Changes in ghrelin mRNA level, plasma growth hormone concentration and performance in different dietary energy and protein levels in broiler chicken

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Abstract

We studied the effects of different energy and protein contents of the diet on performance, growth hormone concentration and ghrelin gene expression in broiler chicken. Two experiments were conducted to determine whether different dietary energy and protein levels alter growth hormone concentration and ghrelin mRNA abundance in broiler chicks. Body weight and feed intake were recorded weekly. Blood, carcass traits and proventriculus samples were collected at 21, 42 and 56 days of age. Blood samples assayed for growth hormone (GH) concentration by radio immuno assay (RIA) and ghrelin gene expression from proventriculus tissue was measured by Real Time polymerase chain reaction (PCR). Feed intake and body weight gain increased in broilers fed on low-energy diets compared with those fed on high-energy diets at 21 days of age (P<0.0001). Also, increasing dietary energy improved feed conversion (FCR) at 22-42 days of age in broiler chicken (P<0.0001). Increasing levels of protein increased feed intake, body weight gain and improved FCR as compared with low level of protein. Carcass percentage and breast percentage increased in broilers fed on high protein diets compared with those fed on low-protein diets during different periods. High-energy and low-protein diets increased abdominal fat percentage in broiler chickens. The result of this experiment indicated that there was no effect of different dietary energy and protein levels on growth hormone concentration and ghrelin gene mRNA expression for broiler chicken.

Introduction

The excess of energy consumed by birds is deposited as fat (Rosebrough and Steele, 1985; Smith and Pesti, 1998). Excessive energy intake is related to the calorie:protein (c:p) ratio in the diet, and consequently, to carcass composition. Iso caloric diets with decreasing protein levels show increasing c:p ratio, which result in carcasses with higher fat percentage (Donaldson, 1985; Rosebrough and Steele, 1985). Armstrong and Britt (1987) showed that changes in energy and protein levels in diets are associated with increased or decreased levels of the concentrations of growth hormone in the blood serum. Chicken ghrelin was isolated from the proventriculus as endogenous ligand for the growth hormone secretagogue receptor (Kojima et al., 1999). There is no study on the effect of dietary energy and protein levels on ghrelin gene expression in broiler chicken. The present studies was designed to examine the influence of different energy and protein contents of the diet on performance, carcass yield, growth hormone concentration, isolation and quantification the ghrelin cDNA from proventriculus.

Materials and methods

Animals and housing

This study took place at Poultry Research Station and biotechnology laboratory in Ferdowsi University of Mashhad, Iran, in 2009. Two hundred and forty day old Ross male broiler chicks were randomly allocated in equal numbers in 24 floor pens. The six diets were prepared daily at each period. Feed ingredients and composition of experimental diets at each period were exhibited in Tables 1, 2 and 3.

In experiment 1, fixed level of dietary energy (3100 kcal ME kg⁻¹) with three different levels of protein offered to the chicken in different growth periods. Protein levels were 22.3, 20.3 and 18.3 % CP in starter period, 19.4, 17.4 and 15.4 % CP in grower period and 17.4, 15.4 and 13.4 % CP in finisher period.

Also, in experiment 2, fixed level of dietary protein with three different levels of energy offered to the chicken in different growth periods. All chickens fed fixed level of dietary protein (22.3% CP) in starter period, 19.4% CP in grower period and 17.4% CP in finisher period with three different levels dietary energy (3100, 2900 and 2700 kcal ME kg⁻¹) in different growth periods. According to the treatment groups, the chickens were arranged in a completely randomized design experiment at 0-56 days of age in two experiments.

Two experiments treatments exhibited in Tables 4 and 5. Each treatment group consisted of 4 replicates of 10 chickens each. The chickens were randomly allocated in cages and light was provided 24 h daily at 0-56 days of age. Experimental diets were formulated to provide similar nutrients content according to the broiler nutrient requirements suggested by NRC (1994), except for protein and energy levels (Tables 1, 2 and 3).

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 d 21, 42 and 56, one chicken from each replicate of each treatment that had body weight close to the mean replicate was selected. Blood samples were collected from wing veins using sterilized and heparinized syringes. Birds were then slaughtered to evaluate carcass quality and proventriculus samples were then collected for evaluation of ghrelin gene expression. Proventriculus samples were immediately soaked in liquid nitrogen and then they were stored at -80°C until use for ghrelin gene expression assay.
Carss 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 (van 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 growth hormone (GH) by RIA. Plasma levels of GH were measured by a homologous double-antibody (PEG-separation method) radio immuno assay (RIA). For GH assay, chicken growth hormone (cGH) (Tabeshyamour Co., Mashhad, Iran) was used for iodination. The rabbit anti cGH was prepared by Tabeshyamour 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 guinea antiserum for GH assay.

