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TUTORIAL REVIEW

Zinc fate in animal husbandry systems

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Zinc (Zn) is considered in animal production systems as both an essential nutrient and a possible pollutant. While it is generally supplemented at low levels in animal diets, with less than 200 mg kg^{-1} in complete feeds, it is under scrutiny due to potential accumulation in the environment. This explains why international regulations limit maximum supplementation levels in animal feeds in a stricter way. This article gives an overview of the current knowledge on the fate of zinc in animal production systems, from animal diets to animal wastes. Some analytical methods can be used for the quantification and qualification of Zn chemical forms: X-ray crystallography, electrospray tandem mass spectrometry, separation techniques, hyphenated techniques... Analysis of chelated forms issued from complex matrices, like hydrolysed proteins, remains difficult, and the speciation of Zn in diluted carriers (premix and feed) is a challenge. Our understanding of Zn absorption has made progress with recent research on ZnT/Zip families and metallothioneins. However, fine-tuned approaches towards the nutritional and metabolic interactions for Zn supplementation in farm conditions still require further studies. The speciation of zinc in pig manure and poultry litter has been a priority as monogastric animals are usually raised under intensive conditions and fed with high quantities of trace minerals, leading to high animal density and elevated quantities of zinc from animal wastes.

Introduction

Zinc (Zn) exists naturally in soils and in plants, but the requirements of livestock are generally higher than the zinc concentration in feed ingredients. Consequently this trace element is supplemented in the diets of animals to fulfil their needs; new scientific findings on zinc in animal nutrition and production facilitate an optimal formulation but trace elements are often

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supplied in excess to enable sufficient safety margins. Growth inhibition and decrease of food intake are associated with Zn deficiency;[1] as a result, this element is usually supplemented in the animal diet, sometimes at high levels for pigs. The pharmacological dosage of ZnO is a supplementation between 2000 and 4000 ppm (usually 2500 ppm of Zn), with a positive impact on the growth performance and on the gut health of weaned piglets in farm conditions,[2] but the mechanisms are not yet well understood. In the last few years, concerns regarding feed safety and the environment have become increasingly important and the maximum authorized levels of trace elements in animal feeds have been reduced in the European Union, according to the Commission Regulation (EC) 1334/2003. Currently, numerous sources of Zn additives are offered by the industry. Some methods are available to characterize these additives at quantitative level, in pure product, in premixes and in the feed; quality controls of the additive in different carriers are therefore possible. These analyses and a better understanding of the absorption mechanisms lead to a finer estimation of Zn excretion, especially in monogastrics. Zn concentration in animal wastes from pig and poultry is, on average, higher than from cattle manure, in connection with the mineral content in feeds[3] and the low zinc retention by the animals.[4,5] As monogastric meat is commonly produced in an intensive system (more than 50% of global pork production, 70% of poultry production),[6] the monogastric wastes require particular attention. In this review, Zn is followed from the animal diet to manure: speciation of zinc additives in various carriers, zinc transport in organism and the fate of zinc in livestock effluents, with a focus on monogastrics, are developed.

A. Zinc in feed

Zinc in the geosphere and in the biosphere

Zinc is naturally present in the different envelopes of the Earth: lithosphere, hydrosphere, cryosphere, atmosphere and biosphere. It can be found in particular in the lithosphere: the content of Zn in this compartment is 70 ppm on average, depending on the location in the world.[7] Earth's crust consists of about 95% of igneous rocks (including metamorphic rocks) and 5% of sedimentary rocks (4% of shale and clays, 0.75% of sandstone and 0.25% of limestone);[8] concentration range of Zn varies between 5 and 250 ppm in the igneous rocks and between 2 and 180 ppm in the sedimentary rocks, with three major Zn-containing minerals: smithsonite ($ZnCO_3$), sphalerite (ZnS) and hemimorphite ($Zn_4(OH)_2Si_2O_7{\cdot}H_2O$).[9]

In the soils and in the hydrosphere, the presence of Zn can be linked to the degradation of the parent materials and to anthropogenic inputs. Chemical and physical weathering of the rocks would represent an important source of Zn in the soils, in the rivers, in the streams or in other aquatic systems. Wind, erosion, freeze–thaw action of water and break-up of the rocks by the plants roots can release Zn in the environment.[7] In addition, human activities represent a major input. In the fields, fertilizers, manures and biosolids increase the metal content in the soil. Industrial activities, such as mining, also have a significant impact on Zn pollution in the environment.

Fossil fuel flux and industrial particulate emissions are the main sources of particulate atmospheric emissions for Zn: they were twenty times higher than dust flux from natural sources (continental and volcanic) during the 1980's, with 840 000 *versus* 36 000 tonnes per year.[7] This phenomenon increases the precipitations of Zn over the seas and on the land.

