Background of the copper and zinc requirements for dairy cattle, growing-finishing pigs and broilers

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Summary

Re-evaluation of the current authorized levels of Cu and Zn in animal rations has been initiated by the European Union (EU). The physiological requirements should be the basis for the future maximum authorized dietary levels for minerals in the EU, resulting in lowering of these maximum levels. Based on an earlier study, the basis of the recommendations for Cu and Zn of dairy cattle, growing pigs, and broilers as used by different countries seemed to be questionable. These recommendations are based on research at relatively low animal production levels as compared with the current Dutch situation. Therefore, a survey of the scientific literature underlying the recommendations of ARC, NRC, DLG, COMV, and INRA was carried out, together with other reports on dietary Cu or Zn requirements of the animal categories mentioned.

Only reports using Cu (< 50 mg/kg), Zn (< 200 mg/kg), and Ca (< 14 g/kg) concentrations comparable with more or less practical levels were included in this study (for cattle, the values reported are expressed on DM basis). Experiments without any added trace elements were excluded from this study, because of a possible deficiency of essential trace minerals. By means of regression analysis and broken line analysis of physiologically relevant response parameters, an attempt was made to calculate dietary total Cu and Zn requirements of the animal categories studied. If broken line analysis yielded insufficiently useful models, dietary requirements were assessed by comparison of the results of the different experiments. Also, dietary levels of Cu and Zn were assessed at which maximal production performance was attained. Special attention was paid to possible differences in effects of intrinsic vs. extrinsic dietary Cu and Zn, to the effects of the Cu or Zn levels during the pre-test period, and to the influence of the duration of the experimental period on mineral concentrations in “storage” tissues, i.e. Cu in liver and Zn in bone.

In general, suitability of the experimental evidence is sometimes limited. Many data sets are incomplete (only very few response parameters determined), inhomogeneous and/or ancient. Results of this study should, therefore, be considered with caution.

In the case of dairy cattle, the low number of suitable experiments for both Cu and Zn precluded any calculation of requirements. Based on production performance in six experiments, requirements for dairy cattle could be roughly assessed to be around 21 mg/kg (DM) for Cu, but it should be noted, however, that due to the limited number of data is was impossible to quantify the Cu requirement in relation to the interaction between Cu, Mo, and S. Maybe, 21 mg Cu/kg DM is marginal at high Mo and S concentrations in the diet because of their negative effect Cu availability. Zinc requirement for dairy cattle was estimated > 25 mg/kg (DM).

Based on serum Cu concentrations, the Cu requirements of growing pigs were calculated to be 4 mg/kg Cu added to a synthetic basal diet. The recommendations of Cu for growing-finishing pigs are in agreement with our results. Therefore, an addition of 4 mg/kg Cu to a complete diet (88% DM) is sufficient. Calculation of a precise Zn requirement was difficult. Based on maximal growth performance total Zn content should be at least 56 mg/kg. Mainly in the case of Zn, feed conversion ratios of the animals used were relatively high. The assessed requirement may, therefore, have to be adapted to the current improved feed conversion ratios. Therefore, a concentration of 67 mg Zn/kg is recommended as was obtained for pigs receiving other types of diets.

The Cu requirement of broilers was estimated to be 6 mg/kg added to a practical diet. Again, the growth rates of the animals used were relatively low, thereby impairing the applicability of the assessed requirement to fast growing broilers of today. However, feed conversion ratios were to a large extent comparable with those in current practice. Based on limited data on bone Zn concentrations and on the Zn level required for maximal growth performance, the Zn requirement was calculated to be 74 mg/kg in practical diets. As it was demonstrated in some experiments that relatively high growth rates were feasible using dietary Zn concentrations below 68 mg/kg, the calculated concentration might even cover these animals’ dietary Zn needs.

In all cases, mostly due to a limited number of suitable data, it was impossible to discriminate between effects of intrinsic and added dietary Cu or Zn, although there were indications for a lower availability of intrinsic Zn. Also, no clear insight could be obtained for the influence of dietary Cu or Zn levels during the pre-test period and the influence of the duration of the experiment. Finally, no conclusions could be drawn with regard to differences between age of the animals.

The requirements as assessed in this study are substantially lower than the maximum allowed concentrations in diets from 26 January 2004 onwards. Therefore, there is no risk for a sub optimal supply of Cu and Zn to the animal categories studied.

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Abbreviations used

The following abbreviations and definitions have been used:

A(F)RC = Agricultural (and Food) Research Council (Great Britain) *
BW = body weight
Cp = ceruloplasmin
C_p = Mallow's C_p
COMV = Commissie Onderzoek Minerale Voeding (The Netherlands)
CuLys = Cu lysine
CuMet = Cu methionine
δ = differences in parameter concentrations or activities as compared with basal level
DIM = days in milk
DLG/GfE = Deutsche Landwirtschaftsgesellschaft/Gesellschaft für Ernährungsphysiologie (Germany)*
DM = dry matter
DMI = dry matter intake
FCR = feed conversion ratio = kg feed/kg gain
INRA = Institut National de Recherche Agronomique (France)
mg/kg = = µg/g
NRC = National Research Council (United States of America) *
ZnLys = Zn lysine
ZnMet = Zn methionine

* the use of these abbreviations in tables refers to the use of that reference for the determination of dietary requirements by ARC, DLG, or NRC

Preface

This project was carried out by the following persons:

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The literature survey was carried out by A.M. van den Top (Adviesbureau VOER-RAAD, Groenekan, The Netherlands)

H. Everts (Department of Nutrition, Veterinary Faculty, Utrecht, The Netherlands) is acknowledged for his help with statistical analysis.

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Age W. Jongbloed

1. General introduction and procedure

1.1. Introduction

In 1999, feed manufacturers and agricultural organizations in The Netherlands agreed amongst others to lower the dietary Cu and Zn supply to livestock compared with the current maximum authorized levels [6]. The reason for this agreement is that Cu and Zn in animal manure can form a considerable environmental hazard in the future. Therefore, avoidable losses to the environment should be prevented as much as possible. Furthermore, in 2003, the European Union (EU) adopted new maximum authorized levels of (all) trace minerals in animal rations [5]. The physiological requirements as mentioned in this document should be the basis for the maximum authorized dietary levels in the EU. The proposed maximum levels in this document are in some cases lower than those mentioned in the Dutch agreement (Table 1.1; [6]).

Nutritional requirements as mentioned in the EU document, however, are based on research at relatively low animal performance levels as compared with current Dutch levels. Therefore, a better insight is necessary into the actual nutritional requirements for Cu and Zn of cattle, pigs, and poultry. Based on the results of an earlier study [3], it was concluded that it was not clear what was the basis for the recommended requirements by AFRC, NRC, DLG, COMV and INRA. Therefore, on behalf of the Dutch Product Board Animal Feed, the Division Nutrition and Food of Animal Sciences Group of Wageningen UR was asked to carry out a survey of the scientific literature concerning the basis for these recommendations for growing pigs, dairy cattle and broilers.

Table 1.1. Overview of maximum allowed concentrations of Cu and Zn in diets for specific categories of animals (mg/kg)

	Copper (mg/kg) total diet			Zinc (mg/kg) total diet		
	EU till 2004	EU 2004	NL 2000	EU till 2004	EU 2004	NL 2000
	total	total	added	total	total	added
Pigs						
Piglets till 12 weeks of age	175	170	160	250	150	100
From 12 - 16 weeks of age	175	25	130	250	150	70
Fatteners from 16 weeks of age	35	25	15	250	150	60
Breeding sows	35	25	20	250	150	65
Birds						
Broilers	35	25	15	250	150	55
Layers	35	25	15	250	150	55
Ovines	15	15	see EU	250	150	see EU
Cattle						
Bovine dairy	35	35	see EU	250	150	see EU
Bovine other	35	35	see EU	250	150	see EU
Calves (before rumination)	30	15	see EU	250	200	see EU
Calves (before rumination)	50	15	see EU	250	150	see EU

1.2. Premises and suppositions

As the results of this study should be applicable in practice, the following premises were chosen. Experiments using total dietary concentrations up to 50 mg/kg for Cu and up to 200 mg/kg for Zn (on approx. 90% DM basis for pigs and poultry, and on a DM basis for cattle) were included in this study. Moreover, for pigs and poultry, experiments using total dietary Ca concentrations only up to 1.4% were included, because of the negative effect of high Ca levels on Zn availability [1]. (Parts of) experiments using concentrations exceeding these values were not included in this study. Furthermore, experiments in which the diets did not contain added essential trace minerals, were excluded.

A reference may comprise more than one experiment, reason why there are more experiments than references.

Copper and Zn concentrations in blood were either expressed in serum, plasma, or whole blood as µmol/L (µM). It is reported that 80-90% of plasma Cu is bound to ceruloplasmin [1;4]. Besides this, a portion is present attached to superoxide dismutase (SOD) in the erythrocytes. About 10-20% of blood-Zn is present in the plasma, mainly bound to albumin [1]. Large differences may, therefore, exist between Zn concentrations in whole blood and serum and/or plasma. For pigs, this is demonstrated by the results of Heigener et al. [2], who found Zn concentrations in whole blood up to 10 times higher than others found in plasma. All Zn concentrations in whole blood were, therefore, excluded from the statistical analysis. As analysed concentrations in the different blood fractions were of the same magnitude, Cu concentrations in whole blood, serum and plasma, as well as Zn concentrations in serum and plasma were considered of similar value for evaluation of dietary requirements.

To optimally characterize the animals used, in the tables the age (all animals), growth rate, feed conversion ratio (pigs and broilers), days in milk, and milk yield (dairy cattle) of the animals are presented. To describe the experimental design, the duration of the experiment, the response parameters measured, as well as dietary added and total Cu or Zn concentrations, and type of basal diet of the literature sources used are given.

For ease of comparison, all dietary mineral concentrations are expressed on the basis of fresh diet as fed (approx. 90% DM in pig and poultry diets) or on a DM basis (cattle). If mineral concentrations had to recalculated and DM content of concentrates was not given, DM content was supposed to be 90%.

1.3. Contents

In the following chapters, the experiments underlying the dietary recommendations for Cu and Zn of the ARC, DLG/GfE, INRA, and NRC are judged. Unsuitable (parts of these) experiments, as well as the reason of their rejection, are listed separately. Chapter 2 deals with the requirements of dairy cows, Chapter 3 with those of growing pigs, and Chapter 4 with those of broilers.

1.4. Summary of requirements of Cu and Zn in different countries

Table 1.2 summarizes the recommendations on requirements of Cu and Zn in different countries [3]. In some countries no discrimination is given between requirements according to physiological status of the animal. This means e.g. the same requirement is given for growing animals and lactating animals. This is indicated in Table 1.2.

Table 1.2. Summarized inventory of requirements of Cu and Zn in different countries (*=same for all categories of the specific animal species; [3])

Lactating cattle (concentrations as mg/kg DM)		
Country	Requirement of Cu	Requirement of Zn
NL	10*	25*
UK	1-15*	30-50*
USA	10	40
D	10	50
F	10*	50*
Piglets and growing-finishing pigs (concentrations as mg/kg fresh diet)		
NL	-	-
UK [7]	6 (added)	60 – 100 (added)
USA	3.0 – 6	50 – 100
D	4 – 5	45 – 90
F	10*	100*
Broilers (concentrations as mg/kg fresh diet)		
NL	-	-
UK	4	35 - 40
USA	8	40
D	6 – 7	40 – 50
F	10	50

Table 1.2. shows large differences in recommendations between the countries for specific animals.

1.5. Literature

[1] Cousins RJ. Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiological Reviews 1985; 65: 238-309.

[2] Heigener H, Michna A, Neumann H-J, Behrens H, Hamann K. Einfluß differenzierter Zinkgaben bei unterschiedlichem Ca/P-Verhältnis auf die Entwicklung wachsender Schweine. Landwirtschaftliche Forschung 1958; 11: 185-201.

[3] Jongbloed AW, Top AMvd, Beynen AC, Klis JDvd, Kemme PA, Valk H. Consequences of newly proposed maximum contents of copper and zinc in diets for cattle, pigs and poultry on animal performance and health. Report ID-Lelystad 2001; 2097.

[4] Todd JR. A survey of the copper status of cattle using copper oxidase (caeruloplasmin) activity of blood serum. In: Mills CF (ed.), Trace element metabolism in animals. Proceedings of WAAP/IBP International Symposium, Aberdeen, Schotland. Edinburgh: E.&S. Livingstone; 1970: 448-451.

[5] EC. Commission regulation (EC) No 1334/203 of 25 July 2003. Amending the conditions for authorisation of a number of additives belonging to the group of trace elements. L 187/11-L 187/15.

[6] PDV. Besluit PDV normen GMP diervoedersector. PDV bundel GMP-regeling diervoedersector, 8 juni 2000.

[7] BSAS, 2003. British Society of Animal Science. Nutrient requirement standards for pigs. p. 18.

2. Copper and zinc requirements of dairy cows

2.1. Introduction

2.1.1. Procedure

In this chapter, references concerning dietary Cu and Zn requirements of dairy cows as quoted by DLG/GfE, A(F)RC and NRC, as well as recent reports from the scientific literature are listed and evaluated. No information concerning suitable scientific references as used by COMV and INRA could be obtained.

2.1.2. Rejected references

Not all references were suitable for this study. There were in total 19 references concerning Cu and nine about Zn, which have been used by DLG/GfE, A(F)RC and NRC, but had to be rejected for this study. The reasons for rejection are listed in Appendix 2.1, e.g. because sheep or calves were used. Among the reports used by DLG/GfE, A(F)RC and NRC some results had to be adapted to be suitable for our statistical analysis. The reference involved, and the adaptations made, are listed in Table 2.1.

Table 2.1. Reference that had to be adapted prior to statistical analysis of dairy cow data

Reference	*Way of adaptation*
[8]	It is assumed that the mineral concentrations in the diets are expressed as g per kg DM. The experiment started on average 3 months before parturition, while the experiment lasted 280 days; therefore, only the composition of the lactation diet was used

2.2. Available literature

The seven references suitable for evaluation of Cu requirements for lactating dairy cows are listed in Table 2.2, and the three suitable for evaluation of Zn requirements are listed in Table 2.3.

Table 2.2. Overview of the experiments used for evaluation of Cu requirements of lactating dairy cattle; D = duration of experiment (d); DIM = days in milk; Cp = ceruloplasmin

Reference	**D**	**DIM**	**Response criteria**	**Cu (mg/kg; DM)**		**S (g/kg DM)**	**Mo (mg/kg; DM)**
				Basal	Added		
[4]	83	97	DMI, milk yield, milk fat and protein content, serum and liver Cu	8, 9	0, 15, 30	2.5	not given
[6]	60	3	Plasma Cu and liver Cu	6	5	2.2	1.7
[7]	300	21	Calf birth weight, milk yield, milk and blood Cu	4.6	0, 15.5	not given	not given
[8]	280		Plasma Cu and Cp	4.5	0, 10	3.0	1.5
[15]	120	preg-nant	Liver Cu	6.1	0, 13.9, 15.7	not given	1.8 – 2.3
[40]	112		Milk yield, blood and liver Cu	9.1	0, 10, 20	3.3	1.7
[7b]	61		DMI, milk yield, milk fat and protein content, serum and liver Cu	8.9	0, 10, 40	2.4	1.1

Table 2.3. Overview of the experiments used for evaluation of Zn requirements of lactating dairy cattle; D = duration of experiment (d)

				Basal Zn	Added Zn
Reference	D	DIM	Response criteria	mg/kg (DM)	
[23]	42	70	Milk yield, fat, protein and Zn content, plasma Zn, bone, liver, kidney, and pancreas Zn	16.6	0, 22.9
[37]	105		DMI, growth, milk yield and fat %	25	0, 32
[38]	105		Growth, milk yield	25	0, 127

2.3. Statistical analysis

Due to the limited number of observations, only relationships between dietary Cu and both (δ) serum and (δ) hepatic Cu concentrations could be subjected to multiple regression analysis. First, possible models were selected based on Mallow's C_p. Then, these selected models were subsequently analysed by forward, backward and stepwise selection of variables. The statistical package Genstat 5, release 3.1 [25] was used throughout.

Only physiologically relevant explaining parameters were included in the statistical analysis. For Cu, these were dietary Cu, S, and Mo concentrations. Both total and added dietary Cu concentrations were included in the statistical analysis throughout to disclose possible differences between the effects of intrinsic and extrinsic Cu in the total ration.

Suitable models should at least comply with the following conditions:

dietary Cu content is an explanatory parameter in the model
$R^2_{adjusted}$ is > 70.0%
C_p is < p + 3 (p = number of explaining parameters in the model) [24]
The number of observations is > 4
The coefficient is significant ($P < 0.05$).

If models, based on these conditions, seemed to be appropriate, data were presented in a graph. Then, the best parameters (showing a visible correlation in the graph) were subjected to broken line analysis to obtain a breakpoint value. This breakpoint discriminates between the first phase of the graph, in which the response parameter increases with each increase in dietary Cu concentrations, and the second phase, a non-responsive plateau of response parameter values. This breakpoint, therefore, suggests the attainment of the dietary Cu requirement.

For Zn, the number of suitable experiments (Table 2.3) was too small (n=3) to carry out any statistical analysis.

2.4. Results and discussion

2.4.1. Dietary total Cu requirements

Data are recent, as 11 out of 12 experiments have been published in or after 1990. We applied regression analysis on the data obtained with lactating cows. In the case of Cu, insight into the different factors influencing dietary requirements (mainly Cu, Mo and S [16]), as well as data on milk yield, are necessary. However, even these data were lacking in several cases. It appeared to be impossible to include Mo as an explanatory parameter in the regression analysis of (δ) liver Cu concentrations, because several data were lacking and moreover, there was a narrow range of Mo contents (mean and sd: 1.7 ± 0.4; range: 1.1 to 2.3 mg/kg DM). Also S content showed a narrow range (mean and sd: 2.6 ± 0.4; range: 2.2 to 3.3 g/kg DM). As the coefficient for dietary Cu content as an explanatory parameter was negative, which means that higher dietary Cu concentrations should be associated with lower hepatic and serum Cu concentrations, results of the regression analysis were omitted.

Graphic presentation of the data showed no visible correlation between (δ) dietary Cu and (δ) liver Cu or (δ) serum Cu concentrations. As an example, the relationship between dietary and serum Cu concentrations is visualised in Figure 2.1.

