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# Physiological Adaptive Indicators in Fasted Neonate Broiler Chicks in Response to Calcium Gluconate Injection

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## Abstract

Four hundred and eighty mixed-sex broiler chicks aged 3 hrs after hatching were allotted according to a completely random design in a 6 × 2 × 2 factorial schedule into 2 groups of 12 replications of 20 chicks each. The main experimental factors were fasting for 0, 6, 12, 24, 36, and 48 hrs after chick placement, calcium gluconate (Ca-glu) injection (0 and 0.6 mL) and sex (male and female). Independent of sex, live body weight (BW) of chicks decreased linearly ($Y=43.36-0.109BW_{0h}$, $r^2=0.876$) as neonatal fasting extended. Injection of 0.6 mL Ca-glu at 3 hrs post hatching did not affect weight loss of chicks. Yolk residuals (YR) utilized linearly ($Y=5.75-0.062YR$, $r^2=0.956$) by 0.062 g/hr in neonate fasted chicks showing no effect for Ca-glu injection. Neonatal fasting periods longer than 12 hrs increased liver weight ($P<0.05$). The mean absolute and proportional (% of $BW_{0h}$) breast and leg weight were reduced linearly as neonatal fasting extended ($P<0.05$). Serum glucose concentration in both sexes increased up to 6 hrs fasting, then reduced linearly to 150 mg/dL after 48 hrs feed withdrawal. The Ca-glu treatment influenced serum glucose level for a short period up to 6 hrs of fasting. Serum Ca concentration sharply increased up to three-fold in the birds received Ca-glu injection resulting in acute hypercalcemia, then decreased to the initial level after 24 hrs feed withdrawal. The mean serum level for creatinine, uric acid, cholesterol, HDL, albumins and total proteins significantly increased during the fasting periods of 6 to 48 hrs and significantly elevated in the birds received 0.6 mL Ca-glu injection compared with the non treated chicks. It was concluded that subcutaneous administration of 0.6 mL Ca-glu in the chick's neck did not suitably support the increased metabolic demands for glucose and calcium in feed deprived neonate chicks.

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**Introduction**

Broiler chickens currently grow to market weight in six to seven weeks and each days accounts for approximately 2.5 percent of their lifetime. However, the placement of broiler chicks at commercial facilities where they have access to feed and water may delay for 24 to 72 hrs. In general, delay before feed access is considered to be the time spent in the hatchery after hatch added to transportation time to the broiler farm. Newly hatched chicks rely mainly on nutritional elements provided via vitelline sac enclosed in their abdominal cavity before they have access to exogenous feeding. Several authors have reported delayed access to feed decreased yolk sac utilization (Noy and Sklan, 2001), retarded early vital development of the gastrointestinal system (Bigot *et al*., 2003, Uni *et al*., 2003) and reduced initial (Noy *et al*., 2001, Halevy *et al*., 2000, 2003) as well as later broiler performance (Gonzales *et al*., 2003).

In depth characterization of nutritional demands in newly hatched chicks indicated nutrient supply via yolk sac is not sufficient to sustain the nutritional requirements of the broiler chicks if they expose to prolong fasting periods (Bhanja *et al*., 2009). Moreover, the yolk contains valuable maternal antibodies that are better used for passive immunity than as a source of amino acids, a phenomenon happens when chicks have no exogenous feeding (Dibner *et al*., 1998).

Many researchers proposed early nutrition strategies to support the metabolism demands in the chicks exposed to unavoidable extended periods of deferred replacement. Nutritional strategies consist of *in ovo* administration of nutrients (Foye *et al*., 2007, Kornasio *et al*., 2011) to provide chicks having greater nutrients reservoirs at hatch, provision of feed (Van de Ven *et al*, 2009) and water (Fairchild *et al*., 2006) at hatchery, enteral (Batal and Parsons, 2002) and paraenteral (Moran, 1990, Shivazad *et al*., 2007) administration of nutrients into the body of newly hatched chicks. Few experiments on subcutaneous or inter muscular injection of nutrients such as glucose (Moran, 1990, Shivazad *et al*., 2007), reported promising influence of paraenteral administration of nutrients on improved immune response and viability, gut development and progressed performance in broiler chicks.

