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# Effect of Feed Restriction and Different Energy and Protein Levels of the Diet on Growth Performance and Growth Hormone in Broiler Chickens

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**Abstract:** The aim of this study was to investigate the effects of feed restriction and different energy and protein contents of the diet on performance and growth hormone concentration in broiler chicken. Five hundred and seventy six day old Ross male broiler chicks were used in a 2×2×3 factorial arrangements in a completely randomized design experiment. Feeding programs consisted of *ad libitum* and Skip-a-Day (SAD) feed restriction, two energy levels (3100 and 2800 kcal ME $kg^{-1}$) and three protein levels (22.3, 19.3 and 16.3% CP). Feed restriction (SAD) was applied during 22-32 d of age. Corn-soybean meal based diets containing vegetable oil were used. Body weight and feed intake were recorded weekly. At 21, 32 and 49 day of age, one bird from four replicate of each treatment was selected randomly to collect blood sample and then carcass, breast and thigh weight were measured. Blood samples assayed for Growth Hormone (GH) concentration by RIA. Feed restriction decreased feed intake and body weight gain ($p<0.001$) of birds during 22-32 and feed intake during 32- 49 day of age, while body weight gain was not affected during this period. Also, feed restriction decreased Feed Conversion Ratio (FCR) ($p<0.01$) and body weight gain ($p<0.001$) during 22-32 day of age. Feed intake ($p<0.001$) and body weight gain increased in broilers fed on low-energy diets compared with those fed on high-energy diets during different periods. Increasing levels of protein increased feed intake ($p<0.001$), body weight gain and improved FCR ($p<0.001$) as compared with least level of protein. Feed restriction decreased carcass percentage ($p<0.001$) and increased thigh percentage ($p<0.01$) at 32 day of age. Carcass percentage ($P<0.05$) and breast percentage increased in broilers fed on low-energy diets compared with those fed on high-energy diets during different periods. The low protein diet decreased carcass percentage ($p<0.01$), breast percentage ($p<0.001$) during different periods and thigh percentage ($p<0.05$) at 21 day of age. The result of this experiment indicated that the lowest protein level had the highest growth hormone concentration at 49 day of age. The low energy diet increased growth hormone concentration ($p<0.05$) at 21 day of age.

**Key words:** Feed restriction, energy, protein, plasma growth hormone, broiler

## INTRODUCTION

In chickens, as well as other species, the animal metabolism is controlled by a variety of hormones that form a complex system which directly affects growth. Among hormones, Growth Hormone (GH) has been reported to be involved in broiler growth control (Scanes *et al*., 1984). Short-term or chronic feed restriction during growth, as well as fasting, increase plasma GH concentrations above those observed in domestic birds fed normally and it could be accompanied by growth delay. The magnitude of the increase in GH concentration reflects the severity of the feed restriction. However, it should be emphasized that the final growth expression is the result of interactions between nutritional, environmental, and genetic factors interacting with endocrine secretions. The present study was designed to examine the influence of feed restriction and different energy and protein contents of the diet on performance and GH concentration in broiler chicken.

## MATERIALS AND METHODS

**Animals and housing:** This study took place at Poultry Research Station in Ferdowsi University of Mashhad, Iran, 2008. Five hundred and seventy six, old Ross male broiler chicks were randomly allocated in equal numbers in 48 floor pens. The six diets were prepared daily and

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Table 1: Feed ingredients and composition of experimental diets