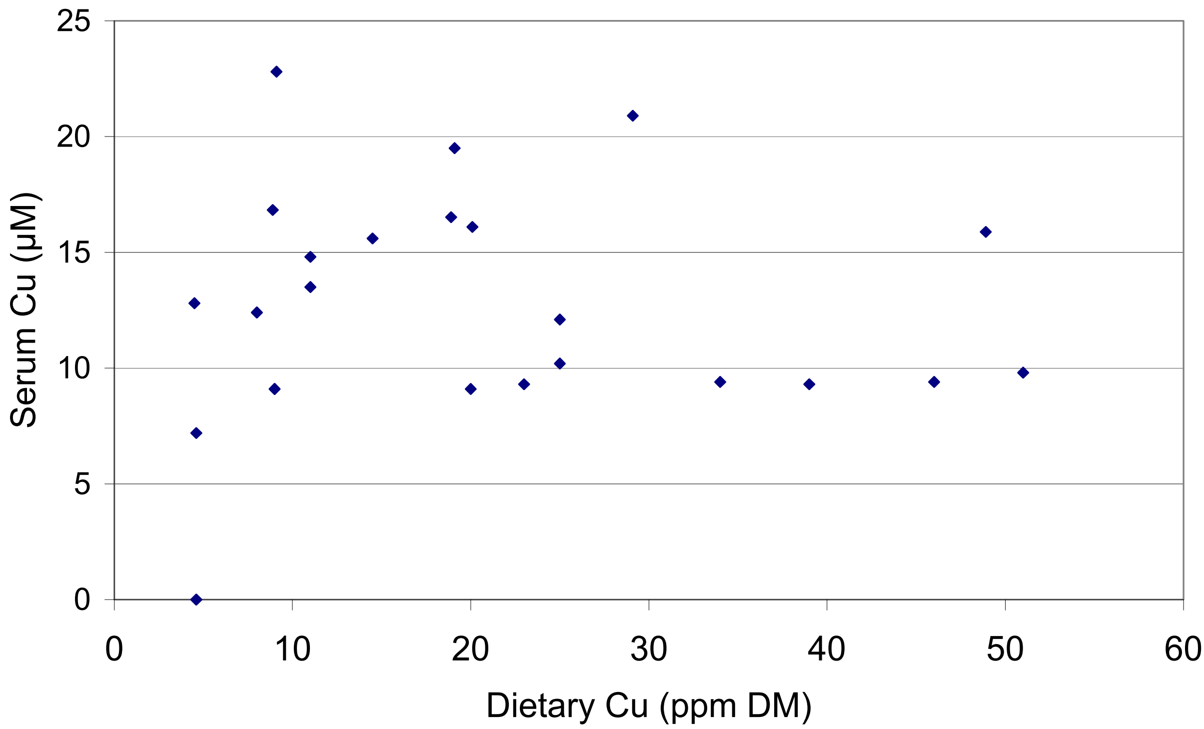


Figure 2.1. Relationship between individual dietary and serum Cu concentrations in dairy cows

Graphic presentation of the data for liver Cu (Figure 2.2) showed some relationship, but broken line analysis of the data did not supply a useful model:

Liver Cu (mg/kg (DM)) = 563.3 – 11.47 x ln (1 + exp $^{(34.2 - \text{dietary Cu (mg/kg DM))}}$)
(R^2 = 26.7%; RSD = 182)

In this model, 563.3 (± 80.3) is the level of the non-responsive plateau in the second phase of the graph, 11.47 (± 5.67) is the slope of the line in phase one, and 34.2 (± 12.6) is the breakpoint value. However, although the breakpoint value nicely represents the relatively high need for Cu of dairy cows, R^2 of this model is too low to be of sufficient value.

Comparison of the different experiments in order to assess dietary Cu requirements based on production performance reveals that Cu concentrations should be (mg/kg in DM): 20 to 25 at a low Fe (135 mg/kg) content [4], 25 to 34 at a high Fe (535 mg/kg) content [4] and 19 [40] (Appendix 2.2). Two experiments supplied only data on Cu liver content and they revealed that dietary Cu level should be >11 [6], >6 [15]. Two more experiments only supplying data on plasma Cu content give values of > 4.6 [7] and >14.5 [8]. A recent study in 2001 [7b] showed in high yielding dairy cows no differences in feed intake, milk yield and milk composition at 9, 19 and 49 mg/kg Cu (DM). It was concluded in this experiment that 49 mg/kg dietary Cu was marginally toxic.

For all experiments listed in Appendix 2.2 a review was made of the total Cu content at which level the highest production of total protein and fat was obtained, without taking into account significant differences. It shows that there is a large variation in Cu levels for maximal performance. Experiments in which no milk production was measured were omitted. It revealed that maximal production was achieved at on average at 24 mg/kg DM. It may be remarked, however, that the values are slightly overestimated, because the maximal performance might have been achieved at a level between this and the lower supplementation level. Therefore, we subtracted the mean of the difference between two supplementary levels of Cu from the level we obtained for maximal milk production. This resulted in (24 – 3 =) 21 mg/kg.

Taking the scarce evidence together (Appendix 2.2), dietary total Cu concentration should be around 21 mg/kg (DM). Evidence for even higher requirements is conflicting.

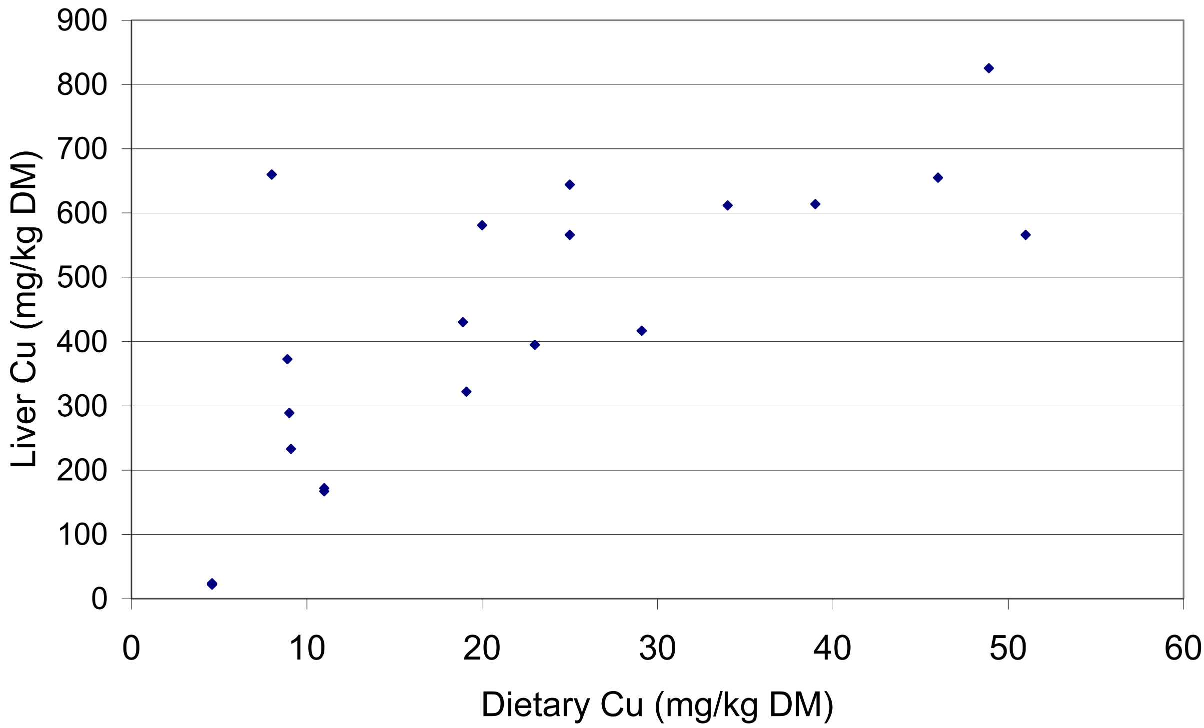


Figure 2.2. Relationship between individual dietary and hepatic Cu concentrations in dairy cattle

As dietary S and Mo concentrations are important determinants of Cu availability in dairy cows [16], the information concerning range and distribution of S and Mo concentrations is insufficient. This hampers a reliable assessment of dietary Cu x S x Mo interactions at various S and Mo levels. In The Netherlands, S concentrations in grass silage may range from 0.6 to 5.3 g/kg DM, and Mo concentrations from 0.2 to 5.3 mg/kg DM [1b]. If both Mo and S content are in the upper range then 21 mg/kg Cu may not be sufficient.

2.4.2. Differences between intrinsic and added dietary Cu

Taking into account only those experiments supplying data on hepatic Cu concentrations (considered to be the most important indicator of Cu status [16]), diets without added dietary Cu (Table 2.2) contained 6-11 mg/kg Cu (DM). In most experiments using diets with added Cu, they contained Cu concentrations of at least 15 mg/kg (DM) and thus Cu concentrations are not of similar magnitude and can, therefore, not be compared. Only two experiments used total (basal + added) Cu concentrations of 11 mg/kg (DM) and, therefore, results can be compared with those with similar basal Cu concentrations. Selected results are given in Table 2.4. As only two values of δ liver Cu were available, this parameter was not included in this table.

Table 2.4. Selected results of references using similar dietary total Cu concentrations from different origin (intrinsic vs. added) and plasma and liver Cu concentrations

Reference	Basal Cu	Added Cu	Total Cu	Plasma Cu	Liver Cu
	mg/kg (DM)			μM/L	mg/kg (DM)
[4]	8	0	8	12.4	660
	9	0	9	9.1	289
[40]	9.1	0	9.1	-	233
[6]	6	5	11	13.5	167
	6	5	11	14.8	172

No relationship is visible between any of the explanatory parameters and plasma or liver Cu concentrations. Concerning the difference between intrinsic vs. added Cu, these data do not supply any evidence for differences in metabolic effects of intrinsic and added Cu in dairy cattle rations. It should also be noted, however, that the previous history of the animals may largely differ among the experiments, resulting in large differences in hepatic Cu content at the start of the experiment.

2.4.3. Dietary total Zn requirements and differences between intrinsic and added Zn

Suitable experimental evidence on Zn requirements of lactating dairy cattle is too scarce even to make an attempt to assess dietary requirements. Besides this, the most recent report was published in 1973. In two cases [37;38], based on animal performance data, dietary Zn requirement was > 25 mg/kg (DM), whereas the third experiment [23] hardly showed any difference in bone Zn and plasma Zn concentrations between dietary Zn concentrations of 17 and 40 mg/kg (DM). The only rough assessment that can be made from the available data is, therefore, that dietary total Zn requirement of dairy cattle is > 25 mg/kg (DM). How much higher the Zn concentration should be can not be assessed due to lack of sufficient information.

As hardly any information is available, any conclusion on differences between metabolic fates of intrinsic or added Zn in dairy cattle rations is precluded.

2.5. Conclusions

2.5.1. Copper

Because few suitable data are available, the only rough conclusion that can be drawn is that based on milk production performance (three references comprising six experiments), dietary total Cu requirement of dairy cows should be 21 mg/kg (DM). Based on these data, quantification of the interaction of Cu with S and Mo is impossible. This may mean that at high Mo and S concentrations in the diet a higher Cu level than 21 may be necessary. The scarcity of data also precludes discrimination between differences in metabolic importance of intrinsic vs. added dietary Cu, or judgement of the effect of Cu, S or Mo levels during the pre-test period. Finally, the dietary Cu recommendations for dairy cattle of several countries seem to be based mainly on experiments with beef cattle.

2.5.2. Zinc

For Zn, the absence of suitable data for dairy cows is even more serious than for Cu. Dietary total Zn requirement is estimated to be >25 mg/kg (DM).

No conclusion can be drawn on different effects of intrinsic or added dietary Zn, or on effects of dietary Zn levels during the pre-test period. Current dietary Zn recommendations for dairy cattle seem to be based mainly on experiments with growing calves.

2.6. Literature

[1] Allcroft R, Lewis G. Relationship of copper, molybdenum and inorganic sulphate content of feeding stuffs to the occurrence of copper deficiency in sheep and cattle. Landbouwkundig Tijdschrift 's-Gravenhage 1956; 68: 711-722.

[1b] BLGG Oosterbeek, The Netherlands, 2003 (adapted by Jongbloed).

[2] Buckley WT. Effect of selenium supplementation on copper metabolism in dairy cows. Canadian Journal of Animal Science 1986; 66: 1009-1018.

[3] Buckley WT. A kinetic model of copper metabolism in lactating dairy cows. Canadian Journal of Animal Science 1991; 71: 155-166.

[4] Chase CR, Beede DK, Van Horn HH, Shearer JK, Wilcox CJ, Donovan GA. Responses of lactating dairy cows to copper source, supplementation rate, and dietary antagonist (iron). Journal of Dairy Science 2000; 83: 1845-1852.

[5] Clawson WJ, Lesperance AL, Bohman VR, Layhee DC. Interrelationship of dietary molybdenum and copper on growth and tissue composition of cattle. Journal of Animal Science 1972; 34: 516-520.

[6] Du Z, Hemken RW, Harmon RJ. Copper metabolism of Holstein and Jersey cows and heifers fed diets high in cupric sulfate or copper proteinate. Journal of Dairy Science 1996; 79: 1873-1880.

[7] Engel RW, Hardison WA, Miller RF, Price NO, Huber JT. Effect of copper intake on concentration in body tissue and on growth, reproduction and production in dairy cattle. Journal of Animal Science 1964; 23: 1160-1163.

[7b] Engle TE, Fellner V, Spears JW. Copper status, serum cholesterol, and milk fatty acid profile in Holstein cows fed varying concentrations of copper. Journal of Dairy Science 2001; 84: 2308-2313.

[8] Gengelbach GP, Ward JD, Spears JW. Effect of dietary copper, iron, and molybdenum on growth and copper status of beef cows and calves. Journal of Animal Science 1994; 72: 2722-2727.

[9] Gooneratne SR, Buckley WT, Christensen DA. Review of copper deficiency and metabolism in ruminants. Canadian Journal of Animal Science 1989; 69: 819-845.

[10] Grift Jvd. Het kopergehalte van lever en bloedserum bij het Fries-Hollandse rund. Verslag Landbouwkundig Onderzoek 1955; 61: 10.

[11] Haaranen S. Some observations on the zinc requirement of cattle for the prevention of itch and hair licking at different calcium levels in the feed. Nordisk Veterinaermedicin 1963; 15: 536-542.

[12] Hartmans J. Copper deficiency in dairy cattle under field conditions. Agri Digest 1969; 18: 42-48.

[13] Hartmans J. Hoe chronische kopervergiftiging bij schapen te voorkomen? Tijdschrift voor Diergeneeskunde 1975; 100: 405-407.

[14] Havre GN, Dynna O, Ender F. The occurrence of conditioned and simple copper deficiency in cattle and sheep in Setesdalen, a valley in the southern part of Norway. Acta Veterinaria Scandinavica 1960; 1: 250-276.

[15] Ivan M, Proulx JG, Morales R, Codagone HCV, Dayrel MS. Copper accumulation in the liver of sheep and cattle fed diets supplemented with copper sulfate or copper chloride. Canadian Journal of Animal Science 1990; 70: 727-730.

[16] Jongbloed AW, Top AMvd, Beynen AC, Klis JDvd, Kemme PA, Valk H. Consequences of newly proposed maximum contents of copper and zinc in diets for cattle, pigs and poultry on animal performance and health. Report ID-Lelystad 2001; 2097.

[17] Kirchgessner M, Grassmann E. The dynamics of copper absorption. In: Mills CF (ed.), Trace element metabolism in animals. Proceedings of WAAP/IBP International Symposium, Aberdeen, Schotland. Edinburgh: E.&S. Livingstone; 1970: 277-285.

[18] Kirchgessner M, Schwarz FJ, Roth HP, Schwarz WA. [Interaction among the trace elements zinc, copper and iron after depletion and repletion of dairy cows with zinc]. Archiv für Tierernährung 1978; 28: 723-733.

[19] Kirchgessner M, Weigand E. [Optimal zinc requirement of lactating dairy cows based on various dose-response relationships]. Archiv für Tierernährung 1982; 32: 569-578.

[20] Koopman JJ, Wijbenga A. Een vergelijkend onderzoek naar het effect van injiceerbare koperpreparaten bij runderen met een verlaagd bloedserumkopergehalte. Tijdschrift voor Diergeneeskunde 1977; 102: 1359-1365.

[21] Miller JK, Miller WJ. Development of zinc deficiency in Holstein calves fed a purified diet. Journal of Dairy Science 1960; 43: 1854-1856.

[22] Miller WJ. Absorption, tissue distribution, endogenous excretion, and homeostatic control of zinc in ruminants. American Journal of Clinical Nutrition 1969; 22: 1323-1331.

[23] Neathery MW, Miller WJ, Blackmon DM, Gentry RP. Performance and milk zinc from low-zinc intake in Holstein cows. Journal of Dairy Science 1973; 56: 212-217.

[24] Oude Voshaar JH. Statistiek voor onderzoekers. Wageningen: Wageningen Pers; 1994.

[25] Payne RW, Lane PW, Digby PGN, Harding SA, Leech PK, Morgan GW, Todd AD, Thompson R, Tunnicliffe Wilson G, Welham SJ, White RP. Genstat 5. Release 3. Reference Manual. Clarendon Press, Oxford, UK: 1993.

[26] Schie Wvd, Assem GHv, Berg Rvd. Breed difference in sheep with respect to the interaction between zinc and the accumulation of copper in the liver. Veterinary Quarterly 1983; 5: 21-26.

[27] Schwarz FJ, Kirchgessner M. [Copper and zinc contents in milk and plasma of cows after high nutritional copper supplements].Kupfer- und Zinkgehalte in der Milch und im Plasma von Kühen nach hoher nutritiver Kupferdosierung. Zeitschrift für Lebensmittel Untersuchung und Forschung 1978; 166: 5-8.

[28] Schwarz WA, Kirchgessner M. [Excretion of zinc in lactating cows receiving various supply of zinc]. Archiv für Tierernährung 1975; 25: 597-608.

[29] Schwarz WA, Kirchgessner M. Zinc metabolism in animals. 14. Changes in zinc content of cow's milk with different zinc supplies
Veränderungen des Zinkgehaltes in der Kuhmilch bei unterschiedlicher Zinkversorgung. 14. Zum Stoffwechsel des Zinks im tierischen Organismus. Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde 1975; 35: 1-8.

[30] Schwarz WA, Kirchgessner M. Zinc metabolism in animals. 15. [Zinc status in bone of dairy cows (Tuber coxae) in zinc depletion and repletion]. Zum Zinkstatus im Knochen von Milchkühen (Tuber coxae) bei Zink-Depletion und -Repletion. 15. Zum Stoffwechsel des Zinks im tierischen Organismus. Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde 1975; 35: 9-17.

