PROTEIN METABOLISM AND VITAMINS AND MINERALS

General References on Protein and Others:

- *Miller, E. R., D. E. Ullrey, and A. J. Lewis. 1991. Swine nutrition. Butterworth-Heinemann, Boston.*
- *Lewis, A. J., and L. L. Southern. 2001. Swine nutrition. 2nd. ed. CRC Press, Boca Raton.*
- *Chiba, L. I. 2013. Sustainable swine nutrition. Willey-Blackwell, A JohnWiley & Sons, Inc., Hoboken, NJ.*

Major References on Protein:

- *Lewis, A. J. 1991. Amino acids in swine nutrition. In: E. R. Miller, D. E. Ullrey, and A. J. Lewis, editors, Swine nutrition. Butterworth-Heinemann, Boston. p. 147-164.*
- *Knabe, D. A. 1991. Bioavailability of amino acids in feedstuffs for swine. In: E. R. Miller, D. E. Ullrey, and A. J. Lewis, editors, Swine nutrition. Butterworth-Heinemann, Boston. p. 327-339.*
- *Lewis, A. J. 2001. Amino acids in swine nutrition. In: A. J. Lewis and L. L. Southern, editors, Swine nutrition. 2nd. ed. CRC Press, Boca Raton. p.131-150.*
- *Gavert, V. M., H. Jørgensen, and C. M. Nyachoti. 2001. Bioavailability of amino acids in feedtuffs for swine. In: A. J. Lewis and L. L. Southern, editors, Swine nutrition. 2nd. ed. CRC Press, Boca Raton. p. 151-186.*
- *Chiba, L. I. 2001. Protein supplements. In: A. J. Lewis and L. L. Southern, editors, Swine nutrition. 2nd. ed. CRC Press, Boca Raton. p. 803-837.*
- *Trottier, N. L, and R. Manjarín. 2014. Amino acids and amino acid utilization in swine. In: L. I. Chiba, editor, Sustainable swine nutrition. Willey-Blackwell, A John Wiley & Sons, Inc., Hoboken, NJ. p. 81-108.*
- *Kil, D. Y., S. K. Cervates-Pahm, and H. H. Stein. 2013. Bioavailability of amino acids, lipids, and carbohydrates in feedstuffs. In: L. I. Chiba, editor, Sustainable swine nutrition. Willey-Blackwell, A John Wiley & Sons, Inc., Hoboken, NJ. p. 317-339.*

PROTEIN IN GENERAL

1. General

A. Protein is a principal constituent of organs & soft structures of the animal body (& obviously others!).
B. Includes an enormous number of closely related, yet physiologically distinct groups of substances.
C. For animals, a liberal & continuous supply of protein is needed throughout the "life" for growth, repair of tissues & organs, and other functions.

2. Functions

A. Enzymes - Almost all known enzymes are protein.
B. Hormones - Insulin, growth hormone, etc.
C. Structural constituents - Collagen, keratin, membrane, etc.
D. Transport - Hemoglobin, myoglobin, transferrin, etc.
E. Protection - Antibodies.
F. Coordination of the motion - Muscle (myosin, actin, etc.).
G. Storage of energy - ?
H. Storage - Casein, albumin, etc.
. . . , etc.

3. Composition (%) of Protein

Carbon	51-55
Hydrogen	6.5-7.3
Nitrogen	15.5-18
Oxygen	21.5-23.5
Sulfur	0.5-2.0
Phosphorus	0-1.5

4. Determination of Protein – "Kjeldahl" Method

A. Estimate the protein content based on the nitrogen content.
B. Advantages - Accurate & repeatable, can be used for liquid or solid samples, and requires no complex equipment.
C. Disadvantages - Time consuming, involves the use of hazardous chemicals, and does not convert nitrate & nitrite N to NH_4SO_4.
D. Calculation of the protein content from the N content?

1) Assumptions:

a) All proteins contain “16% N.”
b) All N is present as proteins.

- % protein = % N x 100/16 or % N x 6.25

2) Assumption (a) is false, but . . .!?

a) Factors for calculating the protein content from the N content of food:” (USDA Agric. Handbook No. 8 by Watt & Merrill)

Food ..... Factor	Food..... Factor
Animal origin:	Navy.....6.25
Eggs..... 6.25	Soybeans 5.71
Gelatin 6.25	Velvetbeans.....6.25
Meat..... 6.25	Peanuts 5.46

Milk	6.38	***Nuts:***	
Grains & cereals:		Almonds	5.18
Barley	5.83	Brazil	5.46
Corn	6.25	Butternuts	5.30
Millets	5.83	Cashew	5.30
Oats	5.83	Chestnuts	5.30
Rice	5.95	Coconuts	5.30
Rye	5.83	Hazelnuts	5.30
Sorghums	6.25	Hickory	5.30
Wheat:		Pecans	5.30
Whole-kernel	5.83	Pinenuts	5.30
Bran	6.31	Pistachio	5.30
Embryo	5.80	Walnuts	5.30
Endosperm	5.70	***Seeds:***	
Legumes:		Cantaloup	5.30
Beans:		Cottonseed	5.30
Adzuki	6.25	Flaxseed	5.30
Castor	5.30	Hempseed	5.30
Jack	6.25	Pumpkin	5.30
Lima	6.25	Sesame	5.30
Mung	6.25	Sunflower	5.30

b) True conversion factors range from 5.3 to 6.38.
c) But, the use of a single factor works well because:

(1) Normal diets contain a mixture of proteins.
(2) Average true conversions are often close to 6.25.

d) Special factors to be used? 5.70 for wheat products & 6.38 for milk products.

3) An assumption (b), "all N is present as proteins," is also false!?

a) "NPN" such as amides, amino acids or AA, glycosides, alkaloids, ammonium salts & others are also present.
b) But, NPN compounds are present in large amounts in only a few feeds such as young grass, silage, and immature root crops.

- Because of these unsound assumptions, the term "crude protein or CP" is used?
- "True protein" can be determined by separating true proteins from non-protein or NPN by precipitation, and then running the Kjeldahl procedure.

5. Direct Determination - The Lowery method

A. Widely used for the analysis of proteins in tissue samples.
B. Involves two steps:

1) "Biuret reaction" - Add Cu to an alkaline solution, which chelates peptide bonds & produces a "blue-violet" color.

2) Add a "Folin-Ciocalteu phenol reagent" & measure a "blue color" spectrophotometrically.

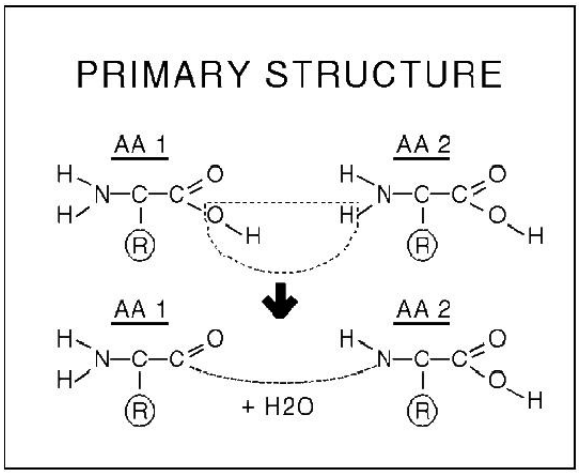
PROTEIN AND AMINO ACIDS

1. Protein Structure

A. Primary structure:

- The linkage of individual amino acids into a long chain by "peptide bonds."

  a) Peptides - 2 to 20 AA residues (e.g., growth hormone; di-, tri-, tetra- . . . oligopeptide).
  b) Polypeptides - 20 to 100 AA residues (e.g., insulin).
  c) Many/most proteins contain at least 100 AA residues.



B. Secondary structure:

1) "Polypeptides" do not exist as straight chains, but they are folded into a specific "three-dimensional" conformation.
2) Most proteins are one of the three forms:

   a) α-Helix - e.g., Found in horns, nails, skin, hair, wool & much of the skeletal muscle proteins (myosin & tropomyosin).

      (1) 3.6 AA residues per turn.
      (2) "R-group" of AA extend outward.
      (3) Stabilized by H-bonds (NH-groups & CO-groups).

   b) β-pleated sheet - e.g., Found in silk (β-keratin) & other insect fibers.

      (1) Peptide chains are arranged side-by-side in sheets.
      (2) Cross-linked by H-bonds.

   c) Triple helix (or collagen helix) - Collagen is the most abundant protein in the body of higher animals - e.g., found in hide, tendons & other connective tissues.

      (1) Consists of 3-polypeptide chains of the same size.
      (2) Contains a high proportion of glycine and proline.
      (3) Also contains OH-proline & OH-lysine, which provide a "rigidity" to protein.

C. Tertiary structure - e.g., Myoglobin:

1) "Polypeptides" are folded & tightly coiled into a globular form.
2) Stabilized by:

a) Disulfide linkages between two cysteine residues (-S-S-).
b) Salt linkages (i.e., basic AA, Arg & Lys + acidic AA, Asp & Glu).
c) Nonpolar ends of No. of AA become "hydrophobic centers" of polypeptide coils.
d) H-bonding.

- A tertiary structure is crucial to enzymatic activity of many proteins!

D. Quaternary structure - e.g., Hemoglobin:

1) Refers to alignment of several tertiary structures into one protein.
2) For instance, "Hb" consists of four single-strand tertiary forms of proteins that are compactly associated into a single globular protein.

2. Classification of Protein

A. Simple proteins - Refer to a protein that yields only AA and(or) its derivatives upon hydrolysis.
B. Conjugated proteins: (e.g.)

1) Nucleoproteins - e.g., Ribosomes/RNA.
2) Phosphoproteins - e.g., Casein/phosphate.
3) Metalloproteins - e.g., Cytochrome oxidase/Fe & Cu.
4) Lipoproteins - e.g., VLDL/phospholipid, fat, cholesterol.
5) Flavoproteins - e.g., succinic dehydrogenase/FAD.
6) Glycoproteins - e.g., γ-globulin/galactose, mannose, hexoamine.

C. Fibrous or globular proteins:

1) Fibrous proteins - Polypeptide chains are coiled into a helix & cross-linked by disulfide & H-bonds:

a) Collagen (in connective tissues) - Heating or treating with acid, yields gelatin.
b) Elastin (part of tendons, arteries & others) - Can stretch in two directions (poorly digested by animals).
c) Keratin - α-keratin is found in hair, horn and wool, whereas β-keratin is found in beaks of birds.
d) Myosin & tropomyosin - Muscle proteins.

2) Globular proteins:

a) Polypeptides are folded & coiled - very compact proteins!
b) Examples include enzymes, protein hormones & oxygen carrying proteins.

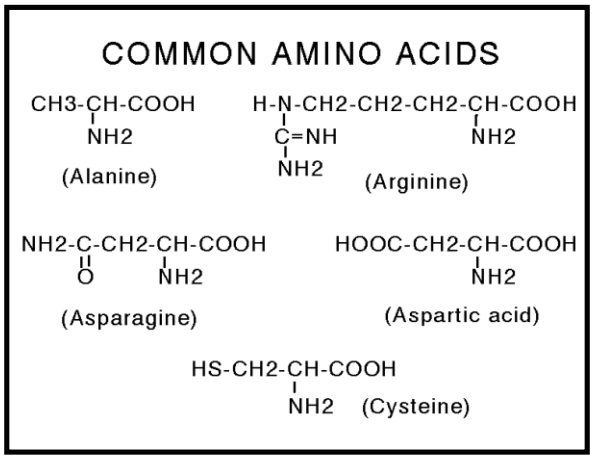
D. Classifications by solubility:

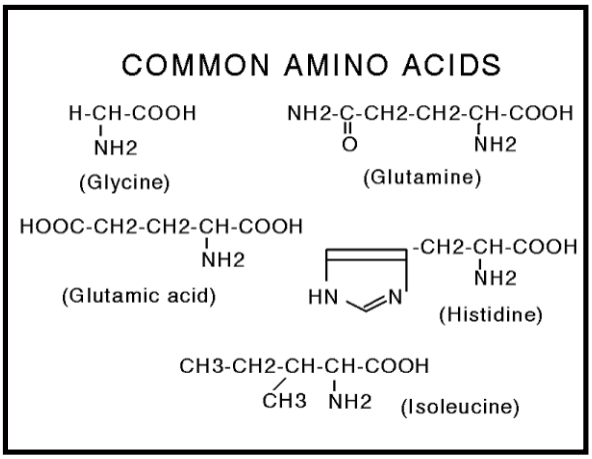
1) Albumins - Soluble in water and salt solutions.
2) Globulins - Insoluble in water, but can ↑ solubility by changing salt concentrations. (Many plant seeds contain globulins!)
3) Protamine - Soluble in a 70-80% ethanol, but insoluble in water & ethanol.
4) Histone - Soluble in salt solutions.
5) Scleroprotein - Insoluble in water or salt solutions.

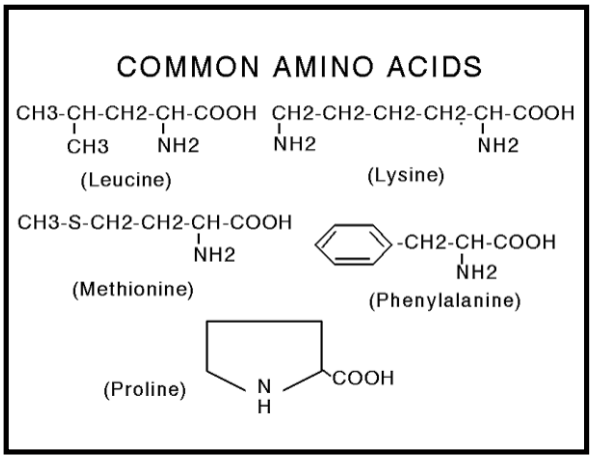
3. Amino Acids

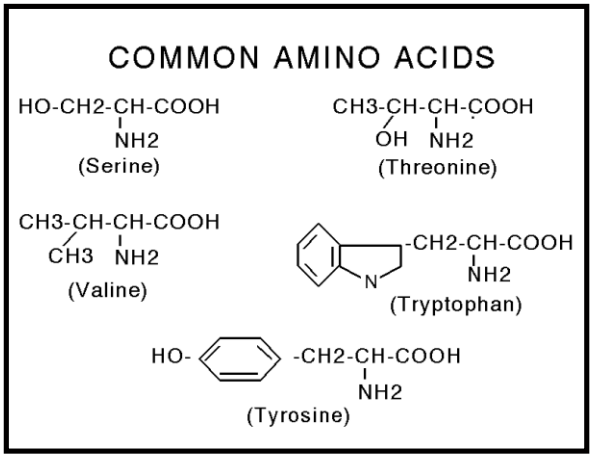
- *References:Nomenclature of α-Amino Acids. 1975. Biochemistry 14:449,Handbook of Chemistry & Physics. 1973-74, and The Merck Index. 1976.*

A. Structures of 20 common amino acids found in protein:









B. Name, systematic name, other name, symbol, formula, molecular weight, and composition of 20 common amino acids:

Symbol	Formula	MW	Composition, % C	H	N	O
Alanine (2-aminopropionic acid)						
Ala	$C_3H_7NO_2$	89.09	40.44	7.92	15.72	35.92
Arginine (2-amino-5-guanidinovaleric acid or 2-amino-5-guanidopentanoic acid)						
Arg	$C_6H_{14}N_4O_2$	174.20	41.36	8.10	32.16	18.37
Asparagine (2-aminosuccinamic acid or aspartic acid β-amide)						
Asn	$C_4H_8N_2O_3$	132.12	36.36	6.10	21.20	36.33
Aspartic acid (aminosuccinic acid or 2-aminobutanedioic acid)						
Asp	$C_4H_7NO_4$	133.10	36.09	5.30	10.52	48.08
Cysteine (2-amino-3-mercaptopropionic acid or β-mercaptoalanine)						
Cys	$C_3H_7NO_2S$	121.16	29.74	5.82	11.56	26.41
Glutamic acid (2-aminoglutaric acid or 2-aminopentanedioic acid)						
Glu	$C_5H_9NO_4$	147.13	40.81	6.16	9.52	43.50
Glutamine (2-aminoglutaramic acid or glutamic acid 5-amide)						
Gln	$C_5H_{10}N_2O_3$	146.15	41.09	6.90	19.17	32.48
Glycine (aminacetic acid or aminoethanoic acid)						
Gly	$C_2H_5NO_2$	75.07	32.00	6.71	18.66	42.63
Histidine (α-amino-1H-imidazole-4-propionic acid or glyoxaline-5-alanine)						
His	$C_6H_9N_3O_2$	155.16	46.44	5.85	27.08	20.62
Isoleucine (2-amino-3-methylvaleric acid or 2-amino-3-methylpentanoic acid)						
Ile	$C_6H_{13}NO_2$	131.17	54.94	9.99	10.68	24.39
Leucine (2-amino-4-methylvaleric acid or α-aminoisocaproic acid)						
Leu	$C_6H_{13}NO_2$	131.17	54.94	9.99	10.68	24.39
Lysine (2,6-diaminohexanoic acid or α,ε-diaminocaproic acid)						
Lys	$C_6H_{14}N_2O_2$	146.19	49.29	9.65	19.16	21.89
Methionine (2-amino-4-(methylthio)butyric acid or α-amino-γ-methylmercaptobutyric acid)						
Met	$C_5H_{11}NO_2S$	149.21	40.25	7.43	9.39	21.45
Phenylalanine (2-amino-3-phenylpropionic acid or α-aminohydrocinnamic acid)						
Phe	$C_9H_{11}NO_2$	165.19	65.43	6.71	8.48	19.37
Proline (2-pyrrolidinecarboxylic acid)						
Pro	$C_5H_9NO_2$	115.13	52.16	7.88	12.17	27.79
Serine (2-amino-3-hydroxypropionic acid or β-hydroxyalanine)						
Ser	$C_3H_7NO_3$	105.09	34.28	6.71	13.33	45.67
Threonine (2-amino-3-hydroxybutyric acid or α-amino-β-hydroxybutanoic acid)						
Thr	$C_4H_9NO_3$	119.12	40.33	7.62	11.76	40.29
Tryptophan (2-amino-3-(3-indolyl)propionic acid or β-3-indolylalanine)						
Trp	$C_{11}H_{12}N_2O_2$	204.22	64.69	5.92	13.72	15.67
yrosine (2-amino-3-(4-hydroxyphenyl)propionic acid or 3-(4-hydroxyphenyl)alanine)						
Tyr	$C_9H_{11}NO_3$	181.19	59.66	6.12	7.73	26.49
Valine (2-amino-3-methylbutyric acid or α-aminoisovaleric acid)						
Val	$C_5H_{11}NO_2$	117.15	51.26	9.46	11.96	27.32

PROTEIN DIGESTION

1. Reasons for Protein Digestion?

A. To get through the membrane (i.e., uptake/absorption).
B. To resynthesize necessary proteins.
C. For the immune process/purpose.

2. Gastric Digestion

A. HCl & pepsin:

1) Are primarily responsible for gastric digestion.
2) Histamine, which is released in response to vagus nerve stimulation & gastrin, stimulates secretion of both.
3) Secretin inhibits acid secretion, but stimulates pepsin secretion.
4) GIP (gastric inhibitory polypeptide) inhibits pepsin secretion.

B. Pepsin:

1) At least four different types exist in both pigs & humans:

	Other name?	Origin	Optimum pH	
A	Pepsin	Fundal mucosa	2.0-2.2	3.5-3.9
D	Pepsin	Fundal mucosa	2.0-2.2	3.5-3.9
B	Parapepsin I	Pyloric mucosa	1.7-1.9	3.2-3.4
C	Parapepsin II	Pyloric mucosa	1.7-1.9	3.2-3.4.

2) Produced & released as inactive precursors (proenzymes or zymogens).
3) Lower optimum pH for B & C, perhaps, reflecting pH in that area!?
4) Pepsinogens are activated at < pH 5 (rapidly at 2 & slowly at 4), and the process is "autocatalytic."

a) Activation by hydrolyzing Glu-Ile bond with subsequent removal of a peptide from the N-terminal end of the molecule.
b) Below pH 3, mostly due to the acidity, but at pH 4, activation due to acid alone is slow & mostly due to pepsin.

5) Has two pH optima, 2 & 3.5 - Usually, a full activity is not achieved because gastric content pH is 3.8 to 4.8 soon after consumption of feed/food.
6) Reduce the activity at pH above 3.5, but depends on the substrate. May not be active at above pH 6.0, at least in pigs.
7) Pepsin is a nonspecific endopeptidase:

a) Rapids if, at least, one side has an aromatic AA.
b) The rate of hydrolysis differs according to the type of bonds (or AA) - e.g., bonds with aromatic AA > Glu > Cys . . . > Gly.

C. Importance of gastric digestion:

1) Conflicting results on overall contributions.
2) But, a total gastrectomy of pigs may decrease an apparent protein digestibility by 17 to 18%.

3. Intestinal Digestion

A. General:

1) 1° site is the duodenum, where feed/food is mixed with pancreatic & duodenal secretions.
2) pH of ingesta increase progressively, and reaches ≈ 7 by the time ingesta get to the end of ileum, ∴ digestion by pancreatic enzymes rather than pepsin.
3) Intestinal digestion consists of two phases: the intraluminal, and membrane & intracellular digestion.
4) Proteolytic enzymes involved:

a) Endopeptidases – Act on a susceptible peptides link in polypeptide/protein.
b) Carboypeptodase – Remove AA residues form the carboxyl end of the chain.
c) Aminopeptidases – Remove AA residues from the amino end of the chain.

B. Intraluminal digestion:

1) Mostly by pancreatic enzymes, but some microbial proteases may be involved.
2) Presence of acid & food in the upper SI results in:

a) Stimulation of pancreas by vagal nerves results in secretion of fluids, bicarbonate & pancreatic enzymes.
b) Release of peptide hormones:

(1) Secretin, which is released in response to "acid," stimulates secretion of fluids & bicarbonate.
(2) Cholecystokinin, which is released in response to the presence of "food," stimulates secretion of pancreatic enzymes.

3) Pancreatic juice contains:

a) Trypsin/trypsinogen:

(1) Trypsinogen is converted to trypsin by enterokinase (hydrolyzes Lys-Ile bond with subsequent removal of a peptide), which is located at the brush border of the duodenal mucosa.
(2) Activation process is also "autocatalytic."
(3) Optimum pH are ≈ 8 to 9.

(4) Trypsin is an endopeptidase & highly specific - acts only on bonds where carboxyl group is contributed by either Lys or Arg.
(5) Perhaps, the most important enzyme?

(a) Present in the greatest quantity.
(b) Activates all pancreatic enzymes - Chymotrypsin, carboxypeptidases, and elastases by spliting an arginyl or lysyl bond to release a small peptide from the N-terminal end!
(c) Activate pancreatic phopholipases.

b) Chymotrypsin/chymotrypsinogen:

(1) Three types, A, B & C, seem to complement each other.
(2) Chymotrypsinogen is activated by trypsin.
(3) Optimum pH is ≈ 8.
(4) Chymotrypsin is an endopeptidase & active against bonds where carboxyl group is contributed by Phe, Tyr, Trp or Leu.
- Other bonds may be susceptible to chymotrypsin, but it's not active against Pro bonds.

c) Elastase(s):

(1) Proelastase is also activated by trypsin.
(2) Only enzyme active against elastin.
(3) Elastase is an endopeptidase, and acts especially on nonpolar AA (Val, Leu, Ser & Ala) & produces peptides.

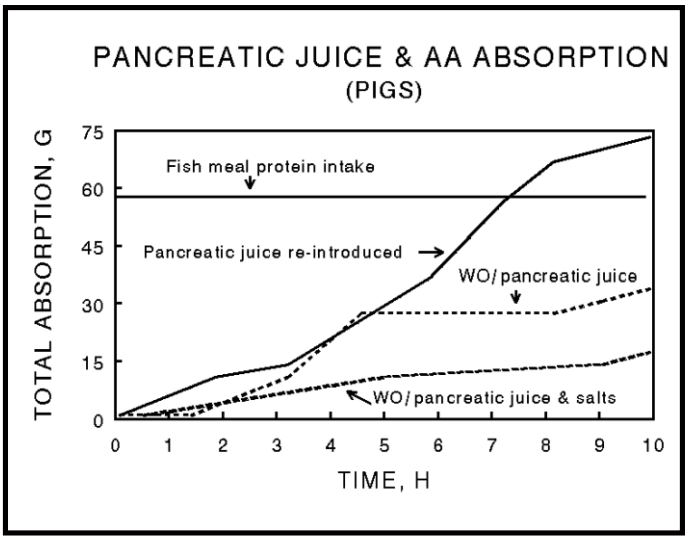
▹ Elastase II - Acts on both typical chymotrypsin substrates and on elastin and other elastin substrates.

d) Carboxypeptidases:

(1) Procarboxypeptidases A & B (exopeptidases) are activated by trypsin.
(2) Optimum pH are ≈ 7.5 to 8.5.
(3) Carboxypeptidase A attacks peptides produced by chymotrypsin & elastase, and hydrolyze AA from the C-terminal end.
(4) Carboxypeptidase B attacks peptides produced by trypsin, and hydrolyze arginyl & lysyl residues from the C-terminal end.

