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# Trend analysis of copper and zinc in animal feed

RIKILT Report 2011.012

P. Adamse, H.J. van Egmond, A. van Polanen, P. Bikker and J. de Jong





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## **RIKILT - Institute of Food Safety**

Wageningen UR (University & Research centre)  
Akkermaalsbos 2, 6708 WB Wageningen, The Netherlands  
P.O. Box 230, 6700 AE Wageningen, The Netherlands  
Tel. +31 317 480 256  
Internet: [www.rikilt.wur.nl](http://www.rikilt.wur.nl)

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# Summary

Copper and zinc are essential trace elements in animal nutrition. Since the inclusion of copper and zinc salts in animal diets is relatively cheap and a deficiency is to be avoided, copper and zinc salts are abundantly included in animal diets. However, their storage in body tissues is relatively low and the majority of copper and zinc is excreted in the faeces. Application of this manure causes high levels of copper and zinc in the soil. Therefore the EC has introduced maximum inclusion levels from 1970 onwards and reduced these levels in recent years.

In this report historical values are used to give insight into the trends in levels of copper and zinc in compound feeds for animals in the Netherlands. The results of these analyses will enable the nVWA (Dutch Food and Consumer Product Safety Authority) to develop a more risk-directed sampling strategy in the National Feed Monitoring program. The data used are collected from the databank of the Program for the Quality of Agricultural Products (KAP). The KAP databank has been filled and managed by the RIKILT and since 2010 by the RIVM. Over 2000 feed samples analysed for this report have been submitted to the databank by the National Feed Monitoring program (VWA, samples taken at the feed industry and some at the farms (liquid feeds)) and the Feed Control program (AID, samples of dry and liquid feed and water taken on (pig) farms). All samples were analysed by RIKILT and used only for monitoring research. The data for this analysis are from the period between 2001 and 2009. The copper and zinc data-set contains mostly feeds for piglets, (older) pigs, sheep and to a lesser extent bovine and other species.

Classification of the samples appeared to be very difficult. The sample description was often too brief, incomplete or not in line with the legislation, to allow a proper classification. Due to this lack of information a large group of complete compound feed samples is classified as 'feed for pigs or piglets'. Testing against a maximum copper amount is impossible for these samples. All complementary feeds for pigs are excluded from the data-analysis due to missing information about the inclusion level in the daily ration (% of the diet). About 20% of the farmers is using these complementary feeds together with wet or dry feedstuffs to obtain a balanced diet for pigs. This means that a significant amount of the total compound feed samples could not be included in this trend-analysis. Addition of the inclusion level to the standard sample description is recommended to solve this problem.

Trend analysis shows that from 2005 onwards, the average copper level in complete feeds for pigs ( $\geq 12$  weeks) is increasing. This clearly indicates that there is a tendency to add copper to the feed up to the maximum allowed content. There is no relevant increase or decrease of copper content in complete feed for piglets ( $< 12$  weeks) during the period studied. In the years 2005-2009 the median and 90percentile values tend to increase with a median value in 2009 that is close to the limit of 170 mg/kg. Liquid feed for pigs ( $\geq 12$  weeks) contained copper levels exceeding the maximum limit for complete feeds in 33 out of 129 samples (26%). The high rate of samples exceeding the limits indicates that there is a need to pay attention to this category. There is a clear increase in average and median copper levels in sheep feed in the last four years with a representative number of samples.

The EC limits for zinc levels in complete feed for pigs (all ages) changed in 2004 from 250 to 150 mg/kg. The data show that the average values for pigs (all ages) are higher in the period from 2004-2009 than in the period from 2001-2003. A likely explanation is that the limits agreed upon in the PDV covenant for the period before 2004 were lower than the EU limit. The average levels of complete feed did not exceed the limit, but individual levels did. During the period from 2006 to 2009, only in 2008 a substantial number of liquid feed samples were taken for zinc. The average values are slightly below the limit for complete feeds (150 mg/kg) but the 90percentile value is higher, indicating the need to pay attention to this category. The zinc content in sheep feeds is very constant during the period of feed sampling, with a mean of approximately 100 mg of zinc/kg of diet. This level is well below the maximum total zinc content of 150 mg/kg.

Complementary feeds with zinc and copper can be added to drinking water to supplement the concentrations of these trace elements in pig(let) diets. In this report at least 2 out of 185 water samples contained complementary feeds with zinc and copper. The total zinc or copper content in the ration of feed plus drinking water should not exceed legal limits. If these drinking waters containing copper and zinc supplements are used together with normal feeds, it may result in an over dosage of zinc or copper. In order to interpret these data it is crucial to know for what age group the drinking water is used (and whether it is all day available). In most cases, this information is lacking in the database. Improvements in the delivery of sample information are necessary. It is recommended that the exact age of pigs/piglets the feed is intended for and the dosage of complementary feeds are registered.

Monitoring copper and zinc supply at the farm rather than at the feed industry seems to be preferred because copper and zinc can also be added to the diet from other sources, e.g. via drinking water. In addition, only at the farm it can be checked whether high copper feed is used only for the intended period of life. This monitoring should also include liquid feeds, especially for pigs younger than 12 weeks.

Samples should be taken after the final daily ration is mixed on the farm using complementary feeds and other wet or dry ingredients to ensure homogeneous distribution of copper within feed batches.

In general it should be verified whether information on the label and instructions for use are complete. Sheep feeds supplemented with copper are likely to contain more than 10 mg Cu/kg and should be labeled with 'the level of copper in this feeding stuff may cause poisoning in certain breeds of sheep'. Sheep feeds supplemented with more than 5 mg Cu/kg are likely to contain more than 15 mg total Cu/kg and should be labeled as supplementary feed with instructions for the percentage in the ration or the daily allowance.

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# 1 Introduction

Copper and zinc are essential trace elements in animal nutrition. These elements have a large diversity of functions in all mammals. They are not only required for production performance, i.e. production of meat, milk and eggs, and reproduction, but also for an adequate immune response to maintain or restore health of the animal.

Copper is a component of a large number of enzymes, including cytochrome c oxidase, superoxide dismutase, ceruloplasmin and lysyl oxidase. These enzymes play a role in energy metabolism, maturation of collagen, anti-oxidant system, iron metabolism and erythropoiesis (production of red blood cells), pigmentation, and other biological processes. Deficiency of copper may result in reduced growth, anaemia, bone abnormalities, and a reduced immune response (NRC, 2005).

Zinc is an essential component of over 200 enzymes including carboxypeptidases A and B, collagenase, copper-zinc superoxide dismutase, and metallothionein. It plays a role in protein, nucleic acid, lipid and carbohydrate metabolism, and is required for DNA replication and transcription (zinc-fingers). Zinc deficiency may result in reduced feed intake and growth, impaired reproduction, parakeratosis, impaired wound healing, alopecia (loss of hair), thymus atrophy and impaired immune function (NRC, 2005).

Compound feeds contain approximately 5 mg copper and 30 mg zinc per kg from normal feed ingredients. This is not enough to meet the nutrient recommendations for the animals and hence these trace elements are added via the premix, mainly in the form of sulphates or oxides. More recent, also organically bound trace elements (chelates) are used in animal diets since their absorbability is assumed to be higher than sulphates. Although the many functions of copper and zinc have been described, the requirements of several animal species have not been precisely determined. Moreover, pharmacological levels of copper between 125 and 250 mg/kg feed stimulate growth and feed efficiency in swine (e.g. Cromwell et al., 1989) and are used for weaned piglets.

