Review:

Zn metabolism of monogastric species and consequences for the definition of feeding requirements and the estimation of feed Zn bioavailability

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Abstract: A major goal of mineral nutrition research is to provide information of feed zinc (Zn) utilization efficiency and gross Zn requirements as affected by changing rearing conditions. This can be achieved only by applying precise experimental models that acknowledge the basic principles of Zn metabolism. This review article summarizes the most important aspects of Zn homeostasis in monogastric species, including molecular aspects of Zn acquisition and excretion. Special emphasis is given to the role of the skeleton as well as the exocrine pancreas for animal Zn metabolism. Finally, we discuss consequences arising from these physiological principles for the experimental design of trials which aim to address questions of Zn requirements and bioavailability.

Key words: Monogastric species; Zinc metabolism; Requirement; Bioavailability; Experimental modelling
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1 Introduction

Zinc (Zn) is one of the most abundant metals within animals. Reported average tissue concentrations are species-dependent and range from 25 mg/kg (rabbit) to 50 mg/kg (swine) of defatted tissue (Klasing et al., 2005). Earlier studies in pigs highlighted total systemic failure of metabolism due to long-term alimentary Zn deficiency, resulting in growth depression, anorexia, tissue necrosis, and other quite unspecific symptoms (Tucker and Salmon, 1955). Therefore, it is obvious that its bioactivity is essential for the development and reproduction of animals, which has been confirmed in other models across biological kingdoms (Raulin, 1869; Sommer and Lipman, 1926; Todd et al., 1934; Bertrand and Bhattacherjee, 1935; Legg and Sears, 1960; O'Dell et al., 1977).

The importance of Zn in cellular systems is based on its role as a structural and catalytic cofactor of specific peptides. Several Zn motifs in peptides have been described, among which the Zn-finger motifs of certain DNA-binding factors are the most prominent (Pace and Weerapana, 2014). Further studies identified a role of free Zn^{2+} ions within cells as second messengers as well as activating and repressing agents of peptide activity (Maret, 2013; Levaot and Hershfinkel, 2018). Andreini et al. (2006) estimated that about 10% of the human genome codes for Zn-metallo-proteins, most of which have been linked to fundamental processes of cell physiology like transcription, replication, and maintenance of DNA. Hence, it is not surprising that endogenous Zn deficiency may lead to systemic problems resulting in unspecific symptoms. In contrast, Zn excess promotes serious toxic effects. This is also due to its interaction with peptides, but in a rather unspecific and uncontrolled manner (Sandstead, 1995). Therefore, evolution gave rise to the formation of a complex and

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finely-orchestrated homeostatic regulation, which aims to maintain a basal Zn load behind the gut barrier and plasma membrane that benefits metabolic activity under changing environmental conditions (Lichten and Cousins, 2009; Colvin et al., 2010).

Although Zn metabolism has been the subject of intense research for more than a century, still there are a lot of unsolved questions. These involve the identification of the master regulators of systemic Zn homeostatic regulation and the precise chemical principles that affect Zn absorption from food and feed under varying conditions. This review presents the basic principles of Zn metabolism in monogastric organisms. Furthermore, the roles of the skeleton and exocrine pancreas in Zn metabolism are discussed. These concepts should be taken into account in the design of in vivo experiments addressing questions of dietary Zn utilization under varying feeding conditions.

2 Basic principles of Zn metabolism

The essential trace elements can be grouped according to the main mechanisms that control their total content within an organism. Whereas iodine and selenium are regulated mainly by renal excretion, the essential transition metals (e.g. Fe, Cu, and Mn) including Zn are regulated by a finely-tuned interplay between luminal absorption from, and endogenous secretion into, the intestines (reviewed by Windisch (2002)). Thereby, Zn homeostasis maintains body Zn at a level that enables proper functioning of metabolic processes. Precise dose-response studies in ^{65}Zn-labelled rats have highlighted the adaption of absorption and excretion pathways under varying Zn feeding conditions in high resolution. These datasets represent the most important source of current knowledge regarding the status-dependent Zn fluxes through the organism (presented in detail below).

