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Concentrations of Metals (Zinc, Copper, Cadmium, and Mercury) in Three Domestic Ducks in France: Pekin, Muscovy, and Mule Ducks

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The role of different factors such as biological material (tissues, organs) and trophic condition (overfeeding or not) in the metal accumulation was studied in three genotypes of ducks (Pekin, Muscovy, and Mule) under breeding conditions. Results showed that overfeeding decreased the concentration in Cd, Cu, and Zn through the dilution process. In contrast, mercury concentration increased with this method. A relation between lipidic metabolism of genotypes and the distribution of this metal in biological material was found. Domestic ducks were little contaminated, but a low chronic contamination in Cd was observed, probably coming from the food. Due to the low levels of contamination observed in these breeding ducks, they can be considered as a good control for further contamination studies and comparison with accumulation levels recorded in the field. The impact of feeding condition on accumulation showed the importance of taking into account the life cycle of birds before studying their contamination and the impact of pollutants.

KEYWORDS: Palmiped; domestic; metals; *Anas platyrhynchos*; *Cairina moschata*; Mule; overfeeding; tissue distribution; hepatic steatosis

INTRODUCTION

In natural environment, palmipeds and birds in general are subject to multiple contamination sources. These species are considered as good integrators of environmental contaminations and good subjects for examination of pollution and its impact on the populations. Indeed, birds feed at different trophic levels, with piscivorous representing the most exposed to a great pressure of contamination (*1, 2*), they can be long-lived, and many are both abundant and widely distributed (*3*).

Nonessential metals such as cadmium and mercury could cause important reproductive effects which include a decrease in fecundity (*4*), decreased egg production and size, decreased hatchability, increased hatchling mortality, and induced eggshell thinning (*3, 5, 6*). Metal can also induce renal damage (*7, 8*), gonad atrophy (*5, 7, 9, 10*), and embryotoxic effects (*11, 12*). Cadmium ingestion induced inflammation of renal interstitium and marked degenerative changes in testes (*13*). Some metals, such as mercury, could reduce food intake and induce weight loss, wing and leg weakness, uncoordination, paralysis, or convulsions (*14*) as well as behavioral problems and even nevrosis for high zinc intake (*15*). Some authors have demonstrated that exposure to metals contributes to poor body condition in ducks (*16, 17*). However, during these experiments involving captive birds orally contaminated by Cd and Hg, no relations were demonstrated between metal exposition and body condition. This lack of relation supports the fact that the inverse correlation found on field study could not be linked to a cause with effect relation and that Cd and Hg concentrations vary seasonally in response to environmental patterns according to weight loss and gain (*18*). On the other hand, some essential metals such as zinc intervene in most of the fundamental biological metabolism (synthesis and degradation of carbohydrates, lipids, and proteins) (*19*). Zinc plays a role in the expression of the genes, stabilization of the structure of proteins, and cellular replication. Copper is an essential trace element which enters in the composition or is a cofactor of numerous enzymes. One of the main functions of Cu is the defense of the body against oxidative stress.

Many factors could be at the origin of variations in contaminant concentrations observed such as sex (*18*), age of individuals, geographic location, and trophic level (*1–3*). During reproduction, changes in physiological condition, involving nutrient intake protein and lipid mobilization (large weight variation), can affect the accumulation of contaminants (*20*). Species and/or taxonomic group, according to absorption and excretion abilities, could also influence metal concentrations (*14*). In wild migrating birds, a particular physiological process, the hepatic steatosis, allows them to store energy before migration. This fast dietary-induced fatty liver of palmipeds

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could enhance the concentration of metals by the large food intake in a short period of time.

The aim of this work was to carry out a preliminary study of the metal accumulation (zinc, copper, cadmium, and mercury) of a reduced sample of breeding ducks to establish a work basis and inventory of pollutants of individual metal concentrations. The chosen biological model concerned breeding palmipeds because of its facility of access and its stable evolution in a controlled system. The number of metals studied was multiplied, and a comparison of accumulation level between captive and field animals was realized because of the interest in judging if captive birds are a good control for further laboratory experiments of contamination.

This study focuses on three important criteria of accumulation variation: genotypes of ducks, biological materials (tissues and organs), and feeding conditions. Genotypes were chosen in relation to their hepatic steatosis ability: Pekin (*Anas platyrhynchos*), Muscovy (*Cairina moschata*), and Mule ducks, a hybrid from the breeding of the two others. Indeed, these genotypes possess divergent metabolisms that allow them to differentially store fat in the liver or in the periphery. The other criteria considered are the distribution of metals according to biological material (tissues and organs) and the impact of feeding condition (overfed vs nonoverfed) on the distribution of pollutants and concentration level.

MATERIALS AND METHODS

Genotypes of Interest. Three breeding genotypes were chosen: Pekin, Muscovy, and Mule ducks. Females of the European native common duck, Pekin (*A. platyrhynchos*), and males of the American native Muscovy duck (*C. moschata*) are crossed to give a hybrid, Mule duck. Muscovy and Mule ducks are more liable to develop hepatic steatosis than Pekin ducks (*21*). This difference between genotypes depends mainly on the imbalance between the intensity of the hepatic lipogenesis and on peripheral activity of LPL (lipoprotein lipase) (*22*).

