ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF POULTRY SCIENCE

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International Journal of Poultry Science 4 (7): 511-517, 2005
ISSN 1682-8356
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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 (µg-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 cm^2 pieces of Whatman nº. 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 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 - 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 ^{239}Np (formed from $^{238}U + n \longrightarrow {}^{239}U \longrightarrow {}^{239}Np$): 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 ^{239}Np 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 C_2/C_1, where C_1 and C_2 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 C_0, C_1 and C_2, 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).

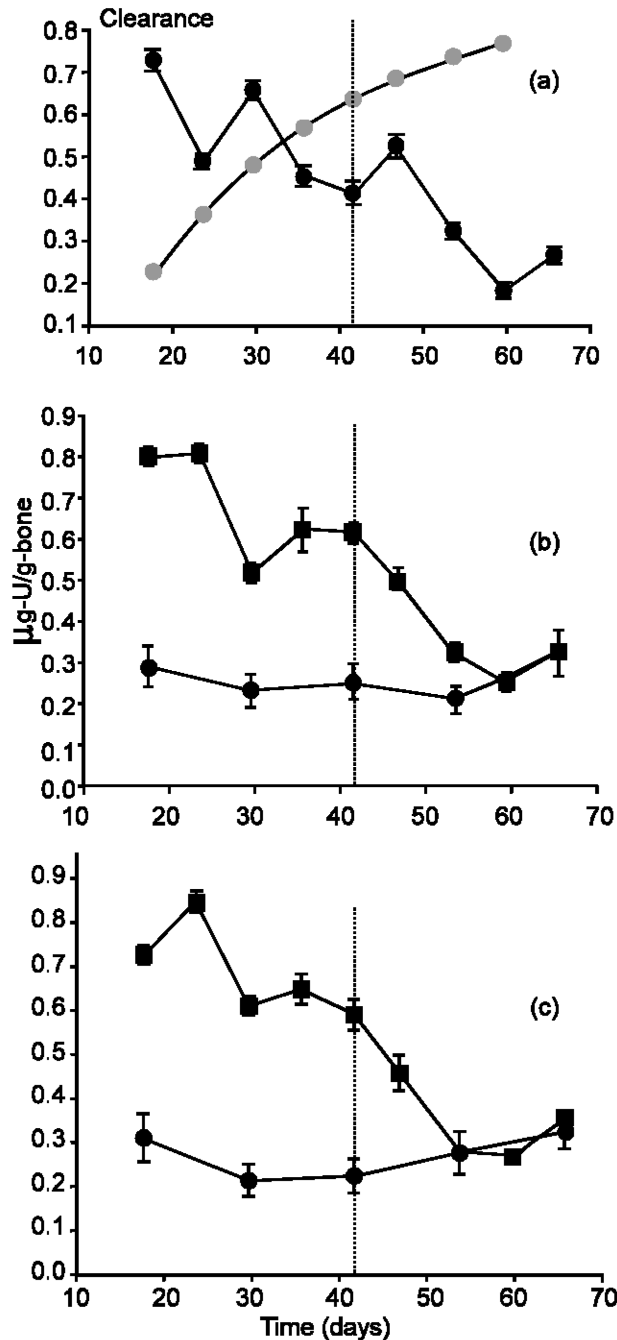

Fig. 1: Concentration of U in the bones of fowls as function of the animals spanned life time, and corresponding to daily diets with (a) no phytase, (b) 120 ppm and (C) 180 ppm of phytase in the food, plus 20 ppm of U for the three groups. The lines connecting the data points are only to guide the eyes, 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:

a) 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;
b) 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