Under conditions of insufficient dietary Zn supply, the system starts quite quickly (within the first hour) to mobilize internal Zn stores predominantly from skeletal tissue (Windisch, 2001, 2003a). Within 3–5 d, absorptive and excretive mechanisms also adapt by increasing the relative absorption capacity at the gut barrier and, simultaneously, decreasing endogenous losses into the gut via pancreatic and bile secretions to a minimal amount (Weigand and Kirchgessner, 1980;

Windisch and Kirchgessner, 1994a, 1994b). This can be considered to represent an attempt to optimize the recycling of luminal Zn, which is represented mainly by endogenous losses under severe Zn malnutrition. The lag time of absorption and excretion directly reflects the half-life of gut mucosal and pancreatic acinar cells (Barker, 2014). Because of this delayed response, the first days of Zn deficiency are characterized by the highest endogenous losses (Windisch, 2003a).

On the other hand, adequate or excessive dietary Zn uptake is associated with reduced Zn absorption efficiency from the intestinal lumen and, at the same time, elimination of excessive Zn levels behind the intestinal barrier by secretion into the gastrointestinal tract (GIT). In this regard, the degree of excretory activity depends on the total amount of Zn in feed. The more Zn is supplemented above the actual requirement, the higher the excretory activity in response (Weigand and Kirchgessner, 1980; Windisch and Kirchgessner, 1994a, 1994b). Note that a sudden parenteral application of ^{65}Zn facilitates excretion primarily via urine not faeces within the first 8 h post-injection (Schwarz and Kirchgessner, 1976). Therefore, it seems like the kidney functions as a "first line of defense" to sudden toxic Zn overload.

Pieper et al. (2015) investigated the porcine pancreatic response to excess dietary supplementation with Zn (about 2500 mg/kg). Apart from an accumulation of pancreatic Zn and upregulation of metallothionein, they found higher synthesis of pancreatic digestive enzymes, which points towards higher exogenous pancreatic activity. At first view, the increase in endogenous Zn in response to over-supplementation appears odd considering the aforementioned down-regulation of active absorption pathways at the gut barrier. However, after down-regulation of active transport, there are still passive mechanisms that remain unaffected by Zn homeostatic regulation (Martin et al., 2013). Several transport proteins for certain divalent cations express some affinity for a variety of metals, although not to the same degree as their major ligands (Sandström, 2001). As soon as the main Zn absorption pathway is down-regulated in response to sufficient or excessive supply, the probability that Zn will compete for different divalent cation transport routes increases. Therefore, the total passively transported amount of Zn strongly correlates with the amount of over-supplemented Zn (Martin et al., 2013).

Increasing evidence suggests that the response patterns discussed above are due to a sophisticated homeostatic regulation which modulates the biosynthesis, degradation, and translocation of specific Zn transporting proteins within the cells of key tissues. These transporters belong to two main groups of solute carriers (SLC families 30 (ZnT) and 39 (ZIP)). In mammals, 10 members of the ZnT family and 14 members of the ZIP family have been described (Lichten and Cousins, 2009; O'Leary et al., 2016). It appears that the activities of specific peptides at plasma or vesicular membranes of enterocytes (ZnT1 and ZIP4) and pancreatic acinar cells (ZnT1, ZnT2, and ZIP5) are important factors for the adaption of an organism to changing alimentary Zn supply levels (Lichten and Cousins, 2009; Fukada and Kambe, 2011; Schweigel-Röntgen, 2014).

ZIP and ZnT peptides can be distinguished by their transport direction. The ZIP family members transport Zn^{2+} from the extracellular space or intracellular compartments towards the cytosol. In contrast, ZnT peptides transport in the opposite direction and decrease cytosolic Zn by transferring it outside of the cell or into vesicles or organelles (Lichten and Cousins, 2009; Fukada and Kambe, 2011; Schweigel-Röntgen, 2014).

ZIP4 is abundant in various cell types and tissues including the intestines. It represents the major active apical transport mechanism for luminal Zn^{2+} into gut mucosal cells (Wang et al., 2002; Dufner-Beattie et al., 2003, 2007; Liuzzi et al., 2004). Data from several labs suggest that intestinal ZIP4 activity is modulated by transcriptional and post-transcriptional processes. Dietary Zn deficiency promotes increased synthesis and presentation of the peptide at the apical plasma membrane. In contrast, repletion of body Zn stores initiates ZIP4 endocytosis and ubiquitination as well as messenger RNA (mRNA) degradation (Mao et al., 2007; Weaver et al., 2007; Kambe and Andrews, 2009). It has been further demonstrated that *ZIP4* mRNA counts are a product of varying mRNA stability and kruppel-like-factor 4 (KLF4) activity (Liuzzi et al., 2009; Curry-McCoy et al., 2013).

