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Zinc and copper in dairy cattle feeding

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Abstract
This literature study shows that dietary supplementation of copper and zinc to dairy cattle above current recommendations does not show convincing health benefits.

Keywords
Dairy cattle, copper, zinc, health

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Preface

The present report is based on a research project initiated by the Dutch Product Board Animal Feed and the Dutch ministry of Economics, Agriculture and Innovation, resulting in an internal report “Effect of dietary zinc and copper on health and fertility in dairy cattle” by Van Laar and Jongbloed, published in September 2010 (WUR Livestock Research, Internal Report 201004). The internal report comprised an extensive literature study that was discussed with the members of the Dutch Commission on Mineral Research in Livestock Feeding, who are acknowledged for their ideas and suggestions. This discussion resulted in the present external report, based on the literature study of Van Laar and Jongbloed with additional information and recommendations.

R.M.A. Goselink
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Samenvatting

Koper (Cu) en zink (Zn) zijn belangrijke spoorelementen in melkveerantsoenen, aangezien beide mineralen wijdverspreid en essentieel zijn in de stofwisseling. Een overmaat aan Cu en Zn wordt uitgescheiden in de mest, wat leidt tot een stapeling in het milieu. Vanuit het milieu bezien is het dan ook gewenst om de Cu- en Zn-niveaus in voedermiddelen terug te dringen, zodat de excretie via de mest geminimaliseerd wordt zonder daarbij de diergezondheid en dierprestaties te schaden. In deze review is onderzocht of de toevoeging van Cu en Zn in rantsoenen boven de fysiologische behoefte een positief effect heeft op de gezondheid en vruchtbaarheid van melkvee.

Vanwege de beperkte hoeveelheid bruikbare data en de variabele onderzoeksresultaten, staat niet vast of supplementatie van Cu en Zn boven de huidige behoeftenormen een effect heeft op gezondheid, vruchtbaarheid of klauwkwaliteit. Er zijn enkele aanwijzingen dat een verhoging van het Zn-niveau in het rantsoen een verlagend effect kan hebben op het celgetal bij melkgevende koeien en de immuunrespons van kalveren kan verbeteren, maar de resultaten zijn variabel. In veel papers is het exacte niveau aan Cu, Zn en andere mineralen die mogelijke een interactie hebben met Cu en Zn niet gerapporteerd. Bovendien is de opzet van een experiment in veel gevallen niet gericht op het vaststellen van gezondheids- en vruchtbaarheidseffecten, waardoor de gemeten resultaten soms twijfelachtig zijn.

Er is daarom dan ook verder onderzoek nodig met nauwkeurige mineralenanalyses en objectieve gezondheids- en vruchtbaarheidskenmerken om vast te stellen of concentraties van Cu en Zn in het rantsoen boven de huidige fysiologische behoeftenormen gerechtvaardigd is. Bovendien zijn meer gegevens nodig om mogelijke interacties tussen (spoor-) elementen te onderzoeken, evenals op het gebied van de fysiologische status van een dier (ziekte, lactatiestadium) en het effect daarvan op de mineralenbehoefte.

Summary

Copper (Cu) and zinc (Zn) are important trace minerals in dairy cattle feeding, as both elements are widely distributed in the body. Excess dietary Cu and Zn will be recovered in faeces, leading to accumulation of these elements in the environment. From an environmental point of view it is therefore desirable to reduce Cu and Zn levels in cattle feeds, minimizing faecal excretion without compromising animal health and production performance. The present review investigates whether there is an effect of the addition of Cu and Zn to dietary levels above the current physiological requirements on health and fertility parameters of cattle.

Due to scarcity of experimental data and the variability in experimental results, the effect of Zn- and Cu-supplementation above required levels on health, fertility and hoof quality is inconclusive considering all the literature reviewed in the present report. There are some indications that Zn supplementation to levels above current recommendations may decrease somatic cell count in lactating dairy cows and stimulate the immune response in calves, but results are variable. In many papers, the exact level of intake of Cu, Zn and other minerals that might interact with these is not reported. Besides that, in many cases the experimental design was not developed to detect effects on health and fertility, leaving the results on that area questionable.

Therefore, further research is needed with good mineral analysis data and objective health and fertility parameters to determine whether increased dietary levels of Cu and Zn above recommendations can be justified. Also more experimental data is needed to investigate possible interactions between (trace) minerals and the effect of the physiological state of an animal (disease, lactation stage) on mineral requirements.

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Preface

Summary

1 Introduction

Copper (Cu) and zinc (Zn) are important trace minerals in dairy cattle feeding. Both elements are widely distributed in the body, as they are part of many enzymes and structural proteins. The dietary intake necessary to maintain health and productivity is defined as the physiological requirement. According to Dutch recommendations (COMV, 2005), Zn, Cu, manganese (Mn) and cobalt (Co) requirements for a dairy cow producing 40 kg of milk are 32.5, 11.1, 40 and 0.1 mg/kg DM, respectively. The actual dietary intake of dairy cattle is far higher than the recommended physiological requirements and approach maximal allowed inclusion limits (EU, 2003). Nevertheless, the beneficial effects of dietary intake levels above physiological requirements are still unclear.

Excess dietary Cu and Zn will be recovered in faeces, leading to accumulation of these elements in the environment. In the Netherlands, the emission of heavy metals such as Cu and Zn to the environment has led to a build-up of these minerals in our soils. On many locations, concentrations of these elements in surface water exceed the limits set by the EU (EU, 2000; De Nijs et al., 2008). The contribution of agriculture to total emission in surface water is around 10% for Cu and 18% for Zn. For total emission to soil, this contribution is even larger: around 75% for Cu and 85% for Zn (data based on Emissieregistratie, 2008). The largest part (80-85%) of the agricultural emission of Cu and Zn to soil can be attributed to animal manure, originating from animal feed. Concentrations of these elements in livestock feeding are therefore important determinants for environmental pollution. Looking at dairy cattle feeding more specifically, Cu and Zn are often added to the ration either by separate mineral supplements or through compound feeds. Approximately 20% of the total estimated supply of Cu and Zn in compound feeds can be found in concentrates produced for cattle (data based on www.lei.nl).

