The effect of humic acid on mercury accumulation in chicken organs and muscle tissues

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ABSTRACT: Forty female chickens were allocated to four groups of ten birds each. The control group (K) was fed a basal diet without supplementation. The second control group received a basal diet with humic acid (HA) at a dose of 0.5 g per chicken/day. The first experimental group was fed the basal diet with methylmercury (MeHg) at a dose of 0.02 mg per chicken/day and the group of chickens MeHg + HA received 0.5 g HA per chicken/day. After slaughter, mercury levels were assessed in liver, kidney, brain and muscle tissue samples. After 10-day treatment of the chickens with MeHg, significantly increased ($P < 0.001$) Hg concentrations were detected in all examined tissues in comparison with the groups K and HA. The average concentrations in liver, kidneys, brain and muscle tissues were 424, 398, 81.6 and 79.2 µg Hg/kg, respectively. After concurrent treatment with HA and MeHg, Hg concentrations were lower by 20.6%, 23.8%, 23.0% and 18.6% in liver, kidneys, brain and muscle tissues ($P < 0.001$). Biological accumulation of Hg was 25.5% and 20.4% in MeHg and MeHg + HA groups, respectively.

Keywords: methylmercury chloride; liver; kidney; brain; muscle tissue; bioavailability

HA are classified among the polyvalent weak organic acids and possess the ability to form complexes. They also have adsorption and ion exchange capabilities (Livens, 1991). A number of studies have failed to identify any toxic, allergic, teratogenic or carcinogenic effects of HA (Klocking, 1994). The use of HA and their sodium salt for the oral treatment of all animals on food production farms is currently permitted (EMEA, 1999). Due to their colloidal characteristics, humic substances have antiphlogistic, adsorption and antibacterial effects on the gastrointestinal tract mucosae, particularly in young animals; it is because of these properties that they are used in farm and domestic animals (Kuhnert et al., 1991). Despite the fact that the use of humic substances as preparations for veterinary purposes has been allowed by EU legislation, their use as feed supplements has not been sanctioned. Recently, humic substances have been proposed as feed supplements to stimulate the growth of animals, to increase the nutritive value of feeds and as a potential replacement for antibiotic growth stimulators (Bailey et al., 1996; Shermer et al., 1998; Kocabagli et al., 2002; Yoruk et al., 2004; Aksu et al., 2005; Islam et al., 2005).

Humic acids have a strong affinity to various substances such as mutagens (Cozzi et al., 1993), herbicides (Negre et al., 2001), monoaromatic (Nanny and Maza, 2001) and polycyclic aromatic compounds (Kollist-Siigur et al., 2001), chemical elements (Elfarissi and Pefferkorn, 2000), heavy metals (Livens, 1991; Herzig et al., 1994, 2007; Madronová et al., 2001; Hammock et al., 2003), mycotoxins (Van Rensburg et al., 2006) and bacteria (Fein et al., 1999). This is due to their varied structures, high content of heterogeneous functional groups and ca-

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pability to form complexes. Livens (1991) reported that the interaction between heavy metal ions and HA takes place largely through the carboxylate and phenolic hydroxyl group and that the adsorption rate reflects the changes in pH and ion concentrations. However, literature data concerning the effect of HA on the accumulation of mercury in organisms is scarce (Hammock et al., 2003).

The purpose of the present study was to verify the effect of short-term HA feeding to mercury treated chickens on the distribution and accumulation of mercury in their organs and muscles and on the characteristics of their metabolism and performance.

MATERIAL AND METHODS

Animals, diets, experimental design

Forty hybrid 9-weeks-old ISA BROWN chickens (pullets) of live weights ranging between 720 and 850 g were used. The experiment was performed under good hygienic conditions in accredited animal facilities at the Veterinary Research Institute in Brno (experiment authorization No. 930/07). All laws were followed and the experiment was approved by an ethical committee. Chickens were housed on grids in boxes (950 cm^2 per bird) equipped with feeders and drinkers. The feed mixture and drinking water were available *ad libitum*. During the experiment, the chickens were kept under a 12 h light – 12 h dark cycle regime.

After the adaptation period, chickens were allocated to 4 groups of 10 birds each according to the following scheme:

Negative control group (K) – chickens were fed the feeding mixture without supplements.

Positive control group (HA) – chickens were fed the feeding mixture and 0.5 g humic acid/chicken/day.

Experimental group (MeHg) – chickens were fed the feeding mixture and treated with 0.016 mg Hg/chicken/day (0.02 mg CH_3HgCl – MeHg).

Experimental group (MeHg + HA) – chickens were fed the feeding mixture and treated with 0.016 mg Hg/chicken/day (0.02 mg CH_3HgCl – MeHg) and 0.5 g humic acid/chicken/day.

