How nutritional Zinc requirements and recommendations for broilers are defined

Yves NYS

with contribution of Catherine JONDREVILLE, Agnès NARY and Patrick SCHLEGEL

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37380 Nouzilly, 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.
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

**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 promoter, 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

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Cu</th>
<th>Fe</th>
<th>Zn</th>
<th>Mn</th>
<th>Se</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced weight gain</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Skeletal disorders</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor feathering</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
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</tr>
<tr>
<td>Anaemia</td>
<td>+</td>
<td>+</td>
<td></td>
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<tr>
<td>Cardiovascular disorder</td>
<td>+</td>
<td></td>
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<td>+</td>
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<tr>
<td>Exudative diathesis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Abnormal thyroid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

- 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
Zn deficiency and bird immunity

- 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
Effect of dietary Zn excess on Broiler Growth Performance

<table>
<thead>
<tr>
<th>Dietary Zn supply (mg/kg)</th>
<th>Body weight gain (g/day)</th>
<th>FCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0*</td>
<td>10.6a</td>
<td>1.56</td>
</tr>
<tr>
<td>500</td>
<td>9.8b</td>
<td>1.57</td>
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<tr>
<td>1000</td>
<td>8.8bc</td>
<td>1.63</td>
</tr>
<tr>
<td>1500</td>
<td>7.9c</td>
<td>1.77</td>
</tr>
</tbody>
</table>

* 74 mg/kg in basal diet  

Henry et al., 1987

- 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

Nys, 2003
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

Nys Y. / Zn Requirements in Poultry
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**

Kratz et al, 2004
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

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Fe</th>
<th>Zn</th>
<th>Cu</th>
<th>Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4</td>
<td>23</td>
<td>14</td>
<td>16</td>
<td>4.9</td>
</tr>
<tr>
<td>0-21</td>
<td>12</td>
<td>6</td>
<td>5</td>
<td>0.6</td>
</tr>
<tr>
<td>0-40</td>
<td>12</td>
<td>6</td>
<td>6</td>
<td>0.3</td>
</tr>
</tbody>
</table>

- 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

Mohanna and Nys, 1998
### Changes in tissue concentration of trace elements with dietary supply

<table>
<thead>
<tr>
<th>mg/kg fresh tissue</th>
<th>Cu</th>
<th>Fe</th>
<th>Zn</th>
<th>Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>3- 5</td>
<td>45-100</td>
<td>22-32</td>
<td>2</td>
</tr>
<tr>
<td>liver</td>
<td>5- 10</td>
<td>60-300</td>
<td>25-120</td>
<td>2- 4</td>
</tr>
<tr>
<td>plasma</td>
<td>0.25</td>
<td></td>
<td>1.8-3.4</td>
<td>4</td>
</tr>
<tr>
<td>Bone (dry matter)</td>
<td>4- 5</td>
<td>97-140</td>
<td>200-400</td>
<td>8- 12</td>
</tr>
<tr>
<td>Feathers*</td>
<td>10-15</td>
<td>80-150</td>
<td>60-300</td>
<td>4-11</td>
</tr>
</tbody>
</table>

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

- 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 approximatively 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)

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

* 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

Nys Y. /bioavailability of Zn sources
Median level of dietary Zn in Europe (mg/kg) (22 countries)

<table>
<thead>
<tr>
<th>Diet category (mg/kg)</th>
<th>N</th>
<th>mg Zn/kg diet</th>
<th>90% percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starter Chicks</td>
<td>75</td>
<td>103</td>
<td>139</td>
</tr>
<tr>
<td>Chick for fattening</td>
<td>433</td>
<td>107</td>
<td>137</td>
</tr>
<tr>
<td>Turkeys for fattening</td>
<td>158</td>
<td>106</td>
<td>144</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Element</th>
<th>Supplied By manure</th>
<th>Plant Requirement**</th>
<th>Excess (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>170</td>
<td>170</td>
<td>0</td>
</tr>
<tr>
<td>P</td>
<td>75</td>
<td>35</td>
<td>115</td>
</tr>
<tr>
<td>Zn</td>
<td>1,52</td>
<td>0,20</td>
<td>660</td>
</tr>
<tr>
<td>Fe</td>
<td>6,41</td>
<td>1,50</td>
<td>330</td>
</tr>
<tr>
<td>Cu</td>
<td>0,41</td>
<td>0,06</td>
<td>580</td>
</tr>
<tr>
<td>Mn</td>
<td>1,93</td>
<td>0,35</td>
<td>450</td>
</tr>
</tbody>
</table>

** 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

Nys, 1999, 12th ESPN, Beckbergen; Mohanna and Nys, 1999, BPS40,108
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.**
Evidence of the antagonist of phytates on zinc availability