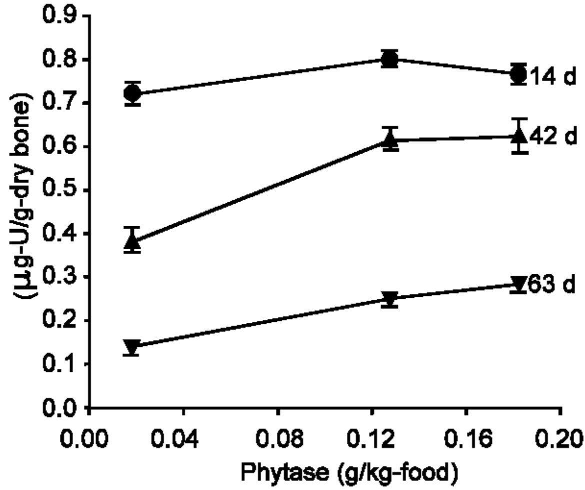

Fig. 2: Concentration of U in the bones of fowls as function of the amount of phytase in the food, and at three selected animal ages.

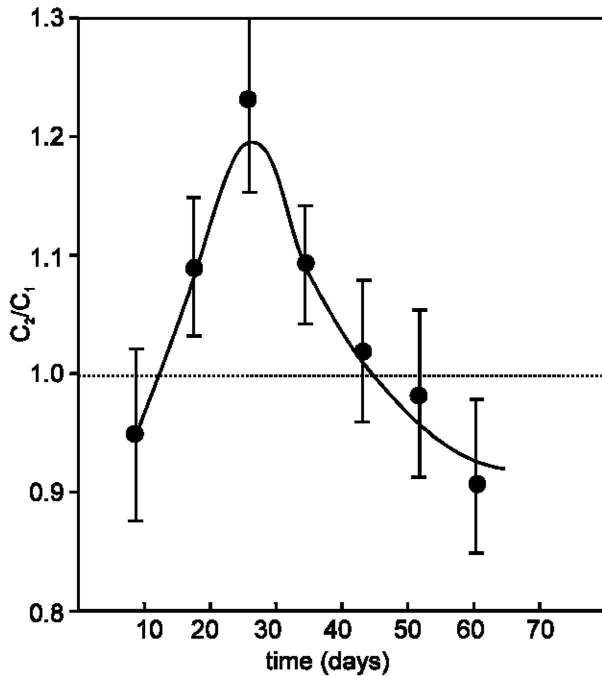

Fig. 3: The ratio C2 / C1 as function of the animal age. The line connecting the data points is only to guide the eyes.

of the uptaken U is C_0 (µg-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

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

where λ^{-1} is the average clearance time. Stevens *et al.* (1980) e.g. found out that λ^{-1}1800 days in Beagles femora. The solution of eqn. (1) is

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

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

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

where C_i has dimensions of µg-U/g-bone per day.
The formal solution of equation 1 is

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

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

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

where

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

is the equilibrium concentration.
The continuous curve drawn across the data points in Fig.1-a (up to t = 42d) was obtained by putting arbitrarily C_{eq} = 0.9 µg-U/g-bone and λ^{-1} = 30 days in eqn.5, just to make salient our results for broilers. For t > 42d 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

$$C_i(t) = C_{io} e^{-\mu t} \qquad (7)$$

where C_{io} is the uptake concentration (µg-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

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

where the functions

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

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

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,

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

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

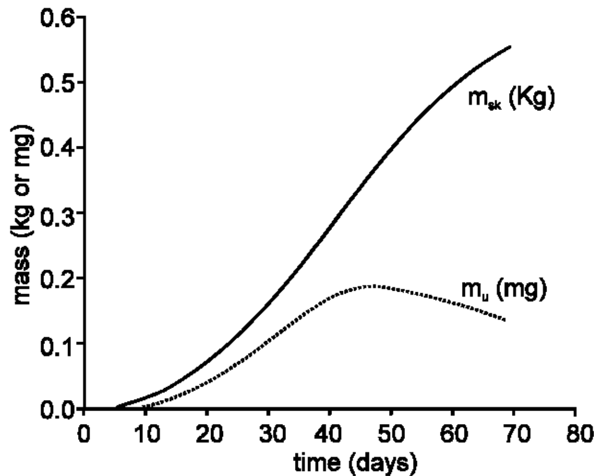

Fig. 4: The average skeleton mass of the Cobb broilers, m_{sk} (kg), and the total uranium content of the skeleton, m_u (mg), as functions of the animal age.

