Biomonitoring of Cadmium in Pig Production

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Abstract


Cadmium is a nephrotoxic metal with increasing levels in arable soils. The non-smoking population is exposed to cadmium mainly from vegetable food, especially cereal products. The major part of pig feed is cereals, and accumulated cadmium in pig kidney could reflect cadmium in the local agricultural environment. In this thesis, the possibility to use pig kidney as a bioindicator of the availability of cadmium in the agricultural environment was evaluated.

There were significant correlations between cadmium levels in soil and wheat, between feed and kidney and between feed and faeces. Cadmium level in feed explained 12% of the variance of cadmium level in kidney. Cadmium levels in barley, the main ingredient in the feeds, were not correlated to feed or kidney.

The non-locally produced feed components rapeseed and soybean meal, vitamin-mineral mixtures and beet fibre contributed to a large extent to the cadmium in feed. The non-locally produced feed components constitute an external source of cadmium to the arable soils when farmyard manure is applied, as most cadmium in feed is excreted in faeces.

Pigs given feeds with less rapeseed and soybean meal and more cereals than controls, had lower cadmium intake, but higher cadmium levels in kidney than control pigs. This can partly be explained by different bioavailability of cadmium in different feed components. Cadmium level in kidney was positively related to age at slaughter and negatively related to kidney weight. No difference in kidney levels of cadmium due to sex was seen. Cadmium levels in kidney differed between breeds given the same feed.

Organically outdoor raised pigs had higher levels in kidneys and faeces than conventional pigs raised indoors, despite a lower cadmium level in the organic feed. The organic pigs were exposed to cadmium from soil via rooting. Differences in feed compositions and bioavailability of cadmium from the feed components may also explain the different kidney levels of cadmium. However, no significant difference in solubility of cadmium from the feeds after in vitro digestion was detected. When soil was added to the feed and digested in vitro, the fractional solubility of cadmium was decreased.

Animals from the same farm and raised under similar conditions had cadmium levels in kidney that could differ several times. This great variation together with the high cadmium contribution from non-locally produced feed components limit the possibilities to use cadmium in pig kidney as an indicator of available cadmium in the agricultural environment.

Key words: animal feed, bioindicator, ecological, environment, food chain, monitoring, porcine, renal, sustainable, swine

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Papers I-IV

The thesis is based on the following papers, which will be referred to in the text by their Roman numerals (I-IV):


III Lindén A, Olsson I-M, Bensryd I, Lundh T, Skerfving S and Oskarsson A. Monitoring of Cd in the chain from soil via crops and feed to pig blood and kidney. (*Submitted*).

IV Lindén A, Eklund G and Oskarsson A. Cd accessible for uptake after *in vitro* digestion of pig feed. (*Submitted*).

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Introduction

Cadmium is a toxic metal without any essential function in plants, animals or humans (Wagner, 1993). Cadmium accumulates in kidney which is the target organ for its toxicity (IPCS, 1992). Plant roots take up cadmium in soil and vegetable food is the main source of cadmium intake in non-smoking humans. Before the Second World War, the anthropogenic use of cadmium was limited (Bergbäck et al., 1994). The rate of total calculated cadmium emissions in Sweden rose from 13 t/year in 1940 to just over 50 t/year in 1970 after which it decreased to 20 t/year in 1990. The major uses of cadmium have been rechargeable nickel-cadmium batteries, pigments, stabilisers in plastics and protective plating for metals. The metal industry, the mining of zinc and lead ores and the manufacturing of phosphorus fertilisers have been the dominant sources of industrial cadmium emissions to the environment. The application of fertilisers contaminated with cadmium has also led to the deposition of significant amounts of cadmium on agricultural land (Bergbäck et al., 1994).

Humans today are exposed to cadmium close to the levels that affect the kidney function. In order to take effective measures to decrease cadmium levels in the environment and foodstuffs, knowledge of the sources and levels of cadmium in the agricultural environment is necessary.

The FAO/WHO Joint Expert Committee on Food Additives and Contaminants (JECFA) has established a provisional tolerable weekly intake of cadmium (PTWI). The PTWI is set to 7 µg/kg body weight, which corresponds to a daily intake of 60 µg (JECFA, 2001). Varying daily dietary intakes of cadmium have been reported, e.g. in US diets 13 µg (Iyengar et al., 2000), Swedish diets 9 µg (Jorhem et al., 1998), Belgian diets 23 µg (Van Cauwenbergh et al., 2000) and UK diet 12 µg (Ysart et al., 2000).

Human diet mostly has varying geographical origin and thus it is difficult to relate the dietary cadmium intake to cadmium levels in a certain area. It is not only important to know the cadmium levels, but also the bioavailability of cadmium – the fraction of cadmium that is taken up e.g. from soil to plants and from food in the gastrointestinal tract. Pigs are mainly fed cereals often locally produced. Cadmium levels in kidneys of growing/finishing pigs increased 2 % year\(^{-1}\) during the period 1984-1992 (Petersson Grawé et al., 1997). The increase could be due to increased cadmium levels in the feed and/or increased availability of cadmium in the feed. The impact of locally produced feed components on the kidney cadmium levels is not known. If the kidney cadmium levels are significantly related to the levels in locally produced feed crops, pig kidneys could be used as indicators of the availability of cadmium in the local agricultural environment.
Bioindicators

The use of living organisms as indicators of environmental pollution has the advantage of not only indicating the presence of a pollutant in the environment, but also the availability of the pollutant. A bioindicator can also provide information on long-term effects as some organisms preserve a continuous record of the environment throughout their lives (Whitfeld, 2001). To be a good bioindicator, changes in a response to a pollutant should be measurable with accuracy and precision and it should be possible to connect the changes to a cause. (Burger and Gochfeld, 2001).

Different organisms have been evaluated for biomonitoring of cadmium in the environment. In Denmark, cadmium levels in cattle kidney were higher in the southern than in the northern part (Gydesen and Rasmussen, 1981). A similar geographical pattern was seen for atmospheric deposition of cadmium, reflected by bulk precipitation and cadmium in epiphytic cryptogams. Milhaud and Mehennaoui (1988) investigated indicators of cadmium exposure in cattle in a polluted area and found that cadmium concentration in kidney was a good post-mortem indicator of exposure, whereas blood and hair cadmium levels were not useful indicators. Holm and González (1992) evaluated the use of goat liver as an indicator of cadmium pollution in Mexico. They found indications of increased contamination of cadmium and regional differences could be detected. Frank and Galgan (1997) found regional differences in cadmium uptake in moose kidney in Sweden and the cadmium levels in kidney could be related to the acidification in the area. They also found a decrease in cadmium levels in moose kidneys from 1982 to 1988. In Sweden, tissues from horse, sheep, lamb and reindeer were analysed for lead and cadmium (Jorhem, 1999). The major feed for reindeers is lichens, which is known to accumulate metals and cadmium levels in reindeer tissues were higher than in most other domestic animals. Dip et al. (2001) found different accumulation patterns of cadmium in kidneys from red foxes living in urban, suburban and rural areas. Urban foxes had lower cadmium levels in kidney than suburban and rural foxes.

