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Original Article

Assessment of ^{47}Ca Distribution and Biological Half-Life in Japanese Quail Chicks

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■**Keywords**

^{47}Ca Distribution, quail chicks, biological half-life.

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ABSTRACT

A study was conducted to assess ^{47}Calcium (^{47}Ca) distribution and biological half-life in different body organs of Japanese quail chicks. A total of 85, 4 week old chicks, were dosed with 1ml/chick volume of 1% (w/v) $CaCl_2$ solution containing 7.51x10^{-08} Ci ^{47}Ca which is equivalent to 1.27x10^{-13} g. ^{47}Ca activity, in terms of decay per minute (DPM), was measured in different body organs at 12 hours time intervals, after 24 hours from ingestion for five days. The total activity of ^{47}Ca for each organ and activity per gram of organ (D.min^{-1}.g^{-1}) was calculated. Data were Statistically analyzed using completely randomized design (CRD), one way analysis of variance (ANOVA) as per the procedure given by SPSS (2002), 9.0 version for Windows. The results obtained indicated that following ^{47}Ca administration most of the total ^{47}Ca concentration was found in the bones with count rates of 110267±550 DPM. While in the fifth day following administration, most of the ^{47}Ca was found in the feathers with a total activity of 13322±760 DPM. The biological half-life time was found to be highest in the heart and kidneys, respectively compared to the other body organs. In conclusion, the current results suggest that the main excretory pathway for Ca is through the skin. Regarding organ dependency on Ca for the normal functioning, the results obtained in our study suggest that the heart is the most dependent organ on Ca.

INTRODUCTION

Calcium (Ca) has been recognized as an essential nutrient in poultry nutrition. It considers the highest abundant mineral in the body (Veum, 2010), required to sustain biological processes of various body functions such as: the ossification of bones and cardiac muscle activity, vascular contraction, activation of several enzymes, transmission of nerve impulses, intracellular signaling, and hormonal secretion, etc., (Pu *et al.*, 2016). Adequate Ca intake is essential for the healthy functioning of bone, heart, muscles, blood and nerves, etc., while, Ca deficiency, Ca metabolism disorders (Weaver, 2014), factors that influence Ca absorption efficiency and thus influence requirements for Ca and also, any change in serum Ca homeostasis can affect one or more of these physiological functions (Shin & Kim, 2015). All these factors are subject to increase the risk of various bone diseases and lead to economic losses to farm holders, (Brini *et al.*, 2013). In order for these processes to function properly, normal Ca ion levels in blood and extracellular Ca homeostasis of various body organs, such as the intestine, muscles, and bone tissue must be maintained within a narrow range (Brini *et al.*, 2013; Brown, 2013). As reviewed above, it is necessary to have a detailed understanding of Ca absorption and metabolism of different body organs. Ca radioactive isotope (^{47}Ca), the most valuable tool for many Ca studies and biological investigations, has been widely used as tracer dietary studies on the uptake, transport and absorption of Ca in biological materials, through the intestinal wall (Reynard *et al.*, 2013; Melin *et al.,* 2014; Heuser, 2016). The importance attributed to ^{47}Ca is due to the special properties of this isotope, it is naturally fractionated between different organs and body fluids (Moynier & Fujii, 2017) and eliminated from the body with a biological half-life of 4.5 days, which is long enough for numerous biological investigations and short enough to ensure that the body subjected to the investigation is not under prolonged irradiation. It emits gamma rays in addition to beta rays, and since the gamma rays are capable of penetrating many centimeters of tissue, determination of ^{47}Ca in the body is possible by external measurements. It is therefore, possible to monitor the Ca movements during different body organs and to investigate the metabolic path of Ca in the body: its absorption through the walls of the intestine into the blood, its deposition in the skeleton, its return to the blood and its excretion, through ^{47}Ca measurements. Since, no reference has been made to trace Ca throughout the body organs of Japanese quail chicks. Therefore, the aim of the present study, was to assess ^{47}Ca distribution and biological half-life in different body organs of Japanese quail chicks and define more closely the specific Ca requirements or the amount of Ca required for each organ using the ^{47}Ca.

MATERIALS AND METHODS

Production of ^{47}Ca

Five grams of Ca chloride, technical grade (Sigma-Aldrich), was placed in a polyethylene vial and irradiated in the thermal irradiation site of the second research reactor (ETRR-2) for 5 hours irradiation at thermal flux of ~ 2 x10^{11} n.cm^{-2}.s^{-1}. After proper cooling time, ^{47}Ca activity was detected and measured using gamma ray spectrometer consisting of a high performance calibrated germanium (HPGe) detector associated with the necessary electronics for data acquisition and processing.