| | Treatment diets | | | | | |
|---|---|---|---|---|---|---|
| Ingredients (%) | 1 | 2 | 3 | 4 | 5 | 6 |
| Corn | 54.50 | 61.87 | 69.38 | 51.98 | 56.38 | 61.73 |
| Soybean meal | 28.60 | 24.92 | 21.00 | 34.83 | 25.17 | 19.80 |
| Corn gluten | 7.95 | 4.71 | 1.61 | 3.00 | 3.00 | 0.57 |
| Vegetable oil | 3.50 | 3.00 | 2.46 | 1.00 | 1.00 | 1.00 |
| Wheat bran | 1.50 | 1.50 | 1.50 | 5.67 | 10.89 | 13.28 |
| Dicalcium phosphate | 1.69 | 1.74 | 1.79 | 1.32 | 1.39 | 1.44 |
| Limestone | 1.17 | 1.21 | 1.24 | 1.16 | 1.17 | 1.20 |
| Vit Min. Premix[1] | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Salt | 0.44 | 0.44 | 0.44 | 0.41 | 0.41 | 0.40 |
| DL-Methionine | 0.09 | 0.08 | 0.07 | 0.13 | 0.09 | 0.08 |
| L-Lysine | 0.06 | 0.03 | 0.01 | - | - | - |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| **Compositions** | | | | | | |
| ME (kcal $kg^{-1}$) | 3100.00 | 3100.00 | 3100.00 | 2800.00 | 2800.00 | 2800.00 |
| Crud protein (%) | 22.30 | 19.30 | 16.30 | 22.30 | 19.30 | 16.30 |
| Calcium (%) | 0.97 | 0.97 | 0.97 | 0.88 | 0.88 | 0.88 |
| Available P (%) | 0.44 | 0.44 | 0.44 | 0.39 | 0.39 | 0.39 |
| Sodium (%) | 0.19 | 0.19 | 0.19 | 0.18 | 0.18 | 0.18 |
| Arginine (%) | 1.27 | 1.12 | 0.97 | 1.41 | 1.18 | 1.01 |
| Lysine (%) | 1.07 | 0.92 | 0.78 | 1.14 | 0.92 | 0.78 |
| Methionine+Cystine (%) | 0.87 | 0.75 | 0.64 | 0.87 | 0.75 | 0.64 |

[1]Provided per kg of diet: vitamin A, 9000 IU; vitamin $D_3$, 2000 IU; vitamin E, 11 IU; vitamin $K_3$, 2 mg; thiamine, 1.775 mg; riboflavin, 6.6 mg; vitamin $B_3$, 9.8 mg; vitamin $B_5$, 29.7 mg; vitamin $B_6$, 1.176 mg; vitamin $B_9$, 1 mg; vitamin $B_{12}$, 0.015 mg; vitamin $H_2$, 0.1 mg; choline chloride, 500 mg, Mn, 76 mg; Zn, 66 mg; Fe, 40 mg; Cu, 4 mg; I, 0.64 mg; Se, 0.2 mg

diets were fed from 1 to 49 day of age. Feed ingredients and composition of experimental diets exhibited in Table 1. Broiler chickens were assigned into two feeding programs from 22 until 32 day of age. One feeding program was given free access to feed (*ad libitum*) and the other fed a limited amount of feed (restricted). The restricted birds were fed *ad libitum* every other day and that no restriction was placed on the birds during the days feed was provided.

Feed restriction treatments were applied during 22-32 day of age. After the feed restriction period, the birds were fed *ad libitum* until end of the experiment (49 day of age). Also, broilers were assigned randomly to either 3100 or 2800 kcal ME $kg^{-1}$ during different periods (0-49 day of age). Protein levels were set to 22.3, 19.3 and 16.3% during different periods (0-49 day of age). According to the treatment groups, the chickens were arranged in a 2×2×3 factorial arrangement in a completely randomized design experiment (two feeding programs, two energy levels and three protein levels) at 22-32 and 32-49 day of age but there was not feed restriction during 0-21 day of age thereby 2×3 factorial arrangements in a completely randomized design experiment at 0-21 day of age (two energy levels and three protein levels).

Experimental treatments exhibited in Table 2. Each treatment group consisted of 4 replicates of 12 chickens each. The chickens were randomly allocated in cages and light was provided 24 h daily at 0-49 day of age.

