EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
C2 - Management of scientific committees; scientific co-operation and networks

Opinion of the Scientific Committee for Animal Nutrition on the use of zinc in feedingstuffs

(adopted on 14 March 2003)

1. Background

Zinc is authorised for all species, including dogs and cats, under Directive 70/524/EEC concerning additives in feedingstuffs under the category "trace elements".

The Commission has the intention to review the maximum content of trace elements authorised in feedingstuffs in order to adapt these levels to the physiological requirements and to minimise negative effects on the environment. In regard with these objectives, particular attention should be paid to the use of Zn where negative effects on the environment have been described.

2. Terms of reference (November 2000)

2.1. What are the real physiological requirements for Zinc of the different animal species referred to in Directive 70/524/EEC in regard to this trace element?

2.2. Has Zinc growth promotion effects for the species referred to in Directive 70/524/EEC? In the affirmative case, at which doses, for the different species?

2.3. Can the use of Zinc at the levels authorised in Directive 70/524/EEC, and considering its use in different animal species, be prejudicial to the environment?

Table 1: Zinc levels authorised in Council Directive 70/524/EEC

No. (or EC No.)	Element	Additive	Chemical formula	Maximum content of the element in mg/kg of complete feedingstuff	Other provisions
E 6	Zinc-Zn	Zinc lactate, trihydrate	$Zn(C_3H_5O_3)_2 . 3H_2O$	250 (total)	-
		Zinc acetate, dihydrate	$Zn(CH_3COO)_2 . 2H_2O$	250 (total)	-
		Zinc carbonate	$ZnCO_3$	250 (total)	-
		Zinc chloride, monohydrate	$ZnCl_2 . H_2O$	250 (total)	-
		Zinc oxide	ZnO	250 (total)	Maximum content of lead: 600mg/kg
		Zinc sulphate, heptahydrate	$ZnSO_4 . 7H_2O$	250 (total)	-
		Zinc sulphate, monohydrate	$ZnSO_4 . H_2O$	250 (total)	-

		Zinc chelate of amino acids hydrate	$Zn(x)_{1-3} \cdot nH_2O$ (x=anion of any amino acid derived from hydrolysed soya protein) Molecular weight not exceeding 1500.	250 (total)	Not more than 80 mg/kg of zinc in the complete feedingstuff may come from zinc chelate of amino acids hydrate

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3. ZINC REQUIREMENTS FOR LIVESTOCK

3.1. Zinc as an essential trace element

Zinc is an essential trace element in all living systems from bacteria, plants and animals to humans. Clear evidence of a biological function for zinc was not available until 1934, when the effect of experimental zinc deficiency in rats were first described (Todd *et al.*, 1934). It took another 20 years before naturally occurring zinc deficiency was recognised as the cause of parakeratotic lesions of the skin of pigs (Tucker and Salmon, 1955). Shortly afterwards, lack of zinc was shown to result in poor growth, feathering and skeletal development of poultry (O'Dell and Savage, 1957; O'Dell *et al.*, 1958).

Clinical deficiency of zinc was first diagnosed in humans in the 1960s (Prasad *et al.*, 1961) and the rare inherited disease "*Acrodermatitis enteropathica"* was related to an inability to absorb adequate zinc from normal diet (Moynahan, 1974). Inherited zinc malabsorption was described also in Friesian cattle and in dogs (Chesters, 1983; Jezyk *et al.*, 1986).

In 1939, recognition of zinc as an integral component of the enzyme carbonic anhydrase established the first biochemical role for zinc (Keilin and Mann, 1939). Subsequently, numerous other zinc enzymes have been discovered (> 200 enzymes) and recently a second group of proteins, probably more numerous than the zinc enzymes have been shown to function in transcription factors (Berg, 1990).

3.2. Zinc metabolism

The absorption of zinc occurs primarily in the small intestine. Only small amounts are absorbed from the stomach. In ruminants endogenous excretion into the rumen and reticulum is higher than absorption from these stomachs. The mechanism and control of zinc absorption are still not fully understood. Studies with rats indicate that absorption occurs by pericellular and carrier-mediated processes, with uptake at the brush border membrane being rate limiting (Rucker *et al.*, 1994). At concentration of 0.2 mmol, zinc uptake by isolated brush border membranes is saturated and occurs via an energy-independent carrier-mediated process. Absorption is markedly affected by other dietary components. In monogastric animals, phytate, for example, decreases zinc absorption, whereas low molecular weight binding ligands such as citrate, picolinate, ethylenediaminetetraacetic acid (EDTA) and amino acids such as histidine and glutamate enhance zinc absorption. (Hambidge *et al.*, 1986).

Zinc in plasma is firmly bound to globulin and loosely bound to albumin. Within the range of homeostatic regulation, the liver pool and storage of zinc is limited except in bone; storage increases only slightly as dietary zinc increases. Zinc concentration in bone has been used as a measure of zinc utilisation and/or zinc status in growing animals, zinc concentration in hairs can be used for diagnostic purpose of zinc supply in the previous period, whereas plasma zinc is not a reliable index.

The liver is the primary organ involved in zinc metabolism. When hepatic zinc content is increased above normal levels, additional zinc is associated with metallothionein, a metal-binding protein thought to have a role in storage and detoxification of zinc, copper, cadmium and other metals.

Zinc is excreted primarily in the faeces as unabsorbed and endogenous zinc. Endogenous excretion varies according to the balance between true absorption and metabolic needs. Variable faecal excretion is one of the primary mechanisms for maintaining zinc homeostasis. Thus, both absorption and faecal excretion are important in regulating zinc balance.

3.3. Bioavailability of zinc

In animal and man, numerous factors have a marked effect on zinc absorption. The kind of chemical compound is one of them.

Most studies on zinc bioavailability determines the relative bioavailability mainly related to zinc sulphate (Wedekind and Baker, 1990) and only a few studies address the absolute apparent bioavailability. Recently the absolute bioavailability was established to be about 22% for zinc oxide, 23% for zinc sulphate and 19% for zinc acetate (Poulsen and Carlson, 2001; Poulsen and Larsen, 1995). Expressed as relative values to zinc sulphate these obtained values correspond well to the published relative values. Surprisingly, the bioavailability was quite low in all tested sources.

Ranking of criteria to assess bioavailability of zinc from different sources were described by Sandoval *et al*. (1997) for broilers and by Swinkels *et al.* (1994) for pigs. They showed that concentration of zinc in bones was closely related to the bioavailability followed by zinc content in liver and pancreas.

Table 2: Relative biological value of zinc sources evaluated by tibia ash content (Jongbloed *et al.*, 2002)

Species	Pig	Ruminants	Broiler
Zinc sulphate	100	100	100
Zinc carbonate	98	58	93
Zinc chloride		42	107
Zinc oxide	92	95	67
Zinc amino acid chelate	102	102	131

Besides the chemical compound numerous interactions between zinc and other feed components exist. Hexa- and penta phosphate derivatives of inositol (phytic acids) affect zinc absorption in non ruminants because insoluble zinc phytate complexes are formed. The absorbability of zinc depends not only on the concentration of phytate, but also of calcium, magnesium and phosphorus. The zinc bioavailability is also influenced by high amounts of copper and iron in the diet. In similar way a nickel oversupply leads to signs of zinc deficiency (Anke *et al.*, 1995, 2002). It seems that an oversupply of divalent cations influences the metabolism of zinc.