In contrast to ZIP4, intestinal ZnT1 activity involves the basolateral transfer of cytosolic Zn^{2+} into the circulation (McMahon and Cousins, 1998; Cousins and McMahon, 2000; Liuzzi et al., 2001; Andrews et al., 2004). Its interplay with ZIP4 activity represents the dominant apical-to-basolateral uptake route for dietary Zn into the organism. Earlier data suggest that free cytosolic Zn^{2+} is the major regulative stimulus of *ZnT1* by activating metal responsive transcription factor 1 (MTF1) (Brugnera et al., 1994; Langmade et al., 2000; Liuzzi et al., 2001). Hence, it is not the overall body Zn status that affects ZnT1 activity, but fluctuations of free cytosolic Zn^{2+} as affected by different Zn transporters.

A major proportion of the endogenous Zn that is excreted into the GIT is of pancreatic origin (Oberleas, 1996; Holt et al., 2012). This highlights the essential role of the pancreas in Zn metabolism. Zn excretion into the intestine via the pancreatic duct occurs predominantly by exocytosis of Zn metallopeptides rather than free Zn^{2+} (Matsuno et al., 1982). This includes, for example, the pancreatic carboxypeptidases A and B, which need Zn^{2+} to bind their catalytic centre (Folk et al., 1960; Folk and Schirmer, 1963; Hsu et al., 1966; Mills et al., 1967; Roth and Kirchgessner, 1974). The transporter ZnT2 has been identified as responsible for loading cytosolic Zn into vesicles of pancreatic acinar cells. It shows a reduced abundance during Zn deficiency, which has been associated with a marked decrease of the Zn concentration within pancreatic zymogen granules. On the other hand, Zn overload promotes the opposite response (Guo et al., 2010). Zn deficiency has also been associated with higher ZnT1 activity at pancreatic basolateral membranes (Liuzzi et al., 2004). This indicates a rerouting of pancreatic Zn into the circulation for the benefit of other tissues during Zn deficiency. In contrast, increased pancreatic Zn absorption from the circulation under Zn-replete conditions is due to basolateral ZIP5 activity, which is evident in many tissues and cell types including pancreatic acinar cells (Dufner-Beattie et al., 2004). Zn deficiency, however, promotes degradation of pancreatic ZIP5 and associated reduction in absorption of circulatory Zn. Weaver et al. (2007) suggest that ZIP5 abundance is regulated at the translational rather than the transcriptomic level. They demonstrated that *ZIP5* mRNA is continuously located at polysomes where it can be quickly translated if necessary. However, our data obtained in the gut mucosa of piglets highlight a regulation of pancreatic *ZIP5* gene expression in response to the dietary Zn supply during short-term subclinical Zn deficiency (Brugger, 2018).

There are currently 24 Zn transporters described in mammals. Furthermore, a whole set of other factors affecting intracellular Zn trafficking and exchange has been identified. Presenting the current knowledge about all currently known molecular key players in Zn homeostasis would exceed this manuscript's capacity. Fortunately, a set of excellent reviews has been published, which address the molecular biology and biochemistry of Zn metabolism in more detail (Lichten and Cousins, 2009; Colvin et al., 2010; Fukada and Kambe, 2011; Schweigel-Röntgen, 2014).