- Also, there are pancreatic nucleases (nucleic acids → mononucleotides) - Ribonuclease & deoxyribonuclease.

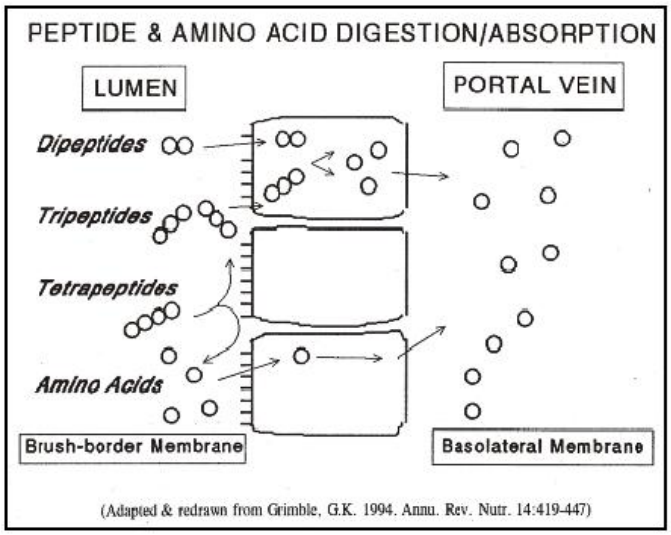
4) Significance of pancreatic digestion:



a) A total pancreatectomy resulted in ≈ 70% normal N absorption rate in dogs (1 h after meal).
b) In pigs, it is affected by age & type of protein fed:

(1) e.g., Pancreatic juice & amino acid absorption in pigs [Rérat et al., 1976. In: Cole et al. (Ed.)] - Measured absorption rate during a 10-h period after ingestion of 500 g of a semisynthetic meal containing 58 g fish meal protein.
(2) Young pigs fed low-quality protein decrease the digestibility by ≥ 50%.
(3) The use of highly digestible protein or older pigs, the reduction in digestibility may be only 15 to 20%.
(4) Preventing pancreatic secretion is detrimental to digestion, but not fatal!

C. Membrane & intracellular digestion:



1) Final stages of protein digestion at the external membrane of intestinal mucosa (brush border) & within the SI columnar cell.

a) Mechanism(s) - Not completely understood.
b) The current view: [See, e.g., Grimble, 1994. Annu. Rev. Nutr. 14:419-447; Also, please see Trottier and Manjarin, 2013]

(1) Larger peptides (3-6 AA residues) are split by aminopeptidases on the luminal surface at the mucosal brush border.
(2) Free amino acids and small peptides are absorbed from the lumen.
(3) Smaller peptides are hydrolyzed by aminopeptidases within mucosal cells.
(4) Metabolism of some absorbed amino acids, including some resynthesis of proteins.

2) Brush border (≈ 10 to 20% of total aminopeptidase activities):

   a) Two oligopeptidases are active against di-, tri- and tetrapeptides.
   b) Dipeptidase has a minimal specificity for larger oligopeptides.
   c) All three are particularly active against glycyl-leucine & leucyl-glycine bonds.

3) Intracellular (≈ 80% of aminopeptidase activities) - Uncertain No. of peptidases, but four to eight may exit:

   a) Leucine aminopeptidase - Active against all bonds adjacent to the N-terminal end, but has a wide range of activity.
   b) Master dipeptidase (or Glycylleucine hydrolase) - Acts on many dipeptides (. . . demonstrated to be active against 65 out of 77 dipeptides tested in one experiment).

      (1) Glycylglycine dipeptidase.
      (2) Imidodipeptidase - Acts on the C-terminal of Pro or Hyp.
      (3) Iminodipeptidase - Acts on the N-terminal of Pro or Hyp.
      (4) Glycylhistidine dipeptidase.
      (5) Arginyl dipeptidase.

D. Digestion at the large intestine:

1) A considerable microbial fermentation at the LI, and some AA may be absorbed?
2) AA not absorbed by the end of the SI are excreted in the feces, being absorbed but excreted in the urine and(or) metabolized within the mucosa, thus, not likely to have any value to the pig. (Zebrowska, 1973. Roczniki Nauk Rolniczych 95B1:115)

4. Digestibility

- Proteins, like any other nutrients, are not completely digested and absorbed!

A. Apparent digestibility:

$$= \frac{\text{Feed protein - Fecal protein}}{\text{Feed protein}} \times 100$$

1) An assumption? - “All proteins in the feces are derived from undigested feed residues (i.e., from an exogenous source).”
2) But, the fecal protein consists of enzymes, cellular materials & others that are not reabsorbed (i.e., contains protein from endogenous sources), thus underestimate digestibility!

B. True digestibility:

$$= \frac{\text{Feed N - (Fecal N - Metabolic fecal N)}}{\text{Feed N}} \times 100$$

1) Makes an adjustment for endogenous sources by estimating metabolic fecal nitrogen (MFN).

   a) Considerable amounts of endogenous N are added in the form of digestive secretions and desquamated/sloughed off mucosal cells during the passage of food – e.g.:

      (1) 20 (e.g., fed a high-protein diet or a high-quality protein) to 30% (e.g., fed a low-protein diet or a low-quality protein) of dietary protein/N. (Estimated using duodenal fistula; Horszczaruk, 1971.)
      (2) Pancreatic secretion in 45-kg pigs - 18.6 +/- 0.6 g protein/d, which represented 11% of dietary protein and 5 to 8% of the AA requirement of the pig. (Corring, 1975. Ann. Biol. Anim. Biochim. Biophys. 12:233-241; Cited by Kidder & Manners, 1978.)
      (3) Secretion of intestinal juice into an isolated jejunal loop in 70-kg pigs - 62 to 73 g CP (normal diet) and 50 to 62 g (protein-free diet). (Horszcxaruk et al., 1974; Cited by Kidder & Manners, 1978).

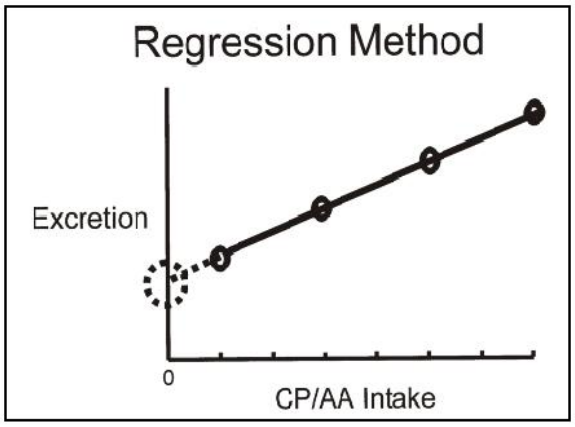
   b) Partly digested and absorbed but less digestible vs dietary sources.

2) Estimation of MFN is a very difficult task!

   a) Two common approaches:

      (1) Feed a N-free diet:

         (a) Since no N in the diet, all fecal N must be metabolic origin!?
         (b) But, a N-free diet is not palatable - Animals may not consume enough feed/day, and(or) may not be able to maintain a certain level of intake during the trial.



      (2) Regression method - Feed various levels of highly digestible protein, and extrapolate back to the zero N intake.

   b) Both methods underestimate MFN because:

(1) Endogenous N increases when protein is fed.
(2) The rate of endogenous N secretion varies with different protein sources. [Also, affected by age – Greater in young animals.]

C. Apparent or true digestibility?

1) The estimation of MFN is not precise.
2) The loss of MFN is inevitable, and must be taken into account when determining the value of feedstuffs to animals.

- Thus, "apparent" digestibility might be a more meaningful and realistic measure of the nutritional value of protein source(s) in question!?

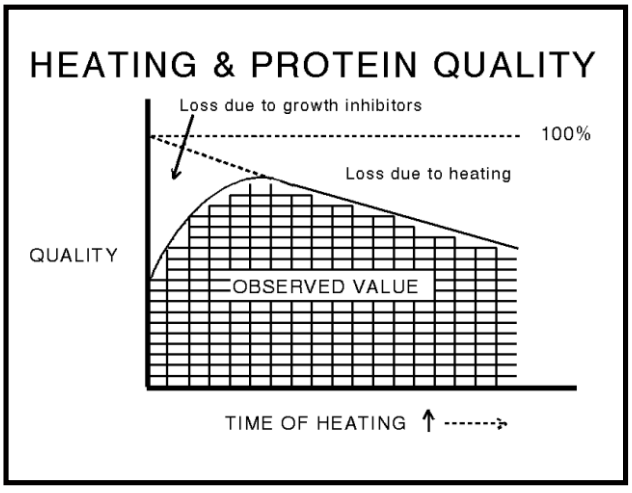
D. Apparent fecal vs ileal digestibility:

1) e.g., Apparent ileal & fecal N digestibilities (%): (Knabe et al., 1989. J. Anim. Sci. 67:441)

Source	Ileal	Fecal
Meat & bone meal	67	81
Canola meal	69	81
Poultry-by-product	79	87
Soybean meal	78	85
Blood meal	87	91
Corn gluten meal	88	92

2) Fecal digestibilities are usually higher than ileal values because of protein degradation by microbial fermentation (∴ less unaltered N in feces).
3) Differences are smaller for highly digestible sources because "less" undigested/ unabsorbed proteins are delivered to the LI for microbial fermentation.

- Thus, better to determine the N-digestibility at the terminal ileum, especially the low-quality source!



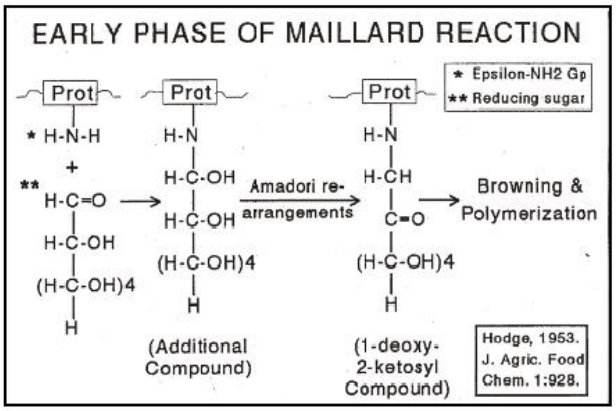
E. Some factors affecting protein digestibility:

1) Presence of enzyme inhibitors (e.g., trypsin inhibitors in soybeans).

- Other growth inhibitors? - Lectin (hemagglutinin), goitrogenic substances, estrogenic substances, allegenic compounds, etc.

2) Heat damage - Mainly due to "Maillard or browning reaction?" [See Hodge (1953)]

a) Reaction between the primary amine of peptide chain (often the ε-amino group of lysyl residue) and reducing sugars such as xylose (most reactive), glucose & galactose → "amino-sugar complex" → isomerization & polymerization.
b) Trypsin cannot work on "lysyl bonds," thus reducing the availability!



- FDNB (fluorodinitrobenzene)/Sanger's agent can bind to the ε-amino group, but not to the complex, thus can be used to assess the availability!

c) Reactions are affected by sugar concentrations, duration of heating, water activity & pH of reaction mixtures.
d) Under the influence of the intestinal microorganisms, a small portion may be released & absorbed, but usually excreted in the urine. According to some data, some "intermediates" may be available to the animal though!

- For ruminant species, may be one way to protect proteins from microbial degradation in the rumen. (See Cleale et al., 1987a,b,c. J. Anim. Sci. 65:1312-1318,1319-1326, and 1327-1335.)

3) Source of protein.
4) Amount of fibers in the diet.
5) Age and(or) development of digestive systems - Perhaps, related to 3) & 4)?

ABSORPTION OF AMINO ACIDS AND PEPTIDES

- *Good references on this topic:*

1) Silk, D.B.A. 1980. Digestion and absorption of dietary protein in man. Proc. Nutr. Soc. 39:61.
2) Collarini, E.J. & D.L. Oxender. 1987. Mechanisms of transport of amino acids across membranes. Annu. Rev. Nutr. 7:75.
3) Christensen, H.N. 1990. Role of amino acid transport and countertransport in nutrition and metabolism. Physiol. Rev. 70:43.
4) Kilberg, M.S. et al., 1993. Recent advances in mammalian amino acid transport. Annu. Rev. Nutr. 13:137.

5) Trottier, N. L, and R. Manjarin. 2014. Amino acids and amino acid utilization in swine. In: L. I. Chiba, editor, Sustainable swine nutrition. Willey-Blackwell, A John Wiley & Sons, Inc., Hoboken, NJ. p. 81-108.

1. Digestion of Protein - Any Exception?

A. Certain neonatal mammals:

1) Have the ability to absorb intact proteins, immunoglobulins (e.g., IgG), directly into the lymphatic system. ("IgA" may not be absorbed, but may be important in protecting the gut!)
2) Accomplished by "pinocytosis."
3) Colostrum contains powerful trypsin inhibitors (+ low acid secretion in baby pigs), ∴ suppressing proteolytic activity in the GI tract.

B. Closure - A loss of ability to absorb intact proteins:

1) Pigs - ≈ < 36 hours.
2) Cattle - < 2-3 days.
3) Humans - Perhaps, shorter than animals, or may not possess the ability to absorb intact proteins?

2. Mechanisms of Absorption

A. General:

1) Current knowledge/understandings on amino acid absorption were obtained mostly during 1950-1970, and little has been added in recent years.
2) Most amino acids are absorbed by the active process involving Na & membrane carriers (probably, protein).

B. In general, can be classified into four distinct amino acid transport systems?

1) Neutral system:

a) Transports monoamino & monocarboxylic amino acids (Ala, Asn, Cys, Gln, His, Ile, Leu, Met, Phe, Ser, Thr, Trp, Tyr, Val).
b) Active process (Na^+-dependent) & very rapid.
c) Amino acids compete with each other for absorption.

2) Basic system:

a) Transports diamino acids (Arg, Lys, Orn & cystine).
b) Active (Na^+-dependent) & fairly rapid (but the rate is only about 10% of the neutral system).

3) Acid system:

a) Transports dicarboxylic amino acids (Asp & Glu).
b) Partially Na^+-dependent, and probably active.

- "Asp & Glu" - Both are rapidly removed by transamination process at the intestine after uptake, ∴ difficult to determine whether they are transported against concentration gradient!

4) Imino acid & glycine system:

a) Transports two imino acids, Pro & Hyp (+ Gly).
b) May not require Na^+, and the rate is slower than three other systems.

- There may be some interactions among these systems - e.g., some neutral AA use carrier(s) for basic AA, and neutral AA may stimulate transport of basic AA.

C. Absorption of peptides:

- Only ≈ 1/3 of total amino acids may exist as free-AA in the intestinal lumen, thus, there must be some "peptide absorption!?"

1) Historical views:

a) 19th century:

(1) Physiologists conducted most of the work on this area.
(2) Assumed that proteins are absorbed in the form of polypeptides.

b) Early 1900's: (Actually, until 1950s!)

(1) Assumed that proteins are completely hydrolyzed to amino acids at the intestinal lumen before being absorbed.
(2) This *classical view* persisted for a long time because:

(a) Extensive work on CH_2O demonstrated a complete hydrolysis before absorption, and assumed a similar mechanism for protein!?
(b) Isolated a considerable amount of AA in the portal blood, but failed to isolate a meaningful amount of peptides.
(c) Observed extensive hydrolytic activity at the brush border & mucosal cells, ∴ assumed a complete hydrolysis before absorption!?

c) In the mid 1950's and early 1960's, people started demonstrating that "peptides" can be absorbed.

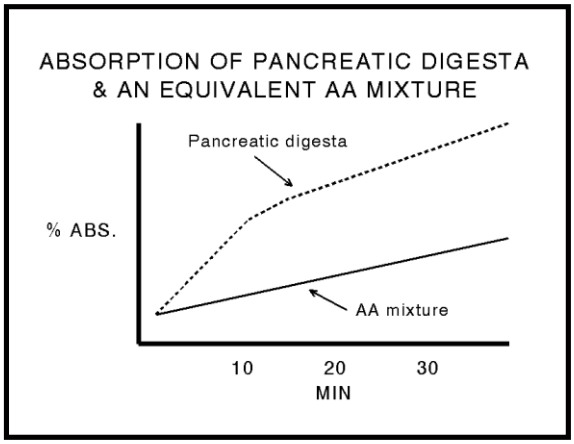
- e.g., Demonstration by one group that dipeptides can be absorbed by intact intestinal preparations (Newey & Smyth, 1962. J. Physiol. 164:527).

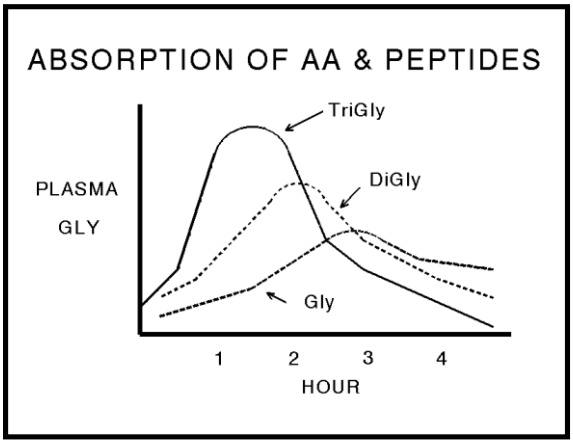
2) Absorption of peptides:

a) Little effect on absorption of free-amino acids, i.e., independent mechanisms for AA & peptides.
b) As in AA absorption, there are competitions among peptides for absorption.

3. Rate of Absorption

A. Absorption of pancreatic digesta (lactalbumin & peptides containing 2-6 AA residues) and an equivalent mixture of amino acids - Figure on the left (Fairclough et al., 1980. Gut 21:829-834).
B. Absorption of amino acid & peptides - Figure on the right (Craft et al., 1968. Gut 9:425).





C. Absorption of peptides:

1) Dipeptides and tripeptides are absorbed faster than free amino acids at the intestinal epithelium.
2) Based on the evaluation with tetraglycine, there has been no evidence of intact absorption of tetrapeptides!?

a) Probably, hydrolyzed to tri- & dipeptides before uptake/absorption?
b) But?

(1) Conclusions based on tetraglycine, which is, perhaps, the simplest form of tetrapeptide.
(2) Considering a possible No. of tetrapeptides (160,000 or 20^4), the possibility of intact tetrapeptide absorption may exist!?

3) A possible mechanism(s)?

a) Uptake of dipeptide is electrogenic and related to H^+ co-transport - Need inwardly directed H^+ gradient!?
b) Protein (127 kDa) - Part of or the entire peptide transport system?

D. Nutritional significance of peptide transport:

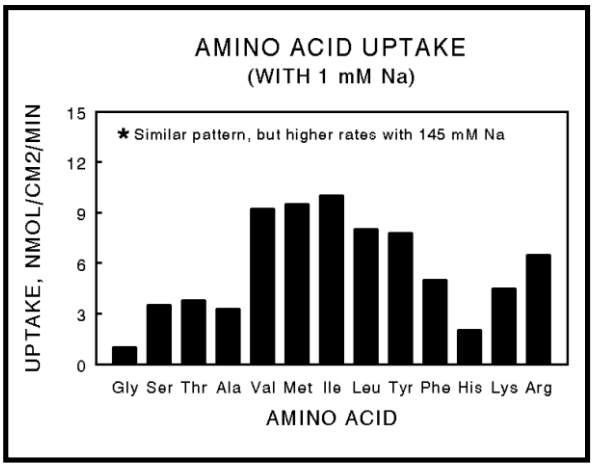
1) ≈ 2/3 of total AA is absorbed as peptides!
2) Absorption of peptides can be very important in some instances of AA absorption defects (e.g., some congenital defects)!

a) *Cystinuria* - Impaired absorption of Cys, Lys, Orn & Arg at the kidneys & intestines. Increase excretion of those AA (e.g., Cys – 20 to 30 times nomal?) and may form Cys calculi or stone.
b) *Hartnup disease* - Impaired absorption of e.g., Thr, Phe, Tyr, Trp, His, Gly, & Ser. For instance, abnormal metabolism of Trp can result in, e.g., pellagra-like skin rash and mental disorder because of the impaired niacin synthesis from Trp.

3) Advantages of peptide absorption?

a) Because of competitions for absorption/transport:

(1) After consumption of a meal, some AA may reach peak concentrations early, while others may lag behind.
(2) Thus, may affect the efficiency of utilization of AA in protein synthesis.



b) Amino acid uptake: [See Sepúlveda & Smith (1978). J. Physiol. (London) 282:73]
c) If AA with slow absorption rate (e.g., Gly & Lys) are provided in the form of peptides instead of free AA, it may ↑ absorption rate of those AA!?

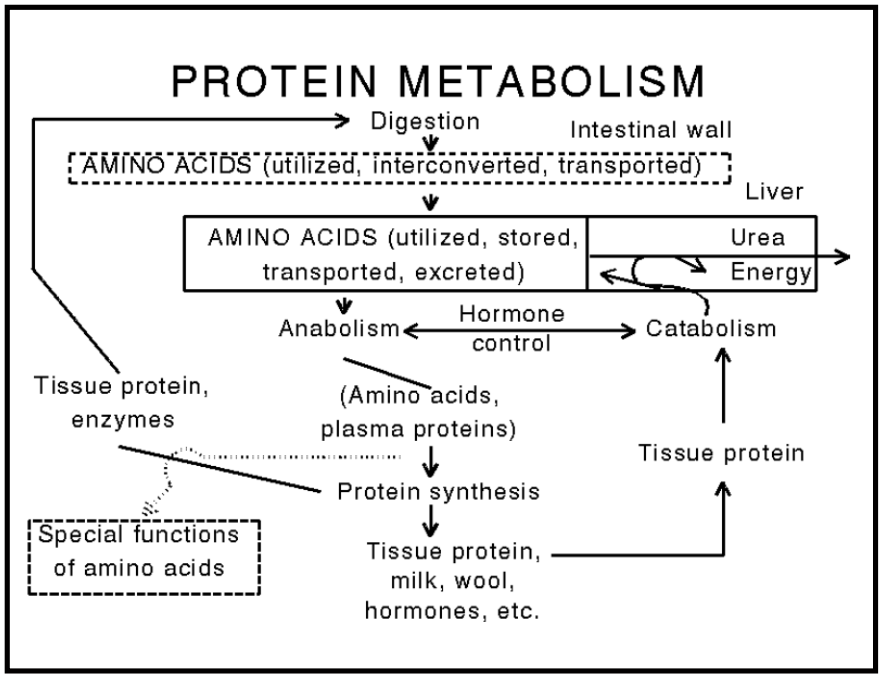
PROTEIN METABOLISM IN GENERAL

1. Amino Acids

A. Protein metabolism - Maynard et al. (1979).
B. Three major points?

1) Protein metabolism is a dynamic state, i.e., a continuous synthesis & breakdown of protein ("protein turnover")!
2) The liver is acting as a buffer or a focal point of regulating protein metabolism.

3) Amino acids have specialized functions.



2. Fate of Absorbed Amino Acids

- Amino acids consumed in excess of that needed for protein synthesis cannot be stored or excreted as such!

A. Anabolism - Protein synthesis (muscle, milk, egg, repair/maintenance, etc.).
B. Catabolism - Catabolized & used as a source of energy (C-skeletons), and N for urea synthesis.
C. Specific function(s) of each individual amino acid - AA are involved in the synthesis of various compounds.