Consequently the reported recommendations for zinc may vary among studies owing to the differences in the absorption of supplemental zinc sources and the use of ingredients that interfer with absorption and/or utilization of zinc under study.

3.4. Requirements and recommendations

Definitions:

Nutrient requirement: the individual demand of a specific nutrient under defined conditions

Recommendation: estimate of the necessary nutrient supply to meet the average gross demand of the population under common conditions plus safety factor considering the individual variability and bioavailability due to the specific chemical zinc compound and interactions between nutrients

Nutrient requirements are not suitable for calculating rations for groups of animals kept under different conditions, therefore, recommendations should be used in practise.

3.4.1. Ruminants (Table 3)

In experiments a dietary zinc concentration of 8 to 9 mg/kg feed dry matter (DM) for growth of suckling calves and 10-14 mg/kg is necessary to maintain normal plasma zinc levels (Mills *et al.*, 1967). In field observations, the zinc requirement is higher. Zinc supplementation of a barley ration containing 29-33 mg/kg dry matter increased the growth rate in heifers and steers (Hambidge, 1986).

Weigand and Kirchgessner (1982) calculated the dietary zinc requirement for a 600 kg dairy cow, producing 30 kg milk per day would be 40 to 50 mg/kg dry matter.

In most attempts zinc requirement was estimated using dose response relationships. Weigand and Kirchgessner (1982) estimated Zn requirements for dairy cows by a factorial method. This was done also by Underwood and Somers (1969) and more recently by the NRC (2001) . The figures differed markedly mainly due to differences in the values for absorption or utilization.

For example Weigand and Kirchgessner (1982) calculated with a utilization for zinc between 0.40 and 0.25, Underwood and Somers (1969) used an absorption coefficient of 0.7 whereas NRC (2001) calculated with an efficiency of absorption of 0.5 for pre-ruminant calves, 0.3 for growing ruminants and 0.2 for adult ruminants.

3.4.2. Horses

The zinc recommendation for horses varies from 35 (GfE, 1994) to 40 mg/kg feed dry matter (NRC, 1989).

Based on a literature review Schwarz and Kirchgessner (1979) estimated a zinc requirement of horses of about 50 mg/kg dry matter.

Zn requirement seems to be higher if there is a high phytate concentration in the total diet or if there is a high calcium or copper content (Meyer and Coenen, 2002).

Harrington *et al.* (1973) demonstrated that 40 mg of zinc/kg of a purified-type diet was sufficient to prevent zinc deficiency in foals. Schryver *et al.* (1974) reported that foals fed 41 mg of zinc/kg of natural diet grew at acceptable rates and maintained normal body stores of zinc. *GfE* (1994) and Jarrige and Martin-Rosset (1981) indicated that 50 mg of zinc/kg DM was adequate for all classes of horses. Mares' milk contains 1.8 to 3.2 mg of zinc/kg of fluid milk. Thus, foals drinking 15 kg of milk/day would consume 27 to 48 mg of zinc/day. This is equivalent to 17 to 30 mg of zinc/kg of dry matter intake. The zinc in milk is assumed to be highly available.

Table3: Zinc requirements (ARC, NRC) of and recommendations (GfE) for ruminants

Species/Category	Requirement/ Recommendation		Reference
	mg/day	mg/kg DM	
Growing calves			ARC (1980)
40 kg bw	28	28	
80 kg bw, ADG = 1 kg	69	34	
Heifer, 300 kg bw ADG = 0.7 kg	202	33	NRC (2001)
	151	25	ARC (1980)
		40-50	GfE (2001)
Heifer, 500 kg bw ADG = 0.5 kg, day 250 gestation	310	31	NRC (2001)
	204	20.4	ARC (1980)
Cow, 650 kg bw 40 kg milk/day	1261	63	NRC (2001)
	946	47.3	ARC (1980)
		50	GfE (2001a)
Cow, 650 kg bw day 270 gestation	274	22.8	NRC (2001)
	178	14.8	ARC (1980)
Beef cattle		30	NRC (2000)
		40	GfE (2001b)
Bullock, 100 – 300 kg bw, ADG = 1 kg	95 - 188	17 - 35	ARC (1980)
Sheep, growth		20	NRC (1985)
Lamb 10 kg bw	9.7	24	ARC (1980)
20 kg bw	20	32 - 36	
40 kg bw	29	29 - 49	
Sheep, reproduction		33	NRC (1985)
Ewe, pregnant	34	20 - 24	ARC (1980)
lactating	46 - 94	24 - 39	
Goat		10	NRC (1981)
		50 – 80	GfE (2003)

bw = body weight; ADG = average daily body weight gain

3.4.3. *Pigs* (Table 4)

The zinc requirement of young pigs consuming a casein-glucose diet is low (15 mg/kg) because such a diet does not contain plant phytates (Smith *et al.*, 1962; Shanklin *et al.*, 1968). For growing pigs fed diets containing significant amounts of phytate the zinc requirement is about 50 mg/kg diet (Lewis *et al.*, 1956, 1957a,b; Luecke *et al.*, 1956; Stevenson and Earle, 1956; Smith *et al.*, 1958, 1962; Miller *et al.*, 1970). Boars have a higher zinc requirement than gilts and gilts have a higher requirement than barrows (Liptrap *et al.*, 1970; Miller *et al.*, 1970). The zinc requirement of breeding animals is not well established, but may be higher than for growing pigs. Kirchgessner *et al.* (1981) estimated the zinc requirement of pregnant sows at 25 mg/kg diet. A level of 33 mg/kg corn-soybean meal diet was adequate for gestation performance, but not for lactation (Hedges *et al.*, 1976).

Table 4: Zinc requirements (NRC, ARC) of and recommendations (GfE, INRA) for pigs

<table>
<tr><th>Category</th><th>NRC (1998)
mg/kg diet
(90 % DM)</th><th>ARC
(1981)
mg/kg DM</th><th>GfE (1987)
mg/kg DM</th><th>INRA
(1984)
mg/kg diet</th></tr>
<tr><td>3 – 10 kg bw</td><td>100</td><td rowspan="6">50</td><td rowspan="2">80 - 100</td><td rowspan="6">100</td></tr>
<tr><td>10 – 20 kg bw</td><td>80</td></tr>
<tr><td>20 – 50 kg bw</td><td>60</td><td rowspan="2">50 - 60</td></tr>
<tr><td>50 – 120 kg bw</td><td>50</td></tr>
<tr><td>Reproduction</td><td>50</td><td rowspan="2">50</td></tr>
<tr><td>Boars, sexually active</td><td>50</td></tr>
</table>

3.4.4. *Dogs and cats* (Table 5)

A concentration of 60 mg zinc per kg in dry natural-ingredient diets appears to meet the requirements for dogs provided Ca concentration is not excessive. Dogs maintained on high-meat or purified diets may be slightly lower. Based on studies with purified diets, it is estimated that the minimum zinc requirement for dogs is 2.32 mg/MJ ME (NRC, 1985). There is no comparable experimental evidence showing the extent to which reproduction and growth influence the zinc requirements of dogs. However, optimal performance during gestation and lactation may be more readily attained when the dietary zinc concentration is increased to near 90 mg/kg (NRC, 1985).

