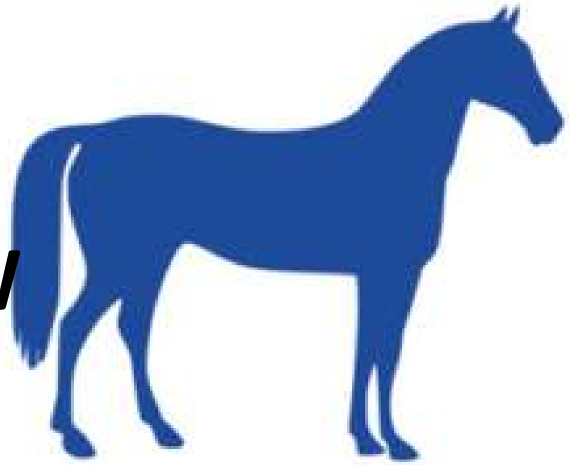
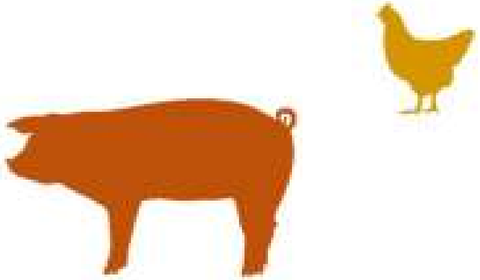


DIETARY COPPER: A REVIEW

Dr Marco Fantinati, DVM, resident ECVCN

Supervisor: Dr Nathalie Priymenko



Introduction

Trace element

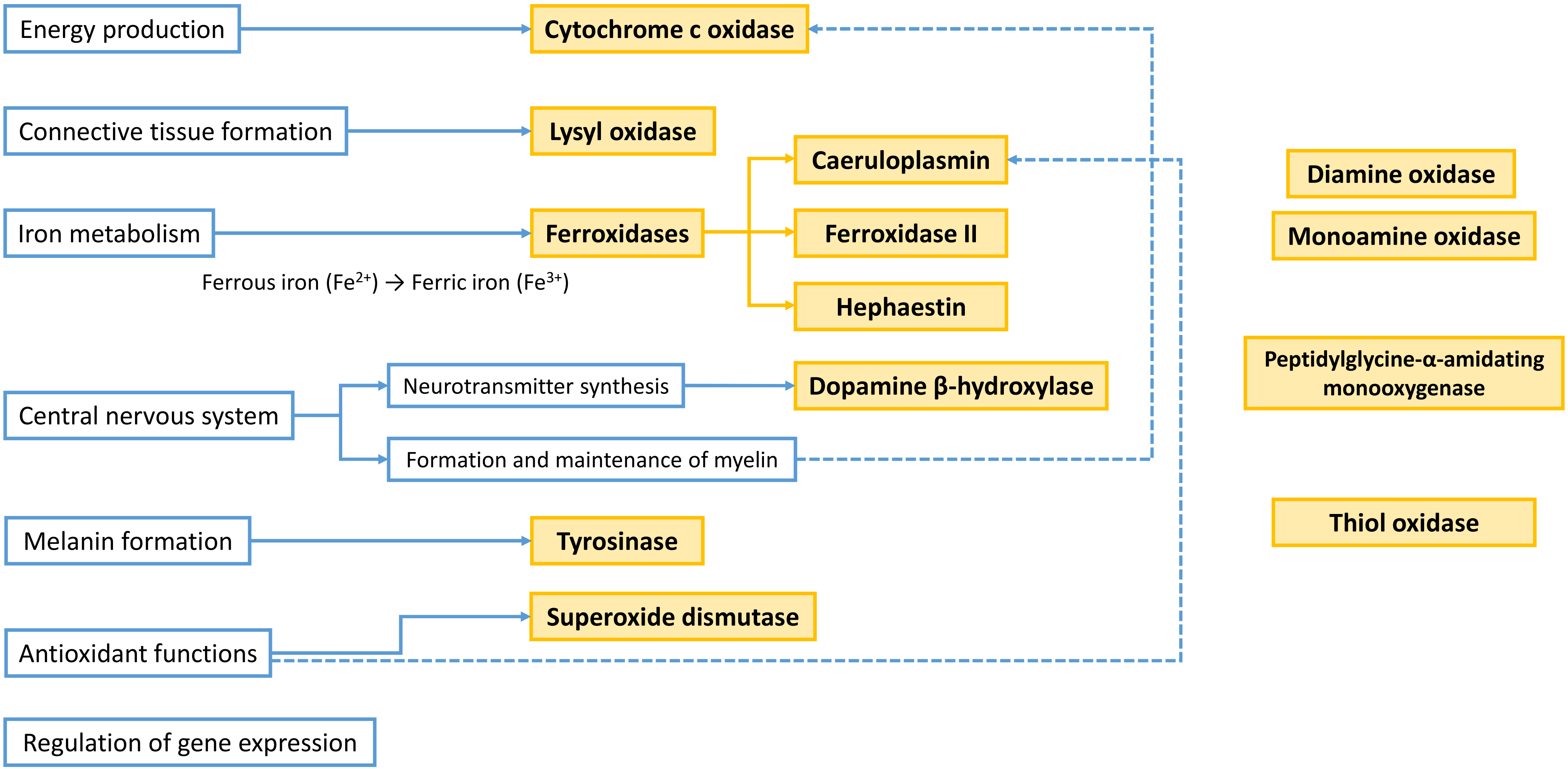
Essential nutrient [20, 21]

Essentiality of Cu depends on its ability to gain and lose an electron to form cuprous (Cu^{+}) and cupric (Cu^{2+}) states

Present in and essential for the activity of numerous enzymes, cofactors and reactive proteins

