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# Pb, Cd, and Cu Play a Major Role in Health Risk from Contamination in Duck Meat and Offal for Food Production in Thailand

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**Abstract**

Zinc, Pb, Cd, Mn, Fe, Cr, and Cu levels in duck meat from large-scale farms have been found to be significantly higher than those from free-grazing duck farms. Zinc, Co, Mn, Cr, and Cu contamination levels in duck liver from large-scale farms were significantly higher than those from free-grazing farms; only Cd in duck liver from free-grazing farms was higher than in liver samples from large-scale farms at $P < 0.05$. Lead, Cd, Fe, and Cr levels in duck intestine samples from free-grazing farms were higher than large-scale farms at $P < 0.001$. Moreover, the average concentrations of Pb in duck meat and liver samples from large-scale farms and Cd levels in duck liver samples from free-grazing farm also exceeded the FAO/WHO and Codex Alimentarius limits by 100% (55/55), 100% (54/54), and 67.6% (23/34), respectively. PCA analysis showed a strong positive relationship between the eight metals in meat, liver, and intestine was > 0.69, > 0.69, and > 0.72, in order. The relationship of the liver combined with the intestine was > 0.65. This study indicated that consumers may incur health risks from long-term consumption of duck due to high Pb and Cd concentrations from both types of farms, particularly from large-scale duck farms.

**Keywords** Meat · Duck · Heavy metals · Offal · Thailand

## Introduction

Heavy metals are dangerous environmental pollutants, particularly in areas with high anthropogenic pressure. In the past, the increase in heavy metal pollution in the environment has been associated with anthropogenic activities in the form of effluent and emissions from mines and smelters. Mercury (Hg), lead (Pb), cadmium (Cd), and arsenic (As) have been determined to be toxic metals, depending on the manner of dosage [1]. Moreover, even trace amounts of heavy metals in plants, the atmosphere, soil, and water can cause serious effects to all biota. Heavy metals are not easily biodegradable and can accumulate in human and animal organs [2, 3]. Lead reduces the growth rate of birds, causes oxidative damage to DNA, and causes reproductive effects such as reduced egg production, leading to growth retardation and increased mortality [4, 5]. In laboratory settings, Cd has been shown to cause kidney toxicity, metallothionein induction, altered avoidance

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behavior, disrupted calcium metabolism, decreased food intake, decreased growth rates, reduced egg production, thin eggshells in response to normal changes in the body, and organ masses during the reproductive time in female elders [6–9]. Female poultry could take up heavy metal from different sources and release these metals into their organ and eggs [10–13]. Meanwhile, ducks are vulnerable to the accumulation of toxic through contaminated feeds. Ducks can serve as a useful bioindicator species for environmental monitoring at higher trophic level of the food chain [14, 15]. Moreover, acute and chronic exposure in duck could be related to environmental contaminants, since they are fed a wide variety of feedstocks [16, 17].

Free-grazing ducks play a major role in the agricultural economy of Eastern Asia in terms of egg and meat production [18]. Most of Thailand's intensive poultry farming is clustered in the central region of the country [19]. In particular, duck production is concentrated along the Chao Phraya River, which also supports multiple rice production cycles per year [20]. Free-grazing ducks are common in South East Asia, particularly in Thailand, where double-crops of rice provide excellent year-round foraging for ducks. They are released into the paddy fields after harvest and feed on leftover rice grains, wild rice, insects, and aquatic animals [18]. In Thailand, the number of ducks was estimated at 26,287,094 animals, whereas only 21.9% were in free-grazing duck production in 2017 [21]. According to the Bureau of Product Standards and Quality Systems, National Bureau of Agricultural Commodity and Food Standards, Ministry of Agriculture and Cooperatives (2016), on the other hand, the average per capita consumption of duck meat reached 0.38 g/day. However, no available information on the average per capita consumption of duck liver and intestines [22].

Free-grazing duck farms were one type of private farm having a high potential for chemical exposure from the environment, particularly from heavy metals and insecticides [23–25]. The contamination in this farm type was found to be higher than other systems since free-grazing entails direct contact with the outdoor environment. In addition, between free-range and broiler chickens, free-range chickens accumulate significantly higher concentrations of Pb and Cd in the liver than broiler chickens [26]. Moreover, home-produced eggs from chickens foraging on soil contaminated with environmental pollution accumulate pollutants, such as dioxins and Pb, which showed higher concentrations than commercially produced eggs [27]. However, this hypothesis has never been proven in the situation of free-grazing ducks and large-scale duck farm systems in Thailand.

The aim of this study was to compare the contamination levels of heavy metals including zinc (Zn), Pb, Cd, cobalt (Co), manganese (Mn), iron (Fe), chromium (Cr), and copper (Cu) in duck meat, liver, and intestine samples from the two types of duck farming systems to find the correlation of metals in organs and to evaluate their attendant health risks.