Trace minerals have generally a low bioavailability in the soils. Zn can be found in three forms: as water-soluble Zn, as absorbed and exchangeable Zn associated with clay particles and as insoluble Zn-complexes. It is distributed in these three fractions according to some factors like pH, soil type, weathering rates, plant uptake... Zn influx into the roots from the soil solution imply Zn as Zn^{2+} or complexed with organic ligands. In fields with a low concentration of zinc in bulk soil solution and high pH, crop zinc deficiency is common.[10] The essentiality of this metal for the maize has been established by Mazé (1915). Sommer and Lipman later obtained the same conclusions for the dwarf sunflower and the barley (1926). The Zn content in plants is highly variable, depending on the species, on the soil type and

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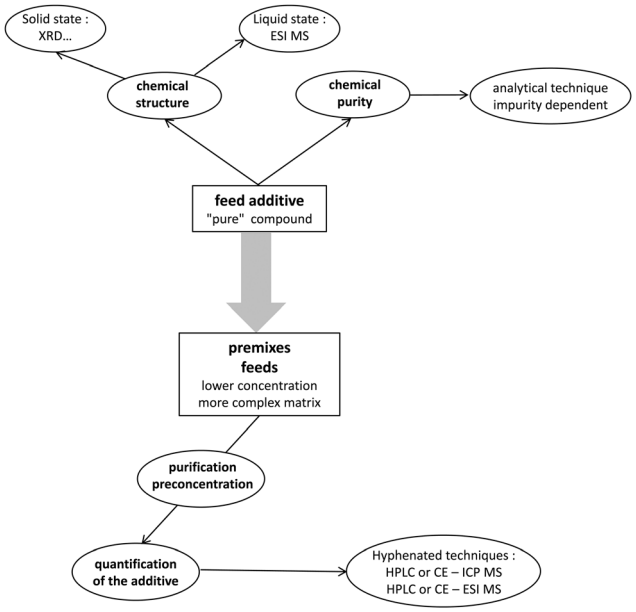

Fig. 1 Summary of the analytical strategies that can be used to characterize Zn additives in the raw materials and in diluted media.

on the localisation of the field: for example, in the Southeastern Mediterranean region, concentration ranges of 20 to 40 ppm in wheat grain and 16 to 130 ppm in corn grain were found in different varieties and regions.[11]

First significant studies with mammals, by Todd, Elevehjem and Hart (1934), involved rodents; the negative effects of Zn deficiency were further described in swine (1955) and in poultry (1958).[12] The Zn content is consequently included in diet formulations, and Zn addition is common. Nevertheless, zinc supplementation is subject to rules and to regulatory changes: in 1970, according to the Council Directive 70/524/EEC, maximum content of the Zn in ppm of the complete feeding stuffs was 250 ppm for all species; nowadays, according to the Commission Regulation (EC) 1334/2003, the maximum content of Zn is 150 ppm for monogastrics. Quantification and speciation of Zn in additives, in premix and in feeds are therefore useful for animal productions.

The industry offers a wide variety of Zn additives for supplementation: zinc acetate, zinc chelate of amino acid, zinc chelate of glycine, zinc chelate of the hydroxy analogue of methionine, zinc chelate of methionine, zinc oxide, zinc sulphate, zinc chloride, zinc chloride hydroxide, according to European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003. The characterization of those feed additives is an important task because too vague regulatory definitions may favour batch to batch variation and induce significant differences between manufacturers. The fine characterization of supplements is also the first step to the understanding of absorption mechanisms that still remain unclear. This characterization should be addressed both at the qualitative and quantitative levels and in the raw additive, the premixes and the feeds according to a scheme that can be summarized as presented in Fig. 1.

Characterization of the Zn-feed additives

Inorganic zinc sources are well recognized chemical compounds, generally identified by CAS international system. Some chelated sources of zinc are also registered: these compounds are based on the principle that the metallic component is bound to one carrier (generally one amino acid). It is highly challenging to qualify the chemical structure of these products and especially to define their chelation ratio. Numerous analytical methods have been proposed by manufacturers, mostly in industry magazines, much more rarely in scientific journals.[13,14] The chelation strength is not predictive of the bioavailability of organic trace minerals.[15] As no analytical assay has been considered sufficiently robust until now, there are no official methods for the determination of chelation rate not only for animal nutrition in the EU, according to the EFSA Journal in 2008, but in any feed and food regulations outside the EU.