Birds' ethics and husbandry

All of the experiments were carried out according to the guidelines of the Institutional Animal Care and Use Committee (IACUC) for animal experiments, which is member in the Egyptian Network of Research Ethics Committee (ENERC). The scientific and ethics committee of the Biological Application Department, Nuclear Research Center, Egypt approved all procedures used in this experiment (protocol number 174; date of approval: 08-03-2018).

The experimental study was conducted on Japanese quail chicks maintained at the Poultry experimental farm of the biological Application Department, Nuclear Research Center, Egypt. All procedures used in this experiment were approved by the Animal Ethics Committee of National Institute of Animal Health and complied with the "Guidelines for the Care and Use of Animals in Research".

Birds, Treatment, Samples and Measurements

A total number of 150 Japanese quail (Coturnix coturnix japonica) chicks, at 4 weeks of age with average body weight of 128±5g/ chick, were used for this study. The chicks were reared in battery cages of 30 × 60 × 150 cm in size and kept under the same environmental conditions of light 23:1 hrs Light: dark cycle, RH was 50±5% and room temperature (30±2°C).

The rearing conditions were 25±2°C, 60±5% relative humidity (RH), with a photoperiod of 14 L: 10 D hours

RH was 50±5% and photoperiod was 14 L: 10 D hours.

Feed and water were provided ad libitum. Chicks, equal in body weight, were evenly distributed into 3 replications, each replicate pen containing 50 quails each. For one week (the treatment period), the quail chicks were fed the basal diet formulated to meet all nutrient requirements of Japanese quail according to (NRC, 1994), with protein level (22 %), metabolizable energy (3350 kcal/kg), Ca level (0.81%) and available phosphorus level (0.31%).

At the beginning of the treatment (time of ingestion), the activity of ^{47}Ca was measured using a gamma ray spectrometer consisting of a calibrated HPGe detector and found to be 7.51μCi/g $CaCl_2$ which is equivalent to 1.27x 10^{-11} g ^{47}Ca since that the ^{47}Ca specific activity is 5.9 × 10^5 Ci/g. The radio activity half life time ($t_{1/2}$) of ^{47}Ca is 4.5 days (Reynard *et al.* 2013).

All chicks were administered orally 1ml volume/ chick of 1% (w/v) $CaCl_2$ solution containing 7.51x10^{-08} Ci ^{47}Ca which was equivalent to 1.27x10^{-13}g ^{47}Ca. After a 24-hour period following the administration, 6 quail chicks (2 chicks /pen) were randomly selected, weighed and slaughtered for carcass analysis: feather, skeletal muscles from breast and thigh, bones, liver, proventriculus, gizzard, intestine, kidney, and heart for each slaughtered chick were calculated and weighed individually. Excreta was also collected. Blood samples were collected from each slaughter chick into test tubes. For assay of the total Ca^{47} content, each body organ and blood per chick was counted immediately for one-minute using gamma-counter in the term of decay per minute per gram (D/min/g) of organ. The same procedure was repeated at 12 hour intervals following the administration for five days. The data obtained were analyzed to estimate the bio-distribution and biological half life time of Ca for each organ.

^{47}Ca detection and amount of ^{47}Ca calculations per gram organ

The ^{47}Ca specific activity was employed to calculate both of the approximate total amount of ^{47}Ca (g) and the approximate amount of ^{47}Ca per gram organ at measuring time intervals.

The calculations of ^{47}Ca effective half life time ($t_{1/2e}$) and biological half-life time ($t_{1/2b}$) were performed according to (Segel, 1976) as follows:

$t_{1/2e}$, in hours, was calculated according to the following equation:

$t_{1/2e}= 0.693x(A-A_o)/2.3logt_o/t$

Where A_o and A are any given activities of ^{47}Ca at time t_o and t

$t_{1/2b,}$ in minutes, of 47Ca in the chicks were calculated using the following equation:

$t_{1/2b}= ((1/ t_{1/2e}) - (1/ (T_{1/2p}\ x24)))x60$

Where $T_{1/2p}$ is the physical half life time of ^{47}Ca which equals 4.5 days.

