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Metabolical Aspects Associated with Incorporation and Clearance of Uranium by Broilers - Case Study and a Biophysical Approach

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Abstract: Groups of seven-day old Cobb broilers were fed with ration doped with uranyl nitrate at a fixed concentration (of 20 ppm), and two concentrations of phytase (120 and 180 ppm). Two animals per group were sacrificed weekly up to their adulthood. Calcium and uranium concentrations in tibia were 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 ~ 40%, in average. Quite surprising too, the concentration of uranium ($\mu\text{g-U/g-bone}$) decreases all along the animal life period of 14-42 days, meaning that the skeleton mass is growing faster than the corresponding accumulation of uranium, while the concentration of calcium remains nearly constant, as expected. This last finding is interpreted as a possible interplay between two metabolical peculiarities, associated both with U transfer to (uptake), and U removed from (clearance) the bones, respectively.

Key words: Uranium uptake, phytase, Cobb broiler, bone, metabolism

Introduction

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), by one hand, can present concentrations of uranium as high as 200 ppm (Arruda-Neto *et al.*, 1997). On the other hand, DCP is extensively used in broilers diet (Junqueira *et al.*, 1993; Lima *et al.*, 1995; Sebastian *et al.*, 1996). Insoluble forms of uranium are greatly solubilized during digestion giving rise to uranyl compounds, particularly uranyl nitrate.

Following uptake through the gastrointestinal tract, uranium is mostly deposited in the skeleton. Actually, bone is one of the most important biological accumulators of uranium (Tandon *et al.*, 1998). It is said that uranium could possibly *mimics* calcium (Legget and Harrison, 1995; Karpas *et al.*, 1998). This fact led us to consider the role played by enzymes in poultry nutrition - phytase in particular, as discussed below.

Phytase is used to improve the availability of phosphorus, minerals and metal ions, like calcium (Leske and Coon, 1999). Thus, our conjecture is: if uranium indeed *mimics* calcium, then, the 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 since 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 consumption by humans.

In fact, it may well be possible that phytase enzymes increase the availability of nutrients other than just phosphorus and calcium, because positively charged molecules, such as lysine, can complex with the negatively charged phytase molecule. Therefore, the uranyl ion UO_2^{++} would follow this same metabolical path. Actually, phytate and citrate have been a long ago identified as soluble plutonium binding species in a wide variety of food crops (Cooper and Harrison, 1982). In view of all these circumstances, we decided to measure the concentration of uranium in bones of broilers fed with uranyl nitrate doped ration (with a fixed doping amount), plus phytase at two different dosages, through a period of time starting at the earlier stages of the animal development and lasting until maturity. We note, in this regard, that uranyl nitrate has long been recognized as a nephrotoxic agent for impairing renal function in growing chicks (Harvey *et al.*, 1986; Mollenhauer *et al.*, 1986). 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.

Materials and Methods

Fifty four, seven-day 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 uranyl nitrate, now referred to simply as *U-doped food*;
- group-2: U-doped food plus 0.12g of phytase per kg of food (or, 600 units of phytase activity);
- group-3: U-doped food plus 0.18g of phytase per kg of food (900 units).

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

Three seven-day old animals were sacrificed with the purpose of obtaining base values, that is, residual uranium transferred to the broilers during the pre- and neonatal phases.

Starting with 14-day old broilers, two animals per group were slaughtered weekly, and the tibiae were immediately removed and frozen at -20°C for a *posteriori* processing and analysis. After 42 days of age, the uranyl nitrate was removed from the diet, and the experiment was finished when the broilers got 70 days of age. The animals were slaughtered accordingly to the Guide of Care and Use of Experimental Animals (Canadian Council on Animal Care, vol.2, 1984).

The bones were individualized in porcelain melting pots, weighed 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 and Ca solutions, with exactly known concentrations, were pipetted onto 2 cm² pieces of Whatman n^o. 40 filter paper and dried in a desiccator. Sets constituted by 6 bone samples and one standard each were wrapped with aluminum foils, and placed in Cd capsule, and irradiated in the IPEN research nuclear reactor (IEA-R1, 2MW) for 8h at a thermal neutron flux of 10¹² n.cm⁻².s⁻¹.