PROTEIN SYNTHESIS AND TURNOVER

1. Protein Synthesis

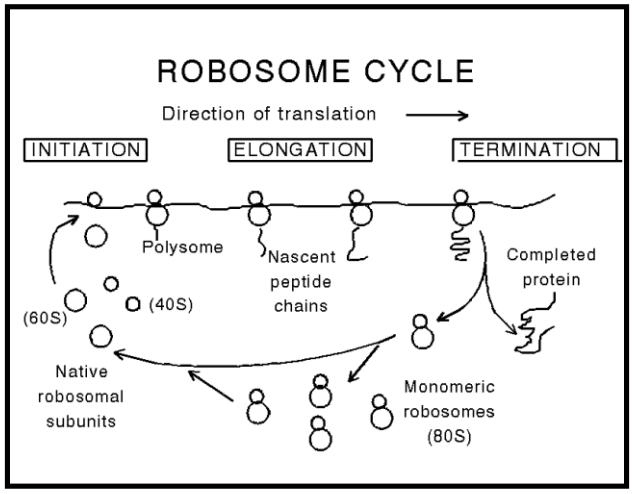
- *A good reference: Pain, V. M. and M. J. Clemens. 1980. Mechanism and regulation of protein biosynthesis in eukaryotic cells. In: P.J. Buttery and D.B. Lindsay (Ed.) Protein Deposition in Animals. pp 1-20. Butterworths, London. (This is also an excellent reference book for the protein metabolism in general!)*

A. General:

1) Transcription - Formation of mRNA from DNA.

2) Translation - All amino acids (protein consists of 22 AA) must be available in adequate amounts simultaneously!

   - Thus, protein digestibility, absorption rate, and protein quality are very important.
   - See, e.g., Pain & Clemens (1980) for the "ribosome cycle."



   a) Initiation - A ribosome & specific initiator tRNA (Met-$tRNA_f$) bind to the beginning of coding sequence of mRNA.
   b) Elongation - A ribosome moves along mRNA, and a polypeptide chain is elaborated from AA in a specific sequence directed by mRNA.
   c) Termination - A ribosome reaches the end of coding sequences, and being released together with a completed protein.

3) The process of protein synthesis is not exact, i.e., many mistakes can be made during the process, thus increasing energy expenditures!

   - In growing animals, protein synthesis may account for ≥ 20 to 30% of total heat production (Reeds eta l., 1982) compared with 1 to 3% for fatty acid synthesis.

B. Rate of protein synthesis:

1) Fractional rate of protein synthesis (rats): [Lewis (Pers. Comm.)]

Tissue	%/day	g/day	% of whole body
SI	136	0.41	9.8
Liver	87	0.55	13.0
Kidney	48	0.069	1.6
Heart	17	0.012	0.3
Brain	17	0.024	0.6
Skeletal muscle	13	0.79	18.7

   a) Over 300 g protein synthesized/d vs requirement of ≈ 56 g/d in adult humans.
   b) In one experiment, the protein synthetic rate was 713 mg/d vs deposition rate of 149 mg/d in a 100-g, rapidly growing rat.

2) The protein synthetic rate is much greater than the protein requirement or accretion rate.

3) Thus, a major focus of protein synthesis seems to be “replacement of old tissues” rather than deposition of new protein!

2. Protein Turnover

A. Possible reasons:

1) Metabolic adaptation - Enzymes, hormones, etc.
2) Restructuring for growth, etc. - e.g., collagen.
3) Repairing cells and tissues.
4) Mobilization/transport of protein - e.g., in the situation of "protein-deficiency."
5) Used as a source of energy.
6) Muscle growth.
7) Removal of faulty molecules or protein . . , etc.

B. Factors influencing the body protein turnover/metabolism:

1) Genetics & sex - species, breeds, lines/strains, etc.
2) Nutrition - Quantity & quality of protein sources (also energy sources).
3) Physiological state - lactation vs dry, pathological state, etc.
4) Age and(or) stage of development:

e.g., Body protein turnover rate in pigs - 13% of body protein/day in a 20-kg pig, 10% in a 60-kg pig, 6% in a 100-kg pig, and 5% in a mature pig.

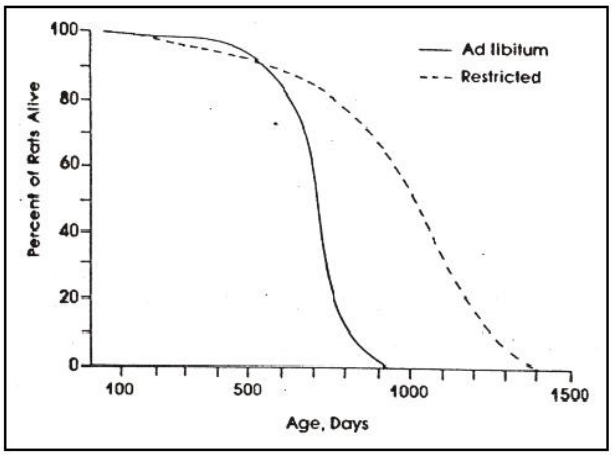
5) Hormones:

e.g., Growth hormone, anabolic; insulin, anabolic; thyroid hormones, anabolic & catabolic; testosterone, anabolic; estrogen, anabolic in ruminants & catabolic in nonruminants; and corticosteroids, catabolic.

C. Protein turnover and longevity?

1) "By restricting what we eat and live longer?" - Aging, dietary restrictions, and longevity?

- e.g., Yu et al. 1982 (.J. Gerontol. 37:130-141). Restriction = 60% of ad lib; Median = ad lib, 714 d & restricted = 1,047 d.



2) Aging is caused by, among others:

a) Worn-out tissues - Not being repaired quick enough?! (Free radicals?!)

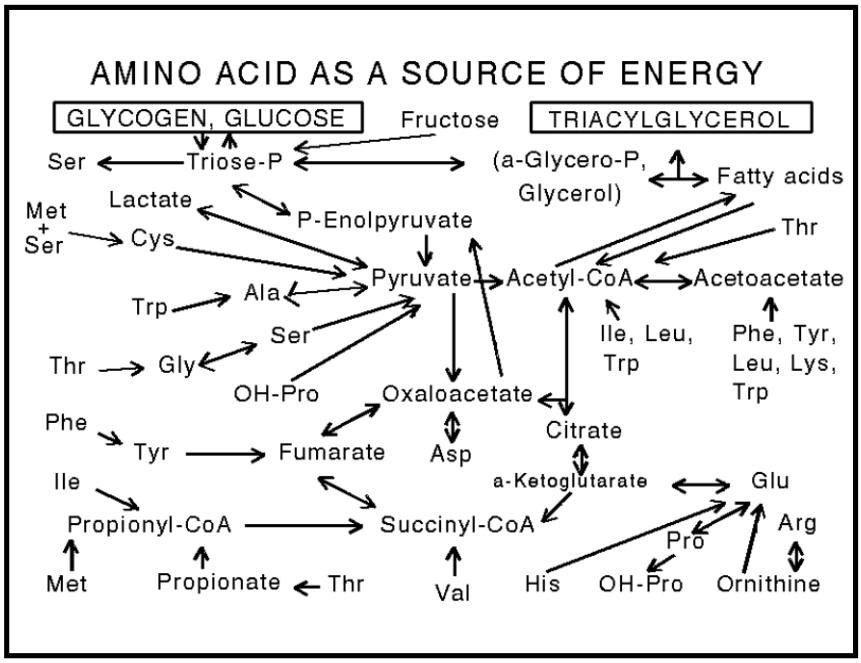
b) Reduced immune response - Not being able to respond quickly to challenges!?

3) Thus, perhaps, associated with the reduced protein turnover, and protein turnover would be reduced with age regardless of species!
4) Can maintain a certain rate of protein turnover as with advancing age, may be able to increase the longevity?!
5) Dietary restrictions can enhance gene expression, which can increase protein turnover!?
6) Similar data on primate, and the dietary restriction is the only known way to increase longevity!?

AMINO ACID AS A SOURCE OF ENERGY

1. General

A. Often, a large part of protein consumed is used as a source of energy.
B. e.g., In human:



1) The RDA for protein (adult male weighing 70 kg) is 56 g/d in the U.S.
2) An average consumption is at least 100 g/d.
3) Excess protein is catabolized - Carbon skeletons are used as a source of energy, and a nitrogen component is converted to urea & excreted in the urine. However, deaminated AA:

a) Are less efficient source of energy vs. carbohydrates or fat!
b) According to one source, have only ½ of the "assumed" energy value, i.e., 50% of the assumed total GE values, which are CH_2O = 4.15 (or 4), lipids = 9.40 (or 9), and protein = 4.40 (or 4) kcal/g!?

2. Metabolic Pathways

☛ Amino acids & entry points: (See Martin et al., 1983 & others).

A Unique for each amino acid.
B. Some amino acids share certain sections of the pathway.

C. All routes converge into a few terminal pathways leading to formation of: 1) pyruvate, 2) acetyl-CoA or 3) intermediates of TCA cycle.

3. Classification of Amino Acids

A. Glucogenic - Some or all C can be converted to glucose via pyruvate & intermediates of TCA cycle (Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser, Thr & Val).
B. Ketogenic - Reactions lead to only acetoacetate & acetyl-CoA (Leu & Lys).
C. Glucogenic & ketogenic - Can be converted into both types of intermediates:

1) Ile - Succinyl-CoA & acetyl-CoA.
2) Phe & Tyr - Fumarate & acetoacetate.
3) Trp - Acetoacetate & pyruvate.

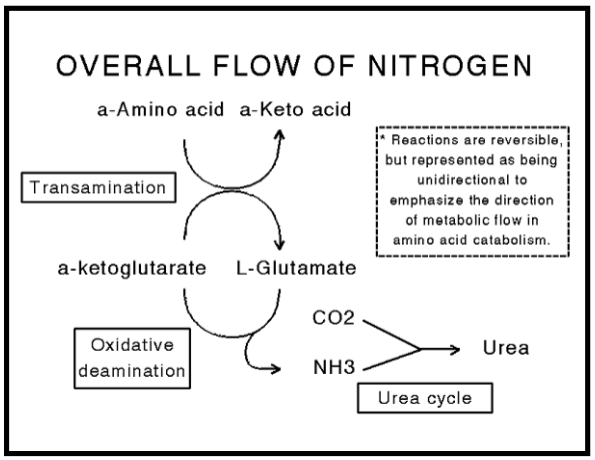
- Most of the AA are glucogenic, ∴ there is a potential for forming glucose from catabolized AA. But, experimental data indicate otherwise!

TRANSAMINATION AND DEAMINATION

1. General

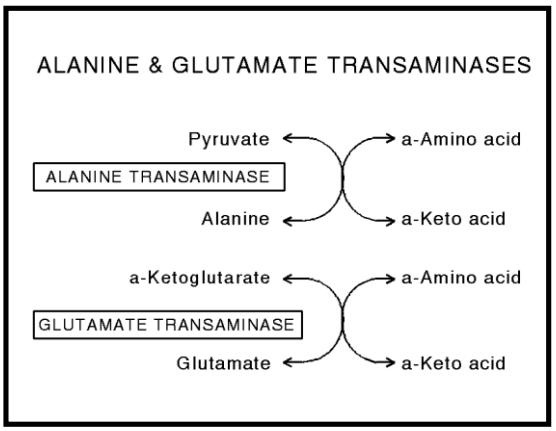
A. Overall flow of N in AA catabolism - See, e.g., Martin et al. (1983).
B. These processes are necessary:

1) To synthesize dispensable amino acids.
2) To oxidize excess amino acids.
3) For gluconeogenesis.



2. Transamination

A. Exchange of ammonia between AA & α-keto acid (reversible reactions).
B. Occurs in the mitochondria & cytoplasm of most cells.
C. Vitamin B_6 is an essential component of these reactions.
D. Lys & Thr do not participate in transamination process (lacking means to add ammonia to their α-keto acids), thus, their analogs cannot be utilized by animals!
E. Catalyzed by transaminases:



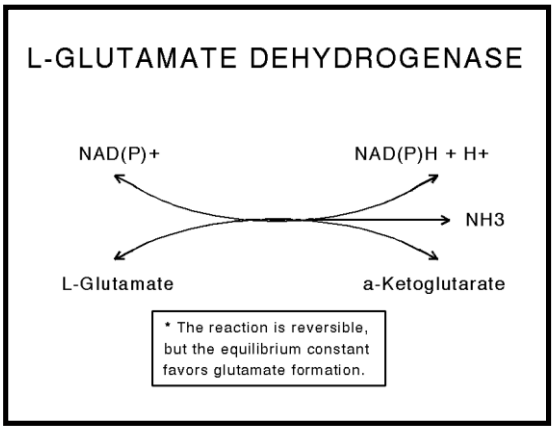
1) Alanine & glutamate transaminases: (Adapted & redrawn from Martin et al., 1983)
2) Also, aspartate transaminase is involved:

Glutamate ⇔ α-ketoglutarate & oxaloacetate ⇔ aspartic acid.

3. Deamination

A. An amino group of most AA is transferred to α-ketoglutarate by transamination process, and a release of ammonia is catalyzed by L-glutamate dehydrogenase (oxidative):

1) Reaction of L-glutamate dehydrogenase (redrawn from Martin et al., 1983) – Vitamin B_6 is necessary.
2) Fate of the end products? Ammonia to urea cycle, and α-ketoglutarate to TCA cycle.



B. Nonoxidative deamination:

1) The α-amino groups of Ser & Thr can be directly converted to NH4+ because each of these amino acids contain an hydroxyl group in its side chain:

1) Ser → Pyruvate + NH4+.
2) Thr → α-ketobutyrate + NH4+.

2) These direct deaminations are catalyzed by serine dehydratase & threonine dehydratase, in which PLP (vitamin B_6) is the prosthetic group.

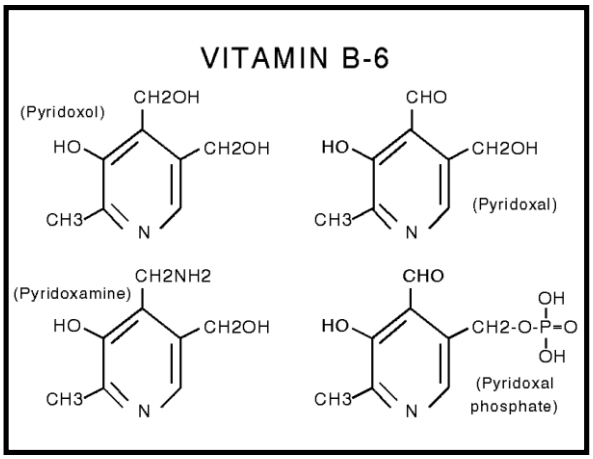
- Called dehydratases because dehydration precedes deamination.

VITAMIN B_6

1. History: [See Maynard et al. (1979), McDowell (1989) & others]

A. Clinical signs of what was later to be known as "vitamin B_6 deficiency" were described by Goldberger & Lillie (1926) in their attempts to produce pellagra in animals.
B. In 1934, Szent-Györgi separated the non-thiamin part of the B complex into riboflavin and a "complementary factor," which he named "vitamin B_6" and defined as "*The factor responsible for the cure of a specific dermatitis developed by young rats on the vitamin-free-diet supplemented with B_1 & riboflavin.*"
C. In 1938, five different labs independently isolated a crystalline form of the vitamin.
D. Szent-Györgi proposed the term "pyridoxine" for the vitamin, which was widely accepted, but subsequent identification of pyridoxal & pyridoxamine led to the use of the original term "vitamin B_6."

2. Chemical Structure



A. Structure of vitamin B_6 compounds (McDowell, 1989).

1) A relatively simple compound with three substituted pyridine derivatives that differ only in functional group in the 4-position:

   a) Pyridoxine (or pyridoxol) is the predominant form found in plants.
   b) Pyridoxal & pyridoxamine are the forms generally found in animal tissues.

2) All three forms are converted in the animal body to the metabolically active form, pyridoxal phosphate (PLP).

   - To a lesser degree, pyridoxamine phosphate (PMP) is also active.

B. Characteristics:

1) Colorless crystals that are soluble in water & alcohol.
2) Stable to heat, acid, alkali, but sensitive to light, especially in neutral or alkaline media.
3) There are several antagonists that compete for reactive sites of apoenzyme or react with pyridoxal phosphate to form inactive compounds: (e.g.)

   a) Deoxy-pyridoxine - a powerful antagonist & being used in experiments to accelerate the vitamin deficiency.
   b) Isonicotinic acid hydrazide (isoniazid) - a binding compound of the vitamin & being used in tuberculosis treatment.
   c) Antihypertensive drugs such as thiosemicarbazide & hydralazine has been shown to interfere with the vitamin.

C. Analysis:

1) A bioassay using the rat or chick growth has been widely used, but expensive & time-consuming.
2) The standard method is microbiological assay - By using *Saccharomyces uvarum* (yeast; most commonly used), along with *Streptococcus faecalis* and *Lactobacillus casei*, it is possible to differentiate alcohol, amine, and aldehyde forms.

   - Possible disadvantages in microbiological methods include: a) time-consuming, b) variations in the growth response to the vitamin, c) a possible mutation of organisms, d) formation of microbiologically unavailable complexes, etc.

3) Other methods include gas-liquid chromatography (mainly for pure standards), a combination of chromatographic separation with fluorometric determination (for enriched foods/feeds) & a HPLC (the method of choice for vitamin B_6 assay?).

3. Metabolism

A. Digestion, absorption & transport:

1) Protein-bound vitamin is split first before absorption, and vitamin B6 compounds are all absorbed in the dephosphorylated forms [SI is rich in alkaline phosphatase (AKP)].
2) Mostly absorbed in the jejunum (& also in the ileum) by passive diffusion.
3) Microorganisms synthesize the vitamin in the colon of nonruminants, but the absorption rate is low in that region.
4) Once absorbed, B6 compounds rapidly appear in the liver where they are converted into mostly PLP (& PMP?)

   - PLP & PMP are interconvertible & the conversion requires FMN, FAD & NAD, ∴ a deficiency of niacin and(or) riboflavin ↓ the active form of B6.

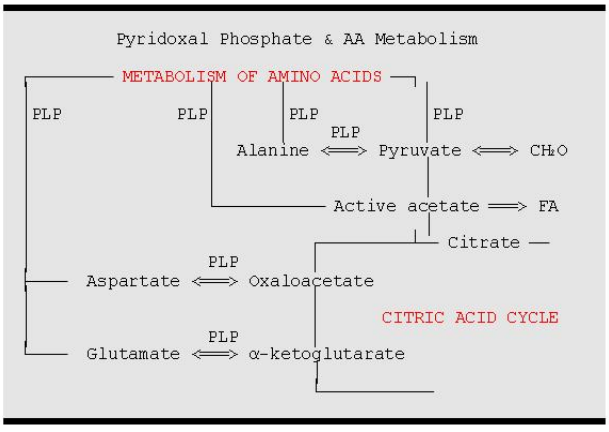
5) "Circulation" - PLP accounts for ≈ 60% of plasma vitamin B_6, and primarily associated with plasma albumin and red blood cells.

B. Storage & excretion:

1) Widely distributed in various tissues mainly as PLP or PMP, but only small amounts are stored in the body.
2) Excess B6-enzymes are dephosphorylated by AKP:

   a) Vitamin is reutilized, or
   b) Oxidized to pyridoxic acid by aldehyde oxidase and(or) NAD-dependent dehydrogenase.

3) Pyridoxic acid is the primary route for elimination of the vitamin (≈ 70%?), and it is excreted in the urine. (Also, small amounts of other forms are excreted in the urine.)



4. Functions

A. PLP (& also PMP):

1) A co-enzyme for many enzymes in the amino acid, carbohydrate and

fatty acid metabolism.
2) Over 50 enzymes are known to be dependent on vitamin B6.

B. Amino acid metabolism -"PLP" functions in practically all the reactions involved in AA metabolism! - "PLP" and amino acid metabolism (adapted & redrawn from McDowell, 1989):

1) "Amino transferases (or transaminases)" - involved in the interconversion of a pair of AA & their corresponding keto acids (AA biosynthesis & catabolism).
2) "Deaminases" - e.g., Ser, Thr & cystathionine.
3) "Desulphydrases & transsulphydrases" - involved in the S-AA metabolism.
4) "Racemases" - involved in utilization of D-AA by microorganisms (. . . to date, not detected in mammalian tissues).
5) Amino acid transport - "three systems" (neutral, basic, and Pro/OH-Pro) seem to require B_6.
6) Formation of antibodies - B_6 deficiency results in inhibition of the synthesis of globulin, which carry antibodies.

. . . , etc.

C. Other functions:

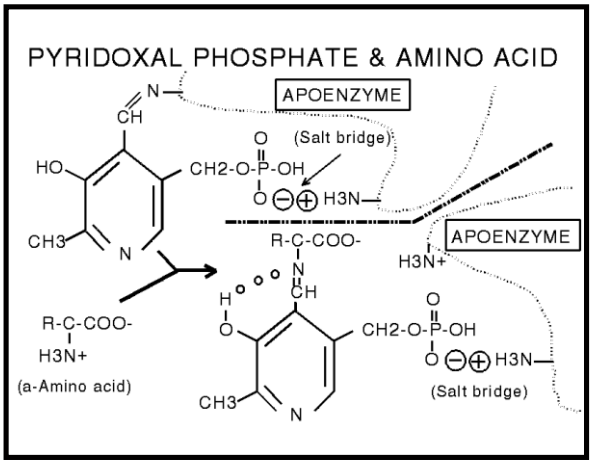
1) Nonoxidative decarboxylation - "decarboxylases" convert AA for the synthesis of many biologically important compounds such as histamine, hydroxytyramine, serotonin, ethanolamine, taurine & γ-aminobutyric acid.
2) Synthesis of niacin from Trp.
3) Formation of δ-aminolevulinic acid from succinyl CoA & Gly, the first step in porphyrin synthesis.
4) May be involved in a conversion of linoleic to arachidonic acid . . . controversial!
5) "Glycogen phosphorylase," which catalyzes glycogen breakdown. (PLP does not seem to be a coenzyme, but rather affects the enzyme's conformation?)
6) Synthesis of epinephrine & norepinephrine from either Phe or Tyr.

. . . , etc.

D. The initial step: Formation of an enzyme-bound Schiff base intermediate (Adapted & redrawn from Martin et al., 1983):

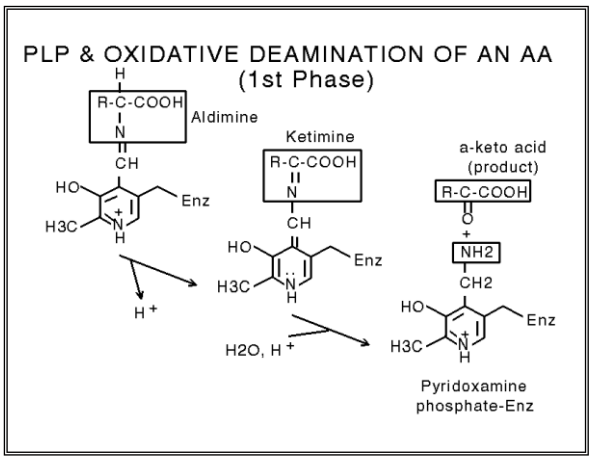
1) PLP binds to its apoenzyme.

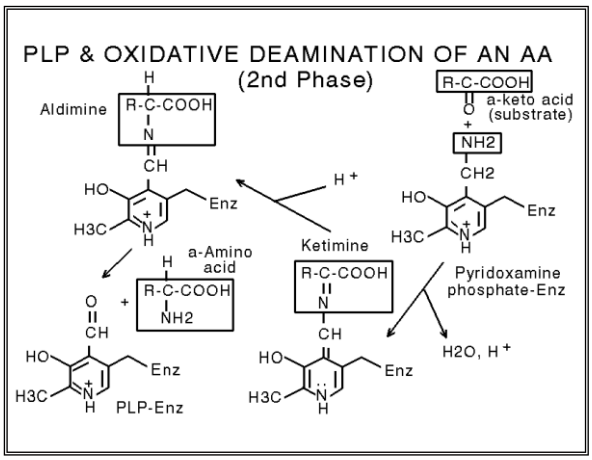
2) When α-amino acid enters, it replaces ε-amino group of apoenzyme=s lysyl residue & forms its own Schiff base with PLP.
3) After a series of rearrangements, substrate is oxidatively deaminated and



PLP would be converted to PMP, and PMP can donate NH2 to keto acids to form other AA.

