

Histidine – a limiting amino acid for dairy cows

Alexander N. Hristov
Distinguished Professor, Department of Animal Science
The Pennsylvania State University

BALCHEM's Real Science Lecture Series, March 4th, 2025

Talk outline

- Feeding reduced-protein diets to dairy cows
- Why Histidine?
- Early research
- Research at Penn State
- Conclusions

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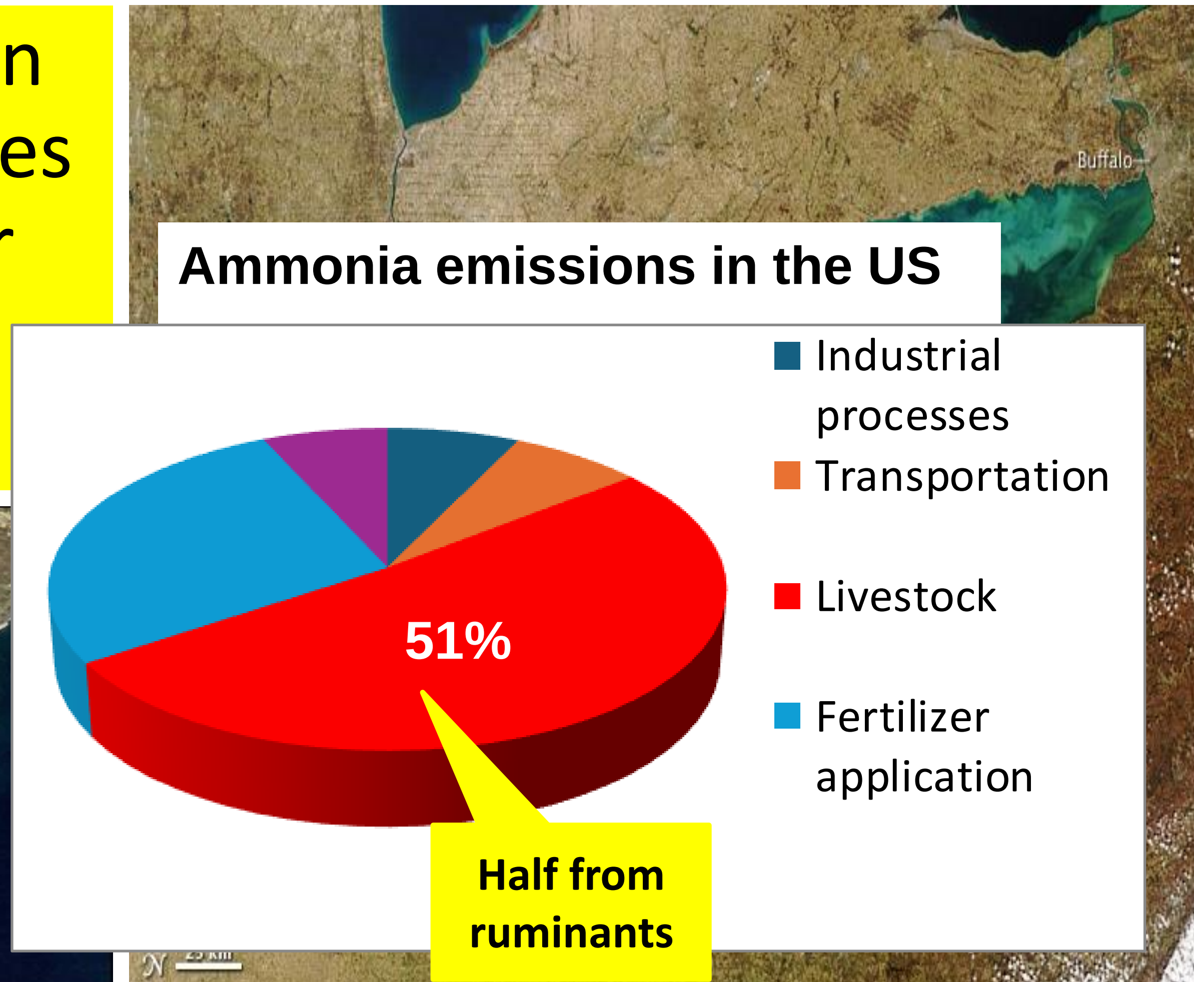
Why feeding low-protein diets?

- Reduced feed cost
- Striving for efficiency
- Reduced N emissions (nitrates, NH_3, N_2O)
- Protein overfeeding and reproduction

It all started with ammonia…

- Eutrophication of water bodies
- Ground water quality
- Air pollution



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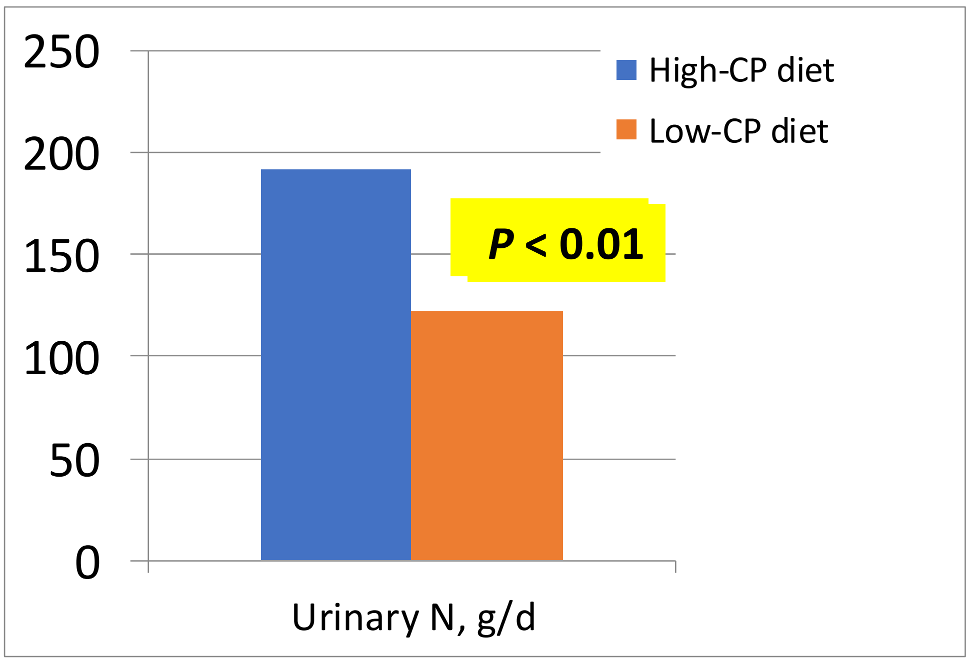
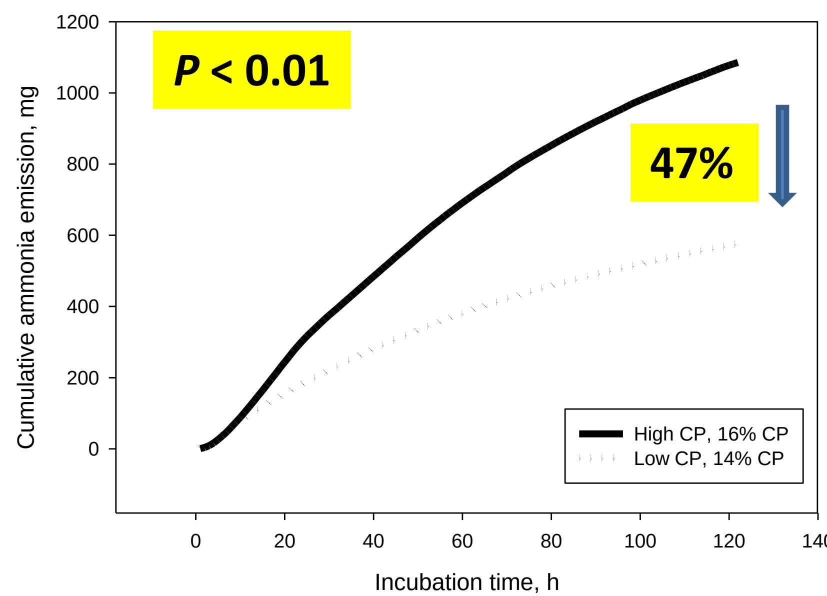
Lee et al., 2010

Decreasing urinary N/urea excretion decreases manure ammonia emissions





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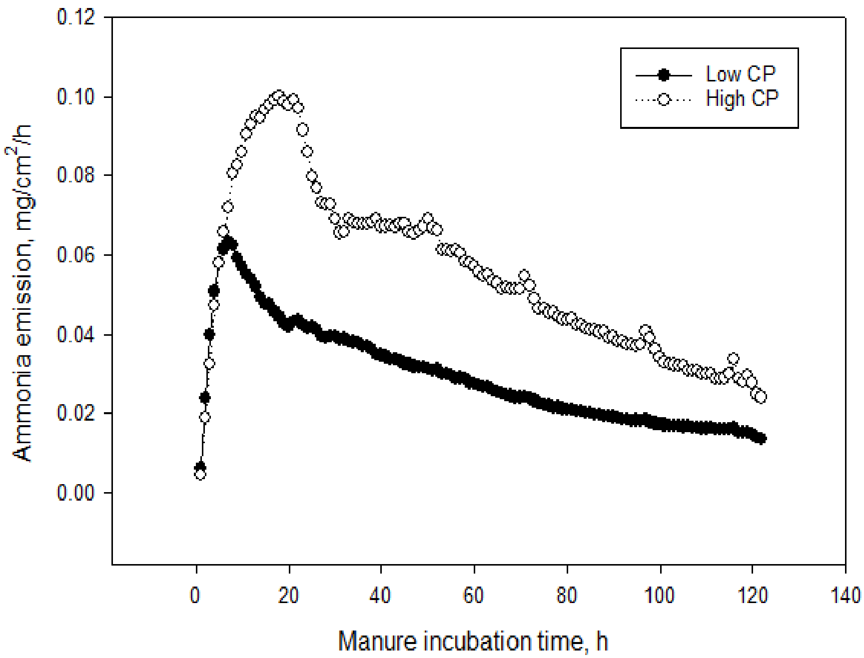
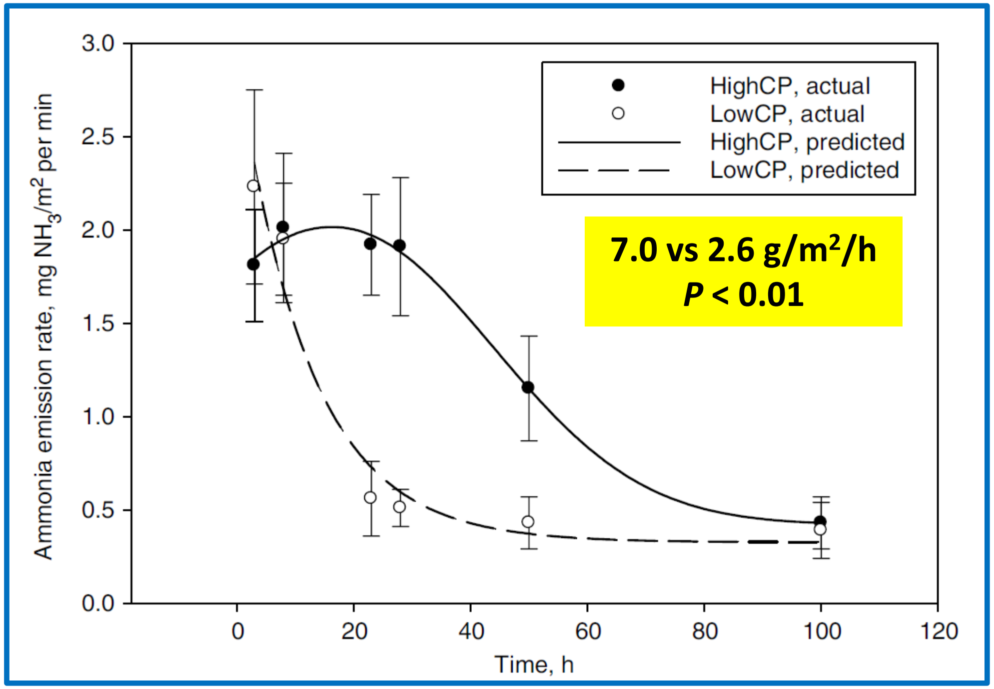
Lee et al., 2016

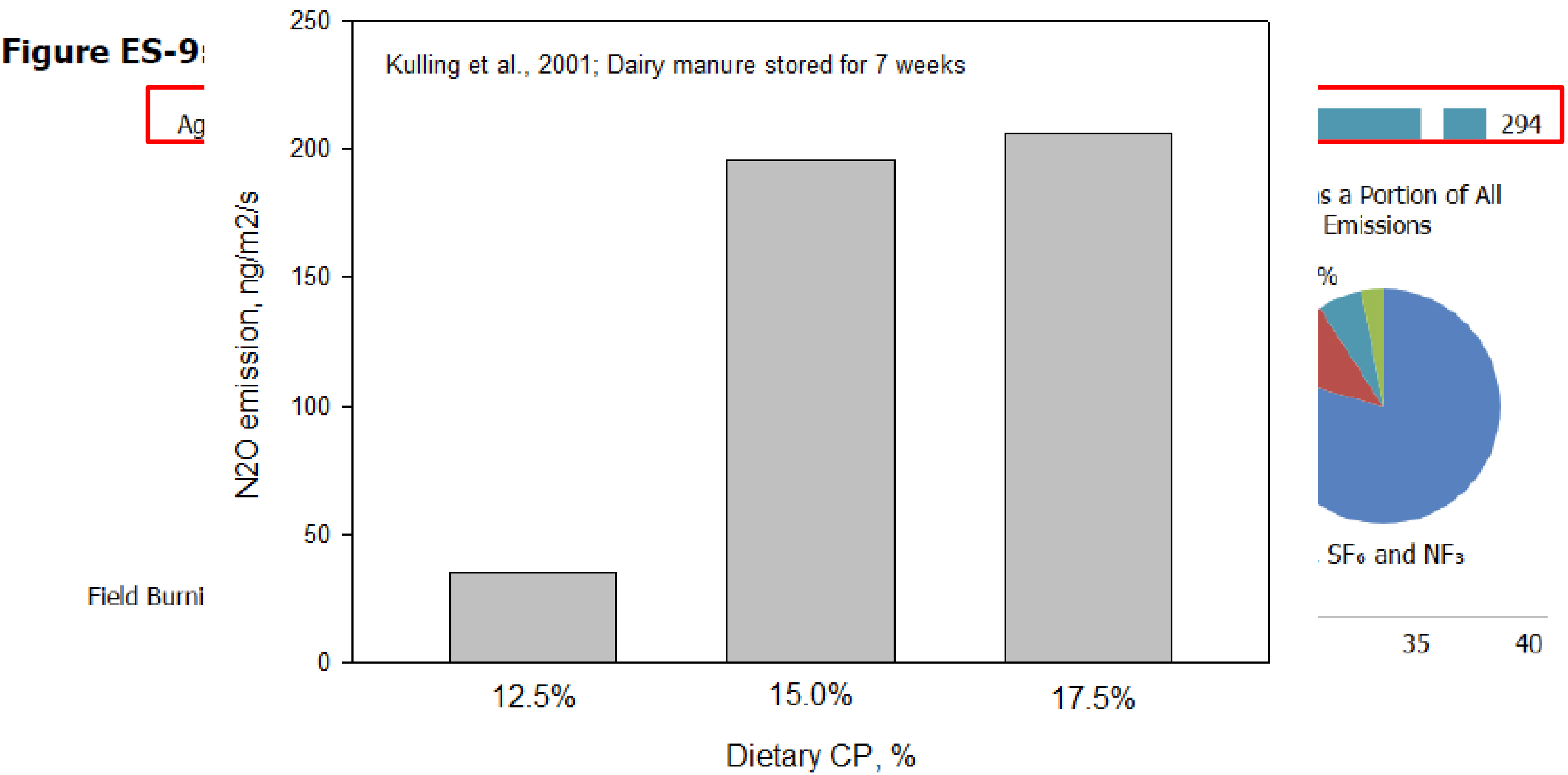
Dietary CP influences soil ammonia emissions as well





USEPA, 2024

Sources of nitrous oxide emissions in the United States & effect of diet CP



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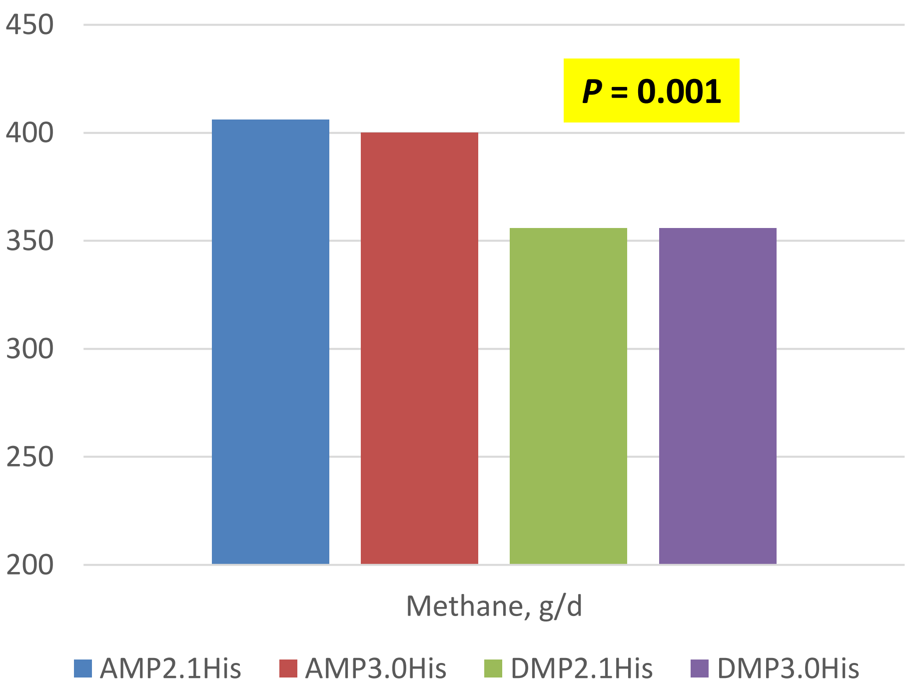
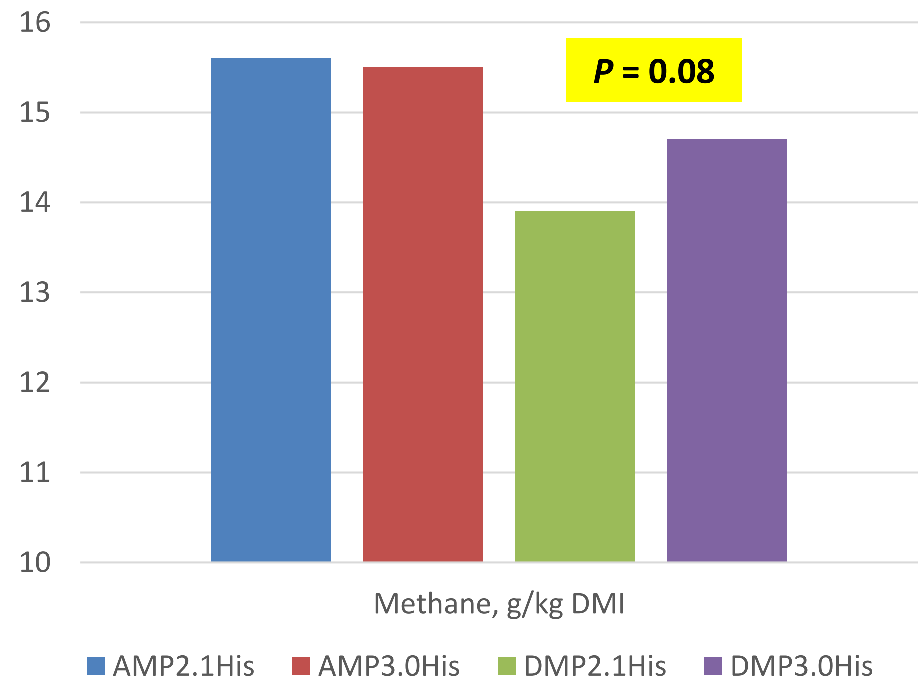
Räisänen et al., 2022

