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Yves Y. Nys, Catherine Jondreville, Agnès Narcy, Patrick Schlegel

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# How nutritional Zinc requirements and recommendations for broilers are defined

Yves NYS

with contribution of Catherine JONDREVILLE, Agnès NARCY and Patrick SCHLEGEL

INRA UR83, Poultry research unit  
37380 Nouzilly, France

France



# Content

## ❖ Context

- Challenge for innovative feeding system in Poultry production

## ❖ Zn Requirements in broilers related to performance, health and environment

- Zn deficiency and excess
- Zn function
- Zn requirement and performance
- Zn and tissue composition
- Zn and immunity
- Zn Recommendations in Poultry

## ❖ Zn and environment

## ❖ bioavailability of Zn

- Phytate, phytase
- Intestinal physiology
- Dietary viscosity
- Source effect

## ❖ Conclusions

# Challenge for animal production



## We must increase food security globally

2050: world population expected to rise by 33%, +2.3 billions,  
Food demand increases with higher inhabitant's income  
Asia-Africa: 80% population but only 36% corn, 13% soybean, 50% wheat



## particular demand from the consumers:

nutritional value, hygienic and sensorial quality,  
Need to take into account welfare in production system



## Sustainability of production, environmental concern

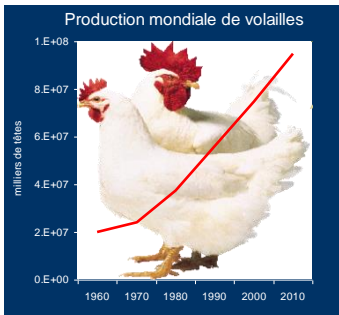
Competition for land  
Shortage of feedstuffs (about 20 years for inorganic Zn)  
Eutrophication, plant phytotoxicity  
Competition with other food industry and low economical margin

→ Production of high quality products for human nutrition taking into account sustainability of production systems and environment.

# CONTEXTS

Availability of high performing birds

Poultry: Animal proteins without consumer restriction



Sustainable agriculture production systems

Control on environmental impact

Competition for land



Competition with other food industry

feedstuffs price volatility

Low economical margin

New regulations:

Ban of antibiotics, of conventional cage system

traceability of products...



**Need to continuously innovate to face constraints and competition**

# Feeding Systems

**Macro-elements:** P, Ca (g/kg)  
**Trace-elements:** Fe, Cu, Zn, I, Se (mg/kg)

## ❖ Requirements

Mineral implicated in numerous functions in animals (organ, tissue, cell levels) :growth, bone formation, enzymatic activities ...

- Variable response to nutrient depending on animal parameters (performance or tissue composition)

## ❖ Requirements established on performance

needs for other functions ?? Growth promotor, immune function, bone, anti-oxidant status, meat quality...

## Environmental concern:

### ❖ Adjusting mineral supply to requirement to limit excretion

- Mineral contents in feedstuffs and mineral sources
- Interactions between dietary components

### ❖ Improve bioavailability of mineral

# Trace element deficiencies

Syndrome	Cu	Fe	Zn	Mn	Se	I
Reduced weight gain	+	+	+	+	+	+
Skeletal disorders	+		+	+		
Poor feathering			+		+	
Anaemia	+	+				
Cardiovascular disorder	+				+	
Exudative diathesis				+		
Abnormal thyroid					+	

- ❖ Zn deficiency: Decreased growth, frizzled feathers, shortening and thickening of long bones, enlarged hocks in chicken (Nielsen, 2012)
- ❖ Zn deficiency: Increased oxidative stress and damage of molecules including DNA (Oteiza, 2012), reduced activity of Zn metalloenzymes

## ❄ Decreased immune response and increased bacterial and viral infection

- Thymus : - 50%, rate : - 60%
- Decreased gamma-globuline level
- Modification in proportion of various immunoglobulins
- Decreased production and maturation of lymphocytes T
- Decreased blood level of thymuline
- Decreased phagocyte activity in macrophage
- Lower neutrophil chimio-tactism

Fletcher et al., 1988



# Dietary excess of Zn

## Effect of dietary Zn excess on Broiler Growth Performance

Dietary Zn supply (mg/kg)	Body weight gain (g/day)	FCI
0*	10.6a	1.56
500	9.8b	1.57
1000	8.8bc	1.63
1500	7.9c	1.77

\* 74 mg/kg in basal diet

Henry et al., 1987

40 à 750 mg Zn



no effect (6/7 trials)

>2000 mg Zn



decreased growth (10 to 50 %),  
higher mortality, gizzard lesions

Nys, 2003

- ❖ Zn Maximum tolerable level set at 500 mg/kg (NRC, 2005)
- ❖ Zn in excess cannot be used as a growth promotor in poultry
- ❖ Reduction in feed intake, weight gain when Zn (sulphate) > 500mg/kg
- ❖ Lesion in pancreas, gizzard at 1000 mg/kg Zn
- ❖ Depressing effect reinforced if diet low in Iron

# Zn Functions in Poultry

\* **Repartition : 60 % muscle, 30 % bone**

\* **Main functions**

- ➔ **Gene Expression (transcription factors) and cellular replication**
- ➔ **Stabilisation of protein (3D), hormone structure, membranes..**
- ➔ **Essential in growth of bones : alkaline phosphatase, collagenase and crystallinity of apatite**

\* **Constituents and cofactors of more than 300 enzymes**

- ➔ **Synthesis and degradation of lipids, proteins and nucleic acids**
- ➔ **Various types of enzymes: oxido-reductase, transferase, hydrolase, isomerase**

\* **Zn essential element for the immune system**

- ❖ **heterophils : phagocytosis, chemo-attractant**
- ❖ **macrophages : bactericidal, antigen presentation**
- ❖ **T-lymphocytes :disruption of virus, bacteria, infected cells**

**Mode of action**

- ❖ **Metalloenzymes (replication, transcription)**
- ❖ **regulation of immunological transcription factors**
- ❖ **cofactor for the thymus hormone, thymulin**

