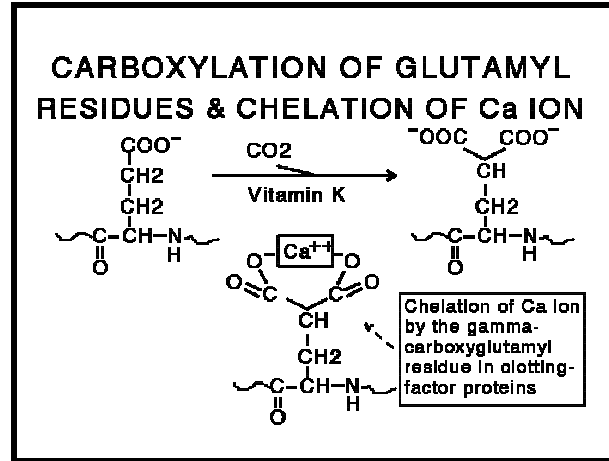


b) γ -Carboxyglutamyl (Gla) residue chelates Ca ion, which is essential for the blood clotting mechanism.

2) Carboxylation of glutamyl residue & chelation of Ca ion: (Adapted & redrawn from Martin et al., 1983)

C. γ -Carboxyglutamyl residues:

- 1) Also identified in a number of proteins in the bone, kidney, lung, placenta, skin & spleen.
- 2) Thus, may have some other functions?!



4. Deficiency

- Essentially, an impairment of blood coagulation!
 - 1) Low prothrombin levels (factor II).
 - 2) Increased blood clotting time.
 - 3) Hemorrhaging - Subcutaneous & internal hemorrhages in severe cases.
 - 4) Anemia.

5. Requirements/Supplementation

A. Most species:

- 1) Generally met by feed sources & microbial synthesis in the hind gut, but may or may not be absorbed efficiently.
- 2) Possible exceptions:
 - a) Use of sulfonamides & other antibiotics - ↓ microbial synthesis of the vitamin. (Also, ↓ synthesis of B-vitamins!)
 - b) Presence of molds in feeds - May produce antagonists such as dicumarol.
 - c) Limited access to feces, i.e., the use of slotted floors & cages.
- 3) Thus, as an insurance, diets are usually supplemented.

B. Vitamin K requirements:

- 1) Poultry (NRC, 1984) - 0.47-0.50 mg/kg for immature chickens & broilers, 0.40-0.60/kg for laying hens, and 0.5-1.75 mg/kg for turkeys.
- 2) Swine (NRC, 1998) - 0.50 mg menadione/kg for all classes of swine.
- 3) Horses (NRC, 1989) - No estimated requirements.
- 4) Fish (. . . Quantitative requirements have not been established!):
 - a) Intestinal synthesis of vitamin K has not been fully evaluated in fish.
 - b) Channel catfish & trout - Need vitamin K for normal blood coagulation, but growth rate is not affected by dietary deletion of the vitamin.
 - c) 0.5 to 1 mg of menadione/kg may be sufficient for fingerling trout, and 10 mg/kg has been suggested for trout & salmon.
 - d) Fish meal & alfalfa are good source of vitamin K.

C. Sources - Water-soluble menadione (vitamin K₃) salts:

- 1) Menadione Na bisulfite (MSB)
- 2) Menadione Na bisulfite complex (MSBC)
- 3) Menadione dimethyl-pyrimidinol bisulfite (MPB)

☞ Vitamin K activity - 50, 33 & 45% of menadione (synthetic form) for MSB, MSBC & MPB, respectively.

IRON

1. General

- A. As a constituent of Hb, Fe plays a central role in life processes.
- B. Occurs as “iron-porphyrin” nucleus, heme:

- 1) Not only in Hb, but also in Mb, cytochromes, peroxidase, catalase & other enzymes.
- 2) Thus, Fe is a constituent of oxygen carriers & oxidation catalysts or enzymes.
- 3) Examples of heme compounds:

Heme compounds	Function
Hb (erythrocytes)	Oxygen transport
Mb (skeletal muscles)	Oxygen transport
Cytochrome oxidase (heart muscle)	Electron transfer
Cytochrome C (heart muscle)	Electron transfer
Peroxidase	Peroxide decomposition
Succinate dehydrogenase (heart)	Electron transfer
Reduced NAD dehydrogenase (heart)	Electron transfer