More recently, enteric methane became a target: low-protein & high-starch diets

Starch replaced RUP; 16.7 vs 15.4% CP; 110% vs 96% of MP requirements; 23.2 vs 25.0% starch

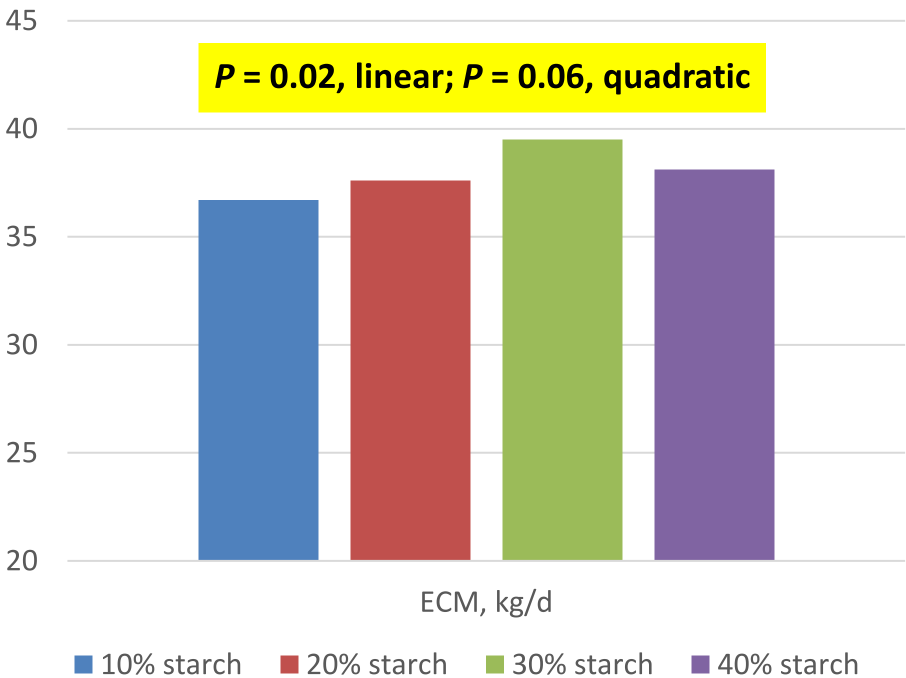


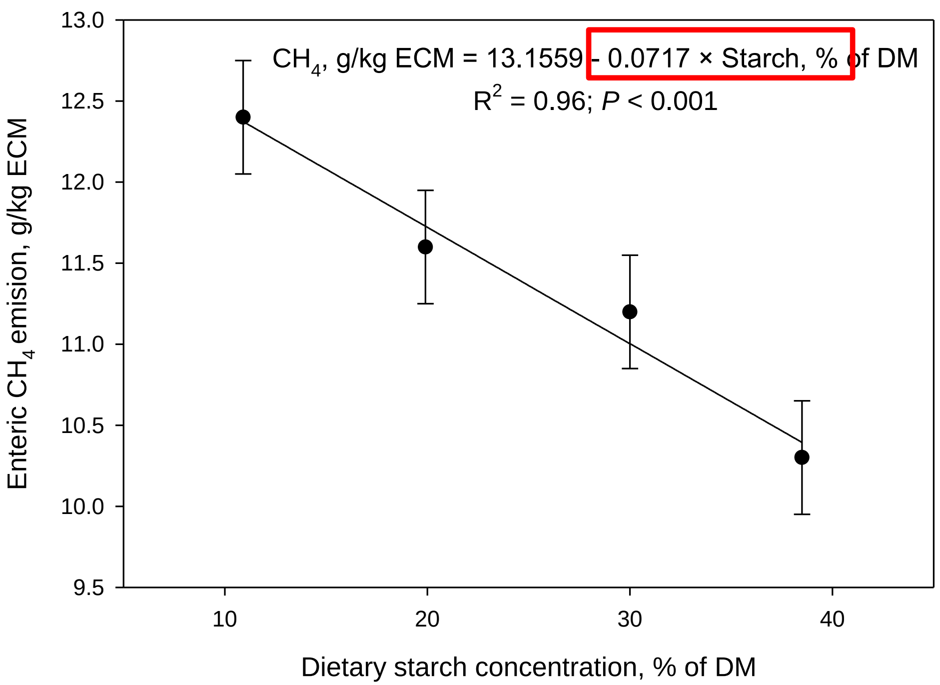


Cueva et al., 2024

Low-protein, high-starch diets?

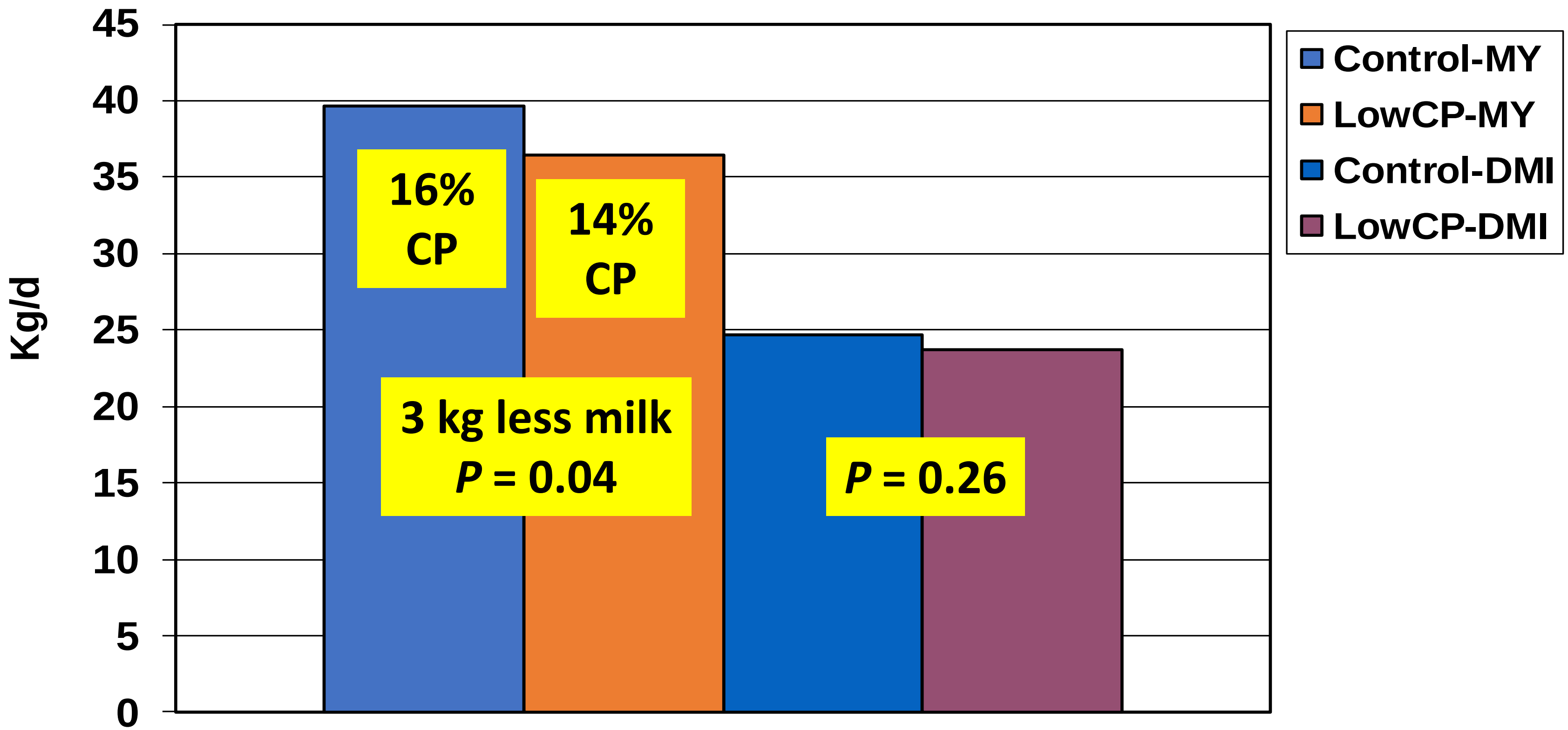
Milk fat % decreased, but milk protein and ECM yields and ECM feed efficiency increased with increasing dietary starch concentration





Penn State data

Severe MP deficiency, however, are likely to decrease DMI, milk yield & components



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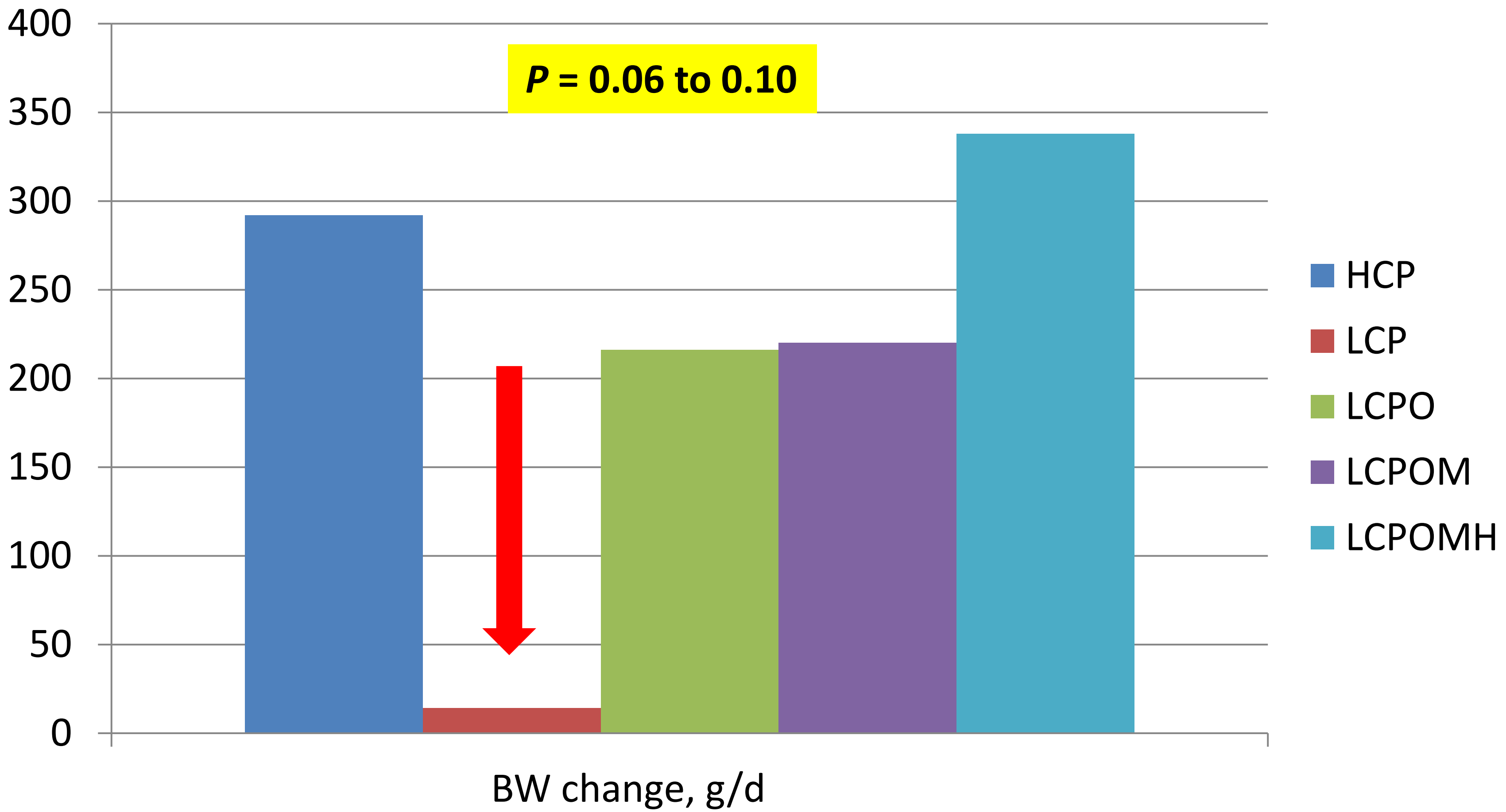
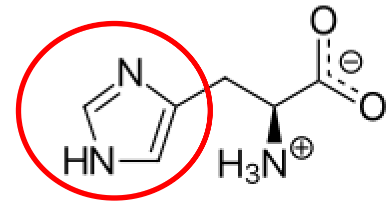
Giallongo et al., 2014

Or cows will lose BW

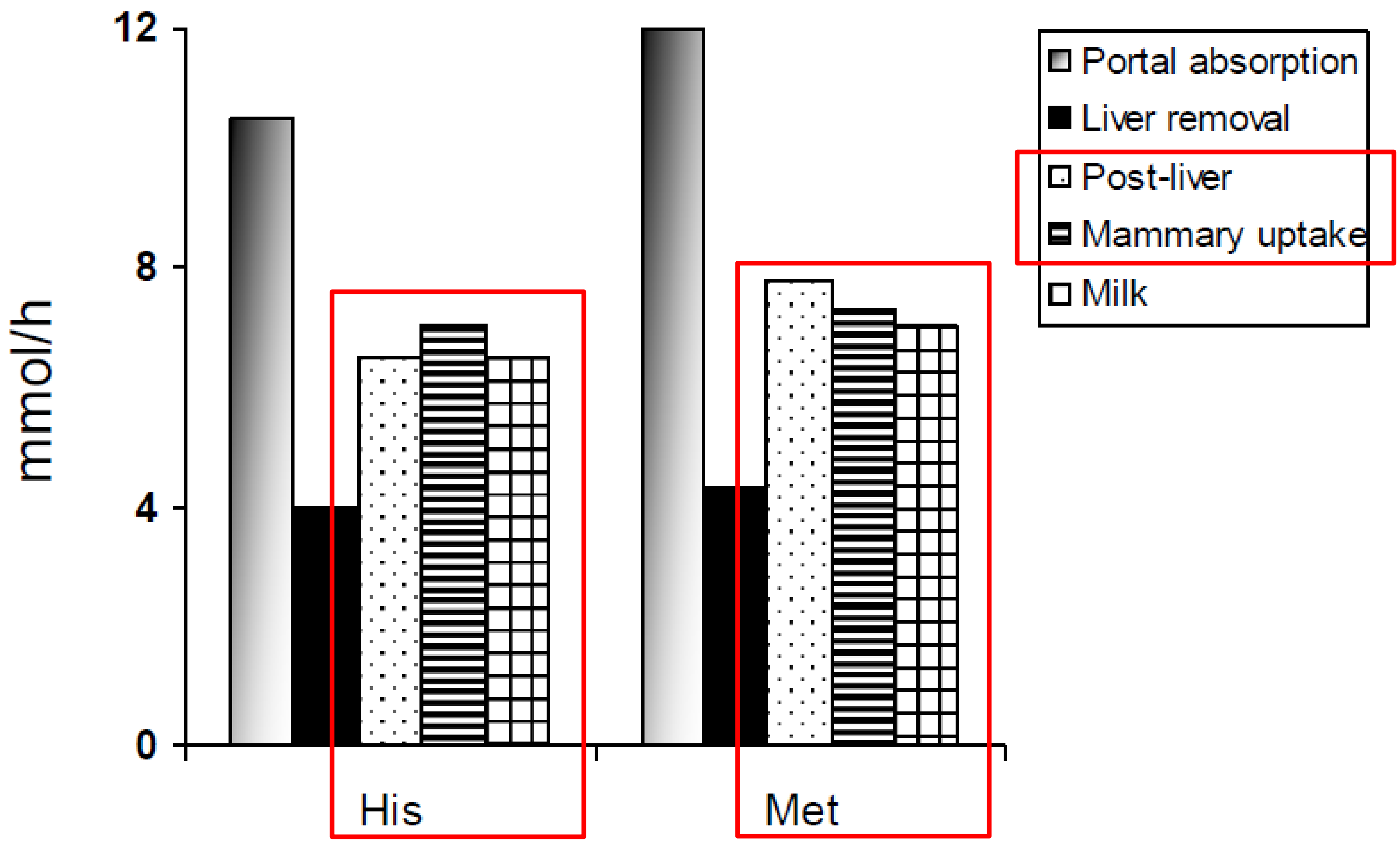


What is Histidine?



- Unique among EAA with an imidazole side chain
- Similar to Met, a Group 1 AA (extracted by the liver with post-liver supply approx. equal to mammary uptake and output in milk)
- Which would suggest that requirements for His should be similar to those for Met
- However, variability in estimates for His requirements have been large: 2.2 to >3.5% of MP
  - Major reasons for this are:
    - endogenous His depots
    - lower His than Met in microbial protein

Net flux of Met and His



Lapierre et al., 2008

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Räisänen et al., 2023

Histidine research over the years

Table 1. Characterization of publications used in the meta-analysis

Source	Design[1]	Method of His supplementation[2]	Basal diet	MP-level[3]	Other supplemental AA
Vanhatalo et al. (1999)	LS	Infusion	Grass silage	MPD	Lys, Met
Kim et al. (1999)	LS	Deletion	Grass silage	MPA	Lys, Met, Trp
Kim et al. (2000)	LS	Infusion	Grass silage	MPA	Lys, Met
Korhonen et al. (2000)	LS	Infusion	Grass silage	MPA	—
Kim et al. (2001)a[4]	LS	Infusion	Grass silage	MPA	—
Kim et al. (2001)b	LS	Infusion	Grass silage	MPA	Lys, Met, Trp
Huhtanen et al. (2002)a	LS	Infusion	Grass silage	MPD	Leu
Huhtanen et al. (2002)b	LS	Infusion	Grass silage	MPD	—
Hadrová et al. (2012)	LS	Deletion	Corn silage	MPD	Leu, Lys, Met
Lee et al. (2012)	RCB	RPHis	Corn silage	MPD	RPLys, RPMet[5]
Giallongo et al. (2015)	RCB	RPHis	Corn silage	MPD	RPLys, RPMet
Giallongo et al. (2016)	RCB	RPHis	Corn silage	MPA	RPLys, RPMet
Giallongo et al. (2017)	RCB	Basal diet[6]	Corn silage	MPA	RPLys, RPMet
Zang et al. (2019)	LS	RPHis	Corn silage	MPA	RPMet
Morris and Kononoff (2020)a	LS	RPHis	Corn silage	MPA	—
Morris and Kononoff (2020)b	LS	RPHis	Corn silage	MPA	RPLys
Lapierre et al. (2021)a	LS	Deletion	Corn silage	MPD	Free AA, casein profile
Lapierre et al. (2021)b	LS	Deletion	Corn silage	MPD	Free AA, casein profile
Räisänen et al. (2021a)	LS	RPHis	Corn silage	MPA	RPLys, RPMet
Räisänen et al. (2021b)	LS	RPHis	Corn silage	MPD	RPLys, RPMet
Räisänen et al. (2022)a	RCB	RPHis	Corn silage	MPA	RPLys, RPMet
Räisänen et al. (2022)b	RCB	RPHis	Corn silage	MPA	RPLys, RPMet

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Milk Production of Cows on Protein-Free Feed

Studies of the use of urea and ammonium salts as the sole nitrogen source open new important perspectives.