## Materials and Methods

### Sample Collection

Thirty-five duck *Anas platyrhynchos f. domestica* samples each of meat, liver, and intestine were collected from seven free-grazing duck farms in Sing Buri, Nakhon Pathom, Pathum Thani, Ayutthaya, Kanchanaburi, and Prachuap Khiri Khan provinces, Thailand. In addition, 55 samples of duck meat, liver, and intestine were collected from 4 large-scale farms (> 5000 ducks) in Nakhon Pathom province, Thailand. All ducks were killed and slaughtered in a slaughterhouse in Nakhon Pathom province between August and December 2015, after which they were recruited for immediate analysis.

### Heavy Metal Analysis

Duck meat, liver, and intestine samples were cleaned with 18.2 MΩ distilled water (Ultrapure Water Purification System, Thermo Scientific™) and then kept at − 20 °C in a freezer (Medium Stainless Steel LLOYD Chest Freezer-Hard Top-LHT565DD). Afterward, they were dried in an oven (Osworld Scientific Equipment Pvt. Ltd) at 60 °C for 24 h [28] and manipulated according to the standard method of analysis following the AOAC [29]. Briefly, 1 g of all the coalesced duck meat, liver, and intestine samples was digested with nitric acid (nitric acid 65%, Grade ISO, MERCK), hydrogen peroxide (hydrogen peroxide 30%, Grade ISO, MERCK), and sulfuric acid (sulfuric acid 98%, AR Grade, Scitrader) at 5:5:1, then heated on a stirring hotplate (Thermo Scientific™ Explosion-Proof SAFE-T SHP9) at 150 °C until all sample solutions became yellowish. After cooling, the sample solutions were filtrated and topped up to 25 ml, then diluted with 20% nitric acid before being analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES), ULTIMA2, Jobin Yvon Holiba, Italy, ICP conditions for generator; solid-state, 40.68 MHz, water-cooled, and continuous wavelength coverage from 120 to 800 nm with far-UV option for improved sensitivity for halogen analysis or alternative wavelengths.

### Method Performance

The spiked used the ICP multi-element standard solution TV 1000 μg $mL^{-1}$ each in 1 mol $L^{-1}$ $HNO_3$ (As, Al, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, K, Li, Mg, Mn, Na, Ni, Pb, Sr, Tl, and Zn). The concentrations of recovered metals were 0.2, 0.5, 1, 5, 20, and 50 mg $kg^{-1}$ in all samples with four replications for each spike level, which showed the following: Zn, percentage recovery = 89.8; Pb, percentage recovery = 84.2; Cd, percentage recovery = 102.1; Co, percentage recovery = 101.5; Mn, percentage recovery = 98.8; Fe, percentage recovery =

85.4; Cr, percentage recovery = 107; Cu, percentage recovery = 95.7. The relative standard deviation (RSD) in the replicates was always < 5%, while the calibration level ranges were 0.5, 1, 5, and 10 mL $L^{-1}$. The analytical detection of limits (LOD) and limit of quantitation (LOQ) were Zn = 0.002409 mg $L^{-1}$; 0.00439 mg $L^{-1}$, Pb = 0.005429 mg $L^{-1}$; 0.0107 mL $L^{-1}$, Cd = 0.001348 mg $L^{-1}$; 0.002454 mg $L^{-1}$, Co = 0.001229 mg $L^{-1}$; 0.002664 mg $L^{-1}$, Mn = 0.006472 mg $L^{-1}$; 0.00895 mg $L^{-1}$, Fe = 0.00583 mg $L^{-1}$; 0.0094 mL $L^{-1}$, Cr = 0.00145 mg $L^{-1}$; 0.003032 mg $L^{-1}$, and Cu = 0.019483 mg $L^{-1}$; 0.02481 mg $L^{-1}$, respectively.

### Statistical Analysis

All the data on the metal contaminants was tested for normal distribution by using the Shapiro-Wilk test, after which it was analyzed and fitted to the non-parametric model. The metal concentration data was analyzed by using the Mann-Whitney *U* test to compare the differences between means by using GraphPad Prism Statistical 5.0, 2012, GraphPad Software, Institute. Principle component analysis (PCA) was conducted to assess the main characteristics of the relationship between the metal levels in tissue by using R studio 7.6 build, R-tools Technology Inc.

