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# Effect of Qualitative and Quantitative Feed Restriction on Growth Performance and Immune Function in Broiler Chickens*

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**ABSTRACT :** The current study was conducted to investigate the effect of early feed restriction (FR, 8 to 14 d of age) on growth performance and immune function in broiler chickens. Birds were fed corresponding diets from 3 to 35 d of age, which consisted of three phases: starter (3 to 7 d of age), FR (8 to 14 d of age) and re-alimentation (15 to 35 d of age) phases. During the FR period, each group of birds was fed the basal diet *ad libitum* (CON), 85% (EN85) and 70% (EN70) of lower calorie diet *ad libitum* (qualitative FR), and 85% (FI85) and 70% (FI70) of voluntary intake on a daily basis (quantitative FR). As a result, there was no statistical difference in weight gain, feed intake and feed conversion ratio between the CON and quantitative or qualitative FR groups during the entire (3 to 35 d) periods. In particular, the EN85 group resulted in a significant ($p<0.05$) increase in weight gain compared with the EN70, FI85 and FI70 groups. Plasma total protein and albumin at 14 d of age (during FR) were significantly ($p<0.05$) lower in the quantitative and qualitative FR groups, but these parameters at 35 d of age (after re-alimentation) were higher ($p<0.05$) in the EN85 and EN70 groups than in the CON group. Plasma IgG level was unaffected by dietary FR procedure. In cytokines, there was no significant difference in the expression of lymphocytic IL-4 and IFN-γ at 14 d of age between the FR and the CON groups, whereas lymphocytic IL-6 and iNOS expression were significantly ($p<0.05$) lower in FI85 and FI70 groups. Moreover, lymphocytic iNOS was also significantly ($p<0.05$) lower in birds fed qualitative and quantitative diets compared with those fed *ad libitum*. In the thymus, IL-4 expression was higher ($p<0.05$) in FI85 and FI70 groups, whereas IL-6 expression was lower ($p<0.05$) in the FI85 and FI70 groups than in the CON group. Thymic iNOS was significantly ($p<0.05$) lower in birds fed qualitatively and quantitatively restricted diets compared with those fed *ad libitum*. At 35 d of age, there was no difference in the expression of IL-4, IL-6 and IFN-γ of lymphocytes and thymus between the FR and CON groups. In conclusion, 85% of quantitative and qualitative FR would have a beneficial effect on the expression of some cytokines including IL-4 and iNOS without change in growth performance of birds. (**Key Words :** Broiler Chickens, Qualitative, Quantitative, Feed Restriction (FR), Growth Performance, Immune, Cytokines)

## INTRODUCTION

Growth performance of broiler chickens has been increased spectacularly over the last 30 years mainly due to the genetic progress, improvements of nutrition and controlled environment so that it takes only 33 days to reach finishing BW of about 2 kg (Wilson, 2005). To maximize the genetic potential for rapid growing broiler, it is nowadays thought to be crucial to provide all nutrients and environmental conditions. However, extremely high density of nutrients and energy render broilers more susceptible to various metabolic diseases including ascites, sudden death syndrome and leg abnormality and subsequently resulted in surging mortality and economic loss (Kestin et al., 1992; Julian, 1993; Olkowski et al., 2008). For these reasons, fast growth rate of broiler has been blamed for welfare concerns and then the broiler industry has attempted to find the solutions to these concerns. Thus, numerous researches have been conducted to find the appropriate ways of solutions such as programs controlling feed intakes and lighting to stay healthy by way of controlling optimal growth rates.

In particular, feed restriction (FR) in broiler has been commonly adopted to control BW, alleviate metabolic diseases and reduce mortality (Zubair and Leeson, 1994; Tolkamp et al., 2005; Zhan et al., 2007). However, the FR

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**Table 1.** Formula of experimental diets manipulated by qualitative feed restriction (FR) procedure to decrease energy and protein during the period of early feed restriction (8 to 14 d)

| Item | CON[1] | EN85[2] | EN70[3] |
|---|---|---|---|
| Ingredients (%) | | | |
| Yellow corn | 55.78 | 49.00 | 36.54 |
| Beef tallow | 3.50 | | - |
| Wheat bran | - | 21.00 | 46.50 |
| Corn gluten meal | 5.00 | 2.00 | - |
| Soybean meal | 30.40 | 22.00 | 11.50 |
| Salt | 0.32 | 0.37 | 0.33 |
| Limestone | 0.80 | 1.20 | 1.30 |
| Dicalcium phosphate | 1.70 | 1.20 | 0.90 |
| Vitamin+mineral premix[a] | 2.50 | 3.23 | 2.93 |
| Calculated analysis | | | |
| ME (kcal/kg) | 3,120 | 2,655 | 2,190 |
| Crude protein (%) | 21.13 | 18.02 | 14.80 |
| Ca (%) | 0.85 | 0.85 | 0.85 |
| P (%) | 0.65 | 0.65 | 0.65 |

[1, 2, 3] Fed for early feed restriction period (8 to 14 d), respectively.