Artturi I. Virtanen

Science, 1966

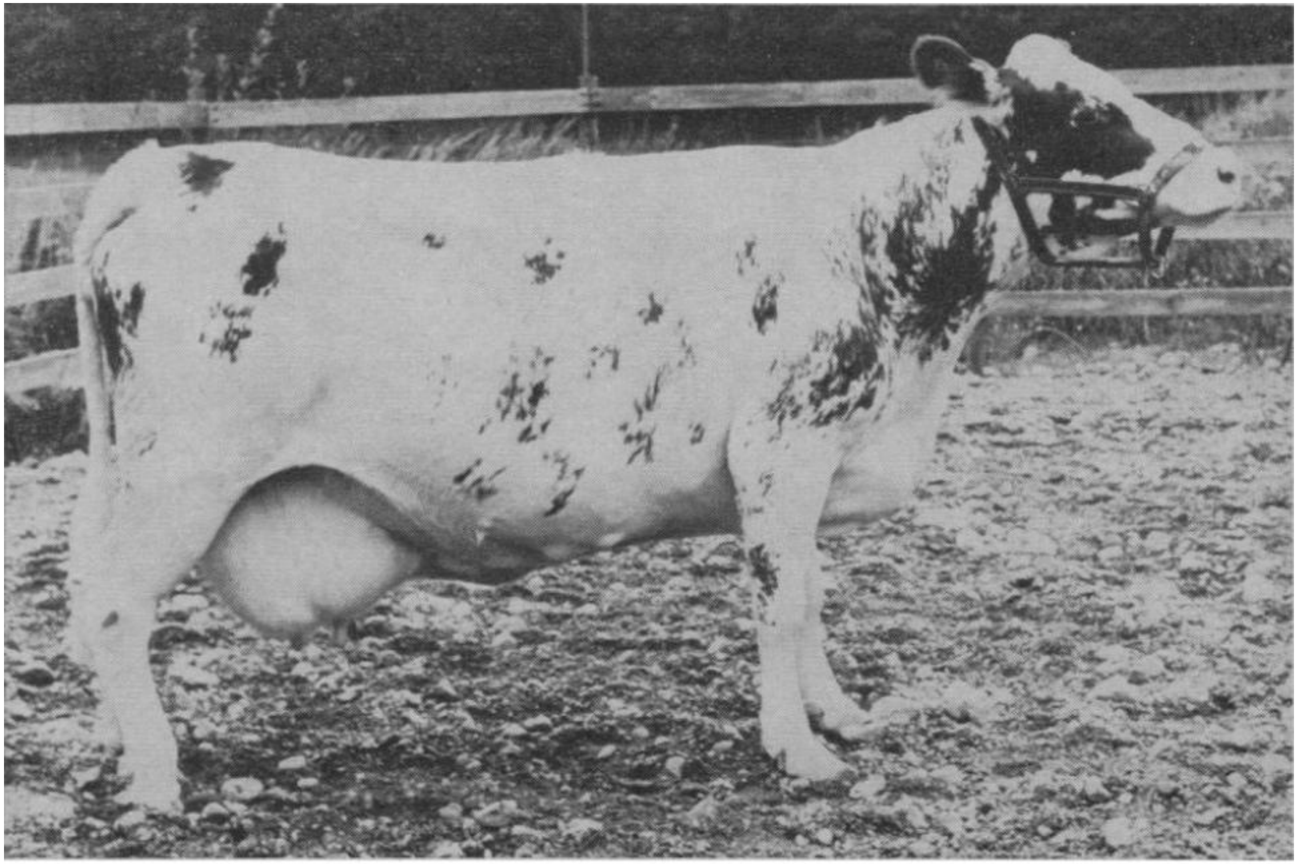


Fig. 3. Test cow Metta after being on test feed 370 days from calving.

A. I. Virtanen; Science, 1966

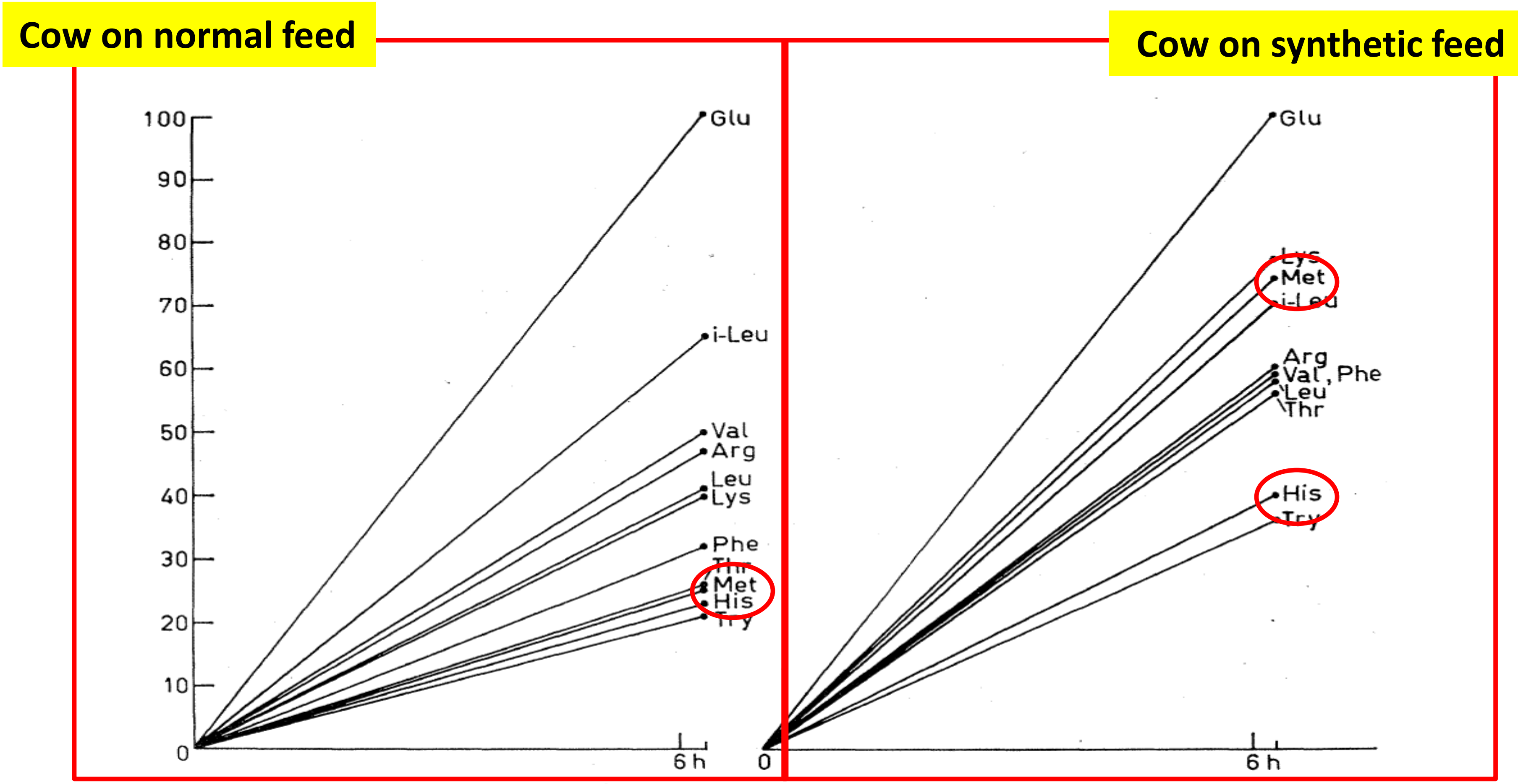
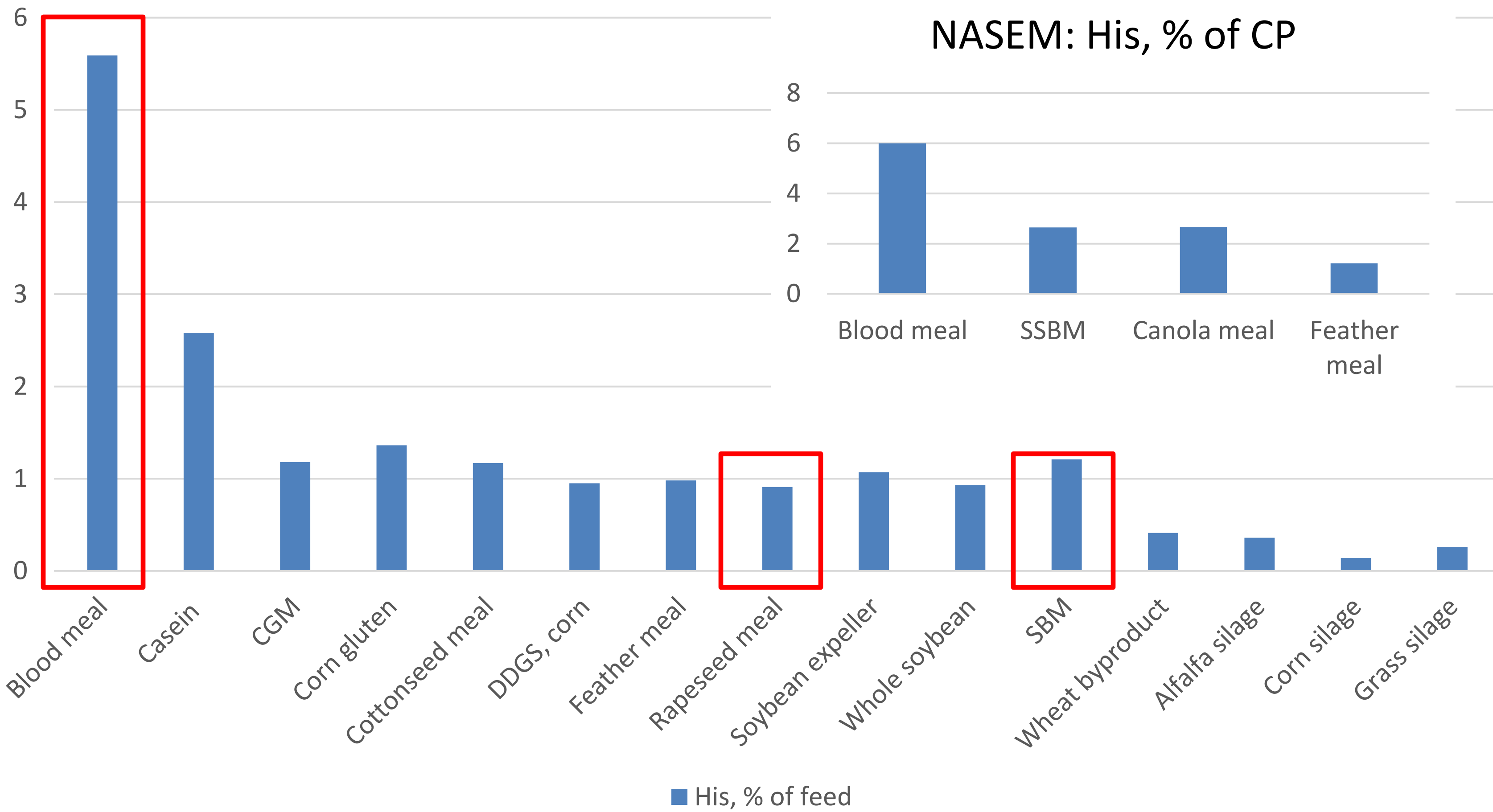


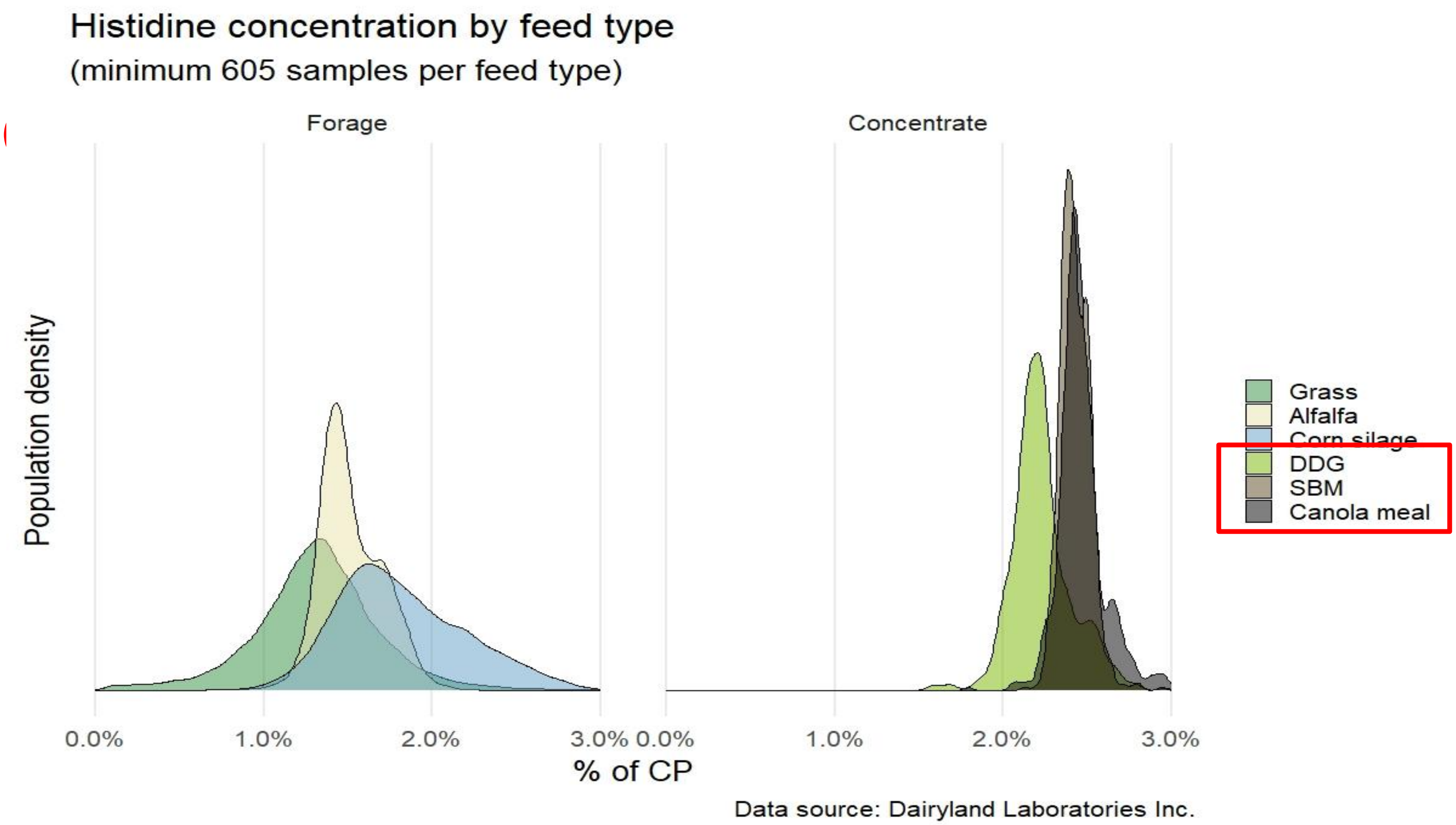
Fig. 1. Labeling of the essential amino acids of total milk protein 6.3 hours after the cow had been fed a single dose of ^{15}N-urea. The results are expressed as a percentage of the labeling of glutamic acid. At left, results of a feeding experiment with a cow on normal feed (17 March 1966); at right, results of a feeding experiment with a test cow (20 October 1962) 6 months after the start of the experimental feeding. Histidine and tryptophan have the lowest labeling in both experiments, but the increase in their labeling in the cow on the experimental feed is remarkable. [Determinations by M. Kreula and T. Moisio]

Evonik AMINODat

Histidine concentration in feeds



His concentration in common forages and protein feeds



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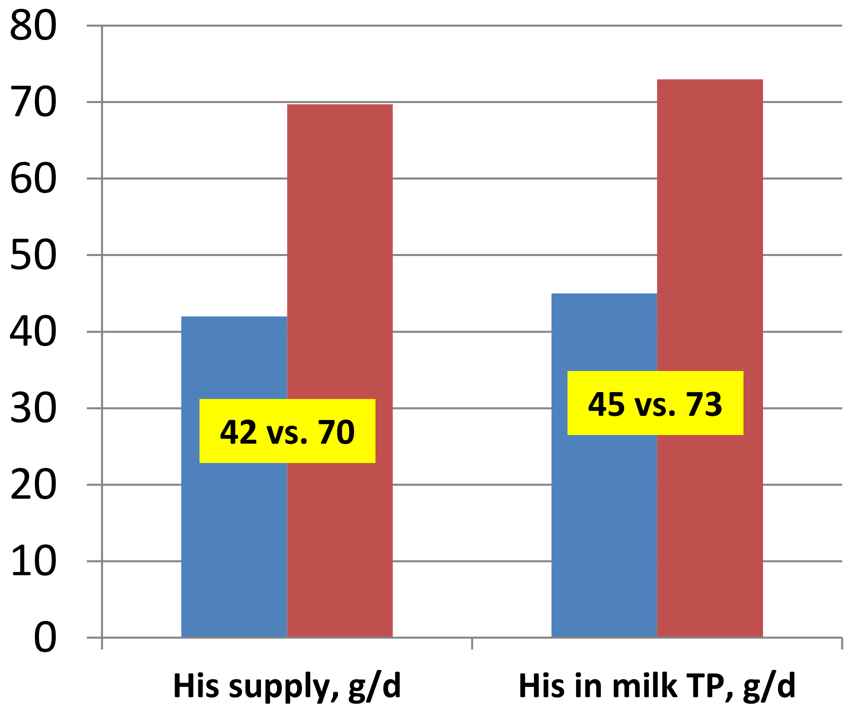
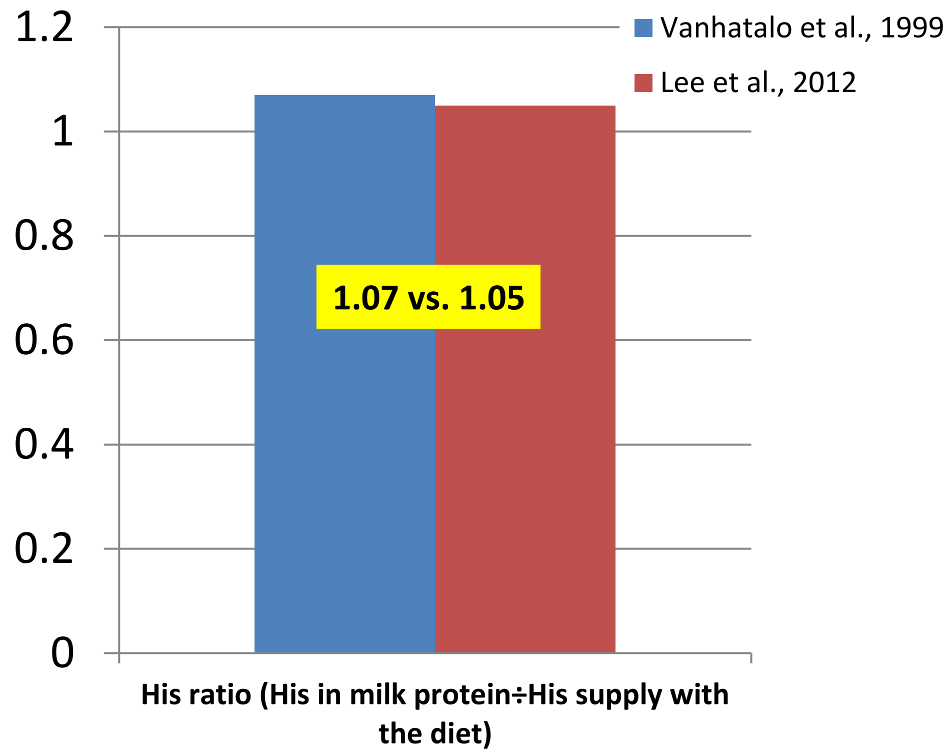
Can His be limiting on CS-based diets?