The zinc requirement of weanling kittens fed a soy-based purified diet was found by Kane *et al.* (1981) not to exceed 15 mg Zn/kg diet based upon growth and lack of gross deficiency signs. Addition of excess Ca (2 % added $CaHPO_4$) to the same diet failed to observe either reduced body weight gains or skin lesions. Male kittens, however, showed evidence of impaired testicular function when fed 15 mg Zn/kg of diet over an 8-month period. This suggests that during growth, male kittens have a higher requirement for zinc than females. Male kittens fed 67 mg Zn/kg diet showed no evidence of testicular degeneration. The same authors also fed kittens a diet based upon EDTA-washed soy protein isolate in an attempt to produce severe zinc deficiency signs. The basal diet contained only 0.7 mg Zn/kg diet. Although classical signs of deficiency were produced in kittens fed this diet for 8 weeks, the parakeratosis and other dermal lesions were less severe than that which has been observed previously in zinc-deficient pigs and dogs. No deficiency signs were observed in kittens fed the EDTA-treated soy diet containing 52 mg Zn/kg diet. Aiken *et al.* (1978) reported skin lesions and reduced growth when a vegetable protein-based diet containing 40 mg Zn/kg was fed to young kittens. Kamphues *et al.* (1999) recommend per kg bw and day: 1mg for maintenance and 2.5 mg for growth.

The requirement of zinc for gestation and lactation has not been determined. A high dietary zinc requirement (50-100 mg/kg diet) is known for several species for fetal development. Kamphues *et al.* (1999) recommend 1.4 mg Zn/kg bw/day during gestation, 1.9, 3.2 and 4.0 mg/kg bw/day for lactating cats with 1-2, 3-4 and >4 kittens, respectively.

Table 5: Zinc requirements of and recommendations for dogs and cats

Requirements						
	Unit	Growth	Maintenance	Gestation	Lactation	Reference
Dog	mg/kg bw/day	1.94	0.72			NRC (1985)
	mg/MJ ME	2.32				
	mg/kg DM (15.4 MJ ME)	35.6				
Cat	mg/kg DM (20.9 MJ ME)	50.0				NRC (1986)
Recommendations						
Dog	mg/kg DM	120	120			AAFCO (1998)
	mg/kg bw/day	1.1-3.7 (<2 months) 3.2-4.1 (3-6 months) 1.3-1.7 (>6 months)	0.9	1.3	2.0 (< 4 puppies) 3.2 (4-6 puppies) 3.7 (>6 puppies)	GfE (1989)
Cat	mg/kg DM	75	75			AAFCO (1998)

3.4.5. *Rabbits*

In rabbits Zn recommendations published vary between 30 and 60 mg/kg diet with higher values proposed for breeders (Mateos and de Blas, 1998). INRA (1984) recommends 50 mg/kg die for fattening and 70 mg/kg diet for breeder rabbits. In practice, these values are considered sufficient for rabbits and commercial diets contain a similar range. However, no trials have been conducted to quantify the requirements (Mateos and de Blas, 1998).

3.4.6. *Poultry* (Table 6)

The zinc requirement of the young broiler is approximately 35 to 40 mg/kg in semipurified diets containing isolated soy protein or casein (Morrison and Sarett, 1958; O'Dell *et al.*, 1958; Roberson and Schaible, 1958). Studies on corn-soybean meal and sesame meal diets suggest that the requirement is in excess of 40 mg/kg (Edwards *et al.*, 1958; Lease *et al.*, 1960; Zeigler *et al.*, 1961). This conclusion was based primarily on small growth responses to zinc supplementation of the basal diets. The estimated zinc requirement is somewhat tenuous, because the estimate was based on calculated values for zinc content of the feed ingredients. A study by Wedekind and Baker (1990) showed that the tibia zinc concentration of chicks fed a corn-soybean meal diet was increased markedly by dietary zinc supplementation but did not provide an estimate of requirements. The source of supplemental zinc used in most of the cited studies was zinc sulphate or zinc chloride. Availability of zinc varies among sources (Wedekind and Baker, 1990). In a diet containing egg white as the primary protein source, the requirement for zinc is only 14 to 18 mg/kg (Southern and Baker, 1983; Dewar and Downie, 1984). Only tentative values are given for chicks after 3 weeks of age. Mineral requirements of layers in production are similar to mineral requirements of other poultry, with the exception of calcium.

Table 6: Zinc requirements of (mg/kg diet, 90 % DM; NRC) and recommendations for poultry (mg/kg diet, 88 % DM; INRA, GfE)

Chicken	*White-egg-laying*		*Brown-egg-laying*		*Reference*
0-6 weeks	40		38		NRC (1994)
6 weeks to first egg	35		33		
Layers, *feed intake, g*	*80*	*100*	*120*		
	44	35	29		
0-6 weeks	44				GfE (1999)
6 weeks to first egg	39				
Layers	44				
Layers	40				INRA (1984)
Broiler	Starter		Finisher		
	40				NRC (1994)
	40		20		INRA (1984)
	44		44		GfE (1999)
Turkey					
weeks	*0-4*	*5-8*	*9-12*	*13-24*	
	70	65	50	40	NRC (1984)
	60		40 - 30		INRA (1984)
	50	40			GfE (2003)
Breeders	*Holding*		*Laying hens*		
	40		65		NRC (1994)
			50		INRA (1984)
Geese	40				NRC (1994)
Ducks	*0-2 weeks*		*Grow*	*Finish*	
	60		40		NRC (1994)
	40		30	20	INRA (1984)
Pheasants	60				

Japanese Quail	*Starter/Grower*	*Breeding*	
	25	50	NRC (1994)
	60		INRA (1984)
Guinea fowl	Starter	Grower	
	40	25	INRA (1984)

Zinc needs of turkey are known to depend on the levels of other dietary constituents. The recommended level of 70 mg/kg was determined with practical diets having phytic acid present, whereas 41 mg/kg were adequate in a purified diet where phytic acid was absent (Dewar and Downie, 1984).

3.4.7. Fish

Fish have the ability to absorb some zinc from water, nevertheless the diet is the predominant uptake route for this mineral (Willis and Sunda, 1984; Spry *et al.*, 1988). Dietary zinc requirements have been established for a number of different fish species fed semi-purified diets: 15-30 mg/kg for rainbow trout (*Oncorhynchus mykiss)* (Ogino and Yang, 1978), 15-30 mg/kg for carp (*Cyprinus carpio*)(Ogino and Yang, 1979) and 20 mg/kg for channel catfish (*Ictalurus punctatus*) (Gatlin and Wison, 1983). The zinc recommendation for Atlantic salmon (*Salmo salar*) has been reported to be 37-67 mg Zn/kg dry diet to maintain whole body zinc and serum zinc concentrations within the physiological range (Maage and Julshamn, 1993).

4. ZINC SUPPLEMENTATION

4.1. Zinc levels in feed materials

Zinc concentrations in plants and plant materials are influenced by soil concentration, soil conditions which influence Zn uptake (pH, ion exchange capacity etc.), fertilization, 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 is taken up by the plant via root uptake, but is added also via air deposition, contamination with soil particles or during processing. Zinc is taken up by the animals also via feedingstuffs of animal origin, mineral supplements and drinking water.

Normally only zinc concentrations were determined without any further specification. It is well known that binding form and other dietary ingredients like phytate, fiber, tannins etc. can influence bioavailability.

In the following table some values about zinc concentrations in different feedingstuffs are summarised.

