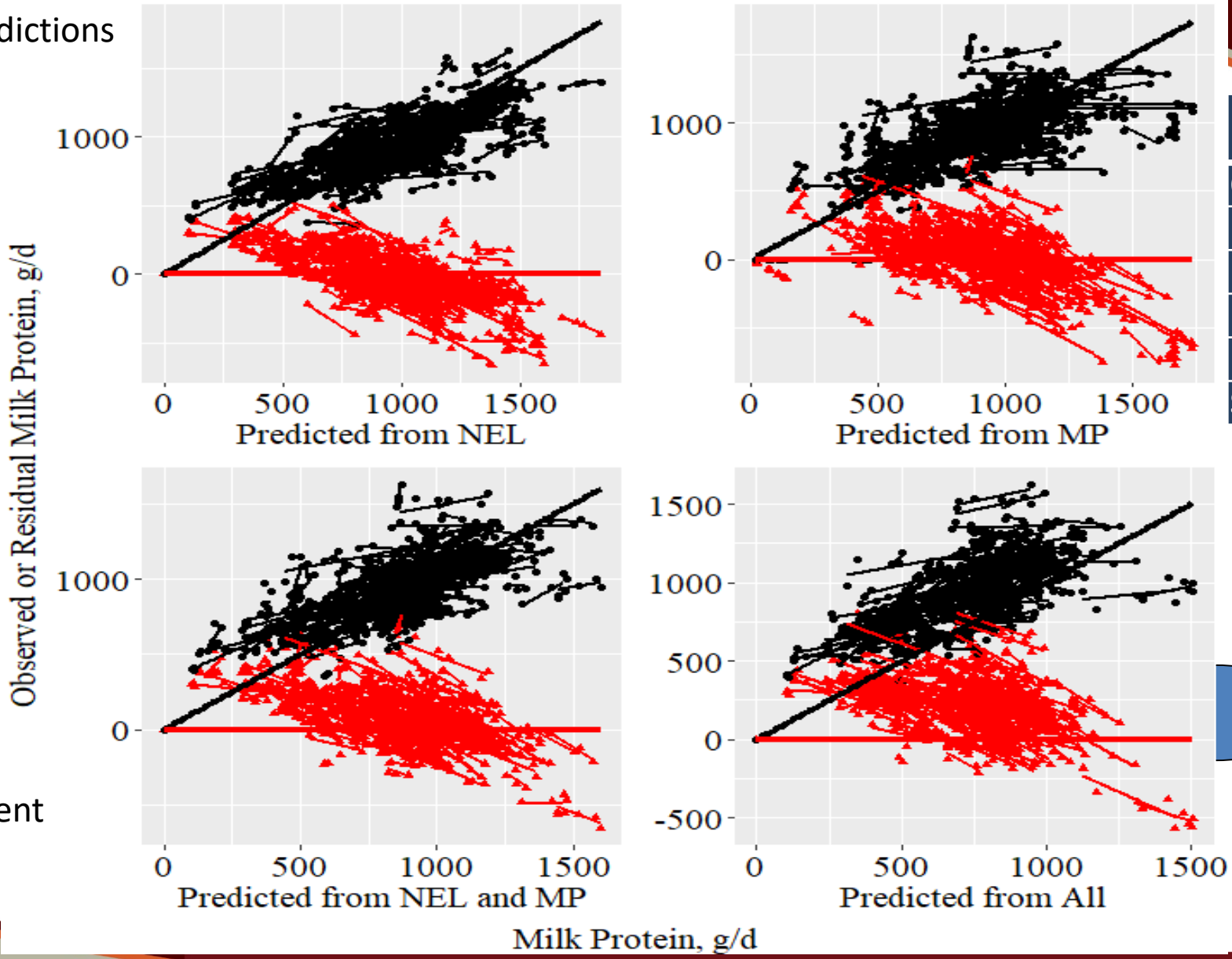


NRC 2001



NRC 2001 Based Predictions using NRC 2021 Supply Predictions

Independent Predictions



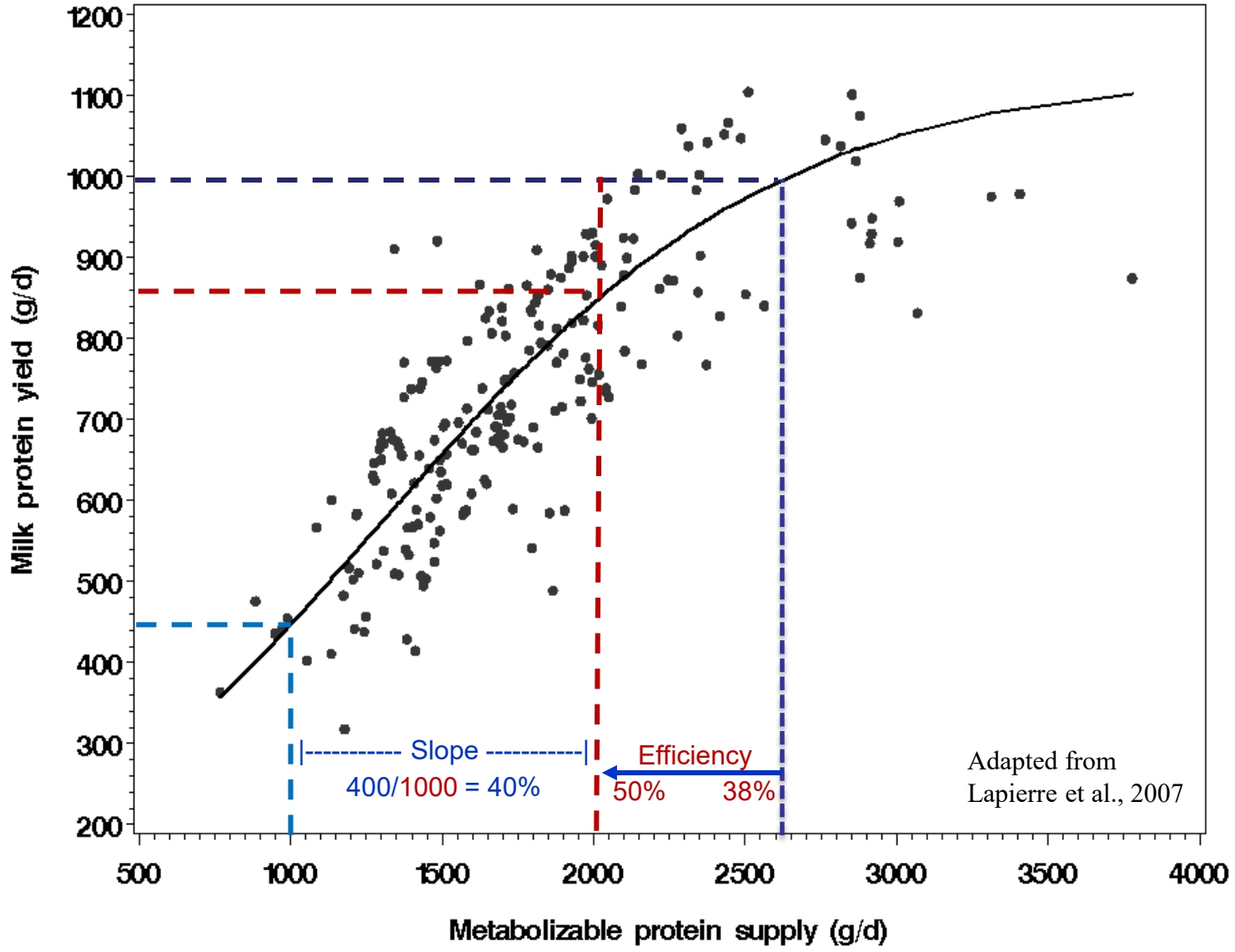
Item	Stat
Observed Mean, g	918
Predicted Mean, g	890
RMSE, % mean	24.9
Mean Bias, % MSE	1
Slope Bias, % MSE	32
Slope Bias, kg/kg	-0.44