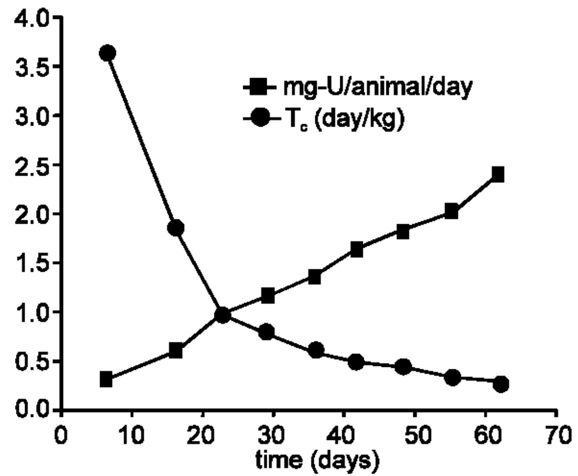

Fig. 5: Uranium transfer coefficient to the bones of fowls (decreasing curve) and the U consumption rate through the feeding (increasing curve).

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)

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

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 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,

$$m_u\,(t) = m_{sk}\,(t).\,C\,(t) \qquad (12)$$

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 Tc, defined as (see Fig. 5)

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

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

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

The fact that Tc 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.

Acknowledgments

Partially supported by FUNDUNESP, FAPESP and CNPq, Brazilian agencies, and by the Latinamerican Physics Center/CLAF.

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International Journal of Poultry Science 4 (7): 476-481, 2005
ISSN 1682-8356
© Asian Network for Scientific Information, 2005

Characterization of GH, GHR and IGF-I in Broiler Lines Selected for Rapid Growth or Improved Feed Efficiency

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Abstract: The aim of this study was to figure out the response of the GH axis to selection criteria in broiler chickens. Plasma profiles of GH, IGF-I along with membrane GHR binding activity were examined during rapid growth phase in experimental lines of broiler chickens. The selection criteria applied influenced the plasma GH levels and the number of hepatic GHR. There was a higher plasma GH level in FC chickens compared to GL chickens while the opposite pattern was observed for GHR. The negative correlation between plasma GH levels and hepatic GHR suggest a down regulatory mechanism for GHR in broiler chickens. The absence of line differences in plasma IGF-I levels between GL and FC lines in spite of clear difference in growth rate and in plasma GH concentrations may support the concept that plasma IGF-I does not appear to be GH-dependent, implying the importance of other factors besides GH in the regulation of IGF-I in chickens. The slower growth rate as function of time in the FC line during rapid growth phase was followed by a phase of compensatory growth. On the basis of this growth curve pattern it can be hypothesized that the dynamics of GH action are shifted or extended to the phase of compensatory growth as an effect of the selection on feed conversion. This may be a causal mechanism for the different growth curve pattern in GL and FC lines. Assuming that the metabolic basis for these differences is better understood it may be possible to device other procedures for improving meat quality in poultry industry.