Table 2: Experimental treatments with and without feed restriction

| | Without feed restriction | | | | | |
|---|---|---|---|---|---|---|
| Treatment | 1 | 2 | 3 | 4 | 5 | 6 |
| Energy level (Kcal ME $kg^{-1}$) | 3100 | 3100 | 3100 | 2800 | 2800 | 2800 |
| Protein level (% CP) | 22.3 | 19.3 | 16.3 | 22.3 | 19.3 | 16.3 |
| | With feed restriction | | | | | |
| Treatment | 7 | 8 | 9 | 10 | 11 | 12 |
| Energy level (Kcal ME $kg^{-1}$) | 3100 | 3100 | 3100 | 2800 | 2800 | 2800 |
| Protein level (%CP) | 22.3 | 19.3 | 16.3 | 22.3 | 19.3 | 16.3 |

Experimental diets were formulated to provide similar nutrients content according to the broilers nutrients requirement suggested by National Research Council (1994), except for protein and energy levels (Table 1).

The experimental diets were based on corn-soybean meal containing vegetable oil. Chickens had free access to fresh water throughout the experiment. Body weight and feed intake were recorded weekly. At 21, 32 and 49 day of age, one chicken from each replicate of each treatment that had body weight close to the mean replicate was selected for blood sampling from wing veins using sterile and heparinized syringes and then slaughtered to evaluate carcass quality.

**Carcass yield assays:** The weights of the carcass, breast and thigh were measured individually. The relative percentages of breast and thigh for individual broilers were calculated by dividing the weight of the carcass parts to the individual carcass weight. The relative percentages of carcass for individual broilers were calculated by dividing the weight of carcass to the individual body weight (Nguyen and Bunchasak, 2005).

**Growth hormone assays:** Blood samples were kept at 4°C until centrifugation. Plasmas were stored at -20°C until assayed for GH by Radio Immuno Assay (RIA). Plasma levels of GH were measured by a homologous double-antibody (PEG-separation method) RIA. For GH assay, chicken growth hormone (cGH) (Tabeshyamoor Co., Mashhad, Iran) was used for iodination. The rabbit anti cGH was prepared by Tabeshyamoor Co., Mashhad, Iran. A seven-point standard curve ranging from 0.50 to 100 ng GH $mL^{-1}$ was used. An average assay binding of 40% was achieved using an initial 1: 20,000 dilution of GH antiserum for GH assay.

**Statistical analysis:** All analyses were conducted using General Linear Model (GLM) procedures of SAS. Significant differences among individual group means were determined with Duncan's multiple range test (SAS Institute, 2001).

## RESULTS

Only main effects of feed restriction, protein and energy contents were considered to determine the effect of dietary protein and energy contents, feed restriction on growth performance, carcass characteristics and GH concentration.

**Growth performance:** The effects of feed restriction, dietary protein and energy levels on growth performance of the broiler chicken are shown in Table 3.

**Feed intake:** Feed intake was decreased in restricted chicks compared with those fed *ad libitum* during 22-32 day of age ($p<0.001$) and 32-49 day of age ($p<0.05$). Feed intake was increased significantly ($p<0.001$) in broiler fed on low-energy diets compared with those fed high-energy diets at 22-32 day of age and 32-49 day of age. Increasing levels of protein increased feed intake in different periods ($p<0.001$) in broiler chicken.

**Body weight gain:** Body weight gain was decreased in restricted chicks compared with those fed *ad libitum* during 22-32 day of age ($p<0.001$) but had no effect on body weight gain in 32-49 day of age. The low energy diet increased body weight gain at 0-21 day of age ($p<0.001$), 22-32 day of age ($p<0.001$) and 32-49 day of age ($p<0.05$). Increasing levels of protein increased body weight gain in different periods ($p<0.001$) in broiler chicken.

**Feed conversion ratio (FCR):** The FCR was improved in restricted chicks compared with those fed *ad libitum* during 22-32 day of age ($p<0.01$) but there wasn't significant difference at 32-49 day of age between restricted chicks and *ad libitum* chicks after refeeding. High energy diet impaired FCR ($p<0.05$) at 0-21 day of age but it was improved at 32-49 day of age and there wasn't significant difference at 22-32 day of age between chicks fed on high-energy diets compared with those fed on low-energy diets and during the studied period. Increasing levels of protein improved FCR in growth period total ($p<0.001$) in broiler chicken.