It was hypothesized that providing calcium gluconate through paraenteral routs into the body may support the increased glucose and calcium demands in feed deprived neonate chicks. A preliminary study showed subcutaneous injection of 0.5 mL into 45 newly hatched chicks divided into 3 groups (non handled control, sham injected control and injected chicks) lowered weight loss rate in neonate chicks subjected to extended periods of post hatch fasting compared with the other two groups (Khosravinia, unpublished data). This study aims to examine the effects on physiological adaptive indicators of calcium gluconate injection in neonate broiler chicks subjected to extended fasting periods up to 48 hrs.

## Materials and Methods

### Birds management

A total of 480 Ross 305 broiler chicks were provided from a commercial hatchery. The chicks were derived from eggs with an average weight of 69.9 ± 1.6 g produced by a breeder flock of 77 wk of age. Chicks were removed from the hatchers at 07:20 hr and left the hatchery at 08:00 hr, arrived at the farm at 09:30 hr. According to our prehatch observation during check out of the hatcher, the chicks may spend an average time of 14 hrs in hatcher baskets before removing from hatcher. Upon arrival at farm, chicks were individually weighed, wing banded and divided into two random groups within an hour after arrival. Within the same time, one group of the chicks were given subcutaneous injection of 0.6 mL calcium gluconate solution (Ca-glu; contained 360, 1.67 and 30 mg/mL of Ca-glu, boric acid and glycol propylen, respectively) in the dorsal area of neck and another group remained intact. The chicks from each group were randomly assigned to 12 replicate box compartments of 20 unsexed chicks each in a commercial house up to 48 hrs of age. Besides experimental chicks, each box contained extra chicks that received the same treatment. These extra chicks were maintained to minimize social interactions caused by removal of experimental chick samples and to adjust the minimum sample size for male and female chicks. House temperature and humidity percent kept constant at 30°C and 68 percent, respectively, during the experimental period.

### Live weight shrinkage and organs weight

Upon arrival of the chicks, at 09:30 hr, corresponding to 0 hr of this experiment, a random sample of one male and one female chicks from each box were weighed in 0.01 g resolution and killed by slicing the chick's carotids arteries and/or its jugular for blood collection. The killed birds were manually processed by removing the skin and gentle opening the abdominal cavity to collect and weigh the yolk sac (at 0.01 g accuracy). The whole breast (skin removed) and left leg (thigh and drumstick) were dissected from each chick and weighed at 0.01 g resolution. The same procedure was repeated using one male and one female chick at 3, 6, 12, 24, 36 and 48 hrs after placement from each box. However, the number of samples for male or female chicks was not always equal but it was below 10 birds at no fasting period. The chicks killed at 3 hrs post placement were used only for blood collection and their blood samples were used for Ca, P and glucose assay.

### Blood parameters

Blood samples collected at 0, 6, 12, 24, 36 and 48 hrs post placement were kept in 35°C temperature for an hour for coagulation and then along with all the killed hatchlings were kept at 4ºC on slush-ice. Samples of coagulated whole blood were centrifuged at 2300 × g for 10 min. The serum was collected and stored at −20°C for considered physiological assays. Concentrations of serum biochemical constituents

including glucose (GLU), creatinine (CRE), total cholesterol (CHOL), high density lipoproteins (HDL), low density lipoproteins (LDL) total triglycerides (TG), uric acid (UA), albumins (ALB), total proteins (TP), calcium (Ca) and phosphorous (P) were determined using an autoanalyzer (Selects E Autoanlyzer, Sr. No. 8-7140, Vital Compny, Netherlands). This analyzer employs enzymatic procedures using SEPPIM Diagnostic Kits (SEPPIM S.A.S., Zone Industrielle, 61500, SEES, France) in two replicates, at 25°C, that have been described by Elliott (1984).

**Statistical analysis**

Data were prepared for analysis according to a completely randomized design with a 6 × 2 × 2 factorial arrangement of 24 treatments and 12 to 15 replications of individual chicks. The main experimental factors were fasting for 0, 6, 12, 24, 36, and 48 hrs after chick placement, calcium gluconate injection (0 and 0.6 mL) and sex. All data were analyzed using PROC GLM of SAS (2002). The LSD test was used for multiple treatment comparisons. The homogeneity of variance was assured for each set of data before analysis using PROC UNIVARIATE in the same software. The REG procedure of SAS (2002) was used to provide regression models for assessment of relation between neonatal fasting and serum glucose, Ca and P concentration. Linear, quadratic and cubic contrasts were used for the effects of post hatch fasting time on the measured variables.