~250 g/d

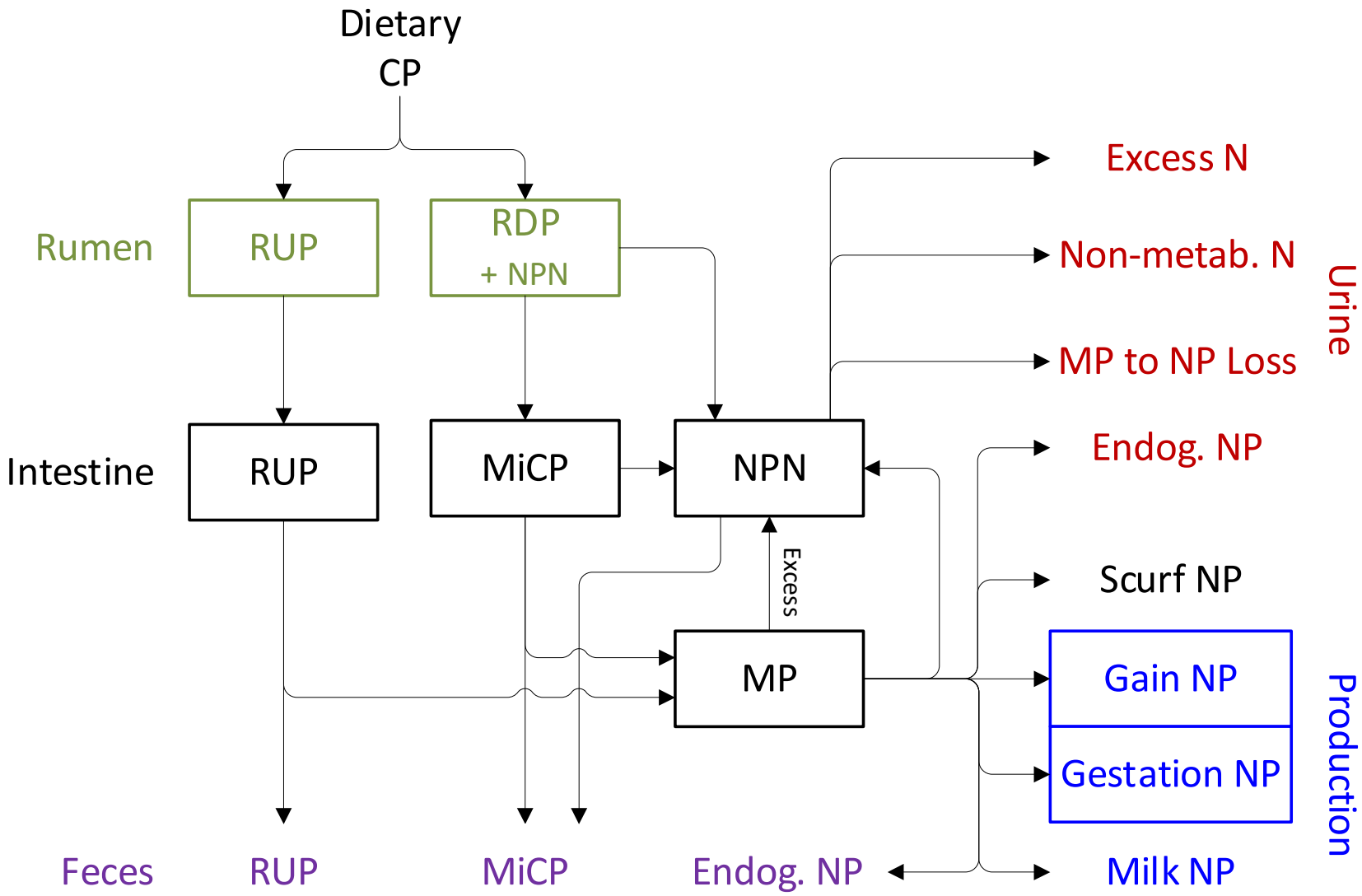
1st Limiting Nutrient

Hanigan et al. in preparation

Milk Protein vs Metabolizable Protein



NASEM 2021 Protein and AA System Derivation

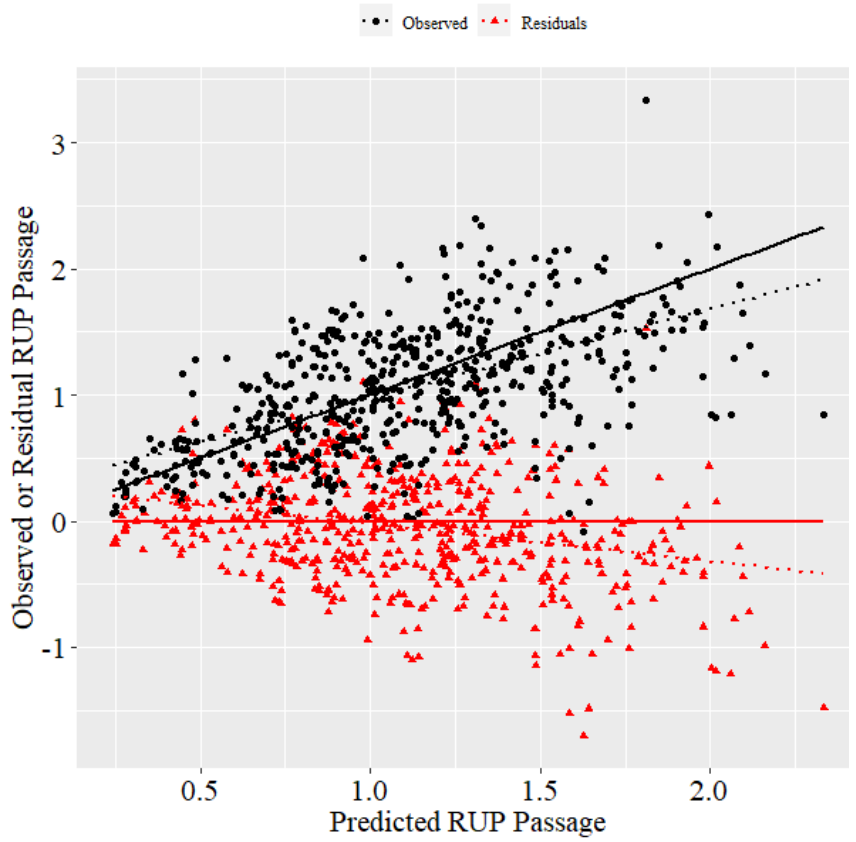


- Track protein and AA through the system
- Add more detail
- Improve accuracy and precision
  - Re-evaluate each component
  - Derive new or revised equations where needed
- Thoroughly test the revised system