Duck Collection. Samples were collected in a breeding structure belonging to the French Research in Agronomy Institute (INRA) in the domain of Artiguères, Benquet, Landes, France. All animals were born in INRA. During the first day of life and 4 weeks of age, males were fed *ad libitum* with small granule (length of 2.5 mm) enriched in protein. Between 4 and 6 weeks of age, ducks were fed *ad libitum* with growth food (granule with length of 4 mm) less enriched in protein. From 6 weeks of life, animals had received one meal per day constituted with 230 g of granule which contained 14% water, 15.7% protein, and 2.3% fat. Fifteen days before overfeeding, ducks only received 210 g of granule per day, and this quantity of food increased progressively the last week before overfeeding.

At 12 weeks of age, two groups were made: (a) the first contained five males of each genotype fed *ad libitum* with granule (14% water, 15.7% protein, and 2.3% fat) during the last 2 weeks and (b) the second contained five males of each genotype overfed for 2 weeks with pounded food constituted of 40% water, 35% cornflour, and 25% grain of maize (10 kg on 2 weeks). At 14 weeks of age, the overfed or not overfed ducks of the three groups (Pekin, Muscovy, and Mule) were sampled and finally analyzed for metals. Consequently, a total of 30 animals were sampled, five overfed and five not overfed per genotypes (10 ducks per genotypes).

Metal Determination. All analyses were performed at the UMR 5805 EPOC, Bordeaux 1 University, GEMA, Arcachon Bay, France.

Four main metals were determined for this study: cadmium (Cd), mercury (Hg), zinc (Zn), and copper (Cu). Metal determinations were made on water and food of ducks and on five replicates per genotype and experimental condition (overfeeding or not). The soft bodies were dissected, and different biological materials were removed and dried (45 °C, 48 h): liver, muscle, kidneys, feathers, and abdominal fat. After 2 days, the dried tissues were weighed to allow the expression of the results of metal concentrations in dry weight (DW). Dried samples were divided in two.

Table 1. Comparison of Total Body Weight (kg ± SD) of Overfed and Nonoverfed Muscovy, Pekin, and Mule Ducks[a]

	weight (kg)		
	nonoverfed	overfed	p level
Muscovy	5.9 ± 0.2	7.2 ± 0.6	**
Pekin	4.2 ± 0.2	5.9 ± 0.5	***
Mule	4.3 ± 0.2	7.6 ± 1.6	*

[a] Comparison between weight of overfed and nonoverfed ducks for all species was notified with p level indication (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). SD = standard deviation.

The first part was used for total Hg determination by flameless atomic absorption spectrometry (Leco Ama 254, Altec, Prague, Czech Republic); the detection limit was 0.01 ng of Hg. This method does not require the utilization of an acid digestion. A maximum of 0.1 g of the different tissues and organs was directly put in nacelles. This technique allows the extraction of total mercury. The samples were dried during 60 s at 200 °C and decomposed during 180 s. The mercury was retained on a gold trap and released by the heating system.

The other part of the samples (0.1−0.2 g) was used for a classical digestion procedure which was carried out with 3 mL of concentrated nitric acid (Merck 65% HNO_3) added in a pressurized medium (borosilicate glass tubes) at 100 °C for 3 h. The digestates were diluted up to 18 mL with ultrapure water (MilliQ plus). Cadmium concentrations were measured on 1 mL of digestate solution by atomic absorption spectrophotometry with Zeeman correction, using a graphite tube atomizer (AAS, Thermoptec M6 Solaar graphite furnace). In order to avoid interference, analyses were carried out in the tube atomizer with a blend of Pd and $Mg(NO_3)_2$. The detection limit was 0.1 μg of $Cd \cdot L^{-1}$ (3 × standard deviation of the reagent blanks). Copper and zinc concentrations were determined on the same samples by flame atomic absorption spectrophotometry (Varian SpectrAA 220 FS). The detection limits were 5 μg of $Zn \cdot L^{-1}$ and 20 μg of $Cu \cdot L^{-1}$. The analytical methods were simultaneously validated for each sample series by the analysis of standard biological reference materials (TORT-2, lobster hepatopancreas; DOLT-3, dogfish liver; NCR/CRNC, Ottawa, Canada). Values were in agreement with the certified ranges (TORT-2, Cd, 26.7 ± 0.6 mg/kg, Cu, 106 ± 10 mg/kg, Zn, 180 ± 6 mg/kg, Hg, 0.27 ± 0.06 mg/kg; DOLT-3, Cd, 19.4 ± 0.6 mg/kg, Cu, 31.2 ± 1 mg/kg, Zn, 86.6 ± 2.4 mg/kg).

Statistical Analysis. Nonparametric analysis of variance (Kruskal–Wallis test, Statistica 7.1) was applied to assess differences in accumulation between genotypes, biological material (tissues and organs), and overfed or not overfed ducks. Two by two comparisons were applied with the nonparametric Mann–Whitney U-test (Statistica 7.1). Indeed, homogeneity of variance was not achieved despite transformation $\log(x + 1)$ (Cochran C test). Comparison between total body mass of genotypes was applied with ANOVA after achieving homogeneity of variance was assessed by the Cochran C test. Finally, a posthoc test was used if significant differences appeared between data to identify which group differed (Tukey HSD test).

The Spearman correlation test was applied to evaluate the relationship between the accumulation of genotypes and the total body mass.