Table 7: Zinc concentrations in some feed materials (mg/kg DM)

Feedingstuff	Levels	Feedingstuff	Levels
Barley	26-38	Potato pulp pressed	46
Brewers grain dehydr.	62-72	Rapeseed	60-66
Citrus pulp	10	Rapeseed extr.	34
Cotton seed extr. non decort.	70	Rye	31-35

Fish meal	90	Skimmed milk powder	41-48
Grass meal	40	Sorghum	15-34
Horsebean	46-49	Soybean extr. non deh.	66-70
Linseed extr.	49-51	Sugar beet pulp	22-24
Lupins	32-59	Wheat	29-49
Maize (Corn)	25-27	Wheat bran	100-112
Maize gluten feed	64-74	Wheat middlings	92-135
Oats	27-41	Whey powder	10-19
Pea	23-37	Whole milk powder	40

CVB, DLG (1991, 1997), NRC (1998, 2001)

4.2. Zinc supplementation

Because of the low zinc content in some feedingstuffs compared with the requirements and recommendations (see Tables 3-6) and varying bioavailability, zinc supplementation (see Table 1) is necessary in general and of common use. In the light of the requirements identified above, the current level of zinc supplementation of 250 mg/kg could be lowered to better reflect the real physiological needs. Adaptation should consider the natural level of zinc present in feedingstuffs and all factors impacting on zinc availability. An appropriate safety margin should therefore be included in the level fixed for this trace element.

4.3. Growth promotion effects

Zinc diet contents are allowed in the European Union up to 250 mg/kg complete feedingstuff (see Table 1). These levels allow to cover largely the requirements and recommendations mentioned previously for all animals. At levels of inclusion of zinc below 250 mg/kg, no significant differences in performance are noted between zinc doses and zinc provided to the animals should only be considered as allowing appropriate coverage of the requirements (Poulsen, 1995).

As far as growth promotion is concerned, among all farm animal species, growth promoting effects have only been reported incidentally by some authors, in piglets, after weaning. When supplementing higher amounts of zinc (zinc oxide fed for up to 5 weeks at levels of 2000 to 6000 mg zinc /kg), positive effects were reported in piglets (Hahn and Baker, 1993; Poulsen, 1995, Carlson *et al.*, 1999, Mavromichalis et al.,2000, 2001); (abstracts: Kavanagh, 1992; LeMieux *et al.*, 1995; McCully *et al.*, 1995; Smith *et al.*, 1995a,b; Hill *et al.*, 1996). Such high levels of zinc should be considered as prophylactic or therapeutical levels and are intended to prevent or overcome physiological disturbance, namely diarrhea, of young animals. Reduction of post-weaning scouring leads to reduced body weight loss by animals. Therefore the body weight gain in piglets should rather be considered as an indirect growth effect. Some studies, however, have failed to observe beneficial effects of therapeutic levels of Zinc (Fryer et al, 1992, Tokach et al, 1992, Schell and Kornegay, 1996).

SCAN therefore considers that the effect of zinc on growth performance remains questionable.

4.4. Excess of zinc

Toxicity of zinc is low. Farm animals, companion animals and fish as well as humans exhibit considerable tolerance to high intakes of zinc. Nonetheless zinc toxicity has been reported to occur under non experimental conditions. The toxicity of zinc clearly depends upon the zinc source, dietary level, the duration of feeding, and the levels of other minerals in the diet.

4.4.1. Ruminants

Ruminants, particularly young and pregnant animals, are more susceptible to zinc toxicity than pigs or poultry. Steers and heifers have been shown to be unaffected by dietary zinc levels of 500 mg /kg feed or less, but 900 mg/kg caused reduced weight gain and lowered feed efficiency (Ott *et al*., 1966b). Dairy cows, however, appear to tolerate zinc of intake of 1300 mg / kg diet with no ill effects, possibly because of the additional route of excretion in milk. The liver zinc concentration was generally increased and copper levels were depressed in liver but elevated in other tissues (Ott *et al., 1966d)*. It is possible that some of their increased sensitivity to zinc relates to direct effects on rumen organisms rather than on the animal itself. In naturally occurring cases of chronic zinc poisoning in calves, animals were in poor condition and anaemic. The estimated, not determined zinc content of the diet was at 500-900 mg/kg.

Zn intoxication also occurred naturally in sheep. Zinc toxicity has been described for growing lambs (Ott *et al*., 1966a, c; Davies *et al*., 1977). 1000 mg/kg diet caused reduced consumption of feed and reduced gain in lambs (Ott *et al*., 1966a). The sheep had subcutaneous oedema, ascites, and proteinuria. The tissue zinc levels suggested that the zinc intakes of these animals, not directly measured, were of the order of 1000 mg/kg diet. These findings were experimentally reproduced in sheep given 800 mg zinc daily (equivalent to 2000 mg/kg diet) for 12 days, followed by 1200 mg zinc daily for up to 10 weeks. Clinical manifestations of zinc toxicity, including loss of appetite, profound weakness and jaundice, in addition to tissue abnormalities, were very similar to the natural case (Allen *et al*., 1983). Zinc intake of 750 mg/kg diet by pregnant ewes caused a high incidence of abortions and stillbirth and feed consumption, body weight gain and feed efficiency were reduced. The animals had copper deficiency manifested by reduced plasma levels of copper, ceruloplasmin and aminooxidase ((Campbell and Mills, 1979).

At high level of zinc intake in lamb, there was a reduction of the volatile fatty acid concentration in the rumen. Cellulose digestion by rumen bacteria *in vitro* is reduced by zinc concentrations of 10 to 20 mg/L.

4.4.2. *Horses*

Horses are the most zinc sensitive farm animals and react with lameness, osteochondrosis (possibly caused by an abnormal collagen metabolism due to an inhibition of lysil oxidase followed by a zinc provoked copper deficiency (Willoughby *et al.*, 1972; Schwarz and Kirschgessner 1979).

A toxic level of zinc for horses of 500 mg/kg diet was set (NRC, 1989). Schwarz and Kirchgessner (1979) mentioned a tolerable level of about 1000 mg/kg DM. It should be pointed out that foals seem to be more sensitive and exhibit shortened tendons and osteochondrosis due to secundary copper deficiency. Also pregnant mares are more sensitive to high zinc intake (Meyer and Coenen, 2002).

In fillies, growth rate decreased markedly after the intake exceeded 90 mg Zn given as oxide per kg of body weight per day. These amounts approximated 3600 mg/kg in the feed on a dry matter basis (Willoughby *et al.*, 1972).

Weaning foals exposed to diet supplemented with 1000 mg/kg zinc became hypocupremic and showed lameness after serum copper concentration had remained at 0.3 µg/ml for more than one week. Copper concentration in the serum remained unaffected at the dose of 250 mg zinc/kg diet (Bridges and Moffitt, 1990).

4.4.3. *Pigs*

Zinc toxicity in growing pigs fed a corn–soybean meal diet supplemented with 2000 to 4000 mg zinc/kg diet from zinc carbonate was manifested by depression of growth, arthritis, hemorrhage in axillae, gastritis, and death. However, a dietary zinc level of 1000 mg/kg diet was not toxic (Brink *et al.*, 1959). But other studies with growing pigs fed 2000 to 4000 mg zinc/kg diet from zinc oxide did not show symptoms of zinc toxicity (Cox and Hale, 1962; Hsu *et al.*, 1975; Hill *et al.*, 1983b). However, pigs became lame and unthrifty within 2 months when they were fed a diet containing 1000 mg zinc/kg diet from zinc lactate (Grimmett *et al.*, 1937). In contrast, pigs fed a diet containing 1000 mg zinc/kg diet from zinc sulphate for 7 months showed no signs of zinc toxicity (Kulwich *et al.*, 1953). High dietary calcium reduces the severity of zinc toxicity (Hsu *et al.*, 1975). A 5000 mg zinc/kg diet as zinc oxide through two parities reduced litter size and pig weight at weaning and caused osteochondrosis in sows (Hill and Miller, 1983; Hill *et al.*, 1983a). Pigs from sows fed high levels of dietary zinc have reduced tissue levels of copper and rapidly develop anaemia when fed a low-copper diet (Hill *et al.*, 1983b,c). In young pig, 2500 mg zinc/kg diet fed as zinc oxide for 2 weeks after weaning stimulated feed intake and growth, whereas piglets fed 4000 mg zinc/kg diet had a reduced feed intake and body weight gain (Poulsen, 1989, 1995). A recent study confirmed that feeding up to 2500 mg/kg diet had no harmful effect on the physiology of weaned pigs but feeding 5000 mg/kg diet or more had detrimental physiological consequences mainly related to the poor feed intake.