Ruminal Protein Outflow: NRC 2001

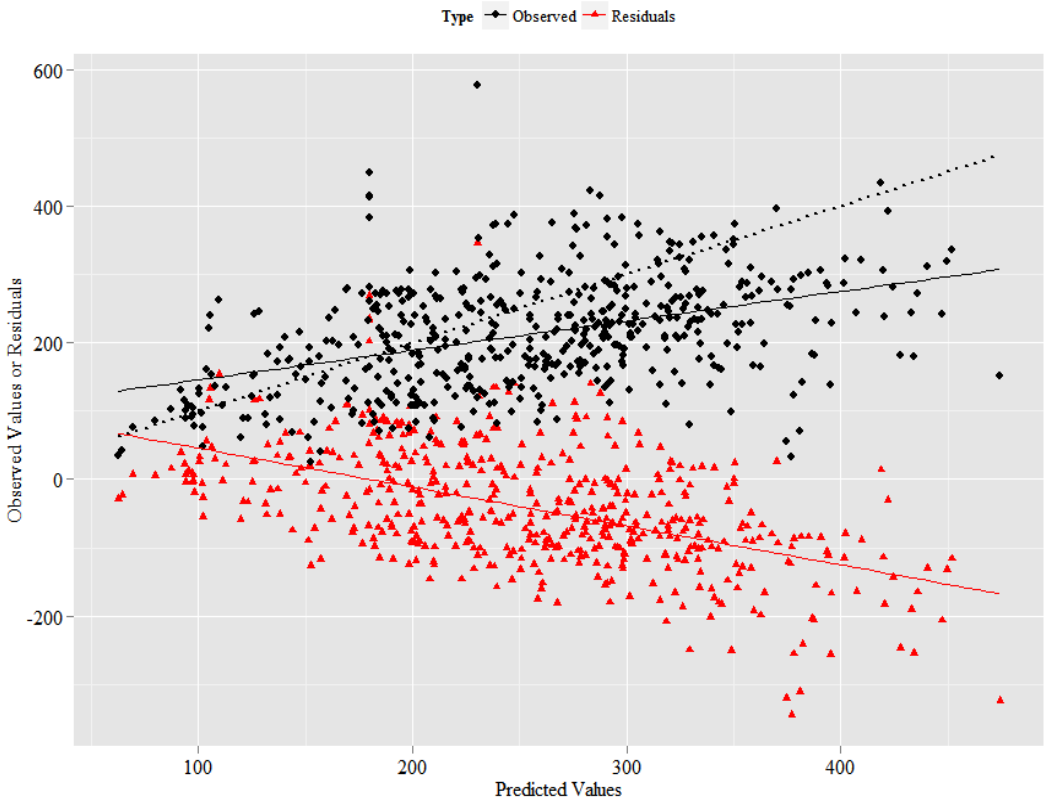
NRC 2001: $RUP = \frac{Kp = fn(Diet)}{Kp + Kd} \times CPB + CPC$

RMSE = 42.0%



NRC 2001: $MiN = \begin{cases} 0.85 \times RDP \\ 0.16 \times TDN \end{cases}$

RMSE ≈ 35%



White et al., 2017.

RUP: Likely Kp and Kd Values

Equation	NRC 2001	Fit Kp[b]		Fit Kp and Adjust Kd		NASEM 2021 Priors from Seo	
Variable[a]	Estimate	Estimate	SE	Estimate	SE	Estimate	SE
Intercept, kg/d		0.304	0.079	0.157	0.078	-0.086	0.066
KpA, kg/kg		0.165	0.045	0.094	0.046	0.064	0.039
$KpBC_{Concentrate}$, %/h	**6.70**	**2.96**	0.44	**6.04**	1.40	5.28	0.63
$KpBC_{Forage}$, %/h	**4.85**	**-1.87**	0.17	**0.70**	1.17	4.87	0.33
$Kd_{Adjustment}$, %/h				**3.89**	1.63		
		Value	%	Value	%	Value	%
AIC		142		83			
CCC	**0.55**	**0.49**		**0.51**		**0.54**	
RMSE, kg/d	**0.438**	**0.427**	**40.8[b]**	**0.410**	**39.2[b]**	**0.428**	**40.9**
Slope Bias, kg/kg	-0.29	-0.12	0.81[c]	0.019	0.02[c]	-0.20	3.8

[a] KpA=fraction of the A protein passing from the rumen
N=581 treatment means
Hanigan et al., 2021

MiN Predictions (N=581)

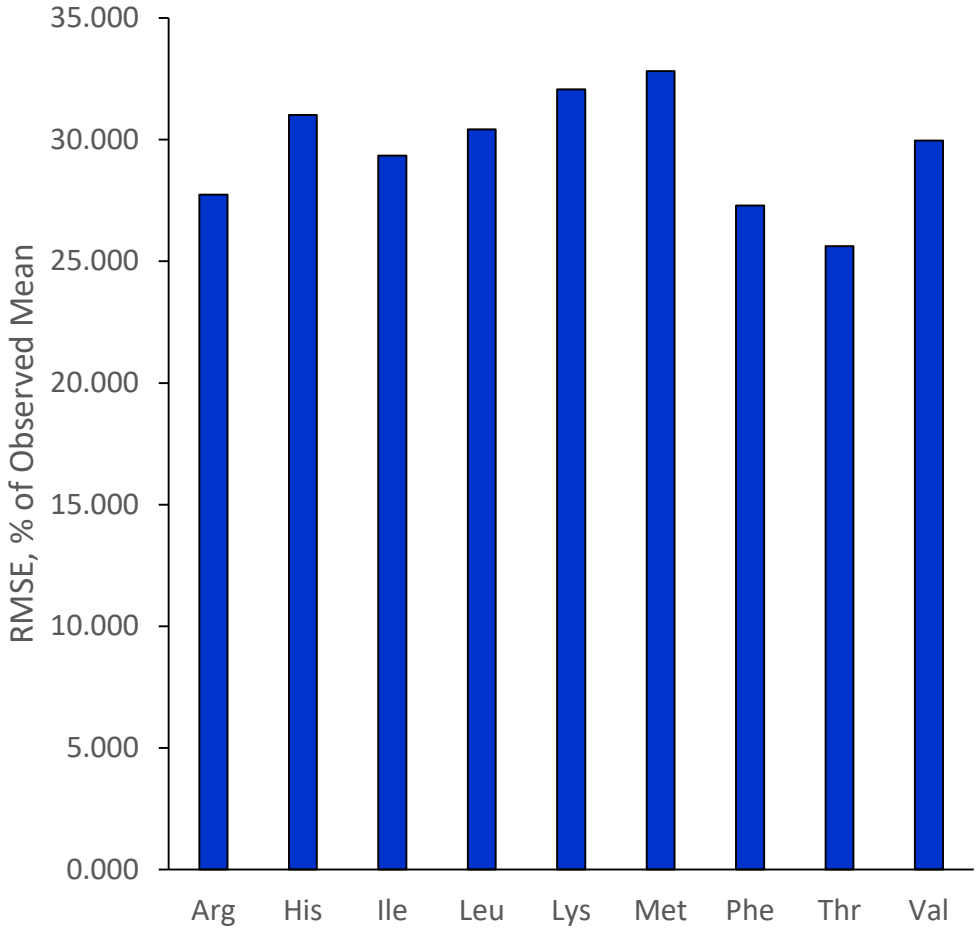
	NRC 2001	Est[a]	SE	2021[b]	Est[a]	SE
Intercept, g/d		-89.9	51	101	13.5	24
Omasal Sample, g/d		62.3**	14		54.4**	14
DMIn, kg/day		16.1**	1.1			
rdSt, g/g		0.630	0.52			
rdSt/rdNDF		0.568	1.7			
rdStIn[d], kg/d				0.027	11.6*	4.4
rdNDFIn[d], kg/d				0.094	29.0**	4.8
RDPIn[e], kg/d				81.6	43.5**	5.3
rOMIn, kg/d					-11.7	12
ForNDFIn, kg/d					8.13*	2.9
rOMIn[2]					2.78*	1.4
rdStIn x rOMIn					5.07**	1.6
rdNDFIn x ForNDFIn					-2.21**	0.74
rdStIn x RDPIn						
RDPIn x rOMIn						
AIC		5966			5935	
RMSE, g/d	81	71		83	70	
RMSE, % mean	29.2	25.5		29.7	25.3	
Mean Bias, % MSE	1.2	0.1			0.1	
Slope Bias, % MSE	2.1	0.3		3.7	0.3	