RESULTS

Difference between Total Body Mass of Genotypes and Metal Determination on Water and Food. Statistical analysis showed that Muscovy ducks displayed higher body weight compared to other genotypes for nonoverfed animals (ANOVA, $p < 0.01$). The mean weight of all duck genotypes is significantly increased by the overfeeding method (**Table 1**). Both Mule and Muscovy ducks had a total body weight significantly higher than Pekin ducks when overfed (ANOVA, $p < 0.05$).

Table 2. Comparison of Mean Zn Concentrations (mg/kg DW) in the Liver, Kidney, Muscle, Feathers, and Abdominal Fat of Nonoverfed (NO) and Overfed (O) Muscovy, Pekin, and Mule Ducks[a]

	organ														
	liver			kidney			muscle			feathers			abdominal fat		
	NO	O	*p* level	NO	O	*p* level	NO	O	*p* level	NO	O	*p* level	NO	O	*p* level
Muscovy	254.7	48.7	**	86.0	100.3	ns	75.5	46.6	*	95.7	135.0	*	5.2	0.7	ns
Pekin	271.9	38.4	**	95.5	138.1	**	67.4	73.9	ns	102.8	164.9	**	6.1	4.9	ns
Mule	278.8	24.6	**	83.0	96.6	ns	77.3	71.2	ns	118.4	195.5	**	4.2	2.3	ns

[a] Not significant results were indicated (ns), and *p* level was notified for overfed vs nonoverfed statistical analysis (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). $n = 5$ per condition.

Water appeared to have low metal concentrations with values <0.2 μg/kg Cd, 0.0056 mg/kg Cu, <0.05 μg/kg Hg, and <0.02 mg/kg Zn. On the contrary, the food appeared to contain higher metal concentrations for Cd with 0.12 mg/kg DW, for Cu with 9.04 mg/kg DW, and for Zn with 103.5 mg/kg DW whereas the concentration of Hg was under detection limits.

Zinc. *Feeding Condition.* Concentration values varied between 278.8 mg/kg DW in the liver of nonoverfed Mule ducks and 0.7 mg/kg DW in the abdominal fat of overfed Muscovy ducks. **Table 2** summaries the Zn concentration values of the biological materials studied for overfed and nonoverfed animals. Comparison between overfed and nonoverfed animals showed a significant decrease in the metal concentrations of liver when the ducks were overfed (Muscovy, 254.7 mg/kg DW for nonoverfed, >48.7 mg/kg DW for overfed; Pekin, 271.9 mg/kg DW for nonoverfed, >38.4 mg/kg DW for overfed; Mule, 278.8 mg/kg DW for nonoverfed, >24.6 mg/kg DW for overfed). In contrast, a significantly higher concentration was observed in the feathers of overfed animals (Muscovy, 95.7 mg/kg DW for nonoverfed, <135.0 mg/kg DW for overfed; Pekin, 102.8 mg/kg DW for nonoverfed, <164.9 mg/kg DW for overfed; Mule, 118.4 mg/kg DW for nonoverfed, <195.5 mg/kg DW for overfed). Concentrations hardly varied between the two feeding conditions for muscle, kidneys, and abdominal fat. However, some differences appeared. Kidney concentrations of Pekin were higher for overfed animals (95.5 mg/kg DW for nonoverfed, <138.1 mg/kg DW for overfed), and muscle accumulation of Muscovy ducks was high in nonoverfed animals (75.5 mg/kg DW for nonoverfed, >46.6 mg/kg DW for overfed).

Intergenotype Comparison. For nonoverfed animals, when comparing genotypes, a similar distribution of metal concentrations was observed. A small difference appeared for feathers. For this biological material, nonoverfed Mule ducks had a significantly higher mean concentration than nonoverfed Muscovy ducks (**Table 2**; Muscovy, 95.7 mg/kg DW, < Mule, 118.4 mg/kg DW). Overfed ducks also displayed the same patterns of accumulation regardless of genotypes. However, statistical analysis between the three genotypes identified Pekin ducks as the group that accumulated the most metal for the kidneys compared to the two others (overfed Pekin, 138.1 mg/kg DW, > overfed Muscovy, 100.3 mg/kg DW, and overfed Mule, 96.6 mg/kg DW). Pekin ducks also showed the highest concentrations in the muscle (overfed Pekin, 73.9 mg/kg DW, > overfed Muscovy, 46.6 mg/kg DW) and in abdominal fat (overfed Pekin, 4.9 mg/kg DW, > overfed Muscovy, 0.7 mg/kg DW) compared to Muscovy ducks only. For these two tissues, Mule ducks displayed intermediate concentrations compared to the two other genotypes. Concerning feathers, Mule ducks possessed a significantly higher Zn concentration than Muscovy ducks (overfed Mule, 195.5 mg/kg DW, > overfed Muscovy, 135.0 mg/kg DW).