Key words: Growth line, feed conversion line, GH, IGF-I, GHR

Introduction

The goals in manipulating growth process in animals are to improve productivity, productive efficiency and the quality of animal products. Growth results from a complex interaction between genotype and environmental factors. Both genetic and environmental factors are largely translated in hormonal signals by which these factors affect growth processes. The hormonal regulation of growth involves a complex sequence of interactions between different hormones. The somatotrophic (growth hormone, GH; growth hormone receptor, GHR: insulin-like growth factor, IGF-I) axis is considered to be one of the most important among them, because of their broad range of effects and central role in growth. The importance of circulating GH in mammalian species is well established. Administration of exogenous GH dramatically increases milk yield in dairy cows (Bauman *et al.,* 1985) and improved carcass protein/fat ratio in sheep (Muir *et al.,* 1983. It has been clearly demonstrated that broiler chickens do not respond to exogenous GH in the same manner as other livestock which show a does-related increase in weight gain, feed efficiency, lean-body mass and plasma IGF-I levels in response to injection of exogenous GH (Cogburn, 1991). In younger broiler chickens no improvement in weight gain and body composition by injecting exogenous chicken growth hormone (cGH) could be obtained, probably because of low expression of GHR (Scanes *et al.,* 1987; Burnside and Cogburn, 1990). Older broiler chickens appear to show slow improvements in feed efficiency, growth rate and body composition in response to pulsatile infusion of cGH (Vasilatos-Younken *et al.,* 1988). Therefore, the pattern of administration and the broiler's age may determine the response to treatment with exogenous cGH. The growth promoting effects of GH were shown to mediated in part by the interaction of GH with its hepatic GHR and the subsequent higher production of IGF-I. It is widely accepted that hormones, growth factors and other agents exert their biological effects on target tissues by binding to specific receptors on the plasma membrane. Certain hormones (steroid and thyroid hormones) bind to receptors in the cytoplasm or nucleus of a target cell. Growth hormone binds with a high affinity and specificity to glycoproteins in the membrane and cytosol fractions of tissue and serum. The cGH resembled the mammalian GHR with the exception of low overall sequence homology and the absence of the exon 3 homologue of the human GHR (Burnside and Cogburn, 1991). Although, GH directly or indirectly through IGF-I is known to stimulate growth by increasing skeletal growth and muscle deposition, it also affected lipid and carbohydrate metabolism and hence body fat stores (Decuypere and Buyse, 1988). The metabolic and physiological effects of IGF-I appear to directly or indirectly mediating anabolic responses associated with

the stimulation of cell growth (Steel and Elasser, 1989). The pattern of IGF-I secretion in rat is highly correlated with pulsatile GH secretion (Bexter *et al.,* 1983). In contrast, no correlation between plasma IGF-I levels and the pulsatile pattern of GH secretion in broiler chickens has been reported (Johnson *et al.,* 1990). The administration of recombinant human IGF-I in broiler chickens significantly reduced abdominal fat content, but not total carcass lipid or growth rate (Huybrechts *et al.,* 1992). The author suggests that a continuous infusion of IGF-I may play a role as a fat repartitioning agent in birds. In broiler chickens, plasma IGF-I are not correlated with age-related changes in plasma GH levels. Furthermore, it has been reported that plasma IGF-I levels of young broiler cockerels do not respond to exogenous cGH (McGuinness and cogburn, 1988). Growth occurs in phases during which the composition of body mass and endocrine regulation varies with the stage of growth. Therefore, it is important to know these stages for the animal species in question because their link with the endocrine system and other metabolic pathways may ultimately determine the ability to manipulate growth. The present work was undertaken to contribute to the clarification of the complex control mechanism of the growth hormone axis related to growth and/or feed conversion pattern under various selection influences in broiler chickens.

Materials and Methods

Rearing management and sample collection: Male and female broiler chickens selected for 6-week body weight (GL) line or for feed efficiency between 3 and 6 weeks of age (FC line) were used. Leenstra (1988) described the history and production traits of these lines. Chickens of both lines were reared in litter pens (3 pens with 12 chickens/sex/pen). Water and commercial pelleted broiler diet (13.2 MJ ME, 210g crude protein/kg) were provided *ad libitum*. Lighting was continuos and all environmental conditions were essentially the same for all birds. Blood samples were taken from the wing vein into heparinized syringes at 4, 5, 6 and 7 weeks of age. Plasma was stored at -20°C until assayed. At 4 and 7 weeks of age 5-9 birds per sex from each line were killed, the liver were removed and stored at -20°C until assayed were performed. GH and IGF-I were measured in plasma and liver was used for quantification of hepatic GHR activity.

Growth hormone radioimmunoassay (RIA): Chicken GH was measured with a homologous RIA as described by Berghman *et al.* (1988). Affinity-purified cGH was iodinated using the chloramine-T method (Greenwood *et al.,* 1963). 20 ml sample or standard GH (2-200 ng/ml) was incubated for 24 h with 100 ml mouse monoclonal GH antiserum (½-106 ascites) and 100 ml tracer. After adding 50 ml goat antimouse antiserum (1/40 dilution) a second 24h incubation period followed. After centrifugation at 2400 g for 10 min, the supernatant was aspirated and the precipitate was counted in a g-counter. The detection limit of the system was 2 ng/ml and no cross-reactivity with other pituitary hormones was observed. The intra- and inter-assay variation coefficient was 4 and 15.5 % respectively.