Samples and standards were analyzed by means of conventional gamma-spectrometry procedures, using a high resolution 75 cm³ - high purity Ge detector operated with a 671 ORTEC amplifier in pile-up rejection mode. For the quantification of uranium 2h counting runs were used, allowing, thus, the determination of the three main gamma lines following the decay of ²³⁸Np (formed from ²³⁸U + n → ²³⁹U → ²³⁹Np): 106 keV, 228 keV and 278 keV.

Results

We show in Fig. 1 our results for U expressed as concentration in the bones (µg/g - bone). Each data point represents an average taken over the 3 gamma rays decay of ²³⁸Np measured in samples of two animals; therefore, it is the average of 6 experimental values for uranium concentrations. Thus, all error bars showing up in our figures represent external standard deviations. Also shown for comparison purposes a few results for Ca, expressed as mg-Ca / mg- bone.

Once only averaging is our data handling procedure, it suffices to calculate the external standard deviation of the averaged values. This simply is a conventional parametric statistics in the normal model (Caria, 2000). The 3 sets of data, namely, Fig. 1a (U and no phytase), Fig. 1b (U and phytase) and Fig. 1c (U and higher concentration of phytase), exhibit the same decreasing trend as a function of time (t). Although it is obvious that the concentration of U decreases for t > 42d, because U administration was interrupted when the animals got 42 days of age, it is quite surprising and unexpected that a decreasing trend is also observed during the period of daily uranium intake. On the other hand, the fractions of calcium remained nearly constant.

The U concentrations measured for the seven-day old animals (base values) are around 2-3 ng-U/g-bone, that is, two orders of magnitude smaller than the lowest values shown in Figs. 1a-c.

Fig. 2 shows the concentration in the bones as 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.

The ratio C₂/C₁, where C₁ and C₂ are the U concentrations corresponding to 0.12 and 0.18g of phytase per Kg of food, respectively, is shown in Fig. 3. In Fig. 4 both the skeleton mass and total uranium incorporated mass are shown as functions of the animal age. Finally, in Fig. 5 we present the U transfer coefficient.

Discussion

The gross role played by phytase: A visual inspection of the results displayed in Fig. 1a, b and c reveals that the administration of phytase does not alter general biokinetics aspects of uranium in the animals bones, since the concentrations C₀, C₁ and C₂, as functions of time, exhibit the same decreasing trend. It seems that the phytase saturating dose should be somewhere in between 0.12 and 0.18g per kg of food (Fig. 2).

The average increase of the U concentration, relatively to the control group (no phytase), ranged from 20 to 35% for the two doses of phytase, suggesting thus that phytase could also improve the availability of uranium. The potential ability of phytate to enhance uptake across the gut has been demonstrated only for transuranics (see e.g. Cooper and Harrison, 1982).

