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THE ROLE PLAYED BY PHYTASE AND METABOLISM IN THE ACCUMULATION OF URANIUM IN THE POULTRY BONES

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ABSTRACT

Groups of seven days old Cobb broilers were fed with feed doped with uranyl nitrate at a fixed concentration of 20 ppm-U, and two concentrations of phytase (120 and 180 ppm). Two animals per group were sacrificed weekly up to their adultness. The uranium content in tibia was measured by neutron activation analysis. It was observed that the biokinetics of U does not change by administration of phytase, but the U concentration in the bones increased by up to a factor of 2, and in a nonexpected periodically time oscillating fashion. Quite surprising too, the concentration of uranium (μg-U/g-bone) is decreasing all along the animal life spanning period of 14-42 days, meaning that the skeleton mass is growing faster than the corresponding accumulation of uranium is. This last finding is interpreted as a possible interplay between two metabolic peculiarities, associated both with U transfer to (uptake), and U removed from (clearence) the bones, repectively.

Keywords**:** uranium, biokinetics, fowl, metabolism

I. INTRODUCTION

From the pathways of entrance of radionuclides in the human body, ingestion is the most effective one because it is closely related to alimentary habits.

The daily intake of uranium through food and water may be regarded as chronic ingestion and it is a much more common occurrence than has generally been appreciated, since uranium is normally present in drinking water and food. Regarding food, uranium concentrations can reach levels above background, depending on regional peculiarities associated with food production and processing.

As far as the food chain in concerned, we note that uranium is a trace constituent in rock phosphate, which is extensively used as source of phosphorus for fertilizers and livestock feed supplements. Dicalcium phosphate (DCP), for example, can present concentrations of uranium as high as 200 ppm [1].

Following uptake through the gastrointestinal tract, uranium is mostly deposited in the skeleton. In fact, bone is one of the most important biological accumulators of uranium [2]. It is said that uranium *mimics* calcium. This fact led us to consider the role played by enzimes in poultry nutrition – phytase in particular, as discussed below.

In fact, phytase is used to improve the availability of phosphorus, minerals and metal ions, like calcium [3]. Thus, our conjecture is: if uranium mimics calcium indeed, then, administration of phytase would improve the availability of uranium too, resulting therefore in a higher accumulation of this radionuclide in bone and, consequently, in other organs. Such a possibility is considerably more important to verify if feed supplements contain appreciable amounts of uranium (see discussion above) and because in this case additional amounts of uranium are introduced in the food chain through poultry consuption by humans.

Therefore, we decided to measure the concentration of uranium in bones of broilers fed with uranyl nitrate doped ration (at one fixed doping amount), plus phytase at two different dosages, by a period of time starting at the earlier stages of the animal development and lasting till maturity. We note, in this regard, that uranyl nitrate has long been recognized as a nephrotoxic agent for impairing renal function in growing chicks [4]. However, almost nothing has been done to evaluate the biokinetics of uranium accumulation in the organs of the animal, and its corresponding radiobiological implications to the animal and to their consumers.

II. MATERIALS AND METHODS

One hundred and fifty, seven days old Cobb broilers were separated into three groups, each receiving different food supplements, namely:

Group-1: basic food (maize and soybean) doped with 20 ppm of U, as uranyl nitrate, now referred to simply as *U-doped food*;

Group-2:U-doped food plus 0.12 g of phytase per kg of food;

Group-3: U-doped food plus 0.18 g of phytase per kg of food.

Food with specific formulation for each distinct period, and following commercial procedures, was provided *ad libitum*.

Starting with 14 days old broilers, two animals per group were slaughtered by decapitation weekly, and the tibiae were immediately removed and frozen at –20 °C for further processing and analysis. After getting 42 days old, the animals had the uranyl nitrate removed from their diet, and the experiment was finished when the broilers got 70 days old.