**Results**

Table 1 shows changes in live weight and yolk residuals (YR) weight during the fasting periods. There was no significant interaction effect ($P>0.05$) between fasting, sex and Ca-gluconate (Ca-glu) for live weight loss. Three hours after removal from the hatchers, considered as 0 hr of placement, males weighed 45.53 ± 0.12 g, and females weighed 44.75 ± 0.11 g. Independent of sex, live weight of chicks decreased linearly ($Y=43.36-0.109BW_{0h'}$ $r^2=0.876$) over increasing neonatal fasting periods. Injection of 0.6 mL Ca-glu at 3 hrs post hatching did not affect weight loss of the chicks. Yolk residuals weighed 4.48 ± 0.33 and 4.62 ± 0.16 g in female and male chicks, respectively, at 0 hr of placement, and utilized linearly ($Y=5.75-0.062YR$, $r^2=0.956$) by 0.062 g/hr of post hatching fasting showing no effect for Ca-glu injection.

Table 2 present changes in absolute and proportional weight (% of $BW_{0h}$ and % of $BW_{fasted}$) of liver, breast (skin removed) and leg (thigh and drumstick skin remover) during the post hatch fasting periods of 6 to 48 hrs of age. There was significant sex and Ca-glu effect for no trait of consideration. A significant interaction ($P<0.05$) was found between fasting duration and Ca-glu for absolute and proportional weight (% of $BW_{0h}$ and % of $BW_{fasted}$) of liver and leg. Neonatal fasting periods up to 12 hrs did not affect liver absolute and proportional weight but longer feed withdrawal periods up to 48 hrs increased liver weight ($P<0.05$).

The mean absolute and proportional (%of $BW_{0h}$) breast and leg weight were reduced linearly as neonatal fasting periods increased (*P<0.05*).

**Table 1. Body weight changes and weight of yolk residuals of broiler chicks subjected to neonatal fasting in response to calcium gluconate injection at 3 hrs after removal from the hatcher**

| | Body weight | | | | Weight of yolk residuals | | |
|---|---|---|---|---|---|---|---|
| | 0 hr (g) | Fasted, (g) | shrinkage (g) | shrinkage (%) | Absolute (g) | % of $BW_{0h}$ | % of $BW_{fasted}$ |
| Fasting (Fast, hr) | | | | | | | |
| 0 | 42.99$^{b}$ | 42.99$^{b}$ | 0.00$^{f}$ | 0.00$^{f}$ | 5.47$^{a}$ | 12.61$^{a}$ | 12.61$^{a}$ |
| 6 | 46.20$^{a}$ | 43.62$^{a}$ | 2.59$^{e}$ | 5.60$^{e}$ | 5.48$^{a}$ | 11.85$^{a}$ | 12.55$^{a}$ |
| 12 | 44.82$^{ab}$ | 41.10$^{c}$ | 3.76$^{d}$ | 8.34$^{d}$ | 5.26$^{a}$ | 11.62$^{a}$ | 12.70$^{a}$ |
| 24 | 46.54$^{a}$ | 41.31$^{c}$ | 5.38$^{c}$ | 11.26$^{c}$ | 4.40$^{b}$ | 9.42$^{b}$ | 10.62$^{b}$ |
| 36 | 45.84$^{a}$ | 39.45$^{d}$ | 6.39$^{b}$ | 13.94$^{b}$ | 3.27$^{c}$ | 7.10$^{c}$ | 8.28$^{c}$ |
| 48 | 45.95$^{a}$ | 37.94$^{e}$ | 8.02$^{a}$ | 17.47$^{a}$ | 2.84$^{c}$ | 6.14$^{c}$ | 7.46$^{c}$ |
| Sex | | | | | | | |
| Female | 44.75 | 40.59 | 4.16 | 9.33 | 4.48 | 9.88 | 10.79 |
| Male | 45.53 | 41.53 | 4.00 | 8.60 | 4.62 | 10.15 | 11.02 |
| Ca-gluconate (mL) | | | | | | | |
| 0 | 45.57 | 41.57 | 4.00 | 8.61 | 4.48 | 9.84 | 10.62 |
| 0.6 | 45.02 | 40.90 | 4.12 | 9.08 | 4.69 | 10.33 | 11.33 |
| *SEM* | 0.292 | 0.298 | 0.213 | 0.459 | 0.144 | 0.300 | 0.304 |
| Source of Variance | | | | | | | |
| Fast | 0.011 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| Sex | 0.325 | 0.274 | 0.264 | 0.598 | 0.804 | 0.130 | 0.156 |
| Ca-g | 0.205 | 0.103 | 0.102 | 0.055 | 0.667 | 0.676 | 0.867 |
| $BW_{0h}$ | | 0.001 | 0.001 | 0.571 | 0.001 | 0.002 | 0.002 |
| Fast × Sex | 0.055 | 0.854 | 0.874 | 0.989 | 0.057 | 0.072 | 0.086 |
| Fast × Ca-g | 0.585 | 0.389 | 0.489 | 0.337 | 0.373 | 0.427 | 0.344 |
| Sex × Ca-g | 0.890 | 0.832 | 0.632 | 0.776 | 0.004 | 0.007 | 0.006 |
| Fast × Sex × Ca-g | 0.673 | 0.824 | 0.724 | 0.747 | 0.073 | 0.066 | 0.107 |
| Orthogonal polynomials | | | | | | | |
| Linear | | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| Quadratic | | 0.129 | 0.229 | 0.049 | 0.018 | 0.027 | 0.007 |
| Cubic | | 0.044 | 0.054 | 0.016 | 0.361 | 0.376 | 0.315 |