[a] Contained per kg: vit. A, 5,500,000 IU; vit $D_3$, 1,500,000 IU; vit E, 15,000 mg; vit K, 800 mg; thiamin, 1,000 mg; riboflavin, 4,000 mg; niacin, 25,000 mg; biotin, 30 mg; folic acid, 500 mg pantothenic acid, 5,000 mg, pyridoxine, 1,500 mg; vitamin $B_{12}$, 15 mg; kg: Cu, 12,000 mg; Fe, 35,000 mg; Zn, 25,000 mg; Co, 150 mg; I. 500 mg; Co, 150 mg; Se, 120 mg; and Mn, 38,000 mg.

aiming at increasing animal health sometimes could cause suppressing the systemic immune system (Cook, 1991) and arise welfare concerns induced by abnormal behaviors such as severe competition, feed pecking, uncomfortable walking, frustration, etc (de Jong et al., 2003). Therefore, numerous attempts have been conducted to find way to improve welfare of broiler by supplying *ad libitum* access to lower caloric diets (qualitative FR; Sandilands et al., 2005) or moderate restriction of voluntary intake (quantitative FR; Lee and Leeson, 2001; Sunder et al., 2007). However, there have been few studies to investigate the effects of FR on cellular immune function in broiler chickens, although FR in birds has been commonly adopted to decrease metabolic disease such as ascites (Ozkan et al., 2006). In addition, a beneficial effect of FR-mediated welfare in broiler production is still a lack of evidence, since several studies addressed that FR itself gave rise to hunger stress (de Jong et al., 2002), which could be associated with impaired immunity.

Therefore, the present study was carried out to assess the effects of early qualitative and quantitative FR program on growth performance, plasma biochemical profiles and several cytokine expressions including IL-4, IL-6 and iNOS in lymphocytes and thymus of broiler chickens.

## MATERIALS AND METHODS

### Animals and experimental design

A total of 200 male 1-d old broiler chicks (ROSS 308) were obtained from Orpum (Co.), Korea and kept in wire cages in a room equipped with temperature (23-33°C) and on a light/dark cycle until 5 wk of age. Immediately after a 2-days adjustment period, all birds were randomly assigned to five treatments. Each group having ten replicate cages with 40 chicks was fed diet according experimental design. Each treatment was given diet from 3 to 35 d of age during the experimental period, which consisted of three phases: starter (3 to 7 d of age), early FR (8 to 14 d of age) and realimentation (15 to 35 d of age) periods. The FR programs were carried out by following procedure. Briefly, birds of qualitative groups had *ad libitum* access 85% (EN85) or 70% (EN70) of lower calorie diet (bulky diet of which the composition was manipulated to decrease energy density and protein) than the control diet as indicated in Table 1. Birds of quantitative groups were given 85% (FI85) or 70% (FI70) of voluntary intake as presented by the technical manual of Ross on a daily basis (not presented). All birds were provided the same commercial starter (3 to 21 d of age) and finisher (22 to 35 d of age) diets throughout the entire experiment period except FR period. During FR period (8 to 14 d of age), the FR groups were daily given the corresponding diet thereafter fed *ad libitum* until the end of trial. Feed intake and BW were monitored on days 3, 7, 14, 21 and 35 d of age to determine growth performance and feed conversion ratio (FCR).

### Tissue harvesting

Effects of FR on plasma profiles and cytokine expression in lymphocytes and thymus were determined at 14 d and 35 d of age. At the end of FR (on 14 d of age) and 3 wk of realimentation (on 35 d of age), five broiler chicks weighing similar to average BW per group were deprived of feed for 12 h and euthanized to harvest blood and thymus. Blood sample was collected into tubes coated with EDTA from the jugular vein. Immediately after bleeding, the thymus was taken and gently soaked into 0.9% ice-cold saline to remove blood. Whole blood was stored on ice for transport to the laboratory to isolate plasma and lymphocytes. The thymus was rapidly frozen in liquid nitrogen and stored at -70°C until further total RNA extraction.

### Assays

To measure plasma biochemical profiles, AST (aspartate aminotransferase), ALT (alanine aminotransferase), total protein and albumin were assayed by the Automatic Serum Analyzer (HITACHI 747, Japan).

The concentration of plasma IgG was assayed by the Chicken IgG plate kit (ECOS Institute, Miyagi, Japan). In brief, 5 μl of standard solutions (A and B) and appropriately diluted plasma were loaded into an individual test well of chicken IgG plate. After firmly securing plate cover, the

plate was placed in a humidified incubator at room temperature for 48 h. After incubation, the external diameter of each precipitin ring was measured. Next, the IgG concentration of each diluted plasma was calculated from the reference curve of standard solutions.