From an environmental point of view it is desirable to reduce Cu and Zn levels in cattle feeds, minimizing faecal excretion without compromising animal health and production performance. Adding mineral supplements to animal feeds can only be justified with a proven effect on practical parameters that reflect animal health and welfare, like for example morbidity, fertility, milk somatic cell count and hoof quality in bovines. The present review investigates whether there is an effect of the addition of Cu and Zn to dietary levels above the current physiological requirements on health and fertility parameters of cattle.

2 Health and immunity

Although results are variable, clearly there are various publications showing that (a deficiency in) Cu and Zn influence health and immunity of bovines. This is witnessed by a large amount of research showing changes in various immunity-related compounds, either in vitro or in vivo, as well as effects on morbidity rates from various viral infections, reviewed by Suttle and Jones (1989), Galyean et al. (1999) and Jongbloed et al. (2001).
Recently, Van Knegsel et al. (2008) showed in a review the importance of a sufficient Cu supply. Cu is involved in the development and maintenance of the immune system, and the Cu status alters several aspects of neutrophil, monocyte, and T-cell function. However, the effect of Cu on the immune response seems to be limited. This is mainly due to the fact that Cu homeostasis is maintained over a wide range of intakes. Zn has been shown to have antioxidant activity in vitro and in vivo as a cofactor of superoxide dismutase (SOD) and through binding and stabilization of protein thiols. Lowering Zn status impaired macrophage functions, neutrophil functions, NK cell activity and complement activity. Zn is an essential cofactor for the thymic hormone thymulin, which induces several T-cell markers, and promotes T-cell function. It also modulates cytokine release and induces the proliferation of the CD8+ T cells that function as cytotoxic cells, able to recognize and kill pathogens.

2.1 Immune function

While the negative effects of Cu and Zn *deficiency* are very clear, any positive effects of additional Cu or Zn *above* the physiological requirements on the immune system are not as clear. Galyean et al. (1999) have reviewed very thoroughly the effect of dietary Zn level on beef cattle health and immunity, especially under conditions of stress such as transportation. A summary of the papers quoted is given in Table 1. In these papers, very few dietary concentrations of other minerals such as Cu, molybdenum (Mo) and sulphur (S) were given. Only two papers reported the level of Cu in the control diet: 10.8 mg Cu/kg DM for Spears et al. (1991) and 7.0 mg Cu/kg DM for Kincaid et al. (1997). Galyean et al. (1999) have quoted several experiments in which increasing dietary Zn level improves immune parameters or performance during infection, also when levels were increased above the recommended Zn requirement. Beside these experiments showing a positive effect of Zn supplementation, other experiments did not show an effect of Zn supplementation on performance or immune parameters. The reason for this variability in the effect of Zn level on immunity-related parameters is not known. Galyean et al. (1999) conclude that diets for stressed (transported) animals should be formulated at mineral levels that compensate for the stress-related decrease in dry matter intake, to ensure that the total mineral intake corresponds to the physiological requirement. However, nutrient fortification with trace minerals above current recommended dietary levels is debatable.

Table 1 Summary of a selection of papers used by Galyean et al. (1999), describing the effect of Zn level (mg/kg DM) on various immune response-related parameters in cattle

Authors cited	T1[1]	T2+more[1]	#T[1]	Zn Sources[2]	Animals	Results[3]
Positive effect						
Spears et al., 1991	26	51	3	ZnMet, ZnO	Steers	High Zn: antibody titres against IBRV tended to be higher in steers.
Blezinger et al., 1992	42	~70	4	ZnMet, ZnProt, $ZnSO_4$	Cattle	High Zn: Lower rectal temperature during IBRV infection.
Engle et al., 1995	17	42	2	-[3]	Heifer calves	Low Zn: PHA skin swelling was decreased (also lower feed efficiency, animals were deficient).
Engle et al., 1995	17	40	4	-	Heifer calves	During low Zn lower skin swelling response to PHA (also lower growth animals were deficient).
Kegley et al., 1997	-	+360 mg/d	3	$ZnSO_4$, ZnAminoAcid	Angus heifers	Cell mediated immunity by injection of PHA greater in high Zn diets.
Mohamed et al., 1995	basal	+35 & +70	4	$ZnSO_4$, ZnMet	Weaned steers	Morbidity from BRD decreased with 70 mg/kg vs basal and 35 mg/kg.
No effect						
Gengelbach et al., 1992	28	53	3	ZnMet, ZnO	Stressed lambs	No difference in immune response.
Droke et al., 1993	deficient	+5 & +40	3	-	Growing lambs	No effect on various immune parameters: lymphocyte blastogenesis in response to PHA, ConA and PWM.
Kincaid et al., 1997	60	150 & 300	4	ZnMet, ZnLys, ZnO	Calves	No effect on various immune parameters: lymphocyte blastogenesis in response to PHA, ConA, PWM, lymphocyte IL-2 production, lymphocyte cytotoxicity, or phagocytic and intracellular killing ability of neutrophils.

[1] T1: Treatment 1 (Control, mg/kg DM); T2: Treatment 2 + other treatments; #T: number of total treatments

[2] type of Zn supplemented with '-' if Zn source was not reported in the paper

[3] IBRV: Infectious Bovine Rhinotracheitis Virus; PHA: Phytohemagglutinin; BRD: Bovine Respiratory Disease; ConA: concanavalin A; PWM: Pokeweed Mitogen

2.1.1 Effect of dietary Zn

Some of the data used by Galyean et al. (1999) are the results of Chirase et al. (1991), on the effect of Zn level on dry matter intake of crossbred steers (230-270 kg) after intentional infection with Infectious Bovine Rhinotracheitis Virus (IBRV). The results are shown in Table 2. Information about the dietary concentrations of Mo and S in the experimental diets was not reported.

From these results it can be concluded that Zn supplementation in the form of Zn methionine, raising Zn levels from 30-35 mg/kg DM to 90 mg/kg DM, reduced the drop in DM intake after infection. The level of Zn supplementation also influenced rectal temperature and body weight development after infection. It should be noted however, that in the Zn-supplemented diet also the Cu concentration was raised when compared with the control diets. When Zn levels were raised from 90 mg/kg DM to 160 mg/kg DM or even higher, the effect on DM intake after infection with IBRV was no longer detected.