3 Role of the skeleton as a reservoir for mobilizable Zn

Weaned piglets develop the first signs of clinical Zn deficiency (feed refusal) after approximately 10 d of low Zn intake (Windisch, 2003b; Ettle et al., 2005). To compensate for the negative side-effects of malnutrition for several days, the body must contain some kinds of Zn storage compartment. Some soft tissues like the liver can accumulate Zn, but these sinks are limited and therefore become exhausted quite quickly (Windisch, 2003a). Precise metabolism trials with ^{65}Zn-labelled rats have demonstrated the central role of the skeleton as a source of mobilizable Zn during times of deficiency. These datasets indicate that under conditions of dietary Zn deficiency, mobilization of stored Zn occurs primarily from the skeleton (Windisch, 2001, 2003a). Furthermore, during subsequent repletion, bone Zn stores undergo a quick and targeted refilling until the original level is replenished (Windisch, 2001). This quick repletion is due to the above described higher ZIP4 activity on the apical gut mucosa of depleted individuals. Interestingly, trabecular bone tissue in particular is involved in Zn exchange mechanisms (Windisch et al., 2002), which is why such bone material (e.g. the femoral head) should be used to monitor the status of Zn storage in studies on Zn metabolism trials. The mobilization and storage of Zn in bone tissue appear to happen independently from general mechanisms of bone turnover, which may point towards a distinct, passive process (Windisch et al., 2002; Brugger et al., 2018). This is in line with data from pigs that show a strong linear correlation between bone Zn and plasma Zn (steady state) in response to different dietary Zn concentrations (Brugger et al., 2014).

In recent years, the opinion has prevailed that bone Zn mobilization is a rather slow process and that for a quick response the soft tissue compartments are more important. However, this contradicts the experimental data which clearly demonstrate that bone Zn mobilization happens within the first 24 h of deficient supply (Windisch, 2003a). Interestingly, our data highlight changes in bone Zn within only 1 h post feeding (Brugger et al., 2018). This adaption is quicker than the changes in absorptive capacity and endogenous secretion at the gut barrier, which need 3–5 d to fully adapt. Hence, bone Zn reservoirs appear to be highly mobile. As soon as the mobilizable fraction of bone Zn is completely exhausted, clinical symptoms arise (Windisch, 2003b). The precise mechanisms of Zn exchange between the blood and skeleton as well as their interactions with bone tissue remain unclear and must be subject to further research.

4 Interaction of exogenous pancreatic activity and Zn metabolism

The adaption of pancreatic Zn excretory behavior points towards a direct interaction between Zn supply and digestive capacity. Indeed, the exogenous pancreas is necessary for providing sufficient digestive capacity to the organism as it is responsible for the production and excretion of a large proportion of necessary digestive enzymes, especially proteases. These include trypsin, chymotrypsin, carboxypeptidases A and B, elastase, α-amylase, and pancreatic lipase (Klein, 2019). An impairment of pancreatic metabolism, e.g. in the course of pathological events like pancreatitis, is therefore associated with impaired digestive function (Lankisch et al., 2015). This may also account for fluctuations in the supply status of nutrients, especially those that, like Zn, are regulated by pancreatic activity.

Earlier studies detected signs of severe digestive depression as a result of clinical Zn deficiency. One of the most illustrative examples is the classic study of Tucker and Salmon (1955). They demonstrated for the first time that Zn was essential for growing pigs. They noticed severe cases of diarrhea and anorexia of pigs being fed with cereal-based diets without Zn

fortification. All symptoms could be cured by generous addition (up to 2% on top of the basal diet) of $ZnCO_3$. Other studies reached similar conclusions in other models (Raulin, 1869; Sommer and Lipman, 1926; Todd et al., 1934; Bertrand and Bhattacherjee, 1935; Legg and Sears, 1960; O'Dell et al., 1977). By controlling for bias due to changes in feed intake behavior, Roth et al. (1992) recognized a significant drop in digestive capacity of clinically Zn-deficient force-fed rats. This was a first hint that growth depression in response to Zn deficiency is due only in part to anorexia. Our data, obtained from restrictively fed subclinical Zn-deficient piglets, confirmed these findings and further highlighted a reduced synthesis of exogenous digestive enzymes within Zn-depleted pancreatic tissue (Brugger and Windisch, 2016a, 2016b). Under conditions of excess Zn supplementation (e.g. when applying pharmacological Zn dosages (about 2500 mg Zn/kg diet)), the response appears to be shifted to the opposite (Pieper et al., 2015). This group also found a higher abundance of exogenous digestive enzymes and Zn in pancreatic tissue of piglets excessively supplemented with Zn. Hence, exogenous pancreatic excretion of Zn may be functionally related to the excretion of pancreatic digestive enzymes. Some of these enzymes (carboxypeptidases) are Zn metalloenzymes (Folk et al., 1960; Folk and Schirmer, 1963; Hsu et al., 1966). It appears plausible that Zn is not excreted as free Zn^{2+} ions considering their strong toxic potential (Bozym et al., 2010). Also, the finding that not only carboxypeptidases but the whole set of pancreatic digestive enzymes is affected during Zn deficiency and excess (Brugger and Windisch, 2016a, 2016b), suggests that the excretion of pancreatic enzymes is not enzyme-specific, but rather involves the complete set of catalytic peptides. A higher necessity for peptide synthesis in times of Zn excess may in part explain the higher abundance of stress peptides in the course of pancreatic Zn accumulation (Pieper et al., 2015).