His supply ÷ output in grass- vs. corn silage-based diets





Histidine work at Penn State

J. Dairy Sci. 99:6702–6713
http://dx.doi.org/10.3168/jds.2015-10673

Effects of slow-release urea and rumen-protected methionine and histidine on mammalian target of rapamycin (mTOR) signaling and ubiquitin proteasome-related gene expression in skeletal muscle of dairy cows

H. Sadri,*†[1] F. Giallongo,‡ A. N. Hristov,‡ J. Werner,§ C. H. Lang,# C. Parys,ǁ B. Saremi,ǁ and H. Sauerwein*
*Institute of Animal Science, Physiology and Hygiene Unit, University of Bonn, 53115 Bonn, Germany
†Department of Clinical Science, Faculty of Veterinary Medicine, University of Tabriz, 516616471 Tabriz, Iran
‡Department of Animal Science, and
§Animal Resource Program, The Pennsylvania State University, University Park 16802
#Department of Cellular and Molecular Physiology, Penn State College of Medicine, Hershey, PA 17033
ǁEvonik Nutrition & Care GmbH, Rodenbacher Chaussee 4, 63457 Hanau, Germany

J. Dairy Sci. 95:6042–6056
http://dx.doi.org/10.3168/jds.2012-5581

Rumen-protected lysine, methionine, and histidine increase yield in dairy cows fed a metabolizable protein-deficient die

C. Lee,* A. N. Hristov,*[1] T. W. Cassidy,* K. S. Heyler,* H. Lapierre,† G. A. Varga,* M. J. and C. Parys#
*Department of Dairy and Animal Science, The Pennsylvania State University, University Park 16802

J. Dairy Sci. 98:3292–3308
http://dx.doi.org/10.3168/jds.2014-8791

Effects of slow-release urea and rumen-protected meth and histidine on performance of dairy cows

F. Giallongo,* A. N. Hristov,*[1] J. Oh,* T. Frederick,* H. Weeks,* J. Werner,† H. La A. Gehman,# and C. Parysǁ
*Department of Animal Science, and
†Animal Resource Program, The Pennsylvania State University, University Park 16802

J. Dairy Sci. 104:9902–9916
https://doi.org/10.3168/jds.2021-20188

Histidine dose-response effects on lactational performance and plasma amino acid concentrations in lactating dairy cows: 1. Metabolizable protein-adequate diet

S. E. Räisänen,[1] C. F. A. Lage,[1,2] J. Oh,[1,3] A. Melgar,[1,4] K. Nedelkov,[1,5] X. Chen,[1,6] M. M
[1]Department of Animal Science, The Pennsylvania State University, University Park 16802
[2]School of Veterinary Medicine, University of California, Davis, Tulare 93274
[3]Cargill Animal Nutrition, Seongnam, South Korea 13630
[4]Agricultural Innovation Institute of Panama (IDIAP), City of Knowledge 07144, Panama
[5]Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria 6000
[6]Livestock Production Science Branch, Agri-food Biosciences Institute, Hillsborough, Co. Down BT26 6DR, UK
[7]Ajinomoto Co. Inc., Kawasaki, Japan 210-8681

J. Dairy Sci. 106
https://doi.org/10.3168/jds.2022-22966

Lactational performance effects of supplemental histidine in dairy cows: A meta-analysis

S. E. Räisänen,[1,2] H. Lapierre,[3] W. J. Price,[4] and A. N. Hristov[1*]
[1]Department of Animal Science, The Pennsylvania State University, State College, PA 16802
[2]ETH Zürich, Department of Environmental Science, Institute of Agricultural Sciences, Zürich 8092, Switzerland
[3]Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada J1M 0C8
[4]Statistical Programs, University of Idaho, Moscow, ID 83844

J. Dairy Sci. 99:4437–4452
http://dx.doi.org/10.3168/jds.2015-10822

Effects of rumen-protected methionine, lysine, and histidine on lactation performance of dairy cows

F. Giallongo,* M. T. Harper,* J. Oh,* J. C. Lopes,* H. Lapierre,† R. A. Patton,‡ C. Parys,§ I. Shinzato,# and A. N. Hristov*[1]
*Department of Animal Science, The Pennsylvania State University, University Park 16802
†Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada J1M 0C8

J. Dairy Sci. 100:2784–2800
https://doi.org/10.3168/jds.2016-11992

Histidine deficiency has a negative effect on lactational performance of dairy cows

F. Giallongo,* M. T. Harper,* J. Oh,* C. Parys,† I. Shinzato,‡ and A. N. Hristov*[1]
*Department of Animal Science, The Pennsylvania State University, University Park 16802

J. Dairy Sci. 104:9917–9930
https://doi.org/10.3168/jds.2021-20189

Histidine dose-response effects on lactational performance and plasma amino acid concentrations in lactating dairy cows: 2. Metabolizable protein-deficient diet

S. E. Räisänen,[1] C. F. A. Lage,[1,2] M. E. Fetter,[1] A. Melgar,[1,3] A. M. Pelaez,[1,4] H. A. Stefenoni,[1] D. E. Wasson,[1] S. F. Cueva,[1] X. Zhu,[1,5] M. Miura,[6] and A. N. Hristov[1*]
[1]Department of Animal Science, The Pennsylvania State University, University Park 16802

J. Dairy Sci. 105:170–187
https://doi.org/10.3168/jds.2021-20800

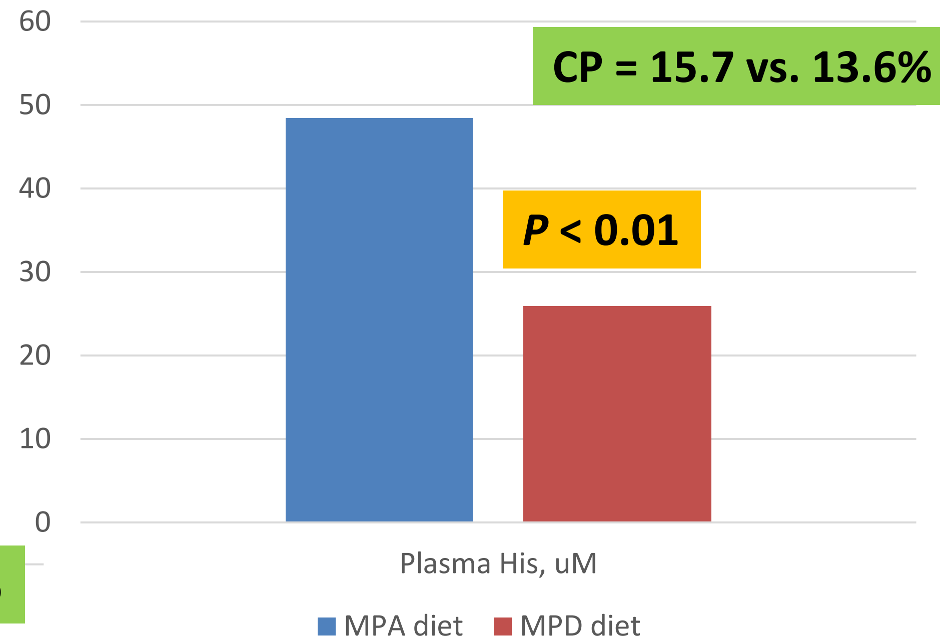
Lactational performance and plasma and muscle amino acid concentrations in dairy cows fed diets supplying 2 levels of digestible histidine and metabolizable protein

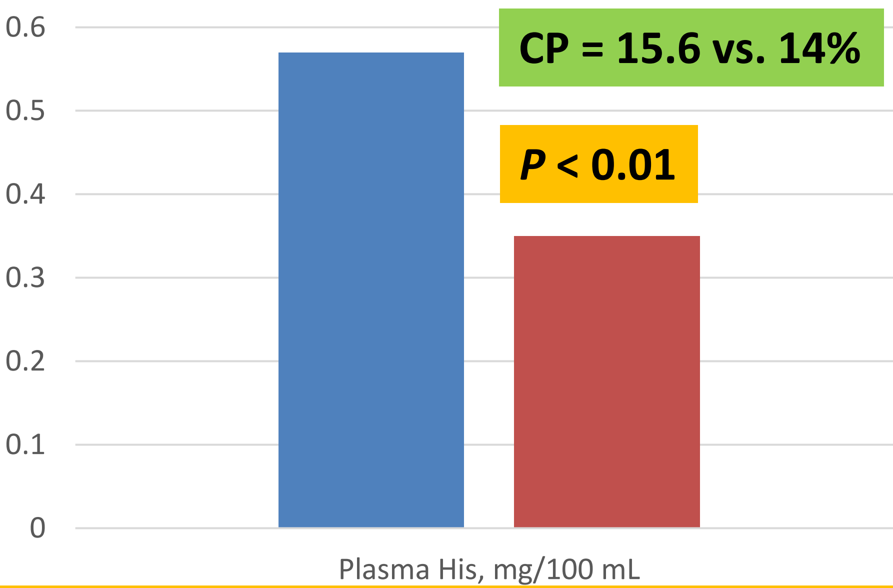
S. E. Räisänen,[1*] C. F. A. Lage,[1,2†] C. Zhou,[1,3] A. Melgar,[1,4] T. Silvestre,[1] D. E. Wasson,[1] S. F. Cueva,[1] J. Werner,[5] T. Takagi,[6] M. Miura,[6] and A. N. Hristov[1‡]
[1]Department of Animal Science, The Pennsylvania State University, University Park 16802
[2]School of Veterinary Medicine, University of California, Tulare 93274
[3]University of Chinese Academy of Sciences, Beijing 100049, P. R. China
[4]Agricultural Innovation Institute of Panama (IDIAP), City of Knowledge 07144, Panama
[5]Animal Resource Program, The Pennsylvania State University, University Park 16802
[6]Ajinomoto Co. Inc., Kawasaki, Japan 210-8681

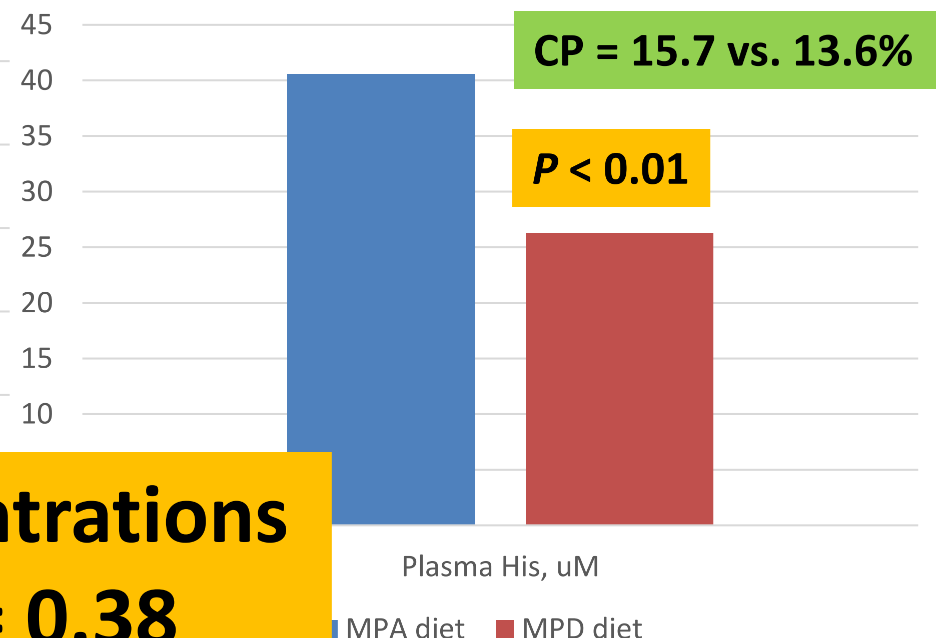
Histidine work at Penn State

- Observed a consistent apparent drop in plasma His with long-term feeding of low-CP diets
- Hypothesis: on low-CP diets, microbial protein is becoming an increasingly important source of AA for the cow
  - However, compared with Met, microbial protein is a poorer source of His

Examples of the effect of dietary CP/MP on plasma His



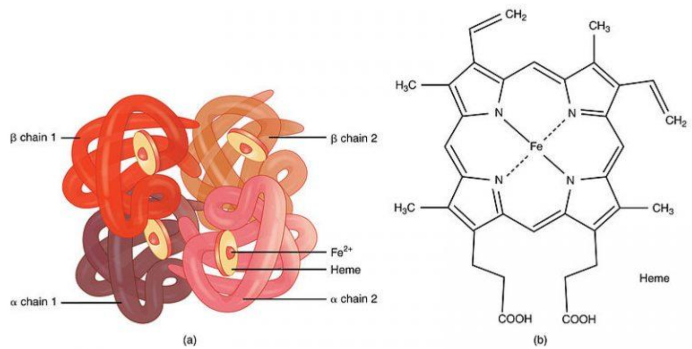




No change in Met concentrations in all experiments; *P* = 0.38

Lee et al., 2012a,b; Giallongo et al., 2016

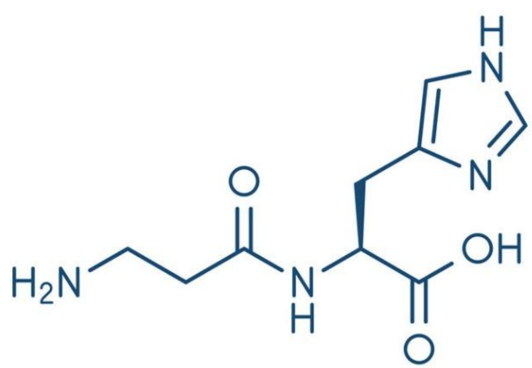
Endogenous sources of His



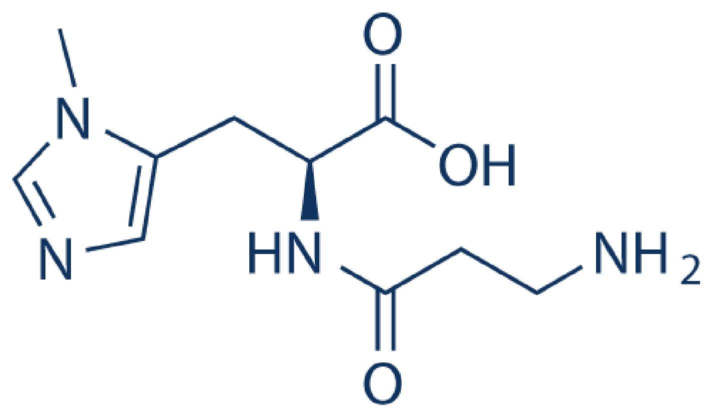
Hemoglobin

Giallongo et al., 2017:

- Blood hemoglobin = 380 g mHis
- Muscle carnosine & anserine = 270 g mHis
- These could supply mHis for about 7 wks (at approx. – 6 g mHis/d deficiency)



