Control of milk protein synthesis by amino acids in dairy cows

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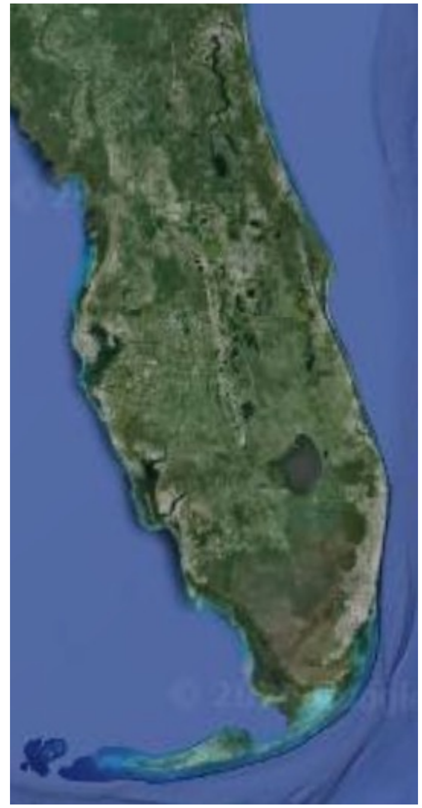
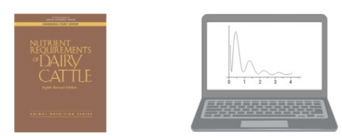


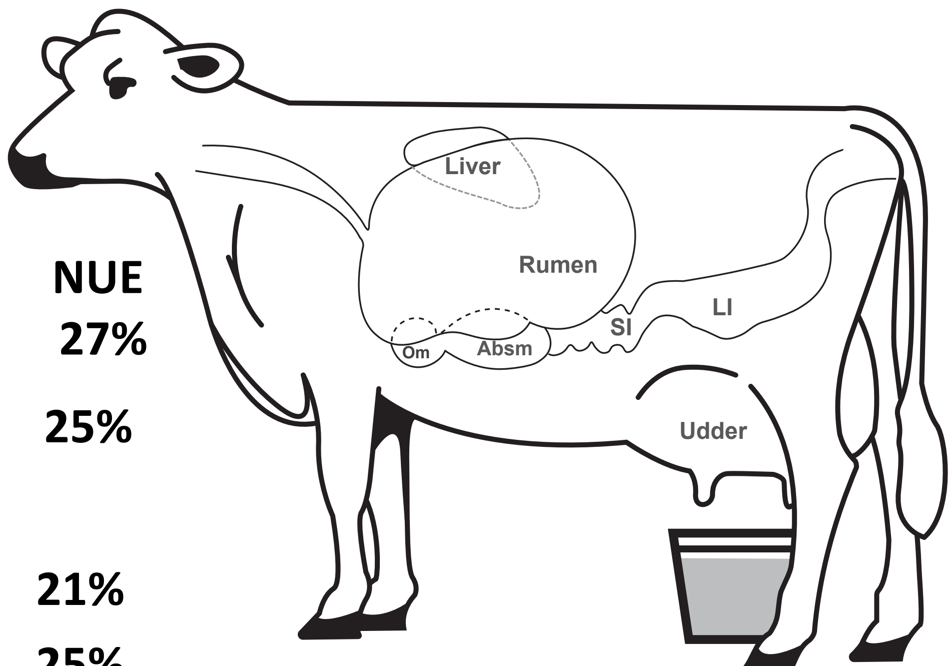
Google earth

OUTLINE

- Nitrogen (N) efficiency and emissions
- Limiting amino acids (AA)
- Regulation of milk protein synthesis, . . . *and beyond*
  - Transcription
  - Translation
  - Insulin role
  - Energy sources
- Model performance

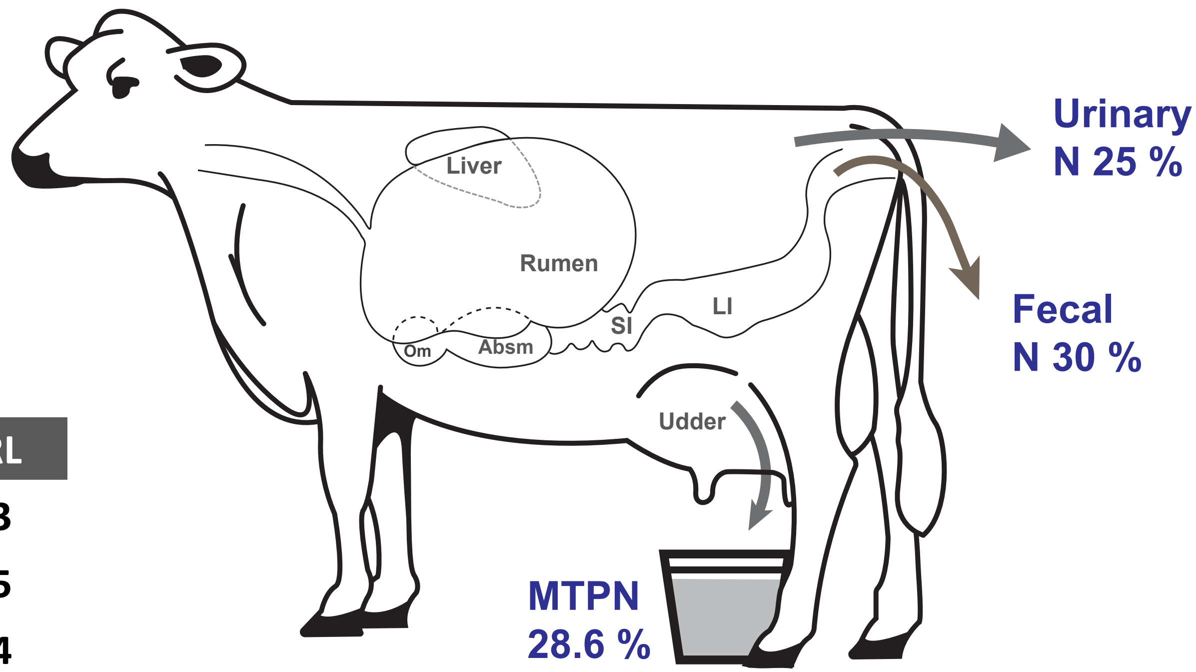


How much N does a lactating cow waste?



	NUE
Spek et al., 2013	**27%**
Arriola Apelo et al., 2014	**25%**
Brito & Silva, 2020	
Jersey - Organic	**21%**
Jersey - Conventional	**25%**
Non-Jersey - Organic	**27%**

Where is the N going?

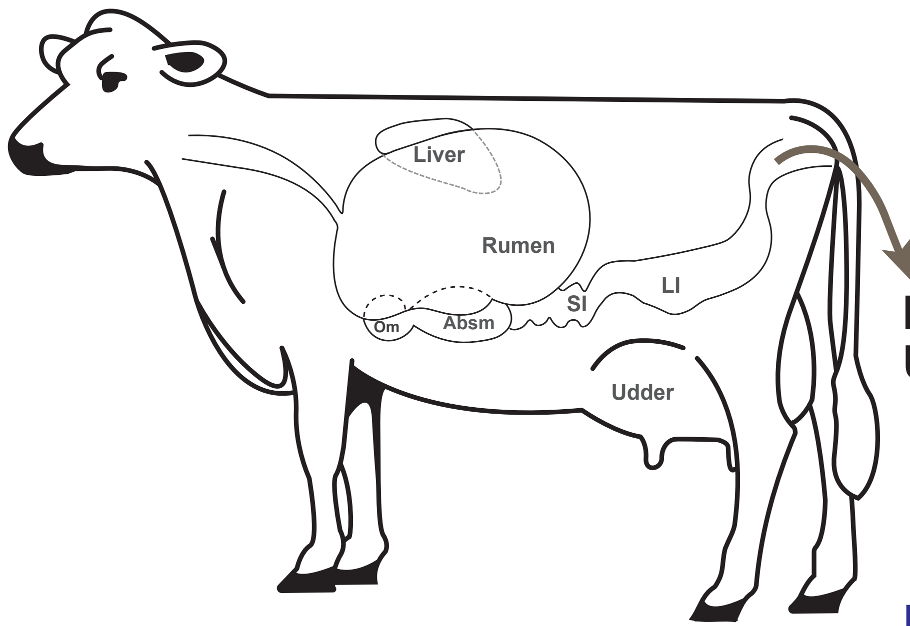


	CTRL
CP %	17.3
DMI, lb/d	48.5
Milk lb/d	83.4
Protein %	2.99
MUN mg/dL	11.9

Adapted from Chowdhury et al. JDS 2024

Is all the N excreted the same?