When crystalline material is available, the elucidation of the chemical structure of the additive can be assessed using direct solid state analytical methods. X-ray crystallography has been used for example for Zn glycinate[16] or Zn bis-glycinate[17] characterization. However XRD can only be performed on crystalline materials with sensitivity in the range of the percent and therefore quantitative data from more complex and diluted matrices than the raw additive, like feeds, can generally not be assessed. A wet extraction of the Zn-species followed by a sensitive and specific detection, like electrospray mass spectrometry, is then necessary.

However the study of Zn complexes with organic ligands in solution is difficult because they do not implicate covalent Zn–carbon bonds but coordinative bonds. Special care should then be paid to the pH of the solution to avoid dissociation of the chelate (when the pH becomes too acidic) but also to the ionization conditions again to avoid the dissociation of the chelate. Electrospray tandem mass spectrometry was for example applied to characterize a Zn glycinate,[18] a Zn bis-glycinate[17] or a Zn chelate of the methionine hydroxyl-analog.[19]

Data on the fine characterization of chelates issued from more complex matrices like proteins hydrolysates are much scarcer. To our knowledge, the only work reported concerned Zn-proteinate from hydrolysed soybeans and the authors considered that the size-exclusion chromatographic pattern was characteristic of the molecular weight Zn distribution and used it for identification.[20] Analytical data on enriched Zn-yeasts are also very scarce due to the complexity of the matrix. To our knowledge, the species present have not yet been identified. In this case, at least in a first step, the speciation could be addressed at the level of fractionation analysis (*e.g.* sequential extraction) as it was proposed for selenized yeasts.[21] This approach allows the distinction of different fractions (water soluble, polysaccharide-bound and proteins-bound fractions).

ZnO is also an increasingly used feed additive. However, due to its low solubility in aqueous media, its characterization requires direct solid state analytical techniques like X-ray diffraction, Fourier transform infrared spectroscopy, UV-visible spectroscopy and transmission electron microscopy.[22] But when included

as a nanoparticle, the ZnO characterization should include the size distribution, the shape and the elemental composition. The hyphenation of separation techniques (size-exclusion liquid chromatography or flow field fractionation) with size (SLS, DLS) or elemental (ICP MS) detectors offers powerful benefits for the physico-chemical characterization of such nanomaterials.

The finalization of the Zn feed additive characterization should be addressed by the evaluation of the impurities. In this case the analytical method to be used is impurity dependent. ICP MS for example can be used to evaluate metallic trace impurities after digestion.

Quantification of the Zn-feed additives

For chelated forms, direct solid speciation analysis would be a method of choice as it should avoid the potential dissociation of the chelates in solution. However most of the techniques available are limited in terms of sensitivity for quantification purpose in diluted media. And the most powerful of them can hardly be used in routine analysis. Quantitative data are therefore preferably obtained after solubilization of the species of interest. Anyway, the stability in solution should be checked before the development of a speciation method can be investigated.

Hyphenated techniques based on the coupling of liquid chromatography (HPLC) or capillary electrophoresis (CE) with ICP MS detection have now become a technique of choice for the sensitive speciation analysis of metallocompounds because of their sensitivity regardless of the matrix. Even if the coupling with HPLC remains the first choice, because of the ease of use and the detection limits reachable, it can be hampered by the instability of the chelates on the stationary phase of the columns. CE can then become an alternative as the separation mechanism is not based on interactions with a stationary phase. However, in both cases, this speciation is modulated by the pH of the solutions used as the chelates can be dissociated at acidic pHs. The CE–ICP MS coupling has for example been successfully used for speciation of Zn-glycinates n.[18] Even if the glycinates studied were prone to rearrangement, the metal glycine moiety was preserved and, in these standard-like conditions, detection limits of 0.2 μg mL^{-1} could be achieved.

Evaluation of the Zn additives in premixes and feeds

Applied to all feed additives, analytical methods used on pure products, and after dilution in premixes and feeds, are assessed by the European Union Reference Laboratory for feed additives at the Institute of Reference Materials and Measurements. An evaluation report (EURL Evaluation report on ''inorganic and organic sources of zinc'', 2011) was released to become publicly available.

The transfer of the methodologies developed for the raw feed additives to diluted media like premixes and feeds is far from being easy due to the decrease of the concentration of the species of interest (from a pure compound, to the g kg^{-1} range in premixes and down to the mg kg^{-1} range in feeds). Moreover the overall chemical composition of the sample may introduce other ligands that can compete with the original ligand of the chelate but also a matrix effect that may degrade the chromatographic or electrophoretic separation.