In conclusion, these data suggest that Zn supplementation to dietary levels above recommendations for steers (up to around 90 mg/kg DM) may have a positive effect on health and performance during an infection with IBRV.

Table 2 Summary of three experiments (Chirase et al., 1991) on the effect of Zn level on DM intake (% relative to day 0, estimated from a graph and rounded to the closest 5%) of crossbred steers (230-270 kg) infected with Infectious Bovine Rhinotracheitis Virus on day 0.

Treatment[1]	**Dietary level (mg/kg DM)**			**DMI on day after infection relative to day 0 (%)**								
	Zn	***Cu***	***Mn***	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**
Control	31	-	-	15	-45	-45	-30	-30	-5	-10	0	20
Zn Met	90	16	-	5	-10	-40	-5	-10	-5	-15	0	20
				ns	*0.05*	*ns*	*0.05*	*0.05*	*ns*	*ns*	*ns*	*ns*
Control	35	-	-	-45	-80	-70	-50	-70	-80	-20	-10	-10
Zn Met	89	17	-	20	-45	-50	20	-20	0	0	60	20
				ns	*ns*	*ns*	*0.05*	*0.05*	*0.05*	*ns*	*0.05*	*ns*
Control	96	20	73	0	-20	-35	-5	-5	20	35	10	55
ZnO	163	26	88	10	-35	-35	-25	-20	0	0	-20	-10
Zn Met	171	26	92	20	-20	-25	20	15	45	35	35	60
				ns	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

[1] Treatments with Zn Met for zinc methionine and ZnO for zinc oxide as Zn source

2.1.2 Effect of dietary Cu

Table 3 summarizes the papers on the effect of Cu on immune response parameters as cited by Galyean (1999). All experiments have been done with calves, but they can be divided into three categories regarding the experimental setup: 1) subcutaneous Cu injection; 2) dietary Cu supplementation; 3) dietary Cu supplementation of dams giving birth to the calves under investigation. A rough representation of the overall effect is indicted by a '–' (negative effect); '0' (no effect); '+' (positive effect) or a '±' (both positive and negative effects, or ambiguous) sign.

The results within and between experimental categories are very variable. The background for these differences in response is not further investigated. In summary, 4 experiments show no effect (0), 2 experiments show a negative effect (-), 2 experiments show a positive effect (+) and 2 experiments show an ambiguous effect of Cu supplementation on immune response parameters. It must be noted that even in situations considered Cu deficient, Dill et al. (1990) did not find a positive effect on the humoral immune response parameters.

Based on the data in Table 3, there is no scientific ground to assume a positive effect of Cu supplementation above recommended levels (approx. 10 mg/kg DM) in calves.

Table 3 Summary of a selection of papers used by Galyean et al. (1999), describing the effect of Cu level on several immune response-related parameters in cattle[1].

Authors cited	Summary of conclusion	Effect[2]
Injection of Cu to animals marginal or deficient in Cu		
Chirase et al., 1994	Calves injected with 120 mg Cu glycinate had reduced DMI and BW change by 30 and 6.9% after challenge with IBRV relative to control at a diet of around 6 mg Cu/kg DM.	-
Dill et al., 1990	No increase in humoral response when injecting Cu to steers fed diets considered deficient in Cu.	0
Dill et al., 1991	Increase in humoral immune response to injected Cu in steers fed diets considered deficient or marginal in Cu.	+
Dietary Cu supplementation to calves through the diet		
Stabel et al., 1993	No consistent immune response in calves with a diet containing 1.5 mg Cu/kg supplemented with either 0 or 10 mg Cu/kg from $CuSO_4$.	0
Saker et al., 1994	Increased monocyte phagocytic activity and oxidative burst in weaned calves (275 kg) when increasing Cu intake from 43 mg/d to 97 mg/d by Cu lysine addition.	+
Arthington et al., 1995	Induced Cu deficiency in heifers with 10 mg Cu/kg DM in diet by feeding Mo and S by which Cu levels in livers were showing deficiency. This had no effect on *in vitro* or *in vivo* neutrophil chemotaxis.	0
Galyean et al., 1995	Addition of 5 mg Cu/kg from Cu lysine to a 3.3 mg Cu/kg control (CuO) negatively influenced dry matter intake and growth, yet tended to decrease the percentage of morbid steers	±
Wright et al., 1996	No effect of a 10 mg Cu/kg addition to a basal diet for transport-stressed calves on haptoglobin concentrations, plasma ceruloplasmin nor on lymphocyte proliferative responses. (Vitamin E and Se did influence live weight gain).	0
Dietary Cu supplementation to heifers during last part of gestation; calves being investigated fed the same diet		
Gengelbach et al., 1997	Calves feeding on diets from their dams with 4.5 mg Cu /kg or a supplemental 10 mg Cu/kg DM were infected with Infectious Bovine Rhinotracheitis Virus and subsequently Pasteurella after weaning. Cu supplemented animals had lower feed intake, higher body temperatures, higher TNF levels. Original authors view this as positive but Galyean et al. (1999) doubt this.	-
Ward et al., 1997	Similar setup and treatments as Gengelbach et al. (1997). Lower secondary antibody response on d14 and 21 with Cu supplementation. Supplemental Cu reduced PHA-induced skinfold response.	±

[1] The following concentrations of Cu, Zn, Mo or S in the control diets were presented (mg/kg DM)
Chirase et al. (1994): Cu = 5.9, Zn = 74;
Stabel et al. (1993): Cu = 1.5, added Zn = 50, added Mo= 4;
Wright et al. (1996): added Cu = 10, Zn = 28;
Gengelbach et al. (1997): Cu = 4.5, added Zn = 22, Mo = 1.5;
Ward et al. (1997): added Zn = 20.