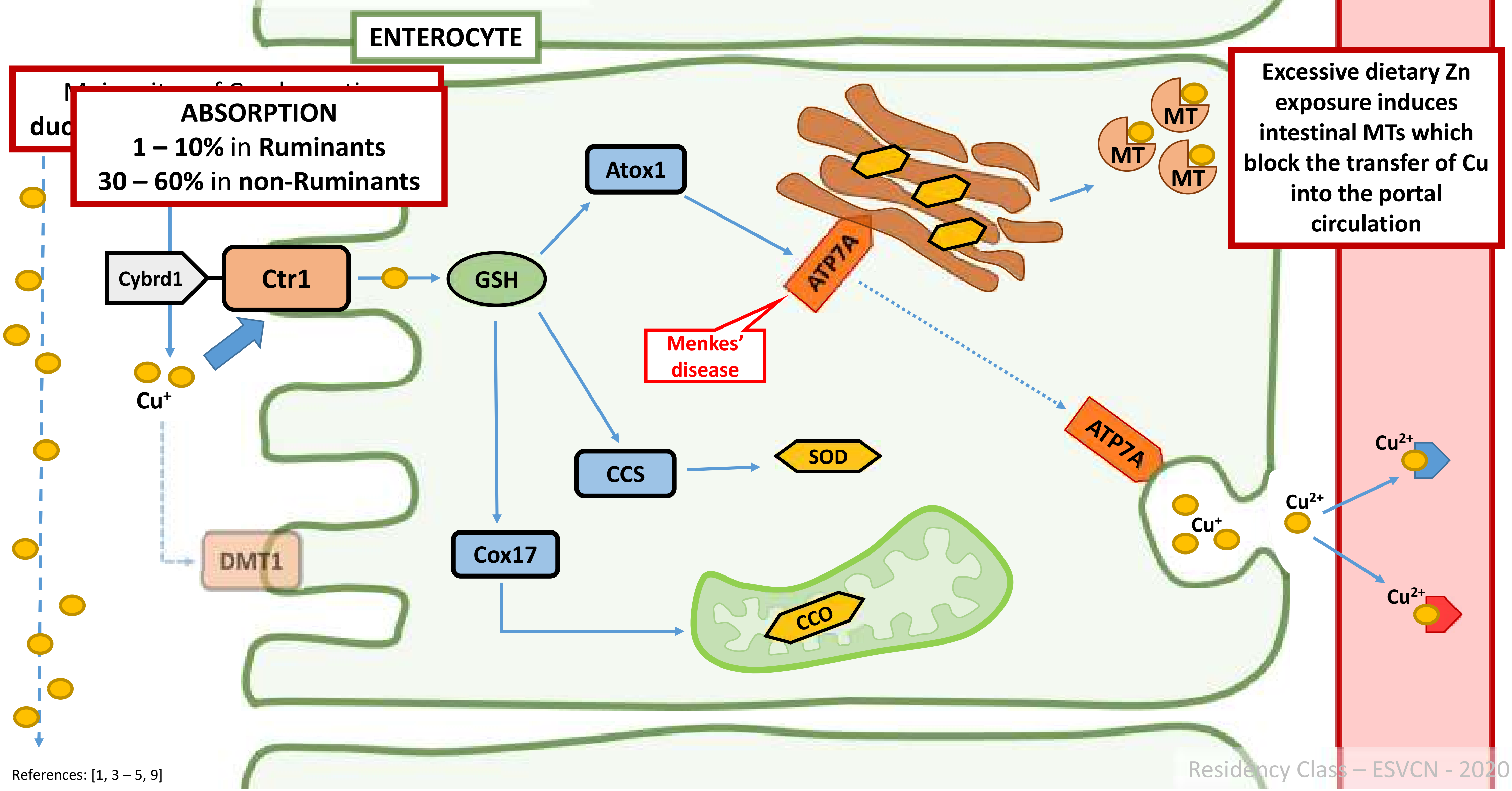
References: [1, 2, 20, 21]

Cu Functions



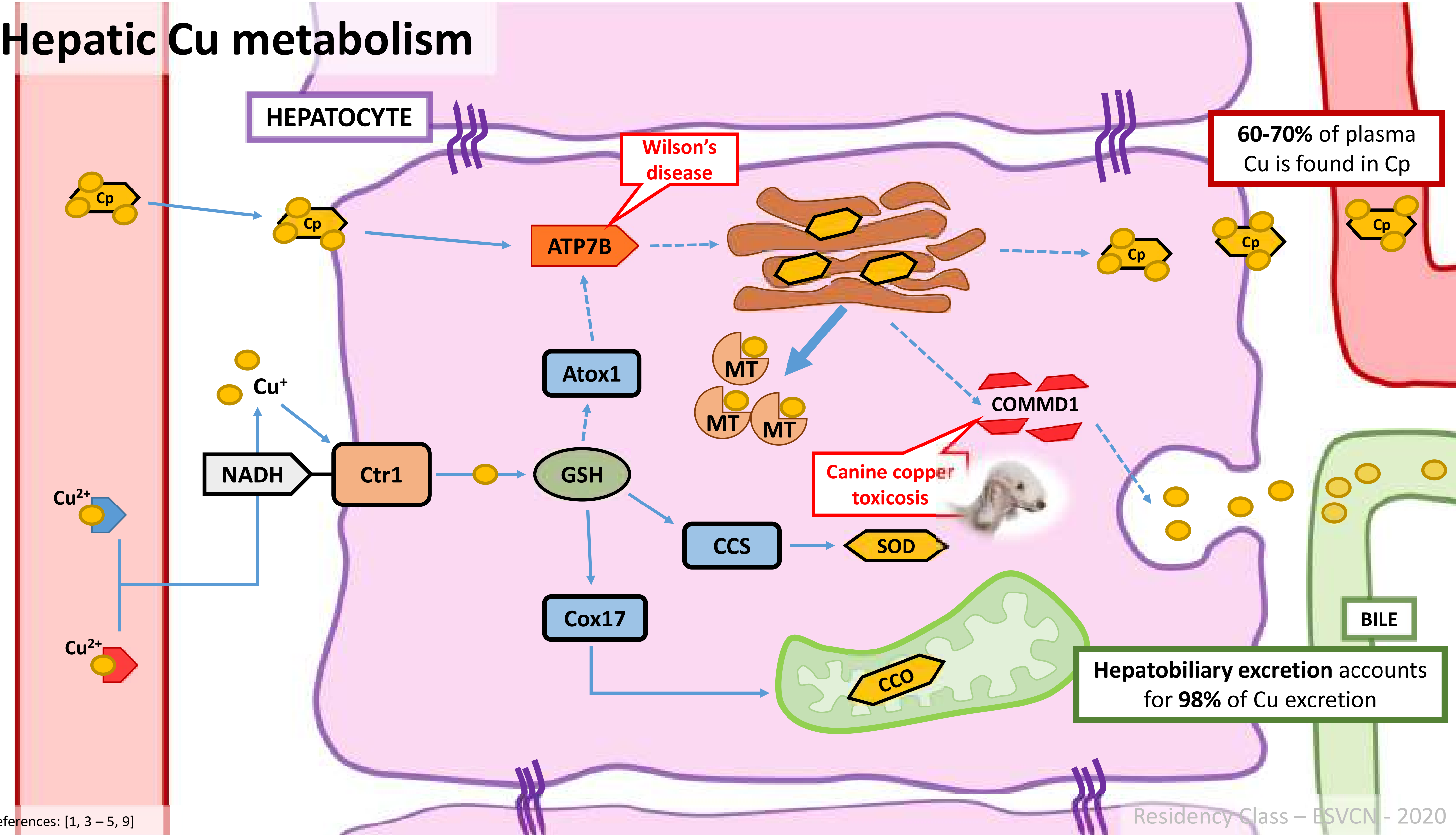
References: [2, 20, 21]