Data reported on premixes are very scarce. But Vacchina *et al.* have obtained a nice correlation between theoretical and experimental concentrations of Zn-glycinate in 4 kinds of premixes by CE–ICP MS.[18] However the transfer of the method developed to feeds necessitated the development of preconcentration and cleaning steps to obtain the same kind of correlation.[23]

B. Zinc in animals

Zinc is an essential trace element and plays crucial roles in the organism; its importance is known for more than a century.[12] It is generally found as a divalent ion (Zn^{2+}) and can bind to complex molecules: 3% to 10% of the genes in the human genome code for proteins contain a zinc-binding domains.[24]

Zinc, an essential trace element

The activity of more than 300 enzymes, in all enzymes classes, depends on Zn, so that this metal takes part in many biological processes: catalysis, cell proliferation, oxidative defence of the plasma membrane, immune defence... Zn is also involved in gene regulation through Zn-finger proteins; these molecules stabilise the binding strength of transcription factors to ADN or ARN.[25] In addition, positive impact of zinc supplementation on growth performance is well known in farm conditions.[1,2]

Concentration of Zn in the diet changes the Zn status in animals. Optimum functions can be maintained by adjusting the rates of Zn absorption and excretion; the Zn status remains approximately constant through homeostasis. Absorption of Zn occurs in several parts of the gastrointestinal tract; endogenous losses correspond to pancreatic exocrine and biliary secretions and to Zn from the sloughed mucosal cells.[26] Molecules and mechanisms involved in this balance are not completely elucidated, but in the last twenty years, significant progress has been made.

Zinc absorption

Biological pathways of dietary Zn have been described in recent reviews, with kinetic analysis and metabolic modeling. Research studies with radioisotopes and stable isotopes contribute to a better knowledge of the fate of Zn in the animal organism;[27] roles and transporters of this trace element are increasingly well documented, even though some points need further investigations. Currently, Zn absorption is considered in all the species.

Some studies mention absorption of Zn anterior to the duodenum in chickens and in dairy cattle, but more recent papers do not support this view; primary sites for Zn absorption in animal would be in the small intestine.[28] Capacity of the ileum for Zn transport may be the highest in the intestinal tract, especially in chickens.[29] The duodenal segment would also be a main site for Zn absorption, and experiments suggest an important role of the jejunal segment in pigs.[30] The proportion of absorbed Zn could be linked with the length of the different intestinal parts: as ileo-jejunum is the largest part of the small intestine, it is the largest site for Zn absorption.

According to some studies, absorption in the large intestine could be significant in rats, pigs and ruminants.[30]

The concentration of trace elements in some key organs is a good criterion for absorption, but enzyme activity is a better option for utilization, in order to know the metabolic activity and the status indicators for Zn. Some methods can be used for the quantification of zinc in animal tissue: flameless atomic absorption spectrophotometry (FAAS), neutron activation analysis, ICP-MS, ICP-AES[31]... Zn sources (chelate, zinc oxide, zinc sulphate...) can modify the Zn content in some storage tissues[32] but not the zinc balance.[33]

Other essential elements in excess (calcium, iron, copper) can reduce zinc absorption.[34] Phytates also have a negative effect on this phenomenon; phytase supplementation can degrade phytate and liberate the endogenous zinc bound to this anti-nutritional factor.[35]

According to O'Dell (1997), bioavailability can be defined as "the proportion of the element consumed that is utilized for a chemical or physiological function". Measuring Zn absorption is insufficient to know Zn bioavailability but can be considered as a first step.

Zinc transporters

Zn transport can be observed in two steps: uptake of Zn from the lumen to the cell is followed by Zn transport from the cell into the circulatory system. The first process could be dependent on active transport and facilitated diffusion; simple diffusion[36] and the paracellular movement[37] of Zn could also be involved for a small proportion of ingested Zn.

ZnT family

In 1995, the first Zn transporter gene, ZnT1, was identified by Palmiter and Findley; before this discovery, Zn transport was supposed to be dependent on an anionic or amino acid complex, or a chelate, with transferrin receptors. The ZnT family belongs to the solute-carrier (SLC) family, subfamily of cation diffusion facilitor (CDF) families. Currently, ten ZnT proteins with a similar topology have been recorded: they have six transmembrane domains, except ZnT5 which has twelve, an intracellular N-terminus and C-terminus and an intracellular loop with several histidine residues.[24] Four of their six transmembrane domains play a role in the translocation of Zn. The intracellular N- and C-termini and the histidine-rich loop are involved in the regulation of Zn removal by the cell. Some ZnT proteins are in the plasma membrane and decrease intracellular Zn *via* efflux from cells; but many of them are in intracellular compartments, like Golgi apparatus and the endoplasmic reticulum, and decrease intracellular Zn *via* influx into intracellular vesicles.[38]

Zn transporter activity has been established for ZnT1, ZnT2, and ZnT4 to ZnT8. Zn transport mechanisms are still unknown.[38–45]

The ZnT1 protein, located in the plasma membrane, takes part in efflux of Zn from the cell and may be found in several parts of the gastrointestinal tract (oesophagus, duodenum, cecum).[46] It seems to be abundant along basolateral membrane of enterocytes in rats[47] and would be a main Zn exporter in the cell membrane of the kidney.[48] According to software calculations, ZnT10 could also be found in the plasma membrane.[24]

Other ZnT proteins belong to organelles.[24,38–49]

Localisations of ZnT proteins in cells are summarized in Table 1.