Since the inclusion of copper and zinc salts in animal diets is relatively cheap and a deficiency is to be avoided, copper and zinc salts are abundantly included in animal diets. However, their storage in body tissues is relatively low and the majority of copper and zinc is excreted in the faeces. The manure is spread over agricultural land and consequently these trace elements from animal diets contribute to the pollution of soil and surface water (Jondreville et al., 2003). Therefore the EC has introduced maximum inclusion levels from 1970 onwards and reduced these levels in recent years. In addition, between July 2000 and January 2004, the Dutch feed industry voluntarily included lower levels of copper and zinc in the diets, based on an agreement between the Commodity Board Animal Feed (PDV) and the Ministry of Agriculture (LNV). The regulations have been included in Table 1.1. The covenant between PDV and LNV used a more detailed distribution of farm animals into different categories than EC regulation, especially with regard to poultry. Therefore in Table 1.1 the classification from the covenant has been used, whereas in EC regulation all poultry is included in one category 'other species'. When the latest EC regulation became effective, January 26th, 2004, the covenant ended because the new EC maximum levels were in good agreement with the covenant. Nevertheless, for zinc, EC levels were higher than the covenant which may explain a possible increase in dietary levels in 2004.

Table 1.1. Maximum total copper and zinc contents<sup>1</sup> of animals diets according to EC regulations<sup>2</sup> and maximum added copper and zinc content according to the agreement between PDV and LNV<sup>3</sup>).

	Copper			Zinc		
	EC 1987-2003	PDV 2000-2003	EC 2004	EC 1987-2003	PDV 2000-2003	EC 2004
Pigs						
Pigs <12 wk	175	160	170	250	100	150
Pigs 12-16 wk	175	130	25	250	70	150
Pigs >16 wk	35	15	25	250	60	150
Breeding pigs	35	20	25	250	65	150
Cattle						
Before rumination						
- milk replacers	30	--	15	250	--	200
- other complete feeds	50	--	15	250	--	150
Other cattle	35	--	35	250	--	150
Sheep	15		15	250	--	150
Poultry <sup>4</sup>						
Broilers	35	15	25	250	55	150
Laying hens	35	15	25	250	55	150
Breeders	35	20	25	250	75	150
Rearing pullets	35	15	25	250	55	150
Turkeys for fattening	35	15	25	250	85	150
Breeding turkeys	35	20	25	250	85	150
Other species	35	--	25	250	--	150

<sup>1</sup> Expressed as mg copper or zinc per kg animal diet with 12% moisture.

<sup>2</sup> Commission Directive 85/520/EEC effective from 3/12/1986 and Commission Regulation (EC) 1334/2003, effective from 26/4/2004. Maximum copper for sheep reduced from 20 to 15 mg/kg diet in 86/300/EEC.

<sup>3</sup> For comparison of EC and PDV data a minimum of 5-10 mg copper and 30-40 mg zinc per kg of diet from the feedstuffs in the compound feeds should be added to the PDV values to calculate the total copper and zinc content in the diet.

<sup>4</sup> In EC regulation all types of poultry are included in the category 'other species'.

In this report historical values are used to give insight into the trends in levels of copper and zinc in compound feeds for animals in the Netherlands. This analysis is performed on request of the VWA (Dutch Food and Consumer Product Safety Authority). The results of these analyses will enable the VWA to develop a more risk-based sampling strategy in the National Feed Monitoring program.

In 2007 and 2009 reports have been published on the trends in levels of aflatoxin B1 and dioxins and dioxin-like PCB's (Adamse et al., 2007) and heavy metals (Adamse et al., 2009) in compound feeds and feed materials. These trend analyses have been carried out in order to use acquired knowledge to reach a more risk-driven sampling in the National Feed Monitoring program. For these reports data have been used from the KAP-database. The Quality Program Agricultural Products (KAP) is a collaboration between agricultural businesses and the Dutch Government. KAP has been designed to focus on continuous monitoring of the level of contaminants and residues in agricultural products such as vegetables, fruit, milk, meat, fish and feed. KAP processes the results of monitoring programmes and has more than 200,000 measurement results per year, from 1989 to date (van Klaveren, et al., 1997).

## 2 Methods and materials

### 2.1 Material

#### 2.1.1 Data from KAP database

The data used are collected from the databank of the Program for the Quality of Agricultural Products (KAP). The KAP databank has been filled and managed by the RIKILT and since 2010 by the RIVM. Over 2000 feed samples analyzed for this report have been submitted to the databank by the National Feed Monitoring program (VWA, samples taken at the feed industry and some at the farms (liquid feeds)) and the Feed Control program (AID, samples of dry and liquid feed and water taken on (pig)farms). All samples are analysed by RIKILT and taken in the framework of monitoring programmes (random selection). The data for this analysis are from the period between 2001 and 2009.

The copper and zinc data-set contain mainly feeds for piglets, (older) pigs, sheep and to a lesser extend bovine and other species. In Table 2.1 the annual number of samples of complementary and complete feeds analysed on copper and zinc are given.

Table 2.1. Numbers of copper and zinc analyses each year in complete and complementary feeds.

Year	Number of Cu samples				Number of Zn samples			
	Complete feeds*	Complementary feeds	Drinking water	Total	Complete feeds*	Complementary feeds	Drinking water	Total
2001	116	32	-	148	116	32	-	148
2002	141	55	-	196	142	55	-	197
2003	140	31	-	171	148	31	-	179
2004	304	36	-	340	403	36	-	439
2005	141	27	-	168	203	27	-	230
2006	87	63	10	149	87	63	10	150
2007	84	67	134	285	85	67	134	286
2008	214	80	41	335	232	80	41	312
2009	171	76	-	247	173	76	-	249
Total	1398	467	185	2050	1589	467	185	2058

\* Includes liquid feeds.

Copper and zinc samples obtained from complementary feeds (for all species) are not included in this report, due to a lack of information about the inclusion level of these feeds. As can be seen from Table 2.1 this excluded 20% of the copper and zinc results from trend analysis.

## 2.2 Methods

### 2.2.1 Grouping of compound feeds

In this report classification of compound feeds is based on the target animal (pig, sheep, cattle, etc.) and type of feed (complete feeds, complementary feeds). Since the maximum inclusion level of copper is higher in diets for pigs until 12 weeks of age than in pigs above 12 weeks of age, this aspect needed to be taken into account in the classification of pigs feeds. This classification is based on the labelling of feeds by the feed supplier and registration of this information in the database. Pigs feeds are classified in three groups: 1) feeds that were obviously made for target animals below 12 weeks of age, further indicated as feed for piglets 2) feeds that were obviously made for target animals above 12 weeks of age, further indicated as feed for pigs and 3) feeds that were not adequately labelled or for which label information as stored in the database was not complete, further referred to as feeds for pigs or piglets. This classification has been illustrated in Figure 2.1. The latter category with feeds for pigs or piglets comprised approximately 47% of all complete feed samples for pigs.