[2] Effects defined as '–' (negative effect); '0' (no effect); '+' (positive effect) or a '±' (both positive and negative effects, or ambiguous)

2.2 Udder health

2.2.1 Effect of Zn on somatic cell count

As already stated ten years ago by Jongbloed et al. (2001), reports on the effect of Zn supply to dairy cows on somatic cell count (SCC) are scarce and conflicting. An often cited review of Kellog (1990), based on 8 experiments, concludes that supplementing diets with Zn-methionine reduces SCC (/ml) up to 50%. Although an impressive number of papers were reported, only one paper (that was actually removed from the analysis) is peer-reviewed and easily accessible. The other 7 papers analysed in the review were 3 internal reports of the manufacturer, 2 papers in Journal of Dairy Science supplements that are not accessible online, and 2 papers published in the proceedings of the Annual meeting of the National Mastitis Council Inc. in Arlington Virginia, USA. Besides the poor traceability of the majority of the papers, basal Zn levels are not reported. Furthermore, milk production was reported to increase, suggesting that control diet composition was below requirements.

Table 4 gives an overview of papers describing the effect of supplementation of Zn above recommended levels on the production performance and SSC in dairy cows, where Zn concentration in the final diet is either reported, or can be estimated from the data supplied. Zn levels of the control diets ranged from 40 to 83 mg/kg DM, showing that some experiments had a control diet composition just below or close to the requirement of 50 mg/kg DM. Three of the four papers use additional supplementation of approximately 20 mg/kg DM (Bioplex) as treatment, while the fourth paper provides additional supplementation of 13 mg/kg DM (Zn methionine). Papers 2 and 3 report experiments with treatment periods for 180 and 140 days, respectively, without a significant effect of Zn supplementation on SSC. Papers 1 and 4 report experiments with treatment periods of 90 days and they found a significant reduction ($P<0.05$) in SCC during the last 2 weeks (paper 1) or the last month (paper 4). These results suggest that an additional dietary Zn supplementation of approximately 20 mg/kg DM may decrease SCC for cows fed on diets close to or even above Zn requirements after approximately two months of supplementation. Averaged over longer time periods, these differences in SCC are not detected.

In conclusion, the scientific basis for a positive effect on SCC with Zn supplementation above recommendations is minimal. Furthermore, an adequate length of the study to show an effect of Zn on SCC is necessary.

Table 4 Overview of experiments on the influence of Zn supplementation above recommended levels in dairy cows on dry matter intake (DMI), milk production, milk composition (fat and protein) and milk somatic cell count (SSC; x 1000 cells/ml)

Treatment	**Zn source**	**B**[1]	**A**[1]	**T**[1]	**DMI kg/d**	**Milk kg/d**	**Fat %**	**Prot %**	**Somatic cell count (SCC)**		
Exp. 1) Atkin et al., 2008 (duration of treatment: 98 days)											
									SCC <wk 12	***SCC wk 12***	***SCC wk 14***
Low Zn	ZnO	35	5	40	23.1	36.0	n.a.	n.a.	57-84	92	84
Low Zn	Bioplex	34	5	39	24.0	35.2	n.a.	n.a.	69-120	59	108
High Zn	ZnO	36	26	62	22.8	35.2	n.a.	n.a.	43-67	56	73
High Zn	Bioplex	34	25	60	23.7	37.6	n.a.	n.a.	41-65	41	32
				SEM							
				Sign.	*n.s.*	*n.s.*			*n.s.*	*<0.05*	*<0.05*
										log (base e)	*log (base e)*
Exp. 2) Lindmark-Månsson et al., 2000 (duration of treatment: 180 days)											
										SCC	
Low Zn	Basal	n.a.	n.a.	41	n.a.	n.a.	n.a.	n.a.		196	
	Bioplex	n.a.	n.a.	50	n.a.	n.a.	n.a.	n.a.		163	
High Zn	Bioplex	n.a.	n.a.	61	n.a.	n.a.	n.a.	n.a.		201	
				SEM						*92*	
				Sign.						*ns*	
Exp. 3) Kellog et al., 1989 (duration of treatment: 140 days)											
									SCC initial	***SCC last 8 wks***	
Low Zn	Basal[3]	62	0	62	n.a.	26.6	3.4	3.2	171	413	
High Zn	Zn-met	62	13	76	n.a.	28.3	3.4	3.2	358	505	
				SEM		*1.3*	*0.06*	*0.04*	*87*	*112*	
				Sign.		*<0.1*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	
Exp. 4) Pechová et al., 2006 (duration of treatment: 90 days)											
									SCC month1	***SCC month2***	***SCC month3***
Low Zn	Basal	83	0	83	n.a.	20.9	n.a.	n.a.	80.7	33.5	209
High Zn	Bioplex	83	22	105	n.a.	22.8	n.a.	n.a.	82.1	55.2	115
				SEM		*4.7*			*45*	*33*	*115*
				Sign.		*n.s.*			*n.s.*	*n.s.*	*<0.05*
											not log based

[1] B = Zn level in Basal feed (mg/kg DM); A = Added Zn level (mg/kg DM); T = Total Zn level (mg/kg DM)

[2] n.a. = not available; n.s. = not significant

[3] The dietary Cu content in the experiment of Kellog et al. (1989) was estimated at 9 mg/kg DM