## Results

### Meat

The average and standard deviation of Pb levels in duck meat from the large-scale farms (3.13 ± 1.13 mg $kg^{-1}$ dry weight) were significantly higher than Pb in the duck meat from free-grazing duck farms (0.06 ± 0.21 mg $kg^{-1}$ dry weight) at $P < 0.001$. Interestingly, many meat samples from the large-scale farms exceeded the levels of Pb cited as safe in the notification by the Ministry of Public Health No. 98 (B.E.2529) of Thailand, Commission Regulation (EC) no. 1881/2006, and FAO/WHO and the Codex Alimentarius standard (2002) at 92.7% (51/55), 100% (55/55), and 100% (55/55), respectively, as shown in Table 1. In addition, the Cd levels in meat from the large-scale farms (0.33 ± 0.14 mg $kg^{-1}$ dry weight) were significantly higher than the Cd levels in the duck meat samples from the free-grazing farms (0.03 ± 0.04 mg $kg^{-1}$ dry weight) at $P < 0.001$, as shown in Table 1. Interestingly, the number of meat samples from the large-scale farms in which the Cd exceeded the limits in the Commission Regulation (EC), FAO/WHO and Codex Alimentarius standard was 96.4% (53/55) and 96.4% (53/55), as shown in Table 1. The concentrations of Zn, Mn, and Cu in duck meat samples from the large-scale farms were significantly higher than Zn, Mn, and Cu levels in the duck meat from the free-grazing ducks at $P < 0.001$, as shown in

**Table 1** Comparison of zinc, Pb, Cd, Co, Mn, Fe, Cr, and Cu levels in duck meat, liver, and intestine from free-grazing and large-scale farms (mg $kg^{-1}$ dry weight)

| Samples | Farm types | | Zn | Pb | Cd | Co | Mn | Fe | Cr | Cu |
|---|---|---|---|---|---|---|---|---|---|---|
| Meat | Free grazing | Min-max level | 11.7–75.9 | ND–1.13 | ND–0.13 | ND–ND | ND–2.97 | 71.6–248 | ND–0.31 | 1.56–19.0 |
| | | Mean + SD | 34.7 ± 13.1 | 0.06 ± 0.21 | 0.03 ± 0.04 | ND | 0.20 ± 0.50 | 129 ± 47.8 | 0.01 ± 0.05 | 9.24 ± 4.28 |
| | Large scale | Min-max level | 13.1–94.0 | 0.55–6.37 | 0.02–0.71 | ND–0.49 | ND–2.09 | 31.7–297 | ND–2.02 | 8.12–21.6 |
| | | Mean + SD | 47.3 ± 16.8** | 3.13 ± 1.13** | 0.33 ± 0.14** | 0.14 ± 0.10 | 0.69 ± 0.66** | 154 ± 68.9* | 0.29 ± 0.44** | 15.28 ± 3.37** |
| Liver | Free grazing | Min-max level | 69.6–215 | ND–10.4 | ND–3.24 | ND–1.05 | 4.20–21.9 | 185–5307 | ND–3.59 | 7.53–240 |
| | | Mean + SD | 116 ± 39.4 | 3.01 ± 2.77 | 0.93 ± 0.85* | 0.27 ± 0.26 | 11.2 ± 4.66 | 1163 ± 966 | 0.36 ± 0.82 | 92.5 ± 61.6 |
| | Large scale | Min-max level | 91.3–542 | 0.23–6.69 | ND–1.06 | ND–1.32 | 7.98–55.9 | 119–3329 | ND–2.25 | 93.7–516.4 |
| | | Mean + SD | 214 ± 91.3** | 3.14 ± 1.49 | 0.48 ± 0.23 | 0.44 ± 0.27* | 21.4 ± 11.2** | 835 ± 573 | 0.52 ± 0.59* | 239 ± 85.8** |
| Intestines | Free grazing | Min-max level | 39.8–132 | 0.95–3.94 | 0.05–1.53 | ND–0.82 | 4.29–64.8 | 86.1–3322 | 0.13–7.61 | 1.81–14.8 |
| | | Mean + SD | 70.8 ± 20.0 | 2.07 ± 0.76** | 0.28 ± 0.33** | 0.23 ± 0.21 | 20.4 ± 18.3 | 359 ± 558** | 1.55 ± 1.54** | 6.36 ± 2.97 |
| | Large scale | Min-max level | 48.1–186 | 0.36–3.71 | ND–0.28 | ND–2.22 | 3.66–104 | 33.3–132 | 0.02–2.43 | 1.63–17.8 |
| | | Mean + SD | 86.8 ± 26.7** | 1.44 ± 0.64 | 0.08 ± 0.09 | 0.51 ± 0.63 | 36.5 ± 26.3** | 71.4 ± 22.6 | 0.61 ± 0.46 | 7.84 ± 4.40 |

*ND* not detected

*Significant difference at $P < 0.05$; **significant difference at $P < 0.001$

Table 1. Fe and Cr levels in the duck meat samples from the large-scale farms were significantly higher than Fe and Cr levels in the duck meat from the free-grazing ducks at $P < 0.05$, as shown in Table 1.