Zinc present in plant feedstuffs is partly linked to phytates

Phytate molecule in feedstuffs

Apparent Zn absorbability (%) (rat)

Examples from Weihenstephan and Giessen, Germany: Weigand et Kirchgessner, 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**

- Ingested phytic acid (g)
- Absorption of Zn (%)

- Rye
- Barley (bread)
- Barley
- Whole wheat
- Triticale (bread)
- Oats
- Triticale

Sandström et al., 1987

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

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

**Supplemented Dietary Zn mg/kg**

Linares et al., 2007

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Nys Y. / Zn Requirements in Poultry
Bioavailability of Zn sources: phytase supplementation

Effect of phytase dietary supplementation on bone Zn


<table>
<thead>
<tr>
<th>Bone Zn (mg/kg MS)</th>
<th>+ Phytase (1250 UP/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>a</td>
</tr>
<tr>
<td>43</td>
<td>b, b</td>
</tr>
<tr>
<td>63</td>
<td>c, c</td>
</tr>
</tbody>
</table>

Bone Zn mg/kg MS

Dietary zinc (mg/kg) vs. Bone Zn mg/kg MS

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

Metaanalysis of 9 trials conducted at INRA

P. Schelgel, 2010

Phytic P concentration: 1.5 to 3.1 g/kg diet
Native zinc: 20 to 38 mg/kg diet
Microbial phytase: 0 to 1300 FTU/kg diet
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

<table>
<thead>
<tr>
<th>Source</th>
<th>ZnSO₄</th>
<th>Zn CO₃</th>
<th>ZnO</th>
<th>ZnCl₂</th>
<th>Zn meth</th>
<th>Zn Lys</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBV</td>
<td>100</td>
<td>105</td>
<td>55</td>
<td>100</td>
<td>125</td>
<td>110</td>
</tr>
</tbody>
</table>

References anterior to 2000

<table>
<thead>
<tr>
<th>Sources</th>
<th>First trial (Zn RBV)</th>
<th>Second, third trial (Zn RVB, additional sources)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight gain</td>
<td>Tibia Zn</td>
</tr>
<tr>
<td>Zn SO</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>ZnO (A)</td>
<td>94</td>
<td>91</td>
</tr>
<tr>
<td>ZnO (B)</td>
<td>32</td>
<td>22</td>
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<td>ZnO (C)</td>
<td>47</td>
<td>44</td>
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<tr>
<td>Zn metal dust</td>
<td>67</td>
<td>36</td>
</tr>
</tbody>
</table>

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
- ZnSO4 Zn, one Zn oxide from US and one from Europe, HiZox (novel source from Animine)

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**

<table>
<thead>
<tr>
<th>source</th>
<th>level</th>
<th>ADG g/d</th>
<th>Plasma Zn</th>
<th>Tibia ash</th>
<th>Pancreas MT RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnSO4</td>
<td>10</td>
<td>29.2</td>
<td>1.55</td>
<td>266</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>30.5</td>
<td>1.61</td>
<td>316</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>29.8</td>
<td>1.65</td>
<td>356</td>
<td>0.61</td>
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<tr>
<td>Zn proteinate</td>
<td>10</td>
<td>30.0</td>
<td>1.65</td>
<td>274</td>
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<tr>
<td></td>
<td>20</td>
<td>31.4</td>
<td>1.66</td>
<td>345</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>30.7</td>
<td>1.72</td>
<td>366</td>
<td>0.63</td>
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**Prob source**

<table>
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</thead>
<tbody>
<tr>
<td></td>
<td>source</td>
<td>0.1</td>
<td>0.05</td>
<td>0.02</td>
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</table>

**Sources**

<table>
<thead>
<tr>
<th>source</th>
<th>level</th>
<th>ADG (11-14)</th>
<th>Tibia ash</th>
<th>Pancreas MT RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnSO4, 7H2O</td>
<td>0-90</td>
<td>19.8</td>
<td>307</td>
<td>0.56</td>
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<tr>
<td>Zn AA</td>
<td>0-90</td>
<td>20.6</td>
<td>320</td>
<td>0.52</td>
</tr>
<tr>
<td>Zn Prot A</td>
<td>0-90</td>
<td>20.0</td>
<td>320</td>
<td>0.56</td>
</tr>
<tr>
<td>Zn Prot B</td>
<td>0-90</td>
<td>20.5</td>
<td>302</td>
<td>0.46</td>
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**Prob source**

<table>
<thead>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Prob</td>
<td>0.26</td>
<td>0.42</td>
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**Prob level**

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td></td>
<td>0.59</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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</tbody>
</table>

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

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Liu et al. 2013

Huang et al., 2014
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)


- 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
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??).
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