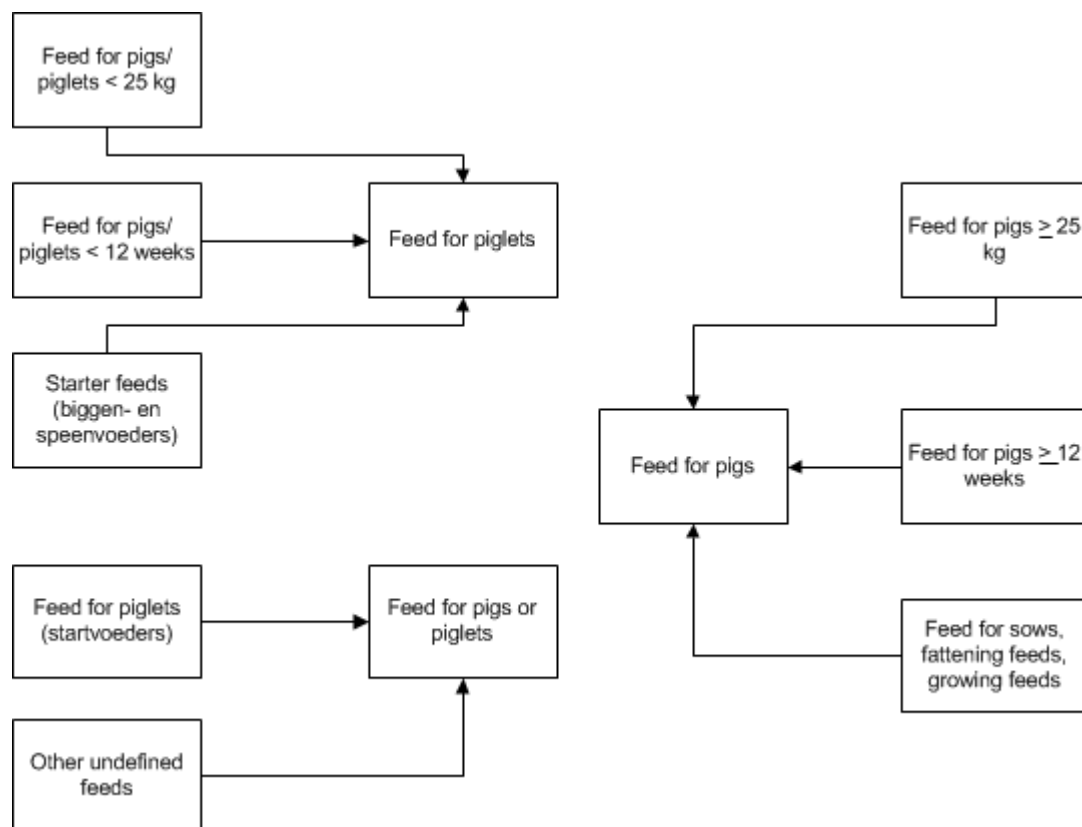


Figure 2.1. Classification of feeds for pigs according to the sample description.

### 2.2.2 Determination of copper and zinc

Digestion of the samples is performed in a temperature controlled microwave oven. The samples are heated with nitric acid (65%) in closed vessels using microwave energy. Water and other polar liquids absorb energy very fast, as a result the sample will be heated quickly. In conjunction with the resulting pressure and temperature the samples are fully digested. With this method contamination is minimized. The destructs can be used for the copper and zinc measurements.

For the copper and zinc measurements a Flame Atomic Absorption Spectrometer (FAAS) with deuterium background correction is used. With compressed air the sample is sprayed into the oxidizing gas flame (air). The aerosol is lead to the burner through an injector-room that serves to catch large drops and to mix the sample with a combustible gas (acetylene). During combustion, atoms of the element of interest in the sample are reduced to free, unexcited ground state atoms, which absorb light at characteristic wavelengths. Because the residence time of the aerosol in the light beam is only 10 ms the aerosol has to be very finely dispersed. To introduce the samples into the flame a sample changer, injector and injector-room are used. In the injector-room large drops of the aerosol are caught by collision with the wall or other parts of the injector-room and disposed as a liquid. The aerosol is then carefully mixed with the combustible gas before exiting from the injector-room into the flame.

With FAAS measurements the light beam of a hollow cathode lamp is lead through the atomised medium. This gas-discharge lamp consist of a tungsten anode and a cylindrical cathode made of, or covered with, the metal to be analysed. The construction is sealed in a glass tube filled with an inert gas such as argon under low pressure. Atomic absorption is a relative assessment method, this means that no absolute data will be obtained. There is always comparison with standard solutions of known concentration. Any increase of the signal by non-atomic absorption will be adjusted with the help of a deuterium background-correction system. Copper is measured at a wave length of 324.75 nm and zinc at a wavelength of 213.86 nm. The limits of quantification (LOQ) for copper and zinc in feeds are 2.0 and 2.5 mg/kg, respectively. The limit of quantification for copper and zinc in water is 0.1 mg/kg. The methods used are accredited and validated and are equivalent to the methods described in Reg 152/2009/EC. The measurement uncertainty is 20% for copper and 10% for zinc. For the trend analysis the measurement uncertainty has not been taken into account because although it is important for legal prosecution of individual lots, it is not relevant when populations of data (lots) are analysed.

### 2.2.3 Expression of results

Maximum total copper and zinc contents of animals diets according to EC regulations are expressed as mg copper or zinc per kg feed with 12% moisture. As from 2005, moisture contents of feeds are determined and the results are converted into 'mg/kg feed with 12% moisture (88% dry matter)'. The RIKILT-data prior to 2005 are reported on:

- mg/kg product (dry compound feeds).
- mg/kg feed with 12% moisture (liquid feeds)

The moisture content of dry compound feeds is approximately 12%. Therefore all data for dry compound feeds are analysed as one data-set.

Copper and zinc results in drinking water are always reported on product base.

#### 2.2.4 Reading instructions

Results are shown as averages, median values and 90-percentile values, with median and 90-percentile absent when less than 5 and 10 samples, respectively, have been analysed. Histograms are used to display the data, with sample year on the X axis and contaminant content on the left Y axis (see as example Figure 2.2). The number of samples is shown as squares connected by a line, with values on the right Y-axis. Furthermore, the limit (legal maximum) of the element analysed is shown by the gray area. When no average values approach the limit the entire plot area will be gray. The limit(s) are presented in the legend. When interpreting the figures it is important to keep in mind the order of magnitude of the left Y-axis. In many cases the measured value is much lower than the limit and the uncertainty of the analysis could be playing a role. The LOQ of the methods used for copper and zinc are 2.0 and 2.5 mg/kg (and 0.1 mg/kg for water), respectively, so if the measured values are in the same order of magnitude, the trends in that measuring range are not reliable.

The average (avg) is the mathematical average of all analyses in a specific year. The median is the level where 50% of the samples in this year had an analysed copper or zinc content above this level and 50% a content below this level. The median is the value in the middle of all values or the average of the two values in the middle with an even number of samples. The median can be more useful than the average when the distribution of values is skewed, because the average is more sensitive to outliers. The 90-percentile (90Per) indicates that 90% of the values are below this 90-percentile value.

In the right-hand corner of the figure the results of the regression analysis are shown. The equation  $y=ax+b$  is the result of a linear regression between the level of the trace element and the year of sampling. The equation algebraically describes a straight line for a set of data with one independent variable, in which  $x$  is the independent variable,  $y$  is the dependent variable,  $a$  represents the slope of the line, and  $b$  represents the y-intercept (the  $y$ -value when  $x = 0$ , the year before the first year shown in the graph).  $R^2$ , the coefficient of determination, indicates how much of the variation can be explained, i.e. whether a low  $x$ -value (year) coincides with a high or low contaminant level. If  $R^2$  is close to 1 there is a close relation. If  $R^2$  is lower than 0.30 there is a weak or (almost) no relation.  $N$  indicates the total number of measurements in the entire period. On the right Y-axis the number of samples per year is shown.