Insulin-like growth factor radioimmunoassay: The plasma concentration of IGF-I was measured with a heterologous radioimmunoassay described by Renaville *et al.* (1993). 400 ml of acid-ethanol buffer was added to 100 ml of plasma sample and incubated for 30 min at room temperature. The mixture was centrifuged at 3000 g for 30 min and the resulted supernatant neutralized afterwards with 80 ml M-Tirs buffer for I h at 20°C. The resulted supernatant after centrifugation during 1 h at 3000 g is used as sample for RIA. 50 ml of the extracted sample is diluted with assay buffer until a final volume of 200 ml. 100 ml antiserum is added and the mixture is incubated for 1 h at 37°C. 100 ml 125I-rbIGF-I is added and the mixture was incubated during 16-24 h at 4°C. At the end of incubation, 50 ml anti-rabbit-gammaglobulines was added and again the tubes were incubated for 24 h. The sample was centrifuged at 3300 g for 15 min and the pellet was counted in the g-counter. The intra- and inter-assay coefficient of variation was 0.09% and 0.13% respectively.

Growth hormone receptor binding activity: The hepatic GHR were determined as described by Vanderpooten *et al.* (1991). Microsomal fractions from individual livers were prepared and stored at 20°C. In order to remove endogenous hormone from the receptors, membranes were treated with a 3 M $MgCl_2$ solution, which resulted in a protein loss of approximately 40%. After dilution with 2 ml ice-cold Tris-HCl buffer, the mixture was centrifuged at 2000 g for 10 min at 4°C and the pellet washed with 1 ml Tris-Hcl buffer. Protein concentration was estimated on these membranes using the method of Lowry, modified by Peterson (1977). Specific binding of cGH was determined by incubating 100 ml 125I-cGH (35 × 103 cpm) with 100 ml membrane fraction, after in the presence or in the absence of an excess of unlabelled cGH (500 ng/100 ml/tube). The radioactivity in pellets was counted in a g-counter. Specific binding was calculated by subtracting nonspecific binding from total binding and expressed as % of total cpm added per mg protein. From these results the receptor-binding parameters (Bmax and Ka) were calculated using the LIGAND program (Munson and Rodbard, 1980).

Statistical analysis: Statistical analyses were performed using the statistical package GLM (General Linear Model) procedure. For all data sets the fixed effect of line,

Table 1: Mean plasma GH levels in male and female broiler chickens from the GL and FC lines. Values are means ± SEM

	Plasma GH levels (ng/ml) ---- Week ----			
Sex	4	5	6	7
GLM	44±5[b]	23±3[b]	22±2.1[b]	18±1.5[b]
GLF	26±3[c]	11±2[c]	9±1.5[c]	8±1.2[c]
FCM	87±9[a]	54±5[a]	45±4.2[a]	28±2.5[a]
FCF	45±6[b]	21±4[b]	18±3.1.2[b]	14±2.1[b]

Means with no common superscripts are significantly different (p<0.05).

Table 2: Mean plasma IGF-I levels in male and female broiler chickens from the GL and FC lines. Values are means ± SEM

	Plasma IGF-I levels (ng/ml) ---- Week ----			
Sex	4	5	6	7
GLM	34±1.3[a]	35±2.8[a]	25±1.3[a]	30±1.3[a]
GLF	33±1.3[a]	29±1.3[b]	18±1.4[b]	25±1.4[b]
FCM	24±1.2[c]	32±1.4[ab]	20±1.5[ab]	32±1.1[a]
FCF	31±1.7[b]	31±1.3[ab]	23±1.1[ab]	27±1.5[ab]

Means with no common superscripts are significantly different (p<0.05).

sex was investigated by ANOVA. The significance of the fixed effect in the ANOVA models was assessed using F-tests for the variance ratio. If a significant effect of variables was calculated, means were contrasted by Duncan's multiple range test.