Table 1 Main localisations of zinc transporters ZnT in organism and in cell

Name	Tissues	Intracellular localisation
ZnT1	Oesophagus, duodenum, cecum, kidney	Plasma membrane (basolateral membrane)
ZnT2	Pancreatic acinar cells, testis, kidney	Endosomes
ZnT3	Brain, testis	Synaptic vesicles
ZnT4	Small intestine, large intestine, mammary gland, brain	Endosomes, transgolgi network
ZnT5	Pancreatic β cells	Secretory granules, Golgi apparatus
ZnT6	Stomach, jejunum, cecum, colon, rectum	Transgolgi network
ZnT7	All parts of gastrointestinal tract	Golgi apparatus
ZnT8	Pancreatic β cells	—
ZnT9	—	Nucleus during mitosis
ZnT10	—	Plasma membrane?

Zip family

The mammalian Zip family has 14 members. Most of them have eight transmembrane domains, extracellular N- and C-termini and a histidine-rich intracellular loop, except Zip14, which has an extracellular loop. They are generally located in the plasma membrane but some of them, like Zip7, belong to the Golgi apparatus or to other intracellular vesicles;[50–65] they increase intracellular Zn *via* influx into the cell, from the lumen and from the intracellular vesicles.

Zn transporter activity has been established for Zip1 to Zip8, and for Zip14.[50–58] Zn transport mechanisms are not well understood.

Localisations of Zip proteins in organisms and in cells are summarized in Table 2.

Zip proteins changes depend on Zn availability and the physiological conditions. For example, in the case of dietary Zn deficit, Zip4 concentration increases in the plasma membrane of enterocytes; when Zn is refed, expression of Zip4 decreases.[53] Similarly, Zip14 protein is up-regulated during an acute-phase reaction like stress or illness; uptake of Zn by liver cells increases and circulating Zn decreases.[48]

Metallothionein

Metallothioneins are cysteine-rich proteins with a high-binding capacity, 7 atoms per mole, and a low molecular weight, approximately 7 kDa.[66] They have two binding domains, α and β; Zn and other divalent metals are bound to the N-terminus in the β-domain which contains 3 binding-sites.[67] The affinity of metallothioneins for Zn is relatively high; consequently the protein can sequester Zn within the enterocytes and plays a role in the regulation of Zn absorption. Located in the cytoplasm, metallothioneins are essentially found in the liver,

Table 2 Main localisations of zinc transporters Zip in organism and in cell

Name	Tissues	Intracellular localisation
Zip1	Various tissues	Plasma membrane
Zip2	Prostate, uterus, peripheral blood mononuclear cell (PBMC), monocytes	Plasma membrane
Zip3	Various tissues	Plasma membrane
Zip4	Stomach, small intestine, colon, cecum, kidney	Apical membrane
Zip5	Spleen, liver, kidney	Basolateral membrane
Zip6	Various tissues	Plasma membrane
Zip7	Various tissues	Golgi apparatus
Zip8	Various tissues	Plasma membrane
Zip9	Various tissues	Transgolgi network
Zip10	Various tissues	Plasma membrane
Zip11	Colon, stomach	Nucleus
Zip12	Brain, lung, testis, retina	Plasma membrane
Zip13	Connective tissues, skin, eye, bone, teeth	Golgi apparatus
Zip14	Various tissues	Plasma membrane

kidneys and intestine,[29] but can be expressed in other tissues, like brain and skin.[67]

Their concentration depends on Zn availability: for example, the concentration of metallothioneins increases in the mucosa of pigs fed with high Zn levels (more than 1000 mg kg^{-1}) compared with the mucosa of pigs fed with nutritional Zn levels (about 100 mg kg^{-1}). A transcriptional regulation by dietary Zn is one explanation for this phenomenon: an excess of Zn leads to an increase of metallothionein induction.[66] Stress conditions can also induce metallothioneins in animals.[67]

In addition, metallothioneins play a role in detoxification: in excess, some metals like cadmium can replace Zn bound to metallothionein. Consequently, cells without metallothionein are more subject to cadmium toxicity.[67]

Zinc transport in the plasma

In the circulatory system, Zn is usually bound to albumin but almost one third is carried by α2-macroglobulin.[68] Transferrin and amino acids can also transport metals in the plasma, but albumin would be the main carrier protein for the Zn distribution in the organism: the presence of albumin is significant in the blood and Zn binding to albumin is relatively weak.[69]