The bones were individualized in porcelain melting pots, weighted and maintained inside an oven at 80 °C for water evaporation. Next, the material was kept by 8 hours on a hot plate at 180 °C for carbonization. After this, the melting pots were inserted in an oven at 600 °C till conversion of the material into ashes.

Approximately 100 mg of bone ashes from each animal were weighed and sealed in polyethylene involucres. Standard aliquots of U solutions, with exactly known concentrations, were pipetted onto 2 cm^2 pieces of Whatman n. 4 filter paper and dried in a dessicator. Sets constituted by 6 bone samples and one standard each were wrapped with thin aluminum foils, and were irradiated in the IPEN research reactor (IEA-R1,4MW, pool type) for 8 h at a thermal neutron flux of 10^{12} $n.cm^{-2}.s^{-1}$.

Samples and standards were analyzed by means of conventional gamma-spectrometry procedures, using a high resolution 75 cm^3 – Ge detector operated with a 671 ORTEC amplifier in pile-up rejection mode for 2 h per counting run, allowing thus the determination of the three main gamma decay of ^{239}Np (formed from $^{238}U + n \rightarrow {}^{239}U \rightarrow {}^{239}Np$): 106, 228 and 278 keV.

III. RESULTS

It shows in Figure 1 our results expressed as concentration of U in the bones. Each datum point represents an average taken over the 3 gamma decay lines of ^{239}Np measured in samples of two animals; therefore, it is the average of 6 uranium concentrations.

The 3 sets of data, namely, Fig. 1-a (U and no phytase), Fig. 1-b (U and phytase) and Fig. 1-c (U and more phytase), exhibit the same decreasing trend as a function of time (t). Although it is obvious that the concentration of U decreases for $t > 42$ d, because U was removed from the diet of those animals getting 42 days old, it is quite surprising and unexpected finding a decreasing trend also during the period of daily uranium intake.

Figure 2 shows the concentration in the bones as a function of the content of phytase in the food, for 3 groups of broilers: young, adult and older, namely, 14, 42 and 63 days old, respectively.

In order to better appraise the role played by phytase in U accumulation in the bones, we show in Figure 3 a plot of the ratios C_1/C_0 and C_2/C_0, as a function of time, where C_0, C_1 and C_2 are the U concentrations corresponding to zero, 0.12 and 0.18 g of phytase per kg of food, respectively.