2.2.2 Effect of Cu on somatic cell count and clinical mastitis

Engle et al. (2001) fed levels of 9, 19 and 49 mg Cu/kg DM to 24 dairy cows for 60 days after a pre-experimental period of 2 weeks. No effect of Cu supplementation on somatic cell count at day 60 was found. Chase et al. (2000) conducted a 2 x 5 factorial trial with Fe and Cu supplementation. There were two levels of Fe supplementation (0 or 500 mg/kg DM) and three levels of Cu supplementation of two different sources (0, 15 or 30 mg/kg DM of either Cu sulphate or Cu lysine). A number of 48 dairy cows were followed in this trial during 83 days, after a 30 day Cu depletion period. Treatments did not have a significant effect on milk somatic cell count (SCC).
Both trials described above were not especially designed for investigating effects on SCC, but SCC was measured as a response parameter. The experimental periods were probably too short to show an effect on SCC. Furthermore, SCC was not analysed or reported on a weekly basis. High SCC and mastitis problems often occur around parturition (Dang et al., 2008; Spain, 2005) when animals are under metabolic and hormonal stress, and naturally have a low vitamin and Zn status (Goff and Stabel, 1990; Meglia et al., 2001). The trials of Engle et al. (2001) and Chase et al. (2000) were carried out in mid-lactation, which may not have been the best period for detecting effects of trace mineral supplementation on SCC.
Scaletti et al. (2003) investigated the effect of Cu supplementation on the resistance to artificial *E. coli* infection (Table 5). In this trial, 23 primiparous Holstein cows were fed on 6.5 mg Cu/kg DM (basal) or 26.5 mg Cu/kg DM, starting 60 days before the expected calving date until 42 days of lactation. The pre-partum diet contained per kg DM 6.7 mg Cu, 34 mg Zn, 1.4 mg Mo and 1.5 g S and the postpartum diet contained per kg DM 6.1 mg Cu, 51 mg Zn, 2.1 mg Mo and 1.9 g S, which means that dietary Cu intake of the control group was below the Dutch recommendations of 24-25 mg Cu/kg DM for the dry period and 11-12 mg Cu/kg DM for lactating dairy cattle (COMV, 2005). At 34 days of lactation, one quarter per animal was artificially infected with *E. coli*. Milk samples during this period were analyzed for bacteriological status. Before the *E. coli* challenge, the Cu-supplemented group had a higher proportion of infected quarters (mostly caused by Coagulase-negative staphylococci) at calving, at day 14 after calving as well as 24 hours before the artificial *E. coli* infection, suggesting that Cu supplementation had a negative effect on immunity. Scaletti et al. (2003) discuss these results and cite papers of Harmon et al. (1994) and Harmon (1998) showing respectively a decrease or an increase of infection at calving with Cu supplementation, and conclude that the effect of Cu supplementation on mastitis incidence is variable. After the artificial *E. coli* infection however, Cu-supplemented animals showed a reduced clinical response as observed by a lower milk bacterial count, SCC and rectal temperature. Scaletti et al. (2003) concluded that although the duration of the infection was not reduced, the clinical response was reduced by Cu supplementation, which is considered a positive effect.

It can be concluded that the effect of Cu supplementation above requirement on SCC and udder health is variable, as reported before in the review of Harmon and Torre (1994). Some papers suggest a beneficial effect of Cu supplementation, but the current data are insufficient to substantiate such a claim.

Table 5 Effect of Cu supplementation (6.5 mg/kg DM vs. 26.5 mg/kg DM) on the percentage of uninfected mammary gland quarters (bacterial growth negative) and quarters positive for Coagulase-negative staphylococci (CNS growth positive). Cows were infected at day 34 of lactation with *E. coli* in one quarter per udder. Bacteriological status was analysed during the first 21 days of lactation (at calving, day 7, day 14 and day 21) as well as 24 hours before and 240 hours after artificial infection (Scaletti et al., 2003).

Sampling time		**Bacterial growth negative**			**CNS growth positive**		
		Cu (mg/kg DM)		*P-value*	**Cu (mg/kg DM)**		*P-value*
		6.5	**26.5**		**6.5**	**26.5**	
Relative to calving	0d	66.7	50.0	*<0.1*	14.6	20.5	*n.s.*
	+7d	89.6	84.1	*n.s.*	2.1	6.8	*n.s.*
	+14d	85.4	70.5	*<0.05*	4.2	15.9	*<0.05*
	+21d	89.6	84.1	*n.s.*	4.2	6.8	*n.s.*
Relative to infection	–24h	91.7	81.4	*<0.1*	4.2	15.9	*<0.05*
	+240h	89.6	84.1	*n.s.*	4.2	13.6	*<0.05*

3 Zinc and hoof quality

The long known relationship between Zn deficiency and claw health was recently reconfirmed by results of Enjalbert et al. (2006), who showed that dairy and beef cattle with an insufficient Zn status (defined by plasma Zn levels below 12 µmol/l), displayed lower milk production, diarrhoea and locomotion problems. Baggott et al. (1988) found that cows with a history of lameness had softer claws with higher water and Mg content, and lower Zn content compared to “healthy cows”. Zn concentration in claws from cows with a history of lameness were lower in both lateral and medial claws, but differences in hardness, water and Mg content were only prominent for the lateral claws, not for the medial claws. Baggott et al. (1988) therefore suggest that water and Mg levels are an indication of a structural difference in horn quality, affecting in this case the lateral claws, while the low Zn levels in lateral as well as medial claws indicate a more general difference in the availability of Zn for the keratin-forming cells. Zn would thus have a role in the synthesis of keratin, rather than a structural role in claw hardness. This hypothesis is supported by Tomlinson et al. (2004) who summarized the role of Zn in the keratin-forming cell into catalytic, structural and regulatory aspects. Zn has a role in many enzyme systems in each cell, also influencing the metabolic and regulatory processes of keratin-forming cells. A deficiency of Zn may therefore disturb the synthesis of keratin. A more structural role for Zn may be through the formation of Zn-finger proteins. Zn-finger proteins regulate the expression of important genes and share many structural characteristics with keratin, which may imply that they are involved in keratin synthesis (Tomlinson et al., 2004).

Well-designed peer-reviewed trials on the effect of specifically Zn supplementation on hoof quality of dairy cows could not be found. Experiments often comprise combinations of effects. Offer et al. (2004) compared a low-fibre control diet with a high-fibre diet supplemented with Zn (+ 2 g/d Zn methionine) and biotin (+20 mg/d) in first and second lactation dairy cows. Diets were fed for 20 weeks starting at calving when animals were turned out to pasture. Claw health was monitored at 42 weeks after calving to investigate the effect of the experimental diet on locomotion and the incidence of sole lesions. Although the treatment combined several factors that have been reported to influence hoof health, no effect of treatment on either sole lesions, white line lesions or locomotion score was found.
Nocek et al. (2000) found a reduction in several claw disorders on five commercial dairy farms when a period without Zn supplementation (around 45 mg Zn/kg DM) was followed by a subsequent period with supplementation of a mineral mixture containing Zn methionine, Cu lysine, Mn methionine and Co glucoheptonate (between 58-86 mg Zn/kg DM). The results therefore indicate that there may be an effect of mineral supply in general (not just Zn) on the incidence of claw problems. However, due to the experimental design, time-related differences other than the mineral supply may have influenced the outcome.