F. Deamination/transamination: (Adapted & redrawn from Martin et al., 1983)





3. Deficiency/Supplementation

A. General:

1) General deficiency signs include retarded growth, dermatitis, epileptic-like convulsions, anemia & partial alopecia (baldness).
2) Least likely to be deficient in the livestock diet because natural energy and protein sources usually provide sufficient amounts of B6
3) Supplementation might be warranted under certain conditions, and possible reasons may include (Perry, 1978. In: Proc. Roche Vitamin Nutr. Update Meeting., Arkansas Nutr. Conf. p 29):

    a) Great variations in the B6 content due to the origin of samples, growing conditions, climate/weather conditions, etc.
    b) Variations in the bioavailability of ingredients - e.g., availability values of 38-45 & 58-62% for corn & soy, respectively, in one chick assay.
    c) Loss of the vitamin during processing - e.g., the availability ↓ as much as 40-50% after heat processing in one study.
    d) Discrepancies in the vitamin activity between the species used for the test & target species.
    e) ↑ vitamin requirements because of marginal levels of dietary Met.

B. Swine:

1) Because of its wide dietary distributions, unlikely to see deficiency signs in pigs fed typical diets - e.g., corn, 5.3 mg/kg & soybean meal, 6.7 mg/kg with the availability being 40 to 60%.

2) Deficiency signs include a poor appetite, slow growth, anemia, epileptic-like convulsions, rough hair coats, diarrhea, scaly skin, brown exudate around the eye, impaired movement, etc.
3) According to the results of some experiments, the protein/N retention in deficient-pigs ↓ to < ½ of those receiving adequate B6.

C. Poultry:

1) As in swine diets, poultry diets are unlikely to be deficient in B6, but growing & breeding diets may have to be supplemented?
2) Deficiency signs include a poor appetite & growth, abnormally excitable, nervous disorder, trembling & vibration of tip of the tail, convulsion, reduced egg production, hatchability, etc.

D. Fish:

1) Expected to have higher vitamin B_6 requirement because of high-protein requirements.
2) Deficiency signs develop quickly - < 4 to 8 wk in channel catfish, salmonids, and carp.
3) Signs include:

    a) Nervous disorders - Hypersensitivity to disturbances, poor swimming coordination, convulsion & tetany.
    b) A greenish-blue sheen to the skin in channel catfish.
    c) Edema, exophthalmos & skin lesions in common carp.

E. Ruminants:

1) Probably adequate amounts of the vitamin would be synthesized by microorganisms in mature animals with fully developed rumen - no deficiency signs have been reported in mature ruminants!
2) Young calves:

    a) Probably need supplementation - e.g., > 2.4 mg/kg of milk substitutes?
    b) Deficiency signs include anorexia, slow growth, scours & convulsive seizures.

F. Humans:

1) Fairly common, especially in young children & lactating women.
2) The use of oral contraceptive & pregnancy ↑ the requirement.
3) Also, alcohol, which may have a negative effect on the formation of PLP, and certain drugs (e.g., antithyroid & antituberculosis drugs) can interfere with metabolic functions of the vitamin.

4) Typical signs include anemia, weight loss, abdominal distress, vomiting, hyperirritability, epileptic-type convulsions in infants, depression & confusion, ↓ in lymphocyte counts, etc.
5) A Mysterious Malady in the Early 1950s?

- Story?

"*All over the country, hundreds of infants (from two- to four-month old) started scaring parents to death by going into generalized "convulsions" several times a day.*

Otherwise, the children were perfectly healthy. And the families were not poor; they could afford to feed their babies manufactured infant formula. Most of the babies were brought by their anxious parents to the hospital, where, as mysteriously as the convulsions had appeared, they disappeared.

The medical sleuthing eventually detected that all the infants had been fed one particular brand of formula. When they were changed to another formula or evaporated milk, they became free of convulsions.

Something in the formula? No, something "not" in the formula, "PYRIDOXINE!" A manufacturer had somehow managed to produce pyridoxine-free formula.

Because of this incident, pyridoxine became a household name back in the early 1950s. This incidence also demonstrates the hazards of relying on "man-made" food, especially when a superior natural food, breast milk in this case, is readily available!"

- "Vitamin B6 and Convulsions?"

  - Pyridoxine deficiency can lead to ↓ GABA (γ-aminobutyric acid), thus, ↑ Neural hyperexcitability!
  - The GABA is a synaptic transmitter agent & inhibitory mediator in the brain, and pyridoxine (or enzyme L-Glu decarboxylase) is involved in the synthesis of GABA!

G. Supplementation in general:

1) Widely distributed in feeds & food.
2) The availability of corn (content, 5.3 mg/kg) & soybean meal (6.7 mg/kg) has been estimated to be 40 to 60% in chick assay, and probably similar availabilities in swine?
3) Swine - Probably not necessary to supplement in practical diets.
4) Birds - Unlikely to see deficiency in adults, but in young birds . . . ?
5) Fish - Most feedstuffs may contain adequate levels, but a common practice to supplement because of variations/uncertainty of the content (& availability?) in feedstuffs & also in processed and(or) stored feeds.

4. Requirements & Sources

A. Requirements: (Also see an appropriate "Nutrition and Feeding" section.)

Animals	mg/kg
Swine: (NRC, 1998)	
Young (3-20 kg)	1.50-2.0
Growing (20-120 kg)	1.0
Adult	1.0
Poultry: (NRC, 1994)	
Immature chickens	2.8-3.0
Laying hens	2.1-3.1
Broilers	3.0-3.5
Turkeys	3.0-4.5
Horses (NRC, 1978)	Microbial synthesis
Fish: (NRC, 1993)	
Channel catfish	3
Rainbow trout	3 (10-15)
Pacific salmon	6 (10-20)
Common carp	6? (5-10)
Beef cattle, sheep, horse & goat	Microbial synthesis
Growing cat	4.0
Growing dog	60 µg/kg BW
Catfish	3.0
Rat	6.0
Human, mg/d: (RDA)	
Infants	0.3-0.6
Children (< 10 yr)	1.0-1.4
Male	1.7-2.0
Female	1.4-1.6
Pregnant/lactating	2.1-2.2

( ) = previous estimates.

B. Sources (B6 is widely distributed in foods and feeds):

1) Foods such muscle meats, liver, vegetables, whole-grain cereals & by-products and nuts are good sources, and only few foods are really poor sources.
2) Cereal grains - B6 is concentrated mainly in bran with the rest containing only small amounts.

D-ISOMERS, α-KETO AND α-HYDROXY ANALOGS

1. General

A. Reasons for the interest:

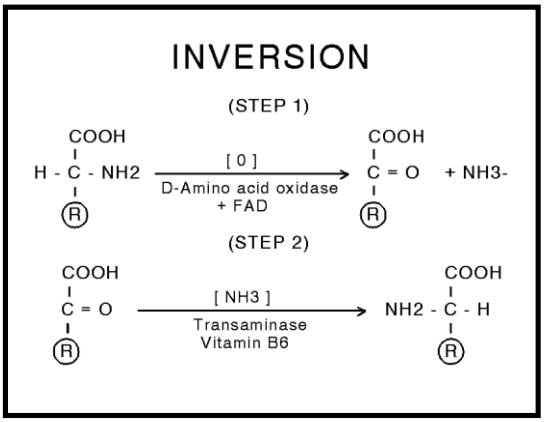
1) Can lower the N content of diets by using analogs - May be useful in various disorders associated with N metabolism such as kidney failure, N-accumulation diseases.

2) May be able to reduce feed costs for animal production - Often, D-forms & analogs are inexpensive vs L-amino acids or intact proteins.
3) Also, would have a positive impact on the environment by reducing excretion of N in animal waste.

B. All natural proteins consist of L-form of amino acids. (Protein synthesized by certain MO "may" contain D-AA though!)
C. For the D-form to have activity or to be utilized, it must be converted to the L-form by the process called "inversion."

2. Inversion

A Keto acids are intermediates of the inversion process, ∴ animals should be able to utilize those acids.
B. Hydroxy analogs - Simply have the OH group instead of amino group, thus animals should be able to utilize those analogs.



3. Utilization

A. Lys & Thr:

1) No inversion for these AA because of the lack of D-AA oxidase & means to incorporate ammonia), thus the D-forms or analogs are not utilized by animals.
2) Thus, must be provided in the diet!
3) Both (L-form) are available commercially - Lys for a long time & Thr (& Trp & Val) fairly recently!

B. Met:

1) D- and L-Met have similar values in rats, mice, dogs & possibly in chicks.
2) α-Keto analog is readily utilized by animals.
3) But, now, a lot of controversies on the effectiveness of some analogs simply because of economical implications, especially in the poultry industry! For instance, based on the weight, OH-analog should have 88% Met activity vs. DL-Met, but only 65% in some data!

C. Phe:

1) D-form is utilized well by species investigated (mice, rats, chicks & humans).
2) α-Keto & hydroxy analogs are utilized by rats and chicks.

D. For others such as Arg, Ile, Leu, Trp & Val, the efficiency of utilization may depend on species & type of AA intermediates, and likely to see considerable variations in their effectiveness.

EXCRETION OF NITROGEN

1. General

A. Mammals - 1° pathway is the urine, and major constituents of urine include: urea (60-80%), ammonia (2-10%), uric acid (2% in humans), amino acids, creatinine (3-10%), protein (< 1%), amino sugars, vitamin residues, etc.

B. Fowl:

1) Excrete N as uric acid - Almost "solid" and also a very low solubility in water, which is different vs. urea.
2) Uric acid:

a) Synthesized from Gln, Gly, and Asp in a very complicated manner. (Uric acid is also an end product of metabolism of purine bases.)
b) The synthetic process is under control of xanthine dehydrogenase or oxidase, which is produced in the liver & kidneys.

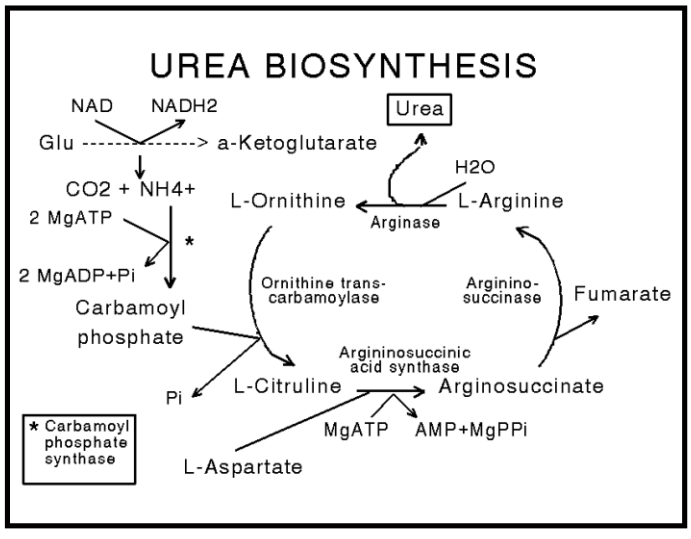
- Gly → (tetra-hydrofolate, Gln, CO_2 . . .) → Adenine → (xanthine oxidase) → uric acid.

2. Urea Cycle

A. Reactions & intermediates of urea biosynthesis (Martin et al., 1983).
B. Presumably requires 3 ATP, but needs additional energy for:

1) Ornithine → mitochondria (crossing membranes).
2) Regeneration of aspartate & release of ammonia from AA.

- Theoretically? $2NH_3 + CO_2 + H_2O + 3\ ATP$ → urea + 2 ADP + AMP + 4 Pi



C. The values determined via experiments range from 4.8 to 5.7 ATP/mole of urea produced (vs. theoretical value of 3 ATP), ∴ a costly process:

1) Account for ≈ 1% of ME intake in growing pigs?
2) Much larger for animals & people consuming large amounts of protein.

- Perhaps, estimates are too low!?

SPECIAL FUNCTIONS OF AMINO ACIDS

1. Arginine

A. A component of creatine, which plays important roles in the storage and transmission of phosphate-bond energy. (Also, Gly & Met are involved in creatine synthesis.)
B. A component of the urea cycle, ∴ involved in the conversion of ammonia to urea.

2. Aspartic acid

A. Involved in biosynthesis of purine - contributes the N atom labeled 1. (Also, Ser, Gln & Gly are involved in purine synthesis.)
B. Pyrimidine biosynthesis - contributes atoms 3 (N), 4, 5 & 6 (C) of the pyrimidine ring.

3. Cysteine

A. A component of tripeptide, glutathione.
B. Taurine is derived from Cys. (Taurine is a component of bile acids & excretory pathway for sulfur, and also may function as a neurotransmitter).

4. Glutamic Acid

A. A component of glutathione.
B. Decarboxylated to γ-aminobutyric acid (GABA), which may be involved in regulation of the CNS activity.
C. Involved in the transamination of amino acids.

5. Glutamine - Contributes two N atoms to the basic structure of purine.

6. Glycine

A. Involved in the synthesis of glutathione, creatine & purine.
B. Involved in conjugation in the liver - Combines with cholic acid to form glycocholic acid & with benzoic acid to form hippuric acid.
C. Contributes the N atom of the pyrrole rings found in porphyrins such as heme.

7. Histidine

A. Histamine (vasodilator & mediates release of HCl & pepsin) is formed by decarboxylation of His.

B. Component of two dipeptides (carnosine & anserine) found in muscle tissues.

8. Serine - Involved in synthesis of purine, pyrimidine, sphingomyelin, ethanolamine & choline.

9. Tryptophan

A. Involved in formation of serotonin, which is a vasoconstrictor & is found in significant amounts in the brain.
B. Involved in formation of melatonin (by further metabolism of serotonin), which may play some role in the regulation of seasonal & diurnal rhythms.
C. Involved in synthesis of niacin - Costly, but it is possible to meet the niacin requirement by providing sufficient Trp.

10. Tyrosine

A. Involved in synthesis of catecholamines - Tyr → dihydroxy-Phe → dopamine →norepinephrine → epinephrine.
B. Involved in synthesis of thyroid hormones - Try → mono-iodotyrosine (T_3) → diiodotyrosine (T_3 & T_4).
C. Involved in synthesis of melanin (pigment of skin & hair).

11. Methionine

A. Plays more than a casual role because it contains sulfur & labile methyl groups - may provide methyl groups to as many as 40 different methyl group acceptors.
B. Involved in synthesis of Cys (∴ taurine), creatine & choline, in conversion of norepinephrine to epinephrine, and in many others.

FOLACIN, VITAMIN B_{12} AND COBALT (& SULFUR)

1. Interrelationships in Metabolism of Methyl Groups

- Metabolically, folacin, vitamin B_{12}/Co, choline and Met are closely related!

A. Folacin:

1) Indispensable in transferring single-carbon units such as methyl, formyl & methylene.
2) One-C units are generated primarily from AA metabolism - e.g., Metabolism of Met, Gly, Ser & His yields 1-C units. (Also, metabolism of nucleic acids.)

- *Folacin is involved in the synthesis of labile methyl groups from a formate carbon!*

B. Vitamin B_{12}:

1) Regulates a proportion of methyl- to nonmethyl-tetrahydrofolate (THF).

2) Necessary for the transport of methyl-THF across cell membranes, and also promotes folacin retention by tissues.

- *Vitamin B_{12} regulates a transfer of the methyl group!*

C. Cobalt is a component of vitamin B_{12} (1°/only biological effect of Co?).

E. Thus, folacin, vitamin B_{12}, and Co:

1) Are involved in the biosynthesis of Met from homocysteine.
2) Choline from ethanolamine.
3) Also, involved in biosynthesis of purine bases, adenine & guanine, and thymine, thus, deficiencies can ↓ nucleic acid synthesis, ∴ impair cell formation & functions.

2. Folacin

A. Introduction [See Maynard et al. (1979, McDowell (1989) & others]

1) General:

a) "Folacin" is a generic term used to describe folic acid & related compounds exhibiting the biological activity of folic acid.
b) Has been estimated that up to one-third of all pregnant women in the world (in both developed & developing countries) may experience a folacin deficiency.
c) Because of its roles in the synthesis of DNA & RNA, it doesn't take much imagination to realize "devastating" effects of the deficiency.

(1) "Hardest-hit" target organs/systems are those that depend on a rapid proliferation of new cells, i.e., bone marrow, immune system, certain mucus membranes & red blood cell.
(2) Are you satisfied with your hair & fingernails?" (Bosco, 1989)

(a) Hair & fingernails are also affected greatly by the deficiency of folacin.
(b) It's not easy to examine the status of bone marrow, red blood cells & other tissues/organs.
(c) But, if your hair and nails are not in as good a condition as you might like them to be ..., then it's possible that a folate deficiency might be impairing those important organ/systems!

d) The folacin needs of animals are generally met by dietary sources, and to some extent by the intestinal bacterial synthesis.

2) History:

a) Willis (1931) first described a disease, "tropical macrocytic anemia," for women patients in India ..... later found to be a "folacin-deficiency" disease:

(1) Often associated with a pregnancy, and an extract from yeast was found to be effective in preventing or alleviating the anemia.
(2) Able to produce a macrocytic anemia in monkeys by feeding similar diets that caused the anemia in humans, which was cured by feeding yeast or liver extracts.

b) Day et al. (1935) obtained similar results in monkeys, and they named an unidentified factor found in brewers' yeast, "vitamin M."
c) In 1938, Stokstad & Manning and Bauernfeind et al. identified factors required for growth & prevention of anemia in young chicks, and termed those factors "factor U" and "factor R."
d) Hogan & Parrot (1939) discovered a factor from the liver that prevented anemia in chicks ("vitamin Bc").
e) In 1940:

(1) Snell & Peterson identified a factor necessary for growth of Lactobacillus casei.
(2) Mitchell, Snell & Williams found a factor required by both L. casei & S. lactis from spinach ("folic acid").

- Confusions existed in the 1940s because L. casei factor & folic acid were active for both MO & animals, but others were not active for MO.
- Subsequently found that folacin exists in nature in both the free and bound forms. (Most species, except MO, can utilize bound forms!)

B. Structure/analysis

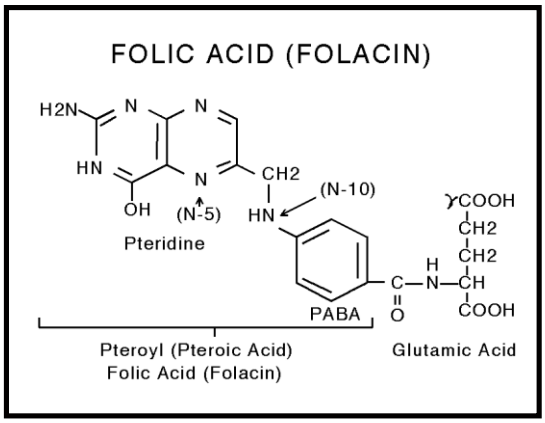
1) Structure:

a) Contains three distinct parts, glutamic acid, ǫ-aminobenzoic acid (PABA) and pteridine nucleus.

- The PABA portion was once thought to be a vitamin, but if the folacin requirement is met, there is no need for dietary PABA.

b) Much of folacin in natural feedstuffs is conjugated with varying numbers of extra Glu, whereas synthetic folacin is "monoglutamate" form.
c) Active forms of folic acid have a formyl or a methyl group attached to "N-5" or "N-10," or a "methylene" group between N-5 & N-10.
d) The THF is the ƭ coenzyme form, and 5-methyl-THF is the ƭ storage form of the vitamin.

- Structure: (Adapted & redrawn from McDowell, 1989)



2) Properties:

a) Tasteless, odorless, yellowish-orange crystalline powder.
b) Insoluble in alcohol, ether & other organic solvents.
c) Slightly soluble in hot water in an acid form, but quite soluble in a salt form.
d) Fairly stable to the air & heat in a neutral and alkaline solution, but unstable in an acid solution.
e) Readily degraded by the light and UV radiation.
f) Cooking can ↓ the folacin content in foods considerably - as much as 50-90%?

3) Antagonists:

a) Since folacin deficiency is more detrimental to "rapidly-growing cells," various antagonists (analogs) have been produced for anticancer & antimicrobial therapies - e.g., aminopterin, tetrahydroaminopterin, etc.
b) Sulfonamides (not folacin analogs, but PABA analogs) are being used as antibacterial agents (sulfonamides compete with PABA, ∴ preventing folacin synthesis).

4) Analysis:

a) Difficult to determine folacin in biological materials because it exists in a number of complexes.
b) The bound form must be freed for the analysis by conjugase enzymes (found in animal tissues), which convert the conjugate to a monoglutamate form.
c) "Microbiological assay" - can use L. casei (respond to all mono-Glu forms), and a differential assay using S. faecalis and L. citrovorum.
d) Various methods have been developed for separation of folacin using the HPLC.

C. Absorption/Excretion

1) Absorption/transport:

a) Poly-Glu forms are digested to pteroylmono-Glu by γ-carboxypeptidase (or folate conjugase ..... probably more than one!) before being transported across the intestinal mucosa.

- Zn deficiency can ↓ hydrolysis, indicating that intestinal conjugase is a Zn-dependent enzyme.

b) Primarily absorbed in the duodenum and jejunum by the active process?
c) After absorption, folates are transported in plasma ↑ as 5-methyl-THF.
d) Specific "folate-binding proteins" are known to exist in body fluids, liver, kidney, brush border membranes & others, but their physiological roles have not been elucidated.
e) Based on several studies, ≈ 79-88% of labeled folacin is absorbed very rapidly - e.g., reaches a peak serum concentration ≈ 2 h after ingestion.

- The average availability in various food items was estimated to be ≈ 50% (ranging from 37 to 72%) in one study.

2) Storage/excretion:

a) A wide distribution in tissues as poly-Glu forms, and body stores have been estimated to be 5-10 mg (≈ ½ in the liver) in humans.
b) Vitamin B12 deficiency can ↓ body stores of folacin because of ↓ in the conversion of mono-Glu to poly-Glu forms.
c) Fecal folacin levels are quite high, whereas urinary excretion represents only a small fraction.

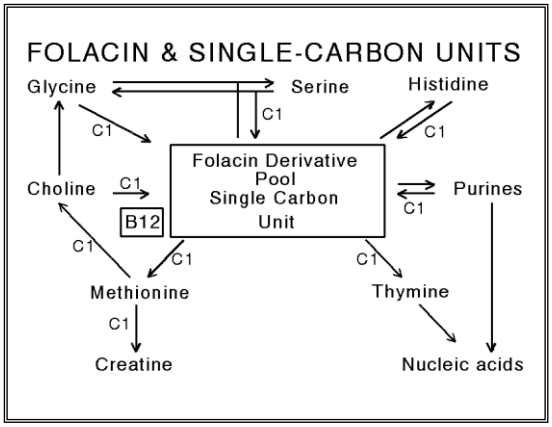
- The fecal excretion rate is often higher than the intake, indicating a considerable bacterial synthetic activity of folacin in the intestine.

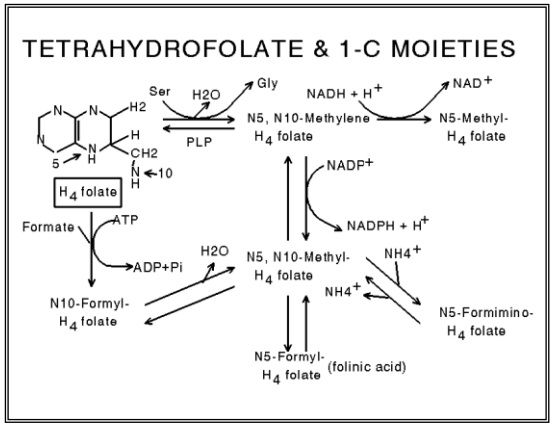
D. Biological Functions

1) The "5,6,7,8-THF" is indispensable in the transfer of single-carbon units in various reactions, and "1-C" units can be a formyl, forminino, methylene or methyl group.

- Analogous to pantothenic acid, which is involved in the transfer of "2-C" units!

2) Folacin metabolism requiring one-carbon units: (Adapted & redrawn from McDowell, 1989)





3) Actions of tetrahydrofolate (THF): (Adapted & redrawn from Martin et al., 1983)
4) Specific reactions (e.g.):

    a) Purine & pyrimidine synthesis.
    b) Interconversion of Ser & Gly.
    c) Gly-α-C as a source of C unit for many biosyntheses.
    d) His degradation.
    e) Synthesis of methyl groups for Met, choline, thymine, etc.