Risk of negative environmental impact of N emissions



Fecal N

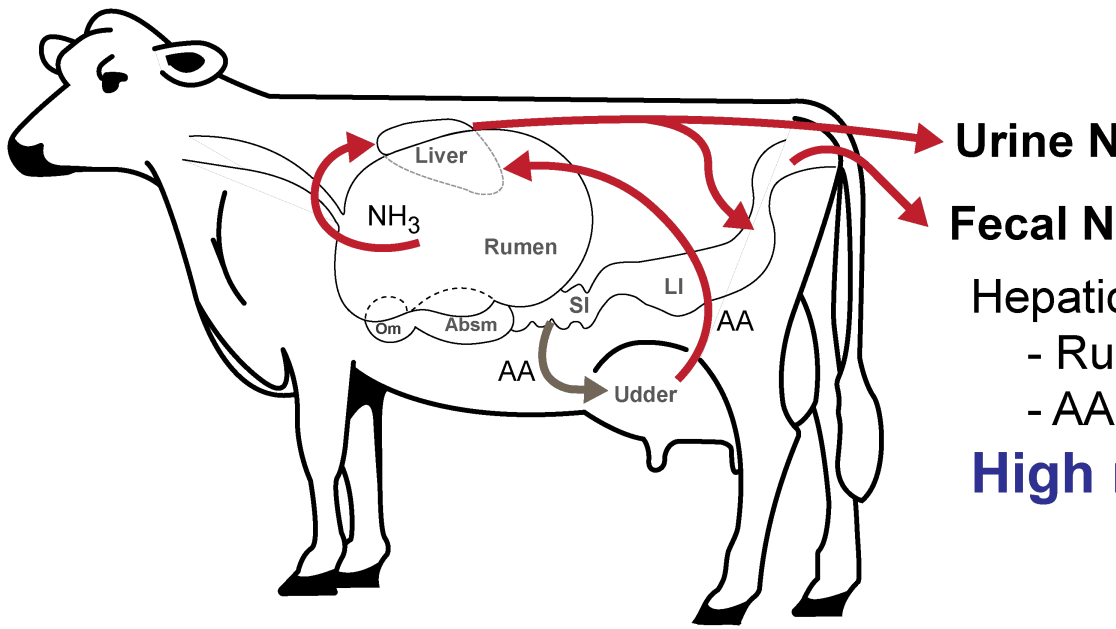
Undigested proteins

- ADIN
- Maillard
- MiCP
- Endogenous

Low risk

Is all the N excreted the same?

Risk of negative environmental impact of N emissions

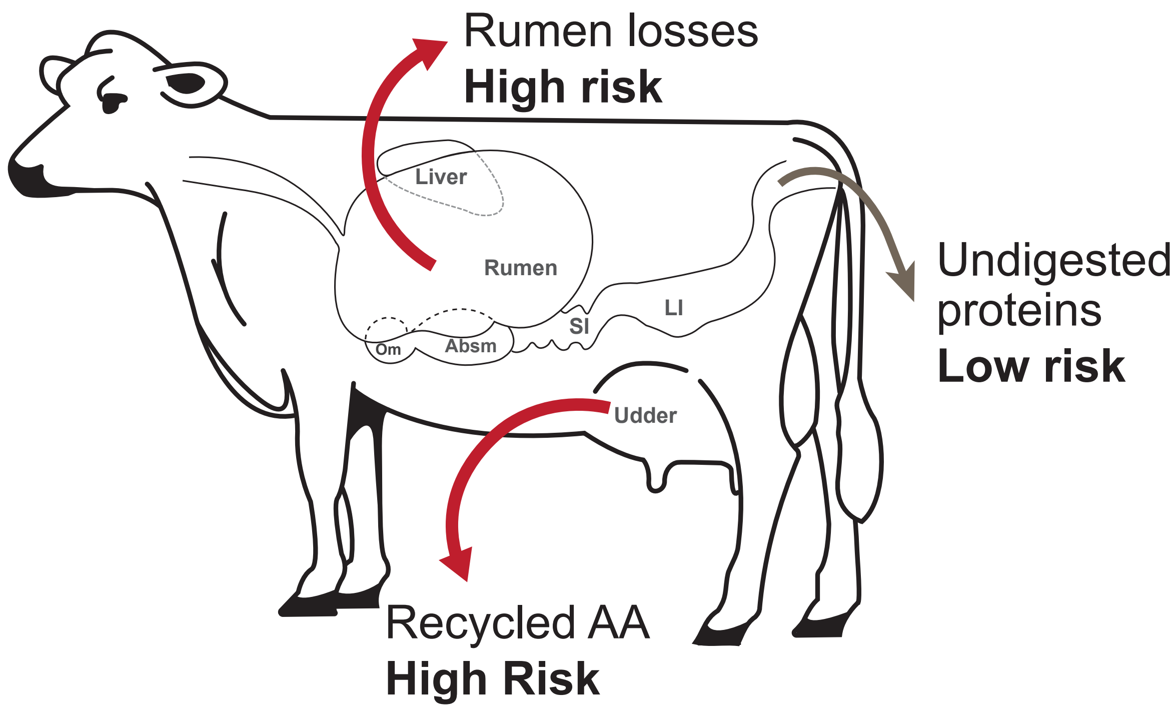


Hepatic ureagenesis

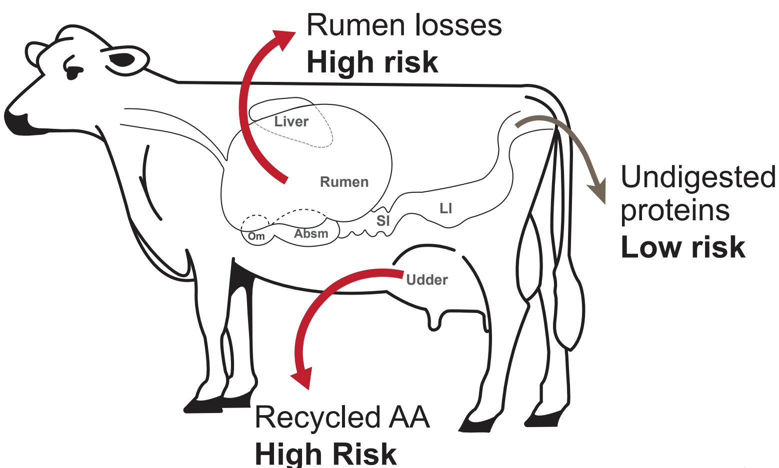
- Rumen ammonia
- AA catabolism

High risk

Risk of negative environmental impact of N emissions



Prediction of N partitioning by NASEM



N losses, % intake	CTL	BAA
[1]Rumen	21.6	18.7
[2] Intestinal	24.9	29.2
[3] Catabolic	13.0	17.6

$$^{1}\text{Rumen} = \frac{\text{RDP\_Bal\_g}}{6.25 \times \text{NIn\_g}}$$

$$^{2}\text{Intestinal} = \frac{(\text{RUPIn\_g} + \text{MiCP\_g} - \text{An\_MPIn\_g})}{6.25 \times \text{NIn\_g}}$$

$$^{3}\text{Catabolic} = \frac{(\text{MPIn\_g} - \text{Mlk\_NP\_g} - \text{Scrf\_NP\_g} - \text{Fe\_NPEnd\_g} - \text{UrNPEnd\_g})}{6.25 \times \text{NIn\_g}}$$

Ruh, . . ., Arriola Apelo, Unpublished data

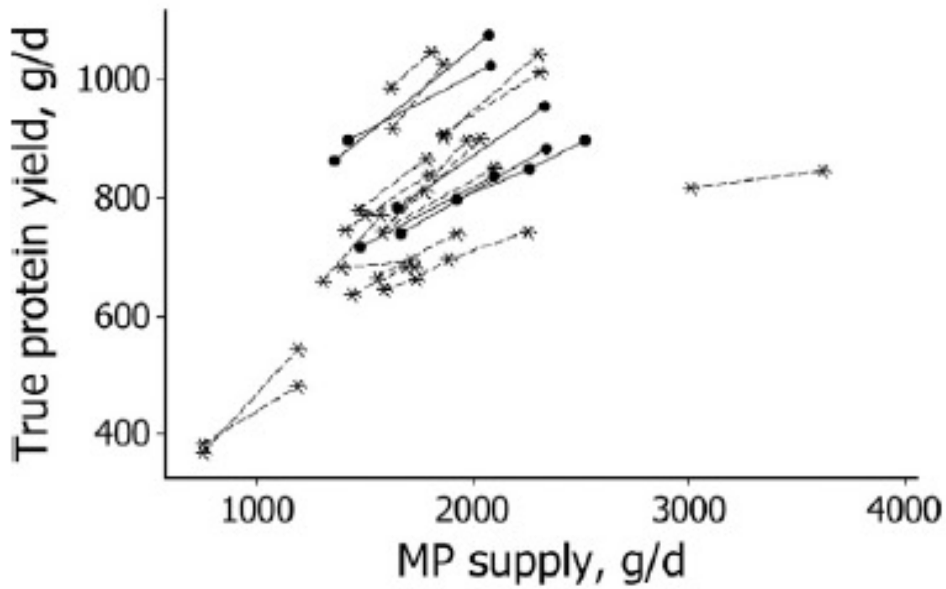
Effect of protein level on N use efficiency

	CTRL	L-CP	*P (n=14)*
CP %	17.3	15.1	
DMI, lb/d	48.5	46.4	0.37
Milk lb/d	83.4	79.9	0.17
Protein %	2.99	3.01	0.4
MUN mg/dL	9.44	6.91	<0.01
NUE %	**28.6**	**33.9**	**<0.01**

– 25% increase in N efficiency
– Relative increase in more stable fecal N
– Absolute and relative decrease in urea-N losses