Statistical analysis

Statistical analysis was done using completely randomized design (CRD), one way analysis of variance (ANOVA) as per the procedure given by SPSS (2002), 9.0 version for Windows. The test was employed to identify the significant differences amongst the different time, organs and feaces. P-value less than 0.05 is considered to be statistically significant.

RESULTS AND DISCUSSION

^{47}Ca distribution throughout the body

^{47}Ca total activity in DPM was tracked in various body organs of quail chick after 24 hours following administration and equivalent calculated amounts of ^{47}Ca in gram at 24-hour time intervals are summarized in Tables (1 and 2). As shown in Figure (1), the results obtained shows that, most of the total ^{47}Ca was found,

Table 1 – Total Calculated Activity of ^{47}Ca (Mean±SD) in different body organs and excreta at 24 hour time intervals measured after 24 hours from the ingestion.

Organ / Time	24h	36h	48h	60h	72h	84h	96h	108h	120h
Blood	3848.7±103.9 Adef	3542.7±223.1 Bd	3034.7±151 Cf	2914.3±79.2 Cde	2167±30.1 Dd	2046±50.5 DEc	2054.7±138.5 DEde	2059±58.9 DEd	1861.6±120 Ec
Feather	49167.7±1320 Ab	46464.8±1306 Bb	43653.3±1050 Cb	44190.3±1344 Cb	46066.3±864 Bb	46638.7±968 Bb	46670±802 Bb	46685.3±1213 Bb	47410±1301 Bb
S.Muscles	19412±653.9 Ac	18344.7±845.6 Bc	17234.3±729.7 Cc	17446.3±912 Cc	18187.3±748.8 Bc	18413.4±868 Bb	18425.9±764.5 Bc	18432±825.8 Bc	18718.2±813 Bc
Bones	56622.3±614.2 Aa	53509.3±789 Ba	50271.7±603.8 Ca	50890.4±760 Ca	53051.1±600 Ba	53709.6±660 Ba	53746.6±678.8 Ba	53763±600.4 Ba	54598.6±758.5 Ba
Intestines	4498±166.9 Cde	4575±131.6 Cd	6541.7±58.1 Bde	6757.3±50.2 Ad	3055.3±173.7 Dd	2289±163.8 Ec	1923±46 Fde	1718.3±114.6 FGd	1527.3±130.2 Gc
Liver	4121±335.3 Ddef	4361±180.5 Dd	7503±394.5 Ad	6518.3±104.9 Bd	5553±150.1 Cd	4329.7±146.7 Dc	4034.6±124.6 Dd	3149±231.8 Ed	1900±360.5 Fc
Kidneys	1523.7±21.5 CDEf	1814.7±30.7 Be	2217.3±226.5 Af	1259±45.2 Fe	1213.3±80.8 Fe	1385±147 EFc	1460.3±69.5 DEde	1628±63.3 BCDd	1715.3±101.6 BCc
Gizzard	4466±246.7 Ade	4635.7±126.3 Ad	4692.3±169 Adef	3289.3±48.8 Bde	2829.7±288.2 Cd	2428.3±111.4 Dc	2109.7±221.8 Ede	2052±93.8 Ed	1992.6±49.6 Ec
Stomach	2076.7±107.8 DEef	3839.7 ±298.6 Ad	3604.7±611.7 ABef	3340.7±142 Bde	2556±76.1 Cd	2224±78.7 CDc	1648.3±45.2 Ede	1815.6±44.4 DEd	1010.3±38.5 Fc
Heart	2010.3±91.9 ABef	1983.7±41 Bd	2160±144 Af	1932.7±76.2 Be	2000.7±96.7 Bd	1746.7±46.2 Cc	2049.3±66.5 ABde	1911.6±78.4 Bd	1664.6±61.2 Cc
Excreta	6239.3±268 Af	4674.7±281 Bd	3652±222 Cef	2374±24 De	1859±67.6 Ed	1470.3±61.1 Fc	1088.3±82.6 Ge	907.3±43.9 Gd	815.3±50 Gc

a, b, c, ..: Means with different superscripts in each row Differ significantly ($p<0.05$).
A, B, C, ...: Means with different superscripts in each column Differ significantly ($p<0.05$).

Table 2 – Approximate total amount of ^{47}Ca in different body organs and excreta at 24 hour time intervals measured after 24 hours from the ingestion.