Results

The overall means of plasma GH concentrations in male and female broiler chickens from GL and FC lines are presented in Table 1. The marked age-dependent pattern in GH concentrations was found in both GL and FC chickens. Comparison of sexes within lines shows a significant sex effect ($p<0.05$), with higher GH concentrations in male compared to female birds. Between line comparison, the FC chickens showed significantly higher GH levels at 4, 5, 6 and 7 weeks of age (Fig. 1). The means plasma IGF-I levels are summarized in Table 2. Male birds showing in general higher plasma GH levels than female in GL birds but this pattern was not uniform within FC chickens. The data on GHR binding activity are presented in Fig. 2-3. A clear age dependent pattern in GHR binding activity was recorded in chickens from both lines (Fig. 2). Since at week 7 the percentage of specific binding activity (%SB) was significantly higher at week 4. No significant sex effect was found within lines in %SB at week 4 but a clear sex effect was found at week 7, since %SB was significantly ($p<0.05$) higher in females compared to males. A clear line effect was evident between lines in both 4 and 7 weeks, since there was a significantly ($p<0.05$) higher %SB in the GL birds than in the FC chickens (Fig. 2). The calculated binding capacity (Bmax) and binding affinity (Ka) are presented in Figure 3. The binding capacity in the GL birds was significantly ($p<0.05$) higher compared to the FC birds. The calculated binding affinity is shown in Fig. 3. No line differences in binding affinity were found.

Discussion

One way of understanding the possible relationship between circulating hormone levels and growth is by measuring these characteristics in animals with widely different of growth rates. It is clear that there is no simple relationship. The results of this study clearly indicate that the GL selected line which had an early rapid growth rate and achieve higher body weight had consistently lower plasma growth hormone levels than the FC line which have been selected for better feed conversion rate. The age-related changes in plasma growth hormone concentrations as well as the higher GH levels found in male broilers compared with females birds during the rapid growth phase confirm earlier data (Decuypere *et al.,* 1993). The existence of significant differences in plasma GH between GL and FC chickens and the absence of any significant differences between high fat and low fat of broiler lines in plasma GH levels are probably linked with differences in the episodic nature of GH releases in these differentially selected lines. The decreased GH mean values in older chickens (week 7) compared with those of the younger ones (week 4) through the lines may be linked with the age-dependently of the episodic nature of GH secretion (Buyse *et al.,* 1994) and secretion rate or metabolic clearance rate who were reported to be significantly lower and higher at adult age compared to those at young age in the domestic fowls (Lauterio *et al.,* 1988). In the present study no line difference in plasma IGF-I was found in spite of a clear difference between GL and

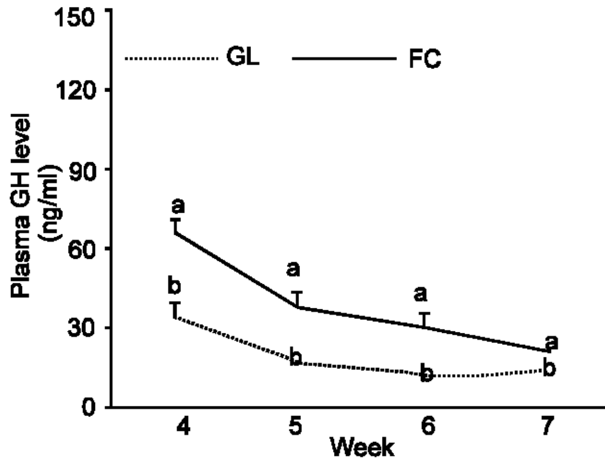

Fig. 1: Mean plasma GH levels in broiler chickens from the GL and FC lines. a, b: groups sharing no common letters are significantly different, p<0.05). Values are means ± SEM.