In summary, the organism is entering a "vicious circle" in the course of Zn deficiency. On the one hand, it must avoid losing too much Zn into the GIT through exogenous pancreatic secretion. On the other hand, the consequent reduction of digestive capacity increases the Zn supply problem, as digestive breakdown of the feed matrix is crucial for the absorption of all nutrients, including Zn.

5 Consequences for the definition of requirements and the estimation of bioavailability

The aforementioned principles of Zn homeostasis should be considered when planning experiments that aim to define Zn feeding requirements and recommendations. The magnitude of body Zn depletion determines the intensity by which a status parameter responds to dietary treatment. In several studies, a higher intestinal ZIP4 activity and transcription were observed in Zn-deficient individuals than in control groups. The response appears to be even more pronounced when comparing clinically to subclinically Zn-deficient individuals (Dufner-Beattie et al., 2003, 2004; Brugger, 2018). Indeed, an organism which is so severely depleted with Zn that obvious degenerative processes occur must temporarily acquire more Zn from the GIT lumen to refill its emptied stores and, simultaneously, supply Zn for processes of maintenance and regeneration (Prasad, 1985). However, such severe events of Zn deficiency are very rare in nature, especially under the terms of practical livestock production (Caulfield and Black, 2004). Therefore, Zn feeding thresholds which have been defined based on studies in clinically Zn-deficient animals are less relevant from a practical point of view. In addition, in these studies comparisons are made between healthy and sick individuals, which further reduces their informative value. In contrast, subclinical Zn deficiencies are more likely to occur in livestock farms (Nielsen, 2012), for example during periods of temporarily reduced feed intake (e.g. in the weanling phase, as described by Lallés et al. (2007)). Therefore, it seems more appropriate to model this specific physiological state in experiments aimed at providing recommendations for practical Zn feeding.

Usually, the term bioavailability is predominantly used as a synonym for absorbability. Although this may be suitable in regard to certain substances, it is not appropriate for Zn. Its solubility kinetics during the gastrointestinal passage is a rather poor indicator of its absorption rate. If this was not so, solubility indices would already be used in practical feeding to predict Zn feeding efficacy under varying dietary conditions. This lack of correlation is evidently explained by the previously discussed metabolic processes as a function of the status of the body's Zn reserves. Rather than aiming to identify or develop Zn

sources with peak solubility values, we should be seeking those that satisfy metabolic demands with the lowest possible dietary Zn concentration under practical conditions. This obviously applies also to other nutrients and nutritive elements. Therefore, for an animal nutritionist the estimation of relative bioavailability is tightly connected to the measurement of gross requirements. In the end, we do not investigate the solubility kinetics of Zn sources, but their efficiency in mediating Zn^{2+} transfer to the mucosal transport peptides.