Adapted from Chowdhury et al. JDS 2024

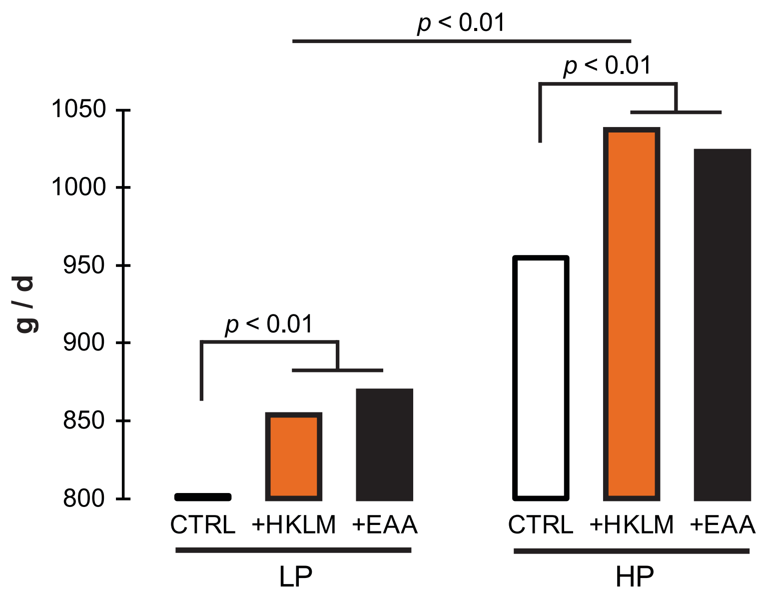
MP effect on MTP yield



POSSITIVE **DIMINISHING** RESPONSE

Lapierre et al. JAS 2012

Balancing for His, Lys, Leu, and Met or all the EAA



Milk protein yield

Haque et al., JDS 2012

Limiting AA theory

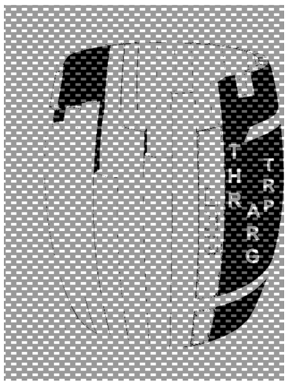
The **first limiting AA** (e.g. Met) limits responses to other AA

Substrate based approach, but . . .

Does the cow runs out of AA?

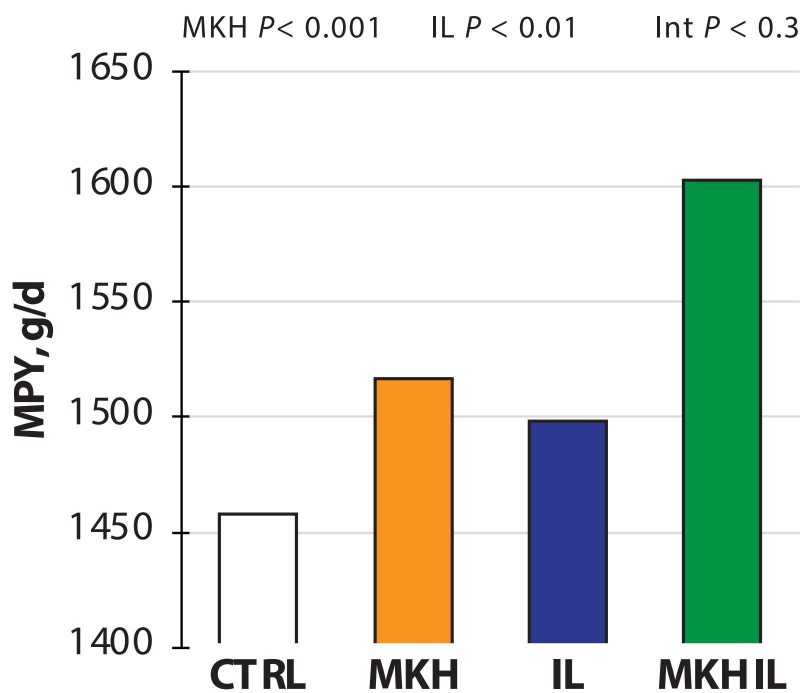
What about fat responses?

Independent AA effects – MPY response to jugular infusion of 5 essential AA



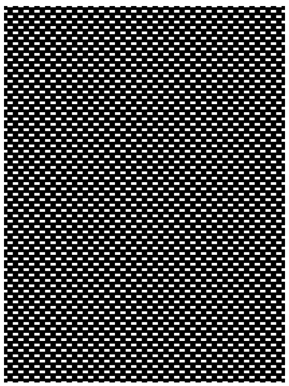
Independent, additive responses to different AA contradicts the idea of a first limiting AA

<u>Met, Lys, His, Ile, and Leu</u> became the 5 NASEM AA with independent, additive effects on MPY

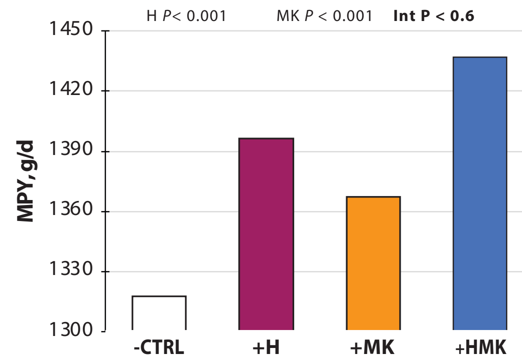


Adapted from Yoder et al. JDS 2020

Independent AA effects – diet approach



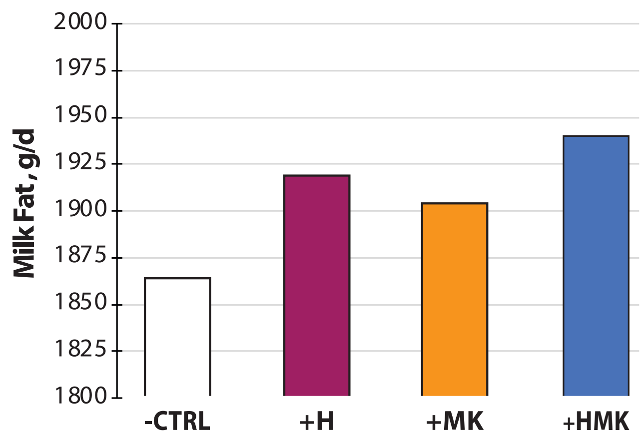
Independent, additive responses using dietary approaches



	-CTRL	+H	+MK	+HMK
His	–	21	3	24
Lys	–	6	42	44
Met	–	-1	14	13
Ile	–	-8	5	-4
Leu	–	15	21	32
EAA	–	81	108	184
MP	–	100	191	272

Killerby, . . . Arriola Apelo, unpublished

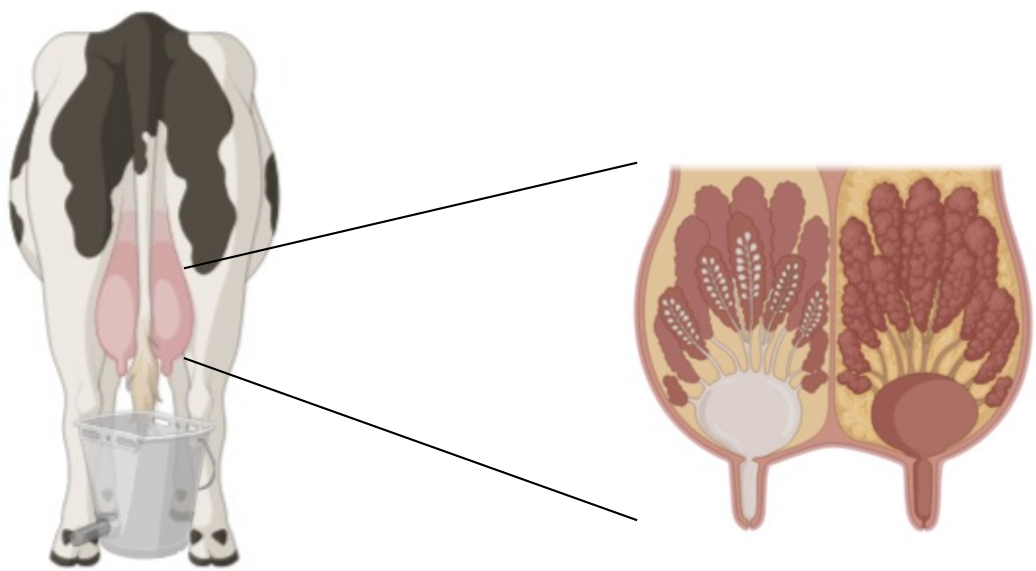
AA effects on milk fat production



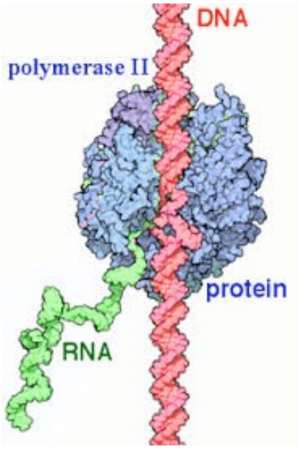
Independent AA effects on milk fat synthesis