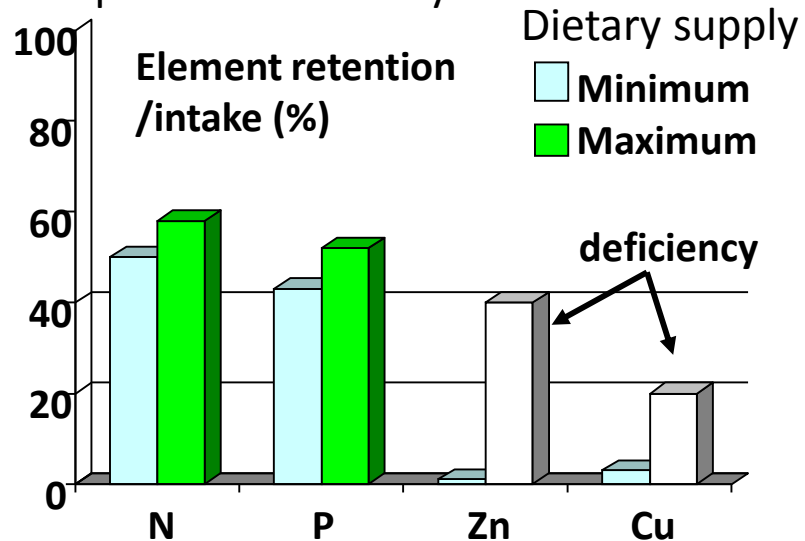
# Intestinal Absorption of Zn

- \* Body Zn regulated mainly through intestinal absorption and faecal excretion (if excess of dietary Zn, higher endogenous pancreatic, liver and gastric Zn secretion)
  - \* Zn absorbed mainly in Ileum (60%) with predominance of a cellular pathway (active process with Zip transporters regulated by dietary Zn) in addition to passive absorption (paracellular)
  - \* Zn interacts with metallothionine in enterocyte and liver. Level of MT stimulated by dietary Zn (allow Zn storage in the cell available in case of any stress)
- Associated with albumin and transferrin in plasma
- \* High variability of Zn absorption with species and dietary Zn level

Range in change of mineral retention associated with its dietary level



**Zn (or Cu) retention cannot be used for measuring efficiency of a dietary source**



# Zn requirement

- ❖ Zn Requirement corresponded to the dietary level of Zn needed to insure bird physiological function associated with Zn.
- ❖ Evaluated by empirical method in chicks and pigs
  - No use of factorial method (maintenance and production requirements X apparent absorption) due to large variability in Zn absorption with bird age, physiology or due to Zn dietary level and diet composition)

yield of body deposition of trace elements with broiler age (% of intake) :

Higher yield of transfer in young broilers

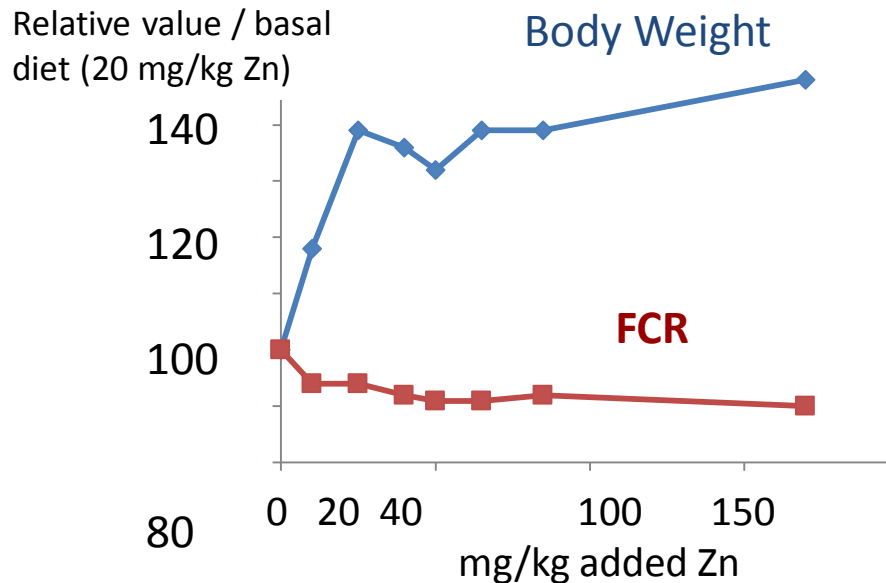
Age (days)	Fe	Zn	Cu	Mn
0-4	23	14	16	4.9
0-21	12	6	5	0.6
0-40	12	6	6	0.3

- Expressed in total dietary Zn or added dietary Zn and influenced by basal diet composition (synthetic or cereal diet)
- Cereals (25mg/kg Zn)-Soybean (45mg/kg Zn) diet contained around 35 mg/kg plant Zn (low availability, <30%) depending on their respective level: need to supply mineral or organic Zn

# Zn requirement

## ❖ Evaluated by empirical method in chicks and pigs

Body weight gain and FCR change with dietary Zn (cereal diet, initial growth period)

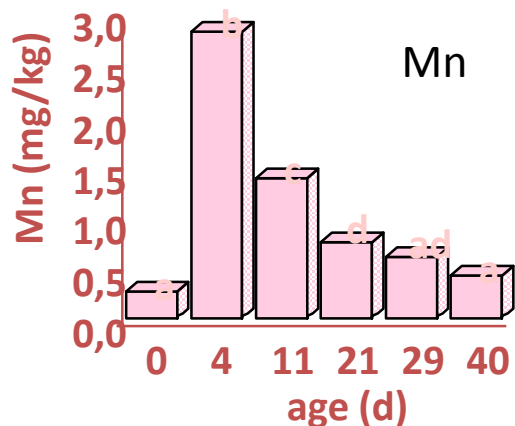
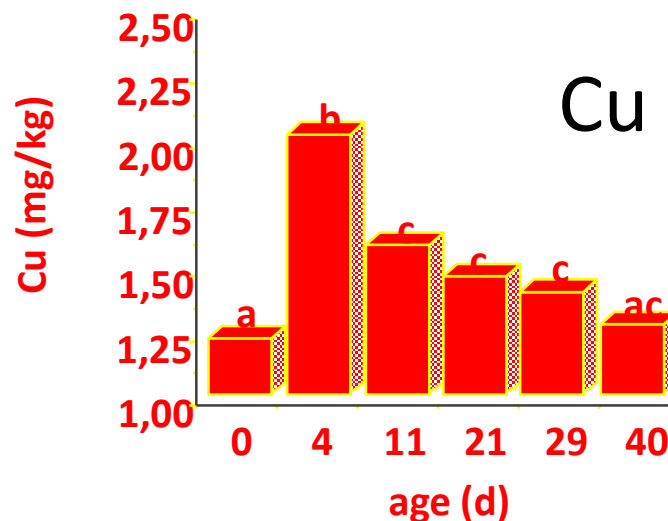


## ❖ Zn Requirements defined on multi-criteria analysis:

- firstly established on broiler performance (feed intake, growth) of young animal, extrapolated to finishing period
- explored for other functions (bone metabolism and composition, immunity, anti-oxidant status, meat or egg quality)
- Based on Zn homeostasis

