SCIENTIFIC OPINION

Scientific Opinion on the potential reduction of the currently authorised maximum zinc content in complete feed[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 22 February 2017, replaces the earlier version published on 5 May 2014.[4]

ABSTRACT

A critical review of (i) the zinc requirements of food-producing and pet animals, (ii) the zinc concentration of feed materials and (iii) the calculated background zinc concentration of complete feed supports the possibility of a considerable reduction of the currently authorised maximum concentration for total zinc in feed. The FEEDAP Panel developed, based on an approximation using zinc requirements and background data, potential new maximum contents, which could replace the current ones. The newly proposed total maximum contents are: 150 mg Zn/kg complete feed for piglets, sows, rabbits, salmonids, cats and dogs; 120 mg Zn/kg complete feed for turkeys for fattening; 100 mg Zn/kg complete feed for all other species and categories. The use of phytase in feeding piglets, pigs for fattening and sows would allow a further reduction of the newly proposed total maximum contents by 30 % (from 150 to 110 mg Zn/kg feed for piglets and sows and from 100 to 70 mg Zn/kg feed for pigs for fattening). The newly proposed total maximum contents ensure health, welfare and productivity of the target species and do not affect consumer safety. The FEEDAP Panel expects that the introduction of the newly proposed total maximum contents, provided they are applied in feeding practices, would result in an overall reduction of zinc emissions from animal production of about 20 %.

KEY WORDS

zinc, zinc requirements, zinc in feed, interactions, maximum content of zinc in feed, safety, environment

[1] On request from European Commission, Question No EFSA-Q-2012-00966, adopted on 8 April 2014.
[2] Panel members: Gabriele Aquilina, Vasileios Bampidis, Maria De Lourdes Bastos, Lucio Guido Costa, Gerhard Flachowsky, Mikolaj Antoni Gralak, Christer Hogstrand, Lubomir Leng, Secundino López-Puente, Giovanna Martelli, Baltasar Mayo, Fernando Ramos, Derek Renshaw, Guido Rychen, Maria Saarela, Kristen Sejrsen, Patrick Van Beelen, Robert John Wallace and Johannes Westendorf. Correspondence: FEEDAP@efsa.europa.eu
[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Revision of Maximum Content of Zinc in Feed, including Noël A. Dierick, Jürgen Gropp, Catherine Jondreville, Marta Lopez Alonso, Anne Katrine Lundebye, Wolfgang Maret and Patrick Schlegel for the preparatory work on this scientific opinion. The Panel also wishes to thank the Authorities of EU Member States and EEA/EFTA countries, and stakeholders who provided information following a call for data on zinc in animal nutrition launched by EFSA.
[4] An editorial amendment was carried out on section 6.3 that does not materially affect the contents nor outcome of this Scientific Opinion. To avoid confusion, the original version has been removed from the EFSA Journal, but is available on request, as is a version showing all the changes made.

Suggested citation: EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2014. Scientific Opinion on the potential reduction of the currently authorised maximum zinc content in complete feed. EFSA Journal 2014;12(5):3668, 77 pp. doi:10.2903/j.efsa.2014.3668

Available online: www.efsa.europa.eu/efsajournal

SUMMARY

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the potential reduction of the currently authorised maximum zinc content in complete feed (250 mg Zn/kg for pet animals, 200 mg Zn/kg for fish and milk replacer and 150 mg Zn/kg for other animal species).

To improve the available information on the use of zinc in animal nutrition, EFSA launched a call for data to EU Member States and EEA/EFTA countries and to stakeholders. The data submitted were used in the current Scientific Opinion.

Zinc in the form of its divalent metal ion, Zn^{2+}, is nutritionally essential for all living organisms. The total amount of zinc in the human body is 2–3 g and its concentrations in tissues are about the same in all mammals. Virtually all its functions are in proteins, in which it is a catalytic, structural, or regulatory cofactor.

A critical review of (i) the zinc requirements of food-producing and pet animals, (ii) the zinc concentration of feed materials and (iii) the calculated background zinc concentration of complete feed supports the possibility of a considerable reduction of the currently authorised maximum concentration for total zinc in feed. The FEEDAP Panel developed, based on an approximation using zinc requirements and background data, potential new maximum contents, which could replace the current ones. The newly proposed total maximum contents are: 150 mg Zn/kg complete feed for piglets, sows, rabbits, salmonids, cats and dogs; 120 mg Zn/kg complete feed for turkeys for fattening; 100 mg Zn/kg complete feed for all other species and categories. The use of phytase, either from endogenous source or from a feed additive, in feeding piglets, pigs for fattening and sows would allow a further reduction of the newly proposed total maximum contents by 30 % (from 150 to 110 mg Zn/kg feed for piglets and sows and from 100 to 70 mg Zn/kg feed for pigs for fattening).

The newly proposed total maximum contents ensure health, welfare and productivity of the target species. The newly proposed total maximum contents do not affect consumer safety. The FEEDAP Panel expects that the introduction of the newly proposed total maximum contents, provided they are applied in feeding practices, would result in an overall reduction of zinc emissions from animal production of about 20 %.

TABLE OF CONTENTS

Abstract 1
Summary 2
Table of contents 3
Background as provided by the European Commission.....5
Terms of reference as provided by the European Commission.....5
Interpretation of the Terms of reference.....5
Assessment.....6
1. Introduction 6
2. Zinc in animal nutrition: requirements, deficiency, tolerance and therapeutic use 8
2.1. Requirements, allowances and recommendations for dietary zinc in target animals 8
2.1.1. Poultry 8
2.1.2. Pigs 9
2.1.3. Ruminants.....10
2.1.4. Horses.....12
2.1.5. Rabbits.....12
2.1.6. Fish 12
2.1.7. Dogs and cats.....13
2.2. Zinc deficiency 13
2.3. Tolerance of animals to dietary zinc.....14
2.3.1. Poultry 15
2.3.2. Pigs 15
2.3.3. Ruminants.....15
2.3.4. Horses.....16
2.3.5. Rabbits.....17
2.3.6. Fish 17
2.3.7. Dogs and cats.....17
2.4. Therapeutic use of zinc in piglets.....18
3. Zinc in feedingstuffs.....19
3.1. Feed materials.....19
3.2. Complete feed – Background zinc levels 19
3.3. Feed additives.....20
3.4. Zinc in compound feed – Data from the control by European countries.....20
4. Bioavailability of dietary zinc 22
4.1. Zinc-containing additives 22
4.2. Interactions of zinc with dietary constituents.....23
4.2.1. Phytates.....23
4.2.2. Iron 24
4.2.3. Copper 24
4.2.4. Calcium.....25
4.2.5. Role of fiber/non-starch polysaccharides 26
4.3. Methods to improve the availability of zinc from feed materials.....27
4.3.1. Use of exogenous microbial phytase 27
4.3.2. Activation of endogenous plant phytase by soaking/fermentation.....27
4.3.3. Use of organic acids 28
5. Newly proposed maximum total zinc contents in complete feed 28
6. Impact of the newly proposed reduced maximum zinc content in feed 30
6.1. Health and welfare of the target animals.....31
6.2. Safety for the consumer.....31
6.2.1. Conclusions on safety for the consumer.....32
6.3. Benefits of the proposed reduction of maximum contents of zinc in feed to the environment .32
7. Conclusions 33
8. Remark.....33
Documentation provided to EFSA 34

References 34
Appendices 55
Abbreviations 76

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Several opinions on applications for the re-authorisation of zinc compounds have already been issued by EFSA and others are pending.

In discussions with Member States on opinions already delivered and considering outstanding opinions, concerns with respect to the maximum content of zinc in feed had been raised.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission asks the European Food Safety Authority to issue and opinion on the maximum content of compounds of zinc in feed considered safe for animals, the consumers and the environment.

INTERPRETATION OF THE TERMS OF REFERENCE

Several opinions on applications for the re-authorisation of zinc compounds as feed additives have been issued by EFSA and others are pending. Namely, two opinions on zinc sulphate monohydrate (EFSA FEEDAP Panel, 2012a, b), two opinions on zinc chelate of amino acids hydrate (EFSA FEEDAP Panel, 2012c, 2013) and one opinion on zinc oxide (EFSA FEEDAP Panel, 2012d) have been delivered to the EC. In these opinions, the FEEDAP Panel specifically remarked that "Current knowledge on the zinc requirements of animals, and the variation in bioavailability of zinc from different sources, indicate the potential to considerably reduce the current maximum content for dietary zinc without affecting animal health and welfare and productivity of animal husbandry. The reduction of the maximum content for zinc would decrease the zinc load in the environment. The simultaneous use of phytases opens further possibilities for the reduction of dietary zinc in animal nutrition. A new assessment of the zinc requirements/allowances of animals would provide the basis to react if a need for action will arise from another relevant field like ecology".

The European Commission (EC) highlighted in its mandate that in discussions with Member States on opinions already delivered and considering outstanding opinions, concerns with respect to the maximum content of zinc in feed had been raised. The EC provided also a report from the Livestock Research Institute of the University of Wageningen (The Netherlands) entitled "Zinc requirements of weaned piglets" (Bikker et al., 2011).

Already in 2000 the Scientific Committee on Animal Nutrition (SCAN) was requested by the EC to deliver an opinion on the total maximum authorised zinc content in feed; this value was at that time 250 mg Zn/kg complete feed for all animal species. The SCAN recommended a reduction to 150 mg Zn/kg complete feed for all animal species except piglets (175 mg zinc in case 175 mg Cu/kg feed would be retained) (EC, 2003a). Regulation (EC) No 1334/2003[5] set the following maximum total zinc contents in feed:

- 250 mg Zn/kg for pet animals,
- 200 mg Zn/kg for fish and milk replacer and
- 150 mg Zn/kg for other animal species.

The European Food Safety Authority considers that the request of the EC refers as to the issuing of an opinion on the potential reduction of the currently authorised maximum zinc content in complete feed, considering safety for animals, consumers and the environment.

[5] Commission Regulation (EC) No 1334/2003 of 25 July 2003 amending the conditions for authorisation of a number of additives in feedingstuffs belonging to the group of trace elements. OJ L 187, 26.7.2003, p. 11.

ASSESSMENT

In its recent opinions on zinc-containing additives, the EFSA FEEDAP Panel concluded that the use of zinc compounds as feed additives in livestock does not pose a direct concern for agricultural soils but the available data were not sufficient to exclude any risk related to drainage and the run-off of zinc to surface water (EFSA FEEDAP Panel, 2012a,b,c,d). The Panel also noted that "problems of high zinc concentrations in drainflow and runoff, once established, would be difficult to remediate."

The EFSA FEEDAP Panel further stated that:

> "Current knowledge on the zinc requirements of animals, and the variation in bioavailability of zinc from different sources, indicate the potential to considerably reduce the current maximum content for dietary zinc without affecting animal health and welfare and productivity of animal husbandry. The reduction of the maximum content for zinc would decrease the zinc load in the environment. The simultaneous use of phytases opens further possibilities for the reduction of dietary zinc in animal nutrition. A new assessment of the zinc requirements/allowances of animals would provide the basis to react if a need for action will arise from another relevant field like ecology".

In order to properly address these concerns, the Commission has now asked the EFSA to issue an opinion on the potential modification of the currently authorised maximum zinc content in feed considered safe for animals, the consumer, and the environment.

EFSA launched a call for data to

> EU Member States and EEA/EFTA countries, concerning national zinc requirements/allowances in animal nutrition and data from official feed control for the zinc content in compound feed. EFSA received contributions from Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Malta, Norway, Poland, Portugal, Slovakia, Slovenia, Sweden, Switzerland, The Netherlands.
>
> Stakeholders, concerning recommendations for the use of zinc in animal nutrition and typical composition of compound feed. Information was submitted by EMFEMA, FEDIAF, FEFAC, FEFANA and Novus Europe S.A.[6]

EFSA commissioned the University of Gent (Belgium) to carry out a study of selected trace and ultratrace elements in animal nutrition, including zinc. The findings were submitted to the EFSA in the form of a technical report (Van Paemel et al., 2010). Information from this report has been used in this opinion.

The following abbreviations are used in the text of this Scientific Opinion: CAMC, Currently Authorised total Maximum Contents of zinc in complete feed and NPMC, Newly Proposed total Maximum Contents of zinc in complete feed.

1. Introduction

Zinc in the form of its divalent metal ion, Zn^{2+}, is nutritionally essential for all living organisms (Rink 2011; Maret, 2013). The total amount of zinc in the human body is 2–3 g and its concentrations in tissues are about the same in all mammals. The cellular concentration is rather high (about 0.5 mM). Virtually all its functions are in proteins, in which it is a catalytic, structural, or regulatory cofactor. It has been estimated that the mammalian genome encodes about 3000 zinc proteins, i.e. 10 % of all proteins are zinc metalloproteins (Andreini et al., 2006). Zinc therefore affects virtually all cellular functions, especially growth and development of organisms, and it seems to be indispensable for the proper functioning of the senses and some critical brain functions (Maret, 2014). Factors that control systemic zinc homeostasis have not been identified, though. Zinc homeostasis is tightly controlled at

[6] To protect the interests of stakeholders that have contributed, they are collectively referred to in this Scientific Opinion as 'European feed industry'.

the cellular level. In mammals, ten proteins of the ZNT family (SLC30A) export zinc from the cytosol, either out of the cell or into cellular vesicles or organelles, 14 proteins of the Zip family (SLC39A) import zinc into the cytsol or into cellular vesicles or organelles, and at least a dozen metallothioneins (MTs) buffer and translocate zinc in the cell. The metal response element-binding transcription factor-1 (MTF-1) elevates cellular zinc levels and directs zinc-dependent gene expression, involving the expression of MTs and ZNT1 (Lichten and Cousins, 2009; Fukada and Kambe, 2011). Some calcium channels are also permeable to zinc ions. The metal specificity of all these transporter and channel proteins is presently being investigated. Transport of manganese, cadmium, and non-transferrin-bound iron has been shown for several of them. Exact mechanisms by which zinc affects the uptake of iron and copper through their specific transporters, DMT-1 (divalent metal transporter 1) and CTR-1 (copper transporter 1) respectively, are not known. However, there is a significant amount of work demonstrating interactions of copper, zinc and iron at the level of absorption (Lönnerdal and Kelleher, 2007), in particular inhibitions of copper and iron availabilities by zinc in humans (Maret and Sandstead, 2006; Olivares et al., 2012).

Zinc homeostasis is a “closed system”. Only about 0.1 % of the total zinc needs to be replenished daily (Maret and Sandstead, 2006). Zinc absorption is a saturable process; thus, assimilation efficiency is inversely related to intake and, within the boundaries of physiological control, giving much more zinc than needed does not result in additional uptake. Therefore, there does not seem to be a condition of zinc overload, unless zinc is massively overdosed or gets into the system by by-passing the intestinal tract. The organism resumes uptake of zinc from the diet only if it needs zinc and it extends this uptake over a period of time rather than taking up a large amount at once. Thus, there is very little acute toxicity, but there may be chronic toxicity as the uptake of other trace metals (copper, iron) is reduced when zinc is in excess. Excess zinc may have additional pharmacological and extracellular effects in the intestinal tract, i.e. being bactericidal or restoring the integrity of the brush border membrane in diarrhea.

Zinc occurs naturally in feed materials of plant and animal origin. Supplementation of animal feeds with zinc has a long history. One of the first signs of zinc deficiency is compromised immunity. In the 1950s and 1960s, it was reported that zinc supplementation cured parakeratosis in swine, cattle, chicken and sheep. It was also found in early studies that zinc deficiency in chicken can cause slow growth, shortened and thickened legs with an enlarged hock, and frizzled feathers, conditions that could all be reversed by zinc supplementation. Part of the problem of relating physiological requirements and zinc in the diet is that many protein sources for animal feeds, such as soy bean and corn, are rich in phytate (inositol hexaphosphate), which binds zinc very strongly and is a potent inhibitor of its absorption. Similarly, some fish feeds contain very high levels of calcium, which may also inhibit zinc absorption. Feed materials are either too low in zinc or show reduced availability of zinc to cover the requirements of target animals. As compensation, animal feeds are routinely supplemented with zinc. In pigs the magnitude of intestinal zinc absorption, as percentage of intake, was reported by Revy et al. (2002) to be 25.5 to 32.4 %, and by Zacharias et al. (2007) to be 15.9 to 25.7 %. If the requirement is markedly exceeded, additional zinc is not absorbed or endogenously secreted, but passes the gut and ends up in the manure, which when spread on fields may enrich soil and drainage water with zinc.

Elevated zinc is an environmental issue. There are many regions in Europe where zinc levels are far above background. Leaching from galvanized steel is the largest source of zinc input into the environment, contributing about 30 % of all zinc emissions in the EU. Industrial point sources add another 10 %, but the remaining 60 % comes from a variety of minor diffuse sources, one of which is farming. The potential problem of high zinc in manure was recognised by the EC over ten years ago, leading to a recommendation by the Scientific Committee for Animal Nutrition (SCAN) to reduce the levels of zinc in feedingstuffs (EC, 2003a), followed by a corresponding amendment of the relevant Regulation[7] to decrease the maximum levels of zinc in animal feeds to the current authorised levels.

[7] Commission Regulation (EC) No 1334/2003 of 25 July 2003 amending the conditions for authorisation of a number of additives in feedingstuffs belonging to the group of trace elements. OJ L 187, 26.7.2003, p. 11.

More recently, EFSA commissioned a study on the environmental impact of zinc and copper used in animal nutrition (Monteiro et al., 2010).[8] The study concluded that the use of zinc as a feed additive at currently authorised levels is not expected to pose a direct concern for the agricultural soil compartment. However, a potential environmental concern was identified relating to groundwater, drainage and the runoff of zinc from arable land to surface water. Acidic sandy soils were predicted to be most vulnerable to these processes.

2. Zinc in animal nutrition: requirements, deficiency, tolerance and therapeutic use

2.1. Requirements, allowances and recommendations for dietary zinc in target animals

The following definitions are used in the context of this Section:

Requirement: the individual demand for zinc under defined conditions;

Allowance/Recommendation: estimate of the zinc supply necessary to meet the average gross demand of the population under common conditions plus a safety factor considering the individual variability, varying bioavailabilities and interactions between nutrients.

Requirements and allowances are provided by scientific bodies; recommendations by the industry or private bodies. Regarding zinc nutrition, the differentiation between requirement and allowances are difficult to distinguish between scientific bodies. Therefore zinc requirements and zinc allowances are not differentiated in Tables 1 to 5.

As zinc homeostasis is regulated through intestinal zinc absorption and zinc excretion, zinc requirements are generally estimated using empirical methods. Urinary zinc excretion plays a minor role in homeostatic zinc regulation. However, the National Research Council (NRC) of the USA and the Centraal Veevoederbureau (CVB) of The Netherlands made an exception on zinc requirements/allowances in dairy cattle and small ruminants by using a factorial approach (NRC, 2001, 2007a; CVB, 2005). The difficulty in the factorial method consists in defining the coefficient of utilization, as it is dependent on the dietary zinc level; the NRC (2001, 2007a) set this coefficient at 15 %, decreasing from 55 % (10 kg body weight (bw) goat kids) to 15 % (adults), and the CVB (2005) at 50 % including a safety margin of 5 % for all ruminants. Zinc requirements and allowances/recommendations are given in dietary concentration (mg Zn/kg diet).

The dietary zinc requirements, allowances and recommendations for a given animal category mainly depend on the definition of the diet, the defined response criteria and the inclusion of safety margins. The diet may influence the daily intake (e.g. by the energy content) and the zinc bioavailability (Chapter 4). The plateau of a dietary zinc dose-response curve is e.g. lower for growth-performance than for bone zinc content in pigs and in poultry (Revy et al., 2006; Bikker et al., 2011; Schlegel et al., 2013). Safety margins are included in some of the published allowances and recommendations. However, the use and magnitude of the safety margins are not always specifically mentioned.

The dietary zinc requirements/allowances and recommendations for the animal species and categories listed in Annex IV of Commission Regulation (EC) No 429/2008[9] are described below. Relevant literature sources published after the latest zinc requirement update of the NRC are also mentioned.

2.1.1. Poultry

The zinc requirement for maximal growth in broilers fed semi-purified diets without phytate is approximately 25 mg/kg (Wedekind and Baker, 1990; Wedekind et al., 1992; Aoyagi and Baker, 1993; Biehl et al., 1995; Edwards et al., 1998; Edwards and Baker, 1999, 2000; Batal et al., 2001). In

[8] The FEEDAP Panel notes that this report contains a typographical error when referring to the units of the maximum total copper and zinc authorised in feed (Background and Introduction of the document).

[9] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

cereal-based diets, without phytase addition, maximal growth in broilers is reached with approximately 30 mg Zn/kg and maximal bone zinc content with 50 mg Zn/kg diet (Mohanna and Nys, 1999a; Ao et al., 2006; Jondreville et al., 2007). The most recent zinc requirements/allowances and recommendations for poultry categories are presented in Table 1. For chicken, the reported values for requirements/allowances vary between 35 and 70, and for recommendations between 70 and 140 mg Zn/kg diet. For turkey, the reported values for requirements/allowances vary between 40 and 120, and for recommendations between 75 and 150 mg Zn/kg diet.

Table 1: Zinc requirements/allowances and recommendations for poultry (mg/kg complete diet). Sources: NRC, USA (1994); GfE, Germany (1999, 2004); MTT, Finland (2013); IFZZ, Poland (2005); European Feed Industry

Species	Category	Age, production stage	Requirements and allowances				Recommendations
			NRC[1]	GfE[2]	MTT	IFZZ[3]	EU Feed Industry[4]
Chicken	Layer	0-6 weeks	40	50	60	50-70	
		6 weeks-first egg	35	40	35-60		75-140
		Laying hen	35	50	60	50-60	70-135
		Breeder hen	45				70-135
	Fattening	0-8 weeks	40				
		Starter	40	50	50-60	60	80-140
		Grower	40	50	50-60	50	75-130
		Finisher	40	50	50-60	40	75-120
Turkey	Fattening	0-4 weeks old	70	50	80	90	95-140
		4-8 weeks old	65	40	80	70	95-140
		8-12 weeks old	50	40	50	70	85-135
		12-24 weeks old	40	40	50	60	75-140
	Breeder	Laying	65		70	120	140-150
		Not laying	40		70	120	140-150
Geese			40			60	75-105
Duck	Fattening	0-2 weeks old	60			70	75-135
		Grower				70	75-135
		Finisher				60	75-135
Pheasant			60				

(1) Requirements. Corn-soybean meal based diets without phytase activity. Layers: 11.9–12.1 MJ metabolisable energy (ME)/kg; broilers: 13.4 MJ ME/kg; turkey: 11.7 - 13.8 MJ ME/kg; pheasant: 11.7 MJ ME/kg.
(2) Requirements. mg/kg dry matter (DM).
(3) Lower value, minimum concentration; higher value, safety margin included.
(4) Data provided by stakeholders following a call for data.

2.1.2. Pigs

The zinc requirement of young pigs consuming a casein-glucose diet without any phytate content is low (15 mg/kg; Smith et al., 1962; Shanklin et al., 1968); however, in conventional diets, zinc requirement is higher. In piglets fed cereal-based diets without added phytase, maximal growth was reached with < 55, 47 and < 60 mg Zn/kg diet, as reported by Revy et al. (2006), Bikker et al. (2011) and Paulicks et al. (2011), respectively. Maximal plasma zinc was reached with 91, 67 and 95 mg Zn/kg diet by Revy et al. (2006), Bikker et al. (2011) and Schlegel et al. (2013), respectively. Requirements are based on corn-soybean meal diets without phytase activity. NRC (2012) mentions that zinc bioavailability is increased with phytase quoting Kornegay (1996) as reference. GfE (2006) also mentions that zinc supplementation can be reduced when diets are supplemented with phytase citing Revy et al. (2006) as reference. Agroscope (2011) includes dietary phytic phosphorus contents and phytase activity as additional parameters to pig body weight (bw) for zinc allowances.

The most recent zinc requirements/allowances and recommendations for pigs are presented in Table 2. Reported values for pigs for requirements/allowances and recommendations vary between 45 and 100 and between 90 and 150 mg Zn/kg diet, respectively.

Table 2: Zinc requirements/allowances and recommendations for pigs (mg/kg complete diet). Sources: NRC, USA (2012); GfE, Germany (2006); Agroscope, Switzerland (2011); MTT, Finland (2013); IFZZ, Poland (1993); European Feed Industry

Category	**Age, production stage**	**Requirements and allowances**					**Recommendations**
		NRC[1]	**GfE[2]**	**Agroscope[3]**	**MTT**	**IFZZ[4]**	**EU Feed Industry[5]**
Piglet	< 11 kg bw	100	100	60-100	100	70-150	140-150
	11-25 kg bw	80	80	60-100	100	70-150	140-150
Pig for fattening	25-50 kg bw	60	50-60	45-80	100	50-80	95-150
	50-135 kg bw	50	50-60	45-80	100	50-80	95-150
Sow	Gestation, lactation	100	50	45-80	100	50-100	90-150
Boar	Mature	50	50	45-80	100		

(1) Requirements. Corn-soybean meal based diet without phytase activity. Growing pig: 14.2 to 13.8 MJ metabolisable energy (ME)/kg; sow and boar: 13.8 MJ ME/kg.
(2) Requirements. mg/kg dry matter (DM).
(3) Level dependent on dietary phytic phosphorus content and phytase activity. Lower values for diets with 1.5 g phytic P/kg and 750 FTU/kg. Upper values for 2.0 g phytic P/kg, and 0 FTU/kg.
(4) Lower value, minimum concentration; higher value, safety margin included.
(5) Data provided by stakeholders following a call for data.

2.1.3. Ruminants

The most recent zinc requirements/allowances and recommendations for ruminants are presented in Table 3. Reported values for requirement/allowances and recommendations vary between 16 and 80 and between 30 and 140 mg Zn/kg diet, respectively. Since the latest published zinc requirements for dairy cattle (NRC, 2001), beef cattle (NRC, 2000) and small ruminants (NRC, 2007a), further data have become available and are described in the next three paragraphs.

Arrayet et al. (2002) observed no improvement in growth performance from newborn dairy calves fed 35 or 75 mg Zn/kg dry matter (DM) for 90 days. Similarly, Wright and Spears (2004) observed no change in plasma and tissue zinc when dairy calves (150 kg bw) were fed 28 or 48 mg Zn/kg diet for 98 days. Finally, Mandal and Dass (2010) observed no differences in the haemato-biochemical profile in calves (226 kg bw) fed a concentrate with 33 or 68 mg Zn/kg. In lactating dairy cows (36 kg milk/day, 610 kg bw), 96 mg Zn/kg diet decreased milk somatic cell counts and amyloid A content compared to a diet with 44 mg Zn/kg (Cope et al., 2009). However, relevant zinc metabolism blood traits (plasma zinc content, superoxide dismutase activity) were not influenced by dietary zinc level.

Ahola et al. (2004) found increased plasma zinc concentration and an improved pregnancy rate when grazing beef cows were supplemented over two years with zinc at the NRC (2000) level compared with cows receiving no zinc supplementation. In feedlot beef cattle (246 kg initial bw, 1200 g/d bw gain), Spears and Kegley (2002) observed increased growth performance and improved carcass quality, but did not observe any effect on plasma zinc nor on immune response when feeding 33 *vs* 25 mg Zn/kg DM. Nunnery et al. (2007) observed no change in overall weight gain and plasma zinc, but a tendency for reduced (receiving phase) and improved (finishing phase) feed efficiency when feeding a diet with 80 mg Zn/kg compared with 53 mg Zn/kg to cattle (220 kg initial bw). Mandal et al. (2007) observed no effect of dietary zinc (33 vs. 68 mg Zn/kg DM) on growth performance of bulls (226 kg initial bw, 500 g/d bw gain), but observed a higher cell mediated immune response in zinc supplemented bulls.

Garg et al. (2008) fed a diet containing 34 or 54 mg Zn/kg DM as $ZnSO_4$ to lambs for 190 days and observed an increase in plasma zinc with the zinc-supplemented diet but no effect on growth performance. Fadayifar et al. (2012) observed increased plasma zinc in lambs when feeding a diet with 42 and 62 mg Zn/kg DM compared with 22 mg Zn/kg DM over 70 days and also measured increased growth performance. Wenbin et al. (2009) fed a diet containing 22 or 42 mg Zn/kg DM to cashmere goats for 60 days and observed an improved growth performance and increased plasma zinc with the zinc-supplemented diet.

The more recent data described above indicate that there would be no improvement on performance and the measured endpoints of the zinc status when providing diets to dairy cows, beef cattle and small ruminants exceeding the requirements (NRC 2000, 2001, 2007a). It is therefore concluded that the requirements set by NRC (Table 3) are still valid.

Table 3: Zinc requirements/allowances and recommendations for ruminants (mg/kg DM complete diet). Sources: NRC, USA (2000, 2001, 2007a); GfE, Germany (1995, 2001, 2003); CVB, The Netherlands (2005); Agroscope, Switzerland (2006, 2009); MTT, Finland (2013); IFZZ, Poland (1994a); European Feed Industry

Species	Category	Age, production stage	Requirements and allowances						Recommendations
			NRC(1)	GfE(2)	CVB	Agroscope	MTT	IFZZ(3)	EU Feed Industry(4)
Cattle	Calf	Pre-ruminating	40	40-50	30	40	50-80	45	30-140
	Dairy heifer	6 months old	32(6)	40	29	40	50		
		12 months old	27(6)	40	26	40	50		
		18 months old	18(6)	40	25	40	50		
		transition to 1st lactation	30(6)	40		40	50		
	Dairy cow	Dry, 270 days gestation	22(6)	50	22	50	50	50-60	70-130
		25 kg milk	43(6)	50	27	50	50	50-60	70-130
		35 kg milk	48(6)	50	30	50	50	50-60	70-130
		45 kg milk	52(6)	50	35	50	50	50-60	70-130
		55 kg milk	55(6)	50		50	50	50-60	70-130
	Beef	100 kg bw, 1000 g ADG(5)	30	40	38	40		40	35-45
		250 kg bw, 1200 g ADG	30	40	30	40		40	35-45
		500 kg bw, 1100 g ADG	30	40	29	40		40	35-45
Goat	Growing	10 kg bw, 100 g ADG	26	50-80		50		45-75	110-130
		20 kg bw, 150 g ADG	21	50-80		50		45-75	110-130
		30 kg bw, 200 g ADG	22	50-80		50		45-75	110-130
		40 kg bw, 200 g ADG	18	50-80		50		45-75	110-130
	Mature	Dry	18(7)	50-80	17	50		45-75	110-130
		Lactating	35(8)	50-80	25	50		45-75	110-130
Sheep	Growing	20 kg bw, 200 g ADG	26	50-80		50		45-75	110-130
		40 kg bw, 400 g ADG	39	50-80		50		45-75	110-130
		60 kg bw, 500 g ADG	38	50-80		50		45-75	110-130
		80 kg bw, 400 g ADG	33	50-80		50		45-75	110-130
	Mature	Dry	36(9)	50-80	23	50		45-75	110-130
		Lactating	46(10)	50-80	16	50		45-75	110-130

(1), (2) Requirements.
(3) Lower value, minimum concentration; higher value, safety margin included.
(4) Data provided by stakeholders following a call for data.
(5) ADG, average daily gain.
(6) Calculated with bw category of 680 kg.
(7) Mature doe, 60 kg bw, early gestation, twin kids.
(8) Mature doe, 60 kg bw, early lactation, twin kids.
(9) Mature ewe, 100 kg bw, early gestation, twin lambs.
(10) Mature ewe, 100 kg bw, early lactation, twin lambs.