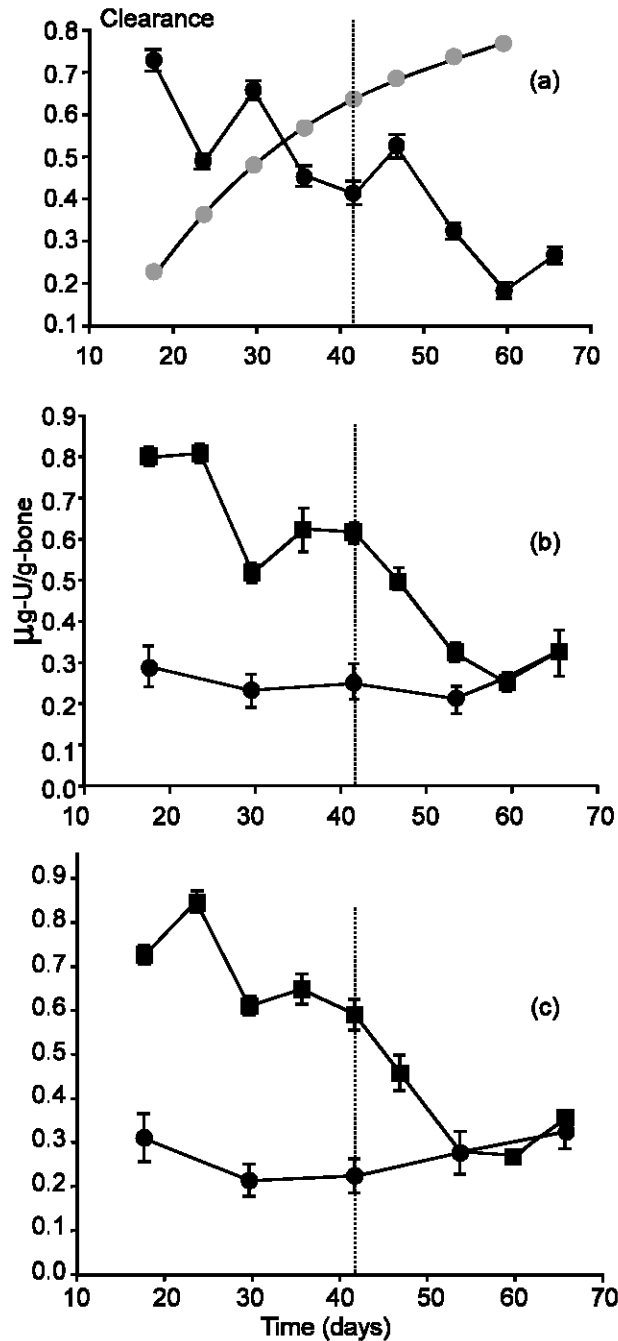


Fig. 1: Concentration of U in the bones of fowls as function of the animals spanned life time, and corresponding to daily diets with (a) no phytase, (b) 120 ppm and (C) 180 ppm of phytase in the food, plus 20 ppm of U for the three groups. The lines connecting the data points are only to guide the eyes, and the error bars represent the external standard deviations. The vertical dashed-lines at $t = 42d$ indicate that for $t > 42d$ uranium was no longer administered to the animals and, therefore, the U clearance process

was expected to take place in this period of the experiment. The continuous line in (a) was obtained from a calculation using a biokinetics model suitable for mammals. The full circles in (b) and (C) represent results for calcium expressed as fractions of the total bone mass (e.g. mg-Ca/mg-bone); in this case, the vertical scale has no unit.

The results shown in Fig. 1a, b and c, their decreasing trend in particular, indicate that the skeleton of the growing fowl is increasing faster than the uranium uptake, resulting therefore in a decreasing concentration in bone (see Fig. 4). Additionally, an equally fast clearance process could contribute to the observed trend.

Finer phytase related aspects of U accumulation: As expected from the well-known fact that broilers exhibit a very fast metabolism, the uranium biokinetics quite fluctuates (see Fig. 1a). Notwithstanding, we observed some regularities we would like to point out, namely:

- The 2 sets of data from 2 different concentrations of phytase in the food (Fig. 1b and 1c), are less fluctuating and quite similar, suggesting that the metabolic process of U accumulation works less erratically with phytase. We emphasize that the 3 sets of data shown in Fig. 1 are independent; so, the similarities are probably neither accidental nor due to artifacts of the detection/measurement techniques;
- Something intriguing seems to be going on at the animal life period around $t = 28d$. The ratio C_2/C_1 , which is independent of growth dilution, exhibits a resonance-like structure around $t = 28d$ (Fig. 3). It seems that younger broilers didn't respond to the 50% increase of the phytase dose, which is consistent with the fact that, usually, the younger the bird the lower the availability of phythase phosphorus.

The biokinetics of U: The results shown in Fig. 1 are surprising and somewhat unexpected, particularly when compared with those obtained in mammals. Uranium was administered daily with a dose 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 systematically decreases 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 us assume that after a single dose the concentration

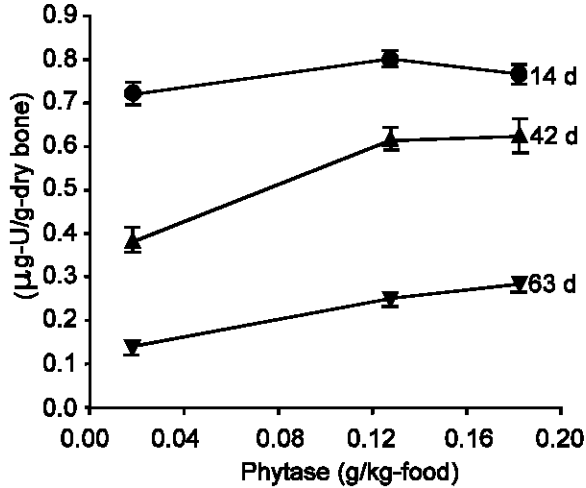


Fig. 2: Concentration of U in the bones of fowls as function of the amount of phytase in the food, and at three selected animal ages.