# Zn requirement

Change in body concentration of trace elements in Broilers with age



➔ Higher level of Zn relative to other trace elements

➔ Increased demand during the first period of growth

Mohanna and Nys, 1998

# Zn requirement

## Changes in tissue concentration of trace elements with dietary supply

mg/kg fresh tissue	Cu	Fe	Zn	Mn
Kidney	3- 5	45- 100	22-32	2
<b>liver</b>	<b>5- 10</b>	<b>60- 300</b>	<b>25-120</b>	<b>2- 4</b>
plasma	0,25		1,8-3,4	<b>4</b>
<b>Bone (/dry matter)</b>	<b>4- 5</b>	<b>97- 140</b>	<b>200-400</b>	<b>8- 12</b>
Feathers*	10- 15	80- 150	60-300	4-11

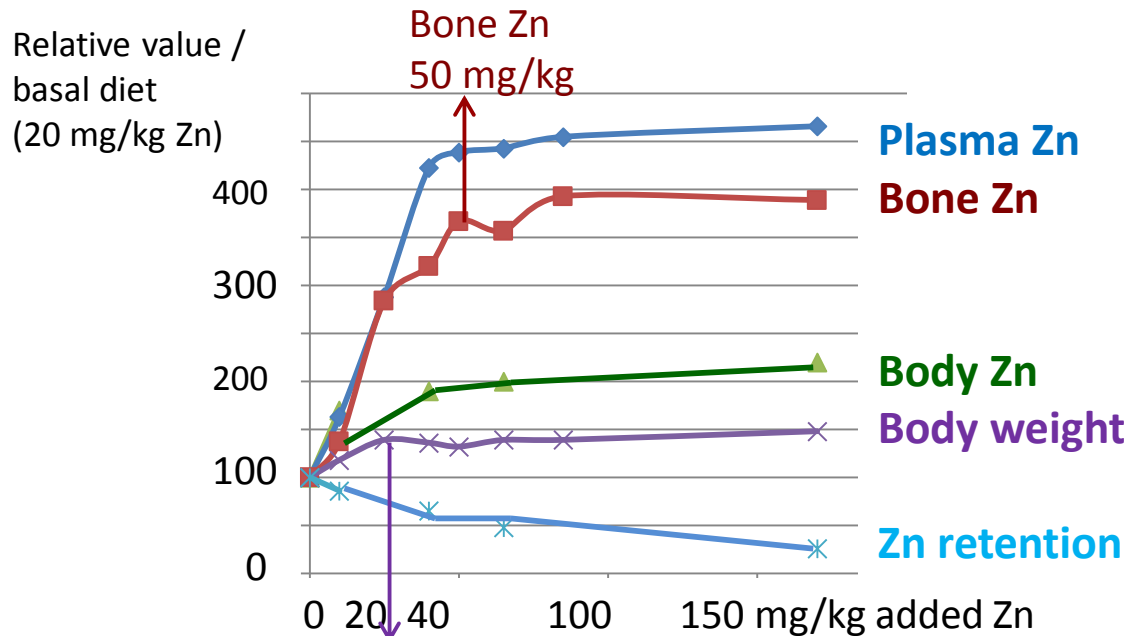


**Concentration of tissue Zn is increasing with dietary supply of Zn  
Can be used for measuring Zn requirement or availability of Zn  
Bone Zn is the most convenient parameter (analysis, magnitude of dose response,  
lower coefficient of variation)**

- ❖ Plasma alkaline phosphatase poorly influenced by dietary Zn in broiler in contrast to pig

# Zn requirement

## Dietary level of Zn on growth, tissue composition and Zn retention in broilers (21 days)



Body weight  
25mg/kg

Semi synthetic diet (isolated soybean protein, starch) with 20 mg/kg Zn supplemented with 10, 25 and 40, 50, 65, 85 and 170 mg/kg Zn (sulphate)

Mohanna and Nys, 1998

- ❖ Higher requirement for bone depletion than for growth or FCR



# Zn requirements and recommendations for broilers mg/kg diet

❄ In semi-synthetic diet (casein, milk powder, low phytate), Zn requirement is approximately 25-30 mg/kg for growth, 50 mg for Max. Bone Zn content, measured in broilers until 21 days of age. (Mohanna and Nys, 1999, Ao et al, 2006, Jondreville et al, 2007)

❄ Current recommendation from various institutes (EFSA journal 2014)

Sources // Category	NRC 1994 USA*	CIE 2004 Germany	MTT 2013 Finland	IFZZ 2005 Poland	INRA 1989 France**	EU feed Industry***
Broiler: Starter	40	50	50-60	60	40	80-140
<b>Broiler: Grower</b>	<b>40</b>	<b>50</b>	50-60	<b>50</b>	<b>40</b>	<b>75-130</b>
Broiler: Finisher	40	<b>50</b>	50-60	<b>40</b>	<b>20</b>	<b>75-120</b>
<b>Turkey 0-4 wks</b>	<b>70</b>	<b>50</b>	<b>80</b>	<b>90</b>	<b>60</b>	<b>95-140</b>
Turkey 5-8 wks	65	40	80	70	60	95-140
Turkey 9-12 wks	50	40	80	70	40	85-135
Turkey 12-24	40	40	50	60	30	75-140

\* semi purified diet (casein)? But > 45 in cereals diets?

\*\* Supplemented Zn (not total!) INRA alimentation des monogastriques, 1989

\*\*\* recommendations

➔ **Age of bird poorly considered in determination of requirement and allowances**

# Zn requirements and recommendations for broilers mg/kg diet

## Median level of dietary Zn in Europe (mg/kg)(22 countries)

Diet category (mg/kg)	N	mg Zn/kg diet	90% percentile
Starter Chicks	75	103	139
Chick for fattening	433	107	137
Turkeys for fattening	158	106	144

EFSA 2014

- ❖ Median Zn content in the range of 103-107 with only 4 % dietary Zn higher than the Currently Authorized total Maximum Content ( 150 mg/kg)
- ❖ Large reduction in Zn dietary supply compared to 20 years ago

## Consequences of Zn and Cu excess on environment

### Contribution of animal manure

- ❖ 19% for Cu (76 % due to phytosanitary treatment)
- ❖ 70% pour le Zn (28 % town dejection)

### Accumulation in surface soils (Coppenet et al., 1993)

- ❖ + 0.41 ppm Zn/year and 0.37 ppm Cu in Brittany from 1975 to 88
- ❖ influence of physical and chemical properties f (pH, level of org matter)  
modification in soil microflora and enzyme activity

### Phytotoxicity

symptoms: chloroses

threshold of 100 ppm for Cu and 300 ppm for Zn (Afnor)

Risk from 35 et 150 ppm (Inra-Aspitet)