Killerby, . . . Arriola Apelo, unpublished

Regulation of milk protein synthesis in the mammary glands



REGULATORY MECHANISMS



DNA

tRNA

Ribosome

Transcription

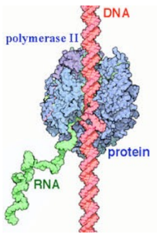
Jak
STAT

Translation

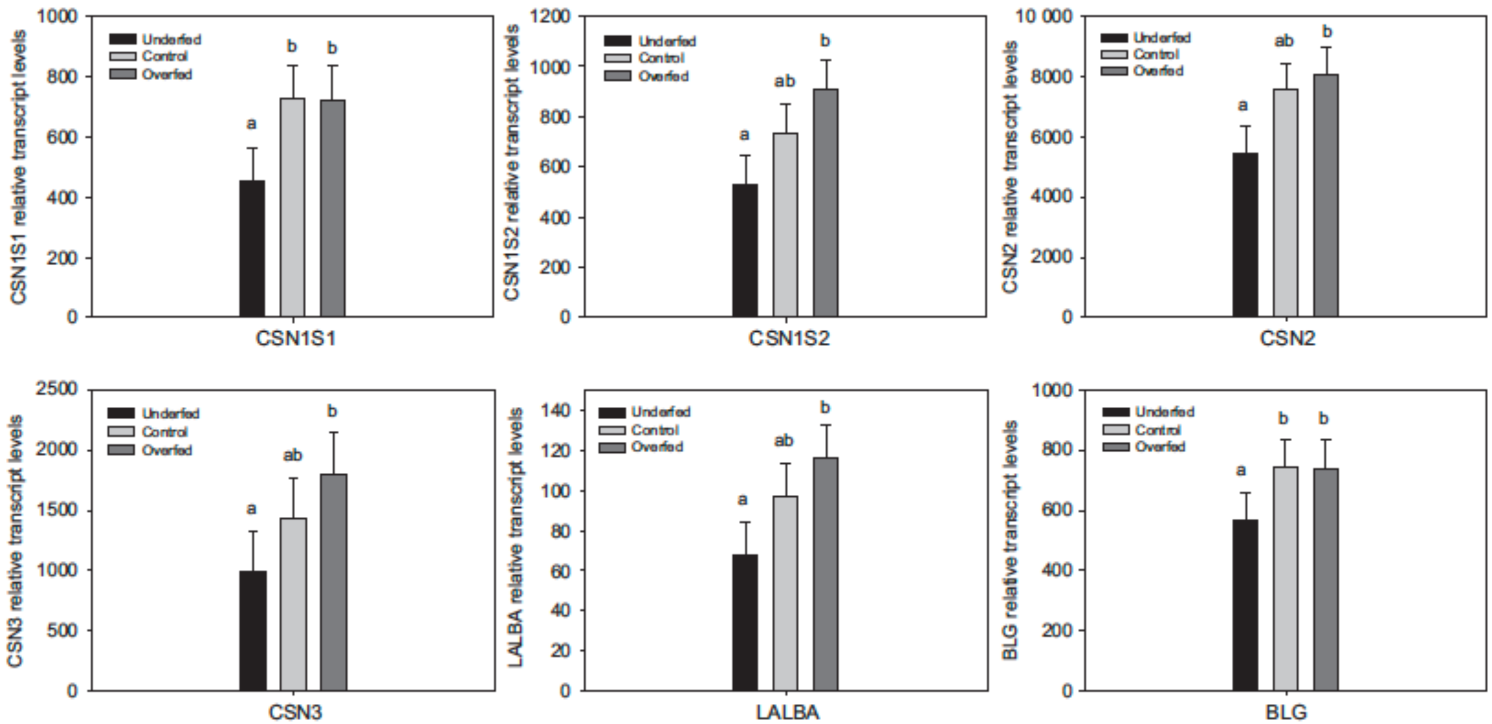
Integrated
Stress
Response

mechanistic
Target **o**f
Rapamycin
Complex 1

Regulation of milk protein's gene transcription



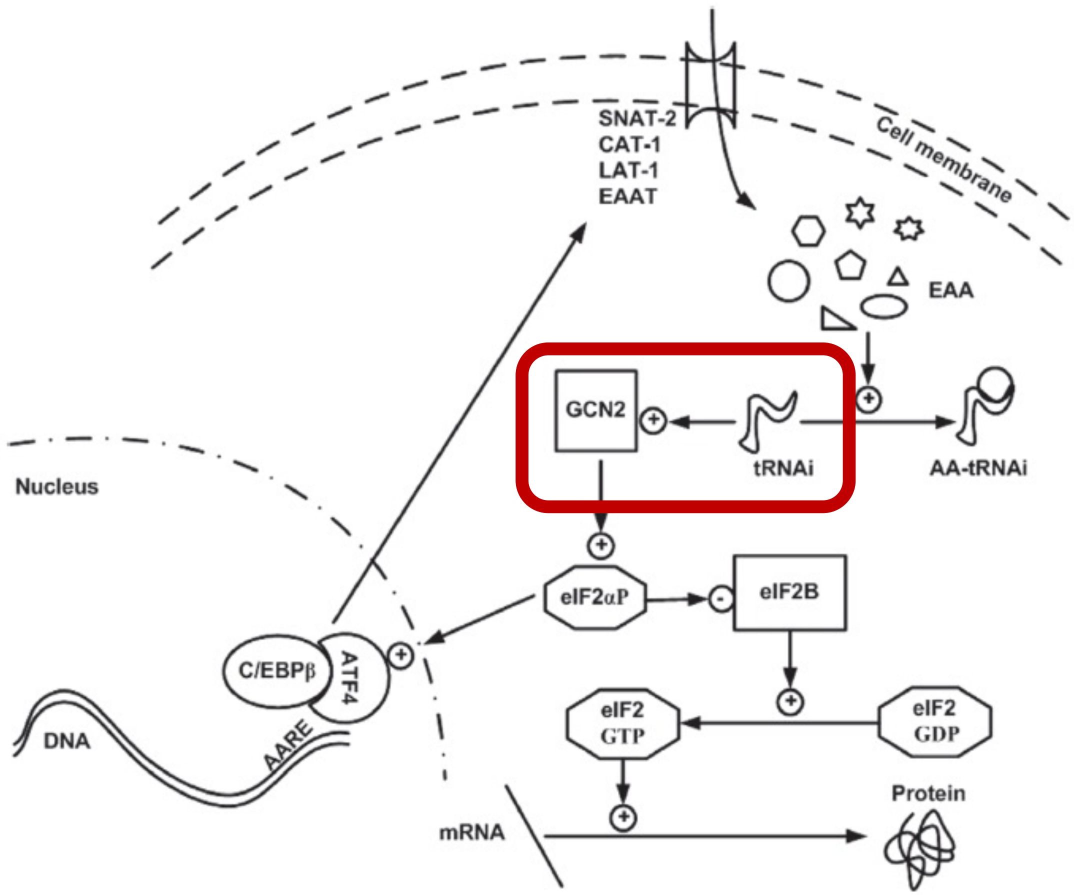
Transcription



Tsiplakou et al., JAPAN, 2015

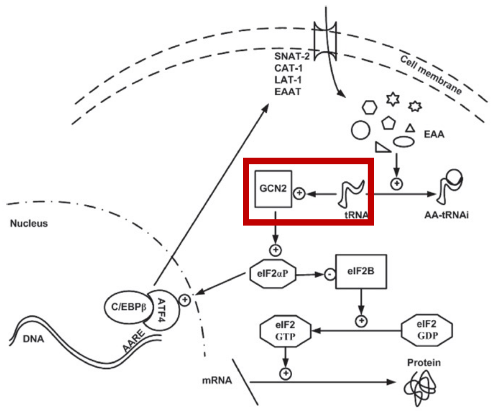
Regulation of milk protein translation - ISR