Intestinal Cu metabolism



References: [1, 3 – 5, 9]

Hepatic Cu metabolism



References: [1, 3 – 5, 9]

Cu homeostasis

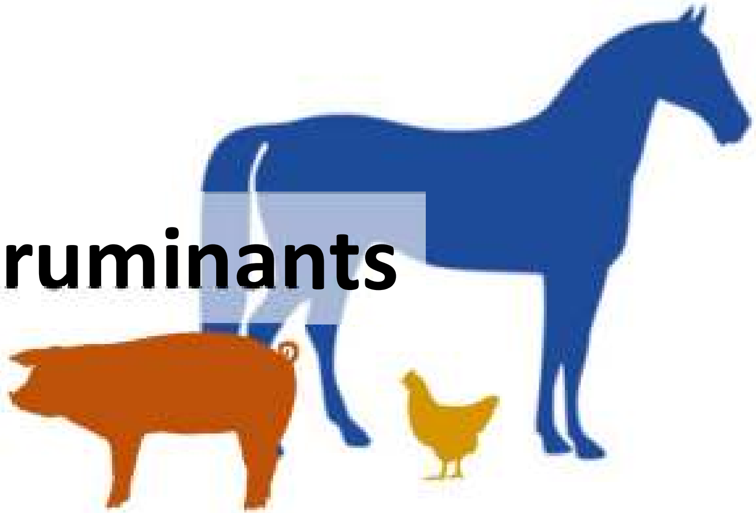
References: [1, 3 – 5, 9, 25]

Interspecific differences

Ruminants

VS

Non-ruminants



↓ capacity of Cu excretion with the bile

↓ Tolerance

Only a small amount of Cu is bound to MT in the hepatocyte

↑ capacity of Cu excretion with the bile

↑ Tolerance

Most of Cu (up to 80%) is bound to MT in the hepatocyte

Cu in feed materials

Geological origin of soil influences Cu concentrations in plants and plant materials

→

Cu concentration in soil
Soil conditions (e.g. pH, ion exchange capacity)
Soil Organic Matter
Fertilisation
Application of Cu as fungicide
Genetic differences in plant species
Part of the plant
Stage of maturity

Forages
3 – 10 mg Cu/kg DM

Cereals, legumes and oilseeds
5 – 35 mg Cu/kg

Oilseed meals
5 – 60 mg Cu/kg

Dried grape pomace
75 mg Cu/kg

Trace minerals supplements
Premixes
Loose mineral mixes
Mineral blocks/licks or buckets

References: [26, 44] http://www.feedipedia.org/

Cu in agriculture

Cu is used in agriculture to control a variety of **fungal and bacterial diseases**, most importantly in vineyards, orchards, and vegetable production (including potatoes)

Cu is the only active ingredient with strong antimicrobial activity approved for use in **Organic Agriculture**

Potato late blight

Apple scab

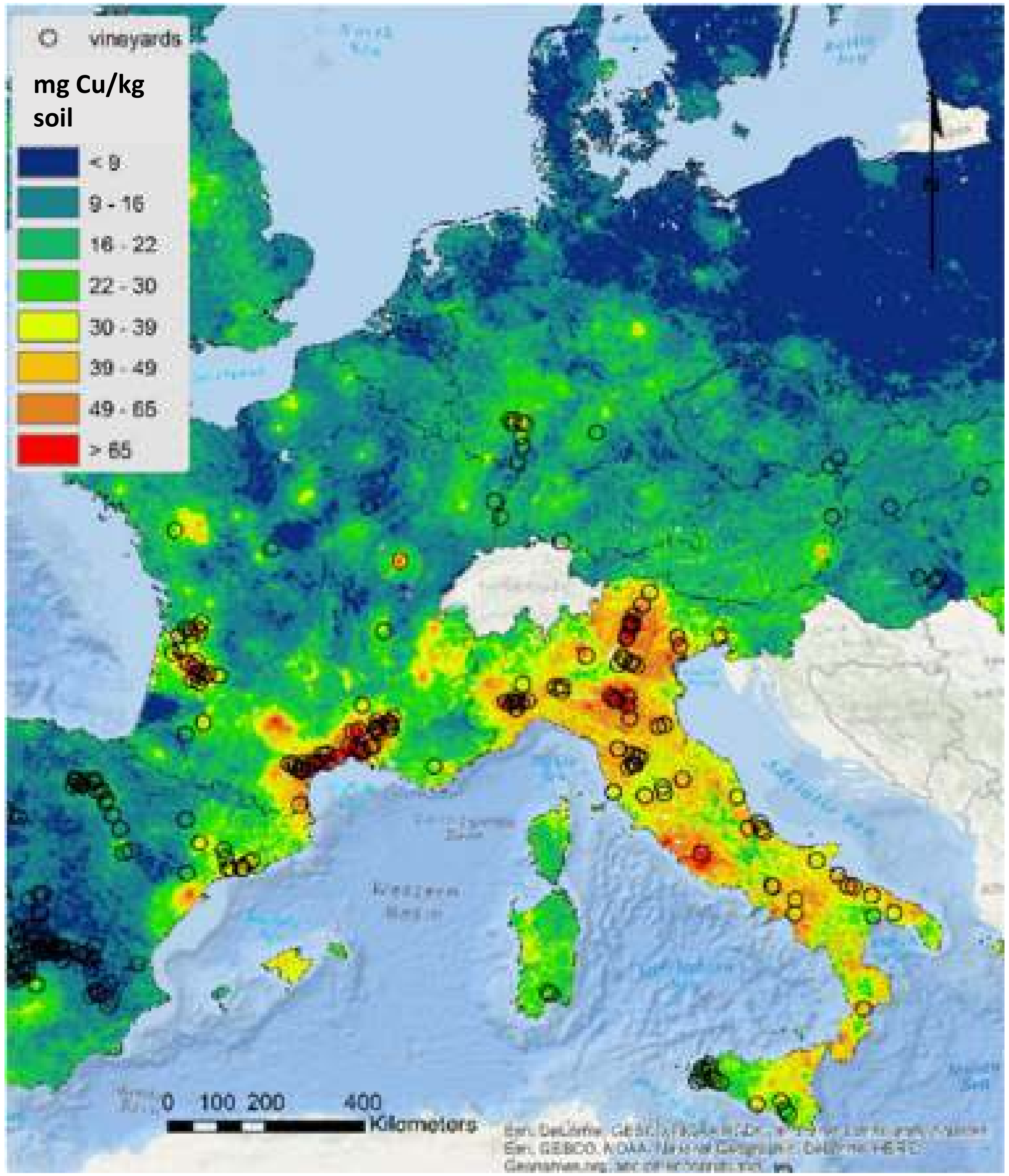
Grape downy mildew

10 – 20 applications per year

Negative environmental effects, notably on soil organisms and crop auxiliary species

