



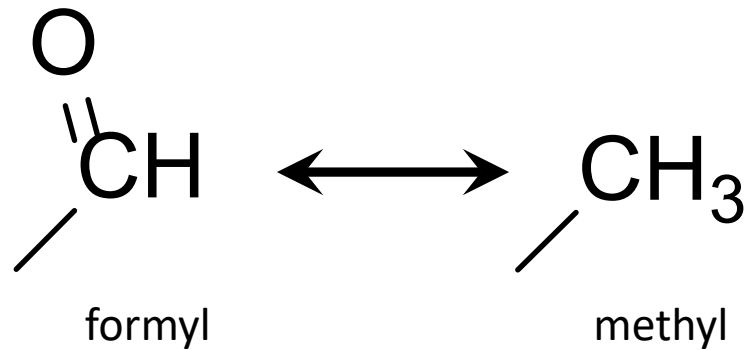
➤ Is there more than nitrogen in low-protein diets?

Jaap van Milgen

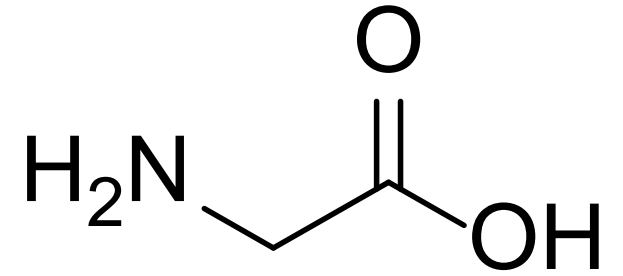
➤ Is there more than nitrogen in low protein diets?



urine: what is in there and how much does it cost to make?