Zinc distribution and excretion

In general, 20 to 30 mg of Zn per kg are found in the whole body of fat free rats, sheep and dairy cows; the average for pigs is 25 mg kg^{-1}.[30] In pigs very few changes are observed in the total Zn content from birth to maturity. Contribution of Zn to the liver increase from birth to weaning, then decrease to the concentration measured at birth. Distribution of Zn in the tissues, bones and integuments is similar for growing pigs, rats, sheep and cows: less than 50 ppm (on a dry matter basis) in the skin, between 50 and 100 ppm in white muscles, brain and the heart, between 100 and 150 ppm in the spleen, bones, red muscles and kidney, and more than 150 ppm in the hair, pancreas and liver.[30] In fact, the largest part of Zn is contained in red muscles: the Zn concentration and the proportion of red muscles in the whole body are relatively high.

Zn retention in monogastrics is low and depends on the dietary Zn content. In general, when the Zn level in the feed is high, the percentage of Zn retention is low. In poultry, according to the NRC (1994), Zn requirements vary between 40 and 75 mg kg^{-1} depending on the life stage of the broiler. The percentage of retention is less than 30% when the dietary Zn content is close to NRC recommendations and less than 10% when the dietary Zn content is about 195 mg kg^{-1}.[4] Similarly, Zn retention in the pig is about 10% with a Zn concentration of 60 mg kg^{-1} in the feed, and less than 5% with a Zn concentration of 150 mg kg^{-1}.[5] A large quantity of the ingested Zn is excreted.

Endogenous losses correspond to pancreatic exocrine and biliary secretions, and to Zn from the sloughed mucosal cells. Under normal conditions, urinary losses are very low; a reabsorption of Zn along kidney proximal tubes is possible.[70] When dietary Zn level is low, intestinal absorption increases and endogenous losses decrease. When the dietary Zn level is high, excretion of Zn and consequently the Zn concentration in animal wastes increases.

C. Zinc in animal wastes

Inventory of Zn in livestock manures

The study of contamination of animal manures by heavy metals began in the mid-1950s.[71] Following pioneer studies,[72] an inventory of trace element contents was regularly being carried out since the 1970s (Table 3). In general the Zn contents of pig manures were higher than in poultry manures reflecting differences in the diet and the range of Zn concentrations was large for both animal categories. The values showed general agreement between inventories conducted in United States, Canada, different European countries (Spain, Switzerland, Austria and England) or Asia (China and Japan). There was no significant difference in the content of Zn measured among manures from farms of different herd sizes.[73] The Zn content in pig slurries showed a dependence on the production stage as a consequence of different Zn contents in the feed. Very few studies addressed the evolution of Zn concentrations in animal manure over time. In China,[74] Zn concentrations in the manures have been significantly increased from the early 1990s to 2003, *i.e.* about 2 times for poultry manure and 6 times for pig manure whereas the increase was only 1.2 times for poultry manure and 1.4 times for pig manure in Austria.[75]

Zn inputs to agricultural soils: importance of livestock manures

It is a challenging task to quantify the relative contribution of livestock to the zinc inputs in the environment, in relation to all human activities in a given area. Various anthropogenic sources need to be considered: atmospheric deposition, livestock manures, sewage sludge, inorganic fertilizers, pesticides and irrigation water. Updated inventories of trace elements inputs to agricultural soils were recently conducted in England and Wales,[76] France[77] and China[74] (Table 4). Livestock manures accounted for 37%, 78% and 51% of the total Zn inputs to agricultural soils in England and Wales, France and China respectively.