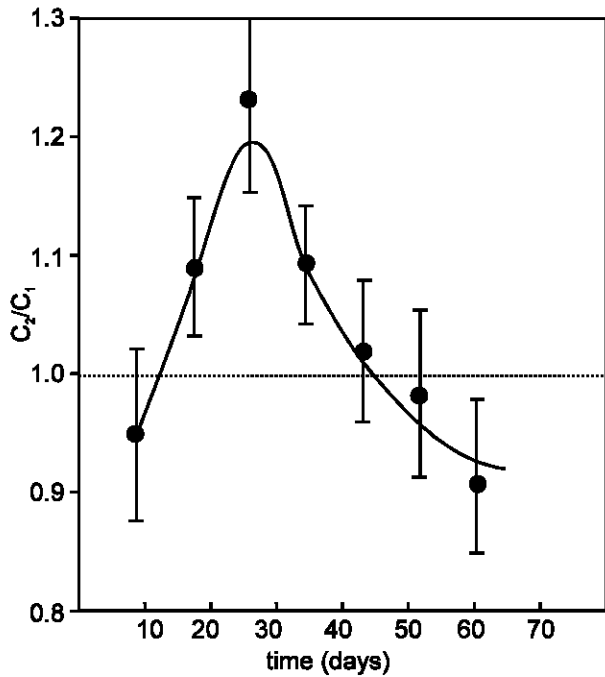


Fig. 3: The ratio C_2 / C_1 as function of the animal age. The line connecting the data points is only to guide the eyes.

of the uptaken U is C_0 ($\mu\text{g-U}$ per g-bone), and that it has occurred at the instant of time $t = 0$. As time evolves ($t > 0$), the skeletal clearance process gradually diminishes the U concentration, such that $C(t) < C_0$, and the clearance equation is

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

where λ^{-1} is the average clearance time. Stevens *et al.*

(1980) e.g. found out that $\lambda^{-1} 1800$ days in Beagles femora. The solution of eqn. (1) is

$$C(t) = C_i e^{-\lambda t} \quad (2)$$

However, for chronic ingestion, where a daily uptake C_i is taking place, eqn. 1 is rewritten as

$$\frac{dC(t)}{dt} = C_i - \lambda C(t) \quad (3)$$

where C_i has dimensions of $\mu\text{g-U/g-bone}$ per day.

The formal solution of equation 1 is

$$C(t) = e^{-\lambda t} \int_0^t C_i e^{\lambda t} dt \quad (4)$$

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

$$C(t) = \frac{C_i}{\lambda} [1 - e^{-\lambda t}] \quad (5)$$

where

$$\lim_{t \rightarrow \infty} C(t) = \frac{C_i}{\lambda} \equiv C_{eq} \quad (6)$$

is the equilibrium concentration.

The continuous curve drawn across the data points in Fig. 1-a (up to $t = 42\text{d}$) was obtained by putting arbitrarily $C_{eq} = 0.9 \mu\text{g-U/g-bone}$ and $\lambda^{-1} = 30$ days in eqn. 5, just to make salient our results for broilers. For $t > 42\text{d}$ and the clearance process (eqn. 2) would be represented by a continuous and decreasing curve. In fact, such a comparison suggests that the uptake parameter C_i in bone is a function of time for the growing fowl. Thus, $C_i(t)$ should be considerably higher for the young animal decreasing gradually as the skeleton grows; therefore, we assume for reasoning purposes only that

$$C_i(t) = C_{i0} e^{-\mu t} \quad (7)$$

where C_{i0} is the uptake concentration ($\mu\text{g-U/g-bone}$) at $t = 0$, that is, at the first day of the animal life. Eqn. 7 is no longer valid when the skeleton size of the adult animal stabilizes.