2.1.4. Horses

The most recent zinc requirements/allowances and recommendations for horses are presented in Table 4. The reported values for requirement/allowances vary between 38 and 50 mg Zn/kg DM.

No articles on this subject has been published since the latest zinc requirement update for horses (NRC, 2007b).

Table 4: Zinc requirements/allowances and recommendations for horses (mg/kg dry matter complete feed). Sources: NRC, USA (2007b); GfE, Germany (1994); INRA, France (2012); MTT, Finland (2013); IFFZ, Poland (1994b); European Feed Industry

Category	Age, production stage	Requirements and allowances					Recommendation
		NRC[1]	GfE[2]	INRA	MTT	IFFZ	EU Feed Industry[3]
Growing	4 -24 months	40	50	50	45	50	80
Stallion		40	50	50		50	80
Mare	pregnant, lactation	38-40	50	50	48	50	80
Adult	No work - heavy work	40	50	50	40	50	80

(1) Requirements. Adult horse with 600 kg bw.
(2) Requirements.
(3) Data provided by stakeholders following a call for data.

2.1.5. Rabbits

Published zinc recommendations for rabbits vary between 30 and 60 mg/kg diet with higher values for breeders (Mateos and de Blas, 1998). The zinc requirements/allowances from INRA (1989) are 50 mg/kg diet in rabbits for fattening and 70 mg/kg diet for breeder rabbits. The EU Feed Industry recommends a dietary zinc level for rabbits of 80–100 mg/kg.

2.1.6. Fish

Fish have the ability to absorb some zinc from water, but the diet is the predominant uptake route (Willis and Sunda, 1984; Spry et al., 1988). However, water quality may affect zinc requirements as channel catfish required about 20 mg Zn/kg DM in hard water (>100 mg $CaCO_3$/L) and 20–40 mg Zn/kg DM when held in soft water (<1 mg $CaCO_3$/L) (Scarpa and Gatlin, 1992).

According to Clearwater et al. (2002), 20 mg Zn/kg DM in a semi-purified diet (0.3–0.4 mg/kg bw and day) are sufficient for a wide range of fish species. NRC (2011) mentions that dietary zinc requirements have been established for a number of different fish species fed semi-purified diets: 15–30 mg/kg for rainbow trout (Ogino and Yang, 1978) and for carp (Ogino and Yang, 1979), 20 mg/kg for channel catfish (Gatlin and Wison, 1983) and 26–29 mg/kg for hybrid tilapia (Lin et al., 2008) and 30 mg Zn/kg for Nile tilapia (Eid and Ghonim, 1994). Tan et al. (2011) and Feng et al. (2011) fed diets with increasing zinc levels (15, 27, 41, 58, 69 and 93 mg Zn/kg) to juvenile Jian carp and found that a levels of 40 mg Zn/kg or higher improved growth performance and decreased lipid peroxidation and protein oxidation and improved antioxidant defense compared with levels below 40 mg Zn/kg diet. These authors estimated the zinc requirement for juvenile Jian carp to be 48 mg Zn/kg diet. Liang et al. (2012) estimated the zinc requirement for juvenile grass carp to be 55 mg Zn/kg. The zinc requirement in Atlantic salmon (*Salmo salar*) fry during start feeding was reported to be 57–97 mg/kg (Maage et al., 1991). Feeding channel catfish, blue tilapia, or Atlantic salmon semi-purified diets fortified with sodium phytate increased their zinc requirements to approximately 100–200 mg Zn/kg DM (Gatlin and Wilson, 1984; McClain and Gatlin, 1988; Gatlin and Phillips, 1989; Gatlin et al., 1989; Maage and Julshamn, 1993). Gatlin and Wilson (1983) found that 20 mg Zn/kg diet was the minimal requirement for channel catfish. Fountoulaki et al. (2010) showed that the optimum dietary zinc level for European sea bass (*Dicentrarchus labrax*) juveniles would be < 91 mg Zn/kg diet.

Using diets based on common feedstuffs, Maage and Julshamn (1993) estimated the dietary requirement for Atlantic salmon to be between 37 and 67 mg/kg. More recently, the same research group (Maage et al., 2001) fed a diet containing either 50 or 180 mg Zn/kg feed to Atlantic salmon for six months and found no differences in growth or mortality but zinc status increased with the higher dietary zinc level. Apines et al. (2001) fed either 55 or 87 mg Zn/kg to rainbow trout fingerlings and found no differences in growth and alkaline phosphatase activity, but increased whole body zinc and bone zinc concentrations. According to Luo et al. (2011), who studied six dietary zinc levels between 8 and 76 mg Zn/kg, yellow catfish juveniles reached maximal specific growth rate and protein efficiency rate at respectively 17 and 21 mg Zn/kg diet. Savolainen and Gatlin (2010) fed 46, 51, 56 or 66 mg Zn/kg diet to fingerling hybrid striped bass for eight weeks and did not observe any dose-response effect of dietary zinc on scale and bone zinc contents.

Recommendations from the European Feed Industry for fish are the following: 300, 250, 200 and 300 mg Zn/kg for larval fish, fry-fingerlings, on-growing fish and broodstock, respectively.[10]

2.1.7. Dogs and cats

The most recent zinc requirements/allowances and recommendations for dogs and cats are presented in Table 5. As published experiments on zinc requirements are very scarce, NRC (2006) published requirements only for puppies, kittens and lactating cats and specifically included allowances for all categories. No paper on this subject has been published since the latest zinc requirement update for dogs and cats (NRC, 2006).

Table 5: Zinc requirements/allowances (mg/kg diet dry matter) for dogs and cats. Sources: NRC, USA (2006); GfE, Germany (1989); FEDIAF (2012)

Species	Category	Requirements and allowances			
		NRC[1]	NRC[2]	GfE[3]	FEDIAF
Dog	Puppies after weaning	40	100	100	100
	Adult, lactation		96	100	100
	Adult, maintenance		60	100	72
Cat	Kittens after weaning	50	75		75
	Adult, lactation	42	60		75
	Adult, maintenance		74		75

(1), (3) Requirements.
(2) Allowances.

2.2. Zinc deficiency

Following early research on experimental zinc deficiency in laboratory animals, signs of zinc deficiency in farm animals were shown to include decreased growth and parakeratosis in swine and ruminants, and decreased growth, frizzled feathers, shortening and thickening of the long bones, and enlarged hocks in chicken (Nielsen, 2012). In dogs, zinc deficiency leads to dermatological disease and immune deficiency (Cunningham and Kovacic, 2009). Data on other animals are relatively scarce but there are reasons to believe that the wide spectrum of clinical signs of severe zinc deficiency in humans, i.e. epidermal, gastrointestinal, central nervous, immune, skeletal and reproductive system disorders (Hambidge, 2000), also applies to the above animals and others. In humans, milder zinc deficiencies are associated with growth defects, diarrhea, increased number of infectious diseases, impaired neuropsychologic performance, prenatal development, pregnancy outcome, and childhood morbidity and mortality (Hambidge, 2000). Without having a biomarker for cellular zinc status, a causative relationship between milder states of zinc deficiency and disease is often difficult to establish and relies largely on improvement of function when zinc is supplemented.

[10] Data submitted to EFSA in 2013 following a call for data.

Zinc deficiency is considered to cause an increased oxidative stress that leads to damage to biomolecules including DNA (Oteiza, 2012). This relationship provides a molecular mechanism for the role of zinc in genomic stability (Sharif et al., 2012) and for the risk of developing degenerative neurological disease, cancer, diabetes and of accelerated ageing, all consequences that are important when considering the long-term quality of livestock and its health.

Reduced activity of various zinc metalloenzymes is another common sign of deficiency in fish (NRC, 2011). Low dietary zinc was studied in an experiment in Atlantic salmon lasting from first feeding until about 30 g body weight (Baeverfjord et al., 2013). The vertebral column of fish fed low-zinc diets was compressed, this finding being comparable to the description of "short body dwarfism" described for zinc deficiency in rainbow trout (Satoh et al., 1987a, b, c). In order to identify long-term impacts of zinc deficiency on bone pathology, salmon with zinc deficiency-induced vertebral deformities were subsequently maintained on a commercial diet through smoltification until the fish weighed on average 1 kg. Even though zinc status was restored by the time of seawater transfer, the vertebral compression was still evident when the fish were 1 kg body weight.

Genetic factors leading to zinc deficiency are also known. Uptake of zinc into the intestinal tissue is mainly determined by the zinc transporter ZIP4. Mutations in this transporter lead to acrodermatitis enteropathica in humans; related mutations and associated disease have been described in cattle (Holstein Friesians, A46 lethal trait) (Kury et al., 2002; Wang et al., 2002; Yuzbasiyan-Gurkan; Bartlett, 2006). The lethal acrodermatitis shows similar symptoms in bull terriers but seems to be refractory to zinc treatment (Grider et al., 2007). Only recently have mutations in swine been detected (Siebert et al., 2013). The extent of these mutations in swine is unknown but indicates that Pietrain pigs have higher zinc absorption rates owing to a mutation in *Zip4* (Siebert et al., 2013). Since there are many other proteins are involved in zinc homeostasis, it is expected that additional mutations affect the requirement of zinc in the diet, mandating higher or lower requirements in genetically susceptible animals. ZIP4 is down-regulated at high doses of zinc (Weaver et al., 2007; Sargeant et al., 2010). Additional inherited diseases caused by poor zinc absorption should be considered in goats and Northern breed group dogs (Hensel, 2010).

Conditioned zinc deficiency elicited by dietary factors is described in the literature, particularly in pigs and fish. It is reviewed in detail in Section 4.2.

2.3. Tolerance of animals to dietary zinc

Zinc is tolerated by animals at relatively high dietary amounts. In general, animals are able to tolerate much higher levels of zinc than those naturally occurring in feed materials and/or in balanced complete/complementary diets supplemented up to the maximum levels permitted in the European Union. EU legal provisions (Regulation (EC) No 1334/2003) established a maximum total zinc content/kg complete feed of 250 mg for pet animals, of 200 mg for fish and milk replacer and of 150 mg for other animal species. Nonetheless, adverse effects of high zinc have been reported to occur under non-experimental conditions.

The most comprehensive review of maximum tolerable zinc concentrations in animals has been carried out by the NRC in its revision of 2005 (NRC, 2005). Most data from which maximum tolerable levels (MTLs) are derived rely on studies conducted some decades ago, when it was generally assumed that zinc had a relatively low toxicity for animals. In the last decade only a few studies have been carried out to better redefine these MTLs. In most of these studies high amounts of zinc were given to animals and tolerance was established considering very general parameters related to feed intake and growth rate. However, even in the absence of negative effects on performance and with lower zinc intakes, zinc interferes with the metabolism of other ions, such as copper and iron, depressing the immune function, at the same time as showing adverse effects on the ratio of low-density lipoprotein to high-density lipoprotein (LDL/HDL)-cholesterol (Fosmire, 1990) while pathological changes are found in the pancreas (exocrine portion), kidney, liver, adrenal gland, rumen, abomasums and small intestine (Allen et al., 1983; NRC, 2005). In addition, it is well established that different compounds of zinc widely differ in bioavailability (see Section 4.1) and probably in toxicity, and this information has not been taken into account. Furthermore, zinc toxicity in animals also depends on the duration of feeding, nutritional history (including other essential minerals, such as calcium, copper and iron), the physiological state and the genotype.

The MTLs seem to be quite well established for intensively grown livestock, e.g. poultry and more particularly pigs, which are routinely supplemented with zinc in fully balanced complete diets. Less information is available for ruminants, which are less tolerant to zinc than swine and poultry (Underwood, 1977). Finally, for horses, rabbits and pets the information is very sparse.

2.3.1. Poultry

The MTL of zinc for poultry was set at 500 mg/kg diet (NRC, 2005). Although studies conducted with diets adequate in all nutrients and published before the 1980s indicated that poultry could tolerate up to 1000 mg Zn/kg without depression of growth rate and feed/gain (NRC, 1980), more recent studies indicate that this level is not safe. Several studies (Sandoval et al., 1997a, 1998, 1999; Cao et al., 2000) have reported reductions in feed intake and weight gain when zinc exceeded 500 mg/kg diet, especially when the source was zinc sulphate. When diets were marginally deficient in iron, 1000 mg Zn/kg diet also resulted in depressed growth (Blalock and Hill, 1988). Lesions in the pancreas and gizzard of chicks fed 1000 mg Zn/kg diet were reported by Dewar et al. (1983). Zinc caused decreased growth and signs of pancreatic pathology when added at 500 mg/kg to purified diets (Lu and Combs, 1988; Lu et al., 1990). Mild histological changes in the thyroid were observed in chicks fed 200 mg Zn/kg diet, and this dose decreased plasma levels of thyroxine in laying hens (Kaya et al., 2001, 2002); however, functional or pathological consequences of these changes have not been shown.

Huang et al. (2007) fed day-old chickens for fattening diets containing zinc concentrations up to 170 mg (corresponding to 140 mg Zn/kg supplemented as zinc sulphate) for 21 days. The authors observed that maximum weight gain and feed intake occurred at a supplemention rate of 20 mg Zn/kg (corresponding to about 50 mg total zinc). Similarly, Jahanian et al. (2008) observed that in broiler chicks, increasing zinc concentration from 105 to 145 mg/kg diet (by supplementing zinc sulphate to a basal diet containing 25 mg Zn/kg) for 42 days significantly decreased average feed intake. In a study by Trindade Neto et al. (2011) in brown layer hens, increasing total dietary zinc (diet supplemented with chelated zinc) from 137 to 655 mg/kg diet reduced bird performance and egg quality parameters (decreased shell weight, percentage of ash, yolk ash deposition and total ash deposition).

2.3.2. Pigs

Pigs are possibly the livestock species with the highest tolerance to zinc. The MTL of zinc for pigs was set at 1000 mg/kg diet (NRC, 2005); this concentration is well above the maximum authorised level established by the EU, which is justified based on environmental concerns. Over the last few years most research has been focused on establishing the zinc requirement based on feed intake and growth responses (see Section 2.1.2) and hardly any information is available for dietary zinc concentrations above 100–150 mg Zn/kg diet that would justify a change of the MTL. However, when evaluating reproductive performance in boars, García-Contreras et al. (2011) found that supplementation of diets to a total of 225 mg Zn (from zinc methionate)/kg diet resulted in adverse effects on the sperm DNA quality which could be related to the ability of the spermatozoa to accumulate zinc during spermatogenesis.

2.3.3. Ruminants

Ruminants, particularly young and gestating animals, show a lower tolerance to dietary zinc than other livestock species. Their susceptibility to zinc seems to be related to the great interference of zinc on copper metabolism (especially in sheep because of their particular susceptibility to copper deficiency; see Suttle, 2010) as well as the effect of high doses of zinc on the rumen metabolism; zinc sulphate supplementation of ruminant diets at levels greater than 1000 mg/kg has shown negative effects on the ruminal flora and feed digestibility (Durand and Kawashima, 1980; Froetschel et al., 1990). Dairy cattle, however, appear to tolerate higher zinc dietary levels (up to twice the amount) because of the additional route of excretion in milk.

The MTL for cattle was set at 500 mg/kg (NRC, 2005); more recent studies support this recommendation. Young calves fed milk replacer tolerated 500 mg Zn/kg diet for five weeks without adverse effects, but 700 mg/kg diet caused a reduction in weight gain, feed intake and feed efficiency (Jenkins and Hidiroglou, 1991). Wright and Spears (2004) administered a diet containing 530 mg Zn (from zinc sulphate, zinc proteinate and a mixture of both zinc sources)/kg diet to Holstein calves for 14 days to evaluate the effects of dose and source on metabolism and zinc tissue concentrations; no adverse effects were recorded. Arelovich et al. (2000) administered zinc chloride by ruminal cannulas to provide the equivalent of an additional 30, 250 and 470 mg Zn/kg diet to heifers that were fed prairie hay and urea. This was administered to evaluate the effect of zinc on ruminal fermentation, forage intake and digestion. It was found that zinc supplementation at a concentration of 250 mg/kg may decrease the likelihood of urea toxicity and increase energetic efficiency of ruminal fermentation, but adding 470 mg Zn/kg tended (P= 0.06) to depress digestibility. Recently, Sobhanirad and Naserian (2012) evaluated the effect of the supplementation of 500 mg Zn (from zinc sulphate monohydrate and zinc methionine)/kg diet to a basal diet containing 42 mg Zn/kg DM on the haematological and biochemical parameters of Holstein dairy cows, and reported no negative effects. Moreover, organic zinc supplementation showed a positive effect on red blood cell parameters, fibrinogen concentration and lactate dehydrogenase and superoxide dismutase activities compared with the control diet.

For sheep, the MTL was set at 300 mg/kg diet (NRC, 1980). In one experiment (Henry et al., 1997) zinc concentrations as high as 2100 mg/kg were fed for as long as 30 days without reducing feed intake, but tissues were not examined for histological lesions. Recent studies in Brazil indicate that feeding weaning lambs diets supplemented with zinc at 200, 400 and 600 mg/kg (information on zinc concentration in the basal diet was not available) from different sources (zinc oxide, zinc proteinate and zinc amino acid) for 114 days did not cause any negative effects on animal performance (Vilela et al., 2012). Wang et al. (2006) did not find any significant difference in body weight gain and feed to gain between lambs given different levels of zinc supplementation (50, 100 and 150 mg/kg diet) for 70 days; zinc content in basal diet was 16 mg/kg feed. However, at the higher level of zinc supplementation, a decrease in vitamin B_{12} concentration was found. It seems likely that the high-zinc diet resulted in an imbalance in the trace element intake, which in turn did not favor the appropriate activity of the ruminal microorganisms, thereby impairing cobalt availability incorporated into vitamin B_{12}.

2.3.4. Horses

Excessive chronic intake of zinc by horses is uncommon but devastating in rapidly growing foals. Zinc is a potent inhibitor of copper absorption, leading to a secondary copper deficiency (Cymbaluk and Smart, 1993). Copper deficiency in foals causes severe degenerative disease of cartilage (because copper is a required co-factor of lysyl oxidase, an enzyme needed for collagen synthesis) characterised by breaking of articular and growth plate cartilage through the zone of hypertrophic cells, resulting in arthritis and periarticular enlargement of the long bones (Eamens et al., 1984; Bridges and Harris, 1988).

A MTL of 500 mg Zn/kg diet has been set for horses by the NRC (1980) and remains unchanged in the absence of new data which may redefine it. Schwarz and Kirchgessner (1979) indicated a tolerable level of about 1000 mg Zn/kg diet, but it should be noted that foals seem to be more sensitive and exhibit shortened tendons and osteochondrosis as a result of secondary copper deficiency. Moreover, gestating mares are more sensitive to high zinc intake (Meyer and Coenen, 2002). Bridges and Moffitt (1990) investigated the influence of variable zinc content (29.1, 250, 1000 and 2000 mg/kg DM) in a basal diet containing 7.7 mg Cu/kg on the ability of weanling foals to maintain normal copper balance. Foals fed the lower-zinc diets (up to 250 mg/kg) maintained normal serum copper and zinc concentrations for 14 to 15 weeks, whereas those fed the two highest zinc diets became hypocupraemic within five to six weeks and were lame within six weeks, owing to cartilaginous disease characteristic of osteochondritis dissecans. Foals fed the high-zinc diets became lame after serum copper concentration had remained at 0.3 µg/mL for more than one week.

2.3.5. Rabbits

For rabbits no MTL has been established by the NRC. No pathological changes related to zinc excess in feed have been described in recent literature. Hossain and Bertechini (1993) administered a diet supplemented withup to 270 mg Zn (from zinc oxide)/kg (to a basal diet containing 16 mg Zn/kg) to 50-day-old New Zealand White (NZW) rabbits for 21 days. Body weight gain was not affected by zinc supplementation and no signs of toxicity were observed. Recently, Nessrin et al (2012) studied the growth response of 5-week-old NZW rabbits to increasing zinc supplementation levels (0, 50, 100, 200 or 400 mg Zn (as zinc oxide)/kg diet, to a basal diet containing 22.3 mg Zn/kg) for eight weeks. Zinc supplementation at up to 200 mg Zn/kg diet significantly improved body weight gain and feed to gain ratio, but 400 mg Zn/kg diet resulted in significantly reduced body weight gain and an increased feed to gain ratio.

2.3.6. Fish

The NRC evaluated the maximum tolerable zinc concentration in several animal species and found this to be 250 mg/kg for fish (NRC, 2005). Regarding the tolerance level for fish, the FEEDAP Panel previously noted that the values reported in the literature (Clearwater et al., 2002) are markedly different for different species, i.e. < 100 mg/kg for tilapia (*Oreochromis niloticus*) and > 2000 mg/kg for carp (several species) and rainbow trout (*Oncorhynchus mykiss*) (EFSA FEEDAP Panel, 2012a, b, c, d).

Using whole-body responses such as growth depression as a parameter of impaired performance, dietary zinc concentrations up to 1700 mg/kg were tolerated by rainbow trout (*Oncorhynchus mykiss*) (Wekell et al., 1983). However, physiological parameters such as blood haematocrit and haemoglobin indicated that dietary zinc concentrations of 1000 mg/kg compromised the health of rainbow trout (Knox et al., 1984). Rainbow trout and turbot (*Scophthalmus maximus*) fed dietary zinc (from zinc sulphate heptahydrate or zinc chloride) at concentrations ranging from 30 to 3000 mg Zn/kg feed in several studies showed no evidence of toxicity in terms of reduced growth or survival (Ogino and Yang ,1978; Wekell et al., 1983, 1986; Knox et al., 1984; Overnell et al., 1988a, b; Mount et al., 1994; Kock and Bucher 1997). This has led to the general impression that dietary zinc is relatively non-toxic to fish. However, owing to the low feeding ratios used in many of the studies, it was calculated that the highest zinc doses were often below 24 mg Zn kg/bw per day (Clearwater et al., 2002). In rainbow trout dietary doses in the range of 20–23 mg Zn/kg bw per day had antagonistic effects on iron and copper tissue levels, and Clearwater et al. (2002) established a threshold level of >30 mg Zn/kg bw per day based on these effects; thus, further emphasizing that growth and/or survival are not the most sensitive endpoints for assessing dietary toxicity of trace elements. The EU maximum content for zinc is set at 200 mg/kg feed; surveillance on Norwegian commercial salmon feeds in the years 2004-2009 revealed average levels ranging from 141 to 168 mg/kg feed, with the highest observed level of 260 mg/kg (Sissener et al., 2013) . With an assumed high feed consumption of 3.5 % per kg bw and day for juvenile Atlantic salmon, the highest observed zinc feed level would correspond to a dose of about 9 mg Zn per kg bw and day, while average feed levels would give a dose of about 5 mg per kg bw and day. For adult fish, with a much lower feed intake (< 1 % per bw and day), the maximum zinc exposure would be about 1.5 mg Zn kg bw and day for the highest observed feed level. These exposure doses would be considerably below the threshold level of > 30 mg Zn per kg bw and day.

Marine fish larvae such as gilthead seabream (*Sparus aurata*), cod (*Gadus morhua*) and Atlantic halibut (*Hipoglossus hipoglossus*) are fed for up to a couple of months on live feed. The species of live feed include artemia, rotifers and copepods which contain 49–570 mg Zn/kg DM (Hamre et al., 2008, 2013).

2.3.7. Dogs and cats

Information on dietary zinc toxicity in dogs and cats is very sparse.

The MTL in cats has been set at 600 mg/kg diet (NRC, 2005) based on a study of Sterman et al. (1986), in which no clinical abnormalities were reported when 600 mg Zn/kg diet was fed to adult cats for six weeks; plasma zinc concentration rose to 1200 µg/L compared to 900 µg/L in cats fed 100 mg Zn/kg. To the FEEDAP Panel's knowledge, no other studies in cats have been published in the recent literature.

No MTL has been established for dogs (NRC, 2005). No experimental data were found for dogs receiving zinc concentrations in their diet above the limits established by the EU, although cases of zinc toxicity have been reported in dogs owing to accidental ingestion of coins and other metallic objects. Zinc supplementation at concentrations of 100–180 mg/kg in commercial diets for puppies showed positive effects compared with controls in terms of growth rate and hair coat properties (Vester et al., 2006; Jamikorn and Preedapattarapong, 2008).

Some dog breeds deserve special attention, particularly the Bedlington Terrier and the Labrador Retriever but also the West Highland White Terrier, the Skye Terrier, Dobermann pinschers and the Dalmatian hound, which have inherited susceptibility to copper-associated chronic hepatitis (Johnson, 2008). Therapeutic doses of zinc of approximately 20 mg/kg body weight per day (equivalent to a concentration of approximately 1400 mg Zn/kg diet) are given for two to three months, followed by maintenance of half of this dose, to block the copper uptake by the enterocyte and decrease copper accumulation in the liver (Brewer et al., 1992; Hoffmann et al., 2009).

2.4. Therapeutic use of zinc in piglets

Zinc oxide has a widespread therapeutic use in piglets. It is considered in many EU countries as an attractive alternative to the use of antibiotics as feed additives, which has been phased out. Pharmacological doses as high as 1000–3000 mg of zinc/kg diet can be given to piglets for up to five weeks to prevent or overcome post-weaning diarrhea and improve pig performance (ANSES, 2013; Sales, 2013). Numerous studies carried out in the last few years have demonstrated the benefits of zinc oxide as a growth promoter in post-weaning piglets at the above mentioned concentrations. However, some studies have failed to observe beneficial effects of therapeutic levels of zinc (review in Sales, 2013), whereas others have found negative effects such as reduced feed intake and growth when given at concentrations of 4000–5000 mg Zn/kg diet (Hill and Miller, 1983; Poulsen, 1989, 1995).

When reviewing other literature (BT Li et al., 2001; Mavromichalis et al., 2001; Hojberg et al., 2005; Han and Thacker, 2010; Hu et al., 2013; Janczyk et al., 2013; Martin et al., 2013), the following outcomes on efficacy, optimum dose and treatment became evident. The therapeutic dose of zinc from zinc oxide is effective in preventing diarrhea and stimulating growth. The optimum dose is about 2500 mg Zn/kg feed, while concentrations of 4000 and 5000 result in adverse effects (see EC, 2003a). The therapeutic concentrations should be applied only in the first two weeks after weaning; extending this treatment may result in adverse effects owing to the toxicity of this high zinc supply, counteracting the beneficial effects on the health status of the 2-week treatment. However, further studies to optimise the zinc dose and treatment duration are considered necessary.

Feeding of high zinc concentrations may stimulate the occurrence of resistance to zinc in the pig gut microbiota (Fard et al., 2011; Vahjen et al., 2011a) and may play a role in the coselection of methicillin-resistant *Staphylococcus aureus* (MRSA) (Aarestrup et al., 2010; Cavaco et al., 2010, 2011; Moodley et al., 2011; Agerso et al., 2012; AMCRA, 2012). This finding requires specific attention and further observations.

Despite several hypotheses, the exact mechanism whereby dietary zinc improves growth of post-weaning pigs is yet to be demonstrated (Heo et al., 2010; Shelton et al., 2011). Antimicrobial properties of zinc oxide were illustrated by changes in the gastrointestinal ecosystem of the piglet (Molist et al., 2011; Slade et al., 2011; Vahjen et al., 2011b; Pieper et al., 2012; Hu et al., 2013), leading to the assumption that high levels of dietary zinc oxide enhanced the growth of weaned pigs by controlling pathogenic bacterial scours. Carlson et al. (1999) suggested a systemic effect via the blood rather a direct influence on the gastrointestinal tract which is supported by more recent findings of Zhang and Guo (2007). Conversely, the effectiveness of zinc oxide despite its relatively low availability compared with other sources of zinc indicated a local effect on the intestine (Pérez et al., 2011). Proposed mechanims also include an increase in barrier functions/properties of the intestinal epithelium (Rodriguez et al,. 1996; Carlson et al., 2006; Feng et al., 2006; Hedemann et al., 2006; Hu et al., 2013; Martin et al., 2013; Sanz Fernandez et al., 2013), immunomodulation (Roselli et al., 2005; Kim et al., 2012; Hu et al., 2013) and reduced activation of cAMP-operated K^+ and Cl^- channels leading to reduced loss of water and other osmolytes (Carlson et al., 2007).

3. Zinc in feedingstuffs

Data on zinc in feedingstuffs are reviewed below in a condensed form. Details are described in the Appendix A for the zinc content of feed materials, Appendix B for phytate and phytase in feed materials and Appendix C on the background concentrations of zinc in complete feed. Appendices D and E refer to the data collected from European countries within the call for data launched by EFSA.

3.1. Feed materials

Zinc concentrations in plants and plant materials are influenced by soil concentration, soil conditions which influence zinc uptake (pH, ion exchange capacity, etc.), fertilisation and genetic differences in plant species, part of plant, stages of maturity, etc. Due to plant processing (milling, extraction processes, etc.) element concentration can be altered (concentration, contamination, dilution, etc.). Zinc concentrations in plant materials are in the range 10–200 mg/kg, with cereals, legumes and oilseeds in the range 15–30 mg/kg, oilseed meals between 30 and 125 mg/kg and germ meals (maize, wheat) and beet leaves between 130 and 190 mg/kg. Forages, depending on the cut, contain between 15 and 35 mg Zn/kg DM. Feed materials of animal origin (meals of bone, meat, feathers) contain high zinc levels (115–160 mg Zn/kg) (Appendix A, Tables A1, A2, A3, A4 and A5). The zinc concentration in fish meal is dependent on the species used to produce the meal from (29–210 mg Zn/kg; Appendix A, Table A6). Contents of zinc in fish meal and meals produced from Arctic krill, Antarctic krill and Arctic amphipod were 80, 51, 81 and 58 mg/kg DM, respectively (Moren et al., 2006).

Dietary phytate is the major limiting factor for zinc bioavailability in rats, broilers and piglets (see Section 4.2.1). Phytate P concentrations of 0.5–1.3 % are noted in wheat by-products, rice bran and maize/wheat gluten feed and of 0.3–0.5 % in triticale and oilseed meals, while in cereals and legumes the content is < 0.3 %, representing between 50 and 80 % of total phosphorous (Appendix B, Table B1). In cereal and oilseed diets, phytate antagonism principally concerns native zinc, already bound to phytates. Zinc content in feed components from plant origin is positively correlated to the phytate P content, with ~10 mg of zinc bound to 1 g phytate P in cereals (Revy et al., 2003). Rodrigues-Filho et al. (2005) determined that two out of the three identified phytate molecules from wheat grains contain zinc. Compiled literature data suggest that on average 80 % of zinc is bound to phytate (Appendix B, Table B2).

3.2. Complete feed – Background zinc levels

Background levels are defined as the trace element concentrations in the complete feedingstuffs delivered by the feed materials. Hence, a background level simulation implies combining data of trace element composition tables of feed materials with complete feedingstuff composition data. The zinc background levels were calculated for a list of animal species/categories complete feed formulations (n= 35; Appendix C, Table C1) using the data from CVB (2007) or INRA (2004) and Batal and Dale (2008). Table C1 does not include zinc from trace element premixtures but includes the zinc element concentrations for mineral sources (considered as feed minerals), according to the data from Batal and Dale (2008).

Differences between the two simulated background level values for the same complete feedingstuff are mainly due to differences in zinc content in the feed materials data from CVB (2007) and INRA (2004) tables. More data are available on zinc content in feed materials in the CVB tables than in the INRA tables. In order to have the same amount of feed materials in both simulations, for feed materials for which no zinc content was available in the INRA tables, CVB values were used to complete the dataset.