Organ \ Time	24h	36h	48h	60h	72h	84h	96h	108h	120h
Blood	2.938E-15	2.705E-15	2.317E-15	2.225E-15	1.654E-15	1.562E-15	1.569E-15	1.572E-15	1.421E-15
Feather	3.754E-14	3.889E-14	3.908E-14	3.512E-14	1.930E-14	1.781E-14	1.682E-14	1.496E-14	1.725E-14
S.Muscles	1.482E-14	1.408E-14	1.392E-14	1.645E-14	1.490E-14	1.477E-14	1.183E-14	9.379E-15	3.286E-15
Bones	4.323E-14	9.034E-14	1.274E-13	1.595E-13	2.731E-13	2.503E-13	2.021E-13	1.575E-13	7.241E-14
Intestines	3.434E-15	3.493E-15	4.994E-15	5.159E-15	2.333E-15	1.748E-15	1.468E-15	1.312E-15	1.166E-15
Liver	3.146E-15	3.330E-15	5.728E-15	4.977E-15	4.240E-15	3.306E-15	3.080E-15	2.404E-15	1.451E-15
Kidneys	1.163E-15	1.385E-15	1.693E-15	9.612E-16	9.263E-16	1.057E-15	1.115E-15	1.243E-15	1.310E-15
Gizzard	3.410E-15	3.539E-15	3.582E-15	2.511E-15	2.160E-15	1.854E-15	1.611E-15	1.567E-15	1.521E-15
Stomach	1.586E-15	2.932E-15	2.752E-15	2.551E-15	1.951E-15	1.698E-15	1.258E-15	1.386E-15	7.713E-16
Heart	1.535E-15	1.515E-15	1.649E-15	1.476E-15	1.527E-15	1.334E-15	1.565E-15	1.459E-15	1.271E-15
Excreta	2.938E-15	2.705E-15	2.317E-15	2.225E-15	1.654E-15	1.562E-15	1.569E-15	1.572E-15	1.421E-15

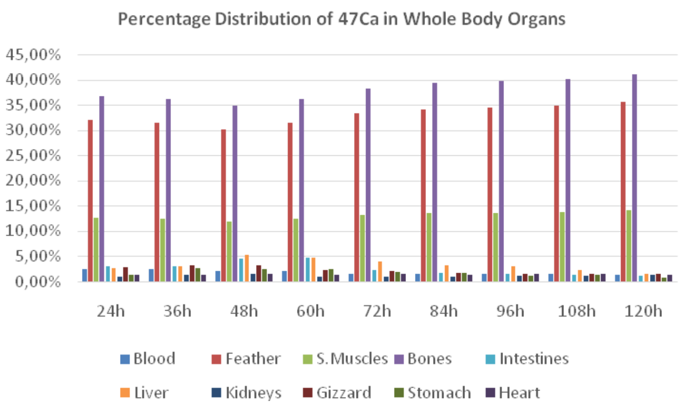

Figure 1 – ^{47}Ca total activity distribution through experimental time of the study.

as expected, in the bones which had ^{47}Ca count rates of 110267±550, 53747±2072, 60416±4188, and 46630±3232 DPM at 24, 48, 72, 96 hours following the administration, respectively. These count rates represented 67%, 48%, 56%, and 56%, in respect to the above mentioned count rates, of the total ^{47}Cadetected at mentioned times. At 120 hours after ingestion it was noticed that the count rate of ^{47}Ca was the highest in the feathers where ^{47}Ca activity recorded 13342±760 DPM and represented about 34% of the total detected activity, while in the bones, the ^{47}Ca recorded 11692±501 DPM and represented about 30% of the total detected activity. These results give obvious evidence that the main site of storage for Ca in terms of ^{47}Ca is in the bones which correlates with most of the studies and references (Taylor & Bushinsky, 2009; Lieben *et al.*, 2010; Carmeli et *et al.,* 2015; Pu *et al.,* 2016). The above results also suggest that the main route of deposition and excretion of Ca may be through the skin and feathers, since the Ca amount, in terms of ^{47}Ca activity, was found to increase in the feathers as experimental time advanced. As a result, feathers can be a reliable indicator to detect the correlations between oral ingestion of ^{47}Ca and its deposition in feathers and skin of quail chicks. Many investigators have used feathers as a simple method for biomonitoring to determine trace element levels, dietary exposure and external contamination in birds (Cardiel *et al.,* 2011; Brait & Antoniosi Filho, 2011; Jenni *et al.*, 2015). In skeletal muscles ^{47}Ca activity appears after 48 h of ^{47}Ca ingestion, recorded 12494±705 DPM and represented about 11% of the total detected activity, then a slight decrease occurred to 3619±190 DPM and represented about 9% of the total detected activity at 120 h of ^{47}Ca ingestion. This result of ^{47}Ca activity in skeletal muscles confirms the essential role of Ca in muscles contraction as shown in many studies (Sun *et al.*, 2009; Brini *et al.*, 2013; Brown, 2013; Weaver, 2014; Pu *et al.*, 2016).