Distribution of Metal Concentrations in Biological Materials. The biological material share of Zn for nonoverfed ducks showed that the liver accumulated the most metal. **Table 2** shows disparities between accumulations of tissues when Pekin ducks were compared to the other genotypes. Pekin ducks had a greater concentration of Zn in kidneys than in muscle (Mann–Whitney U-test, $p < 0.05$), but this difference did not appear for Muscovy and Mule ducks. For the latter, concentration of feathers was higher compared to muscle and kidneys. The abdominal fat significantly had accumulated the less metal for all genotypes and both conditions (overfed or not). For overfed ducks the distribution of metal concentrations was disturbed by the decrease of Zn concentration in the liver. For the three groups, the feathers significantly presented the most important concentration followed by the kidneys. Muscovy and Pekin ducks reached the same level of accumulation for liver and muscle while Mule ducks had a statistically and significantly higher concentration in muscle than in liver.

Relationship with Body Weight and with Cadmium Concentration. A comparison taking into account all individuals of all genotypes showed a negative correlation between the Zn concentration of the kidneys of the overfed animals and the weight of the ducks (Spearman correlation, $p < 0.05$, $r = -0.67$, **Figure 1a**) . Moreover, there was a correlation between the Zn and the Cd concentrations for nonoverfed Pekin ducks in kidneys (Spearman correlation, $p < 0.05$, $r = 0.90$) and nonoverfed Muscovy ducks in liver (Spearman correlation, $p < 0.05$, $r = 0.94$).

Copper. *Feeding Condition.* Concentration values varied between 540.4 mg/kg DW in the liver of nonoverfed Pekin ducks and below detection limits for abdominal fat of overfed Pekin and Mule ducks. **Table 3** represents the comparison between overfed and nonoverfed ducks for all groups. The overfeeding condition significantly reduced the concentrations of the liver of overfed ducks (Muscovy, 168.0 mg/kg DW for nonoverfed, >17.9 mg/kg DW for overfed; Pekin, 540.4 mg/kg DW for nonoverfed ,>36.9 mg/kg DW for overfed; Mule, 352.8 mg/kg DW for nonoverfed, >25.0 mg/kg DW for overfed). The increase in kidney concentrations due to the overfeeding condition was statistically significant (Muscovy, 18.2 mg/kg DW for nonoverfed, <32.2 mg/kg DW for overfed; Pekin, 35.0 mg/kg DW for nonoverfed, <63.7 mg/kg DW for overfed; Mule, 23.4 mg/kg DW for nonoverfed, <45.8 mg/kg DW for overfed). The accumulation of the metal in feathers and abdominal fat remained unchanged with the feeding condition. On the other hand, the muscle results showed some divergences. The level of the concentrations was the same between overfed and nonoverfed ducks for Pekin and Mule ducks, whereas the mean of Cu concentrations decreased for Muscovy ducks when overfed (nonoverfed, 7.1 mg/kg DW, > overfed, 2.8 mg/kg DW).

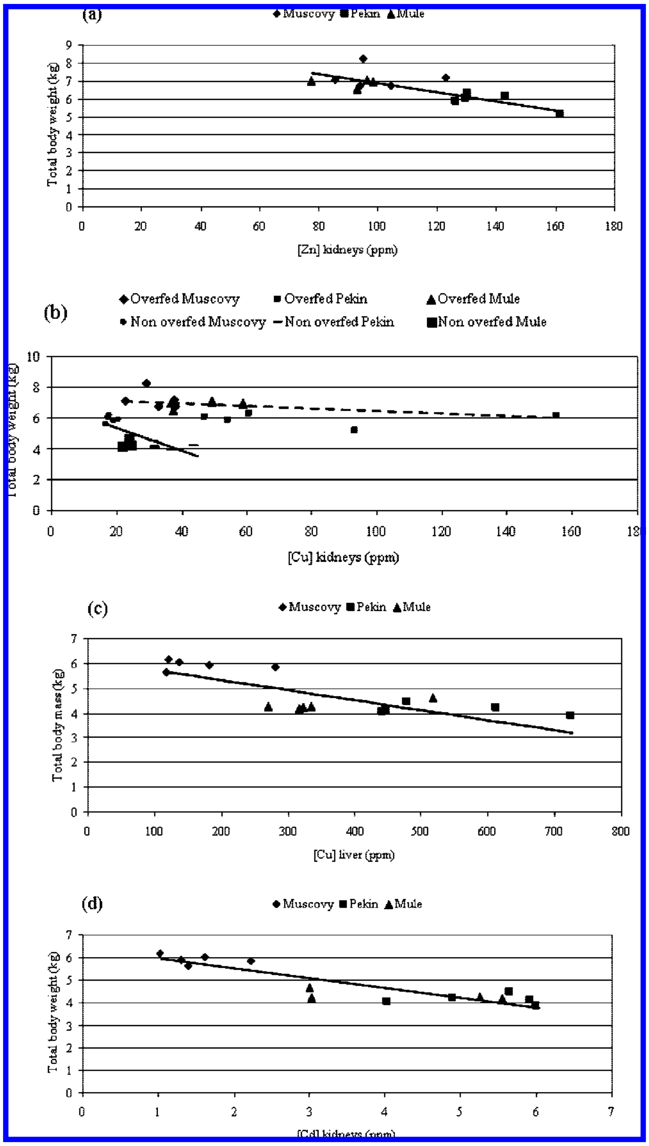

Figure 1. (**a**) Relation between Zn concentrations in the kidneys (mg/kg DW) and the total body weight (kg) for overfed ducks of three species: Muscovy, Pekin, and Mule ducks. (**b**) Relation between Cu concentrations in the kidneys (mg/kg DW) and the total body weight (kg) for nonoverfed and overfed ducks of three species: Muscovy, Pekin, and Mule ducks. (**c**) Relation between Cu concentrations in the liver (mg/kg DW) and the total body weight (kg) for nonoverfed ducks of three species: Muscovy, Pekin, and Mule ducks. (**d**) Relation between Cd concentrations in the kidneys (mg/kg DW) and the total body weight (kg) for nonoverfed ducks of three species: Muscovy, Pekin, and Mule ducks.

