Model trial investigating retention in selected tissues using broiler chicken fed cadmium and humic acid

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ABSTRACT: Forty randomly selected chickens were allocated into four groups: K, HA, Cd and Cd + HA. After a 5-day adaptation period, the experiment was initiated. Group K was fed a diet without supplements. Group HA was fed the same diet with a 0.5 g supplement of humic acid per chicken/day. Group Cd was treated with 0.147 mg Cd per chicken/day (0.3 mg of $CdCl_2 \cdot 2.5H_2O$), and Group Cd + HA was given the same treatment as the latter group, with an additional 0.5 g humic acid per chicken/day. The chickens were given the above mentioned treatment daily, for 10 days. Subsequently, they were slaughtered and samples from livers, kidneys and leg muscle (*m. flexor perforans et perforatus*) were collected and Cd levels determined. The Cd levels detected in Group K are commonly found in market chickens and were assumed to be base background value; these represent 32%, 5.2% and 20% of allowed maximum residual limit (MRL) in kidney, liver and muscle, respectively. The limits are 1.0; 0.5 and 0.05 mg/kg for kidney, liver and muscle, respectively. The ten-day treatment with 0.147 mg Cd/day, i.e. 1.47 mg Cd per 10 days, significantly increased Cd levels in all investigated tissues. Average levels in kidneys, livers and leg muscle were 4.99 ± 1.57, 0.558 ± 0.630 and 0.052 ± 0.008 mg Cd/kg, respectively. When cadmium chloride was given together with humic acids, (group Cd + HA), Cd levels decreased in all investigated tissues by 39.6%, 34.2% and 80.8% in kidney, liver and muscle, respectively. The average levels were 3.012 ± 1.33, 0.361 ± 0.367 and < 0.01 mg/kg in kidneys, livers and leg muscle, respectively.

Keywords: cadmium chloride; liver; kidney; leg muscle; transfer factor; biochemical profile

Cadmium is a highly reactive and toxic element, which is sparsely distributed in most agricultural ecosystems. Once absorbed by animals or humans, however, cadmium is poorly excreted, and increasing efforts are being made to limit the entry of cadmium into the human food-chain (Underwood and Suttle, 1999). The Maximum Residue Limit in broiler chicken diets is 0.5 mg/kg.

Humic acids (HA) are organic compounds arising from the degradation of plant residues, mostly through the activity of microorganisms. They are naturally found as components of drinking water (0.2 mg/l), soil, lignite and brown coal. They are three-dimensional macrocollodial molecules with a polyaromatic centre containing iso- and heterocyclic structures with peripheral side chains. The average molecular weights of HA range between 20 000 and 150 000 and can be extracted easily from lignite (the youngest form of brown coal with a distinct wood structure). They have a large functional surface causing them to be high quality adsorbents (Alvarez-Puebla et al., 2005.)

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HA and its sodium salt are substances that have pharmacological activity and can be given to all animals destined to become foodstuff (EMEA, 1999). They are used in horses, ruminants, pigs and poultry to treat diarrhoea, dyspepsia and acute intoxications. They exert their protective effects on the intestinal mucosa, possessing anti-inflammatory, adsorptive, anti-toxic and antibacterial characteristics (Klocking et al., 1992). The ability for humic acids to bond with metals motivated various authors to study their possible use for excluding undesirable metals from the gastrointestinal tracts of animals, or a decrease in their availability (Beveridge and Pickering, 1980; Marinski et al., 1982; Livens, 1991; Herzig et al., 1994; Glynn, 1995; Hudak et al., 1997; Lind and Glynn, 1999). Ridwan (1977) and Rochus (1983) observed that low concentrations of humic acids (0.1%) in feed were sufficient for a significant decrease of lead and cadmium uptake by rats. Van Rensburg et al. (2006) employed HA (oxihumolite) to decrease the toxic effects of aflatoxins in growing chickens.

HA form colloid solutions, which can protect the digestive tract mucosa from the effects of infectious and toxic agents. Due to their chelate structure, HA can bind various toxic agents, thus forming insoluble and non-resorbable complexes (Alvarez-Puebla et al., 2004); these may be shed through the intestines (Kuhnert et al., 1982a,b). Mechanisms of adaptive and bio-regulative qualities of HA and their salts are associated with their stimulating effect on immunological responses, detoxicating activity within livers and sulphydryl-disulphide balance in protein and saccharide metabolism (Lotosch et al., 1988; Livens, 1991; Glynn, 1995; Lind and Glynn, 1999; Santos et al., 2004).

The purpose of the present short study was to investigate the short term effect of HA supplement on the distribution and retention of Cd in organs and muscle of chicken broilers, their selected intermediary metabolism parameters and their performance under experimental conditions.

