Assessing bioavailability of essential trace minerals in animal nutrition

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Bioavailability of essential trace minerals: restrictions resulting from general definition

**In vitro** studies cannot fully cover bioavailability

Bioavailability = *capability* of metabolic use at the absence of homeostatic regulation

**Bioavailability**

...the **maximum possible** yield of a nutrient that the body may extract from the ingested food and use for its metabolic functions...

(Kirchgessner et al. 1993)
E.g. homeostatic regulation of Zn metabolism: Precise control of Zn uptake from intestinal tract

(Windisch and Kirchgessner 1995)
Metabolic use of dietary trace minerals in relation to homeostatic counter-regulation

- Deficient supply
- Homeostatic regulation at sufficient supply
- Maximum possible use of dietary TM (capability)
- Homeostatically restricted use of dietary TM available in excess to requirement
e.g. Zn homeostasis: Zn concentration in eggs

![Graph showing the relationship between Zn content of feed and egg concentration.](image)

- **Homeostatic regulation at sufficient Zn supply**
- **Onset of homeostatic regulation**
- **Concentration of Zn in the egg**
- **Homeostasis is locked at maximum use, dose-response relationship**

(Paullicks and Kirchgessner 1994)
Example Zn homeostasis:
Zn steady state in products (e.g. milk)

- Deficiency
- Range of homeostatic regulation
- Onset of homeostatic regulation
- Homeostasis is locked at maximum use, dose-response relationship

(Schwarz and Kirchgessner 1975)
### Se Homeostasis: Whole Body Se Retention

The diagram illustrates the concept of homeostasis in selenium (Se) retention, showing how the body maintains a balance between Se intake and retention. At the point of homeostasis, the body's use of Se is maximized, and any further increase in intake leads to an increase in retention, potentially causing excess Se levels.

**Graph Details:**
- **Y-Axis:** Se retention (ng/day) with a logarithmic scale ranging from 100 to 10000 ng/day.
- **X-Axis:** Se intake (ng/day) from 100 to 100000 ng/day.
- **Points:** Various Se intake levels (40 ppb to 3000 ppb) plotted with corresponding retention values.
- **Legend:**
  - **Deficiency:** Intake levels below 40 ppb result in deficiency.
  - **Homeostasis:** Intake levels around 40 ppb to 150 ppb maintain homeostasis.
  - **Excess:** Intake levels above 300 ppb result in excess Se.

**Equation:**
\[ \text{Retention} = \frac{\text{Intake} \times \text{Efficiency}}{\text{Body Mass}} \]

*(Kirchgesner et al. 1997)*

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<table>
<thead>
<tr>
<th>Se Intake (ng/day)</th>
<th>Se Retention (ng/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 ppb</td>
<td>70 ppb</td>
</tr>
<tr>
<td>100 ppb</td>
<td>100 ppb</td>
</tr>
<tr>
<td>150 ppb</td>
<td>200 ppb</td>
</tr>
<tr>
<td>200 ppb</td>
<td>300 ppb</td>
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<tr>
<td>300 ppb</td>
<td>450 ppb</td>
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<tr>
<td>450 ppb</td>
<td>600 ppb</td>
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<tr>
<td>600 ppb</td>
<td>1000 ppb</td>
</tr>
<tr>
<td>1000 ppb</td>
<td>3000 ppb</td>
</tr>
</tbody>
</table>
e.g. Se homeostasis: urinary Se excretion

(Kirchgessner et al. 1997)
Inorganic vs. organic Se compounds

- **Inorganic Se** (selenite, selenate)
  - Recognition as Se source
  - Utilization in Se metabolism
  - Homeostasis controls excretion

- **Seleno amino acid**
  - Recognition as amino acid
  - Protein metabolism
  - Degradation
  - Synthesis
  - Erroneous accumulation in body protein (meat) and product protein (egg, milk)
General principle to assess bioavailability of trace minerals

- Range of dietary supply to assess bioavailability (deficiency)
- Dietary requirement at high bioavailability (onset of homeostatic counter-regulation)
- Different slope = different bioavailability
Bioavailability of essential trace minerals: restrictions resulting from general definition

In vitro studies cannot fully cover bioavailability

Bioavailability = capability of metabolic use at the absence of homeostatic regulation

Bioavailability of essential trace minerals: restrictions resulting from general definition

bio – availability

metabolic (re)actions

dietary properties

...the maximum possible yield of a nutrient that the body may extract from the ingested food and use for its metabolic functions...

(Kirchgessner et al. 1993)

Physiological conditions of metabolism, sensitive response parameter
Metabolic use of dietary trace minerals in relation to homeostatic counter-regulation

- Deficient supply
- Homeostatic regulation at sufficient supply
- Excess \(\rightarrow\) toxicity

- Max. possible use
- Homeostatically restricted use
- Dietary excess overwhelms homeostasis (accumulation)
Short term oral excess demonstrates ability to overwhelm homeostatic counter-regulation (AUC-method)

- Zn glycine
- Zn sulfate
- Zn lactate
- Zn oxide

(Single oral Zn load in horses: Wichert et al. 2001)
Bioavailability of essential trace minerals: restrictions resulting from general definition

*In vitro* studies cannot fully cover bioavailability

Bioavailability = *capability* of metabolic use at the absence of homeostatic regulation

Bioavailability of essential trace minerals:

restrictions resulting from general definition

...the maximum possible yield of a nutrient that the body may extract from the ingested food and use for its metabolic functions...