[31] Stoszek MJ, Mika PG, Oldfield JE, Weswig PH. Influence of copper supplementation on blood and liver copper in cattle fed tall fescue or quackgrass. Journal of Animal Science 1986; 62: 263.

[32] Suttle NF. Safety and effectiveness of cupric oxide particles for increasing liver copper stores in cattle. Research in Veterinary Science 1987; 42: 224-227.

[33] Suttle NF. The interactions between copper, molybdenum, and sulphur in ruminant nutrition. Annual Reviews of Nutrition 1991; 11:121-40: 121-140.

[34] Suttle NF, Abrahams P, Thornton I. The role of a soil x dietary sulphur interaction in the impairment of copper absorption by ingested soil in sheep. Journal of Agricultural Science, UK 1994; 103: 81-86.

[35] Suttle NF, Peter DW. Rumen sulphide metabolism as a major determinant of copper availability in the diets of sheep. Trace element metabolism in man and animals 1985; 5: 367-370.

[36] Todd JR. Chronic copper toxicity of ruminants. Proceedings of the Nutrition Society 1969; 28: 189-198.

[37] Voelker HH, Jorgensen NA, Mohanty GP, Owens M. Zinc supplements. South Dakota Farm and Home Research 1970; 21: 18-20.
[38] Voelker HH, Jorgensen NA, Mohanty GP, Owens MJ. Effects of zinc supplementation to dairy cattle rations. Journal of Dairy Science 1969; 52: 929-930.
[39] Weigand E, Kirchgessner M. Factorial estimation of the zinc requirement of lactating dairy cows. Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde 1982; 47: 1-9.
[40] Xin Z, Waterman DF, Hemken RW, Harmon RJ. Copper status and requirement during the dry period and early lactation in multiparous Holstein cows. Journal of Dairy Science 1993; 76: 2711-2716.

2.7. Appendices dairy cattle

Appendix 2.1. References as used by A(F)RC, NRC, DLG, COMV and INRA and their reasons of rejection for this study

Reference	Element	Part rejected	Reason of rejection
[1;9;17;33]	Cu	Whole reference	Review, no experimental data given
[22]	Zn	Whole reference	Review only; mainly calves
[5;21;31]	Cu [5] Zn [21] Cu [31]	Whole experiment	Non-lactating, growing cattle
[2]	Cu	Whole experiment	Experimental diets only differed in Se content, not in Cu content
[3]	Cu	Whole experiment	Only one group, no control group included
[18;19;28-30]	**Zn**	Whole experiment	Only 5 animals used, each with a different treatment
[12;14]	Cu	Whole reference	Field study only, not a controlled experiment
[11]	Zn	Whole reference	Field study only, no data on milk yield or composition
[27]	Cu	Whole reference	Dietary Cu concentrations exceed 50 mg/kg (DM)
[13;26;34;35]	Cu	Whole reference	Sheep used as experimental animals
[32]	Cu	Whole reference	Steers used as experimental animals
[39]	Zn	Whole reference	Review on factorial estimation of Zn requirements
[10]	Cu	Whole reference	Journal not available
[20]	Cu	Whole reference	Injections of Cu, not a nutritional experiment
[36]	Cu	Whole reference	Focusing on Cu toxicity rather than on Cu requirements

Appendix 2.2. Survey of experiments to assess Cu requirements for dairy cattle

Refer-ence	Basal Cu	Added Cu	Total Cu	Milk yield	Fat+protein	Liver Cu	"Conclusion"total Cu level (mg/kg DM)
	mg/kg(DM)			(kg/d)	(g/d)	mg/kg(DM)	
[4]a	8	0	8	22.7	1553	660	<20
		12	20	20.2	1511	581	
		31	39	16.1	1024	614	
[4]b	9 (high Fe)	0	9	20.3	1401	289	>23
		14	23	20.9	1417	395	
		25	34	23.5	1528	612	
[4]c	8	0	8	22.7	1553	660	<25
		17	25	24.7	1643	644	
		43	51	19.9	1421	566	
[4]d	9 (high Fe)	0	9	20.3	1401	289	~25
		16	25	21.1	1511	566	
		37	46	19.1	1315	655	
[6]a	6	5	11	-	-	167	>11
		80	86	-	-	439	
[6]b	6	5	11	-	-	172	
		80	86	-	-	520	
[15]a	6	0	6	-	-	22	>6
		14	20	-	-	127	
[15]b	4	2	6	-	-	24	
		16	20	-	-	260	
[40]	9	0	9	32.9	-	233	~19
		10	19	33.2	-	322	
		20	29	33.1	-	417	
[7b]	9	0	9	40.0	2640	372	49 is marginally toxic
		10	19	36.7	2386	430	
		40	49	40.3	2620	826	
						blood Cu (μM/L)	
[7b]	9	0	9			16.8	9
		10	19			16.5	
		40	49			15.9	
[7]	5	0	5			7.2	>5
		15	20			16.1	
[8]	14	0	14			12.8	>14
		10	24			15.6	

3. Copper and zinc requirements of growing pigs

3.1. Introduction

3.1.1. Procedure

In this chapter, references concerning dietary Cu and Zn requirements as quoted by A(F)RC and NRC, as well as recent reports from scientific literature are listed and evaluated. No suitable information concerning scientific references as used by DLG/GfE and INRA could be obtained.

3.1.2. Premises, suppositions and calculations

Phytate concentrations in the diets were estimated using the concentrations of phytate P as given for the raw materials in the Dutch Feedstuff Table [8] and are presented as phytate P. When analysed phytate values were given, phytate concentrations were multiplied by 0.282 to obtain phytate P content as based on molar ratios.

Vegetable phytase activity (U/kg) of raw materials was supposed to be 500 U for barley, 1000 U for wheat, and 5000 U for wheat middlings. These activities were multiplied by 35, 80, and 35%, respectively, to obtain comparable figures with that of microbial phytase activity (Kemme, personal communication). So, all phytase activities were expressed as U of microbial phytase activity. From the few references it was not clear whether the diets had been (steam)pelleted or not, so it was assumed that no inactivation of phytase had taken place during feed manufacture.

As in most cases, only few data concerning mineral and phytate concentrations of the diets were given, lacking values were calculated for all diets based on the raw material composition, using a linear programming package (OPTISAM). Furthermore, all hepatic Cu and Zn concentrations were calculated on a DM basis. When values were expressed as mg/kg fresh tissue, DM content of pig liver was supposed to be 27.8% [13]. Zinc content of the pancreas was calculated as mg/g protein. If values were expressed as mg/g fat-free DM, values were recalculated supposing a non-protein DM content of the pancreas of 10% (Jongbloed, pers. comm.). If feed intake of piglets was not given, feed intake was assumed to be 4.5% of their BW (Jongbloed, pers. comm.).

3.1.3. Rejected references

Not all references were suitable for this study. Several reports as used by A(F)RC and NRC had to be rejected for the scope of this study. The reasons of rejection are listed in Appendix 3.1. Besides the references used by AFRC and NRC, other publications were used to assess Cu and Zn requirements. Unsuitable parts of these references, as well as the reasons of their rejection, are listed in Table 3.1.

Table 3.1. Parts of other references not suitable for this study

Reference	Element	Part rejected	Reason of rejection
[1]	Zn	21-day growth experiment	Shortage of digestible P (2.6 g/kg)
[25]	Zn	Experiment 1	Shortage of digestible P (0.9 g/kg)
[16;23;25]	Zn	Blood Zn values	Blood Zn values were up to 10 times the normal values for plasma and were, therefore, considered insufficiently reliable to be used for statistical analysis

Finally, among the remaining reports several results had to be adapted to be suitable for statistical analysis. These references, and the adaptations made, are listed in Table 3.2.

Table 3.2. References that had to be adapted prior to statistical analysis of pig data

Reference	Element	Way of adaptation
[6]	Zn	In this experiment, a Zn deficient group (fed *ad libitum*), a group pair-fed an adequate Zn diet and a group fed an adequate Zn diet *ad libitum* were used. These groups were considered to be three different treatments
[14]	Cu	No separate results of the growing phase were presented; only combined results for the growing and finishing phase were given. Therefore, Cu content of the feed used during the whole experiment was assumed to be 1/3 of the Cu content of the growing feed and 2/3 of that of the finishing feed; only the feed composition of the finishing feed was used
[31]	Zn	Besides a control group (low Zn), two sources of additional Zn were investigated, both using two levels of additional Zn. As the results of these Zn additions were significantly different, the two sources were considered to be separate experiments. In both experiments, the control group was included.
[40]	Zn	As the finishing feed was responsible for the majority of the final result, only the composition of this feed was used. Besides a control group (low Zn), two sources of additional Zn were used, both using different levels of additional Zn. As the results of these Zn additions were significantly different, the two sources were considered to be separate experiments. In both experiments the control group was included.
[45]	Cu	For analysis of the 3rd experiment, BW as based on age was assessed; feed intake was supposed to be 4.5% of BW; dietary Cu concentrations on a 90% DM basis were assessed supposing the liquid feed contained 13% DM
[49]	Zn	Growing and finishing phase were considered to be separate experiments

3.2. Available literature

The nine references suitable for evaluation of Cu requirements are listed in Table 3.3, and the 29 references suitable for evaluation of Zn requirements are listed in Table 3.4. For calculation of the average animal performance, performance data are excluded for groups of animals that showed to be severe deficient in Cu or Zn.

Table 3.3. Overview of the experiments used for evaluation of Cu requirements of pigs (D= duration of experiment; FCR=feed conversion ratio; Cp = ceruloplasmin; Hb = hemoglobin; SOD = superoxide dismutase)

Refer-ence	Age (d) or weight (kg) at start	D (d)	Growth (g/d)	FCR	Response criteria	Type of diet[A]	Basal Cu	Added Cu
							(mg/kg; fresh)	
[11]	4 d	17	-	-	serum Cu and Cp, liver Cu	milk		0, 20
[45]	9 d	98	-	-	Hb, blood Cu	milk	0.6	0, 2 mg/pig/d (= ± 22 mg/kg)
[14]	34 kg	119	-	-	performance, Hb, serum Cu and Cp, liver Cu	S	0	1.5, 4.5
[18]	10 d	35	406	-	performance, Hb, serum Cu and Cp, liver and kidney Cu	S	0.7	0, 4.2, 9,3
[35]	11 d	28 – 65	275	-	performance, plasma Cu and Cp, liver and kidney Cu	S	0.6, 0.9, 1.3	0, 1.1, 1.3, 1.9, 2.2, 3.1, 4.0, 4.3, 8.0
[52]	28 kg	28	-	-	serum Cu	S	4.8	0, 5.2
[15]	9 kg	63	552	2.20	performance, Hb, serum Cu and Cp, liver and kidney Cu	CS	7	0, 18
[13]	70 d	112	630	3.20	performance, liver Cu	O	7	0, 32
[24]	25 kg	78	966	2.19	performance, Hb, blood SOD, liver Cu	O	15	0, 35

[A] S = synthetic or semi-synthetic diet; CS = corn-soybean meal diet; O = other mixed diets

3.3. Statistical analysis

The data were subjected to multiple regression analysis. First, possible models were selected based on Mallow's C_p. Then, these selected models were subsequently analysed by forward, backward and stepwise selection of variables. The statistical package Genstat 5, release 3.1 [38] was used throughout.

Only physiologically relevant explanatory parameters were included in the statistical analysis. For Cu, these were dietary Cu, Ca and Zn, and phytate P concentrations, whereas phytase activities were excluded from the analysis. For Zn, these were dietary Zn, Ca, Cu, Fe, and phytate P concentrations, as well as dietary phytase activities.

Both total and added dietary Cu or Zn concentrations were included in the statistical analysis throughout, to disclose possible differences between the effects of intrinsic and extrinsic minerals in the total ration.

Suitable models should at least comply with the following conditions:

Dietary Cu or Zn content is an explanatory parameter in the model
R^2 adjusted is > 70.0%
C_p is < p + 3 (p = number of explaining parameters in the model) [37]
The number of observations is > 4
The coefficient is significant ($P < 0.05$).

If models, based on these conditions, seemed to be appropriate, data were presented in a graph. Then, the best parameters (showing a visible correlation in the graph) were subjected to broken line analysis to obtain a breakpoint value. This breakpoint discriminates between the first phase of the graph, in which the response parameter increases with each increase in dietary Cu or Zn concentrations, and the second phase, a non-responsive plateau of response parameter values. This breakpoint, therefore, suggests the attainment of the dietary Cu or Zn requirement.

Table 3.4. Overview of the experiments used for evaluation of Zn requirements of pigs (D= duration of experiment; GR=growth rate; FCR=feed conversion ratio; P = performance; AP = alkaline phosphatase)

Reference	Age (d) or weight (kg) at start	D (d)	GR (g/d)	FCR	Response criteria	Type of diet[A]	Basal	Added Zn
							(mg/kg; fresh)	
[1]	31 d	21	412	2.21	Performance, plasma Zn	CS	27.5	0, 100
[2]	16 kg	91, 119	450	2.98	Performance	O	51	0, 100
[3]	14 d	42	304	-	Performance	S	0	0, 90
[4]	21 kg	?	737	2.89	Performance	CS	29	0, 71
[5]	77 d	84	569	-	P, plasma Zn, liver Zn	CS		0, 100
[6]	35 d	42	357	4.48	P, serum Zn and AP, liver, pancreas and kidney Zn	S	7.3	0, 120
[9]	21 d	63	506	2.25	Performance	S	28	0, 100
[12]	14 d	28	320	1.15	P, bone Zn, plasma Zn, Zn digestibility	S	6 (5)	5, 10, 15, 20, 25, 30, 35, 115
[16]	22 kg	68	500	2.90	Performance, blood Zn	O	33, 38	0, 12, 17, 67
[20]	49 d	91	-	-	Performance, serum AP	CS	28.1, 34.5 35.7, 38.7	0, 50, 75
[21]	21 d	49	399	2.12	Performance	S	16	0, 50
[23]	35 kg	34	-	-	Plasma Zn	O	37	0, 26, 55, 113
[25]	28 d	28	541	1.91	Performance, plasma Zn, serum AP	CS	25	0, 22
[26]	weanling	84	544	-	Performance, liver Zn	CS	22	0, 100
[27]	weanling	84	500	-	P, bone Zn, serum Zn, liver and pancreas Zn	CS	25	0, 100
[28]	weanling	84	331	-	Performance	CS	35	0, 50
[29]	15 kg	112	700	3.36	Performance, serum Zn and AP	CS	22.4, 28.7	0, 19.4, 41.1
[30]	42 d	56	524	2.94	Performance	CS	29/31	0, 20
[31]		35	459	1.73	Performance, serum Zn	S	20	0, 25, 50
[32]	7 d	28	282	1.43	P, serum Zn and AP, liver and pancreas Zn	S	12	0, 87.6
[34]	16 kg	70	586	2.68	P, serum AP, liver Zn	CS	28	0, 44, 49
[39]	25 kg	50	-	-	Plasma Zn, serum AP	O	37	0, 26, 55, 113
[40]	weanling	105	767	3.19	P, bone Zn, serum Zn and AP	O	31-46	0, 43.8, 84.4
[41]	weanling	84, 126	621	3.67	Performance	O	28, 29, 31	0, 100
[42]	5 kg 18 kg	88 28	345 721	2.48 2.23	Performance	CS	37.8	60.5
[43]	58-67	42	555	-	Performance, serum AP	O	32	0,12, 48
[49]	63 d	84, 98	787	2.64	P, plasma Zn, bone Zn	CS	32, 27	0, 7.5, 10, 15, 20, 40, 80
[50]	10 d	18	63	2.00	Performance, serum Zn	S	12	0, 88
[51]	28 d	28	342	-	Performance	O	34	0, 106

[A] S = synthetic or semi-synthetic diet; CS = corn-soybean meal diet; O = other mixed diets

3.4. Results and discussion

3.4.1. Copper

3.4.1.1. Dietary total Cu requirements

Selected results of the regression analysis on Cu are given in Table 3.5. More data are presented in Appendix 3.2. Unfortunately, suitable evidence for a reliable relationship is scarce and results should, therefore, be regarded with caution. Furthermore, analytical difficulties and differences between experiments may be responsible for the inhomogeneous results. Finally, taking into account the relatively small number of experiments, large differences in initial BW of the pigs and length of the experiments, and possible differences in Cu content in the tissues before the start of the experiment, it is impossible to discriminate further between age of the pigs or types of diet (Table 3.3).

Table 3.5. Selected results of regression analysis of Cu data (Number = number of observations (total number = 13); C_p = Mallow's C_p [37]; Hb = hemoglobin concentration; serum Cp = serum ceruloplasmin concentration; δ = difference as compared with basal level)

Response parameter	Number	Cu (mg/kg)	Ca (g/kg)	Zn (mg/kg)	Phytate P (g/kg)	$R^2_{adjusted}$	C_p
Feed intake	5	13.00	12.10	-	-10.83	98.2	4.00
Hb	6	8.16	9.23	-9.29	11.16	99.0	5.00
Serum Cu	11	9.28	0.40	-0.79	-0.74	95.6	5.00
Serum Cp	8	10.78	-	-	-	94.3	2.06
Kidney Cu	6	6.89	-	-	-5.53	98.6	2.01
δ growth	7	24.58	-20.09	-21.63	-24.73	99.3	5.00
δ Hb	6	3.23	5.66	-	-6.42	96.2	3.51
δ liver Cu	10	10.43	-2.59	-1.36	-8.56	95.9	5.00
δ serum Cp	8	10.39	-14.77	-	-25.31	99.1	3.38

The results of the experiments were also judged according to the response criteria as given by Jongbloed et al. [22]. As data on Cu absorption, superoxide dismutase activity and hepatic ceruloplasmin content were lacking in all experiments, only hepatic Cu content and animal performance data (i.e. feed intake and δ growth; Table 3.5) could be used. Unfortunately, graphic presentation of these data did not reveal any useful relationship. For example, the relationship between dietary and hepatic Cu concentrations is presented in Figure 3.1. The three values around a liver Cu content of 60 mg/kg, and that one at 1 mg Cu/kg diet are from milk-fed baby piglets [11;18]. Furthermore, one value representing a dietary Cu content of 20 mg/kg and a Cu liver content of 893 mg/kg was omitted, because this value came also from milk-fed piglets [11]. The other response parameters classified as suitable indicators of the accuracy of dietary Cu supply showed similar graphs.