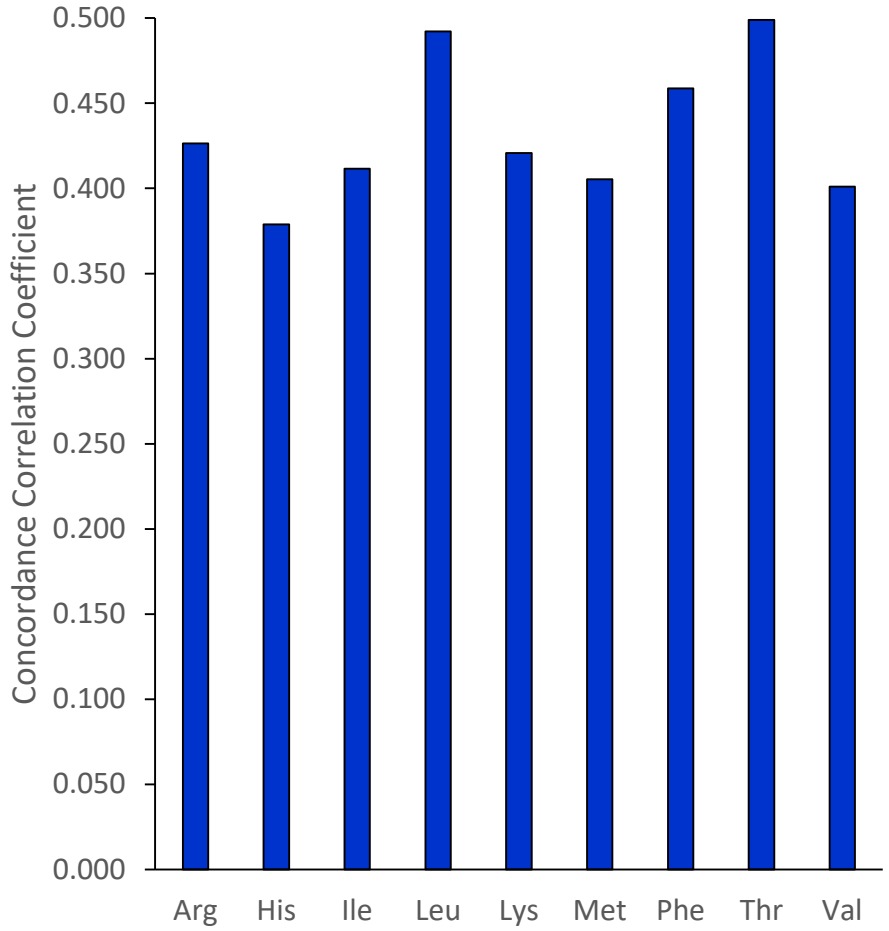
[a]Hanigan et al., 2021

[b]NASEM 2021 M-M equation from White et al. 2017

Prediction Errors for Duodenal AA Flows

with Updated RUP, MiCP, EndoCP, & AA Composition





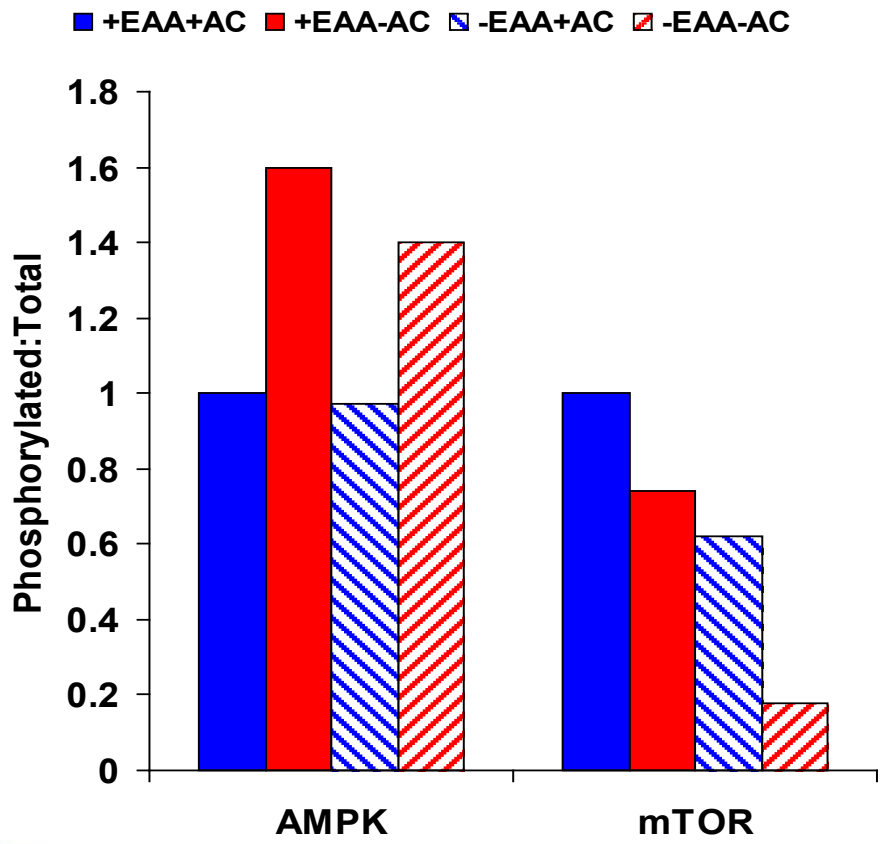
Fleming et al, 2019

Study effects excluded