Table 3 Selected inventory of zinc concentrations in poultry and pig manures

		Mean	Range (min–max) or standard deviation (±)	Publication year	Location	Ref.
Pioneer study	Various manures (n = 44)	96.2	43–274	1951	Canada	72
Poultry manure	Poultry litter (n = 238)	—	105–713	1967–1971	US	74 and 75
	Chicken manure (n > 22)	159.6	±101.3	1990	China	88
	Poultry manure (n = 10)	534	±18	1996	Canada	89
	Poultry litter (n = 10)	425.3[a]	379–533	1998	Switzerland	90
	Poultry litter (n = 43)	511.5[a]	237–789	1998	Switzerland	90
	Poultry litter (n = 40)	372.7	132.8–594.8	2003	US	91
	Chicken manure (n = 16)	153.8	35.8–399.4	2004	China	92
	Poultry dung	314	92–739	2007	Austria	75
	Chicken manure (n = 70)	308.9	±189.3	2009	China	74
	Poultry litter (n = 2)	386	346–426	2009	Japan	85
	Chicken manure – small farms (herd size <2000, n = 8)	268.2	203.4–394	2012	China	73
	Chicken manure – large farms (herd size >20 000, n = 11)	384.2	152.2–1063	2012	China	73
	Poultry manure (n = 65)	432.3	73.0–1827	2013	China	93
Pig manure	Pig manures (n = 27)	—	128–981	1975	US	94
	Pig manure (n > 33)	137.2	±81.2	1990	China	88
	Pig slurry (n = 194)	746.5[a]	337–2490	1998	Switzerland	90
	Pig slurry (n = 81)	517.5[a]	269–1112	1998	Switzerland	90
	Pig slurry (n = 198)	553.8[a]	146–5832	1998	Switzerland	90
	Pig FYM (n = 7)	431	206–716	1999	England	3
	Pig slurry (n = 12)	575	<5–2500	1999	England	3
	Pig manure (n = 7)	144.2	35.3–320.2	2004	China	92
	Pig dung	710	48–1439	2007	Austria	75
	Pig manure	1156	214–1693	2007	Austria	75
	Pig slurries (n = 36)	172	±176	2008	Spain	95
	Pig manure (n = 61)	843.3	±504.2	2009	China	74
	Pig manure – small farms (herd size <200, n = 8)	674.7	332.5–901.8	2012	China	73
	Pig manure – large farms (herd size >800, n = 19)	691.6	63.4–1622.8	2012	China	73
	Pig manure (n = 80)	599.1	39.5–11 379	2013	China	93

[a] Median values.

Table 4 Annual Zn inputs to agricultural soils in England and Wales,[76] France[77] and China[74]

	England and Wales		France		China	
	tons year^{-1}	%	tons year^{-1}	%	tons year^{-1}	%
Livestock manures	1858	36.9	11 848	78	95 668	51
Atmospheric deposition	2457	48.8	1671	11	78 973	42
Sewage sludge and industrial wastes	430	8.5	608	4	669	0.4
Inorganic fertilisers	266	5.3	760	5	7874	4.2
Pesticides	21	0.4	303	2	125	<0.1
Irrigation water	5	0.1	—	—	4432	2.4
Total	5038		15 190		187 741	

Therefore, livestock manures are the predominant sources of Zn to agricultural soils.

Zn speciation in livestock effluents

A selection of studies dealing with Zn speciation in livestock effluents is summarized in Table 5.

In pig slurry, studies concerning Zn speciation are not consistent. The study using sequential chemical extraction observed a majority of Zn bound to Fe/Mn oxides and carbonates,[78] while the study using X-ray absorption spectroscopy (XAS) observed a majority of Zn bound to organic matter, Zn hydroxides and Zn sulfides.[79] Nevertheless, the low proportion of labile-Zn (4%) observed by sequential chemical extraction is not contradictory to the speciation of Zn observed using XAS.

In pig manure, a large part of labile-Zn is observed[80–82] even though the proportion varies greatly from 27%[80] to 59%[81] of the total Zn. Besides, organic matter has been identified as the predominant bearing phase of Zn, with a proportion that can vary from 37%[80] to 77%[81] of the total Zn.

In poultry litter, labile-Zn ranges from 0.7% to 24% of the total Zn. The largest proportions of labile-Zn were observed in poultry litter containing large particles. Indeed, 24% of labile-Zn was observed in poultry litter mixed with wood shavings, waste feed and feathers[83] and 14% in the fraction inferior to 2 mm.[84] In contrast, the lowest proportion of labile-Zn (0.7%) was observed in a thinner fraction (inferior to 250 μm) of poultry litter.[85] Therefore, it seems that the thinner fraction of poultry litter presents a lower proportion of labile-Zn. In addition, this thinner fraction is principally composed of Zn bound to the carbonate and Zn bound to the residual matrix,[85] which is consistent with its low lability.

The negative environmental impact of spreading of animal effluents can be mitigated by treatment. Indeed, composting,[83]