Bioindicators are valuable tools for studying the biological implication of temporal and spatial trends and evaluate measures for reduction of pollution. A bioindicator of cadmium in the agricultural environment should also be relevant for the food production and human exposure of cadmium. Pig kidney could be such an indicator, as cereals are the main feed ingredient in pig feed and also the main source for cadmium exposure in humans (JECFA, 2001). In order to evaluate the suitability of a bioindicator an understanding of the factors that influence the levels of the pollutant in the animal is needed. Age is one factor that is of importance for cadmium monitoring and diet is another. Using pigs for biomonitoring has several advantages. There is a narrow range in age at slaughter for growing/finishing pigs. Pigs are raised
indoors and given feed mainly produced within a limited local area. Moreover, cadmium in pig kidney is analysed routinely as a part of the control program in many countries.

**Cadmium bioavailability from diet**

Oral bioavailability is the fraction of a dose absorbed systemically, determined by comparing the plasma area under curve (AUC) after intravenous and peroral dosing (Medinsky and Valentine, 2001). A large proportion of ingested cadmium passes through the gastrointestinal tract without being absorbed. Toxicokinetic studies with inorganic cadmium salts have shown that cadmium retention varies in the range of 3-7% in humans and monkeys (Groten and van Bladeren, 1994). The dietary uptake of cadmium occurs in two steps: Uptake from the intestinal lumen into the mucosa and passage across the basolateral membrane into the blood stream (Groten and van Bladeren, 1994), the second step being the rate-limiting one.

Retention and absorption of cadmium in the gastrointestinal tract is higher in younger than in older animals (Engström and Nordberg, 1979; Kostial et al., 1983; Sullivan et al., 1984; Eklund et al., 2001). The gastrointestinal absorption in pigs was 4% in neonatal and <1% in juvenile (11 weeks old) pigs (Sasser and Jarboe, 1980). Cadmium is mainly retained in the duodenum (Sørensen et al., 1993; Eklund et al., 2001). Iron status influences the intestinal absorption of cadmium. Women with low body iron stores have higher cadmium levels in blood and urine (Berglund et al., 1994; Olsson et al., in manuscript). High levels of calcium in diet decrease cadmium absorption (Brzoska and Moniuszko-Jakoniuk, 1998; Walter et al., 2000). Cadmium levels in blood are also influenced by genetic factors (Björkman et al., 2000).

Phytic acid (Myo-inositol 1,2,3,4,5,6, hexakis dihydrogen phosphate), has the ability to form metal-ion complexes, inhibiting the absorption of metals in the gastrointestinal tract (Torre et al., 1991). Phytic acid is present in plant seeds for storage of phosphate (Pallauf and Rimbach, 1997). In grain, phytic acid is mainly localised in the bran, and in legume seeds phytic acid accumulates in the cotyledon. Cadmium is one of the metals forming complexes with phytic acid (Nolan and Duffin, 1987; Persson et al., 1998; Wise and Gilburt, 1981). The binding of cadmium to phytic acid is dependent on pH with no binding occurring *in vitro* at pH below 3.5 (Persson et al., 1998). *In vitro* intestinal absorption of cadmium was reduced in the presence of phytic acid and calcium (Turecki et al., 1994). However, no reduction in availability of cadmium was seen *in vivo* in rat when phytic acid and cadmium was added to the diet (Rose and Quaterman, 1984; Turecki et al., 1995). The fractional accumulation of cadmium in liver and kidney was lower in rats fed wheat bran diets compared to those fed wheat endosperm (Moberg Wing,
1993) and also lower in liver of mice fed wheat bran compared to mice fed diets containing sugar-beet fibre, carrots or CdCl$_2$ where the cadmium levels in the diets were similar (Lind et al., 1998).

The enzyme phytase catalyses the stepwise removal of phosphate groups from phytic acid, decreasing the binding of cadmium. Phytases are present in plants, microorganisms and intestinal mucosa (Lei and Stahl, 2000). The significance of intestinal phytase activity is discussed. Hu et al. (1996) found the highest intestinal phytase activity in pig in jejunum and the activity was greater for hydrolysis of lower inositol phosphates. Lopez et al. (2000) reported an enhancement of mucosal phytase activity in rat small intestine from rats fed diets supplemented with phytic acid or wheat bran. Iqbal et al. (1994) found a low phytase activity in human intestines, with a limited ability to degrade phytates.

Microbial phytase added to pig feed has been shown to increase the gastrointestinal availability of phosphorus and other minerals such as Mg, Zn, Ca, and Cu (Adeola et al., 1995; Kemme et al., 1999; Jongbloed et al., 2000). The availability of cadmium could also increase. Pigs fed diets with phytase and reduced phosphorus and calcium content (Rimbach et al., 1996) and pigs fed diets with addition of phytase and cadmium (Zacharias et al., 2001), accumulated more cadmium in kidney than pigs fed diets without phytase. However, studies in rat showed no increased cadmium accumulation when phytase was added (Rimbach et al., 1998) The mechanisms responsible for changing the availability of cadmium due to dietary phytate and phytase are not clear (Pallauf and Rimbach, 1997).

**Cadmium in soil**

Cadmium has a natural occurrence in soil. The background level depends on bedrock and degree of weathering (McLaughlin et al., 1999). Besides the natural occurrence, there is an input of cadmium to soil through atmospheric deposition, and from application of phosphate fertilisers, lime, sewage sludge and farmyard manure. Long-term increasing trends have been reported from England (Jones et al., 1987), Norway (Jeng and Sing, 1995) and Sweden (Andersson, 1992). The cadmium concentration in Swedish arable soil varies widely and the mean level is 0.23 mg kg$^{-1}$ (n=3067, SD=0.17) (Eriksson et al., 1997). According to calculations by Eriksson (2000), there is a yearly increase in topsoil content of cadmium of 0.05%, at a farm with pig production. The sources of input are feed through farmyard manure, (38%), deposition (57%) and lime (5%). The calculated annual increase of cadmium in arable soil has decreased from 0.2% in 1992 (Andersson, 1992) and 0.1% in 1997 (Hedlund et al., 1997). There is still an increase, but a balance between input and output is approaching.
Phosphate fertilisers contain cadmium as a contamination originating from the parent rock. The cadmium levels vary widely depending on origin. In Sweden, maximum allowed cadmium concentration in phosphate fertilisers is 100 mg/kg phosphorus and when exceeding 5 mg/kg an environmental charge must be paid. Because of restrictions, the cadmium input from P-fertilisers has decreased and is now below 1 g ha\(^{-1}\) year\(^{-1}\) (Hedlund et al., 1997).

Sewage sludge is also a source of cadmium input to soil. There are restrictions concerning application of sewage sludge to arable soil. Maximum allowed level of cadmium is 2 mg/kg and maximum application of cadmium from sewage sludge is 0.75 g ha\(^{-1}\) year\(^{-1}\). In Sweden the mean cadmium level in sewage sludge is 1.4 mg/kg dry weight. (n=48, SD=1.5) (Eriksson, 2001).