From Table C1 (Appendix C) it becomes evident that for pigs, poultry and ruminants, zinc background levels in complete feeds are in the range 25–45 mg/kg feed, while in feeds for rabbits, fish and pets, the levels are in the range 45–75 mg/kg. The latter range is confirmed for dogs and cats by data submitted by the industry (Appendix C; Table C2). In contrast, food for other pets may have a lower zinc content: food for dwarf rabbits, hamsters and guinea pigs has a zinc content in the range 9–42 mg, that for fish (goldfish, tropical fish) contains 15–31 mg and that for ornamental birds 15–43 mg Zn/kg food. These wide ranges reflect also the variety of feed materials used in formulating pet foods.

3.3. Feed additives

Several compounds of zinc (zinc lactate, trihydrate; zinc acetate, dihydrate; zinc carbonate; zinc chloride, monohydrate; zinc oxide; zinc sulphate, heptahydrate; zinc sulphate, monohydrate; zinc chelate of amino acids, hydrate; zinc chelate of glycine, hydrate; zinc chloride hydroxide monohydrate (minimum zinc content 54 %);[11] zinc chelate of hydroxy analogue of zinc (zinc content 17.5–18 %);[12] methionine-zinc, technically pure (zinc content 17.5–18.5 %)[13]) are currently authorised as nutritional feed additives in the EU.

3.4. Zinc in compound feed – Data from the control by European countries

EFSA launched a call for data throughout the EFSA's Focal Points to collect data on the official feed control on zinc monitoring. In total, data from 22 European countries were received, covering a total of 13618 feed samples; the bulk of the data refers to feed for pigs, poultry and ruminants, followed by feed for horses, pets and rabbits. These raw data were submitted to a validation procedure:

- The first criterion consisted in the inclusion/exclusion of samples considering the type of feed. For most animal species/categories only *Complete feed* samples were considered. *Complementary fee*d, which was labelled to be as 100 % of the daily ration was attributed to complete feed. For cattle, dairy cows and horses only complementary feed/concentrate was considered, since the number of samples of complete feed was not representative. Dog and cat wet food samples were excluded since the dry matter content was not reported.
- As a second criterion, the content of zinc was taken. Descriptive parameters of data distribution would be biased by unlikely low zinc concentrations as well as by excessive levels which may be driven by intentions other than the production of standard feed (e.g. disease prevention). Therefore, zinc concentrations below 30 mg/kg (background level; see Section 3.2) were not considered. Maximum cut-off values were built for (i) complete feed with zinc concentrations exceeding the requirements[14] by a factor of 4 and (ii) complementary feed with values higher than 1000 mg Zn/kg (characteristic of mineral feed).

A remaining total of 9842 samples were submitted to descriptive statistical analysis (see Table D1 in Appendix D). The results are summarised in Table 6; for more details see Appendix E.

[11] Commission Implementing Regulation (EU) No 991/2012 of 25 October 2012 concerning the authorisation of zinc chloride hydroxide monohydrate as feed additive for all animal species. OJ L 296, 26.10.2012, p. 18.
[12] Commission Regulation (EU) No 335/2010 of 22 April 2010 concerning the authorisation of zinc chelate of hydroxy analogue of methionine as a feed additive for all animal species. OJ L 102, 23.4.2010, p. 22.
[13] Commission Implementing Regulation (EU) No 636/2013 of 1 July 2013 concerning the authorisation of zinc chelate of methionine (1:2) as a feed additive for all animal species. OJ L 183, 2.7.2013, p. 3.
[14] For dogs and cats, allowance data were taken since there is not a complete set of requirements data.

Table 6: Zinc in compound feed. Descriptive statistics of the control data submitted by 22 European countries

Animal Group	Category/Species[1]	n	mg Zinc/kg complete feed[2] Median	90th percentile	10th percentile	Samples above CAMC[3] (%)
Poultry	Starter Chicks	75	103.0	139.0	68.0	1.3
	Chickens reared for laying	52	95.3	121.3	74.0	0
	Laying hens	545	89.0	128.0	64.5	1.8
	Chicken for fattening	433	107.0	137.0	77.7	1.6
	Turkeys for fattening	158	106.0	144.0	82.0	3.8
Pig	Piglets	2098	137.0	178.0	99.0	30.2
	Pigs for fattening	3124	117.0	151.9	76.0	10.6
	Sows	636	129.0	169.0	90.0	20.1
Bovid	Calves	41	79.0	106.0	68.0	0
	Calves milk replacer	191	86.0	136.0	44.0	0
	Cattle	250	105.0	246.5	44.0	Not Applicable
	Dairy cows	830	113.0	271.5	62.0	Not Applicable
	Sheep	280	97.0	144.0	70.1	6.8
	Goat	20	81.6	154.5	42.5	10.0
Horse	Horse	314	132.3	314.0	67.0	Not Applicable
Rabbit	Rabbit	205	98.4	130.2	73.0	2.4
Fish	Salmonids	109	131.0	200.0	107.0	9.2
Dog	Dog	162	157.5	240.0	65.6	7.4
Cat	Cat	76	154.0	222.0	87.8	1.3

(1) The following grouping was applied:
“Laying hens”: Includes the data labelled as feed for laying hens, layer phase I and layer phase II
“Chickens for fattening”: Includes the data labelled as feed for chickens for fattening, broiler starter, grower and finisher
“Turkeys”: Includes the data labelled as feed for turkeys for fattening, starter, grower and finisher
“Piglets”: Includes the data labelled as feed for piglets weaned, piglets starter I and piglets starter II
”Pigs for fattening”: Includes the data labelled as feed for pigs for fattening, pig grower and pig finisher
”Sows”: Includes the data labelled as feed for sows, sows gestating and sows lactating
“Rabbit”: Includes the data labelled as feed for rabbit, rabbit breeder and rabbit grower/finisher.

(2) Except for cattle, dairy cows and horses in which complementary feed and/or concentrate has been used, and therefore the calculation of the amount of samples above the CAMC is not applicable.

(3) CAMC, Currently Authorised total Maximum Contents of zinc in complete feed.

The median zinc content in poultry complete feeds is in the range 89–107 mg/kg; only about 4 % of the samples showed values above the CAMC (150 mg/kg). The median zinc content in pig complete feeds is in the range 117–137 mg/kg; between 10 and 30 % of the samples showed values above the CAMC (150 mg/kg). The median of zinc content in feed for calves and milk replacer is in the range 79–86 mg/kg.

No data are available on total zinc content in total mixed ration (TMR) for cattle, dairy cows and horses. The median of zinc content in complementary feed and/or concentrate for cattle, dairy cows and horses is in the range of 105–132 mg/kg.

The median zinc content in complete feed for rabbits, salmonids, dogs and cats amounted to 98, 131, 157 and 154 mg/kg, respectively; only between 1.3 and 9.2 % of the samples showed values above the CAMC. Data from the Norwegian Fish Feed Surveillance Programme (Appendix D, Table D2) identified for the years 2001–2011 mean values between 122 and 224 mg Zn. The range of the mean was from 31 to 308 mg without an annual trend.

4. Bioavailability of dietary zinc

According to Ammerman et al. (1998), bioavailability is defined as the proportion of an ingested nutrient that is absorbed in a form that can be utilised in the metabolism by a normal animal. This definition stresses that the mineral must be available not only at the dietary level but also at the tissue level. Bioavailability is thus the result of successive phases: accessibility in the intestinal lumen, absorption through the intestinal mucosa, retention and incorporation in a functional form (e.g. cofactor of an enzyme).

To properly evaluate dietary zinc availability, care should be taken on the level of zinc supply and on the criteria to assess the zinc status of the animal.

A suitable criterion to evaluate the bioavailability of a mineral should be specific and sensitive enough to respond rapidly to variations in dietary mineral supply. Owing to the down-regulation of the true absorption and of the endogenous secretions of zinc, absolute availability strongly depends on the status of the animal (e.g. Nockels et al., 1993) and, in turn, on the level of zinc dietary supply. Therefore, the bioavailability of a source of zinc is usually assessed relative to a reference, what is called “relative bioavailability” (RBV). This method allows the ranking of the sources of zinc. In relative availability experiments, criteria used should respond linearly to zinc ingested; thus, experiments should be designed at suboptimal levels of zinc supply.

Although performance may be impaired in case of zinc deficiency, this criterion is usually not considered sensitive enough to assess the availability of minerals. At suboptimal zinc supply, the amount of zinc absorbed (true or apparent absorption) and retained responds linearly to zinc supply. In contrast, at levels exceeding the requirement, the amount of zinc absorbed is optimised so that the zinc absorbed and retained expressed as a percentage of zinc ingested decreases as zinc ingested increases. For example, apparent absorption of zinc decreased from 47 to 22 % of zinc intake as zinc concentration in a milk replacer for calves was increased from 40 to 1000 mg/kg DM (Jenkins and Hidiroglou, 1991). When the dietary zinc content was decreased from 190 to 65 mg/kg, the relative body zinc retention was increased from 8 % to 20 % (Mohanna and Nys, 1999a). The concentration of zinc, metalloproteins or zinc-dependent enzymes in different fluids and tissues are often used as indicators of zinc status. The most used criteria are bone zinc and plasma zinc concentrations. Circulating alkaline phosphatase activity and serum 5'-nucleotidase activity are also a relevant criterion in pigs (e.g. Revy et al., 2002) and in broilers (Huang et al., 2007), respectively. Other criteria, such as zinc or metallothionein concentrations in liver, kidney and intestine, are also responsive to dietary zinc supply but do not reach a plateau. Indeed, above the physiological requirements, accumulation of zinc in these tissues allows the regulation of zinc homeostasis by trapping zinc in excess.

4.1. Zinc-containing additives

The most commonly used sources of zinc to supplement diets are the oxide (ZnO) and the feed-grade sulphate heptahydrate ($ZnSO_4.7H_2O$). Bioavailability of these feed-grade sources relative to zinc sulphate (analytical-grade) is variable. The RBV of feed-grade sulphate was reported to be 94 % based on bone zinc concentration in chicks (Sandoval et al., 1997a), and 86 to 100 % based on liver zinc concentration in sheep (Sandoval et al., 1997b). The RBV of ZnO was reported to be 22, 44, 61, 74 and 91 % in chicks based on bone zinc concentration (Wedekind and Baker, 1990; Wedekind et al., 1992; Sandoval et al., 1997a; Edwards and Baker, 1999), 69 % based on bone zinc concentration in piglets (Wedekind et al., 1994), and 87 and 79 % based on liver zinc concentration in sheep (Sandoval et al., 1997b). Recent data on chickens for fattening on RBV of zinc oxide compared to zinc sulphate show values between 31 and 99 % (Sahraei et al. 2013). According to Edwards and Baker (1999), high temperature (1200°C) in the production process of some feed grade sources of zinc oxide (e.g. Waelz process) may contribute to the lower bioavailability of these sources. It should be noticed that in most of the above mentioned studies, only one level of zinc oxide was tested, so that the estimated RBV should be taken with caution. Based on growth performance, tetrabasic zinc chloride is considered to be as available as zinc sulphate in chicks (Batal et al., 2001).

Many studies deal with the comparison between organic sources of zinc to a reference, often zinc sulphate. As mentioned by Suttle (2010), although studies were published in peer-reviewed journals, conclusions on the effectiveness of organic sources of zinc sometimes do not fit with the experiments, and one should carefully check the experimental design and the results. In particular, although organic and inorganic zinc were not provided at similar dietary levels, many studies conclude that using organic zinc would reduce zinc excretion, unfairly suggesting that organic sources would be more available. Nevertheless, previous literature reviews on zinc availability in poultry, pigs and ruminants estimated organic and inorganic zinc sources as equivalent (Ammerman et al., 1998; Jongbloed et al., 2002). The same conclusion is drawn from a recent meta-analysis conducted by Schlegel et al. (2013) for pigs (based on 13 experiments with 54 treatments) and poultry (based on 11 experiments with 72 treatments).

4.2. Interactions of zinc with dietary constituents

There are considerable interactions in homeostasis as well as functions of essential elements and in particular between zinc, calcium, copper and iron. For the definition of optimal amounts of zinc in diets, it is therefore necessary to consider the ratios of elements in feeds. Optimal utilisation of iron requires copper, as the latter is for example involved in the reoxidation of ferrous to ferric ions. Copper and iron affect the absorption (and functions) of zinc, and vice versa. These examples illustrate that optimal intake of one metal needs to be discussed in the context of the intake of several other. Other dietary components that enhance or inhibit uptake, not only of zinc but also of these other constituents, need to be considered. In particular, the phytate content of the diet is a major modifier of zinc absorption. Interactions are considered here at the level of absorption. Much less is known for interactions that affect utilisation and retention.

4.2.1. Phytates

Phytates are identified as the major dietary factor limiting zinc availability in non-ruminants, because of the formation of insoluble phytate-zinc complexes. Phytic acid (known as inositol hexakisphosphate (IP6), or phytate when in salt form) is the hexaphosphoric ester of the hexahydric cyclic alcohol meso-inositol. It is the principal storage form of phosphorus in many plant tissues. The lower inositol phosphate esters, inositol penta-(IP5), tetra-(IP4), and tri-(IP3) phosphate, are also called phytates.

Sodium phytate strongly reduces zinc availability added as sulphate in semi-synthetic diets given to rats (Rimbach et al., 1995; Windisch and Kirchgessener, 1999), pigs (Oberleas et al., 1962), broilers (O’Dell and Savage, 1960) and fish (Satoh et al., 1989). These observations strongly indicate that sodium phytates would interact with added zinc and limit its bioavailability to non-ruminants. From such studies on the interaction between a source of phytates devoid of zinc (sodium phytates) and zinc added as sulphate or as oxide, the molar ratio phytate:zinc was suggested as a good indicator of available zinc. However, in practical diets, phytates are not present as sodium phytates. Rather, it is believed that most zinc present in feedstuffs containing phytates (cereals and cereal by-products, oil seeds and meals) is bound to phytates, with around 10 mg of zinc for 1 g of phytic phosphorus (Appendix B, Table B2; Revy et al., 2003). Nevertheless, there is also evidence of a negative effect of plant phytates on dietary zinc availability.

Dephytinisation of soybean meal increased bone zinc concentration in pigs (Matsui et al., 1998a). Cultivars of barley, maize and soya were selected for their low phytic phosphorus concentration, mainly in order to improve phosphorus availability (e.g. YC Li et al., 2001; Veum et al., 2001, 2002). Zinc in low-phytate cultivars of maize, barley and rice was reported to be more available to rats than in conventional cultivars (Lönnerdal et al., 2011). Linares et al. (2007) showed that, at similar concentration (23–24 mg/kg), zinc in low-phytate barley was more available to chickens than zinc in conventional barley, with a retention coefficient of zinc which increased from 42 to 63 %. Similarly, in rainbow trout, Sugiura et al. (1999) observed improvements in zinc availability from low-phytate dent corn compared with a conventional cultivar.

Through a meta-analysis, Schlegel et al. (2013) also investigated the question of whether phytates negatively impact the availability of zinc present in plant feedstuffs only or whether they also depress the availability of supplemented zinc. In both broilers and pigs, these authors observed that the increase in bone zinc concentration in response to zinc supplemented as zinc sulphate to basal diets containing suboptimal levels of zinc was independent of the phytate or phytase concentrations. They concluded that phytates negatively affect the availability of zinc present in plant feedstuffs only and, in contrast to sodium phytate, do not interact with supplemented zinc. Conversely, phytase enhances the availability of zinc present in plant feedstuffs only.

There is no evidence that phytates reduce zinc availability to ruminants under practical conditions. Indeed, phytates are readily degraded by microbial phytase in the rumen, and availability of zinc in forages is believed to be quite high (Suttle, 2010). However, in ruminants fed high-concentrate diets, undegraded phytate may pass into the duodenum; these residual phytates may impair zinc availability. In sheep consuming high-grain diets, low-phytate barley decreased the amount of undegraded phytate passing into the duodenum compared with conventional barley and increased zinc absorption and retention. This effect was not seen in lambs (Leytem et al., 2007).

According to Suttle (2010), horses are sensitive to the interaction between zinc and phytates, whereas they would be as efficient as ruminants in extracting zinc from roughages.

Phytate in vegetable ingredients reduces phosphorus availability in fish (Storebakken et al., 2000) and has been shown to reduce the availability of other minerals, including zinc (Gatlin and Phillips, 1989; Satoh et al., 1989; Denstadli et al., 2006) and magnesium (Denstadli et al., 2006). Catfish fed a diet containing 2.2 % phytic acid had significantly reduced feed efficiency and weight gain compared with fish fed a diet containing 1.1 % phytic acid. In fish fed diets containing 50 mg Zn/kg feed, the zinc concentration in vertebrae was significantly reduced from 133 mg/kg in control fish compared with 59 mg/kg in fish fed 2.2 % phytic acid (Helland et al., 2006).

4.2.2. Iron

Dietary zinc levels and organismal zinc status influence iron absorption. At the brush border membrane of the intestine, zinc inhibits divalent metal transporter-1 (DMT1), which is the principal uptake pathway for non-haem iron (Gunshin et al., 1997). However, at least in a cell model (Caco-2), this inhibition leads to a compensatory up-regulation of DMT1 expression (Yamaji et al., 2001). Additional free iron can be absorbed across zinc channels (SLC39), but again in competition with zinc (Jeong and Eide, 2013). At the systemic level, iron uptake is limited by hepcidin, a peptide synthesised and secreted by the liver in response to high iron levels (Fleming and Sly, 2001). Hepcidin decreases movement of iron across the enterocytes by causing ubiquitination and subsequent proteasomal degradation both of DMT1 (Brasse-Lagnel et al., 2011) and of ferroportin, which is the basolaterally located iron exporter that transports iron from the enterocyte to the circulation (Donovan et al., 2000). Therefore, a high dietary zinc intake is expected to reduce iron uptake, and low zinc to increase iron absorption. This interaction between zinc and iron has been confirmed *in vivo* for rainbow trout (Wekell et al., 1986), zebrafish (Zheng et al., 2013), rats (Kelleher and Lönnerdal, 2006), sheep (Grün et al., 1978) and humans (Olivares et al., 2012).

4.2.3. Copper

Copper inhibits zinc uptake in the intestine and zinc also inhibits absorption of copper (Hall et al., 1979; Hogstrand, 2011). This effect can be explained molecularly by strong interactions on expression and functions of metal-regulatory proteins. For example, copper is a potent inhibitor of zinc influx through SLC39 (Zrt-, Irt-like Protein, ZIP) zinc channels (Gaither and Eide, 2000; Qiu and Hogstrand, 2005). Also, an increase in cellular copper causes activation of the zinc-sensing transcription factor, MTF1, resulting in down-regulation of zinc importers, such as SLC39A10 (Zheng et al., 2008; Lichten et al., 2011), up-regulation of the zinc exporter SLC30A1 (Langmade et al., 2000) and up-regulation of the zinc-buffering protein, metallothionein (Westin and Schaffner, 1988).

Copper was found to be an inhibitor of zinc absorption in the rat (van Campen, 1969). In rats fed a semi-purified diet with a relatively low zinc content of 12 mg/kg and copper concentrations of 3, 24, 120 or 300 mg/kg, intestinal absorption of ^{65}Zn was decreased by 20 % when dietary copper was raised from 3 to 24 mg/kg with no further effect at higher concentrations (Hall et al., 1979). The interaction between copper and zinc was known at a practical level for pigs much earlier than this. Feeding diets with a high level of copper (250 mg Cu/kg diet) for growth stimulation (O'Hara et al., 1960; Suttle and Mills, 1966) results in parakeratosis and even mortality. Zinc supplementation was known to counteract parakeratosis in swine (Tucker and Salmon, 1955) and increasing zinc in the diet to 60 mg/kg together with iron solved the adverse effects caused by the 250 mg Cu/kg diet (O'Hara et al., 1960). The interaction between zinc and copper can also be seen if high dietary zinc concentrations are applied. Feeding sows with a diet containing 5000 mg Zn/kg resulted in the production of Cu-defficient piglets which could be cured by providing supplemental copper (Hill et al., 1983). In summary, high zinc levels in the diet can cause copper deficiency, and high copper levels can cause zinc deficiency.

4.2.4. Calcium

It is well established that zinc can and does permeate a variety of calcium channels, including voltage-gated calcium channels, transient receptor potential (TRP) channels and glutaminergic receptors (Bouron and Oberwinkler, 2013). In terms of calcium uptake, TRPV6 is the most relevant as it is responsible for intestinal calcium absorption in mammals (Hoenderop and Bindels, 2008) and branchial calcium absorption in fish (Qui and Hogstrand, 2004). TRPV6 from pufferfish was originally found to be highly permeable to Zn^{2+}, and this finding was recently confirmed to apply to mammalian TRPV5 and -6 orthologs (Qiu and Hogstrand, 2004; Kovacs et al., 2013). In fish, TRPV6 is expressed at the apical surface of gill cells and competition between zinc and calcium for this channel is the principal determinant of water chemistry modulation of zinc uptake and toxicity (Hogstrand, 2011). Furthermore, expression of TRPV6 in vertebrates is up-regulated by vitamin D_3 and intraperitoneal administration of 1α,25-$(OH)_2$-cholecalciferol (10 µg/kg) to rainbow trout increased zinc uptake and TRPV6 mRNA in gill tissue (Qiu et al., 2007). Supplementation of vitamin D increases dietary zinc uptake in chicken further supporting this overlap between calcium and zinc homeostasis (Roberson and Edwards, 1994; Biehl et al., 1995).

In addition to the competition between calcium and zinc for cellular access through calcium channels, there are some more complex interactions. For example, zinc appears to be a physiological regulator of the activity of the plasma membrane Ca^{2+}-ATPase (PMCA; Hogstrand et al., 1999) and the zinc exporter SLC30A1 is a regulator of plasma membrane residence of both L-type and T-type calcium channels (Levy et al., 2009; Mor et al., 2012). Thus, there are without doubt extensive interactions between calcium and zinc in biology including ion transport as well as cell signalling processes. Whether or not these interactions translate into a practically important influence of calcium on dietary zinc absorption is in many cases more debatable.

In 1955, nutritional zinc deficiency was induced in swine by a diet low in zinc; the resulting parakeratosis and poor growth were cured by supplementing the diet with zinc (Tucker and Salmon, 1955). High calcium and phytate content exacerbates parakeratosis (Lewis et al., 1956), suggesting that zinc uptake in pigs is inhibited by a high calcium:zinc ratio. Poultry is commonly considered less vulnerable to high calcium (Underwood, 1977; Suttle, 2010).

Findings regarding the consequences of increasing dietary calcium on the zinc availability in lambs are controversial. At low calcium levels, an influence of calcium on zinc availability was not found (5 to 8 g Ca/kg; Pond, 1983), while at even lower calcium levels serum zinc concentration was depressed with increasing calcium (2.5 to 5 g Ca/kg; Perry et al., 1968).

A significant body of literature exists on the bioavailability of zinc in fish feed; deficiency symptoms appear at either low dietary zinc levels or in cases of strong antagonistic effect of calcium and phosphorus on zinc absorption (Watanabe et al., 1997). Growth retardation and short body dwarfism, as well as cataracts are among the classical deficiency signs in rainbow trout, which can be induced by (i) omitting zinc supplementation of fish meal diets (Satoh et al., 1987a), (ii) using less available chemical zinc salts (Satoh et al., 1987c) or (iii) supplementing tri-calcium phosphate (Satoh et al., 1987b).

The addition of calcium to diets containing sodium phytate has been reported in rats and catfish to aggravate the antagonistic effect of phytates on zinc availability, whereas it does not influence zinc availability in the absence of sodium phytates (Oberleas et al., 1966; Satoh et al., 1989). Thus, the negative effect of calcium on zinc availability in phytate-containing diets is believed to be mediated by the formation of insoluble Ca-phytate-Zn complexes.

However, in practical diets, an effective role of calcium on phytates solubility is questionable. In humans given typical diets, excluding semi-synthetic diets, Miller et al. (2007) indicated that calcium probably does not affect zinc absorption. In pigs, Matsui et al. (1998b) observed that phytates in soybean flour do not interact with calcium added as carbonate. Through meta-analyses, Letourneau-Montminy et al. (2010, 2012) assessed the interaction of calcium with phytates and phytases in pigs and broiler diets. They concluded that calcium added to pigs or broilers diets up to 10 g/kg diet does not cause the insolubilisation of phytates, since calcium equally depresses the availability of phosphorus in diets with or without phytase. Rather, the negative effect of calcium on phosphorus availability is probably due to the formation of insoluble calcium-phosphate precipitates in the small intestine (Létourneau-Montminy et al., 2011). Consequently, in practical diets, the negative impact of calcium on zinc availability through the formation of these Ca-phytate-Zn complexes is probably limited.

Larsen and Sandström (1993) did not observe any modulation of zinc absorption in pigs given cereals-soybean meal diets containing 3 to 11 g Ca/kg. Similarly, bone zinc concentration of chickens was not modified when calcium was increased from 6 to 9 g/kg diet (Rama Rao et al., 2006). The negative effect of calcium on zinc availability was observed at calcium concentrations far above the usual levels of supplementation. Shafey et al. (1991) reported that 15 to 23 g Ca/kg diet increased pH in gizzard and in small intestine and reduced zinc solubility in the intestine in broilers. Similarly, in pigs given maize-soybean meal diets, increasing calcium concentration from 5 to 15 g/kg reduced liver zinc and serum alkaline phosphatase activity (Morgan et al., 1969). Thus, the interactions between calcium and zinc in the intestine are complex and far from well understood. It appears that very high levels of dietary calcium are required to impair zinc absorption; however, under most practical conditions it is unlikely that calcium in the diet substantially alters zinc uptake.

4.2.5. Role of fiber/non-starch polysaccharides

Fibers may impair zinc availability through the formation of insoluble chelates. They may also increase zinc endogenous losses by increasing cell sloughing in the intestine. However, the effect of fibers on zinc availability is sometimes difficult to investigate because some fibrous feedstuffs are also rich in phytates (e.g. cereals bran). In chicks, the incorporation of 8 % alfalfa cell walls in the diet depressed bone zinc and plasma zinc concentrations by 12 % (van der Aar et al., 1983); such an effect was not observed with pectin and cellulose. According to Mohanna et al. (1999), zinc availability in chicks may be depressed when intestinal viscosity is increased because of the presence of water-soluble non-starch polysaccharides (NSPs) in the diet. However, the introduction of 0.75 % gum guar reduced zinc availability by less than 4 %. Nevertheless, the authors concluded that the dietary addition of NSP-degrading enzymes may improve nutrient availability for broilers fed diets rich in NSPs, accompanied by an increase in zinc bioavailability (Mohanna et al., 1999). In pigs, there is no evidence of a significant effect of fibres (non soluble or water soluble) on zinc availability. In this context, it should be noted that the viscosity of digesta in poultry is about 10 times higher than in pigs (Bedford and Schultze, 1998). Spears (2003) indicated that the impact of fibres, to which most zinc in roughages is associated, on zinc availability is not well characterised.

4.3. Methods to improve the availability of zinc from feed materials

Ways to improve zinc bioavailability in broilers and piglets need to be focused on native zinc. Dietary interventions involve feed supplementation with microbial phytases (mainly 3-phytase, E.C. 3.1.3.8; optimal pH range 3–8; Nayini and Markakis, 1986; Eeckhout and Paepe, 1994), and the activation of endogenous phytases by germination/fermention of plant materials (Urbano et al., 2000; Masud et al., 2007). Another possibility consists in the use of organic acids to lower the pH of the digesta (Jongbloed et al., 2000). The use of feed materials low in phytic P, obtained either by classic or by modern plant breeding/selection techniques, should be mentioned as a further possibility.

4.3.1. Use of exogenous microbial phytase

Most studies dealing with the effect of microbial phytase on zinc availability were conducted with 3-phytase from *Aspergillus niger*. Microbial phytase may hydrolyse up to 35 and 50 % of the phytates in poultry and pig diets, respectively (Selle and Ravindran, 2007), liberating zinc from phytate. There is evidence of improved zinc availability by the use of microbial phytase in broilers (e.g. Thiel et al., 1993; Biehl et al., 1995; Yi et al., 1996; Mohanna and Nys, 1999b; Jondreville et al., 2007), in pigs (Pallauf et al., 1992, 1994; Lei et al., 1993; Adeola et al., 1995; Revy et al., 2004, 2006; Jondreville et al., 2005; Bikker et al., 2012a, b; Blank et al., 2012) and in fish (Cheng and Hardy, 2003; Laining et al., 2012). These experiments indicate that improvements in zinc availability are far lower in broilers than in pigs, with an equivalency of 5 mg zinc in broilers and 27–30 mg zinc from sulphate in piglets at 500 FTU as 3-phytase from *Aspergillus niger* (Jondreville et al., 2005, 2007; Revy et al., 2006). The low figure for chickens is confirmed by another recent meta-analysis conducted by Schlegel et al. (2013). In a meta-analysis based on 22 experiments with piglets and growing pigs, Jongbloed and Thissen (2010) reported that the equivalency values based on digested zinc at 500 and 1250 FTU/kg diet ranged from 9 to 25 mg Zn/kg and from 12 to 32 mg Zn/kg as zinc sulphate, respectively.

Schlegel et al. (2010, 2013) investigated the origin of the difference between pigs and broilers. Based on the amount of soluble zinc in the stomach/gizzard of animals given diets containing different amounts of phytic phosphorus with and without phytase, the authors concluded that, owing to the low pH, zinc dissociates from phytates in gizzard of broiler chickens, even if phytates are not hydrolysed. In contrast, the higher pH in pig stomach does not allow this dissociation and phytates must be hydrolysed before zinc can be released from phytates. As a consequence, zinc in plant feedstuffs would be naturally more available in broiler chickens than in pigs, even in the absence of phytase. This is in agreement with the low zinc requirements and with the low effect of phytase on zinc availability in chickens compared with pigs.

In summary, the effect of microbial phytase on zinc availability in broiler chicks, and probably in all poultry species, is not great enough to contribute to a proposal for reduced maximum contents of zinc in poultry feed. In contrast, in piglets, the effect of phytase is significant enough to support further reductions in the maximum zinc content. Most studies were conducted with piglets. Quantitative relations between phytase and available phytate zinc are not known for pigs for fattening and sows; however, the mode of action of phytase is the same as in piglets.

In fish, phytase activity is highly dependent on the pH of the gut. Unlike mammals, fish are either gastric or agastric, and hence, the action of dietary phytase varies from species to species. In comparison with poultry and swine production, the use of phytase in fish feed is still in an unproven stage (Kumar et al., 2012).

4.3.2. Activation of endogenous plant phytase by soaking/fermentation

It has been shown that soaking/fermentation of feed or feed ingredients, or the use of fermented liquid feeding (FLF), can initiate mobilisation of phosphorus from phytate by activation of endogenous/intrinsic grain phytase (Ilyas et al., 1995; Skoglund et al., 1997; Larsen et al., 1999; Carlson and Poulsen, 2003; Blaabjerg et al., 2010; Rojas and Stein, 2012). However, endogenous phytase activity (mainly 6-phytase, E.C. 3.1.3.26, optimal pH range 5–8; Appendix B, Table B1) as found in some feed materials (> 2500 FTU/kg in wheat, rye and cereal brans, 500–2500 FTU/kg in barley and triticale, 50–500 FTU/kg in maize, oats, sorghum, malt spouts and legumes, < 50 FTU/kg in cereal glutenfeeds and oilseed meals), is more susceptible to low pH and pepsin in the stomach and is rather heat labile, resulting in a reduced or even eliminated activity in heat-processed feeds (Pointillart, 1988; Jongbloed and Kemme, 1990). Therefore, only processes suvh as germination, soaking and FLF can fully exploit the potential of endogenous phytase activity.