By substituting eqn. 7 in eqn. 4 we obtain

$$C(t) = \frac{C_{i0}}{(\lambda - \mu)} e^{-\mu t} - \frac{C_{i0}}{(\lambda - \mu)} e^{-\lambda t} \equiv F_u(t) - F_c(t) \quad (8)$$

where the functions

$$F_u(t) = \frac{C_{i0}}{\lambda - \mu} e^{-\mu t} \quad (9a)$$

$$F_c(t) = \frac{C_{i0}}{\lambda - \mu} e^{-\lambda t} \quad (9b)$$

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

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

$$\frac{dC}{dt} = \frac{C_{i0}}{\lambda - \mu} [\lambda e^{-\lambda t} - \mu e^{-\mu t}] \quad (10)$$

We note that $dC/dt < 0$ for $t > 20\text{d}$ and any λ and μ ($\lambda > \mu$ or $\lambda < \mu$), which qualitatively describes our findings (see Fig. 1). The result $dC/dt > 0$, for $t < 20\text{d}$, is highly expected for younger animals (see last paragraph of Section 5).

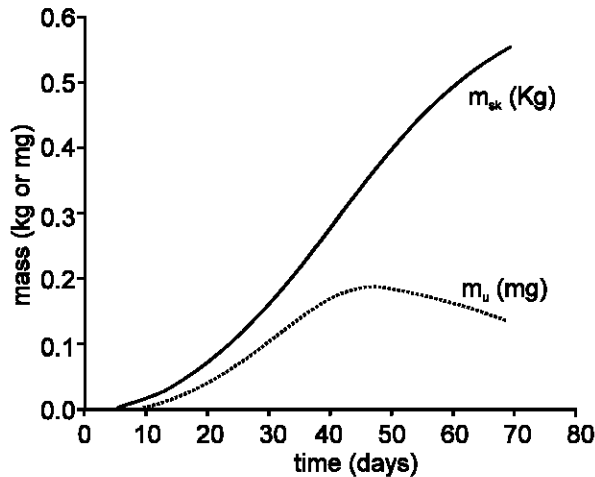


Fig. 4: The average skeleton mass of the Cobb broilers, m_{sk} (kg), and the total uranium content of the skeleton, m_u (mg), as functions of the animal age.

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 time constant equal to μ^{-1} , and *bone clearance*, where λ^{-1} is its mean time. In this sense, a gradually decreasing uptake of uranium, relatively to the skeleton mass makes the difference $F_u(t) - F_c(t)$ a decreasing function of time (eqn.8) 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, studied since their post-weaning period (Arruda-Neto *et al.*, 2004), we also observed an increasing U-concentration starting at the time period of the very young animal, and keeping positive its derivative all the way long until adult age, as described by eqn.5 and illustrated by the continuous solid curve depicted in Fig.1a. This behavior, 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, that is, $\mu \approx 0$ (see eqn.7). Thus, from eqn.9a we have that for Beagles (see also eqn.8)

$$F_u = \frac{C_i}{\lambda} \approx \text{Constant} \quad (11)$$

and, off course, eqn. 5 is rendered.

The situation for fowls is the opposite, as shown in Fig. 4. The curves labeled with m_u and m_{sk} represent the total uranium content of the skeleton and skeleton

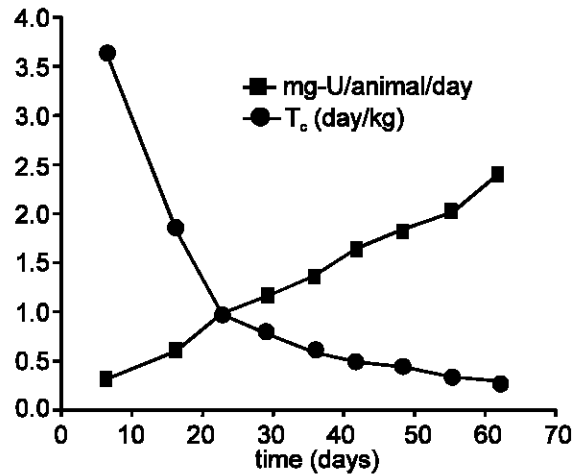


Fig. 5: Uranium transfer coefficient to the bones of fowls (decreasing curve) and the U consumption rate through the feeding (increasing curve).