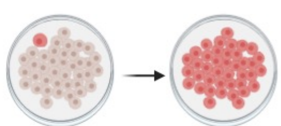
tRNA

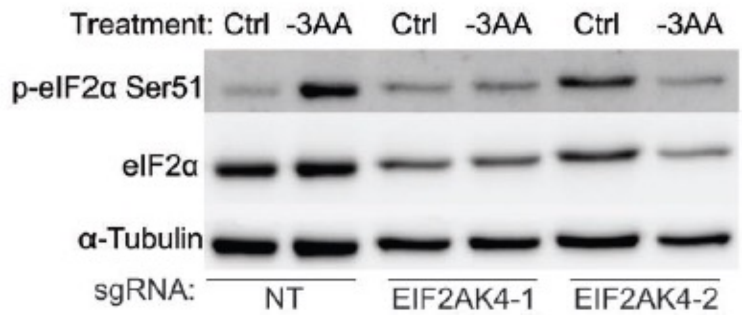


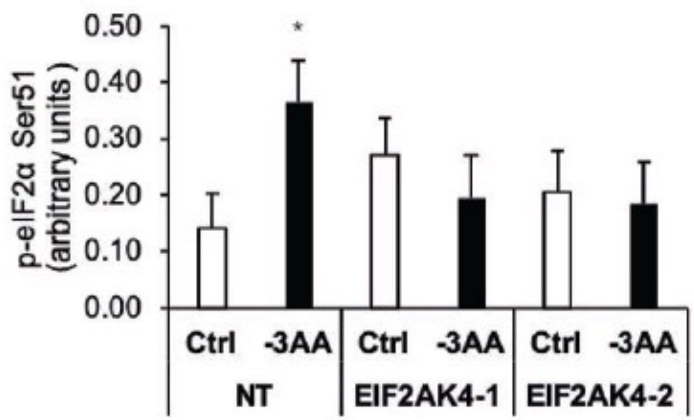
Arriola Apelo et al., JDS 2014

GCN2 sensing of AA in BMEC



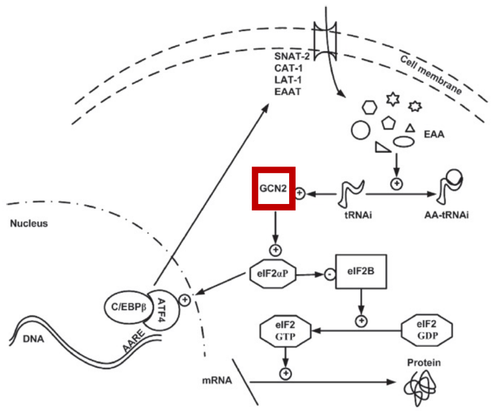




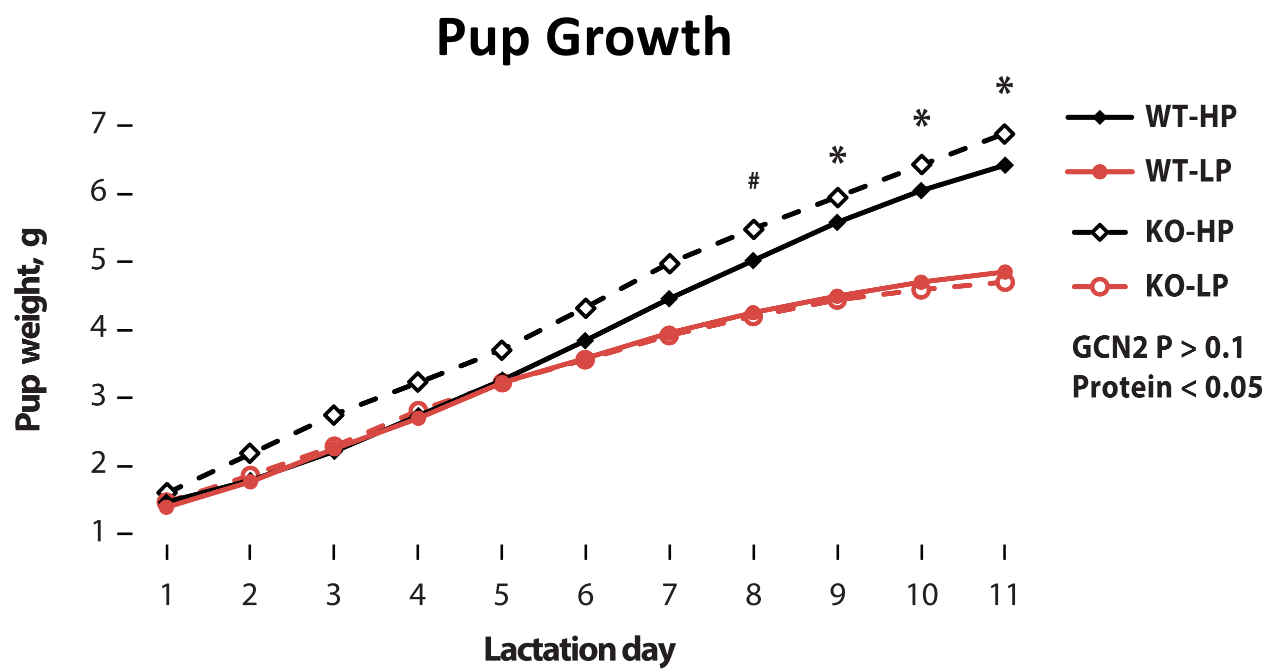


Edick et al., JDS 2021

GCN2 regulation of lactation

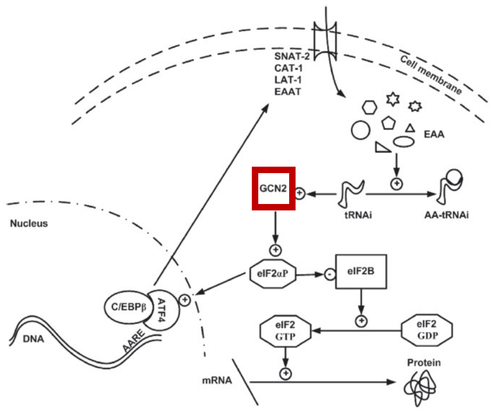


WAP-cre GCN2



Arriola Apelo, unpublished

GCN2 regulation of lactation



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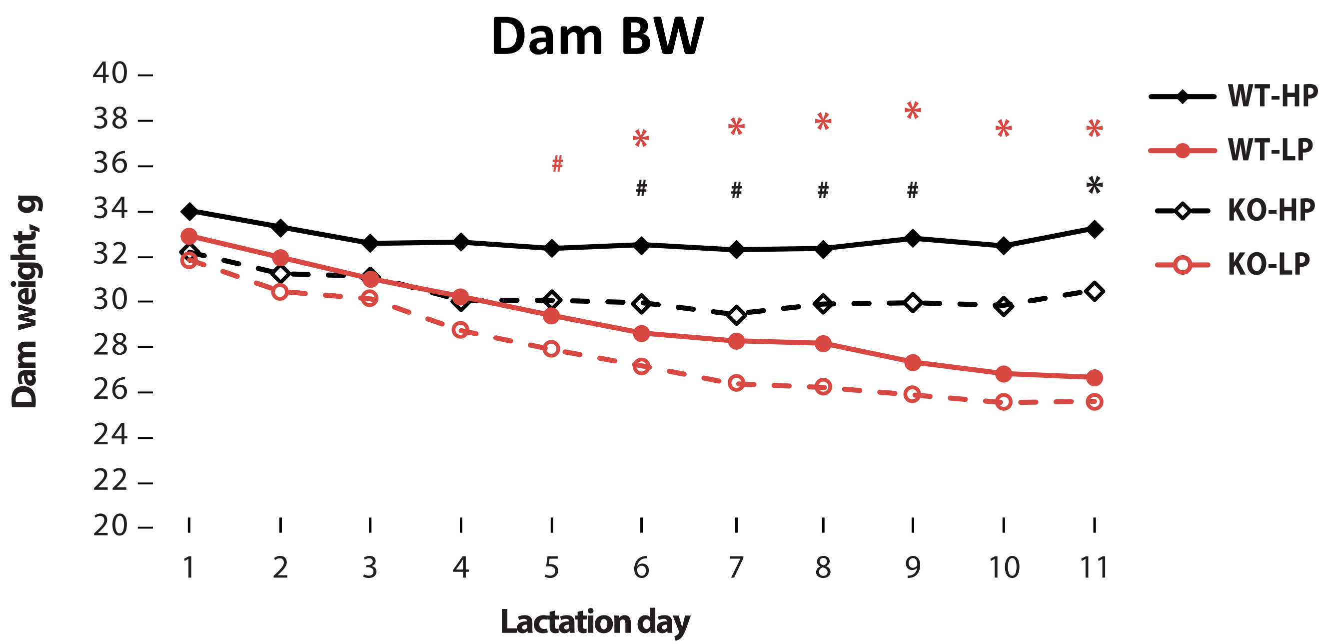

Arriola Apelo, unpublished

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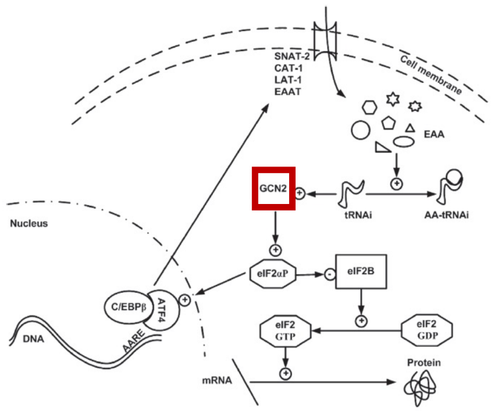