^{47}Ca amount per gram tissue of each body organs

In this part of the study, the correlation between ^{47}Ca activity in DPM per gram organ and equivalent calculated amounts in gram per gram organ at 12 hr time intervals until 120 hours of the administration time are presented in Tables (3 and 4) and in Figures (2 and 3).

It was found that the picture changed when we studied the Ca requirements in terms of ^{47}Ca per gram in the studied organs, as shown in Figures (1 and 3) and detailed hereafter. The ^{47}Ca count rates in DPM per gram of organ at 24 hours after administration in the blood, feather, skeletal muscles, bones, intestine, liver, kidneys, gizzard, proventriculus, heart and excreta was found to be 661±46.24, 2891.3±241.79, 258±11.04, 1078.3±14.39, 460.3±11.57, 1317±48.7, 1364±55.8, 1326.7±80.38, 3651.7±134.1, 2101±188.5 and 556±16.4, respectively. As a result, the highest ^{47}Ca activities per gram organ were detectable at 24 h after ingestion in the proventriculus, feather and heart

Table 3 – Activity of ^{47}Ca per gram (Mean±SD) in different body organs and excreta at 12 hour time intervals measured after 24 hours from the ingestion.

Organ / Time	24h	36h	48h	60h	72h	84h	96h	108h	120h
Blood	661±46.240 Ade	539±14.16 Bf	456.3±18.90 Cgh	427±12.72 Cf	330±13.01 Dg	312.7±12.43 Dg	293.7±10.99D Eh	278±7.23 Deg	234.3±10.35 Ef
Feather	2891.3±241.79 Ba	3273±80.92 Aa	3049±172.14ABa	2748±71.68 Bb	1591±29.83 Cd	1506.3±11.16 Cd	1383.3±25.90 Ce	1282.7±23.93 Cd	1433±97.28 Cd
S.Muscles	258±11.04 Abe	244.7±3.82 Bh	245±7.63 Bi	266.7±3.87 Ag	259.3±2.60ABgh	237.7±4.28 Bgh	208±7.28 Chi	158.3±7.28 Dh	58.3±7.69 Eg
Bones	1078.3±14.39 Hcd	2043.3±66.07 Gc	3123±42.37 Fa	4044.7±84.57 Da	7056±74.74 Aa	6270.6±89.88 Ba	5110.7±82.83 Ca	3673.3±90.35 Ea	2059.3±87.8 Ga
Intestines	460.3±11.57 Be	423±8.51 Cg	550.3±12.49 Ag	552±8.16 Af	236.7±2.40 Dgh	204.3±5.37 Ehi	170.3±4.92 Fi	139±4.92 Gh	111±4.92 Hg
Liver	1317±48.7 Dc	1184.7±85.5 Ee	1837.7±131.6 Ae	1667±58 Bd	1432.7±29.1 Ce	1184.7±29.9 Ee	976±35.6 Ff	863.3±29.1 Ge	523.7±8.2 He
Kidneys	1364±55.8 Fc	1685.3±88.7 BCd	1965.7±53.6 Ad	1771±41.7 Bd	1605.3±93.1 CDd	1496±42.4 Ed	1554.7±36.2 DEd	1638±48.7 CDc	1759.3±50.3 Bb
Gizzard	1326.7±80.38 Ac	1109.7±34.1 Be	992.3±65.3 Cf	861.3±22.03 De	797.3±43 Df	659±29 Ef	604.3±27.1 EFg	571±29 Ff	553±29 Fe
Stomach	3651.7±134.1 Aa	3254.7±178.5 Ba	2833.7±143.3 Cb	2485±65 Dc	2162.3±76.1 Eb	1872±62.7 Fc	1759±37.3F Gc	1662.3±49.6 GHc	1553±62.5 Hc
Heart	2101±188.5 Bb	2247.3±51.1A Bb	2257±197.7 ABc	2360.7±43.9 Ac	1876.7±36.1 Cc	2080±53.8 Bb	2082.7±53.1 Bb	2096±51.1 Bb	2116±52.8 Ba
Excreta	556±16.4 Ae	439±11.6 Bg	340±10 Chi	242±6.24 Dg	185.7±6.6 Eh	135.3±6.1 Fi	98±2.6 Gi	81.7±2.9 Hh	75±5 Hg

a, b, c, ..: Means with different superscripts in each row Differ significantly ($p<0.05$).