**Plasma GH concentration:** The effect of treatments on growth hormone concentration in broiler chicken is presented in Table 3 and Fig. 1-3. Feed restriction didn´t have effect on GH concentration at 32 and 49 day of age. The low energy diet increased GH concentration ($p<0.05$) only at 21 day of age. Chicks fed high-protein diet had

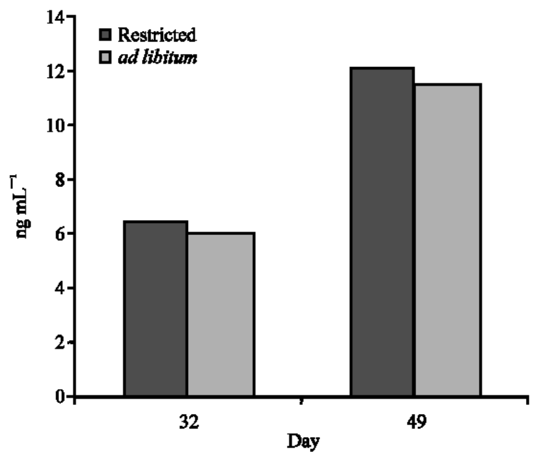


Fig. 1: Effect of feed restriction on GH concentration

Table 3: Effect of treatments on growth performance of broiler chickens in different periods

| | 0-21 days | | | | 22-32 days | | | | 32-49 days | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | FI (g) | BWG (g) | FCR (g g⁻¹) | GH (ng mL⁻¹) | FI (g) | BWG (g) | FCR (g g⁻¹) | GH (ng mL⁻¹) | FI (g) | BWG (g) | FCR (g g⁻¹) | GH (ng mL⁻¹) |
| **Feeding program** | | | | | | | | | | | | |
| Feed restricted | - | - | - | - | 509$^b$ | 279$^b$ | 1.84$^b$ | 6.43 | 1943$^b$ | 818 | 2.40 | 12.08 |
| Ad libitum | - | - | - | - | 765$^a$ | 397$^a$ | 1.98$^a$ | 5.96 | 2064$^a$ | 855 | 2.43 | 11.45 |
| ±SEM | - | - | - | - | 11.1 | 5.90 | 0.03 | 0.36 | 36.5 | 21.20 | 0.04 | 0.69 |
| p-values | | | | | 0.001 | 0.001 | 0.01 | 0.37 | 0.05 | 0.2359 | 0.53 | 0.54 |
| **Energy (kcal ME kg$^{-1}$)** | | | | | | | | | | | | |
| 3100 | 683 | 371$^b$ | 1.9$^a$ | 6.85$^b$ | 598$^b$ | 319$^b$ | 1.90 | 6.55 | 1839$^b$ | 800$^b$ | 2.32$^b$ | 12.18 |
| 2800 | 712 | 402$^a$ | 1.8$^b$ | 9.08$^a$ | 676$^a$ | 358$^a$ | 1.91 | 5.85 | 2168$^a$ | 873.3$^a$ | 2.50$^a$ | 11.58 |
| ± SEM | 10.5 | 5.17 | 0.02 | 0.59 | 11.1 | 5.90 | 0.03 | 0.36 | 36.5 | 21.20 | 0.04 | 0.69 |
| P values | 0.0545 | 0.001 | 0.05 | 0.0123 | 0.001 | 0.001 | 0.837 | 0.16 | 0.001 | 0.05 | 0.01 | 0.50 |
| **Protein (%)** | | | | | | | | | | | | |
| 22.3 | 818$^a$ | 512$^a$ | 1.6$^c$ | 9.5$^a$ | 791$^a$ | 436$^a$ | 1.81$^b$ | 6.83 | 2311$^a$ | 970$^a$ | 2.40$^{ab}$ | 12.28$^a$ |
| 19.3 | 685$^b$ | 376$^b$ | 1.8$^b$ | 7.94$^{ab}$ | 644$^b$ | 354$^b$ | 1.80$^b$ | 5.9 | 2164$^b$ | 930$^b$ | 2.33$^b$ | 8.17$^b$ |
| 16.3 | 589$^c$ | 272$^c$ | 2.2$^a$ | 6.91$^b$ | 476$^c$ | 226$^c$ | 2.10$^a$ | 5.85 | 1535$^c$ | 610$^c$ | 2.51$^a$ | 14.41$^a$ |
| ±SEM | 12.9 | 6.3 | 0.03 | 0.73 | 13.6 | 7.2 | 0.04 | 0.44 | 44.7 | 26 | 0.05 | 0.84 |
| p-values | 0.001 | 0.001 | 0.001 | 0.0621 | 0.001 | 0.001 | 0.001 | 0.20 | 0.001 | 0.001 | 0.05 | 0.0001 |