## Liver

Zinc levels were found to be the highest in duck liver, followed by the intestine and meat, respectively. Moreover, Zn levels in duck livers from the large-scale farms ($214 \pm 91.3$ mg kg$^{-1}$ dry weight) were significantly higher than the levels in duck livers from the free-grazing duck farms ($116 \pm 39.4$ mg kg$^{-1}$ dry weight) at $P < 0.001$, as shown in Table 1. The highest levels of Pb were found in the liver followed by the intestine and meat, respectively. The number of liver samples from the large-scale farms in which the level of Pb exceeded limits in the notification of the Ministry of Public Health of Thailand [30], Commission Regulation (EC) [31], and FAO/WHO and Codex Alimentarius standards [32] was 94.4% (51/54), 100% (54/54), and 100% (54/54) of the samples respectively, as shown in Table 2. In the case of Cd, the duck liver samples contained the highest levels, more than both intestines and meat. The study found that the Cd levels in the duck liver samples from free-grazing farms ($0.93 \pm 0.85$ mg kg$^{-1}$ dry weight) were significantly higher than the Cd levels in the duck livers from large-scale farms ($0.48 \pm 0.23$ mg kg$^{-1}$ dry weight) at $P < 0.05$, as shown in Table 1. In fact, a number of liver samples from the free-grazing duck farms exceeded the Commission Regulation (EC) and FAO/WHO and Codex Alimentarius standard for Cd levels in 67.6% (23/34) and 67.6% (23/34) of samples, as shown in Table 2.

Cobalt levels in the duck liver samples from the large-scale farms ($0.44 \pm 0.27$ mg kg$^{-1}$ dry weight) were significantly higher than the Co levels in the duck livers from the free-grazing ducks ($0.27 \pm 0.26$ mg kg$^{-1}$ dry weight) at $P < 0.05$. Moreover, the concentration of Mn in the duck livers from large-scale farms ($21.4 \pm 11.2$ mg kg$^{-1}$ dry weight) was significantly higher than Mn levels in the duck liver from the free-grazing ducks ($11.2 \pm 4.66$ mg kg$^{-1}$ dry weight) at $P < 0.001$, as shown in Table 1. In the three organs investigated, the highest levels of Fe were found in the liver, followed by the intestines and meat, respectively. Cr levels in the duck livers from large-scale farms ($0.52 \pm 0.59$ mg kg$^{-1}$ dry weight) were significantly higher than Cr levels in the duck livers from the free-grazing ducks ($0.36 \pm 0.82$ mg kg$^{-1}$ dry weight) at $P < 0.05$, as shown in Table 1. The highest levels of Cu were contained in the liver samples, followed by the meat and intestine samples, respectively. Cu levels in the duck livers from the large-scale farms ($239 \pm 85.8$ mg kg$^{-1}$ dry weight) were significantly higher than the Cu levels in the duck livers from the free-grazing ducks ($92.5 \pm 61.6$ mg kg$^{-1}$ dry weight) at $P < 0.001$, as shown in Table 1.

## Intestine

The concentration of Pb in the duck intestines from the free-grazing duck farms ($2.07 \pm 0.76$ mg kg$^{-1}$ dry weight) was significantly higher than the Pb levels in duck intestines from the large-scale farms ($1.44 \pm 0.64$ mg kg$^{-1}$ dry weight) at $P < 0.001$, as shown in Table 1. In 97.1% (33/34) of the intestine samples from the free-grazing duck farms and 76.0% (38/50) of the intestine samples from the large-scale farms, Pb exceeded the limits set by the notification of the Ministry of Public Health of Thailand, as shown in Table 2. Zn, Mn, and Cr levels in the duck intestines from the large-scale farms were significantly higher than Zn, Mn, and Cr levels in the duck intestines from the free-grazing duck farms at $P < 0.001$, as shown in Table 1. However, Cd and Fe levels in the duck intestine samples from the free-grazing ducks were significantly higher than the Cd and Fe levels in the duck intestine from the large-scale farms at $P < 0.001$. Cobalt, Mn, and Cr levels were the highest in the intestine samples, followed by the liver and meat samples, respectively, as shown in Table 1.

## Principal Component Analysis

The PCA analysis was used to identify the two main groups of metals in meat duck. Both components accounted for 68.9% of the total variance and had a strong positive relationship (> 0.69). Component 1 accounted for 43.4%, while component 2 accounted for 25.5% of the total variance. The loading plot showed a significant relationship for Pb and Cd, Zn and Mn, Fe and Zn, and Pb and Co in the meat samples as shown in Fig. 1a.

Whereas the principal components in the liver and intestine were accounted for at 66.3% and 60.2%, respectively, the total variance for components 1 and 2 were 47.7% and 18.64% in the liver and 39.0% and 21.1% in the intestine samples, respectively. The loading plot showed a highly positive relationship (> 0.69) for Zn and Co, Zn and Mn, Zn and Cr, and Co and Cr in the liver samples, as shown in Fig. 1b, and a positive relationship (> 0.72) for Zn and Co, Zn and Cu, and Co and Mn in the intestine samples, as shown in Fig. 1c. In addition, the combination of principal components in both the liver and intestine accounted for 65.5% of the total variance (components 1 and 2 explaining 38.8% and 26.7%, respectively). Factor loading had a highly positive relationship (> 0.65) for Zn and Fe, Zn and Cu, Pb and Fe, and Co and Mn, as shown in Fig. 1d.

