Uranium incorporation biokinetics in poultry bones as function of phytase doses

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Neutron activation analysis has been used to study uranium incorporation in poultry bones as function of chow doped with: (a) uranium (20 ppm); (b) U-doped food (20 ppm) plus phytase (120 ppm) and (c) U-doped food (20 ppm) plus phytase (180 ppm). To investigate this situation experiments involving several groups of Cobb broilers was performed. Two animals per group were sacrificed weekly up to their adultness and uranium concentration in the tibia was measured. It was observed that the concentration of uranium (µg U/g bone) is decreasing all along the animal life spanning period of 14–42 days. This behavior suggests that the skeleton mass is growing faster than the corresponding accumulation of uranium. The administration of phytase seems not to alter this scenario.

Introduction

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.

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.² On the other hand, enzymes in poultry nutrition – phytase in particular, are 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. Such a possibility is considerably more important to verify if feed supplements contain appreciable amounts of uranium, and because in this case additional amounts of uranium are introduced in the food chain through poultry consumption by humans.

To check these possibilities, this work presents measurements of uranium concentration in bones of

broilers fed with uranyl nitrate doped chow (at one fixed doping amount), plus phytase at two different dosages, for a period of time starting at the early 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 their consumers.

Experimental

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 and consumption was monitored.

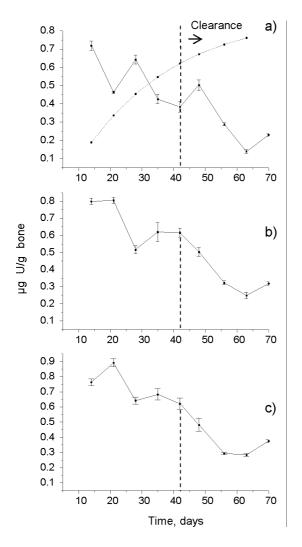


Fig. 1. Concentration of U in the bones of fowls as a function of the spanned life time, and corresponding to daily diets with no phytase (a), 120 ppm (b) and 180 ppm of phytase (c) in the food

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

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 for 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.

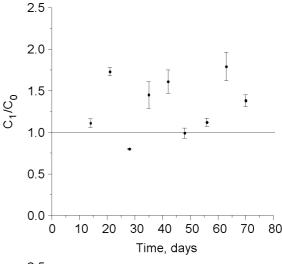
Approximately 100 mg of bone ash from each animal was weighed and sealed in polyethylene bags. Standard aliquots of U solutions, with known concentrations, were pipetted onto 2 cm² pieces of Whatman n.4 filter paper and dried. Each bone sample

and the standard were irradiated in the IPEN research reactor for 8 hours at a thermal neutron flux of $10^{12}~\rm n\cdot cm^{-2}\cdot s^{-1}$.

Samples and standards were analyzed by means of conventional gamma-spectrometry procedures, using a high resolution 75 cm³ HPGe detector operated with a 671 Ortec amplifier in pile-up rejection, allowing thus the determination of the three main gamma decay energies for ^{239}Np (formed from $^{238}\text{U+n} \rightarrow ^{239}\text{U} \rightarrow ^{239}\text{Np}$): 106, 228 and 278 keV.

Results

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



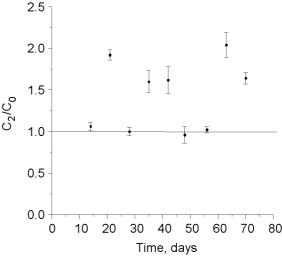


Fig. 2. Ratios of U concentrations in the bones as a function of the animal age: C_0 , C_1 and C_2 stand for diets with no phytase, 120 ppm and 180 ppm of phytase, respectively

The three sets of data, namely, Fig. 1a (U and no phytase), Fig. 1b (U and phytase) and Fig. 1c (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 after 42 days old, it is quite surprising and unexpected finding a decreasing trend also during the period of daily uranium intake.

In order to better appraise the role played by phytase in U accumulation in the bones, we show in Fig. 2 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.

Discussion

It is clear that 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; the phytase saturating dose should be 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 (Fig. 2). The general trend of C_1 and C_2 (Figs 1b and 1c, 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. 1a), 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 result in Fig. 1 was

obtained by averaging results from 2 animals (and three gamma lines per animal).

The data points 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 between 21 and 42 days.

Conclusions

The concentration of uranium ($\mu gU/g$ bone) decreases 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. This last finding is interpreted as a possible interplay between two metabolic peculiarities, associated both with U transfer to (uptake), and U removed from (clearance) the bones, respectively.

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References

- 1. J. D. T. ARRUDA-NETO, M. V. TAVARES, M. FILADELFO, J. Radioanal. Nucl. Chem., 221 (1997) 97.
- L. TANDON, G. V. IYENGAR, R. M. PARR, Appl. Radiation Isotopes, 49 (1998) 903.
- 3. D. A. ROLAND, Publ. Mallinckrodt Feed Ing., 1995.
- R. B. HARVEY, L. F. KUBENA, T. D. PHILLIPS,
 N. D. HEIDELBAUGH, Bull. Environ. Contam. Toxicol., 36 (1986) 67.