A, B, C, ….: Means with different superscripts in each column Differ significantly ($p<0.05$).

Table 4 – Approximate amount of ^{47}Ca in different body organs and excreta at 12 hour time intervals measured after 24 hours from the ingestion.

hours from ingestion	Amount of 47Ca per gram organ (g)										
	Blood	Feather	Skeletal muscles	Bones	intestine	Liver	Kidneys	Gizzard	Proventriculus	Heart	Excreta
24	5.04657E-16	4.11513E-16	3.48374E-16	3.26004E-16	2.51947E-16	2.38739E-16	2.24233E-16	2.12246E-16	1.78882E-16	5.04657E-16	4.11513E-16
	2.20744E-15	2.49885E-15	2.32784E-15	2.09803E-15	1.21469E-15	1.15002E-15	1.05612E-15	9.7931E-16	1.09406E-15	2.20744E-15	2.49885E-15
48	1.96977E-16	1.86822E-16	1.87051E-16	2.03619E-16	1.97969E-16	1.81478E-16	1.58803E-16	1.20858E-16	4.45106E-17	1.96977E-16	1.86822E-16
60	8.23255E-16	1.56001E-15	2.38433E-15	3.08803E-15	5.38708E-15	4.78745E-15	3.90189E-15	2.80447E-15	1.57222E-15	8.23255E-16	1.56001E-15
72	3.51428E-16	3.2295E-16	4.2014E-16	4.21438E-16	1.80715E-16	1.55978E-16	1.3002E-16	1.06123E-16	8.47458E-17	3.51428E-16	3.2295E-16
84	1.0055E-15	9.04489E-16	1.40304E-15	1.27271E-15	1.09383E-15	9.04489E-16	7.45152E-16	6.59108E-16	3.99832E-16	1.0055E-15	9.04489E-16
96	1.04138E-15	1.28668E-15	1.50076E-15	1.35211E-15	1.22561E-15	1.14216E-15	1.18698E-15	1.25057E-15	1.34318E-15	1.04138E-15	1.28668E-15
108	1.0129E-15	8.47229E-16	7.57597E-16	6.57581E-16	6.08719E-16	5.0313E-16	4.61368E-16	4.35944E-16	4.22202E-16	1.0129E-15	8.47229E-16
120	2.78798E-15	2.48488E-15	2.16346E-15	1.89724E-15	1.65086E-15	1.42923E-15	1.34295E-15	1.26913E-15	1.18568E-15	2.78798E-15	2.48488E-15

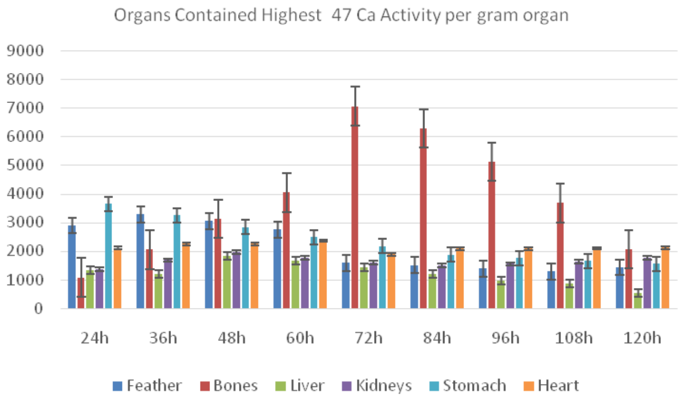

Figure 2 – distribution of ^{47}Ca activity, per gram organ, in organs which found to have the highest per gram organ expressed as percentage from total 47 Ca activities throughout the time of the study.

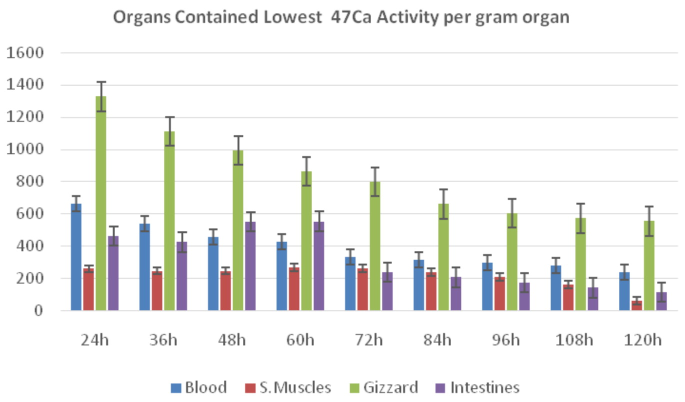

Figure 3 – distribution of ^{47}Ca activity, per gram organ, in organs which found to have the lowest expressed as percentage from total ^{47}Ca activities throughout time of the study.