The separation of lymphocytes was performed according to the manufacturer's instructions (Mediatech, Inc, Manassas, VA). Briefly, 2 ml whole blood with an equal vol of 0.9% saline was carefully layered onto the lymphocyte separation medium (LSM). The mixed solution was centrifuged at 400×g for 30 min at room temperature. The buffy coat was carefully aspirated with Pasteur pipette and added an equal vol of buffered saline to harvested lymphocyte layer. The mixed solution was then centrifuged at 200×g for 20 min. The obtained cells were washed 2 times to remove LSM. QIAmp mini kit (Qiagen Inc, Valencia, CA) was applied for extracting total RNA from lymphocytes. Briefly, lymphocytes were mixed with EL buffer and centrifuged at 4°C for 10 min. The supernatant was then discarded and the harvested cells were resuspended in RLT lysis buffer. The lysed lymphocytes were transferred to QIAshredder spin column and homogenized at 14,000 rpm for 2 min. The harvested homogenized lysate was mixed with 70% of ethanol and transferred to QIAshredder spin column. The column was then centrifuged (8,000×g for 1 min at 4°C), washed with RW1 buffer and centrifuged (8,000×g for 1 min at 4°C). The total RNA was extracted from the homogenized lysate by the centrifugation with RNase-free water and stored at -80° until further assay.

In order to extract total RNA from the thymus, the method of RNAsol$^{TM}$ B (Tel-Test Inc, Friendswood, TX) was applied. Briefly, 100 mg of tissue was removed from each organ and added to 1 ml of RNAsol solution. The samples were cut by scissors and homogenized using glass-glass homogenizer. The lysate was transferred to microcentrifuge tube and added to 1/10 vol of chloroform to remove protein extract. The aqueous phase was separated by centrifugation for 15 min at 15,000 rpm. Total RNA was precipitated with the same vol of isopropanol and centrifuged for 15 min at 15,000 rpm. The precipitated total RNA washed with 75% ethyl alcohol, dried and diluted with DEPC treated water. The concentration of isolated total mRNA was determined by spectrophotometer and confirmed on a 1.0% agarose gel stained with EtBr. Semiquantification of mRNA using RT-PCR was performed to quantify mRNA of the cytokines such as IL-4, IFN-γ, IL-6 and iNOS.

In brief, for synthesis of first strand cDNA, 1.0 μg of total RNA was incubated at 62°C for 10 min with 1 μg of oligo dT (Invitrogen Inc, Carlsbad, Ca). And then the resulting solution was continuously incubated at 42°C for 50 min in a reaction mixture containing 2.5 mM dNTP and 200 units reverse transcriptase (Takara Inc, Shiga, Japan). After that, 3.2 unit RNAase H was treated to remove RNA hybridized with cDNA for 30 min at 37°C. The amplification of lymphocytic RNA or thymic RNA was performed for 32 cycles of denaturation at 94°C for 30 sec, annealing at 62°C for 30 sec and extension at 72°C for 10 min. The amplification of the thymus was performed for 32 cycles of denaturation at 94°C for 30 sec, annealing at 59°C for 30 sec and extension at 72°C for 10 min. The reaction mixture consisted of 10 pmol primer, 2.5 μg cDNA, 2.5 mM dNTP and 1 unit Taq polymerase (Takara Inc, Shiga, Japan). The cDNA primers to amplify each gene were as follows: 5'- GCTCGCCGGCTTCGA -3' and 5'- TGACT CATAGCAGAGACGTG -3' for IL-6 (Gene bank accession no AJ250838, 188 bp), 5'- TTCCTTCATTTTCCTCTTGA -3' and 5'- ACTGGAAAACACAAGGTCAC -3' for IFN-γ (Gene bank accession no Y07922, 294 bp), 5'- AACATG CGTCAGCTCCTGAAT -3' and 5'- TCTGCTAGGAAC TTCTCCATTGAA -3' for IL-4 (Gene bank accession no AJ621735, 350 bp), 5'- GCATCCAAAATATGAGTGGT -3' and 5'- AAGCACAGCCACATTTATCT -3' for iNOS (Gene bank accession no U34045, 274 bp). The internal standard primers were 5'- CAAAGCGCTCGATTTC ATCGC -3' and 5'- TCTCTTCCACGGAGATGTCCT -3' for β-actin (Gene bank accession no NM205518, 180 bp). We determined the number of cycles and kept the products within the exponential phase. The density of each product in Agarose gel electrophoresis (1.5%) containing EtBr was quantified using densitometer (Kodak EDAS 120, CT). Levels of all mRNAs were expressed as the ratio of signal intensity for genes relative to that for β-actin.