Although Jongbloed et al. [22] characterized serum/plasma Cu concentration to be less suitable for the assessment of Cu requirements (it is only valuable within a very limited range of Cu supply), serum Cu concentration was the only response parameter showing a visible correlation with the dietary Cu content. Data are presented in Figure 3.2. Broken line analysis supplied the following model to describe the relation between dietary Cu and serum Cu concentrations:

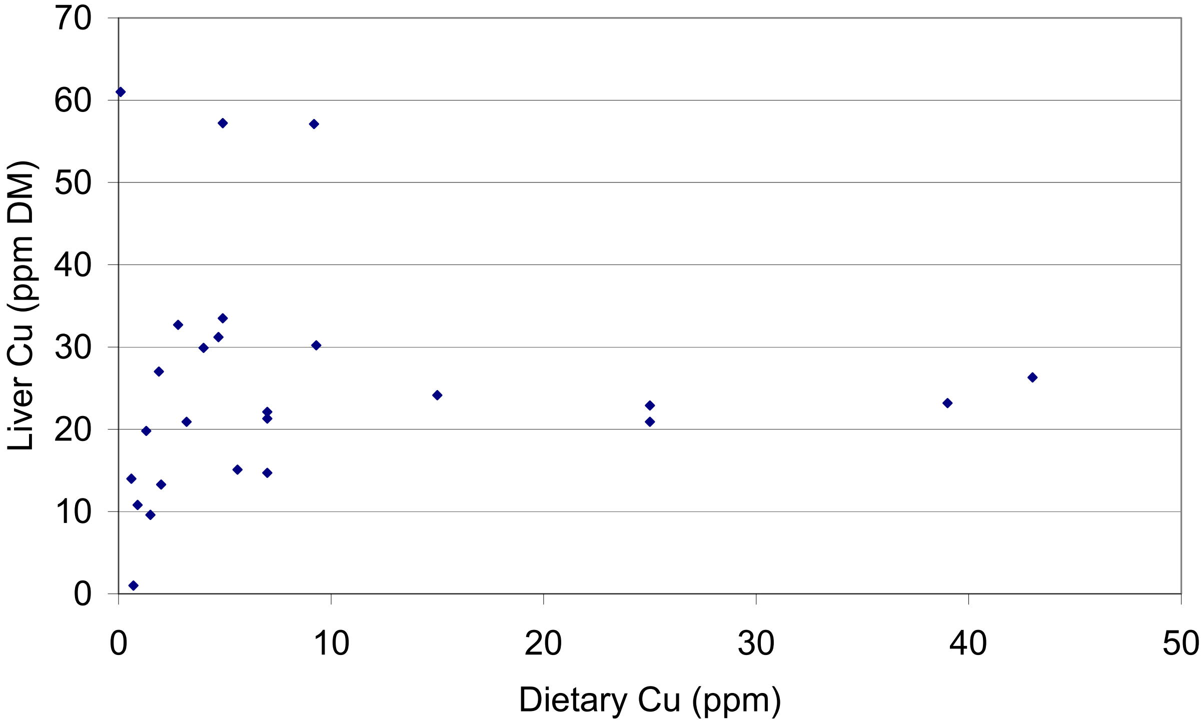


Figure 3.1. Relationship between dietary and liver Cu concentrations in growing pigs

Serum Cu (µM) = 32.99 – 8.69 x ln (1 + exp $^{(3.958 - \text{dietary Cu (mg/kg)})}$); R^2 adj. = 83.0%; RSD = 5.29

The value of 32.99 (± 1.51) is the level of the non-responsive plateau in the second phase of the graph, 8.69 (± 2.10) is the slope of the line in the first phase, and 3.958 (± 0.793) is the breakpoint value. A concentration of 4.0 mg/kg can, therefore, be assessed as the dietary total Cu requirement of growing pigs.

Among the suitable experiments there is only one recent report [24] using fast-growing pigs (± 950 g/d), while in another experiment [13] on growing-finishing pigs growth rate and FCR were substantially worse than in practice today in The Netherlands. In piglets, growth rates were between 275 and 550 g/day, which is more or less comparable with current practice. Therefore, it is not clear as to what extent the assessed requirement also applies to fast growing-finishing pigs.

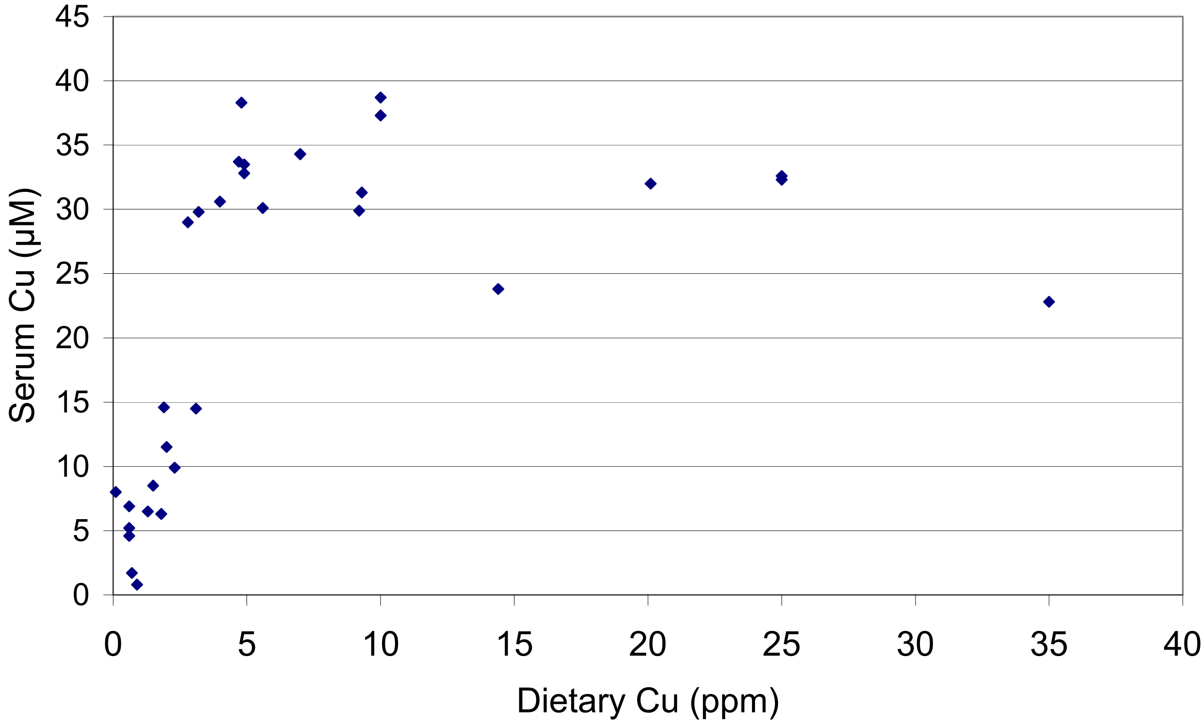


Figure 3.2. Relationship between dietary and serum Cu concentrations of references used for the assessment of dietary Cu requirements in growing pigs

3.4.1.2. Differences between intrinsic and added Cu

Due to the scarcity and inhomogeneity of data it is difficult to assess as to what extent differences exist between diets containing approximately the same amount of total Cu, but of different origin (intrinsic vs. added). An attempt to compare some similar data (all data on diets containing a total Cu concentration of 5-10 mg/kg) is made in Table 3.6.

Table 3.6. Comparison of results obtained on rations with similar total dietary Cu concentrations (5-10 mg/kg) from different origin (intrinsic vs. added)

Reference	Basal Cu (mg/kg)	Added Cu (mg/kg)	Total Cu (mg/kg)	Duration experiment (d)	Serum Cu (µM)	Liver Cu (mg/kg; DM))
[13]	7	0	7	112	-	15
[15]	7	0	7	63	34.3	22.1
[15]	7	0	7	63	34.3	21.3
[35]	1.3	4.3	5.6	56	30.1	15.1
[35]	1.3	8	9.3	56	31.3	30.2
[18]	0.7	9.3	10	35	29.9	57.1

The first three diets in Table 3.6 contain only intrinsic Cu, whereas the second three contain mainly added Cu. Serum Cu concentrations are hardly different, while liver Cu concentrations seem to be rather correlated with total Cu concentrations than with basal or added Cu. Observing no differences is not surprising, as total Cu content already exceeds 5 mg/kg. Therefore, this comparison does not supply convincing evidence for different metabolic fates of intrinsic vs. added dietary Cu.

3.4.2. Zinc

3.4.2.1. Dietary total Zn requirements

For Zn, statistical analysis revealed a significant correlation between the differences in dietary Zn content and differences in bone Zn concentrations (n = 6; $R^2_{adjusted}$ = 81.6%; C_p = 1.10; coefficient for δ Zn = 4.04). However, graphic presentation of the data (Figure 3.3) showed only a poor relationship. It shows a large effect of the experiment on the increase of bone Zn content. Another graph (not shown) was constructed taking into account the number of days pigs were in the experiment. It showed that the increase of bone Zn content per day by [12] were substantially higher than for the other experiments. For the other parameters analysed, no significant correlation was detected.

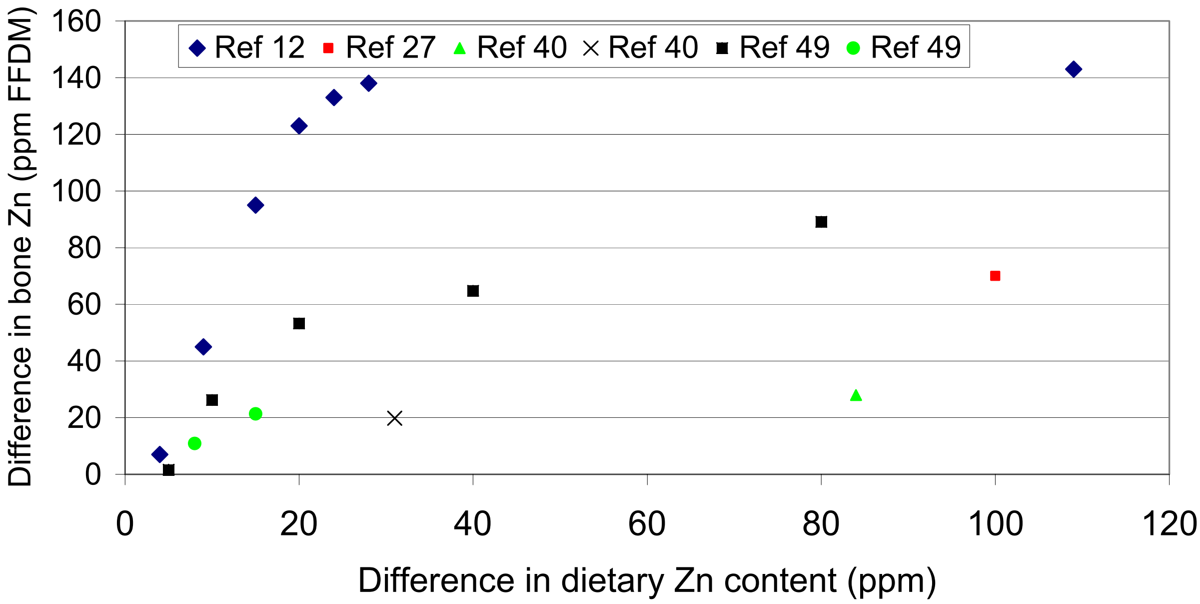


Figure 3.3. Relationship within some studies between differences in dietary Zn (δ dietary Zn) and differences in bone Zn concentrations (δ bone Zn); δ = compared with unsupplemented basal diet

Although the number of suitable experiments for Zn is much larger (54) than for Cu (13), evidence is inhomogeneous and partially old. More selected results of statistical analysis are given in Appendix 3.3.

As statistical analysis and graphic presentation of the data did not show a suitable indicator, Zn requirements had to be assessed by comparison of the results of the separate experiments. In several experiments using very low dietary Zn concentrations, it could be observed that performance and plasma and bone Zn concentrations improved after Zn addition. In some of these experiments also the occurrence of a Zn-responsive parakeratosis in the low Zn groups was reported. On the other hand, based on deteriorating performance and blood Zn values associated with higher dietary Zn concentrations, also upper limits of dietary Zn requirements were detected. Finally, some authors could not reveal significant differences in performance within a certain range of dietary Zn concentrations. From two references [16] and [25] it was shown that dietary Zn concentrations should not exceed 94 to 100 mg/kg, because performance was less than at the lower level used.

For all experiments listed in Table 3.4, we made a review of the total Zn content at which numeric level the best growth performance was obtained, without taking into account significant differences

(Table 3.7). Per experiment per treatment an overall production parameter was calculated, which was done as follows: the growth rate and feed intake of pigs receiving the diet without supplementary Zn was put on 100, and relative values for other supplementary levels were calculated and averaged for growth rate and feed intake. Furthermore, we ranked according to type of diets.

Table 3.7. Overview of maximal performance in the experiments used for evaluation of Zn requirements of pigs (D= duration of experiment; GR=growth rate; FCR=feed conversion ratio; see also text)

Refe-rence	Age (d) or weight (kg) at start	D (d)	GR (g/d)	FCR	Type of diet[A]	Basal Zn	Added Zn	Total Zn content at best growth performance [B]
						mg/kg; fresh		
[3]	14 d	42	304	-	S	0	0, 90	90
[6]	35 d	42	357	4.48	S	7	0, 120	127
[9]	21 d	63	506	2.25	S	28	0, 100	128, 128
[21]	21 d	49	399	2.12	S	16	0, 50	66
[32]	7 d	28	282	1.43	S	12	0, 88	100, 100, 100
[50]	10 d	18	63	2.00	S	12	0, 88	100
[31]		35	459	1.73	S	20	0, 25, 50	70
[12]	14 d	28	320	1.15	S	6 (5)	5, 10, 15, 20, 25, 30, 35, 115	35
[1]	31 d	21	412	2.21	CS	28	0, 100	28
[4]	21 kg	?	737	2.89	CS	29	0, 71	100, 100
[5]	77 d	84	569	-	CS		0, 100	30
[26]	weanling	84	544	-	CS	22	0, 100	122
[27]	weanling	84	500	-	CS	25	0, 100	125
[28]	weanling	84	331	-	CS	35	0, 50	85
[30]	42 d	56	524	2.94	CS	31	0, 20	31, 51
[42]	5 kg 18 kg	88 28	345 721	2.48 2.23	CS	38	60	98, 98
[20]	49 d	91	-	-	CS	28-39	0, 50, 75	78, 86, 114, 110
[25]	28 d	28	541	1.91	CS	25	0, 22, 69	47
[29]	15 kg	112	700	3.36	CS	22, 29	0, 19.4, 41.1	80, 48
[34]	16 kg	70	586	2.68	CS	28	0, 44, 49	77, 28
[49]	63 d	84, 98	787	2.64	CS	32, 27	0, 7.5, 10, 15, 20, 40, 80	37, 32, 40, 35
[2]	16 kg	91, 119	450	2.98	O	51	0, 100	151, 51, 151
[41]	weanling	84, 126	621	3.67	O	28, 29, 31	0, 100	120, 122, 122
[51]	28 d	28	342	-	O	34	0, 106	34, 140
[16]	22 kg	68	500	2.90	O	33, 38	0, 12, 17, 67	50, 100
[39]	25 kg	50	-	-	O	37	0, 30, 60, 120	37 not enough
[40]	weanling	105	767	3.19	O	31-46	0, 43.8, 84.4	120
[43]	58-67	42	555	-	O	32	0,12, 48	44, 80, 80

[A] S = synthetic or semi-synthetic diet; CS = corn-soybean meal diet; O = other mixed diets

[B] two or more values represent the results of more experiments

Of the synthetic diets it is clear that supplementation with Zn results in better growth performance, as well as for the other type of diets. In all but two synthetic diets only one supplementary level of Zn was used, which cannot be used for a proper evaluation on the level for assessing optimal performance. In the two experiments [31] and [12] total Zn content for maximal performance was 70 and 35 mg/kg, respectively. Only five authors used experiments with maize-soybean meal diets with more than two supplementary levels of Zn. For each separate author the mean level of Zn was calculated if more than one experiment was done. It shows that there is a large variation in Zn levels for maximal growth performance (Table 3.7). It revealed that in the maize-soybean meal diets maximal performance was achieved on average at 59 mg/kg. For the other mixed diets (three authors) this value was 88 mg/kg, which is substantially higher than for the maize-soybean meal diets. It may be remarked that the values are slightly overestimated, because the maximal performance might have been achieved at a level between this and the lower supplementation level. Therefore, we subtracted for each type of diet, the mean of the difference between two supplementary levels of Zn from the level we obtained for maximal growth performance. This resulted in (52 – 7 =) 45 mg/kg for the synthetic diets, (59 – 15 =) 45 mg/kg for the maize-soybean meal diet and (88 – 21 =) 67 mg/kg for the other mixed diets. It is surprising that the same Zn level for both the synthetic and maize-soybean meal diets were obtained. It may be speculated that the level for the maize-soybean meal diet is underestimated. On average for the maize-soybean meal diets and other mixed diets the mean level of Zn at which maximal performance was obtained was 56 mg/kg.

Concerning the growth performance, it can be remarked that for growing-finishing pigs average growth rate (g/d) and FCR were 628 ± 123 and 2.96 ± 0.28, respectively, while for weaned piglets these were 369 ± 153 and 2.43 ± 0.93, respectively. These performance data are 10 to 20% worse than in practice in The Netherlands today. For Zn, only 8 out of 52 experiments used pigs growing faster than 700 g/d. Applicability of the assessment of the dietary Zn requirement to currently fast-growing pigs is, therefore, questionable. However, the highest growth rate (882 g/d from 55 to 104 kg) and a feed conversion ratio of 3.12, reported relatively recently (1994), was attained at dietary Zn levels of 36 mg/kg [49] in a maize-soybean meal diet.

3.4.2.2. Differences between intrinsic and added Zn

Similar to Cu, differences between dietary Zn from intrinsic or extrinsic origin cannot be excluded. As (differences in) bone Zn concentrations can be considered to be the most reliable response parameters, only this group of references was considered. An attempt was made to compose groups of references using similar total dietary Zn concentrations. Ranges of total dietary Zn concentrations (25-35 mg/kg and 35-45 mg/kg) were chosen arbitrarily. As dietary total Zn concentrations are similar, the differences originate from the partition between intrinsic and added Zn. However, also the length of the experimental period and the possible differences in Zn content of the tissues when the experiment started may play a part in the differences obtained (Table 3.4). The results are presented in Table 3.8.