To assess Zn bioavailability and requirements, the response of appropriate status parameters in standardized high-resolution dose-response studies must be observed. It is of utmost importance to choose the range of applied dosages in a way that allows discrimination between Zn-deficient and sufficiently supplied animals. Furthermore, these dosages should cover the range from potentially deficient to sufficient supply levels in high resolution, to allow a mapping of the gross Zn requirement threshold. The state-of-the-art approach is to use linear and non-linear regression models to estimate dietary thresholds, below or above which significant differences are evident in the response behavior of suitable status parameters to varying Zn supply (Robbins et al., 2006). These "breakpoints" may mark the minimum dosage to satisfy the gross Zn requirement. However, the question remains, what is a "suitable status parameter"? On the one hand, it must express a highly significant responsiveness to varying dietary Zn supply. On the other hand, the intensity or direction of the response should change when the dietary Zn intake falls below, meets, or exceeds the gross requirement. Apart from its relationship to changes in body Zn status, a Zn status parameter should also meet some functional prerequisites. Zn is important for basic mechanisms in all cell types. Hence, a lot of parameters would respond to a change in the Zn supply status, including some not directly related to Zn homeostatic regulation. Prominent examples involve key players of redox metabolism and immune function (reviewed by Eide (2011) and Haase and Rink (2014)). Indeed, parameters related to these metabolic pathways have been shown to be affected by Zn homeostatic regulation (Brugger and Windisch, 2017a; Kloubert et al., 2018). However, this also implies that under more practical experimental conditions, environmental stressors like

infections might bias the results. Furthermore, some data suggest that certain parameters of Zn homeostasis respond less to the body Zn status itself, but more to the stress or inflammatory status of a tissue (Brugger, 2018). Hence, monitoring these factors to highlight homeostatic regulation in the context of body Zn losses appears questionable. A preferable parameter to estimate Zn requirements or availability should reflect Zn homeostatic regulation in relation to changes in body Zn. Based on our experience, the apparent digestion of dietary Zn has proved to be a robust indicator of the status of Zn homeostatic regulation (Brugger et al., 2014). Earlier studies clearly demonstrated it to be highly and significantly correlated with the truly absorbed dietary Zn (Weigand and Kirchgessner, 1980; Windisch and Kirchgessner, 1999). Hence, this parameter directly reflects the Zn homeostatic regulation at the gut barrier, which plateaus over sufficiently supplied groups. This allows a differentiation between animals fed diets with insufficient or sufficient Zn concentrations under experimental conditions. Furthermore, it can be measured in vivo without killing animals, as only feed and fecal samples are needed. The sole use of partial digestibility data (e.g. from the end of the ileum) would not be sufficient, as it has been shown that the colon absorbs significant amounts of luminal Zn and its *ZIP4* gene expression is regulated depending on the Zn supply status (Hara et al., 2000; Brugger, 2018). Consequently, ignoring the quantitative role of the colon in Zn absorption inevitably leads to information being lost and the data quality of studies on Zn metabolism being impaired (for a more comprehensive discussion on suitable status parameters, see Brugger et al. (2014)).

The chemical conformation in the intestinal lumen, together with the physiological status of the animal, determines the net amount of ingested feed Zn. The former is a product of the chemical ligands associated with Zn (chemical Zn species) under luminal conditions, and the extent and diversity of their interactions with other feed components and endogenously excreted substances (Windisch, 2002). Active Zn transporters accomplish Zn transfer through biological membranes exclusively with divalent cations (Zn^{2+}) (reviewed by Holt et al. (2012), Lichten and Cousins (2009), and Schweigel-Röntgen (2014)). Therefore, the absorption of luminal Zn depends on the total

content of free Zn^{2+} ions (usually very low) and above all on the amount of Zn loosely bound to respective chemical ligands (Windisch, 2002). Significant levels of luminal Zn appear to be unavailable in practical swine diets due to their association with phytic acid in the course of gastrointestinal passage (Cosgrove, 1980). Therefore, growing piglets without appropriate Zn supply from complete feed develop clinical Zn deficiency within about 10 d (Windisch, 2003b; Ettle et al., 2005). Previous data indicate the gross Zn requirement of weanling piglets at 50–60 mg/kg in modern high phytate diets without addition of exogenous phytase (Lewis et al., 1956, 1957a, 1957b; Luecke et al., 1956; Stevenson and Earle, 1956; Smith et al., 1958, 1962; Shanklin et al., 1968; Liptrap et al., 1970; Brugger et al., 2014). Assuming Zn requirements of 15–20 mg/kg under semi-synthetic dietary conditions without phytic acid (Smith et al., 1962; Shanklin et al., 1968) and native Zn levels of cereal-based diets of 30–40 mg/kg (Brugger et al., 2014), we conclude in context to the defined requirements (about 50 mg/kg) of growing piglets (Lewis et al., 1956, 1957a, 1957b; Luecke et al., 1956; Stevenson and Earle, 1956; Smith et al., 1958, 1962; Shanklin et al., 1968; Liptrap et al., 1970; Brugger et al., 2014) that in practical feeding systems a high proportion of supplemented Zn is needed to saturate phytic acid Zn-binding sites, rather than the actual net requirement. Therefore, the essence of Zn supplementation strategies for pigs and poultry is to increase the total amount of luminal Zn to provide an excess of non-phytate Zn that can meet the body's Zn requirements. Not only does this underscore the need for elevated dietary levels of Zn in the presence of phytate-rich diets, but it also suggests the ideal range of dietary Zn doses intended for dose-response studies to modulate Zn metabolism. These should cover deficient (<50 mg/kg diet) and adequate intake levels (≥50 mg/kg diet) to reflect the dose-dependent adaptation of Zn homeostasis, which allows estimation of the respective Zn requirement threshold.