Intergenotype Comparison. Some differences exist with regard to genotypes. For nonoverfed ducks, Muscovy displayed lower concentrations in the liver and kidneys than Pekin ducks (**Table 3**). The comparison between overfed individuals of the three genotypes showed that kidneys of Muscovy ducks had significantly less accumulated than those of Pekin ducks (**Table 3**; Muscovy, 32.2 mg/kg DW, < Pekin, 63.7 mg/kg DW). Muscovy had also less accumulated the element in the muscle than the two other groups (overfed Muscovy, 2.8 mg/kg DW, > overfed Pekin, 6.5 mg/kg DW, and overfed Mule, 5.8 mg/kg DW). For feathers, Pekin ducks were less contaminated than Mule ducks (overfed Pekin, 9.1 mg/kg DW, < Mule, 19.8 mg/kg DW).

Distribution of Metal Concentrations in Biological Materials. Copper was mainly accumulated in the liver for nonoverfed animals (**Table 3**). In this organ, the concentrations reached 540.4 ± 123.5 mg/kg DW for Pekin, and the minimum was obtained for Muscovy with 168.0 ± 68.7 mg/kg DW (**Table 3**). The concentrations of metal were different between biological materials: liver > kidneys > feather > muscle > abdominal fat for all genotypes (Kruskal–Wallis test) (**Table 3**). Accumulation of abdominal fat was low and often below detection limits especially for overfed animals (**Table 3**). The distribution of metals was affected by the overfeeding condition. For all genotypes, the liver and kidneys, which did not show statistically different concentrations, remained the main organs of accumulation. Feathers were the third biological material that accumulated the most for all genotypes. The muscle of Pekin reached the same Cu concentration as feathers.

Relationship with Body Weight. Cu concentrations in the liver of nonoverfed ducks were negatively correlated to body weight (Spearman correlation, $p < 0.05$, $r = -0.71$, **Figure 1c**). Moreover, the kidney concentrations of nonoverfed and overfed ducks were negatively correlated to body weight (Spearman correlation, $p < 0.05$; overfed, $r = -0.54$; nonoverfed, $r = -0.68$; **Figure 1b**).

Cadmium. *Feeding Condition.* **Table 4** presents the Cd concentration values of the biological materials studied for overfed and nonoverfed animals of the three different genotypes. Data dispersion was relatively high: from 5.295 mg/kg DW in the kidneys of nonoverfed Pekin ducks to 0.001 mg/kg DW in abdominal fat of overfed Pekin ducks. The comparison between nonoverfed and overfed individuals of the three genotypes studied showed significant higher Cd concentrations in the liver of nonoverfed animals (Muscovy, 0.547 mg/kg DW for nonoverfed, >0.280 mg/kg DW for overfed; Pekin, 1.884 mg/kg DW for nonoverfed, >0.176 mg/kg DW for overfed; Mule, 1.840 mg/kg DW for nonoverfed, >0.137 mg/kg DW for overfed). The kidneys and muscle underwent no changes in their levels of concentration with regard to feeding condition. On the other hand, disparities exist for feathers and abdominal fat. Muscovy duck feathers accumulated more of the metal when animals were overfed (0.004 mg/kg DW for nonoverfed, >0.011 mg/kg DW for overfed). Pekin ducks displayed lower concentrations in fat for overfed individuals (0.019 mg/kg DW for nonoverfed, >0.001 mg/kg DW for overfed).

Intergenotype Comparison. Statistical comparison (Kruskal–Wallis, $p < 0.05$) between genotypes showed that Muscovy ducks possess the lower accumulation for the liver in all of the conditions (nonoverfed, Muscovy, 0.547 mg/kg DW, < Pekin, 1.884 mg/kg DW, and Mule, 1.840 mg/kg DW; overfed, Muscovy, 0.028 mg/kg DW, < Pekin, 0.176 mg/kg DW, and Mule, 0.137 mg/kg DW) and kidney (nonoverfed, Muscovy, 1.514 mg/kg DW, < Pekin, 5.295 mg/kg DW, and Mule, 4.207 mg/kg DW; overfed, Muscovy, 1.237 mg/kg DW, < Pekin, 4.398 mg/kg DW, and Mule, 4.486 mg/kg DW).