Before the initiation of the experiment, the content of selected chemical elements (mg/kg) was assessed in the feeding mixtures: Hg 0.0002, Pb 0.1, Cu 15.2, Zn 61.8, Se 0.215 and Fe 102. Capsules containing HA or MeHg were wetted with oil and administered to the tongue root every morning. The feed consumption of the respective chicken groups was noted throughout the whole experimental period and health status was monitored every day. After 10 days, the chickens were euthanized with a narcotic overdose (ketamine and xylazine).

Body weight assessment and sample collection

The chickens were weighed at the beginning and at the end of the trial. Groups of chickens were always weighed in the same sequence and at the same time. Blood samples for biochemical analysis were collected following euthanasia. On necropsy, the organs were taken; liver, kidney and brain were weighed and leg muscle samples were collected (*m. biceps femoris*). The samples from each chicken were stored at −18°C before analysis.

Specification of the active substance

The humic acid, batch No. B03A1, was a generous gift from the Research Institute of Inorganic Chemistry (Ústí nad Labem, Czech Republic). The sample contained (%): 90.6 dry matter, 86.9 humic acid and 3.74 ash. HA was prepared by sedimentation and centrifugation of potassium humate with sulphuric acid at pH 1.5–1.7 and dried. The sample analysis of mercury content showed 0.222 mg/kg.

Chemical analysis

Mercury content was assessed in the samples of feed, HA, liver, kidneys, muscles and brain; the weight of each sample ranged between 0.05 and 0.1 g. Homogenized solid samples were directly weighed into pre-cleaned combustion boats and inserted into the Advanced Mercury Analyser AMA 254 (Altec, Prague, Czech Republic). The samples were dried at 120°C for 90 s and thermally decomposed at 550°C for 180 s under oxygen flow. The selectively trapped Hg was released from the amalgamator by briefly heating and was finally quantified as Hg^0 by cold-vapour atomic absorption spectroscopy at a wavelength of 253.65 nm. The detection limit was 0.1 μg/kg. The validity of the analysis was checked by the use of certified reference Milk Powder material (BCR®-151, Belgium).

Total protein (TP), albumin (Alb), glucose (Glu), triacylglycerols (TG), cholesterol (Chol), aspartate aminotransferase (AST), calcium (Ca), phosphorus (P), magnesium (Mg), iron (Fe) and copper (Cu) blood plasma levels were assessed spectrophotometrically using Bio-La-Tests (PLIVA – Lachema Brno, Ltd., Czech Republic).

Statistical analysis

The significance of between-group differences in mean values was assessed by one-way analysis of variance (ANOVA) followed by Tukey's test or by the nonparametric Kruskal-Wallis test. Statistical analysis was performed with STAT Plus software (VRI, Brno, Czech Republic).

RESULTS AND DISCUSSION

The mercury (Hg) content found in the feeding mixture was 0.2 μg/kg, which is a level near the detection limit; it was 500 times lower than the maximum residue limit (MRL), i.e. 0.1 mg/kg (Anonymous, 2004). The analysis of HA revealed a concentration of 0.222 mg Hg/kg. Experimental groups of chickens daily ingested 0.02 mg MeHg (0.016 mg of elementary Hg); with regard to the volume of ingested feed, they received about 60% more Hg than allowed by the MRL. The Hg intake of experimental chickens was 800 and 125 times higher in comparison with the K and HA groups of chickens, respectively.

The highest average body weight gain during the entire experimental period was observed in the HA group of chickens (231 ± 23.5 g) and is consistent with the already described favourable effect of humic substances on efficiency parameters (Lotosch, 1991; Yoruk et al., 2004). In the MeHg group of chickens, a stress caused by Hg was manifested by growth depression and the average body weight gain was 23.7% lower in comparison with K group. However, if chickens were concurrently treated with HA, the growth activity was reduced by 4.8% only (Table 1). A positive effect of HA on feed conversion was noted (4.11 kg). Comparable effects of HA on efficiency and on feed conversion were reported by Kocabagli et al. (2002), who tested the effects of humic substances on fattened broilers.

During the MeHg treatment of chickens, health status was monitored and no clinical manifestations of disturbed vital functions were observed compared to K and HA groups. In MeHg + HA group, a significantly higher concentration of P ($P < 0.01$) and a lower concentration of Cu ($P < 0.05$) compared with MeHg group or control chickens was detected (Table 2). These changes can be caused by metal-humic complexes (Livens, 1991). The other monitored parameters of metabolism were within the physiological range (Meluzzi et al., 1992) and were in accordance with the findings of Demeterová and Mariščaková (2006), who studied the effects of humic substances on chickens.