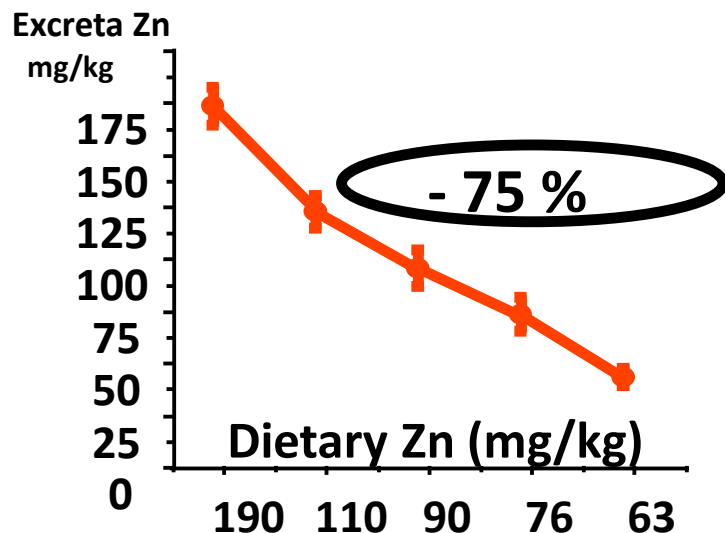
# Environment: Reducing contamination due to poultry manure

Comparison of manure supply (170kg N/ha following legislation) to mean plant requirement: Risk of eutrophication (P) and plant toxicity (Zn Cu, Mn)

Element	Supplied By manure	Plant Requirement**	Excess (%)
N	170	170	0
P	75	35	115
Zn	1,52	0,20	660
Fe	6,41	1,50	330
Cu	0,41	0,06	580
Mn	1,93	0,35	450



\*\* Coïc et Coppenet, 1989



## Means to reduce Zn excretion

- Adjusting dietary level to broiler requirements
- Improving availability of plant Zn
- Use of source with high Zn availability

# Bioavailability of Zn sources

## ■ Bioavailability

- Part absorbed and used for normal metabolic functions in healthy bird (can include Zn storage, bone , liver for some authors)
- Depends on sources, diets, physiological parameters (intestinal solubility), experimental test.

## ■ Measure of bioavailability

- Comparison the slopes of response (growth, Zn deposition) to different levels of a source of dietary Zn relative to a reference source

## ■ Reduction of Zn availability by phytic acid, reinforced by Ca.

- High correlation between phytate and Zn in feedstuffs
- phytase decreases this negative interaction (10mg)

## ■ Variability between Zn sources (inorganic, organic)

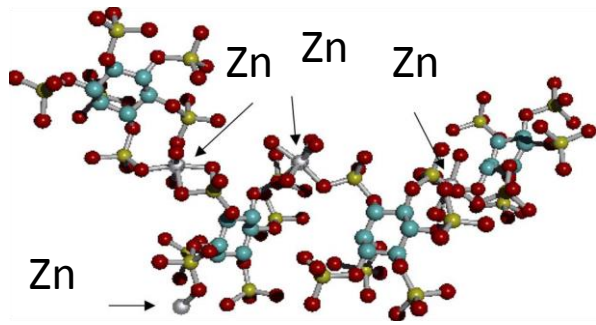
## ■ Diet viscosity and Zn retention

## ■ Antagonism between trace elements when chicks fed on marginal dietary levels Cu-Zn.

# Bioavailability of Zn sources

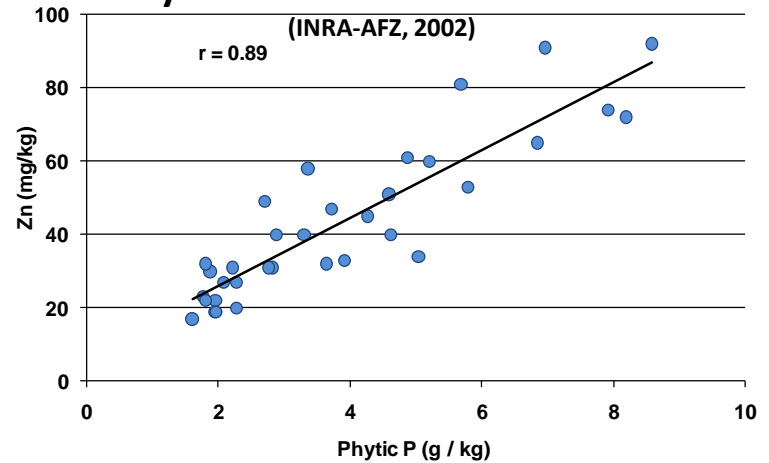
## Evidence of the antagonist of phytates on zinc availability

Zinc present in plant feedstuffs is partly linked to phytates



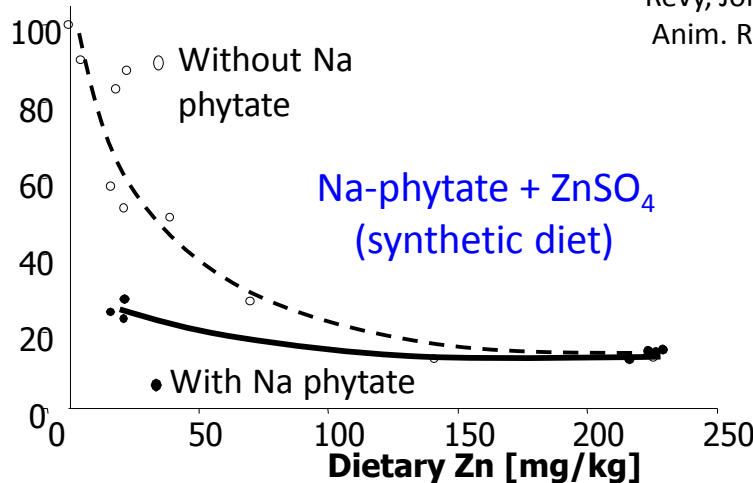
Rodrigues-Filho *et al.*, 2005

## Phytate molecule in feedstuffs



Revy, Jondreville, Dourmad, Guinotte, Nys 2002, Anim. Res. 51, 315

Apparent Zn absorbability (%) (rat)