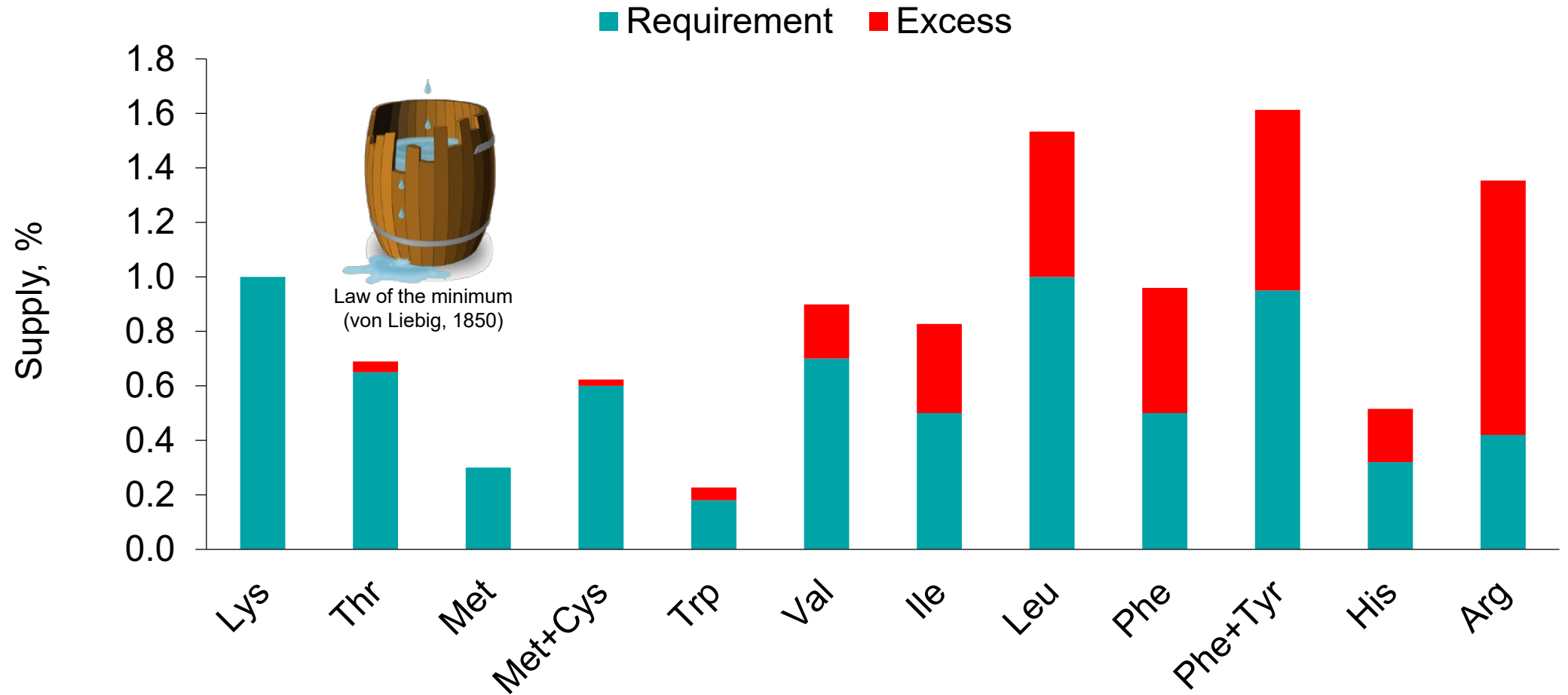


the role of amino acids in 1-carbon metabolism

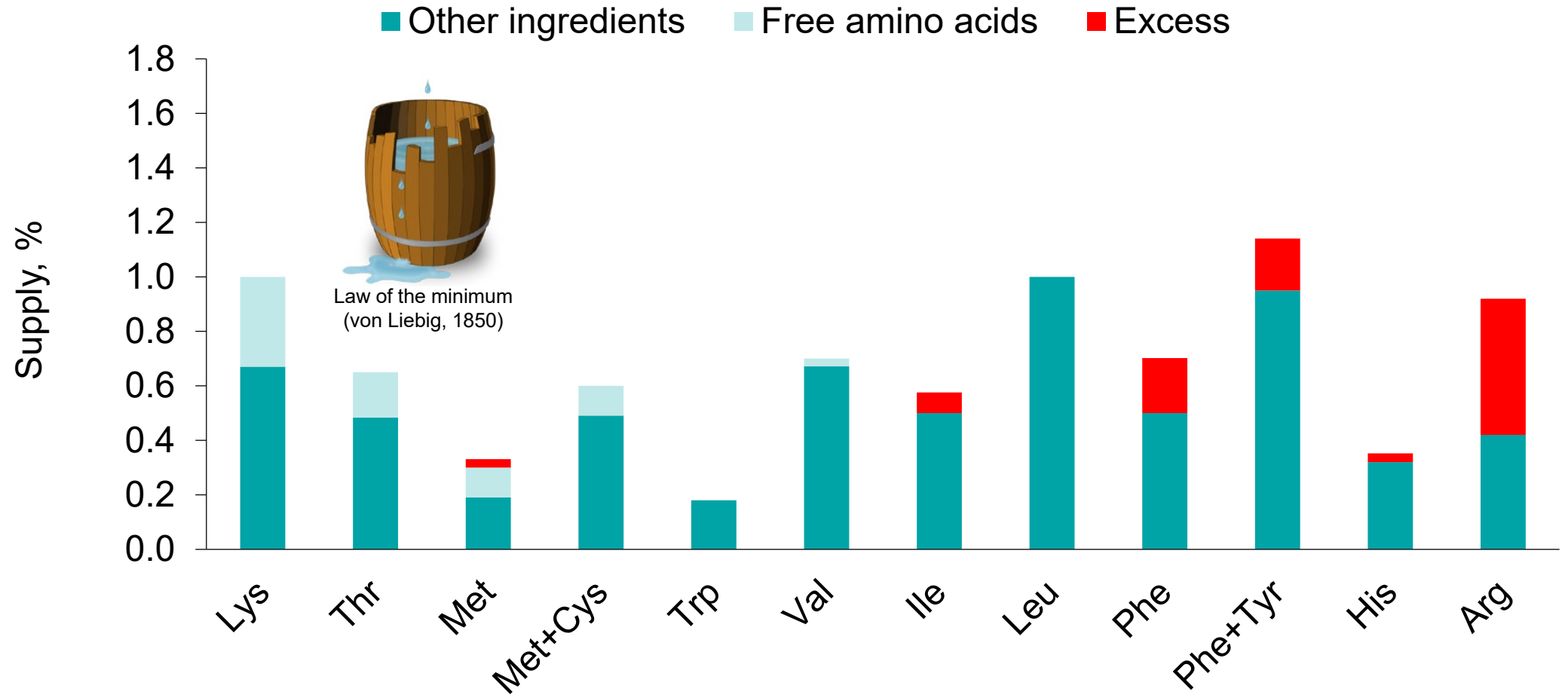


glycine: an essential non-essential amino acid

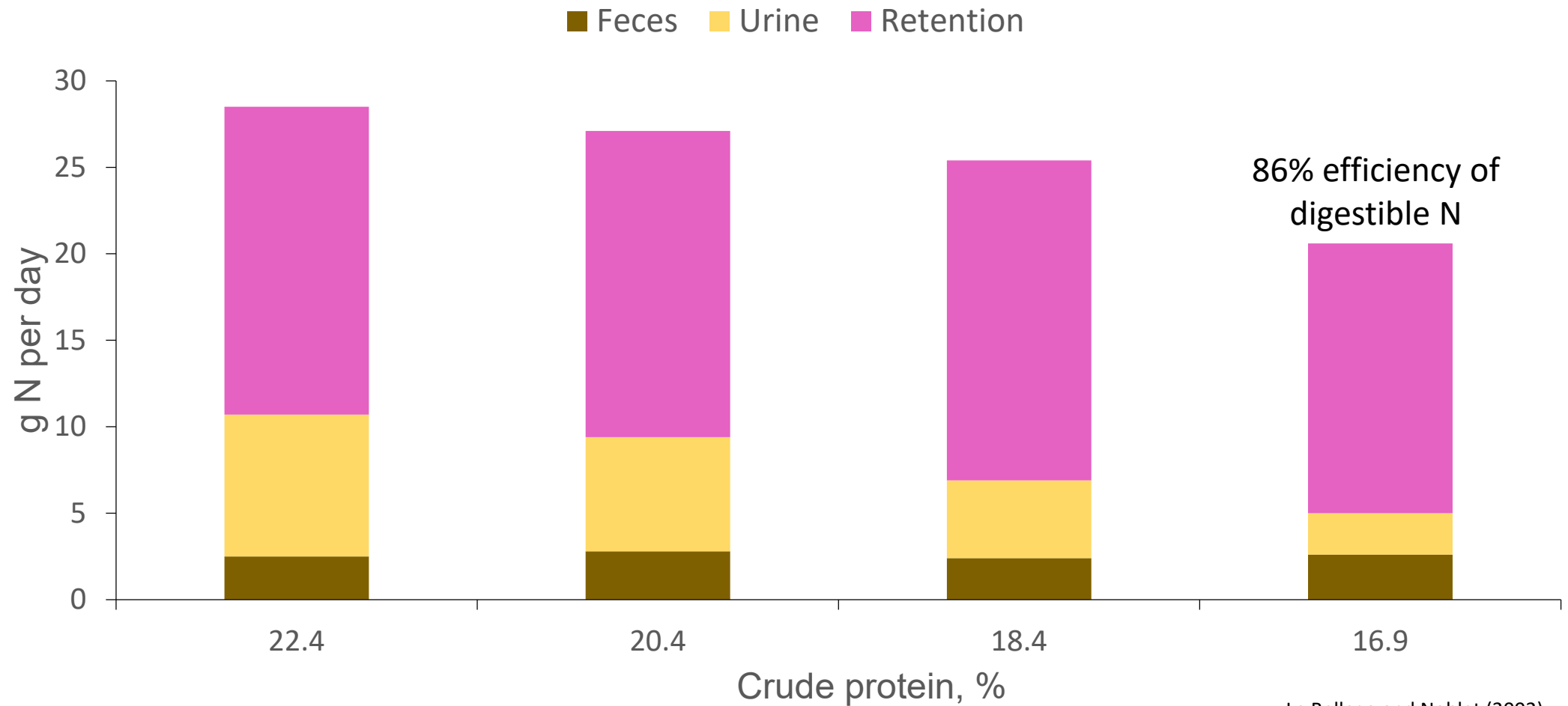
➤ With low-protein diets, more amino acids become co-limiting



➤ With low-protein diets, more amino acids become co-limiting



➤ Nitrogen balance in 15-kg piglets



86% efficiency of digestible N

Le Bellego and Noblet (2002)



INRAE

Is there more than nitrogen in low-protein diets?

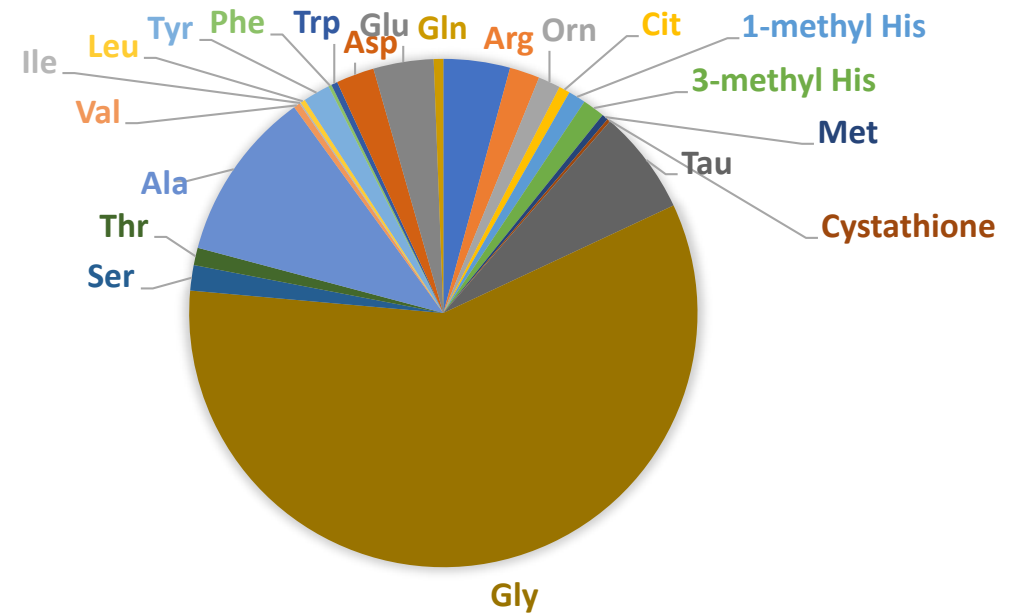
Advancia, May 17, 2022 / Jaap van Milgen

➤ Urinary nitrogen excretion in young pigs



Source	Excretion (g/d)
Total-N	6.37
Urea-N	4.23
NH ₃ -N	0.40
Creatinine-N	0.17
Amino acid-N	0.09

Southern and Baker (1984)



➤ What a lockdown can do to people ...

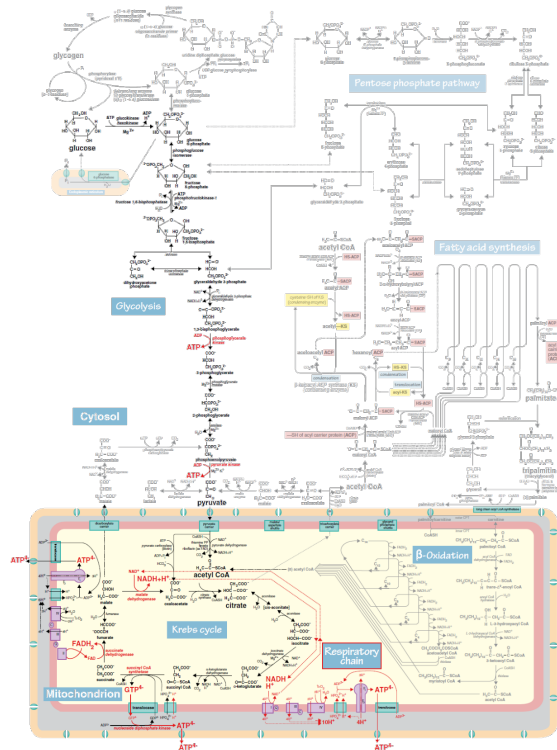


Nutritional Models

Modeling Biochemical Aspects of Energy Metabolism in Mammals¹

Jaap van Milgen²

INRA, Unité Mixte de Recherches sur le Veau et le Porc, 35590 Saint-Gilles, France



- Construct complete pathways from individual reactions (e.g., using Kegg).
- It quantifies how it happens; it does not indicate if it happens



INRAE

Is there more than nitrogen in low-protein diets?

Advancia, May 17, 2022 / Jaap van Milgen

➤ What a lockdown can do to people ...



Supplementary Table S1

Stoichiometric reactions of urea synthesis from amino acid catabolism in mammals.

Kegg ID¹ Reaction

1.1 Urea cycle

- R01398 1 ornithine + 1 carbamoyl phosphate → 1 citrulline + 1 P_i
 R01954 1 citrulline + 1 aspartate + 1 ATP → 1 argininosuccinate + 1 AMP + 1 PP_i
 R01086 1 argininosuccinate → 1 arginine + 1 fumarate
 R00551 1 arginine + 1 H₂O → 1 ornithine + 1 urea
 R00127 1 AMP + 1 ATP → 2 ADP
 R00004 1 PP_i + 1 H₂O → 2 P_i
 Subtotal 1 carbamoyl phosphate + 1 aspartate + 2 ATP + 2 H₂O →
 1 urea + 1 fumarate + 2 ADP + 3 P_i

1.2 Synthesis of carbamoyl phosphate from NH₄⁺ and CO₂

- R00149 1 NH₄⁺ + 1 CO₂ + 2 ATP + 1 H₂O → 1 carbamoyl phosphate + 2 ADP + 1 P_i

1.3 Synthesis of aspartate from fumarate and NH₄[±]

- R01082 1 fumarate + 1 H₂O → 1 malate
 R00342 1 malate + 1 NAD⁺ → 1 oxaloacetate + 1 NADH
 R00355 1 oxaloacetate + 1 glutamate → 1 aspartate + 1 α-ketoglutarate
 R00243 1 α-ketoglutarate + 1 NH₄⁺ + 1 NADH → 1 glutamate + 1 NAD⁺ + 1 H₂O
 Subtotal 1 NH₄⁺ + 1 fumarate → 1 aspartate

Stoichiometry of urea synthesis

- Total 2 NH₄⁺ + 1 CO₂ + 4 ATP + 3 H₂O → 1 urea + 4 ADP + 4 P_i

AMP = adenosine monophosphate; P_i = phosphate; PP_i = diphosphate.

¹ Reaction identifier in the Kegg database (www.kegg.jp).