Using linear regression analysis, a trend line through the average annual levels is calculated and displayed (dotted line). This line reflects the evolution of the copper or zinc content in the years analysed. However, no extensive statistical methods have been used to determine the significance of the trends observed. For this statistical analyses, prerequisites would have to be supplied that are not available. The way the results are presented in this report will give sufficient information for discussing sampling strategies and suggest additional trend analyses of other elements and products.

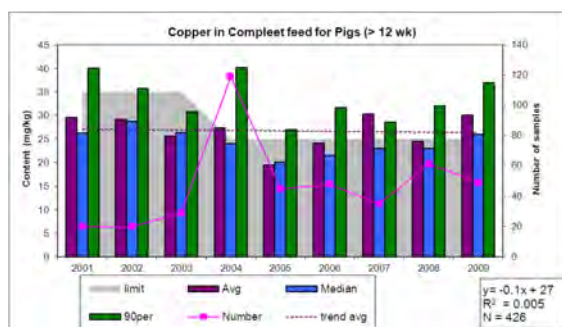


Figure 2.2. Copper content of complete feed for Pigs; limit = 35 (before 2004) to 25 mg/kg (12% moisture) (from 2004 on).

Using the reading manual above, Figure 2.2 can be interpreted as follows: The number of samples analysed each year fluctuates between 60 and 120. The average copper content in feed for pigs in the period 2001 through 2009 decreases with approximately 0.1 mg/kg each year. However, the  $R^2$  value indicates that this decrease is not significant ( $<0.3$ ). The median values (blue bars) remain below the limit (grey area), but both average and 90percentile values sometimes exceed the limit. When the median is just below the limit, it means that (almost) 50% of the samples are above the limit. For example in 2009, it means that around 25 samples are above the limit. When the 90percentile is above the limit this means that at least 10% of the samples (in 2009 26 samples) is above the limit. Further discussion of these results can be found in 3.1.1.



## 3 Results

### 3.1 Trends for copper

#### 3.1.1 Trends for copper in feed for piglets (<12 weeks)

Complete feed for piglets younger than 12 weeks of age is allowed to contain 175 (before May 2004) to 170 mg/kg (from May 2004 onwards). Figure 3.1A shows that the average content is usually close to this limit. In 8 out of 104 samples (8%) taken between 2001 and 2004 the copper content is between 175 and 224 mg/kg and in 29 out of 108 samples (27%) from 2005-2009 the copper content is between 170 and 292 mg/kg. There is no relevant increase or decrease of copper content during the period studied. However, within the copper levels in the years 2001-2004 (Figure 3.1B), a significant decrease of the average copper content can be observed. In the years 2005-2009 (Figure 3.1C), no relevant change in the average values is observed, but the median and 90percentile values tend to increase. In 2009 the median value is close to the limit of 170 mg/kg.

In liquid feed for piglets (Figure 3.1D) one sample out of sixteen (6%) contained copper above the limit (181 mg/kg, expressed in feed with 12% moisture).

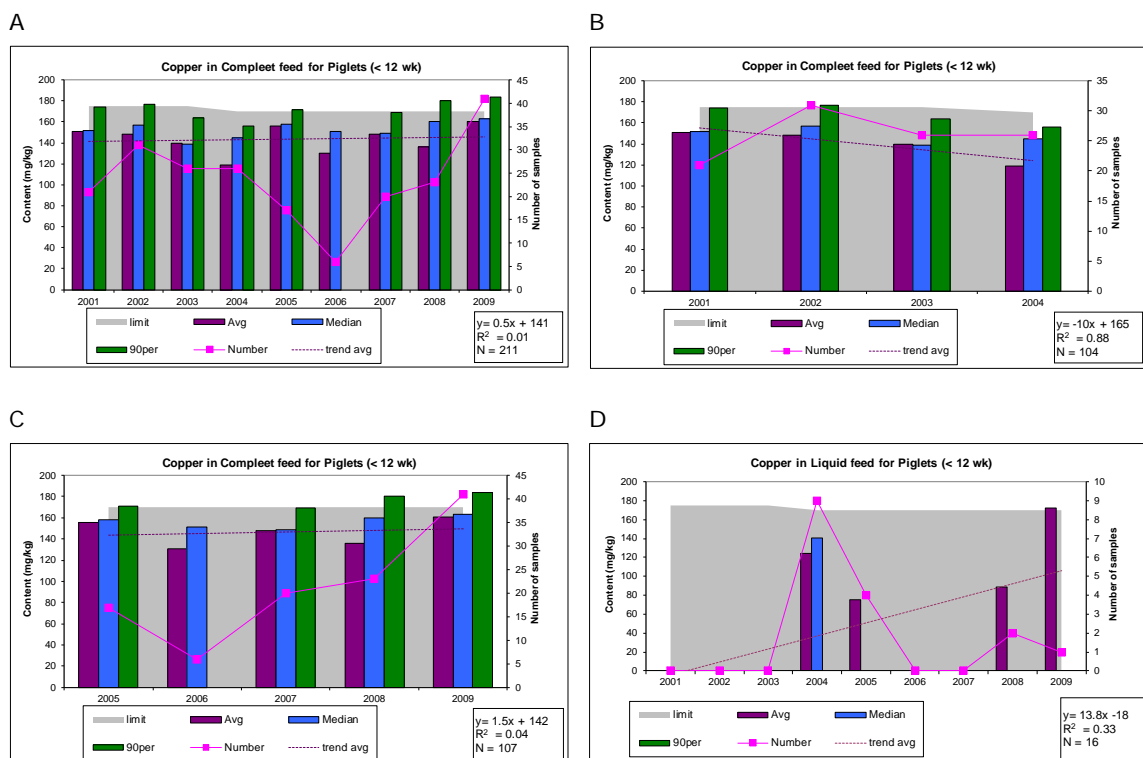


Figure 3.1. Copper content of feed for Piglets, A = Complete (2001-2009); B = Complete (2001-2004); C = Complete (2005-2009); D = Liquid; limit = 175 (before 2004) to 170 mg/kg (12% moisture) (from 2004).

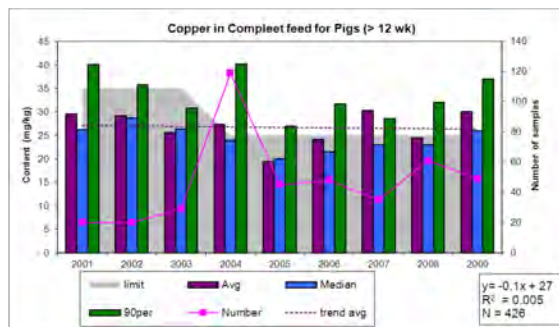
### 3.1.2 Trends for copper in feed for pigs (>12 weeks)

The maximum inclusion level (=limit) for copper in complete feed for pigs (>12 weeks) is 35 mg/kg (until April 2004) or 25 mg/kg (from May 2004 onwards). In the samples the average copper content is often close to this limit (Figure 3.2A). In the period 2001-2004 23 out of 188 samples (12%) exceeded 35 mg/kg. Starting in 2004 the 90percentile value is always above the limit. This means that in these years at least 10% of the samples exceed the limit. With regard to the copper levels in the years 2001-2004 (Figure 3.2C) it is clear that average and median values remain below the limit, but in 2005-2009 (Figure 3.2D) the levels (average, median and 90 percentile) often exceed the limit. From 2005 onwards, the average copper level increases, resulting in 26 out of 49 samples (= 53%) above 25 mg/kg in 2009.