The cadmium concentration in animal manure depends on the levels in feed and animal species (Steineck et al., 1999). Cadmium concentration in pig manure was significantly higher than from dairy cow manure. In Austria, a mean cadmium level of 0.39 mg/kg dry weight was reported in pig manure (Spiegel et al., 1999) and Eriksson (2001) reported levels of 0.25 mg/kg dry weight.

**Organic farming**

In Sweden, the Certification Organisation for Organic Production (KRAV) sets up standards for organic farming, which follow the European Council Regulations of organic farming (EC Regulation 2092/91 and 1804/99). In organic farming, the use of mineral fertilisers is restricted. Mineral fertilisers can be applied only in natural form (stone meal, raw phosphate, apatite, limestone meal, calcified seaweed, wood ash and dolomitic limestone). The EC regulation has a maximum limit for cadmium in raw phosphate of 90 mg cadmium/kg \(P_2O_5\), corresponding to 206 mg cadmium/kg phosphorus. The use of many different types of organic fertilisers is permitted e.g. farmyard manure, straw, green plant material and compost. The total input of cadmium allowed in average over a five year period is 0.75 g ha\(^{-1}\) year\(^{-1}\) ([http://www.krav.se/regler2002/regler2002.pdf](http://www.krav.se/regler2002/regler2002.pdf): Accessed 11-march-2002).

**Cadmium in plants**

Cadmium that has weathered from the parent material or dissolved from solid phase in fertilisers or sludge exists in solution mostly as the divalent cation, \(Cd^{2+}\) (McLaughlin et al., 1999). Compared to many other metals, cadmium is readily taken up by plants and plant uptake is influenced by many factors. Cadmium concentration in soil is one factor. In Sweden, cadmium level in winter wheat doubled from 1918 to 1980 (Andersson and Bingefors, 1985). The calculated increase of cadmium level in arable soil from 1900 to 1990 was 33% (Andersson 1992) Other factors than cadmium level in soil are of importance for cadmium uptake in plants. Soil pH influence the uptake, with
higher uptake in plants at lower pH (Van Bruwaene et al., 1986; Alloway et al., 1990; Öborn et al., 1995; Eriksson et al., 1996; Oliver et al., 1998; Wenzel et al., 1996; Mench et al., 1997; Puschenreiter and Horak, 2000). Organic matter content is negatively associated with the uptake of cadmium in plants (Eriksson, 1988; Mench et, 1997). The soil solution composition is of importance. Cadmium uptake in crop is enhanced by Cl\(^-\) and decreased by Ca\(^{2+}\) (Wenzel et al., 1996). Cadmium levels differ widely between plant species. In general, cereal grain has low cadmium content (30-90 µg/kg) compared to root crops (100-700 µg/kg) grown in parallel (Sillanpää and Jansson, 1991) and wheat grain has higher cadmium levels than barley grain which also was found by Gray et al. (1999). Cultivar can also influence cadmium level in a species. Wenzel et al. (1996) reported cadmium levels in winter wheat grain to vary up to a factor of 1.8 and a factor of 2.4 in spring durum wheat. Cadmium uptake in maize cultivars grown on a contaminated soil varied by a factor of 20 (Kurz et al., 1999).

Mean cadmium levels in winter wheat (n=606), oats (n=208) and barley (n=327) in Swedish samples from 1992 to 1998 were 44, 36 and 19 µg/kg dry weight, respectively (Eriksson et al., 2000). Varying levels of cadmium have been reported in organically produced wheat, 21-66 µg/kg (Jorhem and Slanina, 2000; Salomonsson et al., 1995).

**Pig feed**

Pig feed consists of cereals that often are grown at the farm complemented with protein rich components, vitamins, minerals and amino acids. These ingredients can be purchased as ready-made mixtures, so called concentrates. In Sweden, common feed crops are barley, wheat, oats and triticale, and protein rich components used are e.g. rapeseed meal, soybean meal and peas (Anonymous, 2001). The maximum allowed cadmium level in feed is 0.5 mg/kg in feed mixture, 1 mg/kg in vegetable feed raw material, 10 mg/kg in phosphates and 5 mg/kg in mineral mixtures (SJVFS 1993:177).

Cadmium levels in pig feed reported in the literature vary widely from 25 to 18,000 µg/kg (Ostertag and Kreuzer, 1980; Sharma et al., 1982; Amodio-Cocchieri and Fiore, 1987; Bache et al., 1987; Nicholson et al., 1999; Ulrich et al., 2001; Sapunar-Postruznik et al., 2001). However, there is often a lack in information on feed composition, cadmium levels in the separate feed components, or the limit of detection is high, which makes it difficult to compare feeds and draw conclusions on the sources of contamination.

The phytic acid concentrations vary in different feed components. Barley and wheat have relatively low phytic acid levels (3.8-8.2 and 4.9-9.3 g/kg, respectively) and soybean meal and wheat bran have higher phytic acid levels (25-50 and 13-21 g/kg, respectively) (Garcia-Estepa et al., 1999; Kasim and
Edwards, 1998; Helander *et al.*, 1994). The phytic acid concentration in rapeseed meal varies depending on cultivar and phosphorus supply (2.8-40 g/kg) (Lickfett *et al.*, 1999). Wheat and barley have high phytase activities (400-1600 units/kg). Wheat bran has phytase activity of about 4600 units/kg, while soybean and rapeseed meal have low activities (0-50 units/kg) (Eeckhout and De Paepe, 1994; Viveros *et al.*, 2000).

**Cadmium in pig kidney**

In most experimental studies on cadmium in pig kidney, the feeds have been supplemented with very high levels of cadmium. The uptake of cadmium from feed and distribution to tissues in swine has been investigated by Sharma *et al.* (1982). A control feed (0.2 mg Cd/kg) and two feeds supplemented with cadmium (2 and 10 mg/kg) were given to pigs for three or six months. Cadmium was accumulated in kidney and liver with highest levels in kidney. The levels in kidney remained nearly at the same level throughout a 3 month depletion period (Sharma *et al.*, 1982). Cadmium added to pig feed as cadmium chloride or rock phosphate did not affect growth rate at levels up to 4.4 mg Cd/kg feed (King *et al.*, 1992). The concentration of cadmium in kidney and liver showed a linear relationship to dietary cadmium level. The retention of cadmium from cadmium chloride tended to be greater than from rock phosphate. The impact of copper, zinc and vitamin C in feed on cadmium level in kidney has been investigated when 1 mg cadmium/kg feed was added as cadmium chloride. Copper supplement (50 to 200 mg/kg feed) resulted in higher cadmium levels in kidney and vitamin C counteracted the copper induced increase of cadmium in kidney. Zinc supplement did not counteract the effect of copper (Rothe *et al.*, 1994). Effects of phytic acid and phytase on cadmium levels in kidney have been described in the section *Cadmium availability from diet*.