Animal 15 (2021) 100213



Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

Animal

The international journal of animal biosciences



The role of energy, serine, glycine, and 1-carbon units in the cost of nitrogen excretion in mammals and birds

J. van Milgen

Pegase, INRAE, Institut Agro, Le Clos, 35590 Saint Gilles, France

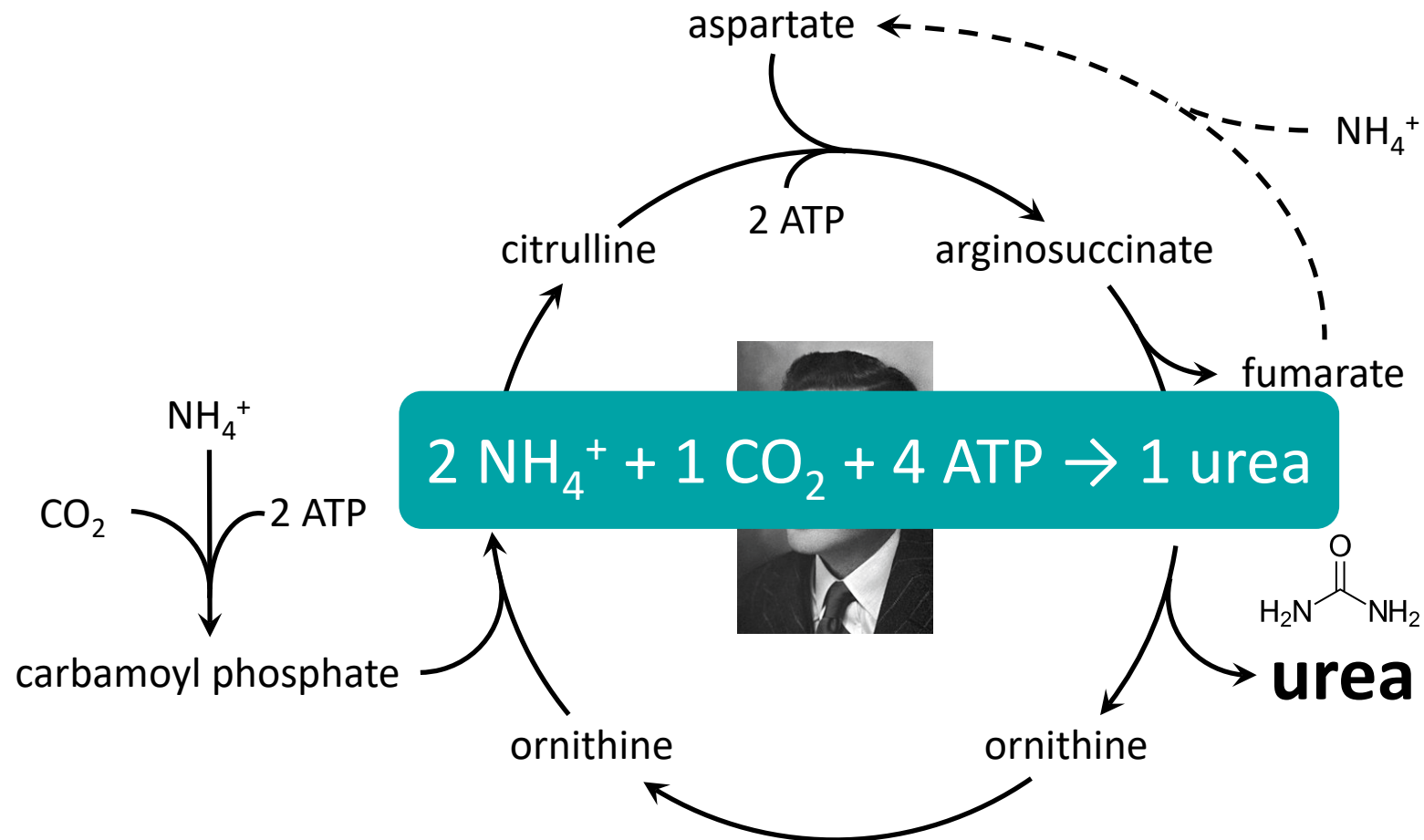


INRAE

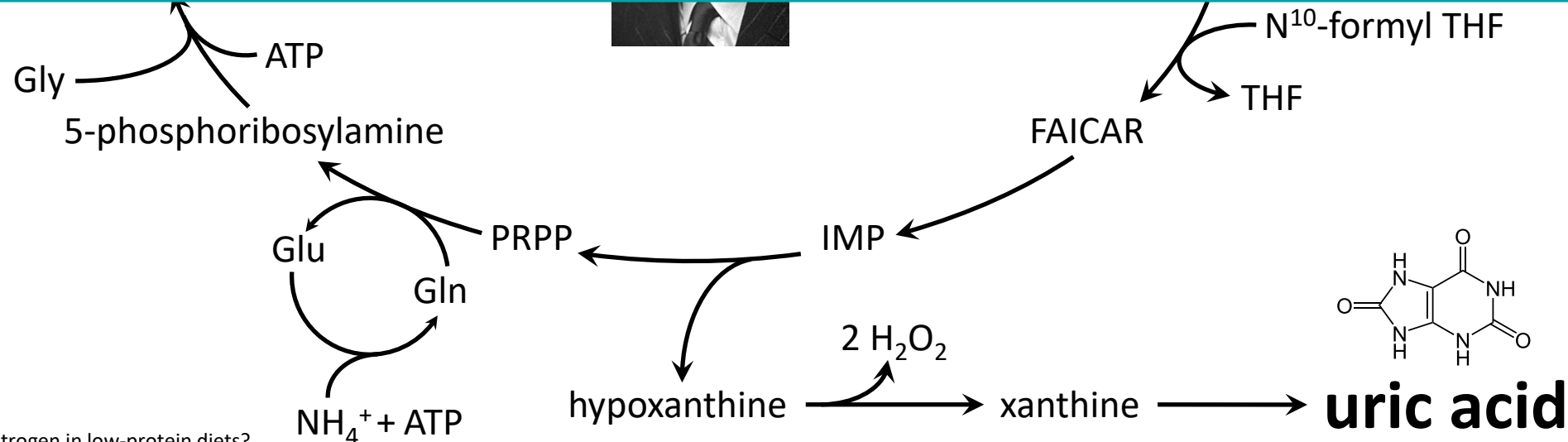
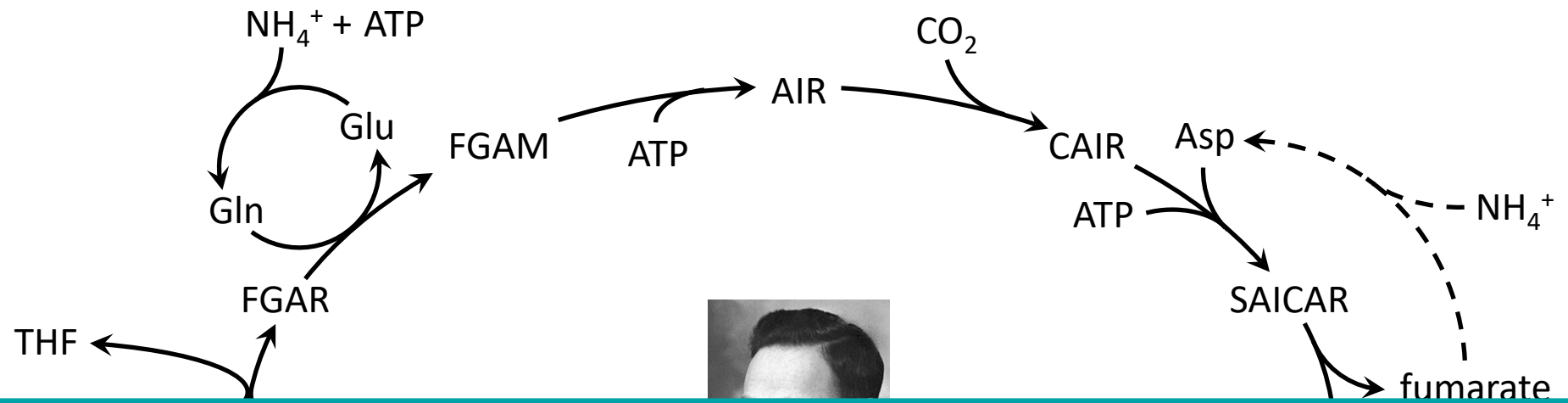
Is there more than nitrogen in low-protein diets?

Advancia, May 17, 2022 / Jaap van Milgen

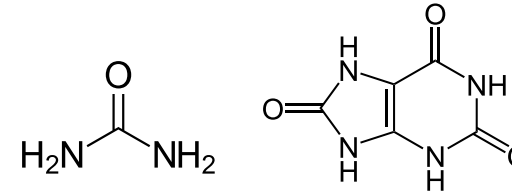
➤ Urea cycle (Krebs and Henseleit, 1932)



➤ Uric acid cycle (Mapes and Krebs, 1978)