[a-f] Means within a column with no common superscript differ significantly (*P<0.05*).

**Table 2. Organs weight of broiler chicks subjected to neonatal fasting in response to calcium gluconate injection at 3 hrs after removal from the hatcher**

| | Liver weight | | | Breast weight | | | Leg weight | | |
|---|---|---|---|---|---|---|---|---|---|
| | Absolute (g) | % of $BW_{0h}$ | % of $BW_{fasted}$ | Absolute (g) | % of $BW_{0h}$ | % of $BW_{fasted}$ | Absolute (g) | % of $BW_{0h}$ | % of $BW_{fasted}$ |
| Fasting (Fast, hr) | | | | | | | | | |
| 0 | 1.02$^{c}$ | 2.39$^{b}$ | 2.39$^{c}$ | 1.16$^{ab}$ | 2.71$^{a}$ | 2.71 | 2.79$^{a}$ | 6.51$^{a}$ | 6.51$^{a}$ |
| 6 | 1.12$^{b}$ | 2.42$^{b}$ | 2.57$^{c}$ | 1.24$^{a}$ | 2.68$^{ab}$ | 2.84 | 2.60$^{cb}$ | 5.64$^{cd}$ | 5.98$^{b}$ |
| 12 | 1.04$^{c}$ | 2.33$^{b}$ | 2.55$^{cd}$ | 1.17$^{ab}$ | 2.62$^{abc}$ | 2.86 | 2.67$^{ab}$ | 5.97$^{b}$ | 6.52$^{a}$ |
| 24 | 1.25$^{a}$ | 2.69$^{a}$ | 3.03$^{b}$ | 1.13$^{ab}$ | 2.44$^{bc}$ | 2.75 | 2.72$^{ab}$ | 5.85$^{bc}$ | 6.58$^{a}$ |
| 36 | 1.24$^{a}$ | 2.71$^{a}$ | 3.15$^{ab}$ | 1.12$^{b}$ | 2.24$^{c}$ | 2.81 | 2.61$^{cb}$ | 5.67$^{cbd}$ | 6.75$^{a}$ |
| 48 | 1.24$^{a}$ | 2.70$^{a}$ | 3.27$^{a}$ | 0.99$^{c}$ | 2.14$^{d}$ | 2.59 | 2.49$^{c}$ | 5.40$^{d}$ | 6.54$^{a}$ |
| Sex | | | | | | | | | |
| Female | 1.13 | 2.53 | 2.81 | 1.15 | 2.55 | 2.81 | 2.58 | 2.64 | 6.35 |
| Male | 1.14 | 2.52 | 2.76 | 1.15 | 2.52 | 2.75 | 2.70 | 5.97 | 6.50 |
| Ca-gluconate (mL) | | | | | | | | | |
| 0 | 1.15 | 2.54 | 2.79 | 1.17 | 2.57 | 2.81 | 2.73 | 6.01 | 6.54 |
| 0.6 | 1.12 | 2.50 | 2.77 | 1.12 | 2.49 | 2.73 | 2.58 | 5.74 | 6.39 |
| *SEM* | 0.013 | 0.026 | 0.036 | 0.019 | 0.038 | 0.039 | 0.028 | 0.054 | 0.062 |
| Source of Variance | | | | | | | | | |
| Fast | 0.001 | 0.001 | 0.001 | 0.005 | 0.003 | 0.443 | 0.001 | 0.001 | 0.012 |
| Sex | 0.589 | 0.750 | 0.655 | 0.101 | 0.122 | 0.116 | 0.143 | 0.112 | 0.406 |
| Ca-g | 0.544 | 0.608 | 0.277 | 0.167 | 0.197 | 0.261 | 0.072 | 0.055 | 0.434 |
| $BW_{0h}$ | 0.001 | 0.004 | 0.010 | 0.001 | 0.284 | 0.205 | 0.001 | 0.513 | 0.068 |
| Fast × Sex | 0.018 | 0.029 | 0.039 | 0.467 | 0.557 | 0.466 | 0.238 | 0.219 | 0.806 |
| Fast × Ca-g | 0.038 | 0.038 | 0.018 | 0.851 | 0.843 | 0.875 | 0.044 | 0.018 | 0.011 |
| Sex × Ca-g | 0.225 | 0.024 | 0.023 | 0.845 | 0.932 | 0.988 | 0.912 | 0.896 | 0.829 |
| Fast × Sex × Ca-g | 0.387 | 0.382 | 0.457 | 0.010 | 0.015 | 0.011 | 0.138 | 0.074 | 0210 |
| Orthogonal polynomials | | | | | | | | | |
| Linear | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.238 | 0.001 | 0.001 | 0.004 |
| Quadratic | 0.710 | 0.694 | 0.631 | 0.165 | 0.190 | 0.088 | 0.257 | 0.163 | 0.769 |
| Cubic | 0.540 | 0.550 | 0.830 | 0.910 | 0.893 | 0.947 | 0.051 | 0.038 | 0.038 |