E. Deficiency, Supplementation, and Sources

1) Deficiency:

    a) Symptoms are mostly associated with reduced synthesis of purines & pyrimidines, ∴ nucleic acid synthesis.
    b) Tissues with a rapid rate of cell growth or regeneration, such as epithelial lining of the GI tract, epidermis & bone marrow, are affected most.

2) Supplementation:

    a) Folacin requirements for livestock are generally met by good practical diets & substantial amounts of folacin produced by microbes in most species.
    b) For some animals such as chicks, guinea pigs & monkeys, an adequate dietary folacin is must!

        • Can readily induce deficiency signs in those animals!

    c) For others such as rats, dogs & pigs, folacin produced by microbes (+ dietary) is usually adequate to meet the requirement.

        • ∴ the deficiency signs do not develop unless a bacterial growth is somehow depressed - e.g., the use of antibiotics.

3) Ruminants:

    a) Probably folacin synthesis in the rumen is adequate to meet the requirement of mature animals (+ green forages are a very good source of folacin).
    b) Although a deficiency has not been clearly demonstrated, young animals may experience a deficiency:

        • e.g., lambs fed synthetic diets developed deficiency signs, such as leukopenia followed by diarrhea, pneumonia & death, in one experiment.

4) Swine:

a) The folacin deficiency can be produced only in a combination with sulfa drugs, indicating the intestinal synthesis may be adequate.

- Deficiency signs were not observed when young pigs were fed diets containing natural ingredients (or even with purified diets).
- But, probably need to consider the difference between no apparent deficiency signs vs "optimum" performance of animals!

b) Deficiency signs include anemia (↓ hematopoiesis), listlessness, ↓ feed intake & growth rate, diarrhea & poor reproductive performance.

5) Poultry:

a) More susceptible to a lack of folacin vs others, i.e., can produce deficiency signs very easily.
b) Sings include anemia, poor feathering/growth, cervical paralysis & others in young birds, and poor hatchability & ↑ embryonic mortality in breeding birds.

6) Humans:

a) Probably the most common vitamin deficiency in the world, and infants, adolescents, elderly & pregnant women are especially vulnerable.
b) Signs include megaloblastic red cell maturation/macrocytic anemia, gastrointestinal lesions/malabsorption & diarrhea, weakness, forgetfulness, sleeplessness, sterility, etc.
c) Often associated with chronic alcoholism:

(1) e.g., 40-87% of alcoholics have low serum folacin level, and 40-61% of alcoholics show signs of megaloblastic anemia.
(2) May be associated with inhibition/reduction of hematopoiesis & ↓ in the absorption of folacin with alcohol?

F. Sources:

1) Widely distributed in nature, almost exclusively as THF derivatives, which generally possess three or more Glu residues.
2) "Abundant sources" - e.g., green leafy materials (e.g., 5.5 mg/kg in alfalfa meal) & organ meats (e.g., 8.4 mg/kg in the liver).
3) "Good sources" - e.g., beans, nuts, some animal products & citrus fruits.
4) "Poor sources" - e.g., cereal grains (e.g., 0.3 mg/kg in corn), milk (0.7 mg/kg) & eggs.

G. Folic acid requirements:

Animal	mg/kg or µg/d
Poultry: (NRC, 1994)	

Immature chickens	0.23–0.55
Laying hens	0.21-0.31
Broilers	0.50-0.55
Turkeys, growing	0.70-1.00
Turkeys, breeding	1.00
Swine: (NRC, 1998)	
Growing pigs	0.30
Sows & boars	1.30
Horses, adult (NRC, 1978; No established requirement, but may respond to)	20 mg/d
Fish: (NRC, 1993)	
Channel catfish	1.5
Rainbow trout	1.0
Pacific salmon	2
Adult cat	.80
Growing dog	.20
Fish	1.00-1.50
Rat	1.00
Beef & dairy cattle, sheep, goats	Microbial synthesis
Humans, µg/d): (RDA)	
Infants	25-35
Children (< 14 yr)	50-150
Males	200
Females	180
Pregnant	400
Lactating	260-280

1) Folacin in common ingredients (corn, 0.30 & soybean meal, 0.70 mg/kg) + microbial synthesis may be adequate to meet the requirements of animals fed practical diets.
2) Folic acid is presently added to most fish diets.
3) Many factors can affect the requirement - Other vitamins (e.g., B_{12}, choline), use of antimicrobial agents & molds in feeds, dietary amino acids (Met, Gly & His), etc.

H. Toxicity

1) There is no report on adverse effects of large amounts of folacin in animals, and the vitamin is generally regarded as "nontoxic."
2) No adverse effects with 400 mg/d for 5 mo or 10 mg/d for 5 yr in adult humans.

I. Folacin supplementation - Examples in pigs:

- A good review paper - Lindemann, M. D. 1993. Supplemental folic acid: A requirement for optimizing swine reproduction. J. Anim. Sci. 71:239-246.

1) Effects of supplemental folic acid (FA) & sulfamethazine (S) on sow performance: (Lindemann & Kornegay, 1989. J. Anim. Sci. 67:459)

Item	0 ppm FA/ 0 ppm S	0 ppm FA/ 110 ppm S	1 ppm FA/ 0 ppm S	1 ppm FA/ 110 ppm S
Litter size:				

Total born[a]	9.76	10.70	11.04	11.31
Born alive[a]	9.51	10.22	10.64	10.93
At d 21	8.95	9.15	9.29	9.46
At weaning	8.92	9.10	9.24	9.44
Pig wt, kg:				
Birth, all	1.47	1.50	1.47	1.44
Birth, live	1.48	1.51	1.48	1.44
At d 21	5.82	5.69	5.68	5.52
At weaning	7.68	7.56	7.44	7.52
Breedings/litter farrowed[b]	1.17	1.16	1.10	1.04
Days to estrus after weaning	7.30	5.20	6.66	6.09

[a]Folic acid effect, $P < .05$ & [b]folic acid effect, $P = 0.12$.

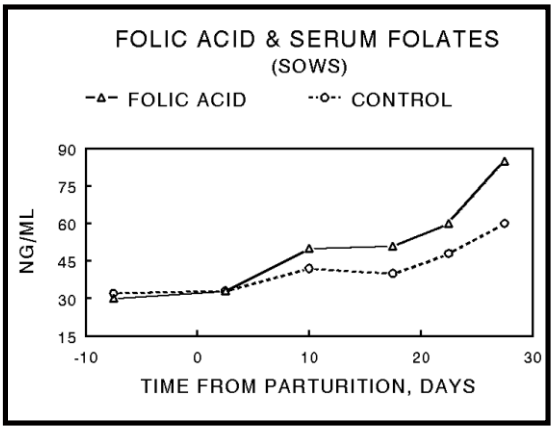
2) Effects of folic acid on litter criteria: (Thaler et al., 1989. J. Anim. Sci. 67:3360)

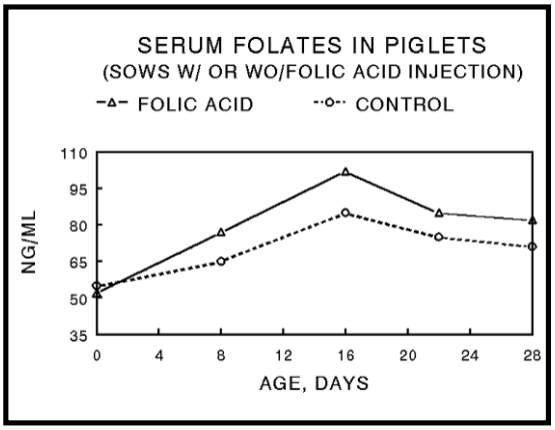
	Supplemental folacin, mg/kg		
Item	0	1.65	6.62
Total pigs born[a]	8.86	9.84	9.41
Pig born alive[a]	7.93	8.88	8.21
Pigs on d 21[b]	7.79	8.66	7.91
Birth wt, kg	1.54	1.52	1.51
21-d wt, kg[c]	5.96	5.63	5.88

[a]Quadratic effect, $P < .05$; [b]Quadratic effect, $P < .001$; [c]Quadratic effect, $P < .01$.

3) Effects of injection of folic acid (15 mg/wk from d 2 postpartum to weaning) to sows on serum folate: (Matte & Girard, 1989. J. Anim. Sci. 67:426)

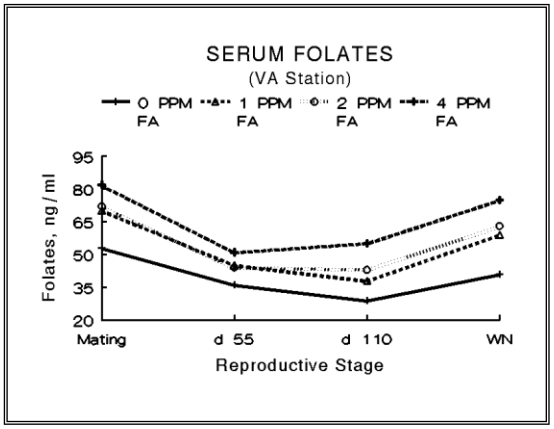
- Folic acid supplementation of sow diets increased serum folate in sows & their pigs, but it had no effect on the growth rate of piglets from birth to 8 wk of age!





4) Dietary folic acid supplementation & reproductive performance of sows (A cooperative S-145 regional study): [Harper et al., 1994. JAS 72:2338]

a) Folic acid supplementation had beneficial effects on litter size & weights at one station (Auburn), but had no effect at other stations.
b) Blood folate concentrations ↓ progressively from weaning to late gestation & this depression can be attenuated by supplemental folic acid (data collected at Virginia only), but it had no effects on reproductive performance of sows.
c) Conclusion? - "A litter size response to folic acid supplementation may not be elicited under all circumstances!"



☛ The bottom line?

a) Folic acid supplementation to corn-soy diets can improve reproductive performance of sows. The 1988 NRC requirement for sows may be too low, but the optimum supplementation level has not been established.
b) Can enhance the folate status of piglets by treating sows, but had no effect on growth performance of piglets.
c) Dietary supplementation of baby pig diets may be unwarranted. (Also, folic acid supplementation of starter to finisher diets has been resulted in inconsistent responses!)

J. Folacin for Humans? (Bosco, 1989)

1) Folacin & mental illness/functions?

a) Folacin can alleviate signs of polyneuropathy (inflammation of nerves → weakness in extremities, loss of reflexes & feeling, etc.).
b) Folacin can improve everyday mental capacity:

(1) One researcher reported that a folacin deficiency can result in an atrophy of the brain & mental disorders.
(2) May have beneficial effects on forgetfulness, apathy, irritability, insomnia, depression, dementia, etc.
(3) Also, folacin may help schizophrenia patients.

c) The neurological impairment caused by a folacin deficiency may be labeled inappropriately as "senility" (according to one British study).

B. Also, may have beneficial effects on heart disease, psoriasis, gingivitis, cancer & others.

3. Vitamin B_{12}

A. Introduction [See Maynard et al. (1979, McDowell (1989) & others]

1) General:

a) The last known vitamin to be discovered, and it is the most potent vitamin.
b) Formally known as a "chick growth factor" or an "animal protein factor," and three seemingly unrelated conditions led to its identification:

(1) A fatal anemia in humans.
(2) A potent growth factor for nonruminant species.
(3) Lack of Co & wasting diseases in ruminants.

- Contributing factors?

(1) Before WW II - Fed many protein sources to animals.
(2) Limited resources led to the use of plant based diets for animals - e.g., corn-soy.
(3) Found that animals would not perform well without some animal protein sources.
(4) Believed that the animal sources have some “unidentified growth factor/UGF!”
(5) UGF? - Found to be vitamin B12, thus the name, "chick growth factor" or "animal protein factor!"

c) Synthesized in nature only by microorganisms, ∴ usually not found in plant feedstuffs.
d) Cobalt is an integral part of the molecule.
e) Vitamin B_{12} is a generic name for a group of compounds having the B_{12} activity.

2) History:

a) In 1824, Combe described a fatal anemia & suggested its relation to a disorder in the digestive tract.
b) In 1926, Minot & Murphy showed that a large amount of raw liver would alleviate pernicious anemia, ∴ recognized the existence of an unknown factor that can be used for treatment.
c) During the World War II, efforts were made to eliminate expensive & scarce animal proteins sources from the diet of farm animals:

(1) All-vegetable diets ↓ gain in pigs & chickens and hatchability of eggs.
(2) The deficiency was corrected by an unidentified factor in the liver, fish meal or cow manure.

- Cornell workers called/named the substance "Animal Protein Factor!"

d) Cary et al. (1946) demonstrated the presence of a factor, which was necessary for the growth of rats, in purified liver extracts, and named it "factor X!"
e) Zucker & Zucker (1948) named a rat factor "Zoopherin."

- Shortly thereafter, it became apparent that these factors were identical, and probably synthesized by the microbes because it was present in the cow manure.

f) Between 1951 & 1953, two groups reported an isolation of vitamin B_{12}:

(1) Folkers et al. (U.S.) isolated the "animal protein factor," which was aided by a microbiological assay developed by Shorb in 1947.
(2) Smith et al. (U.K.) isolated the "antipernicious anemia factor" after testing all fractions associated with "relapse" of pernicious anemia patients.

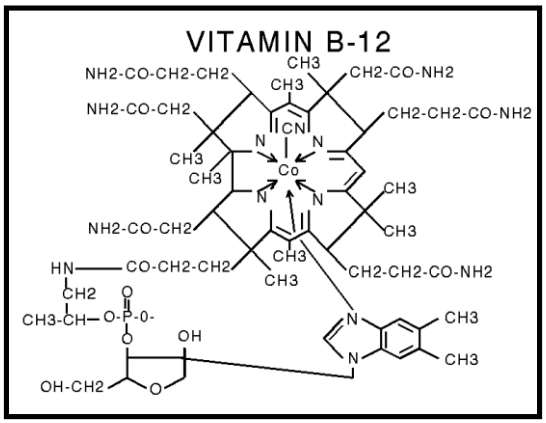
- Smith was the first one to identify Co as a constituent of the "factor!"

B. Structure/analysis

1) Structure & properties:

a) Structure: (Adapted & redrawn from Martin et al., 1983 & McDowell, 1989)
b) Resembles a porphyrin structure, i.e., consisting of four pyrrole nuclei with each inner N atom coordinated with a single atom of Co (≈ 4.5% Co in the vitamin).
c) The name "cobalamin" is used for a compound, in which the Co atom is the center of the corrin nucleus.
d) Cyanide can be replaced by others such as OH (hydroxycobalamin), H2O (aquacobalamin), NO2 (nitrocobalamin) & CH3 (methylcobalamin) ← all active!

- "Cyanocobalamin" is not a naturally occurring form of the vitamin, but an artifact of the isolation procedure - most commonly used form because of its relative availability & stability!



e) A hygroscopic, crystalline, dark-red substance.
f) Soluble in water & alcohol, but insoluble in acetone, chloroform or ether.
g) The exposure to oxidizing & reducing agents and sunlight tends to destroy its activity.

h) Losses during cooking are not excessive because it is stable at 250°C or less.

2) Analysis:

a) "Chemical methods" - spectrophotometric & colorimetric procedures have been developed, but not sensitive enough for the vitamin in natural materials.
b) "Microbiological methods" - sensitive & can be applied to crude materials, but may also respond to several pseudovitamins. [Lactobacillus leichmannii & Euglena gracilis, and Ochromonus malhamensis (protozoan).]
c) "Isotope dilution methods" - based on a competition with radioactive cyanocobalamin for binding sites on protein after liberation. (Being widely used in recent years.)

C. Metabolism

1) Absorption/transport:

a) The vitamin in the diet is bound to food proteins, and it can be released by "low pH" & peptic digestion.
b) To be absorbed, must be bound to the "intrinsic factor" (a glycoprotein), which is synthesized & secreted by parietal cell of the gastric mucosa.

- There is evidence that both active & passive (diffusion?) absorption mechanisms exist for this vitamin!

c) Absorption takes place at the terminal ileum.
d) After absorption, the vitamin is bound to specific transport protein called "transcobalamins," and three binding proteins have been identified in a normal human serum.

2) Distribution/excretion:

a) In most species, B_{12} is found ↑ in the liver (1.5 mg in humans), and also in heart, spleen & brain (≈ 20-30 µg in humans).

- In pigs, 20-30% of oral dose can be retained, and ⅔ of retained vitamin can be found in the liver.
- Although it is a water-soluble vitamin, a tissue half-life is ≈ 32 d, indicating a considerable degree of tissue storage.

b) "Conversion" - B_{12} must be converted to "active" form, which takes place ↑ in the liver, but also in the kidney:

(1) Two forms in humans:

(a) Methylcobalamin - 60-80% of total plasma cobalamin.
(b) Adenosylcobalamin (cellular tissues) - 60-70% of the vitamin in the liver & 50% of the vitamin in other organs.

(2) Cyanocobalamin is converted within cells to "methyl-from" by methyltransferase or adenosyl-form by mutase.

c) Excretion:

(1) Total body loss ranges from 2 to 5 µg/d in humans.
(2) Urinary excretion of the intact vitamin is minimal.
(3) Biliary excretion via the feces is the ↟ route.

D. Biological Functions

1) Two types of cobalamin coenzymes that participate in biochemical reactions:

a) Adenosylcobalamin - a 5'-deoxyadenosine linked covalently to the Co atom.
b) Methylcobalamin - a methyl group attached to the central Co atom.

2) Most reactions involve transfer or synthesis of one-C units (e.g., methyl groups):

a) Metabolism of labile methyl groups - e.g., synthesis of Met by 5-methyl-THF methyltransferase, "5-methyl-THF + homocysteine ⇄ Met + THF."
b) Conversion of propionate to succinate by methylmalonyl CoA isomerase (mutase):

- Propionate + ATP + CoA ⇄ propionyl-CoA
  Propionyl-CoA + CO2 + ATP ⇄ methylmalonyl-CoA
  Methylmalonyl-CoA (inactive) ⇄ methylmalonyl-CoA (active)
  Methylmalonyl-CoA (active) ⇄ succinyl-CoA

- A special interest for ruminant species because of the production of large amounts of propionate during CH2O fermentation.

3) Known enzymatic functions: (Weissbach & Taylor, 1968. Vitamin & Hormones 26:395)

a) Synthesis of methyl groups for Met & choline synthesis.
b) Protein metabolism (AA incorporation).
c) Transmethylation (Met ↔ homocysteine ↔ choline).
d) Purine biosynthesis & nucleic acid formation.
e) Conversion of carbohydrates to lipids.

E. Deficiency/Supplementation

1) General:

    a) Deficiency signs in humans are megaloblastic anemia & neurological lesions, which can lead to weakness, tiredness, progressive paralysis, mental disorders, diarrhea, loss of appetite & weight, etc.
    b) The vitamin functions primarily as a growth factor in nonruminant species:

        (1) Very inconsistent results in animal studies - perhaps due to various factors such as initial body stores, other dietary nutrients & factors that can influence microbial synthesis.
        (2) "Supplementation" - normally add B_{12} to swine and poultry diets because they have less access to feces in today's production systems.

2) Ruminants:

    a) A deficiency can occur in calves < 6 wk-old that receive no dietary animal protein, and signs include poor appetite & growth, muscular weakness, demyelination of peripheral nerves & poor general condition.
    b) A Co-deficiency can causes B_{12} deficiency in adults:

        (1) Co-deficiency signs are not specific & difficult to distinguish from malnutrition, diseases or parasite infestations.
        (2) Signs may include lack of appetite, rough hair coat, thickening of the skin, "wasting away" and death.
        (3) Subclinical Co-deficiencies or "borderline" Co status are fairly common, but often unnoticed, ∴ can be costly (& may not realize!).

3) Swine:

    a) "Growing pigs" - deficiency signs include loss of appetite, ↓ gain, rough skin & hair coat, vomiting & diarrhea, voice failure, slight anemia & nervous disorders.
    b) "Reproducing animals" - additional signs include ↓ litter size, survival rate & birth weight, abortions, inability to rear the young, delayed estrus, etc.

4) Poultry:

    a) "Growing birds" - signs include ↓ feed intake, gain & feed efficiency, nervous disorders, defective feathering, anemia, gizzard erosion & fatty liver, heart & kidneys.
    b) "Breeding animals:" (May take 2-5 mo to deplete the vitamin!)

        (1) A great ↓ in hatchability.
        (2) Embryos die ≈ 17th d, and show leg myoatrophy, malposition of the head, multiple hemorrhages, enlarged heart & thyroid & fatty liver.

(3) A high mortality rate for those hatched.

c) Also, "perosis" may occur in conjunction with inadequate choline, Met or betaine.

5) Factors that can influence the vitamin B_{12} status in humans: (McDowell, 1989)

a) Inadequate dietary intake, especially among vegetarians.
b) "Absorption/transport failure" - inadequate secretion of carrier proteins because of digestive disorders, excessive intake of alcohol, ingestion of certain drugs & others.
c) "Hereditary" - pernicious anemia can be inherited as an autosomal dominant trait, which affect mainly persons past the middle age. (Associated with an inadequate production of intrinsic factor, glycoprotein.)

- Affects ≈ 1-2 per 1,000 in a general population & 25 per 1,000 among relatives of pernicious anemia patients.

F. Sources, Requirements & Toxicity

1) Sources:

a) Synthesized by many bacteria, but not by yeasts or most fungi.
b) No convincing evidence for vitamin production by higher plants or animals.

- Higher plants contain a small amount of B_{12}, but the vitamin is probably produced by "soil-microbes" & absorbed by plants?

c) Synthesis in the GI tract by microbes:

(1) Microbial vitamin can be used to satisfy a portion of the needs in animals - e.g., via coprophagy/absorption in swine & absorption in poultry.

- But, the rate of absorption is unreliable, ∴ must be supplemented!

(2) With consumption of sufficient amounts of Co, ruminants are independent of external source of B_{12}.

d) Foods/feeds of animal origin are good sources of the vitamin, and kidneys & liver are excellent sources.

- "Ruminant-sources" are generally higher in the B_{12} contents than most "nonruminant-sources."

e) The B_{12} is commercially produced by fermentation & available as cyanocobalamin, which is highly stable in premixes & also during the pelleting process.

2) Requirements:

a) Affected by dietary levels of choline, folacin, Met, etc.
b) Requirements (animals need very small amounts, i.e., B_{12} is the most potent vitamin!):

Animal	μg/kg
Poultry: (NRC, 1994)	
Immature chickens	3-9
Laying hens	4
Broilers	7-10
Turkeys	3
Swine: (NRC, 1998)	
3-20kg	15-20
20-120kg	5-10
Sows/boars	15
Horses (1978)	Microbial synthesis
Fish: (NRC, 1993)	
Channel catfish	Required, but not determined
Rainbow trout	0.01 mg/kg (estimated)
Pacific salmon	Required, but not determined
Common carp & Tilapia	No dietary requirement
Beef cattle, adult dairy cattle, sheep, goat, rabbit	Microbial synthesis
Dog & cat	20-26
Fish	2-3
Rat	50
Humans, µg/d: (RDA)	
Infants	0.30-.50
Children	0.70-1.40
Adult	2.00
Pregnant/lactating	2.20-2.60

3) Toxicity:

(1) Based on the study using mice, high doses of B_{12} given i.p. or i.v. seem to be innocuous (at least several hundred times the requirement?!).
(2) Other species ...?

4. Cobalt

A. General

1) "Nonruminant species" - probably no requirement for Co or no beneficial effect of dietary Co if their diets contain adequate levels of vitamin B_{12}, i.e., serving as a cofactor for B_{12} (≈ 4.5% Co) might be the only role of Co!

2) "Ruminant species" - Co is a dietary essential nutrient!

B. Co Deficiency

1) Several forms of Co deficiency in sheep & cattle have been given various "local names:"

a) Bush-sickness in New Zealand.
b) Coast disease & wasting disease in Australia.
c) Salt sick, neck ail & Grand Traverse disease in U.S.
d) Pining disease in U.K. ....., etc.

- A suggested designation of all these conditions is "enzootic marasmus" or "muscular wasting."

2) Cattle & sheep in those areas:

a) Were unable to thrive unless periodically moved to "healthy" areas.
b) Ones remaining in the "deficient" area stopped eating, became weak & emaciated, and often died.

- Because these conditions were cured by moving to the other areas, it was assumed that those were nutritional deficiency diseases.