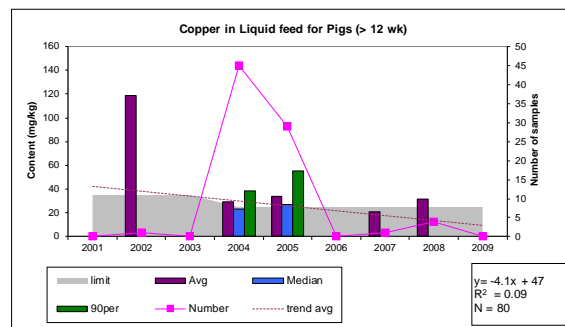
Five out of all complete feed samples (n= 426) contain more than 60 mg/kg copper. In one of these five samples the copper content even exceeds the limit for feed for piglets with a value of 182 mg/kg. Four of these samples may be intended for piglets, however the indicated age of these piglets is not in correspondence with the legislation (on the label referred to as <17 weeks (sample from 2009), <4 months, 10-16 weeks, 10-15 weeks (samples from 2004)). This seems to be a labelling problem. The fifth feed is a feed for fattening pigs.

Liquid feed for pigs (Figure 3.2B) contains copper levels comparable to complete feed. In total 33 out of 129 samples (26%) exceed the limit, viz. 1 sample in 2002, 4 samples in 2003, 15 samples in 2004, 6 in 2005 and 6 samples in 2008 exceeding 35 mg/kg but below 175 mg/kg . One sample in 2008 is above 175 mg/kg: 227 mg/kg. These deviations may be caused by incorrect labelling of the feed by the manufacturer or feeding of feeds for 'piglets <12 weeks' to older pigs by the farmer.

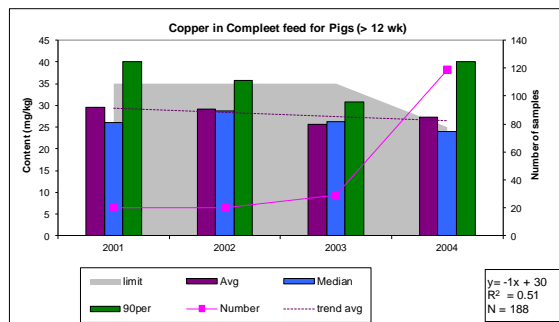
A



B



C



D

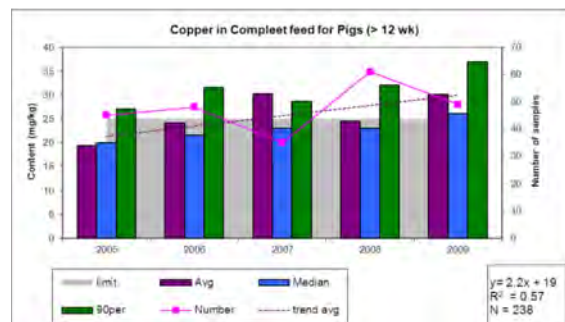


Figure 3.2. Copper content of feed for Pigs, A = Complete; B = Liquid; C = Complete (2001-2004); D = Complete (2005-2009); limit = 35 (before 2004) to 25 mg/kg (12% moisture) (from 2004 on).

### 3.1.3 Trends for copper in feed for pigs or piglets (age unknown)

The average copper content of unspecified samples of complete feed for pigs/piglets (Figure 3.3A) generally remains below the limit for feed for piglets (175 or 170 mg/kg ) but exceeds the limit for pig feed (35 or 25 mg/kg ), see Table 3.1. Eleven samples have levels above 175 (between 179 and 263 mg/kg). A number of samples (viz. 7 out of 109 'unspecified' in 2008 and 9 out of 80 unspecified in 2009) contain levels between 30 and 50 mg/kg. Most probably these products were intended for pigs ( $\geq 12$  weeks) but contained too high copper contents. Moreover, some samples (3 in 2008 and 3 in 2009) contain levels between 50 and 130 mg/kg which is a range that is rather unusual because it is between the normal content for piglets and pigs.

In liquid feed for pigs/piglets (Figure 3.3B) similar levels are detected with three samples above 175 (177 and 227 mg/kg).

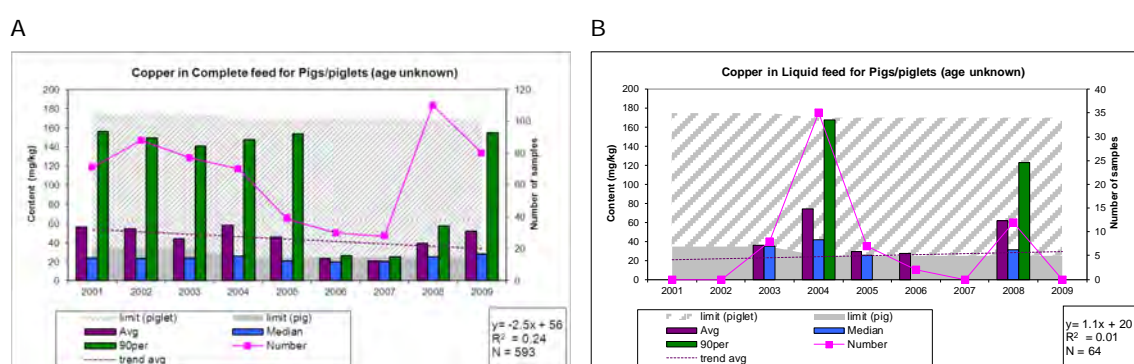


Figure 3.3. Copper content of feed for Pigs/Piglets, A = Complete ; B = Liquid; limit = 175 or 35 (before 2004) to 170 or 25 mg/kg (12% moisture) (from 2004 on).

Table 3.1. Number of unspecified samples of feeds for pigs or piglets classified in various ranges of copper contents.

	2001	2002	2003	2004	2005	2006	2007	2008	2009
pig/piglet complete									
total #	71	88	77	70	39	30	28	109	80
<30	46	60	63	42	31	29	27	79	49
$\geq 30 < 35$	1	1	1	4	-	-	1	12	6
$\geq 35 < 50$	3	2	-	-	1	-	-	7	9
$\geq 50 < 130$	8	9	2	11	1	-	-	3	3
$\geq 130 < 175$	11	14	10	13	5	1	-	7	10
$\geq 175$	2	2	1	-	1	-	-	2	3
Avg (mg/kg)	56	55	44	58	46	23	21	39	52
pig/piglet-liquid									
total #	0	0	8	35	7	2	0	12	0

	2001	2002	2003	2004	2005	2006	2007	2008	2009
< 30	-	-	-	16	5	1	-	4	-
> 30 < 35	-	-	4	-	1	1	-	3	-
> 35 < 50	-	-	4	3	-	-	-	1	-
> 50 < 130	-	-	-	7	1	-	-	3	-
> 130 < 175	-	-	-	7	-	-	-	-	-
> 175	-	-	-	2	-	-	-	1	-
Avg (mg/kg)	-	-	36	74	29	28	-	61	-

### 3.1.4 Trends for copper in feed for other animals

In feed for sheep average copper levels remained below the limit of 15 mg/kg (Figure 3.4A). In 21 out of 286 (7%) individual samples this limit is exceeded with values between 15.4 to 32 mg/kg. One sample of feed for sheep in 2002 contained 136 mg/kg. No significant increase or decrease in average copper levels could be detected in the time period studied. In the period 2006-2009, years with higher numbers of samples, there is a clear increase in average copper content (Figure 3.4B). This increase is largely affected by a very few number of samples containing a copper content above 20 mg/kg.