$^{a-d}$Means within a column with no common superscript differ significantly ($P<0.05$).

Table 3 gives serum concentration of major biochemical constituents in male and female broiler chicks subjected to neonatal fasting in response to calcium gluconate injection at 0 hr of placement. Serum GLU concentration was 213 mg/dL in the chicks killed at 0 hr of placement, with no difference between males and females, increased to 240 mg/dL after 6 hrs fasting and reduced linearly to 150 mg/dL after 48 hrs feed withdrawal. The Ca-glu treatment influenced serum glucose level for a short period up to 6 hrs post hatch (Figure 1).

**Table 3. Blood parameters of broiler chicks subjected to neonatal fasting in response to calcium gluconate injection at 3 hrs after removal from the hatcher (as mg/dL)**

| | GLU[1] | CRE[2] | CHOL[3] | HDL[4] | LDL[5] | TG[6] | UA[7] | ALB[8] | TP[9] |
|---|---|---|---|---|---|---|---|---|---|
| Fasting (Fast, hr) | | | | | | | | | |
| 0 | 213[b] | 0.21[b] | 453[b] | 174[b] | 250 | 90 | 7.48[c] | 0.99[bc] | 2.61[c] |
| 6 | 240[a] | 0.29[a] | 443[b] | 175[b] | 254 | 84 | 23.45[a] | 0.88[c] | 2.62[c] |
| 12 | 163[c] | 0.17[b] | 445[b] | 205[a] | 279 | 84 | 11.45[bc] | 1.09[b] | 2.83[bc] |
| 24 | 152[c] | 0.17[b] | 489[a] | 200[a] | 256 | 84 | 7.57[c] | 1.05[b] | 2.54[c] |
| 36 | 148[c] | 0.10[c] | 502[a] | 207[a] | 272 | 89 | 10.81[bc] | 1.41[a] | 3.23[a] |
| 48 | 150[c] | 0.18[b] | 515[a] | 205[a] | 252 | 90 | 14.40[b] | 1.42[a] | 3.12[ab] |
| Sex | | | | | | | | | |
| Female | 173 | 0.19 | 478 | 191 | 260 | 86[a] | 12.57 | 1.15 | 2.80 |
| Male | 183 | 0.19 | 482 | 194 | 261 | 83[b] | 12.46 | 1.12 | 2.82 |
| Ca-gluconate (mL) | | | | | | | | | |
| 0 | 182 | 0.17[b] | 469[b] | 191[b] | 254[b] | 88 | 10.10[b] | 1.13 | 2.74 |
| 0.6 | 178 | 0.21[a] | 495[a] | 196[a] | 268[a] | 80 | 15.13[a] | 1.13 | 2.90 |
| *SEM* | 3.378 | 0.009 | 7.223 | 2.480 | 4.093 | 1.617 | 0.727 | 0.025 | 0.059 |
| Source of Variance | | | | | | | | | |
| Fast | 0.001 | 0.001 | 0.047 | 0.001 | 0.617 | 0.061 | 0.001 | 0.001 | 0.004 |