4.4.4. Dogs and cats

Although relatively non-toxic, excess dietary zinc can interfere with other trace elements (iron, copper) thus excess should be avoided. The only reported cases of zinc intoxication in dogs or cats have been due to dietary indiscretion (*e.g.*, consumption of die-cast nuts from animal carriers or pennies).

4.4.5. Rabbits

No pathology related to zinc excess in feed has been described. No level known to induce signs of toxicity in the rabbit has been determined. In an experiment rabbits were fed, at 50 days old and for 21 days, a diet containing 16 mg zinc/kg and supplemented with 270 mg zinc/kg diet. Body weight gain was not affected by zinc supplementation and no signs of toxicity were observed (Hossain and Bertechini, 1993). In rabbits no maximum acceptable level of zinc is known.

4.4.6. Poultry

In young chickens the following concentrations (mg/kg diet) caused reduced growth: 800 mg as ZnO (Berg and Martinson, 1972), 1500 mg as $ZnSO_4$ or $ZnCO_3$ (Roberson and Schaible, 1960), 3000 as $ZnSO_4$ (Jensen, 1975) and as ZnO (Johnson *et al.*, 1962). Jensen (1975) observed also exudative diathesis and muscular dystrophy in his study at 2000 mgZn/kg feed as Zn sulphate. In immature turkeys 4000 mg Zn/kg diet as ZnO caused reduced growth (Vohra and Kratzer, 1968).

4.4.7. Fish

Physiological parameters such as blood haematocrit and haemoglobin levels indicated that dietary zinc concentrations of 1000 mg/kg compromised health in rainbow trout (*Oncorhynchus mykiss)* (Knox *et al.*, 1984). Furthermore, high dietary levels of zinc may negatively affect the status of other elements such as iron (Wekell *et al.*, 1986). However, when whole body responses such as growth depression are used as a parameter of impaired performance, dietary zinc concentrations up to 1700 mg/kg have been shown be tolerated by rainbow trout (Wekell *et al.*, 1983).

5. IMPACT ON ENVIRONMENT

The use of zinc in animal nutrition as dietary supplement contributes to the overall environment exposure to that trace element. Farm animal zinc intake is almost entirely excreted and slurry is spread onto arable and grass lands, as common agricultural practice. In the case of aquaculture, zinc comes from fish excreta but also from the supplementation itself as the feed is in direct contact with the aquatic environment.

Adequate models should be used to establish this impact.

In the particular case of fish, well-designed and suitable models are necessary to reflect the European production systems. One ton fish consumes circa 10 kg feed per day. Based on the current legislation, and considering that all zinc consumed is released, 2 500 mg zinc are released in the environment per ton fish and day. This should be considered with different aquaculture production scenarios in Europe: pond, raceways, sea-cages, etc. and will not be further addressed in the present document.

For farm animals, the model is based on the calculation of the net quantities of zinc finally added to the environment and implies therefore consideration of

– the zinc quantity excreted by animals, based on their respective intake,
– the quantity spread on lands
– the disappearance due to crop uptake or transport within soil

Data on zinc release from soil by crops or mobilisation are scarce. The available data would not allow consideration of crop sequence, influence of harvest and of climate as well as the degree of erosion. Therefore the worst case scenario was chosen by the Committee. All data for the calculation of element emissions from terrestrial animals are reported in annex.

The values of zinc concentration in feed authorised under Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs[1] were used *i.e.* 250 mg Zn/kg feed, for all animals. This value does not consider the dry matter content of feedingstuffs. Considering a dry matter content of 88% of the feedingstuffs, the following corrected values have been used for the calculations: for all species, 284 mg/kg dry matter.

5.1. Methodology

For soils, the assessment of impact of zinc application has been calculated on the basis of zinc/nitrogen ratio in manure and is based on the Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources ("European nitrate directive")[2] which establishes the nitrogen amount applicable on sensitive agricultural soil (170 kg N/ha y). The zinc/nitrogen ratio in manure has been calculated as the ratio between the total zinc and the total nitrogen excreted in the life cycle period taken into account for each animal and assuming a nitrogen loss in manure of 25%, mainly due to ammonia volatilisation. For non sensitive soils, the maximum nitrogen amount allowed in European member states has been used (France: 350 kg

1 E.C.O.J. n° L 270 of 14/12/1970, p.1

2 E.C.O.J. n° L 375 of 31/12/1991 p.1

N/ha y) (Spaepen *et al.*, 1997)[3]. The soil concentration is calculated after one-year application, for the top soil layer (5 cm thick), assuming a default soil density of 1.5 g/cm³. For long term application of manure (20 years), maximum accumulation of zinc in soil is referred to a depth of 20 cm of soil (the minimum layer involved in tillage) assumed as a more realistic scenario. However, as a worst case, losses due to crop intake and transport within soil are not taken into account.

Calculated values for annual application on soil and resulting soil concentrations were compared with limit values for zinc fixed in Council Directive 86/278/EEC of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture[4].

5.2. Results

On the basis of the diets established by SCAN (see table under 10), the annual load of zinc spread onto soil could be calculated in table 8 on the basis of the amount of nitrogen excreted by the animals and considering the levels of nitrogen allowed per hectare.

Table 8. Values of zinc annual load and resulting metal concentrations in soil after one year and 20 years application of animal manure for different animals. Calculations are performed for two levels of nitrogen application on soil: 170 and 350 kg/ha/y, respectively for vulnerable and not vulnerable areas.

	Calculation based on application on soil of two levels of nitrogen : 170 and 350 kg/ha/y, respectively, for vulnerable and non vulnerable areas					
	170	350	170	350	170	350
Animals	Zinc annual load on soil (g/ha/y)		Increase of zinc concentration in soil (mg/kg)			
			Over one year		Over 20 years	
Veal calves	3682	7580	4.9	10.1	24.5	50.5
Replacement calves	2863	5895	3.8	7.9	19.1	39.3
Fattening steers	3819	7864	5.1	10.5	25.46	52.4
Replacement heifers	3800	7824	5.1	10.4	25.3	52.2
Dairy cow	3744	7708	5.0	10.3	25.0	51.4
Piglets	3477	7159	4.6	9.6	23.2	47.7
Fattening pigs	3434	7071	4.6	9.4	22.9	47.1
Sows	2179	4487	2.9	6.0	14.5	29.9
Sheep-goats	3704	7626	4.9	10.2	24.7	50.8
Fattening lambs[1]	5166	10637	6.9	14.2	34.4	70.9
Broilers 5 weeks	4575	9420	6.1	12.6	30.5	62.8

3 In the Spaepen *et al.* paper a value of 600 kg N/ha is reported for Italy. This value, quoted as "Personal communication", is not reliable. A maximum value of 350 kg N/ha is recommended for the Po valley by the Po Basin National Authority and lower value are recommended for other Italian agricultural areas.