Cadmium in food producing animals is analysed within the food control programme in many countries. Levels in pig kidney from 147 to 13000 µg/kg have been reported during the period from 1975-1998 in Europe and the US (Ostertag and Kreuzer, 1980; Penumarthy *et al.*, 1980; Vos *et al.*, 1986; Amodio-Cocchieri and Fiore, 1987; Niemi *et al.*, 1991; Coleman *et al.*, 1992; Falandysz 1993; Soares *et al.*, 1995; Doganoc 1996; Sinigoj-Gancnik and Doganoc, 2000; Sapunar-Postruznik *et al.*, 2001; Ulrich *et al.*, 2001). Animal age and sampling of kidney varied, or information was lacking, which makes it difficult to compare the results from the different studies. The importance of sampling technique of kidney on cadmium levels has been demonstrated by Olsson and Oskarsson (2001). Most studies did not have information on feed composition or cadmium levels in the feed.
Aims of the thesis

The overall aim of the thesis was to evaluate the possibility to use pig kidney as a bioindicator of available cadmium in the agricultural environment. To do that, the following parameters were investigated:

• Correlation of cadmium levels between steps in the chain from soil, via crops and feed to pig blood and kidney and impact of geographical location.

• Cadmium levels in pig feed and feed ingredients and the cadmium contribution from separate feed ingredients.

• The impact of cadmium concentration in feed, feed composition, age at slaughter, pig breed, sex and kidney weight on cadmium level in pig kidney.

• Comparison of cadmium levels in organic pigs raised outdoors and conventional pigs raised indoors.

• The solubility of cadmium from different feeds and feed components after \textit{in vitro} digestion.
Materials and methods

Animal husbandry and pig feed

Paper I
Ninety-six pigs were raised indoors in boxes, eight animals per box, at the Funbo-Lövsta Research Station, Swedish University of Agricultural Sciences, Uppsala, Sweden. 48 pigs were given a conventional feed (control feed) and 48 were given a feed with adjustment of the protein content to the need of the pigs (phase feed) (Table 1). The phase feed consisted of two feed mixtures, one with high and one with low protein content. The proportions of the feed mixtures changed during the growing period. In each feed group 19 pigs were purebred Swedish Yorkshire and 29 pigs were crossbred Swedish Yorkshire/Hampshire. The pigs were slaughtered after reaching 105 kg live weight at the age of five to six months. The feed composition of the control feed is shown in Table 1. The composition of the phase feed changed during the experiment and is presented in Paper I.

Paper II
Eighty pigs were raised at the Swedish University of Agricultural Sciences Experimental Station in Bjertorp, in the county of Västergötland, Sweden, from approximately 28 to 107 kg live weight. [(Swedish landrace x Swedish Yorkshire) x Hampshire] were used in the study. Forty pigs were organically raised outdoors together in one group in an area of about 6000 m² with a brook, and given a feed fulfilling the criteria for organic pig production. Wheat, oats and peas were KRAV certified. The 40 conventional pigs were raised indoors, eight animals per box, and were given a conventional feed. The organic pigs were supplied from one herd and the conventional pigs came from six different herds. The organic pigs were weaned at the age of 8-9 weeks and the conventionally raised pigs at about 5 weeks of age. The compositions of the dietary mixtures are presented in Table 1.

Paper III
Eight hundred growing/finishing pig producers were randomly selected from a total of 2,400 producers in Skåne, registered at Statistics Sweden (SCB), July 1, 1997. A first questionnaire about the farm and the residents’ food and smoking habits was sent to the farmers in February 1998. Complete answered questionnaires were returned from 465 (58%) farmers, of which 224 volunteered to participate in a more detailed study. From this group, 51 farms were selected. The selection criteria were a) production of more than 50% of the feed at the farm, b) both man and woman at the farm willing to participate and c) both being non-smokers. The two latter criteria were chosen because of a parallel study on cadmium exposure in men and women living on the farms.
(Olsson et al., in manuscript). Two farms dropped out from the study during the sampling period, resulting in 49 farms participating throughout the study. The participants answered a second questionnaire with detailed questions about the feed and pig production. Five to ten pigs from each of the 49 farms in Skåne were sampled. All pigs were raised indoors. At 45 farms, the pigs were given feeds partly produced at the farm and four farms had no own feed production. The pigs were three breed crossings. The average composition of the feeds is shown in Table 1.

**Paper IV**

In paper IV, feeds from Bjertorp (Paper II) were used. The feed mixtures, wheat, vitamin-mineral premixes, potato protein, beet fibre, rape seed meal and wheat bran were selected.

<table>
<thead>
<tr>
<th>Feed component</th>
<th>Control feed, Funbo-Lövsta Paper I</th>
<th>Conventional feed, Bjertorp Paper II</th>
<th>Organic feed, Bjertorp Paper II</th>
<th>Feed average Skåne Paper III</th>
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<tr>
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<tr>
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<tr>
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<tr>
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<td>5</td>
<td></td>
</tr>
</tbody>
</table>
Sample collection

All vessels used to collect samples were acid washed before use to avoid contamination.

*Feed, straw, faeces, soil and water*

Paper I and II

Samples of feed components and the feed mixtures were supplied directly from the feed factories. Straw, water, faeces, and soil were sampled at the research stations.

Paper III

Feed, feed components, straw, faeces and water were sampled at the farms. It was not possible to collect all the separate feed ingredients at the farms. At most farms, cereals and concentrates were collected. Four feeds were liquid feeds. Two faeces samples were taken from the pen were the pigs for the study were held at each farm. Cadmium levels in soils were interpolated values from a mapping of arable soils in Skåne (Eriksson et al., 1997) by the geographical location of the farm building.

*Blood, kidney and liver*

Blood from five to ten pigs per farm was sampled in heparinised Vacutainer® sample tubes (Paper III). Blood samples were taken within a week before slaughter. At slaughter, one kidney per pig was sampled (Paper I-III) and about 250 g liver (Paper II).

Sample preparation

*Paper I-III*

Before sample preparation and analysis, all vessels and utensils were acid washed to avoid contamination. Feed, dried faeces, kidney and liver samples were microwave digested in a temperature controlled closed vessel system with a time-effect programme (Milestone mls 1200 mega). Duplicate or triplicate samples (0.2-2 g) were weighed into Teflon digestion vessels and 3.0 mL concentrated nitric acid and 2.0 mL hydrogen peroxide, were added (feed and faeces) or 5 mL concentrated nitric acid (kidney and liver). The liver samples were taken in the middle of one lobe and the kidney samples were taken in the outer part of cortex (Figure 1, Paper I). The digested samples were transferred into 50 mL polypropylene sample tubes and the Teflon vessels and lids were rinsed with 0.1 M nitric acid. The rinse solution was also added to the sample tubes. The samples were then diluted to 10-50 mL with 0.1 M nitric acid. The digested and diluted samples were weighed. To calculate the volume of the samples, the densities of the digested samples were determined by weighing a definite volume.
Two extraction methods were used for the soil samples: extraction in 7 M nitric acid and extraction in 0.5 M NH₄Ac + 0.02 M EDTA, pH 4.65.