**Statistical analysis**

Effects of FR on growth performance, blood biochemical profiles and cytokine expression were analyzed by Proc GLM (SAS Institute Inc., 1989). When the treatment effect was significant at $p<0.05$, Tukey's test was applied to identify significant differences among groups. The level of probability for statistical difference was established at $p<0.05$.

## RESULTS

**Growth performance**

Effects of FR manipulated by qualitative (energy dilution) or quantitative (feed intake) procedure on weight gain, feed intake and FCR in broiler chickens are presented in Table 2. In weight gain, as expected, birds assigned to the FR groups resulted in a significant ($p<0.05$) lower gain during FR periods (8 to14 d) than those assigned to the CON group. The EN85 and EN70 groups of qualitative FR gained about 81% and 47%, respectively, of weight gain that the CON group achieved. The FI85 and FI70 groups of quantitative FR showed about 83% and 63% gains,

**Table 2.** Growth performance, feed intake and feed conversion ratio (FCR) in broiler chickens fed the diets manipulated by quantitative and qualitative feed restriction (FR) procedure

| Item | Treatment[1] | | | | |
|---|---|---|---|---|---|
| | CON | EN85 | EN70 | FI85 | FI70 |
| Days 3-7 | | | | | |
| Initial wt (g) | 34.2±0.03 | 34.2±0.03 | 34.3±0.03 | 34.1±0.04 | 34.3±0.03 |
| Gain (g) | 133.5±1.64 | 134.0±1.01 | 133.7±1.40 | 137.1±1.85 | 133.2±2.26 |
| FI (g) | 121.5±1.41 | 128.0±1.71 | 123.9±1.78 | 124.3±1.29 | 126.6±2.08 |
| FCR | 0.91±0.01 | 0.96±0.01 | 0.93±0.02 | 0.91±0.01 | 0.95±0.01 |
| Days 8-14 | | | | | |
| Gain (g) | 237.0±4.03$^{a}$ | 192.0±2.45$^{b}$ | 112.1±2.16$^{d}$ | 197.0±2.02$^{b}$ | 148.7±2.57$^{c}$ |
| FI (g) | 365.0±7.34$^{a}$ | 339.8±3.73$^{ab}$ | 315.5±1.26$^{bc}$ | 283.1±0.00$^{c}$ | 233.1±0.00$^{d}$ |
| FCR | 1.54±0.02$^{b}$ | 1.77±0.01$^{b}$ | 2.81±0.06$^{a}$ | 1.45±0.02$^{b}$ | 1.57±0.03$^{b}$ |
| Days 15-35 | | | | | |
| Gain (g) | 1,574.3±14.71$^{ab}$ | 1,669.0±19.22$^{a}$ | 1,553.9±7.96$^{ab}$ | 1,513.9±13.89$^{b}$ | 1,558.4±11.32$^{ab}$ |
| FI (g) | 2,422.0±16.96$^{ab}$ | 2,521.6±28.14$^{ab}$ | 2,252.7±6.14$^{b}$ | 2,674.1±75.03$^{a}$ | 2,557.6±39.70$^{ab}$ |
| FCR | 1.54±0.01$^{b}$ | 1.52±0.02$^{b}$ | 1.45±0.01$^{b}$ | 1.76±0.03$^{a}$ | 1.64±0.03$^{ab}$ |
| Days 3-35 | | | | | |
| Final BW (g) | 1,979.1±17.39$^{ab}$ | 2,029.1±19.90$^{a}$ | 1,834.0±8.99$^{b}$ | 1,882.2±13.93$^{b}$ | 1,874.2±11.41$^{b}$ |
| Gain (g) | 1,944.9±17.42$^{ab}$ | 1,995.0±19.88$^{a}$ | 1,800.0±9.01$^{b}$ | 1,848.1±13.92$^{b}$ | 1,839.9±11.40$^{b}$ |
| FI (g) | 2,908.5±23.60 | 2,989.5±29.11 | 2,692.0±5.51 | 3,081.5±76.09 | 2,917.2±41.37 |
| FCR | 1.50±0.01 | 1.51±0.02 | 1.50±0.01 | 1.66±0.03 | 1.59±0.02 |

[1] Feed restriction programs were carried out by qualitative 85% (EN85) and 70% (EN70) of lower energy diet than standard diet) or by quantitative 85% (FI85) and 70% (FI70) of lower supply of voluntary feed intake.

Means (means±SE) with different superscripts within a row differ ($p<0.05$).

respectively, that the CON group achieved. During 3 wk of realimentation (15 to 35 d), the EN85 group resulted in the highest compensatory growth among the FR groups. Overall period (3 to 35 d), there was no statistical difference in weight gain and final BW between the FR and the CON group, although the EN70 and FI70 groups tended to numerically decrease in weight gain. In particular, the EN85 group resulted in a significant ($p<0.05$) increases in weigh gain and final BW compared with those in the EN70, FI85 and FI70 groups on the cumulative basis.