MATERIAL AND METHODS

The experiment was carried out at the experimental animal facility in the Veterinary and Pharmaceutical University Brno from 4–19 May, 2006. Forty hybrid Ross 308 chickens were selected with a body weight of about 1.5 ± 0.193 kg, aged 32 days and allocated into four groups (10 in each group):

Negative control group (K) chickens received a mixed diet without additional supplements.

Positive control group (HA) chickens received a mixed diet and 0.5 g of humic acid per chicken/day.

Experimental group (Cd) chickens received a mixed diet and were treated with 0.147 mg Cd (0.3 g $CdCl_2 \cdot 2.5H_2O$) per chicken/day.

Experimental group (Cd + HA) chickens received a mixed diet and were treated with 0.147 mg Cd per chicken/day and humic acid (0.5 g chicken/day).

The content of the mixed diet containing basic nutrients, ash (g/kg) and gross energy:

	K	HA
Dry matter	1 000	1 000
Nitrogenous substances	239.1	248.2
Fat	76.7	78.2
Fibre	29.3	26.8
Ash	41.1	44.1
Gross energy (MJ/kg)	18.73	18.62
Cadmium (mg/kg)	0.02	

The experiment started after a five-day adaptation period. Cd or HA containing these capsules were moisturised with oil and put on the base of the tongue of the chickens every morning. The Cd amount was the same for each animal regardless of their initial body weight. They were fed these for 10 consecutive days, after which the chickens were slaughtered, and samples from livers, kidneys and leg muscle *(m. flexor perforans et perforatus)* were collected and Cd levels assessed. At slaughtering blood was also sampled for the analysis of selected biochemical parameters. The chickens were fed the same diet, and drinking water was available *ad libitum* over the entire experimental period.

Specifications of the active substance

HA was prepared by sedimentation and centrifugation of potassium humate after coagulation with sulphuric acid at pH 1.5 to 1.7, and dried at room temperature. HA (Vyzkumny ustav anorganicke chemie, a.s., Usti nasd Labem, Czech Republic – batch BO3A1) contained 90.63% dry matter, 86.89% humic acid and 3.74% ash per sample.

Cd assessment

Cadmium level was determined in samples from livers, kidneys and muscle as follows: a sample of

about 0.5 g with 4 ml of nitric acid (Suprapur Merck) and 0.5 ml of hydrogen peroxide (Suprapur Merck) was decomposed in a microwave – 300 W/5 min, 600 W/5 min (Milestone 1200 MEGA). The determination was performed using the AAS GF method – wave length 228.8, matrix modificator Pd/Mg, atomisation temperature 1 500°C (Solaar 939, ATI Unicam, Cambridge). The detection limit was 0.1 µg/1. The analysis was checked for reliability using the reference material Seronorm Trace Elements Serum L-1 (REF 201 405). The values presented in Table 1 are the means of two measurements.

Assessment of selected metabolic parameters

Total protein (TP), albumin (Alb), uric acid, cholesterol, triacylglycerols (TG), glucose (Glu), lactose (Lact), aspartate aminotransferase (AST), calcium, magnesium and phosphorus blood serum levels were determined by means of the biochemical analyser Cobas EMira, using commercial kits (Biovendor – Laboratorni medicina, a.s, Brno, Czech Republic).

Determination of chicken body weight

The body weight of the live chickens was taken during their allocation into the experimental groups and after completing the experiment. They were weighed with an accuracy of 0.1 g, always at the same time and in the same sequence. Relative body weight gains (BWG) were calculated using the differences in initial body weight, as compared to the final weighing on completion of the experiment.

Health status

During the experiment the health status of chickens was monitored. No clinical signs of disease were observed, except for one chicken from group Cd that died during the experiment (ascites).

Statistical evaluation

The results obtained were evaluated by Unistat 5.1 software. For processing data with a homogenous scatter, ANOVA was used and subsequently

Table 1. Cd levels in chicken kidneys, livers and leg muscle (mg/kg)

	K	HA	Cd	Cd + HA
Kidneys				
n	10	10	9	10
$\bar{x}$	0.32^{A}	0.83^{A}	4.99^{B}	3.012^{C}
SD	0.12	0.99	1.57	1.33
V%	39.1	118.8	31.4	44.1
Livers				
n	10	10	9	10
$\bar{x}$	0.026^{a}	0.11^{b}	0.558^{b}	0.361^{b}
SD	0.014	0.11	0.630	0.367
V%	53.5	98.3	112.9	101.7
Leg muscle				
n	10	10	9	10
$\bar{x}$	< 0.01	0.012	0.052	< 0.01
SD	0	0.007	0.008	0
V%	0	57.0	208.9	0

^{A,B,C}significant differences ($P < 0.01$)

^{a,b}significant differences ($P < 0.05$)

n = number of examined samples, $\bar{x}$ = average, SD = standard deviation, *V*% = variation coefficient

multiple comparisons were performed using the Tukey-HSD test (Zar, 1999) to find pairs of groups with significant differences. For data with a non-homogeneous scatter, Kruskal-Wallis ANOVA and subsequent multiple comparisons by means of a non-parametric test (Tukey type multiple comparisons; Zar, 1999) were used.