Regulation of Protein Synthesis in Mammary Cells

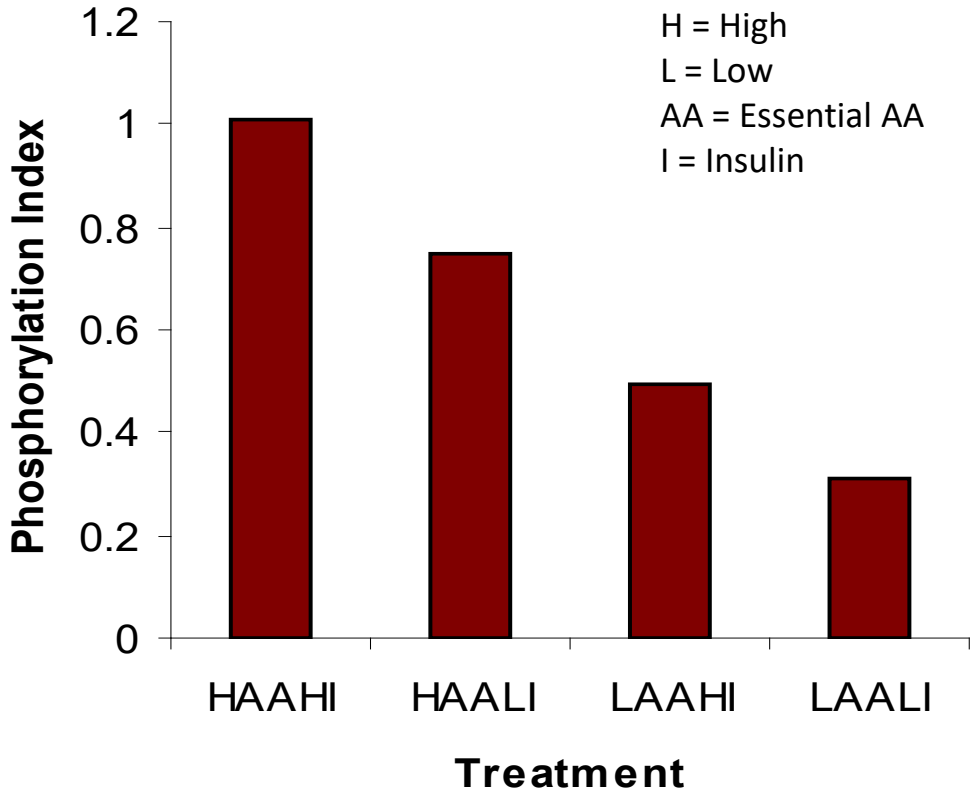
Acetate and EAA Effects on Cell Signaling

Appuhamy et al., 2009

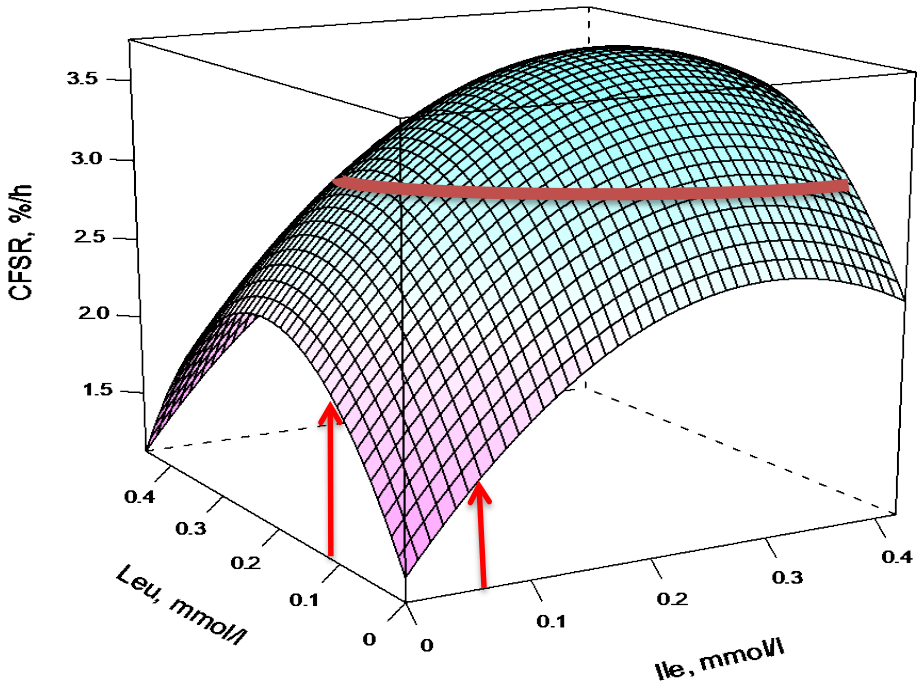


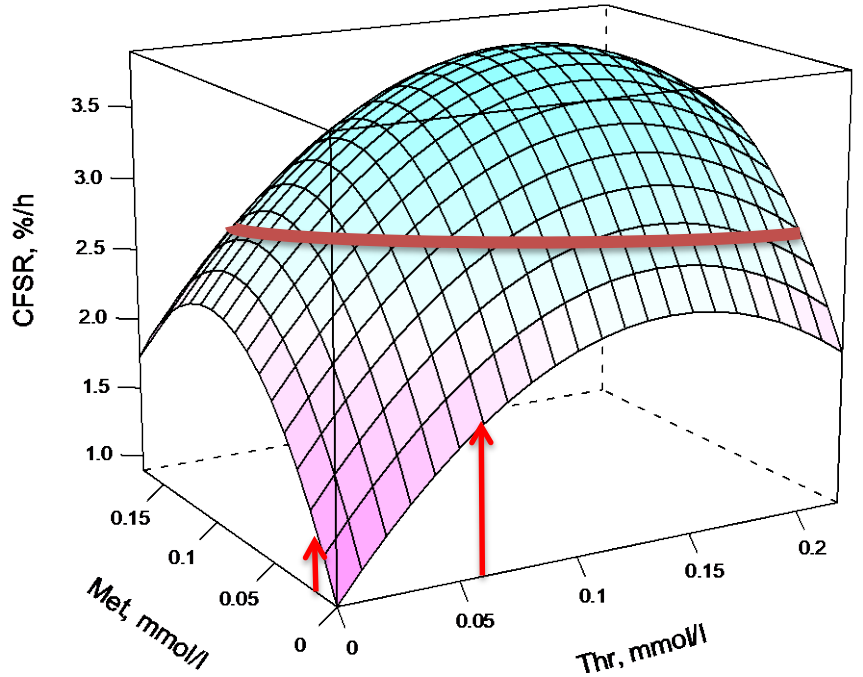
Insulin and EAA Effects on mTOR Signaling