4.3.3. Use of organic acids

The pH of digesta, especially in the proximal part of the digestive tract (stomach, gizzard), influences zinc solubility and, in turn, zinc availability. Thus, any method reducing the pH in the stomach may improve zinc availability in non-ruminant animals. In particular, the question arises whether organic acids would enhance the positive effect of phytase on zinc availability as they do for phosphorus in pigs (Kemme et al., 1999; Blank et al., 2012). However, published information on the effects of organic acids introduced in pig or chicken diets, with or without phytase, is inconclusive. Either organic acids do not improve (Brenes et al., 2003; Bikker et al., 2011; Blank et al., 2012; Swiatkiewicz and Arczewska-Wlosek, 2012) or improve only slightly (Höhler and Pallauf, 1993, 1994; Roth et al., 1998) zinc availability.

5. Newly proposed maximum total zinc contents in complete feed

The details of nutrient requirement data for the different target animals are usually taken into consideration when formulating a complete feed. Feed business operators base calculations for nutrients in diets on averages/medians —intended primarily not to fall below the animal's requirements—, and consider the maximum contents set by legislation. Maximum contents in feed are set for trace elements for different reasons, which include safety for the consumer, the target species and the environment. The request of the European Commission is mainly driven by environmental concerns and covers the appropriateness of the CMPC when compared with the requirements.

The proposal for a potential reduction of the CMPC has to consider the different aspects discussed in this document. The NPMC shall be high enough to ensure health, welfare and performance of healthy target animals. The NPMC must therefore be above requirements considering age, genetics and physiological state (growth, pregnancy, lactation, work).

The requirement (the individual demand under defined conditions (see Section 2.1); requirements for the target animals are described in Sections 2.1.1 to 2.1.7 and summarised in Table 7) is a mean. Thus, it would cover the demand of half the healthy individuals in a particular life stage and gender group. At this level of intake, the needs of other half of the individuals in any specified group would not be met. Therefore, the requirement alone does not suffice for use as the NPMC.

a) The principle of allowances in animal nutrition is equivalent to derivation of a Population Reference Intake (PRI)/Recommended Dietary Allowances (RDA) in humans. A PRI/RDA is the average daily dietary intake level that is sufficient to meet the nutrient requirement of nearly all (97.5 %) healthy individuals at a particular life stage and gender group (EFSA NDA Panel, 2010). Therefore, this approach can also be used to derive the NPMC.

   - If the distribution of requirements in the group is assumed to be normal, then the PRI/RDA can be calculated from the requirement and the standard deviation (SD) of requirements as follows: PRI/RDA= Requirement + 2 SD (EFSA NDA Panel, 2010; Health Canada[15]). However it is not possible to calculate an inter-individual variation in zinc requirement for all animal species and therefore variability has to be estimated.
   - If data about variability in requirements are insufficient to calculate a SD, a coefficient of variation (CV) for the requirements of 10 to 20 % can be assumed (EFSA NDA Panel, 2010). If 10 % is assumed to be the CV, then twice that amount when added to the requirements is defined as equal to the PRI/RDA. The resulting equation for the PRI/RDA is then

[15] Health Canada, Official webpage. Food and Nutrition. Dietary Reference Intakes. http://www.hc-sc.gc.ca/fn-an/nutrition/reference/table/index-eng.php

Allowance= 1.2 × requirement. This level of intake, statistically, covers the requirements of 97.5 % of the population.

- It is concluded that the zinc allowance is calculated from 1.2 times the requirement.

b) Allowances also have to take into consideration variation in the availability of zinc sources used for feed supplementation (see Section 4.1. Zinc-containing additives). Zinc oxide is one of the most commonly used forms of zinc supplementation in animal feed. Relative to zinc sulphate, availability of zinc oxide ranges from 22 to 99 %, with a CV of 35 %.

- It is concluded that zinc allowance in animal feed needs to include an additional 35 % of the requirement to account for differences in bioavailability, arriving at a final factor of 1.5 (1.2 + 0.35~ 1.5).

c) The NPMC should also consider the interactions of zinc with other nutrients, minerals or additives, if these have not already been taken into account when establishing requirements.

Because zinc in feed materials interacts with other nutrients and minerals, it is difficult to estimate bioavailabilty of zinc in the basal diet. Therefore, in practical feed formulations, it is assumed that zinc in the background feed is not available.

- It is concluded that the NPMC should allow for an additional zinc content in the magnitude of the zinc background. The background levels are derived from Section 3.2 (and the tables in Appendix C).

d) The NPMC should be feasible under the practical conditions of the feed manufacturing industry.

- This is considered by rounding the mathematically-derived NPMC to practical figures and thus, establishing three groups of NPMC.

e) The NPMC could also take into account intentionally improved zinc availability, e.g. by the action of phytases.

- This is considered by the introduction of lower NPMCs in case zinc availability is intentionally improved as a result of phytase addition or activation.

The NPMCs are summarised in Table 7; only the animal categories listed in this table, for which requirement data are reported in Section 2.1, could be considered.

Table 7: The newly proposed maximum zinc contents in complete feed for target animals. All figures are expressed in mg Zn/kg complete feed

Target species, animal category	R[1]	1.5 × R	Background	NPMC[2]
Chickens for fattening, reared for laying	40-50	60-75	30	100
Laying hens, breeder hens	45	67.5	30	100
Turkeys for fattening, 0-8 weeks of age	70	105	30	120
Turkeys for fattening, from 8 weeks of age onwards	50	75	30	120
Other poultry	40, 60, 60	80	30	100
Piglet, until 11 kg body weight	100	150	30	150
Piglet weaned, from 11 kg body weight onwards	80	120	30	150
Pigs for fattening	60	90	30	100
Sow	50-100	75-150	30	150
Calves – milk replacer	40	60	30	100
Cattle for fattening	35*	53	30	100
Dairy cows, dairy heifer	44*	66	30	100
Sheep	40*	60	30	100
Goat (dairy)	31*	47	30	100
Horses	44*	66	30	100
Rabbits	70	105	50	150
Salmonids	50	75	60	150
Other fish	20	30	60	100
Dogs		100**	70	150
Cats		75**	70	150

(1) R, requirement.
(2) NPMC, Newly Proposed total Maximum Contents of zinc in complete feed.
* Adjusted from dry matter to complete feed with 88 % dry matter.
** Allowance, taken as 1.5 times the requirement (see Table 5).

A substantial increase in zinc availability as a result of phytase action (either from supplemented or from endogenous and activated phytase) has been observed in pigs fed vegetable-based diets (see Section 4.3). If these options are considered in feed formulation/preparation (phytase activity of 500 FTU/kg feed), the NPMC could be further reduced, e.g. for pigs for fattening from 100 to 70 mg Zn and for piglets and sows from 150 to 110 mg Zn/kg complete feed.

6. Impact of the newly proposed reduced maximum zinc content in feed

Appendix E shows in graphs and tables the statistical analysis of the data collected by official feed controls in 22 European countries, in terms of zinc concentration in compound feed (on an *as is* basis). Chicken feed (Tables E1, E2 and E4) was in about 50 % of samples above the NPMC; for layers (Table E3) and turkeys for fattening (Table E5), it was about 30 %. The percentage of piglets (Table E6) and pigs for fattening (Table E7) feeds with concentrations above the NPMC is considerably higher (70 %). These figures for the commercially most relevant complete feed in food production indicate a high potential for zinc reduction in animal feed.

This potential reduction will be higher in pigs when phytases would be used; about 80–90 % of all feed samples showed zinc concentrations above the further reduced NPMC. All other complete feeds considered indicate a potential for reduction in zinc levels of a comparable magnitude to that described for chickens. Finally, it should be noted that the zinc concentration in about half of all pet food was about the NPMC.

Independent of the potential effect of NPMC on environmentally relevant zinc emissions, the NPMC would reduce the quantities of limited zinc-containing resources used in animal nutrition. This includes both food-producing and pet animals.

6.1. Health and welfare of the target animals

The introduction of the NPMC in the practice of the feed formulation will reduce the absolute zinc supply to animals (see above). The NPMC are markedly above the requirements and mostly in the range of industrial recommendations for the zinc content in complete feed. Consequently, no negative impact on health, welfare and productivity of target animals is expected.

Interactions with minerals, other trace elements and certain dietary constituents deserve increased attention when the use levels of dietary zinc are reduced. However, feed business operators have full access to the relevant databases which are used to calculate feed formulations on the basis of the most updated information.

6.2. Safety for the consumer

Dietary Reference Values (DRVs) have been established for zinc by various bodies, with the range 7−11 mg/day for adult males and 6–9 mg/day for adult females (UK Department of Health (DH, 1991); Netherlands Food and Nutrition Council, (1992); EC (1993); IOM (2001); D-A-CH (2013); Nordic Nutrition Recommendations (Nordic Council of Ministers, 2013)).

The Scientific Committee on Food (SCF) described the mean zinc intake of the European population as between 7.5 and 12 mg Zn/day, based on nutritional surveys (EC, 2003b). The 97.5th percentile in some countries (i.e. Austria and Ireland) was estimated to be higher than 20 mg and close to the tolerable upper intake level (UL), but this was not considered a matter of concern by the SCF. The SCF data, although collected in the 1990s, appear to describe a currently valid scenario when compared with more recent data (Flynn et al., 2009; Rubio et al., 2009; Turconi et al., 2009).

A consumption survey conducted in Germany in 2008 (Bundesministerium für Ernährung, Landwirstchaft und Verbraucherschutz, 2008) found that the median daily zinc intake among adult Germans was 11.6 and 9.1 mg in men and women, respectively. The corresponding 95th percentiles were 20.2 and 15.1 mg. The data for intake in children indicated that the median zinc daily intake of boys aged 6−11 years was 7.4–8.7 mg and that for girls of the same age group was 7.1–8.3 mg (Mensink et al., 2007; Ernährungsstudie als KiGGS: Der Kinder- und Jugendgesundheitssurvey-Modul (EsKiMo)). The upper 95th percentiles were 13.2 and 12.6 mg for boys and girls, respectively; the FEEDAP Panel notes that the figure for boys equals the UL set for 7- to 10-year-old children by the SCF (13 mg per person and day; EC, 2003b).

In all consumer groups, tissues and products of animal origin contributed to about 40–50 % of total zinc intake, with meat and milk being the two main items (Walsh et al., 1994; Mensink et al., 2007; Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz, 2008). On average, among all consumer groups, the contribution of milk and meat to the total zinc intake is nearly the same. The practice of supplementing animal feed with zinc-containing compounds has not essentially changed during the last decade. It is therefore reasonable to assume that food of animal origin recorded in the above-mentioned consumption surveys derived from animals fed zinc-supplemented diets. Zinc is regulated at the intestinal level in the target animals. With the exceptions of liver and kidney (Eisemann et al., 1979; Jenkins and Hidiroglou, 1991; Cao et al., 2000; Gallaher et al., 2000; Wright and Spears, 2004; see also review of Schlegel et al., 2013), zinc concentrations exceeding the requirements up to about 200 mg/kg feed will not result in a change of zinc concentrations in animal tissues (Jenkins and Hidiroglou, 1991), and other products including milk (Schwarz and Kirchgessner, 1975; Miller et al., 1989; Wiking et al., 2008; Peters and Mahan, 2008; Peters et al., 2010). Consequently, a reduction in dietary zinc in the range between requirements and 150 mg/kg feed will affect zinc concentration only in liver and kidney.

The estimated consequences of reducing dietary zinc in feed of animals on human intake can therefore be based on the zinc content of liver only. The differences in the zinc content of liver expected at dietary levels of the current maximum content and the NPMC are calculated following a linear regression equation, describing the relationship between dietary zinc and zinc deposition in pig liver

(Schlegel et al., 2013). Taking 60 g liver/person per day as the 95th percentile of the intake by a liver consumer (see Guidance on consumer safety; EFSA FEEDAP Panel, 2012e), 3 mg zinc would be consumed from liver obtained by feeding 150 mg zinc/kg diet (CAMC) and 2.4 mg zinc when feeding diets with 100 mg zinc (NPMC). The difference amounts to 0.6 mg/person per day. The average daily intake of adult consumers was 11.6 mg Zn for males and 9.1 mg for females (Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz, 2008). Reducing the values by 0.6 mg, the zinc intake would still be above the Population Reference Intake (as DRV, see above). There would be no influence on toddlers since there is no relevant liver consumption. This scenario contains several conservative elements: (i) high liver intake, (ii) maximum contents are taken to calculate the difference in zinc liver instead of average values, and (iii) maximum contents are assumed to persist over the period of feeding to be applied for consumer risk assessment.

6.2.1. Conclusions on safety for the consumer

The FEEDAP Panel concludes that the reduction of the CAMC for zinc for food-producing animals to the NPMC values has marginal consequences on the zinc intake and is of no concern for the safety of the consumer.

6.3. Benefits of the proposed reduction of maximum contents of zinc in feed to the environment

The FEEDAP Panel found no consolidated inventory of zinc environmental emission sources in the EU. The EU Risk Assessment Report on zinc (EU RAR Zinc metal, 2010), gives the zinc inputs from animal nutrition in the environmental system (14 MSs) with 14599 tonnes/year. In agricultural areas, spreading of manure on land appears to be a major source of zinc emission; according to the RAR, zinc emissions average 174 g Zn per hectare utilised agricultural area, with a range from 36 g (Austria) to 694 g/ha (The Netherlands). Agricultural activities cause more than 80 % of the emissions to soil in The Netherlands (Bodar et al., 2005). In England and Wales, livestock manure is responsible for an estimated 37 % of all zinc input across the whole agricultural land area (Nicholson et al., 2003). Bodar et al. (2005) additionally refer to data showing that in The Netherlands agricultural emissions to soil significantly contribute to zinc emissions to surface water.

The percentages of feed samples that presently have zinc contents higher than the NPMC range from about 15 (calves) to 70 % (pigs for fattening, Appendix E). Thus, reducing the CAMC to the NPMC would have an impact on the future dietary zinc concentration.

An estimate was made of the reduction in zinc input to the EU environment following the potential implementation of the NPMC for the highest production volume farm animals, namely poultry, pigs and bovines. The zinc input into animal production was calculated by using compound feed production data (FEFAC report, 2011[16]) and the mean zinc concentration of feed samples. The expected mean of the use levels after introduction of NPMC was calculated by ommiting all samples with values above the NPMC. It was further assumed that the zinc content in animal tissues and products was not substantially affected by the NPMC (see also Section 6.2). In consequence, the absolute reduction in the zinc input is equal to the quantity of the zinc output via manure.

The calculation had to be restricted to poultry and pigs only since the monitoring data collected on zinc levels in compound feed for dairy cows and cattle referred predominantly to complementary feed. The NPMC refer to complete feed and do not allow calculations with complementary feed.

It was calculated that the amount of zinc entering the EU environment per year via farm animal manure from pigs and poultry would be reduced by about 2300 tonnes (see Appendix F). Reducing zinc in feed for pigs for fattening would have the greatest impact, resulting in a 31 % reduction in zinc

[16] FEFAC statistics on Compound Feed Production in the EU: http://www.fefac.eu/publications.aspx?CategoryID=2061

emission from this animal category.[17] It deserves particular attention that the use of phytase, either from endogenous source or from a feed additive, in feeding pigs for fattening, which would allow a reduction of the NPMC from 100 to 70 mg, would result in a 53 % reduction in zinc emissions.[18]

Because of the assumptions made, these calculations might be considered a best-case scenario. On the other hand, reductions in zinc input to the environment would result also from the animal categories not included in the above estimate. Compound feed production from cattle and dairy cows amounted to about 25 % of total compound feed production. Assuming that the complementary feed would how a comparable reduction of zinc concentrations as introduced by the NPMC for complete feed, another 750 tonnes of zinc ((2300×25)/(100–25)) would not be used for feed supplementation. Comparing the total reduced zinc output of about 3000 tonnes with the total output of 14599 tonnes/year (EU RAR Zinc metal, 2010), a reduction of zinc emissions from animal production of about 20 % could be expected.

7. Conclusions

A critical review of (i) the zinc requirements of food-producing and pet animals, (ii) the zinc concentration of feed materials and (iii) the calculated background zinc concentration of complete feed supports the possibility of a considerable reduction of the currently authorised maximum contents for total zinc in feed.

The FEEDAP Panel developed, based on an approximation using zinc requirements and background data, potential new maximum contents, which could replace the current ones. The newly proposed total maximum contents of total zinc in complete feed (NPMC) are:

- 150 mg Zn/kg complete feed for piglets, sows, rabbits, salmonids, cats and dogs
- 120 mg Zn/kg complete feed for turkeys for fattening
- 100 mg Zn/kg complete feed for all other species and categories

The use of phytase, either from endogenous source or from a feed additive, in feeding piglets, pigs for fattening and sows would allow a further reduction of the NPMC by 30 % (from 150 to 110 mg Zn/kg feed for piglets and sows and from 100 to 70 mg Zn/kg feed for pigs for fattening).

The NPMC ensure health, welfare and productivity of the target species, and do not affect consumer safety.

The FEEDAP Panel expects that the introduction of the NPMC, provided they are applied in feeding practices, would result in an overall reduction of zinc emissions from animal production of about 20 %.

8. Remark

Interactions with minerals, other trace elements and certain dietary constituents deserve increased attention when formulating feed with reduced zinc content.

[17] It should be noted that the total zinc emissions of a pig during the production life time (6 to 110 kg bw) would decrease by 15 % if the piglet is not administered 2500 mg Zn/kg feed for the first two weeks post-weaning (AMCRA, 2012).

[18] These figures do not consider that probably more than 1000 tonnes of zinc/year are used for medical purpose in piglets production. For calculations: pig production in Europe (EURO-25 in 2008) was 248 millions heads; 30 % of which is produced in the basin between Denmark and Belgium. These piglets are assumed to be extensively fed and therefore feed with 2.5 g Zn/kg is used in the first 14 days after weaning. Daily feed consumption is given as 0.4 kg in the first week and 0.5 kg in the second week: zinc consumed amounts to 1312 tonnes per year.

DOCUMENTATION PROVIDED TO EFSA

1. Report on Zinc requirements of weaned pigs. Bikker et al. (2011).[19] Submitted by the European Commission.

2. Data from European Countries concerning "Allowance/Requirements levels of zinc for animal species, defined by national scientific bodies" and "Analyses of compound feed for all animal species/categories obtained during national official controls" received as reply to the ad-hoc questionnaires submitted to the Focal Points of the EFSA's Advisory Forum.

3. Data from Stakeholders concerning "Industry recommendation of zinc supplementation and zinc use level in all animal species categories in the EU" and "Typical composition of complete/complementary feed for all animal species/categories" received as reply to the ad-hoc questionnaires submitted to the stakeholders via the EFSA's stakeholder platform.

4. EFSA Internal Report. Dietary and Chemical Monitoring Unit. Technical assistance "Assistance in Data processing from Questionnaires received from Member States and Stakeholders in the context of Zinc in Feed". December 2013.

REFERENCES

Aarestrup FM, Cavaco L and Hasman H, 2010. Decreased susceptibility to zinc chloride is associated with methicillin resistant *Staphylococcus aureus* CC398 in Danish swine. Veterinary Microbiology, 142, 455–457.

Adeola O, Lawrence BV, Sutton AL and Cline TR, 1995. Phytase-Induced Changes in Mineral Utilization in Zinc-Supplemented Diets for Pigs. Journal of Animal Science, 73, 3384–3391.

Agersø Y, Hasman H, Cavaco LM, Pedersen K and Aarestrup FM, 2012. Study of methicillin resistant *Staphylococcus aureus* (MRSA) in Danish pigs at slaughter and in imported retail meat reveals a novel MRSA type in slaughter pigs. Veterinary Microbiology, 157, 246–250.

Agroscope, 2006. Apports alimentaires recommandés et tables de la valeur nutritive pour les ruminant. Available online: http://www.agroscope.admin.ch/futtermitteldatenbank/04834/index.html?lang=fr (accessed on June 2013).

Agroscope, 2009. Apports alimentaires recommandés et tables de la valeur nutritive pour les ruminant. Available online: http://www.agroscope.admin.ch/futtermitteldatenbank/04834/index.html?lang=fr (accessed on June 2013). Chapter 11, Apports alimentaires recommandés pour le mouton.

Agroscope, 2011. Apport en zinc chez le porc. ALP-Actuel n°40.

Ahola JK, Baker DS, Burns PD, Mortimer RG, Enns RM, Whittier JC, Geary TW and Engle TE, 2004. Effect of copper, zinc, and manganese supplementation and source on reproduction, mineral status, and performance in grazing beef cattle over a two-year period. Journal of Animal Science, 82, 2375–2383.

Allen JG, Masters HG, Peet RL, Mullins KR, Lewis RD, Skirrow SZ and Fry J, 1983. Zinc toxicity in ruminants. Journal of Comparative Pathology, 93, 363–377.

AMCRA (AntiMicrobial Consumption and Resistance in Animals), 2012. L'usage d'oxyde de zinc (ZnO) chez les porcelets sevrés en belgique en prévention de la diarrhée de sevrage. Available online: http://www.amcra.be/sites/default/files/bestanden/avis%20ZnO%20porcelets%20sevre%CC%81s-%20final%20-%20FR.pdf

[19] Refer to References list

Ammerman CB, Henry PR and Miles RD, 1998. Supplemental organically-bound mineral compounds in livestock nutrition. In: Recent advances in animal nutrition Eds Garnsworthy PC and Wiseman J. Nottingham University Press, Nottingham, UK.

Andreini C, Banci C, Bertini I and Rosato A, 2006. Counting the zinc-proteins encoded in the human genome. Journal of Proteome Research, 5, 196–201.

ANSES (French Agency for Food, Environmental and Occupational Health & Safety), 2013. Opinion of the on the use of zinc oxide in the diet of piglets at weaning to reduce the use of antibiotics. ANSES Opinion. Request No. 2012-SA-0067 Available online: http://www.anses.fr/sites/default/files/documents/ALAN2012sa0067Ra.pdf

Ao T, Pierce JJ, Power R, Dawson KA, Pescatore AJ, Cantor AH and Ford MJ, 2006. Evaluation of Bioplex Zn as an organic zinc source for chicks. International Journal of Poultry Science, 5, 808–811.

Aoyagi S and Baker DH, 1993. Nutritional evaluation of copper-lysine and zinc-lysine complexes for chicks. Poultry Science, 72, 165–171.

Apines MJ, Satoh S, Kiron V, Watanabe T, Nasu N and Fujita S, 2001. Bioavailability of amino acids chelated and glass embedded zinc to rainbow trout, *Oncorhynchus mykiss*, fingerlings. Aquaculture Nutrition, 7, 221–228.

Arelovich HM, Owens FN, Horn GW and Vizcarra, JA, 2000. Effects of supplemental zinc and manganese on ruminal fermentation, forage intake, and digestion by cattle fed prairie hay and urea. Journal of Animal Science, 78, 2972–2979.

Arrayet JL, Oberbauer AM, Famula TR, Garnett I, Oltjen JW, Imhoof J, Kehrli ME and Graham TW, 2002. Growth of Holstein calves from birth to 90 days: the influence of dietary zinc and BLAD status. Journal of Animal Science, 80, 545–552.

Baeverfjord G, Fjelldal PG, Albrektsen S, Hatlen B, Denstadli V, Ytteborg E, Takle H, Lock E-J, Berntssen MHG, Lundebye A-K, Åsgård T and Waagbø R, 2013. Mineral nutrition and bone health in farmed salmonids – a review. Aquaculture Nutrition (submitted).

Batal AB and Dale N, 2008. Feedstuffs September 10, p. 16. Available online: http://fdsmagissues.feedstuffs.com/fds/Reference_issue_2010/Reference_issue_2009/Section2_2008.pdf

Batal AB, Parr TM and Baker DH, 2001. Zinc bioavailability in tetrabasic zinc chloride and the dietary zinc requirement of young chicks fed a soy concentrate diet. Poultry Science, 80, 87–90.

Bedford MR and Schulze H, 1998. Exogenous enzymes for pigs and poultry. Nutrition Research Reviews, 11, 91–114.

Biehl RR, Baker DH and de Luca HF, 1995. α-Hydroxylated cholecalciferol compounds act additively with microbial phytase to improve phosphorus, zinc and manganese utilization in chicks fed soy-based diets. The Journal of Nutrition, 125, 2407–2416.

Bikker P, Jongbloed AW, Verheijen R, Binnendijk GP and van Diepen H, 2011. Zinc requirements of weaned pigs. Report 274. Wageningen UR Livestock Research, p. 31.

Bikker P, van Diepen JTM, Binnendijk GP and Jongbloed AW, 2012a. Phytase inclusion in pig diets improves zinc status but its effect on copper availability is inconsistent. Journal of Animal Science, 90 (Suppl. 4), 197–199.

Bikker P, Jongbloed AW and Thissen JTNM, 2012b. Meta-analysis of effects of microbial phytase on digestibility and bioavailability of copper and zinc in growing pigs. Journal of animal science, 90 Suppl 4, 134–136.

Blaabjerg K, Jørgensen H, Tauson A and Poulsen H, 2010. Heat-treatment, phytase and fermented liquid feeding affect the presence of inositol phosphates in ileal digesta and phosphorus digestibility in pigs fed a wheat and barley diet. Animal, 4, 1–10.

Blalock TL and Hill CH, 1988. Studies on the role of iron in zinc toxicity in chicks. Biological Trace Element Research, 17, 17–29.

Blank R, Naatjes M, Baum C, Kohling K, Ader P, Roser U and Susenbeth A, 2012. Effects of formic acid and phytase supplementation on digestibility and use of phosphorus and zinc in growing pigs. Journal of Animal Science, 90 (Suppl. 4), 212–214.

Bodar CWM, Pronk MEJ and Sijm DTHM, 2005. The European Union Risk Assessment on Zinc and Zinc Compounds: The Process and the Facts. Integrated Environmental Assessment and Management, 1, 301–319.

Bouron A and Oberwinkler J, 2013. Contribution of calcium-conducting channels to the transport of zinc ions. Pflügers Archiv - European Journal of Physiology. Published online: 30 May 2013.

Brasse-Lagnel C, Karim Z, Letteron P, Bekri S, Bado A and Beaumont C, 2011. Intestinal DMT1 cotransporter is down-regulated by hepcidin via proteasome internalization and degradation. Gastroenterology, 140, 1261–1271.

Brenes A, Viveros A, Arija I, Centeno C, Pizarro M and Bravo C, 2003. The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. Animal Feed Science and Technology, 110, 201–219.

Brewer GJ, Dick RD, Schall W, Yuzbas, Thomas J and Padgett G, 1992. Use of zinc acetate to treat copper toxicosis in dogs. Journal of American Veterinary Medical Association, 201, 564–568.

Bridges CH, and Harris ED, 1988. Experimentally induced cartilaginous fractures (osteochondritis dissecans) in foals fed low-copper diets. Journal of American Veterinary Medical Association, 193, 215–221.

Bridges CH and Mofitt PG, 1990. Influence of variable content of dietary zinc on copper metabolism of weanling foals. American Journal Veterinary Research, 51, 275–280.

Bundesministerium für Ernährung, Landwirstchaft und Verbraucherschutz, 2008. Nationale Verzehrs Studie II. Max Rubner-Institut. Available online: http://www.was-esse-ich.de/uploads/media/NVSII_Abschlussbericht_Teil_2.pdf

Cao J, Henry PR, Guo R, Holwerda RA, Toth JP, Littell RC, Miles RD and Ammerman CB, 2000. Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. Journal of Animal Science, 78, 2039–2054.

Carlson D and Poulsen H, 2003. Phytate degradation in soaked and fermented liquid feed – effect of diet, time of soaking, heat treatment, phytase activity, pH and temperature. Animal Feed Science and Technology, 103, 141–154.

Carlson D, Sehested J and Poulsen HD, 2006. Zinc reduces the electrophysiological responses in vitro to basolateral receptor mediated secretagogues in piglet small intestinal epithelium. Comprehensive Biochemistry and Physiology 144, 514–519.

Carlson D, Sehested J, Feng Z and Poulsen HD, 2007. Zinc is involved in regulation of secretion from intestinal epithelium in weaned piglets. Livestock Science, 108, 45–48.

Carlson MS, Hill GM and Link JE, 1999. Early- and traditionally weaned nursery pigs benefit from phase-feeding pharmacological concentrations of zinc oxide: effect on metallothionein and mineral concentrations, Journal of Animal Science, 77,1199–1207.

Cavaco LM, Hasman H, Stegger M, Andersen PS, Skov R, Fluit AC, Ito T and Aarestrup FM, 2010. Cloning and occurrence of czrC, a gene conferring cadmium and zinc resistance in Methicillin-Resistant *Staphylococcus aureus* CC398 isolates. Antimicrobial Agents and Chemotherapy, 54, 3605–3608.

Cavaco LM, Hasman H and Aarestrup FM, 2011. Zinc resistance of *Staphylococcus aureus* of animal origin is strongly associated with methicillin resistance. Veterinary Microbiology, 150, 344–348.

Cheng ZJJ and Hardy RW, 2003. Effects of extrusion and expelling processing, and microbial phytase supplementation on apparent digestibility coefficients of nutrients in full-fat soybeans for rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 218, 501–514.

Clearwater SJ, Farag AM and Meyer JS, 2002. Bioavailability and toxicity of dietborne copper and zinc to fish. Comparative Biochemistry and Physiology - Part C: Toxicology & Pharmacology 132, 269-313.

Cope CM, Mackenzie AM, Wilde D and Sinclair LA 2009. Effects of level and form of dietary zinc on dairy cow performance and health. Journal of Dairy Science, 92, 2128–2135.

Cunningham JE and Kovacic JP, 2009. The ubiquitous role of zinc in health and disease. Journal of Veterinary and Emergency Critical Care, 19, 215–240.

CVB (Centraal veevoederbureau), 2005. Handleiding Mineralenvooziening Rundvee, Schapen, Geiten. Centraal veevoederbureau, Lelystad, The Netherlands.

CVB (Centraal veevoederbureau), 2007. Feed Tables. Productschap Diervoeding. Centraal veevoederbureau, Lelystad, The Netherlands.

Cymbaluk NF, Smart ME, 1993. A review of possible metabolic relationships of copper to equine bone diseases. Equine Veterinary Journal, Suppl. 16, 19–26.

D-A-CH (Deutsche Gesellschaft für Ernährung, Österreichische Gesellschaft für Ernährung, Schweizerische Gesellschaft für Ernährungsforschung, Schweizerische Vereinigung für Ernährung), 2013. Referenzwerte für die Nährstoffzufuhr. Neuer Umschau Buchverlag, Neustadt an der Weinstraße, Germany, 292 pp.

Denstadli V, Skrede A, Krogdahl Å, Sahlstrøm S and Storebakken T, 2006. Feed intake, growth, feed conversion, digestibility, enzyme activities and intestinal structure in Atlantic salmon (*Salmo salar* L.) fed graded levels of phytic acid. Aquaculture, 256, 365–376.

Dewar, W A, Wight PA, Pearson RA and Gentle MJ, 1983. Toxic effects of high concentrations of zinc oxide in the diet of the chick and laying hen. British Poultry Science, 24, 397–404.

DH (Department of Health), 1991. Dietary reference values for food energy and nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy. HM Stationary Office, London, UK, 212 pp.