4 E.C.O.J. n° L 181 of 04/07/1986 p.6

Replacement pullets	3500	7205	4.7	9.6	23.3	48.0
Layers	3742	7704	5.0	10.3	25.0	51.4
Turkey poults[2]	3036	6250	4.1	8.3	20.2	41.7
Turkey female[3]	3300	6795	4.4	9.1	22.0	45.3
Turkey male[4]	3658	7530	4.9	10.0	24.4	50.2

[1] 2 runs/year; [2] average of both sexes, middle and heavy breeds, age at slaughter: 11 weeks; [3] females, age at slaughter: 16 weeks; [4] males, age at slaughter: 22 weeks

The table 9 hereafter shows how much of the total load of zinc allowed is consumed for the different animal species and categories, respectively for vulnerable areas and non sensitive soils. Results are expressed in percentage of the maximum amount that may be added annually to agricultural land, in compliance with the current legislation. In addition, two simulations are proposed to anticipate possible reduction of the maximum load of zinc on soil. Two figures have been arbitrarily chosen: a fifth and a tenth of the current limit i.e. respectively 6 kg and 3 kg per hectare and per year.

The Directive 86/278/EEC fixed the following limit values for zinc:

- amount which may be added annually to agricultural land, based on a 10 year average: 30 kg/ha/y
- concentration in soil: 150-300 mg/kg dm

Table 9: Contribution in percentage of the maximum zinc load allowed in soil to the contamination of lands, for vulnerable and non vulnerable areas, with zinc. The contribution is evaluated against the current legal limit and two alternative reduced limits (20% and 10% of the existing limit)

	Vulnerable areas	Non vulnerable areas	Legal situation: Based on current maximum zinc soil concentration		Alternative scenario with lower limit: Based on a fifth of the current maximum zinc soil concentration		Alternative scenario with lower limit: Based on a tenth of the current maximum zinc soil concentration	
	Zinc applied (g/ha and year)		Percentage of copper allowed per hectare					
			Max. 30 kg		Max. 6 kg		Max. 3 kg	
Animals	170 kg N/ha	350 kg N/ha	170 kg N/ha	350 kg N/ha	170 kg N/ha	350 kg N/ha	170 kg N/ha	350 kg N/ha
Veal calves	3682	7580	12%	25%	61%	**126%**	**123%**	**253%**
Replacement calves	2863	5895	10%	20%	48%	98%	95%	**197%**
Fattening steers	3819	7864	13%	26%	64%	**131%**	**127%**	**262%**
Replacement heifers	3800	7824	13%	26%	63%	**130%**	**127%**	**261%**
Dairy cow	3744	7708	12%	26%	62%	**128%**	**125%**	**257%**
Piglets	3477	7159	12%	24%	58%	**119%**	**116%**	**239%**
Fattening pigs	3434	7071	11%	24%	57%	**118%**	**114%**	**236%**
Sows	2179	4487	7%	15%	36%	75%	73%	**150%**
Sheep-goats	3704	7626	12%	25%	62%	**127%**	**123%**	**254%**
Fattening lambs[1]	5166	10637	17%	35%	86%	**177%**	**172%**	**355%**
Broilers 5 weeks	4575	9420	15%	31%	76%	**157%**	**153%**	**314%**
Broilers 8 weeks	4134	8511	14%	28%	69%	**142%**	**138%**	**284%**
Replacement pullets	3500	7205	12%	24%	58%	**120%**	**117%**	**240%**
Layers	3742	7704	12%	26%	62%	**128%**	**125%**	**257%**
Turkey poults[2]	3036	6250	10%	21%	51%	**104%**	**101%**	**208%**
Turkey female[3]	3300	6795	11%	23%	55%	**113%**	**110%**	**227%**
Turkey male[4]	3658	7530	12%	25%	61%	**126%**	**122%**	**251%**

[1] 2 runs/year
[2] average of both sexes, middle and heavy breeds, age at slaughter: 11 weeks
[3] females, age at slaughter: 16 weeks
[4] males, age at slaughter: 22 weeks

The zinc load appears to be within the limit of 30 kg/ ha and year, and averages at approximately 13% of the total amount allowed for vulnerable soils and 25% for non vulnerable areas, across animal species and categories, the highest being for lambs. So it can be concluded that on the basis of the present limits, the impact of zinc use on environment is not of concern.

Although the current legal limit is complied with, in the case where this limit would be reviewed and reduced by risk managers, then the current levels of zinc load could exceed maximum zinc concentration of soil. Reduction of the limits for soil concentration would then probably mean revision of the amounts allowed in animals or appropriate controls of the amount of zinc present in the slurry before spreading onto a land.

Attention should be drawn to the known use of zinc at higher levels in piglets. Levels of 2500 to 5000 mg/kg complete feedingstuff might be used and are intended to prevent or limit diarrhoea. Such levels are not in accordance with the present legislation on feed additives and are not considered in this assessment. However, they certainly impact on release in the environment. If such use is validated by the risk manager, its impact should then be further considered as zinc limits for spreading of slurry could be overcome for this animal category.

6. Conclusion

Zinc is an essential trace element necessary to all animals. It has to be provided to animals to cover their requirements.

Currently, zinc is authorised for all species including fish at the level of 250 mg/ kg feed (see Council Directive 70/524/EEC). This concentration exceeds the requirement for farmed livestock, fish, dogs and cats.

In terms of growth promotion and improvement of animal performances, SCAN considers that the effect of zinc remains questionable.

No particular risk for the environment has been identified consecutive to the use of zinc in animals' diets at the current allowed levels.

7. Recommendations

(1) Current zinc levels allowed in diets should be reviewed to better reflect animal requirements.

(2) If reviewed, the current levels of zinc in animal feed should consider the natural level of zinc present in feedingstuffs and all factors impacting on zinc availability. An appropriate safety margin should therefore be included in the levels fixed for this trace element. A total zinc of 150 mg/kg complete feedingstuff would appear appropriate for all animals considered.

(3) As long as 175 mg copper/kg feed remains authorised, comparably high levels of zinc and iron should be kept to prevent adverse effects of copper.

(4) The impact on aquatic environment of the use of zinc in fish feed should be considered and assessed using adequate models for different fish production systems in Europe.

(5) Levels of zinc presently allowed in feed of farm animals should be kept under scrutiny in the light of the possible evolution of the authorised load of zinc on soil. For some animal categories, zinc may indeed replace nitrogen as limiting factor for spreading of manure onto lands, if the allowed load of zinc on soil was to be reduced.

(6) Zinc is known to be used in some animals, for prophylactic or therapeutic purposes, at far higher levels than those authorised as additive in feed, in particular in piglets. Levels can reach 10 to 20 times those allowed in the feed additive legislation. This other use may invalidate the assessment of the impact on environment done for the animal categories considered If an authorisation of high zinc levels for piglets and for a short time should be considered - *e.g.* under the control of the medicated feed legislation as a prophylactic or therapeutic agent or as a characteristic for a feedingstuff for particular purposes - the environmental impact should be reevaluated.