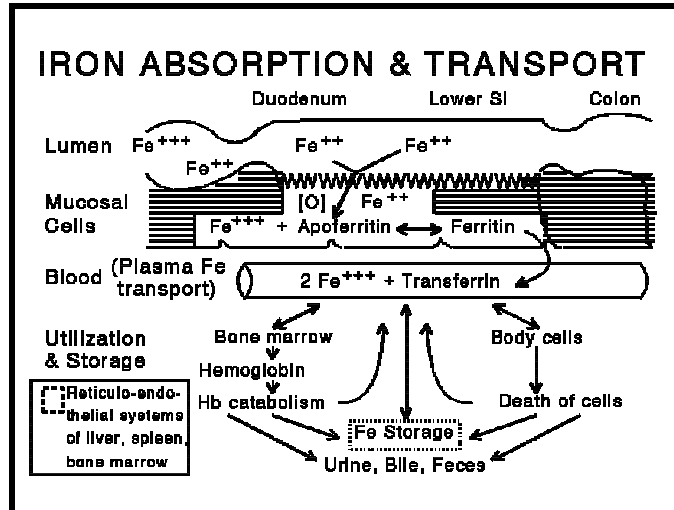
C. Iron metabolism: (Adapted & redrawn from McDowell, 1992)

1) Absorption:

- a) Fe in ferrous state (Fe^{2+}) is absorbed much more efficiently than that in "ferric" state (Fe^{3+}).

▷ Vitamin C (& others) can reduce Fe from 3^+ to 2^+ , ∴ having beneficial effects on Fe absorption.

- b) Generally, Fe is poorly absorbed, but the efficiency increase in the deficient state - e.g., Only 7-10 % in Fe-adequate rats vs \approx 80% in Fe-deficient rats.



2) Once absorbed, Fe is not readily lost from the body:

- a) e.g. - Fe released from Hb → liver → secreted into the bile → reabsorbed & used again. (Retained with a great tenacity!)
- b) Exception - hemorrhages!

- 3) Fe is stored as: a) "Ferritin & hemosiderin" in the liver, b) "Transferrin" in serum, c) "Uteroferrin" in placenta, and d) "Lactoferrin" in milk.
- 4) Excretion - Feces (1° unabsorbed Fe) & urine, and also a continual loss in sweat, hair & nails.

D. Fish:

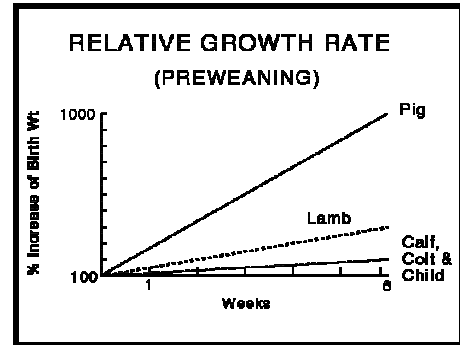
- 1) Relatively little information on absorption/metabolism of Fe, but probably similar to other species.
- 2) Absorption of Fe takes place across the gill, but the intestinal mucosa is the major site.
- 3) Dissolved Fe (e.g., ferrous sulfate) in water may serve as a source in certain warm-water fish, but may precipitate out as ferric hydroxide.

E. Natural feed ingredients usually supply enough Fe to meet the postweaning requirements of pigs & poultry, and Fe-deficiency is unlikely to occur under practical conditions in fish.

2. Iron Deficiency Anemia in Nursing Pigs

A. Reasons?

- 1) Low body storage in newborn pigs - Born with only about 40-50 mg of Fe, and with the daily requirement of 7-16 mg/day, deplete rapidly!
- 2) Low Fe content in sow's milk - About 2 ppm in colostrum & 1 ppm in milk, thus piglets receive only about 1 mg/day from milk!
- 3) Rapid growth rate vs other species - See Figure.
- 4) No access to iron sources (e.g., soil) in the confinement.



B. Fe deficiency:

- 1) Characterized by pale mucous membranes (around eyes, ears, nose & mouth).
- 2) Slow growth, rough hair coat, wrinkled skin, and may be listless.
- 3) Labored breathing or “thumps” (spasmodic jerking of the diaphragm muscles), and sudden death from anoxia.
- 4) More susceptible to infectious diseases.

C. Hb or hematocrit levels - The best index of the Fe status in pigs:

	Normal	Anemic
Hb, g/100 ml	12	5
Hematocrit, % RBC	35	17

D. Prevention of baby pig anemia:

1) Treatment of sows with oral or injectable Fe during gestation:

- ▶ Injection of 22 mg Fe-dextran/kg BW (divided among 5 injections at d 40, 45, 50, 55 & 60 of gestation): (Ducsay et al., 1984. J. Anim. Sci. 59:1303)

Item	Control	Treated
Fetal liver:		
Fe, mg/g DM	1.3	1.2
Total Fe, mg	3.7	3.5
Placenta:		
Uteroferrin, mg/g	1.6	2.4
Uteroferrin, mg	287	427
Allantoic:		
Fe, µg/ml	5	7
Fe, mg	204	1,327

- ▶ No appreciable effect on body stores of newborn pigs!