Table 3.8. Comparison of results obtained on rations with similar total dietary Zn concentrations (25-35 mg/kg and 35-45 mg/kg, respectively) from different origin (intrinsic vs. added); δ = difference as compared with the basal level

Reference	Basal Zn (mg/kg)	Added Zn (mg/kg)	Total Zn (mg/kg)	Bone Zn (mg/kg in fat-free DM)	δ bone Zn mg/kg (in fat-free DM)
[12]	6.6	19.6	26.2	160	123
	6.6	28.1	34.7	175	138
[27]	25	0	25	64	-
[49] expt. 1	27	0	27	107.1	-
expt. 1	27	5	32	108.7	1.6
expt. 2	27	0	27	66.9	-
expt. 2	27	7.5	34.5	77.8	10.9
[12]	6.6	30.1	36.7	170	133
[40]	38.5	0	38.5	83.8	-
[49] expt. 1	27	10	37	133.3	26.2
expt. 2	27	15	42	88.2	21.3

In the first group (25 – 35 mg/kg Zn) the bone Zn concentrations of [12] are much higher than of [27] and [49]. In the second group (37 – 42 mg/kg Zn) also bone Zn content of [12] is higher than of [40] and [49]. This implies that intrinsic Zn has a lower Zn availability than added Zn. Based on these data it is impossible to quantify intrinsic Zn compared with an amount of supplementary Zn.

3.5. Conclusion

3.5.1. Copper

Based on serum Cu concentrations, dietary total Cu requirement in synthetic diets should be around 4 mg/kg. No differences could be observed between effects of either intrinsic or added dietary Cu. In practice this may mean that for practical diets, which have an intrinsic Cu content of 6 to 10 mg/kg, an addition of 4 mg/kg Cu will be sufficient. Furthermore, no differences between pigs depleted or fed normal Cu levels before the start of the experiment could be observed. Finally, the duration of the experiment had no visible influence on liver Cu concentrations.

3.5.2. Zinc

Based on the available data, it is clear that diets should be supplemented with a Zn source. Maximal growth performance for pigs receiving maize-soybean meal diets or other mixed diets (no synthetic diets) was achieved at 56 mg/kg, which may be slightly underestimated. The reason for this is that in the first place the Zn level in the maize-soybean meal diet seems to be underestimated. Secondly, the data presented in Table 3.4 show that growth performance of the pigs used in the experiments is ± 20% lower than current Dutch performance levels. Higher feed conversion ratios of pigs used in the experiments mean that feed consumption is higher at the same growth rate. Therefore, it should be kept in mind that at current - lower - feed consumption levels per kg live weight gain, dietary total Zn concentrations should be higher than the assessment made above. The assessed requirement may, therefore, have to be adapted to the current improved feed conversion ratios. Therefore, a concentration of 67 mg Zn/kg is recommended as was obtained for pigs receiving other diets than maize-soybean meal.

From the available data it was shown that intrinsic Zn has a lower availability than added dietary Zn. Finally, also the duration of the experiment had no visible effect on bone Zn concentrations.

3.6. Literature

[1] Adeola O, Lawrence BV, Sutton AL, Cline TR. Phytase-induced changes in mineral utilization in zinc-supplemented diets for pigs. Journal of Animal Science 1995; 73: 3384-3391.

[2] Babatunde GM. Optimum levels of zinc in the diets of pigs in the tropics as influenced by the addition of graded levels of lard. Journal of the Science of Food and Agriculture 1972; 23: 113-120.

[3] Beardsley DW, Forbes RM. Growth and chemical studies of zinc deficiency in the baby pig. Journal of Animal Science 1957; 16: 1038.

[4] Berry RK, Bell MC, Grainger RB, Buescher RG. Influence of dietary calcium and zinc on calcium-45, phosphorus-32 and zinc-65 metabolism in swine. Journal of Animal Science 1961; 20: 433-439.

[5] Bremner I. The relationship between the zinc status of pigs and the occurrence of copper- en Zn-binding proteins in liver. Journal of Animal Science 1976; 35: 245-252.

[6] Burch RE, Williams RV, Hahn HKJ, Jetton MM, Sullivan JF. Serum and tissue enzyme activity and trace-element content in response to zinc deficiency in the pig. Clinical Chemistry 1975; 21: 568-577.

[7] Carter JH, Miller RF, Brooks CC. The effect of copper and calcium levels on the performance of growing swine. Journal of Animal Science 1959; 18: 1502.

[8] Centraal Veevoeder Bureau. Veevoedertabel 1999. CVB-reeks 1999.

[9] Conrad JH, Beeson WM. Effect of calcium level and trace minerals on the response of young pigs to unidentified growth factors. Journal of Animal Science 1957; 16: 589-599.

[10] Crook NE, Clem RJ, Miller LK. An apoptosis-inhibiting baculovirus gene with a zinc finger-like motif. J. Virol. 1993; 67: 2168-2174.

[11] Gomez-Garcia GG, Matrone G. Copper metabolism in the early postnatal period of the piglet. Journal of Nutrition 1967; 92: 237-244.

[12] Hankins CC, Veum TL, Reeves PG. Zinc requirement of the baby pig when fed wet-autoclaved spray-dried egg albumen as the protein source. Journal of Nutrition 1985; 115: 1600-1612.

[13] Hansen V, Bresson S. Copper sulphate as a feed additive to bacon pigs. Acta Agriculturae Scandinavica A 1975; 25: 30-32.

[14] Hartmann S, Eder K, Kirchgessner M. Marginal copper and iron supply of pigs. 1. Influence on haematological parameters, enzymes and copper and iron concentrations in various tissues
Marginale Kupfer- und Eisenversorgung beim Schwein. 1. Einfluss auf hamatologische Parameter, Enzyme und Kupfer- und Eisenkonzentrationen in verschiedenen Geweben. Archives of Animal Nutrition 1994; 47: 91-106.

[15] Hedges JD, Kornegay ET. Interrelationship of dietary copper and iron as measured by blood parameters, tissue stores en feedlot performance of swine. Journal of Animal Science 1973; 37: 1147-1154.

[16] Heigener H, Michna A, Neumann H-J, Behrens H, Hamann K. Einfluß differenzierter Zinkgaben bei unterschiedlichem Ca/P-Verhältnis auf die Entwicklung wachsender Schweine. Landwirtschaftliche Forschung 1958; 11: 185-201.

[17] Hennig A, Kracht W, Anke M. Die Parakeratose des Schweines. 2. Mitteilung. Der Einfluß von Öl- und Cholesterinzulagen auf den Verlauf der Krankheit und den Zinkgehalt einiger Körperteile. Jahrbuch für Tierernährung und Futtermittelkunde 1965; 5: 286-296.

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

[19] Hoefer JA, Liptrap DO, Miller ER, Ullrey DE, Schmoll RJ. Bentonite additions to swine diets with varying levels of calcium and zinc. Journal of Animal Science 1968; 27: 1776.

[20] Hoefer JA, Miller ER, Ullrey DE, Ritche HD, Luecke RW. Interrelationships between calcium, zinc, iron and copper in swine feeding. Journal of Animal Science 1960; 19: 249-259.

[21] Jeter DL, Conrad JH, Plumlee MP, Beeson WM. Effect of organic and inorganic sources of unidentified growth factors on the growing pig. Journal of Animal Science 1960; 19: 226-237.

[22] Jongbloed AW, Top AMvd, Beynen AC, Klis JDvd, Kemme PA, Valk H. Consequences of newly proposed maximum contents of copper and zinc in diets for cattle, pigs and poultry on animal performance and health. Report ID-Lelystad 2001; 2097.

[23] Larsen T, Poulsen HD. The relationship between mineral and nitrogen balances in growing pigs fed diets supplemented with zinc oxide. Canadian Journal of Animal Science 1996; 76: 409-415.

[24] Lauridsen C, Højsgaard S, Sørensen MT. Influence of dietary rapeseed oil, vitamin E, and copper on the performance and the antioxidative status of pigs. Journal of Animal Science 1999; 77: 906-916.

[25] Lei X, Ku PK, Miller ER, Ullrey DE, Yokoyama MT. Supplemental microbial phytase improves bioavailability of dietary zinc to weanling pigs. Journal of Nutrition 1993; 123: 1117-1123.

[26] Lewis PK, Grummer RH, Hoekstra WG. The effect of method of feeding upon the susceptibility of the pig to parakeratosis. Journal of Animal Science 1957; 16: 927-936.

[27] Lewis PK, Hoekstra WG, Grummer RH. Restricted calcium feeding versus zinc supplementation for the control of parakeratosis in swine. Journal of Animal Science 1957; 16: 578-588.

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

[29] Liptrap DO, Miller ER, Ullrey DE, Whitenack DL, Schoepke BL, Luecke RW. Sex influence on the zinc requirement of developing swine. Journal of Animal Science 1970; 30: 736-741.

[30] Luecke RW, Hoefer JA, Brammell WS, Thorp F. Mineral interrelationships in parakeratosis of swine. Journal of Animal Science 1956; 15: 347-351.

[31] Miller ER, Ku P, Hitchcock JP, Magee WT. Availability of zinc from metallic zinc dust for young swine. Journal of Animal Science 1981; 52: 312-315.

[32] Miller ER, Luecke RW, Ullrey DE, Baltzer BV, Bradley BL, Hoefer JA. Biochemical, skeletal and allometric changes due to zinc deficiency in the baby pig. Journal of Nutrition 1968; 95: 278-286.

[33] Miller ER, Ullrey DE, Brent BE, Bradley BL, Hoefer JA, Luecke RW. Zinc requirement of baby pigs on soya protein diet. Journal of Animal Science 1966; 25: 1260.

[34] Morgan DP, Young EP, Earle.I.P., Davey RJ, Stevenson JW. Effects of dietary calcium and zinc on calcium, phosphorus and zinc retention in swine. Journal of Animal Science 1969; 29: 900-905.

[35] Okonkwo AC, Ku PK, Miller ER, Keahey KK, Ullrey DE. Copper requirement of baby pigs fed purified diets. Journal of Nutrition 1979; 109: 939-948.

[36] Okonkwo AC, Miller ER, Brady PS, Ku PK, Ullrey DE. Copper requirement of baby pigs on purified diets. Journal of Animal Science 1975; 41: 324.

[37] Oude Voshaar JH. Statistiek voor onderzoekers. Wageningen: Wageningen Pers; 1994.

[38] Payne RW, Lane PW, Digby PGN, Harding SA, Leech PK, Morgan GW, Todd AD, Thompson R, Tunnicliffe Wilson G, Welham SJ, White RP. Genstat 5. Release 3. Reference Manual. Clarendon Press, Oxford, UK: 1993.

[39] Poulsen HD, Larsen T. Zinc excretion and retention in growing pigs fed increasing levels of zinc oxide. Livestock Production Science 1995; 43: 235-242.

[40] Rupic V, Ivandija L, Luterotti S, Dominis KM. Influence of inorganic and organic dietary zinc on its concentration in blood serum, bones and hair and on catalytical activity of some serum enzymes in pigs. Acta Veterinaria Brno 1997; 66: 75-85.

[41] Smith ID, Grummer RH, Hoekstra WG, Phillips PH. Effects of feeding an autoclaved diet on the development of parakeratosis in swine. Journal of Animal Science 1960; 19: 568-579.

[42] Smith WH, Plumlee MP, Beeson WM. Effect of source of protein on zinc requirement of the growing pig. Journal of Animal Science 1962; 21: 399-405.

[43] Stevenson JW, Earle.I.P. Studies on parakeratosis in swine. Journal of Animal Science 1956; 15: 1036-1045.

[44] Sullivan JF, Burch RE, Quigley HJ, Magee DF. Zinc deficiency and decreased pancreatic secretory response. American Journal of Physiology 1974; 227: 105-108.

[45] Teague HS, Carpenter LE. The demonstration of a copper deficiency in young growing pigs. Journal of Nutrition 1951; 43: 389-399.

[46] Top AMvd, Beynen AC, Blok MC, Jongbloed AW, Kemme PA, Verdonk J, Wensing T. Literatuuronderzoek naar nieuwe statusindicatoren voor Cu en Zn bij vleeskalveren. 2001.

[47] Tucker HF, Salmon WD. Parakeratosis or zinc deficiency disease in the pig. Proceedings of the Society for Experimental Biology and Medicine 1955; 88: 613-616.

[48] Ullrey DE, Miller ER, Thompson OA, Zutaut CL, Schmidt DA, Ritche HD, Hoefer JA, Luecke RW. Studies of copper utilization by the baby pig. Journal of Animal Science 1960; 19: 1298.

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

[50] Whitenack DL, Whitehair CK, Miller ER. Influence of enteric infection on zinc utilization and clinical signs and lesions of zinc deficiency in young swine. American Journal of Veterinary Research 1978; 39: 1447-1454.

[51] Whiting F, Bezeau LM. The calcium, phosphorus, and zinc balance in pigs as influenced by the weight of pig and the level of calcium, zinc, and vitamin D in the ration. Canadian Journal of Animal Science 1958; 38: 109-117.

[52] Zoubek GL, Peo ER, Moser BD, Stahly T, Cunningham PJ. Effects of source on copper uptake by swine. Journal of Animal Science 1975; 40: 880-884.

3.7. Appendices growing-finishing pigs

Appendix 3.1. References as used by A(F)RC and NRC and their reasons of rejection for this study

Reference	Element	Part	Reason of rejection
[6]	Zn	All	As the control group receiving a diet containing 7.3 mg/kg of Zn lost instead of gained weight during the experiment, the data from this experiment were unsuitable for statistical analysis
[41]	Zn	Experiment 4	Pigs fed severely Zn-deficient rations (33 mg/kg Zn) for over 7 months were considered to be unsuitable to reliably assess Zn needs
[45]	Cu	Experiment 1	Insufficient information for statistical analysis
[47]	Zn	All	No control group included, Ca content of the ration was 1.55%
[7;17;19;33; 36;44;48]	Cu[7;36;48] Zn[17;19;33;44]	All	Insufficient information for statistical analysis

Appendix 3.2. Selected results of regression analysis of Cu data (Number = number of observations; Cu = dietary Cu concentration; C_p = Mallow's C_p [37]; FCR = feed conversion ratio; Hb = hemoglobin concentration; serum Cp = serum ceruloplasmin concentration; δ = difference as compared with basal level)

Response parameter	Number	Cu	δ Cu	Ca	Zn	Phytate P	$R^2_{adjusted}$	C_p
Growth	8	-2.52		-4.91	5.39	6.09	99.6	5.00
Feed intake	5	13.00		12.10	-	-10.83	98.2	4.00
FCR	4	-17.04		-	-	18.48	99.3	3.00
Hb	6	8.16		9.23	-9.29	11.16	99.0	5.00
Serum Cu	11	9.28		0.40	-0.79	-0.74	95.6	5.00
Serum Cp	8	10.78		-	-	-	94.3	2.06
Kidney Cu	6	6.89		-	-	-5.53	98.6	2.01
δ growth	7		24.58	-20.09	-21.63	-24.73	99.3	5.00
δ Hb	6		3.23	5.66	-	-6.42	96.2	3.51
	6		1.87	-	6.57	-10.90	97.1	3.13
δ liver Cu	10		10.43	-2.59	-1.36	-8.56	95.9	5.00
	10		10.33	-6.21	-	-12.95	95.4	4.86
δ serum Cp	8		10.39	-14.77	-	-25.31	99.1	3.38
	8		8.29	-2.30	0.61	-4.71	99.0	5.00
δ kidney Cu	6		-5.40	6.19	-	5.92	93.8	4.00

Appendix 3.3. Selected results of regression analysis of Zn data (Number = number of observations; C_p = Mallow's C_p [37]; FCR = feed conversion ratio; serum AP = serum alkaline phosphatase activity; δ = difference as compared with basal level)

Response parameter	**Number**	**Zn**	**δ Zn**	**Ca**	**Cu**	**Fe**	**Phytate P**	**Phytase**	**$R^2_{adjusted}$**	**C_p**
Growth	50	2.02		-1.48	1.68	2.75	2.46		32.3	5.01
Feed intake	39	0.43		-	-	1.77	3.55	-1.79	35.8	4.05
FCR	41	0.39		-	-	-3.21	-3.14	-	22.6	2.34
Plasma Zn	20	3.98		-	-	2.66	-	1.13	56.4	4.6
	20	1.51		1.55	1.18	3.44	1.75	-	60.6	5.11
Serum AP	19	0.24		0.76	-	-	-	4.76	53.6	3.10
Bone Zn	6	2.53		-	-	-	-	-	52.0	0.42
δ growth	50	-3.70	1.50	0.99	-2.32	-	2.46	-2.37	21.1	6.27
	50	-3.61	1.43	-	-2.28	-	-2.29	-2.36	21.2	5.23
δ feed intake	39	-1.24	0.71	-	-	-2.50	-	-	9.0	2.22
δ FCR	41	1.35	-1.78	-	-1.59	2.62	2.89	-	47.1	4.55
δ plasma Zn	20	-	3.65	-	-0.50	-1.89	-	-	48.2	0.61
δ serum AP	19	-	1.63	-	-0.87	-	-	1.84	8.8	0.93
δ bone Zn	6	-	3.83	0.73	-	-	-	-	72.2	1.67
	6	-	3.43	-	-	-	-0.65	-	71.3	1.72

Appendix 3.4. Lower and upper limits and indifferent ranges of Zn requirements of pigs (mg/kg) as given by different authors

Lower limit (mg/kg)	**Reference**	**No difference in performance between (mg/kg)**	**Reference**	**Upper limit (mg/kg)**	**Reference**
12	[32;50]	25 – 47	[25]	100	[16]
16	[21]	26 – 31	[12]	94	[25]
20	[31;44]	28 – 123	[1]		
22	[26]	28 – 105	[9]		
25	[5;27]	34 – 140	[51]		
28	[9;19;34]	37 – 42	[49]		
31	[30;41]	39 – 114	[19]		
33	[16;17]	51 – 151	[2]		
35	[19;28]	53 – 105	[23]		
36	[19]	56 – 127	[4]		
38	[42]	92 – 150	[39]		
44	[43]	82 – 122	[40]		
46	[40]				
47	[49]				
53	[29]				

4. Copper and zinc requirements of broilers

4.1. Introduction

4.1.1. Procedure

In this chapter, references concerning dietary Cu and Zn requirements of chicks as quoted by A(F)RC and NRC, as well as recent reports from scientific literature are listed and evaluated. No further information concerning suitable scientific references as used by DLG/GfE and INRA could be obtained.

4.1.2. Premises, suppositions and calculations

In most cases, only few data concerning mineral and phytate concentrations of the diets were given. Lacking values that were regarded necessary for evaluation of the requirements, were calculated for all diets based on the raw material composition, using a linear programming package (OPTISAM).

Frequently, only the final BW of the chicks was given. To calculate growth performance of chicks starting the experiment at the age of one day, initial BW was assumed to be 34 g.

All hepatic Cu and Zn concentrations were calculated on a DM basis. When values were expressed as mg/kg fresh tissue, DM content of chick liver was supposed to be 25% [31]. If bone Zn concentrations were given as mg/kg (ash weight basis) [43], these results were recalculated and expressed as mg/kg (fat-free DM); if ash % of the DM was not given, it was supposed to be 54.4% [44].