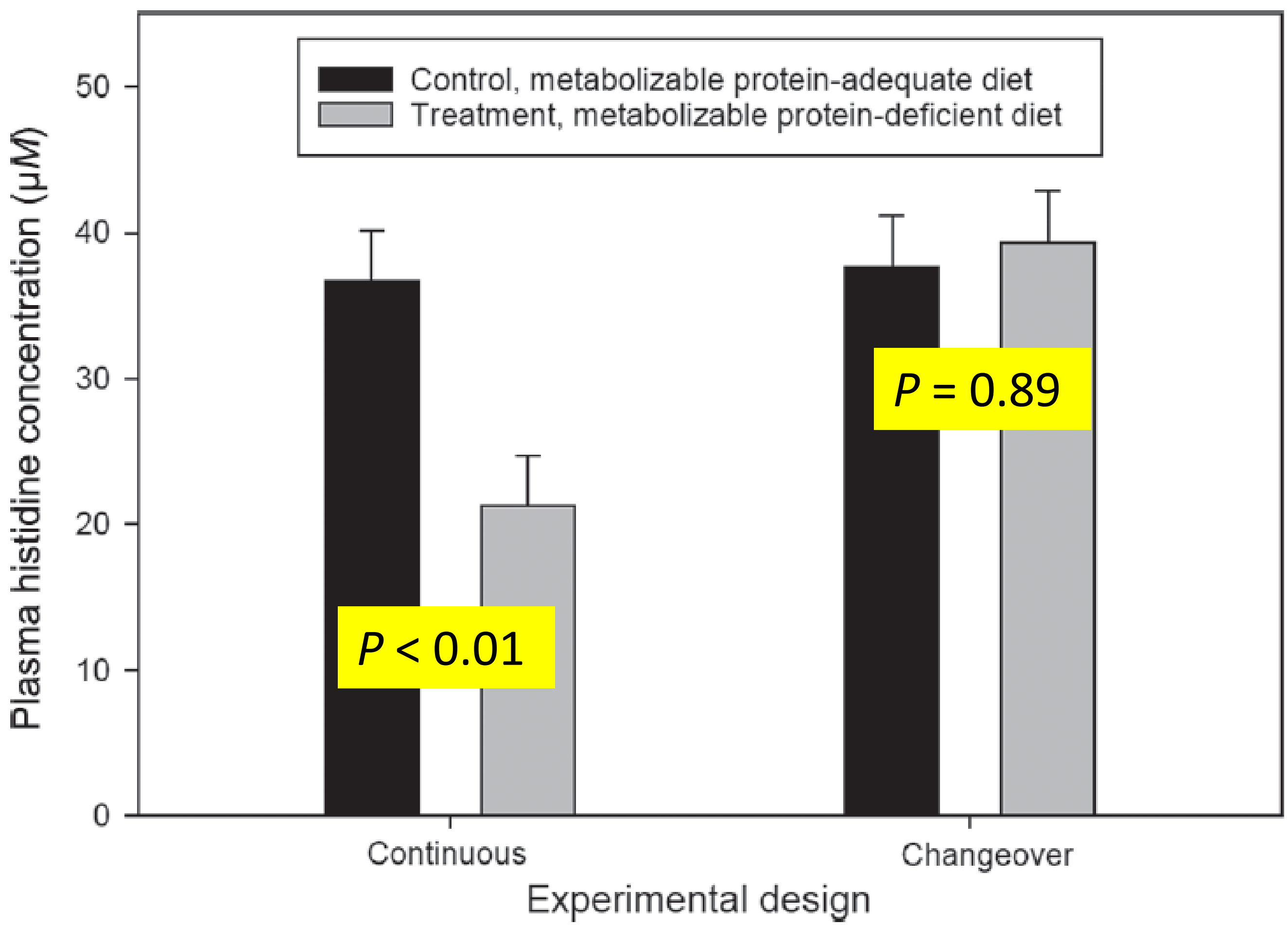
Carnosine



Anserine

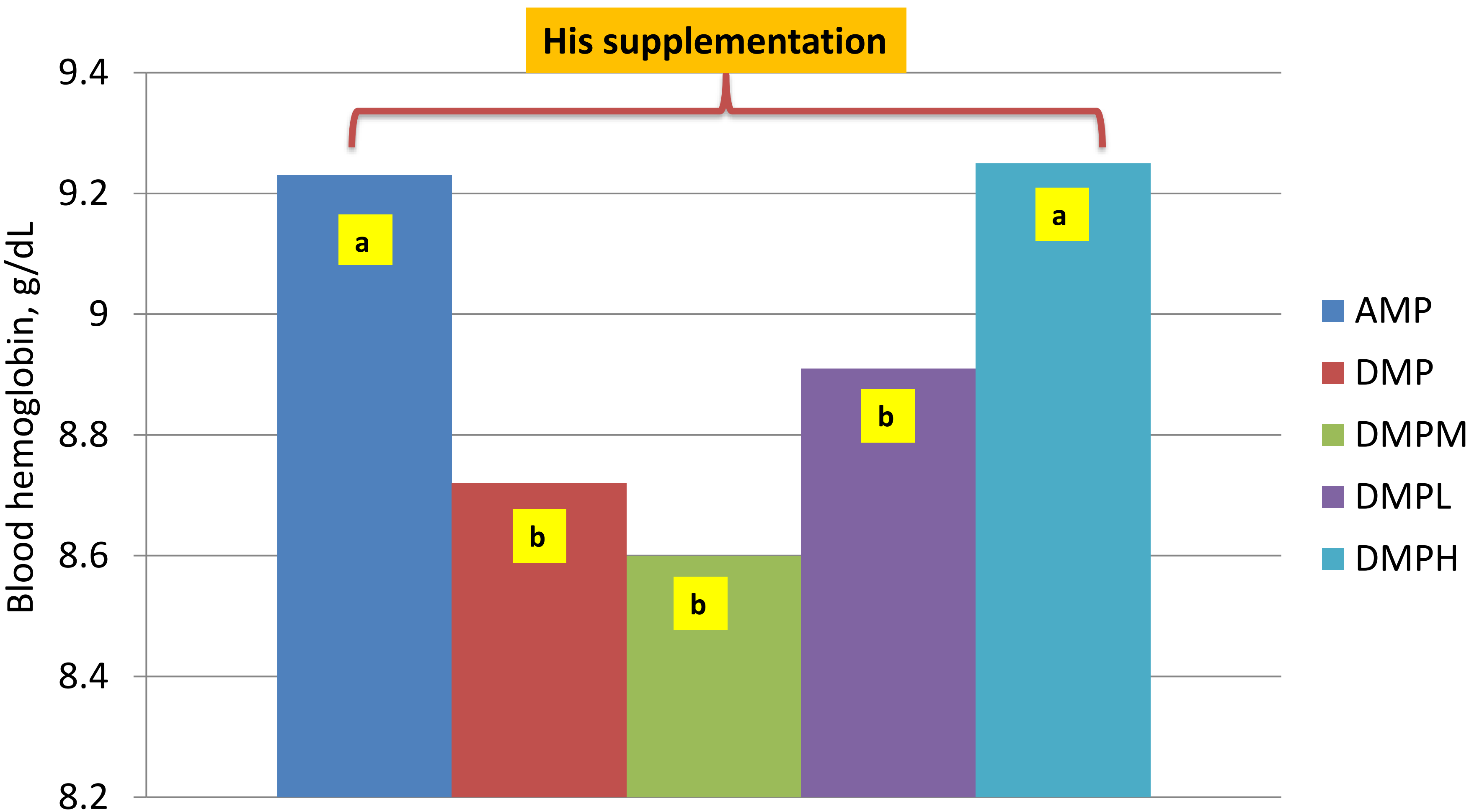
Hristov et al., 2019 (data from Lee et al., 2012, 2015)

Body reserves can hide temporary His deficiencies



Giallongo et al., 2015

His and blood hemoglobin



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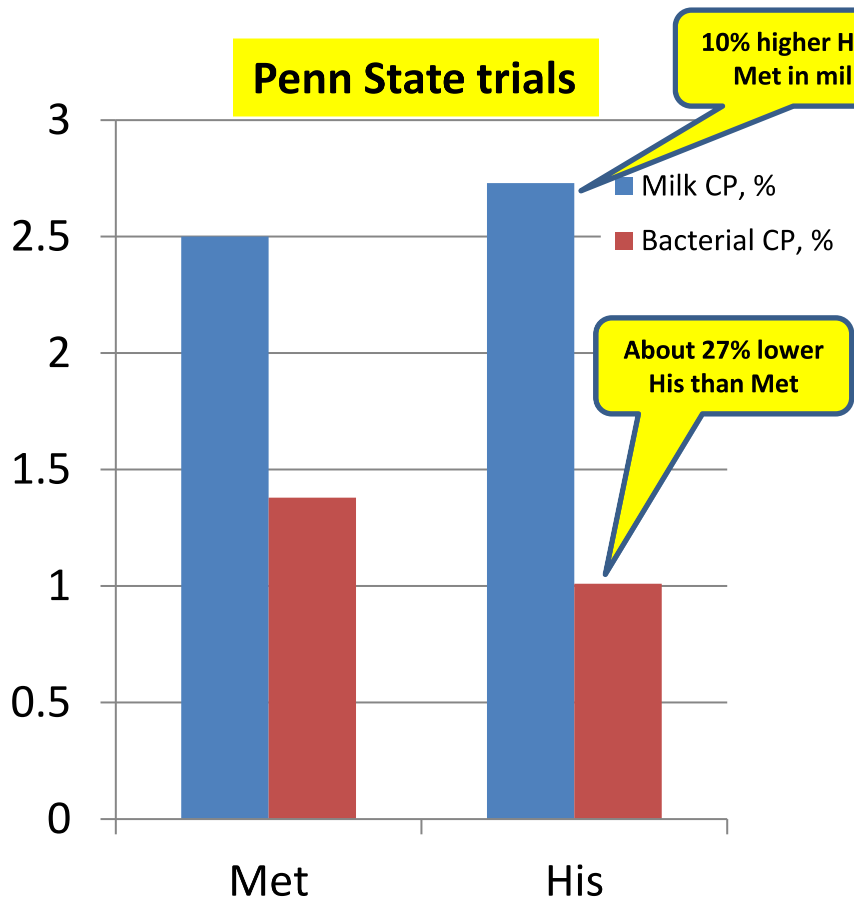
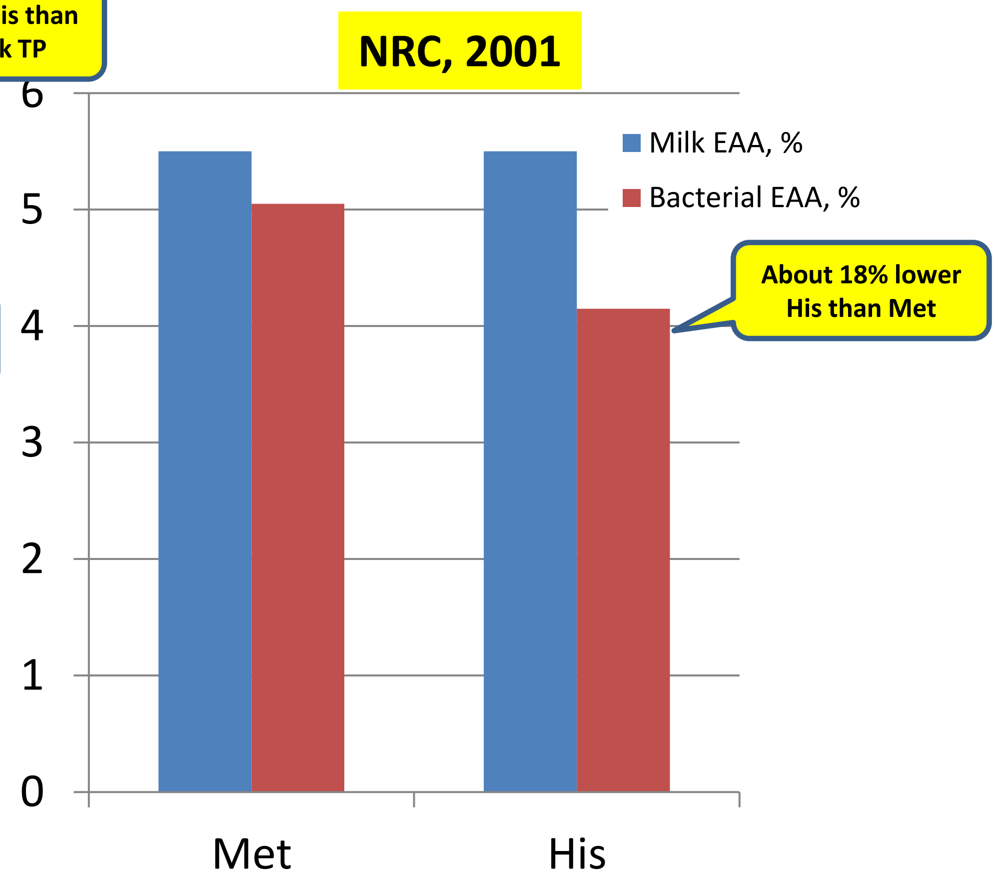
Met and His in milk protein vs. bacteria





NASEM (2021) AA composition of microbial protein

	$g\ AA_{corr}/100\ g$ CP	$g\ AA_{corr}/100\ g$ TP[a]				$g\ AA_{calc}/100\ g$ TP[b]
AA	Duodenal Endogenous	Microbial[c]	Scurf	Whole Empty Body	Metabolic Fecal	Milk
Ala	4.69	7.38	[illegible]	[illegible]	6.32	3.59
Arg	4.61	5.47	[illegible]	[illegible]	5.90	3.74
Asx	4.75	13.39	[illegible]	[illegible]	7.56	8.14
Cys	2.58	2.09	[illegible]	1.74	3.31	0.93
Glx	11.31	14.98	14.69	15.76	15.67	22.55
Gly	5.11	6.26	21.08	14.46	8.45	2.04
His	2.90	2.21	1.75	3.04	[illegible]	2.92
Ile	4.09	6.99	2.96	3.69	[illegible]	6.18
Leu	7.67	9.23	6.93	8.27	[illegible]	10.56
Lys	6.23	9.44	5.64	7.90	7.61	8.82
Met	1.26	2.63	1.40	2.37	1.73	3.03
Phe	3.98	6.30	3.61	4.41	5.28	5.26
Pro	4.64	4.27	12.35	9.80	8.43	10.33
Ser	5.24	5.40	6.45	5.73	7.72	6.71
Thr	5.18	6.23	4.01	4.84	7.36	4.62
Trp	1.29	1.37	0.73	1.05	1.79	1.65
Tyr	3.62	5.94	2.62	3.08	4.65	5.83
Val	5.29	6.88	4.66	5.15	7.01	6.90

16% lower His than Met

Only 4% difference

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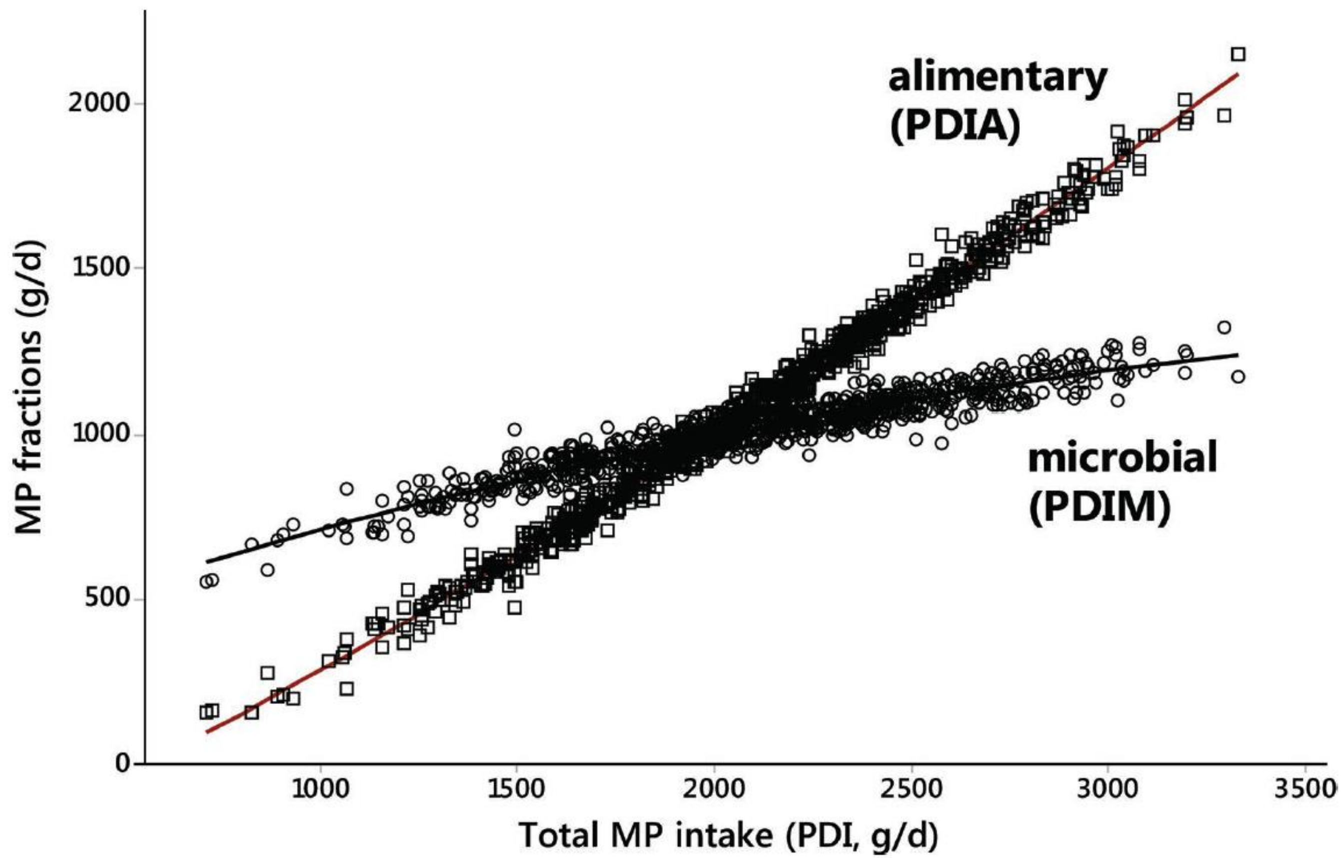
INRA data from Hristov et al., 2019

The relative contribution of microbial protein to the total MP supply increases with decreasing dietary MP

INVITED REVIEW: NITROGEN IN RUMINANT NUTRITION



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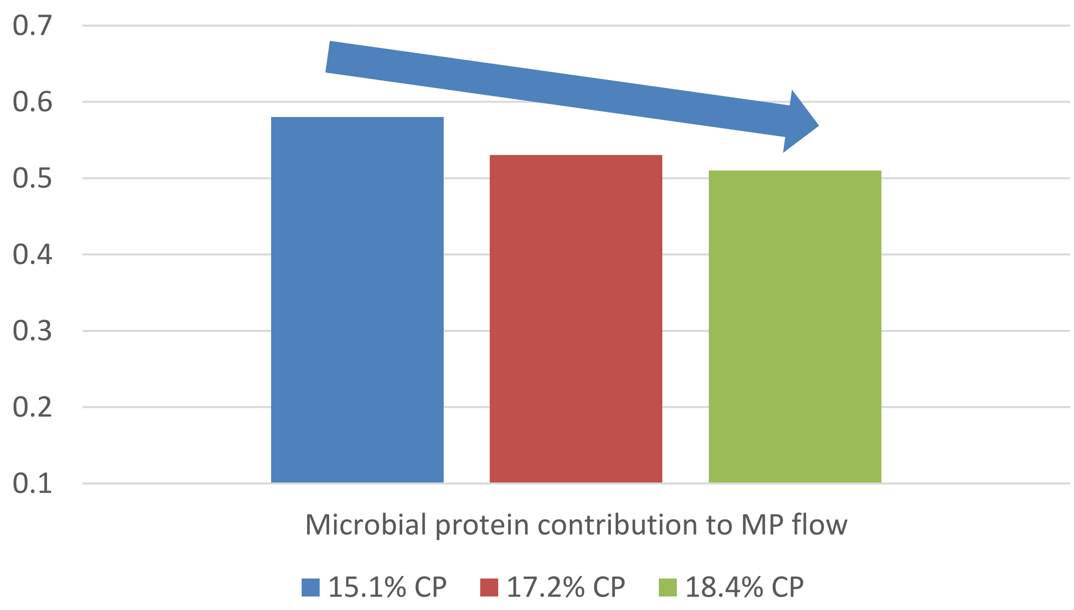
NASEM 2021 simulations

Mature, 700 kg BW Holstein cow, 100 DIM, 55 kg milk/d, 3.30% fat, 2.80% TP, 28 kg/d DMI

Diet CP, %	Proportion of microbial MP	Total mHis, g/d	mHis efficiency (target is 0.75)	N excretions, g/d
15.1	0.58	56	1.04	402
17.2	0.53	67	0.87	488
18.4	0.51	73	0.80	539



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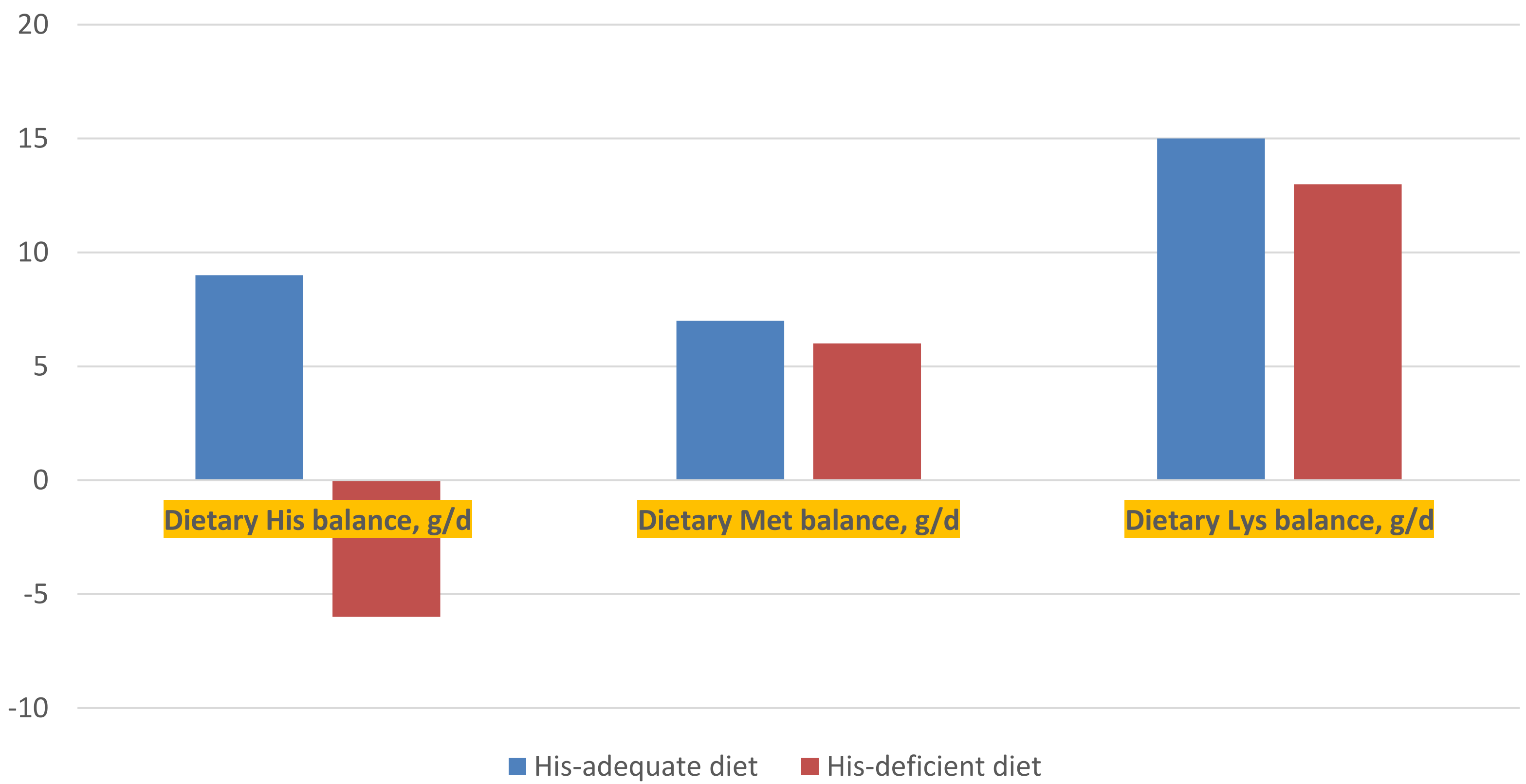
*Department of Animal Science, The Pennsylvania State University, University Park 16802
†Evonik Nutrition and Care GmbH, 63457 Hanau, Germany
‡Ajinomoto Co. Inc., Tokyo, Japan 104