(23.31, 18.46 and 13.41%), respectively, of total estimated ^{47}Ca activity, with intermediate activities in kidney, gizzard, liver and bones (8.71, 8.47, 8.41, and 6.88%), respectively, and low activities in intestine, blood, skeletal muscles and excreta (2.94, 4.22, 1.65 and 3.55%), respectively. With progress in time, starting from the 24th h until the 72nd h of the administration time, gradual decreases were recorded for the percentage of ^{47}Ca activity per gram in all organs and excreta, except the bones in which the ^{47}Ca reached 40% of total activity in the studied organs. The observed decreases in ^{47}Ca activity with progress of time, as observed in many studies (Khanal & Nemera, 2008; Geibel & Hebert, 2009; Christakos, 2012), confirmed the intestinal absorption and Ca metabolism, which refers to the movements and regulation of Ca into and out of various body compartments of quail chick, such as the intestine, the blood plasma, the extracellular and the intracellular fluid, and the bone tissue. The observed patterns of ^{47}Ca in bones bone tissue acts as a Ca storage center for deposits and withdrawals needed by the blood, via continual bone remodeling (Morgan *et al.*, 2012; Brini *et al.,* 2013; Brown, 2013; Carmeli et *et al.*, 2015). Most studies on the physiology of muscles showed that the muscles require Ca to contract, by increasing the amount of free intracellular Ca into the muscle cells (Ewing & Charlton, 2007; Pu *et al.*, 2016; Shin & Kim, 2015; Heuser, 2016). In Skeletal muscles, liver, gizzard, intestine and excreta, it was noticed that, with progress of time starting from the 24th h towards the end of the measuring time at the 120th h of administration, ^{47}Ca activity per gram these organs decreased gradually and reached 0.56, 5, 5.28, 1.06, and 0.72%, respectively, of the total estimated ^{47}Ca activity. While, a slight increase was detected gradually in the behavior of ^{47}Ca activity per gram of kidney, starting from the 84th h towards the end of measuring time at the 120th h of administration time, and reached 16.79% of the total estimated ^{47}Ca activity. Similarly, a slight increase was detected gradually in the behavior of ^{47}Ca activity per gram of heart from 10% at the 84th h to 20.2% of the total estimated ^{47}Ca activity at the 120th h of administration time. Depending on the previous results, it can be concluded that the observed decrease in ^{47}Ca activity per gram of liver, gizzard, intestine and excreta after 24 h following the administration in this study is logically since the majority of the Ca, approximately 65%, is absorbed throughout the intestine but varies by region to provide a Ca pool to maintain serum levels (Pu *et al.*, 2016; Heuser, 2016). Furthermore, most studies on Ca^{2+} homoeostasis in hepatic tissue have suggested a dynamic equilibrium between separate Ca^{2+} influx and Ca^{2+} efflux pathways (Reinhart *et al.*, 1984). Moreover, these results may indicate that the quail´s body reached the Ca balance state which is determined by the relationship between Ca intake and Ca absorption and excretion. Ewing & Charlton (2007) reported that, the Ca requirement is generally recognized to be the intake required to maintain Ca balance and therefore skeletal integrity. Examination of the data clearly detected a constant behavior of ^{47}Ca activity per gram of blood at approximately 3% of the total estimated ^{47}Ca activity which occur for 5 days following the administration. As a result, the regulation of Ca in the blood plasma is within narrow limits. As such, this may explain why an important aspect of Ca metabolism is plasma Ca homeostasis (Pu *et al.*, 2016).

The biological half life time ($t_{1/2b}$) of ^{47}Ca

The calculated biological half life time ($t_{1/2b}$) in minutes of ^{47}Ca and the highest $t_{1/2b}$ for ^{47}Ca activity, are shown in Figure (4), and are found to be 101.1, 48.2, and 10.9, 6.2 minutes in the heart, kidney, bones and feathers respectively. The calculated biological half-life

Table 5 – effective half life time ($t_{1/2e)}$ and biological half life time ($t_{1/2b}$) of ^{47}Ca in Japanese quail body organs under study.