RESULTS AND DISCUSSION

The Cd levels detected in kidneys, livers and muscle from group K chickens (Table 1) were considered as the background level, commonly found in market chickens; these represented 32, 5.2 and 20% of the allowed MRL in kidney, liver and muscle, respectively. These values affected the levels detected for all experimental groups. The maximum levels of cadmium in kidney, liver, and meat of chickens allowed by the Commission Directive (EC) 466/2001 are 1.0, 0.5 and 0.05 mg/kg of fresh weight, respectively (Anonymous, 2001).

The ten-day treatment with 0.147 mg Cd/day, i.e. 1.47 mg Cd/10 days, significantly increased Cd levels in all investigated tissues. The average values found were 4.99 ± 1.57, 0.558 ± 0.630 and 0.052 ± 0.008 mg Cd/kg in kidney, liver and leg muscle, respectively. The levels allowed by the EU Commission were exceeded in all tissues from this group.

When cadmium chloride together with humic acid was given to chickens (group Cd + HA), Cd was decreased in all of the investigated tissues by 39.6%, 34.2% and 80.8% in kidney, liver and muscle, respectively. The average levels were comparable with controls: 3.012 ± 1.33, 0.361 ± 0.367 mg/kg and < 0.01 in kidney, liver and muscle respectively.

Cadmium mainly accumulates in the kidney and liver (Bokori et al., 1996; Akyolcu et al., 2003). The results confirmed significantly higher levels of cadmium in the parenchymatous organs when compared with muscle. Bokori et al. (1996) documented significantly more hypertrophy of livers, hearts and impaired growth of testes in chickens fed Cd (doses of 25–75 ppm in the form of $CdSO_4$), in comparison to a control group. Chronic changes of kidney tissue were observed in the chickens with the highest concentration of Cd (724 mg/kg). Similar results were obtained by Akyolcu et al. (2003). The average body weight of chickens exposed to Cd was significantly lower ($P < 0.001$) compared to the control group.

In the present study, regardless of experimental treatment, the highest Cd levels of all groups were found in kidney, followed by liver; and the lowest Cd levels were found in leg muscle (Table 1). Kramarova et al. (2005) investigated Cd levels in livers and kidneys from wild animals. The highest levels were found in the kidneys (0.213–2.387 mg per kg) of various species. Cd levels in livers ranged from 0.032–0.258 mg/kg. Brzoska et al. (2004) observed histopathological and biochemical changes in the kidneys of rats fed 5 mg and 50 mg Cd/l in their drinking water. The function of nephrons was disturbed by Cd. Damaged kidney structure and impaired function was observed when 5 mg/l Cd was administered, such levels of Cd is commonly present in the human environ-

Table 2. Evaluation of body weight and growth rate in chickens (g)

	K (n = 10)	HA (n = 10)	Cd (n = 9)	Cd + HA (n = 10)
RGR (%)	54.4	54.4	55.1	63.0
Body weight, Day 0	2 284 ± 302.5	2 423 ± 213.8	2 167 ± 241.6	2 247 ± 272.7
V%	13.2	8.8	11.1	12.1
Body weight, Day 15	3 527 ± 408.8	3 742 ± 425.3	3 362 ± 436.5	3 662 ± 499.1
V%	11.6	11.4	13.0	13.6
Growth rate	1 242 ± 269.7	1 321 ± 245.7	1 200 ± 201	1 415 ± 262.7
V%	21.7	18.6	16.8	18.6
Min.	858	958	874	982
Max.	1 735	1 733	1 485	1 839

RGR = relative growth rate

Table 3. Weight of chicken organs (g)

	K (n = 10)		HA (n = 10)		Cd (n = 9)		Cd + HA (n = 10)	
	liver	kidney	liver	kidney	liver	kidney	liver	kidney
Average weight	67.3	6.8	72.2	8.4	63	7.3	67.1	8.1
Index	100	100	107.3	123.5	93.6	107.4	99.7	119.1
Min. weight	52	6	58	6	50	5	55	5
Max. weight	83	9	87	10	75	10	77	11
SD	9.4	1.2	9.7	1.3	8.3	1.6	7.7	1.5
V%	14	18.1	13.4	15.1	13.2	21.6	11.5	18.8

ment. Gonzales-Weller et al. (2006) compared Cd levels in various meat types and meat products, finding the mean Cd concentrations in chicken and turkey meat were 1.68 µg/kg and 5.49 µg/kg, respectively. The mean Cd concentrations in chicken and turkey products were 4.15 µg/kg and 5.98 µg per kg, respectively.