Demertzis and Mills (1973) describe two experiments in which Zn treatment cured infectious pododermatitis in young bulls (around 5 months of age) that they developed shortly after a change to a diet of Swede turnips and bruised barley, containing 30-35 and 48-56 mg Zn/kg DM, respectively. The bulls were treated by supplementing 1.5 – 2.4 mg Zn per kg live weight (live weights are not reported) and after 16 to 27 days the claws were healed. Although the change of diet may also have contributed to the claw problems, the effect of Zn supplementation suggests that basal levels of 30 to 56 mg Zn/kg DM in the ration, which are in the range of Zn requirements for bulls of 100 kg and heavier (37.8 mg/kg DM; COMV, 2005), were insufficient to maintain claw health.
Kessler et al. (2003) investigated the effect of dietary Zn level on claw health of red Holstein fattening bulls in a 284-days feeding trial (Table 6). A control diet with 35 mg Zn/kg DM was compared with treatment diets of 45 mg Zn/kg DM, by adding 10 mg Zn/kg DM to the control diet in the form of either Zn oxide, Zn proteinate or Zn polysaccharide. No information was reported on the concentrations of Mo and S in the diet. There was no effect of Zn level or form of supplementation on any growth parameter, nor on Zn content of muscle, liver, bone, hair or claws. Nevertheless, supplementation of Zn proteinate or Zn polysaccharide reduced the score for macroscopic claw lesions, but not for histological lesion score, as shown in Table 6.

In conclusion, there is substantial support for the role of Zn in claw health of cattle, but the lack of scientific data complicates the evaluation of the effect of Zn supplementation on claw health at dietary Zn levels above the generally established requirements (COMV, 2005; NRC, 2001).

Table 6 Effect of Zn dose (35 or 45 mg/kg) and form of additional Zn-supplementation (10 mg/kg DM ZnO, Zn-proteinate or Zn-polysaccharide) on the performance and claw characteristics of fattening bulls (Kessler et al., 2003).

	Treatment				*SEM*
	Control	ZnO	Zn-prot	Zn-poly	
Dietary Zn level (mg/kg DM)	35	45	45	45	
Performance					
DMI (kg/d)	6.7	6.8	6.5	6.7	*0.12*
Initial LW (kg)	146	146	146	146	*1.3*
Final LW (kg)	514	531	509	525	*7.9*
ADG (g/d)	1297	1354	1277	1338	*26.9*
Claw characteristics					
Claw Zn (mg/kg DM)	114	117	114	111	*2*
MS Score Initial[1]	0.87	0.95	0.88	0.97	-
MS Score Final[1]	1.10^a	1.08^a	0.88^b	0.57^c	-
Histological Score[1]	0.87	0.89	0.69	0.74	-
Tensile strength (kp/mm)	5.81	5.72	6.16	5.95	-

DMI: dry matter intake
LW: live weight
ADG: average daily gain
MS: macroscopic scoring of the claw
^{a,b,c}: different superscripts within the same row indicate significant differences ($P<0.05$)
[1]: scoring system: 0=unchanged; 1=slight alteration; 2=moderate alteration; 3=severe alteration

4 Fertility

4.1 Effect of multiple trace mineral supplementation

A strategy to avoid trace mineral deficiencies in practical dairy nutrition is the supplementation of multiple trace minerals. Due to this multi-mineral strategy, it is not possible to determine which trace mineral may have caused a certain improvement in milk yield or fertility parameters. Nevertheless, several peer-reviewed papers using this strategy have been published. Although the value of these data for the current analysis is limited, three papers with diets containing different levels of multiple minerals have been summarized in Table 7. Control diets were reported to have Zn levels varying from 40 to 64 mg/kg DM, Cu levels varying from 12 to 17 mg/kg DM, manganese (Mn) levels varying from 15 to 55 mg/kg DM and cobalt (Co) levels varying from 0.04 to 1.0 mg/kg DM. According to Dutch recommendations (COMV, 2005), Zn, Cu, Mn and Co requirements for a dairy cow producing 40 kg of milk are 32.5, 11.1, 40 and 0.1 mg/kg DM, respectively. This implies that control diets in all three experiments should have supplied sufficient Zn and Cu to cover physiological requirements. In the control diet of experiment 3, Mn requirements were not met, whereas Co level was marginal. Furthermore, Co level was below COMV recommendations in experiment 1.

The fertility parameter which was most influenced by multi-mineral supplementation of trace minerals was the number of days to first oestrous, which was significantly reduced by multi-mineral supplementation in experiments 1 and 3.
Interestingly in experiment 1, only the Co level was below COMV requirements and multi-mineral supplementation with Zn, Cu, Mn and Co decreased number of days to first oestrous. Considering the fact that levels of Zn, Cu and Mn in the control diet were adequate, it is likely that this effect is caused by raising dietary Co intake to a level above requirement.
In experiment 2, levels of all reported minerals were above COMV recommendations, making the experiment usable for analysis of the effect of supplementation above the recommended levels. From the results it can be concluded that additional supplementation of Zn, Cu, Mn and phosphorus did not improve fertility.
In experiment 3, the only treatment that significantly influenced days to first oestrous was treatment 4, which had higher Mn and Co levels when compared with the other treatments. Increased Zn, Cu and a low increase in Mn supplementation, either by organic or inorganic sources (treatment 1 to 3), had no effect on reproduction parameters. The low levels of Mn and Co in the control treatment compared with the recommended dietary intake suggest that the supplementation of Mn and/or Co reduced days to first oestrus for treatment 4, while additional Zn and Cu supplementation (treatments 2 and 3) was ineffective.

From the available data on multi-mineral supplementation only experiment 2 in Table 7 is suitable to analyse the effect of additional Zn and Cu above requirements; the other experiments had a suboptimal supply of Co and/or Mn in the control diet. In experiment 2 no effects of additional supplementation of Zn, Cu, Mn and P on fertility parameters were found. In conclusion, there is insufficient data to decide whether multi-mineral trace element supplementation above recommended levels has an effect on dairy cow fertility.

Table 7 Overview of experiments investigating the influence of multi-mineral supplementation (mineral levels in mg/kg DM) of dairy cows during the dry period as well as lactation on dry matter intake (DMI, kg/d), milk production (kg/d), milk composition (%), somatic cell count (SSC, $*10^3$/ml) and the fertility parameters days to first oestrus (D2FE), days to first insemination (D2FI), days open (DO) and services per conception (S/C).