**Paper IV**

Feed samples were weighed into 50 mL polypropylene sample tubes (0.2-2g). An extra set of replicates of organic feed was prepared with an addition of 12% (w/w) soil to simulate the organic pigs soil ingestion via rooting (Fries et al., 1982). After addition of 10 mL freshly prepared gastric juice to each feed sample, the tubes were incubated on rocking tables at 37°C for 4 hours. The pH was kept below 3.5 by adding 4.75 M HCl. After 4 hours, half of the twelve replicates were centrifuged and the supernatants were analysed for cadmium. To the remaining sample tubes, saturated NaHCO₃ solution was added to adjust pH to 7.4 after which 10 mL freshly prepared intestinal juice was added. The samples were incubated at 37 °C for 4 hours and after centrifugation the supernatants were analysed for cadmium. The rapeseed meal and wheat bran samples were treated in the same way, except that phytase also was present in the gastric juice, corresponding to an addition of 1000 U/kg. Five and ten mL of gastric and intestinal supernatant respectively, were transferred to Teflon digestion vessels. Two mL concentrated nitric acid and 1 mL 30% H₂O₂ were added and the samples were microwave digested.

**Cadmium analysis**

*Feed, kidney, liver, faeces (Paper I-III) water (Paper I-II) and in vitro digest samples (Paper IV)*

The sample solutions of feed, kidney, liver, faeces, soil and digests were analysed for cadmium by atomic absorption spectrometry, graphite furnace technique with Zeeman background correction (Perkin Elmer 4100 ZL). In paper I, the feed and kidney samples were analysed by a standard addition technique and in the other studies linear calibration was used for all types of samples. Peak area was measured at 228.8 nm for five seconds.

The water samples were analysed for cadmium directly without any sample preparation. The atomic absorption spectrometer was programmed to inject water samples three times and to dry the sample after each injection to concentrate the water sample.

**Blood and water, Paper III**

Blood and water samples were analysed by inductively coupled plasma mass spectrometry (ICP-MS) at the Department of Occupational and Environmental Medicine, University Hospital, Lund, Sweden. Pig blood was analysed according to Barany et al., (1997). The blood was diluted ten times with a solution containing 5 g/L of 25% ammonia, 0.5 g/L Triton X-100 and 0.5 g/L EDTA in ultra pure water. To a 2 mL water sample, 50 µL concentrated nitric
acid was added. Internal standards, (Sc, In, Bi, and Ga) were added to all samples. All samples were prepared in duplicate.

The instrument was a PQ2+ from Thermo Elemental (Winsford, Cheshire, UK) with a Gilson 222 autosampler (Gilson, Villiers, France). Sample introduction was in a segmented-flow mode. The samples were analyzed in the peak-jumping mode (3 points per peak). Interference corrections were made for $^{114}$Cd (corrected for spectral overlap from Sn, measured at 118 m/z). For calibration, the procedure in the PQ2+ software was used. One-point calibration curves were obtained using outdated human blood from donors and 2% nitric acid. For all sample results, a reagent blank was subtracted.

**Quality control and limit of detection**

The accuracy of the cadmium determinations was checked on a wheat flour reference sample (Certified Reference Material, Wheat flour GBW8503; Cereal and Oil Chemistry Institute, Ministry of Commerce, Beijing, China), a pig kidney reference sample (Certified Reference Material, Lyophilized pig kidney, BCR 186; Community Bureau of Reference - BCR, Brussels, Belgium), a bovine liver reference sample (Certified Reference Material, Lyophilized bovine liver, BCR 185; Community Bureau of Reference - BCR, Brussels, Belgium), Riverine Water Reference Material for Trace Metals (SLRS-2; National Research Council, Ottawa, Canada) and human blood reference samples (Seronorm 404107 and 404108). The results are shown in Table 2.

<table>
<thead>
<tr>
<th>Table 2. Results from analysis of certified reference materials</th>
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<tbody>
<tr>
<td>GBW 8503 (µg/kg)</td>
</tr>
<tr>
<td>Certified</td>
</tr>
<tr>
<td>Paper I</td>
</tr>
<tr>
<td>Paper II</td>
</tr>
<tr>
<td>Paper III</td>
</tr>
</tbody>
</table>

*Recommended values

The limits of detection (three standard deviations of at least 20 reagent blanks) were 0.09-0.12 µg/L for GFAAS-analyses. The detection limits for water and blood analysed with ICP-MS were 0.007 and 0.05 µg/L, respectively.

Our laboratories have participated in proficiency testing of trace elements in foods, organised by the Swedish National Food Administration, with a mean Z-score of –0.7 (n=5) (Jorhem and Merino, 1997; Jorhem and Engman, 1999; Sundström and Jorhem, 1999; Åstrand and Jorhem, 2000, 2001) and the laboratory in Lund participated once every month in an intercomparison programme from United Kingdom external quality assurance scheme.
(UKNEQAS, Birmingham UK). The median divergence from the target values were 3% (range 0.2% - 8%, n=17) for values ranging from 1.65 to 9.51 μg Cd/L. The precision for kidney concentrations expressed as the RSD, were 3.9, 3.7 and 5.4% in paper I, II and III, respectively.

Statistics

The Statview 5.0 software was used for statistical evaluation of the data. All results were included in the calculations, even the results below the formal detection limit, so that the distributions would not be distorted. Unpaired t-test was used for two-group comparison (Paper I-III) and Mann-Whitney U-test (Paper IV). Homogeneity of variance was tested with Bartlett’s test and Analysis of variance (ANOVA) was used in cases with homogeneous variance and Welch's test in cases with heterogeneous variance. Post hoc testing was performed with Sheffé's F where p<0.05 in ANOVA or Welch's test. In paper III, the individual kidney cadmium levels from each farm were used in the calculations and treated as a block variable in the ANOVA. Correlation was tested with Fisher's r to z. In paper III, the variables cadmium levels in kidney, blood, water and soil were log transformed. Furthermore, software Unscrambler 7.0 was used for multivariate evaluation of data in Paper III by principal component analysis.

Results and discussion

Cadmium in the chain from soil via crops and feed to pig blood, kidney and faeces

Correlations

Significant correlations between cadmium in soil and wheat, wheat and barley, as well as between feed and kidney and feed and faeces were found (Paper

![Diagram of cadmium levels in the chain from soil to faeces.](image)

Figure 1. Correlations of cadmium levels in the chain from soil to faeces. Full lines indicate significant correlations and dotted lines no correlation (p-value within parenthesis).
Cadmium level in concentrate was correlated to cadmium levels in feed and kidney. Cadmium levels in barley, the main ingredient in the feeds, were not correlated to soil, feed or kidney (Figure 1).

**Principal component analysis**

The same variables, except for water and blood, were analysed together in a principal component analysis. Blood and water were excluded because the levels were low and many samples were below the detection limit. The loading plot from the PCA showed that soil, wheat and barley were associated and mainly explained by principal component 2 (PC 2) (Figure 2). These variables were separated from and not correlated to cadmium levels in kidney, concentrate, feed and faeces, mainly explained by principal component 1 (PC 1). PC 1 and 2 explained 38 and 26% of the variation, respectively.