In the majority of EU countries the use of Cu is restricted to a maximum of **6 kg/ha/year**

Figure from: [37]



Disposal of Cu-enriched manure

In the EU, animal manure can be used as **organic fertiliser**

In **pig** farming, **faecal Cu** accounts for up to **72-80% of the amount ingested**

Nitrate Directive 91/676/EEC poses a limit of 170 kg/ha/year of nitrogen (N) from organic manure

Nicholson *et al.* reported that an application of 250 kg/ha of N with pig slurry made a contribution of 1.6 kg Cu/ha
Moral *et al.* reported that an application of 210 kg/ha of N with pig slurry made a contribution of 3.8 kg Cu/ha
Provolo *et al.* reported that an application of 200 kg/ha of N with pig slurry made a contribution of >0.5 kg Cu/ha

There is no legal threshold for Cu in animal manure, this is indirectly controlled by limitations in animal feed

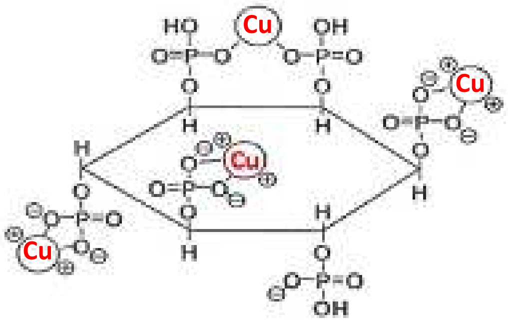
In piglets	**Until August 13th 2019**	**As of August 13th 2019**
Up to 4 weeks post-weaning	170 mg Cu/kg feed	150 mg Cu/kg feed
5 to 8 weeks post weaning	170 mg Cu/kg feed	100 mg Cu/kg feed

References: [26, 35, 36, 38, 39]

Cu interactions with dietary constituents

Presence of **PHYTATE** in cereal grains and oilseeds may depress the availability of Cu in monogastric animals

No consistent effect of **microbial phytase** addition on Cu status of pigs and poultry

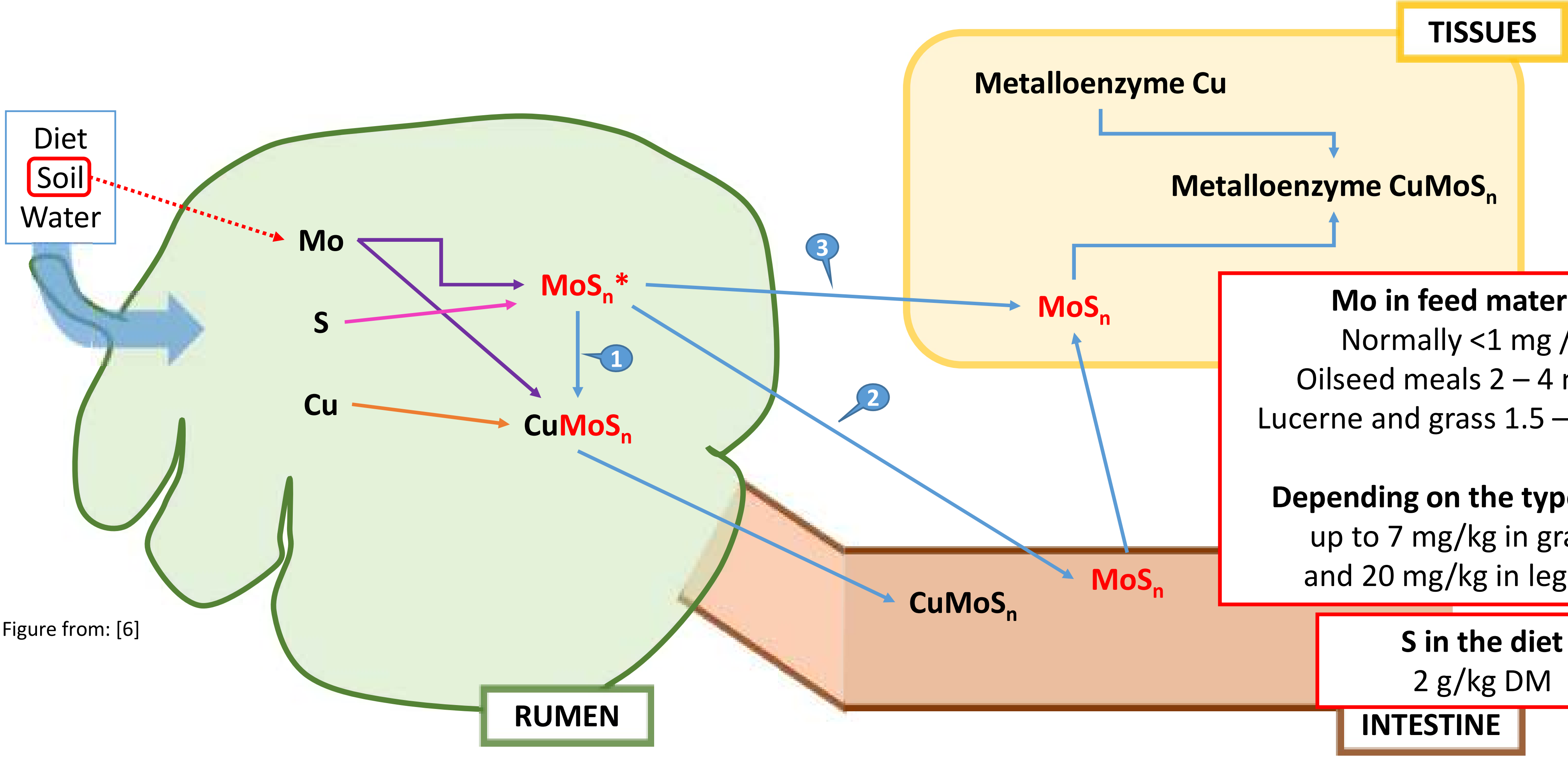


High exposure to **ZINC** may block the transfer of Cu from the gut by inducing enterocytic metallothionein which sequesters Cu in the intestinal epithelium
Induction of hepatic MTs promotes sequestering of free Cu in the non-toxic MT-bound form