2) Treatment of sows with Fe during lactation:

- ▶ Hemoglobin levels in young pigs (g/100 ml): (Univ. of Kentucky data)

Treatment	Birth	Wk 1	Wk 3
None	9.2	7.7	5.9
Pigs injected	9.0	9.3	9.1
Sows fed 700 ppm Fe	9.6	8.4	8.9

- ▶ No effect on the Fe content of milk!

☞ ∴ limited placental & mammary transfer of Fe in pigs, and it is a common practice to inject pigs with 100-150 mg of Fe as Fe-dextran or Fe-dextrin at 1-3 days of age.

3. Iron Requirements/Supplementation

A. Requirements:

Animal	mg/kg
Poultry (NRC, 1994):	
Immature chickens	56-80
Laying hens	38-56
Broilers	80
Turkeys	50-80
Swine (NRC, 1998):	
3-120 kg	40-100
Sows/boars	80
Horses (NRC, 1989; DM)	40-50
Fish (NRC, 1993):	
Channel catfish	30
Rainbow trout	60
Pacific salmon	Not tested
Common carp	150
Tilapia	Not tested

B. Bioavailability:

- 1) Ferrous sulfate (FeSO₄) - Highly available.
- 2) Ferrous carbonate (FeCO₃) - Variable.
- 3) Ferrous oxide (FeO) - Poorly available.
- 4) Ferric oxide (Fe₂O₃) - Totally unavailable.

☞ Others such as ferrous ammonia sulfate, ferrous chloride, ferrous fumarate, ferrous gluconate, ferric chloride, ferric citrate, ferric choline citrate & ferric sulfate are available, but not commonly used!

4. Fe Toxicity

A. Excess Fe (pigs):

- 1) 600 mg Fe/kg BW - Develop toxic signs within 3 h (incoordination, shivering, heavy breathing, convulsion, diarrhea, etc.).
- 2) Injection of > 200 mg Fe/day - May increase bacterial growth, thus become susceptible to infections & diarrhea.

C. Toxicity in general:

- 1) Chronic - Reduced feed intake, growth rate and feed efficiency.
- 2) Acute - Anorexia, diarrhea, hypothermia, shock, metabolic acidosis, vascular congestion of various organs & death.

D. Maximum tolerable levels - 500 ppm for sheep, 1,000 ppm for cattle & poultry, and 3,000 ppm for swine.

COPPER

1. Deficiency/Functions

A. Anemia:

- 1) Can result from poor Fe mobilization, abnormal hemopoiesis, etc.
- 2) Cu enhances transport of Fe, and catalyzes incorporation of Fe into Hb.

☞ Ceruloplasmin has ferrioxidase activity, thus can be involved in conversion of the ferrous (Fe^{2+}) to ferric (Fe^{3+}) state, which can be incorporated into transferrin.

- 3) Cu also assists maturation of erythrocytes, and may be involved in the retention of erythrocytes.

B. Abnormal bone development:

- 1) “Bowling” of the leg, spontaneous fractures & others (low osteoblastic activity).
- 2) Cu is involved in the collagen synthesis - Is a component of lysyl oxidase, thus, Lys to allysine → desmosine & isodesmosine → cross-linking of collagen?!

C. Cardiac and vascular disorders - e.g., Aortic rupture in turkeys, and falling disease in cattle (heart failure, atrophy of myocardium, etc.).

- 1) A component of lysyl oxidase, and involved in elastin synthesis (cross-linking).
- 2) Ceruloplasmin (6 Cu/molecule) - Inhibiting peroxidation?
- 3) A component of superoxide dismutase, which converts superoxide to hydrogen peroxide & oxygen?

D. Abnormal pigmentation (not in pigs) - Due to loss or lack of melanin synthesis:

- 1) Possibly due to a ↓ in activity of tyrosinase (polyphenyl oxidase, which contains Cu).
- 2) Tyrosinase is involved in conversion of Tyr to dopa (dihydroxy-Phe), and dopa is converted to melanin.

E. Also, Cu is a component of many oxidases and other enzymes, and a activator of a number of enzymes, thus likely to be involved in the CNS functions, reproduction, immune system, lipid metabolism, etc.