## The Calculation of Average Daily Intake for Metals from Duck Meat for Consumption

The average daily intake of Pb, Cd, and Cu was calculated and compared with the World Health Organization-Food Agriculture Organization provisional tolerated daily intake

**Table 2** Comparison of zinc, Pb, Cd, Co, Mn, Fe, Cr, and Cu levels in duck meat, liver, and intestine, plus average daily intakes of metals from duck meat consumption with standard

| Samples | Standard limit | Farm types | Zn | Pb | Cd | Co | Mn | Fe | Cr | Cu |
|---|---|---|---|---|---|---|---|---|---|---|
| Meat | NMPHT | | – | 1.0 | – | – | – | – | – | – |
| | | Free grazing | – | 2.86% (1/35) | – | – | – | – | – | – |
| | | Large farm | – | 92.7% (51/55) | – | – | – | – | – | – |
| | EC | | – | 0.1 | 0.05 | – | – | – | – | – |
| | | Free grazing | – | 14.3% (5/35) | 34.3% (12/35) | – | – | – | – | – |
| | | Large farm | – | 100% (55/55) | 96.4% (53/55) | – | – | – | – | – |
| | FAO/WHO | | – | 0.1 | 0.050 | – | – | – | – | – |
| | | Free grazing | – | 14.3% (5/35) | 34.3% (12/35) | – | – | – | – | – |
| | | Large farm | – | 100% (55/55) | 96.4% (53/55) | – | – | – | – | – |
| Liver | NMPHT | | – | 1.0 | – | – | – | – | – | – |
| | | Free grazing | – | 64.7% (22/34) | – | – | – | – | – | – |
| | | Large farm | – | 94.4% (51/54) | – | – | – | – | – | – |
| | EC | | – | 0.10 | 0.50 | – | – | – | – | – |
| | | Free grazing | – | 70.6% (24/34) | 67.6 (23/34) | – | – | – | – | – |
| | | Large farm | – | 100% (54/54) | 42.6 (31/54) | – | – | – | – | – |
| | FAO/WHO | | – | 0.10 | 0.50 | – | – | – | – | – |
| | | Free grazing | – | 70.6% (24/34) | 67.6% (23/34) | – | – | – | – | – |
| | | Large farm | – | 100% (54/54) | 42.6% (31/54) | – | – | – | – | – |
| Intestines | NMPHT | | – | 1.0 | – | – | – | – | – | – |
| | | Free grazing | – | 97.1% (33/34) | – | – | – | – | – | – |
| | | Large farm | – | 76.0% (38/50) | – | – | – | – | – | – |
| The average daily intakes of metals from duck meat consumption (mg day$^{-1}$) | WHO provisional tolerated daily intakes in mg/day per capita [33] | | – | 0.429 | 0.05–0.071 | – | – | – | – | – |
| | Nogawa et al. (1989) and ATSDR (1999) | | – | – | 0.0002 | – | – | – | – | – |
| | Recommended Daily Intake (RDI) | | – | – | – | – | – | – | – | 2 |
| | ATSRD (2004) | | – | – | – | – | – | – | – | 0.01 |
| | | Free grazing | – | 0.00002 | 0.00001 | – | – | – | – | 0.0035 |
| | | Large farm | | 0.00119 | 0.00013 | | | | | 0.0058 |

"–" no standard limit, *NMPHT* Notification of Ministry of Public Health No. 98 (B.E.2529) of Thailand [30], *EC* (EC) Commission Regulation No. 1881/2006 [31], *FAO/WHO* FAO/WHO 2002 and Codex Alimentarius [32]

[33] and Recommended Daily Intake (RDI) of minerals [34]. In Thailand, the average per capita consumption of duck meat during 2016 was 0.38 g/day according to the Bureau of Product Standards and Quality Systems, National Bureau of Agricultural Commodity and Food Standards, Ministry of Agriculture and Cooperatives, Thailand [22]. Therefore, the daily intakes of Pb, Cd, and Cu from duck meat were calculated using the following formula:

$$\text{Average daily intakes}\left(\text{mg day}^{-1}\right) = \text{Level of metal in duck}\left(\text{mg kg}^{-1}\right) \times \text{Average per capita consumption of duck meat}\left(\text{kg day}^{-1}\right)$$

The calculated data for the average daily intake of metals revealed that concentrations of Pb, Cd, and Cu from all the farms studied did not exceed the standard limits as shown in Table 2.