**Geographical variations**

Geographical variations of the variables in the loading plot (Figure 2) analysed with PCA are shown in the score plot (Figure 3). The 49 farms were divided into four groups according to their geographical location in Skåne. Each marking represents one farm and the geographical location (NE=Northeast, NW=Northwest, SE=Southeast, SW=Southwest). It is shown that some farms in the Southeast had high cadmium levels in soil and crop (positive value of PC 2). However, the cadmium levels in feed and kidney of Southeastern farms were not generally high, but are spread along PC 1 from negative to positive. The Northeastern farms had generally high levels in kidney (negative PC 1) and generally low levels in crop and soil (negative PC 2). The other farms had no clear tendencies and were more evenly distributed in the score plot.
Cadmium from soil to crop

The cadmium levels in soils were not analysed values from the farms, but interpolated values from a mapping of arable soils in Skåne (Eriksson et al., 1997). The significant correlation between cadmium levels in soil and wheat ($p=0.02$, $r=0.46$) indicate that the interpolated values are relevant. However, barley was not correlated to the soil ($p=0.8$). The lack of correlation could be due to that other factors than cadmium concentration in soil are of importance for the levels in crops, or a statistical Type II error. Different cultivars of barley might have been used that can explain why there was no significant correlation between cadmium in soil and barley. However, there was an association between cadmium in barley and wheat ($p=0.0002$, $r=0.67$). Only 26 wheat samples were analysed compared to 44 barley samples. When correlation was tested between barley and soil from the same 26 farms as wheat and soil correlation was tested, still no correlation was found between cadmium in barley and soil. Thus, the different results of correlation to soil for wheat and barley were not due to a different sample selection. However, when all cadmium level variables (soil, wheat, barley, feed, concentrate, kidney and faeces) were analysed together in the PCA, barley, wheat and soil were grouped together, indicating a correlation.

Cadmium contribution from feed components to feed

Cadmium levels in feed mixtures from Funbo-Lövsta, Bjertorp and Skåne ranged from 13 to 84 µg/kg. The main part of the feeds consisted of cereals (Figure 4). The cereal part was about 70-80%. There was no correlation in cadmium levels between feed and barley or between feed and wheat ($p=1.0$) (Paper III).
The type of cereals and the supplementing ingredients varied in the feeds. Barley, oats, triticale and wheat had generally low cadmium levels (4-69 µg/kg) compared to other ingredients in the feeds. The lowest cadmium level in cereal was found in barley, and the highest in wheat. The other crop products (soybean meal, rapeseed meal, potato protein and beet fibre), usually not produced at the farm, had cadmium levels from 14 to 370 µg/kg. In paper III, most farms supplemented the cereals with concentrate. High cadmium levels were found in the concentrates, ranging from 42-630 µg/kg. In Figure 4, the components are grouped into three categories: cereals, concentrate (protein feedstuff, vitamins, minerals, amino acids, lime and phosphate) and beet fibre (in Bjertorp conventional only). The results from paper III are based on mean values from 10 farms where it was possible to calculate cadmium contribution from different feed components. The cadmium contribution from the cereals varied from 30 to 60%. The conventional feed in paper II contained beet fibre, which contributed 38% of the cadmium content. Due to the high cadmium levels in concentrates compared to cereals, the concentrates had a significant contribution of cadmium to the feeds and the cadmium levels in concentrates were correlated to cadmium in feed (p=0.009, r=0.44) (Paper III).

Figure 4. Proportional composition of feed mixture and cadmium contribution of feed components

Cadmium from feed to blood and from blood to kidney
There were no significant relationships, either between cadmium levels in feed and in blood, or between cadmium in blood and in kidney. One reason may
be the very low levels in blood and the limitations of the analytical method, as many samples were below the detection limit. The lack of correlation between feed and blood may also be due to the relatively low cadmium levels in feed and the low and varying gastrointestinal absorption of cadmium. Because of the slow turnover of cadmium in kidney, the kidney reflects long-term exposure, which also may explain the lack of correlation between cadmium in blood and kidney.

**Cadmium from feed to pig kidney**

There was a significant linear relationship between the cadmium level in feed and cadmium level in kidney ($p<0.0001$) (Paper III). The cadmium level in feed explained 12% of the variance of cadmium level in kidney. However, there was no correlation between cadmium level in kidney and barley ($p=0.7$) or between kidney and wheat ($p=0.7$), explained by the low contribution of cadmium from cereals to the feed and the lack of correlation between cereals and feed. Concentrates had a higher contribution of cadmium to the feed than cereals and cadmium levels in concentrates were significantly correlated to levels in kidney ($p=0.04$).

Figure 5 shows a regression plot of cadmium level in kidney vs cadmium level in feed, with mean values from paper I-III and literature data. The relationship between cadmium level in feed and kidney was described by the equation: $Y=79+1.4X$ ($p<0.0001$, $R^2=0.52$). That equation could be compared to the equation of the relationship between cadmium in feed and kidney from Skåne using unlogged values from kidney: $Y=93+1.2X$ ($p<0.0001$, $R^2=0.09$).

![Figure 5. Regression plot of cadmium in kidney vs cadmium in feed. Data from paper I-III and literature.](image-url)
The cadmium levels in kidneys were significantly higher in pigs from Skåne than from Funbo-Lövsta and Bjertorp (Welch’s test p=0.0000, Sheffé’s F p<0.0001), despite that the cadmium levels in feed did not differ much (Figure 6). This can partly be explained by the relatively low impact of cadmium levels in feed on levels in kidney. Ulrich et al. (2001) found cadmium levels in pig kidney comparable to the levels found in paper I, despite that cadmium levels in the feed given to the pigs in that study was twice as high as in paper I. Figure 6 shows a boxplot of cadmium levels in kidneys from paper I-III.

Cadmium from feed to faeces and back to soil
There was a significant relationship between cadmium levels in faeces and feed (p<0.0001), with 63% explained variance. Due to the low gastrointestinal absorption, most cadmium in feed components is excreted in faeces, explaining the strong relationship. The cadmium levels in faeces were 266, 223 and 280 µg/kg dry weight from Bjertorp organic, conventional and Skåne pigs, respectively. These levels are in accordance with the levels reported by Eriksson (2001) of 250 µg/kg dry weight pig manure.
In organic farming, the maximum limit of cadmium application to arable soil is 0.75 g ha\(^{-1}\). A maximum of 2.8 tonnes dry weight manure ha\(^{-1}\) with a cadmium level of 266 µg/kg dry weight, can be applied to the soils in order not to exceed the maximum limit.

Manure application of 5 tonnes dry weight year\(^{-1}\) ha\(^{-1}\) to arable soil has been reported from Italy (Saviozzi et al., 1999). That application of manure with a cadmium concentration of 266 µg/kg dry weight would correspond to 1.3 g cadmium ha\(^{-1}\).

The cadmium levels in the feeds in these studies were far below (about ten times) the maximum permitted limit in Sweden (SJVFS 1993:177). If the cadmium levels in feed were as high as the permitted 500 µg/kg, and the intestinal absorption in pigs would be the same as from the feeds used in the present studies, cadmium levels in faeces would be ten times higher. Application of that manure to soils would make a considerable contribution of cadmium to the soils.

