HEAVY METAL LEVELS IN PLASMA AND FECAL MATERIAL SAMPLES OF THE BLACK VULTURE (*Coragyps atratus*)

Nivel de metales pesados en muestras de plasma y materia fecal del Zamuro Cabeza Negra (*Coragyps atratus*)

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ABSTRACT

The level of cadmium (Cd), copper (Cu), lead (Pb), vanadium (V), and zinc (Zn) were determined in blood plasma samples of healthy black vultures (*Coragyps atratus*) (n = 10). The correlation between metal levels in plasma samples and metal vs total plasmatic protein level, also calculated. Cd, Cu, Zn, Ni and Fe concentrations determined in a pooled sample of fecal material from the black vulture (n = 5) and compared with levels determined in a pooled sample of Hen fecal material (*Gallus gallus domesticus*) (n = 5). Metal levels determined through flame atomic absorption spectrophotometry (FAAS). Analysis of vulture plasma samples yielded concentrations (mg/L) of 7.25 ± 3.01; 14.50 ± 8.60; 6.85±4.85; 47.33 ± 24.74 and 3.50 ± 2.73, for Cd, Cu, Pb, V and Zn, respectively. No correlation established between plasmatic levels of the various metals, or between metals and total protein levels (P > 0.05). In vulture feces, metal levels (µg/g) corresponded to 13.93 ± 1.18; 20.26 ± 0.41; 15.19 ± 1.33; 4,823.76 ± 8.99; and 202.57 ± 1.65 for Cd, Cu, Ni, Fe, and Zn, respectively. In hen fecal samples, metal levels (µg/g) corresponded to 38.85 ± 0.70; 700.84 ± 11.09; and 478.18 ± 5.83, for Cu, Fe, and Zn, respectively. Under assay conditions Cd or Ni were not detected in hen feces. The physiological and environmental significance of elevated plasmatic and fecal metal levels in the black vulture, are discussed.

Key words: *Coragyps atratus*, black vulture, heavy metals, plasma, feces.

RESUMEN

El nivel de cadmio (Cd), cobre (Cu), plomo (Pb), vanadio (V), y zinc (Zn) fue determinado en muestras de plasma sanguíneo provenientes de (n = 10) zamuros negros sanos (*Coragyps atratus*). La correlación entre el nivel de metales en muestras plasmáticas, y concentración de proteína plasmática total fue calculada. Concentraciones de Cd, Cu, Zn, Ni y Fe fueron determinadas en muestras de material fecal de zamuro negro (n = 5) y gallina (*Gallus gallus domesticus*) (n = 5); ambos fueron comparados. Las determinaciones realizadas a través de espectrofotometría de absorción atómica con llama (FAAS). Los niveles de metales en muestras de plasma de zamuro correspondió a (mg/L) 7,25 ± 3,01; 14,50 ± 8,60; 6,85 ± 4,85; 47,33 ± 24,74 y 3,50 ± 2,73, para Cd, Cu, Pb, V y Zn, respectivamente. Para los mismos metales, presentes en heces de zamuro, los niveles correspondieron a (µg/g) 13,93 ± 1,18; 20,26 ± 0,41; 15,19 ± 1,33; 4.823,76 ± 8,99; y 202,57 ± 1,65. En muestras fecales de gallina, los niveles (µg/g) correspondieron a 38,85 ± 0,70; 700,84 ± 11,09; y 478,18 ± 5,83, Cu, Fe, y Zn, respectivamente. En las condiciones de ensayo, no se encontró Cd o Ni en muestras fecales de gallina No se encontró correlación entre los niveles de metales plasmáticos y concentración de proteína total (P > 0,05). La importancia fisiológica y ambiental de niveles plasmáticos y niveles metálicos en heces elevados para zamuro, es discutida.

Palabras clave: *Coragyps atratus,* zamuro negro, metales pesados, plasma, heces.