$t_{1/2e}$(hr)									
Blood	Feather	Skeletal muscles	Bones	intestine	Liver	Kidneys	Gizzard	Proventriculus	Heart
13.0	8.8	11.9	5.2	15.1	11.8	0.6	11.0	10.7	0.6
$t_{1/2b}$ (min)									
4.0	6.2	4.5	10.9	3.4	4.5	48.2	4.9	5.0	101.1

values tended to increase with the increase in the Ca retention in the heart which may suggest that the heart muscle is the most dependent organ on Ca for proper functioning. In this respect, (Kranias *et al.*, 2007; Bers, 2008; Sun *et al.*, 2009) showed that Ca has an essential role in cardiac muscle physiology (Excitation contraction coupling), allowing the heart to function as a whole to contract properly and pump the blood efficiently into the arteries throughout the body. It was also showed that ^{47}Ca $t_{1/2b}$ is higher in the kidneys than int he bones, this finding may be related to the primary mechanism of kidney for rapid release or absorption of Ca through the filtration and excretion functions. (Toka *et al.*, 2015) showed that extra intestinal Ca can be processed through the kidneys and removed from the body through excretion, however, when correlated to the above total findings of total ^{47}Ca activity of feather, we found that the main secretion pathway of Ca is through the skin rather than the kidneys due to the feather´s much greater size compared to the kidneys. Finally, the calculated $t_{1/2b}$ suggest that, although Ca is apparently mainly deposited and utilized by the bones of quail chick, this is not the case on the specialized tissue levels of body organs where it was revealed using ^{47}Ca measurements and $t_{1/2b}$ that the Ca retention in the heart is the highest, therefore there is minimal turnover and reduced exchange of calcium molecules between heart muscle cells and blood which may suggest that the heart muscle it the most dependent organ on Ca for proper functioning.

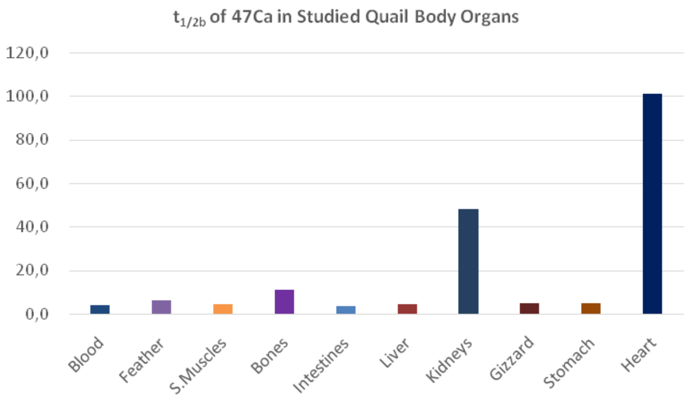

Figure 4 – $t_{1/2b}$ (min) of ^{47}Ca in different Quail body organs under study
*($t_{1/2b}$): biological half-life time

CONCLUSION

The results of the present study have provided a means for closely monitoring ^{47}Ca distribution throughout body organs which may occur following the administration in quail chicks and furthermore they allow a realistic estimation of the Ca content to be made and examine the ability of body organs to maintain a given ^{47}Ca load. Most of the total ^{47}Ca was found in the bones. While in the fifth day following the administration most the ^{47}Ca was found in the feathers. The highest biological half-life time was found to be in the heart and kidney, respectively. As a result, the heart is the most dependent organ on Ca requirements for the normal functioning and the main excretory pathway for Ca is through the skin.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

CONTRIBUTION OF AUTHOR'S

- Walaa F. Hassanin, hatamahmoud@yahoo.com, Principal Author, participated in the conception, design, planning methodology–of the research, performed analysis on all samples, helped in data interpretation and manuscript evaluation.
- Nashat Saeid Ibrahim, nashaat1977@yahoo.com, Co-Author, participated in the conception, participated in the design, data collection, statistical analysis, interpretation of the results, wrote the manuscript and acted as corresponding author.
- Emad. Eldien El-Barkouky, Emad1949@hotmail.com, Co-Author, participated in the planning methodology, biological materials, contributed substantially to the revising of the manuscript.
- Adel Mohamed. Abu-Taleb, adelabutaleb@gmail.com, Co-Author, participated in the data collection, management and reporting, processing, materials (reagents).

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