Since sheep are sensitive to high copper levels, the fact that 7% of the feeds monitored exceeds the limit for copper seems something to be concerned about. On the other hand, since grass is the main feed stuff for sheep, most of the feeds containing high copper levels are probably complementary feeds and only represent part of the daily ration.

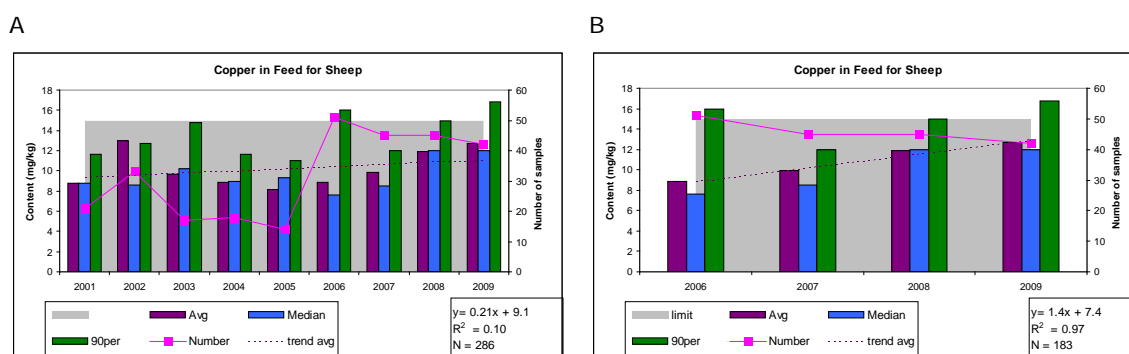


Figure 3.4. Copper content of feed for Sheep; A = 2001-2009; B=2006-2009; limit = 15 mg/kg (12% moisture).

Other feeds are analysed for copper content as well. However, the number of samples per year is not sufficient for trend analysis. Table 3.2 shows the average and maximum copper levels in a number of feeds. The milk replacers remain below the limit for milk replacers for calves (30 mg/kg). All samples remain below the legal limit.

Table 3.2. Copper content (mg/kg (12% moisture)) of several animal feeds.

Animal	# samples	Cu (mg/kg, 12% moisture)				Years
		Average	Stdev	Max	Limit	
bovine-milk replacer	14	7.1	5.0	16	15	2004, 2005
duck-complete	1	19	-	19	25	2008
goat-complete	3	25.8	6..6	31.3	35	2001, 2002
poultry-complete	1	42	-	-	35	2001
ruminant-complete	1	3.2	-	322	15	2008
undefined -complete	1	42	-	42	35	2001

### 3.1.5 Trends for copper in drinking water

Copper can also be fed to animals by adding it in a supplementary feed to drinking water. In Table 3.3 the copper content of water for several animals is shown. Highest levels are found in water for pigs (22 mg/kg) and poultry (23 mg/kg). In eight samples (4%) levels are between 1.6 and 23 mg/kg and in the remaining 177 samples the copper content is below 1 mg/kg.

Table 3.3. Copper content (mg/kg) of water for animals.

Animal-Feed	# samples	Cu (mg/kg)			Years
		Average	Stdev	Max	
bovine	2	0	0	0	2007
goat	29	0.03	0.1	0.4	2006, 2007
pig	48	0.53	3.2	22	2006, 2007, 2008
poultry	105	0.36	2.5	23	2007
undefined	1	0	-	0	2008

Four out of eight samples with copper levels above 1 mg/kg also contained zinc levels above 1 mg/kg (see next chapter).

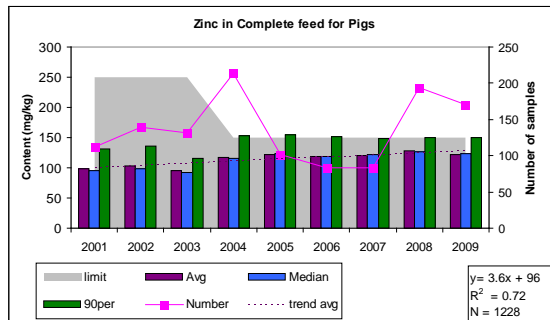
## 3.2 Trends for zinc

### 3.2.1 Trends for zinc in feed for pigs (all ages)

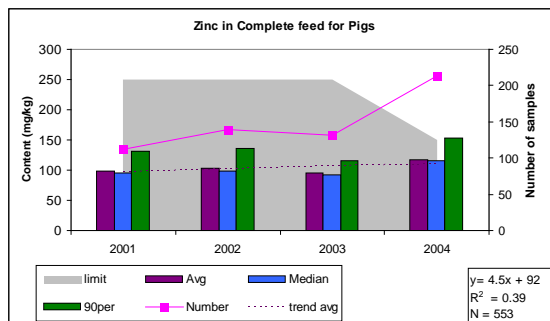
The legal maximum limit for zinc levels in complete feed for pigs of all ages (and other species besides pet animals and fish) was reduced in 2004 from 250 to 150 mg/kg. This reduction is not reflected in the average zinc levels in either complete feed (Figure 3.5A-C) or liquid feed (Figure 3.6A and B). On the contrary, the average levels of zinc in complete feed consistently increase in the time period studied. A likely explanation is that the limits agreed upon in the PDV covenant for the period before 2004 were lower than the EU limit. The average levels of complete feed do not exceed the limit, but some individual levels do. Four samples out of 1229 contain more than 250 mg/kg zinc (427 and 372 (2004), 356 (2006), 627 (2008) mg/kg) and another 94 (8%) have zinc levels between 150 and 250 mg/kg. From the trend in the period 2001-2004

a slight increase can be observed (Figure 3.5B). In the period 2005-2009 there is no relevant increase of the average zinc level (Figure 3.5C).

A



B



C

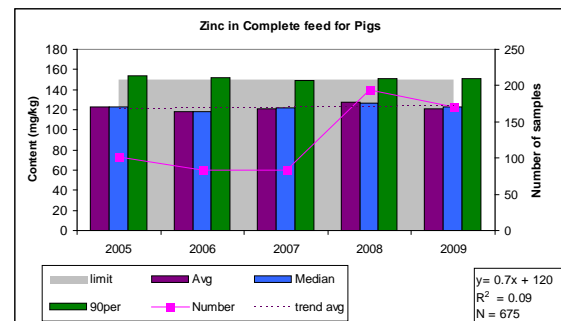
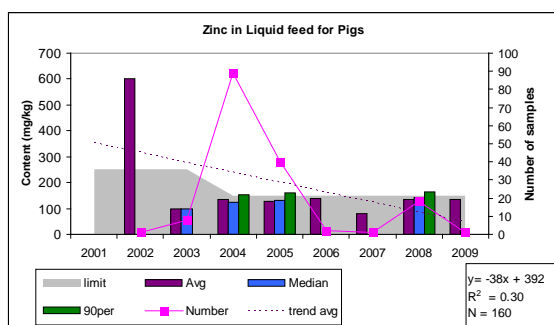


Figure 3.5. Zinc content of feed for Pigs, A = Complete; B =Complete (2001-2004 ; C = Complete (2005-2009) limit = 250 (before 2004) to 150 mg/kg (12% moisture) (from 2004 on).