Means within column having different superscripts are significantly different ($p<0.05$). FI: Feed intake, BWG: Body weight gain, FCR: Feed conversion. There is not feed restriction at 0-21 days of age thereby there is not feed restriction data at 0-21 days of age. It explain in material and methods part.There is not feed restriction at 0-21 days of age thereby there is not feed restriction data at 0-21 days of age

Table 4: Effect of treatments on relative carcass characteristics of broiler chickens in different period

| | 21 days | | | 32 days | | | 49 days | | |
|---|---|---|---|---|---|---|---|---|---|
| Treatment | Carcass (%) | Breast (%) | Thigh (%) | Carcass (%) | Breast (%) | Thigh (%) | Carcass (%) | Breast (%) | Thigh (%) |
| **Feeding program** | | | | | | | | | |
| Feed restricted | - | - | | 66.86$^{b}$ | 18.47 | 24.97$^{a}$ | 80.57 | 21.76 | 25.22 |
| Ad libitum | - | - | - | 82.35$^{a}$ | 17.80 | 23.85$^{b}$ | 79.65 | 22.03 | 25.56 |
| ±SEM | - | - | - | 0.47 | 0.34 | 0.24 | 0.67 | 0.38 | 0.25 |
| p-values | | | | 0.001 | 0.1706 | 0.01 | 0.3453 | 0.6165 | 0.3426 |
| **Energy (kcal ME kg$^{-1}$)** | | | | | | | | | |
| 3100 | 76.59 | 16.18$^{b}$ | 22.21 | 73.88$^{b}$ | 17.06$^{b}$ | 24.69 | 79.46 | 21.06$^{b}$ | 25.77$^{a}$ |
| 2800 | 77.98 | 17.31$^{a}$ | 21.47 | 75.33$^{a}$ | 19.21$^{a}$ | 24.13 | 80.76 | 22.74$^{a}$ | 25.00$^{b}$ |
| ±SEM | 0.50 | 0.36 | 0.26 | 0.47 | 0.34 | 0.24 | 0.67 | 0.38 | 0.25 |
| p-values | 0.0568 | 0.05 | 0.0568 | 0.05 | 0.001 | 0.1082 | 0.1445 | 0.01 | 0.05 |
| **Protein (%)** | | | | | | | | | |
| 22.3 | 78.05 | 19.06$^{a}$ | 22.19$^{a}$ | 74.63$^{ab}$ | 20.81$^{a}$ | 24.54 | 82.23$^{a}$ | 24.57$^{a}$ | 25.04 |
| 19.3 | 77.25 | 17.26$^{b}$ | 22.21$^{a}$ | 75.67$^{a}$ | 18.10$^{b}$ | 24.69 | 79.95$^{b}$ | 22.72$^{b}$ | 25.24 |
| 16.3 | 76.55 | 13.91$^{c}$ | 21.12$^{b}$ | 73.53$^{b}$ | 15.50$^{c}$ | 24.00 | 78.14$^{b}$ | 18.40$^{c}$ | 25.88 |
| ±SEM | 0.61 | 0.45 | 0.32 | 0.58 | 0.41 | 0.30 | 0.81 | 0.47 | 0.30 |
| p-values | 0.2372 | 0.001 | 0.05 | 0.05 | 0.001 | 0.2374 | 0.01 | 0.001 | 0.1404 |