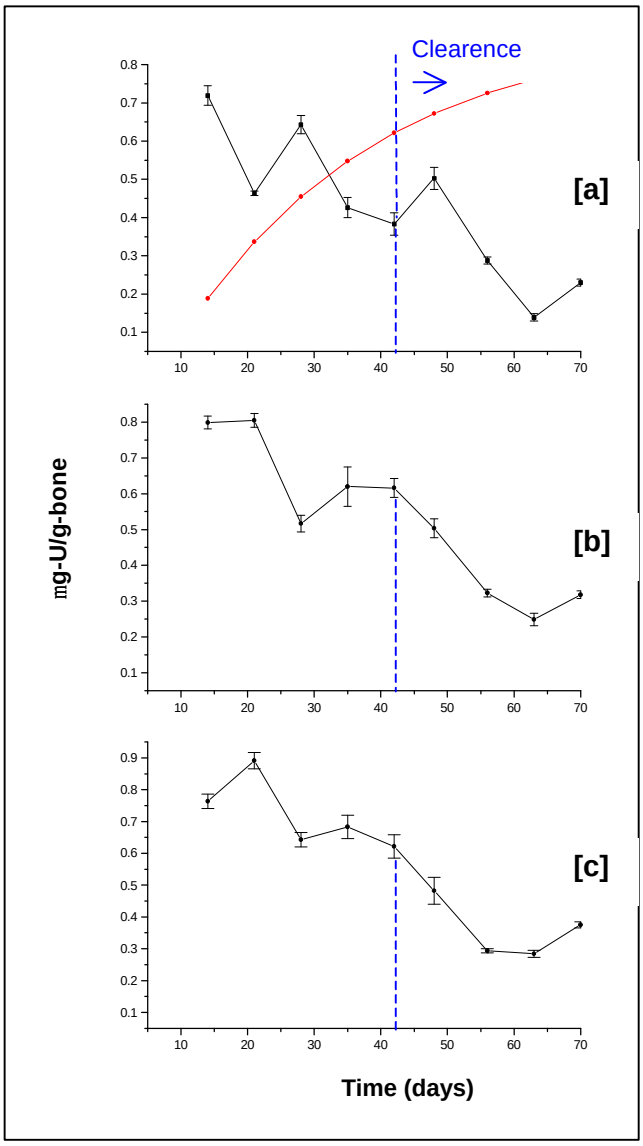

Figure 1. Concentration of U in the bones of fowls as a function of the spanned life time, and corresponding to daily diets with no phytase (a), 120 ppm (b) and 180 ppm of phytase (c) in the food.

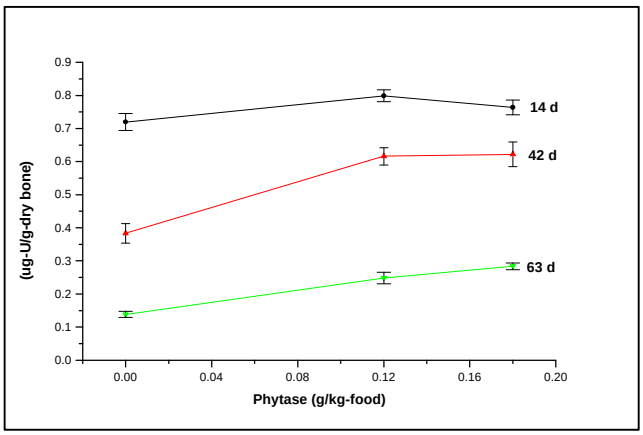

Figure 2. Concentration of U in the bones of fowls as a function of the amount of phytase in food, and at 3 selected animal ages.

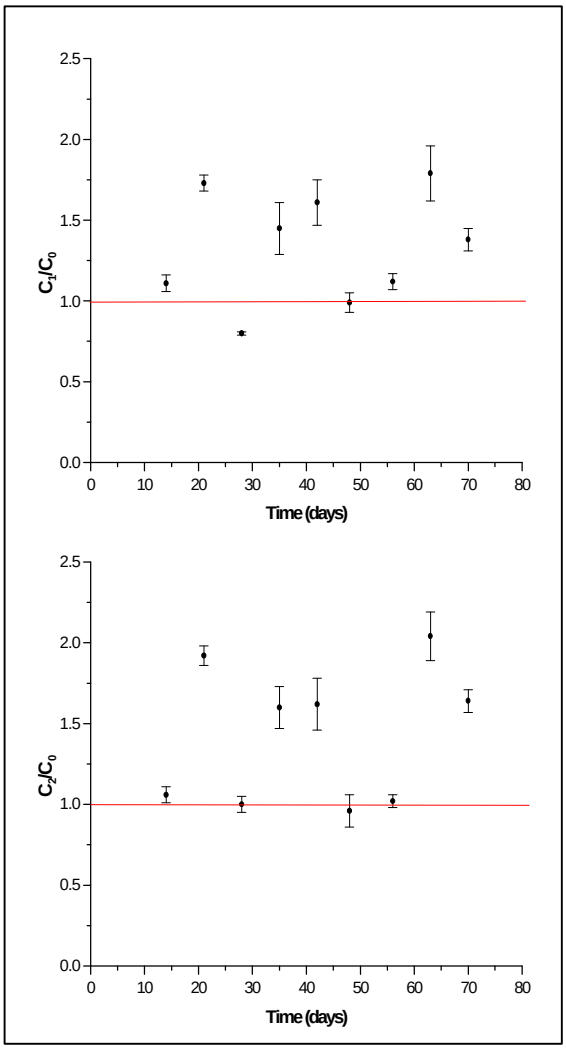

Figure 3. Ratios of U concentrations in the bones as a function of the animal age: C_0 , C_1 and C_2 stand for diets with no phytase, 120 ppm and 180 ppm of phytase, respectively. See text for details .