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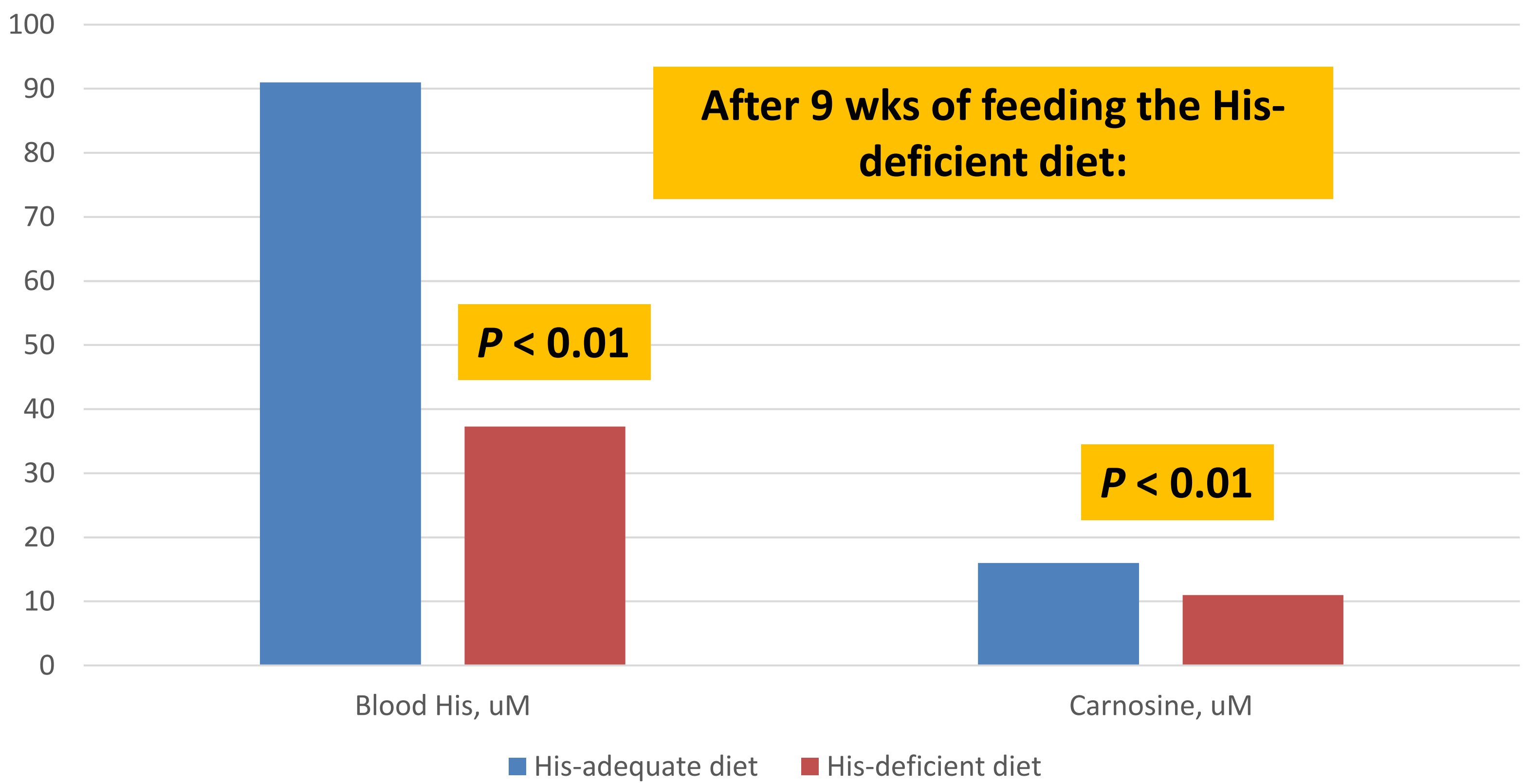
J. Dairy Sci. 100:2784–2800
https://doi.org/10.3168/jds.2016-11992

Histidine deficiency has a negative effect on lactational performance of dairy cows

F. Giallongo,* M. T. Harper,* J. Oh,* C. Parys,† I. Shinzato,‡ and A. N. Hristov*[1]
*Department of Animal Science, The Pennsylvania State University, University Park 16802
†Evonik Nutrition and Care GmbH, 63457 Hanau, Germany
‡Ajinomoto Co. Inc., Tokyo, Japan 104



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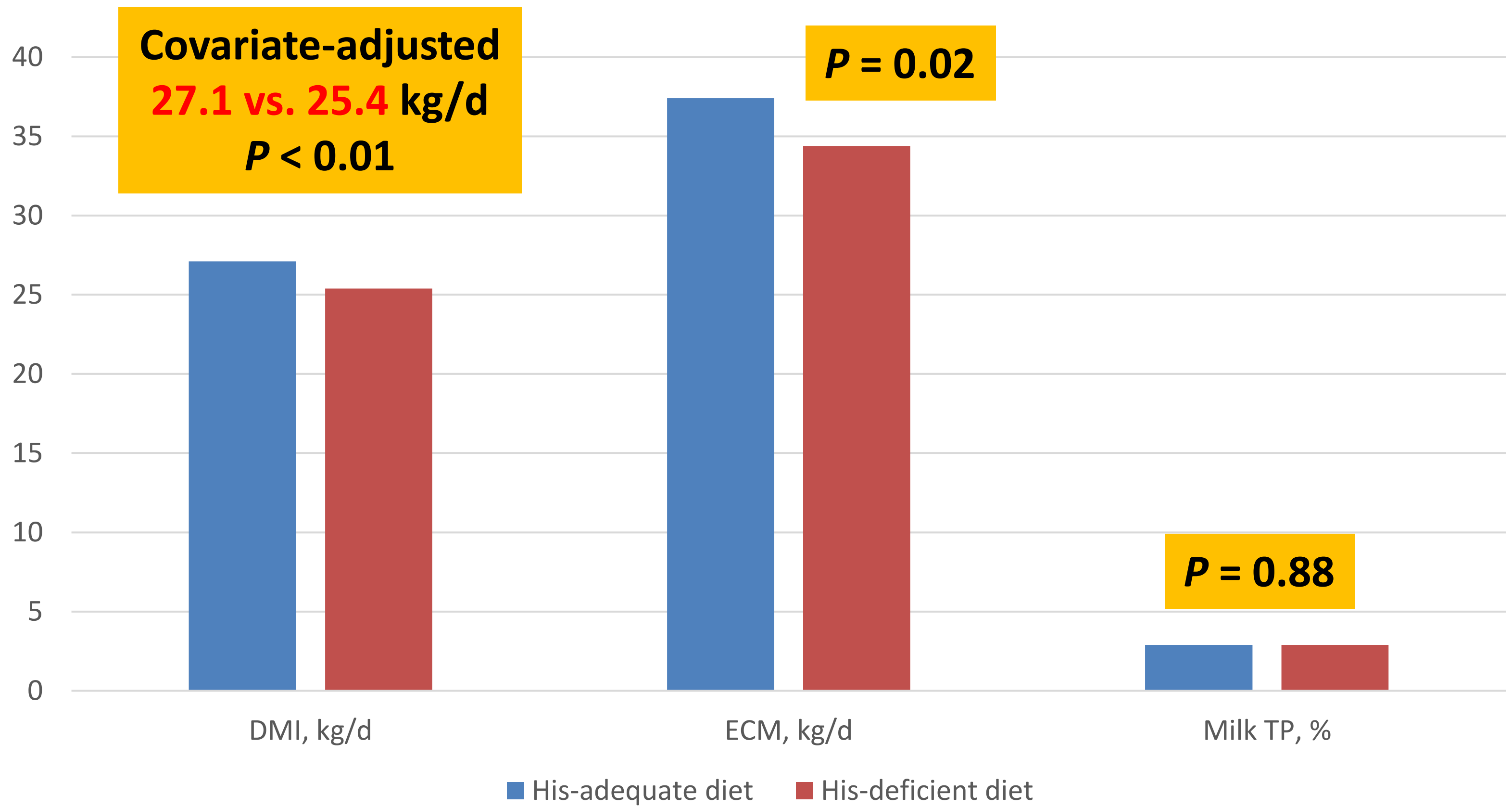

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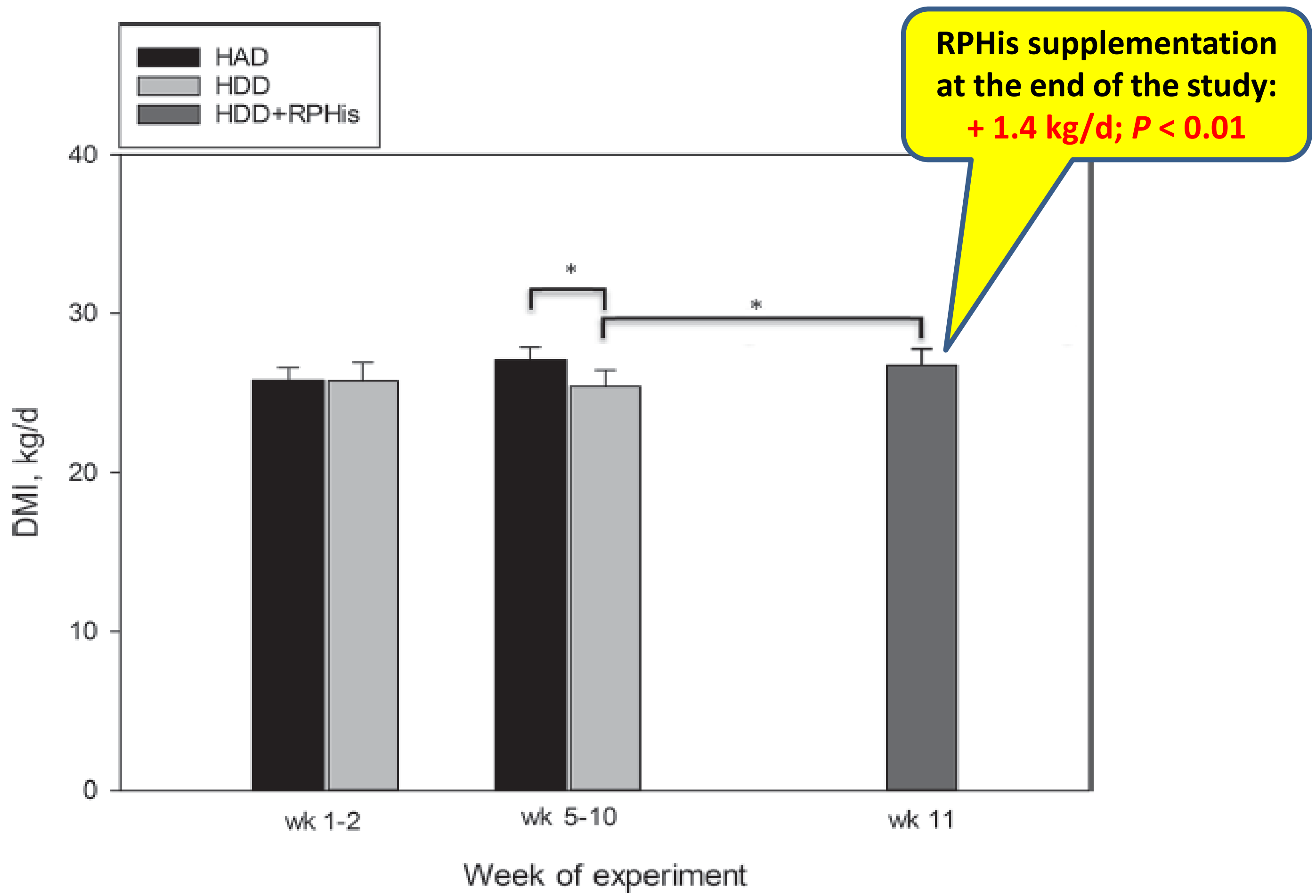

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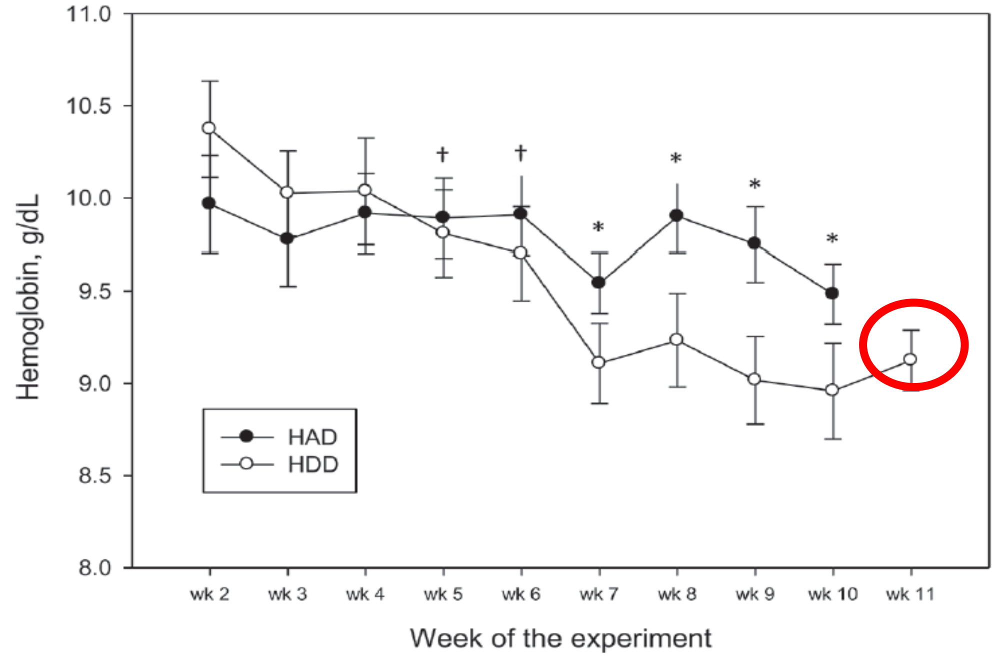
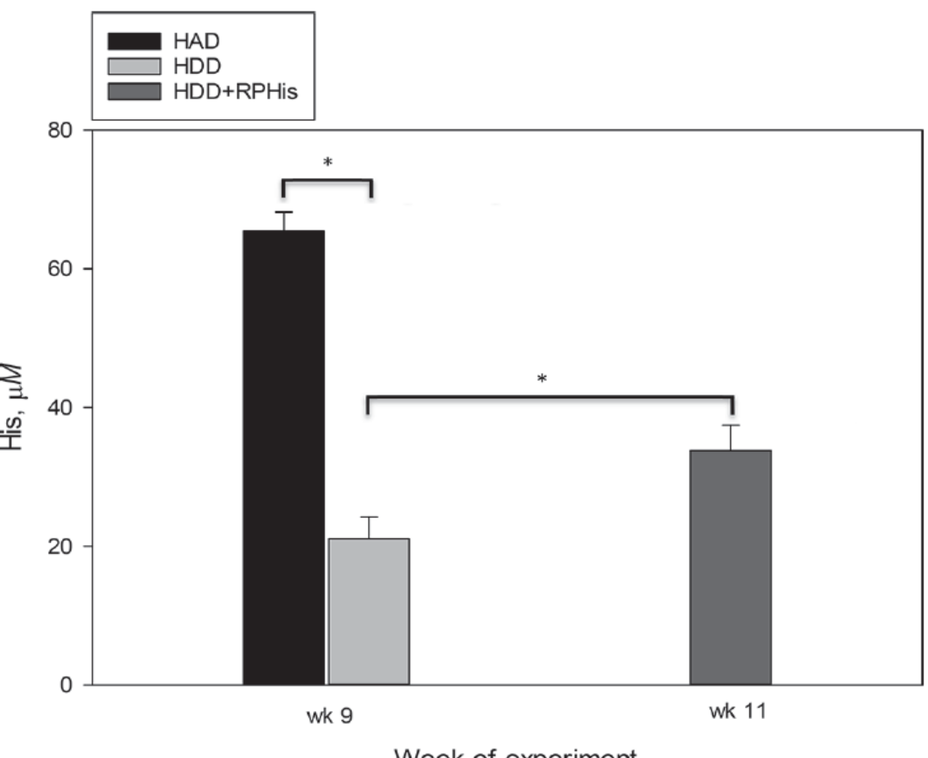
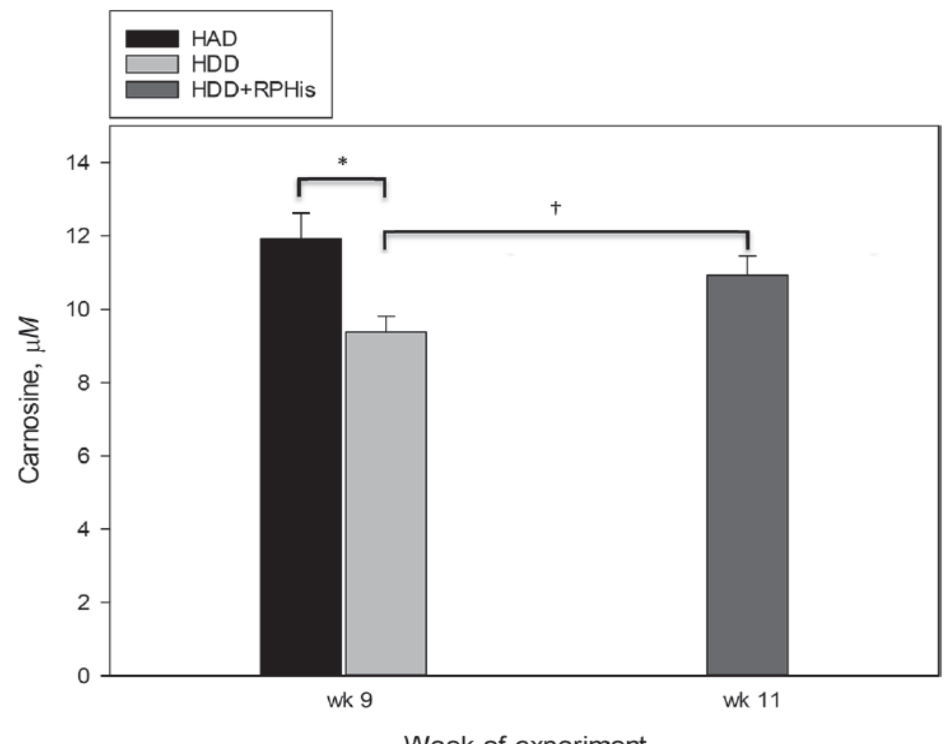
Giallongo et al., 2017