Body weight ratios for the livers and kidneys of chickens are presented in Table 3. The organ weight differences between the short-term mercury treated and untreated (K, HA) groups of chickens were not significant. The average liver weight of K, HA, MeHg and MeHg + HA groups was 20.8, 20.0, 22.2 and 21.1 g, respectively, and the weight of kidneys ranged between 3.9 and 5.0 g.

The trace amounts of mercury detected in the liver, kidneys, brain and muscle tissue of control chickens showed the current status and the background Hg concentrations in chicken organs. In the above-mentioned organs and tissues, the values represented only 0.6 to 1.4% of the MRL. In accordance with Commission Regulation (EC) 1881/2006 the maximum permissible concentration in the

Table 1. Evaluation of body weight and growth rate and feed efficiency in chickens (g)

Parameters	K	HA	Hg	Hg + HA
Body weight day 0	884 ± 43.9	918 ± 66.2	878 ± 76.3	901 ± 92.6
Body weight days 10	1 093 ± 61.3	1 149 ± 78.3	1 038 ± 47.8	1 100 ± 97.7
BWG[1]	209 ± 32.3	231 ± 23.5	159 ± 32.9	199 ± 18.1
FCR[2] (kg/kg)	4.40	4.11	5.27	4.72

[1]body weight gain
[2]feed conversion rate
$\bar{x}$ ± SD; n = 10

Table 2. Selected biochemical characteristics of blood sera of chickens

Parameters	K	HA	Hg	Hg + HA
Total protein (g/l)	39.1 ± 3.88	37.9 ± 1.77	36.3 ± 4.66	39.2 ± 1.72
Albumin (g/l)	14.0 ± 1.09	13.8 ± 0.75	13.2 ± 1.62	13.3 ± 0.82
Glucose (mmol/l)	12.1 ± 0.60	12.1 ± 0.60	11.3 ± 1.77	12.2 ± 0.93
Triacylglycerol (mmol/l)	0.35 ± 0.03	0.40 ± 0.08	0.41 ± 0.13	0.43 ± 0.15
Cholesterol (mmol/l)	2.78 ± 0.34	2.77 ± 0.30	2.51 ± 0.42	2.80 ± 0.24
Calcium (mmol/l)	2.78 ± 0.15	2.77 ± 0.17	2.59 ± 0.38	2.53 ± 0.10
Phosphorus (mmol/l)	2.17 ± 0.10	2.18 ± 0.11	1.92 ± 0.59[A]	2.55 ± 0.37[B]
Magnesium (mmol/l)	0.83 ± 0.03	0.79 ± 0.06	0.86 ± 0.24	0.82 ± 0.05
Iron (mmol/l)	17.7 ± 1.55	17.1 ± 3.01	16.1 ± 3.63	18.4 ± 1.87
Copper (mmol/l)	2.73 ± 0.95[a]	2.83 ± 0.21	1.93 ± 0.39	1.74 ± 0.48[b]

[A,B]significant differences ($P < 0.01$)
[a,b]significant differences ($P < 0.05$)
$\bar{x}$ ± SD; $n = 10$

organs and meat of chickens is 0.05 mg/kg fresh sample.

Mercury levels in the organs and muscle tissues of humic acid treated chickens were twice and three times higher compared with control chickens. This increase was due to the Hg content in HA itself, as its analysis showed that it contained 0.222 mg Hg/kg.

The ten-day treatment of the experimental group (MeHg) caused a significant increase in Hg concentration ($P < 0.001$) in all monitored organs and tissues (Figures 1 and 2). In liver, kidneys, brain and muscle tissue 424, 398, 81.6 and 79.2 µg Hg/kg (RSDs < 10%) were detected, respectively. The limits established by the European Commission were exceeded by 748, 696, 63.2 and 58.2%, respectively. The detected Hg levels in chicken organs are in accordance with those reported by other authors (Pribilincová et al., 1997; Marettova et al., 2003; Cabanero et al., 2005). The highest Hg concentrations were detected in liver, which is considered a target organ. In contrast to inorganic mercury, MeHg is almost completely absorbed from the gastrointestinal tract and its excretion from the organism is very limited (Clarkson, 1997; Morel et al., 1998).