3) By 1935, Australian workers had demonstrated that Co was effective in preventing coast & wasting diseases.
4) In 1937, Co deficiency in cattle was established in Florida, i.e., Co deficiency was responsible for the condition known in that region as "salt sick."
5) The action of Co in the body & reason for its necessity were not discovered until 1948:

a) Smith (U.K.) and Rickes et al. (U.S.) discovered vitamin B_{12}.
b) Both research reported that their compounds contained Co, which were effective against pernicious anemia in humans.

C. Properties, metabolism & Excretion

1) Properties/distribution:

a) A gray, hard, magnetic metal, and hydrated salts are red.
b) Co concentrations in soils and plants are highly variable.
c) Widely distributed in the animal body, with high concentrations in the liver, bone & kidney.

(1) The total Co content ≈ 1.5 mg in normal adult humans, with .11 mg in the liver, 0.2 mg in the skeletal muscle, 0.28 mg in the bone, 0.31 mg in hair & 0.36 mg in the adipose tissue (Smith, 1986. In: Mertz, 1986).
(2) A similar distribution of Co in the tissues of other species (mice, rats, rabbits, pigs, dogs, chicks, sheep & cattle), with little distinction among species.

- A large variation in values reported because tissue levels of Co are very low compared to other elements & also differences in the analytical method.

2) Absorption, storage & excretion:

a) Absorption site/rate:

(1) In most species, the absorptive site is the lower portion of the SI.
(2) Orally administered Co is well absorbed by small Lab animals & humans - e.g., 26.2% of oral dose of labeled Co in normal mice & 20-97% in humans (based on balance studies).
(3) Much less efficient in ruminant species:

(a) A Co to B_{12} conversion rate was only ≈ 3% in the rumen with an adequate Co intake, and it ↑ to ≈ 13% on those on a Co-deficient diet (Smith & Marston, 1970. Br. J. Nutr. 24:857).
(b) Following oral or intraruminal administration of labeled Co to sheep or cattle, 84-98% appeared in the feces within 5-14 d (Smith, 1987. In: Mertz, 1986).

- Of the total B_{12} produced, only 1 to 3% may be absorbed.

b) Body storage:

(1) ≈ 43% of the body Co is stored in muscles, ≈ 14% in the bone, and the kidney & liver contain most of the remaining Co.
(2) Even though, vitamin B_{12} is water-soluble, a tissue half-life has been reported to be 32 d, indicating a considerable tissue storage of this vitamin.

c) Excretion:

(1) In ruminants, both Co & B_{12} are mainly excreted in the feces - e.g., 86 to 87.5% of absorbed Co excreted in the feces (↓ via the bile), 0.9 to 1% with urine & 11.5 to 12.5% with milk in lactating cows fed a normal diet.
(2) In humans, the major route of excretion is the urine, and small amounts in the feces, sweat & hair.

D. Function, Deficiency & Requirements

1) Function & deficiency:

    a) Only known function of Co is its participation in the metabolism as a component of vitamin B_{12}.
    b) ∴ deficiency signs are reflection of the vitamin deficiency.
    c) Other functions? - Co can partly substitute Zn in carboxypeptidase and alkaline phosphatase.

2) Ruminants:

    a) Have higher requirements for B_{12} vs nonruminants because of "propionic acid" production.
    b) Young ruminants (i.e., < 6-8 wk of age) need dietary source of B_{12} - e.g., calves may need 0.34-0.68 µg/kg BW.
    c) Requirements:

        (1) Beef cattle, daily cattle (& horses) - .10 ppm on the DM basis.
        (2) Sheep - 0.10 to 0.20 ppm.

        • On the average, grasses in healthy areas contain ≈ > .10 ppm vs .004 to .07 ppm Co for those in deficient areas.

3) Nonruminants:

    a) Co deficiency per se has never been clearly demonstrated in nonruminant species.
    b) Most species have a limited ability to synthesize B_{12} in the lower tract, and their absorption capacity is probably minimal.
    c) There are some indications that the synthesis & subsequent absorption of B_{12} are more efficient in horses & rabbits vs other nonruminants & ruminants.

E. Toxicity

1) Co or vitamin B_{12} has low toxicity in all species studied.
2) The maximum dietary tolerable level for common livestock species is 10 ppm, but:

    a) 150 ppm Co (1,000 times the normal levels) for many weeks had no adverse effects on sheep.
    b) Pigs fed corn-soy diets can tolerate up to 200 ppm.

3) Signs may include ↓ feed intake & weight, emaciation, anemia & debility in most species, and excessive urination, defecation & salivation & shortness of breath in cattle.
4) The toxicity is partly due to the mineral antagonism - e.g., anemia is the result of ↓ Fe absorption caused by excess Co.

5. Sulfur

A. Introduction

1) General & functions:

a) Sulfur is one of more abundant elements in nature, but a shortage of S-AA is a worldwide problem in the animal nutrition.
b) Found in nature both in the elemental state and as sulfides [sulfur pyrites (FeS2), copper pyrites (CuFeS2) ....] & sulfates [gypsum (CaSO4•2H2O), anhydrite (CaSO4) .....].
c) Sulfur is a component of many compounds, ∴ involved in a wide range of functions in the body:

(1) "S-amino acids" - constituents of tissue proteins & various biologically active substances such as hormones & vitamins:

(a) "Cysteine" - e.g., presents as "-SH" groups in many compounds, participates in glutathione synthesis, a precursor of coenzyme A, etc.
(b) "Cystine" - presents as a "-S-S-" group in many compounds.
(c) "Met" - a source of methyl groups for choline, acetylcholine, etc.

(2) Hormones - e.g., insulin, prolactin, ACTH, oxytocin, vasopressin, etc.
(3) Vitamins - e.g., thiamin & biotin.
(4) Sulfate compounds - e.g., thiols (-SH group), disulfides (-S-S- group) & chondroitin sulfates (important component of cartilage, tendons, bones & wall of blood vessels).
. . . , and many others.

2) Distribution:

a) Most animals contain ≈ .16 to .23% S on the live weight basis.
b) Concentrations ↑ with age, probably because of ↑ muscle protein & accumulation of S in hair or feathers - e.g., ↑ from 2.03 g (d 10) to 2.85 g S/kg fresh tissue (d 70) in chickens.
c) Distribution of S in the animal body:

(1) ≈ 50% in muscle tissues, ≈ 15-17% in hide, hair & horny tissues, ≈ 9-10% in bones & cartilage, ≈ 6-7% in blood, ≈ 5-6% in liver, etc.
(2) Except in the cartilage, S is present in all tissues as AA.
(3) In plasma, a protein fraction constitutes 80-90% of the total S, and non-protein fraction contains oxidized (e.g., organic sulfates) & neutral S (e.g., derivatives of the thiol group).

B. Absorption & Excretion

1) Absorption takes place in the SI:

a) Free S-AA, thiamin, biotin & others are probably absorbed intact.
b) Bound S-AA are absorbed following the cleavage of proteins.
c) Inorganic sulfates can be absorbed, but only to a small extent.

2) Excretion:

a) Products of the S metabolism (free or esterified sulfates, taurine, thiosulfates & others) are mostly eliminated in the urine.
b) Forms present in the urine:

(1) Mineral (sulfate) - mostly from oxidized AA, but partly from absorbed & unutilized sulfates from feeds.
(2) SO_4^{2-} ions - excreted together with Na^+, K^+ & NH_4^+ cations (equivalent amount).
(3) Esterified - phenol & cresol sulfates (Phe & Tyr derivatives, respectively), or indoxyl & skatoxyl sulfates (Trp derivatives).
(4) Neutral - S-AA, taurine, mercaptan, thiamine, biotin & urochrome.

- ↑ neutral S might be an indication of ↑ degradation of endogenous protein!

C. Ruminants & Sulfur

1) Sulfur metabolism in ruminants - very complex/extensive! (See Georgievskii, 1982. In: Georgievskii et al., 1982)
2) General:

a) Sulfur is essential for the microbes for digestion of cellulose, utilization of NPN & synthesis of B-vitamins.
b) Forages contain significant amounts of inorganic sulfate, and certain rumen microbes can utilize it for the synthesis of S-AA & proteins.

- Sulfur in the form of sulfide (sulfate is reduced to sulfide) may be the 1̊ source of S in the microbial protein synthesis.
- (• Also, the microbes in the hind gut of nonruminants can utilize inorganic sulfate, but only to a small extent.)

c) ≈ 80% of labeled S can be found in protein of milk after infusion of radioactive 35S into the rumen of sheep & goats.

3) Sulfur requirement of rumen microbes:

a) With insufficient S, may see ↓ digestibility of feedstuffs, N retention & synthesis of B-vitamins.
b) Sulfur is required by all rumen bacteria, but not all bacteria can utilize inorganic S - e.g., only 5 of 10 strains studied utilized inorganic S for the synthesis of S-compounds in one study.

4) Dietary S requirement:

a) Established by supplementing diets with Met, Cys, sulfate salts or elemental S.
b) Na- & Ca-sulfate, dl-Met & Met hydroxy analogs are similar in promoting cellulose digestion (in vitro).

- The optimum dietary level of S was estimated to be .16 to .24% in one study, and the estimates by others were very close to this range.

5) Dietary "N:S ratio:"

a) Natural protein feedstuffs normally contain sufficient S to meet the requirement for rumen microbes, but may need additional S for microbial protein synthesis when NPN sources are used, i.e., additional N has no value unless additional S is provided.
b) Loosli (1952. Feed Age 2:44) suggested the N:S ratio of 15:1 based on observation that the ratio in animal tissues is relatively constant.

6) Relation to other elements:

a) Cu & Mo:

(1) Mo ↓ liver Cu storage.
(2) S potentiates Cu-Mo antagonism & influences Mo excretion in the urine.

- Thus, an excess of one of these elements may influence the requirement of other(s).

b) Selenium:

(1) Seleno-AA & S-AA have similar chemical structures, and they compete each other for reactive sites on enzymes.
(2) At the same time, S-AA can reduce the toxic effects of Se.

D. Poultry & Sulfur

1) Poultry can satisfy a part of the total S requirements (including S-AA) with inorganic S (Gordon & Sizer, 1955. Science 122:1270.):

a) An addition of .50% Na sulfate to a diet deficient in Cys (0.08% Cys & 0.51% Met) resulted in 31.4% growth response over the basal diet.
b) An addition of 0.50% Na sulfate & .22% Met resulted in 66.1% growth response over the basal diet.

2) A three-way interaction in Met, choline & S:

a) "Inadequate choline" can ↑ "Met requirement" because more Met would be used as a methyl group donor.
b) "Inadequate S" can ↑ "Met requirement" because Met can be used as a source of S.

E. Deficiency & Toxicity

1) Deficiency signs include a loss of appetite, ↓ weight gain & production (e.g, eggs & wool), lacrimation, dullness, weakness, emaciation & others, and death.
2) Toxicity:

a) Signs include anorexia, weight loss, constipation, diarrhea & depression, and pulmonary emphysema, cardiac petechiation, congestion of the CNS, acute catarrhal enteritis & hepatic necrosis in fatal cases.
b) "Upper safe levels" - insufficient data to establish levels!

(1) .40% might be the maximum for ruminants (based on the data using sheep & Na sulfate).
(2) Feeding .28% S for 16 wk had no adverse effects on dogs.

- The optimum total S level for rats has been estimated to be .69%, so . . . ?

F. Requirements

1) The animal's requirements for S are generally met by S-containing AA, and partly by heterocyclic compounds (e.g., biotin & thiamin).
2) Ruminant species may need a dietary supplementation for optimum protein synthesis when using "NPN" as indicated before.
3) Requirements: (NRC)

Animal	%
Beef cattle	0.10
Dairy cattle:	
Growing, mature bulls & dry-cow	0.16
Lactating	0.20
Calf milk replacer	0.29
Sheep	0.14-0.26
Horses	0.15
Others	Not established

6. Molybdenum

A. Introduction

1) General:

a) Molybdenum was discovered ≈ 1782, but the interest for this element in animals started ≈ 1938:

(1) Ingestion of forages high in Mo caused a debilitating scouring disease of cattle in the U.K.
(2) Large doses of Cu sulfate was effective in prevention & cure of disorder caused by Mo, and conversely Mo limited Cu retention, indicating a Mo-Cu interaction!
(3) The effect of Mo on Cu was exhibited only in the presence of adequate amounts of inorganic sulfate.

- These findings indicate a three-way interaction among Cu, Mo & S.

b) Essentiality of Mo in animals:

(1) Two groups of workers independently discovered that a flavoprotein enzyme, "xanthin oxidase," is a Mo-containing enzyme. (DeRenzo et al., 1953. Arch. Biochem. Biophys. 45:247; Richert & Westerfield, 1953. J. Biol. Chem. 203:915)
(2) Other Mo-containing enzymes were subsequently discovered, and direct evidence was then obtained that Mo is essential for chicks, poults and lambs.

- Included in this section because of the three-way interaction among Cu, Mo, and S, and also its involvement in xanthine oxidase.

2) Properties/distribution:

a) A dark-gray or black powder with metallic luster, or a coherent mass of silver-white color.
b) The total concentration in soils bear little or no relationship to the level of Mo in plants, and variations are due to the Mo content, soil pH & season of the year.
c) Mammals contain 1-4 mg Mo/kg live weight, with 60-65% in skeleton, 10-11% in hide, 5-6% in wool (sheep), 5-6% in muscles & 2-3% in liver.
d) On the average, ≈ 1-2 µg/dL in blood (70% in erythrocytes & the rest in plasma).

B. Absorption, Metabolism & Excretion

1) Absorption/excretion:

a) Readily and rapidly absorbed from most diets (& supplements) in the GI tract, but the exact site of "intensive" absorption is unknown.
b) The extent of absorption depends on species, age & dietary levels, but probably around 20-30% of the intake.
c) Mo is not only rapidly absorbed, but also rapidly excreted (f in the urine & partly via the bile).

- Absorption & deposition in the tissue & excretion rate are determined by complex interrelationships of Cu, Mo & inorganic sulfate.

2) Molybdenum-sulfur-copper interrelationships:

a) The three-way interaction among Cu, Mo, and S is complex and not fully understood, but may be summarized as follows: (Georgievskii, 1982. In: Georgievskii et al., 1982; McDowell, 1992)
b) Mo, especially in the presence of sulfate, ↓ the deposition of Cu in the organ and synthesis of ceruloplasmin.

- Thus, Cu excretion with the bile ↓, but its excretion in the urine ↑.

c) An ↑ in dietary Cu ↓ deposition of Mo in the liver, even though its intake remains the same.
d) When S level is ↑, the excretion of Mo in the urine ↑ considerably, and its deposition in the tissue ↓ correspondingly.

- Some interactions take place in the GI tract, whereas others at the site of metabolism!

C. Functions, Deficiency & Requirements

1) Functions:

a) Mo-containing enzymes:

Enzyme	Other co-factors	Substrate	Product
Xanthine oxidase	FAD & Fe	Xanthine & purine	Uric acid
Aldehyde oxidase	FAD & Fe	RCHO	RCOOH
Assimilatory nitrate reductase	FAD & cytochrome B	$NO_{3\_}$	$NO_{2\_}$
Respiratory nitrate reductase	FAD & cytochrome C	$NO_{3\_}$	$O_{2\_}$
Nitrogenase	?	N_2	NH3

b) Functions:

(1) Xanthine oxidase catalyzes the oxidation of reduced diphosphopyridine nucleotide, hypoxanthine & acetaldehyde, and plays important role in the metabolism of purine.

(2) Aldehyde oxidase is involved in the e-transport chain, and may be involved in the niacin metabolism.

- In plants, Mo improves N fixation - Involved in the reduction of nitrates to ammonia, which is subsequently utilized for the synthesis of protein.

2) Deficiency:

a) Birds metabolize all the N components to uric acid, thus Mo may be more critical, but ...?
b) No characteristic syndrome of Mo deficiency has been recognized, and animals perform normally even with extremely low dietary Mo.
c) According to some, "Mo" may be just on a borderline between an "essential" & "conditionally essential" element.

3) "Requirements" - Probably < .2 ppm for rats & chicks, and < 2 ppm for ruminants. (Because of interactions with Cu & S, almost impossible to estimate the exact requirement.)

D. Toxicity

1) Adverse effects of excess Mo are largely due to Cu deficiency, but:

a) Excess Mo (13.3 ppm) fed to steers ↓ CP entering the SI.
b) Relatively low levels of Mo can ↓ growth & cause infertility in heifers (seem to be independent of alterations in the Cu metabolism).
c) If diets contain adequate levels of Cu & S, substantial amount of Mo can be tolerated!?

2) Upper safe levels:

a) Nonruminants are much more resistant to "Mo toxicity," and only ruminants are affected by excess Mo under practical conditions.
b) Safe levels vary greatly from 6.2 ppm for growing cattle to ≈ 1,000 ppm for swine & adult mule deer (NRC, 1980).

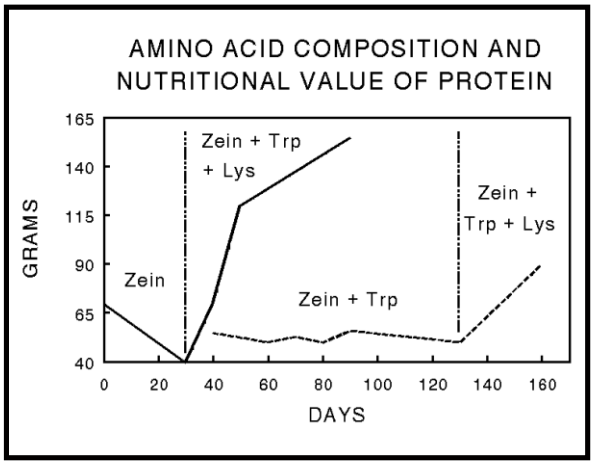
ESSENTIALITY OF AMINO ACIDS

1. Brief History [Please see Maynard et al. (1979) & others]

A. Magendie (1816) fed diets of pure carbohydrates & fats to animals, and concluded that N is essential!
B. Mulder (1839) used a term “protein,” meaning the “first” or the most important.
C. Kjeldahl (1883) developed the method for the nitrogen analysis.
D. Identified 13 amino acids in 1900.

E. Hopkins (1906) reported that feeding only zein (nothing more than the storage form of N or protein) to mice resulted in ↓ weight, but weight was maintained by adding Trp to the zein-based diet.

F. Osborne & Mendel (1914) evaluated the effect of supplementing zein with Trp and Lys: See Maynard et al.(1979). "Amino acid composition and nutritional value of protein."



1) Zein – Prolamines, which forms protein/N bodies. Nothing more than the N-storage, and for instance in corn, about 80% of protein in endosperm & ~ 50, 35, and 10 to 15% would be zein, glutelin, and albumin/globulin, respectively.
2) The amino acid composition influences the nutritional value of protein, and animal body may not synthesize many amino acids adequately!

G. Rose (1930):

1) Used semipurified diets (AA as sole N sources) and investigated the effect of addition or deletion of each amino acid.
2) Classified AA into essential or nonessential dietary constituents – Indispensable & dispensable amino acids.

2. Essential or "Indispensable" Amino Acids

A. Definition:

1) Commonly used definition - "*One which cannot be synthesized by the species in question from materials ordinarily available to the cells at a rate commensurate with the needs for the optimum growth!*"
2) The definition implies:

a) Species involved.
b) Optimum performance and(or) well-being of the animal.
c) The stage of the life cycle.

B. Indispensable & dispensable amino acids (Lewis, A.J. Pers. Comm.): [(-) = dispensable & (+) = indispensable]

Species	Ala	Arg	Asn	Asp	Cys	Glu	Gln	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
Salmon	-	+	-	-	-	-	-	-	+	+	+	+	+	+	-	-	+	+	-	+
Chick	-	+	-	-	-	-	-	+[a]	+	+	+	+	+	+	-	+[a]	+	+	-	+

Turkey	-	+	-	-	-	-	-	+[a]	+	+	+	+	+	+	-	+[a]	+	+	-	+
Mouse	-	+	-	-	-	-	-	-	+	+	+	+	+	+	-	-	+	+	-	+
Rat	-	+	+[b]	-	-	+[b]	-	-	+	+	+	+	+	+	+[b]	-	+	+	-	+
Rabbit	-	+	-	-	-	-	-	+[b]	+	+	+	+	+	+	-	-	+	+	-	+
Cat	-	+	-	-	-	-	-	-	+	+	+	+	+	+	-	-	+	+	-	+
Dog	-	+	-	-	-	-	-	-	+	+	+	+	+	+	-	-	+	+	-	+
Pig	-	+[c]	-	-	-	-	-	-	+	+	+	+	+	+	-	-	+	+	-	+
Human	-	+	-	-	-	-	-	-	+[d]	+	+	+	+	+	-	-	+	+	-	+

[a]Gly or Ser is indispensable; [b]Required for maximal growth; [c]Not required by adult animals; [d]May not be required by adults.

1) Ile, Leu, Lys, Met, Phe, Thr & Trp - Required by all species.
2) Arg, His & Val - Required by most. [(1) + (2) = "PVT TIM HALL"]
3) Chicks & turkeys - 10 AA + Gly or Ser (. . . can be synthesized & interconversible; the synthetic rate may not be fast enough for a rapid growth).
4) Swine - Arg is not essential for adults.
5) Met & Cys:

    a) Cys can be synthesized from Met, but Met cannot be synthesized from Cys.
    b) Cys can satisfy at least 50% of the need for total S-AA.
    c) Met can satisfy the need for total S-AA.

6) Phe & Tyr:

    a) Tyr can be synthesized from Phe, but Phe cannot be synthesized from Tyr.
    b) Tyr can satisfy at least 50% of the need for Phe + Tyr.
    c) Phe can satisfy the need for Phe + Tyr.

3. Nonessential Amino Acids or Nonessential N?

- May want to read *"Harper, A.E. 1974. Nonessential amino acids. J. Nutr. 104:965."*

A. From physiological, biochemical & nutritional viewpoints:

1) Clearly, all of the AA that occur in proteins are essential to the animal.
2) Thus, the term, nonessential, might be a misnomer!
3) The term, dispensable, might be a better description.

B. The term, nonessential N or amino acids:

1) Nonessential (or dispensable) AA can be synthesized from a nonspecific source of N (Glu, Ala, diammonium citrate, etc.).
2) Nonessential N becomes essential or a limiting factor in certain situations:

a) A mixture of free indispensable AA (only source of N!) does not support a maximum growth of rats or chicks, and the diet can be improved by addition of a mixture of nonspecific N.
b) No single N-source is as effective as a mixture of AA that can be synthesized by the body.

3) The term, nonspecific N (nitrogen):

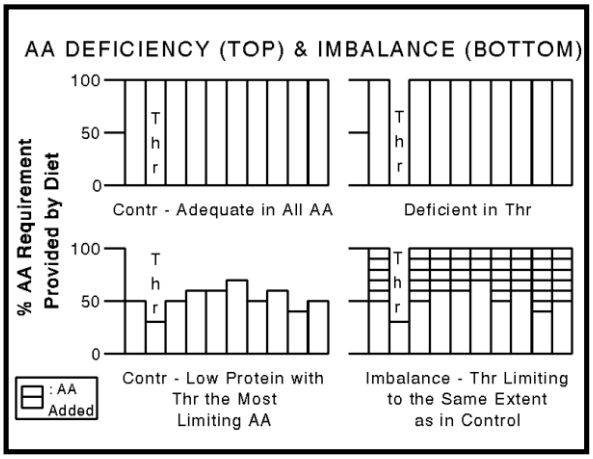
a) Does not imply essentiality or dispensability.
b) Thus, might be a better term to describe N that does not have to be provided by specific compounds.

AMINO ACID DISPROPORTION

- An excellent reference on the topic: Harper, A.E., N.J. Benevenga, and R.M. Wohlhueter. 1970. Effects of ingestion of disproportionate amounts of amino acids. Physiol. Rev. 50:428-558.

- Because of an increased availability/use of crystalline AA in recent years (feed grade Met, Lys, Trp, Thr, and Val are currently available), a chance of formulating diets containing disproportionate amounts of AA may increase in the future!

1. Amino Acid Deficiency & Imbalance



A. Difference between the “deficiency” & “imbalance” (Redrawn from Harper et al., 1970).
B. Deficiency:

1) Simply, a protein or diet is deficient in any one of the indispensable AA.
2) Animals can only utilize various AA for protein synthesis up to the level of the deficient AA in the diet/protein.
3) Observe a prompt reduction in feed intake.
4) Can be referred to as a “naturally” unbalanced protein or diet.