In feed intake, the EN70 group fed bulky diet of which the composition was manipulated to decrease energy density to 70% showed a significant ($p<0.05$) reduction in feed intake during FR period. During 3 wk of realimentation period, the FR groups consumed similar amount of feed intake compared with the CON group, although the quantitative FR groups including the FI85 and FI70 resulted in numerically increased feed intake. For the entire period, there was no statistically difference in feed intake between the FR and CON group.

In FCR, the EN70 group showed a significantly ($p<0.05$) higher FCR value among all treatments during FR period, while the other FR groups appeared to be shown similar FCR. During 3 wk of realimentation period, the FI85 group resulted in a significantly ($p<0.05$) higher FCR compared with the CON group. On the cumulative basis (3 to 35 days), however, there was no difference in FCR among birds fed various dietary regimens, although the quantitative FR groups tended to be higher.

**Plasma biochemical profiles and IgG level**

Changes in plasma biochemical profiles and IgG level in birds at 14 d (during FR) and 35 d of age (after 3 wk of realimentation) in response to dietary FR are shown in Table 3. At 14 d of age, plasma AST and ALT level significantly ($p<0.05$) decreased in the FI70 group compared with those in the CON group. Total protein, albumin and creatinine were also significantly ($p<0.05$) lower in the quantitative and qualitative FR groups compared with those in the CON group, whereas IgG level was not different among dietary treatment groups

At 35 d of age, plasma AST level noticeably ($p<0.05$) increased in the EN70, FI85 and FI70 groups compared with the CON group. Total protein and albumin were significantly ($p<0.05$) higher in the EN85 and EN70 groups than the CON group, whereas IgG level did not change between the FR and CON group after 3 wk of realimentation.

**Expression of cytokines in lymphocytes and thymus**

The expressions of IL-4, IFN-γ, IL-6 and iNOS from lymphocytes and thymus in birds aged 14 d (during FR) and 35 d (after 3 wk of realimentation) are presented in Figure 1 and 2, respectively. At 14 d of age (Figure 1), no significant difference in the expression of lymphocytic IL-4 and IFN-γ was observed among dietary treatment groups. However, lymphocytic IL-6 expression was significantly ($p<0.05$) lower in FI85 and FI70 groups than that in the CON group.

**Table 3.** Plasma biochemical components and IgG in broiler chickens fed the diets manipulated by quantitative and qualitative feed restriction (FR) procedure

| Item | Treatment[1] | | | | |
|---|---|---|---|---|---|
| | CON | EN85 | EN70 | FI85 | FI70 |
| At 14 days | | | | | |
| AST (U/L)[2] | 228.0±71.66[a] | 209.4±58.87[ab] | 184.80±22.87[ab] | 175.4±52.4[ab] | 145.6±18.08[b] |
| ALT (U/L) | 4.40±1.52[a] | 2.80±0.84[bc] | 3.60±0.89[ab] | 1.60±1.14[c] | 1.80±0.45[c] |
| Total protein (g/dl) | 3.26±1.00[a] | 2.16±0.61[b] | 2.20±0.47[b] | 2.18±0.37[b] | 1.96±0.13[b] |
| Albumin (g/dl) | 1.26±0.42[a] | 0.88±0.23[b] | 0.88±0.23[b] | 0.86±0.13[b] | 0.82±0.08[b] |
| Creatinine (mg/dl) | 0.68±0.20[a] | 0.42±0.08[b] | 0.40±0.07[b] | 0.42±0.04[b] | 0.38±0.05[b] |
| IgG (mg/ml) | 6.34±0.67 | 6.21±0.62 | 6.05±0.58 | 6.15±0.59 | 6.07±0.82 |
| At 35 days | | | | | |
| AST (U/L) | 161.2±35.97[c] | 195.2±49.27[bc] | 272.2±69.35[a] | 230.6±33.45[ab] | 193.6±29.26[ab] |
| ALT (U/L) | 0.30±0.30 | 0.20±0.45 | 0.6±0.55 | 0.60±0.55 | 0.30±0.25 |
| Total protein (g/dl) | 2.12±0.45[b] | 3.02±0.65[a] | 2.98±0.30[a] | 2.76±0.48[ab] | 2.76±0.40[ab] |
| Albumin (g/dl) | 0.90±0.16[b] | 1.12±0.22[a] | 1.16±0.13[a] | 1.12±0.13[ab] | 1.08±0.13[ab] |
| Creatinine (mg/dl) | 0.26±0.05 | 0.26±0.05 | 0.32±0.04 | 0.30±0.02 | 0.26±0.02 |
| IgG (mg/ml) | 6.81±0.58 | 6.87±0.61 | 6.78±0.59 | 6.69±0.69 | 6.78±0.72 |

[1] Feed restriction programs were carried out by qualitative 85% (EN85) and 70% (EN70) of lower energy diet than standard diet) or by quantitative 85% (FI85) and 70% (FI70) of lower supply of voluntary feed intake.