Appuhamy et al., 2011



AA Effects on αS1-Casein Synthesis



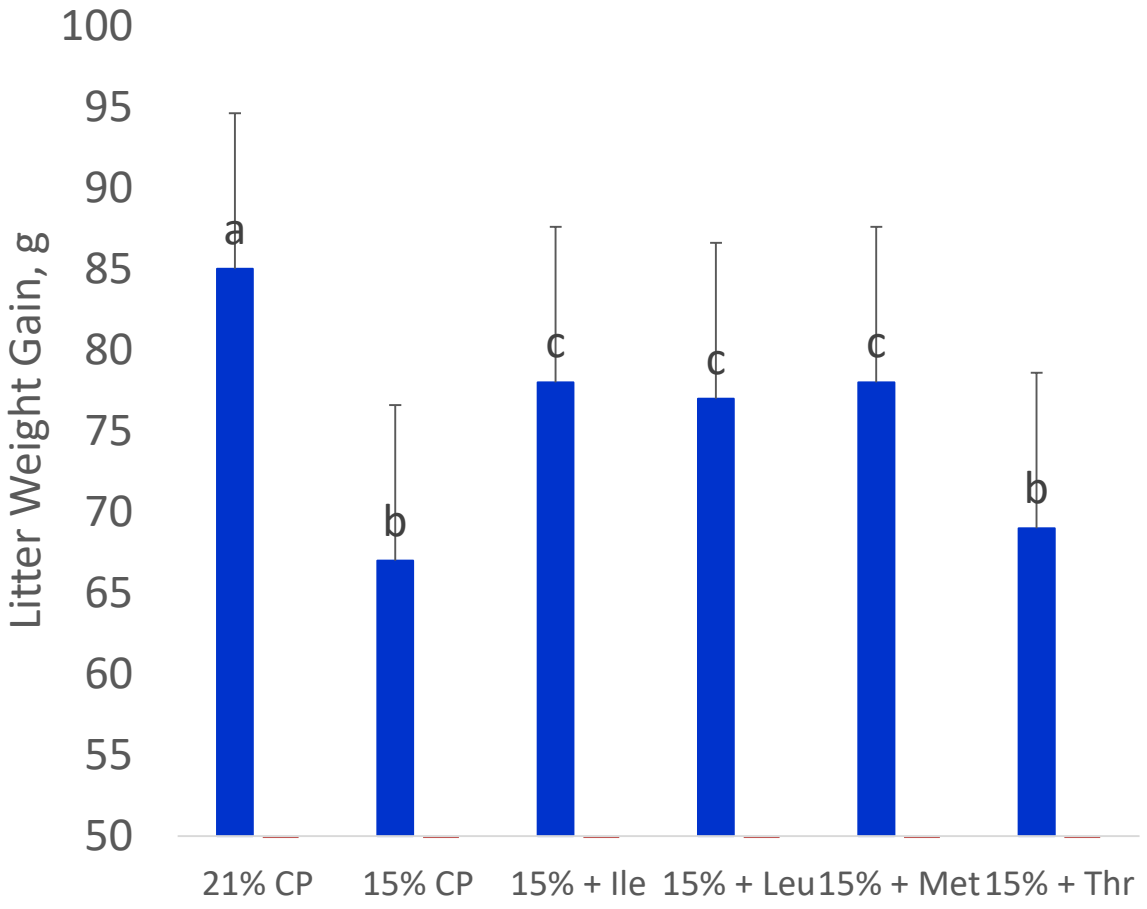


Arrows indicate high cow in vivo concentrations (Swanepoel et al., 2016 and Yoder, 2019)

Arriola, 2014

Additive Responses to EAA in Mice and Cows

Liu et al., 2017

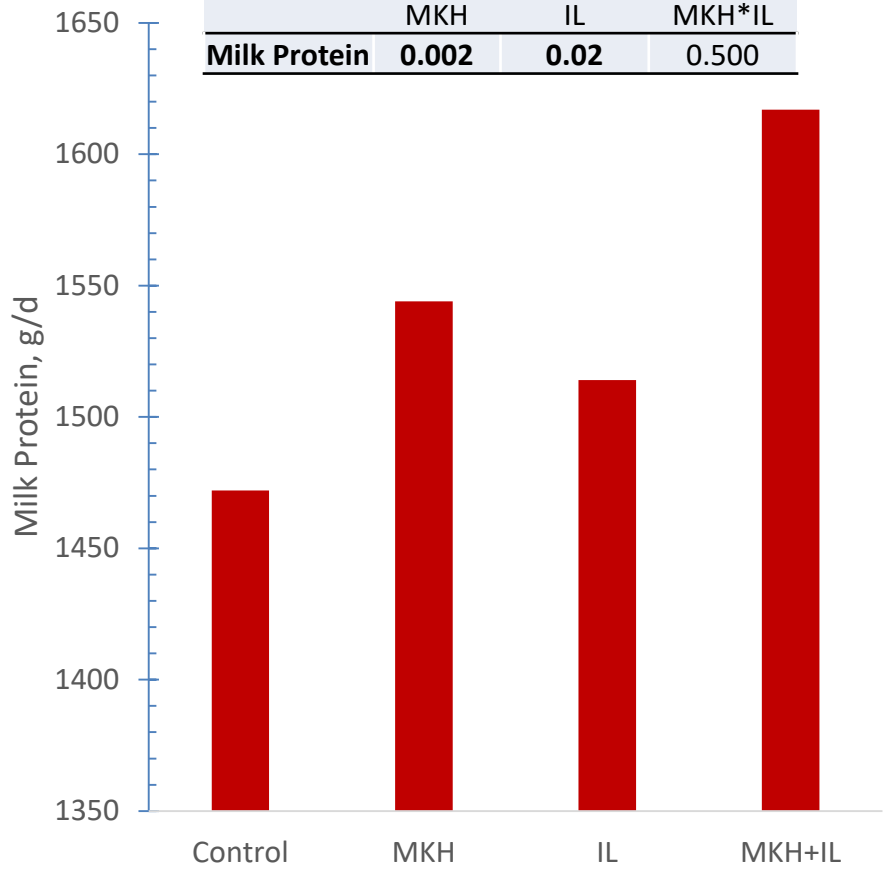


Yoder et al., 2020

15% CP Diet

38% Gross N Efficiency

	Effect (P-values)		
	MKH	IL	MKH*IL
Milk Protein	**0.002**	**0.02**	0.500



H=His, I=Ile, K=Lys, L=Leu, M=Met

Integrated Milk Protein Predictions using NASEM 2021 Nutrient Supply

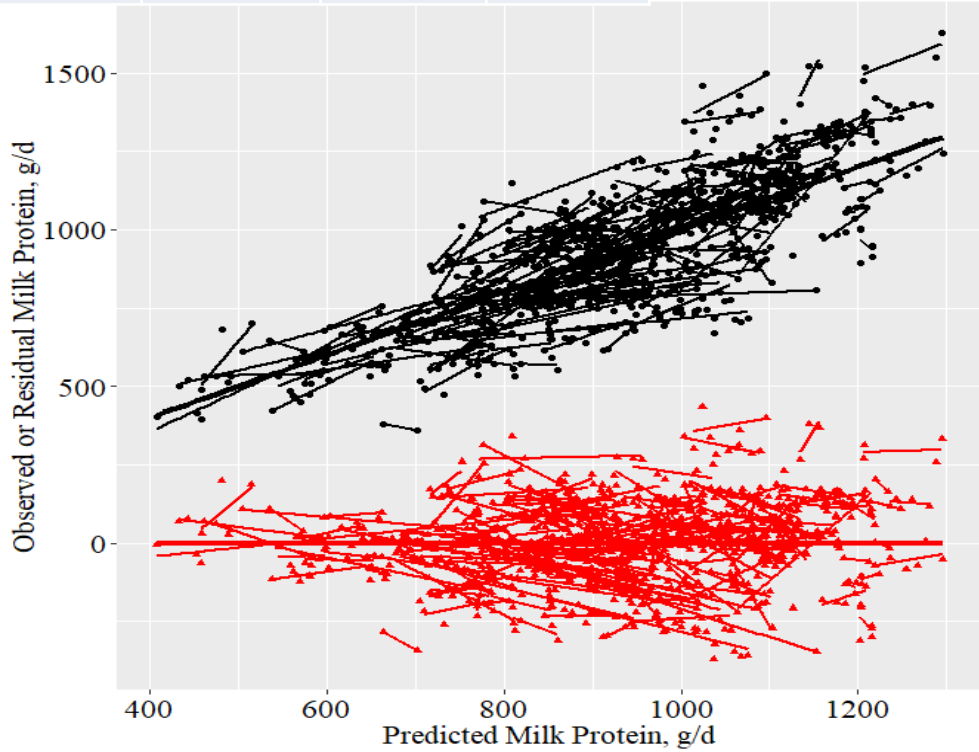
$$mPrt = Int + \alpha Arg + \beta His + \chi Ile + \delta Leu + \varepsilon Lys + \phi Met + \psi Phe + \varphi Thr + \mu Trp + \theta Val + \lambda OthAA + \varpi \sum EAA^2$$
$$+\kappa DEInp + \eta dNDF + \gamma dSt + \pi dFA + \mu BW$$