Distribution of Metal Concentrations in Biological Materials. Considering all genotypes and two conditions (overfed or not), kidneys were significantly the main biological material for Cd accumulation, followed by the liver (Mann–Whitney U-test, $p < 0.05$). The distribution of concentrations between muscle, feathers, and abdominal fat varies following genotypes and conditions. For nonoverfed hybrid ducks, Mule, feathers contained a significantly higher concentration of Cd than muscle and abdominal fat (Mann–Whitney U-test, $p < 0.05$, kidneys > liver > feathers > muscle and abdominal fat). For overfed Muscovy ducks, the same level of metal concentration was observed between the liver,

Table 3. Comparison of Mean Cu Concentrations (mg/kg DW) in Liver, Kidney, Muscle, Feathers, and Abdominal Fat of Nonoverfed (NO) and Overfed (O) Muscovy, Pekin, and Mule Ducks[a]

	organ														
	liver			kidney			muscle			feathers			abdominal fat		
	NO	O	*p* level	NO	O	*p* level	NO	O	*p* level	NO	O	*p* level	NO	O	*p* level
Muscovy	168.0	17.9	**	18.2	32.2	**	7.1	2.8	**	13.2	12.1	ns	1.3	0.6	ns
Pekin	540.4	36.9	**	35.0	63.7	*	5.9	6.5	ns	12.9	9.1	ns	1.8	nd	ns
Mule	352.8	25.0	**	23.4	45.8	*	6.5	5.8	ns	16.0	19.8	ns	2.7	nd	ns

[a] Not significant results were indicated (ns), and *p* level was notified for overfed vs nonoverfed statistical analysis (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Two abdominal fat values are below detection limits of 20 μg of Cu$\cdot$L^{-1} (nd). $n = 5$ per condition.

Table 4. Comparison of Mean Cd Concentrations (mg/kg DW) in the Liver, Kidney, Muscle, Feathers, and Abdominal Fat of Nonoverfed (NO) and Overfed (O) Muscovy, Pekin, and Mule Ducks[a]

	organ														
	liver			kidney			muscle			feathers			abdominal fat		
	NO	O	*p* level	NO	O	*p* level	NO	O	*p* level	NO	O	*p* level	NO	O	*p* level
Muscovy	0.557	0.028	**	1.514	1.237	ns	0.004	0.003	ns	0.004	0.011	*	0.020	0.004	ns
Pekin	1.884	0.176	*	5.295	4.398	ns	0.005	0.007	ns	0.008	0.008	ns	0.019	0.001	*
Mule	1.840	0.137	*	4.207	4.486	ns	0.005	0.025	ns	0.016	0.015	ns	0.005	0.003	ns

[a] Not significant results were indicated (ns), and *p* level was notified for overfed vs nonoverfed statistical analysis (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). $n = 5$ per condition.

Table 5. Comparison of Mean Hg Concentrations ($\times 10^{-5}$ mg/kg DW) in Liver, Kidney, Muscle, Feathers, and Abdominal Fat of Nonoverfed (NO) and Overfed (O) Muscovy, Pekin, and Mule Ducks[a]

	organ														
	liver			kidney			muscle			feathers			abdominal fat		
	NO	O	*p* level	NO	O	*p* level	NO	O	*p* level	NO	O	*p* level	NO	O	*p* level
Muscovy	3.4	12.3	**	0.27	1.2	*	0	1.0	**	1.8	8.4	**	33.0	48.3	ns
Pekin	12.8	21.4	ns	1.5	141.0	**	1.2	42.0	*	7.8	35.1	**	71.2	1673.0	*
Mule	7.5	73.8	**	1.1	172.2	*	4.8	57.0	*	3.2	126.2	**	41.8	492.5	**

[a] Not significant results were indicated (ns), and *p* level was notified for overfed vs nonoverfed statistical analysis (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). $n = 5$ per condition.

muscle, feathers, and abdominal fat. For overfed Pekin and Mule ducks abdominal fat showed the lowest Cd concentrations in comparison with other tissues and organs studied.

Relationship with Body Weight. A comparison taking into account all individuals of all genotypes (**Figure 1d**) showed that Cd concentrations in kidneys of nonoverfed ducks were negatively correlated to body weight (Spearman correlation, $p < 0.05$, $r = -0.85$).

Mercury. *Feeding Condition.* The concentrations varied from 71.2×10^{-5} mg/kg DW in the abdominal fat of nonoverfed Pekin to 0.016 mg/kg DW (1673×10^{-5} mg/kg DW) in the same tissue of overfed Pekin, which represented a very low value (**Table 5**). Statistical analysis showed the impact of overfeeding condition on the accumulation of all biological materials and genotypes. The concentrations of Hg significantly increased in the overfed condition with two exceptions for Pekin ducks in the liver and Muscovy ducks in the abdominal fat.

Intergenotype Comparison. The statistical analysis concerning the genotype effect shows that overfed Mule ducks had accumulated more of the metal than overfed Muscovy ducks for all biological material (**Table 5**). Pekins displayed superior concentrations in abdominal fat than Muscovy (**Table 5**). In the case of nonoverfed animals, Muscovy also appeared to be the less contaminated genotypes, especially for the liver and feathers compared to Pekin (**Table 5**).

Distribution of Metal Concentrations in Biological Materials. Statistical analysis showed that Hg was greatly accumulated in abdominal fat of all genotypes and that Pekin ducks possessed the strongest concentration (**Table 5**). The distribution of metal concentrations was not the same for all genotypes. For nonoverfed Muscovy and Pekin ducks (**Table 5**), the liver and feathers possessed the second highest concentrations after abdominal fat. Kidneys and muscle had the lower accumulation level. Mule ducks presented a significant difference of Cu concentration between liver and kidneys.

Relationship with Body Weight. No correlation between the weight of the total soft body and the contamination of the liver or the kidneys appeared.