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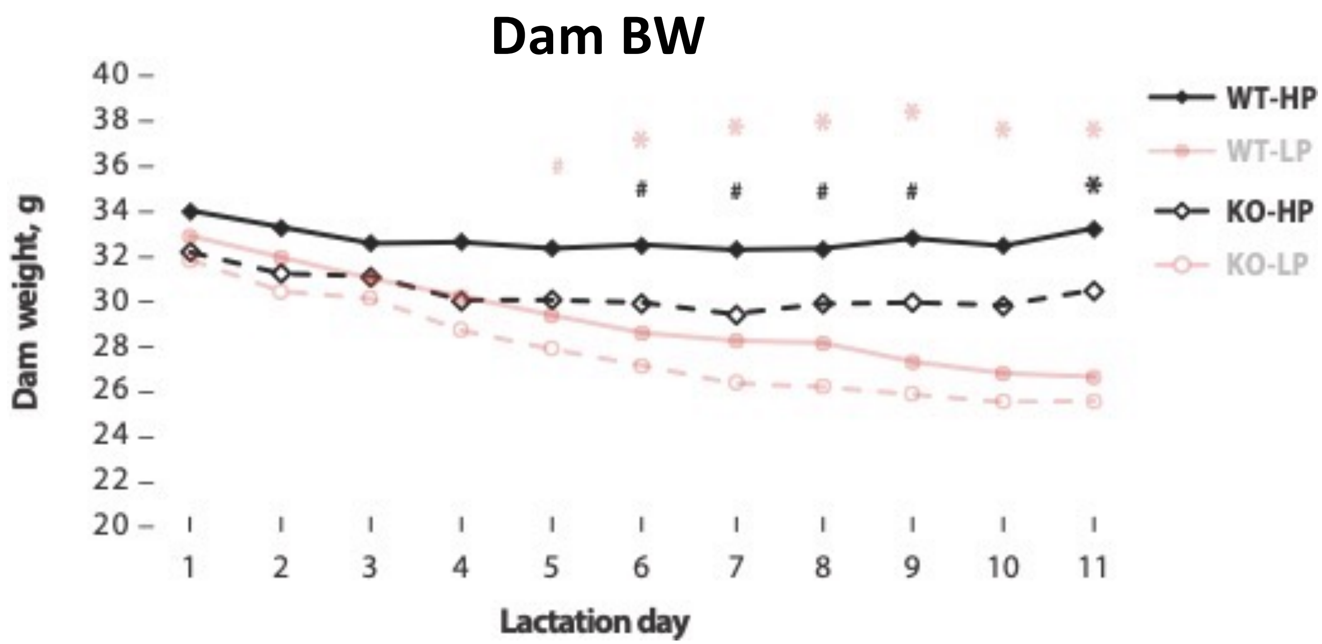
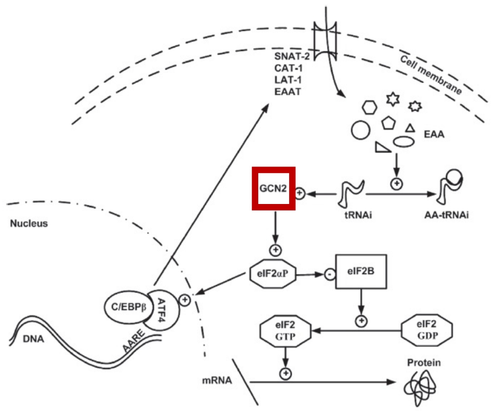
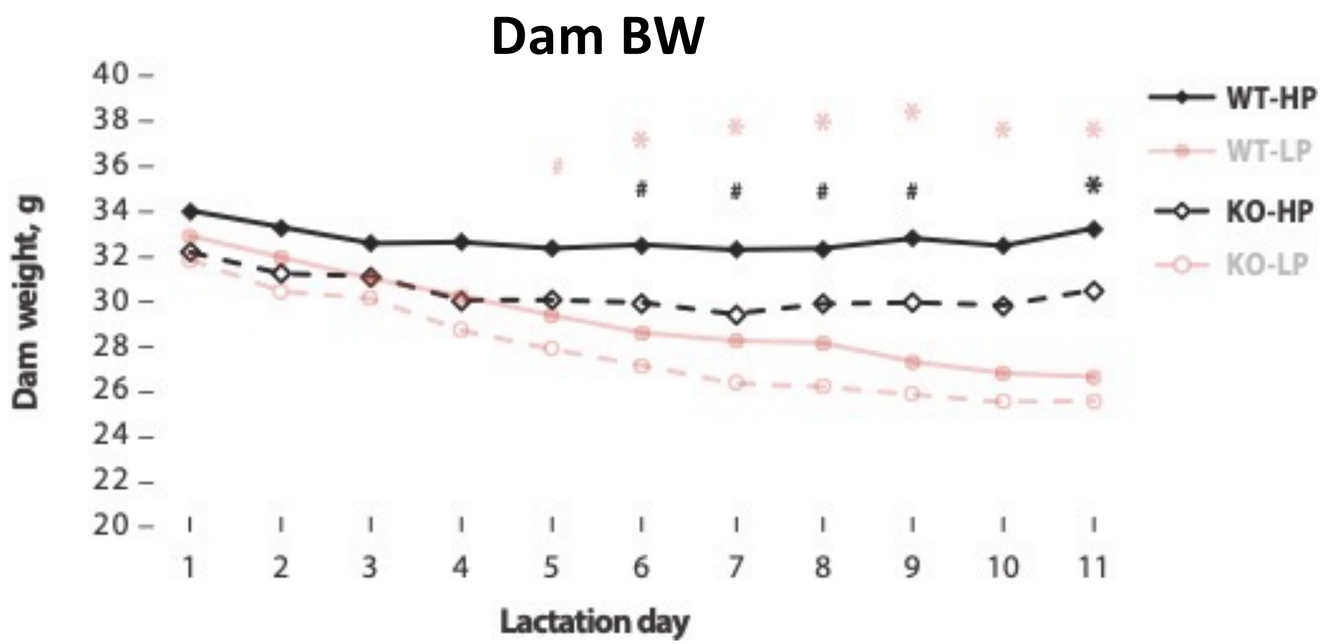


Arriola Apelo, unpublished

GCN2 regulation of lactation



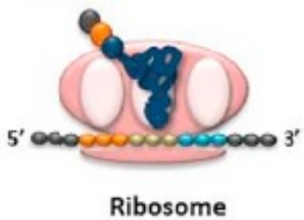
WAP-cre GCN2

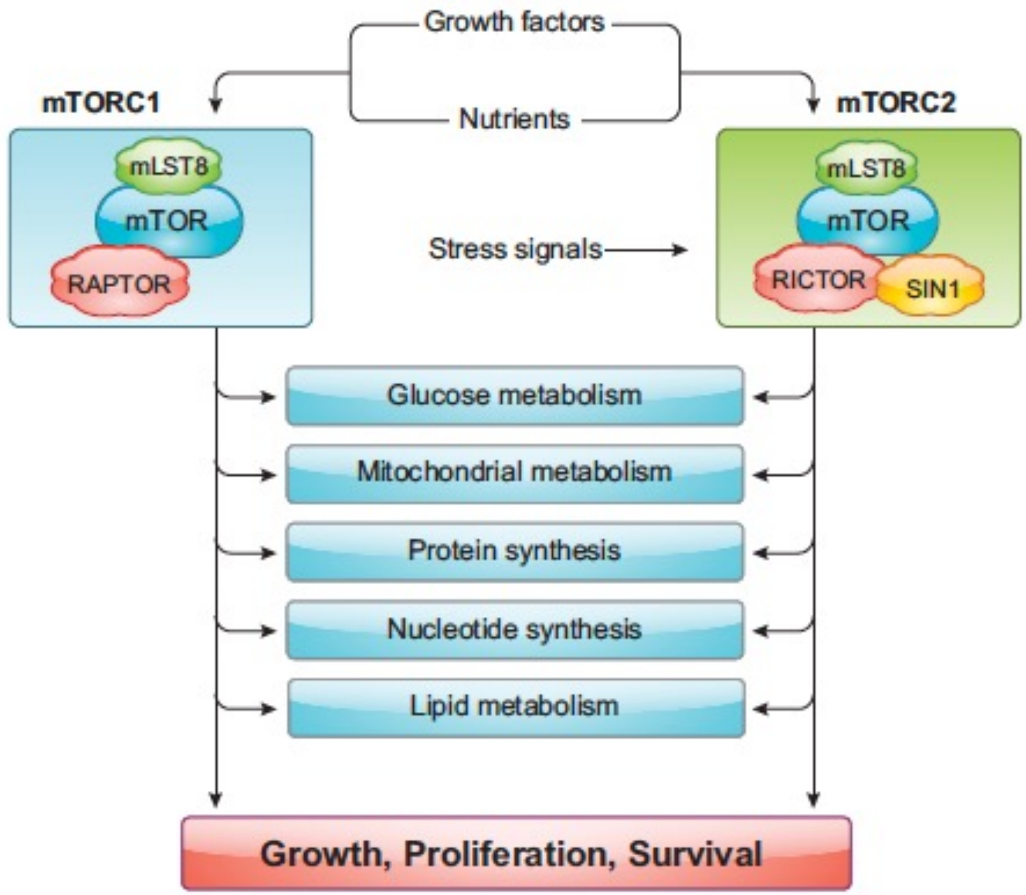


- Limited evidence in vitro and other species
- Probably more relevant under strong AA imbalance

Arriola Apelo, unpublished

mTORC1 regulation of translation, . . . *and beyond*





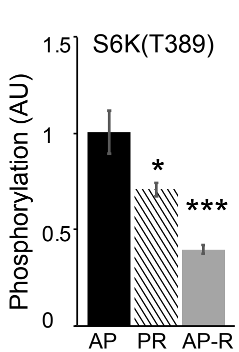
Swed et al., PR 2021

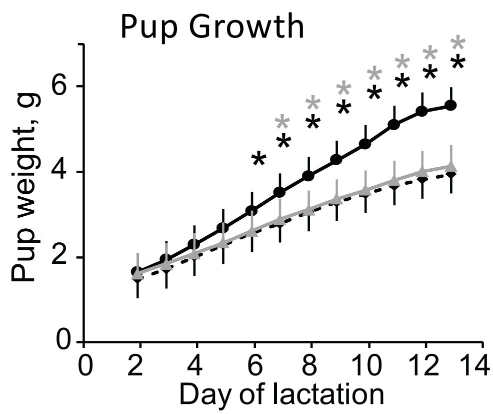
Review

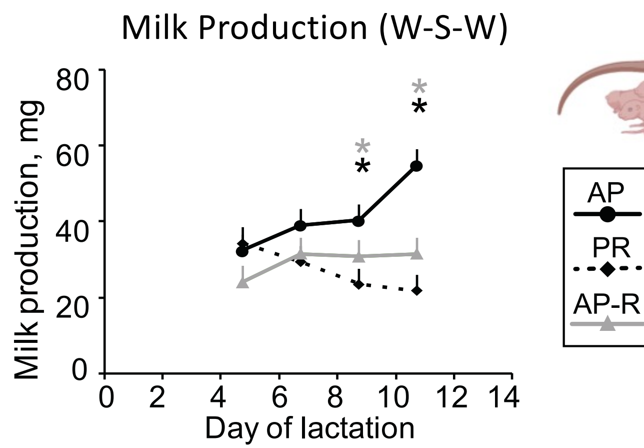
Rapamycin: An InhibiTOR of Aging Emerges From the Soil of Easter Island

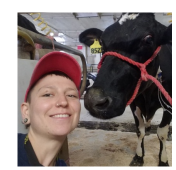
Sebastian I. Arriola Apelo and Dudley W. Lamming

Department of Medicine, University of Wisconsin–Madison and William S. Middleton Memorial Veterans Hospital, Madison, Wisconsin.