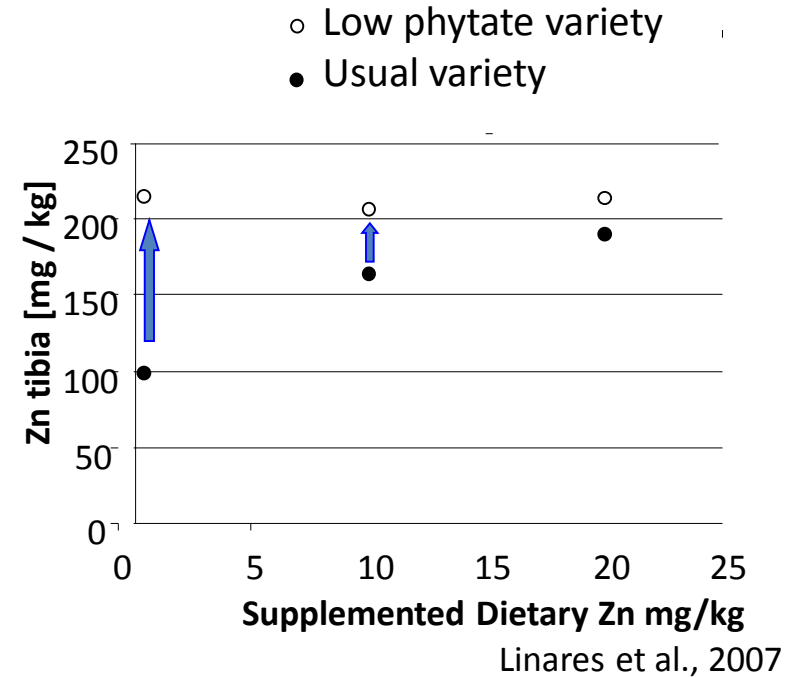
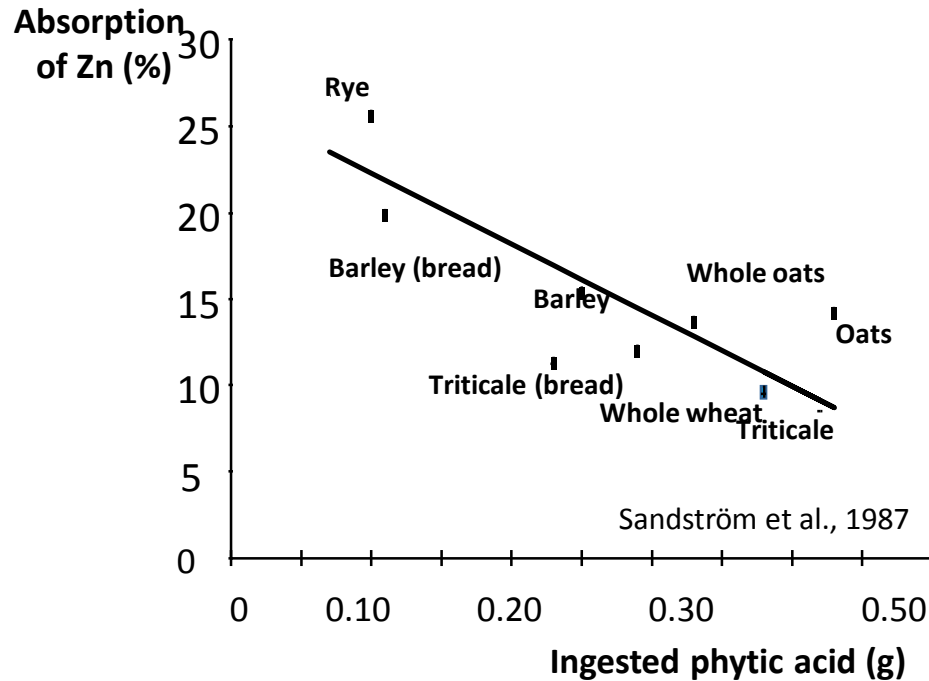


Examples from Weihenstephan and Giessen, Germany: Weigand et Kirchgesner, 1980; 1992; Rimbach et Pallauf, 1992; 1997; Rimbach et al., 1995

# Bioavailability of Zn sources

## Evidence of the antagonist of phytates on zinc availability

### Phytates reduce zinc absorption



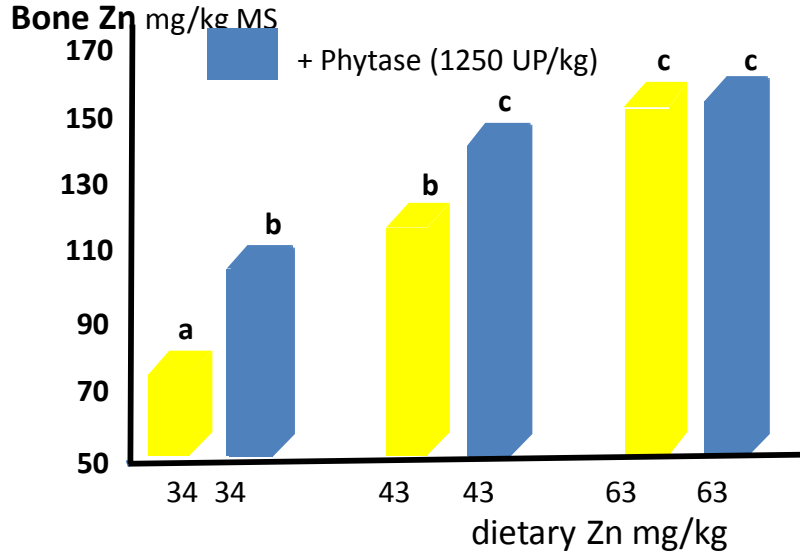
➡ **Phytates present in plant feedstuffs limit plant zinc availability**

➡ Different strategies: Withdraw antagonism (low phytate diet)  
Destroy antagonism (phytase)  
or avoid interaction (chelated Zn sources)

# Bioavailability of Zn sources: phytase supplementation

## Effect of phytase dietary supplementation on bone Zn

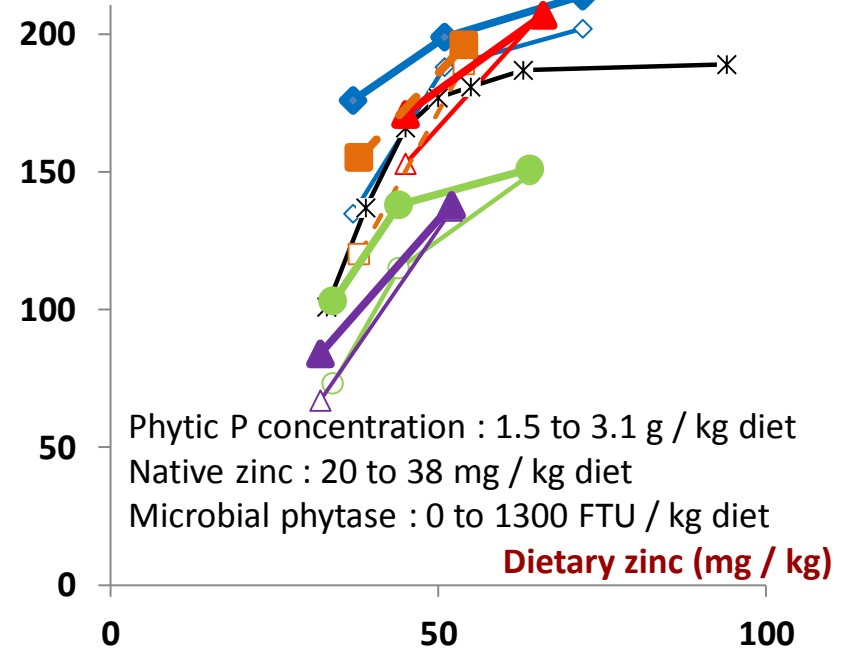
Mohanna and Nys, 1999, An. Feed Sci. Tech.77