4.1.3. Rejected references

Several reports as used by A(F)RC and NRC had to be rejected for this study due to the fact that they did not respond to the criteria set for this study. The reasons for rejection are listed in Appendix 4.1.

Besides the references used by AFRC and NRC, also other reports were used to assess Cu and Zn requirements. Unsuitable parts of these references, as well as the reasons of their rejection, are listed in Table 4.1.

Table 4.1. Parts of other references than used by AFRC and NRC not suitable for this study

Reference	Element	Part	Reason of rejection
[1]	Cu	Parts of experiment 2, 3	Cuprous and cupric oxide used as Cu sources
[2]	Cu	Experiment 2	Cu levels exceeding 50 mg/kg
[4]	Cu	Experiment 1 Experiment 2 Experiment 4	Similar to reference [2] No negative control group Cu levels exceeding 50 mg/kg
[13]	Zn	Experiment 1, 3	Zn levels exceeding 200 mg/kg
[16;39]	Zn	Whole reference	Limited value because insufficient information was given
[20]	Cu	Experiment 2	During the pretest period (8 days), the chicks were fed a diet containing 1.1 mg/kg Cu
[29]	Zn	Whole reference	In all experiments Zn and Mn were added together to the ration, so no separate dietary Zn effect could be determined
[44]	Zn	Bone Zn concentrations	Bone Zn concentrations were already as high as 158 mg/kg (fat-free DM) on a ration containing < 1 mg/kg total Zn and increased up to 306 mg/kg when the total Zn concentration was 21 mg/kg. These values were considered to be erratically high
[43;44]	Zn	Experiment 1, 2	Dietary Zn levels exceeded 200 mg/kg

4.2. Available literature

The references suitable for evaluation of Cu requirements are listed in Table 4.2, and those suitable for evaluation of Zn requirements are listed in Table 4.3.

Table 4.2. Overview of the experiments used for evaluation of Cu requirements of chicks; D = duration of the experiment; FCR = feed conversion ratio; Cp = ceruloplasmin; P = performance; SOD = superoxide dismutase; Hb = hemoglobin concentration

Refer-e	Age of bir start (d)	D (d)	Growth (g/d)	FCR	Response criteria	Type of d	Basal Cu	Added Cu
							ppm in fresh	
[1]	8	15	14.3	1.54	P, plasma and liver Cu	S	0.56	0, 0.5, 1, 2, 4, 8, 12, 16
[2]	8	13	18.4	1.51	Performance	S	0.6	0, 0.5, 1
[3]	8	14	15	1.46	Performance	S	0.6	0, 0.5, 1
[4]	8	13	18.6	1.37	Performance	S	0.6	0, 0.5, 1
[5]	8	13	20.8	1.44	Performance	S	0.6	0, 1
[15]	1	42	26.6	-	P, Hb, serum Cp, liver Cu	S	0.9, 3.2 (2.3) [B]	0, 0.8, 1.3, 1.7, 2.2, 3.0; 3.9, 6.5,10.5
[17]	1	22, 19, 30	-	-	Hb	S	-	0.8, 1.5, 1.8, 2.2, 3.2, 3.5, 4.8, 5.2, 5.5
[20]	10	6	23.7	1.43	P, plasma Cu, SOD and Cp	S	1.1	0, 5,10,15
[24]	1	21	11.6	-	Growth, Hb	S	0.8	2, 4, 6, 8, 10, 12
[25]	1	21	9.5	-	Growth, Hb, liver Cu	S	-	1, 2
[35]	1	21	8.3	-	Performance	S	0.3	0, 2, 4, 8, 16

[A] S = synthetic or semi-synthetic diet

[B] figure within brackets is added inorganic Cu to the basal diet

Table 4.3. Overview of the experiments used for evaluation of Zn requirements of chicks; D= duration of the experiment; FCR = feed conversion ratio, P = performance

Reference	Age (d) of bird at start	D (d)	Growth (g/d)	FCR	Response criteria	Type of diet[A]	Basal Zn	Added Zn
							ppm in fresh	
[3]	8	14	11.6	1.88	P, bone Zn	S	13	0, 3, 4, 6, 8
[7]	8	14	15.7	1.76	Performance	S	13.5	0, 5, 6, 11, 15, 20
[9]	1	21	17	1.47	P, bone, serum and liver Zn	S	7	0, 4, 8, 12, 16, 20
[12]	8	12	19.3	-	P, bone Zn	S	13.5	0, 5, 9, 10
[13]	8	12	21	-	Performance	S	0	0, 5, 10, 15, 20, 25
[21]	1	28	13.1	-	Performance	S	9.3(5.5) [B]	0, 60
[22]	1	21	5.7	-	Performance	S	10.5	0, 40
[31]	12	14	17.4	1.44	Performance	S	30(5) [B]	0, 5, 25, 100
[33]	1	28	12.8	-	Performance	S	6.6(6) [B]	0, 52
[34]	1	28	18.4	-	Performance	S	11, 12, 13, 17, 18	0, 55
[39]	1	28, 21, 49	6.7, 31.6	1.84	Performance	S	8	10, 17, 20, 30, 40, 50, 117
[41]	1	28	13.2	1.52	Performance	S	10	0, 10, 20, 40, 80, 100
[42]	-	28	-	1.77	Performance	S	16, 36(20) [B]	0, 20, 80
[44]	8	14	12.9	1.79	Performance, bone Zn	S	0	0, 4, 7, 10, 14, 21
[45]	3	35	40.4	1.54	Performance	S	-	13, 20, 27, 34, 45
[48]	8	14	18.4	1.23	P, bone and plasma Zn	S	13	0, 2.5, 5, 7.5, 10, 15, 20, 40
[50]	1	10, 21	7.1-9.3	1.85, 1	Performance	S	15	0, 10, 20, 40, 60, 80, 160
[51]	1	28	18.5	1.68	Performance	S	4.8, 5.2, 6.5, 8.6, 9.0	0, 5, 10, 15, 20, 25, 30, 60, 90, 120
[11]	1	14	9.6	1.68	Performance	CS	37, 41	0, 10, 20, 40, 80, 100
[11]	1	42	17.2	-	Performance	CS	37, 41	0, 10, 20, 40, 80, 100
[6]	8	14	19.2	1.55	P, plasma and liver Zn	CS	33	0, 52
[19]	-	29	23.1	1.73	Performance	CS	100(75)B	0, 40
[21]	1	28	13.1	-	Performance	O	45.8(5.8);17.1 (5.5) [B]	0, 30, 60
[26]	1	63	18.7	2.62	Performance	CS	36	0, 15, 36, 66, 108
[30]	5	16	36.5	1.48	P, bone and plasma Zn	O	20, 65(40) [B]	0, 10, 20, 40, 50, 65, 85
[40]	1	14	27.8	1.32	Performance	CS	42	0, 40
[43]	-	19	-	-	P, bone, liver and pancreas Zn	CS	31	0, 40, 80, 120
[46]	-	21, 28	-	-	Performance	CS	44, 50	0, 26, 52, 104
[46]	-	56	-	-	Performance	CS	44, 50	0, 26, 52, 104
[51]	1	28	18.5	1.68	Performance	CS	36, 37, 43	0, 100
[16]	1	15	-	1.47	Performance	O	40	0, 60

[A] S = synthetic or semi-synthetic diet; CS = corn-soybean meal diet; O = other mixed diets
[B] figure within brackets is added inorganic Zn to the basal diet

4.3. Statistical analysis

The data were subjected to multiple regression analysis. First, possible models were selected based on Mallow's C_p. Then, these selected models were subsequently analysed by forward, backward and stepwise selection of variables. The statistical package Genstat 5, release 3.1 [37] was used throughout.

Only physiologically relevant explaining parameters were included in the statistical analysis. For Cu, these were dietary Cu, Ca and Zn concentrations, whereas phytase P and phytase activities could not be used because of S diets. For Zn, the explaining parameters were dietary Zn, Ca, Cu, Fe, and phytate P concentrations, as well as dietary phytase activities.

Both total and added dietary Cu or Zn concentrations were included in the statistical analysis to disclose possible differences between the effects of intrinsic and extrinsic minerals in the total ration.

Suitable models should at least comply with the following conditions:

Dietary Cu or Zn content is an explanatory parameter in the model
$R^2_{adjusted}$ is > 70.0%
C_p is < p + 3 (p = number of explaining parameters in the model) [36]
The number of observations is > 4
The coefficient is significant ($P < 0.05$).

If models, based on these conditions, seemed to be appropriate, data were presented in a graph. Then, the best parameters (showing a visible correlation in the graph) were subjected to broken line analysis to obtain a breakpoint value. This breakpoint discriminates between the first phase of the graph, in which the response parameter increases with each increase in dietary Cu or Zn concentrations, and the second phase, a non-responsive plateau of response parameter values. This breakpoint, therefore, suggests the attainment of the dietary Cu or Zn requirement.

4.4. Results and discussion

4.4.1. Copper

4.4.1.1. Total Cu requirements

It was not possible to include either phytate P concentration (only synthetic diets) as an explanatory parameter or feed intake, FCR, and plasma Cu as response parameters in the regression analysis. A significant correlation was shown between δ serum Cp and δ dietary Cu ($R^2_{adjusted}$ = 93.2; C_p = 2.00; coefficient for Cu = 5.33). However, the number of observations (n = 3) was too small to be of sufficient value. Furthermore, graphic presentation of the data did not show any visible relationship. Therefore, these results had to be ignored. More selected results of statistical analyses are presented in Appendix 4.2.

As statistical analysis of Cu data did not show significant correlations between dietary Cu and any of the parameters measured, Cu requirements had to be assessed by comparison of the different experiments. This indicated that for obtaining maximal growth performance, dietary Cu concentrations for growing chicks ranged from 1.1 to 16.3 mg/kg of (synthetic) diet. Calculation of the mean dietary Cu concentration for maximal growth performance, as described in Chapter 3.4.2.1, could be assessed for six references. This was 7.5 and ranged from 1.1 to 13.6 mg Cu/kg diet and seemed thus not very useful. However, omitting the high values of 13.6 and 12.3, the average was 4.7 mg Cu/kg of diet (n = 4).

As far as possible, the indicators as listed by Jongbloed et al. [18] were used to judge the relative importance of the different results. Unfortunately, in only few cases suitable parameters were reported (performance and liver Cu content). Therefore, although the number of reports is already small, the three reports [1;15;25] giving hepatic Cu concentrations may be more important. This means that dietary Cu concentrations should be (mg/kg) 2.6 – 4.6, 4.6, or 2.4 – 3.1. Considering these results, 4.8 mg/kg, rounded to 5 mg/kg, may be recommended as dietary total Cu requirement for the growing chick receiving synthetic diets.

Growth rate, final BW and FCR determined in the experiments were compared with the feeding scheme of male Ross broilers, which are most commonly used in The Netherlands. Male broilers were chosen because the scheme originates from 1998 and meanwhile growth performance of broilers has been improved. It appeared that in all experiments using (semi)synthetic diets, final weights of the birds were at least 50% less than according to the Ross 208 scheme (Appendix 4.3). The FCR, however, was almost the same. Maybe the breed of the birds is to a large extent responsible for the difference in growth rate. At the same FCR, per kg growth the same amount of Cu is provided. As the Cu content of the body is very low (1 mg/kg), it may be speculated that at the same supply of Cu per kg growth the estimated Cu requirement is also valid for fast-growing chicks.

4.4.1.2. Differences between intrinsic and added Cu

Nearly all experiments used (semi)synthetic basal diets containing ± 1 mg/kg Cu or less, which is well below the recommended requirement. Thus, by far most of the Cu in the diets is of extrinsic origin. Therefore, it is not possible to discriminate between effects of basal and added Cu.

4.4.2. Zinc

4.4.2.1. Dietary total Zn requirements

It appeared to be impossible to include phytase activity as an explanatory parameter in the regression analysis. This is not surprising. Apart from the synthetic and maize-soybean meal diets there were only two diets containing some phytase activity. It may be assumed that intrinsic phytase activity in the bird itself does not play any role, because the supply of available P was sufficient. For Zn, significant correlations were shown between dietary and bone Zn concentrations. When calculating relationships between dietary and plasma Zn concentrations, we noticed very low plasma Zn concentrations in the publication by Wedekind and Baker (1990). As the results in our data file showed to be correct, we concluded that their data were at least a factor 10 too low and therefore, their data on plasma Zn were omitted. The regression coefficients of Ca and phytate content for estimation of plasma Zn are opposite to what may be expected. This may be a.o. due to the low number of observations.

Selected results on bone Zn are presented in Table 4.4. More selected results are presented in Appendix 4.4.

Table 4.4. Selected results of regression analyses of Zn data in chicks (n = number of observations; Zn = dietary Zn concentrations; C_p = Mallow's C_p [36]).

Response parameter	**n**	**Zn (mg/kg)**	**Ca (g/kg)**	**Phytate P (g/kg)**	**Cu (mg/kg)**	**Fe (mg/kg)**	$R^2_{adjusted}$	C_p
Bone Zn	13	1.10	-15.5	-13.7	-4.8	-	97.7	4.1
	13	1.05	-14.3	-12.7	-1.4	0.24	97.4	6.0

Table 4.4. shows that dietary Ca and phytate P contents have a negative effect on bone Zn content as well as Cu.

Broken line analysis showed a proper estimation with a breakpoint value of 65 mg Zn/kg of diet. However, further exploration of the data from the six references showed that the line was based predominantly on data from a maize-soybean meal diet [43] and an other type of diet [29]. This is visualized in Figure 4.1.

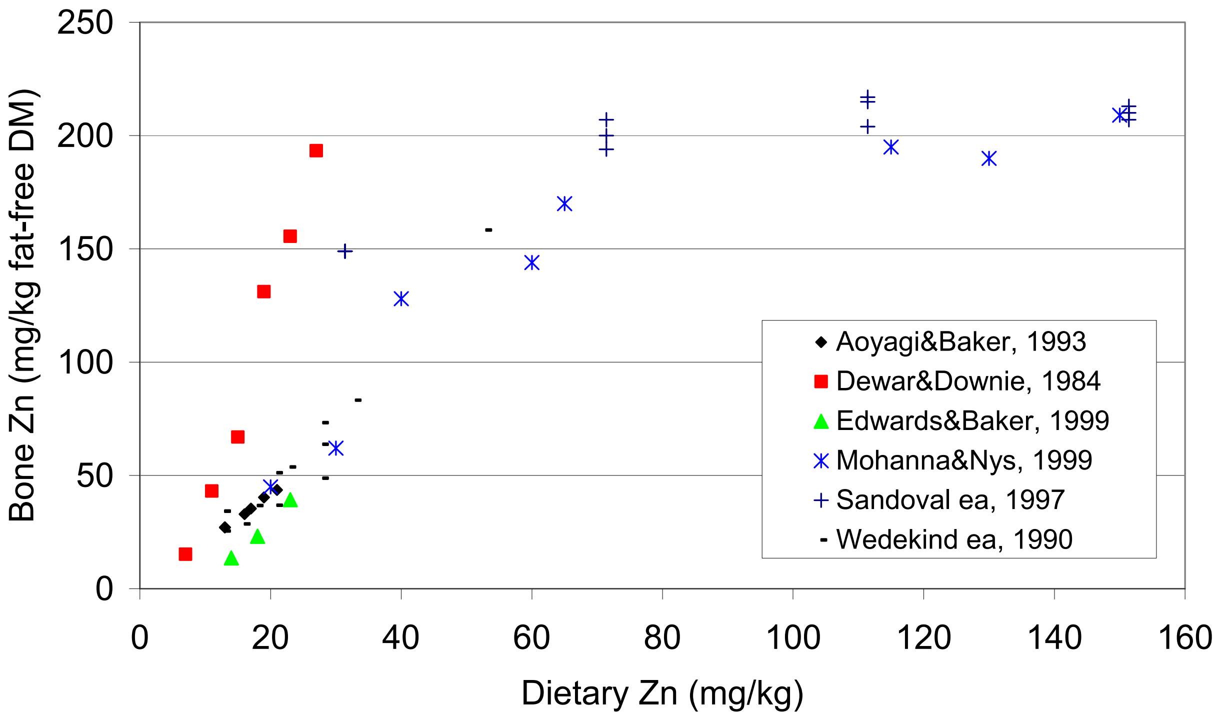


Figure 4.1. Relationship between individual dietary Zn concentrations and bone Zn concentrations in growing broilers

The relationship between dietary and plasma Zn concentrations could not be properly estimated due to the deletion of three experiments of Wedekind and Baker (1990), so that only four experiments were left (see also Figure 4A as Appendix 4.6). Figure 4.2 shows the relationship between total Zn and total plasma Zn of broilers.

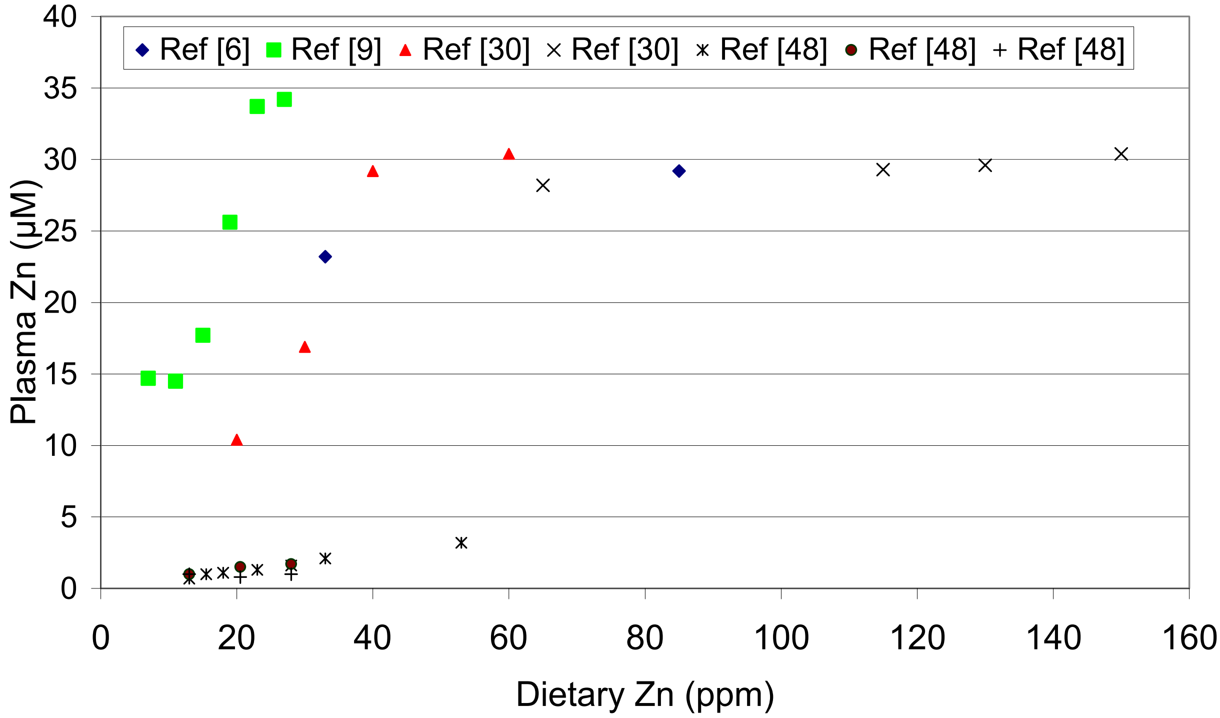


Figure 4.2. Relationship between dietary and total plasma Zn concentrations in growing chicks

Figure 4.2 shows that, apart from the values by [48], at a concentration around 60 mg Zn/kg of diet a plateau in total plasma Zn has been achieved.