6 Conclusions and outlook

A crucial cornerstone for more sustainability in animal nutrition is to establish precision feeding. This applies especially to micromineral supplementation.

Indeed, high-yielding livestock animals must be supplied with sufficient amounts to maintain productivity and wellbeing through all stages of the production cycle. However, excessive supplementation far above the requirement thresholds should be avoided as this has been associated with environmental concerns as well as impaired animal and human health (reviewed for Zn in more detail by Brugger and Windisch (2015) and Brugger and Windisch (2017b)). European authorities recently acknowledged these issues by decreasing allowed upper limits for total Zn concentrations in feed (The European Commission, 2016). However, the allowed maximum of 150 mg/kg for piglets is still about three times their requirement (about 50 mg/kg diet) (Lewis et al., 1956, 1957a, 1957b; Luecke et al., 1956; Stevenson and Earle, 1956; Smith et al., 1958, 1962; Shanklin et al., 1968; Liptrap et al., 1970; Brugger et al., 2014). This generosity in terms of safety margins reflects uncertainty regarding the magnitude of Zn utilization as well as the parameters that affect it under practical conditions. To refine current Zn feeding recommendations, there is a set of research questions that first have to be answered.

There is an urgent need for studies comprising modern genotypes highlighting the changes in Zn requirements under changing dietary and environmental conditions. This must be done by applying appropriate and precise experimental models, the cornerstones of which have been described above. For weaned piglets such a model is already available (Brugger et al., 2014), but for further species they are yet to be developed. Furthermore, we still lack a comprehensive understanding of Zn chemistry along the GIT. Closing this gap is crucial to facilitate a targeted development of efficient Zn supplements for dietary intervention. Therefore, state-of-the-art analytical methods of metal speciation analysis ("metallomics") must be implemented in mineral nutrition research (Michalke, 2016). Furthermore, there is no solid diagnostic marker that indicates the Zn status of an individual with sufficient sensitivity under field conditions (especially in the case of subclinical events). This reflects our limited understanding of the body's regulation of Zn metabolism, and especially of the molecular transmitters and receivers of information on the body's Zn supply status in and between tissues. Hence, more basic research on the function of vertebrate Zn homeostasis should be conducted.

Contributors

Daniel BRUGGER performed the literature research, wrote the article, and had primary responsibility for the final content. Wilhelm M. WINDISCH contributed to the writing of the article. Both authors have read and approved the final manuscript.

Compliance with ethics guidelines

Daniel BRUGGER and Wilhelm M. WINDISCH declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by either of the authors.

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中文概要

题　目：单胃动物锌代谢及其对营养需要量和饲料锌生物利用率估算的影响

概　要：矿物质营养研究的主要目标是为猪饲养者提供饲料锌利用率和总锌需要量信息，这些需要量信息受到饲养条件的影响。这只能通过应用精确实验模型来阐明锌代谢的基本原理。本综述总结了单胃动物体内锌稳态的最重要方面，包括锌的获取和排泄的分子机理，尤其强调骨骼以及外分泌胰腺在这种稳态调节中的作用。最后，文章讨论了上述生理学原理对试验设计产生的影响，旨在解决锌需要量和生物利用率的问题。

关键词：单胃动物；锌代谢；需要量；生物利用率；实验模型

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