(Kirchgessner et al. 1993)

Interactions with other dietary components

Physiological conditions of metabolism, sensitive response parameter
Phytic acid is a strong chelator to trace minerals

Inositol 1,2,3,4,5,6-Hexakis-dihydrogenphosphat

Phytic acid (phytate)

Phytic acid is a strong chelator to trace minerals (Lantzsch 1990)
Dietary phytate may massively reduce maximum possible Zn absorption measured at Zn deficiency

Almost 100% absorption from diets without chelators

Range of phytate content in common diets to pigs/poultry

(Windisch and Kirchgessner 1999)
Added phytase may significantly improve Zn bioavailability (e.g. from inorganic sources, Zn sulfate)

(Ettle et al. 2006)
Se retention from organic Se depends on supply status with methionine (growing rat model)

Basal diet: marginal in Met, 800 ppb dietary Se as SeMet

(Butler et al. 1989)
Assessing bioavailability of essential trace minerals in animal nutrition

\textit{In vitro} methods do not fully cover bioavailability (BA).

BA = \textit{capability} of metabolic trace mineral use
It is not fully realized at normal feeding conditions

Assessment of BA at deficient trace mineral supply
(no interference with homeostasis).

BA cannot be assessed independent from dietary composition.

Example to Zinc
Example: Quantifying Zn bioavailability with a radiotracer study

Bioavailability = true absorption of dietary Zn
= influx of Zn from diet into the inside of the organism
x metabolic utilization of absorbed dietary Zn
...for tissue retention, endogenous faecal and renal excretion, surface losses, ...

measured in a radiotracer study at Zn deficiency using a purified diet added with Na$_{12}$phytate (8g/kg)

(Schlegel and Windisch 2006)
Example: Quantifying Zn bioavailability in a radiotracer study
(Schlegel and Windisch 2006)

<table>
<thead>
<tr>
<th>Treatment group: Added dietary Zn</th>
<th>positive control (52µg/g)</th>
<th>negative control (12µg/g)</th>
<th>Test group (12µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>suffice Zn</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn flux (µg/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>intake</td>
<td>516</td>
<td>108</td>
<td>109</td>
</tr>
<tr>
<td>truly absorbed from diet</td>
<td>159&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>endogenous faecal excretion</td>
<td>48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>urine</td>
<td>4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>retention</td>
<td>107&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Max. absorption (%)</td>
<td>44.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>50.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Metabolic utilization (%)</td>
<td>94.7</td>
<td></td>
<td>95.7</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>41.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>48.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
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Experimental model to assess Zn bioavailability in practical pig feeding

<table>
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<th>Diet?</th>
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<tr>
<td>“Worst case” diet (corn &amp; soybean extracts): rich in phytate, low in native Zn, no phytase activity (pelleted with steam). Graded levels of added Zn (sulfate) from deficient to sufficient supply</td>
</tr>
</tbody>
</table>

Zn supply before the onset of study: depletion or adequate?

(Brugger et al. 2012)
Mobilization and refilling of mobilized bone Zn is highly regulated by homeostasis (rat model)

(Windisch 2001)
Experimental model to assess Zn bioavailability in practical pig feeding

Diet?

“Worst case” diet (corn & soybean extracts): rich in phytate, low in native Zn, no phytase activity (pelleted with steam). Graded levels of added Zn (sulfate) from deficient to sufficient supply

Zn supply before the onset of study: depletion or adequate?

Adequate Zn supply before the onset of study

Duration of Zn deficiency?
(no Zn deficiency symptoms)

(Brugger et al. 2012)
Symptoms of Zn deficiency in piglets fed a soya-corn-based diet without Zn supplementation

Feed intake of piglets at „native“ Zn supply

Maximum duration of physiologically tolerable Zn deficiency

Final exhaustion of mobilizable Zn stores

(Windisch et al. 2003)
## Experimental model to assess Zn bioavailability in practical pig feeding

<table>
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<th>Question</th>
<th>Answer</th>
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</tr>
<tr>
<td>Zn supply before the onset of study: depletion or adequate?</td>
<td>Adequate Zn supply before the onset of study</td>
</tr>
<tr>
<td>Duration of Zn deficiency? (no Zn deficiency symptoms)</td>
<td>Maximum 8 days</td>
</tr>
</tbody>
</table>
| Response parameter?                                                     | • Apparently absorbed dietary Zn (mg/day)  
• Blood plasma: total Zn, AP activity  
• Bone Zn  
• mRNA of metallothioneine in intestinal tissues |

*(Brugger et al. 2012)*
Reaction of apparently absorbed dietary Zn indicates absence/presence of homeostatic counter-regulation

- Theoretical max. slope of 1.0 (100% BA)
- Measured slope = 0.23
- Break point at 59mg/kg

Relevant range of dietary Zn to compare Zn sources of unknown bioavailability.

(Brugger et al. 2012)
Assessing bioavailability of essential trace minerals in animal nutrition

In vitro methods do not fully cover bioavailability (BA).

BA = capability of metabolic trace mineral use.
It is not fully realized at normal feeding conditions.

Assessment of BA at deficient trace mineral supply (no interference with homeostasis) and absence of deficiency disorders

BA cannot be assessed independent from dietary composition.

Comparison of dietary trace mineral sources for BA should be done on base of a well defined standard “worst case” diet.