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9. FARM ANIMAL DIETS USED BY SCAN AS A BASIS FOR ITS ASSESSMENT CALCULATIONS

* average of both sexes, middle and heavy breeds
** two runs/year

Animal category	Body weight				Production period (days)	Feed intake			Feed conversion (feed/gain)	Protein g / kg feed	Protein intake			CP/product	
	Range (kg)	Gain (kg)	ADG (g)	kg FCM /year		kg DM / day	kg DM / animal	kg / year			kg / day	kg / animal	kg / year	g/kg	g/kg gain or
Veal calves	45 - 200	155	1100		141	1,9	263,5	682	1,7	220	0,411	57,97	150		187,5
Replacement calves	45 - 125	80	702		114	1,83	209	669		202	0,370	42,22	135	160	
Fattening steers	125 - 600	475	1100		432	7	3023	2555		130	0,910	392,95	332	160	
Replacem. heifers	125 - 500	375	543		690	6,5	4485	2373		120	0,780	538,20	285	160	
Dairy cows	600		*Milk*	*5000*	365	14	5110	5110		140	1,960	715,40	715		34
	and calf	45												180	
			Total												
Dairy cows	650		*Milk*	*8000*	365	18	6570	6570		150	2,700	985,50	986		34
	and calf	45												180	
			Total												
Suckler cows	not calculated, because of no extra input on pasture or arable land except 20 kg mineral feed/year														
Piglets	8 - 25	17	378		45	0,600	27	219	1,6	227	0,136	6,13	50	170	170
Fattening pig	25 - 110	85	700		121,4	1,85	224	675	2,6	182	0,337	40,77	123	170	170
Sows, ear. wean.		25			49	4,400	215,6			204,5	0,900	44,10		170	170
		160			316	2,381	752,4	968		136	0,323	102,10	146,2	170	170
Sows, conv. wean.		25			80	4,400	352			204,5	0,900	72,00		170	170
		200			285	2,47	704	1056		136	0,336	95,76	167,76	170	170
Sheep - Goats	70	30	1,5 lambs		365	1,78	650	650		120	0,213		78		180
		4	wool												444
Fattening lambs	20 - 40	20	357		56	1,23	68,88			125	0,154	8,62			170
Broiler 5 weeks	40 - 1750	1,71	49		35	0,078	2,736	29	1,60	220	0,017	0,602	6,3	214	
Broiler 8 weeks	40 - 2650	2,61	47		56	0,093	5,2	34	1,99	205	0,019	1,066	6,9	214	
Replac. Pullets	38 - 1600	1,562	11		140	0,054	7,5	20	4,80	160	0,009	1,200	3,1	218	
Layers	2000		*Eggs*	*308*	365	0,118	43,0	43		160	0,019	6,880	6,9		121
Turkey 11 weeks*	50 - 5300	5,25	68		77	0,129	9,9	47	1,89	250	0,032	2,475	11,7	218	
Turkey fem. 16 w	50 - 9900	9,85	88		112	0,220	24,6	80	2,50	210	0,046	5,166	16,8	218	
Turkey male 22 w	50 - 20700	20,65	134		154	0,369	56,8	135	2,75	190	0,070	10,792	25,6	218	

Animal category	Product g N/kg	N-intake			N-retained		N in product		**N-excreted**		P	P	P-intake	**P-excretion**	
		kg / day	kg / animal	kg / year	kg / animal	kg / year	kg / animal	kg / year	kg / animal	kg / year	g/kg feed	g/kg gain g/kg	kg / animal	kg / animal	kg / year
Veal calves	30	0,066	9,275	24	4,65	12			4,625	**12**	6	7	1,581	0,496	**1,28**
Replacement calves	25,6	0,059	6,755	22	2,048	7			4,707	**15**	4,8	7	1,003	0,443	**1,42**
Fattening steers	25,6	0,146	62,873	53	12,16	10			50,713	**43**	3,5	7	10,580	7,255	**6,13**
Replacem. heifers	25,6	0,125	86,112	46	9,6	5			76,512	**40**	3,5	7,5	15,698	12,885	**6,82**
Dairy cows	5,44	0,314	114,464	114							3,5	1	17,885		
	28,8				1,296	1,296						7			
					1,296	1,296			113,168	**113**				12,57	**13**
Dairy cows	5,44	0,432	157,680	158			43,52	43,52			3,5	1	22,995		
	28,8				1,296	1,296						7			
					44,816	44,816			112,864	**113**				14,68	**15**
Suckler cows	not calculated, because of no extra input on pasture or arable land except 20 kg mineral feed/year														
Piglets	27,2	0,022	0,981	8	0,4624	3,751	0,462	3,751	0,518	**4**	7	4,9	0,21	0,127	**1,03**
Fattening pig	27,2	0,054	6,523	20	2,312	7			4,211	**13**	6,5	6	1,658	1,148	**3,45**
Sows, ear. wean.	27,2	0,144	7,056		0,68					**0**	6	6			
	27,2	0,052	16,331	23,4	4,352	5,032		5,032	18,4	**18,4**	6	4,9	6,6		**5,67**
Sows, conv. wean.	27,2	0,144	11,520		0,68						6	6			
	27,2	0,054	15,390	26,91	5,44	6,12		6,12	20,79	**20,79**	6	4,9	7,2		**6,07**
Sheep - Goats	28,8	0,034		12,44	1,148		0,864				3	5,2			
	160						0,284	1,148	11,29	**11,29**		0,3	1.915	1,76	**1,76**
Fattening lambs	27,2	0,025	1,400		0,544				0,856	**1,7****	3,5	5,2	0,241	0,137	**0,27****
Broiler 5 weeks	34,24	0,003	0,096	1,00	0,059	0,61			0,038	**0,394**	6,50	5,5	0,018	0,008	**0,087**
Broiler 8 weeks	34,24	0,003	0,171	1,11	0,089	0,58			0,081	**0,529**	6,25	5,5	0,033	0,018	**0,118**
Replac. Pullets	34,88	0,001	0,192	0,50	0,054	0,14			0,138	**0,359**	5,50	5,5	0,041	0,033	**0,085**
Layers	19,37	0,003	1,101	1,10			0,358	0,358	0,743	**0,743**	6,00	6,9	0,258	0,130	**0,130**
Turkey 11 weeks*	34,88	0,005	0,396	1,88	0,183	0,87			0,213	**1,009**	6,50	5,5	0,064	0,035	**0,168**
Turkey fem. 16 w	34,88	0,007	0,827	2,69	0,344	1,12			0,483	**1,574**	6,50	5,5	0,160	0,106	**0,345**
Turkey male 22 w	34,88	0,011	1,727	4,09	0,720	1,71			1,006	**2,385**	6,50	5,5	0,369	0,256	**0,606**