[2] One U/L of AST or ALT is defined as the liberation of 1mM of pyruvate per minute at 37°C incubation per L of plasma.

[a, b, c] Means (means±SE) with different superscripts within a row differ ($p<0.05$).

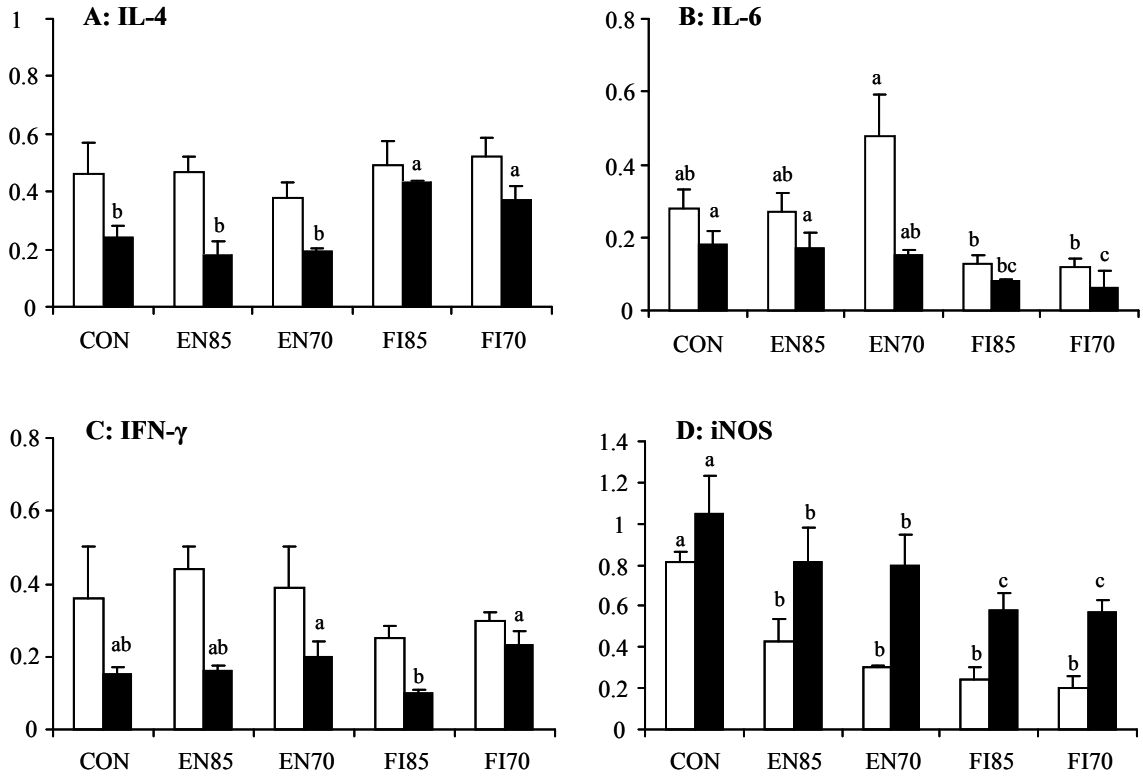


**Figure 1.** Semi-quantification of mRNA expressions of IL-4 (A), IL-6 (B), IFN-γ (C) and iNOS (D) in lymphocytes (□) and thymus (■) in 14-d old broiler chicks fed the diets manipulated by quantitative and qualitative feed restriction (FR) procedure. Levels of all mRNA were expressed as the value of signal intensity for genes relative to that for β-actin. Values are means per group with standard errors shown by vertical bars.