## Discussion

In this study, it was found that Zn, Mn, Co, Cr, and Cu concentrations in the meat and liver samples from the large-scale duck farms were significantly higher than in ducks from the free-grazing farms. Moreover, Pb, Cd, and Fe levels in duck meat samples from the large-scale farms were significantly higher than those from the free-grazing farms. Our previous study found that 83.33% (5/6) of large farms used commercial feeds; in contritely, 62.5% (5/8) of free-grazing farms treated the ducks by grazing. Moreover, the commercial feeds from the large-scale farms had significantly higher levels of Cd ($0.41 \pm 0.1$ mg kg$^{-1}$) and Pb ($3.58 \pm 0.56$ mg kg$^{-1}$) than the commercial feeds and the self-mixed feed from small-scale farms (< 5000 animals) and free-grazing farm in Thailand. Meanwhile, we found that canal water was the main drinking water source for free-grazing farms at 87.5% (7/8). In

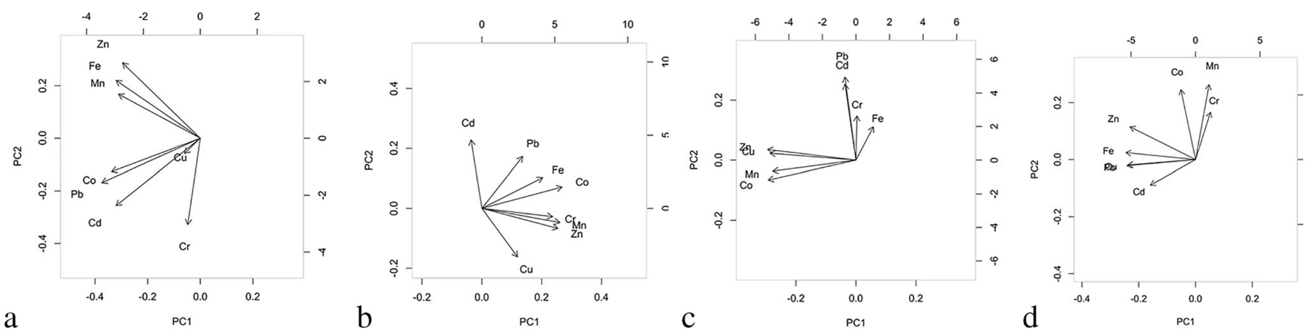


**Fig. 1** PCA plot showing metal loadings on components from meat (**a**), liver (**b**), intestine (**c**), and combined between liver and intestine (**d**)

addition, Pb concentration in the drinking water used by free-grazing farms was $0.16 \pm 0.02$ mL $L^{-1}$, which exceeds the water standards for animal consumption (0.10 mL $L^{-1}$). Further, the drinking water had a high correlation on the free-grazing duck farms between Pb levels in the egg and the drinking water [35]. Therefore, this report may link to the high contamination of Cd and Pb in duck tissues for both large farms and free-grazing farms. There have been many reports on heavy metals in duck meat and liver, while very few studies have been done on metal levels in duck intestine. According to current data, the levels of Pb, Cd, Zn, and Cu in the duck samples from both types of farms tend to be higher than the meat previously reported in ducks, geese, chickens, hens, rabbits, and sheep slaughtered in the northern part of Poland [36], in cocks from Chittagong city, Bangladesh [37], and beef in Korea [38]. However, the levels of Mn and Co from ducks on the large-scale farms in the present study were higher than the reported levels in the liver samples of mallards at Gimpo, Korea [39], ducks, chickens, geese, hens, rabbits, and sheep in Poland [36], and duck meat from Taipei, Taiwan [40], while the levels of Cr in the meat were not reported. Moreover, Cd levels in the duck livers from the free-grazing farms were found to be significantly higher than those from the large-scale farms at $P < 0.05$. Cadmium induces changes in the organs, particularly in the gonads, kidney, and adrenal glands [41]. For example, Cd and Pb induced apoptosis and a decrease in the number of erythrocytes in mallard (*Anas platyrhynchos*) blood cells [42]. Moreover, young animals are more susceptible to Cd than adults [43]. In this study, data on the average daily intake of duck meat revealed that Cd in the duck meat samples from both types of farms was lower than the standard limits. According to Nogawa et al. and ATSDR, the chronic duration of oral consumption of Cd with minimal risk levels for renal effects in humans was 0.0002 mg $kg^{-1}$ $day^{-1}$ [44, 45]. In addition, long-term low-dose Cd exposure could disrupt DNA methylation in human embryo lung fibroblast cells [46]. In addition, women are highly vulnerable to Cd because of their relatively higher absorption rate compared to men, which causes harm to both the mother and new-born child. Cadmium partially crosses the placental barrier and can potentially impact fetal development through epigenetic mechanisms, causing changes to fetal gene expression [47]. Therefore, the calculation of the provisional tolerable daily intake presented suggests that consumers may incur a health risk from Cd contamination from long-term consumption of duck meat from both types of farms studied, as the data shows in Table 2. In addition, health risks from contamination can derive from the Cd concentrations in duck meat as well as in contaminated liver and intestines.