Increased Cd levels in organs and leg muscle of the HA group chickens that were treated with humic acid only were surprising. Increased levels were detected in all the examined samples (kidney 0.83 ± 0.99, liver 0.11 ± 0.11, muscle 0.012 ± 0.007 mg/kg) versus the control group. This finding is difficult to explain, because Cd was not detected in the HA, and we did not encounter a comparable finding in the literature.

Various studies deal with the binding of dangerous metals by humic acids (Stevenson, 1977; Kerdorff and Schnitzer, 1980; Klocking, 1980, 1994; Lind and Glynn, 1999; Alvarez-Puebla et al., 2006; Hizal and Apak, 2006). The changes in the toxicological qualities of metal ions bound to humic acids are particularly important. The route of administration also plays a significant role. While the toxicity of metal ions is reduced by their binding to humic acids after oral administration, the toxicity of metal-humic acid compounds administered parenterally is increased (Klocking, 1980).

Transfer factors were calculated from the obtained results; these express the ratio of cadmium concentration in the animal product to the received amount. The highest values were detected for kid-

Table 4. The levels of selected biochemical parameters in blood sera of chickens ($\bar{x}$ ± SD)

	K (n = 10)	HA (n = 10)	Cd (n = 9)	Cd + HA (n = 10)
Total protein (g/l)	31.6 ± 2.62	32.8 ± 3.56	31.1 ± 4.65	34.69 ± 2.69
Albumin (g/l)	20.79 ± 1.91	21.40 ± 2.32	20.32 ± 2.34	22.07 ± 1.55
Uric acid (µmol/l)	426.4 ± 98.7	527.5 ± 201.9	445.4 ± 193.9	410.7 ± 107.7
Cholesterol (mmol/l)	2.82 ± 0.25^{a}	3.13 ± 0.31^{b}	2.78 ± 0.31^{a}	3.38 ± 0.32^{b}
Triglyceride (mmol/l)	0.48 ± 0.15	0.72 ± 0.32	0.44 ± 0.20	0.32 ± 0.12
Glucose (mmol/l)	13.07 ± 0.83	12.99 ± 1.07	12.4 ± 21.05	13.12 ± 0.69
Lactose (mmol/l)	5.98 ± 1.36	6.09 ± 2.19	4.79 ± 1.07	4.68 ± 1.23
AST (µkat/l)	6.86 ± 1.68	6.83 ± 2.16	6.44 ± 1.69	6.85 ± 2.64
Calcium (mmol/l)	2.67 ± 0.14^{b}	2.73 ± 0.15^{b}	2.49 ± 0.17^{a}	2.74 ± 0.15^{b}
Phosphorus (mmol/l)	2.06 ± 0.16	2.03 ± 0.19	1.86 ± 0.13	2.03 ± 0.10
Magnesium (mmol/l)	0.76 ± 0.08	0.76 ± 0.10	0.74 ± 0.09	0.78 ± 0.17

AST = aspartate aminotransferase

^{a,b}significant differences ($P < 0.05$)

ney (HA 0.35, Cd 3.18, Cd + HA 1.83), lower in liver (0.057, 0.36, 0.23) and the lowest in leg muscle (0.0014, 0.029, 0.0).

The difference in the body weight of chickens at the beginning of the experiment was eliminated by the calculation of a relative growth rate over the monitored period. The values obtained for groups K, HA and Cd were comparable (54.4, 54.4, 55.1). The highest value was noted for group Cd + HA (63.0), where the highest absolute body weight gain was detected (1 415 g) (Table 2).

The proportion of liver and kidney to the total live body weight (%) of chickens (Table 3) were 1.91 and 0.19 in the control group, and 1.93 and 0.22 % in the HA group. It was higher in the Cd and Cd + HA groups: 1.87 and 0.22 and 1.83 and 0.22 %, respectively.

The levels of selected parameters of the chicken metabolic profile (Table 4) ranged within the reference limits (Meluzzi et al., 1992). The levels detected in the present study are in accordance with data obtained after administration of humic substances (Humex®) by Demeterova and Mariscakova (2006). Significant differences between experimental and control groups were exceptionally detected (cholesterol, calcium). Cadmium is also involved in interactions with macrominerals and the lowering of calcium absorption (Underwood and Suttle, 1999). Akahori et al. (1994) noted slightly decreased cholesterol levels in monkeys given cadmium.

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