DISCUSSION

Distribution of Metals. For all of the metals considered, the results concerning nonoverfed animals showed the same pattern of accumulation as those previously established. For Cd, the kidneys, followed by the liver, represented the main organs of accumulation. This finding agreed with the results of other studies regarding the differences between metal accumulations in bird tissues and organs (*23–27*). This result allowed us to conclude that further measures could be focused on these particular organs. Concentration of zinc was also greatly higher in the liver than in other tissues and organs (*26–28*). This demonstrates the role of this organ in detoxification and storage of metals. For copper, the liver represented the biological material that accumulated the most as observed by other authors (*26–28*), and a graduation of accumulation appeared: liver > kidneys > feathers > muscle > abdominal fat. In the

Table 6. Metal Concentrations in Cadmium, Copper, Zinc, and Mercury in Liver, Kidney (μg/g Dry Weight), and Muscle (μg/g Wet Weight)

				Cd		Cu		Zn		Hg		
species	common name	geographic location	ref	liver	kidney	liver	kidney	liver	kidney	liver	kidney	muscle
Anas platyrhynchos	mallard	Donana National Park (Spain)	*1*	nd−2.178		5.069–81.5		163				
		Alaska	*3*									0.0893
Anas crecca	teal	Donana National Park (Spain)	*1*	0.466		35.2		83.91				
		Alaska	*3*									0.0283
Anas clypeata	shoveler	Donana National Park (Spain)	*1*	0.21		29.9		65.32				
		Alaska	*3*									0.1512
Anas strepera	gadwall	Donana National Park (Spain)	*1*	1.269		114.5		269.2				
Somateria fischeri	spectacled eiders	St. Lawrence Island (Alaska)	*4*	33.8	95.55	558.7	67.5	157.64	129.63	1.13	0.73	
Somateria mollissima	common eiders	Nunavut (Canada), 1998	*37*		162.6					3.7		
		eastern Canadian arctic	*18*		74[a]–164[b]					2[a]–3.5[b]		
Anas acuta	northern pintail	Alaska	*3*									0.0385
Clangula hyemalis	oldsquaw	Alaska	*3*									0.1512

[a] Ducks in prenesting period. [b] Nesting period.

case of mercury, the abdominal fat possesses the most important concentrations, especially in Pekin ducks. The presence of mercury in abdominal fat can be explained by the lipophile attribute of this metal especially in its organic form, which is generally more toxic to birds (*2–5*). It represents the most bioavailable chemical species of Hg and can be bioaccumulated through the food chain (*29*).

Overfeeding Impact on Accumulation Related to Duck Metabolism. The overfeeding method reduced the concentration level of the liver for Cd, Zn, and Cu through a dilution process. This dilution of metal concentrations can result from the important weight increase in ducks during the overfeeding period, considering that accumulation by ducks of food remains unchanged during this period.

On the contrary, overfeeding increased Hg concentration for all genotypes and biological materials. Three different patterns emerged: (a) mercury concentrations of Pekin ducks increased in their abdominal fat with overfeeding but not in the liver, (b) Muscovy ducks displayed a Hg increase in the liver but not in abdominal fat, and (c) there was an augmentation in both biological materials in Mule ducks. This is strongly coherent with the previous results concerning physiological lipid mobilization processes in these species. Previous study comparing storage of fat between Muscovy and Pekin ducks showed that before overfeeding (a) Muscovy ducks exhibited a lower subcutaneous adiposity and a higher muscular development and that (b) in both genotypes hepatic composition was similar (*30*). But in Muscovy ducks the overfeeding response induces a higher degree of hepatic steatosis and a lower increase in plasma triglycerides (peripherical accumulation). Some genotypes may be more responsive to the dietary induction of fatty liver because of differences existing in the channeling of hepatic lipids toward secretion into plasma and adipose storage (*30, 31*). Higher plasma insulin concentrations measured in Pekin ducks after overfeeding could be responsible for the preservation of LPL (lipoprotein lipase) activity and, thus, allow the storage of extrahepatic fattening (*22*). When providing an amount of food in proportion to their body weight, Muscovy ducks developed a significant liver steatosis, whereas it was not very pronounced in the Mule ducks and even less in the Pekin ducks (*22*). For the Pekin ducks, the increase of Hg concentration (and Cd dilution) in abdominal fat probably corresponds to a large amount of fat accumulated at the periphery. On the opposite, Muscovy ducks store fat in the liver inducing a higher degree of hepatic steatosis. Mule ducks display intermediate capacities of fat storage which induced an Hg increase in the liver and abdominal fat.

The difference in lipidic metabolism ability conducts genotypes to a differential level of accumulation between tissues and organs. In the case of migrating ducks, this reversible (*32, 33*) natural phenomenon of hepatic steatosis (seasonal variations of body weight) has to be taken into account in order to evaluate the accumulation level. When animals are exposed to large seasonal variations in both quality and quantity of food, it is essential to relate element concentration to the physiological condition of the animal (*34*).