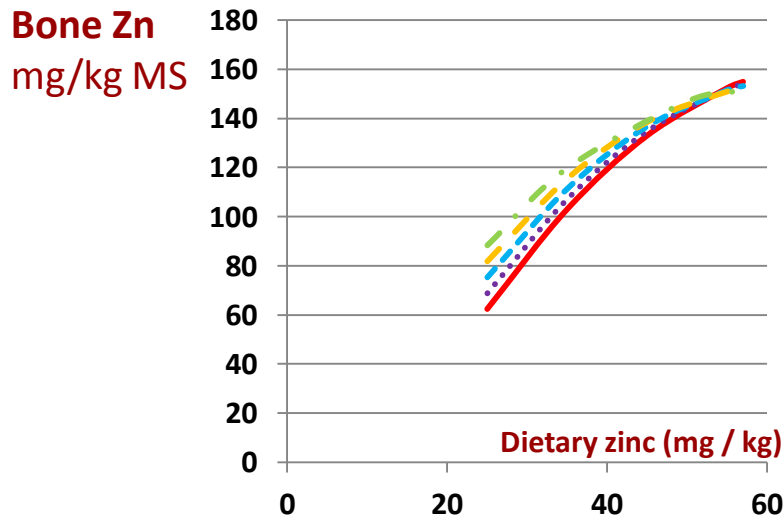
## Bone Zn mg/kg MS

Metaanalysis of 9 trials conducted at INRA

P. Schelgel, 2010



P. Schelgel, 2010



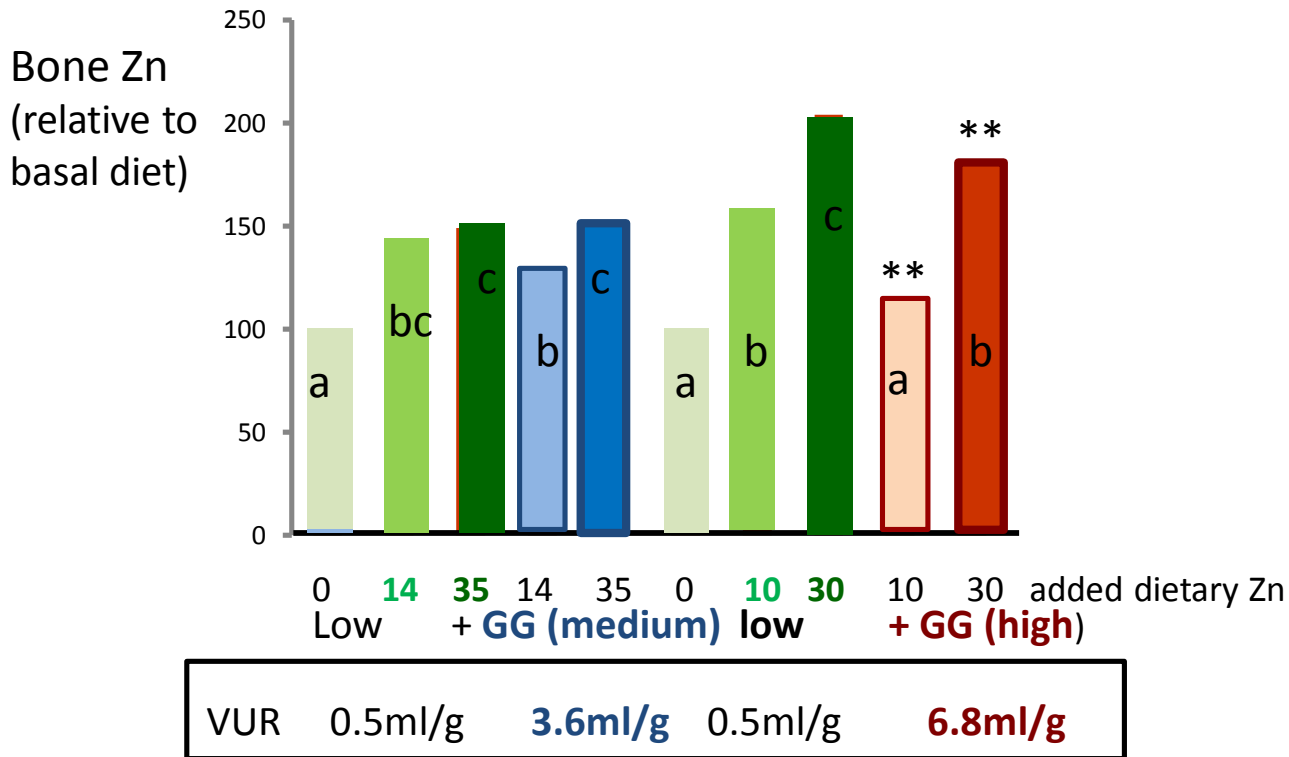
P. Schelgel, 2010

➔ **Phytase affected Zn retention but at a relatively low magnitude when dietary Zn increased**



# Zn and dietary viscosity

Effect of a high dietary viscosity (by addition of Guar Gum) on Bone Zn content in broiler compared to 0,5ml/g) on Bone Zn



➔ Lower Zn retention in diet with very high viscosity

❖ Diets rich in fiber (non starch fiber, luzerne) or rich in phytate (Whey) decreased Bone Zn (van der Aar et al., 1983)

# Bioavailability of Zn: comparison of inorganic sources

Source	ZnSO <sub>4</sub>	Zn CO <sub>3</sub>	ZnO	ZnCl <sub>2</sub>	Zn meth	Zn Lys
RBV	100	105	55	100	125	110

References anterior to 2000

Sources	First trial (Zn RBV)		Second , third trial (Zn RVB, additional sources)	
	Weight gain	Tibia Zn	Weight gain	Weight gain
Zn SO	100	100	100	100
ZnO (A)	94	91	89	93
ZnO (B)	32	22	41	39
ZnO (C)	47	44	86	84
Zn metal dust			67	36