For all experiments listed in Table 4.3, we made a review of the total Zn content at which numeric level the best growth performance was obtained, without taking into account significant differences (Appendix 4.7; see also Chapter 3.4.2.1). Furthermore, we ranked according to the type of diet. No distinction was made between the maize-soybean meal and other type of diets, because the latter also contained a large proportion of maize and soybean meal. The mean content at which maximal performance was achieved (corrected for the difference of two successive supplementary levels of Zn) for broilers receiving (semi)synthetic diets was 35 mg Zn/kg (n = 8). For the maize-soybean meal and other types of diets this was 69 mg Zn/kg of diet (n = 9). It can be calculated that the amount of Zn that had been added to the basal diets was 23 and 31 mg Zn, respectively, for the (semi)synthetic and other diets.

Surprisingly, the values of 65 mg/kg for maximal bone Zn content and 69 mg/kg for maximal performance are rather close to each other. However, it may be speculated that in general the dietary Zn concentration for maximal bone Zn content (only three references) is higher than required for maximal growth performance. A mean concentration of 67 mg/kg, may be recommended as dietary total Zn requirement of growing chicks, which may be sufficient. As in none of the suitable experiments varying levels of dietary phytase were investigated, it is not possible to calculate the influence of phytase on Zn metabolism of broilers. The only report employing different levels of phytase [29] had to be omitted (Table 4.1).

The reports used for the assessment of the dietary requirement of Zn originate from 1984-1999. Growth rate, final BW and FCR determined in the experiments were compared with the feeding scheme of male 208 Ross broilers. It appeared that except for three experiments, all final weights of the birds were at least 50% less than according to the Ross scheme (Appendix 4.5). The FCR, however, was except for three experiments, almost similar to the Ross scheme. Maybe the type of the birds is to a large extent responsible for the difference in growth rate. At the same FCR, however, per kg growth the same amount of Zn is provided. The question remains if applicability of the assessed requirement to modern, fast-growing chicks is still valid.

Furthermore, only 9 experiments reported bone Zn concentrations and were, therefore, involved in the assessment. However, the most recent report [30] with the highest growth rates (28 – 39 g/day) obtained maximal performance at 40 mg Zn/kg in one experiment, while there was hardly any difference between 65 and 150 mg Zn/kg in the other experiment. This may mean that the recommended value of 67 mg/kg of diet may be even applicable to fast-growing broilers.

4.4.2.2. Differences between intrinsic and added Zn

Concerning the difference between metabolic effects of intrinsic vs. added Zn, some selected results are given in Table 4.5. As (differences in) bone Zn concentrations were indicated by Jongbloed et al. [18] to be the most important indicators of the accuracy of dietary Zn supply in poultry, initially only references reporting bone Zn data were used. Diets containing similar total Zn, but from different origin (intrinsic vs. added) are compared with regard to their effects on (δ) bone Zn concentrations. As dietary total Zn concentrations are similar, occurring differences may originate from differences in the partition between intrinsic and added Zn. Ranges of total dietary Zn (25-35 and 35-45 mg/kg, respectively) were chosen arbitrarily, as for these ranges a reasonable number of observations was available.

Table 4.5. Selected results of references using similar dietary total Zn concentrations from different origin (intrinsic vs. added) and supplying data on bone Zn concentrations

Reference	Basal Zn	Added Zn	Total Zn	Duration expt.	Bone Zn	δ bone Zn
	mg/kg			(d)	mg/kg (fat-free DM)	
[3]	13	2.9	15.9	14	33	6
	13	4	17	14	35	8
	13	5.8	18.8	14	40	13
	13	8	21	14	44	16
[9]	7	12	19	21	131	116
	7	16	23	21	156	140
[12]	13.5	4.8	18.3	12	23	10
	13.5	9.9	23.4	12	39	26
[30]	20	0	20	16	45	-
[48]	13	2.5	15.5	14	29	3
	13	2.5	15.5	14	51	17
	13	2.5	15.5	14	37	3
	13	5	18	14	37	11
	13	5	18	14	73	39
	13	5	18	14	49	15
	13	10	23	14	54	28
[9]	7	20	27	21	193	178
[48]	13	15	28	14	69	38
[30]	20	10	30	16	62	17
[48]	13	20	33	14	83	58
[43]	35	0	35	19	149	-

Graphic presentation of the relationship between added dietary and total bone Zn concentrations, however, did not show a clear relationship (15-25 mg/kg total dietary Zn; Figure 4.3).

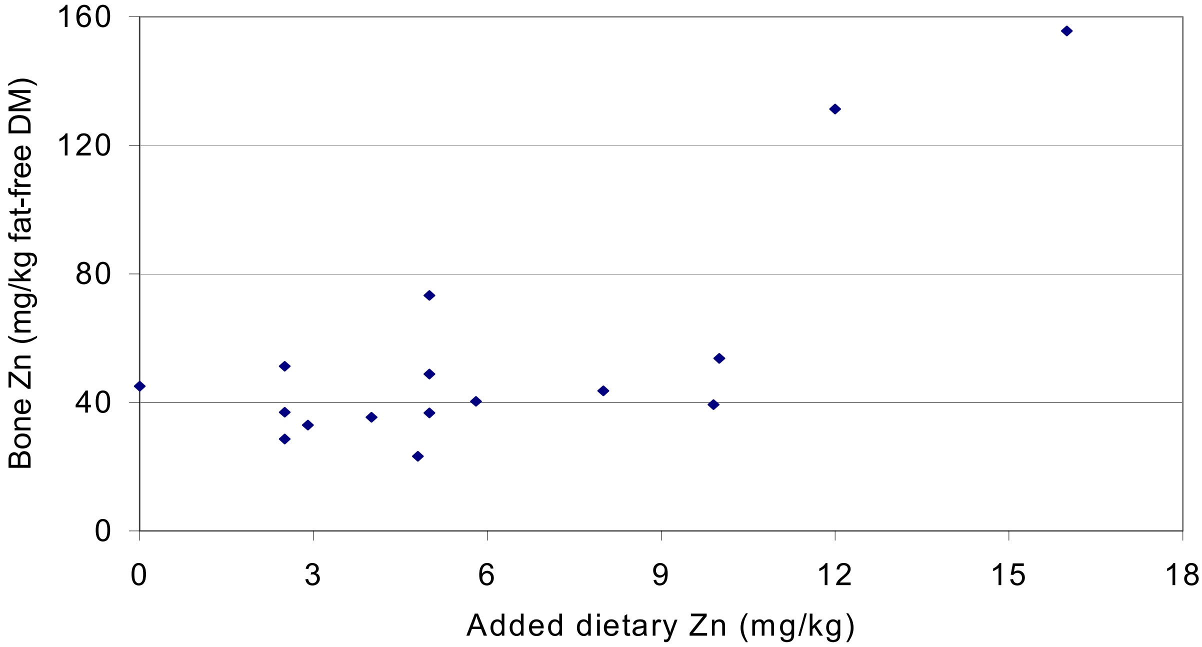


Figure 4.3. Relationship between added dietary Zn and total bone Zn concentrations in growing chicks (total dietary Zn 15-25 mg/kg Zn)

The relationship between added dietary Zn and δ bone Zn concentrations showed a similar graph, whereas no visible relationships between either basal or added dietary Zn and δ or total bone Zn concentrations could be observed. The same was the case for data from the total dietary Zn range of 25-35 mg/kg, mainly due to the limited number of observations within this range. As Jongbloed et al. [18] indicated plasma/serum Zn concentrations to be the second best indicator of dietary Zn status in chicks when dietary Zn supply was below their requirements, attention was also paid to this parameter. However, there were too few observations to make a proper comparison. However, based on maximal performance data, it was shown that total dietary Zn content of (semi)synthetic diets was 35 mg Zn/kg, while for the maize-soybean meal and other diets this was 69 mg Zn/kg of diet. It was calculated that the amount of Zn that had been added to the basal diets was 23 and 31 mg Zn, respectively, for the (semi)synthetic and other diets. This is an indication for differences in metabolic effects between intrinsic and extrinsic Zn

In summary, neither relationships between dietary Zn and bone Zn concentrations nor between dietary Zn and plasma Zn concentrations supply convincing evidence for any difference in metabolic effects of intrinsic and extrinsic dietary Zn in poultry rations, but based on required dietary Zn concentrations for maximal performance there are indications for differences in metabolic effects between intrinsic and extrinsic Zn.

4.5. Conclusions

4.5.1. Copper

Dietary total Cu requirement of growing chicks in (semi)synthetic diets can be assessed to be 5 mg/kg Cu as based on hepatic Cu content. Based on the estimates for maximal growth performance, this was 7 mg Cu/kg diet. For practical diets this may mean that an addition of 5 mg Cu/kg of diet will be sufficient. As growth rate of the chicks used in the experiments was substantially lower than but FCR was almost the same as current Dutch performance levels, applicability of this assessment to modern chicks is questionable. It can be recommended to supplement broiler diets with 6 mg Cu/kg of diet (mean of 5 and 7 mg/kg diet).

Due to a lack of suitable data no conclusion can be drawn on differences between the effects of intrinsic vs. added dietary Cu. The same is the case for effects of dietary Cu level during the pre-test period and for the duration of the experiment.

4.5.2. Zinc

Based on bone Zn data and maximal performance dietary total Zn requirement can be assessed to be on average 67 mg/kg for maize-soybean meal diets. Because today broilers have a much higher growth rate and a better feed conversion ratio than in the experiments reviewed, and also other diets than maize-soybean meal diets are provided, a 10 percent higher Zn requirement may be recommended, being 74 mg Zn/kg. Unfortunately, plasma Zn data did not yield a sufficiently reliable model to supply extra information on Zn requirements. There is an indication for differences between the effects of intrinsic vs. added dietary Zn. Neither dietary Zn levels during the pre-test period nor the duration of the experiment appeared to have any influence on the bone Zn concentrations measured. As starting day of the experiment (day 1 vs. day 8) and dietary Zn level during the pre-test period were interrelated, also no effect of starting day on bone Zn levels could be observed.

4.6. Literature

(1) Aoyagi S, Baker DH. Bioavailability of copper in analytical-grade and feed-grade inorganic copper sources when fed to provide copper at levels below the chick's requirement. Poultry Science 1993; 72: 1075-1083.

(2) Aoyagi S, Baker DH. Nutritional evaluation of a copper-methionine complex for chicks. Poultry Science 1993; 72: 2309-2315.

(3) Aoyagi S, Baker DH. Nutritional evaluation of copper-lysine and zinc-lysine complexes for chicks. Poultry Science 1993; 72: 165-171.

(4) Aoyagi S, Baker DH. Copper-amino acid complexes are partially protected against inhibitory effects of L-cysteine and L-ascorbic acid on copper absorption in chicks. Journal of Nutrition 1994; 124: 388-395.

(5) Aoyagi S, Baker DH. Effect of microbial phytase and 1,25-dihydroxycholecalciferol on dietary copper utilization in chicks. Poultry Science 1995; 74: 121-126.

(6) Bafundo KW, Baker DH, Fitzgerald PR. The iron-zinc interrelationship in the chick as influenced by Eimeria acervulina infection. Journal of Nutrition 1984; 114: 1306-1312.

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

(8) Davis PN, Norris LC, Kratzer FH. Interference of soybean proteins with the utilization of trace minerals. Journal of Nutrition 1962; 77: 217-223.

(9) Dewar WA, Downie JN. The zinc requirements of broiler chicks and turkey poults fed on purified diets. British Journal of Nutrition 1984; 51: 467-477.

(10) Edwards HM. The effect of protein source in the diet on Zn^{65} absorption and excretion by chickens. Poultry Science 1966; 45: 421-422.

(11) Edwards HM, Dunahoo WS, Fuller HL. Zinc requirement studies with practical rations. Poultry Science 1959; 38: 436-439.

(12) Edwards HM, III, Baker DH. Bioavailability of zinc in several sources of zinc oxide, zinc sulfate, and zinc metal. Journal of Animal Science 1999; 77: 2730-2735.

(13) Emmert JL, Baker DH. Zinc stores in chickens delay the onset of zinc deficiency symptoms. Poultry Science 1995; 74: 1011-1021.

(14) Englert SI, Jeffers TK, Sunde ML, McGibbon WH. Differences among inbreds with respect to dietary zinc. Poultry Science 1966; 45: 1082-1083.

(15) Hampel G, Grassmann E, Kirchgessner M. Zum Kupferbedarf wachsender Küken. Archiv für Tierernährung 1970; 20: 329-340.

(16) Hegazy SM, Adachi Y. Comparison of the effects of dietary selenium, zinc, and selenium and zinc supplementation on growth and immune response between chick groups that were inoculated with Salmonella and aflatoxin or Salmonella. Poultry Science 2000; 79: 331-335.

(17) Hill CH, Matrone G. Studies on copper and iron deficiencies in growing chickens. Journal of Nutrition 1961; 73: 425-431.

(18) Jongbloed AW, Top AMvd, Beynen AC, Klis JDvd, Kemme PA, Valk H. Consequences of newly proposed maximum contents of copper and zinc in diets for cattle, pigs and poultry on animal performance and health. Report ID-Lelystad 2001; 2097.

(19) Kidd MT, Anthony NB, Lee SR. Progeny performance when dams and chicks are fed supplemental zinc. Poultry Science 1992; 71: 1201-1206.

(20) Koh TS, Peng RK, Klasing KC. Dietary copper level affects copper metabolism during lipopolysaccharide-induced immunological stress in chicks. Poultry Science 1996; 75: 867-872.

(21) Lease JG, Barnett BD, Lease EJ, Turk DE. The biological unavailability to the chick of zinc in a sesame meal ration. Journal of Nutrition 1960; 72: 66-70.

(22) Martin WG, Patrick H. Radionuclide mineral studies 3. The effect of breed and dietary zinc, calcium, and vitamin D_3 on the retention of zinc^{65} in chicks. Poultry Science 1961; 40: 1004-1009.

(23) McNaughton JL, Day EJ. Iron and copper requirements of broiler chicks. Poultry Science 1977; 56: 1737.

(24) McNaughton JL, Day EJ. Effect of dietary Fe to Cu ratios on hematological and growth responses of broiler chickens. Journal of Nutrition 1979; 109: 559-564.

(25) McNaughton JL, Day EJ, Dilworth BC, Lott BD. Iron and copper availability from various sources. Poultry Science 1974; 53: 1325-1330.

(26) Mehring AL, Brumbauch JH, Titus HW. A comparison of the growth of chicks fed diets containing different quantities of zinc. Poultry Science 1956; 35: 956-958.

(27) Miller JK, Jensen LS. Effect of dietary protein source on zinc absorption and excretion along the alimentary tracts of chicks. Poultry Science 1966; 45: 1051-1053.

(28) Moeller MW, Scott HM. Studies with purified diets 3. Zinc requirement. Poultry Science 1958; 37: 1227-1228.

(29) Mohanna C, Nys Y. Changes in zinc and manganese availability in broiler chicks induced by vegetal and microbial phytases. Animal Feed Science and Technology 1999; 77: 241-253.

(30) Mohanna C, Nys Y. Effect of dietary zinc content and sources on the growth, body zinc deposition and retention, zinc excretion and immune response in chickens. British Poultry Science 1999; 40: 108-114.

(31) Morrison AB, Sarett HP. Studies in zinc deficiency in the chick. Journal of Nutrition 1958; 65: 267-280.

(32) O'Dell BL, Newberne PM, Savage JE. Significance of dietary zinc for the growing chicken. Journal of Nutrition 1958; 65: 503-518.

(33) O'Dell BL, Savage JE. Potassium, zinc and distillers dried solubles as supplements to a purified diet. Poultry Science 1957; 36: 459-460.

(34) O'Dell BL, Yohe JM, Savage JE. Zinc availability in the chick as affected by phytate, calcium and ethylenediaminetetraacetate. Poultry Science 1964; 43: 415-419.

(35) Opsahl W, Zeronian H, Ellison M, Lewis D, Rucker RB, Riggins RS. Role of copper in collagen cross-linking and its influence on selected mechanical properties of chick bone and tendon. Journal of Nutrition 1982; 112: 708-716.

(36) Oude Voshaar JH. Statistiek voor onderzoekers. Wageningen: Wageningen Pers; 1994.

(37) Payne RW, Lane PW, Digby PGN, Harding SA, Leech PK, Morgan GW, Todd AD, Thompson R, Tunnicliffe Wilson G, Welham SJ, White RP. Genstat 5. Release 3. Reference Manual. Clarendon Press, Oxford, UK: 1993.

(38) Pensack JM, Henson JN. The effects of calcium and phosphorus on the zinc requirements of growing chickens. Poultry Science 1958; 37: 1232-1233.

(39) Pimentel JL, Cook ME, Greger JL. Immune response of chicks fed various levels of zinc. Poultry Science 1991; 70: 947-954.

(40) Roberson KD, Edwards HM. Effects of 1,25-Dihydroxycholecalciferol and phytase on zinc utilization in broiler chicks. Poultry Science 1994; 73: 1312-1326.

(41) Roberson RH, Schaible PJ. The zinc requirement of the chick. Poultry Science 1958; 37: 1321-1323.

(42) Roberson RH, Schaible PJ. The effect of elevated calcium and phosphorus levels on the zinc requirement of the chick. Poultry Science 1960; 39: 837-840.

(43) Sandoval M, Henry PR, Ammerman CB, Miles RD, Littell RC. Relative bioavailability of supplemental inorganic zinc sources for chicks. Journal of Animal Science 1997; 75: 3195-3205.