Predictors	Intercept	His	Ile	Leu	Lys	Met	OthAA	$\sum(EAA^2)$	DEInp	dNDFIn	BW
	g/d	---------- g/g ----------							Mcal/d	% DM	kg
Estimates	-97	1.68	0.89	0.47	1.15	1.84	0.077	-0.0024	10.8	-4.06	-0.42
SE	45	0.50	0.27	0.16	0.17	0.19	0.055	0.0002	8	3	0.04

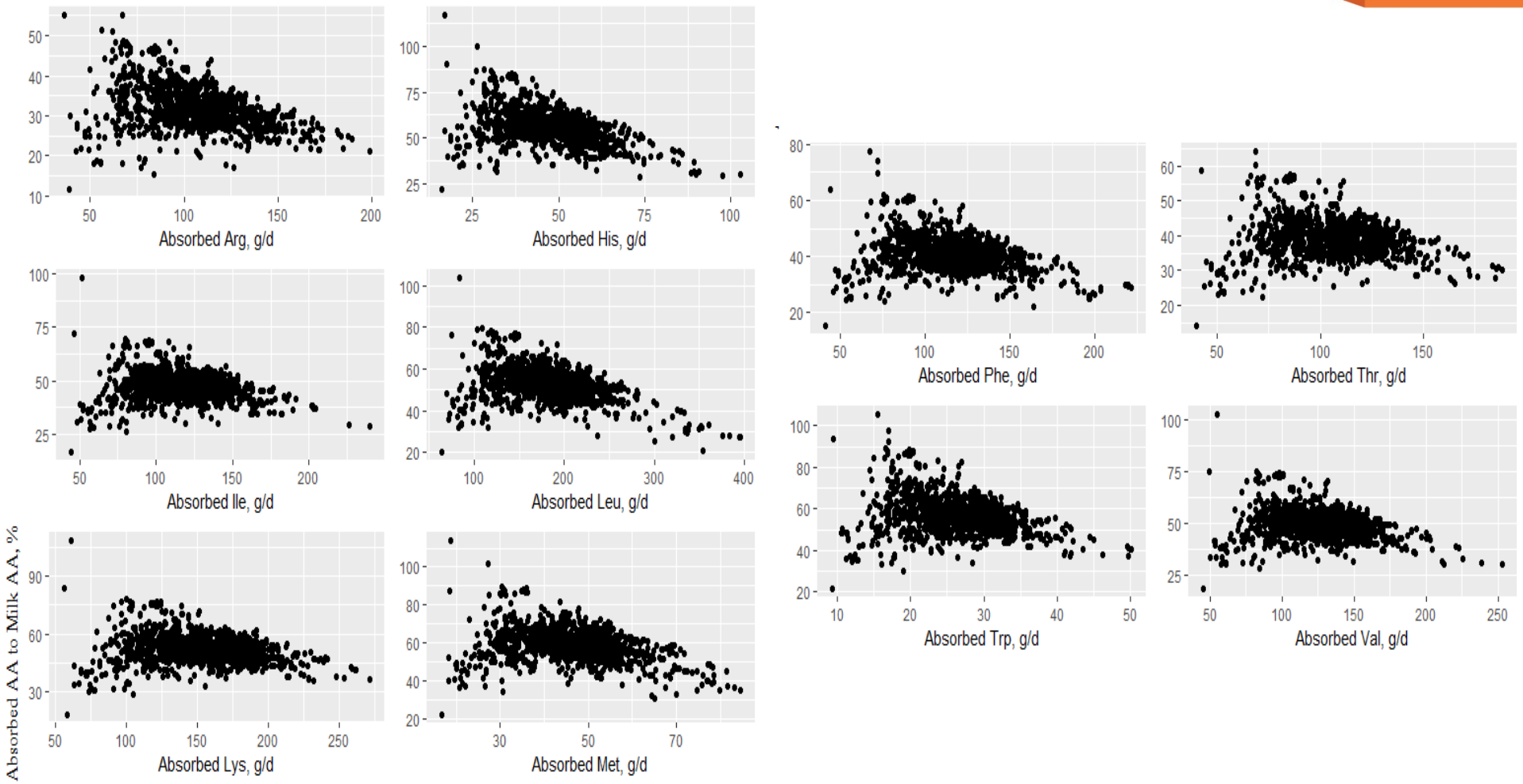
Cross Evaluation Results – 500 Iterations

Variable	NRC 2001	NRC 2021	SE
Observed Mean, g/d	918	921	17
Predicted Mean, g/d	890	923	12
RMSE	228	131	7
RMSE, % mean	**24.9**	**14.3**	**0.8**
Mean Bias, % MSE	2	0.7	1.0
Slope Bias, % MSE	32	4	3
CCC	0.65	0.75	0.03

- Arg, Leu, Thr, & NEAA trends
- Trp, Phe, and Val → inadequate data



Efficiency of Absorbed EAA Conversion to Milk EAA



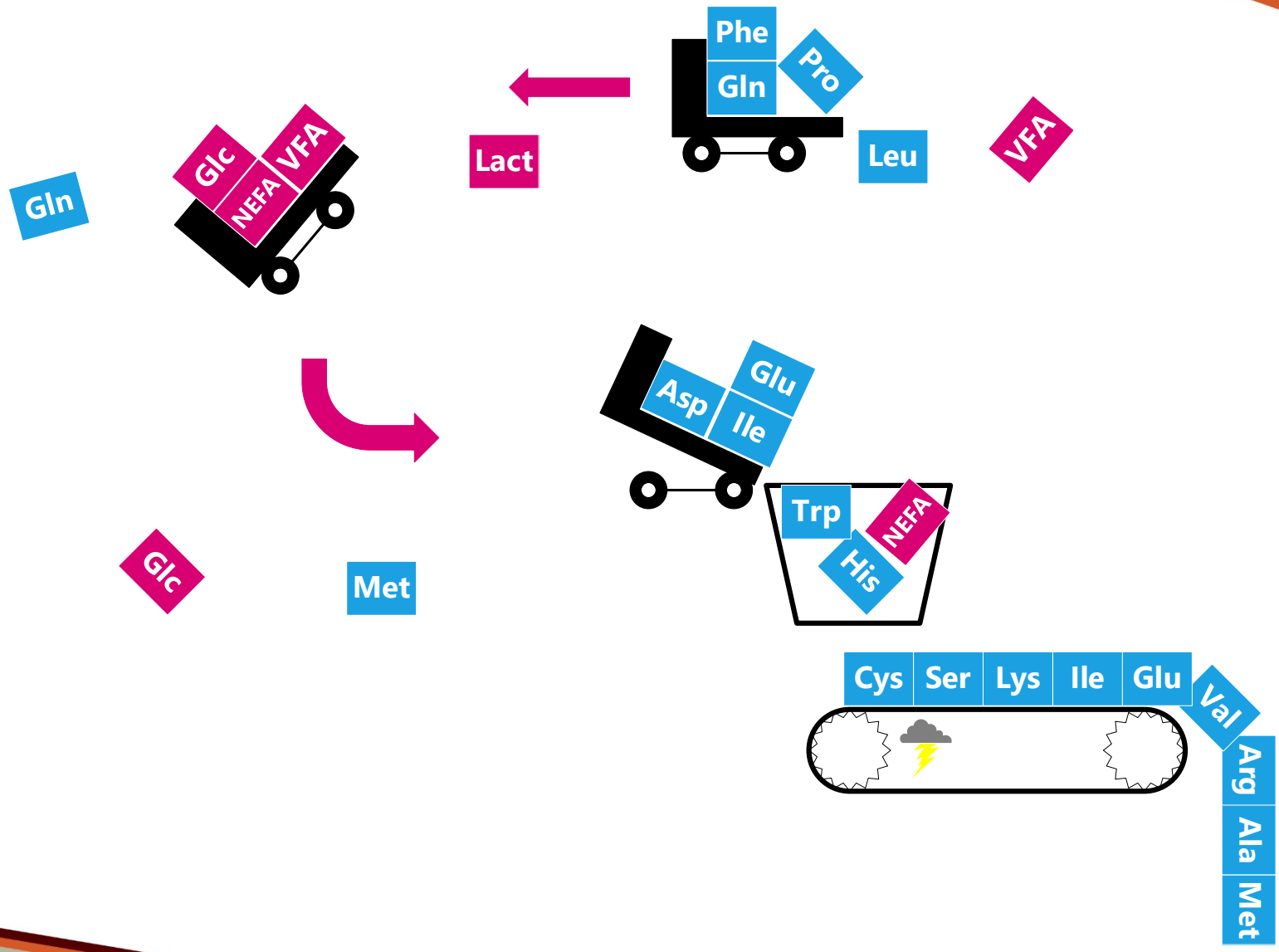
Using Total AA Efficiency as a Guide: Table 6.3