Recibido: 26 / 11 / 2004. Aceptado: 04 / 05 / 2005.

INTRODUCTION

Metal level and concentration of other elements in organ and animal tissues, are indicative of the overall body chemical and mineral status. The homeostasis of a particular mineral involves different mechanisms depending on the organ involved; with participation of body tissue fluids, subject to modulation and high mobilization. The study of biological fluids and other materials, such as blood plasma and fecal material, is relevant as function indicative of underlying normal biochemical processes, living conditions and potential diagnostic tool to identify a disease state [19, 26].

It has been determined that humans need nearly 72 trace elements, including very low concentrations of heavy metals, such as Cu, Sn, V, Cr, Mo, Mn and Co. Most metals are toxics at high concentrations, while other provoke deleterious effects at low concentrations [27]. Cd, Pb and V [16] are pollutants of biological interest due to their biotoxicity [3, 10, 28].

Several reports on dietary habits, resistance and adaptation to adverse external factors of the black vulture *Coragyps atratus*, comprise an interesting array of biological aspects; currently subjected to intense research. This beneficial carrion eating fowl plays a particular cleaning role in the environment [2, 13, 14, 15, 24, 31, 35] and theretofore constantly exposed to noxious materials and substances [33]. In general, little is reported on basal tissue and body fluid metal levels, present on biological samples of cathartid birds [38], now *Cicconids* [9]. Research related with analysis and interpretation of metal content in plasma and fecal material of the black vulture are unknown. However, it is important to investigate these animals in spite of they immunological responses to contaminated areas.

The aim of this work focus on the determination of heavy metals levels, Cd, Cu, Ni, Fe, Pb, V and Zn, in blood plasma and fecal samples of the *C. atratus*. Correlation between heavy metal levels, and metal concentration vs total plasmatic protein determined. Results were analyzed and compared with human and published animal reference values.

MATERIALS AND METHODS

Reagents and solutions

All chemical reagents corresponded to analytic grade, from Fisher Scientific Co., Riedel-de Haën or Merck, Sodium salt of porcine heparin (1,000 units/mL), were purchased from Eli Lilly & Co., Venezuela. The protein assay kit (Lot. 046H6079) was purchased from Sigma Chemical Co., USA [34], and used according to manufacturer instructions.

Study population

The animal sample comprised black vultures (*Coragyps atratus)* specimens that roost the neighboring grounds of a slaughterhouse, located in municipio Santa Rita, Zulia state, Venezuela. For comparation regarding fecal material analysis, domestic hens (*Gallus gallus domesticus*) were also studied.

Animal sample

Ten (n = 10) active young-adult black vultures were sampled for the blood plasma metal analysis and five (n = 5) vultures sampled for fecal material. Healthy appearing vultures selected without discrimination for sex [37]. The comparative animal sample consisted in five (n = 5) mature and reproductively active hen specimens, approximately 40 weeks old.

Avian capture and handling

Vultures collected following a stratified sampling. The size of the study sample corresponded to approximately 10% of the overall vulture population on site. Capture and immobilization techniques performed as reported previosly by Salas *et al.* [32]. Hens acquired locally from a commercial breeding farm. Once immobilized, the animals were transferred to the laboratory in mobile cages.

Maintenance and captivity conditions

Vultures were housed in permanent external cages (size 1.75 × 1.75 × 2 m). Animals labeled and distributed to a maximum of five birds per cage, arranged according to collection date. Hens were maintained under similar conditions, in separate quarters. Water was provided *ad libitum*. Vultures were fed a fresh or discomposed ground bovine viscera, every 36 h. Hens fed daily with a commercial diet (Protinal®). The animals remain undisturbed three days prior initiation of the study, to minimize the negative effects caused by capture, transfer and environment change. Animals were handled by the same person, when possible [11].