Means followed by different superscripts within a column are significantly different ($p<0.05$). There is not feed restriction at 0-21 days of age thereby there is not feed restriction data at 0-21 days of age

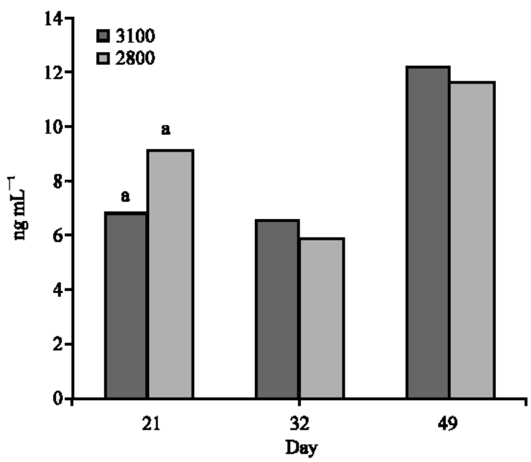


Fig. 2: Effect of dietary energy levels on GH concentration

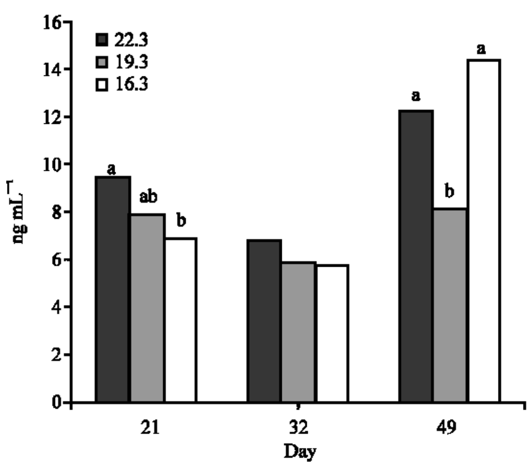


Fig. 3: Effect of dietary protein levels on GH concentration

higher GH concentration at 21 day of age ($p<0.06$). There wasn´t significant difference on GH concentration at 32 day of age. The lowest and the highest protein diet increased GH concentration at 49 day of age ($p<0.0001$).

**Carcass characteristics:** The effect of treatments on carcass characteristics of broiler chicken is presented in Table 4. Feed restriction decreased carcass percentage ($p<0.001$) and increased thigh percentage ($p<0.01$) at 32 day of age. The low energy diet increased carcass percentage at 32 day of age ($p<0.05$) and breast percentage in different periods. Increasing levels of energy increased thigh percentage in different periods but it was significant at 49 day of age ($p<0.05$). The low protein diet decreased carcass percentage at 32 ($p<0.05$) and 49 day of age ($p<0.01$), breast percentage in different periods ($p<0.001$) and thigh percentage at 21 day of age ($p<0.05$) in broiler chicken.

## DISCUSSION

**Effect of feed restriction on performance:** Feed restriction decreased feed intake at 22-32 day of age and 32-49 day of age and body weight gain at 22-32 day of age. In final period, body weight gain and FCR were similar between feed restriction and *ad libitum* groups. Results of the present study generally are in agreement with McGoven *et al.* (1999) found that body weight at 40 day of age was significantly greater in the birds fed *ad libitum* birds than in the feed-restricted birds. Vo *et al.* (1998) reported that feed restriction to 70% of the full-fed control for 2 weeks significantly reduced weight gain. Payawal (1996) reported that feed removal improved feed conversion efficiency and had no effect on carcass composition. Improvement in feed efficiency noted with the use of feed restriction programs, is due to reduced overall maintenance requirements (Payawal, 1996). This reduction seems to be due to a transient decrease in basal metabolic rate of feed-restricted birds (Zubair and Leeson, 1996) and is linked with a smaller body weight during early growth, leading to less energy requirement for maintenance. Also, in this study, feed restriction decreased carcass percentage and increased thigh percentage ($p<0.01$) at 32 day of age.