Table 5 Selected references on Zn speciation in livestock effluent

Effluent type	Treatment	Method	Zn speciation	Ref.
Pig slurry	Centrifuged 1 h at 3000 rpm	Sequential extraction[96]	Zn-carbonate 27% Zn–Fe/Mn oxides 67% Zn-exchangeable 4%	78
Pig slurry	Dried 24 h at 60 °C	XAS	Zn-organic matter 49% $Zn(OH)_2$ 37% ZnS 4%	79
Pig slurry	Sieved, composted 122 days	Sequential extraction[96]	Zn-carbonate 44–54% Zn–Fe/Mn oxides 35–49% Zn-organic matter 4–10%	97
Pig manure	Mixed with saw dust	Sequential extraction[96]	Zn-exchangeable 27% Zn-organic matter 37% Zn-residual 32%	80
	Mixed with saw dust composted 42 days	Sequential extraction[96]	Zn-exchangeable 35% Zn-organic matter 40% Zn-residual 21%	
Pig manure	Mixed with saw dust/coffee dregs composted 180 days	Sequential extraction[96]	Zn-organic matter 70%	82
Pig manure	Air dried, milled <2 mm; dried 100 °C	EDTA extraction XAS	Zn-EDTA 59% Zn-organic matter 77% $Zn(OH)_2$ 12% $ZnCO_3$ 11%	81
	Air dried, milled to <2 mm; pyrolysed 2 h at 300 °C OR 500 °C	EDTA extraction XAS	Zn-EDTA 43% OR 31% Zn-organic matter 69 OR 61% ZnS 7% OR 22% $ZnCO_3$ 14% OR $Zn(OH)_2$ 16%	
Pig manure	Mixed with sawdust air dried, milled to <2 mm	DTPA extraction	Zn-DTPA 43%	98
	Air dried, milled to <2 mm; composted 21 then 84 days	DTPA extraction	Zn-DTPA 42% then 39%	
	Air dried, milled to <2 mm; pyrolyzed 2 h at 400 °C OR 700 °C	DTPA extraction	Zn-DTPA 2% OR 6.8%	
Poultry manure		Water extraction	Zn–water 1.3%	89
	Mixed with peat OR straw	Water extraction	Zn–water 8.2% OR 6.2%	
Poultry litter		Water extraction	Zn–water 5.5 to 8.8%	87
Poultry litter	Air dried, crushed <250 μm	Eight different extraction methods	Zn-extractable 0.7 to 4%	86
	Air dried, crushed <250 μm Ashed 2 h at 600 °C	Eight different extraction methods	Zn-extractable 0.1 to 0.8%	
Poultry litter	Air dried and sieved	Water extraction	Zn–water 6%	91
Poultry litter	Freezed dried, ground, sieved <2 mm	Water extraction	Zn–water 13.8%	84
Poultry litter	Air dried, crushed <250 μm	Sequential extraction[85]	Zn-exchangeable 4.8–6.2% Zn-carbonate 31–36% Zn-residual 31–48%	85
Poultry litter	Mixed with wood shavings, waste feed, feathers	Water extraction	Zn–water 24%	83
	Mixed with wood shavings, waste feed, feathers composted 168 days	Water extraction	Zn–water 2.7%	

ashing[86] or pyrolysing[87] an effluent can decrease the labile-Zn up to 10-fold. However, the results concerning the Zn speciation in livestock effluents (treated and not treated) are not consistent because studying Zn speciation in livestock effluents is a difficult task. First, livestock effluents are complex matrices, which can be liquid (*e.g.* slurry) and/or solid (*e.g.* manure or litter). They can contain both organic and inorganic phase and their composition can vary dramatically. Second, there is so far no method of reference to study Zn speciation in this type of complex matrices that is fully reliable as it is for the measurement of Zn concentration. The results obtained using the chemical approach (*e.g.* chemical extraction) and the physical approach (XAS) are complementary but are still difficult to compare, because the results are very dependent on the method. For example, eight different extracting agents were used to evaluate the labile-Zn in poultry manure and the results vary up to 5-fold.[86] Similarly, changing the solid/liquid ratio from 1/10 to 1/100 in a chemical extraction can double the labile-Zn.[87]

Finally, the current inconsistency of the Zn speciation results makes it difficult to predict the fate of Zn of the livestock effluent in the environment. That is why it is of primary importance to standardize the methods used for Zn speciation in complex matrices. We therefore consider that assessment of: (i) the speciation of Zn in livestock effluents, and (ii) the fate of Zn in soils following livestock effluents in long-term field experiments are major future complementary lines of research.

Conclusion

A large variety of zinc additives are available for livestock and poultry nutrition, from different sources, with different properties, but the same claims: satisfying animal requirements and ameliorating growth performance. A fine characterization of these additives is sometimes difficult but the analytical techniques continue to improve. As the retention is low in monogastrics, when dietary zinc is above animal requirements, the maximal zinc content is more strictly regulated to get closer to the real requirements of livestock. Studies in the last few decades have improved our knowledge of the mechanism of zinc absorption but some points are still not well understood. Intensive husbandry can cause soil pollution; speciation of zinc in animal wastes could lead to a better prediction of zinc fate in the soil and to a better use of manure on arable lands. Before the ingestion of the zinc by the animal, further investigations regarding zinc in premixes and in feeds will facilitate the quality control of diets and their compliance with regulation. A better understanding of the fate of the metal in the diet, in animals and in manure is in fact a key step towards sustainable livestock production.

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