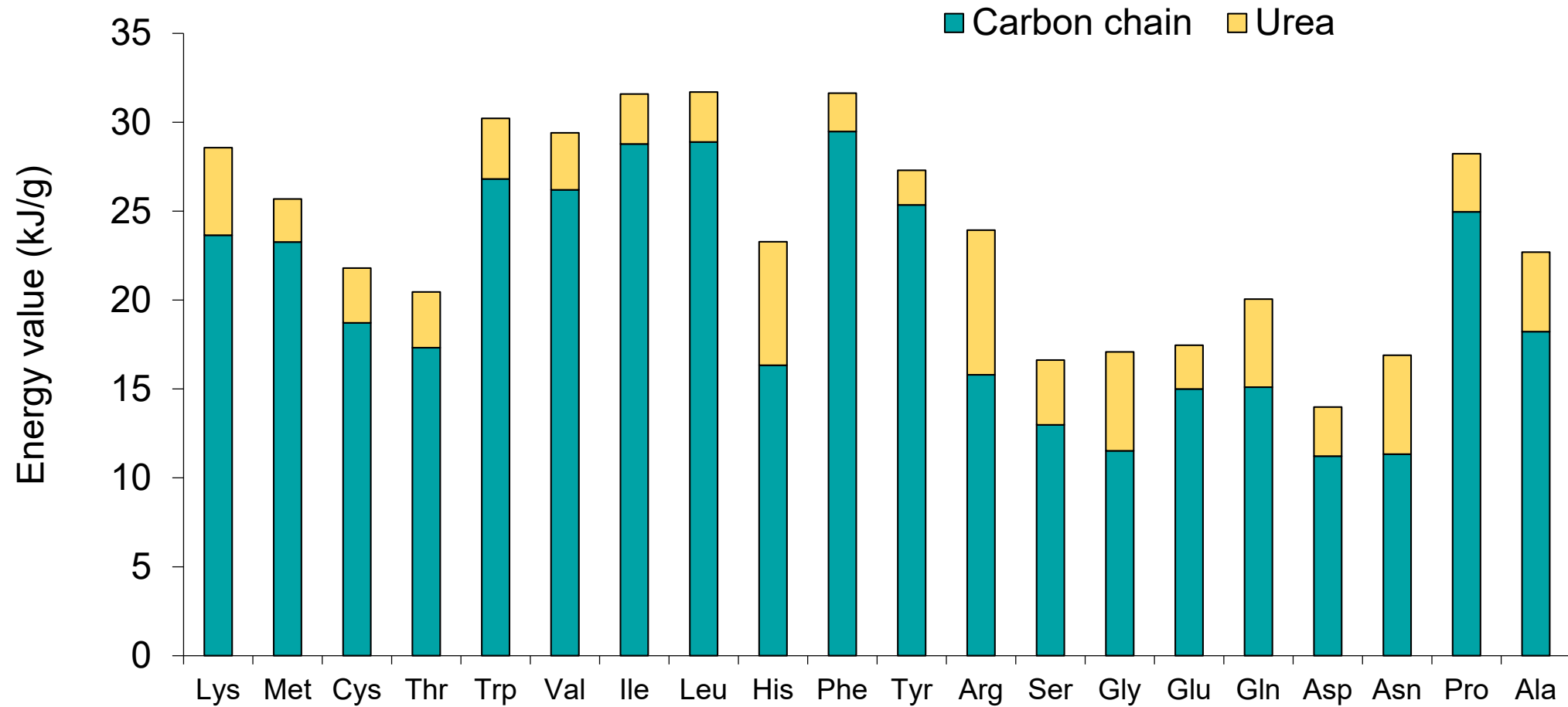
➤ The energy cost of nitrogen excretion



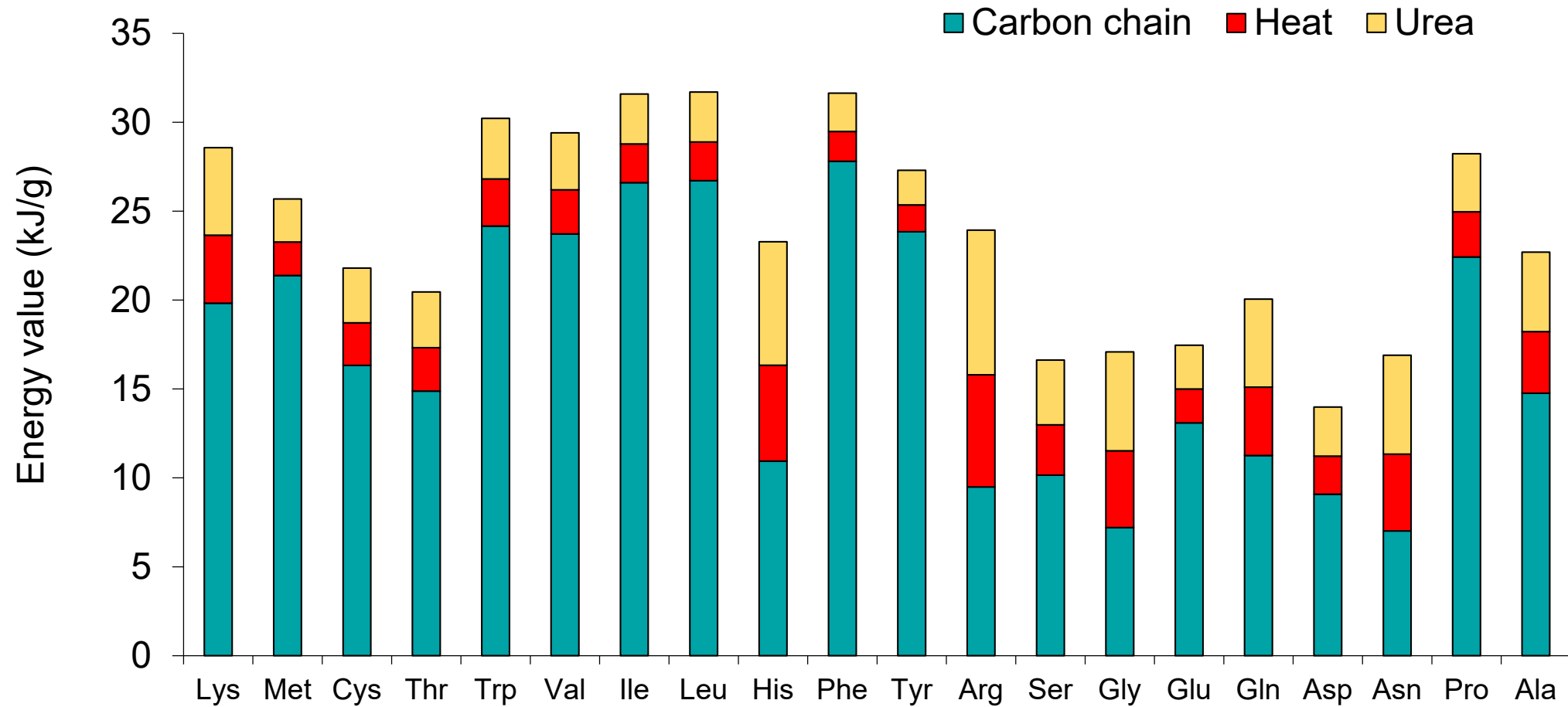
	Urea	Uric acid*
Cost to produce, kJ/mol N	564	850
Energy in product, kJ/mol N	318	480
Heat, kJ/mol N	247	370
DE, kJ/g digestible protein	23.8	23.8
ME*, kJ/g excess protein	20.2	18.3
NE*, kJ/g excess protein	17.4	14.1

* Maximum values based on stoichiometry

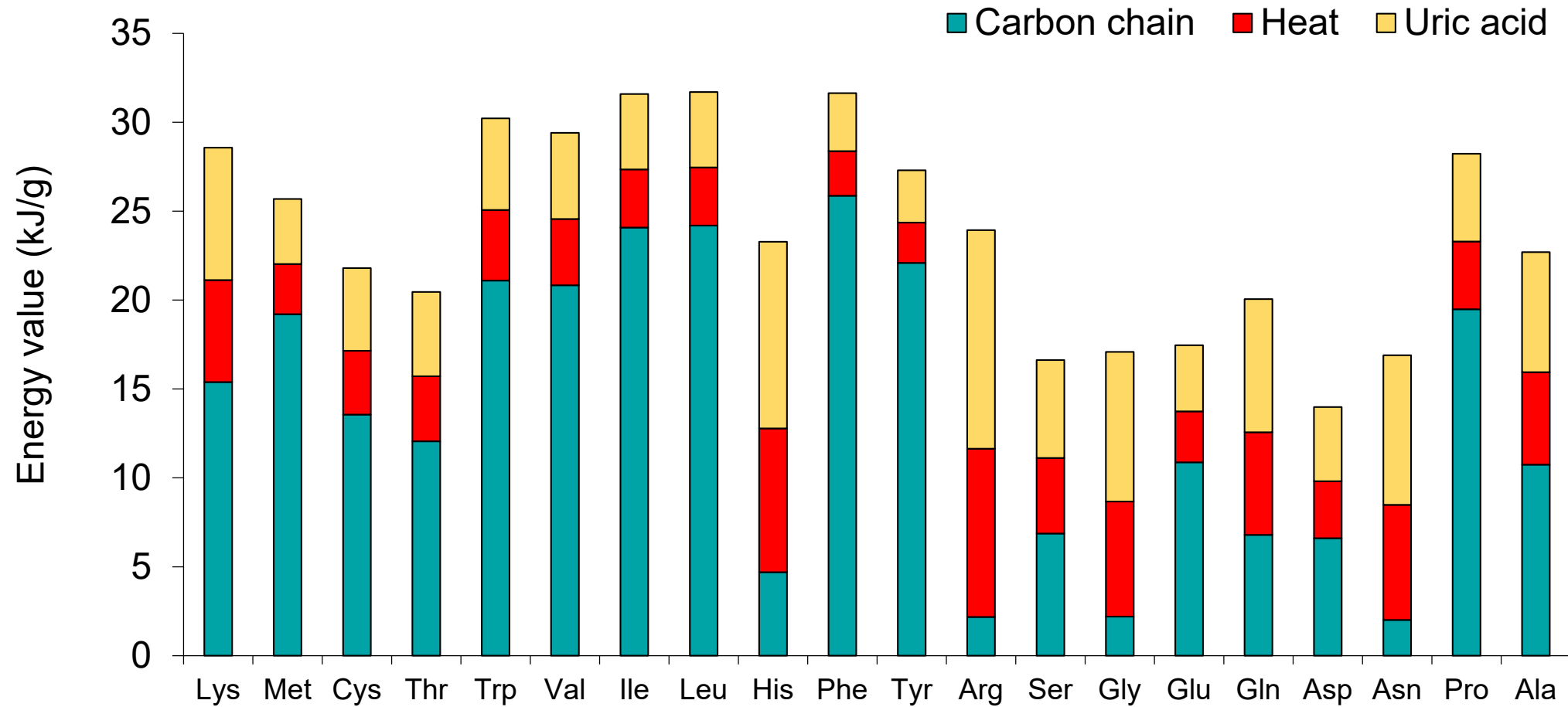
➤ Metabolizable energy values of excess amino acids