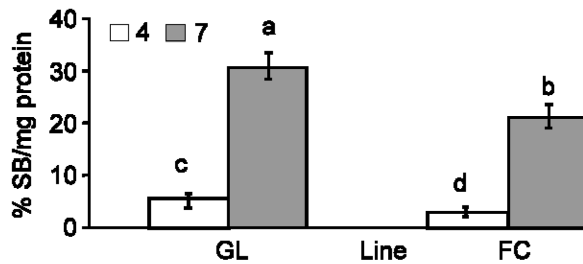

Fig. 2: Specific binding of 125-IcGH (% of total cpm/mg protein) to MgCl2 treated liver membranes of GL and FC lines at week 4 and 7 weeks of age. a, b, c, d: groups sharing no common letter are significantly different (p<0.05). Values are means ± SEM.

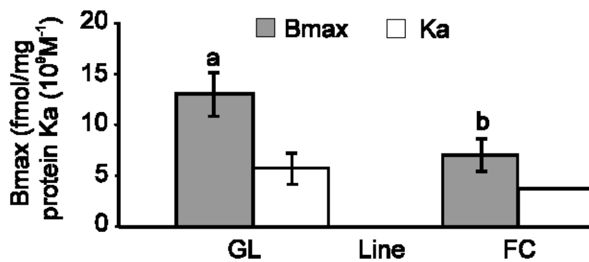

Fig. 3: Binding capacity (Bmax) and binding affinity (Ka) of broiler chickens from GL and FC lines at 7 weeks of age. a, b: groups sharing no common letter are significantly different (p<0.05). Values are means ± SEM.

FC chickens in plasma GH concentrations during the rapid growth phase. No uniform trend was found for plasma IGF-I level in relation to age and sex. However, no sex differences in plasma IGF-I levels have been reported by other authors (Scanes *et al.,* 1989) In mammals the circulating IGF-I is a GH-dependent peptide showing a positive correlation with growth rate, circulating GH, hepatic GH-binding activity and plasma IGF-I concentrations (Etherton, 1987). In contrast, in broiler chickens plasma IGF-I does not appear to be GH-dependent at least not to a large extent. No response of plasma IGF-I levels to exogenous cGH has been reported in young broiler cockerels (McGuinness and Cogburn, 1988). The data on plasma IGF-I show no correlation between plasma IGF-I levels and body weight gain in this genetic model of broiler chickens. In the present study no line difference in plasma IGF-I was found in spite of a clear difference between GL and FC chickens in plasma GH concentrations and body weight gain. The results of this study clearly indicate that the GH binding activity to its receptor was increased by age. The increase of the hepatic cGH receptor binding with age during the rapid growth phase has been previously observed (Leung *et al.,* 1987). The plasma GH concentration data indicated a negative correlation between serum and GH profile and GH binding with age. The same inverse pattern was observed between GH concentrations in the circulation and the number of GHR has already been observed with GH administration to hypophysectomized chickens (Vanderpooten *et al.,* 1991). In this study the GH concentrations in females were lower than those in males while the opposite was found for the GH binding activity. The difference at this stage was probably due to a down regulation of the receptors by the significantly higher plasma GH concentrations in male chickens. The low binding activity in the FC chickens compared to the GL birds was in agreement with the previous report (Vanderpooten *et al.,* 1993). The lower GHR binding activity in the liver of FC birds could be explained by the hypothesis that higher GH peaks in these lines lead to a greater occupancy of GH receptors, hence to an increased down-regulation and subsequently higher receptor degradation, leaving fewer receptors available to be bound by GH (Vanderpooten *et al.,* 1993). The present data showed that circulating GH-IGF-I could be uncoupled to a large extent, implying the importance of other factors besides GH in regulation of IGF-I in chicken. On the basis of this study it can be suggested that endocrine parameters may offer alternative approaches of genetic variability with the aim to stabiles underlying causal mechanisms. It can be concluded that, if the metabolic basis for differences between different genetic lines is better understood, it may be possible to devise other procedures for improving poultry productions. This may have further consequences for the poultry industry.

Acknowledgments

Grateful thanks are extended to Prof. E. Decuypere at

KUL in Belgium for his help, support and encouragement.

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