Blood hemoglobin, His, and carnosine as affected by His deficiency







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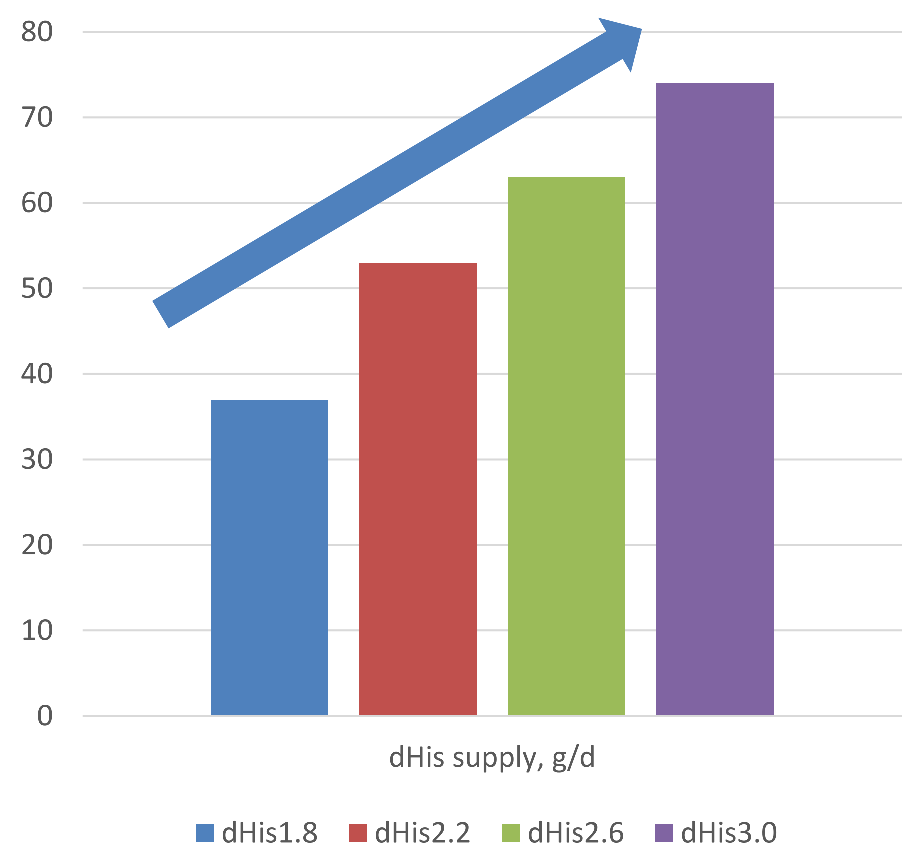
J. Dairy Sci. 104:9917–9930
https://doi.org/10.3168/jds.2021-20189

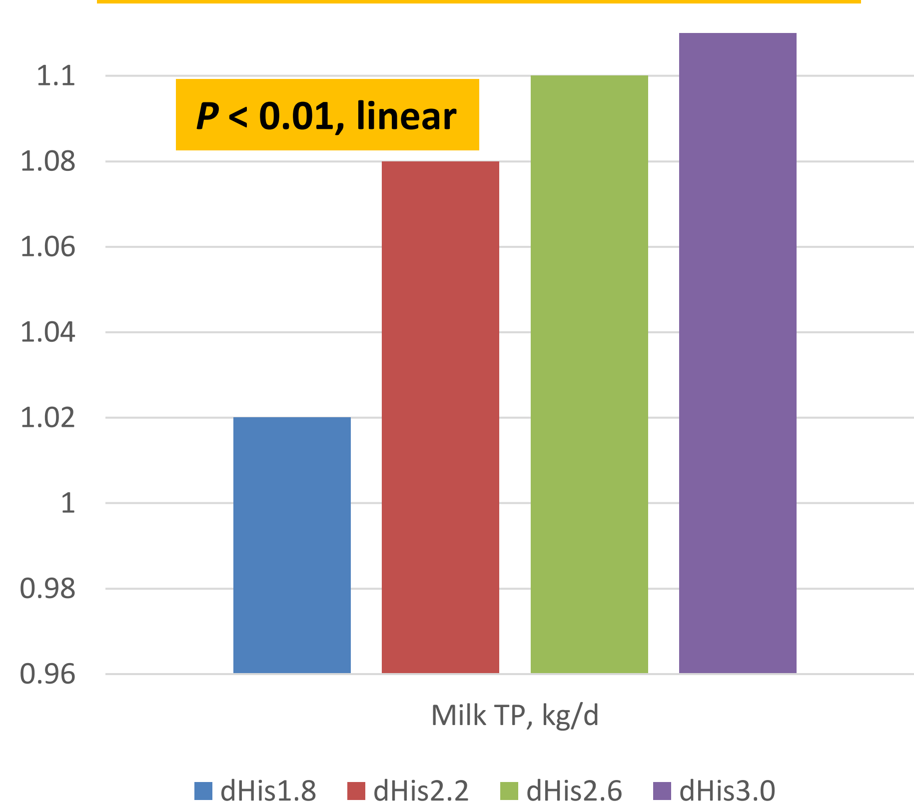
Histidine dose-response effects on lactational performance and plasma amino acid concentrations in lactating dairy cows: 2. Metabolizable protein-deficient diet

S. E. Räisänen,[1] C. F. A. Lage,[1,2] M. E. Fetter,[1] A. Melgar,[1,3] A. M. Pelaez,[1,4] H. A. Stefenoni,[1] D. E. Wasson,[1] S. F. Cueva,[1] X. Zhu,[1,5] M. Miura,[6] and A. N. Hristov[1]*
[1]Department of Animal Science, The Pennsylvania State University, University Park 16802
[2]School of Veterinary Medicine, University of California, Davis, Tulare 93274
[3]Agricultural Innovation Institute of Panama (IDIAP), City of Knowledge 07144, Panama
[4]Wageningen University and Research, PO Box 338, 6700 AH Wageningen, the Netherlands
[5]University of Chinese Academy of Sciences, Beijing 100049, P. R. China
[6]Ajinomoto Co. Inc., Kawasaki, Japan 210-8681

Lactational performance was optimized at dHis supply of 74 g/d (or 3.0% of MP)
No effect on MTP in the MPA diet trial



Linear increase in MY and FE, no effect on DMI



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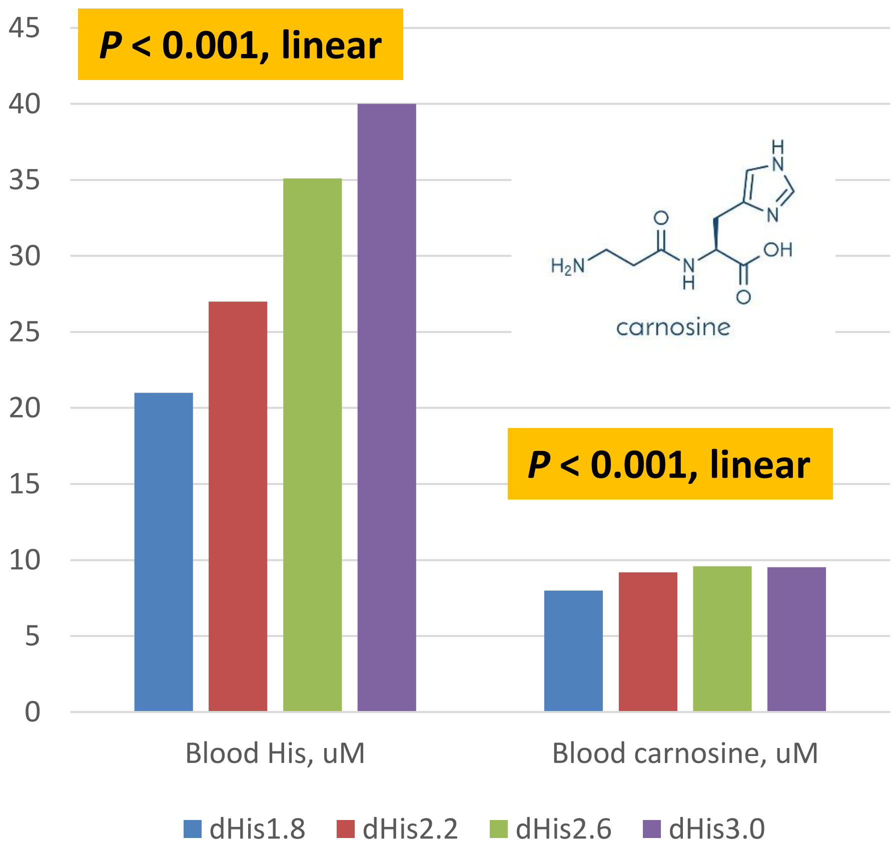
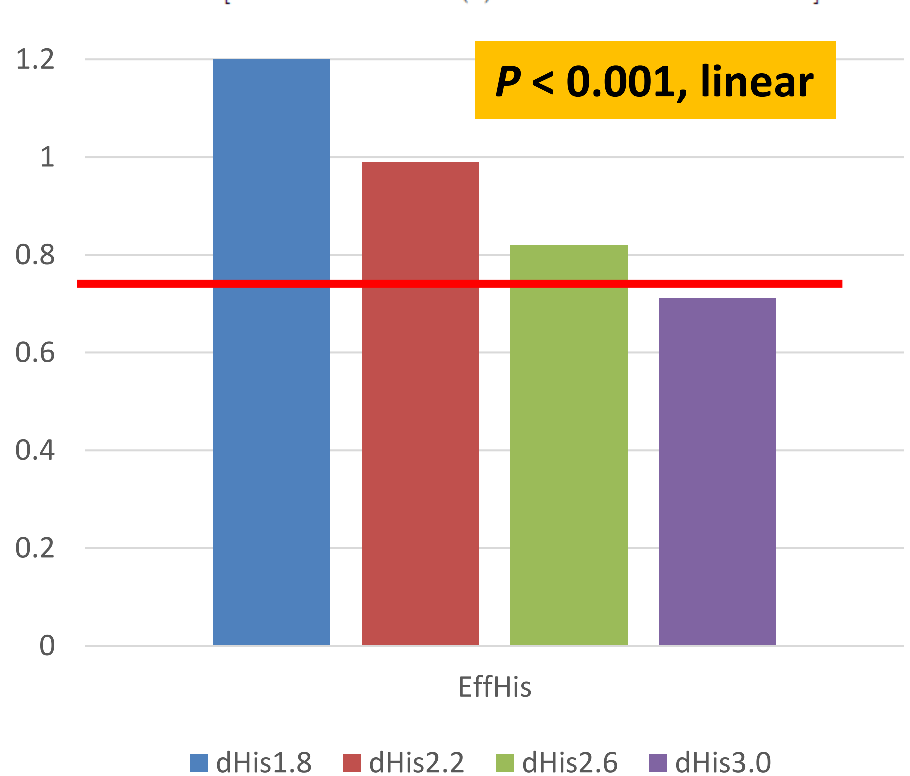


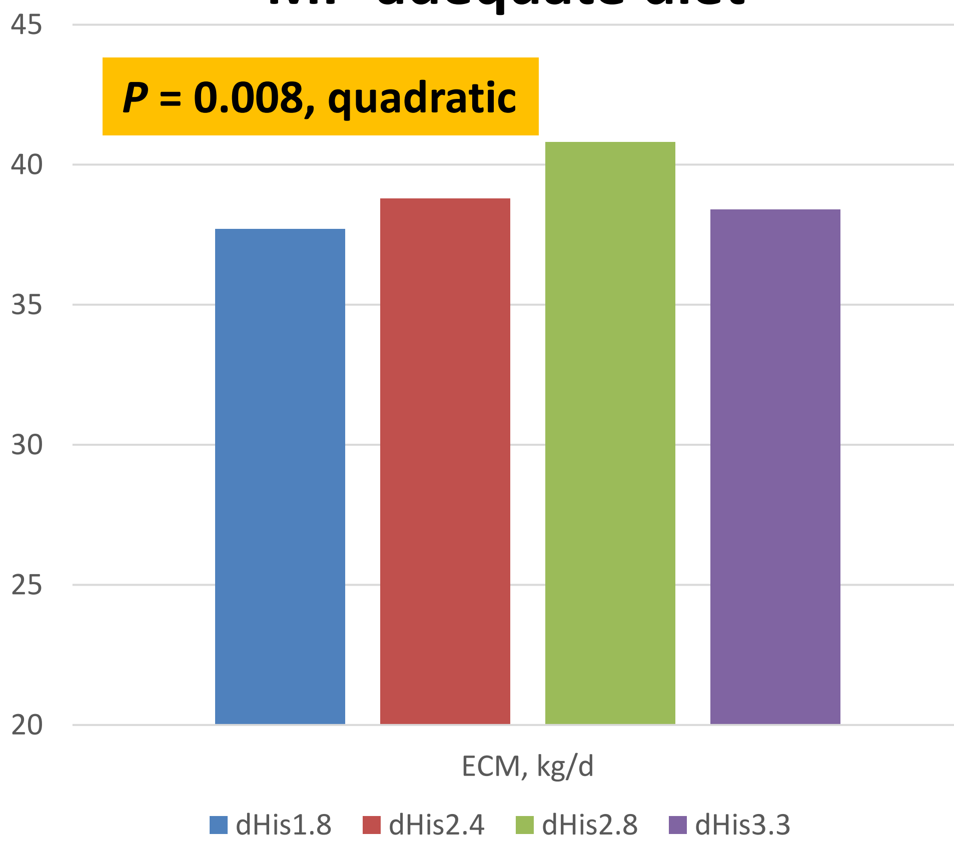
$$\mathrm{Eff}_{\mathrm{His}} = \frac{\text{sum of His in true exported proteins (g)}}{[\text{digestible His flow (g)} - \text{endogenous urinary loss}]},$$



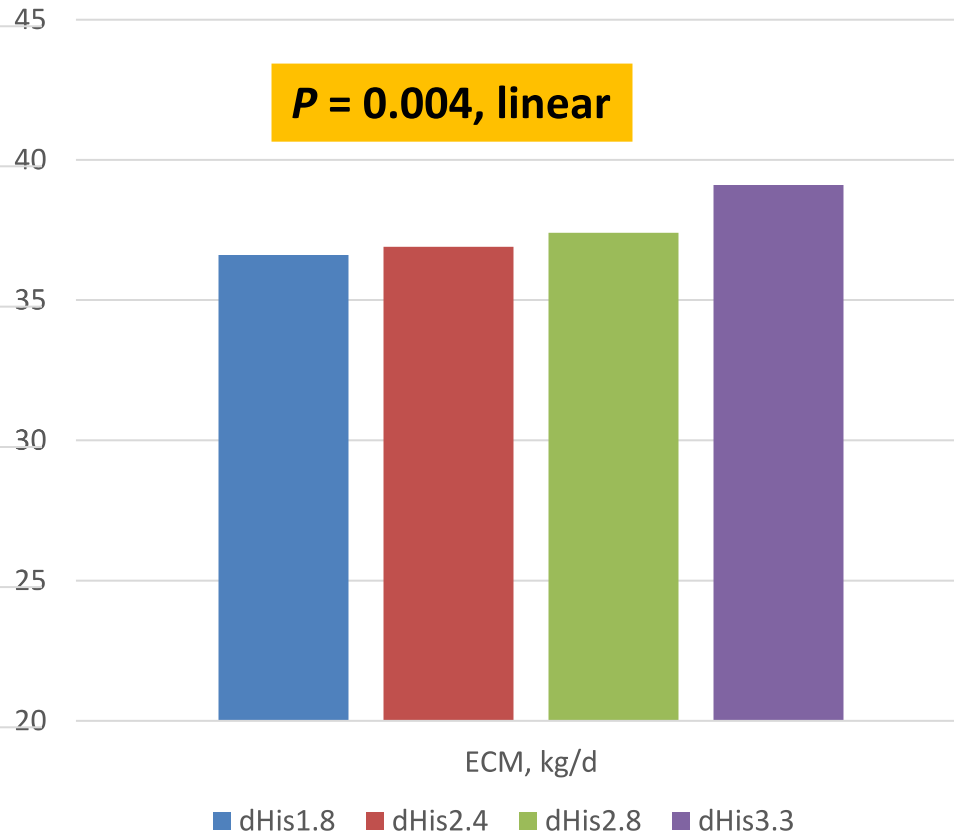
Räisänen et al., 2021; 2022

Dose-response studies with RPHis: ECM yield effect with MP-adequate and -deficient diet

MP-adequate diet



MP-deficient diet



J. Dairy Sci. 106
https://doi.org/10.3168/jds.2022-22966

Lactational performance effects of supplemental histidine in dairy cows: A meta-analysis

S. E. Räisänen,[1,2] H. Lapierre,[3] W. J. Price,[4] and A. N. Hristov[1]*
[1]Department of Animal Science, The Pennsylvania State University, State College, PA 16802
[2]ETH Zürich, Department of Environmental Science, Institute of Agricultural Sciences, Zürich 8092, Switzerland
[3]Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada J1M 0C8
[4]Statistical Programs, University of Idaho, Moscow, ID 83844

Table 1. Characterization of publications used in the meta-analysis

Source	Design[1]	Method of His supplementation[2]	Basal diet	MP-level[3]	Other supplemental AA
Vanhatalo et al. (1999)	LS	Infusion	Grass silage	MPD	Lys, Met
Kim et al. (1999)	LS	Deletion	Grass silage	MPA	Lys, Met, Trp
Kim et al. (2000)	LS	Infusion	Grass silage	MPA	Lys, Met
Korhonen et al. (2000)	LS	Infusion	Grass silage	MPA	—
Kim et al. (2001)a[4]	LS	Infusion	Grass silage	MPA	—
Kim et al. (2001)b	LS	Infusion	Grass silage	MPA	Lys, Met, Trp
Huhtanen et al. (2002)a	LS	Infusion	Grass silage	MPD	Leu
Huhtanen et al. (2002)b	LS	Infusion	Grass silage	MPD	—
Hadrová et al. (2012)	LS	Deletion	Corn silage	MPD	Leu, Lys, Met
Lee et al. (2012)	RCB	RPHis	Corn silage	MPD	RPLys, RPMet[5]
Giallongo et al. (2015)	RCB	RPHis	Corn silage	MPD	RPLys, RPMet
Giallongo et al. (2016)	RCB	RPHis	Corn silage	MPA	RPLys, RPMet
Giallongo et al. (2017)	RCB	Basal diet[6]	Corn silage	MPA	RPLys, RPMet
Zang et al. (2019)	LS	RPHis	Corn silage	MPA	RPMet
Morris and Kononoff (2020)a	LS	RPHis	Corn silage	MPA	—
Morris and Kononoff (2020)b	LS	RPHis	Corn silage	MPA	RPLys
Lapierre et al. (2021)a	LS	Deletion	Corn silage	MPD	Free AA, casein profile
Lapierre et al. (2021)b	LS	Deletion	Corn silage	MPD	Free AA, casein profile
Räisänen et al. (2021a)	LS	RPHis	Corn silage	MPA	RPLys, RPMet
Räisänen et al. (2021b)	LS	RPHis	Corn silage	MPD	RPLys, RPMet
Räisänen et al. (2022)a	RCB	RPHis	Corn silage	MPA	RPLys, RPMet
Räisänen et al. (2022)b	RCB	RPHis	Corn silage	MPA	RPLys, RPMet

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S. E. Räisänen,[1,2] H. Lapierre,[3] W. J. Price,[4] and A. N. Hristov[1]*
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[3]Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada J1M 0C8
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Table 4. Effect size[1] and heterogeneity for the effect of His supplementation on lactational performance of dairy cows

		Effect size and 95% CI					Heterogeneity	
Item	N[2]	Random	SE	Lower limit	Upper limit	*P*-value	Q-value[3]	*P*-value
DMI, kg/d	22	0.241	0.097	0.050	0.432	0.01	21.4	0.44
Milk yield, kg/d	22	0.888	0.192	0.512	1.26	<0.001	69.4	<0.001
ECM yield,[4] kg/d	14	0.187	0.115	−0.039	0.413	0.11	8.78	0.85
Milk true protein, %	22	0.246	0.104	0.041	0.450	0.02	23.9	0.30
Milk true protein, kg/d	22	0.674	0.147	0.386	0.962	<0.001	42.8	0.003
Milk fat, %	22	−0.427	0.119	−0.660	−0.195	<0.001	29.7	0.10
Milk fat, kg/d	22	−0.009	0.096	−0.197	0.178	0.92	12.6	0.92
Milk lactose, %	20	0.004	0.121	−0.234	0.241	0.97	27.1	0.10
Milk lactose, kg/d	20	0.425	0.101	0.227	0.623	<0.001	43.7	0.001
Plasma His, m*M*	22	1.81	0.251	1.39	2.37	<0.001	92.3	<0.001

[1]Computed as standard mean difference = raw mean difference of treatment and control means divided by the pooled SD of the means; values of <0.2, 0.2 to 0.7, and >0.7, were considered small, moderate, or large, respectively.