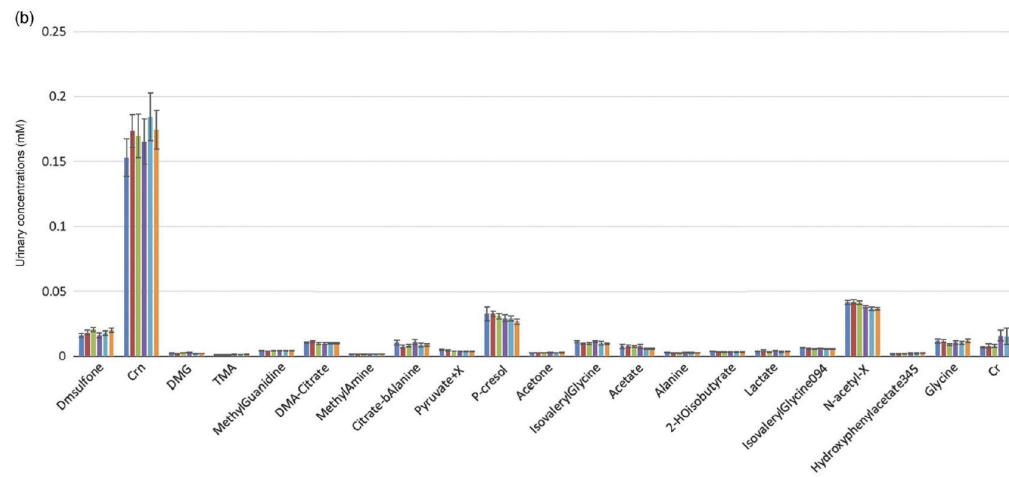
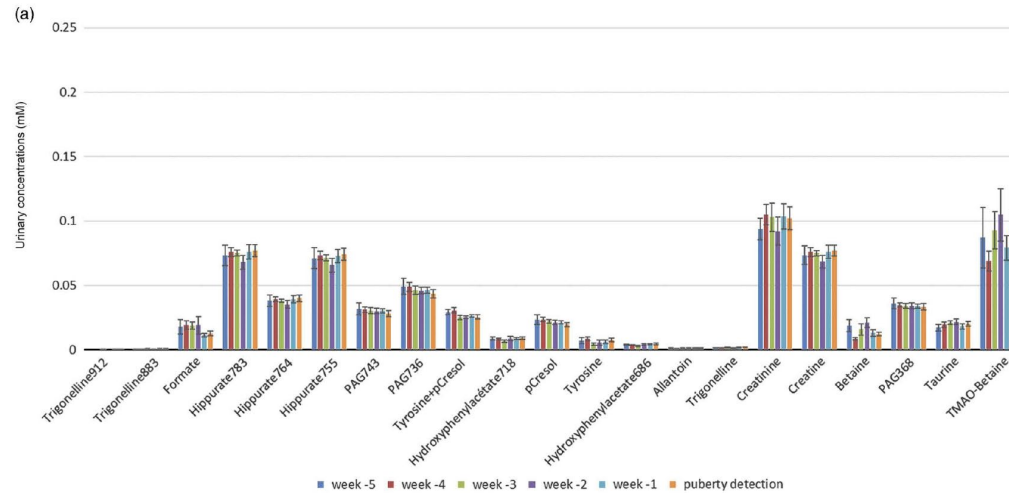
➤ Net energy values of excess amino acids (in mammals)



➤ Net energy values of excess amino acids (in birds)



➤ There is more than urea in the urine



Goudet *et al.* (2019)

Source	N	1-carbon
Creatinine	X	
Creatine	X	
Betaine	X	X
Allantoin	X	X
Formate		X
Hippurate	X	
P-Cresol		X
Glycine	X	X
Taurine	X	
Alanine	X	
Tyrosine	X	
IsovalerylGlycine	X	
PhenylacetylGlycine	X	X
Citrate-β-alanine	X	
Trigonelline	X	X
Trimethylamine oxide	X	X
Dimethylsulfone		X
Dimethylglycine	X	X
Trimethylamine	X	X
Methylguanidine	X	X
Dimethylamine-citrate	X	X
Methylamine	X	X
Pyruvate		
Lactate		
Acetone (dimethyl ketone)		
Acetate		
N-acetyl-X		
2-hydroxybutyrate		
Hydroxyphenylacetate		

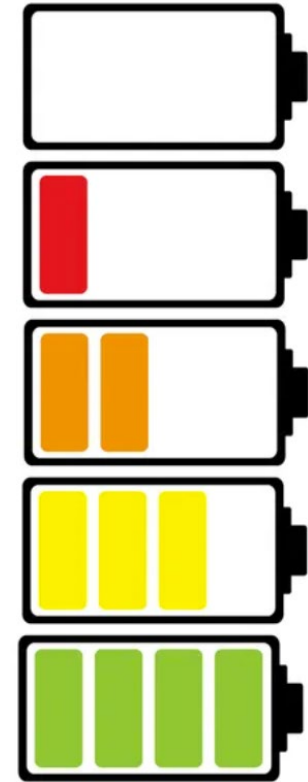
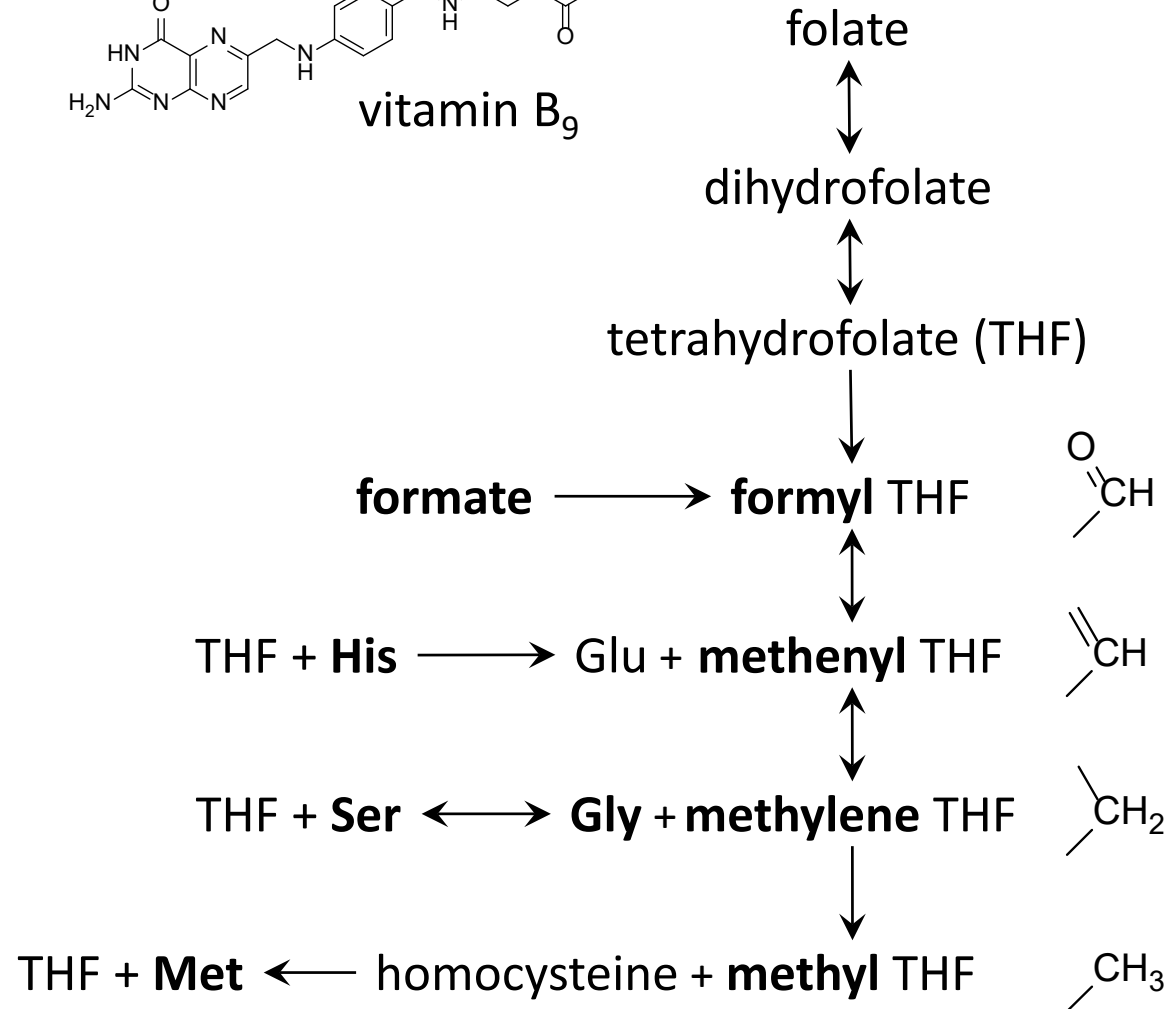
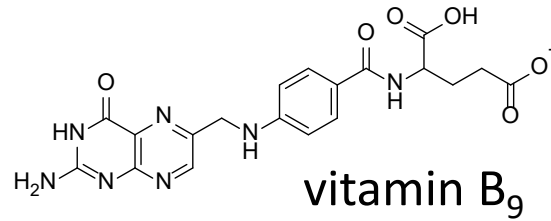