**Cadmium levels in non-cereal feed components**

The highest concentrations of cadmium were found in the vitamin-mineral premixes. Totally six vitamin-mineral premixes have been analysed and the cadmium concentrations varied from 500-1000 µg/kg except for one mixture containing 70 µg/kg. Lately, a few studies have drawn attention to cadmium levels in feed additives such as minerals and phosphates and reported very high cadmium levels: 800 µg/kg (Nicholsson et al., 1999), 480 and 898000 µg/kg (Sapunar-Postruznik et al., 2001), 16000 µg/kg (King et al., 1992). The premix analysed by Sapunar-Postruznik et al. (2001) contained heavily contaminated Zn-salt. Median cadmium levels of lime and different phosphates added to feed have been reported: 230, 1560, 330, 3270 and 5320 µg/kg in lime, dicalciumphosphate, dimagnesiumphosphate, magnesium-phosphate and monocalciumphosphate, respectively (Spiegel et al., 1999). With the exception for the premixes containing 898,000 µg/kg and 16,000 µg/kg, the ingredients have levels below the Swedish maximum permitted level (SJVFS 1993:177). Cadmium from these components is an input of cadmium to the farm and to the arable soils when farmyard manure is applied as discussed above.

It was found that cadmium levels in rapeseed and soybean meal could vary up to a factor 3 and 7, respectively (Paper I and II). The differences could be due to conditions at the growing area and cultivar. These variations show that it is possible to decrease the cadmium levels in feed by choosing products from crops with lower cadmium levels.
Feed components with generally low cadmium levels were: peas, fat, amino acids, molasses and salt. These products constituted a minor part of the feeds. The cadmium levels in water were also low. Many of these samples had levels below the limit of detection. Cadmium in water did not significantly contribute to the cadmium intake in pigs.

Impact of age, breed, sex and kidney weight on cadmium levels in kidney

Age at slaughter

In paper I, the age of the pigs at slaughter was known. A significant linear relationship between cadmium levels in kidneys of all pigs and age at slaughter was found (p=0.01), R²=0.07. For each additional week of survival, the cadmium levels in kidney increased 2.8 µg/kg wet weight.

Breed

Analysis of variance showed that both feed and breed affected the cadmium levels in kidney (p=0.05 and 0.0005, respectively) (Paper I). The differences between breeds could be an effect of age at slaughter, as the purebred Yorkshire had a somewhat slower growth rate than the crossbreds. However, there may be genetical differences in the gastrointestinal absorption and renal accumulation of cadmium. Björkman et al., (2000) found that genetic factors, possibly related to uptake and storage, were of importance for cadmium levels in blood in humans.

Max/min ratios of cadmium levels in kidney for pigs that have been given the same feed and grown up in the same environment was calculated and are shown in Table 3. The 95%ile/5%ile ratio was also calculated and did not differ to a high extent from the max/min ratio, indicating that no extreme individuals were included in the max/min ratio. Pigs from the same litter, given the same feed in Paper I had lower max/min ratios than the pigs in the whole groups. The mean ratio was 1.4 ranging from 1.0 to 1.9. Totally, pigs from 12 litters were used in the study and the groups of pigs from the same litter given the same feed were small (2-5 pigs per group). The comparisons of max/min ratios indicate that genetic factors could influence cadmium levels in kidney. However, it should be noted that the groups were small. Pigs from Skåne were heterogeneous concerning both feed and breed and are not included in the table. However, from each farm a max/min ratio was calculated. The ratios ranged from 1.2 to 3.6 and were on average 2.1.
Table 3. Max/min and 95%ile/5%ile ratios of cadmium levels in kidney from pigs given the same feed.

<table>
<thead>
<tr>
<th>Feed</th>
<th>Breed</th>
<th>n</th>
<th>Paper</th>
<th>Max/min ratio</th>
<th>95%ile/5%ile ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Yorkshire</td>
<td>19</td>
<td>Paper I</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>Control</td>
<td>Yorkshire/Hampshire</td>
<td>29</td>
<td>Paper I</td>
<td>2.5</td>
<td>2.1</td>
</tr>
<tr>
<td>Phase</td>
<td>Yorkshire</td>
<td>19</td>
<td>Paper I</td>
<td>1.9</td>
<td>1.6</td>
</tr>
<tr>
<td>Phase</td>
<td>Yorkshire/Hampshire</td>
<td>24</td>
<td>Paper I</td>
<td>2.7</td>
<td>2.0</td>
</tr>
<tr>
<td>Conventional</td>
<td>3-breed</td>
<td>40</td>
<td>Paper II</td>
<td>2.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Organic</td>
<td>3-breed</td>
<td>37</td>
<td>Paper II</td>
<td>2.2</td>
<td>1.9</td>
</tr>
</tbody>
</table>

**Sex**

No significant differences of cadmium levels in kidney between castrated male and female pigs were found (Paper I and II). Castrated male pigs and female pigs evidently did not differ in gastrointestinal uptake and retention of cadmium in kidney. Differences of cadmium level in kidney between sex have been reported for cattle, with higher levels in kidneys from female than from male calves aged 6 to 10 months (López Alonso, et al., 2000).

**Kidney weight**

The weights of the kidneys were registered in paper II and III. The weights ranged from 118 to 250 g in paper II and from 78 to 299 g in paper III. There were negative significant relationships between cadmium level in kidney and kidney weight ($R^2=0.28$ and $R^2=0.14$ for organic and conventional pigs, respectively) and a positive relationship in Paper III, $R^2=0.01$. The organic pigs came from the same herd, the conventional came from six different herds and in paper III the pigs came from many different herds, which might explain the low coefficient of variance in that study.

**Impact of feed composition on cadmium availability**

The two feeds in Paper I were composed of the same ingredients, with the exception of wheat that was included in phase feed only. The proportions of the feed ingredients differed. Totally, the phase fed pigs had more cereals and less rapeseed and soybean meal than the control fed pigs. The phytic acid content and phytase activities varies in different feed components. Barley and wheat have quite low phytic acid content and soybean meal has higher phytic acid content (García-Estepa et al., 1999; Kasim and Edwards, 1998; Helander et al., 1994). Wheat and barley have high phytase activities while soybean and rapeseed meal have low phytase activities (Eeckhout and De Paepe, 1994). A high intrinsic phytase activity in the feed may partly explain why the phase fed pigs had higher cadmium levels in kidney despite a lower dietary intake compared to the control pigs. However, in Paper IV, treatment with microbial phytase resulted in unchanged and decreased solubility of cadmium from
rapeseed meal and wheat bran, respectively. The effect of microbial phytase addition to feed on cadmium availability is not clear. Increased cadmium accumulation in pigs fed phytase supplemented feed have been reported (Rimbach et al., 1996; Zacharias et al., 2001) but no effect on cadmium accumulation in rats was seen (Rimbach et al., 1998). Phytases from different microbial origins have different properties (Igbasan et al., 2000) and it is possible that intrinsic plant phytase differs from added microbial phytase. The pigs given phase feed had a higher cadmium intake in the beginning of the growing/finishing period compared to controls. A possible higher absorption of cadmium at younger age could also explain the higher cadmium levels in kidney.