High **IRON** can negatively influence Cu absorption, especially in the presence of high sulphur → Fe-Cu-S complexes in the rumen

References: [26, 44]

Thiomolybdates in Ruminants



Mo in feed materials
Normally <1 mg /kg
Oilseed meals 2 – 4 mg/kg
Lucerne and grass 1.5 – 3 mg/kg

Depending on the type of soil
up to 7 mg/kg in grasses
and 20 mg/kg in legumes [45]

S in the diet
2 g/kg DM [26]

Figure from: [6]

*thiomolybdate series $MoS_nO^{2-}_{(4 - n)}$ where n is 1 to 4

References: [2, 6 – 8, 10, 17 – 19, 26, 45]

	BOVINE	OVINE	PIG	POULTRY	HORSE
Requirements	**6 – 25 mg Cu/kg DM feed** Highest requirements are meant for **dairy cows** in the **close-up period** and in the **first weeks after parturition**	**5 – 14 mg Cu/kg DM feed** **CAPRINE** **5 – 15 mg Cu/kg DM feed**	**Piglets** and **growing-finishing pigs** **6 mg Cu/kg feed** **Reproducing sows** **8 – 10 mg Cu/kg feed**	**All poultry** species and categories **5 – 10 mg Cu/kg feed**	Vary according to breed, age, sex and pregnancy status ranging from **7 – 12.5 mg Cu/kg DM feed** In France **10 mg Cu/kg DM feed** for all ages, regardless of the degree of work and stage of production
Maximum Tolerable Levels	**40 mg/Cu kg DM diet**	Ovine **15 mg Cu/kg DM diet** Caprine **40 mg Cu/kg DM diet**	**250 mg Cu/kg diet**	**250 mg Cu/kg diet**	**250 mg Cu/kg diet**
Currently authorised maximum Cu content in feed in the European Union (EU) **[in complete feed with a moisture content of 12%]**	Before the start of rumination **15 mg Cu/kg feed** Other bovines **30 mg Cu/kg feed**	Ovine **15 mg Cu/kg feed** Caprine **35 mg Cu/kg feed**	Piglets suckling and weaned up to 4 weeks after weaning, **150 mg Cu/kg feed** From the 5th week after weaning up to 8 weeks after weaning, **100 mg Cu/kg feed** Other pigs **25 mg Cu/kg feed**	All poultry categories **25 mg Cu/kg feed**	Horses **25 mg Cu/kg feed**
	[2, 22 – 24, 26]	[2, 22 – 24, 26]	[26, 28]	[26, 27, 29]	[26, 28, 30]

Deficiency

VS

Toxicosis

Copper deficiency

Primary — Naturally Cu-deficient soils or Cu-deficient diet

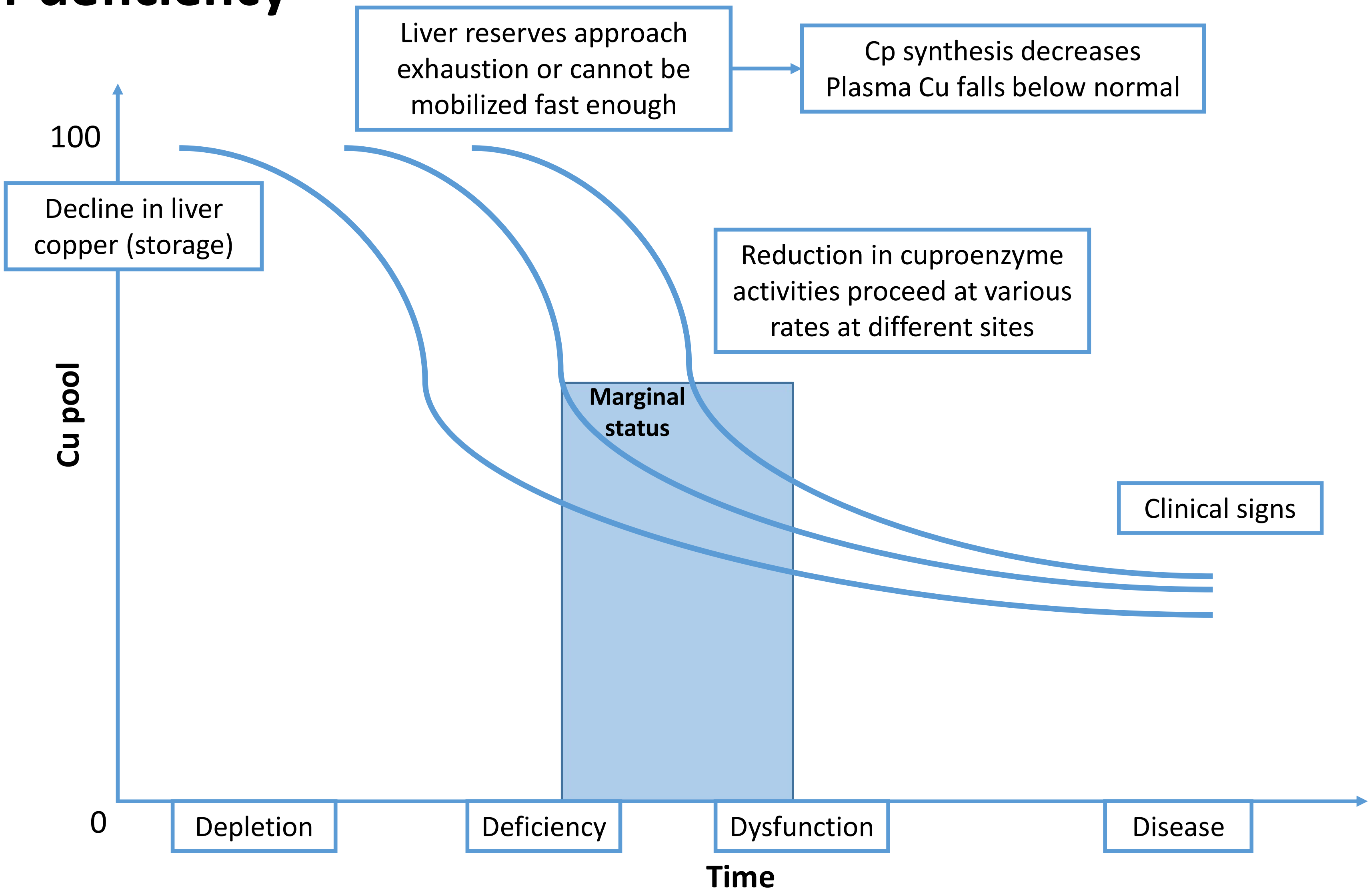
Secondary — Antagonism by Mo (and S) in Mo-rich soils

5-7 mg Mo/kg DM

Susceptible of Cu deficiency

References: [2]

Copper deficiency



References: [1, 2, 4]   Figure modified from: Suttle NF. *Mineral nutrition of livestock: Fourth edition*. 2010.