Blood and fecal sample collection

Whole blood samples collected during early morning from fasting animals to minimized interference of postpandrial blood lipids, obtained plasma and activity of humoral components [2, 19]. Blood samples collected near the cages from immobilized animals on a custom designed wooden board. The blood samples taken from the basilic vein after area disinfection with 70% (v/v) isopropyl alcohol; double syringe method and 23G × ¾" needle with 5 mL syringe used. Collected blood placed in sterile polyethylene tubes, containing a heparin solution (75 units/mL of blood), and processed immediately. Plasma obtained after blood centrifugation at 2,500 r.p.m., 4°C, for 20 min. Fecal samples also collected during early morning from fasting animals, using a clean polyvinyl sheet placed on the cage floor for 24 h. The collected fecal material suspended in dionized distilled water, pooled, homogenized and lyophilized. Plasma aliquots and feces distributed in 1.5 mL Eppendorf vials, sealed and maintained at –40°C and at room temperature, respectively. Chemical and biological analysis performed within fifteen consecutive days.

Blood and fecal sample preparation for FAAS

Calibration standards for atomic absorption spectrophotometry prepared by serial dilutions from stock solutions containing 500 mg/L of Zn, and 1,000 mg/L of Cd, Cu, Fe, Ni, Pb and V; dionized water used for each dilution. Calibration solutions and blood plasma aliquots acidified with 1 mol/L nitric acid (*e.g.*, 100 mL per each 10 mL of final sample volume).

Blood plasma samples

Blood plasma samples diluted as required with deionized water, according to the apparatus detection limit for the target metal. Cd and Cu detected in 200-fold blood plasma dilution aliquot; Zn and V detected in 20-fold diluted samples; Pb analyzed on undiluted samples and on a 20-fold dilution sample. Seronorm™ (Batch Nº 31089) Trace element serum was used to establish trace elements concentration accuracy (TABLE I).

Fecal samples

Lyophilized dry vulture (0.19746 g) and hen (0.16730 g) material digested with concentrated nitric acid, at 110°C for 6 h, in a pressure pump, Parr type. Final volume solution adjusted to 25 mL with deionized water.

FAAS apparatus

Samples analyzed in a Perkin Elmer flame atomic absorption spectrophotometer, Model 3110, equipped with a hollow cathode lamp, according to analyte. The operational parameters for FAAS are represented in TABLE II.

Determination of the total protein level in blood plasma

The total protein level (g/L), determined according to Lowry's modified technique [34]. The protein values correlated with the detected plasmatic metal levels.

Statistical analysis

The data analyzed using commercial software STATISTICA program (Statsoft®). Mean, standard deviation and range values determined for each metal detected. Lineal correlations also calculated with this program.

RESULTS AND DISCUSSION

Metal levels in blood plasma samples

The mean levels of Cd, Cu, Pb, V and Zn in blood plasma samples of the black vulture *Coragyps atratus*, are included in TABLE III. Toxic metals Cd and V were conspicuously high in samples analyzed. No correlation was obtained (TABLE IV) between the plasmatic levels of the various metals or between metals and total plasmatic protein ($P > 0.05$). The hen metal concentrations determined were under the equipment detection limits.

Metal level in fecal material samples

The mean level of Cd, Cu, Fe, Ni and Zn in pooled fecal material of *C. atratus* and *G. gallus domesticus* are represented in TABLE V. Levels of Cd and Ni were found to be particularly high in the vulture feces, when compared with hen. In

TABLE I
STANDARD SERUM TRACE ELEMENT ASSAY*

Metal (mgL^{-1})	Expected	Found
Fe	(1.47-1.26)	(1.37-1.28)
Ni**	(2.10-2.94)	(2.10-2.20)
Zn	(1.44-1.64)	(1.55-1.67)
Cu	(1.26-1.27)	(1.20-1.25)

*Note: number of samples, five (n = 5). Seronorm™ Trace element serum test.
**Concentration in µg L^{-1}). This metal was determined by Graphite Furnace Atomic absorption Spectrometry (GFAAS).