| Sex | 0.298 | 0.631 | 0.699 | 0.231 | 0.765 | 0.081 | 0.452 | 0.717 | 0.354 |
| Ca-g | 0.928 | 0.030 | 0.010 | 0.014 | 0.049 | 0.010 | 0.006 | 0.728 | 0.171 |
| $BW_{0h}$ | 0.856 | 0.268 | 0.036 | 0.004 | 0.039 | 0.929 | 0.297 | 0.052 | 0.069 |
| Fast×Sex | 0.232 | 0.016 | 0.024 | 0.005 | 0.217 | 0.307 | 0.163 | 0.964 | 0.810 |
| Fast×Ca-g | 0.468 | 0.605 | 0.120 | 0.023 | 0.180 | 0.670 | 0.006 | 0.011 | 0.134 |
| Sex×Ca-g | 0.188 | 0.607 | 0.134 | 0.055 | 0.906 | 0.991 | 0.146 | 0.267 | 0.924 |
| Fast×Sex×Ca-g | 0.460 | 0.491 | 0.004 | 0.013 | 0.008 | 0.113 | 0.563 | 0.686 | 0.764 |
| Orthogonal polynomials | | | | | | | | | |
| Linear | 0.001 | 0.001 | 0.178 | 0.001 | 0.909 | 0.007 | 0.713 | 0.001 | 0.001 |
| Quadratic | 0.006 | 0.070 | 0.005 | 0.123 | 0.078 | 0.004 | 0.862 | 0.025 | 0.182 |
| Cubic | 0.001 | 0.006 | 0.820 | 0.753 | 0.840 | 0.043 | 0.001 | 0.085 | 0.640 |

[1]Glucose; [2]Creatinine; [3]Cholesterol; [4]High density lipoproteins; [5]Low density lipoproteins; [6]Triglycerides; [7]Uric acid; [8]Albumins; [9]Total proteins.

[a-c] Means within a column with no common superscript differ significantly (P<0.05).

Serum creatinine and uric acid levels were significantly increased after 6 hrs fasting followed by nonlinear changes over the extended periods of fasting up to 48 hrs (*P<0.05*). The mean triglycerides level in serum was significantly greater in female than in male chicks and did not change by post hatching periods of feed withdrawal (*P>0.05*). Serum cholesterol and HDL concentration remained unchanged up to 12 and 6 hrs fasting, respectively, but they were reclined significantly as fasting lasted for 48 hrs (*P<0.05*). Serum total protein and albumins concentrations were significantly increased in the chicks experienced 6 to 48 hrs

neonatal fasting with no difference between males and female chicks. Administration of 0.6 mL Ca-glu significantly increased serum concentration of creatinine, cholesterol, HDL, LDL and uric acid compared with the non treated chicks (*P<0.05*).