Level of Accumulation Compared to Field Studies and Other Domestic Ducks. Generally, in our study the three genotypes used were little contaminated (**Tables 2−5**) according to the relative protection to which these animals were subjected within the framework of breeding compared to wild birds. Similarities exist between previous results on domestic Pekin ducks (*35*) and ours. For nonoverfed individuals, Zn concentrations of the muscle were at the same level (79 mg/kg DW compared to 67.4 mg/kg DW in our study). The same observation can be made for Zn in kidneys (114.6 mg/kg DW compared to 95.5 mg/kg DW in our study) (*35*). This metal is present at natural rates relatively high in the body, 25 mg/kg wet weight (*36*), with an increase when expressed in dry weight. However, the concentrations recorded in this study were more important. This result can be linked to the correlation between Zn and Cd concentrations. Indeed, the more the accumulation of Cd increased, the more the accumulation of Zn increased.

On the other hand, the results for liver concentrations in this work are higher than those of Dressel et al. (*35*), who found only 154 mg/kg DW of Zn (compared to 271.9 mg/kg DW) and 255 mg/kg DW of Cu (compared to 540.4 mg/kg DW). In contrast, the contamination was lower in the case of Cu for muscle (5.9 mg/kg DW in our study compared to 38.4 mg/kg DW) and for kidneys (35 mg/kg DW in our study compared to 76.7 mg/kg DW). The physiological level previously recorded reached 4 mg/kg wet weight for Cu (*36*). The levels measured in this study correspond for the major part with that observation.

Field studies concerning diverse sorts of ducks done in diverse geographical locations have shown levels of metal accumulation appreciably higher than our results (**Table 6**).

The Cd concentrations observed in literature for the liver can rise up to 34 mg/kg (*4*) but remains more generally around 1 mg/kg (*1–3*). The values found in this study for the liver, on average 1.884 mg/kg DW for Pekin and 1.840 mg/kg DW for Mule, thus stay for the greater part below levels observed in the field but are not unimportant especially for kidneys. The concentrations of metals, Cd but also Cu and Zn, can be explained by the levels observed in food. When the ratio Cd liver/Cd kidneys was included between 0.09 and 0.61, animals were subjected to a low chronic exposure in Cd (*5*). In our study, this ratio for overfed ducks was included between 0.02 and 0.04 for Pekin, 0.01 and 0.04 for Mule, and 0 and 0.04 for Muscovy. Nonoverfed had a ratio comprised between 0 and 0.42 for Pekin, 0.32 and 0.53 for Mule, and 0.15 and 0.45 for Muscovy. These values allow us to say that the contamination is very low for overfed ducks due to the dilution induced by fast weight increase and low but noticeable for nonoverfed ducks. For the latter, the values are closer to the limits defined and demonstrate that the individuals are not subjected to an acute contamination in Cd but can be considered submitted to a low chronic contamination.

Very low Hg concentrations are observed compared to field studies (*4, 18,*) (*37*). Indeed, 0.11−4.2 mg/kg in feathers are listed according to the species studied on the Chilean coast (*14*). These concentrations are greatly higher than those of the Muscovy ($1.8 \times 10^{-5} \pm 0.9 \times 10^{-5}$ mg/kg DW), Pekin ($7.8 \times 10^{-5} \pm 2.2 \times 10^{-5}$ mg/kg DW) and Mule ($3.2 \times 10^{-5} \pm 1.4 \times 10^{-5}$ mg/kg DW) studied here. This study shows that mercury does not represent a problem for breeding ducks of this station.

Copper and zinc displayed higher concentration levels than cadmium and mercury because of their involvement in the metabolism of animals. Thus, the observed values get closer to those observed elsewhere (**Table 6**). On average, ducks reached a liver concentration in Cu of 353.7 ± 186.2 mg/kg DW and in Zn of 268.4 ± 12.4 mg/kg DW.

The thresholds leading to perturbation in animals are very variable according to organs and authors. Our study revealed detectable concentrations of metals that can be of consideration, especially for Cd, but none at levels that are considered a health hazard for domestic ducks or for humans.

Influence of Body Weight on Metal Concentrations. Negative correlations were observed between total body weight and accumulation of cadmium, zinc, and copper. This observation was made for kidneys of nonoverfed ducks in the case of Cd and for kidneys and liver of nonoverfed ducks in the case of Cu. Overfed ducks presented the same tendency especially for Zn and Cu in the kidneys. This relationship was already described for wild common eider ducks in the eastern Canadian arctic (*18, 38*): the heavier the animal, the less the accumulation. This result must be considered cautiously because it integrates three genotypes at the same time and not only one as the previous author did. Moreover, levels of metals appeared to be influenced by genotypes that had previously been described in environmental studies (*1, 3, 14*). Muscovy ducks often appeared as the genotype that accumulated the less for all metals and biological materials. This can be linked to the weight of the individuals of this species which possess the highest total body mass.

Due to the low levels of the accumulation observed, domestic ducks are good control animals for further contamination studies. This can be envisaged to contaminate these genotypes, to compare the level of accumulation to the level found in this study, and to evaluate the impact of metals on some biological parameters. In this study, we detect a low chronic contamination concerning cadmium and assume that it comes from food. We have established that genotype, feeding condition, and biological material such as organs and tissues influence accumulation levels and distribution of metals. In particular, the differential lipidic metabolism leads to differential accumulation of metals and to dilution of pollutants except for mercury. The comparison between nonoverfed and overfed ducks, which represents food intakes during the natural migrating cycle of the ducks, showed the importance of taking into account the life cycle of birds before studying their contamination and the impact of pollutants.

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