2. Requirements/Toxicity

A. Requirements:

Animal	mg/kg
Poultry (NRC, 1984):	
Immature chickens	4-5
Laying hens	?
Broilers	8
Turkeys	6-8
Swine (NRC, 1998):	
Growing	3-6
Adults	5
Horses (NRC, 1989; DM)	10
Fish (NRC, 1993):	
Channel catfish	5
Rainbow trout & common carp	3
Others	Not tested or determined

B. Toxicity:

- 1) Signs include loss of appetite, ↑ thirst, apathy, ↑ breathing rate, intensified heart beat, jaundice, hemolysis, necrosis of liver & death.
- 2) Maximum tolerable levels:

Sheep	25 ppm
Cattle	100 ppm
Chickens & turkeys	300 ppm
Rats	1,000 ppm
Swine	250 ppm

3. The Use of Copper as a Growth Stimulant

- A. A high dietary level of Cu (100-250 ppm) has antimicrobial activity, and acts like an antibiotic.
- B. Widely used as a growth promotant for pigs in the U.K. and Europe.
- C. Dietary copper supplementation?

- 1) Effect of Cu on performance of starter pigs (a summary of seven 7 28-d studies with 4-wk old pigs): (Cromwell. Pers Comm.)

Cu, ppm:	0	125	250	500	
ADG, g		245	291	305	236
G:F		.49	.52	.54	.45
Liver Cu, ppm		23	24	191	349

- 2) The most effective supplementation seems to be 250 ppm Cu.

D. Dietary copper and age of pigs:

- 1) A summary of 12 starter and 18 grower/finisher experiments: (Cromwell. Pers. Comm.)

Item	0	250	% ↑
Starter:			
ADG, g	227	286	26
G:F	.49	.54	9
Grower:			
ADG, g	659	714	6.9
G:F	.36	.37	3.6
Finisher:			
ADG, g	714	736	3.1
G:F	.31	.32	2.5
Liver Cu, ppm (DM)	22	244	

- 2) Dietary Cu supplementation is the most effective during the starter phase.

E. Sources:

- 1) Copper oxide & sulfide - Totally unavailable.
- 2) Copper sulfate - Available and most commonly used.

☞ 2 lb of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ /ton of diet provide 250 ppm Cu.

- 2) Copper carbonate & chloride, sequestered copper & chelated copper Met are all effective, but costly.

F. Cu & Fe:

- 1) When using 250 ppm Cu, may have to provide \approx 50% more dietary Fe (\approx 150 ppm), or even more?

2) Supplemental Cu and Fe on performance & hematology of weanling pigs: (Dove & Haydon, 1991. J. Anim. Sci. 69:2013)

	5 ppm Cu			250 ppm Cu			
	+ Fe, ppm ^a :	100	200	300	100	200	300
Hematocrit, % ^b		37.2	39.9	40.5	39.9	39.3	41.4
Hb, g/dL ^b		10.6	11.2	10.8	11.2	11.3	11.9
Plasma Fe, µg/dL ^b		245	248	233	218	233	241
Gain, g/d ^c		320	360	330	410	400	430
Feed:gain ^c		1.62	1.54	1.65	1.55	1.59	1.59

^aBasal diet contained 169 ppm Fe & added 50, 100, 150, 200, 250 & 300 ppm; ^bCu x Fe interaction, *P* = 0.05 to 0.06; ^cEffect of Cu, *P* < 0.01.

G. Mode of action:

- 1) Not well defined, but probably similar to the action of antibiotics - Unclear, but some suggestions?
 - a) Metabolic effects - Direct influences on metabolic processes such as ↓ FA oxidation, ↑ protein synthesis, etc.
 - b) Nutritional effects (cannot separate completely from the metabolic effect):
 - (1) May be ↓ undesirable microbes & ↑ desirable microbes, i.e., ↑ the population of microbes that synthesize vitamins & amino acids.
 - (2) Inducing changes in the GI tract - e.g., Thinner wall of the gut, thus may ↑ absorption rate & ↓ energy expenditures.
 - c) Disease controlling effects.
- 2) Effect of Cu is “additive” to antimicrobial agents.

H. Some concerns regarding the use of high levels of copper:

- 1) Toxicity in pigs - The optimum level for growth promoting effect & toxicity level are similar!
- 2) Adverse effects on humans - e.g., Consumption of high-Cu liver.
- 3) Rapid deterioration of galvanized metals (buildings & equipment).
- 4) Reduced microbial decomposition of wastes in lagoons.
- 5) Can be an environmental pollutant.