10. CALCULATION OF THE AMOUNT OF ZINC SPREAD ONTO LANDS

Time	Weight range	Feed intake		Zinc in feed		Total zinc excretion [6]		Nitrogen excreted			Zinc / Nitrogen ratio per animal	**Zinc applied, in g/ha and year, on**	
days	kg	kg dm per animal	kg dm per year	mg / kg	mg / kg dm [1]	g / animal	g / year	kg / animal	kg / year	kg / year [2]	g / kg [3]	**vulnerable areas [4]**	**non sensitive soils [5]**
141	45-200	263	682	250,0	284,1	74,716	193,750	4,6	12,000	9,000	21,657	**3682**	**7580**
114	45-125	209	669	250,0	284,1	59,375	190,057	4,7	15,000	11,250	16,844	**2863**	**5895**
432	125-600	3025	2555	250,0	284,1	859,375	725,852	51	43,000	32,250	22,467	**3819**	**7864**
690	125-500	4485	2373	250,0	284,1	1274,148	674,148	76	40,000	30,000	22,353	**3800**	**7824**
365	650	6570	6570	250,0	284,1	1866,477	1866,477	113	113,000	84,750	22,023	**3744**	**7708**
45	8-25	27	219	250,0	284,1	7,670	62,216	0,5	4,000	3,000	20,455	**3477**	**7159**
121,4	25-110	224	675	250,0	284,1	63,636	191,761	4,2	13,000	9,750	20,202	**3434**	**7071**
80+285		704	1056	250,0	284,1	200,000	300,000	20,8	20,8	15,600	12,821	**2179**	**4487**
365	70	650	650	250,0	284,1	184,659	184,659	11,3	11,300	8,475	21,789	**3704**	**7626**
56	20-40	69	70	250,0	284,1	19,602	19,886	0,86	1,700	1,275	30,391	**5166**	**10637**
35	0.04 - 1.75	2,7	29	250,0	284,1	0,767	8,239	0,038	0,390	0,293	26,914	**4575**	**9420**
56	0.04 - 2.65	5,2	34	250,0	284,1	1,477	9,659	0,081	0,530	0,398	24,317	**4134**	**8511**
140	0.04 - 1.6	7,5	20	250,0	284,1	2,131	5,682	0,138	0,350	0,263	20,586	**3500**	**7205**
365	2	43	43	250,0	284,1	12,216	12,216	0,74	0,740	0,555	22,011	**3742**	**7704**
77	0.05 - 5.3	9,9	47	250,0	284,1	2,813	13,352	0,21	1,000	0,750	17,857	**3036**	**6250**
112	0.05 - 9.9	24,6	80	250,0	284,1	6,989	22,727	0,48	1,570	1,178	19,413	**3300**	**6795**
154	0.05 - 20.7	56,8	135	250,0	284,1	16,136	38,352	1	2,390	1,793	21,515	**3658**	**7530**

[1] 88% dry matter

[2] considering an average 25% loss

[3] the calculation includes the 25% nitrogen losses in accordance with [2]

[4] calculation based on the limit of 170 kg N / ha and year fixed in Council Directive 91/676/EEC

[5] calculation based on the limit of 350 kg N / ha and year extracted from the publication of Spaepen *et al.*

[6] It is assumed that the amount excreted corresponds to 100% of the zinc ingested

11. DATA OF STUDIES ON THERAPEUTIC LEVELS OF ZINC SUPPLEMENTATION

11.1. Influence of dietary zinc on performance of weaned piglets (Poulsen, 1995)

Zn mg/kg feed	60	160	260	1060	2560	4060
No of animals	34	35	36	36	35	36
ADG 4-7 weeks[1]	325	324	321	338	376**	345
7-9 weeks[1]	592	631	625	630	642	605
4-9 weeks[1]	432	447	443	455	483*	449
FI 4-7 weeks[1]	11.1	11.4	11.1	11.6	12.2	12.1
7-9 weeks[1]	15.8	16.4	15.6	16.3	16.7	16.0
4-9 weeks[1]	26.9	27.8	26.7	27.9	28.9	28.1
FC 4-7 weeks[1]	1.69	1.79	1.72	1.69	1.57	1.71
7-9 weeks[1]	1.97	1.87	1.81	1.88	1.88	1.91
4-9 weeks[1]	1.80	1.80	1.74	1.77	1.72	1.80

[1] age of piglets; means different from those of 60 mg group ** $P \leq 0.01$; * $P \leq 0.05$
ADG = average daily body weight gain (g); FI = feed intake (kg/piglet);
FC = feed intake/body weight gain

11.2. Performance and plasma Zn values of weaned piglets (5 replicate groups of 6 pigs; age at beginning: 35 days, duration of experiment: 21 days; Hahn and Baker, 1993)

Treatment	bw gain (g/day)	Feed intake (g/day)	Gain/Feed (g/kg)	Plasma Zn (mg/L)
Basal diet (B)	463	794	584	0.85
B + ZnO 3000	530	901	587	1.30
B + ZnO 5000	556	892	623	1.99
B + $ZnSO_4$ 3000	540	871	620	2.78
B + $ZnSO_4$ 5000	456	751	609	3.27
Pooled SEM	16	21	14	0.16

Basal diet: 125 mg Zn/kg feed; 3000 and 5000 = mg Zn supplement/kg feed
bw gain: Basal vs Zn, and Zn sources x Zn level interaction were significant ($P<0.05$)

11.3. Performance and plasma Zn values of weaned piglets (5 replicate group of 6 pigs; age at beginning: 35 days; duration of experiment: 14 days; Hahn and Baker, 1993)

Treatment	bw gain (g/day)	feed intake (g/day)	Gain/Feed (g/kg)	Plasma Zn (mg/L)
Basal diet (B)	445[ab]	678[a]	659[ab]	1.29[a]
B + ZnO	500[c]	765[b]	652[a]	2.20[b]
B + $ZnSO_4$	414[a]	635[a]	651[a]	4.01[c]
B + Zn met	437[ab]	635[a]	690[b]	4.82[d]
B + $CuSO_4$	451[b]	692[a]	654[ab]	1.22[a]
Pooled SEM	12	21	12	0.21

Means with different superscripts are different ($P<0.05$)
Basal diet: 125 mg Zn/kg feed; Zn supplementation of 3000 mg/kg feed;
Zn supplementation of 250 mg/kg feed

11.4. Effect of phase feeding pharmacological zinc doses on growth performance (Carlson *et al*., 1999)

Variable	Weeks feeding rations with 3 000 mg Zn supplementation /kg feed as ZnO						P
	0	1	2	1-2	2-3	1-4	
Experiment 1: Early weaning (11.5 days)							
ADG wk 1	128^{b}	120^{b}	120^{bc}	147^{a}	98^{c}	125^{b}	0.04
wk 2	234^{c}	260^{b}	273^{b}	310^{a}	237^{c}	315^{a}	0.005
wk 3	354^{a}	320^{b}	320^{b}	350^{a}	287^{c}	335^{ab}	0.05
wk 4	280^{a}	278^{a}	250^{b}	269^{a}	230^{c}	295^{a}	0.03
Overall	250^{b}	255^{b}	245^{bc}	270^{a}	220^{c}	275^{a}	0.04
ADFI overall	360	352	354	370	351	367	0.41
Experiment 2: Traditional weaning (24.5 days)							
ADG wk 1	155^{c}	216^{a}	175^{b}	235^{a}	176^{b}	210^{a}	0.005
wk 2	252^{e}	285^{d}	328^{c}	350^{b}	351^{b}	416^{a}	0.0001
wk 3	436^{b}	428^{b}	471^{a}	453^{a}	455^{a}	480^{a}	0.05
wk 4	511^{c}	510^{c}	495^{c}	530^{b}	497^{c}	575^{a}	0.02
Overall	340^{c}	350^{b}	360^{bc}	395^{a}	375^{b}	420^{a}	0.001
ADFI overall	472	506	484	518	510	548	0.08

ADG = average daily body weight gain (g); ADFI = average daily feed intake (g)
Basic diet: 100 mg Zn supplement as ZnO
Means in the same row with different superscript differ ($P<0.05$)
Number of pigs per treatment in Experiment 1: wk 1: 23, wk 2: 22, wk 3: 21, wk 4: 20;
in Experiment 2: wk 1: 41, wk 2: 40, wk 3: 39, wk 4: 38