IV. DISCUSSION

The Role Played by Phytase

A mere visual inspection of the results displayed in Figures 1, 2 and 3 reveals that:

(i) The administration of phytase does not alter the biokinetics of U in the animals bones, since the concentrations C_0, C_1 and C_2, as a function of time (Fig.1), exhibit the same general trend.

(ii) The phytase saturating dose should be in between 0.12 and 0.18 g per kg of food, because the ratios C_1/C_0 and C_2/C_0 are similar within the uncertaintes (see Fig.3).

(iii) Quite intriguing, however, is the nonexpected periodically time oscillating behavior of the ratios C_1/C_0 and C_2/C_0 (Fig.3). Structures show up in the two sets of incorrelated data and at the same time positions t = 21, 42

and 63 days. A closer examination of Fig.1-a, C_0, reveals three minima at these same time positions. On the other hand, the general trend of C_1 and C_2 (Figs. 1-b and 1-c, respectively) is similar and reasonably nonfluctuating. Therefore, the structures observed in both C_1/C_0 and C_2/C_0 are due only to the irregularities present in the U biokinetics of animals receiving no phytase (Fig. 1-a), and probably not to the action of phytase itself. It goes beyond the scope of this work the setting up of conjectures on the physiological nature of such irregularities, but we are quite sure on their statistical significance. In fact, each datum point in Fig.1 was obtained by averaging results from 2 animals (and 3 gamma lines per animal). In the particular case of the minima at t=21 and 42 days (Fig. 1-a) the dispersion of the averaged data is smaller than 2 %.

(iv) The data points in between the structures show that $C_1/C_0 \approx C_2/C_0 \approx 1$ implying, thus, that phytase plays no significant role in the accumulation of U in the bone at these specific animal life periods, particularly in between 21 and 42 days.

The Biokinetics of U

The results shown in Figure 1 are surprising and somewhat unexpected, particularly when compared to those obtained with mammals. Uranium was administered daily at a dose rate of 20 ppm (20 mg of U per kg of food), during the broilers life time period from 7 to 42 days. The concentration of U in their bones is systematically decreasing in this period of time.

In the case of mammals (sheeps, dogs, cows, etc.) the transfer of most contaminants from food to their organs is quite rapid (within a few days). Thus, for the purpose of assessing concentration levels of uranium in bone, in quasi-equilibrium with a constant rate of U ingestion, a detailed understanding of the animal metabolism is unnecessary, and a single bone-compartment suffices as a mathematical model of the transfer.

Let's assume that after a single dose the concentration of the uptaken U is C_0 (μg-U per g-bone), and that it has occurred at the instant of time t=0. As time evolves (t >0), the skeletal clearence process gradually diminishes the U concentration, such that $C(t) < C_0$, and the clearence equation is

$$\frac{dC(t)}{dt} = -\lambda C(t) \qquad (1)$$

where λ^{-1} is the average clearence time. Stevens et al. [5] found out that $\lambda^{-1} \approx$ 1800 days in Beagles femora. The solution of Eq. (1) is

$$C(t) = C_0 . e^{-\lambda t} \qquad (2)$$

However, for chronic ingestion, where a daily uptake C_0 is taking place, Eq. (1) is rewritten as

$$\frac{dC(t)}{dt} = C_0 - \lambda C(t) \qquad (3)$$

where now C_0 has dimensions of μg-U/g-bone per day.

The formal solution of Equation 1 is

$$C(t) = e^{-\lambda t} \int_0^t C_0 . e^{\lambda t'} dt', \qquad (4)$$

Since Co is constant (time independent) in this approach, we easily get that

$$C(t) = \frac{C_0}{\lambda}\left[1 - e^{-\lambda t}\right] \qquad (5)$$

where

$$\lim_{t\to\infty} C(t) = \frac{C_0}{\lambda} \equiv C_{eq} \qquad (6)$$

is the equilibrium concentration.