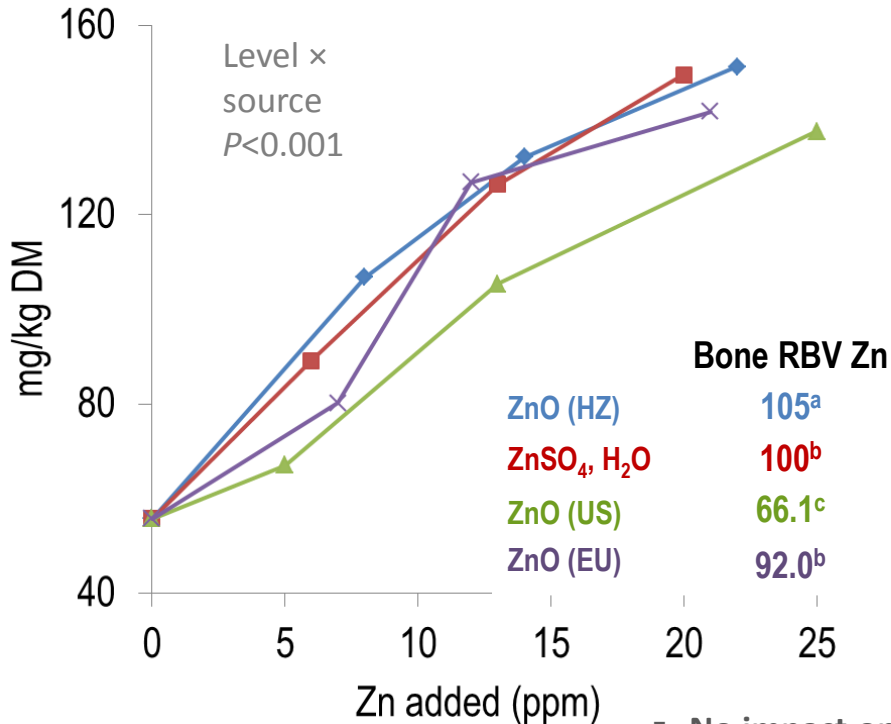
Edwards and Baker, 1999

➔ **High variability of Zn availability between various inorganic Zn sources**

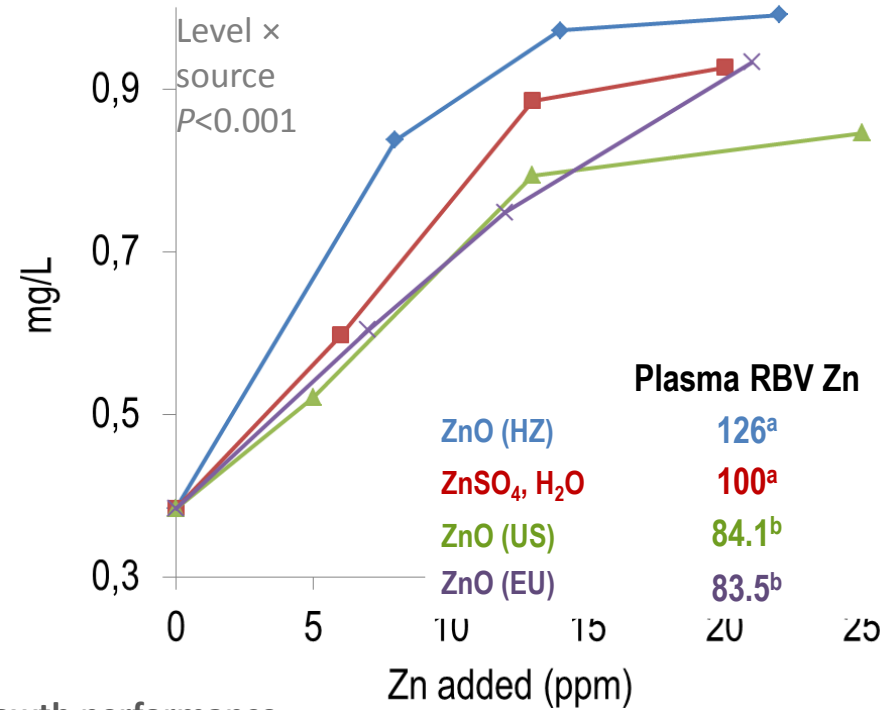
# Bioavailability of Zn: comparison of inorganic sources Zn in broilers

- ❖ Semi synthetic diet (corn, potatoes proteins and casein) with 22 mg/kg Zn
- ❖ ZnSO<sub>4</sub> Zn, one Zn oxide from US and one from Europe, HiZox (novel source from Animine)

### Bone Zn



### Plasma Zn



NARCY et al. ESPN - Prague, 24-27 August, 2015



**Importance of evaluating the Zn sources because of the high variability amongst sources of similar chemical composition**

# Bioavailability of Zn: comparison of organic /inorganic sources

source	level	ADG g/d	Plasma Zn	Tibia ash	Pancreas MT RNA
ZnSO4	10	29,2	1,55	266	0,49
	20	30,5	1,61	316	0,56
	40	29,8	1,65	356	0,61
Zn proteinate	10	30,0	1,65	274	0,52
	20	31,4	1,66	345	0,57
	40	30,7	1,72	366	0,63
<b>Prob source</b>		<b>0,1</b>	<b>0,05</b>	<b>0,02</b>	<b>0,7</b>

Liu et al. 2013

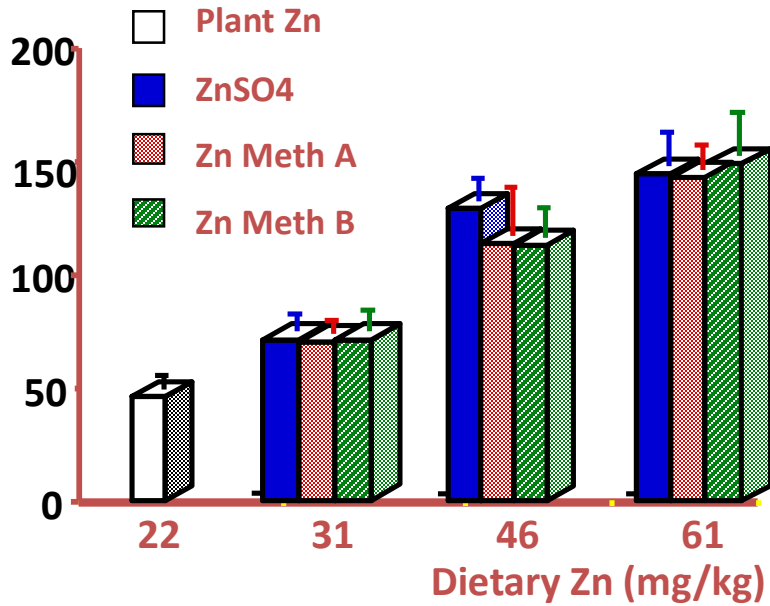
sources	level	ADG (11-14)	Tibia ash	Pancreas MT RNA
ZnSO4, 7H2O	0-90	19,8	307	0,56
Zn AA	0-90	20,6	320	0,52
Zn Prot A	0-90	20,0	320	0,56
ZnProt B	0-90	20,5	302	0,46
<b>Prob source</b>		<b>0,26</b>	<b>0,42</b>	<b>0,06</b>
<b>Prob level</b>		<b>0,59</b>	<b>&lt;0,001</b>	<b>&lt;0,001</b>