TABLE II
OPERATIONAL PARAMETERS FOR FLAME ATOMIC ABSORPTION SPECTROPHOTOMETRY

Metal	Wavelength (nm)	Spectral width (nm)	Flame
Cadmium	228.8	0.7	A/Ac
Copper	324.8	0.7	A/Ac
Iron	248.3	0.2	A/Ac
Lead	283.3	0.7	A/Ac
Nickel	232.0	0.2	A/Ac
Vanadium	318.4	0.7	N/Ac
Zinc	213.9	0.7	A/Ac

A/Ac: air/acetylene. N/Ac: nitric oxide/acetylene.

TABLE III
PLASMATIC LEVELS OF Cd, Cu, Pb, V AND Zn IN THE *Coragyps atratus*

Metal	Level (mg/L)*	Range (mg/L)
Cd	7.25 ± 3.01	2.0-12.0
Cu	14.50 ± 8.60	6.0-30.0
Pb	6.85 ± 4.85	0.3-15.0
V	47.33 ± 24.74	10.0-80.0
Zn	3.50 ± 2.73	0.4-9.0

*Mean ± standard deviation (n = 10).

TABLE IV
CORRELATION ANALYSIS BETWEEN METAL LEVELS AND METALS VS TOTAL PROTEIN, IN PLASMA SAMPLES OF *Coragyps atratus*

Material assayed	r	R2	P*
Cd vs Cu	0.479698	0.230110	0.229021
Pb vs V	0.267958	0.071802	0.454146
Pb vs Zn	0.011636	0.000135	0.968509
V vs Zn	0.604398	0.365297	0.064197
Cd vs total protein	0.584721	0.341899	0.127911
Cu vs total protein	0.127267	0.016197	0.763939
Pb vs total protein	0.090239	0.008143	0.759003
V vs total protein	0.010636	0.000113	0.976736
Zn vs total protein	0.162747	0.026487	0.578280

*P > 0.05 non significant. r: correlation coefficient. R^2: variation coefficient. P: significance.

TABLE V
LEVELS OF Cd, Cu, Ni, Fe and Zn IN FECAL SAMPLES FROM *Coragyps atratus* AND *Gallus gallus domesticus*

Metal	Level (µg/g)*	
	Coragyps atratus	*Gallus gallus domesticus*
Cd	13.93 ± 1.18	ND**
Cu	20.26 ± 0.41	38.85 ± 0.70
Fe	4,823.76 ± 8.99	700.84 ± 11.09
Ni	15.19 ± 1.33	ND**
Zn	202.57 ± 1.65	478.18 ± 5.83

*Mean ± standard deviation (n = 5). ** Non detectable (ND).

the last such heavy metals remain undetected, under current sample processing and assay conditions.

Human exposure to heavy metals and derivative compounds at toxic levels may produce biological adverse effects; of particular interest are those elicited by a chronic low-level exposure in the human population, involving the general environment and occupational risks. The consequence has been termed “critical effect” for its relation to preventive actions, aimed at limiting exposure to safe levels [22]. In general, reports on basal levels of trace metals and possible effects on wild birds are scarce, particularly regarding the black vulture, *Coragyps atratus*. The information derived from this study is of basic relevance, perhaps as reference for further studies on the subject.

In blood plasma samples from *C. atratus,* the Cd level corresponded approximately to 2,000-fold greater than reported values for normal whole human blood; with Cd levels of 0.003-0.004 ± 0.0005 mg/L [12]. Humans and animals, including domestic varieties can accumulate toxic metals to harmful concentrations, such as Cd. Despite increasing awareness efforts and attempts to control environmental pollution, changes in the distribution and bioavailability of toxic metals, still occurr.

Notwithstanding the natural processes, other contributing factors include the gradual spread of industrialization, the use of sewage sludge as fertilizer and the acidification of groundwater [21]. Regarding to this study, Cd was found in vultures at high concentrations; a highly toxic metal with an extremely long biological half-life capable of accumulating as a toxin. It has been shown to have sterilizing, teratogenic and carcinogenic effects [3].