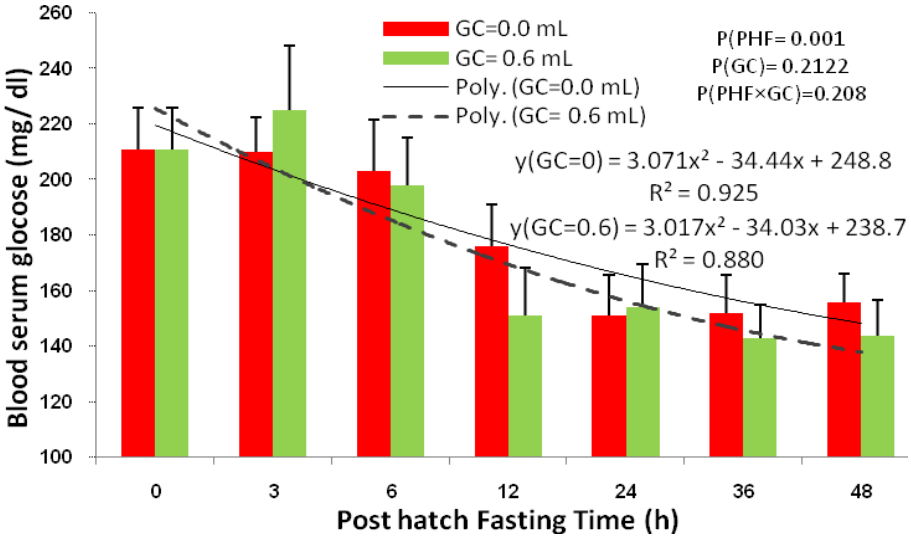


**Figure 1. Serum glucose concentration in chick broilers subjected to post hatch fasting (PHF) in response to calcium gluconate (GC) injection at 3 hrs after removal from the hatcher.**

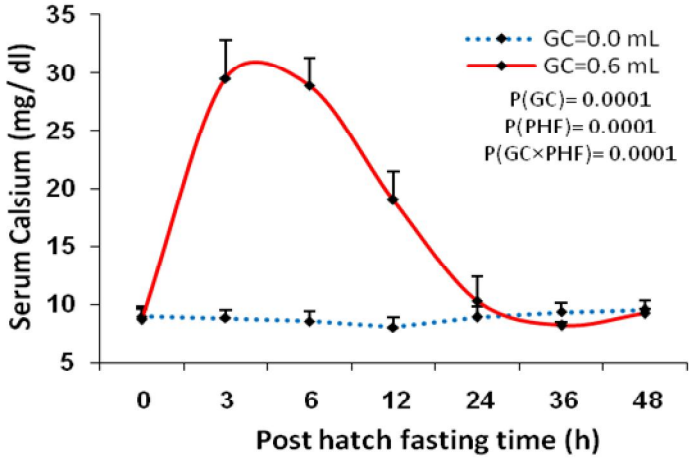


**Figure 2. Serum Ca concentration in chick broilers subjected to post hatch fasting (PHF) in response to calcium gluconate (GC) injection at 3 hrs after removal from the hatcher.**

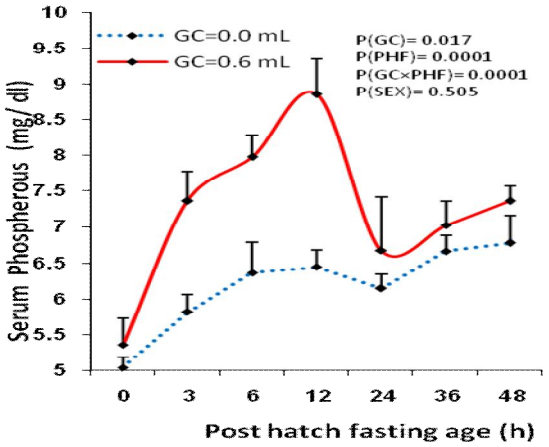


**Figure 3. Serum P concentration in chick broilers subjected to post hatch fasting (PHF) in response to calcium gluconate (GC) injection at 3 hrs after removal from the hatcher.**

The mean serum Ca concentration sharply increased up to three folds in the birds received Ca-glu injection resulting in acute hypercalcemia, then decreased to the initial level after 24 hrs post hatching fast ($P<0.05$; Figure 2). Serum P concentration in chick broilers subjected to Ca-glu injection showed a similar trend of change as serum Ca level during post hatch fasting periods of 6 to 48 hrs. However, responses in serum P level were delayed by 6 hrs ($P<0.05$; Figure 3).

**Discussion**

According to the stress models proposed by Puvadolpirod and Thaxton (2000a,b) and Post *et al.* (2003) in broiler chickens, increased serum glucose creatinine, cholesterol, HDL, triglyceride, uric acid, albumin, total protein and liver weight in the male and female chicks exposed to post hatch fasting time of 6, 12, 24, 36 and 48 hrs should be considered as elicited adaptive responses caused by delayed feeding. However, serum glucose concentration, in contradiction of the above mentioned stress models, was decreased in a non linear trend during the extended fasting times of 6 to 48 hrs in both sexes.