Clinical signs of Cu deficiency

"Swayback"

Enzootic ataxia — CCO

- Neonatal
- Delayed
- Atypical

Abnormal wool and hair — TO

Anaemia — HEP, Cp, SOD

Bone disorders — MAO, CCO

LOX

Connective tissue disorders

Depigmentation — TY

Diarrhoea

Cardiovascular disorders

Infertility

Growth retardation

Susceptibility to infection

References: [2, 22 – 24, 25, 29, 30]

Diagnosis of Cu Deficiency

Presence of clinical symptoms

Biochemical evidence of subnormal tissue or plasma Cu

Improvement after treatment with Cu

Species	Plasma Cu (µmol/l)	Liver Cu (µmol/kg DM)
Sheep and Cattle	3 – 9	100 – 300
Goats	5 – 8	180 – 300
Horse	8 – 12	100 – 200
Pig	14 – 16	100 – 1000
Poultry	3 – 6	100 – 500

Dietary Cu
Dietary Cu:Mo
Dietary Fe:Cu

Alternative criteria

Caeruloplasmin
Erythrocyte CuZnSOD
Hair or wool Cu → **Not a reliable diagnostic criterion**
Neutrophil Cu and SOD1
Plasma MAO
Liver CCO
Plasma diamine oxidase
…

References: [2, 7, 19, 26]

Prevention and Control

Pasture fertilizers ($CuSO_4$) [EU 2019/1009 application by 2022]

Dietary supplementation → Inorganic sources / Organic sources

EU register feed additives

Inorganic sources:
- Cu diacetate monohydrate (min 31% Cu)
- Cu carbonate dihydroxy monohydrate (min 52% Cu)
- Cu chloride dehydrate (min 36% Cu)
- Cu oxide (min 77% Cu)
- **Cu sulphate pentahydrate** (min 24% Cu)

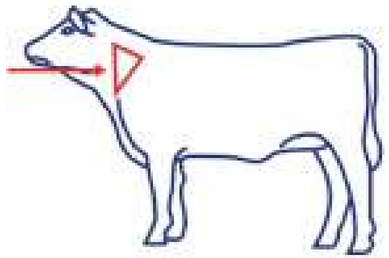
Organic sources:
- Cu chelate of amino acids hydrate (min 10% Cu)
- Cu chelate of protein hydrolysates (min 10% Cu)
- Cu chelate of glycine hydrate [solid] (min 15% Cu)
- Cu chelate of glycine hydrate [liquid] (min 6% Cu)

Slow-release boluses → Slow-release oral supplementation of Copper oxide wire particles (CuOWP) - - → Anthelmintic activity (*Haemonchus contortus*)

2 g CuOWP in lambs

References: [11, 12, 33]

Parenteral administration → SC injections



References: [2, 11 – 16, 33]

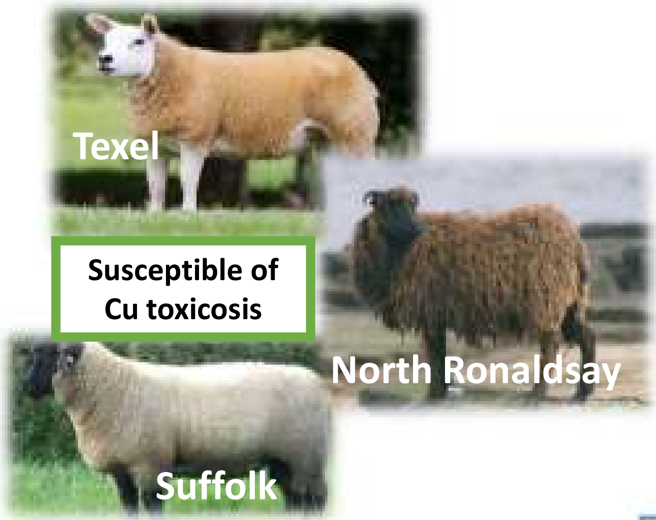
Chronic Cu Poisoning

Clinical and subclinical CCP represent nowadays the **main toxicological problem** in ruminants, especially sheep

CCP is a two-stage process → 1st stage – prehaemolytic phase → Cu accumulates in the liver without clinical signs of disease

Up to 1000 – 1500 mg Cu/kg DM

↑ GLDH and GMT



Liver's capacity is overloaded

Liver degeneration

Cu is released from the liver

2nd stage – haemolytic crisis

CCP results from
- Excessive supplementation of Cu
- Use of feeds contaminated with Cu from agricultural or industrial sources
- Grazing on pastures grossly contaminated with Cu-enriched pig slurry

References: [2, 22 – 24, 26, 32]

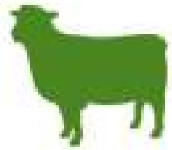
Diagnosis of CCP

Clinical evidence of haemolysis → **Jaundice Haemoglobinuria**

Confirmed by **high level of liver and kidney Cu** and **kidney Fe**

Species	Liver Cu (mg/kg DM)	Kidney Cu (mmol/kg DM)	Kidney Fe (mmol/kg DM)
Sheep	405 – 1015	0.6 – 0.8	16 – 18
Cattle	405 – 1015	0.6 – 0.8	16 – 18
Goat	405 – 1015	0.6 – 0.8	16 – 18
Pig	2029 – 3043	32.0 – 48.0	-
Poultry	63 – 127	-	-

Surviving, clinically normal animals - - → ↑ liver enzymes (serum AAT, GLDH, GGT) - - → Confirmation of pre-haemolytic liver injury



References: [2, 22 – 24, 26, 32]

Prevention and Treatment of CCP

Antagonists of Cu can be used to prevent CCP

- Dietary supplementation with Mo and S
  - Cu-deficiency
  - S intoxication
- Dietary supplementation with Zn
  - Low capacity of MT induction in sheep prevents Zn from producing an increase in biliary Cu excretion

Transfer animals to a diet low in available Cu

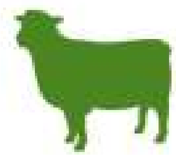
Ammonium Tetrathiomolybdate (ATTM)

Inhibition of Cu absorption in the GIT

Complex with Cu in the blood stream

Enters the liver and binds up to 6 Cu ions

- **PREVENTION**: IV injections, each of 1.7 mg ATTM/kg LW, every other day for 6 days [49]
- **TREATMENT**: Three SC injections, each of 3.4 mg ATTM/kg LW, given on alternate days [48]