➤ 1-carbon metabolites

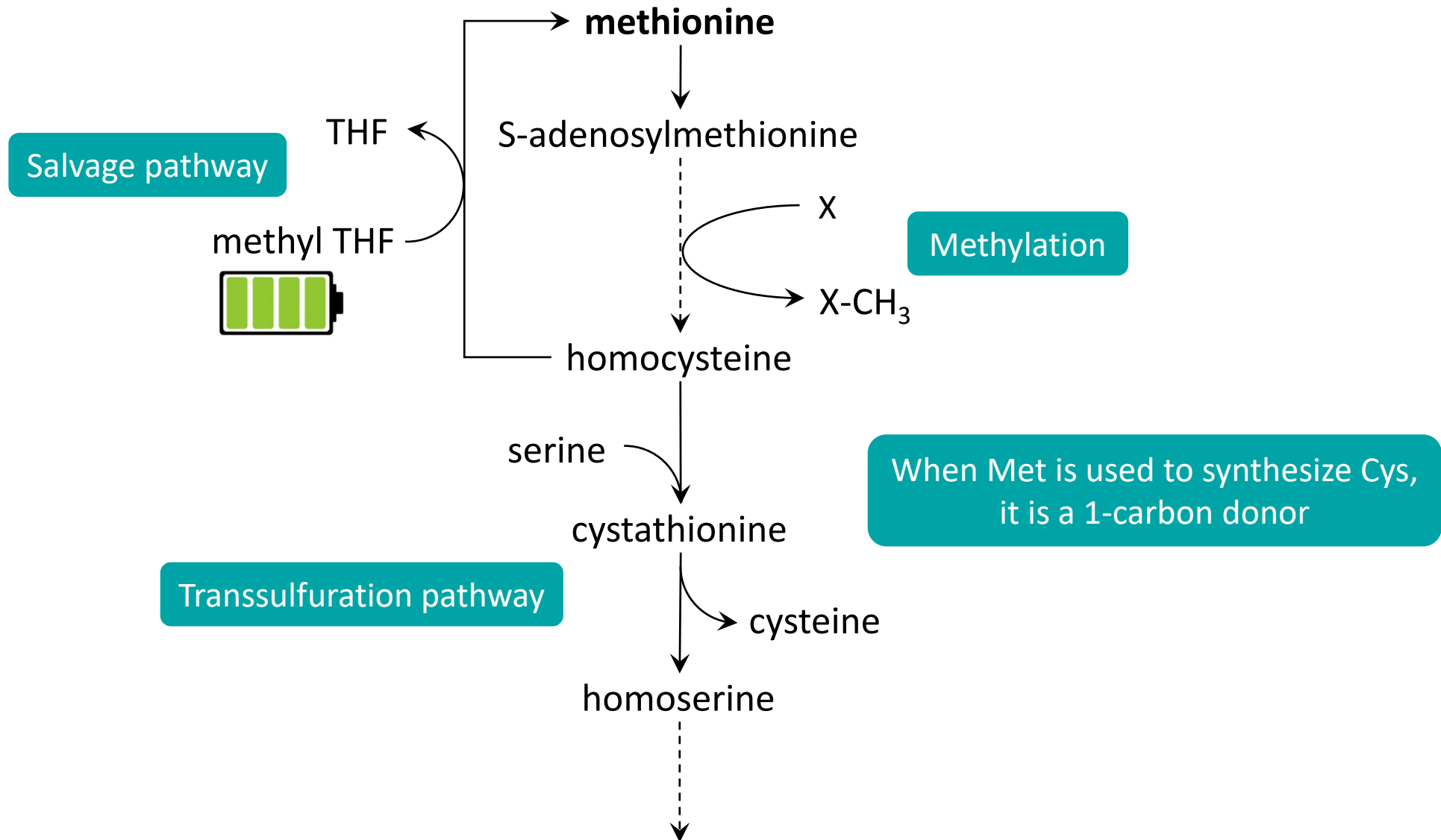
- Are used in the synthesis of:
 - Inosine monophosphate (IMP) → ATP, GTP, DNA, RNA
 - Uric acid
 - Allantoin
- Result from the catabolism of:
 - Histidine
 - Tryptophan (through formic acid)
 - Choline
 - Betaine
- Result from the catabolism and are used in the synthesis of:
 - Serine
 - Glycine
 - Methionine



➤ 1-carbon comes in different flavors



➤ 1-carbon and methionine metabolism

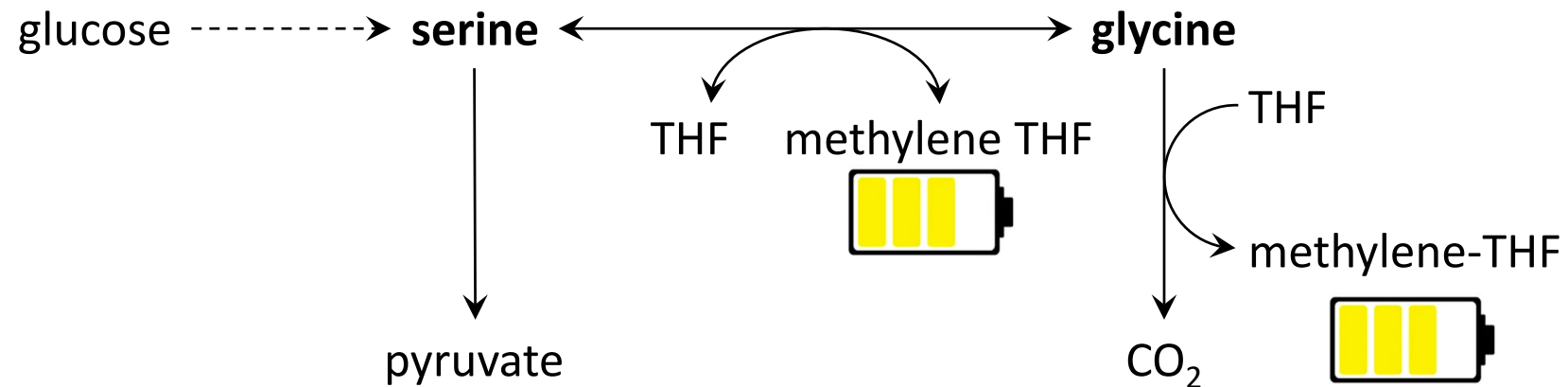


➤ What can be methylated via methionine?

- DNA (epigenetics):
 - Adenine
 - Cytosine
- Histones (with positive or negative effects on gene transcription):
 - Arg: 1 or 2 methyl-groups
 - Lys: 1, 2 or 3 methyl-groups
- Some proteins (e.g., myosin, actin, myosin light-chain kinase)
 - His: on 2 positions (N^π or N^τ)
 - His in myosin and actin can be methylated at the N^τ position
 - Post-translationally, but role unknown
- Histidine-containing dipeptides:
 - Anserine (β -Ala- N^π -His; mostly in birds)
 - Balenine (β -Ala- N^τ -His; in whales, somewhat in pigs)
 - pH buffer, antioxidant



➤ Glycine, serine, and 1-carbon metabolism

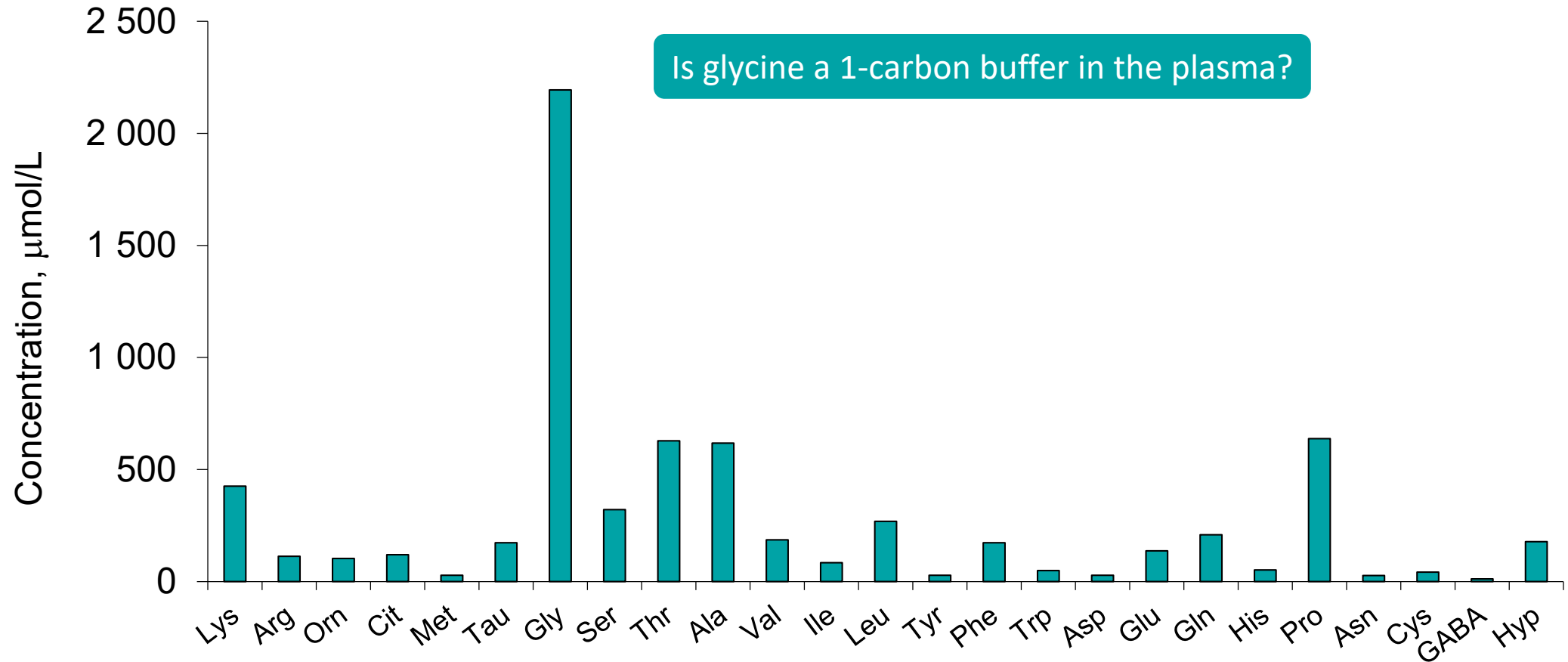


Serine can act as a donor of 1-carbon groups
Glycine can act as a donor and as a receptor of 1-carbon groups

Serine and glycine can be synthesized *de novo* from glucose
Excess serine can be catabolized to pyruvate or to glycine



➤ Amino acid concentrations in the plasma



Bertolo *et al.* (2000)



INRAE

Is there more than nitrogen in low-protein diets?