**Effect of dietary energy and protein levels on performance:** In present study, broilers fed low energy diet (2800 kcal ME $kg^{-1}$) tended to show a higher feed intake and body weight gain compared with broilers fed high energy diet. In addition, by increasing levels of protein in the diet, feed intake and body weight gain increases and FCR improves in broiler chicken. Results of the present study are in agreement with those of Buyse *et al.* (1992). They found that decreasing the crude protein content of isocaloric diets resulted in depressed body weight gain and impaired feed efficiency. Malheiros *et al.* (2003) observed that chickens fed low crude protein diet showed a reduction in body weight, feed intake and FCR compared to those fed low lipid and low carbohydrate diets. Nguyen and Bunchasak (2005) indicated that lower protein diet (17% CP) reduced live body weight and daily weight gain at early ages. Dietary protein levels higher than 17% CP did not show any significant effect on growth performance, although increasing dietary protein levels positively improved growth performance and feed utilization. Increasing the energy content of the diet also results increasing metabolic rate, more oxygen consumption and decreasing feed to gain ratio (Khazali and Moravej, 2003). It was found that broiler chickens reared on the low protein diet produced more heat per kg of metabolic body weight ($kgW^{0.75}$) than broiler chickens fed on the high protein diet (Khazali and Moravej, 2003). Energy restriction has also been shown to result in a reduction in metabolic energy loss leading to a reduced requirement for maintenance. In addition, in this study, the low-energy diet increased carcass percentage at 32 day of age and breast percentage and decreased thigh percentage in different periods that it was significant at 49 day of age ($p<0.05$). The low protein intake decreased carcass percentage, breast percentage and thigh percentage in different periods.

**Effect of diet manipulation on growth hormone concentration:** In this experiment, feed restriction had no effect on GH concentration at 32 day of age (Fig. 1). Scientists reported that in all bird species studied, plasma GH concentrations were high during the rapid growth period. Then, plasma GH concentrations declined during the slow growth period to reach low concentrations in adult birds (Darras *et al.*, 2000; Vasilatos-Younken *et al.*, 1999). In this experiment, GH concentration decreased in feed restriction period (22-32 day of age) but after refeeding, GH concentration increased (32-49 day of age). This result confirm above mention.

Also, in this study, decreasing dietary energy increased GH concentration only at 21 day of age ($p<0.05$) (Fig. 2). Chicks fed the lowest and highest protein diet had higher GH concentration at 49 day of age ($p<0.001$) (Fig. 3). Results of the present study are in agreement with Armstrong and Britt (1987) who have shown that change in energy and protein levels in diets are associated with increased or decreased levels of the concentrations of GH concentration in the blood serums of mammals. As with the mammals, birds are also susceptible to imbalanced metabolic hormone levels associated with changes of energy and protein content in their diets. There are a number of studies (Darras *et al.*, 2000; Harvey *et al.*, 1984; Vasilatos-Younken *et al.*, 1999) that show the effect of different energy and protein levels on mean plasma concentrations of GH concentration in broilers. From the study of Scanes *et al.* (1981) apparently a reduction in dietary content was associated with higher plasma concentrations of GH, which plays an important role in avian growth. Broilers fed low protein diet had higher overall mean, amplitude, baseline and peak frequency of plasma GH than broiler chickens fed high protein diet. Moravej and Khazali (2000) and Moravej *et al.* (2006) indicated that different protein levels during starting, grower and finishing periods in broilers may not change the plasma concentration of GH. The low energy intake may increase mean plasma concentrations of growth hormone.

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