Donovan A, Brownlie A, Zhou Y, Shepard J, Pratt SJ, Moynihan J, Paw BH, Drejer A, Barut B, Zapata A, Law TC, Brugnara C, Lux SE, Pinkus GS, Pinkus JL, Kingsley PD, Palis J, Fleming MD, Andrews NC and Zon LI, 2000. Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. Nature, 403, 776–781.

Durand M and Kawashima R, 1980. Influence of minerals in rumen microbial digestion. In: Digestive Physiology and metabolism in the ruminant. Eds Ruckenbush Y and Thivend P. AVI Publ. Co., Westport, CT, USA, 375–408.

Eamens GJ, Macadam JF, Laing EA, 1984. Skeletal abnormalities in young horses associated with zinc toxicity and hypocuprosis. Australian Veterinary Journal, 91, 205–207.

EC (European Commission), 1993. Reports of the Scientific Committee for Food of the European Community. Thirty-first series. Nutrient and energy intakes for the European Community. Commission of the European Communities, Luxembourg. Available online: http://ec.europa.eu/food/fs/sc/scf/out89.pdf

EC (European Commission), 2003a. Opinion of the Scientific Committee for Animal Nutrition on the use of zinc in feedingstuffs. Available online: http://ec.europa.eu/food/fs/sc/scan/out120_en.pdf

EC (European Commission), 2003b. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Zinc. Available online: http://ec.europa.eu/food/fs/sc/scf/out177_en.pdf

Edwards HM and Baker DH, 1999. Bioavailability of zinc in several sources of zinc oxide, zinc sulphate and zinc metal. Journal of Animal Science, 77, 2730–2735.

Edwards HM and Baker DH, 2000. Zinc bioavailability in soybean meal. Journal of Animal Science, 78, 1017–1021.

Edwards HM, Boling SD, Emmert JL and Baker DH, 1998. Bioavailability of zinc in two zinc sulphate by-products of the galvanizing industry. Poultry Science, 77,1546–1549.

Eeckhout W and Paepe M, 1994. Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. Animal Feed Science and Technology, 47, 19–29.

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition, and Allergies), 2010. Scientific Opinion on principles for deriving and applying Dietary Reference Values. EFSA Journal 2010; 8(3):1458, 30 pp. doi:10.2903/j.efsa.2010.1458

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012a. Scientific Opinion on safety and efficacy of zinc compounds (E6) as feed additive for all species: zinc sulphate monohydrate, based on a dossier submitted by Grillo-Werke AG/EMFEMA. EFSA Journal 2012;10(6):2734, 23 pp. doi:10.2903/j.efsa.2012.2734

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012b. Scientific Opinion on safety and efficacy of zinc compounds (E6) as feed additive for all species: zinc sulphate monohydrate, based on a dossier submitted by Helm AG. EFSA Journal 2012;10(2):2572, 22 pp. doi:10.2903/j.efsa.2012.2572

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012c. Scientific Opinion on safety and efficacy of zinc compounds (E6) as feed additives for all animal species: Zinc chelate of amino acids hydrate, based on a dossier submitted by Zinpro Animal Nutrition Inc.. EFSA Journal 2012;10(3):2621, 22 pp. doi:10.2903/j.efsa.2012.2621

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012d. Scientific Opinion on safety and efficacy of zinc compounds (E6) as feed additive for all animal species: Zinc oxide, based on a dossier submitted by Grillo Zinkoxid GmbH/EMFEMA. EFSA Journal 2012;10(11):2970, 24 pp. doi:10.2903/j.efsa.2012.2970

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2012e. Guidance for establishing the safety of additives for the consumer. EFSA Journal 2012;10(1):2537, 12 pp. doi:10.2903/j.efsa.2012.2537

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), 2013. Scientific opinion on the characterisation of zinc compound 'Zinc chelate of amino acids, hydrate (Availa®Zinc)' as a feed additive for all animal species. EFSA Journal 2013;11(10):3369, 7 pp. doi:10.2903/j.efsa.2013.3369

Eid AE and Ghonim SI, 1994. Dietary zinc requirement of fingerling *Oreochromis niloticus*. Aquaculture, 119, 259–264.

Eisemann JH, Pond WG and Thonney ML, 1979. Effect of dietary zinc and copper on performance and tissue mineral and cholesterol concentrations in swine. Journal of Animal Science, 48, 1123–1128.

EU RAR (European Union Risk Assessment Report) Zinc metal, 2010. Available online: http://publications.jrc.ec.europa.eu/repository/bitstream/111111111/15064/1/lbna24587enn.pdf

Fadayifar A, Aliarabi H, Tabatabaei MM, Zamani P, Bahari A, Malecki M and Desfoulian AH, 2012. Improvement in lamb performance on barley based diet supplemented with zinc. Livestock Science, 144, 285–289.

Fard R, Heuzenroeder M and Barton M, 2011. Antimicrobial and heavy metal resistance in commensal enterococci isolated from pigs. Veterinary Microbiology, 148, 276–282.

FEDIAF (European Pet Food Industry Federation), 2012. Nutritional guidelines for complete and complementary pet food for cats and dogs. Brussels, Belgium.

Feng L, Tan LN, Liu Y, Jiang J, Jiang WD, Hu K, Li SH and Zhou XQ, 2011. Influence of dietary zinc on lipid peroxidation, protein oxidation and antioxidant defence of juvenile Jian carp (*Cyprinus caprio* var. Jian). Aquaculture Nutrition, 17, 875-882.

Feng Z, Carlson D and Poulsen HD, 2006. Zinc attenuates forskolin-stimulated electrolyte secretion without involvement of the enteric nervous system in small intestinal epithelium from weaned piglets. Comparative Biochemistry and Physiology, 145, 328–333.

Fleming RE and Sly WS, 2001). Hepcidin: A putative iron-regulatory hormone relevant to hereditary hemochromatosis and the anemia of chronic disease. Proceedings of the National Academy of Sciences of the USA, 98, 8160–8162.

Flynn A, Hirvonen T, Mensink GBM, Ocké MC, Serra-Majem L, Stos K, Szponar L, Tetens I, Turrini A, Fletcher R and Wildeman T, 2009. Intake of selected nutrients from foods, from fortification and from supplements in various European countries. Food and Nutrient Research, 53, 8–51.

Fosmire GJ, 1990. Zinc toxicity. American Journal of Clinical Nutrition, 51, 225–227.

Fountoulaki E, Morgane H, Rigos G, Antigoni V, Mente E, Sweetman J and Nengas I, 2010. Evaluation of zinc supplementation in European sea bass (*Dicentrarchus labrax*) juvenile diets. Aquaculture Research, 41, 208–216.

Froetschel MA, Martin AC, Amos HE and Evans JJ, 1990. Effects of zinc sulphate concentration and feeding frequency on ruminal prtotozoal numbers, fermentation patterns and amino acid passage in steers. Journal of Animal Science, 68, 2874–2884.

Fukada T and Kambe T, 2011. Molecular and genetic features of zinc transporters in physiology and pathogenesis, Metallomics, 3, 662–674.

Gaither LA and Eide DJ, 2000. Functional expression of the human hZIP2 zinc transporter. The Journal of Biological Chemistry, 275, 5560–5564.

Gallaher DD, Gallaher CM, Shulman S, McElhome A, Brokken KA and Shurson G, 2000. Biovailability of different sources of protected zinc. In: Trace elements in man and animals 10. Eds Roussel et al. Kluwer Academic/Plenum Publishers, New York, USA.

Garcia-Contreras A, De Loera Y, Garcia-Artiga C, Palomo A, Guevara JA, Herrera-Haro J, López-Fernández A, Johnston S and Gosálvez J, 2011. Elevated dietary intake of Zn-methionate is associated with increased sperm DNA fragmentation in the boar. Reproductive Toxicology, 86, 2582–2589.

Garg AK, Mudgal V and Dass RS 2008. Effect of organic zinc supplementation on growth, nutrient utilization and mineral profile in lambs. Animal Feed Science and Technology, 144, 82–96.

Gatlin DMI and Phillips HF, 1989. Dietary calcium, phytate and zinc interactions in channel catfish. Aquaculture, 79, 259–266.

Gatlin DMI, Phillips HF and Torrans EL, 1989. Effects of various levels of dietary copper and zinc on channel catfish. Aquaculture, 76, 127–134.

Gatlin DMI and Wilson RP, 1983. Dietary zinc requirement of fingerling channel catfish. Journal of Nutrition 113, 630–635.

Gatlin DMI and Wilson RP, 1984. Zinc supplementation of practical channel catfish diets. Aquaculture, 41, 31–36.

GfE (Gesellschaft für Ernährungsphysiologie), 1989. Energie- und Nährstoffbedarf Hunde. DLG Verlag, Frankfurt am Main, Deutschland.

GfE (Gesellschaft für Ernährungsphysiologie), 1994. Empfehlungen zur Energie- und Nährstoffversorgung der Pferde. DLG Verlag, Frankfurt am Main, Deutschland.

GfE (Gesellschaft für Ernährungsphysiologie), 1995. Empfehlungen zur Energie- und Nährstoffversorgung der Mastrinder. DLG Verlag, Frankfurt am Main, Deutschland.

GfE (Gesellschaft für Ernährungsphysiologie), 1999. Empfehlungen zur Energie- und Nährstoffversorgung der Legehennen und Masthühner. DLG Verlag, Frankfurt am Main, Deutschland.

GfE (Gesellschaft für Ernährungsphysiologie), 2001. Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. DLG Verlag, Frankfurt am Main, Deutschland.

GfE (Gesellschaft für Ernährungsphysiologie), 2003. Empfehlungen zur Energie- und Nährstoffversorgung der Ziegen. DLG Verlag, Frankfurt am Main, Deutschland.

GfE (Gesellschaft für Ernährungsphysiologie), 2004. Empfehlungen zur Energie- und Nährstoffversorgung der Mastputen. Proceedings of the Society of Nutrition Physiology 13, 199–233.

GfE (Gesellschaft für Ernährungsphysiologie), 2006. Empfehlungen zur Energie- und Nährstoffversorgung von Schweinen. DLG Verlag, Frankfurt am Main, Deutschland.

Grider A, Mouat MF, Mauldin EA and Casal ML, 2007. Analysis of the liver soluble proteome from bull terriers affected with inherited lethal acrodermatitis. Molecular Genetics and Metabolism, 92, 249–257.

Grün M, Anke M, Hennig A, Seffner W, Partschefeld M, Flachowsky and Groppel B, 1978. Überhöhte orale Eisengaben an Schafe. Archiv für Tierernaehrung, 28, 341–347.

Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, Nussberger S, Gollan JL and Hediger MA, 1997. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature, 388, 482–488.

Hall AC, Young BW and Bremner I, 1979. Intestinal metallothionein and the mutual antagonism between copper and zinc in the rat. Journal of Inorganic Biochemistry, 11, 57–66.

Hambidge M, 2000. Human zinc deficiency. Journal of Nutrition, 130, 1344S–1349S.

Hamre K, Srivastava A, Rønnestad I, Mangor-Jensen A. and Stoss J, 2008. Several micronutrients in the rotifer *Brachionus* sp. may not fulfil the nutritional requirements of marine fish larvae. Aquaculture Nutrition, 14, 51–60.

Hamre K, Yufera M, Rønnestad I, Boglione C, Conceicao LEC and Izquierdo M, 2013. Fish larval nutrition and feed formulation: knowledge gaps and bottlenecks for advances in larval rearing Reviews in Aquaculture, 5, 26–58.

Hamre K, Yufera M, Rønnestad I, Boglione C, Conceicao LEC and Izquierdo M, 2013. Fish larval nutrition and feed formulation: knowledge gaps and bottlenecks for advances in larval rearing Reviews in Aquaculture, 5, 26–58.

Han Y-K and Thacker PA, 2010. Effects of antibiotics, zinc oxide or a rare earth mineral-yeast product on performance, nutrient digestibility and serum parameters in weanling pigs. Asian-Australian Journal of Animal Science, 23, 1057–1065.

Hedemann MS, Jensen BB and Poulsen HD, 2006. Influence of dietary zinc and copper on digestive enzyme activity and intestinal morphology in weaned pigs. Journal of Animal Science, 84, 3310–3320.

Helland S, Denstadli V, Eckhard Witten P, Kjelde K, Storebakken T, Skrede A, Åsgård A and Bæverfjord G, 2006. Hyper dense vertebrae and mineral content in Atlantic salmon (*Salmo salar* L.) fed diets with graded levels of phytic acid. Aquaculture, 261, 603–614.

Henry PR, Littell RC and Ammerman CB, 1997. Effect of high dietary zinc concentration and length of zinc feeding on feed intake and tissue zinc concentration in sheep. Animal Feed Science and Technology, 66, 237–245.

Hensel P, 2010. Nutrition and skin diseases in veterinary medicine. Clinics of Dermatology, 28, 686–693.

Heo JM, Kim JC, Hansen CF, Mullan BP, Hampson DJ and Pluske JR, 2010. Effects of dietary protein level and zinc oxide supplementation on performance responses and gastrointestinal tract characteristics in weaner pigs challenged with an enterotoxigenic strain of *Escherichia coli*. Animal Production Science, 50, 827–836.

Hill GM and Miller ER, 1983. Effect of dietary zinc levels on the growth and development of the gilt. Journal of Animal Science, 57, 106–113.

Hill GM, Ku PK, Miller ER, Ullrey DE, Losty TA and O'Dell BL, 1983. A copper deficiency in neonatal pigs induced by a high zinc maternal diet. The Journal of Nutrition, 113, 867–872.

Hoenderop JGG and Bindels RJM, 2008. Calciotropic and Magnesiotropic TRP Channels. Physiology, 23, 32–40.

Hoffmann G, Jones PG, Biourge V, van den Ingh TSGAM, Mesu SJ, Bode P and Rothuizen j, 2009. Dietary management of hepatic copper accumulation in Labrador Retrievers. Journal of Veterinary Internal Medicine, 23, 957–963.

Hogstrand C, 2011. Zinc. In: Homeostasis and toxicology of essential metals. Eds Wood CM, Farrell AP, and Brauner CJ. Book Series: Fish Physiology, 31A. Academic Press, New York, USA, 135–200.

Hogstrand C, Verbost PM and Wendelaar Bonga SE, 1999. Inhibition of human erythrocyte Ca^{2+}-ATPase by Zn^{2+}. Toxicology, 133, 139–145.

Höhler D and Pallauf J, 1993. Effect of citric-acid added to a maize-soya-diet with or without Zn-supplementation on the availability of minerals. Journal of Animal Physiology and Animal Nutrition-Zeitschrift Fur Tierphysiologie Tierernahrung Und Futtermittelkunde, 69, 133–142.

Höhler D and Pallauf J, 1994. Effects of Zn-supply and addition of citric-acid to a maize-soya diet on the nutritional efficiency and absorption of minerals in piglets. Journal of Animal Physiology and Animal Nutrition-Zeitschrift Fur Tierphysiologie Tierernahrung Und Futtermittelkunde, 71, 189–199.

Højberg O, Canibe N, Poulsen HD, Hedemann MS and Jensen BB, 2005. Influence of dietary zinc oxide and copper sulfate on the gastrointestinal ecosystem in newly weaned piglets. Applied and Environmental Microbiology, May, 2267–2277.

Hossain S and Bertechini AG, 1993. Requirement of zinc for growing rabbits. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 45, 323–329.

Hu CH, Xiao K, Song J and Luan ZS, 2013. Effects of zinc oxide supported on zeolite on growth performance, intestinal microflora and permeability, and cytokines expression of weaned pigs. Animal Feed Science and Technology, 181, 65– 71.

Huang YL, Lu L, Luo X G, Liu B, 2007. An optimal dietary zinc level of broiler chicks fed a corn-soybean meal diet. Poultry Science, 86, 2582–2589.

Huang YL, Lu L, Xie JJ, Li SF, Li XL, Liu SB, Zhang LY, Xi L and Luo XG, 2013. Relative bioavailabilities of organic zinc sources with different chelation strengths for broilers fed diets with low or high phytate content. Animal Feed Science and Technology, 179, 144–148.

IFZZ (Instytut Fizjologii I Zywienia Zwierzat), 1993. Pigs Feeding Standards. Nutrition value of fodders. Wartość pokarmowa pasz. IFŻZ PAN Jabłonna, Omnitech Press, Warszawa.

IFZZ (Instytut Fizjologii I Zywienia Zwierzat), 1994a. Standards feeding of carnivorous and herbivorous animal fur nutritional value of feed: collective work. Wartość pokarmowa pasz. IFŻZ PAN Jabłonna, Omnitech Press, Warszawa.

IFZZ (Instytut Fizjologii I Zywienia Zwierzat), 1994b. Equine nutrition standards: dietary advice and nutritional value of feed: collective work. Wartość pokarmowa pasz. IFŻZ PAN Jabłonna, Omnitech Press, Warszawa.

IFZZ (Instytut Fizjologii I Zywienia Zwierzat), 2005. Poultry nutrition standards. Dietary advice and nutritional value of feed S., Rutkowski A. (ed). Wartość pokarmowa pasz. IFŻZ PAN Jabłonna, Omnitech Press, Warszawa.

Ilyas A, Hirabayashi T, Matsui H, Yano F, Yano T, Kikushima M, Takebe, Hakayaka K, 1995. A note on the removal of phytate in soybean meal using *Aspergillus usami*. Asian-australasian Journal of Animal Science, 8, 135–138.

INRA (Institut National de la Recherche Agronomique), 1989. L'alimentation des animaux monogastriques: porcs, lapins, volailles (2e edition), INRA Editions, France.

INRA (Institut National de la Recherche Agronomique), 2004. Tables of composition and nutritional value of feed materials. Wageningen Academic Publishers, The Netherlands & INRA, Paris, France.

INRA (Institut National de la Recherche Agronomique), 2012. Nutrition et alimentation des chevaux. Editions Quae, Versailles, France.

IOM (Institute of Medicine, Food and Nutrition Board), 2001. Dietary Reference Intakes: Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. National Academy Press. Washington DC, USA.

Jahanian R, Moghaddam HN and Rezaei A, 2008. Improved broiler chick performance by dietary supplementation of organic zinc sources. Asian-Australasian Journal of Animal, 21, 1348–1354.

Jamikorn U and Preedapattarapong T, 2008. Comparative effects of zinc methionylglycinate and zinc sulfate on hair coat characteristics and zinc concentration in plasma, hair, and stool of dogs. Thai Journal of Veterinary Medicine, 38, 9–16.

Janczyk P, Kreuzer S, Assmus J, Nöckler K and Brockmann GA, 2013. No protective effects of high-dosage dietary zinc oxide on weaned pigs infected with *Salmonella enterica* Serovar Typhimurium DT104. Applied and Environmental Microbiology, 79, 2914–2921.

Jenkins KJ and Hidiroglou M, 1991. Tolerance of the preruminant calf for excess manganese or zinc in milk replacer. Journal of Dairy Science, 74, 1047–1053.

Jeong J and Eide DJ, 2013. The SLC39 family of zinc transporters. Molecular Aspects of Medicine, 34, 612–619.

Jondreville C, Hayler R and Feuerstein D, 2005. Replacement of zinc sulphate by microbial phytase for piglets given a maize-soya-bean meal diet. Animal Science, 81, 77–83.

Jondreville C, Lescoat P, Magnin M, Feuerstein D, Gruenberg B and Nys Y, 2007. Sparing effect of microbial phytase on zinc supplementation in maize–soya-bean meal diets for chickens. Animal, 1, 804–811.

Jongbloed AW and Kemme P, 1990. Effect of pelleting mixed feeds on phytase activity and the apparent absorbability of phosphorus and calcium in pigs. Animal Feed Science and Technology, 28, 233–42.

Jongbloed A and Thissen J, 2010. Meta-analysis on quantification of the effect of microbial phytase on the bioavailability of copper and zinc in growing pigs and broilers. Internal Report 201003, Livestock Research Wageningen, The Netherlands, 39 pp.

Jongbloed AW, Mroz Z, van der Weij-Jongbloed R and Kemme PA, 2000. The effects of microbial phytase, organic acids and their interaction in diets for growing pigs. Livestock Production Science, 67, 113–122.

Jongbloed AW, Kemme PA, De Groote G, Lippens M and Meschy F, 2002. Bioavailability of Major and Trace Minerals. 1st ed. Brussels: EMFEMA-International Association of the European (EU) Manufacturers of Major, Trace and Specific Feed Mineral Materials, 113 pp.

Johnson SE, 2008. Breed-specific hepatopathies. Available from http://www.cincyvma.com/files/BreedHepatopathies.pdf

Kaya S, Kececi T and Haliloglu S, 2001. Effects of zinc and vitamin a supplements on plasma levels of thyroid hormones, cholesterol, glucose, and egg yolk cholesterol of laying hens. Research in Veterinary Science, 71, 135–139.

Kaya S, Ortatatli M and Haliloglu S, 2002. Feeding diets supplemented with zinc and vitamin A in laying hens: effects on histopathological findings and tissue mineral contents. Research in Veterinary Science, 73, 251–257.

Kelleher SL and Lönnerdal B, 2006. Mammary gland copper transport is stimulated by prolactin through alterations in Ctr1 and Atp7A localization. American Journal of Physiology, Regulatory, Integrative and Comparative Physiology, 291, 1181–1191.

Kemme PA, Jongbloed AW, Mroz Z, Kogut J and Beijnen AC, 1999. Digestibility of nutrients in growing-finishing pigs is affected by *Aspergillus niger* phytase, phytate and lactic acid levels. 2. Apparent total tract digestibility of phosphorus, calcium and magnesium and ileal degradation of phytic acid. Livestock Production Science, 58, 119–127.

Kim JC, Hansen CF, Mullan BP and Pluske JR, 2012. Nutrition and pathology of weaner pigs: nutritional strategies to support barrier function in the gastrointestinal tract. Animal Feed Science and Technology, 173, 3–16.

Knox D, Cowey CB and Adron JW, 1984. Effects of dietary zinc intake upon copper metabolism in rainbow trout (*Salmo gairdneri*). Aquaculture, 40, 199–207.

Kock G and Bucher F, 1997. Accumulation of zinc in rainbow trout (*Oncorhynchus mykiss*) after waterborne and dietary exposure. The Bulletin of Environmental Contamination and Toxicology, 58, 305-310.

Kornegay ET, 1996. Nutritional, environmental and economical considerations for using phytase in pig and poultry diets. In: International symposium on nutrient management of food animals to enhance the environment. Ed. Kornegay ET. CRC Press, 279–304.

Kovacs G, Montalbetti N, Franz MC, Graeter S, Simonin A and Hediger MA, 2013. Human TRPV5 and TRPV6: key players in cadmium and zinc toxicity. Cell Calcium, 4, 276–286.

Kumar V, Sinha AK, Makkar HPS, De Boeck G and Becker K, 2012. Phytate and phytase in fish nutrition. Journal of Animal Physiology and Animal Nutrition 96, 335–364.

Kury S, Dreno B, Bezieu S, Giraudet S, Kharfi M, Kamoun R and Moisan J, 2002. Identification of SLC39A4, a gene involved in acrodermatitis enteropathica. Nature Genetics, 31, 239–240.

Laining A, Ishikawa M, Koshio S, Lideman and Yokoyama S, 2012. Dietary inorganic phosphorus or microbial phytase supplementation improves growth, nutrient utilization and phosphorus mineralization of juvenile red sea bream, *Pagrus major*, fed soybean-based diets. Aquaculture Nutrition, 18, 502–511.

Langmade SJ, Ravindra R, Daniels PJ and Andrews GK, 2000. The transcription factor MTF-1 mediates metal regulation of the mouse ZnT1 gene. The Journal of Biological Chemistry, 275, 34803–34809.

Larsen T and Sandstrom B, 1993. Effect of dietary calcium level on mineral and trace-element utilization from a rapeseed (*Brassica napus* L.) diet fed to ileum-fistulated pigs. British Journal of Nutrition, 69, 211–224.

Larsen T, Skoglund E, Sandberg A and Engberg R, 1999. Soaking and pelleting of pig diets alters the apparent absorption and retention of minerals. Canadian Journal of Animal Science, 79, 477–483.

Lei XG, Ku PK, Miller ER, Ullrey DE and Yokoyama, M.T., 1993. Supplemental microbial phytase improves bioavailability of dietary zinc to weanling pigs. Journal of Nutrition, 123, 1117–1123.

Letourneau-Montminy MP, Narcy A, Lescoat P, Bernier JF, Magnin M, Pomar C, Nys Y, Sauvant D and Jondreville C, 2010. Meta-analysis of phosphorus utilisation by broilers receiving corn-soyabean meal diets: influence of dietary calcium and microbial phytase. Animal, 4, 1844–1853.

Letourneau-Montminy MP, Narcy A, Lescoat P, Magnin M, Bernier JF, Sauvant D, Jondreville C and Pomar C, 2011. Modeling the fate of dietary phosphorus in the digestive tract of growing pigs. Journal of Animal Science, 89, 3596–3611.

Letourneau-Montminy MP, Jondreville C, Sauvant D and Narcy A, 2012. Meta-analysis of phosphorus utilization by growing pigs: effect of dietary phosphorus, calcium and exogenous phytase. Animal, 6, 1590–1600.

Levy S, Beharier O, Etzion Y, Mor M, Buzaglo L, Shaltiel L, Gheber LA, Kahn J, Muslin AJ, Katz A, Gitler D and Moran A, 2009. Molecular basis for zinc transporter 1 action as an endogenous inhibitor of L-type calcium channels. The Journal of Biological Chemistry, 284, 32434–3243.

Lewis PK, Hoekstra WG, Grummer RH and Phillips PH, 1956. The effect of certain nutritional factors including calcium, phosphorus, and zinc on parakeratosis in swine. Journal of Animal Science, 15, 741–751.

Leytem AB, Taylor JB, Raboy V and Plumstead PW, 2007. Dietary low-phytate mutant-M 955 barley grain alters phytate degradation and mineral digestion in sheep fed high-grain diets. Animal Feed Science and Technology, 138, 13–28.

Li BT, Van Kessel AG, Caine WR, Huang SX and Kirkwood RN, 2001. Small intestinal morphology and bacterial populations in ileal digesta and feces of newly weaned pigs receiving a high dietary level of zinc oxide. Canadian Journal of Animal Science, 81, 511–516.

Li YC, Ledoux DR and Veum TL, 2001. Low phytic acid barley improves performance, bone mineralization, and phosphorus retention in turkey poults. Journal of Applied Poultry Research, 10, 178–185.

Liang JJ, Yang HJ, Liu YJ, Tian LX and Liang GY, 2012. Dietary zinc requirement of juvenile grass carp (*Ctenophayngodon idella*) based on growth and mineralization. Aquaculture Nutrition, 18, 380–387.

Lichten LA and Cousins RJ, 2009. Mammalian zinc transporters: nutritional and physiologic regulation. Annual Review of Nutrition, 29, 153–176.

Lichten LA, Ryu MS, Guo L, Embury J and Cousins RJ, 2011. MTF-1-mediated repression of the zinc transporter Zip10 is alleviated by zinc restriction. PLoS One, 6(6), e21526.

Lin Y-H, Jiang LC and Shiau S-Y, 2008. Dietary zinc requirements of juvenile hybrid Tilapia, *Oreochromis niloticus* × *O. aureus*. Journal of the Fisheries Society of Taiwan, 35, 117-125.

Linares LB, Broomhead JN, Guaiume EA, Ledoux DR, Veum TL and Raboy V., 2007. Effects of low phytate barley (*Hordeum vulgare* L.) on zinc utilization in young broiler chicks. Poultry Science, 86, 299–308.

Lönnerdal B and Kelleher SL, 2007. Iron metabolism in infants and children. Food and Nutrition Bulletin, 28, S491–S499.

Lönnerdal B, Mendoza C, Brown KH, Rutger JN and Raboy V, 2011. Zinc absorption from low phytic acid genotypes of maize (*Zea mays* L.), barley (*Hordeum vulgare* L.), and rice (*Oryza sativa* L.) assessed in a suckling rat pup model. Journal of Agricultural and Food Chemistry, 59, 4755–4762.

Lu J and Combs GFJr, 1988. Effect of excess dietary zinc on pancreatic exocrine function in the chick. Journal of Nutrition, 118, 681–689.

Lu JX, Combs GFJr and Fleet JC, 1990. Time-course studies of pancreatic exocrine damage induced by excess dietary zinc in the chick. Journal of Nutrition, 120, 389–397.

Luo Z, Tan XY, Zheng JL, Chen QL and Liu CX, 2011. Quantitative dietary zinc requirement of juvenile yellow catfish *Pelteobagrus fulvidraco*, and effects on hepatic intermediary metabolism and antioxidant responses. Aquaculture, 319, 150–155.

Maage A and Julshamn K, 1993. Assessment of zinc status in juvenile Atlantic salmon (*Salmo salar*) by measurement of whole body and tissue levels of zinc. Aquaculture, 117, 179–191.

Maage A, Lorentzsen M, Bjornevik M and Julshamn K, 1991. Zinc requirement of Atlantic salmon (*Salmon salar*) fry during start feeding. In: Fish Nutrition in Practice, ed. INRA, Paris, Les Colloques no. 61.

Maage A, Julshamn K and Berge GE, 2001. Zinc gluconate and zinc sulphate as dietary zinc sources for Atlantic salmon. Aquaculture Nutrition, 7, 183–187.

Mandal GP and Dass RS, 2010. Haemato-biochemical profile of crossbred calves supplemented with inorganic and organic sources of zinc. Indian Journal of Animal Research 44, 197–200.

Mandal GP, Dass RS, Isore DP, Garg AK and Ramb GC, 2007. Effect of zinc supplementation from two sources on growth, nutrient utilization and immune response in male crossbred cattle (*Bos indicus*×*Bos taurus*) bulls. Animal feed Science and Technology, 138, 1–12.

Maret W, 2013. Zinc biochemistry: From a single zinc enzyme to a key element of life. Advances in Nutrition 4, 82–91.

Maret W, 2014. Zinc and human disease. In: Interrelations between essential metal ions and human diseases. Volume 13 of Metal Ions in Life Sciences. Eds Sigel A, Sigel H and Sigel RKO. Springer Science + Business Media B.V., Dordrecht, The Netherlands, 389–414.

Maret W and Sandstead HH, 2006. Zinc requirements and the risks and benefits of zinc supplementation, Journal of Trace Elements in Medicine and Biology, 20, 3–18.

Martin L, Lodemann U, Bondzio A, Gefeller E-M, Vahjen W, Aschenbach JR, Zentek J and Pieper R, 2013. A high amount of dietary zinc changes the expression of zinc transporters and metallothionein in jejunal epithelial cells in vitro and in vivo but does not prevent zinc accumulation in jejunal tissue of piglets. The Journal of Nutrition 143, 1205–1210.

Masud T, Mahmood T, Latif A, Sammi S, Hameed T, 2007. Influence of processing and cooking methodologies for reduction of phytic acid content in wheat (*Triticum aestivum*) varieties. Journal of Food Processing and Preservation, 31, 583–594.

Mateos GG and de Blas C, 1998. Minerals, vitamins and additives. In: C de Blas and J Wiseman (eds.). Nutrition of the rabbit. CABI Publishing, Wallingford, UK, 145–175.

Matsui T, Susaki H, Tamura A, Yano H, Nakajima T, Matsuda M. and Yano F, 1998a. The improvement of zinc bioavailability in fermented soybean meal in growing pigs. Animal Feed Science and Technology, 69, 589–591.