In liquid feed 3 samples out of 160 exceed 250 mg/kg. In 2002 one sample contains 600 mg zinc/kg feed and in 2004 two samples contain 762 and 783 mg/kg zinc, respectively. Twenty-five liquid feed samples(16%) contain between 150 and 250 mg/kg zinc (Figure 3.6A). The sample from 2002 causes a steep decrease in the period 2001-2009. In Figure 3.6B) the range 2004-2009 has been analysed to give a more realistic view on the trend. In this period there is no in- or decrease of the average zinc content.

A



B

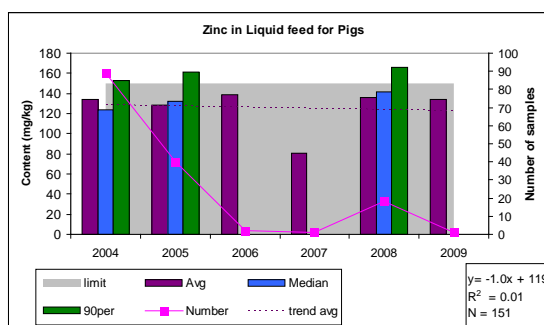


Figure 3.6. Zinc content of feed for Pigs, A = Liquid (2001-2009); B = Liquid (2004-2009); limit = 250 (before 2004) to 150 mg/kg (12% moisture) (from 2004 on).

### 3.2.2 Trends for zinc in feed for other animals

The legal limit for zinc in feed for sheep decreased in 2004 from 250 to 150 mg/kg. The average zinc content remains below this limit, except in 2004. One sample of complementary food for sheep from 2004 contains 2036 mg/kg. The other samples remain below the limit. In 2009 a similar feed contains 4390 mg/kg. This feed is described as a mineral mix to be mixed into feed at 10 kg/1000kg feed. No significant increase or decrease can be observed in the period analysed.

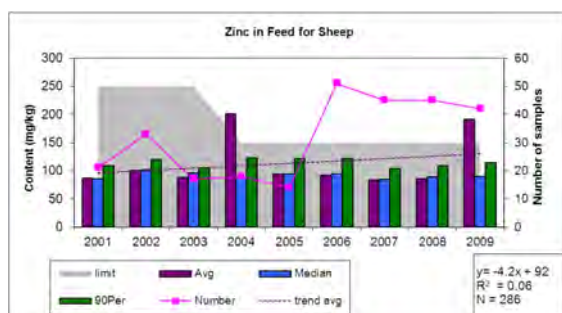


Figure 3.7. Zinc content of feed for Sheep; limit = 250 (before 2004) to 150 mg/kg (12% moisture) (from 2004 on).

The limit for zinc in milk replacer is higher than the limit for complete feed; 200 versus 150 mg/kg (since 2004). The average zinc content per year in the few samples analysed remained below this limit (Table 3.4). One sample of milk replacer for calves from 2005 contained 222 mg/kg. This is above the limit for milk replacer. The amount of samples and years is too small to analyse a trend.



Table 3.4. Zinc content(mg/kg (12% moisture)) of milk replacer for bovines.

Year	# samples	Zinc (mg/kg, 12% moisture)			
		Average	Stdev	Max	Limit
2004	6	108	22	134	200
2005	8	107	51	222	200

In the other feeds in Table 3.5 the average zinc content did not exceed any of the zinc limits.

Table 3.5. Zinc content (mg/kg)) of several animal feeds.

Animal-Feed	# samples	Zinc (mg/kg)				Years
		Average	Stdev	Max	Limit	
undefined-complete	1	116	-	116	250	2001
poultry-complete	1	92	-	92	250	2001
goat-complete	3	89.5	34.2	129	250	2001, 2002
duck-complete	1	86	-	86	150	2008
ruminant-complete	1	55	-	55	150	2008

### 3.2.3 Trends for zinc in drinking water

Zinc could be added to the animal diet through additional zinc in the water. However, the samples analysed in 2006-2008 (Table 3.6) in general did not contain high zinc levels. Only five samples of water showed levels of zinc above 1 mg/kg (3x water for poultry, 1x water for pigs and 1x water for goats). In 2006, one water sample for pigs contained 85 mg/kg zinc. This sample also contained a relatively high copper level (22 versus max. 1.6 mg/kg for similar samples). In water for poultry one sample contained 19 mg zinc per kg where the other 102 samples remain below 1 mg/kg.

Table 3.6. Zinc content (mg/kg) of water for animals.

Animal-Feed	# samples	Zinc (mg/kg)			Years
		Average	Stdev	Max	
undefined	1	0	-	0	2008
poultry	105	0.2	1.9	19	2007
pig	48	2.3	14.0	85	2006, 2007, 2008
bovine	2	0	0	0	2007
goat	29	0.1	0.3	1.4	2006, 2007

### 3.3 Complementary feeds

Trend analysis in complementary feed samples has not been performed due to unavailability of the inclusion level in the daily ration. To demonstrate the importance of the dosage, four examples with given inclusion level have been selected from the data-set (see Table 3.7).

*Table 3.7. The measured amount of copper and zinc , dosage and calculated final concentration in complete feed.*

Sample	Measured amount (mg/kg, 12% moisture)		Max. dosage (%) in final feed	Final concentration in feed (mg/kg, 12% moisture)	
	Cu	Zn		Cu	Zn
Complementary feed for pigs $\geq$ 12 wk	979	3050	0.5	5	15
Complementary feed for pigs $\geq$ 12 wk	55	280	38	21	106
Complementary feed for pigs < 12 wk	5310	2660	3	159	80
Complementary feed for pigs $\geq$ 12 wk	94	624	20	19	125

These examples illustrate that the amount of copper and zinc in complementary feeds can be very high, and dosages very low. For all four samples, the copper and zinc concentrations in the final feeds are below the maximum inclusion levels. Without information about the dosage, this could not have been evaluated.

## 4 Conclusions

Classification of the samples appeared to be very difficult. The sample description is often too brief, incomplete or not in line with the legislation, to allow a proper classification. Due to this lack of information a large group of complete compound feed samples (30% of the total) has been classified as 'feed for pigs or piglets'. Testing against a maximum copper level is impossible for these samples. For future purposes it seems required to add information about the exact age of the pigs for which the feed is meant to the label of each sample (description). Distinction between feeds for sows (breeding) and feeds for fattening pigs would also be useful.

All complementary feeds for pigs are excluded from the data-analysis due to missing information about the dosage (% of the diet). About 20% of the farmers is using these complementary feeds together with wet or dry feedstuffs to obtain a balanced diet for pigs. This means that a significant amount of the total compound feed production could not be included in this trend-analysis.

Addition of the dosage to the standard sample description is recommended to solve this problem. According to Regulation 767/2009, feed suppliers are obliged to include this information on the label.

### 4.1 Conclusions copper

From 2005 onwards, the average copper level in complete feeds for pigs (>12 weeks) is increasing, resulting in 26 out of 49 samples (= 53%) above 25 mg/kg in 2009. Moreover, average values are close to or above 25 mg/kg in the period 2007-2009. This clearly indicates that there is a tendency to add copper to the feed up to the maximum allowed content.