mass, respectively, as functions of time. We calculated m_u by assuming that the uranium concentrations we measured from samples of tibia, $C(t)$, do not vary appreciably for other parts of the skeleton; then, $m_u(t) = m_{sk}(t) \cdot C(t)$ (12) As we observe in Fig. 4 (where the curves only represent the general trend of the results), the curve $m_{sk} = m_{sk}(t)$ is steeper than the curve $m_u = m_u(t)$ explaining, thus, why the ratio $C(t) = m_u(t) / m_{sk}(t)$ shown in Fig. 1 is a decreasing function of time.

Growth dilution and transfer coefficients: This study involved animals between 14 and 70 days, where the mass of the skeleton increases by a factor 20-30 over this period. Our taking into account of growth dilution was partially accomplished when we expressed our results as “concentrations”, namely, when we divided the measured amount of U by the organ mass (tibiae). However, the growth dilution issue is compounded because the amount of food ingested by the animal as it grows to maturity changes, with a consequent change in the amount of uranium ingested each day as shown in Fig. 5. Moreover, radiobiological investigations of feed contaminants transfer must rely on a sound knowledge of the amount of a radionuclide that is ingested. In our approach, eqn. 7 is a tentative way to describe such a circumstance.

The usual way to partially circumvent this drawback is by calculating the U transfer coefficient T_c , defined as (see Fig. 5)

$$T_c(t) = \frac{C(t)}{m(t)} \text{ [d.kg}^{-1}\text{]} \quad (13)$$

where $C(t)$ is the uranium concentration displayed in Fig.1-a, and $m(\text{kg.d}^{-1})$ is the amount of uranium daily ingested (also shown in Fig. 5). T_c measures the

incorporation efficiency relatively to the daily intake. For example, if $T_c = 0.02$ ($d \cdot kg^{-1}$), it means that 1 mg of organ is accumulating 0.02 mg of U per each kg of U daily ingested.

The fact that T_c decreases very fast as a function of the animal age is interesting. While the skeleton of the young bird is avid for both calcium and uranium, the metabolism of the mature animal prevents the incorporation of uranium in the bones. Actually, the fraction of ingested U that crosses the gut may also decrease as the animal gets older. For mammals we know that young animals are often in positive uranium balance (between uptake and excretion) due to the build up of uranium in the growing skeleton. Moreover, the gastrointestinal tract of newborn animals is orders of magnitude more permeable to a number of radio nuclides, including uranium, than is that of the adults (Sullivan and Gorham, 1982).

Conclusions:

- 1 The administration of phytase to growing fowls improved the availability of uranium. Therefore, uranium seems to mimic calcium also in this aspect. Otherwise, this could indicate that uranium does fulfill some role in the fowl biochemistry, or that its behavior is typical of an essential element.
- 2 The action of phytase does not alter the overall trend of the uranium biokinetics in bone of fowls along the growing period.
- 3 The decreasing trend of C_0 , C_1 and C_2 with time is biokinetically explained as the interplay between metabolic peculiarities driving the transfer and clearance processes of U in the bones.
- 4 As a result of metabolic peculiarities, it was experimentally found out that the uranium accumulation rate is slower than the skeleton growing rate.
- 5 Regarding radiological implications to poultry consumers, our study indicates that they would be subject to a much smaller radioactive burden comparatively to the consumers of mammals. In fact, uranium is fastly cleared out from the bones of poultries, while it is continuously accumulated till saturation in the bones of mammals (see Fig.1a). It should be noted that the skeleton works as a reservoir of U to the rest of the body, and that the U accumulation in other organs is proportional to the U content in bones (Garcia *et al.*, 1999 and references therein). Finally, the administration of phytase seems not to change the picture drawn in this paragraph.

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Arruda-Neto *et al.*: Metabolical Aspects Associated with Incorporation and Clearance of Uranium by Broilers

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