Our data shows that Pb, Cd, Zn, Mn, Fe, Cr, and Cu concentrations in the liver samples from both types of farm were higher than other reports in mallards, sea ducks, gadwall, canvasback, American wigeon, lesser scaup, greater scaup, bufflehead, black duck, pintail, ring-necked duck, and ruddy duck as well as other reports in Spain, the USA, Poland, Korea, and Greece [14, 48–52]. In addition, it has been reported that Cd levels in gizzards of 0.24 mg $kg^{-1}$ dry weight in mallards [51] were higher than our findings on large-scale farms ($0.08 \pm 0.09$ mg $kg^{-1}$ dry weight), but similar to Cd levels found in the intestine samples from ducks on free-grazing farms ($0.28 \pm 0.33$ mg $kg^{-1}$ dry weight). Meanwhile, Pb, Cd, Fe, and Cr levels in duck intestine from free-grazing duck farms were found to be higher than those from large-scale farms at $P < 0.001$. On the contrary, Zn and Mn levels in the duck intestine samples from large-scale farms were higher than in intestine samples from free-grazing duck farms at $P < 0.001$. This finding is similar to other studies which indicated that free-grazing systems have a high potential for chemical contamination attributable to environmental contamination exposure as the duck is in direct contact with the outdoor environments [23–25, 27]. This finding is similar to Yabe et al. who reported that Cd accumulation in the liver of free-range chickens was greater than in broiler chickens at $P < 0.05$ [25]. However, this finding is similar to our previous study that reported that the levels of Pb, Cd, Cr, and Cu in eggs from large-scale farms were significantly higher than from free-grazing farms, and this might be the same contamination pattern in meat and liver [35].

Interestingly, it was found that most of the intestine samples, particularly from free-grazing farms, had higher levels of Pb than the limits set by the regulation of the Ministry of Public Health of Thailand, despite the body and liver weight of chickens typically decreasing after consuming a diet of Pb-contaminated feeds [53]. Mateo and Guitart reported that birds died after exposure to Pb concentrations of more than 5 mg $kg^{-1}$ dry weight [54]. Average levels of Pb in duck livers from the free-grazing and large-scale farms were close to this level, as shown in Table 1. In duck species, Pb concentration was significantly different among tissue samples, with mean concentrations of Pb being greater in the bones than in the livers and kidneys of spot-billed ducks and mallards but not in geese. Moreover, Pb concentration was correlated between liver, kidney, and bone [55]. Data on the average daily intake of duck meat revealed that Pb in duck meat samples from both types of farms did not exceed the standard limits. However, the JECFA (Joint FAO/WHO Expert Committee on Food Additives) reported in 2011 that exposure to Pb of 0.003 mg $kg^{-1}$ body weight $day^{-1}$ affects the cardiovascular system and increases the systolic blood pressure by 2 mmHg (0.3 kPa) [56]. Furthermore, Pb exposure in humans leads to adverse health effects, particularly in young children and pregnant women [57]. Fetal and childhood lead exposure has been associated with attention deficit hyperactivity disorder (ADHD), IQ deficits, and behavioral disorders, as well as decreased brain volume in adults [58–60]. Moreover, children consuming 0.002 mg $kg^{-1}$ body weight $day^{-1}$ of Pb will display neurodevelopmental effects associated with a decrease of 3 IQ points. Therefore, consumers may incur health risks from long-term consumption of duck meat contaminated with Pb at levels similar to those found in this study, as seen in Table 2. Furthermore, there is a possibility of health risks due to contamination with Pb from a dietary source of duck meat as well as duck liver and intestine.

Clinical symptom results showed Zn poisoning in the livers of mallards at a contain rate of 1100 mg $kg^{-1}$ dry weight [61]. However, Zn levels from all the samples in our study were much lower than Zn toxicity levels for ducks. Puls [62] reported that hepatic levels of Mn considered as high and toxic in poultry were 4.0–6.0 mg $kg^{-1}$ wet weight (approximately 15–23 mg $kg^{-1}$ dry weight), which, interestingly, is the same as in our study. It was found that 64.8% (35/54) of samples from the large-scale farms had levels of Mn higher than 15 mg $kg^{-1}$ dry weight, which may have an adverse impact on the health of ducks. Isanhart et al. [63] reported that acute Cu toxicity was observed in mallard livers with concentrations of between 270 and 1300 mg $kg^{-1}$ dry weight. In our study, it was found that 33.3% (18/54) of samples had Cu levels of 270–1300 mg $kg^{-1}$ dry weight. In humans, the gastrointestinal tract is the most sensitive target for Cu toxicity. Exposure to Cu at doses of 0.011–0.03 mg $kg^{-1}$ could induce adverse gastrointestinal effects such as nausea, vomiting, abdominal pain, and diarrhea [64, 65]. Moreover, ATSRD [66] reported that the minimal risk level of Cu is 0.01 mg $day^{-1}$, derived from acute oral exposure to Cu. Our findings indicated that consumers may incur health risks from long-term consumption of duck meat from large-scale farms contaminated with Cu, as shown in Table 2. In addition, health risks may result from contamination from dietary sources of Cu, not only in duck meat but also from contaminated duck livers and intestines. Conversely, the five other elements were found to be below the RDI (Recommended Daily Intake), which has not been found to pose risk to health from consumption.