Led levels in vulture blood plasma, corresponded to 60-fold higher the normal value of 0.115 ± 0.05 mg/L, reported for human samples [29]. This element was also found to be 40-fold greater than the value of 0.16 mg/L, reported in whole blood samples for humans, not exposed to the metal. This metal was also found to be 15-fold greater than reported levels of 0.46 mg/L, found in blood samples of exposed workers [20]. Lead, an environmental toxin is known to impair the functional properties of osteocalcin, a small active protein in bone mineralization and resorption [10]. Lead residues in bone may result from either acute high level exposure or chronic low level exposure [18]. Lead toxicosis has been reported in a captive Andean Condor [17]. The presence of Pb and Cd in the daily diet, represent the principal non occupational source of contamination for humans. Both metals may induce aortic atherosclerosis and hypertension in pigeons [26]. Recently, there has been an increase in interest regarding toxicological and biological activities of V, due to high concentrations found in the environment, as a consequence of indiscriminate industrial use. V containing compounds and materials known for their genotoxicity, are extensively used in modern industry and occupational exposure to high doses of this element is quite common [28]. V levels detected by our group in vulture blood plasma exceeded the toxic concentration TC50 of 16-25 mg/L, reported by Owusu-Yaw et al. [23] for animal cellular systems. This value was approximately 10,000-fold greater than the normal level of 0.0047 ± 0.0026 mg/L, reported for human blood plasma [29].

The Cu and Zn levels in vulture plasma samples were slightly higher with respect to reported values for normal human plasma. The obtained Cu concentration was almost 5-fold greater than the level of 3.19 ± 0.83 mg/L, reported for normal human plasma. The Zn value obtained was nearly 4-fold the human plasma level of 0.96 ± 0.14 mg/L, reported previously by Ranaudo et al. [25]. However, these differences were less conspicuous than the results reported herein for levels of Cd, Pb and V, in vulture samples. Zn is an essential element for growth, development and functioning of all living cell [3]. The liver is the metabolic center for Cu; in this organ the element is removed from the blood, excreted partially in bile, associated with ceruloplasmin and partially stored. Both Cu and Zn are essential microelements for the biological activity of numerous metal-bound enzymes and other proteins [19].

Methallothionein, is an important metal-binding protein that occurs in varying amounts, in a wide range of tissue, particularly in liver, kidneys, intestine and pancreas. Synthesis of this protein is induced by Zn, Cu, Ca and many other non-essential elements. The protein tissue concentration of Cu and Zn is dependent and related to body homeostasis. A variety of stress factors stimulate methallothionein synthesis, particularly hepatic. The turnover rate of this protein in tissue is relatively high, but depends to a large extent on its metal content. There has been much speculation as to the function of the protein and its potential role in the cellular detoxification of Cu, Zn and other metals. The protein appears in small amounts in blood and urine, and concentrations assays may be used to assess the trace element status [7]. Methallothionein, to be determined in futures studies may be present at high levels in fluids and tissues of the black vulture partially responsible for its tolerance; its activity and concentration may differ when compared with other animal systems.

Heavy metals have been directly related with the induction of hematological alterations in various organisms, including man. These effects determined by biochemical changes, are translated in level alterations of organic macromolecules, affecting metabolical centers, such as liver [5, 8]. In addition, decreased in protein level, induced by toxic metals has been already documented by Tamburini [36]. Considering this observation, the potential correlation of plasmatic metal levels and total protein concentration was studied. Analysis of the results obtained indicated that the protein levels in vultures are not influenced by the presence of such metals, including those valued as toxic. The levels for this metal were found to be higher than the subletal dosage reported for other animal species [5]. Total plasmatic protein levels in vulture, determined here, corresponded to a normal avian range of 30-50 g/L, including values reported for other Falconiformes birds [11].