[2]Number of studies.

[3]Chi-squared (Q) test for heterogeneity and variation among the study level.

[4]Six studies were excluded from the analysis due to lack of ECM data and respective SD in the publication.

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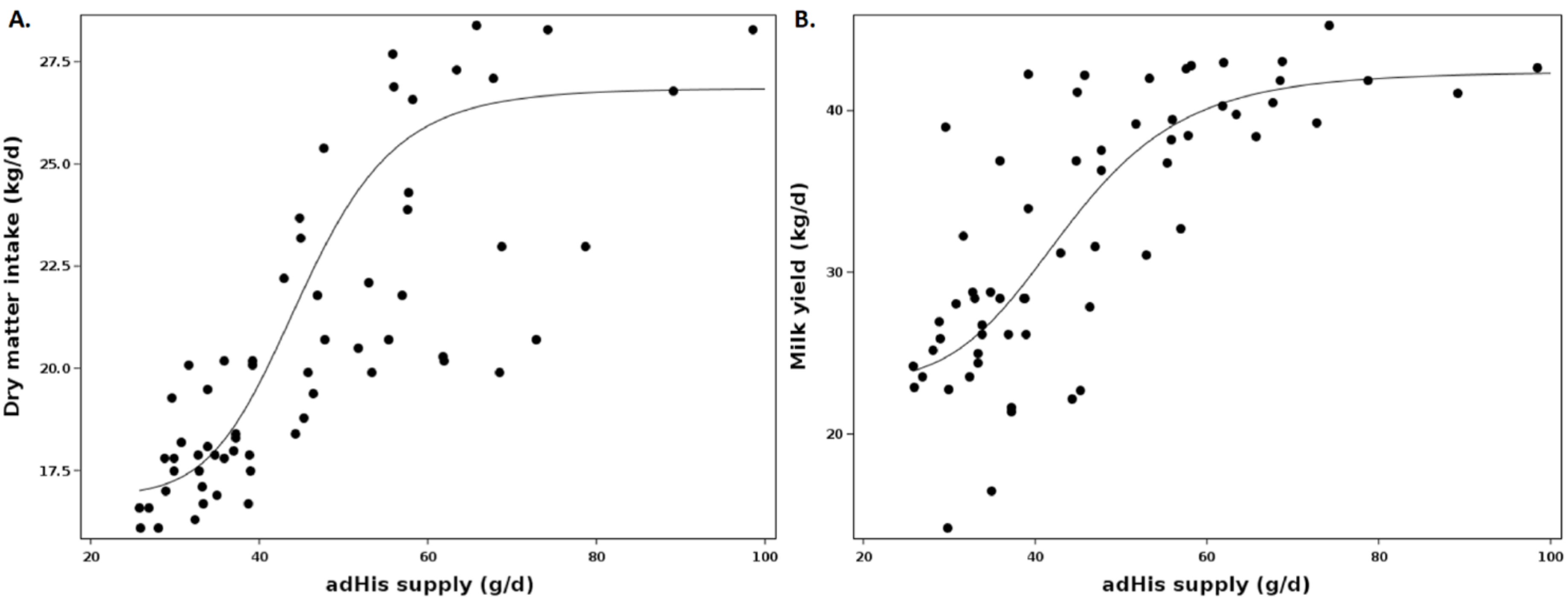
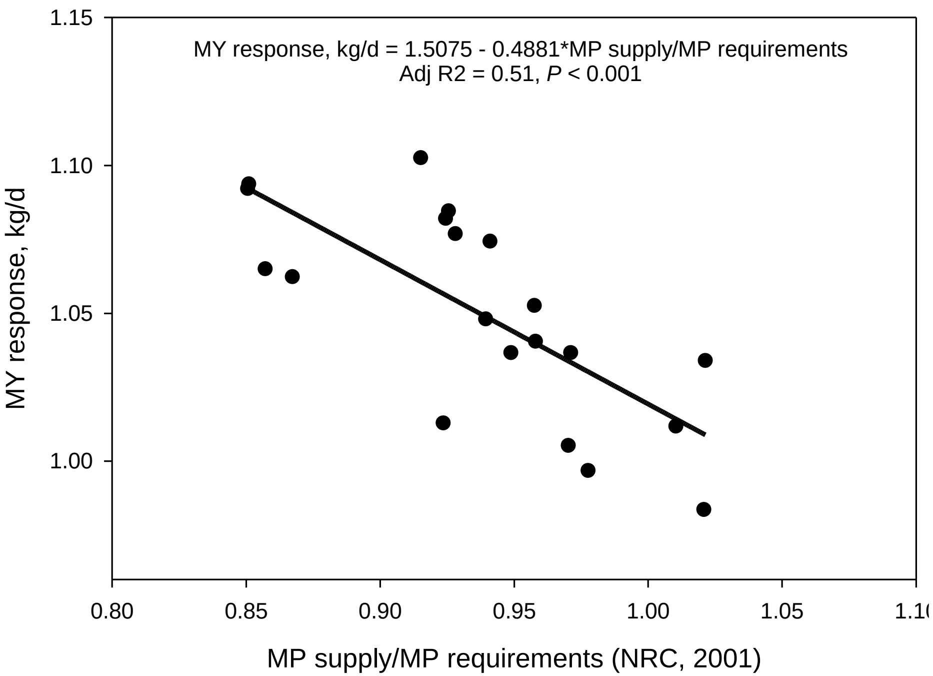


Dry matter intake and milk yield across adjusted digestible His (adHis) supply

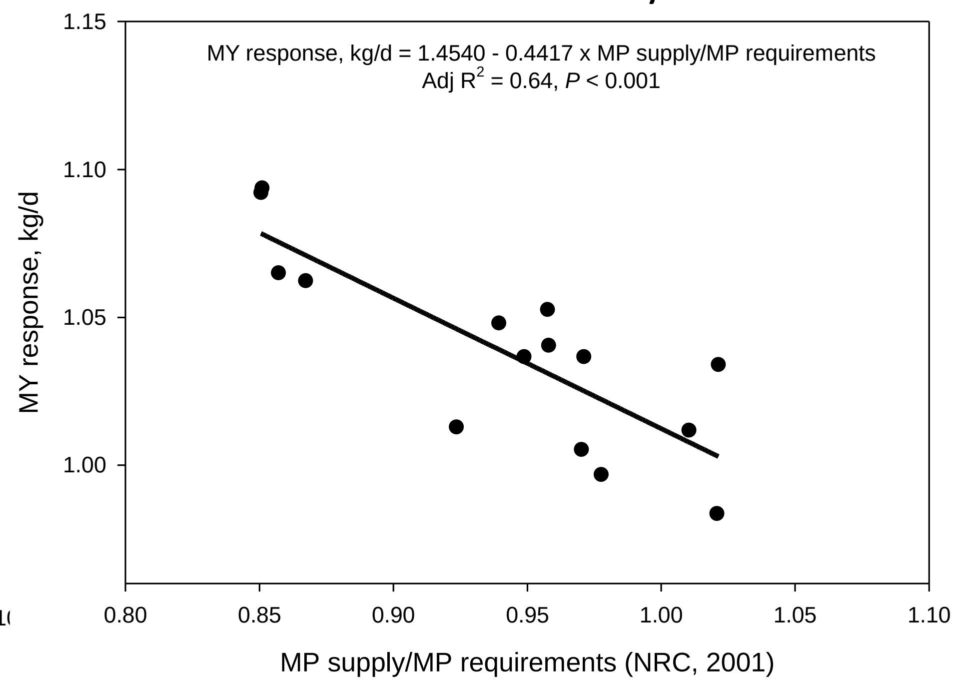
Räisänen et al., 2022

Responses to RPHis supplementation depend on MP supply

All studies (RPHis and infusion)



RPHis studies only



Production responses increase as MP deficiency increases

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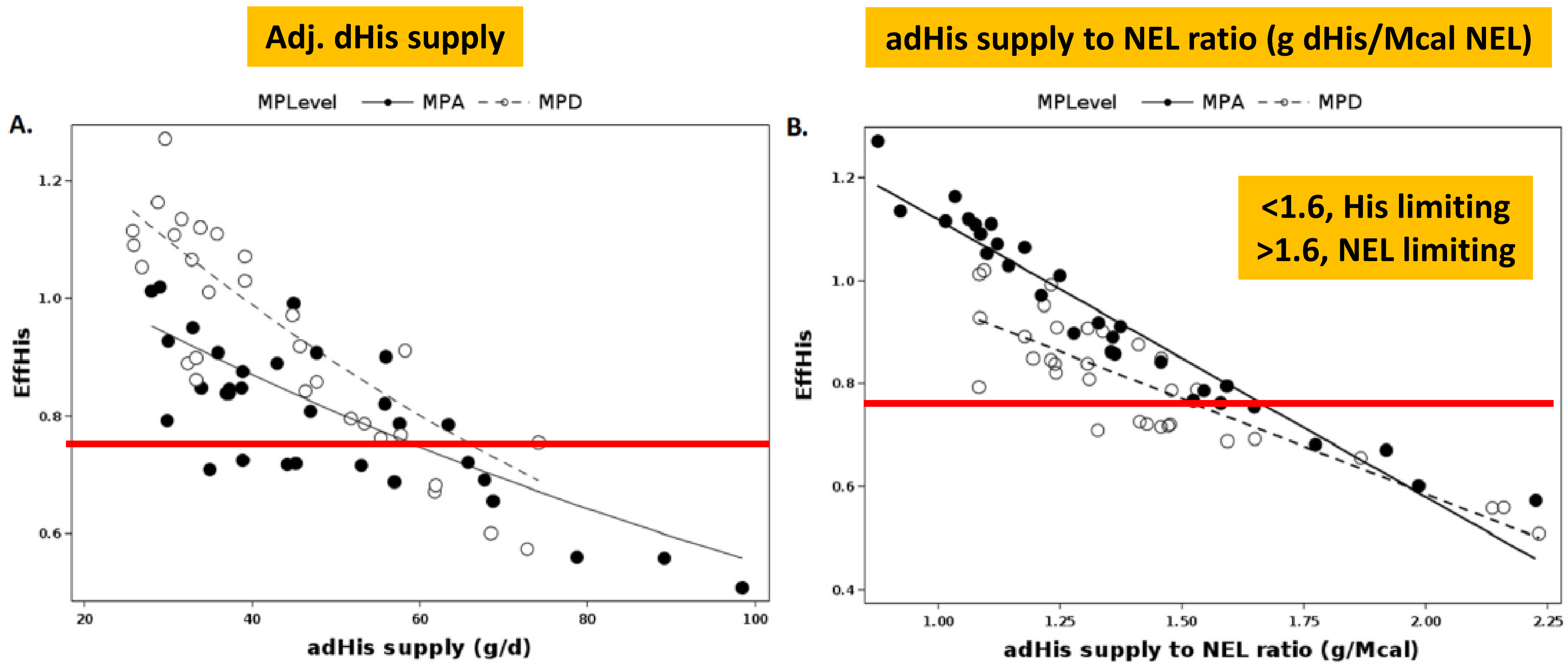

Figure 7. Efficiency of His utilization (Eff_{His}) observations by MP-level and across (A) increasing adjusted digestible His (adHis) supply or (B) ratio of adjusted digestible His (adHis) to NE_L supplies. Metabolizable protein requirements and supply were calculated based on NRC (2001); MP-deficiency (MPD) was defined as MP supplied at or below 95% of NRC (2001) requirements, and MP-adequacy (MPA) above 95% supply of requirements.

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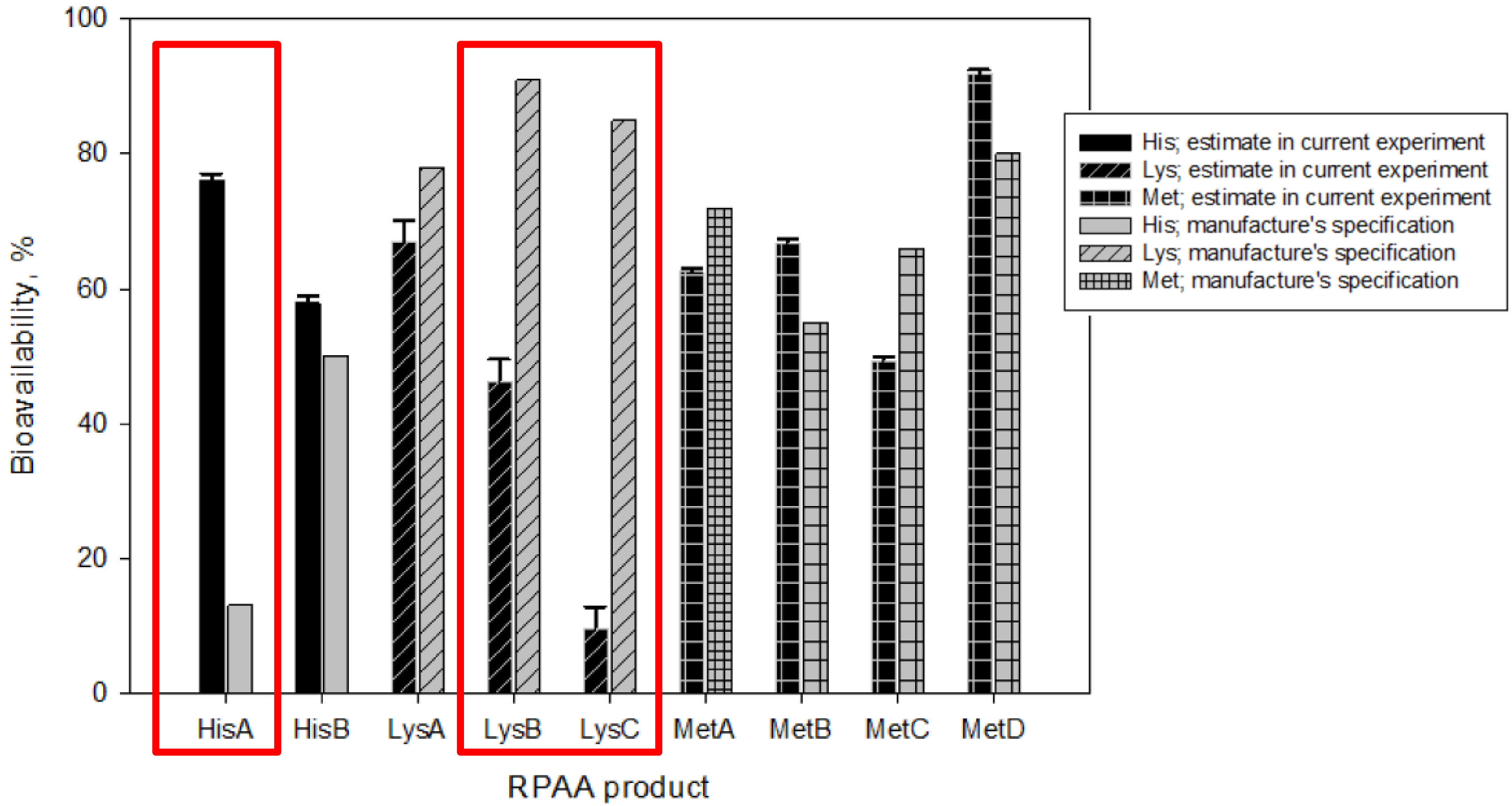
Räisänen et al., 2020

Be aware of incorrect bioavailability data for RPAA!



Take-home message

- Careful reduction in dietary protein intake will increase milk nitrogen efficiency in dairy cows and will decrease urinary nitrogen losses, nitrate leaching and ammonia and nitrous oxide emissions from dairy manure
- Earlier studies with grass silage-based diets and more recently studies with corn silage-based diets conducted at Penn State indicate that His may be a limiting AA in dairy cow fed low-protein (< 16% CP) diets
  - Long-term trials showed that supplementation of such diets with rumen-protected His increased or tended to increase milk yield and milk protein percent and yield, including through increasing DMI
  - Our data suggest dHis recommendations for MP-deficient diets at around 3.0% of MP, or 74 g/d
  - Watch for false bioavailability data

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Advance access publication 28 November 2022
Non ruminant nutrition

OXFORD

AMERICAN SOCIETY OF ANIMAL SCIENCE

The effects of standardized ileal digestible His to Lys ratio on growth performance, intestinal health, and mobilization of histidine-containing proteins in pigs at 7 to 11 kg body

SID His to Lys ratios of 26%, 32%, 38%, 43%, and 49% fed to growing pigs……. It was concluded that SID His to Lys ratio between 35% and 41% in diets fed to nursery pigs at 7 to 11 kg enhanced intestinal health and maximized concentrations of His-containing proteins, indicating that His containing proteins are effective response criteria when determining His requirement

32%, 38%, 43%, and 49% and fed to pigs for 14 d (SID Lys = 1.22%). Feed intake and BW were recorded at d 0, 7, and 14 to measure growth performance. Blood samples were collected on d 12. Pigs were euthanized on d 14 to collect pancreas, longissimus dorsi muscles, mid-jejunum, and jejunal mucosa. Data were analyzed using the Proc Mixed of SAS. Growth performance was not affected, whereas varying SID His to Lys ratio affected hemoglobin ($P < 0.05$, max: 12 g/dL at 36%), immunoglobulin A (IgA, $P < 0.05$, min: 1.25 µg/mg at 35%) in jejunal mucosa, villus height ($P = 0.065$, max: 536 µm at 40%) in jejunum, trypsinogen ($P = 0.083$, max: 242 pg/mg at 41%) in pancreas, and carnosine ($P = 0.051$, max: 4.7 ng/mg at 38%) in muscles. Varying SID His to Lys ratios linearly increased ($P < 0.05$, from 1.95 to 2.80 nmol/mg) protein carbonyl in muscles and decreased ($P < 0.05$, from 29.1% to 26.9%) enterocyte proliferation. In conclusion, SID His to Lys ratio between 35% and 41% in diets fed to nursery pigs at 7 to 11 kg enhanced intestinal health and maximized concentrations of His-containing proteins, indicating that His-containing proteins are effective response criteria when determining His requirement.

QUESTIONS?