C. Imbalance:

1) Caused by addition of one or more amino acids other than the one that is limiting the growth of animals.
2) Pigs & rats prefer a protein-free diet to an imbalanced-diet.
3) Can be referred to as a “man-made” unbalance or disproportion.

- Both can be corrected by supplying the “growth-limiting” amino acid!

2. Amino Acid Toxicity

A. Caused by addition of a large amount of individual amino acid(s).
B. Depending on AA, may see specific gross or histopathological lesions.
C. Cannot be corrected by simply adding other amino acids.
D. Order of toxicity? - Met (most toxic) → Trp → His → Tyr → Phe → Cys → Leu → Ile → Val → Lys → Thr (least toxic). (Signs? - e.g., with 2% Met, may see liver & pancreas damages, darker spleen, etc.)

3. Amino Acid Antagonism

A. One AA can affect the requirement of another by interfering with the metabolism.
B. Example? Antagonisms among structurally similar AA - Between Lys and Arg (basic AA), and among Val, Leu & Ile (branched-chain AA).
C. Can be corrected/prevented by adding only a structurally similar AA(s).

4. Amino Acid Interactions?

A. e.g., Effects of amino acid interactions on chicks[a]:

	Ad libitum			Force fed		
Diet	Gain, g/d	Feed, g/d	F:G	Gain, g/d	Feed, g/d	F:G
Thr imbalance:						
Basal	9.1	18.8	2.05	9.3	17.8	1.91
+ 30 g Ser/kg	7.6*	16.2*	2.15	9.9	17.8	1.80
BCAA antagonism:						
Basal	9.8	21.7	2.67	9.6	21.5	2.23
+ 38 g Leu/kg	6.8*	18.4*	2.20*	8.7	21.5	2.47
Lys-Arg antagonism:						
Basal	9.9	18.4	1.86	8.6	17.4	2.02
+ 12 g Lys/kg	5.5*	15.2*	2.76*	7.4*	17.5	2.37*

[a]Data compiled by Austic, 1986. Biochemical description of nutrient effects. In: C. Fisher & K.N. Boorman (Ed.). Nutrient Requirements of Poultry and Nutritional Research. pp 59-77. Butterworths, London.

B. Most amino acid interactions can cause a reduced feed intake! - Perhaps, ↓ limiting AA in plasma may be a biochemical signal leading to ↓ feed intake because the infusion of small amounts of some AA prevented feed intake depression associated with the imbalance.
C. Imbalances:

1) "Force-feeding" generally results in normal growth rate & body composition?
2) Thus, "poor" performance associated with the imbalance seems to be due almost exclusively to the reduced feed intake?

D. Antagonisms - The biochemical basis for BCAA antagonism is not known, but it seems to have specific effects in addition to effects on feed intake:

1) Excess Leu:

a) ↑ the activity of muscle BCAA amino transferase in chicks, hepatic BC α-ketoacid dehydrogenase in rats.
b) ↑ the oxidation of Ile & Val in chicks & Ile in rats.
c) A possible loss of significant amounts of Ile & Val via catabolism may explain the failure of force-feeding to prevent the adverse effects!?

2) Lys-Arg antagonism in chicks:

a) Signs? ↓ creatine synthesis, ↑ activity of kidney arginase & urea excretion, and ↑ urinary excretion of Arg when Lys levels are highly excessive.
b) Lys & Arg share a membrane-bound carrier, and excess Lys inhibits the binding of Arg to the carrier, ∴ ↓ reabsorption (kidneys) & ↑ excretion. (But, a moderate excess of Lys may not ↑ Arg excretion!)
c) ↑ urea excretion is probably due to Arg degradation via kidney arginase because the chick lacks a functional urea cycle.

5. Factors Affecting Amino Acid Disproportion?

A. Degree of disproportion:

1) Imbalance - Caused by as little as 1/5 of the requirement?
2) Antagonism & toxicity - With at least twice the requirememnt?

B. Age - Older animals can tolerate better.
C. Adaptation - Some can show some adaptation, but can be dependent on the degree of amino acid disproportion.

PROTEIN QUALITY

- *A good reference: "Hackler, L.R. 1977. Methods of measuring protein quality: A review of bioassay procedures. Cereal Chem. 54:984. Also, see Maynard et al. (1979) & Jurgens (2002)."*

1. Protein Quality

A. Simply refers to the amount and ratio of indispensable amino acids present in a protein.
B. Protein quality & satisfying the requirement

Protein quality	Requirement[a]
100	1 x

50	2 x
25	4 x
12.5	8 x
0	Infinite

[a]The amount of protein needed to satisfy the requirement.

- Low- to med-quality protein sources would have a significant impact on the amount needed to satisfy the requirement!

2. Protein Quality is not Synonymous with Efficiency of Utilization

A. Protein quality is "inherent" feature of protein, and Protein quality determined by one set of conditions/situations may be different from the one determined by others. Thus, important to state conditions of the test!
B. On the other hand, the efficiency of utilization can be affected by many factors such as:

1) Protein quality & intake.
2) Dietary energy content, and other nutrient concentrations.
3) Environment.
4) Species, breeds, strains, and sex.
5) Previous nutrition.
6) Age,
7) Health status.

. . . , etc.

3. Some Methods to Assess the Protein Quality - Examples

A. Choosing an Assay?

1) Need to consider validity, precision, proportionality, costs, simplicity, etc.
2) An assay has no value unless it can relate back to the target species!

B. Biological value (BV):

1) A measure of the relationship of protein/N retention to protein/N/ absorption.
2) $$\text{BV (\%)} = \frac{\text{N intake - (Fecal N - Metabolic fecal N) - (Urinary N - endogenous urinary N)}}{\text{N intake - (Fecal N - Metabolic fecal N)}} \times 100$$
3) Egg protein is considered to have the highest BV of natural sources (94% +).
4) Generally, BV is higher for animal sources (60 to 80% +) vs plant sources (40 to 65%).

C. Net protein value (NPV):

1) BV does not take into account differences in digestibility from one protein to another.

2) When digestibility as well as BY data are used, a NPV can be computed, "NPV = BV x Digestion coefficient."
3) NPV is corrected for a very low or very high digestibility and is a more useful!?

D. Net protein utilization (NPU):

1) Similar to BV (i.e., NPU = Retained N/Food N), but measures by comparing body N contents - Feed a test protein to one group & a protein-free diet to other group.
2) $$\text{NPU} = \frac{\text{(Body N with test group) - (Body N of non-protein group)}}{\text{N intake by test group}}$$
3) An advantage? - A brief test period (7 to 10 days) with a minimum of measurements. (But, measuring body N can be a problem for some!)

E. Protein efficiency ratio (PER)

1) Use a feeding trial to compare protein sources in terms of gain in animal body weight per gram of protein or nitrogen fed.
2) $$\text{PER} = \frac{\text{Body weight gain, g}}{\text{Protein consumed, g}}$$
3) Problem? Does not make an allowance for maintenance - A protein may meet the maintenance needs, but may not promote growth?

F. Net protein ratio (NPR):

1) Simply the weight loss of a negative control group added to the weight of gain of the test group, divided by the protein consumed by the latter - Similar to PER.
2) $$\text{NPR} = \frac{\text{Weight gain on test group + Weight loss of non-protein group}}{\text{Weight of test protein group}}$$
3) Problem? Among others, feeding a protein-free diet!?

G. Slope ratio assay (Relative Protein Value, RPV):

1) Feed several concentrations protein or N from the test & standard/reference protein sources & measure response criterion/criteria.
2) Only use the linear portion a curve in computation of the slope assay value, i.e.,
$$\frac{\text{b (Test source)}}{\text{b (Standard/reference source)}}$$
3) Problems? Perhaps, the use of many diets/animals & may not have a common intercept would be the main ones, but it is a very reliable method.

4. Assessing Protein Quality for Ruminants

A. Assuming for years that protein leaving the rumen and entering the lower digestive tract was of good quality and adequate to meet the essential amino acid needs.
B. But, a feedstuff protein of good quality may be reduced via microbial protein degradation and synthesis.
C. Some instances, protein leaving the rumen could be inadequate to meet the amino acid needs of the high producing ruminant.
E. Dietary protein:

1) Either degraded in the rumen with partial or total conversion to microbial protein, or escape breakdown as undegraded protein - “Bypass” or “undegraded” protein.
2) Toe major factors influencing the rate of protein degradation in the rumen, “solubility of feedstuff protein” and the “rate of passage through the rumen.”
3) Approximately 60% of the various feed proteins are broken down in the rumen by microbes their component amino acids and then to ammonia (NH3), and the other 40% passes on to the omasum.
4) Methods to protect high quality protein from excessive ruminal degradation? - e.g., a) Heat treatment, b) treatment with formaldehyde or tannins, c) encapsulation of amino acids, d) use of amino acid analogs, and e) control microbial metabolism in the rumen.

F. Metabolizable protein or amino acids (also called “absorbable protein”):

1) Can be defined as the quantity of protein digested or amino acid(s) absorbed in the postruminal portion of the digestive tract of cattle and other ruminants.
2) In nonruminants, it has the same meaning as apparent digestible protein or absorbable amino acids.
3) But, in ruminants, metabolizable protein differs from digestible protein in that it reflects:

a) The quantity of feed protein consumed that escapes degradation in the rumen.
b) The quantity of degraded protein reformed into rumen microbial protein.

4) Major advantages of using the metabolizable protein system?

a) Its greater accuracy in predicting protein needs of cattle.
b) It gives a better insight into the individual amino acid needs of cattle and how these needs can best be supplied by a combination of performed protein and NPN.

PROTEIN AND(OR) AMINO ACID REQUIREMENTS

1. Introduction

A. Protein requirements:

1) Pigs & poultry do not have “protein” requirements!

2) Instead, they require amino acids!
3) e.g., In general, pigs depend on diets for 10 indispensable AA & sufficient amounts of nonspecific N to synthesize dispensable AA.

B. The use of "crude protein" values:

1) Often used for characterizing feedstuffs & expressing the dietary requirement for a "nitrogen fraction."
2) Reasons? Perhaps, tradition, convenience & ease of analysis (vs AA).
3) May be appropriate to use when:

a) Using a certain combination of feed ingredients (e.g., corn & soy) or
b) Using the "ideal protein" or balanced protein.

C. Ways to express the requirement:

1) % of diet - Works well if variations in the energy density are minimal.
2) g/day - Works well when intake is restricted, and if a certain intake level can be assured.
3) g/kg/day or g/kg$^{.75}$/day - Accounts the body size or metabolic body size, ∴ theoretically the best.
4) g/Mcal DE or ME - Important, or even necessary, when large differences in the energy density exist.
5) % crude protein for AA - Have to know the exact protein requirement.

- Primary objective? - "*To make sure that the animal can consume adequate daily nutrient allowances for optimum performance!*"

2. Estimating the Requirement

- Can be estimated in one of the two ways, a factorial or empirical method (or a combination of two in some instances).

A. Factorial method:

1) Estimation by the sum of requirements for maintenance and growth.
2) A simple example for a 50-kg pig - Assuming a pig is growing at a rate of 450 g of lean tissues/day:

a) Maintenance:

- 50 kg x 16% protein x 12% recycled/d x 6% loss of recycled protein = **58 g protein** (maintenance requirement)

b) Growth:

- 450 g lean x 23% protein = **104 g protein/d** (needed for growth)

c) Thus, the requirement is **162 g protein/d** (58 g + 104 g).
d) Then, must consider:

(1) Efficiency of utilization of absorbed nitrogen or protein (e.g., BV = N retained/N absorbed) - assume corn-soy = 0.60 or 60% in this example.
(2) Digestibility - Assume corn-soy = 0.75 or 75% in this example.

e) Thus,

- 162 ÷ 0.60 = 270 g digested protein/d.
- 270 ÷ 0.75 = 360 g dietary protein/d.

f) Conversion to a percentage of diet:

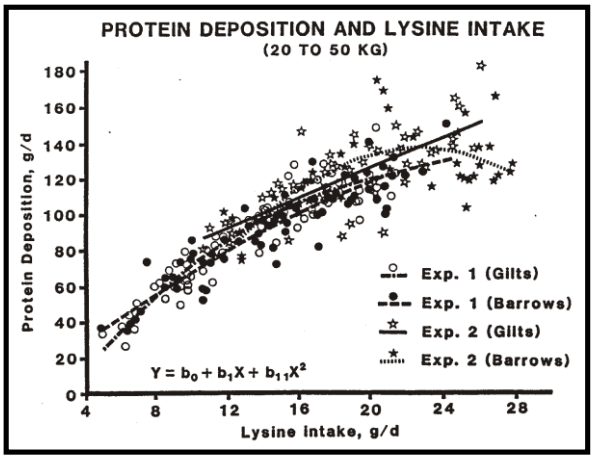
- Energy intake = 8 Mcal DE/d.
- 8 ÷ 3.4 (Mcal DE/kg) = 2.35 kg feed/d.
- Thus, 360 ÷ 2.35 = 153.2 g/kg diet or **15.32%** ⇐ Protein requirement!

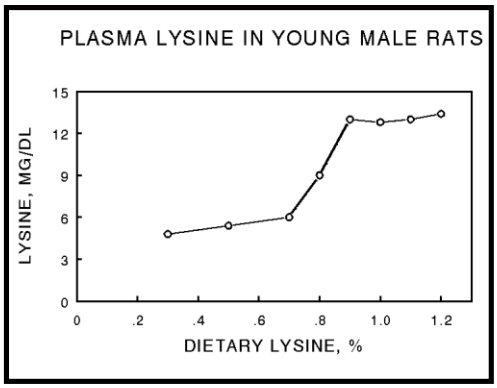
▸ Factorial method - Not absolute, i.e., many assumptions/estimations are involved in the process, ∴ influencing the requirement estimate!

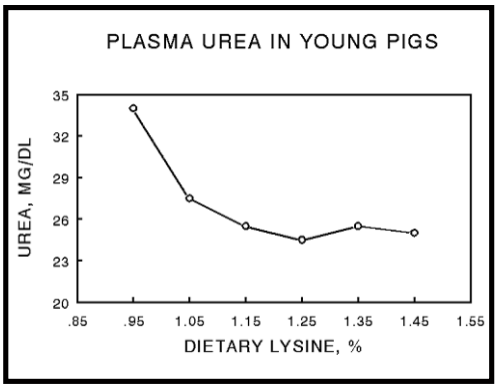
B. Empirical method:

1) Examine response patterns of animals to various levels (graded levels or intakes) of protein or amino acids.
2) Various response criteria have been used in the past - e.g., Weight gain, feed efficiency, nitrogen retention, protein deposition, plasma amino acid & urea, etc.
3) Some examples:

a) Protein deposition rate: (Chiba et al., 1991. J. Anim. Sci. 69:708)
b) Plasma amino acid (lysine): (Redrawn from Morrison et al., 1961. Can. J. Biochem. Physiol. 39:1675)
c) Plasma urea: (Redrawn from Lewis et al., 1980. J. Anim. Sci. 51:361)
d) Uric acid excretion or plasma uric acid for the bird - e.g., Miles and Featherston, 1974. Proc. Soc. Exp. Biol. Med. 145:686-689.

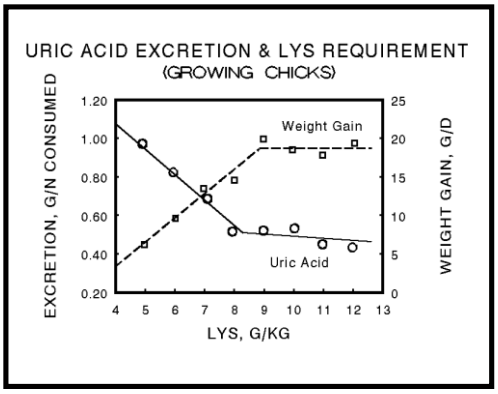






4) Difficulties associated with the empirical method?

   a) Defining a point of maximum response? - Can be done easily mathematically or statistically, but, perhaps, very difficult to do that "biologically!?"
   b) The response can be dependent on a number of factors such as . . . availability of amino acids, status of energy and other nutrients, sex and(or) genetics, environment, response criterion used, etc.



C. Alternative approach – Use modeling!?

- Please see NRC, 1998, 2012; Chiba, 2000 [In: M. K. Theodorou and J. France. Feeding systems and feed evaluation models. p. 181-209 (brief description)], Miller and Calvert. 2001 (In: A. J. Lewis and L. L. Southern. Swine nutrition. 2nd ed. p. 867-879); and others on the topic.

3. Requirements

A. Amino acid requirements for nonruminant species (see appropriate "Nutrition & Feeding" sections for details) - Lysine and(or) sulfur-amino acids are likely to be the first or second limiting amino acid in many practical diets for nonruminant animals.

Species	CP, %	Lys, %	S-AA, %
Swine (NRC, 1998):			
Growing	13-26	0.60-1.50	0.35-0.86
Gestating sows/boars	11-14	0.52-0.60	0.36-0.42
Lactating sows	16-19	0.82-1.03	0.40-0.49
Poultry (NRC, 1994):			
Chickens, growing	15-18	0.45-0.85	0.42-0.62
Chickens, laying	12.5-18.8	0.58-0.86	0.48-0.73
Broilers	18-23	0.85-1.10	0.60-.90

Turkeys, growing	14-28	0.65-1.60	0.45-1.05
Horses (NRC, 1989): (In total diet, DM)			
Growing (up to 2 yr-old)	10.4-14.5	0.42-0.68	-
Working	9.8-11.4	0.35-0.40	-
Pregnant	10.0-10.6	0.35-0.37	-
Lactating	11.0-13.2	0.37-0.46	-
[Pagan (1998) - Growing, g/Mcal DE	42.5-50.0	1.7-2.1	-]
Fish (NRC, 1993):			
Channel catfish	32	1.43	0.64
Rainbow trout	38	1.80	1.00
Pacific salmon	38	1.70	1.36
Common carp	35	1.74	0.94
Tilapia	32	1.43	0.90

B. Variations in estimates - e.g., NRC (1988) vs. ARC (1981; UK) & SCA (1987; Australia):

1) ARC & SCA estimates - Tend to be greater than the NRC, which are requirements, not allowances!? Also, perhaps, a reflection of numerous factors that are used to estimate the requirements!?

- Please see Lewis (1991) in Miller et al. (1991) and Chiba (2000) in Theodorou & France (2000) for the comparison of NRC & ARC.

2) e.g., Lysine requirements (%):

	ARC	SCA(♂)	SCA(♀)	NRC
15 (or 20)-50 kg	1.19	1.07	0.95	0.75
50-90 (or 110) kg	0.85	0.85	0.85	0.60
Gestating sows[a]	0.43		0.43	0.43
Lactating sows	0.63		0.63	0.60

[a]Based on 2 kg/d (ARC & SCA) or 1.9 kg/d (NRC).

C. Factors that affect the requirement:

1) Stage & levels of production - Growth, gestation, lactation, etc.
2) Genetic capacity - Breeds, strains, sex, etc.
3) Response criteria used - e.g., Protein or AA requirement for a maximum leanness is higher than that required for a maximum rate of weight gain.
4) Health/disease.
5) Any factors that affect feed intake such as energy density & environmental temperatures, . . . , etc.

D. Satisfying the requirement (e.g., pigs):

1) Amino acids (%) in corn and corn + soybean meal (44% CP) vs amino acid requirements of a 25-kg pig (. . . based on 1988 NRC):

Amino acid	Corn	Corn+SBM[a]	Requirement
Arg	0.43	0.95	0.25
His	0.27	0.43	0.22
Ile	0.35	0.66	0.46
Leu	1.19	1.58	0.60
Lys	0.25	0.75	0.75
Met + Cys	0.40	0.54	0.41
Phe + Tyr	0.84	1.35	0.66
Thr	0.36	0.61	0.48
Trp	0.09	0.19	0.12
Val	0.48	0.76	0.48

[a]Formulated to meet the lysine requirement.

2) Cereal grains (70 to 80% of the diet) provide over half of the total AA:

   a) The Lys content in grain is low, thus nearly always the first limiting AA in swine diets.
   b) Analysis is important because of considerable variations in nutrient contents of grains - e.g., corn, 0.20 to 0.30% Lys vs. the NRC value of 0.25% Lys.

3) Soybean meal:

   a) High in Lys (& other AA) content.
   b) Thus, complement corn very well in meeting the AA requirements.

4. Protein/Amino Acid Sources

A. The nutritional value of ingredients depends on “digestibility of protein” and “biological value (efficiency of utilization)” of digested protein. [See Chiba (2001) in Lewis & Southern (2001) for “Relative feeding values and maximum incorporation rates of some protein sources.”]
B. Proportion of Lys in protein: [Compiled by Cromwell, Univ. of Kentucky; Also, see Chiba (2001) in Lewis & Southern (2001)]

Feedstuff	CP, %	Lys, %	Lys, % CP
Blood meal	86	7.4	8.7
Dried skim milk	33	2.6	8.2
Dried whey	13	1.05	8.1
Fish meal, menhaden	61	4.80	7.9
Brewers dried yeast	45	3.20	7.1
SBM, dehulled	48	3.10	6.5
SBM	44	2.80	6.4
Canola meal	38	2.30	6.1
Meat & bone meal	50	2.90	5.8
Meat meal	56	3.10	5.5
Alfalfa meal	17	0.80	4.7

Wheat middlings	16	0.70	4.4
Cottonseed meal	41	1.70	4.1
Sunflower meal	47	1.70	3.6
Brewers dried grains	26	0.90	3.5
Wheat, hard	12	0.42	3.5
Wheat, soft	11	0.35	3.2
Triticale	16	0.50	3.2
Barley	11	0.35	3.2
Coconut meal	20	0.64	3.2
Peanut meal	49	1.45	3.0
Oats	12	0.36	3.0
Sesame meal	45	1.30	2.9
Distillers grains	27	0.70	2.6
Corn gluten feed	23	0.60	2.6
Sorghum	9	0.24	2.6
Corn	9	0.24	2.6
Feather meal	84	1.65	1.9

1) The proportion of Lys in protein gives a crude assessment of the biological value because it is the first limiting AA in many swine diets (& others?).
2) Possible problems in using these values:

    a) The differences in digestibility.
    b) The differences in availability (e.g., heat damaged feedstuffs).
    c) May be limiting in other AA (e.g., Trp in meat meal).

C. Other factors affecting the nutritional value of protein sources?

- e.g., Stability, presence of anti-nutritional factors, interactions among nutrients and with non-nutrient factors, palatability, etc.

5. Amino Acid Digestibility or Availability

A. Apparent ileal digestibility (%) of AA in swine feedstuffs: (NRC, 1998)

Feedstuff	Lys	Trp	Thr	Met
Barley	68	70	66	80
Blood, meal, spray/ring dried	91	88	86	85
Blood, plasma	87	92	82	64
Canola meal	74	73	69	82
Corn	66	64	69	86
Cottonseed meal, sol. ext.	61	67	63	73
Fish meal, menhaden	89	79	85	88
Meat & bone meal	74	60	70	79
Oat groats	79	80	76	85
Oats	70	72	59	79
Peanut meal, sol. ext.	78	73	74	85

Rye, grain	64	67	59	76
Sorghum	62	75	68	81
SBM, dehulled	85	81	78	86
SBM	85	80	78	86
Triticale	76	74	69	76
Sunflower meal, without hulls	74	76	71	87
Wheat, soft red winter	73	81	72	85

B. Recent trends?

1) Poultry industry – Has been using the available AA values for a while.
2) Swine industry:

a) Moving toward expressing the requirement & formulating diets based on the AA availability or often in terms of “ileal digestibility.”
b) Started using "standardizes ileal digestibility or SID" in recent years, and it’s likely that it's use would increase in the future!?

IDEAL PROTEIN

1. Introduction

A. To deposit “1 g” of protein or lean, relative amounts or proportions of different (indispensable) AA needed to deposit that amount should be the same. (Must make an assumption that age, sex & others have no effect on proportions of AA in tissue proteins or lean tissues!)
B. It is possible that some protein or a mixture of proteins may supply AA in exactly those proportions required by the animal.
C. For different classes (i.e., differences in the body wt, sex, breed, etc.):

1) Different amounts of the “balanced AA” may be required.
2) But, the quality (or AA balance/proportion) of protein would be the same!