Huang et al., 2014

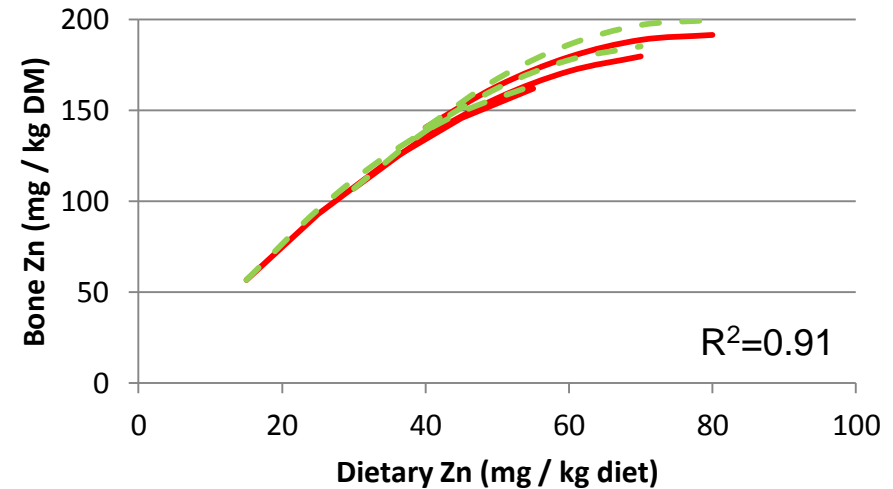
➔ **Limited change in Zn availability between organic/inorganic Zn sources**

# Bioavailability of Zn: effect of Zn sources, inorganic/organic

Effect of dietary Zn level and source origine on Bone Zn level



Metaanalysis comparing inorganic and organic sources (Schlegel, 2010)



Acetate (1),  
oxide (2),  
Sulfate (6)

Lysine, methionine,  
glycine, hydrolysed  
protein

From Cao et al., 2002; Pancosma, 2002; Gebert et al., 2002; Mohanna et Nys, 1999; Schlegel et al., 2010; Swiatkiewicz et al., 2001; Wedekind et al., 1992)

- ❖ Limited difference between organic and reference inorganic sources compared to the effect of dietary level of Zn on Zn bone retention
- ❖ Properties of Organic source (solubility, stability in solution) remained poorly defined in literature
- ❖ Issue remained controversial

Higher availability of Zn Methionine mentioned by Wedekind et al, 1992 (=30%) and by Swiatkiewicz, et al, 2001 (hydrolysed soy protein), Cao et al, 2002 and Ao et al, 2006.

# Conclusions

## ❖ Requirements and allowances in broilers

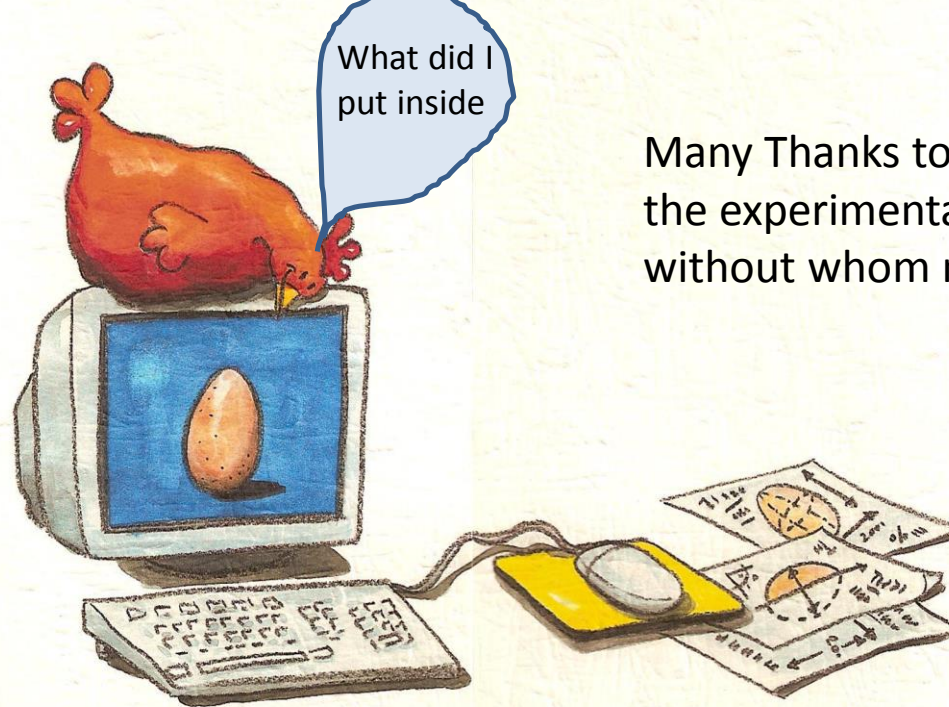
- + 25 mg/kg for growth, + 50 mg/kg for optimising bone content (cereal diets)
- Established at initial phase of growth, no experimental data on requirement for finishing period

## ❖ bioavailability of mineral in broilers

- Predominant role of dietary Zn level because of low pH in bird gastro intestinal tract
- Source origin (mainly amongst inorganic origin as organic source are highly available) has large impact and need to be well characterized
- Phytate, fiber, diet viscosity have negative effect of low magnitude

## ❖ Environmental concern

- Mainly controlled in broilers by adjusting dietary mineral supply to bird requirement
- Phase feeding should be implemented to reduce Zn excretion during finishing period
- EU project limiting at 100 mg/kg dietary Zn feasible when using sources with high availability. A maximum level defined for each growth period will be more pertinent (120 at initial period and 80 in finishing??).



Many Thanks to my colleagues for their contribution to the experimental strategy and work without whom nothing would have been possible.....

My direct and efficient collaborators **Catherine Jondreville** and **Agnès Narcy**

And the enthusiastic and active PHd students

**Chadi Mohanna (1998)**

**Pierre-Stéphan Revy (2003)**

and **Patrick Schlegel (2010)**