The average and median values in complete feed for piglets (<12 weeks) are lower than the limit. In 8 out of 104 samples (8%) taken between 2001 and 2004 the copper content is between 175 and 224 mg/kg and in 29 out of 108 samples (almost 29%) from 2005-2009 the copper content is between 170 and 292 mg/kg (12% moisture). There is no relevant increase or decrease of the copper content during the period studied. In the years 2005-2009 the median and 90 percentile values tend to increase with a median value in 2009 that is close to the limit of 170 mg/kg.

Liquid feed for pigs (>12 weeks) contain copper levels exceeding the maximum limit for complete feeds in 33 out of 129 samples (26%). In some cases quite high values are measured, e.g. one sample in 2008 contains 227 mg/kg. This product has been sampled by the AID in the framework of a project where the correct application of feeds was checked. Most probably this is not a labelling problem but a case where liquid feed for piglets has been fed to pigs >12 weeks. During the period from 2006 to 2009, only in 2008 a substantial number of liquid feed samples was taken. The high rate of samples exceeding the limits indicates that there is a need to pay attention to this category.

Complementary feeds with copper (and zinc) can be added to drinking water to supplement the concentrations of these trace elements in pig(let)diets. In this report at least 2 of the 185 water samples contained complementary feeds with copper. The total copper content in the ration of

feed plus drinking water should not exceed legal limits. If these drinking waters with supplements containing high copper are used together with normal feeds, it may result in over dosage of copper. In order to interpret these data it is crucial to know for what age group the drinking water is used (and whether it is all day available). In most cases, this information is lacking in the database.

In 2008 the General inspection service (AID) investigated the diet of piglets between 12-16 weeks old. This was done by visiting pig farms and taking faeces samples and samples of the feed and water supplied. It was shown that 40% of the farmers used feeds with high copper content, only allowed for piglets younger than 12 weeks of age, for older pigs. This indicates that communication and official control of the application of high copper feeds for piglet needs attention. One extra week of feeding high copper feeds to each pig results in approximately 24.000 kg extra copper pollution of soil and surface water in the Netherlands, calculated as:  $18 \cdot 10^6 \text{ pigs} \cdot 1.3 \text{ kg feed/d} \cdot (170-25) \text{ additional copper} \cdot 7 \text{ days}$ .

There is a clear increase in average and median copper levels in sheep feed in the last four years with a representative number of samples. The increase in the average value is largely affected by a very few number of samples containing a copper content above 20 mg/kg. The increase in the median value may suggest that the contribution from feedstuffs commonly included in sheep diets slightly increased during the last few years.

Sheep are particularly sensitive to a high copper intake. Therefore, sheep feeds generally do not contain additional copper included via the premix. The copper from common feed ingredients in sheep feeds would be approximately 5-10 mg/kg. Presumably higher levels may occur due to natural variation in copper content of feedstuffs. Most samples of sheep feed had a copper content below 10 mg/kg, a substantial number of samples had a copper content between 10 and 15 mg/kg and a small number of samples contained above 15 mg of copper/kg. It is difficult to judge in which samples copper was additionally added via the premix. It seems likely that levels up to 15 mg/kg can be explained by variation in copper content of feed ingredients.

According to Commission Regulation EC 1334/2003 feedingstuffs with a copper content exceeding 10 mg/kg should contain additional information in the labelling and accompanying documents declaring that 'the level of copper in this feeding stuff may cause poisoning in certain breeds of sheep.' This would imply that if copper is additionally added to the diet as copper salt, e.g. copper sulphate, in the premix, this declaration needs to be included. From the information in the database we cannot judge whether this declaration was adequately applied in the labelling of the respective sheep feeds. Hence, this aspect would require further attention when taking feed samples. It can be easily verified from the label information since according to Regulation EC 767/2009 the inclusion level of copper should be declared on the label from September 2009 onwards.

When the copper content of the sheep feeds is in excess of the maximum levels established for complete feed, i.e. 15 mg/kg, the instructions for proper use of complementary feed containing additives shall state the maximum quantity in kg per animal per day, as portion of the complete feed or daily ration (EC Regulation 767/2009, Annex 2). In general, compound sheep feeds are intended for use as complementary feed in addition to pasture grazing or conserved roughage. It is particularly relevant to include this on the label when copper exceeds 15 mg/kg. This would likely be the case in all feeds containing additionally supplemented copper. This can easily be

included in regular control of sheep feeds. From the database it seems that all but one sample with copper exceeding 15 mg/kg is labelled as supplementary feed.

## 4.2 Conclusions zinc

The legal limit for zinc levels in complete feed for pigs of all ages was reduced in 2004 from 250 to 150 mg/kg. The data show that the average values for pigs (all ages) are higher in the period from 2004-2009 than in the period from 2001-2003. A likely reason is that the limits agreed upon in the PDV covenant for the period before 2004 were lower than the EU limit. The average levels of complete feed did not exceed the limit, but levels of individual samples did. Four samples out of 1228 (2 after 2004) contain more than 250 mg/kg and another 94 (8%, 65 after 2004) between 150 and 250 mg/kg. In the period 2005-2009 there is no relevant increase of the average zinc level.

During the period from 2006 to 2009, only in 2008 a substantial number of liquid feed samples were taken for zinc determination. The average values are slightly below the limit for complete feeds (150 mg/kg) but the 90percentile value is higher, indicating the need to pay attention to this category.

The zinc content in sheep feeds is very constant during the period of feed sampling, with a mean of approximately 100 mg of zinc/kg of diet. This level is well below the maximum total zinc content of 150 mg/kg.

Complementary feeds with zinc (and copper) can be added to drinking water to supplement the concentrations of these trace elements in pig(let) diets. In this report at least 2 of the 185 water samples contained complementary feeds with zinc. The total zinc content in the ration of feed plus drinking water should not exceed legal limits. If these drinking waters with complementary feeds containing a high zinc level are used together with normal feeds, it may result in over dosage of zinc. In order to interpret these data it is crucial to know for what age group the drinking water is used (and whether it is all day available). In most cases, this information is lacking in the database.

## 4.3 Recommendations

1. Improvements in the delivery of sample data are necessary. Required are:
  - a. The exact age of pigs/piglets the feed is intended for
  - b. The recommended inclusion level of complementary feeds
2. Monitoring copper inclusion in the diet at the farm is more logical than at the feed industry because:
  - a. copper can also be added to the diet from other sources, such as water
  - b. only at the farm it can be verified whether high copper feed is being used in the intended period of life

This monitoring should also include liquid feeds, especially for pigs ( $\geq 12$  weeks).

3. It is advisable to investigate the homogeneous distribution of copper and zinc within feed batches. This includes:
  - a. The distribution of copper and zinc within a complete dry compound feed production.
  - b. The distribution of copper and zinc within the final feed, when using complementary feeds at the farm.
4. For copper, it is also useful to analyse the trends for feeds for sows (breeding) and feeds for fattening pigs separately.
5. Labelling
  - a. In general it should be monitored whether information on the label and instructions for use are complete.
  - b. Sheep feeds supplemented with copper are likely to contain more than 10 mg Cu/kg and should be labelled with 'the level of copper in this feeding stuff may cause poisoning in certain breeds of sheep'.
  - c. Sheep feeds supplemented with more than 5 mg Cu/kg are likely to contain more than 15 mg total Cu/kg and should be labelled as supplementary feed with instructions for the percentage in the ration or the daily allowance.

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