- mTORC1 mediates AA effect in murine lactation

Pszczolkowski et al., JASB 2020

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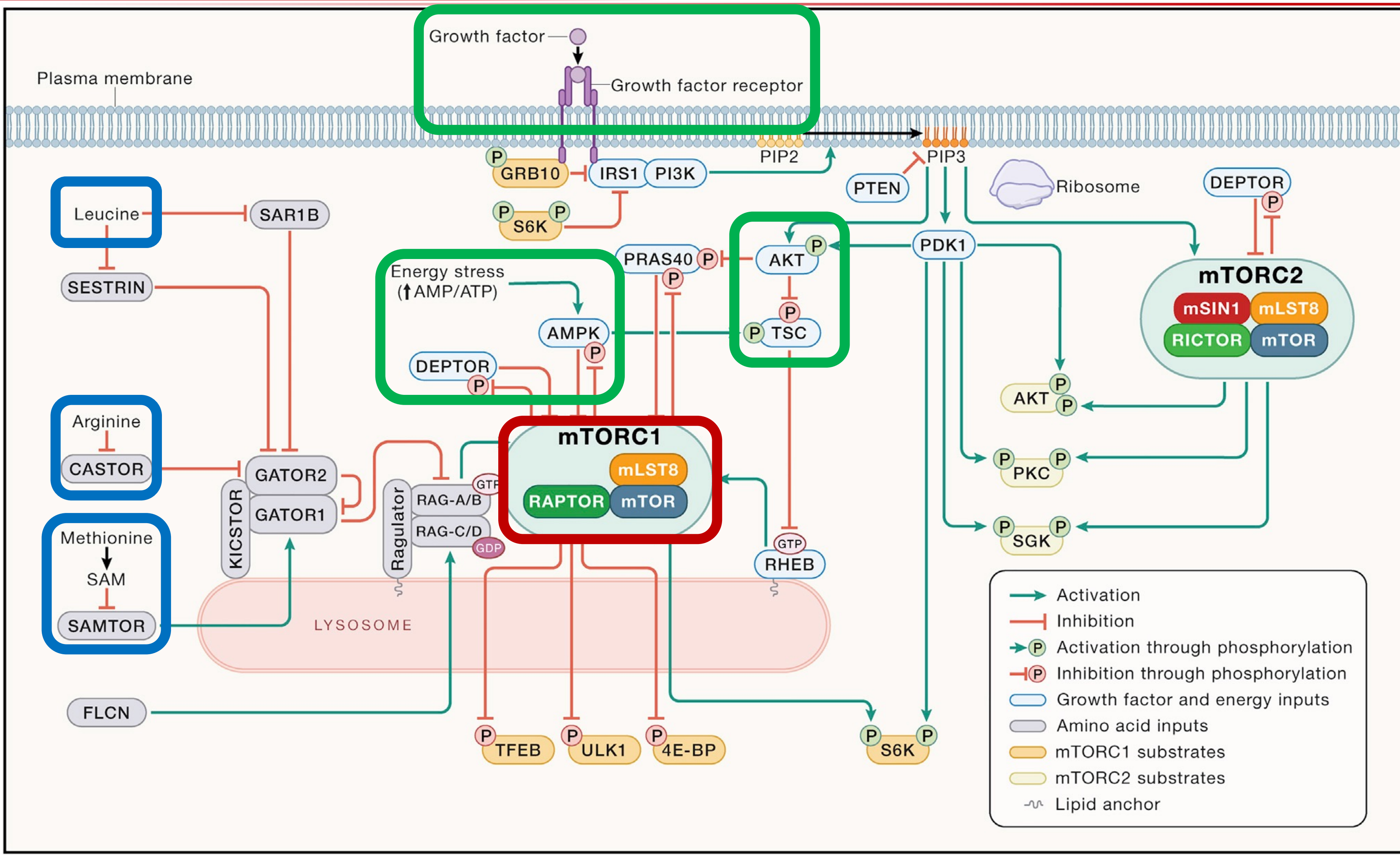
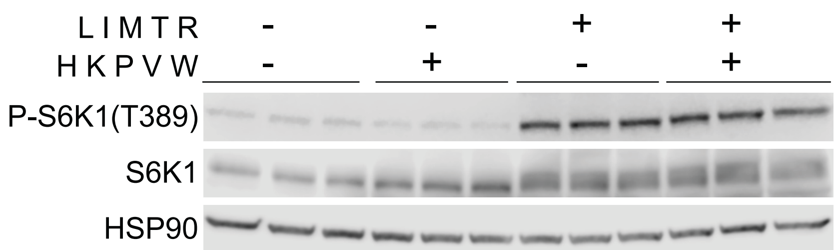
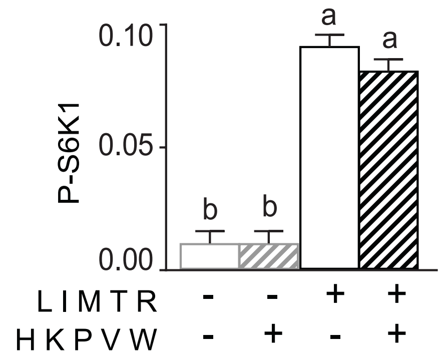
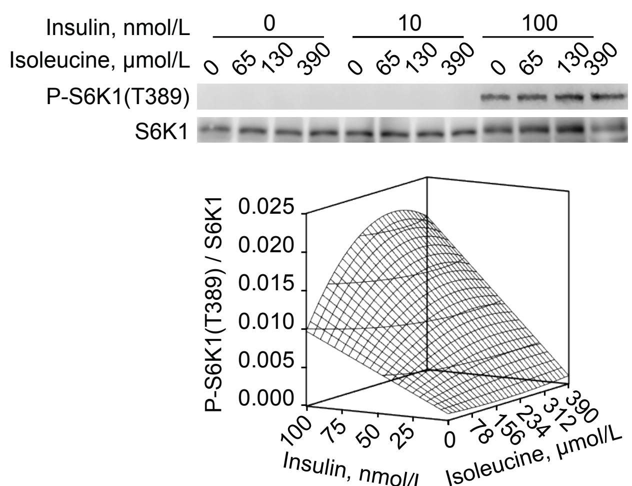
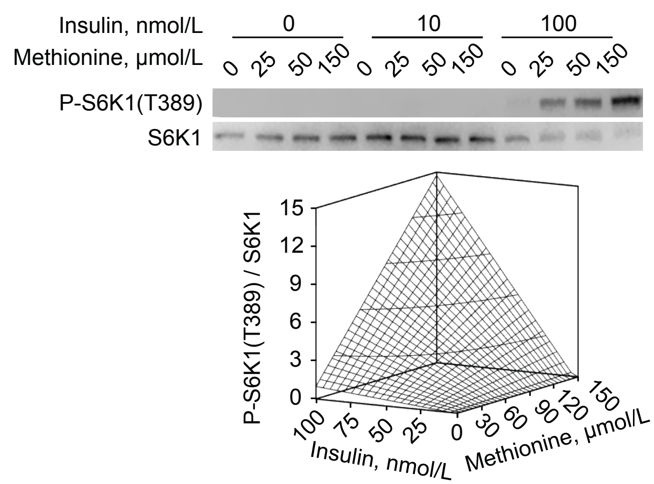


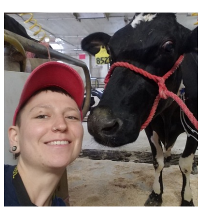
Specific AA regulation of mTORC1





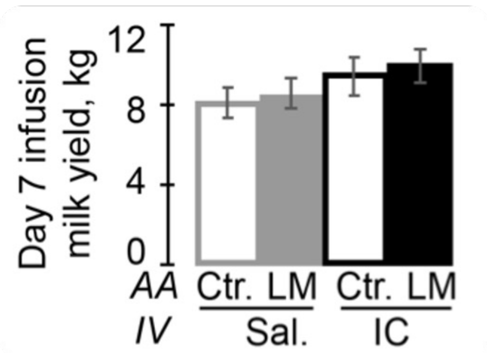


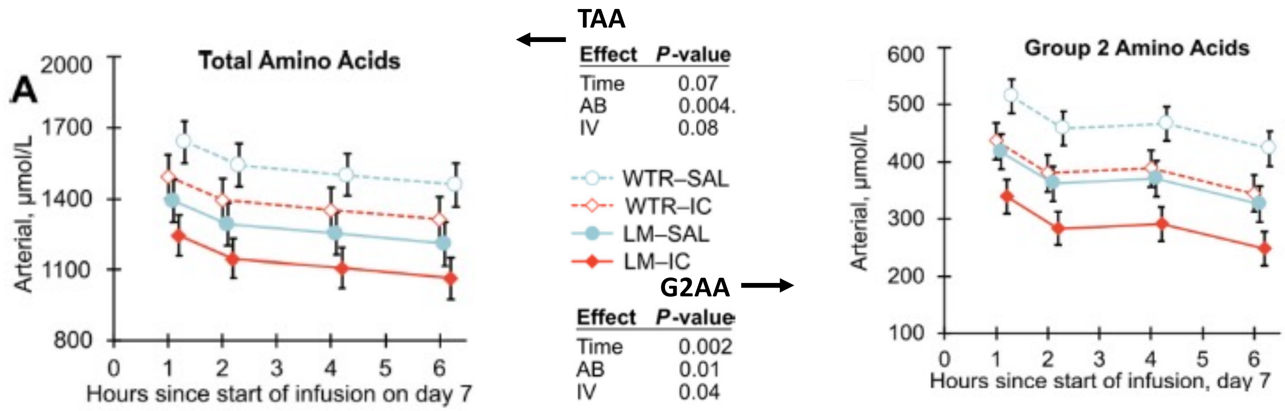




Pszczolkowski, Zhang et al., 2020

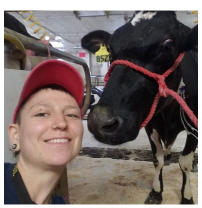
Insulin role in AA regulation of milk production