The continous curve drawn across the data points in Fig.1 was obtained by putting arbitrarily C_{eq} = 0.9 μg-U/g-bone and $\lambda^{-1} \cong$ 30 days in Eq. (5), just to make salient our results for fowls. In fact, such a comparison suggest that the uptake parameter C_0 in bone is a function of time for the growing fowl. Thus, $C_0(t)$ should be considerably high for the young animal decreasing gradually as the skeleton grows; therefore we assume for reasoning purposes only that

$$C_0(t) = C_{00} e^{-\mu t} \qquad (7)$$

where C_{00} is the uptake concentration (μg-U/g-bone) at t = 0, that is, at the first day of the animal life. By substituting Eq.(7) in Eq. (4) we obtain

$$C(t) = \frac{C_{00}}{(\lambda-\mu)} e^{-\mu t} - \frac{C_{00}}{(\lambda-\mu)} e^{-\lambda t} \equiv F_u(t) - F_c(t) \qquad (8)$$

where the functions

$$F_u(t) = \frac{C_{00}}{\lambda-\mu} e^{-\mu t} \qquad (9\,a)$$

and

$$F_c(t) = \frac{C_{00}}{\lambda-\mu} e^{-\lambda t} \qquad (9\,b)$$

represent the amounts of U concentration transferred to, and removed from the bone by the uptake and clearence processes, respectively.

Taking the time derivative of C(t), Eq.(8), we obtain the slope of the curve C=C(t), namely,

$$\frac{dC}{dt} = \frac{C_{00}}{(\lambda-\mu)}\left[\lambda e^{-\lambda t} - \mu e^{-\mu t}\right] \qquad (10)$$

We note that $dC/dt < 0$ for any λ and μ ($\lambda>\mu$ or $\lambda<\mu$), which qualitatively describes our findings (see Fig.1).

The Role Played by Metabolism

Although simple, the approach suggested and discussed above makes salient the role played by the fowl metabolic process at two distinct stages of the U-transfer biokinetics: uptake, characterized by a decay mean time equal to μ^{-1}, and bone clearence, where λ^{-1} is its mean time. In this sense, a gradually decreasing uptake of uranium, relatively to the skeleton mass, combined with a "slower"

clearence, makes the difference $F_u(t) - F_c(t)$ a decreasing function of time (Eq. (8)) too in the animal life period studied in this work ($t \geq 14$ days). However, for the period 0 – 14 days we can make only an educate guess: the concentration function would be increasing steeply from C(t=0)=0 up to some point around or below t=14 days where a change of sign of its derivative takes place, giving rise to a decreasing C(t) from t=14 days on.

In the case of mammals as Beagles, recently studied by us since their post-weaning period [6], we also observe an increasing U-concentration starting at the time period of the very young animal, and keeping positive its derivative all the way long till adultness age, as described by Eq.(5) and illustrated by the continuous solid curve depicted in Fig. 1-a. This behaviour, in our approach, is a consequence of a nearly constant, time independent uptake concentration, meaning that the amount of U transferred to the bones increases proportionally with the mass of the growing skeleton. In fact, from Eq. (5) we have that for Beagles (see also Eq. (8))

$$F_u = \frac{C_0}{\lambda} \approx \text{cons tan t} \qquad (11)$$

The situation for fowls is the opposite, because fowls grow much faster.

V. CONCLUSIONS

Our findings point to the necessity of more and specific studies on the physiological and metabolical aspects driving uptake/clearence of not only U (which mimics a nutrient), but also of essential elements present in the diet of fowls.

This study poses, for the first time, an alternative methodology to the study of metabolism by the use of a fissile tracer, demonstrating, moreover, the potentialities of interdisciplinary research.

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