In our study, the concentrations of essential metals like Fe, Zn, and Cu were higher than nonessential metals like Pb and Cd. The level of Zn > Cu>> Mn in both the liver and brain tissue, while Pb > Cr in the brain and > Cd in the liver tissue [52]. From our study, it can be concluded that the concentration of Fe > Zn > Cu > Pb > Mn > Cd > Cr > Co in the duck meat, while Fe > Cu > Zn > Mn > Pb > Cd > Cr > Co in the liver and Fe > Zn > Mn > Cu > Pb > Cr > Co > Cd in the intestinal tissue, which is similar to the findings of Aloupi et al. [52]. Since the liver is the target tissue for the accumulation of both essential and nonessential heavy metals in animals and metabolites from the body [67], it is suggested that the liver may be the main target organ for heavy metal determination.

Metallothionein (MT) protein levels and Cd bioaccumulate predominantly in the kidney then liver tissue [68]. Metallothionein is a cysteine-rich protein approximately 7 kDa, with a high binding capacity and a low molecular weight 7 atom per mole [69]. It is found in the liver, kidney, and intestine, but can also be expressed in the brain and skin. In birds, Cd can accumulate in many organs and tissue, including the lungs, spleen, bone, and urinary bladder [70]. It adversely affects the functions of these organs, but it is mostly accumulated in the liver and kidney [71, 72]. Moreover, the liver is the main storage site and an important target organ for Cu toxicity [73, 74]. However, exposure to metals may, in general, contribute to poor physical condition in ducks. However, it has been found that meat is a minor site for the accumulation of metals, which is similar to our finding that heavy metal concentrations in duck meat from both types of farm were generally lower than levels found in the liver and intestinal tissue [75, 76]. In addition, chicken gizzards contained the highest concentrations of Pb compared to chicken kidney and liver tissue [77]. In this study, Co, Mn, and Cr levels were found to be the highest in the duck intestine.

The correlation of Pb or Cd with essential element concentrations in tissues has been reported in sea birds from Russia [78] and in mallards from Korea [79]. Interestingly, it was found that Pb levels in the duck meat in our study were positively correlated ($r = 0.93$) with Cd. This result was similar to the result of the relationship of the metal-metal relationship in the muscle tissue of dairy product ($r = 0.63$) reported by Kim et al. [38], and Kim and Oh [79] found positive correlations in mallard liver tissue between Zn and Pb, Cd and Pb, Fe and Cd, and Zn and Cd. Moreover, Levengood and Skowron [80] reported a highly positive correlation between Cu and Zn in the liver, while positive correlations were found in our study between Zn and Co, Zn and Mn, Zn and Cr, and Co and Cr in the liver samples, whereas Zn and Co, Zn and Cu, and Co and Mn in the intestine sample were found to have a positive correlation. The interaction between toxic and essential metals occurs when they are similarly metabolized [79]. Therefore, an understanding of the absolute relationship for the metals distributed in the tissue of each matrix must be further elucidated and defined to determine the toxic mechanism for the safety of food.

## Conclusion

Our findings indicated that consumers may incur health risks from long-term consumption of duck meat with high Pb, Cd, and Cu concentrations from both types of farms (free range and large scale), particularly duck meat from large-scale duck farms. With respect to the determination of heavy metals in the three organs, it was found that the highest levels of Zn, Pb, Cd, Fe, and Cu contamination were found in the liver. As a result, consumers should avoid the health risks related to the consumption of contaminated duck liver, especially those posed by Cd and Pb in duck livers from free-grazing duck farms, whereas Co, Mn, Cr, and Cu were also found in the intestines of these ducks. Thus, the consumption of different internal duck organs could result in exposure to heavy metals. Long-term monitoring of domestic duck production in Thailand must be implemented. The information gained from this study will be useful for authorized bodies to standardize the levels of heavy metal contamination in duck tissues and prevent the negative health effects of metal toxicity in human consumers who could contract it through the food chain.

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### Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflicts of interest.

**Ethical Approval** The protocol of laboratory animal uses has been approved by ethical committees of Kasetsart University.

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