Example Diet 3: 35 kg milk , 24.9 kg DM/d, 14.7% CP

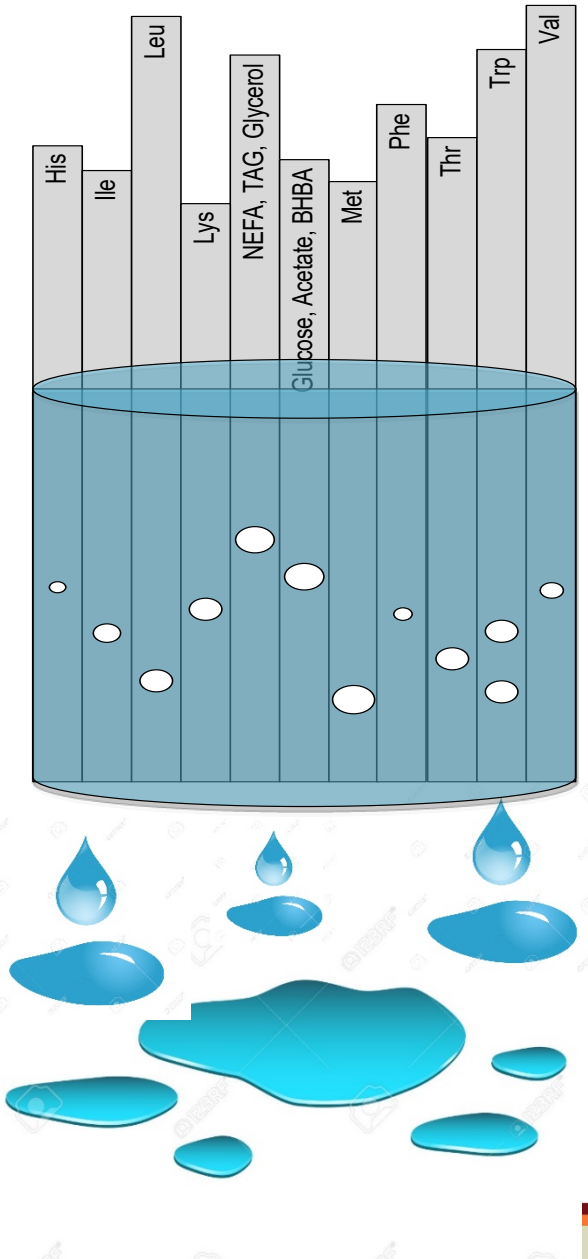
MP Supply: 2117 g, Target MP: 2320 g
NE Allow Milk: 40.9 kg

	Trg Milk NP	Trg Effic	Trg Suppl	Pred Suppl	Pred Effic	Regr Coeff	Milk NP
Int_BW_NDF							-115
DEInp				62		10.79	665
Arg	41			130	0.47	0	0
His	32	0.75	60	54	0.81	1.675	91
Ile	67	0.71	121	133	0.64	0.885	117
Leu	115	0.73	204	205	0.71	0.466	96
Lys	96	0.72	174	170	0.72	1.153	196
Met	33	0.73	55	49	0.80	1.839	91
Phe	57	0.60	127	130	0.57	0	0
Thr	50	0.64	118	118	0.62	0	0
Trp	18	0.86	28	29	0.82	0	0
Val	75	0.74	135	138	0.71	0	0
EAA2	582		1021	1156	0.66	-0.00215	-202
AA_other				1976		0.0773	153
Nutr_Allow	1085				0.69	NA	1092
Milk, kg/d							33.5

Conceptual Change for Milk Protein vs 2001



Summary



- Milk Protein
  - Responds Additively to:
    - Individual EAA supplies
    - Energy supply
    - Hormonal signals
  - Has some nonlinearity
  - New equations representing those are far superior
- Implications
  - substrate available
  - barrel is leaking not spilling over
  - no such thing as first-limiting nutrient for protein
  - no unique requirements for protein synth substrates
  - infinite substrate combinations yield similar output

Acknowledgements

Final model, publication, and software commissioned by NASEM, but extensive prior work supported the effort!

Funding

- UK Government
- Purina Animal Nutrition
- England and Wales Milk Marketing Board
- Nutreco
- AFRI Grant: 2012-67015-19464
- AFRI Grant: 2017-67015-26539
- Balchem
- Evonik
- Perdue Ag Solutions
- Papillon
- Adisseo
- Ajinimoto
- Virginia State Dairymen's Association
- Virginia Agricultural Council
- The VT Pratt Endowment
- VT College of Agriculture and Life Sciences
- USDA under Regional Research Project NC-1040/NC-2040

Collaborators

- Jim France, U. of Guelph
- Brian Bequette, UMD
- Les Crompton, U. Reading
- Chris Reynolds, U. Reading
- John Mecalf , U. Reading
- Gerald Lobley, Rowett
- John MacRae, Rowett
- John Sutton , U. Reading
- Nate Smith, Purina
- Larry Reutzel, Purina
- Helene Lapierre, Ag Canada
- Alex Hristov, Penn State Univ.
- Jeffery Escobar, VT/Novus
- Mike Akers, VT
- Mike McGilliard, VT
- Ondrej Becvar, VT
- Hollie Schramm, VT
- Danijela Kirovski, Univ. of Belgrade
- Xueyan Lin, Shandong Ag Univ.
- Zhonghua Wang, Shandong Ag Univ
- Jeff Firkins, Ohio State.
- Niels Kristensen, Aarhus Univ.
- Adam Storm, Aarhus Univ.
- Mogens Larsen, Aarhus Univ.
- Tatiana Ruiz-Cortes, Univ. Antioquia
- Chong Wang, Zhejiang Ag. & Forestry
- Ning Jiang, Heilongjiang Bayi Ag. Univ

Students/Technicians

- Tara Pilonero
- Agustin Rius
- Ranga Appuhamy
- Ashley Bell
- Sebastian Arriola
- Michelle Aguilar
- Ashley Felock
- Rebecca Garnett
- Tyler McGill
- Juan Castro
- Kari Estes
- Adelyn Myers
- Robin White
- Xinbei Huang
- Guimei Liu
- Xin Feng
- Veridiana Daley
- Meng Li
- Peter Yoder
- Jacquelyn Prestegaard
- Alexis Hruby
- Alvaro Morales
- Leticia Marra Campos
- Vinicius de Souza