Treatment (Trt)	n	Zn	Cu	Mn	Co	P	DMI	Milk	Fat	Prot	SCC	D2FE	D2FI	DO	S/C
Exp. 1) Campbell et al., 1999 (total cows: 60)															
0 to 154 days in milk															
Trt 1: Adequate	30	64	17	55	0.04	n.a.[2]	n.a.	34.6	n.a.	n.a.	n.a.	67.6	82	91.7	1.4
Trt 2: 4-plex Zn	30	78	22	63	0.23	n.a.	n.a.	35.7	n.a.	n.a.	n.a.	46.9	74	80.2	1.4
SEM								*1.1*				*10.2*	*4.7*	*10.6*	*0.2*
P								*0.38*				*0.02*	*0.23*	*0.45*	*0.89*
Exp. 2) De Boer et al., 1981 (total cows: 33)															
-98 to 154 days in milk															
Trt 1: 100% of NRC[3]	11	40	12	41	n.a.	3.4	20.6	28.0	3.67	3.15	n.a.	44.7	66.1	74.4	1.5
Trt 2: 150% of NRC	11	58	17	59	n.a.	5.1	20.7	27.2	3.69	3.26	n.a.	54.4	74.1	80.0	1.0
Trt 3: 200% of NRC	11	79	21	79	n.a.	6.9	21.7	27.4	3.66	3.37	x	32	62.2	72.9	1.5
SEM							*0.1*	*1.3*	*0.05*	*0.02*		*11.4*	*6.4*	*8.1*	*0.3*
P										*0.05*		*ns*	*ns*	*ns*	*ns*
Exp. 3) Nocek et al., 2006 (total cows[1]: 573)															
year 1: start d-55, whole 1st lact															
Trt 1: Complex	2	58	12	15	0.09	n.a.	22.2	36.2^{b}	3.95^{ab}	3.06	255	56^{a}	66	120	2.3
Trt 2: Sulfates	2	78	16	21	0.11	n.a.	22.0	35.0^{c}	4.02^{a}	3.07	241	54^{a}	66	118	2.4
Trt 3: Complex	2	77	16	20	0.11	n.a.	22.6	36.6^{b}	4.00^{a}	3.07	258	54^{a}	65	115	2.2
Trt 4: Complex+Sulfate	2	75	15	68	1.0	n.a.	22.4	37.6^{a}	3.91^{b}	3.04	214	47^{b}	65	104	1.9
SEM								*0.2*	*0.02*	*0.1*	*19*	*1.7*	*1*	*6.4*	*0.2*
P								*0.01*	*0.01*	*ns*	*ns*	*0.01**	*ns*	*ns*	*ns*
year 2: -55 to 200 days in milk															
Trt 1: Complex	2	58	12	15	0.09	n.a.	25.7	41.5^{b}	3.93^{b}	2.95	447^{a}	57^{a}	65	129	2.6
Trt 2: Sulfates	2	78	16	21	0.11	n.a.	24.4	41.5^{b}	3.99^{ab}	2.93	437^{a}	57^{a}	64	132	2.5
Trt 3: Complex	2	77	16	20	0.11	n.a.	25.4	43.8^{a}	4.03^{a}	2.94	234^{b}	57^{a}	65	135	2.7
Trt 4: Complex+Sulfate	2	75	15	68	1.0	n.a.	25.6	43.5^{a}	3.98^{ab}	2.92	243^{b}	50^{b}	64	116	2.2
SEM								*0.2*	*0.02*	*0.01*	*30*	*1.5*	*0.6*	*6.1*	*0.2*
P								*0.01*	*0.02*	*ns*	*0.01*	*0.001*	*ns*	*0.02*	*ns*

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[1] cows were housed in 2 groups: high number of cows but only 2 experimental units

[2] n.a.: means not given in paper

3 NRC (1978) recommendations for P, Cu, Zn and Mn

4.2 Effect of Cu or Zn supplementation

Unfortunately, peer reviewed scientific papers reporting well-designed experiments investigating the effect of a relatively low supplementation level of single minerals like Cu or Zn above physiological requirements on fertility are not available. Two experiments with some limitations concerning the analysis of individual mineral effects will be described below.

Campbell and Miller (1998) performed a 2 x 2 x 2 factorial design with 136 cows with the factors vitamin E (alpha-tocopheryl acetate), Zn (Zn-methionine), and Fe (iron sulphate) supplied daily in gelatine capsules starting 42 days before expected parturition until calving. Treatments did not have an effect on the incidence of retained foetal membranes. Days to first oestrous were reduced for all treatments; however, Zn supplementation did not reduce days to first oestrous in combination with Fe supplementation, as Fe-supplemented cows already had lower days to first oestrous. It must be mentioned though, that unsupplemented animals in this trial had an unusual high number of days to first oestrous of 82 days, suggesting unconventional conditions. No actual Zn or Fe levels in the total prepartum rations were given, but these can be calculated by estimating dry matter intake of the forage. Prepartum dietary Zn and Fe levels are thus estimated to be respectively 100 and 175 mg/kg DM for the control treatment and 170 and 400 mg/kg DM for the Zn- and Fe-supplemented treatments. No further information was presented on the dietary concentrations of Mo and S. A lack of data on the feed supply after parturition impedes the estimation of post-partum mineral levels.

Ingraham et al. (1987) supplemented 204 Holstein dairy cows with either Cu (as $CuSO_4$ to levels of 15 mg Cu/kg DM), magnesium (as MgO to levels of 3 g Mg/kg), or both, starting 30 days before expected calving. Cows were grazed on pasture; additional feedstuffs were deficient in Cu (concentrate contained 10 mg Cu/kg DM, other feedstuffs lower) and marginal in Mg (feedstuffs contained between 2.0 and 2.9 g Mg/kg DM). Rations were low in Mo with feedstuffs containing less than 0.9 mg Mo/kg DM. Cu:Mo ratios in all rations were higher than 3, thus expecting no interaction of Mo with Cu intake (COMV, 2005). Supplementation with Cu or Mg separately did not influence any of the fertility parameters reported (Table 8). Only the combined supplementation with Cu and Mg was effective in increasing the number of cows pregnant at a defined number of days in milk. No further information was presented on the dietary concentrations of Zn and S.