(44) Southern LL, Baker DH. Zinc toxicity, zinc deficiency and zinc-copper interrelationship in *Eimeria acervulina*-infected chicks. Journal of Nutrition 1983; 113: 688-696.

(45) Steinruck U, Kirchgessner M. [Estimation of the zinc requirement for broilers using their ability for selective zinc absorption and by dose-response relations]
Schätzung des Zinkbedarfs für Broiler anhand ihrer Fähigkeit zur selektiven Zinkaufnahme und mittels Dosis-Wirkungsbeziehungen. Archives of Animal Nutrition 1993; 43: 27-43.

(46) Sunde ML. Zinc requirement for normal feathering of commercial Leghorn-type pullets. Poultry Science 1972; 51: 1316-1322.

(47) Turk DE, Lease JG. Variations in the feeding value of sesame meals for chicks. Federation Proceedings 1962; 21: 311.

(48) Wedekind KJ, Baker DH. Zinc bioavailability in feed-grade sources of zinc. Journal of Animal Science 1990; 68: 684-689.

(49) Wedekind KJ, Hortin AE, Baker DH. Bioavailability of zinc in a zinc-methionine chelate. Poultry Science 1990; 6 (S1): 142.

(50) Young RJ, Edwards HM, III, Gillis MB. Studies on zinc in poultry nutrition. 2. Zinc requirement and deficiency symptoms of chicks. Poultry Science 1958; 37: 1100-1107.

(51) Zeigler TR, Leach-RM J, Norris LC, Scott ML. Zinc requirement of the chick: factors affecting requirement. Poultry Science 1961; 40: 1584-1593.

4.7. Appendices broilers

Appendix 4.1. References as used by A(F)RC and NRC and their reasons of rejection for this study

Reference	Element	Part	Reason of rejection
[10;27]	Zn	Whole experiment	Only Zn^{65} absorption determined, no performance data given
[42]	Zn	Exp. 1 (groups 4, 5); exp. 2	Dietary Ca content exceeded 1.4%
[34]	Zn	Exp. 1 (G-L)	
[22]	Zn	Replicates 1, 2; other diets containing 1.6% Ca	
[8;24;25;32]	Zn/Cu [8]; Cu[24;25];Zn[32]	Whole experiment	
[31]	Zn	Experiment 3; second part of experiment 4	
[51]	Zn	Experiments E, F, G	
[14;23;28;38; 47;49]	Zn[14;28;38;47;49]; Cu[23]	Whole reference	Insufficient information
[17]	Zn	Parts of experiment 1; whole experiments 2, 3	Fe deficient diets (20 mg/kg Fe and lower)

Appendix 4.2. Selected results of regression analysis of Cu data (n = number of observations; Cu – dietary Cu concentration; C_p = Mallow's C_p [36]; FCR = feed conversion ratio; Hb = hemoglobin concentration; serum Cp = serum ceruloplasmin concentration

Response parameter	n	Cu	δ Cu	Ca	Zn	$R^2_{adjusted}$	C_p
growth	17	2.55		1.71	-	29.5	10.60
Hb	5	-0.44		4.27	-	99.5	4.00
δ growth	18		-0.71	-	-	a	2.00
δ feed intake	15		-1.79	-	-	13.68	2.00
δ FCR	15		0.68	-	-	b	2.00
δ Hb	5		-0.10	-	-	c	2.00
δ serum Cp	3		5.33	-	-	93.2	2.00
δ liver Cu	3		-0.08	-	-	d	2.00

Appendix 4.3. Comparison of broiler performance in literature receiving different dietary Cu levels with that of current Ross broilers

							Ross 208 scheme	
Reference number	**Age start**	**Days in expt**	**Growth (g/d)**	**FCR**	**Weight at end expt (g)**	**Age end expt**	**Weight (g)**	**FCR**
15	1	42	26.6	-	1157	43	2447	1.74
17	1	22	-	-	-	23	949	1.42
24	1	21	11.6	-	284	22	884	1.40
25	1	21	9.5	-	240	22	884	1.40
35	1	21	8.3	-	214	22	884	1.40
1	8	15	14.3	1.54	315	23	949	1.42
2	8	13	18.4	1.51	339	21	820	1.39
3	8	14	15.0	1.46	310	22	884	1.40
4	8	13	18.6	1.37	342	21	820	1.39
5	8	13	20.8	1.44	370	21	820	1.39
20	10	6	23.7	1.43	242	16	533	1.28

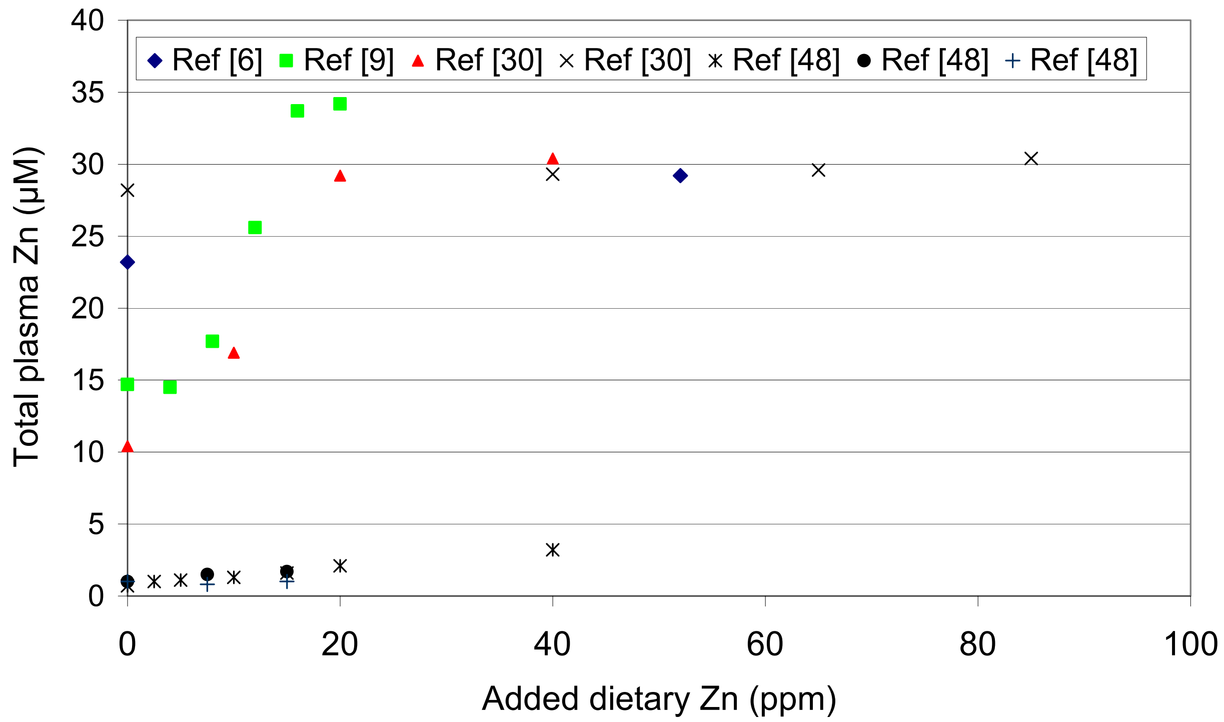
Appendix 4.4. Selected results of regression analyses of Zn data (n = number of observations; Zn = dietary total Zn concentration; C_p = Mallow's C_p [36]; FCR = feed conversion ratio

Response parameter	**n**	**Zn**	**δ Zn**	**Ca**	**Phytate P**	**Cu**	**Fe**	**$R^2_{adjusted}$**	**C_p**
growth	57	0.62		1.51	-	-	-	0.9	1.14
feed intake	36	1.26		-	-	-1.82	1.83	5.4	13.76
FCR	35	-1.42		-3.65	-1.36	-	-	40.0	2.19
	35	-2.33		-3.25	-	-1.05	-	38.6	2.88
	35	-3.31		-3.44	-	-	-	38.4	1.95
	35	-2.36		-3.33	-	-	-0.93	38.1	3.11
bone Zn	13	1.10		-15.51	-13.69	-4.77	-	97.7	4.06
	13	1.05		-14.27	-12.67	-1.44	0.24	97.4	6.00
	13	0.87		-13.81	-12.00	-	-3.99	97.0	6.07
plasma Zn	7	79.43		13.87	8.28	-81.31		100.0	5.00
δ growth	57	-	-0.22	-	-2.48	1.73	-0.94	8.5	3.80
δ feed intake	36	-1.40	1.16	-	-2.74	-	-	42.0	4.35
δ FCR	34	1.57	-2.36	4.17	1.67	-	1.01	59.6	5.55
	34	1.71	-2.39	4.38	2.34	-	-	59.5	4.56

Appendix 4.5. Comparison of broiler performance in literature receiving different dietary Zn levels with that of current Ross broilers

Parameters observed in experiments reviewed							**Ross 208 scheme**	
Reference number	**Age start (d)**	**Age end expt**	**Days in expt**	**Growth (g/d)**	**FCR**	**Weight end (g)**	**Weight (g)**	**FCR**
[[50]	1	11	10	7.1	1.85	111	301	1.09
[11]	1	15	14	9.6	1.68	174	481	1.25
[40]	1	15	14	27.8	1.32	429	481	1.25
[16]	1	16	15	-	1.47	-	533	1.28
[22]	1	22	21	5.7	-	160	884	1.40
[50]	1	22	21	9.3	1.64	235	884	1.40
[46]	1	25	24	8.4	-	242	1084	1.46
[21]	1	29	28	13.1	-	407	1370	1.52
[33]	1	29	28	12.8	-	398	1370	1.52
[34]	1	29	28	18.4	-	555	1370	1.52
[39]	1	29	28	6.7	1.84	228	1370	1.52
[41]	1	29	28	13.2	1.52	410	1370	1.52
[42]	1	29	28	-	1.77	-	1370	1.52
[51]	1	29	28	18.5	1.68	558	1370	1.52
[19]	1	30	29	23.1	1.73	710	1444	1.54
[11]	1	43	42	17.2	-	762	2447	1.74
[39]	1	50	49	31.6	-	1588	2991	1.85
[46]	1	57	56	9.8	-	589	3530	1.96
[26]	1	64	63	18.7	2.62	1218	4067	2.07
[45]	3	38	35	40.4	1.54	1454	2058	1.66
[30]	5	21	16	36.5	1.48	624	820	1.39
[12]	8	20	12	19.3	-	332	759	1.37
[13]	8	20	12	21	-	352	759	1.37
[3]	8	22	14	11.6	1.88	262	884	1.40
[6]	8	22	14	19.2	1.55	369	884	1.40
[7]	8	22	14	15.7	1.76	320	884	1.40
[44]	8	22	14	12.9	1.79	281	884	1.40
[48]	8	22	14	15	1.70	310	884	1.40
[9]	8	29	21	17	1.47	457	1370	1.52
[31]	12	26	14	17.4	1.44	344	1153	1.47
[43]	1	20	19	-	-	-	736	1.38

Appendix 4.6. Figure 4A. Relationship between added dietary and plasma Zn concentrations



in growing chicks

Appendix 4.7. Overview of maximal growth performance in the experiments used for evaluation of Zn requirements of broilers (D = duration of experiment; FCR = feed conversion ratio

Reference	Age of bird at start (d)	D (d)	Growth (g/d)	FCR	Type of diet[A]	Basal Zn	Added Zn	Zn level at maximal performance for each separate experiment
						mg/kg in fresh		
[3]	8	14	11.6	1.88	S	13	0, 3, 4, 6, 8	21; 19
[7]	8	14	15.7	1.76	S	13.5	0, 5, 6, 11, 15, 20	28.6; 24.3; 24.3
[9]	1	21	17	1.47	S	7	0, 4, 8, 12, 16, 20	27
[12]	8	12	19.3	-	S	13.5	0, 5, 9, 10	24.3; 23.6; 22.6;
[13]	8	12	21	-	S	0	0, 5, 10, 15, 20, 25	15
[21]	1	28	13.1	-	S	9.3 (5.5) [B]	0, 60	69.3
[22]	1	21	5.7	-	S	10.5	0, 40	50.5
[31]	12	14	17.4	1.44	S	30(5) [B]	0, 5, 25, 100	55; 55; 55
[33]	1	28	12.8	-	S	6.6(6) [B]	0, 52	58.6
[34]	1	28	18.4	-	S	11-18	0, 55	66; 68; 67; 72; 73
[39]	1	28, 21, 49	6.7, 31.6	1.84	S	8	10, 17, 20, 30, 40, 50, 117	125; 48; 48; 58; 88
[41]	1	28	13.2	1.52	S	10	0, 10, 20, 40, 80, 100	52.5; 110
[42]	-	28	-	1.77	S	16, 36(20)[B]	0, 20, 80	96; 96
[44]	8	14	12.9	1.79	S	0	0, 4, 7, 10, 14, 21	21
[45]	3	35	40.4	1.54	S	-	13, 20, 27, 34, 45	45
[48]	8	14	18.4	1.23	S	13	0, 2.5, 5, 7.5, 10, 15, 20, 40	33; 20.5; 20.5
[50]	1	10, 21	7.1-9.3	1.85, 1.64	S	15	0, 10, 20, 40, 60, 80, 160	25; 25
[51]	1	28	18.5	1.68	S	4.8, 5.2, 6.5, 8.6, 9.0	0, 5, 10, 15, 20, 25, 30, 60, 90, 120	20; 10; 12; 24; 24; 143; 136; 137
[11]	1	14	9.6	1.68	CS	37, 41	0, 10, 20, 40, 80, 100	121, 57, 61
[11]	1	42	17.2	-	CS	37, 41	0, 10, 20, 40, 80, 100	77, 51
[6]	8	14	19.2	1.55	CS	33	0, 52	33
[19]	-	29	23.1	1.73	CS	100(75)[B]	0, 40	100
[21]	1	28	13.1	-	O	45.8 (5.8); 17.1 (5.5)[B]	0, 30, 60	105.8; 69.3; 77.1
[26]	1	63	18.7	2.62	CS	36	0, 15, 36, 66, 108	105.8; 77.1
[30]	5	16	36.5	1.48	O	20, 65(40) [B]	0,10,20,40,50,65,85	40; 130
[40]	1	14	27.8	1.32	CS	42	0, 40	42
[43]	-	19	-	-	CS	31	0, 40, 80, 120	31.4; 31.4; 151
[46]	-	21, 28	-	-	CS	44, 50	0, 26, 52, 104	96; 44; 76
[46]	-	56	-	-	CS	44, 50	0, 26, 52, 104	96; 154; 50
[51]	1	28	18.5	1.68	CS	36, 37, 43	0, 100	136; 137; 143
[16]	1	15	-	1.47	O	40	0, 60	100

[A] S = (semi)synthetic diet; CS = corn-soybean meal diet; O = other type of diet
[B] value in brackets represent amount of added inorganic Zn in basal diet

5. Summary of requirements of Cu and Zn

Table 5.1 summarizes the recommendations on requirements of Cu and Zn in different countries [2] and those obtained in our study. In some countries no discrimination is given between requirements according to physiological status of the animal. This means e.g. that the same requirement is given for growing and lactating animals. This is indicated in Table 5.1.

Table 5.1. Summarized inventory of requirements of Cu and Zn in different countries and the results of our study (* = same for all categories of the specific animal species; [1])

Dairy cattle (concentrations as mg/kg DM)				
	Requirement of Cu		Requirement of Zn	
Country		This study		This study
NL	10*	21	25*	> 25
UK	1-15*		30-50*	
USA	10		40	
D	10		50	
F	10*		50*	
Piglets and growing-finishing pigs (concentrations as mg/kg fresh diet)				
Country		This study		This study
UK [3]	6 (added)	4 (added)	100 (added; to 60 kg) 80 (added; from 60 – 90 kg) 60 (added; from 90 – 120 kg)	> 67
USA	3.0 – 6		50 – 100	
D	4 – 5		45 – 90	
F	10*		100*	
Broilers (concentrations as mg/kg fresh diet)				
Country		This study		This study
UK	4	6 (added)	35 – 40	74
USA	8		40	
D	6 – 7		40 – 50	
F	10		50	

Comparison of the recommendations of Cu for dairy cattle in different countries shows that our recommendation of 21 mg/kg (DM) is substantially higher. Our recommendation is mainly based on limited information on maximal production performance (three references comprising six experiments). Maybe, 21 mg Cu/kg DM is even marginal at high Mo and S contents in the diet because of their negative effect Cu availability. There are too few references to make a reliable recommendation of Zn for dairy cattle. At least 25 mg Zn/kg DM should be present in the diet, which is lower than most recommendations. How much higher the Zn concentration should be can not be assessed due to lack of sufficient information.

The recommendations of Cu for growing-finishing pigs are in agreement with our results. An addition of 4 mg/kg Cu to a complete diet (88% DM) is sufficient. The recommendations of Zn in different countries for growing-finishing pigs are intermediate with our results. Based on maize-soybean meal diets 57 mg Zn/kg seems to be sufficient. The assessed requirement, however, should be adapted to the current improved feed conversion ratios. Therefore, a concentration of 67 mg Zn/kg is recommended as was obtained for pigs receiving other types of diets than maize-soybean meal.

The recommendations of Cu for broilers are in agreement with our results. An addition of 6 mg/kg Cu to a complete diet (88% DM) is sufficient. The recommendations for Zn in different countries for broilers are lower than obtained in this study. Our suggestion is 74 mg/kg in a complete diet (88% DM), because it is unknown how much higher the level should be as a compensation for the better

growth rate and feed conversion ratio in broilers of today compared with those of the references used.
When the results presented in Table 5.1 are compared with the maximum allowed concentrations from 26 January 2004 onwards [1], it can be seen that the requirements are substantially lower than the maximum allowed levels. Therefore, there is no risk for a sub optimal supply of Cu and Zn to the categories listed in Table 5.2.

Table 5.2. Allowed maximum content in diets (mg/kg) from 26-01-2004 onwards [1]

Animal category	Cu	Zn
Dairy cattle	35	150
Piglets up to 12 weeks	170	150
Other pigs	25	150
Broilers	25	150

5.1. Literature

[1] EC. Commission regulation (EC) No 1334/203 of 25 July 2003. Amending the conditions for authorisation of a number of additives belonging to the group of trace elements. L 187/11-L 187/15.
[2] Jongbloed AW, Top AMvd, Beynen AC, Klis JDvd, Kemme PA, Valk H. Consequences of newly proposed maximum contents of copper and zinc in diets for cattle, pigs and poultry on animal performance and health. Report ID-Lelystad 2001; 2097.
[3] BSAS, 2003. British Society of Animal Science. Nutrient requirement standards for pigs. p. 18.