Advancia, May 17, 2022 / Jaap van Milgen

➤ Low-protein diets may induce a requirement for Gly in broilers

	PC	Low CP	Low CP + NEAA	Low CP + EAA	Low CP + NEAA + EAA
Weight gain, g/d	29.3	25.6*	28.3	26.6*	27.0*
Feed intake, g/d	36.8	35.0	35.1	35.1	33.2*
Gain:Feed	0.80	0.73*	0.81	0.74*	0.81

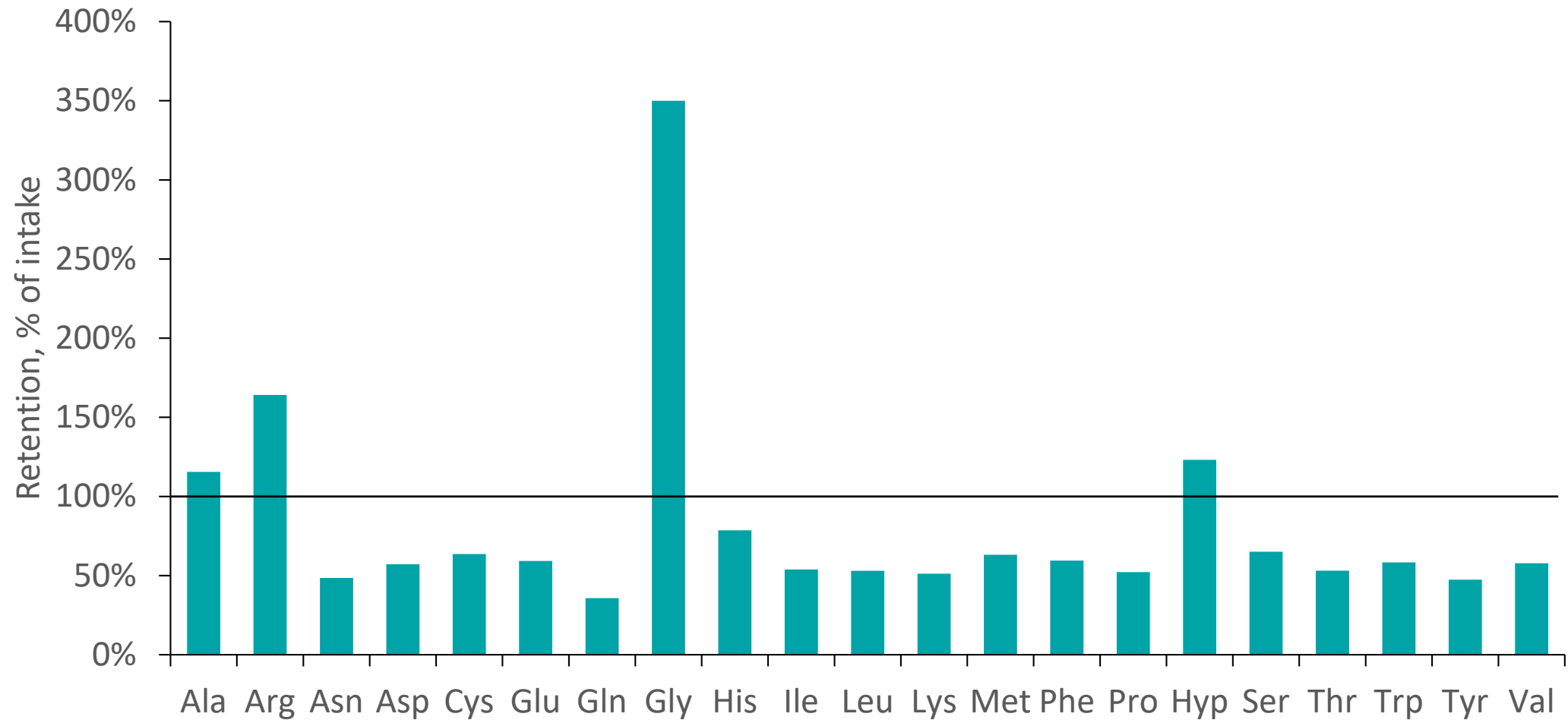
Dean *et al.* (2006)

PC = 22.2% CP; Low CP = 16.2% CP

* Values differ from the PC

	PC	Low CP	Low CP + Gly	Low CP + Glu	Low CP + Ala	Low CP + Asp	Low CP + Pro	Low CP + NEAA
Weight gain, g/d	28.7	24.9*	28.5	26.2*	24.2*	25.6*	25.2*	27.8
Feed intake, g/d	37.5	35.0*	35.6	35.1*	32.2*	34.5*	34.0*	34.5*
Gain:Feed	0.77	0.71*	0.80*	0.75	0.75	0.74	0.74	0.81*

➤ Nursing piglets synthesize large quantities of glycine



Wu (2013)

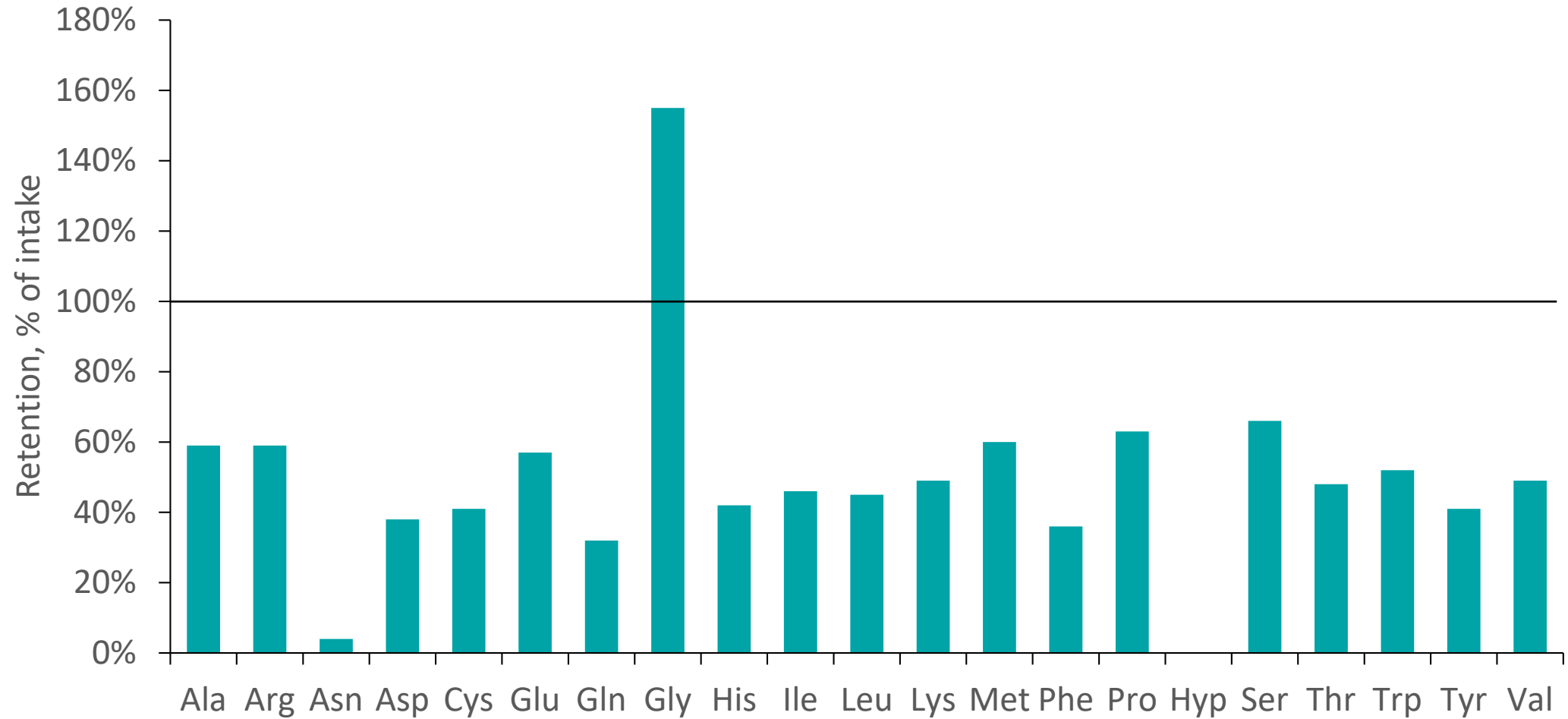


INRAE

Is there more than nitrogen in low-protein diets?