Results on weight loss and yolk sac utilization, in agreement with previous reports (Noy *et al.*, 2001, Halevy *et al.*, 2003, Uni *et al.*, 2003), reveal accelerating effects of neonatal fasting on live weight shrinkage and yolk sac retraction in broiler chicks. Lack of access to feed and water as an environmental stressor induce metabolic changes toward an increased energy demand and dehydration of chicks caused by increased evaporative heat loss (Mitchell *et al.*, 2003). With longer times of feed withdrawal (36 to 48 hrs), resorption of the remaining yolk is accelerated and with no access to water for facilitating the absorption of nutrient elements, yolk is resorbed in a greater rate (Noy and Sklan, 1998, Bhanja *et al.*, 2009)

presumably through the yolk sac wall directly into the blood to increase its usability (Noy *et al*., 1996, Sklan, 2003). When yolk reservoirs get close to end and still energy requirements are not satisfied, the chick's body probably utilizes resorbed glycogenic amino acids from the skeletal muscles (Moran, 2007). It was shown that breast muscle receives priority for the same purpose (Halevy *et al*., 2000, Bigot, 2003, Kornasio *et al*., 2011). In the current study leg and, in particular, breast absolute and proportional weight decreased significantly with extended fasting periods suggesting post hatching feeding delay may distort early muscle development in broilers most likely by masking the expression of genetic potential and disturbing the initial cell muscles proliferation (Kornasio *et al*., 2011).

The results on liver absolute weight percentage revealed no significant challenge on liver metabolism in both male and female chicks in short post hatching fasting periods up to 12 hrs. However, longer feed withdrawal periods up to 48 hrs increased liver weight conceivably due to increased demands for glycogeneisis metabolic pathway and synthesis of certain stress related proteins (Puvadolpirod and Thaxton, 2000a).

The aforesaid discussion on weight loss, yolk resorption rate, organ weights and serum blood parameters confirmed collaborative physiological adaptive responses aiming at preserving chick's life suffering from feed and water withdrawal. Based on the hypothesis considered, in the current study it was anticipated that subcutaneous injection of 0.6 mL Ca-glu provide a slowly-released source of glucose and Ca to the stressed chicks followed by a degree of relief from fasting associated metabolic disturbances. Nevertheless, close monitoring of serum Ca and glucose levels showed that subcutaneous injection of Ca-glu resulted in a rapid absorption of Ca and glucose within 3 hrs post injection. The increased serum glucose level slowed down in a short time (after 6 hrs) but the elevated serum Ca concentration was lasted for 24 hrs post injection, leading to acute hypercalcemia symptoms (weakness and reduces activity).

Calcium plays two important physiological roles in birds by providing the structural strength of the avian skeleton and playing vital roles in many biochemical reactions within the body via its concentration in the extracellular fluid (Dacke, 2000). Serum Ca concentration ranges from 9 to 10 mg/dL in neonate chicks with no difference between males and females (Khosravinia, 2010). Hypercalcemia as a laboratory value is defined as a serum total calcium level greater than 10.3 mg/dL in human subjects. Severe hypercalcemia (>14 mg/dL) or symptomatic moderate hypercalcemia (>12 mg/dL) requires urgent treatment, mainly rehydration, and possible referral to a nephrologist. Moreover, an elevated serum P concentration in association with serum Ca levels was observed in Ca-glu injected chicks. This tightly-connected metabolism of P and Ca, is postulated to also be in part responsible for severe hypercalcemia symptoms in the fasted chicks by disruption in acid-base balance followed by failure in function of kidney, heart and respiratory system function.

The results of the current study demonstrate that physiological adaptive responses were realized in feed deprived newly hatched male and female chicks. These responses are not necessarily similar to those exhibits in feed deprived broiler chick at later ages having plenty body fat depots, great mass of muscles, well developed behavioral activities and a realized experience in challenge with environmental stressors. Its contradiction with what hypothesized, providing calcium gluconate through subcutaneous injection to the chick's body did not properly supported the increased metabolic demands for glucose and calcium in feed deprived neonate chicks.

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