Mammary gland extraction of AA at h 6 of clamp

	WTR		LM			p-value	
%	SAL	IC	SAL	IC	SEM	AB	IV
Total AA	22.9	24.2	23.2	24.5	5.07	0.95	0.79
EAA	39.5	37.8	38.8	37.1	6.12	0.34	0.69
Group 1 AA	34.5	36.7	31.0	33.2	6.30	0.43	0.64
Group 2 AA	38.9	35.5	46.6	43.2	5.30	0.05	0.39
NEAA	14.2	16.5	11.8	14.1	6.05	0.68	0.70

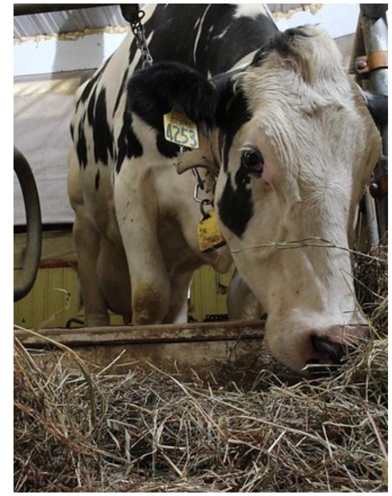


Pszczolkowski et al., DAE 2022

Starch role in milk production

Item	Diets (NDF : starch ratios)			
	T1	T2	T3	T4
Ingredient, % DM				
Alfalfa	15.0	15.0	15.0	15.0
Corn silage	20.0	25.0	30.0	35.0
Oat hay	0.0	5.0	10.0	15.0
Corn	35.0	25.0	15.0	5.0
CP	17.5	17.6	17.6	17.6
NDF	29.8	34.0	37.7	41.2
ADF	18.1	20.5	25.0	27.7
Starch	34.4	28.8	23.2	17.6
NEL†, Mcal/kg	1.81	1.73	1.65	1.57
DMI, kg/day	23.2[a]	21.7[a]	20.1[b]	18.3[c]
MP¶, g/day	3,029[a]	2,831[ab]	2,614[bc]	2,462[c]
Milk yield, kg/day	33.2[a]	33.0[a]	31.4[b]	28.3[c]
FCM†, kg/day	32.2[a]	32.5[a]	32.0[a]	29.2[c]
ECM‡, kg/day	34.2[a]	34.1[a]	33.4[b]	30.2[c]
Protein, kg/day	1.06[a]	1.02[b]	0.96[c]	0.85[d]

Adapted from Zhao et al. ASJ 2016



Substituting starch decreases:

- Dietary energy density
- Dry matter intake
- VFA production
- MiCP & MP supply
- Lactose, protein, and fat yield

Isocaloric substitution of starch with non-pNDF (+fat) in AA balanced diets

Ingredient, % DM	HS-DAA	HS-BAA	LS-DAA	LS-BAA
Corn silage	37.8	37.8	38.0	38.0
Haylage	33.5	33.5	33.6	33.6
Corn grain	14.7	14.3	8.0	7.6
Soybean hulls	10.7	8.1	14.8	12.2
80:10 C16C18:1	0.0	0.0	1.5	1.5
Soybean meal	0.8	0.8	1.6	1.6
SE-SBM	0.4	0.8	0.4	0.8
Corn gluten meal	0.0	2.4	0.0	2.4
RP-Met/Lys	0.0	0.2	0.0	0.2

Isocaloric substitution of starch with non-pNDF (+fat)

Ingredient, % DM	HS-DAA	HS-BAA	LS-DAA	LS-BAA
Corn silage	37.8	37.8	38.0	38.0
Haylage	33.5	33.5	33.6	33.6
Corn grain	14.7	14.3	8.0	7.6
Soybean hulls	10.7	8.1	14.8	12.2
80:10 C16C18:1	0.0	0.0	1.5	1.5
Soybean meal	0.8	0.8	1.6	1.6
SE-SBM	0.4	0.8	0.4	0.8
Corn gluten meal	0.0	2.4	0.0	2.4
RP-Met/Lys	0.0	0.2	0.0	0.2
RDP	**9.0**	**9.0**	**9.0**	**9.0**
MP	**7.5**	**8.8**	**7.8**	**9.0**
NDF	**34.5**	**31**	**39.6**	**36.0**
Starch	**28.0**	**28.2**	**20.5**	**20.7**
FA-H	**3.4**	**3.4**	**5.7**	**5.6**

=MP

Isocaloric substitution of starch with non-pNDF (+fat) in AA balanced diets

	HS		LS		*P* - values		
Item	DAA	BAA	DAA	BAA	ES	AA	ES x AA
DMI, kg/d	31.38	33.97	31.32	33.91	0.86	< 0.001	1.00
Milk kg/d	41.7	45.2	44.0	46.7	< 0.001	< 0.001	0.36
ECM, kg/d	42.4	46.0	46.4	49.4	< 0.001	< 0.001	0.61
Fat, g/d	1567	1674	1794	1878	< 0.001	< 0.001	0.67
Protein, g/d	1188	1356	1235	1380	0.03	< 0.001	0.47
Fat, %	3.85	3.80	4.10	4.05	< 0.001	0.39	0.97
Protein, %	2.87	3.05	2.87	2.95	0.12	< 0.001	0.11

Isocaloric substitution of starch with non-pNDF (+fat) in AA balanced diets

Item	HS		LS		*P* - values		
	DAA	BAA	DAA	BAA	ES	AA	ES x AA
Allantoin, mmol/d	445	440	493	465	0.19	0.56	0.68
MiCP, g/d	2164	2610	2251	2638	0.005	< 0.001	0.14
Urine N, g/d	149	195	174	237	< 0.001	< 0.001	0.048
Fecal N, g/d	274	310	262	318	0.81	< 0.001	0.24
PUN, mg/dL	8.4	11.3	10.8	14.1	< 0.001	< 0.001	0.44
MUN, mg/dL	8.3	11.0	9.8	13.3	< 0.001	< 0.001	< 0.01

Conclusions

- There is room to reduce N emission by dairy cows, specifically at **rumen** and post-absorptive levels
- Balancing for specific AA improves milk protein and **milk fat responses**, and . . .
- The mechanisms for the regulation of milk components synthesis have been largely elucidated
- Energy plays a critical role in milk protein synthesis regulation
- However, the mammary has the plasticity to use different energy sources
- Peripheral roles of insulin, post peak-lactation could shadow the effect of glucogenic energy sources

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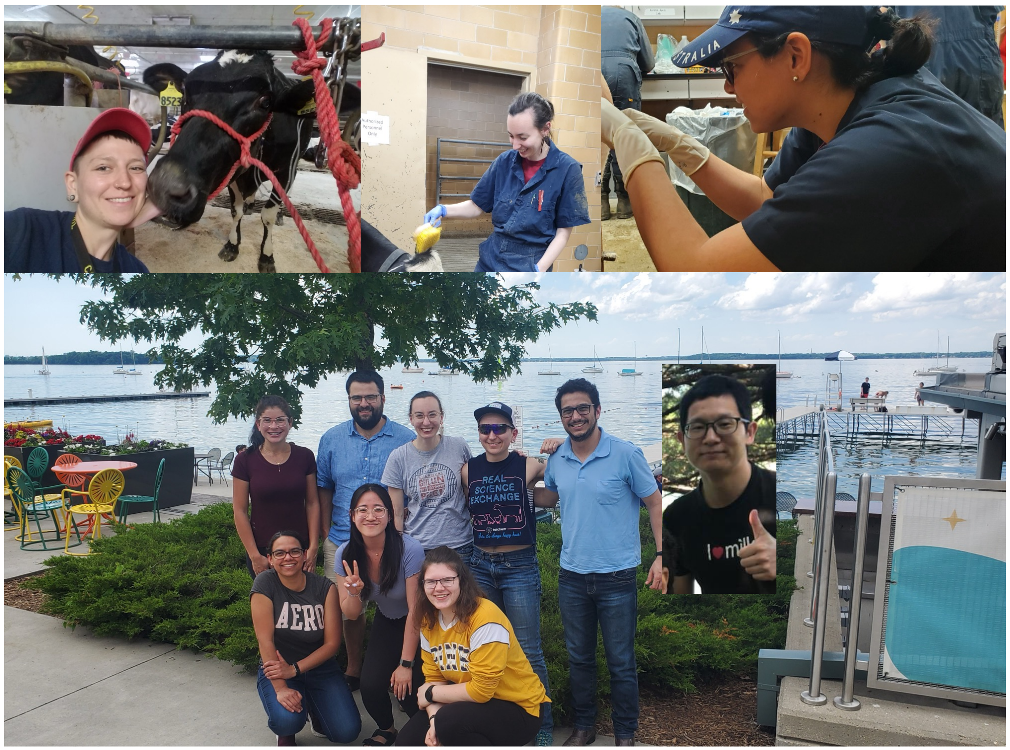

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