D. Definition of ideal protein?

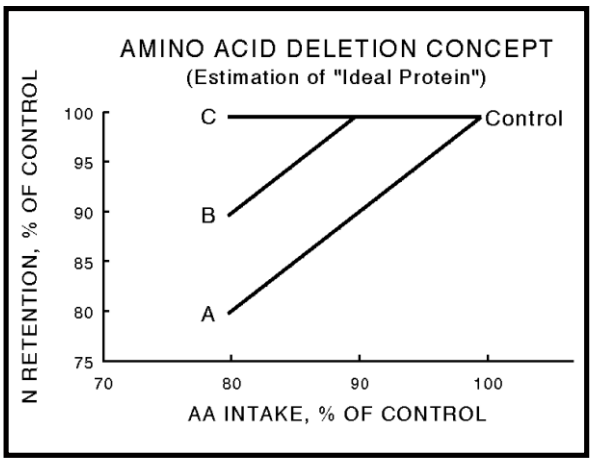
“*A protein which supplies AA in exactly the proportions required by the pig or poultry and which may, therefore, be utilized fully under appropriate circumstances!*”

E. Should be the most efficient protein or diet because there would be no deficiency or excess of AA.

2. Estimation of "Ideal Protein"

A. Estimation of AA requirements by a "dose-response" type experiment - e.g., Found to be 6-8 g Lys /100 g protein, thus Lys should be ≈ 7 g/100 g of protein.

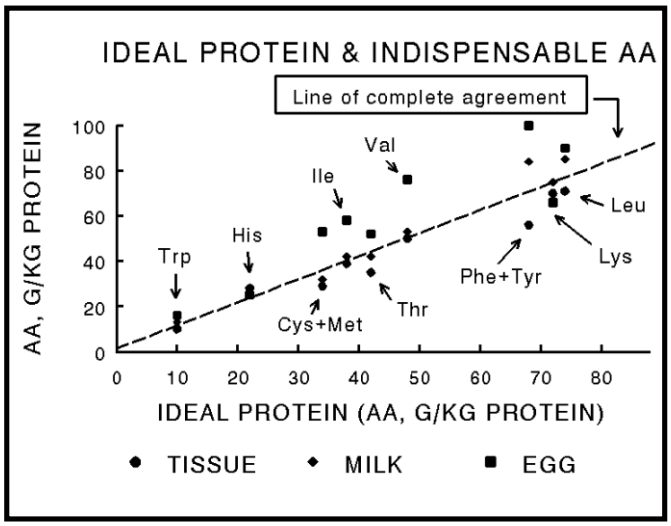
B. Estimation via experiment designed for that purpose - e.g., Wang & Fuller, 1989. Br. J. Nutr. 62:77.



- This method is based on the assumption that the removal of a non-limiting AA has no effect on N-retention (NR):

  a) The removal of the first limiting AA reduces NR (as **A** in a figure).
  b) If the removal of AA does not reduce NR (as in **C**), the quantity removed was excess relative to the first limiting AA.
  c) If the removal of AA results in reduced NR (as in **B**), then the proportion that could have been removed without reducing NR can be interpolated proportionately.

C. Consider both A (dose-response) & B (via experiments), and also the AA composition of body tissues:



1) Why body tissues?

   a) Assuming a complete utilization of protein, a major end product should be body protein, thus, body AA pattern may dictates AA pattern required.
   b) Proteins with high biological value tend to resemble in their AA composition in tissues - "Ideal Protein & Indispensable AA" [Fuller and Chamberlain, 1985. In: Cole & Haresign (Ed.)].

   - Biological value? - Sow's milk = 0.90 or 90%, and egg protein = 1.00 or 100%.

2) AA requirements for maintenance & protein accretion:

   a) Proportions needed differ, but the N requirement for maintenance is a small proportion of overall needs (about 30% in normally growing pigs).
   b) Thus, likely to have no or small influence for overall pattern needed.

3. Ideal Protein

A. Ideal protein or balanced protein (relative to Lys in %) in pigs:

Item	ARC (1981)/SCA (1987))	NRC (1998)[a]
Indispensable AA:		
Arg	-	48
Lys	100	100
Met + Cys	50	55
Trp	14	18
Thr	60	60
Ile	54	54
Leu	100	102
His	33	32
Phe + Tyr	96	93
Val	70	68
Total indispensable A, g/kg CP	404	
Dispensable AA (total), g/kg CP	596	

[a]For protein accretion.

B. Ideal protein for poultry (relative to Lys)*:

	ARC, 1975	NRC, 1977	NRC, 1994	AEC, 1978[a]	Scott, 1982[b]	SCA, 1983	Authors
Lys	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Arg	0.94	1.20	1.10	1.05	1.00	0.90	1.05
Gly & Ser	1.27	1.25	1.14	1.41	-	-	1.31
His	0.44	0.29	0.32	0.39	0.40	0.35	0.40
Ile	0.78	0.67	0.73	0.72	0.80	0.50-0.76	0.72
Leu	1.33	1.13	1.09	1.33	1.20	1.03-1.72	1.25
S-AA	0.83	0.78	0.72	0.76	0.72	0.75	0.75
Phe & Tyr	1.44	1.12	1.22	1.23	1.28	1.20	1.21
Thr	0.67	0.63	0.74	0.62	0.64	0.60	0.63
Trp	0.19	0.19	0.18	0.17	0.18	0.19	0.18
Val	0.89	0.68	0.82	0.79	0.64	0.68-0.94	0.79

*Based on: Boorman, K. N., and A. D. Burgess. 1986. Responses to amino acids. In: C. Fisher and K. N. Boorman (Ed.). Nutrient Requirements of Poultry and Nutritional Research. pp. 99-123. Butterworths, London. Added the data (based on 3- to 6-wk old broilers) from NRC (1994).] [a]AEC = Document No. 4, Animal Feeding. Commentary, France, AEC; [b]Scott et al., 1982. Nutrition of the Chicken.

C. Once established the “ideal balance,” the next step is to determine the “ideal protein requirement” for each class of swine.

4. Deviations from the Ideal Protein/Pattern

A. Theoretically:

1) Amino acids are likely to be utilized less efficiently.
2) Can create amino acid disproportions such as deficiency, imbalance, antagonism, and possibly toxicity.

B. Effect of moderate oversupply (. . . assuming that all AA are supplied to meet or exceed the requirements)?

1) Excess Arg on performance of G-F pigs: (Anderson et al., 1984. J. Anim. Sci. 58:362)

Item	% NRC for Arg: / Arg:Lys (27-44 kg): / Arg:Lys (44-97 kg):	500 / 1.43 / 1.48	400 / 1.14 / 1.18	300 / 0.86 / 0.89	200 / 0.57 / 0.59
27-44 kg:					
Feed, kg/d[a]		1.77	1.92	1.87	1.84
Gain, kg/d		0.59	0.64	0.63	0.63
Gain:feed		0.338	0.335	0.335	0.344
44-97 kg:					
Feed, kg/d		3.09	3.16	3.19	3.03
Gain, kg/d[a]		0.77	0.81	0.82	0.74
Gain:feed[b]		0.251	0.257	0.257	0.244

[a]Quadratic effect, $P < .05$; [b]Quadratic effect, $P = 0.10$.

- Reduction of the Arg content of typical diets resulted in little or no improvement in performance of pigs.

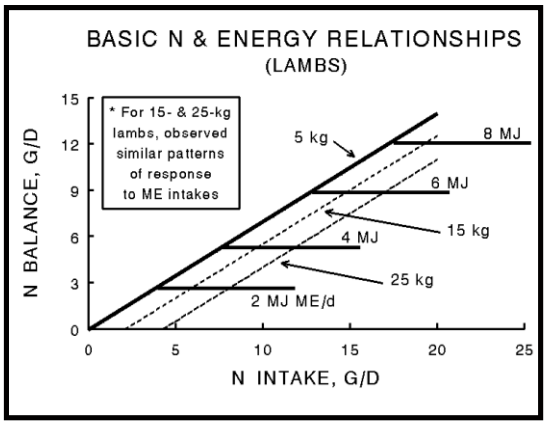
2) Similar results with Arg & Leu have been observed by others - e.g., Southern & Baker, 1982. J. Anim. Sci. 55:857, and Cromwell et al., 1982. J. Anim. Sci. 55(Suppl. 1):41 (Abstr.).
3) The bottom line?

a) Moderate excesses are unlikely to produce adverse effects on performance under practical conditions (e.g., when using corn-soy type diets).
b) Using many protein sources (especially, byproducts) or crystalline AA, then may need to pay an attention!

AMINO ACIDS AND ENERGY

1. The Basic N & Energy Relationships

- Nitrogen retention and N intake in lambs: (Redrawn from Black and Griffiths, 1975. Br. J. Nutr. 33:399)



1) In the initial phase:

a) N retention - Dependent on N but independent of energy intake.
b) A slope of line is a measure of the BV of protein.

c) A line shifts to the right as the weight increases because of the increase in endogenous N losses.

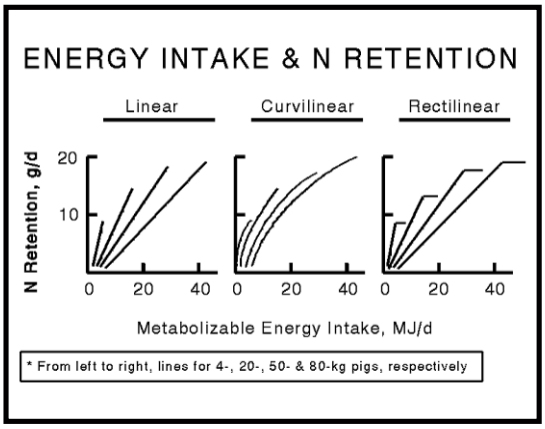
2) In the second phase:

a) N retention is dependent on energy intake and live weight.
b) i.e., at a given wt, an additional N intake has no effect unless additional energy is supplied.

2. Three Possible N & Energy Relationships

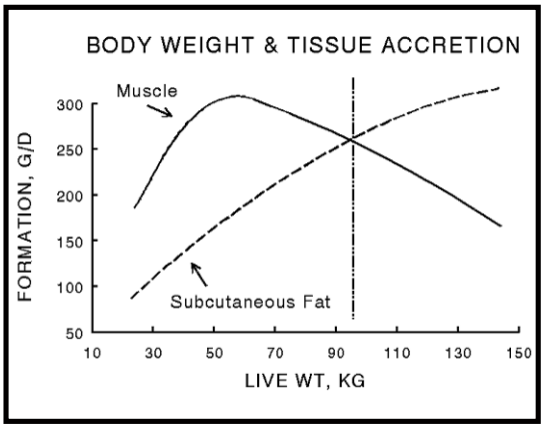
A. Linear, curvilinear or rectilinear:

- Energy intake and nitrogen retention: (Redrawn from Williams, 1980. Proc. Aust. Soc. Anim. Prod. 13:126 & ARC, 1981)



B. Body weight and tissue accretion in pigs: (Just, 1984. J. Anim. Sci. 58:740)

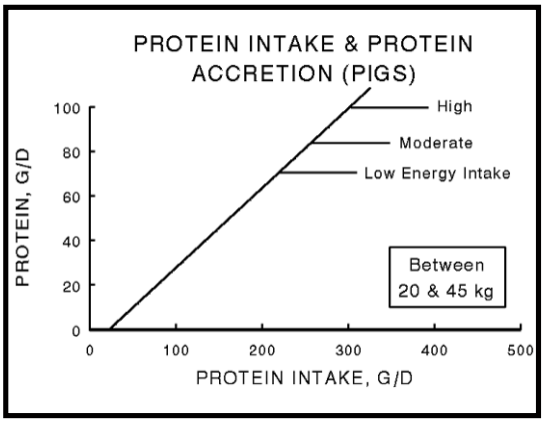
1) Up to ≈ 50 kg, the rate of muscle deposition is almost linear.
2) Thus, protein (AA) and energy requirements probably increase linearly during this phase.



C. Young animals (e.g., pigs up to 50 kg or so):

1) Protein/energy intake & protein deposition in young pigs: (Campbell et al., 1985. Anim. Prod. 40:489)

a) As the energy intake increases, protein deposition (PD), and also the protein requirement increase.
b) Increases in PD & protein need/unit ↑ in energy intake are constant.

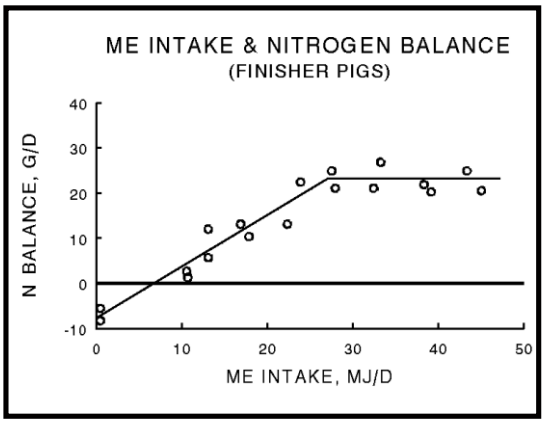


2) The bottom line? - The relationship between energy intake & PD (& protein intake) in young animals (e.g., pigs ≤ 50 kg) is a linear!

D. Implication(s) of a linear relationship in young animals (e.g., pigs ≤ 50 kg):

- *"The pig's potential for protein growth from birth to 50 kg seems to lie beyond the upper limit of appetite (SCA, 1987)."*

1) Diets of high energy density can be fed ad libitum without excessive fat deposition or reducing the efficiency of feed utilization.
2) A single diet can be fed to meet both energy and amino acid requirements.
3) Expressing amino acid requirements in terms of energy is appropriate.



E. Older animals (e.g., finishing pigs):

1) ME intake and N balance in finisher pigs: (Dunkin et al., 1984. Proc. Aust. Soc. Anim. Prod. 15:672)

   a) Protein/AA intake was not a limiting factor, and avg wt was 73.8 kg.
   b) N balance ↑ up to 27.6 MJ ME/d (6.6 Mcal ME/d) & no response with further increases.

2) Energy intake and protein & fat accretions in ♀ (g/d): (Campbell et al., 1985. Anim. Prod. 40:497.)

MJ/d	Protein	Fat
23	63.4	125
27.5	84.5	208
33	103.0	279
37.5	102.0	332
39.2	99.0	371

- The bottom line?

   a) The relationship between energy intake and protein deposition in larger/older animals may not be a linear!
   b) Larger animals consume more energy than they need for maximum/optimum protein accretion, and excess energy can be deposited as fat!
   c) Can reduce energy intake of larger pigs without adverse effect on protein accretion . . i.e., can/should use a “restricted energy or feeding” practice!?

SOME FACTORS AFFECTING AMINO ACID REQUIREMENTS

1. Effect of Thermal Environment

A. Dietary lysine & temperatures: [Stahly & Cromwell, 1987. J. Anim. Sci. 65 (Suppl. 1):299)]

		10°C				22.5°C			
Item	Lys, %:	0.50	0.65	0.80	0.95	0.50	0.65	0.80	0.95
Lys intake, g/d[a]		13.6	17.6	21.8	25.0	11.7	15.3	17.8	22.6
Growth (26-92 kg), g/d:									
Live wt[b]		762	788	792	806	691	751	748	802
Water[c]		316	337	334	352	269	325	325	358
Protein[d]		112	116	120	122	101	112	113	121
Fat		307	306	309	302	299	286	281	291
Fat, %[e]		32.8	31.9	31.4	30.4	34.7	31.1	30.8	30.1

[a]Temperature & Lys effects, $P < 0.01$; [b]Temperature x Lys, $P = 0.15$; [c]Temperature x Lys, $P < 0.05$; [d]Temperature x Lys, $P = 0.10$; [e]Temperature x Lys, $P = 0.13$.

1) At low environmental temperatures:

   a) Pigs increase feed intake to meet energy requirement, ∴ increasing amino acid intakes.
   b) Thus, can reduce amino acid content of the diet.

2) At high environmental temperatures:

   a) Pigs reduce feed intake, ∴ reducing amino acid intakes.
   b) Thus, need to increase amino acid content of the diet.

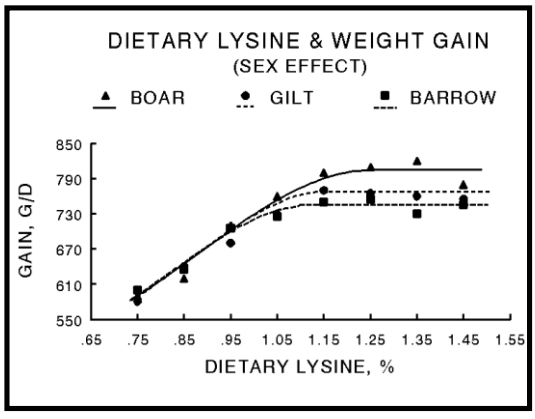
B. The bottom line?

1) Adequate daily amino acid intakes is the "key" to achieve optimum performance of animals.
2) Thus, AA levels must be adjusted for any factors that influence feed intake.

- Adjusting amino acids is necessary for changes in dietary energy densities too, not just for changes in temperatures!

2. Effect of Sex

A. Dietary lysine and weight gain for boars, gilts and castrates: "Fuller & Chamberlain, 1985. In: Cole & Haresign (1985)."
B. Potential for lean deposition - Boars > gilts > castrated males (barrows).

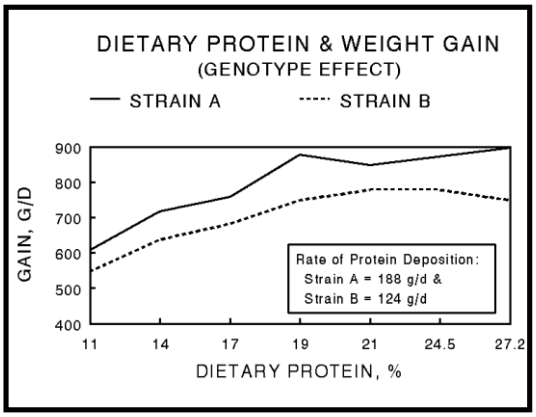


- Because of this (& others - e.g., animal welfare issue), raising boars for meat

production is popular in Europe and Australia . . . Not in the US for various reasons though!

C. Protein or AA requirements are reflection of the potential for protein or lean accretion.

2. Effect of the Type of Pigs



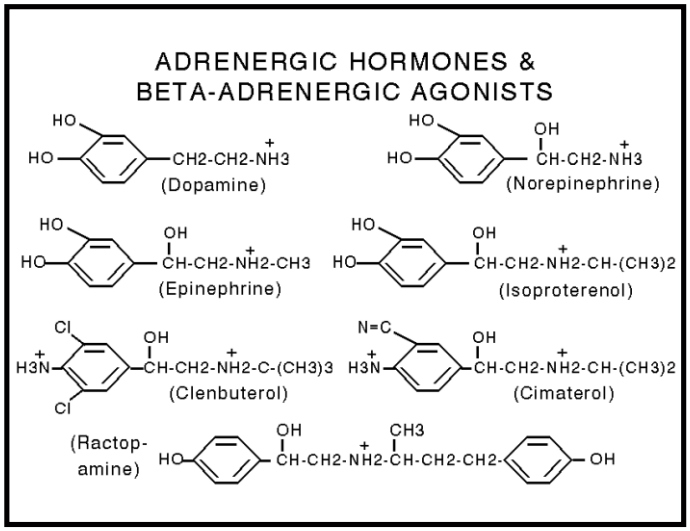
A. Dietary lysine and weight gain: "Campbell, 1988. Hog Farm Management 25(5):34."

☛ Strain A, which has a higher potential for protein deposition, responded to higher dietary protein concentrations, thus, they have a higher protein requirement!

B. The bottom line?

1) A wide variation in the genetic potential for growth (lean growth) exist in today's swine industry.
2) Amino acid requirements are dependent on the genetic potential for protein (or lean) deposition.
3) Thus, for the efficient utilization of amino acid(s) (& also for optimum lean accretion), may have to formulate diets accordingly for sex, type of pigs, etc.

3. Effect of Repartitioning Agents



A. Repartitioning agents:

1) Partition nutrients away from fat deposition & toward lean muscle (or protein) accretion!
2) Examples:

a) pST (porcine somatotropin):

(1) Can increase muscle deposition & reduce fat deposition.
(2) Must be injected daily or use some implant, which must be inserted weekly or monthly?

b) β-adrenergic agonists (dietary supplement):

(1) Similar to catecholamines, which may function as hormones or neurotransmitters.

(2) May or may not increase muscle deposition, but ↓ fat deposition.

B. Effects of repartitioning agents - e.g., pigs

Item	β-agonists	pST
Weight gain	+ 8	+ 22
Feed to gain	- 10	- 28
Loin muscle area	+ 12	+ 14
Backfat	- 12	- 26
Carcass lean	+ 8	+ 21
Protein accretion		+ 31
Heat production		+ 8
MEm		+ 17

C. Effects of dietary Lys

1) pST and dietary Lys: [Goodband et al., 1989. J. Anim. Sci. 67(Suppl. 2):122 & 123]

		4 mg pST				8 mg pST			
Item Lys, %:	0 .80	0.80	1.00	1.20	1.40	0.80	1.00	1.20	1.40
Lys, g/d[abcd]	25.1	23.3	27.0	32.6	38.7	22.5	26.4	30.1	36.5
Feed, kg/d[abc]	3.14	2.92	2.70	2.72	2.77	2.82	2.64	2.51	2.61
Gain, kg/d[ab]	1.14	1.27	1.30	1.23	1.31	1.24	1.27	1.30	1.43
Feed:gain[e]	2.76	2.29	2.09	2.22	2.11	2.28	2.08	1.93	1.83
PU, mg/dL[acd]	35.6	21.0	17.8	19.7	22.1	17.0	12.0	12.9	12.0
LMA[a]	35.4	39.8	37.7	37.7	39.8	40.2	41.7	45.1	43.8
BF[a]	2.62	2.32	2.38	2.31	2.27	2.24	2.05	2.11	1.85

[a]Linear effect of pST, $P < 0.05$; [b]Linear effect of Lys, $P < 0.05$; [c]Quadratic effect of Lys, $P < 0.05$; [d]Quadratic effect of pST, $P < 0.05$; [e]pST x Lys, $P < 0.05$.

2) The bottom line:

1) Repartitioning agents increase the rate of protein accretion.
2) Dietary amino acid contents may need to be adjusted concomitantly.

CRYSTALLINE AMINO ACIDS

1. Reasons for Interest

A. Soybean meal - A major protein supplement for nonruminant species:

1) The cost fluctuates considerably.
2) May not be available in the future since it is a byproduct of soybean oil production.
3) Thus, important to make efforts to find alternative protein supplements!

B. "Feed-grade" amino acids are currently available commercially (Lys, Trp, Thr, and Val; Met has been available for a long time!), and they might be economically viable alternatives.

2. Replacing SBM with Crystalline AA

A. Effect of a mixture of AA: (Kephart & Sherritt, 1990. J. Anim. Sci. 68:1999)

Item (CP:)	Contr. (16.9%)	corn + AA (10.9%)	Corn + AA + Glu (3.5%) (13.0%)
Gain, g/d	797	693	660
Feed Intake, g/d	1,805	1,765	1,696
Feed:gain	2.27	2.55	2.57

B. Addition of crystalline AA to corn: Lewis, 1989. NE Swine Rep."

Item	Corn-soy positive control	Corn negative control	Corn + Lys & Trp	Corn + Lys, Trp & Thr
Initial wt, kg	60.2	60.0	60.1	59.8
Final wt, kg	112.2	96.9	108.5	107.8
Feed, kg/d	3.00	2.15	2.69	2.52
Gain, kg/d	0.80	0.32	0.54	0.59
Feed:gain	3.77	6.79	4.98	4.28
Backfat, mm	31.0	35.1	34.0	32.8
% lean	54.9	53.0	53.4	54.1

C. The bottom line?

1) Pigs fed low-protein diets supplemented with crystalline AA do not perform well compared to those fed corn or milo-soy diets.
2) But, the performance of pigs improves progressively as "limiting" AA are added to low-protein diets sequentially.
3) Thus, may have the potential in the future depending on other pertinent information:

    a) The order of limiting amino acids in grains.
    b) The amount of dietary nonspecific N needed for the synthesis of dispensable amino acids.