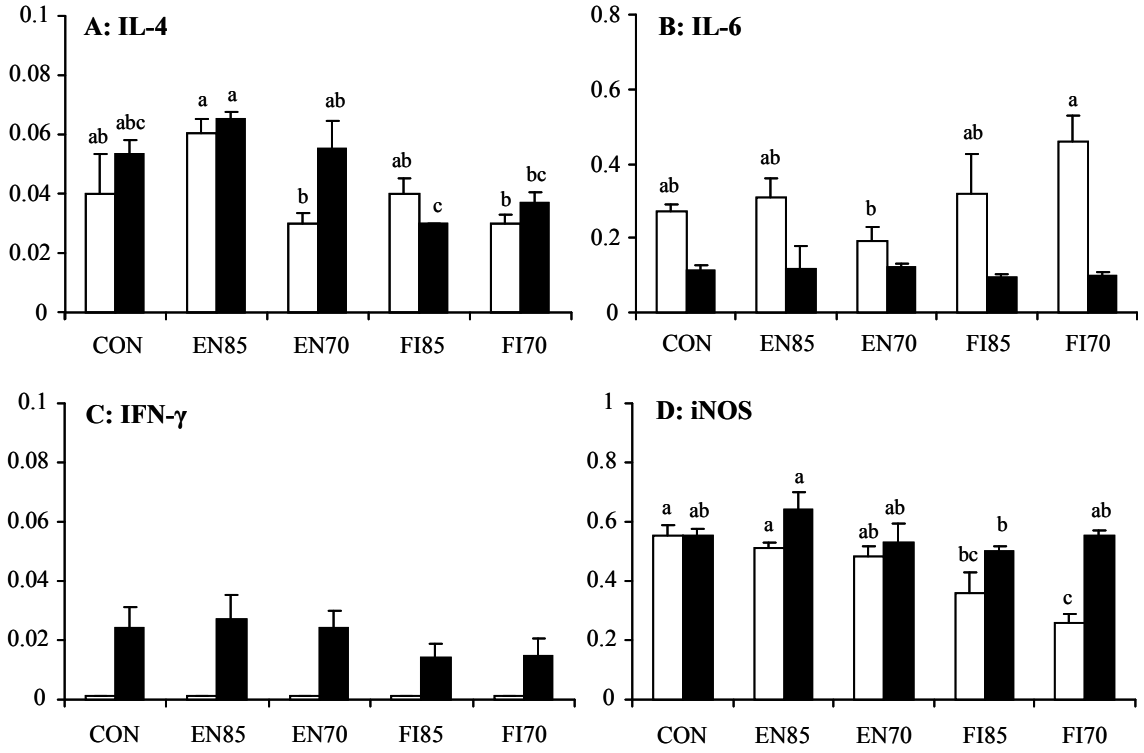


**Figure 2.** Semi-quantification of mRNA expressions of IL-4 (A), IL-6 (B), IFN-γ (C) and iNOS (D) in lymphocytes (□) and thymus (■) in 35-d old broiler chickens after 3wk of realimentation. Levels of all mRNA were expressed as the value of signal intensity for genes relative to that for β-actin. Values are means per group with standard errors shown by vertical bars.

Moreover, lymphocytic iNOS was also significantly ($p<0.05$) lower in birds fed qualitatively and quantitatively restricted diets (EN85, EN70, FI85 and FI70) compared with those fed *ad libitum* diet (CON). In the thymus, the expression of IL-4 was greater ($p<0.05$) in FI85 and FI70 groups, whereas the expression of IL-6 was lower ($p<0.05$) in the FI85 and FI70 groups than that in the CON group. There was no difference thymic IL-4 and IL-6 expression between the CON and qualitative FR groups (EN85 and EN70). Thymic iNOS was significantly ($p<0.05$) lower in birds fed qualitatively and quantitatively restricted diets (EN85, EN70, FI85 and FI70) compared with those fed *ad libitum* diet (CON).

At 35 d of age (Figure 2), there was no difference in the expression of IL-4, IL-6 and IFN-γ of lymphocytes between the FR and CON group. Lymphocytic iNOS expression was significantly ($p<0.05$) lower in the quantitative FR groups (FI85 and FI70) than the CON group. In the thymus, we could not observe any significant different expression of IL-4, IL-6 and IFN-γ between the FR and CON group. In addition, thymic iNOS expression was not altered by both qualitative and quantitative FR after realimentation.

## DISCUSSION

In broiler chickens, compensatory growth following early moderate FR has been reported by numerous studies (Plavnik and Hurwitz, 1988; Leeson and Zubair, 1997; Lee and Leeson, 2001). Moderate FR which may avoid malnutrition can be achieved by a 20 to 60% reduction of balanced decreased in calories and protein compared with *ad libitum* feed intake in rodents (Weindruch and Walford, 1988; Yu, 1996). In the present study, 15 to 30% of both quantitative and qualitative FR groups during realimentation (15 to 35 d) and the whole (3 to 35 d) periods did not show a statistical difference in final BW, weight gain and FCR compared with the CON group, indicating that early FR could be overcome by compensatory growth during the later stage of realimentation. In particular, the EN85 group exhibited significantly higher weight gain compared with the FI85 and FI70 groups, suggesting that there seems to be an advantage of qualitative FR over quantitative FR in terms of growth performance and FCR according to the present study. According to study of Zubair and Leeson (1994), most weight loss during early FR in birds can be normally compensated by 20 to 25 d of the refeeding period.