The concurrent treatment with HA and MeHg in the second experimental group of chickens caused a significant decrease in Hg concentrations in all investigated organs and tissues in comparison with the MeHg group ($P < 0.001$) (Figures 1 and 2): the mercury content was lower by 20.6%, 23.8%, 23.0% and 18.6% in liver, kidneys, brain and muscle tissue, respectively. When investigating the effect of HA on the distribution and retention of cadmium in organs and muscle tissue of broiler chickens, Herzig et al. (2007) obtained comparable results with a more pronounced effect. They found that Cd content decreased by up to about 80.8%, above all in muscle tissue. If humic acid is fed concurrently, the concentration of Hg in organs and tissues is decreased; it leads to an assumption that HA influences, in particular, the absorption of mercury. It

Table 3. Weight of chicken organs (g)

Groups	K		HA		Hg		Hg + HA	
Organs[1]	L	K	L	K	L	K	L	K
x	20.80	3.90	20.00	4.00	22.20	4.90	21.10	5.00
SD	1.83	1.10	1.26	1.26	1.75	0.74	2.23	1.15
CV (%)	8.80	28.00	6.30	31.50	7.90	15.10	10.60	23.00

[1]L – liver; K – kidney; $n = 10$

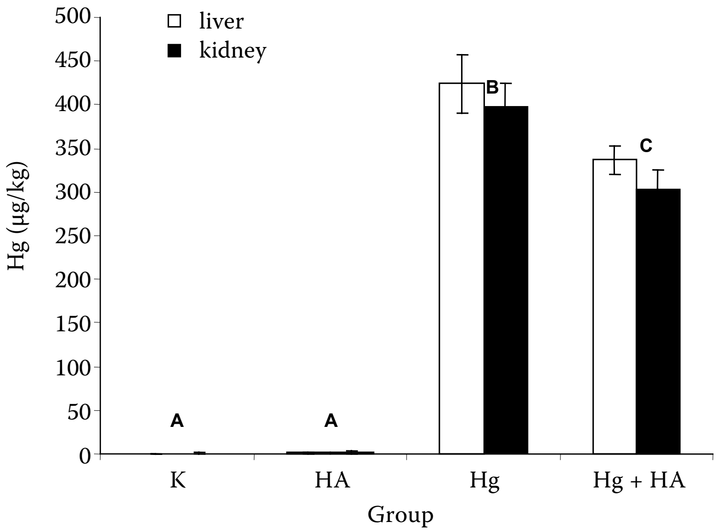

Figure 1. Hg levels in chicken liver and kidneys (μg/kg)

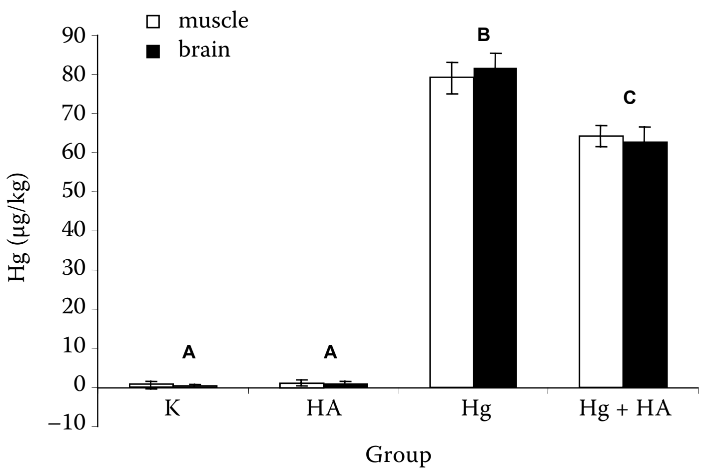

Figure 2. Hg levels in chicken muscle and brain (μg/kg)

follows from our results that if the ratios of muscle tissue, liver and kidneys are 50%, 21% and 0.5% of the slaughter weight, respectively, 41.3 μg of total ingested Hg was accumulated in the MeHg group and 33.1 μg in the MeHg + HA group. In this case, the bioavailability of mercury was 25.5% and 20.4% for both experimental groups and revealed a 20% decrease in biological accumulation caused by the concurrent treatment with humic acids. Cabanero et al. (2005) discovered a 20.7% bioavailability of methylmercury in chickens; however, it was low for inorganic Hg (1.3%). Hammock et al. (2003) detected a 44% reduction of Hg in salmon ova after HA treatment in comparison with an untreated group. They concurrently observed a slower migration of Hg from the chorion to the yolk sac compared with Cd and Zn.

The ability of humic substances to form complexes which are less easily absorbed was described by Kuhnert et al. (1991), Hampl et al. (1994) and other authors. Since the dioxin contamination episode in Belgium in 1999, increased attention has been paid to the investigation and use of non-nutrient adsorptive substances in diets with the aim of decreasing absorption or antagonising toxic effects, especially of methylmercury (Lysenko, 2000; Cabanero et al., 2005). Feeding fish meal as a source of a high quality protein poses a considerable risk of Hg contamination to poultry. Recent experiments with fish meal fed to fowl showed a high correlation between mercury content in fish meal and poultry meat (Bjornberg et al., 2003).

In conclusion, we can say that HA reduced the adverse effects of Hg on chicken performance parameters. After HA treatment, we observed a significantly decreased accumulation of Hg in the organs and meat of chickens exposed to highly toxic methylmercury.

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