References: [2, 22 – 24, 26, 32, 48, 49]

Growth promotion

Mechanism behind the growth-promoting effect are still not fully understood

Hypothesis

Antimicrobial effect in the gut

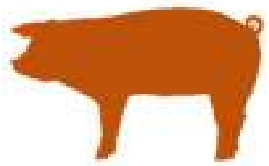
Less microbial load and less microbial toxic metabolites

Foregut

↓ lactic acid bacteria

↓ clostridia and coliforms

More energy and nutrients available for the pig itself



Systemic effect

125 – 250 mg Cu/kg diet [40]

↑ hypothalamic NPY concentration and expression

Up-regulation of appetite

↑ feed intake

Growth promotion seems to be greater in young pigs fed **organic chelates** (e.g. Cu-Lys, Cu-Met) compared to those fed cupric sulphate

References: [26, 40, 41]

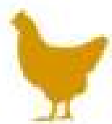
Cu and lipid metabolism

Results are inconsistent and poorly understood

Supplementation of 20 – 40 mg Cu/kg DM to high-concentrate finishing diets [42]

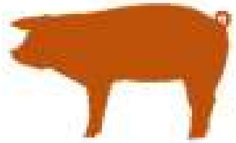
↓ subcutaneous adipose tissue deposition
↓ cholesterol concentration
↑ UFA in beef

Effects on biohydrogenation, esterification and mobilisation of triglycerides [43]



Diets containing 125 – 200 mg Cu/kg diet [29]

↓ egg cholesterol by 7 – 25%



Diets containing 250 mg Cu/kg diet [26]

↑ UFA and peroxide value of the backfat

Above EU maximum Cu content

References: [26, 42, 43]

Cu and antibiotic-resistant bacteria in the soil

Metal-driven co-selection of antimicrobial resistance

Simultaneous resistance to Cu and antibiotics has been observed for several bacterial species

When bacteria encounter adverse conditions, such as high levels of potential toxicants, dormant survival mechanisms are triggered

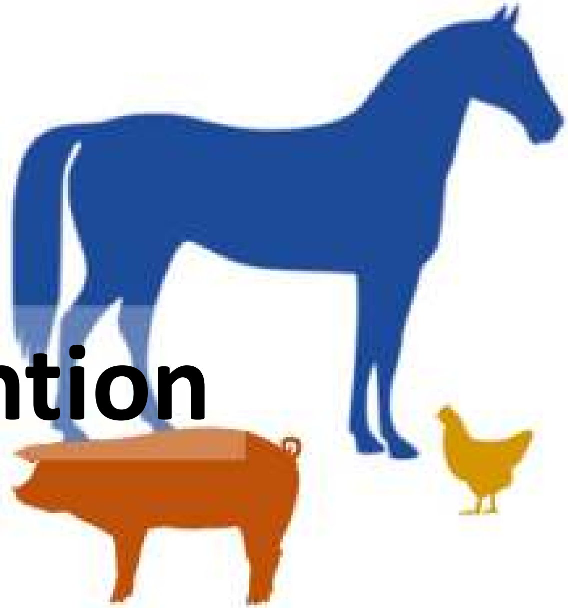
→ High Cu content in manure represents a challenge to bacterial survival

→ May be followed by increased resistance to antibiotics in these areas

Sufficiently clear demonstration of this chain of events is currently not available

References: [26, 46, 47]

Thank you for your attention



References

[1] Prohaska JR. Copper. In: *Present Knowledge in Nutrition*. Oxford, UK: Wiley-Blackwell, pp. 540–553.
[2] Suttle NF. *Mineral nutrition of livestock: Fourth edition*. 2010.
[3] Maryon EB, Molloy SA, Kaplan JH. Cellular glutathione plays a key role in copper uptake mediated by human copper transporter 1. *Am J Physiol Physiol* 2013; 304: C768–C779.
[4] Clarkson AH, Paine S, Martín-Tereso J, et al. Copper physiology in ruminants: trafficking of systemic copper, adaptations to variation in nutritional supply and thiomolybdate challenge. *Nutr Res Rev* 2020; 33: 43–49.
[5] Lin C, Zhang Z, Wang T, et al. Copper uptake by DMT1: a compensatory mechanism for CTR1 deficiency in human umbilical vein endothelial cells. *Metallomics* 2015; 7: 1285–1289.
[6] Gould L, Kendall NR. Role of the rumen in copper and thiomolybdate absorption. *Nutr Res Rev* 2011; 24: 176–182.
[7] Dick AT, Dewey DW, Gawthorne JM. Thiomolybdates and the copper–molybdenum–sulphur interaction in ruminant nutrition. *J Agric Sci* 1975; 85: 567–568.
[8] El-Gallad T. Thiomolybdates in rumen contents and rumen cultures. *J Inorg Biochem* 1983; 18: 323–334.
[9] Rucker RB, Fascetti AJ, Keen CL. Trace Minerals. In: *Clinical Biochemistry of Domestic Animals*. 2008. Epub ahead of print 2008. DOI: 10.1016/B978-0-12-370491-7.00022-2.
[10] Spears JW. Trace Mineral Bioavailability in Ruminants. *J Nutr*. Epub ahead of print 2003. DOI: 10.1093/jn/133.5.1506s.
[11] Burke JM, Miller JE, Brauer DK. The effectiveness of copper oxide wire particles as an anthelmintic in pregnant ewes and safety to offspring. *Vet Parasitol* 2005; 131: 291–297.
[12] Soli F, Terrill TH, Shaik SA, et al. Efficacy of copper oxide wire particles against gastrointestinal nematodes in sheep and goats. *Vet Parasitol* 2010; 168: 93–96.
[13] Balemi S, Grace N, West D, et al. Accumulation and depletion of liver copper stores in dairy cows challenged with a Cu-deficient diet and oral and injectable forms of Cu supplementation. *N Z Vet J* 2010; 58: 137–141.
[14] Bohman VR, Drake EL, Behrens WC. Injectable Copper and Tissue Composition of Cattle. *J Dairy Sci*. Epub ahead of print 1984. DOI: 10.3168/jds.S0022-0302(84)81463-0.
[15] Pogge DJ, Richter EL, Drewnoski ME, et al. Mineral concentrations of plasma and liver after injection with a trace mineral complex differ among Angus and Simmental cattle. *J Anim Sci* 2012; 90: 2692–2698.
[16] Pate RT, Cardoso FC. Injectable trace minerals (selenium, copper, zinc, and manganese) alleviate inflammation and oxidative stress during an aflatoxin challenge in lactating multiparous Holstein cows. *J Dairy Sci* 2018; 101: 8532–8543.
[17] Raisbeck MF, Siemion RS, Smith MA. Modest Copper Supplementation Blocks Molybdenosis in Cattle. *J Vet Diagnostic Investig* 2006; 18: 566–572.
[18] Bailey JD, Ansotegui RP, Paterson JA, et al. Effects of supplementing combinations of inorganic and complexed copper on performance and liver mineral status of beef heifers consuming antagonists. *J Anim Sci* 2001; 79: 2926.
[19] Tompsett SL. The copper content of blood. *Biochem J* 1934; 28: 1544–1549.
[20] Hart EB, Elvehjem CA, Waddell J, et al. Iron in nutrition IV. Nutritional anemia on whole milk diets and its correction with the ash of certain plant and animal tissues or with soluble iron salts. *J Biol Chem* 1927; 72: 299.
[21] Hart EB, Steenbock H, Waddell J, et al. Iron in nutrition VII. Copper as a supplmenet to iron for hemoglobin building in the rat. *Nutr Rev* 1928; 77: 797.
[22] *Nutrient Requirements of Small Ruminants*. Washington, D.C.: National Academies Press. Epub ahead of print 22 December 2007. DOI: 10.17226/11654.
[23] *Nutrient Requirements of Beef Cattle, 8th Revised Edition*. 2015. Epub ahead of print 2015. DOI: 10.17226/19014.
[24] *Nutrient Requirements of Dairy Cattle*. Washington, D.C.: National Academies Press. Epub ahead of print 9 January 2001. DOI: 10.17226/9825.
[25] FEEDAP. Revision of the currently authorised maximum copper content in complete feed. *EFSA J*; 14. Epub ahead of print August 2016. DOI: 10.2903/j.efsa.2016.4563.