An inverse correlation has been reported between the level of various trace metals in human tissues [3], while a direct correlation has been shown in fluids [30]. However, in this study, no correlation was established between analyzed metals present in vulture plasma samples. Though plasmatic levels of toxic metals determined in vulture samples was particularly high, no typical or evident pathophysiological alterations detected.

Studies regarding the chemical compositional characterization of fecal material are important, since modifications of chemical processes in a diseased organism usually induce modifications in the quantity and composition of feces. In certain cases, this contributes to the presence and subsequent detection of biological and chemical of substances, absent under normal conditions. The composition of normal feces depends at large on the quality and quantity of the food substance consumed [19]. Reports related to the detection of metals in vulture feces, were not found.

In the fecal material analysis, iron level was approximately 7-fold higher in vultures than in hens. These results could be attributable to the differences in the dietary content of the two animals species studied. Iron normal values excreted in human urine oscillate in the range of 60-100 µg/24 h [19]. The levels of Cu and Zn were approximately 2-fold lower in vul-

ture than in hen sample. In *Falconiformes* species, such as the California Condor, values of 87.0 and 7.4 µg/g have been reported for Cu, in liver and kidney, respectively [38]. The minimal level toxic of dietary Cu is of 500 µg/g for domestic chickens. In normal humans, Cu and Zn are excreted in urine at a rate of 15.4-36.6 µg/24 h and 240-415 µg/24 h, respectively [19].

Under assay conditions, Cd or Ni were not detected in the hen fecal samples. However, the levels of both metals in the vulture samples were elevated. It is striking that the Cd concentration obtained in vulture feces samples represent a toxic level, acquired by bioaccumulation. This particular aspect has not been documented in the literature. The Cd levels encountered exceeded the limit permitted by the World Health Organization in food material for human consumption [6]. In addition, vulture Cd level corresponded to nearly 40-fold the minimum value of 0.31 (µg/g) reported for tissue of various animal species, currently used as bioindicators of environmental contamination [1]. The Cd level determined in this study represented approximately a 1,400-fold and 180-fold, the respective reported values of 0.01 and 0.08 (µg/g) for liver and kidney tissue in the California condor [38].

The potential Cd contamination of eggs, and the accumulation dynamics of this metal, have been studied in Japanese quails, housed nearby batteries and supplied with dietary Cd. A high metal excretion in eggs was reported, relating the phenomenon with the absence of metal protein carriers, lack of metal absorption and excretory capacity expressed in the oviduct. However, the value found in vulture feces exceeded 18-fold the level of 0.777 (µg/g), reported in a previous study, corresponding to higher Cd exposure [4].

Regional petroleum and petrochemical industries engaged in production and processing of mostly heavy crude, an electrical power plant partially operated on heavy metal-rich Bunker–C fuel and daily discharge of heavy-metal rich gas exhausts, the operation of open pit city sanitary landfills completed with unclassified refuse and air pollution, may be largely responsible for an overall heavy metal environmental contamination. While ground feeding in city dumps as ready food source or breathing polluted air when high flying may be likely responsible for the high heavy metal concentrations found in the study animals. Further studies on specific sources of contamination for the said metals, the metabolic tolerance and assimilation of the black vultures to the pollutants are to follow. The *Coragyps atratus* may be interesting biological indicators of contamination by heavy metals and other anthropogenic pollutants. Similar studies in other parts of the world should aid to further document this findings.

CONCLUSIONS

Result analysis on the study of heavy metals present on the black vulture blood plasma and fecal samples indicate that these wild birds live and feed in highly contaminated areas, rich in toxic heavy metals, particulary Cd, Pb and V. The black vultures subject of study appears to have developed an effective detoxification or tolerance mechanism, contributing to a stable homeostasis. Basal concentrations of the metals in biological samples of the *C. atratus* are unreported in the international literature.

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