As generally known, one of crucial factors contributing to immune function is nutritional status (Benson et al. 1993; Kidd, 2004). In order to investigate effect of FR on metabolic status and immunity, we examined plasma biochemical components and the expression of cytokines in

lymphocytes and thymus. In particular, it is important to examine the effect of FR on the acquired immunity associated with two different cell types; B-lymphocytes (the production of IgG for humoral immunity) and T-lymphocytes (CD4 and CD8 which are associated with anti-inflammatory and pro-inflammatory cytokines for cellular immunity, Janeway et al., 2001). T-helper2-cell (Th2) CD4- T lymphocytes give rise to the production of IL-4 and IL-6 cytokines, while T-helper1-cell (Th1) CD4- T lymphocytes produce IL-2 and IFN-γ (Jolly, 2004). IL-4 cytokine is known as anti-inflammatory cytokine and plays a significant role in the suppression of pro-inflammatory cytokines including IL-6, IFN-γ, etc (Jolly, 2004).

As expected in our study, there was a significant reduction in plasma total protein and albumin but not IgG during FR periods. However, plasma total protein and albumin in the FR groups appeared to be normal after 3 wk of realimentation. Our observation is in agreement with an earlier report that plasma total protein and albumin in birds fed diet containing one-third amino acid, protein and calorie was markedly reduced, while IgG were not affected (Glick et al., 1981; 1983). The moderate FR (54% of *ad libitum*) and realimentation in birds did not affect weights of immune organs and IgA production (Fassbinder-Orth and Karasov, 2006). Liew et al. (2003) also reported that antibody production and weights of immune organs did not differ between broiler chicks fed *ad libitum* and those fed 60% of *ad libitum*. In the present data, lymphocytic and thymic IL-6, pro-inflammatory cytokine, was significantly lower in the FI85 and FI70 groups than that in the CON group, whereas thymic IL-4 was greater in the FI85 and FI70 groups during FR period. Moreover, lymphocytic iNOS was significantly lower in birds fed both qualitatively and quantitatively restricted diets compared with those fed *ad libitum* diet. However, after 3 wk of realimentation, most cytokine expression in the FR groups appeared to be similar to that of the CON group, although there was a lower lymphocytic iNOS expression in the FI85 and FI70 groups compared with that in the CON group. From our observation, it could be speculated that moderate FR had in part a beneficial impact on increased cellular immunity. This result is somewhat supported by Khajavi et al. (2003) study reporting that early FR showed greater CD4- and antibody titer, but lower CD8- T lymphocytes when birds were exposed to heat stress later in life, suggesting that FR have some beneficial effects on the immune system of broiler chickens. However, little is investigated to see the effect of FR on the expression of cytokines in birds so far. Numerous studies with rodents demonstrated that FR is beneficial in promoting the immune systems, although it still remains to be determined whether the positive effect of FR is similar to the result obtained from rodent model. It is well reported that FR in mice and rats was associated with decrease in pro-inflammatory cytokines including IL-6, IL-10, IL-12, TNF-α and IFN-γ mRNA level (Muthukumar et al., 2000; Bhattacharya et al., 2006).

iNOS is another important molecule that might be associated with the inflammatory process, since nitric oxide (NO) formed by iNOS play a crucial role in the activation of pro-inflammatory cytokines such as IL-1 and TNF-α (Guzik et al., 2003). Our observation is in agreement with the previously reported study that 60% of *ad libitum* intake resulted in the suppression of iNOS expression in the kidney of mice (Kim et al., 2006).

In general, the level of FR is a crucial factor to assess its effect on immunity in birds. A study conducted by Praharaj et al. (1999) reported that broiler chicks fed moderately differing level of energy (2,800, 2,650 and 2,500 kcal/kg of ME) did not show any significant difference in antibody response and weights of immune organs by dietary energy levels. However, several studies have been reported that severe (one-third of protein restriction) or prolonged FR in birds showed impairment of systemic immune function (Payne et al., 1990; Cook, 1991; Hangalapura et al., 2005).

Taken together, it could be concluded that quantitative and qualitative 85% of early FR would had a beneficial effect on the expression of cytokines including IL-4 and iNOS. It seemed that even 70% of FR in the present study would be enough to meet their requirements for keeping birds growing steadily and comfortably. In fact, *ad libitum* feeding or feeding at higher level of nutrients does not always mean that birds are good status of health and welfare. At present, broiler chickens are extremely overfed to maximize growth rate than those they are needed throughout the life. Therefore, it should not be ignored that a significantly increased cytokine expression in the FR groups could have positive impact on health and welfare of birds even when birds consumed a restricted feed amounts as low as 75% of *ad libitum* in quantitative and qualitative manners. In conclusion, more desirable broiler production in terms of increased immunity without affecting growth performance could be attributed by the moderate FR procedure, although detailed studies with respect to the optimal level of restriction under various circumstances are still needed to explore ways of improving health and welfare of birds.

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