Matsui T, Kawakita Y and Yano H, 1998b. Phytate in soybean flour does not affect ionized calcium concentration in the digesta of small intestine of piglets. Nutrition Research 18, 1907–1914.

Mavromichalis I, Webel DM, Parr EN and Baker DH, 2001. Growth-promoting efficacy of pharmacological doses of tetrabasic zinc chloride in diets for nursery pigs. Canadian Journal of Animal Science, 81, 387–391

McClain WR and Gatlin DMI, 1988. Dietary zinc requirement of *Oreochromis aureus* and effects of dietary calcium and phytate on zinc bioavailability. Journal of The World Aquaculture Society, 19, 103–108.

Mensink GBM, Heseker H, Richter A, Stahl A, Vohmann, Fischer J, Kohler S and Six J, 2007. Ernährungsstudie als KiGGS-Modul (EsKiMo). Robert Koch-Institut and Univertität Paderborn, Germany.

Meyer H and Coenen M, 2002. Pferdefütterung, 4th edn. Blackwell Wissenschaftsverlag, Berlin, Germany, 59.

Miller WJ, Amos HE, Gentry RP, Blackmon DM, Durrance RM, Crowe CT, Fileding AS and Neathery MW, 1989. Long-term feeding of high zinc sulphate diets to lactating and gestating dairy cows. Journal of Dairy Science, 72, 1499-1508.

Miller LV, Krebs NF and Hambidge KM, 2007. A mathematical model of zinc absorption in humans as a function of dietary zinc and phytate. Journal of Nutrition, 137, 135–141.

Mohanna C and Nys Y, 1999a. Effect of dietary zinc content and sources on the growth, body zinc deposition and retention, zinc excretion and immune response in chickens. British Poultry Science, 40, 108–114.

Mohanna C and Nys Y, 1999b. Changes in zinc and manganese availability in broiler chicks induced by vegetal and microbial phytases. Animal Feed Science and Technology, 77, 241–253.

Mohanna C, Carre B and Nys Y, 1999. Incidence of dietary viscosity on growth performance and zinc and manganese bioavailability in broilers. Animal Feed Science and Technology, 77, 255–266.

Molist F, Hermes RG, Gómez de Segura A, Martín-Orúe SM, Gasa J, Garcia Manzanilla E and Pérez JF, 2011. Effect and interaction between wheat bran and zinc oxide on productive performance and intestinal health in post-weaning piglets. British Journal of Nutrition, 105, 1592–1600.

Monteiro SC, Lofts S and Boxall ABA, 2010. Pre-assessment of environmental impact of zinc and copper used in animal nutrition. Scientific/Technical Report submitted to EFSA. Available from http://www.efsa.europa.eu/en/supporting/pub/74e.htm

Moodley A, Søren Saxmose Nielsen SS and Guardabassi L, 2011. Effects of tetracycline and zinc on selection of methicillin-resistant *Staphylococcus aureus* (MRSA) sequence type 398 in pigs. Veterinary Microbiology 152, 420–423.

Mor M, Beharier O, Levy S, Kahn J, Dror S, Blumenthal D, Gheber LA, Peretz A, Katz A, Moran A and Etzion Y, 2012. ZnT-1 enhances the activity and surface expression of T-type calcium channels through activation of Ras-ERK signaling. American Journal of Physiology - Cell Physiology, 303, 192–203.

Moren M, Suontama J, Hemre G-I, Karlsen O, Olsen RE, Mundheim H and Julshamn K, 2006. Element concentrations in meals from krill and amphipods. Possible alternative protein sources in complete diets for farmed fish. Aquaculture, 261, 174–181.

Morgan DP, Young EP, Earle IP, Davey RJ and Stevenson JW, 1969. Effects of dietary calcium and zinc on calcium, phosphorus and zinc retention in swine. Journal of animal science, 29, 900–905.

Mount DR, Barth AK, Garrison TD, Barten KA and Hockett JR, 1994. Dietary and waterborne exposure of rainbow trout (*Oncorhynchus mykiss*) to copper, cadmium, lead and zinc using a live diet. Environmental Toxicology and Chemistry, 13, 2031–2041.

MTT (Maa- ja elintarviketalouden tutkimuskeskus), 2013. Available online: https://portal.mtt.fi/portal/page/portal/Rehutaulukot/feed_tables_english/nutrient_requirements (Accessed on 30.10.2013).

Nayini N and Markakis P, 1986. Phytases. In: Phytic acid: chemistry and applications. Ed. EGraf E. Pilatus Press, Minneapolis, MN, USA, 101–118.

Nessrin S, Abdel-Khalek AM and Gad SM, 2012. Effect of supplemental zinc, magnesium or iron on performance and some physiological traits of growing rabbits. Asian Journal of Poultry Sciences, 6, 23–30.

Netherlands Food and Nutrition Council, 1992. Recommended dietary allowances 1989 in The Netherlands. The Hague, The Netherlands, 115 pp.

Nicholson FA, Smith SR, Alloway BJ, Carlton-Smith C and Chambers BJ, 2003. An inventory of heavy metals inputs to agricultural soils in England and Wales. The Science of the Total Environment, 311, 205–219.

Nielsen FH, 2012. History of zinc in agriculture. Advances in Nutrition 3, 783–789.

Nockels CF, Debonis J and Torrent J, 1993. Stress Induction Affects Copper and Zinc Balance in Calves Fed Organic and Inorganic Copper and Zinc Sources. Journal of Animal Science, 71, 2539–2545.

Nordic Council of Ministers, 2013. Nordic Nutrition Recommendations 2012. Part 1. Summary, principles and use. 85 pp.

NRC (National Research Council), 1980. Mineral Tolerance of Domestic Animals. National Academies Press, Washington, DC, USA.

NRC (National Research Council), 1994. Nutrient Requirements of Poultry. 9th revised edition. National Academy Press, Washington, DC, USA.

NRC (National Research Council), 2000. Nutrient Requirements of Beef Cattle. 7th revised edition. National Academy Press, Washington, DC, USA.

NRC (National Research Council), 2001. Nutrient Requirements of Dairy cattle. 7th revised edition. National Academy Press, Washington, DC, USA.

NRC (National Research Council), 2005. Mineral Tolerance of Domestic Animals. 2nd revised edition. National Academy Press, Washington, DC, USA.

NRC (National Research Council), 2006. Nutrient Requirements of Dogs and Cats. National Academy Press, Washington, DC, USA.

NRC (National Research Council), 2007a. Nutrient Requirements of Small Ruminants. National Academy Press, Washington, DC, USA.

NRC (National Research Council), 2007b. Nutrient Requirements of Horses. 6th revised edition. National Academy Press, Washington, DC, USA.

NRC (National Research Council), 2011. Nutrient Requirements of Fish and Shrimp. National Academy Press, Washington, DC, USA.

NRC (National Research Council), 2012. Nutrient Requirements of Swine. 11th revised edition. National Academy Press, Washington, DC, USA.

Nunnery GA, Vasconcelos JT, Parsons CH, Salyer GB, Defoor PJ, Valdez FR and Galyean ML, 2007. Effects of source of supplemental zinc on performance and humoral immunity in beef heifers. Journal of Animal Science, 85, 2304–2313.

Oberleas D, Muhrer ME, and O’Dell BL, 1962. Effects of phytic acid on zinc availability and parakeratosis in swine. Journal of Animal Science, 21, 57–61.

Oberleas D, Muhrer ME and O'Dell BL, 1966. Dietary metal-complexing agents and zinc availability in the rat. The Journal of Nutrition, 90, 56–62.

O'Dell BL and Savage JE, 1960. Effect of phytic acid on zinc availability. Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, N.Y.), 103, 304–306.

Ogino C and Yang GY, 1978. Mineral requirements in fish .4. Requirement of rainbow trout for dietary zinc. Bulletin of the Japanese Society for the Science of Fish, 44, 1015–1018.

Ogino C and Yang GY, 1979. Requirements of carp for dietary zinc. Bulletin of the Japanese Society for the Science of Fish, 45, 967–969.

O'Hara PJ, Newman AP and Jackson R, 1960. Parakeratosis and copper poisoning in pigs fed a copper supplement. The Australian Veterinary Journal, 36, 225.

Olivares M, Pizarro F, Ruz M and de Romaña DL, 2012. Acute inhibition of iron bioavailability by zinc: studies in humans. Biometals, 25, 657–664.

Oteiza PI, 2012. Zinc and the modulation of redox homeostasis. Free Radical Biology and Medicine, 53, 1248–1259.

Overnell J, Fletcher TC and McIntosh R, 1988a. The apparent lack of effect of supplementary dietary zinc on zinc metabolism and metallothionein concentrations in the turbot, *Scophthalmus maximus* (Linnaeus). Journal of Fish Biology, 33, 563–570.

Overnell J, Fletcher TC and McIntosh R, 1988b. Factors affecting hepatic metallothionein levels in marine flatfish. Marine Environmental Research, 24, 155–158.

Pallauf J, Hohler D and Rimbach G, 1992. Effect of microbial phytase supplementation to a maize-soya-diet on the apparent absorption of Mg, Fe, Cu, Mn and Zn and parameters of Zn-status in piglets. Journal of Animal Physiology and Animal Nutrition-Zeitschrift Fur Tierphysiologie Tierernahrung Und Futtermittelkunde, 68, 1–9.

Pallauf J, Rimbach G, Pippig S, Schindler B, Hohler D and Most E, 1994. Dietary-effect of phytogenic phytase and an addition of microbial phytase to a diet based on field beans, wheat, peas and barley on the utilization of phosphorus, calcium, magnesium, zinc and protein in piglets. Zeitschrift Fur Ernahrungswissenschaft, 33, 128–135.

Paulicks BR, Ingenkamp H and Eder K, 2011. Bioavailability of two organic forms of zinc in comparison to zinc sulphate for weaning pigs fed a diet composed mainly of wheat, barley and soybean meal. Archives of Animal Nutrition, 65, 320–328.

Pérez VG, Waguespack AM, Bidner TD, Southern LL, Fakler TM, Ward TL, Steidinger M and Pettigrew JE, 2011. Additivity of effects from dietary copper and zinc on growth performance and fecal microbiota of pigs after weaning. Journal of Animal Science, 89, 414–425.

Perry TW, Beeson WM, Smith WH and Mohler MT, 1968. Value of zinc supplementation of natural rations for fattening beef cattle. Journal of Animal Science, 27, 1674–1677.

Peters JC and Mahan DC, 2008. Effects of dietary organic and inorganic trace mineral levels on sow reproductive performances and daily mineral intakes over six parities. Journal of Animal Science, 86, 2247–2260.

Peters JC, Mahan DC, Wiseman TG and Fastinger ND, 2010. Effect of dietary organic and inorganic micromineral source and level on sow body, liver, colostrum, mature milk, and progeny mineral compositions over six parities. Journal of Animal Science, 88, 626–637.

Pieper R, Vahjen W, Neumann K, Van Kessel AG and Zentek J, 2012. Dose-dependent effects of dietary zinc oxide on bacterial communities and metabolic profiles in the ileum of weaned pigs, Journal of Animal Physiology and Animal Nutrition, 96, 825–833.

Pointillart A, 1988. Phytate phosphorus utilisation in growing pigs. In: Digestive Physiology in the Pig. Proceedings of the Fourth International Seminar. Eds Buraczewska L, Buraczewska S and Zebrowska T. Polish Academy of Science, Jablonna, Poland, 192–196.

Pond WG, 1983. Effect of dietary calcium and zinc levels on weight-gain and blood and tissue mineral concentrations of growing Columbia-sired and Suffolk-sired lambs. Journal of Animal Science, 56, 952–959.

Poulsen HD, 1989. Zinc oxide for weaned pigs. Proceedings of the 40th Annual Meeting of the European Association of Animal Production, Dublin, Ireland. EAAP Publications, 265–266.

Poulsen HD, 1995. Zinc oxide for weanling pigs. Acta Agriculturae Scandinavica Section A, 45, 159−167.

Qiu A and Hogstrand C, 2004. Functional characterisation and genomic analysis of an epithelial calcium channel (ECaC) from pufferfish, *Fugu rubripes*. Gene, 342, 113–123.

Qiu A and Hogstrand C, 2005. Functional expression of a low-affinity zinc uptake transporter (FrZIP2) from pufferfish (Takifugu rubripes) in MDCK cells. Biochemical Journal 390, 777−786.

Qiu A, Glover CN and Hogstrand C, 2007. Regulation of branchial zinc uptake by 1alpha,25-(OH)(2)D(3) in rainbow trout and associated changes in expression of ZIP1 and ECaC. Aquatic Toxicology, 84, 142−152.

Rama Rao SV, Raju M, Reddy MR and Pavani P, 2006. Interaction between dietary calcium and non-phytate phosphorus levels on growth, bone mineralization and mineral excretion in commercial broilers. Animal Feed Science and Technology, 131, 133–148.

Revy PS, Jondreville C, Dourmad JY, Guinotte F and Nys Y, 2002. Bioavailability of two sources of zinc in weanling pigs. Animal Research, 51, 315–326.

Revy PS, Jondreville C, Dourmad JY and Nys Y, 2003. [Zinc in pig nutrition: the essential trace element and potential adverse effect on environment]. INRA Productions Animales, 16, 3–18.

Revy PS, Jondreville C, Dourmad JY and Nys Y, 2004. Effect of zinc supplemented as either an organic or an inorganic source and of microbial phytase on zinc and other minerals utilisation by weanling pigs. Animal Feed Science and Technology, 116, 93–112.

Revy PS, Jondreville C, Dourmad JY and Nys Y, 2006. Assessment of dietary zinc requirement of weaned piglets fed diets with or without microbial phytase. Journal of Animal Physiology and Animal Nutrition, 90, 50–59.

Rimbach G, Pallauf J, Brandt K and Most E, 1995. Effect of phytic acid and microbial phytase on cd accumulation, Zn status, and apparent absorption of Ca, P, Mg, Fe, Zn, Cu, and Mn in growing rats. Annals of Nutrition and Metabolism, 39, 361–370.

Rink L, 2011. Zinc in Human Health. IOS Press, Amsterdam, The Netherlands.

Roberson KD and Edwards HM Jr, 1994. Effects of ascorbic acid and 1,25-dihydroxycholecalciferol on alkaline phosphatase and tibial dyschondroplasia in broiler chickens. British Poultry Science, 35, 763–773.

Roberson KD and Edwards HM, 1995. Effects of 1,25-dihydroxycholecalciferol and phytase in zinc utillisation in broiler chicks. Poultry Science, 73, 1312–1326.

Rodrigues-Filho U, Vaz S, Felicissimo M, Scarpellini M, Cardoso D, Vinhas R, Landers R, Schneider J, Mc Garvey B, Andersen M and Skibsted L, 2005. Heterometallic manganese/zinc-phytate complex as a model compound for metal storage in wheat grains. Journal of Inorganic Biochemistry, 99, 1973–1982.

Rodriguez P, Darmon N, Chappuis P, Candalh C, Blaton MA, Bouchaud C and Heyman M, 1996. Intestinal paracellular permeability during malnutrition in guinea pigs: effect of high dietary zinc. Gut, 39, 416–422.

Rojas O and Stein H, 2012. Digestibility of phosphorus by growing pigs of fermented and conventional soybean meal without and with microbial phytase. Journal of Animal Science, 90, 1506–1512.

Roselli M, Finamore A, Britti MS, Bosi P, Oswald I and Mengheri E, 2005. Alternatives to in-feed antibiotics in pigs: Evaluation of probiotics, zinc or organic acids as protective agents for the intestinal mucosa. A comparison of in vitro and in vivo results. Animal Research, 54, 203–218.

Roth FX, Windisch W and Kirchgessner M,1998. Mineral metabolism (P, K, Ca, Mg, Zn, Mn, Cu) of piglets supplied with potassium diformate (FormiTM LHS). Agribiological Research, 51, 177–183.

Rubio C, Gutiérrez AJ, Revert C, Reguera JI, Burgos A and Hardisson A, 2009. Dietary intake of iron, copper, zinc and manganese in a Spanish population. International Journal of Food Sciences and Nutrition, 60, 590–600.

Sahraei M, Janmmohamadi H, Taghizadeh A, Moghadam GA and Rafat SA, 2013. Estimation of the relative bioavailability of several zinc sources for broilers fed a conventional corn-soybean meal diet. Journal of Poultry Science, 50, 53–59.

Sales J, 2013. Effects of pharmacological concentrations of dietary zinc oxide on growth of post-weaning pigs: A meta-analysis. Biological Trace Element Research, 152, 343–349.

Sandoval M, Henry PR, Ammerman CB, Miles RD and Littell RC, 1997a. Relative bioavailability of supplemental zinc sources for chicks. Journal of Animal Science, 75, 3195–3205.

Sandoval M, Henry PR, Littell RC, Cousins RJ and Ammerman CB, 1997b. Estimation of the relative bioavailability of zinc from inorganic zinc sources for sheep. Animal Feed Science and Technology, 66, 223–235.

Sandoval M, Henry PR, Luo XG, Littell RC, Miles RD and Ammerman CB, 1998. Performance and tissue zinc and metallothionein accumulation in chicks fed a high dietary level of zinc. Poultry Science, 77, 1354–1363.

Sandoval M, Henry PR, Littell RC, Miles RD, Butcher GD and Ammerman CB, 1999. Effect of dietary zinc source and method of oral administration on performance and tissue trace mineral concentration of broiler chicks. Journal of Animal Science, 77, 1788–1799.

Sanz Fernandez MV, Pearce SC, Gabler NK, Patience JF, Wilson ME, Socha MT, Torrison JL, Rhoads RP and Baumgard LH, 2013. Effects of supplemental zinc amino acid complex on gut integrity in heat-stressed growing pigs. Animal, 29, 696–702.

Sargeant H., McDowall KJ, Miller HM and Shaw M-A, 2010. Dietary zinc oxide affects the expression of genes associated with inflammation: transcriptome analysis in piglets challenges with ETEC K88. Veterinary Immunology and Immunopathology, 137, 120-129.

Satoh S, Takeuchi T and Watanabe T, 1987a. Availability to rainbow trout of zinc in white fish meal and of various zinc compounds. Bulletin of the Japanese Society for the Science of Fish, 53, 595–599.

Satoh S, Tabata K, Izume K, Takeuchi T and Watanabe T, 1987b. Effect of dietary tricalcium phosphate on availability of zinc to rainbow trout. Nippon Suisan Gakkaishi, 53, 1199–1205.

Satoh S, Izume K, Takeuchi T and Watanabe T, 1987c. Availability to rainbow trout of zinc contained in various types of fish meals. Nippon Suisan Gakkaishi, 53, 1861–1866.

Satoh S, Poe WE and Wilson RP, 1989. Effect of Supplemental Phytate and or Tricalcium Phosphate on Weight-Gain, Feed-Efficiency and Zinc Content in Vertebrae of Channel Catfish. Aquaculture, 80, 155–161.

Savolainen LC and Gatlin DM, 2010. Evaluation of sulfur amino acid and zinc supplements to soybean-meal-based diets for hybrid striped bass. Aquaculture, 307, 260–265.

Scarpa J and Gatlin DMI, 1992. Dietary zinc requirements of channel catfish, *Ictalurus punctatus*, swim-up fry in soft and hard water. Aquaculture, 106, 311–322.

Schlegel P, Nys Y and Jondreville C, 2010. Zinc availability and digestive zinc solubility in piglets and broilers fed diets varying in their phytate contents, phytase activity and supplemented zinc source. Animal, 4, 200–209.

Schlegel P, Sauvant D and Jondreville C, 2013. Bioavailability of zinc sources and their interaction with phytates in broilers and piglets. Animal, 7, 47-59.

Schwarz WA and Kirchgessner M, 1975. [Excretion of zinc in lactating cows receiving various supply of zinc] In German. Archiv Fur Tierernahrung, 25, 597–608.

Schwarz FJ and Kirchgessner M, 1979. Spurenementbedarf und-versorgung in der Pferdefütterung. Übers. Tierernährung 7, 257–276.

Selle PH and Ravindran V, 2007. Microbial phytase in poultry nutrition. Animal Feed Science and Technology, 135, 1–41.

Shafey TM, McDonald MW and Dingle JG, 1991. Effects of dietary calcium and available phosphorus concentration on digesta pH and on the availability of calcium, iron, magnesium and zinc from the intestinal contents of meat chickens. British Poultry Science, 32, 185–194.

Shanklin SH, Miller ER, Ullrey DE, Hoefer JA and Luecke RW, 1968. Zinc requirements of baby pigs on casein diets. Journal of Nutrition, 96, 101–108.

Sharif R, Thomas P, Zalewski P and Fenech M, 2012. The role of zinc in genomic stability. Mutation Research, 733, 111-121.

Shelton NW, Tokach MD, Nelssen JL, Goodband RD, Dritz SS, DeRouchey JM and Hill GM, 2011. Effects of copper sulphate, tri-basic copper chloride, and zinc oxide on weanling pig performance, Journal of Animal Science, 89, 2440–2451.

Siebert F, Lühken G, Pallauf J and Erhardt G, 2013. Mutation in porcine Zip4-like zinc transporter is associated with pancreatic zinc concentrations and apparent zinc absorption. British Journal of Nutrition, 109, 969-976.

Sissener NH, Julshamn K, Espe M, Lunestad BT, Hemre G-I, Waagbø R and Måge A, 2013. Surveillance of selected nutrients, additives and undesirables in commercial Norwegian fish feeds in the years 2000 to 2010. Aquaculture Nutrition (accepted).

Skoglund E, Larsen T and Sandberg S, 1997. Comparison between steeping and pelleting a mixed diet at different calcium levels on phytate degradation in pigs. Canadian Journal of Animal Science, 77, 471–477.

Slade RD, Kyriazakis I, Carroll SM, Reynolds FH, Wellock IJ, Broom LJ and Miller HM, 2011. Effect of rearing environment and dietary zinc oxide on the response of group-housed weaned pigs to enterotoxigenic *Escherichia coli* O149 challenge. Animal, 5, 1170–1178.

Smith WH, Plumlee MP and Beeson WM 1962. Effect of source of protein on zinc requirement of the growing pig. Journal of Animal Science, 21, 399–405.

Sobhanirad S and Naserian AA, 2012. Effects of high dietary zinc concentration and zinc sources on hematology and biochemistry of blood serum in Holstein dairy cows. Animal Feed Science and Technology, 177, 242–246.

Spears JW, 2003. Trace mineral bioavailability in ruminants. Journal of Nutrition, 133, 1506S–1509S.

Spears JW and Kegley EB, 2002. Effect of zinc source (zinc oxide and zinc proteinate) and level on performance, carcass characteristic, and immune response of growing and finishing steers. Journal of Animal Science, 80, 2747–2752.

Spry DJ, Hodson PV and Wood CM, 1988. Relative contributions of dietary and waterborne zinc in the rainbow trout, *Salmo gairdneri*. Canadian Journal of Fisheries and Aquatic Sciences, 45, 32–41.

Sterman M, Shouse M, Fairchild M and Belsito O, 1986. Kindled seizure induction alters and is altered by zinc absorption. Brain Research, 383, 382–386.

Storebakken T, Shearer KD and Roem AJ, 2000. Growth, uptake and retention of nitrogen and phosphorus, and absorption of other minerals in Atlantic salmon *Salmo salar* fed diets with fish meal and soy-protein concentrate as the main sources of protein. Aquaculture Nutrition, 6, 103–108.

Sugiura SH, Raboy V, Young KA, Dong FM and Hardy RW, 1999. Availability of phosphorus and trace elements in low-phytate varieties of barley and corn for rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 170, 285–296.

Suttle NF, 2010. Zinc, Mineral nutrition of livestock, 4th edn. CABI, Wallingford, UK, 426–456.

Suttle NF and Mills CF, 1966. Studies on the toxicity of copper to pigs. I. The effects of oral supplements of zinc and iron salts on the development of copper toxicosis. British Journal of Nutrition, 20, 135.

Swiatkiewicz S and Arczewska-Wlosek A, 2012. Bone quality characteristics and performance in broiler chickens fed diets supplemented with organic acids. Czech Journal of Animal Science, 57, 193–205.

Tan LN, Feng L, Liu Y, Jiang J, Jiang WD, Hu K, Li SH and Zhou XQ 2011. Growth, body composition and intestinal enzyme activities of juvenile Jian carp (*Cyprinus caprio* var. Jian) fed graded levels of dietary zinc. Aquaculture Nutrition, 17, 338–345.

Thiel U, Weigand E, Hoppe PP and Schoner FJ, 1993. Zinc retention of broiler chickens as affected by dietary supplementation of zinc and microbial phytase, Trace elements in man and animals - TEMA 8: Proceedings of the Eighth International Symposium on Trace Elements in Man and Animals, 658–659.

Trindade Neto MA, Pacheco BHC, Albuquerque R and Rodriguez-Lecompte JC, 2011. Dietary effects of chelated zinc supplementation and lysine levels in ISA Brown laying hens on early and late performance, and egg quality. Poultry Science, 90, 2837–2844.

Tucker HF and Salmon WD, 1955. Parakeratosis or zinc deficiency disease in the pig. Proceedings of the Society for Experimental Biology and Medicine, 88, 613.

Turconi G, Minoia C, Ronchi A and Roggi C, 2009. Dietary exposure estimates of twenty-one trace elements from a Total Diet Study carried out in Pavia, Northern Italy. British Journal of Nutrition, 101, 1200–1208.

Underwood E, 1977. Trace Elements in Humans and Animal Nutrition, 4th edn, Academic Press, New York.

Urbano G, López-Jurado M, Aranda P, Vidal-Valverde C, Tenorio E and Porres J, 2000. The role of phytic acid in legumes: antinutrient or beneficial function? Journal of Physiology and Biochemistry, 56, 283–294.

Vahjen W, Pieper R and Zentek J, 2011a. Increased dietary zinc oxide changes the bacterial core and enterobacterial composition in the ileum of piglets, Journal of Animal Science, 89, 2430–2439.

Vahjen W, Liedtke J, Zentek J, 2011b. Growth of porcine intestinal bacteria in the presence of ZnO. Proceedings of the Society of Nutrition Physiology, 21, Abstract 139.

van Campen DR, 1969. Copper interference with the intestinal absorption of zinc-65 by rat. Journal of Nutrition, 97, 104–108.

van der Aar PJ, Fahey GC, Ricke SC, Allen SE and Berger LL, 1983. Effects of dietary-fibers on mineral status of chicks. Journal of Nutrition, 113, 653–661.

Van Paemel M, Dierick N, Janssens G, Fievez V and De Smet S, 2010, online. Selected trace and ultratrace elements: Biological role, content in feed and requirements in animal nutrition – Elements for risk assessment. Technical Report submitted to EFSA. Available online: http://www.efsa.europa.eu/en/supporting/pub/68e.htm.

Vester BM, Karr-Lilienthal LK, Tomlinson D, Swanson KS and Fahey GC, 2006. Evaluation of zinc methionine for use in weanling pappy diets. Compendium on Continuing Education for the Practicing Veterinarian, 28, 76–82.

Veum TL, Ledoux DR, Raboy V and Ertl DS, 2001. Low-phytic acid corn improves nutrient utilization for growing pigs. Journal of Animal Science, 79, 2873–2880.

Veum TL, Ledoux DR, Bollinger DW, Raboy V and Cook A, 2002. Low-phytic acid barley improves calcium and phosphorus utilization and growth performance in growing pigs. Journal of Animal Science, 80, 2663–2670.

Vilela FG, Zanetti MA, Netto AS, Netto AS, Rennó FP, Venturelli BC and Canaes TS, 2012. Supplementation of diets for Santa Ines sheep with organic and inorganic zinc sources. Revista Brasileira De Zootecnia-Brazilian Journal of Animal Science, 41, 2134–2138.

Walsh CT, Sandstead HH, Prasad AS, Newberne PM and Fraker PJ, 1994. Zinc: health effects and research priorities for the 1990s. Environmental Health Perspectives, 102, 5–46.

Wang K, Zhou B, Kuo YM, Zemansky J and Gitschier J, 2002. A novel member of a zinc transporter family is defective in acrodermatits enteropathica. American Journal of Human Genetics, 71, 66–73.

Wang R, Zhu X, Guo F, Zhang W and Jia Z, 2006. Influence of different dietary levels of zinc on performance, vitamin B-12, and blood parameters in lambs. International Journal for Vitamin and Nutrition Research, 76, 353–358

Watanabe T, Kiron V and Satoh S, 1997. Trace minerals in fish nutrition. Aquaculture, 151,185–207.

Weaver BP, Dufner-Beattie J, Kambe T and Andrews GK, 2007. Novel zinc-responsive post-transcriptional mechanisms reciprocally regulate expression of the mouse Slc39a4 and Slc39a5 zinc transporters (*Zip4* and *Zip5*). Biological Chemistry, 388, 1301–1312.

Wedekind KJ and Baker DH, 1990. Zinc bioavailability in feed-grade sources of zinc. Journal of Animal Science, 68, 684–689.

Wedekind KJ, Hortin AE and Baker DH, 1992. Methodology for assessing zinc bioavailability: efficacy estimates for zinc-methionine, zinc sulphate, and zinc oxide. Journal of Animal Science, 70, 178–187.

Wedekind KJ, Lewis AJ, Giesemann MA and Miller PS, 1994. Bioavailability of zinc from inorganic and organic sources for pigs fed corn-soybean meal diets. Journal of Animal Science, 72, 2681–2689.

Wekell JC, Shearer KD and Houle CR, 1983. High zinc supplementation of rainbow trout diets. The Progressive Fish-Culturist, 45, 144–147.

Wekell JC, Shearer KD and Gauglitz EJ Jr, 1986. Zinc supplementation of trout diets: tissue indicators of body zinc status. The Progressive Fish-Culturist, 48, 205–212.

Wenbin J, Xiaoping Z, Wie Z, Jianbo C, Cuihua G and Zhihai J, 2009. Effects of source of supplemental zinc on performance, nutrient digestibility and plasma mineral profile in Cashmere goats. Asian-Australasian Journal of Animal Science, 22, 1648–1653.

Westin G and Schaffner W, 1988. A zinc-responsive factor interacts with a metal-regulated enhancer element (MRE) of the mouse metallothionein-I gene. The EMBO Journal, 12, 3763-3770.

Wiking L, Larsen T and Sehested J. 2008. Transfer of dietary zinc and fat to milk—evaluation of milk fat quality, milk fat precursors, and mastitis indicators. Journal of Dairy Science, 91, 1544–1551.

Willis JN and Sunda WG, 1984. Relative contributions of food and water in the accumulation of zinc by two species of marine fish. Marine Biology, 80, 273–279.

Windisch W and Kirchgessner M, 1999. Zinc absorption and excretion in adult rats at zinc deficiency induced by dietary phytate additions: I. Quantitative zinc metabolism of Zn-65-labelled adult rats at zinc deficiency. Journal of Animal Physiology and Animal Nutrition-Zeitschrift Fur Tierphysiologie Tierernahrung Und Futtermittelkunde, 82, 106–115.

Wright CL and Spears JW, 2004. Effect of zinc source and dietary level on zinc metabolism in Holstein calves. Journal of Dairy Science, 87, 1085–1091.

Yamaji S, Tennant J, Tandy S, Williams M, Srai SKS and Sharp P, 2001. Zinc regulates the function and expression of the iron transporters DMT1 and IREG1 in human intestinal Caco-2 cells. Febs Letters, 507, 137–141.

Yi Z, Kornegay ET and Denbow DM, 1996. Supplemental microbial phytase improves zinc utilization in broilers. Poultry Science, 75, 540–546.