The conventional and organic pigs in paper II were given different feeds. Again, the pigs with the lowest cadmium level in feed had highest levels in kidneys. This difference can partly be due to the different feed ingredients. Wheat bran has been shown to decrease intestinal cadmium absorption in rats (Moberg Wing, 1993; Lind et al., 1998). The conventional feed contained 10% wheat bran, which contributed 14% of dietary cadmium intake. It is possible that cadmium in wheat bran has a low availability and may decrease cadmium absorption from other dietary sources. The conventional feed also contained beet fibre and beet fibre pectin has been shown to decrease levels of divalent metals in rat serum after six weeks of administration (El-Zoghbi and Sitohy, 2001). In paper IV, the solubility of cadmium was highest from beet fibre. However it is possible that cadmium in the supernatant bound to soluble fibre, not available for uptake, was analysed. No difference in solubility of cadmium after in vitro digestion between organic and conventional feed could be detected (p=0.90 and p=0.85 after gastric and intestinal digestion, respectively). With the in vitro digestion method, cadmium accessible for uptake was analysed, not the actual uptake. The gastrointestinal absorption is a complex processes that is difficult to simulate.

Impact of production system on cadmium levels in kidney and liver

In paper II an organic and a conventional pig feed were used. The organic feed contained organically produced wheat, oats and peas. These ingredients had higher cadmium levels than the corresponding conventionally produced crops. A Swedish pilot study of cadmium levels in conventionally and organically produced wheat showed contradicting results (Jorhem and Slanina, 2000). In one area higher cadmium levels were found in the organically produced wheat and from another area, organic wheat had lower levels than conventional wheat. Other factors than cultivation system is of importance for the levels of cadmium in grain and more long-term studies are needed to elucidate the influence of organic farming on cadmium levels in crops. The
total cadmium level in the conventional feed was higher mainly due to high cadmium levels in beet fibre (Figure 4).

The organic pigs had significantly higher levels of cadmium in kidney and faeces than the conventional pigs (p=0.005 and p=0.02, respectively) despite lower cadmium level in organic feed, indicating that the organic pigs were exposed to cadmium from other sources than the feed, e.g. soil through rooting. Hansen et al (1981) reared pigs on sewage sludge amended soil and confirmed that animals rooting in soil also ingested soil and Fries et al. (1982) reported the mean and the 95% confidence interval of soil intake in pigs as 8 and 12% respectively, of dry matter intake. Soil samples from the area where the organic pigs were reared had a mean cadmium level of 155 µg/kg. *In vitro* digestion of feed with added soil showed a significantly lower solubility of cadmium (p=0.01) compared to organic feed without soil. Decreased availability of cadmium in feed containing soil has also been shown both *in vitro* (Sheppard *et al.*, 1995) and *in vivo* in rat (Schilderman *et al.*, 1997). Although the solubility of cadmium was reduced, the amount of soluble cadmium was not diminished. Ingestion of soil contributes to the cadmium intake, but can not entirely explain the higher levels in kidneys from organic pigs. Another possible source of cadmium intake in the organic pigs is from plants growing in the area. However, that is probably not a significant source of cadmium explaining the different kidney levels. The cadmium levels in liver did not differ significantly between organic and conventional pigs.

**Pig kidney as a bioindicator**

Monitoring of toxic substances in the environment is a valuable tool in order to detect temporal and spatial trends in contamination levels and to evaluate progress of preventive strategies to reduce pollution. The advantages of using cadmium in pig kidney as an indicator is that it reflects long-term exposure and that it is routinely analysed in the programmes for food control of contaminants. Cadmium level in feed explained only 12 % of the variance of cadmium level in kidney. Animals from the same farm and raised under similar conditions had cadmium levels in kidney that could differ several times. This great variation together with the high contribution from non-locally produced feed components limits the possibilities to use pig kidney as a bioindicator of cadmium in the local agricultural environment. Furthermore, feed composition, age at slaughter, kidney weight and breed were factors also influencing cadmium levels in kidney. However, cadmium in pig kidney reflects cadmium in the feed as well as in faeces and data on kidney cadmium levels from the food control programmes could be used as an indicator of cadmium input to the local agricultural system. The factors influencing cadmium levels in kidney must be considered in the interpretation of data.
Conclusions

Significant correlations of cadmium levels between steps in the chain from soil via crops and feed to pig blood and kidney were found: between soil and wheat, between feed and kidney and between feed and faeces. Cadmium level in feed explained approximately 12% of the variance of cadmium level in kidney. No correlations between feed and blood or between blood and kidney were found. Cadmium in barley, the main ingredient in the feeds, was not correlated to cadmium in soil, feed or kidney.

Median levels of cadmium in kidney cortex varied from 64 to 140 µg/kg wet weight in the present studies. Compared to literature data both kidney and feed cadmium levels were relatively low. However, certain feed ingredients of non-local origin contributed to a large part of the cadmium in the feed. Such ingredients were protein-rich components, vitamin-mineral mixtures and beet fibre. Locally produced cereals, which are the main part of the feed, had a low cadmium contribution to the feeds. The non-local feed components are not a part of the cadmium circulation at the farm and due to a low gastrointestinal absorption most cadmium in feed is excreted in faeces. Thus, cadmium from the non-local components constitutes an external source of cadmium to the arable soils when farmyard manure is applied.

Pigs given feeds with less rapeseed and soybean meal and more cereals than controls had a lower dietary intake of cadmium, and significantly higher cadmium levels in kidney than control pigs. The different bioavailability could be due to different phytic acid content and phytase activity in the feed components. Another explanation for the different kidney levels could be that the pigs with the highest cadmium levels in kidney had a higher intake of cadmium in the beginning of the growing/finishing period when the absorption of cadmium could be higher than at older age.

A significant relationship between age at slaughter and cadmium level in kidney was detected despite the narrow age range. There were differences in cadmium levels in kidney between breeds, which partly could be due to different growth rates. Cadmium levels in kidney were negatively related to kidney weight in a study with pigs raised together at the same farm. No significant difference between castrated male pigs and female pigs was seen.

Cadmium levels in pig kidney and faeces were higher from organic pigs than from conventional pigs, despite lower cadmium level in the organic feed. The feeds had different compositions that might influence the bioavailability of cadmium from the feeds and partly explain the different cadmium levels in kidney. However, no difference in solubility of cadmium after in vitro digestion, was found between the feeds. The organic pigs were reared outdoors
and exposed to cadmium from the outdoor environment. One extra source of cadmium intake is soil when pigs are rooting. When soil was added to the feed and digested in vitro, the fractional solubility of cadmium decreased, compared to feed without soil. However, the amount of cadmium in solution did not decrease compared to feed without soil, because of the addition of cadmium from soil.

Animals from the same farm and raised under similar conditions had cadmium levels in kidney that could differ several times. This great variation together with the high cadmium contribution from non-locally produced feed components limit the possibilities to use cadmium in pig kidney as an indicator of available cadmium in the agricultural environment. However, cadmium in pig kidney reflects the total feed as well as the faeces levels of cadmium. Thus, data on kidney cadmium levels from the food control programmes could be used as an indicator of cadmium input to the agricultural system. The factors influencing cadmium levels in kidney must be considered in the interpretation of data.
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