Table 8 Effect of Cu and Mg supplementation of 204 Holstein dairy cows on fertility data: days to first oestrous (D2FE), days to first service (D2FS), days open (DO), services per conception (S/C) and pregnancy rate at 75, 100, 125 and 150 days in milk (Ingraham et al., 1987)

	Cu	**Mg**	**Milk**	**D2FE**	**D2FS**	**DO**	**S/C**	**pregnancy rate (%) at day**			
	mg/kg DM	***g/kg DM***	***kg/d***	***d***	***d***	***d***		***75***	***100***	***125***	***150***
Control	<10	<3.0	24.9	52	81	107	1.9	22^{a}	39^{a}	45^{a}	59^{a}
+Cu	15	<3.0	24.3	42	77	108	1.9	16^{a}	34^{a}	53^{a}	62^{a}
+Mg	<10	3.0	24.8	53	83	116	2.0	16^{a}	35^{a}	49^{a}	63^{a}
+Cu+Mg	15	3.0	25.0	51	74	92	1.6	39^{b}	65^{b}	73^{b}	84^{b}
SEM			*2.0*	*16*	*11*	*23*	*0.7*				

[a] rows with different subscripts differ (P<0.01) in the chi-square test

It can be concluded that the two experiments found in literature dealing with single supplementation of Zn or Cu are insufficient to evaluate the effect of supplementation of either Cu or Zn above recommended dietary levels on dairy cow fertility.

5 Risk of dietary overload

5.1 Zinc excess

Dietary Zn overload is especially deleterious for the environment. Raising Zn in the diet to levels above the physiological requirement will result in increased Zn excretion and Zn accumulation in the environment.
Zn is relatively safe for the animal itself up to concentrations of approximately 500 mg/kg DM, while recommendations reach 20-35 mg/kg DM depending on stage of lactation (COMV, 2005). At 500 mg Zn/kg DM, Zn will have a negative effect on Cu and iron (Fe) absorption, resulting in symptoms of Cu or Fe deficiency (COMV, 2005) as well as degenerative reactions in liver, kidney and pancreas. Besides acute intoxication, a chronic Zn overload will also result in degenerative processes and Zn accumulation in liver, kidney and pancreas (Allen et al., 1983), causing health problems. High levels of Zn also affect rumen metabolism, probably via a toxic effect on ruminal microorganisms (Ott et al., 1966). At current feeding and management standards in the Netherlands, Zn levels in livers of approximately 50% of the dairy calves under 75 days of age have reached levels over 500 mg Zn/kg DM, which are considered to be deleterious for animal health (J. Veling, Animal Health Services Deventer, personal communication).

5.2 Copper excess

Dietary Cu overload can have toxic effects in ruminants (sheep are highly susceptible). Excess Cu is stored in the liver and released when plasma Cu decreases. When Cu accumulation reaches its maximum in liver, hepatic damage will occur. Maximum Cu concentration for dairy cattle is approximately 40 mg/kg DM, while recommended dietary intake varies between 10-25 mg/kg DM depending on stage of lactation (COMV, 2005).
Feeding above required intake levels will also result in undesirable Cu excretion and accumulation in the environment.

6 General conclusions

Minerals are involved in all aspects of bovine metabolism. It is very well established that *deficiencies* of Cu and Zn can cause problems in health and fertility. This review focused on the effect of supplementation of Zn and Cu to levels *above* the currently recommended levels (COMV, 2005), on immunity, fertility, udder health and hoof quality in predominantly dairy cattle. If additional Cu or Zn above recommendations does not seem relevant for health and fertility of dairy cattle, dietary intake levels should be reduced to recommendations to prevent unnecessary accumulation in the environment.

In general, due to scarcity of experimental data and the variability in experimental results, the effect of Zn- and Cu-supplementation *above* required levels is inconclusive. The results of the literature review are summarized in table 9. There are some indications that Zn supplementation to levels around 100 mg/kg DM (while current recommended levels are about 50 mg/kg DM) may decrease somatic cell count in lactating dairy cows and stimulate immune response parameters in calves. Results are variable though and an adequate length of experiments may be crucial.

To provide the scientific background necessary to justify the supplementation of Cu and Zn above recommended dietary levels based on the physiological requirements, well-designed dose-response studies are needed. Papers that have been published in the past often lack data on several subjects that require further research:

The exact level of intake and excretion of Cu and Zn

The exact level of intake should be analysed and reported. Some papers only report supplementation levels without the intake level of the control diet, which may be directly or indirectly deficient for Cu or Zn. Additionally, measuring excretion levels during experiments may contribute to determine the variation in absorption of Cu and Zn, and the possible effect on the environment.

The dietary intake of other (trace) minerals (e.g. Mo, S)

Many papers do not report intake levels of other minerals in the diet. Control diets may have been deficient for some minerals, or have been oversupplied with minerals that interact with Cu and Zn uptake in the gastrointestinal tract. Mo and S have been proven to reduce Cu uptake through precipitation.

Objective parameters on health, immunity or fertility

The experimental design should aim at objectively registering functional parameters of health, immunity and fertility to determine the effect of additional Zn or Cu. For some papers, the experimental design was not developed to measure effects on health or fertility, and the results on these areas are questionable.

There are some other subjects of interest that have not been investigated sufficiently. There are indications suggesting that potassium (K) may negatively affect Zn uptake in ruminants (Bonomi et al., 2000). This interaction and possibly other interactions between (trace) minerals need further investigation. Finally, the physiological state of an animal (e.g. disease) may influence mineral requirements and should be considered when defining the optimal dietary intake levels for dairy cattle.

Table 9 Summary of the effects of Zn or Cu supplementation *above* currently recommended levels.

	Zn	**Cu**
Immunity in calves	Potential effect of supplementation up to 100 mg/kg DM	No indication for a significant positive effect.
Fertility in dairy cows	Insufficient data	Insufficient data
Somatic cell count in dairy cows	Some indications for positive effect of long term supplementation	Insufficient data
Hoof quality in dairy and beef cows	Insufficient data	-

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Literature

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