References

[26] Cano-Sancho G, Rovira J, Perelló G, et al. Extensive Literature Search on the bioavailability of selected trace elements in animal nutrition: Incompatibilities and interactions. *EFSA Support Publ*; 11. Epub ahead of print March 2014. DOI: 10.2903/sp.efsa.2014.EN-565.
[27] Dale N. National Research Council Nutrient Requirements of Poultry – Ninth Revised Edition (1994). *J Appl Poult Res* 1994; 3: 101.
[28] NRC. *Nutrient Requirements of Swine*. Washington, D.C.: National Academies Press. Epub ahead of print 2 July 2012. DOI: 10.17226/13298.
[29] Leeson S. Copper metabolism and dietary needs. *Worlds Poult Sci J* 2009; 65: 353–366.
[30] Geor RJ, Harris PA, Coenen M. *Equine Applied and Clinical Nutrition: Health, Welfare and Performance*. 2013. Epub ahead of print 2013. DOI: 10.1016/C2009-0-39370-8.
[31] Malinak CM, Hofacre CC, Collett SR, et al. Tribasic Copper Chloride Toxicosis in Commercial Broiler Chicks. *Avian Dis* 2014; 58: 642–649.
[32] López-Alonso M. Trace Minerals and Livestock: Not Too Much Not Too Little. *ISRN Vet Sci* 2012; 2012: 1–18.
[33] Burke JM, Miller JE. Evaluation of multiple low doses of copper oxide wire particles compared with levamisole for control of Haemonchus contortus in lambs. *Vet Parasitol* 2006; 139: 145–149.
[34] Andrivon D, Bardin M, Bertrand C, et al. *Can organic agriculture give up copper as a crop protection product?* 2018.
[35] Provolo G, Manuli G, Finzi A, et al. Effect of Pig and Cattle Slurry Application on Heavy Metal Composition of Maize Grown on Different Soils. *Sustainability* 2018; 10: 2684.
[36] Sommer SG, Christensen ML, Schmidt T, et al. *Animal Manure Recycling*. Chichester, UK: John Wiley & Sons, Ltd. Epub ahead of print 9 September 2013. DOI: 10.1002/9781118676677.
[37] Ballabio C, Panagos P, Lugato E, et al. Copper distribution in European topsoils: An assessment based on LUCAS soil survey. *Sci Total Environ* 2018; 636: 282–298.
[38] Nicholson F., Chambers B., Williams J., et al. Heavy metal contents of livestock feeds and animal manures in England and Wales. *Bioresour Technol* 1999; 70: 23–31.
[39] Moral R, Perez-Murcia MD, Perez-Espinosa A, et al. Salinity, organic content, micronutrients and heavy metals in pig slurries from South-eastern Spain. *Waste Manag* 2008; 28: 367–371.
[40] Yang G, Su J, Yao Y, et al. The regulatory mechanism of neuromedin S on luteinizing hormone in pigs. *Anim Reprod Sci* 2010; 122: 367–374.
[41] Zhu D, Yu B, Ju C, et al. Effect of high dietary copper on the expression of hypothalamic appetite regulators in weanling pigs. *J Anim Feed Sci*. Epub ahead of print 2011. DOI: 10.22358/jafs/66158/2011.
[42] Engle TE. Copper and lipid metabolism in beef cattle: A review. *Journal of Animal Science*. Epub ahead of print 2011. DOI: 10.2527/jas.2010-3395.
[43] Netto AS, Zanetti MA, Claro GR Del, et al. Effects of Copper and Selenium Supplementation on Performance and Lipid Metabolism in Confined Brangus Bulls. *Asian-Australasian J Anim Sci* 2014; 27: 488–494.
[44] Batal A, Dale N, Persia M. Ingredient Analysis Table: 2012 Edition. *Ingredients Byprod*.
[45] Kabata-Pendias A. *Trace Elements in Soils and Plants*. CRC Press. Epub ahead of print 18 October 2010. DOI: 10.1201/b10158.
[46] Berg J, Thorsen MK, Holm PE, et al. Cu Exposure under Field Conditions Coselects for Antibiotic Resistance as Determined by a Novel Cultivation-Independent Bacterial Community Tolerance Assay. *Environ Sci Technol* 2010; 44: 8724–8728.
[47] Seiler C, Berendonk TU. Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. *Front Microbiol*; 3. Epub ahead of print 2012. DOI: 10.3389/fmicb.2012.00399.
[48] Humphries W, Morrice P, Bremner I. A convenient method for the treatment of chronic copper poisoning in sheep using subcutaneous ammonium tetrathiomolybdate. *Vet Rec* 1988; 123: 51–53.
[49] Ledoux DR, Henry PR, Ammerman CB. Response to high dietary copper and duration of feeding time on tissue copper concentration of sheep. *Nutr Res* 1996; 16: 69–78.