Yuzbasiyan-Gurkan V and Bartlett E, 2006. Identification of a unique splice variant in SLC39A4 in bovine hereditary zinc deficiency, lethal trait A46: An animal model of acrodermatitis enteropathica. Genomics, 88, 521–526.

Zacharias B, Pelletier W and Drochner W, 2007. Availability of inorganic and organic bound copper and zinc fed at physiological levels to fattening pigs. Žemės Ūkio Mokslai, 14, 45–50.

Zhang B and Guo Y, 2007. Beneficial effects of tetrabasic zinc chloride for weanling piglets and the bioavailability of zinc in tetrabasic form relative to ZnO. Animal Feed Science and Technology, 135, 75–85.

Zheng D, Feeney GP, Kille P and Hogstrand C, 2008. Regulation of ZIP and ZnT zinc transporters in zebrafish gill: zinc repression of ZIP10 transcription by an intronic MRE cluster. Physiological Genomics, 34, 205–214.

Zheng D, Feeney GP, Handy RD, Hogstrand C and Kille P, 2013. Uptake epithelia behave in a cell-centric and not systems homeostatic manner in response to zinc depletion and supplementation. Metallomics, 18, 154-165.

APPENDICES

APPENDIX A. Zinc concentration in feed materials

Table A1: Zinc concentration in feed materials according to CVB[1] feed composition tables (in mg/kg feed material *as is*) and in mineral feed materials according to Batal and Dale (2008)[2]

Feed materials	mg/kg	Feed materials	mg/kg
Alfalfa meal	21-27	Barley	23
Barley feed (residue of polishing)	67	Barley milling byproduct	35
Beans (phaseolus) heat treated	32	Biscuits	8-11
Blood meal spray dried	37	Bone meal	118
Bread meal	16	Brewers' grains dried	65
Brewers' yeast dried	49	Buckwheat	9
Canary seed	31	Carob	6
Casein	36	Chicory pulp dried	31
Citrus pulp dried	9	Coconut expeller	46
Coconut extracted	53	Cotton expeller with hulls	71-72
Cotton extracted with hulls	68	Distillers grains and solubles	61
Fat from animals	9	Feather meal hydrolysed	140
Fish meal	83-84	Grass meal	34-47
Horsebeans	41	Horsebeans white	40
Lentils	33	Linseed	50
Linseed expeller	69	Linseed extracted	52
Lupins	37-52	Maize	21
Maize chemically-heat treated	18	Maize feed meal extracted	46
Maize feed flour	4	Maize germ meal expeller/extracted	62-63
Maize gluten feed	57-68	Maize gluten meal	19
Malt culms	39	Meat and bone meal	99-104
Meat meal	114-156	Milk powder skimmed	45
Milk powder whole	50	Millet	25
Nigerseed	42	Oats grain	25
Oats grain peeled	28	Oats husk meal	21
Palm kernels	20	Palmkern expeller	42-44
Peanut expeller	64-65	Peanut extracted	50-51
Peas	31	Potatoes sweet dried	6
Potato pulp	35	Potato starch	2-3
Potato protein	3-29	Rapes meal	60
Rapeseed	40	Rapeseed expeller	62
Rapeseed extracted	60	Rice bran meal extracted	93
Rice feed	56-73	Rice with hulls	16
Rye	29	Sesameseed meal extracted	91
Sesameseed expeller	126	Sorghum	19
Soybean meal	47-51	Soybean expeller	46
Soybean hulls	50	Soybeans	38
Sugarbeet pulp	16-30	Sugarbeet/sugarcane molasses	9
Sunflowers with hulls/dehulled	42	Sunflower expeller with hulls/dehullsed	70-71
Sunflower meal	79-100	Tapioka	8-10
Triticale	34	Vinasse sugarbeet	15-40
Wheat	23	Wheat bran	99
Wheat feed meal	74	Wheat feed flours	54
Wheat germ	169	Wheat germ feed	86
Wheat gluten meal	36	Wheat gliuten feed	47
Wheat middlings	85	Whey powder	13
Whey powder partially delactosed	10-32		

[1] Centraal Veevoederbureau (CVB). 2007. Feed Tables. Produktschap Diervoeding, The Netherlands.

Table A1 (continued): Zinc concentration in feed materials according to CVB[1] feed composition tables (in mg/kg DM) and in mineral feed materials (in mg/kg *as is*) according to Batal and Dale (2008)[2]

Moisture rich feed materials	mg/kg DM	Moisture rich feed materials	mg/kg DM
Beet pulp fresh/ensiled	34	Brewers grains	98-99
Brewers yeast	65	Corn cob meal	28-30
Whey	29-37	Chicory pulp fresh/ensiled	41
Maize gluten feed fresh/ensiled	45	Maize solubles	226
Potato starch, different products	19-32	Potato cut, raw	15
Potato juice concentrate	111	Potato pulp	11-18
Wheat starch	27-33		
Roughages and comparable products	**mg/kg DM**	**Roughages and comparable products**	**mg/kg DM**
Beet leaves ensiled	189	Chicory roots frcsh cleaned	14
Clover red silage	24	Cucumber fresh	65
Fodderbeets cleaned	100	Gras, average	43
Gras silage, average	42	Green cereals silage	41
Lucerne (alfalfa) ad	28	Lucerne silage	45
Maize (fodder) ad	38	Maize Cobs with leaves silage	31
Maize, fresh	38	Maize silage	38
Sunflower silage	57	Whole crop silage (cereals)	48

[1] Centraal Veevoederbureau (CVB). 2007. Feed Tables. Produktschap Diervoeding, The Netherlands.

Mineral feed materials	mg/kg	Mineral feed materials	mg/kg
Bone meal (steamed)	424	Diammonium phosphate	300
Difluorinated phosphate	44	Dicalcium phosphate	220
Mono-dicalcium phosphate	210	Monoammonium phosphate	300

[2] Batal and Dale. 2008. Feedstuffs September 10, p. 16.

Table A2: Zinc concentration in feed materials according to INRA[1] feed composition Tables (in mg/kg feed material *as is*)

Cereals	**mg/kg ± SD**	**Cereals**	**mg/kg ± SD**
Barley	30 ± 8	Maize	19 ± 6
Oats	23 ± 4	Oats groats	26
Rice, brown	17	Rye	22
Sorghum	19 ± 7	Triticale	20 ± 9
Wheat, durum	15	Wheat, soft	27 ± 8
Wheat byproducts	**mg/kg ± SD**	**Wheat byproducts**	**mg/kg ± SD**
Wheat bran	74 ± 25	Wheat middlings	91 ± 20
Wheat shorts	81	Wheat feed flour	40
Wheat gluten feed, starch 25 %	62	Wheat gluten feed, starch 28 %	61
Maize byproducts	**mg/kg ± SD**	**Maize byproducts**	**mg/kg ± SD**
Corn distillers	65	Corn gluten feed	53 ± 15
Corn gluten meal	33 ± 16	Maize bran	2
Maize germ meal, solvent extracted	131	Hominy feed	45
Other cereal byproducts	**mg/kg ± SD**	**Other cereal byproducts**	**mg/kg ± SD**
Barley rootlets, dried	78	Brewers' dried grains	82 ± 28
Rice bran, extracted	73	Rice bran, full fat	60 ± 22
Rice, broken	16		
Legume and oil seeds	**mg/kg ± SD**	**Legume and oil seeds**	**mg/kg ± SD**
Chickpea	22	Cottonseed, full fat	34 ± 3
Faba bean, coloured flowers	31 ± 6	Faba bean, coloured flowers	31
Linseed, full fat	45	Lupin, blue	31
Lupin, white	27	Pea	32
Rapeseed, full fat	40	Soybean, full fat, extruded	40
Soybean, full fat, toasted	40	Sunflower seed, full fat	51
Oil seed meals	**mg/kg ± SD**	**Oil seed meals**	**mg/kg ± SD**
Copra meal, expeller	49	Cottonseed meal, CF 7-14 %	72
Cottonseed meal, CF 14-20 %	58	Grapeseed oil meal, solvent extracted	15
Groundnut meal, detoxified, CF < 9 %	58	Groundnut meal, detoxified, CF > 9 %	57 ± 11
Linseed meal, expeller	66	Linseed meal, solvent extracted	60
Palm kernel meal, expeller	32 ± 20	Rapeseed meal	65 ± 17
Sesame meal, expeller	125	Soybean meal	47
Sunflower meal, partially decorticated	69	Sunflower meal, undecorticated	92 ± 11
Other plant byproducts	**mg/kg ± SD**	**Other plant byproducts**	**mg/kg ± SD**
Beet pulp, dried	19 ± 9	Beet pulp dried, molasses added	13
Beet pulp, pressed	4 ± 1	Brewers' yeast, dried	64
Carob pod meal	7	Citrus pulp, dried	12 ± 13
Grape marc, dried	25	Liquid potato feed	7
Molasses, beet	17	Molasses, sugarcane	13 ± 15
Potato protein concentrate	21	Potato pulp, dried	40
Soybean hulls	40 ± 11	Vinasse, from yeast production	97
Dehydrated forages	**mg/kg**	**Dehydrated forages**	**mg/kg ± SD**
Alfalfa, dehydrated, protein	19 - 26	Grass, dehydrated	32 ± 7
Wheat straw	19		
Dairy products	**mg/kg**	**Dairy products**	**mg/kg**
Milk powder, skimmed	43	Milk powder, whole	33
Whey powder, acidic	64	Whey powder, sweet	20
Fish meals and solubles	**mg/kg ± SD**	**Fish meals and solubles**	**mg/kg ± SD**
Fish meal, protein 62 %	89 ± 5	Fish meal, protein 65 %	85 ± 14
Fish meal, protein 70 %	88	Fish solubles, condensed, defatted	78
Other animal byproducts	**mg/kg ± SD**	**Other animal byproducts**	**mg/kg ± SD**
Blood meal	23 ± 2	Feather meal	130 ± 18
Meat and bone meal, fat <7.5 %	109	Meat and bone meal, fat >7.5 %	110

[1] INRA. 2004. Tables of composition and nutritional value of feed materials. Wageningen Academic Publishers, The Netherlands & INRA, Paris, France.

Table A3: Zinc content in feed materials according to DLG-Futtermitteldatenbank[1]

Feed material[2]	Number of samples	Zn mean, (mg/kg DM)	SD
Grassland 1-2 uses (late 1st use)	97	33.7	13.3
Grassland 2-3 uses	70	30.2	13.5
Grassland 4 uses	85	36,2	11.3
Lucerne (Alfalfa)	5	54.6	30.1
Jerusalem artichoke, roots	12	15.5	1.3
Winter barley, grain seeds	135	25.4	13.2
Winter wheat, grain seeds	51	26.9	18.4
Common vetch, grains	11	46.8	2.8
Stillage (from barley)	6	72.8	31.4
Stilage sludge (from barley)	5	78.8	22.1
Brewers' grain	11	72.0	11.6
Stillage (from wheat)	18	75.9	6.7
Stillage from maize	10	69.5	17.1
Citrus pulp	6	14.6	20.1
Rapeseed expeller	10	62.5	19.2
Extracted rape seed	15	65.2	18.4
Extracted soya bean meal, partially decorticated	14	62.4	11.3
Extracted soya bean meal, hulled	45	58.3	12.4
Vinasse from sugar beet	5	76.7	22.3
Acid whey, mineral-acidic	9	28.2	20.0
Fish meal	11	115.2	30.7
Brown algae	5	71.3	22.0
Red algae	13	74.6	23.1
Carob, seeds	18	47.4	8.1
Oat grains	21	31.6	8.7

[1] Source: DLG (2014) DLG-Futtermitteldatenbank <http://datenbank.futtermittel.net>
[2] Feed materials with less than five samples analysed have not been listed.

Table A4: Zinc content in feed materials according to Agroscope[1]

Feed material	Number of samples	Dry matter (g/kg)	Zn mean (mg/kg DM)	SD
Barley	51	882	25.6	3.1
Wheat	54	885	26.6	4.1
Triticale	25	896	32.1	3.2
Oats	20	909	26.9	3.9
Maize	46	887	18.2	1.2
Millet	13	915	30.9	2.4
Sorghum	19	905	20.3	3.9
Oat hulls	7	939	10.8	2.0
Barley offal	5	912	48.1	7.9
Oat offal	9	934	29.8	10.1
Wheat middlings	8	894	122.8	15.5
Wheat feedmeal	6	889	40.0	11.2
Oat feedmeal	6	916	37.6	4.3
Wheat bran	11	889	103.6	12.7
Wheat starch	10	917	4.6	7.0
Maize gluten	10	924	23.2	7.2
Cereal aftermeal	11	888	82.1	15.3
Distiller dried grain	8	938	61.5	5.0
Brewery by-product	7	919	93.3	3.1
Horse beans	5	902	47.2	3.6
Lupin white	5	905	38.3	2.5
Lupin blue	5	901	32.6	1.6
Rapeseed meal cake	10	916	60.9	2.5
Rapeseed meal	9	901	65.5	3.5
Soybean meal cake	10	893	55.2	2.0
Soybean meal	14	892	52.2	3.4
Sunflower meal cake	11	922	82.6	6.9
Lineseed meal cake	10	912	70.7	11.0
Sugarbeet pulp	8	892	16.6	5.3
Sugarbeet molasses	8	811	23.5	7.8
Potato protein	8	915	23.4	4.2
Milk powder skimmed	7	954	42.2	4.8
Whey powder	6	963	3.7	1.5

[1] Source: Schlegel P, 2013. Teneurs en minéraux des matières premières destinées aux animaux de rente. Internal Agroscope Research Report. Unpublished.

Table A5: Zinc content in forages according to Agroscope[1]

	Growth stage[2]	Zn (mg/kg DM)	
		First cut	Further cuts
G, graminea rich population	1	30	34
	2	27	31
	3	25	29
	4	23	27
	5	22	26
	6	22	26
	7	22	25
GR, graminea rich population with raygrass	1	28	32
	2	26	29
	3	23	27
	4	22	26
	5	21	24
	6	20	24
	7	20	24
E, graminea and legume rich population	1	30	34
	2	27	31
	3	25	29
	4	23	27
	5	22	26
	6	22	26
	7	22	25
ER, graminea and legume rich population, with raygrass	1	28	32
	2	26	29
	3	23	27
	4	22	26
	5	21	24
	6	20	24
	7	20	24

Modeled data: N= 205, year 2008-2012, non conserved.
[1] Source: Schlegel P, 2013. Teneurs en minéraux des herbages. Internal Agroscope Research Report. Unpublished.
[2] Growth stage 1: begin elongation, 2: elongation, 3: begin heading, 4: heading, 5: end heading, 6: flowering, 7: seeding.

Table A6: Concentrations of zinc (mean and range, mg/kg) in fish meal

Year	Number of samples	Zinc	
		Mean (mg/kg)	Range (mg/kg)
2003	10	77	65-93
2004	10	70	51-90
2005	8	55	45-64
2006	13	70	50-96
2007	13	73	29-210
2008	4	69	64-74

Source: Norwegian Food Safety Authority's Annual Fish Feed Surveillance Programme.

APPENDIX B. Content of P, phytate P and phytase activity in feed materials

Table B1: Content of total P (%), phytate P (%) and phytase activity (FTU/kg) in feed materials

Reference	**1**	Total P	Phytate P	Phytase activity	**2**	Total P	Phytate P	**3**	Total P	Phytate P	Phytase activity	**4**	Total P	Phytate P	Phytase activity	**5**	Total P	Phytate P	**6**	Total P	Phytate P
Feed material	n	% (88% DM)	% (88% DM)	FTU/kg (88% DM)	n	% DM	% DM	n	% (88% DM)	% (88% DM)	FTU/kg (88% DM)	n	% DM	% DM	FTU/kg DM	n	% DM	% DM	n	% (88% DM)	% (88% DM)
Wheat	13	0.33 (0.31-0.38)	0.22 (0.19-0.27)	1193 (915-1581)				5	0.33	0.18	1565	18	0.40	0.29	2886	22	0.42	0.25		0.37	0.24
Barley	9	0.37 (0.34-0.39)	0.22 (0.20-0.24)	582 (408-882)								15	0.42	0.26	2323					0.36	0.19
Maize	11	0.28 (0.25-0.35)	0.19 (0.16-0.26)	15 (0-46)	4	0.26	0.22	5	0.25	017	24					133	0.32	0.19		0.28	0.20
Oats	6	0.36 (0.33-0.40)	0.21 (0.16-0.28)	42 (0-108)								6	0.37	0.25	496					0.27	0.22
Sorghum	5	0.27 (0.20-0.33)	0.19 (0.14-0.24)	24 (0-76)				5	0.26	0.17	24										
Sorghum dark colour					2	0.41	0.27														
Sorghum light colour					3	0.36	0.23														
Buckwheat																				0.32	0.20
Millet					13	0.25	0.17													0.32	0.20
Rye	2	0.36 (0.35-0.36)	0.22 (0.20-0.23)	5130 (4132-6127)								13	0.36	0.24	6016					0.32	0.26
Rice brown					3	0.38	0.28	5	0.12	0.08	112										
Rice polished					2	0.31	0.17	5	1.57	1.13	134									0.08	0.05
Triticale	6	0.37 (0.35-0.40)	0.35 (0.22-0.28)	1688 (1475-2039)								12	0.40	0.28	2799					0.30	0.20
Peas	11	0.38 (0.36-0.40)	0.17 (0.13-0.21)	116 (36-183)								18	0.41	0.24	262						
Soybeans	4	0.57 (0.55-0.59)	0.26 (0.23-0.28)	55 (0-188)	3	0.60	0.37														
Field beans	1	0.50	0.23	81								11	0.57	0.39	290						
Groundnut					4	0.49	0.40														
Lupins	1	0.25	0.05	0				5	0.64	0.49	51	14	0.57	0.35	324						
Cottonseed																					
Lentils					2	0.31	0.20														
Wheat bran fine	6	0.95 (0.088-1.03)	0.72 (0.60-0.81)	4601 (3485-5345)	2	1.15	0.57	5	0.92	0.63	928	3	0.88	0.79	9945					1.15	0.95
Wheat bran pellets	15	1.01 (0.88-1.17)	0.78 (0.62-0.88)	2573 (1206-4230)																	

Wheat middlings pellets	5	0.80 (0.53-1.20)	0.53 (0.33-0.71)	4381 (2825-5042)												31	1.31	0.80			
Wheat feed	11	0.56 (0.26-0.91)	0.39 (0.15-0.64)	3350 (1007-4708)																0.49	0.35
Wheat shorts																15	1.25	0.72			
Wheat bran	5	1.16 (1.03-1.36)	0.97 (0.77-1.27)	2957 (1180-5208)																	
Rye bran												3	0.58	0.49	9241						
Malt sprouts	4	0.60 (0.52-0.73)	0.01 (0.0-0.05)	877 (605-1174)																	
Corn distillers	3	0.90 (0.86-0.96)	0.19 (0.17-0.21)	385 (141-850)				5	1.22	0.30	39					89	0.96	0.26		1.27	0.10
Rice bran	2	1.71 (1.37-1.74)	1.10 (1.08-1.11)	122 (108-135)	4	1.34	1.03													1.50	1.23
Maize glutenfeed	9	0.87 (0.63-1.00)	0.47 (0.35-0.54)	48 (0-177)																0.50	0.36
Maize glutenfeed pellets	5	0.89 (0.75-0.99)	0.52 (0.40-0.60)	5 (0-15)																	
Maize germs	1	0.65	0.42	16				10	0.93	0.60	49										
Maize feed flour	2	0.23 (0.22-0.24)	0.14 (0.12-0.16)	5 (3-6)																	
Maize feed flour USA	5	0.50 (0.45-0.55)	0.27 (0.20-0.36)	37 (0-78)																	
Rice feed	1	0.32	0.23	0																	
Rice bran extracted	4	1.89 (1.57-2.21)	0.79 (0.69-1.07)	45 (0-145)																	
Wheat glutenfeed	6	0.78 (0.71-1.87)	0.56 (0.44-0.69)	25 (0-150)																	
Peanut extracts	3	0.68 (0.65-0.70)	0.32 (0.30-0.34)	3 (0-8)																0.63	0.50
Coconut expeller	4	0.53 (0.47-0.58)	0.18 (0.14-0.20)	24 (0-80)	5	0.59	0.33	5	0.43	0.24	37										
Lineseed expeller	4	0.75 (0.73-0.78)	0.42 (0.39-0.43)	5 (0-12)																	
Lineseed extracted	1	0.82	0.47	41																	
Rapeseed extracted	5	1.12 (1.07-1.17)	0.40 (0.34-0.48)	16 (0-36)												21	1.35	0.70		1.17	0.87
Palmkernel expeller	6	0.59 (0.55-0.62)	0.39 (0.33-0.41)	37 (0-91)				4	0.51	0.29	34										
Sunflower extracted	11	1.00 (0.86-1.28)	0.44 (0.32-0.51)	40 (0-185)																1.00	0.84
Soybeanmeal expeller					3	0.63	0.38														
Soybeanmeal 44 extracted	15	0.66 (0,61-0,71)	0.35 (0.33-0.39)	40 (0-120)	2	0.63	0.38	5	0.57	0.37	62					114	0.84	0.40		0.65	0.38

Soybeanmeal 48 extracted	5	0.61 (0.59-0.62)	0.32 (0.28-0.33)	8 (0-20)																	
Soybeanmeal 50 extracted	9	0.71 (0.67-0.73)	0.38 (0.37-0.40)	31 (0-149)																	
Cottonseed meal								5	1.34	0.84	36									0.97	0.75
Safflourmeal																				1.29	0.90
Sesam meal																				1.37	1.03
Maize ensiled	7	0.30 (0.24-0.38)	0.13 (0.11-0.18)	12 (0-30)																	
Beet pulp pellets	18	0.10 (0.08-0.11)	0	3(0-13)																	
Potato	1	0.10	0.00	0,00	2	0.24	0.05														
Potato starch	1	0.10	0.00	0,00																	
Cassava roots chips	11	0.09 (0.06-0.12)	0.00	6 (0-40)	2	0.16	0.04														
Cassava roots pellets	7	0.08 (0.06-0.12)	0.00	9 (0-21)																	
Potatoes sweet	3	0.11 (0.10-0.13)	0.00	26 (0-73)	2	0.21	0.05														
Citrus pulp	4	0.10 (0.09-0.11)	0.00	3(0-12)																	
Cocoa shells	1	0.40	0.00	65																	
Soybean hulls	5	0.19 (0.17-0.21)	0.00	99 (0-150)																	
Flax chaff	1	0.10	0.00	58																	
Mycelium	2	0.14 (0.13-0.15)	0.00	77 (22-131)																	
Alfalfa	7	0.23 (0.11-0.33)	0.00	60 (15-250)																0.2	0	
Maize cobbs	1	0.05	0.00	58																	

1. Eeckhout W and de Paepe M, 1994. Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. Animal Feed Science and Technology, 47, 19–29.
2. Ravindran V, Ravindran G and Sivalogan S, 1994. Total and phytate phosphorus contents of various foods and feedstuffs of plant origin. Food Chemistry, 50, 133–136.
3. Godoy S, Chicco C, Meschy F and Requena F, 2005. Phytic phosphorus and phytase activity of animal feed ingredients. Interciencia, 30, 24–28.
4. Steiner T, Mosenthin R, Zimmermann B, Greiner R and Roth S, 2007. Distribution of phytase activity, total phosphorus and phytate phosphorus in legume seeds, cereals and cereal by-products as influenced by harvest year and cultivar. Animal Feed Science and Technology, 133, 320-334.
5. Tahir M, Shim M, Ward N, Smith C, Foster E, Guney A and Pesti G. 2012. Phytate and other nutrient components of feed ingredients for poultry. Poultry Science, 91, 928-935.
6. NRC (National Research Council). 1994. Nutrient requirements for Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

FTU: One unit of phytase activity is defined as the amount ofenzyme which sets free1 micromol of inorganic phosphorus per minute from 0.0015M sodium phytate solution at 37°C and pH 5.5. (ISO 30024/2009).

Table B2: Content of phytate P (% and g/kg), Zn (mg/kg) and Zn bound to phytate (%)[1]

Feed material	Phytate P %						Phytate-P Mean %	Phytate-P g/kg	Zn content mg/kg	Zn bound to phytic acid mg/kg	Bound Zn % of total Zn
	Ref 1	Ref 2	Ref 3	Ref 4	Ref 5	Ref 6					
Wheat	0.22		0.18	0.29	0.25	0.24	0.24	2.36	23	24	103
Barley	0.22			0.26		0.19	0.22	2.23	23	22	97
Maize	0.19	0.22	0.17		0.19	0.20	0.19	1.93	21	19	92
Oats	0.21			0.25		0.22	0.23	2.27	25	23	91
Sorghum	0.19		0.17				0.18	1.80	19	18	95
Buckwheat						0.20	0.20	2.00	9	20	222
Millet		0.17				0.20	0.19	1.85	25	19	74
Rye	0.22			0.24		0.26	0.24	2.40	29	24	83
Rice brown		0.28	0.08				0.18	1.80	16	18	113
Triticale	0.35			0.28		0.20	0.28	2.77	34	28	81
Peas	0.17			0.24			0.21	2.05	31	21	66
Soybeans	0.26	0.37					0.32	3.15	38	32	83
Field beans	0.23			0.39			0.31	3.10	41	31	76
Groundnut		0.40					0.40	4,00	50	40	80
Lupins	0.05		0.49	0.35			0.30	2,97	45	30	66
Wheat bran fine	0.72	0.57	0.63	0.79		0.95	0.73	7.32	99	73	74
Wheat bran pellets	0.78						0.78	7.80	99	78	79
Wheat middlings pellets	0.53				0.80		0.66	6.65	85	66	78
Wheat feed	0.39					0.35	0.37	3.70	54	37	69
Wheat shorts					0.72		0.72	7.20	86	72	84
Wheat bran	0.97						0.97	9.70	99	97	98
Corn distillers	0.19		0.30		0.26	0.10	0.21	2.12	61	21	35
Rice bran	1.10	1.03				1.23	1.12	11.20	93	112	120
Maize glutenfeed	0.47					0.36	0.42	4.15	68	42	61
Maize glutenfeed pellets	0.52						0.52	5.20	68	52	76
Maize germs	0.42		0.60				0.51	5.10	63	51	81
Maize feed flour	0.14						0.14	1.40	46	14	30
Maize feed flour USA	0.27						0.27	2.70	46	27	59
Rice feed	0.23						0.23	2.30	65	23	35
Rice bran extracted	0.79						0.79	7.90	93	79	85

Wheat glutenfeed	0.56						0.56	5.60	47	56	119
Peanut extracts	0.32					0.50	0.41	4.10	60	41	68
Coconut expeller	0.18	0.33	0.24				0.25	2.50	46	25	54
Lineseed expeller	0.42						0.42	4.20	69	42	61
Lineseed extracted	0.47						0.47	4.70	52	47	90
Rapeseed extracted	0.40				0.70	0.87	0.66	6.55	60	66	109
Palmkernel expeller	0.39		0.29				0.34	3.40	42	34	81
Sunflower extracted	0.44					0.84	0.64	6.40	90	64	71
Soybeanmeal expeller		0.38					0.38	3.80	46	38	83
Soybeanmeal 44 extracted	0.35	0.38	0.37		0.40	0.38	0.38	3.75	48	38	78
Soybeanmeal 48 extracted	0.32						0.32	3.20	48	32	67
Soybeanmeal 50 extracted	0.38						0.38	3.80	48	38	79
Cottonseed meal			0.84			0.75	0.80	7.95	68	80	117
Sesam meal						1.03	1.03	10.30	91	103	113
Maize ensiled	0.13						0.13	1.30	38	13	34
							Mean	**4.28**	**53**	**43**	**82**

[1] According to the assumption: 1g Phytate-P binds 10 mg Zn in cereals (Revy et al., 2003; Rodrigues-Filho et al., 2005).

Ref 1. Eeckhout W and de Paepe M, 1994. Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. Animal Feed Science and Technology, 47, 19–29.

Ref 2. Ravindran V, Ravindran G and Sivalogan S, 1994. Total and phytate phosphorus contents of various foods and feedstuffs of plant origin. Food Chemistry, 50, 133–136.

Ref 3. Godoy S, Chicco C, Meschy F and Requena F, 2005. Phytic phosphorus and phytase activity of animal feed ingredients. Interciencia, 30, 24–28.

Ref 4. Steiner T, Mosenthin R, Zimmermann B, Greiner R and Roth S, 2007. Distribution of phytase activity, total phosphorus and phytate phosphorus in legume seeds, cereals and cereal by-products as influenced by harvest year and cultivar. Animal Feed Science and Technology, 133, 320-334.

Ref 5. Tahir M, Shim M, Ward N, Smith C, Foster E, Guney A and Pesti G. 2012. Phytate and other nutrient components of feed ingredients for poultry. Poultry Science, 91, 928-935.

Ref 6. NRC (National Research Council). 1994. Nutrient requirements for Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

References

Rodrigues-Filho U, Vaz S, Felicissimo M, Scarpellini M, Cardoso D, Vinhas R, Landers R, Schneider J, Mc Garvey B, Andersen M, Skibsted L, 2005. Heterometallic manganese/zinc-phytate complex as a model compound for metal storage in wheat grains. Journal of Inorganic Biochemistry, 99, 1973–1982.

Revy R, Jondreville C, Dourmad JY, Nys Y, 2003. [Zinc in pig nutrition: the essential trace element and potential adverse effect on environment]. INRA Productions Animales, 16, 3–18.

APPENDIX C. Background concentration of zinc in complete feed, from several sources

Table C1: Background concentration of zinc in a representative complete feedingstuff for a list of animal species/categories using CVB[1] and INRA[2] trace element composition tables[3]

	Number of feed materials in the formula	Total zinc background concentration (mg/kg) in complete feedingstuff	
		CVB	**INRA[4]**
Starter Chicks (complete feed)	10	35.9	38.3
Chicken reared for laying (complete feed)	11	39.7	41.8
Layer Phase I (complete feed)	10	32.1	33.7
Layer Phase II (complete feed)	10	33.2	35.6
Broiler Starter (complete feed)	8	29.9	30.9
Broiler Grower (complete feed)	9	30.3	31.9
Broiler Finisher (complete feed)	8	29.5	31.9
Turkey Starter (complete feed)	7	38.6	39.0
Turkey Grower (complete feed)	7	35.9	36.9
Turkey Finisher (complete feed)	6	34.3	35.3
Turkey Breeder (complete feed)	5	25.1	23.4
Duck, grower/finisher (complete feed)	6	32.7	35.8
Geese, grower/finisher (complete feed)	5	31.0	31.5
Piglet Starter I (from weaning)	8	27.6	31.1
Piglet Starter II (complete feed)	13	32.2	36.2
Pig Grower (complete feed)	12	34.5	37.0
Pig Finisher (complete feed)	12	36.0	39.4
Sows, gestating (complete feed)	14	45.9	44.4
Sows, lactating (complete feed)	14	37.4	40.5
Calf, milk replacer (complete feed)	8	16.7	20.2
Calf concentrate (complete feed)	14	41.7	35.3
Calf concentrate (complementary feed)	13	41.5	38.5
Cattle concentrate (complete feed)[5]	8	33.1	33.6
Cattle concentrate (complementary feed)	7	29.3	30.0
Dairy cows TMR (based on corn silage)[5]	13	41.3	41.4
Dairy cows TMR (based on grass silage)[5]	13	41.5	41.4
Dairy concentrate (complementary feed)	11	43.2	42.3
Dairy cows mineral feed (min. 40% crude ash)	7	38.6	38.6
Rabbit, breeder (complete feed)	5	53.1	49.3
Rabbit, grower/finisher (complete feed)	8	44.6	42.7
Salmon feed (wet)[5]	4	50.1	52.9
Salmon feed (dry)	4	55.0	57.2
Trout feed (dry)	6	39.9	39.4
Dog food (dry)	9	73.0	53.2
Cat food (dry)	12	46.3	75.0

[1] CVB. 2007. Feed Tables. Productschap Diervoeding, The Netherlands.
[2] INRA. 2004. Tables of composition and nutritional value of feed materials. Wageningen Academic Publishers, The Netherlands & INRA, Paris, France.
[3] For mineral sources element concentrations were used from Batal and Dale. 2008. Feedstuffs September 10, p. 16.
[4] For feed materials without Zn content in the INRA tables, CVB values were used to complete the dataset.
[5] On DM basis.