Advancia, May 17, 2022 / Jaap van Milgen

➤ Weaned piglets also need to synthesize glycine



Wu (2013)

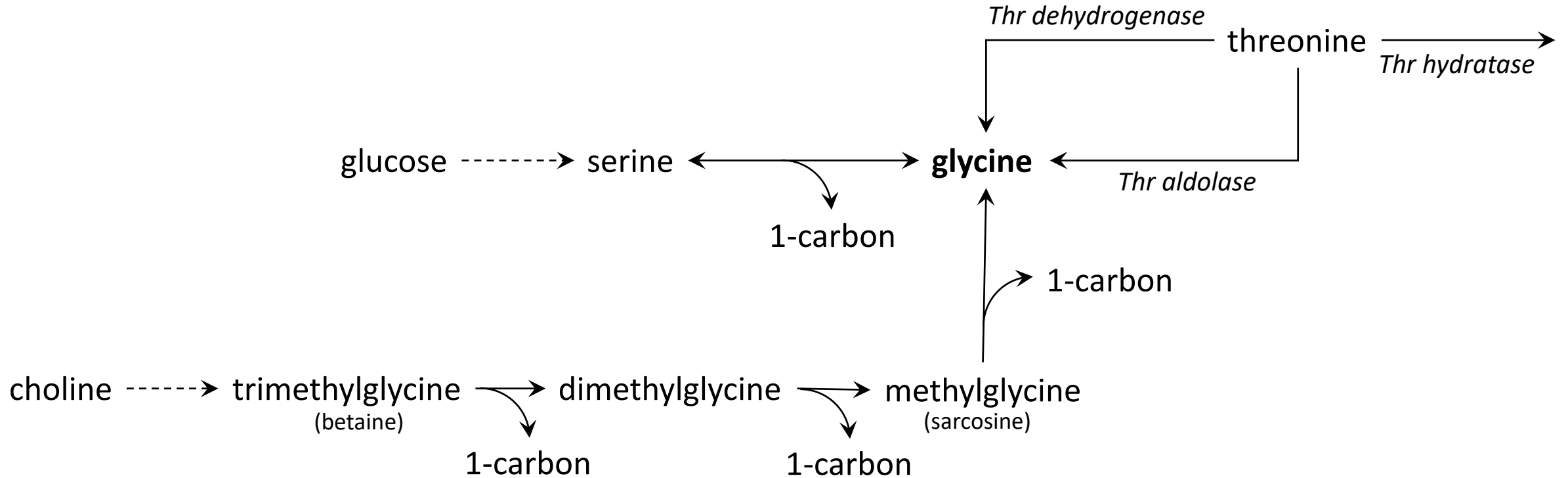


INRAE

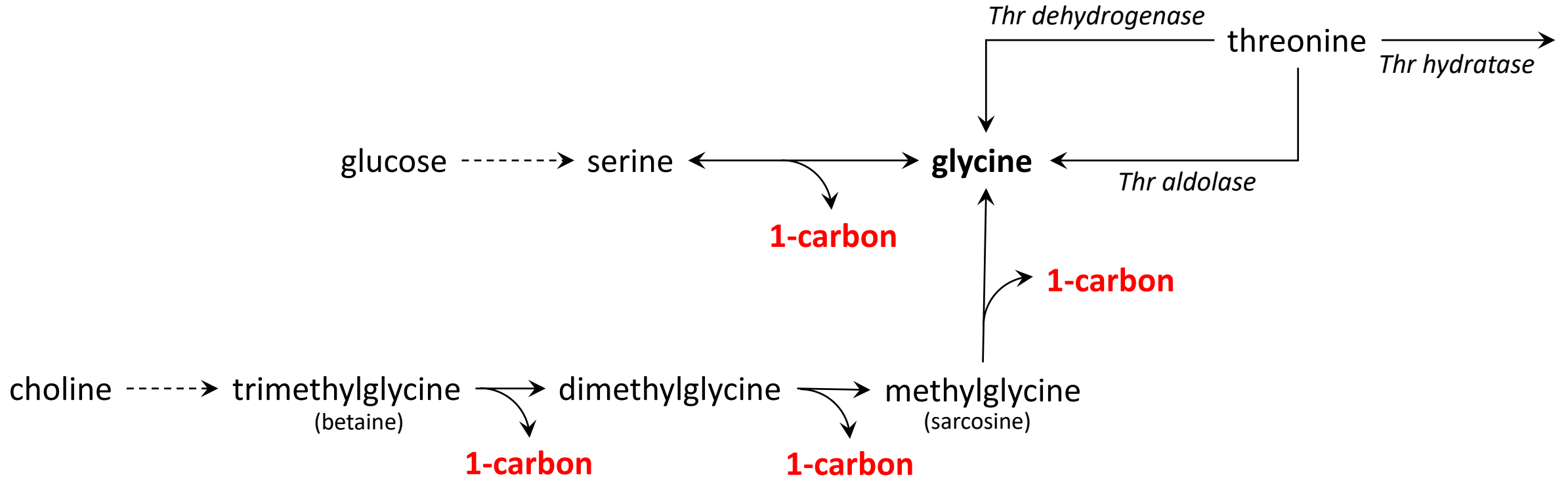
Is there more than nitrogen in low-protein diets?

Advancia, May 17, 2022 / Jaap van Milgen

➤ Sources of glycine



➤ Sources of glycine



➤ A weak link in metabolism?

Branch-point stoichiometry can generate weak links in metabolism: the case of glycine biosynthesis

ENRIQUE MELÉNDEZ-HEVIA* and PATRICIA DE PAZ-LUGO

*Instituto del Metabolismo Celular, Carretera Vecinal de El Sauzal, nº 160 A, El Sauzal, 38360 Tenerife,
Canary Islands, Spain*

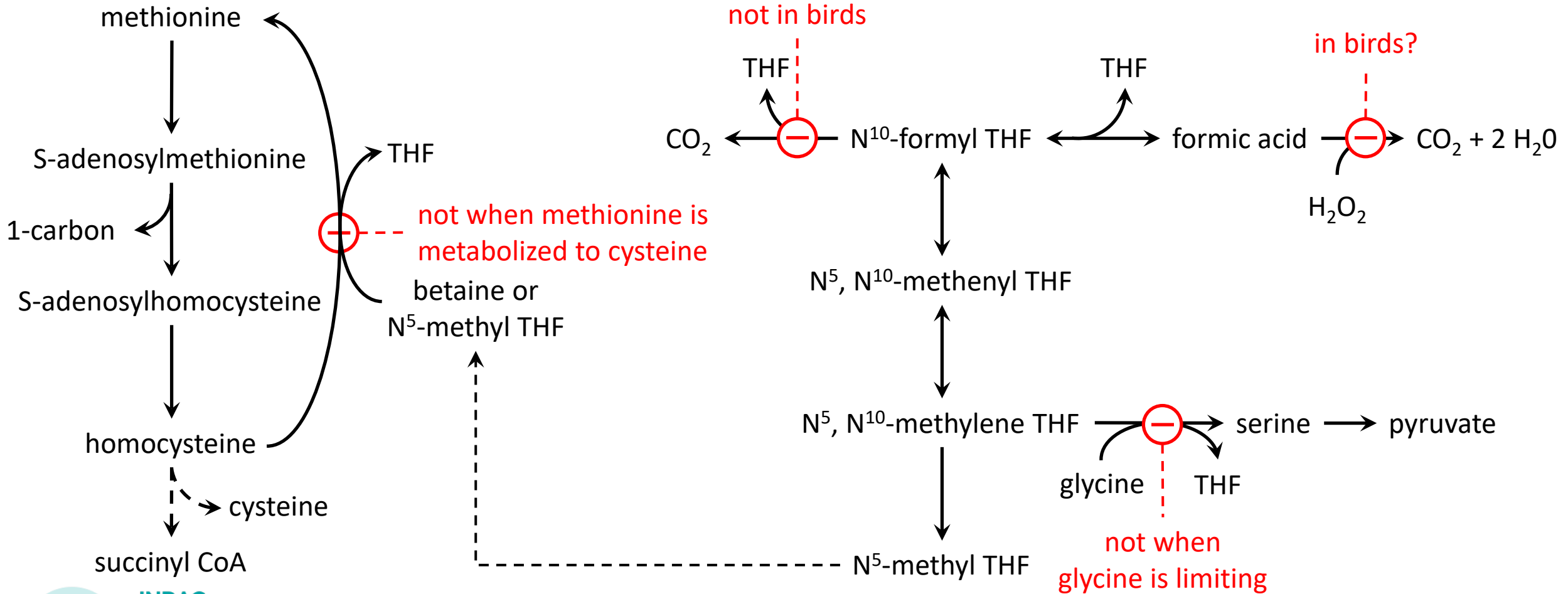
**Corresponding author (Fax, +34-922-571078; Email, emelhevia@metabolismo.ws)*

J. Biosci. (2008)



De novo synthesis of glycine always produces a 1-carbon

➤ How to handle excess 1-carbon (in birds)?



➤ 1-carbon and glycine

- Making more 1-carbon may not be a metabolic problem:
 - From *de novo* Gly synthesis
 - From choline, betaine, Ser, Gly, His, Trp, and Met catabolism
- Can too much 1-carbon be a metabolic problem?
 - Gly is needed to metabolize 1-carbon to Ser (and subsequent catabolism to pyruvate)
 - Catabolism to formic acid and urinary excretion?
- Low-protein diets and excess 1-carbon:
 - Limited dietary Gly
 - Limited dietary excess Thr to make Gly
 - Larger supply of 1-carbon through Cys synthesis from Met
- Why is performance reduced when Gly is limiting?
 - Uric acid can be a 1-carbon disposal method (two 1-carbon, one Gly)
 - ↓ protein retention = ↑ uric acid excretion



➤ Conclusions

- The energy values of (excess) protein and amino acids vary considerably:
 - The GE of digestible protein is 23.8 kJ/g
 - The ME value of excess protein is 20.2 kJ/g in mammals and 18.3 kJ/g in birds
 - The NE value of excess protein is 17.4 kJ/g in mammals and 14.1 kJ/g in birds
 - It varies with the nitrogen and carbon contents of the amino acid
- 1-carbon metabolism has not received much attention in animal nutrition
 - 1-carbon is required for uric acid synthesis
 - The joint *de novo* synthesis of Gly and 1-carbon has been considered a “weak link” in metabolism
 - Excess 1-carbon may become more important in in diets low-protein
- Glycine retention exceeds glycine intake, especially in young animals
 - Dietary glycine may be required to metabolize excess 1-carbon