Table C2: Zinc in feed extracted from data submitted from the Industry as response to a questionnaire on "Typical composition of complete/complementary feed for all animal species/categories"

Animal-Food	Zinc in feed (mg/kg) Contribution from feed ingredients
Complete Dry Dog Food	56.5
Complementary Dry Dog Food	49.2
Completementary Semi-moist Dog Food	68.6
Complete Dry Cat Food	59.1
Typical Dry Food for dogs	37.2
Typical Dry Food for cats	41.4
Parakeet	43.4
Dwarf rabbit (without cereals)	22.7
Dwarf rabbit (with cereals)	42.0
Goldfish	14.8
Tropical fish	30.5
Ornamental birds (Canary, Budgie, Exotic)	14.6
Pet rabbits and Guinea pigs	8.9
Hamsters	10.0
Complete Premium Dry Food for cats	42.6
Complete Super-Premium Dry Food for cats	59.2
Complete Premium Dry Food for dogs	6.8
Complete Super-Premium Dry Pet Food for dogs	45.8

Comment of the FEEDAP Panel to Table C2. The data cannot be compared with the values of Table C1 for several reasons: (i) Table C1 is calculated on the basis of feed materials, Table C2 on the basis of ingredients (e.g. cereals, meat and animal derivatives), (ii) Table C1 considers the contribution from phosphorus and calcium sources to total zinc, while Table C2 does not, (iii) Table C2 obviously operated twice with ranges for the zinc content: for the feed material given in the formula and for the zinc content in the specific feed material.

APPENDIX D. Data of zinc in compound feed from monitoring activities in European countries

Table D1: Data submitted from European Countries as response to a questionnaire on "Analyses of Compound Feed for all animal species/categories obtained during National Official Controls": raw data, data validation and analysed data

Compound feed[(1)]	All data	Excluded data based on feed type	Excluded data based on zinc amount	Data analysed
Poultry	**1841**	**397**	**43**	**1401**
Starter chicks	81	6	0	75
Chickens reared for laying	61	8	1	52
Laying hens	862	294	23	545
Chickens for fattening	512	62	17	433
Turkeys	176	16	2	158
Other poultry: ducks and geeses	149	11	0	138
Pigs	**7740**	**1581**	**301**	**5858**
Piglets	2821	526	197	2098
Pigs for fattening	4032	811	97	3124
Sows	887	244	7	636
Bovids	**2936**	**634**	**690**	**1612**
Calves	234	166	27	41
Calf milk replacer	229	16	22	191
Cattle	1019	417	352	250
Dairy cow	1097	35	232	830
Sheep, concentrate	331	0	51	280
Goat, concentrate	26	0	6	20
Horses	421	59	48	314
Rabbits	243	33	5	205
Fish	116	5	2	109
Dogs	207	20	25	162
Cats	114	14	24	76
TOTAL	**13618**	**2743**	**1138**	**9737**

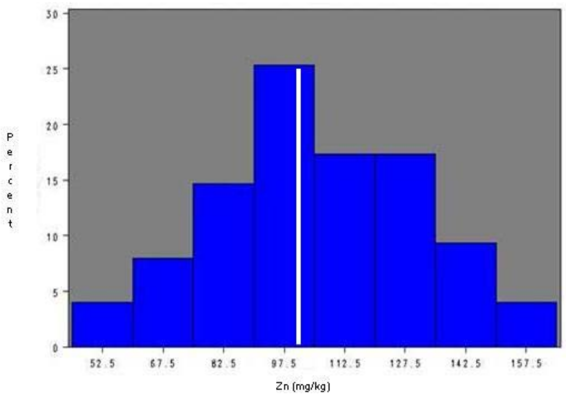
[1] The following grouping was applied:
"Laying hens": Includes the data labelled as feed for laying hens, layer phase I and layer phase II
"Chickens for fattening": Includes the data labelled as feed for chickens for fattening, broiler starter, grower and finisher
"Turkeys": Includes the data labelled as feed for turkeys for fattening, starter, grower and finisher
"Piglets": Includes the data labelled as feed for piglets weaned, piglets starter I and piglets starter II
"Pigs for fattening": Includes the data labelled as feed for pigs for fattening, pig grower and pig finisher
"Sows": Includes the data labelled as feed for sows, sows gestating and sows lactating
"Rabbit": Includes the data labelled as feed for rabbit, rabbit breeder and rabbit grower/finisher.

Table D2: Zinc in fish feed (mg/kg feed DM). Data from Norwegian Food Safety Authority's Annual Fish Feed Surveillance Programme

Year	Samples (n)	Mean	Range
2001	23	224	40 - 308
2003	40	165	44 - 235
2004	40	148	96 - 191
2005	23	122	31 - 254
2006	49	141	68 - 241
2007	22	144	100 - 190
2008	21	162	61 - 260
2009	25	168	110 - 230
2010	23	157	110 - 210
2011	25	162	109 - 242

APPENDIX E. Zinc in feed (mg/kg) per animal category[20] (figures[21] and tables). Data from European countries (2010-2012)[22]

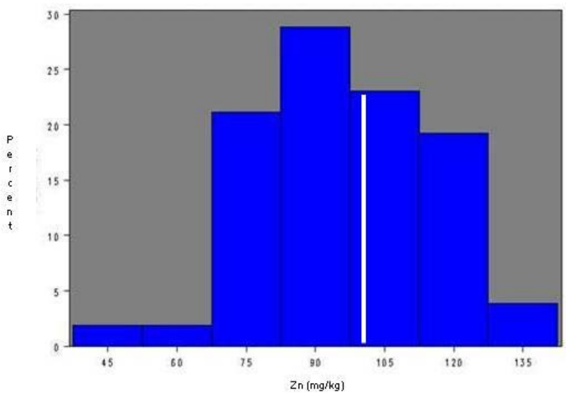
Figure and Table E.1: Starter Chicks



No of samples	75
Mean of zinc in feed (mg/kg)	105.2
P90	139.0
Median	103.0
P10	68.0
Range used	30-160
% samples above limit (100 mg/kg)	53.3
% samples below limit (100 mg/kg)	46.7

Number of samples per country: CH, 24; CZ, 3; DE, 42; EE, 3; LV, 1; PL, 2.

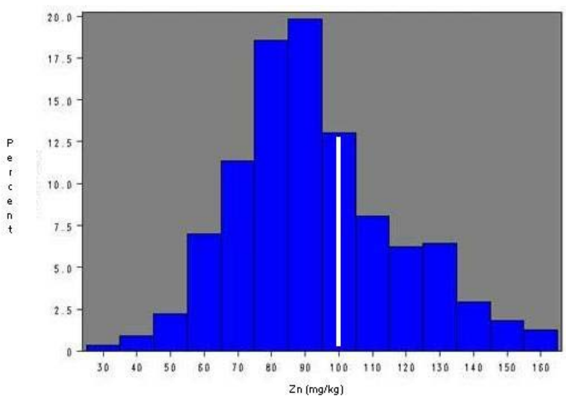
Figure and Table E.2: Chickens reared for laying



No of samples	52
Mean of zinc in feed (mg/kg)	96.8
P90	121.3
Median	95.3
P10	74.0
Range used	30-160
% samples above limit (100 mg/kg)	42.3
% samples below limit (100 mg/kg)	57.7

Number of samples per country: BE, 2; BG, 1; CH, 2; CZ, 7; DE, 18 (2013:1); DK, 8; HU, 2; PL, 10; SK, 2.

Figure and Table E.3: Laying hens

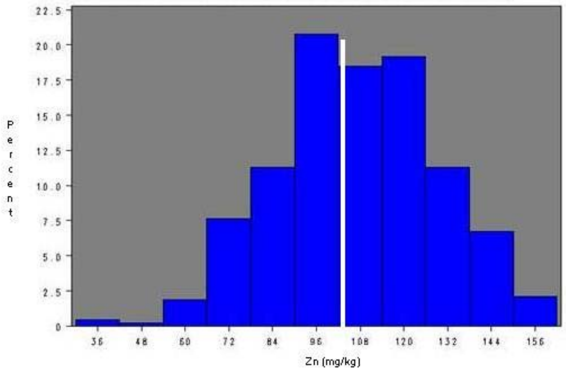


No of samples	545
Mean of zinc in feed (mg/kg)	92.7
P90	128.0
Median	89.0
P10	64.5
Range used	30-160
% samples above limit (100 mg/kg)	31.0
% samples below limit (100 mg/kg)	69.0

Number of samples per country: BE, 8; BG, 7; CH, 90; CZ, 100; DE, 184 (2013: 5); DK, 23; EE, 7; FI, 1; HU, 14; IT, 5; LV, 1; PL, 76; SI, 4; SK, 25.

[20] Unless otherwise indicated, data refer to complete feed.
[21] The white line drawn in some figures indicates the new maximum limit proposal for total Zinc in feed.
[22] Samples from 2009 or 2013 are specified in the "number of samples per country".

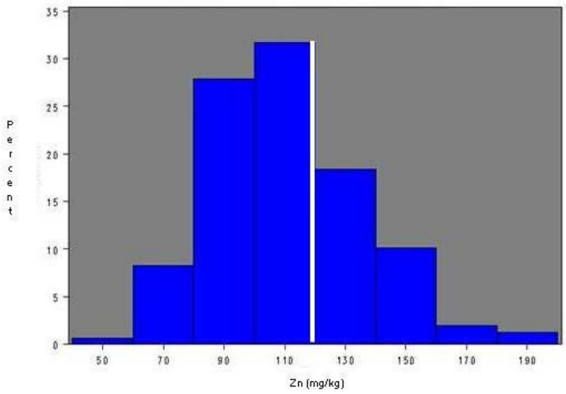
Figure and Table E.4: Chickens for fattening



No of samples	433
Mean of zinc in feed (mg/kg)	106.9
P90	137.0
Median	107.0
P10	77.7
Range used	30-160
% samples above limit (100 mg/kg)	60.3
% samples below limit (100 mg/kg)	39.7

Number of samples per country: BE, 5; BG, 5; CH, 62; CY, 2; CZ, 67; DE, 121 (2013: 1); DK, 3; EE, 1; FI, 5; FR, 7; HU, 23; IT, 7; MT, 18; PL, 73; SI, 5; SK, 29.

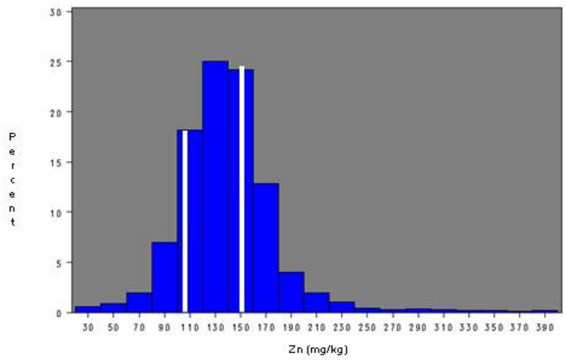
Figure and Table E.5: Turkeys for fattening



No of samples	158
Mean of zinc in feed (mg/kg)	110.6
P90	144.0
Median	106.0
P10	82.0
Range used	30-280
% samples above limit (120 mg/kg)	30.4
% samples below limit (120 mg/kg)	69.6

Number of samples per country: BG, 1; CH, 8; CZ, 19; DE, 106; HU, 7; PL, 14; SI, 2; SK, 1.

Figure and Table E.6: Piglets

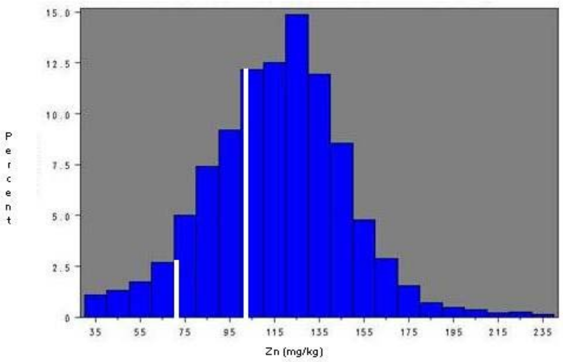


No of samples	2098
Mean of zinc in feed (mg/kg)	139.4
P90	178.0
Median	137.0
P10	99.0
Range used	30-400
% samples above limit-1[23] (150 mg/kg)	30.2
% samples below limit-1 (150 mg/kg)	69.8
% samples above limit-2 (110 mg/kg)	82.0
% samples below limit-2 (110 mg/kg)	18.0

Number of samples per country: BE, 39; BG, 29; CH, 363; CY, 3; CZ, 35; DE, 867 (2013:2); DK, 156; EE, 39; FR, 1; GR, 1; HU, 201; LV, 5; NL, 53; PL, 151; PT, 125; SI, 7; SK, 23.

[23] Limit-1 is for the use of feed without phytases and limit 2 is for the use of feed with phytases.

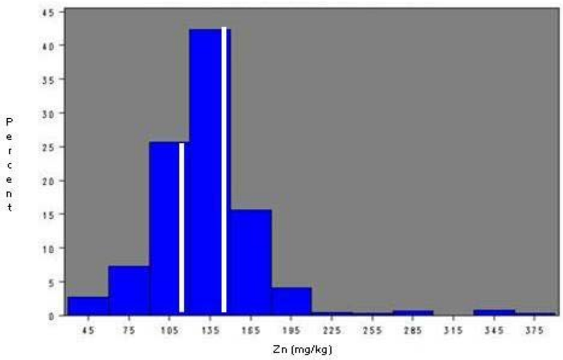
Figure and Table E.7: Pigs for fattening



No of samples	3124
Mean of zinc in feed (mg/kg)	115.7
P90	151.9
Median	117.0
P10	76.0
Range used	30-240
% samples above limit-1[24] (100 mg/kg)	70.5
% samples below limit-1 (100 mg/kg)	29.5
% samples above limit-2 (70 mg/kg)	93.0
% samples below limit-2 (70 mg/kg)	7.0

Number of samples per country: BE, 41; BG, 41; CH, 421; CY, 1; CZ, 338; DE, 692 (2013:6); DK, 190; EE, 34; FR, 2; GR, 11; HU, 266; IT, 43; LV, 7; MT, 2 (2009:1); NL, 364; PL, 200; PT, 303; SE, 1 (2013:1); SI, 45; SK, 122.

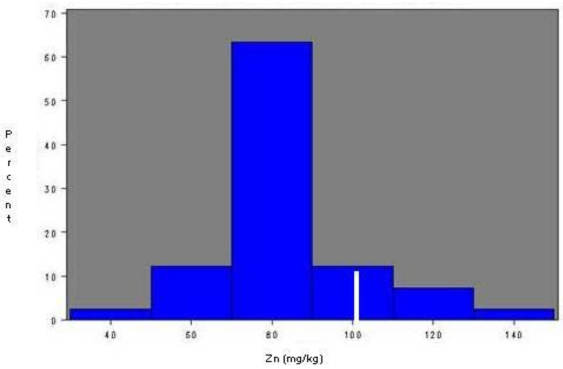
Figure and Table E.8: Sows



No of samples	636
Mean of zinc in feed (mg/kg)	131.4
P90	169.0
Median	129.0
P10	90.0
Range used	30-400
% samples above limit-1[25] (150 mg/kg)	20.1
% samples below limit-1 (150 mg/kg)	79.9
% samples above limit-2 (110 mg/kg)	75.1
% samples below limit-2 (110 mg/kg)	24.9

Number of samples per country: BE, 6; BG, 3; CH, 170; CY, 1; CZ, 51; DE, 243 (2013: 3); DK, 30; EE, 12; FI, 1; FR, 2; GR, 1; HU, 53; IT, 2; LV, 2; NL, 7; PL, 33; PT, 1; SE, 1 (2013:1); SI, 5; SK, 12.

Figure and Table E.9: Calves

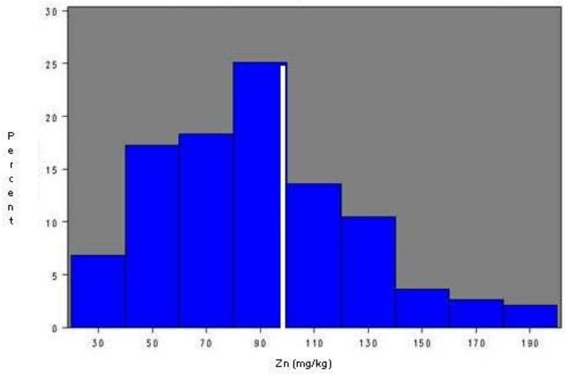


No of samples	41
Mean of zinc in feed (mg/kg)	82.7
P90	106.0
Median	79.0
P10	68.0
Range used	30-160
% samples above limit (100 mg/kg)	14.6
% samples below limit (100 mg/kg)	85.4

Number of samples per country: BE, 1; DE, 3; DK, 35; IT, 2.

[24] See Footnote in Table of Piglets.
[25] See Footnote in Table of Piglets.

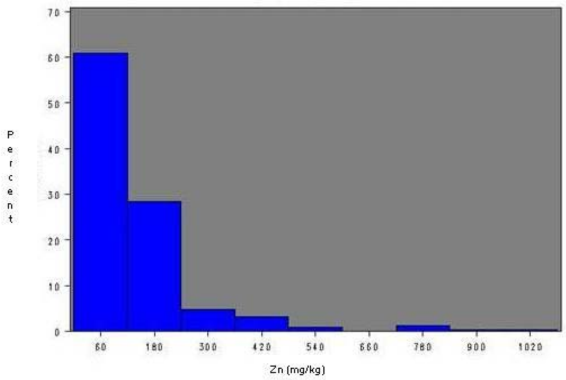
Figure and Table E.10: Calves milk replacer



No of samples	191
Mean of zinc in feed (mg/kg)	88.5
P90	136.0
Median	86.0
P10	44.0
Range used	30-200
% samples above limit (100 mg/kg)	31.4
% samples below limit (100 mg/kg)	68.6

Number of samples per country: CH, 8; CZ, 16; DE, 53 (2013: 5); FI, 1; NL, 82; PL, 6; SK, 24; SI, 1.

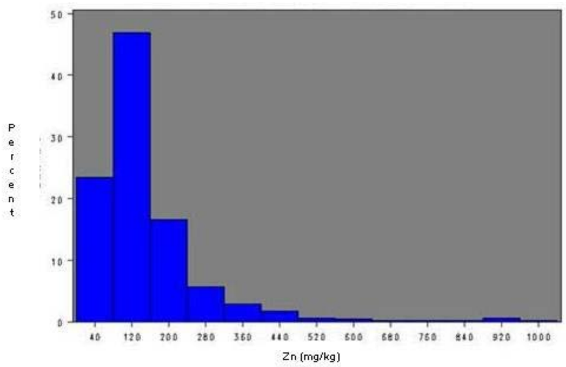
Figure and Table E.11: Cattle[26]



No of samples	250
Mean of zinc in feed (mg/kg)	140.7
P90	246.5
Median	105.0
P10	44.0
Range used	30-1000

Number of samples per country: BE, 11; CH, 8; CZ, 4; DE, 95; DK, 78; EE, 9; FI, 8; IT, 5; LV, 1; NL, 6; PL, 17; SI, 5; SK, 3.

Figure and Table E.12: Dairy cows[27]

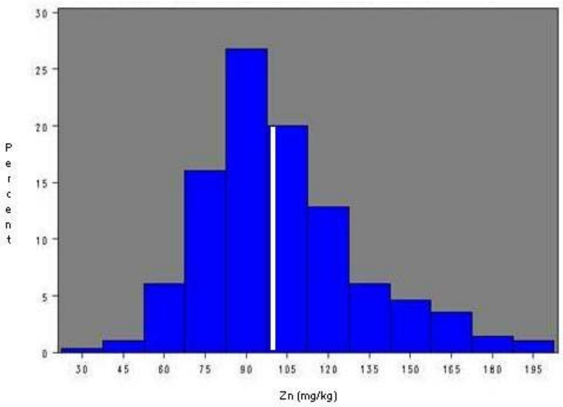


No of samples	830
Mean of zinc in feed (mg/kg)	151.7
P90	271.5
Median	113.0
P10	62.0
Range used	30-1000

Number of samples per country: BE, 22; BG, 2; CH, 254; CZ, 33; DE, 421 (2013: 4); EE, 5; FI, 25; GR, 1; HU, 10; IT, 1; MT, 5 (2013: 5); PL, 34; SE, 3 (2013: 3); SK, 5; SI, 9.

[26] Includes only data of feed labelled as Complementary feed and Concentrate.
[27] Includes only data of feed labelled as Complementary feed and Concentrate.

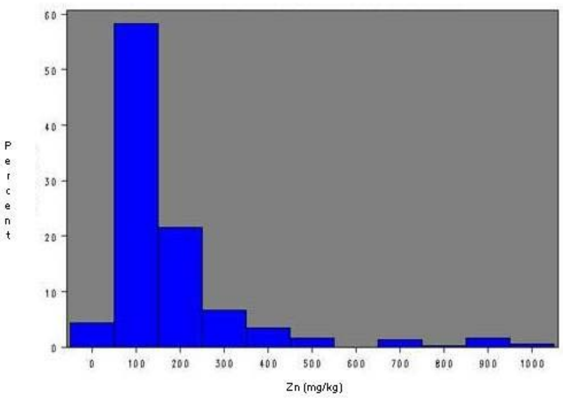
Figure and Table E.13: Sheep concentrate



No of samples	280
Mean of zinc in feed (mg/kg)	102.3
P90	144.0
Median	97.0
P10	70.1
Range used	30-200
% samples above limit (100 mg/kg)	45.7
% samples below limit (100 mg/kg)	54.3

Number of samples per country: BE, 2; BG, 2; CH, 6; DE, 105; DK, 11; FI, 2; GR, 3; HU, 1; IT, 2; NL, 138; SI, 1; SK, 7.

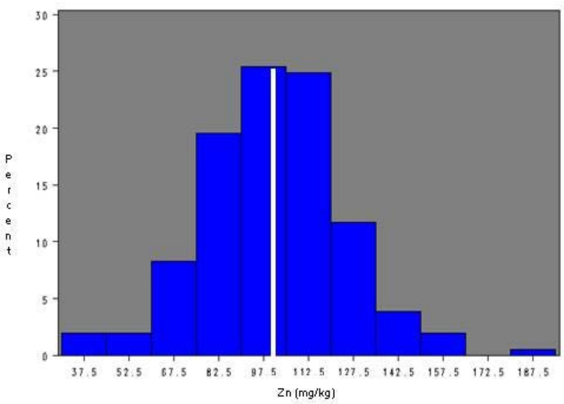
Figure and Table E.14: Horses[28]



No of samples	314
Mean of Zinc in feed (mg/kg)	175.8
P90	314.0
Median	132.3
P10	67.0
Range used	30-1000

Number of samples per country: BE, 1; CH, 37; CZ, 13; DE, 206 (2013: 1); DK, 49; FI, 8.

Figure and Table E.15: Rabbits

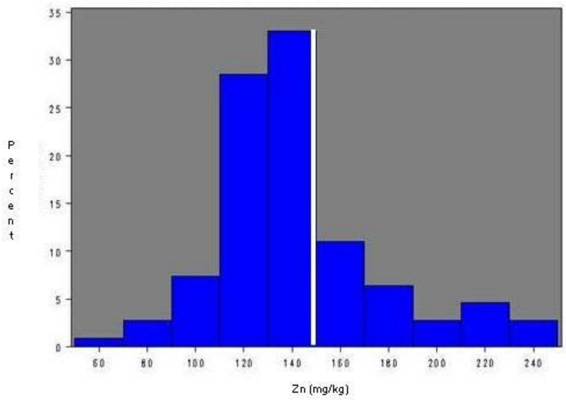


No of samples	205
Mean of zinc in feed (mg/kg)	100.3
P90	130.2
Median	98.4
P10	73.0
Range used	30-200
% samples above limit (100 mg/kg)	47.3
% samples below limit (100 mg/kg)	52.7

Number of samples per country: BE, 1; BG, 2; CH, 2; CZ, 54; DE, 120 (2013: 3); DK, 4; FI, 1; FR, 6; HU, 2; IT, 5; PL, 2; SI, 2; SK, 4.

[28] Includes only data of feed labelled as Complementary feed and Concentrate.

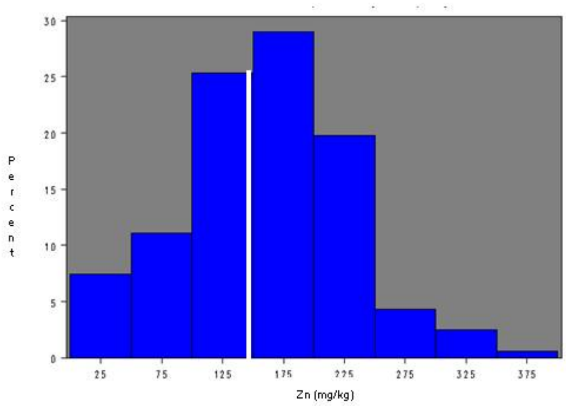
Figure and Table E.16: Fish



No of samples	109
Mean of zinc in feed (mg/kg)	140.0
P90	200.0
Median	131.0
P10	107.0
Range used	30-240
% samples above limit (150 mg/kg)	23.9
% samples below limit (150 mg/kg)	76.1

Number of samples per country: CH, 37; DE, 11; DK, 21; FI, 7; NO, 33 (2013: 33).

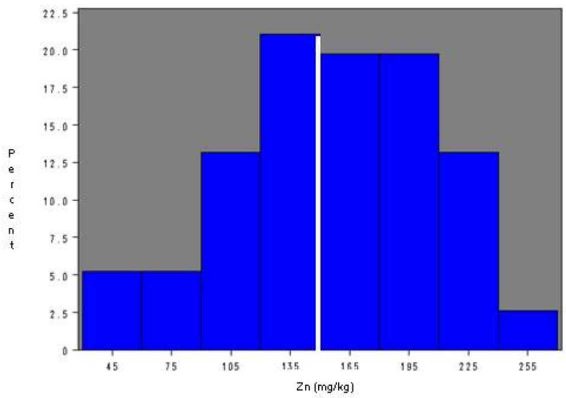
Figure and Table E.17: Dogs[29]



No of samples	162
Mean of zinc in feed (mg/kg)	159.0
P90	240.0
Median	157.5
P10	65.6
Range used	30-400
% samples above limit (150 mg/kg)	55.6
% samples below limit (150 mg/kg)	44.4

Number of samples per country: BE, 4; CZ, 48; DE, 48 (2013: 1); DK, 43; FI, 2; FR, 6; HU, 2; PL, 1; SI, 3; SK, 5.

Figure and Table E.18: Cats[30]



No of samples	76
Mean of zinc in feed (mg/kg)	154.5
P90	222.0
Median	154.0
P10	87.8
Range used	30-300
% samples above limit (150 mg/kg)	53.9
% samples below limit (150 mg/kg)	46.1

Number of samples per country: BE, 1; CZ, 20; DE, 11; DK, 36; FI, 3; FR, 2; PL, 2; SI, 1.

[29] Contains 9.9 % Complementary feed
[30] Contains 2.6 % Complementary feed

APPENDIX F. Calculations derived from poultry and pig feed data to obtain estimations of savings of zinc emissions to the environment

Types of compound feed	**Column A** Feed produced in 2011 (t/year)[1]	**Column B** Mean zinc in feed (mg/kg)[2]	**Column C** Mean zinc in the samples below the NPMC (mg/kg)[3]	**Column D** Benefit for environment (t zinc/year)[4]
Poultry	**50947000**			
Chicken for fattening	26288652	107	85	578.35
Chick and layers	16965351	98	82	271.45
Other	7692997	111	97	107.70
Pigs	**50256000**			
Piglets	7287120	139	121	131.17
Pigs for fattening	31962816	116	80	1150.66
Breeding Pigs	7538400			
Other	3467664			
TOTAL				2239.39

[1] Figures from FEFAC 2011 statistics.
[2] Calculations based on the data submitted by European countries following a call for data by EFSA in 2013.
[3] Expected average after the introduction of NPMC following the same disttribution as shown in the data sub submitted by European countries following a call for data by EFSA in 2013.
[4] Figures in Column D have been calculated using the following formula, where applicable:
[(Column A*Column B)−(Column A*Column C)]/1000000

ABBREVIATIONS

°C	degree Celsius (centigrade)
ADG	average daily gain
AMCRA	antimicrobial consumption and resistance in animals
ANSES	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (French Agency for Food, Environmental and Occupational Health & Safety)
bw	body weight
CAMC	currently authorised total maximum contents of zinc in complete feed
cAMP	cyclic adenosine monophosphate
CTR	copper transporter
CV	coefficient of variation
CVB	Centraal Veevoederbureau
DM	dry matter
DMT	divalent metal transporter
DNA	deoxyribonucleic acid
DRV	Dietary reference value
EC	European Commission
EEA	European Economic Area
EFSA	European Food Safety Authority
EFTA	European Free Trade Association
EMFEMA	International Association of the European Manufacturers of Major, Trace and Specific Feed Mineral Materials
EsKiMo	Ernährungsstudie als KiGGS-Modul
EU	European Union
FEDIAF	European Pet Food Industry Federation
FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
FEFAC	European Feed Manufacturers' Federation
FEFANA	European Association for the Producers of specialty Feed Ingredients and their Mixtures
FLF	fermented liquid feeding
FTU	phytase units
GfE	Gesellschaft für Ernährung (German nutrition society)
HDL	high-density lipoprotein-cholesterol
IFZZ	Instytut Fizjologii I Zywienia Zwierzat (Institute of animal physiology and nutrition)
IOM	Institute of Medicine, Food and Nutrition Board
INRA	Institut National de la Recherche Agronomique (French National Institute for Agricultural Research)
IP	inositol phosphate
kg	kilogram
KiGGS	Der Kinder- und Jugendgesundheitssurvey
LDL	low-density lipoprotein-cholesterol
mg	milligram
mL	milliliter
MJ ME	megajoules metabolisable energy
MRSA	methicillin-resistant *Staphylococcus aureus*
MT	metallothionein
MTF	metal transcription factor
MTL	maximum tolerable levels
MTT	Maa- ja elintarviketalouden tutkimuskeskus (Agrifood research Finland)
NPMC	newly proposed total maximum contents
NRC	National Research Council
NSP	non-starch polysaccharides
NZW	New Zealand White
PMCA	Plasma membrane Ca^{2+}-atpase

PRI	population reference intake
RAR	Risk Assessment report
RBV	relative bioavailability
RDA	recommended dietary allownaces
SCAN	Scientific Committee on Animal Nutrition
SCF	Scientific Committee on Food
SD	standard deviation
TMR	total mixed ration
TRP	transient receptor potential
UL	tolerable upper intake level
USA	United States of America
ZIP	Zrt-, Irt-like Protein