RNA extraction and reverse transcription-polymerase chain reaction assay for ghrelin gene expression
Total RNA was extracted from 100 mg of chicken proventriculus tissue using the Trizol reagent procedure (Invitrogen/Life Technologies, Isogene Co, Russia) according to the manufacturer’s instructions. The quantity and integrity of isolated RNA were determined for each sample by using both UV absorbances (260/280) as well as by 1% agarose gel electrophoresis. Then RNA samples were stored at -80°C until use. RNA were treated with DNase using Ambions DNA - free kit (Fermentas/Life Science/Isogene Co, Russia) to remove any possible DNA contamination. Samples were stored at -80°C until use. Reverse transcription (RT) PCR was performed using a RevertAid first strand cDNA synthesis kit (Fermentas/Life Science/Isogene Co, Russia) in a final volume of 20 μL containing RNA (5 μg), 2 μL gene-specific primer (20 pmol) and DEPC-treated water up to 12 μL. The mixture incubated at 65°C for 5 min. Then 4 μL SX reaction buffer, 1 μL RiboLock RNase Inhibitor (20 Units), 2 μL dNTP mix (10 mM) and 1 μL RevertAid M-MuLV Reverse Transcriptase (200 Units) added to above mixture. After incubation (42°C, 60 min), the mixture was heated at 70°C for 5 min. A chicken ghrelin fragment

| Table 1. Feed ingredients and composition of experimental diets for broiler from 0 to 21 days of age (starter period). |
|---------------------------------------------------------------|
| **Ingredients**               | 1       | 2       | 3       | 4       | 5       | 6       |
|-----------------------------|--------|--------|--------|--------|--------|--------|
| Corn, %                    | 54.50  | 59.88  | 63.82  | 54.50  | 55.89  | 47.63  |
| Soybean meal, %            | 28.60  | 25.41  | 24.5   | 28.60  | 33.58  | 34.00  |
| Corn gluten, %             | 7.95   | 6.22   | 3.16   | 7.95   | 4.38   | 2.79   |
| Vegetable oil, %           | 3.50   | 3.00   | 3.02   | 3.50   | 1.00   | 1.00   |
| Wheat bran, %              | 1.50   | 1.50   | 1.50   | 1.50   | 1.50   | 11.24  |
| Dicalcium phosphate, %     | 1.69   | 1.73   | 1.75   | 1.69   | 1.47   | 1.24   |
| Limestone, %               | 1.17   | 1.19   | 1.22   | 1.17   | 1.15   | 1.09   |
| Vit Min. Premix*          | 0.50   | 0.50   | 0.50   | 0.50   | 0.50   | 0.50   |
| Salt, %                    | 0.44   | 0.44   | 0.44   | 0.44   | 0.41   | 0.38   |
| DL-Methionine, %           | 0.09   | 0.08   | 0.09   | 0.09   | 0.12   | 0.13   |
| L-Lysine, %                | 0.06   | 0.05   | 0.06   | 0.06   | 0.06   | 0.06   |
| Total                      | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |

*Provided per kg of diet: vitamin A, 9000 IU; vitamin D₃, 2000 IU; vitamin E, 1 IU; vitamin K₃, 2 mg; thiamine, 1.775 mg; riboflavin, 6.6 mg; vitamin B₁₂, 9.8 mg; vitamin B₆, 29.7 mg; vitamin B₉, 1.176 mg; vitamin B₃, 1 mg; vitamin B₁₀, 0.015 mg; vitamin H₂, 0.1 mg; choline chloride, 500 mg; Mn, 76 mg; Zn, 66 mg; Fe, 40 mg; Cu, 4 mg; I, 0.04 mg; Se, 0.2 mg.

| Table 2. Feed ingredients and composition of experimental diets for broiler from 21 to 42 days of age (grower period). |
|-----------------------------------------------|
| **Ingredients**               | 1       | 2       | 3       | 4       | 5       | 6       |
|-----------------------------|--------|--------|--------|--------|--------|--------|
| Corn, %                    | 61.69  | 66.28  | 70.95  | 61.69  | 60.54  | 52.07  |
| Soybean meal, %            | 24.94  | 23.00  | 20.93  | 24.94  | 26.00  | 25.50  |
| Corn gluten, %             | 4.87   | 2.42   | -      | 4.87   | 3.37   | 2.31   |
| Vegetable oil, %           | 3.00   | 2.78   | 2.56   | 3.00   | 1.00   | 1.00   |
| Wheat bran, %              | 1.50   | 1.50   | 1.50   | 1.50   | 5.38   | 15.74  |
| Dicalcium phosphate, %     | 1.74   | 1.76   | 1.79   | 1.74   | 1.53   | 1.30   |
| Limestone, %               | 1.20   | 1.23   | 1.26   | 1.20   | 1.17   | 1.11   |
| Vit Min. Premix*          | 0.50   | 0.50   | 0.50   | 0.50   | 0.50   | 0.50   |
| Salt, %                    | 0.44   | 0.44   | 0.44   | 0.44   | 0.41   | 0.38   |
| DL-Methionine, %           | 0.08   | 0.07   | 0.07   | 0.08   | 0.09   | 0.10   |
| L-Lysine, %                | 0.04   | -      | -      | 0.04   | -      | -      |
| Total                      | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |

*Provided per kg of diet: vitamin A, 9000 IU; vitamin D₃, 2000 IU; vitamin E, 11 IU; vitamin K₃, 2 mg; thiamine, 1.775 mg; riboflavin, 6.6 mg; vitamin B₁₂, 9.8 mg; vitamin B₆, 29.7 mg; vitamin B₉, 1.176 mg; vitamin B₃, 1 mg; vitamin B₁₀, 0.015 mg; vitamin H₂, 0.1 mg; choline chloride, 500 mg; Mn, 76 mg; Zn, 66 mg; Fe, 40 mg; Cu, 4 mg; I, 0.04 mg; Se, 0.2 mg.
was amplified with a sense primer (5'-CCT TGG GAC AGA AC TGC TC-3') and an anti-sense primer (5'-CAC CAA TTT CAA AAG GAA CG-3') reported by Richards et al., 2006. Chicken 18S as an internal control (fragment size: 148 bp): sense primer (5'-CGG GTG CAT TTA TCA GAC CA-3') and an anti-sense primer (5'-ACC CGT GGT CAT CAC GGT A-3') reported by Paczoska-Eliasiewicz et al., 2003. A 20 μL master mix containing 10 μL SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK), 0.7 μL forward primer (10 pmol), 0.7 μL reverse primer (10 pmol), 3 μL cDNA, 5.6 μL water was prepared to perform real-time PCR. The following PCR protocol was used on the ABI (Applied Biosystems) 7300 apparatus. Initial steps contain 2 min at 50°C and 10 min at 95°C, followed by two-step amplification program (15 s at 95°C followed by 1 min at 61°C), repeated 40 times. Quantification was performed using ABI integrated software as previously described (Pfäffli, 2001). As reference gene, 18S ribosomal RNA was chosen. Each PCR run included a no template control and replicates of control and unknown samples. Runs were performed in duplicate. The chicken ghrelin (203 bp) and 18S (148 bp) cDNA were run on a 1% agarose gel stained with ethidium bromide. Also, we carried out sequencing PCR product ghrelin fragment (203 bp) to confirm amplification fragment.

Statistical analysis
All analyses were conducted using general linear model procedures (GLM) of SAS. Significant differences among individual group means were determined with Duncan’s multiple range test (SAS, 2001). Relative expression of Ghrelin mRNA determined using sample delta CT to control delta CT ratio method and then were conducted using GLM of SAS.

Results and discussion

Growth performance
The results indicated that different protein and energy levels of diet affected feed intake, body weight gain and feed conversion (PCR) in broiler chickens. The effects of dietary energy and protein levels on growth performance of the broiler chicken are shown in Table 6 and 7. In experiment 1, reducing dietary protein with fixed level of energy (3100 kcal ME kg⁻¹) decreased feed intake during different periods at 21-42 (P<0.05), 21-42 (P<0.001) and 42-56 (P<0.001) days of age and body weight gain at

Table 3. Feed ingredients and composition of experimental diets for broiler from 42 to 56 days of age (finisher period).

| Ingredients         | Treatment |
|---------------------|-----------|
|                     | 1         | 2         | 3         | 4         | 5         | 6         |
| Corn, %             | 66.19     | 70.73     | 77.93     | 66.19     | 64.68     | 55.8      |
| Soybean meal, %     | 23.00     | 21.12     | 15.02     | 23.00     | 25.74     | 23.34     |
| Corn gluten, %      | 2.54      | -         | -         | 2.54      | -         | -         |
| Vegetable oil, %    | 2.77      | 2.59      | 1.43      | 2.77      | 1.00      | 1.00      |
| Wheat bran, %       | 1.50      | 1.50      | 1.50      | 1.50      | 4.83      | 16.43     |
| Dicalcium phosphate, % | 1.76   | 1.79      | 1.85      | 1.76      | 1.55      | 1.32      |
| Limestone, %        | 1.23      | 1.26      | 1.27      | 1.23      | 1.21      | 1.14      |
| Vit Min. Premix*, % | 0.50      | 0.50      | 0.50      | 0.50      | 0.50      | 0.50      |
| Salt, %             | 0.44      | 0.44      | 0.44      | 0.44      | 0.41      | 0.38      |
| DL-Methionine, %    | 0.07      | 0.07      | 0.05      | 0.07      | 0.09      | 0.09      |
| L-Lysine, %         | 0.02      | -         | -         | -         | -         | -         |
| Total               | 100.00    | 100.00    | 100.00    | 100.00    | 100.00    | 100.00    |
| Compositions (calculated) |             |           |           |           |           |           |
| ME, kcal kg⁻¹       | 3100      | 3100      | 3100      | 3100      | 2900      | 2700      |
| Crude protein, %    | 17.4      | 15.4      | 13.4      | 17.4      | 17.4      | 17.4      |
| Calcium, %          | 0.97      | 0.97      | 0.97      | 0.97      | 0.91      | 0.84      |
| Available P, %      | 0.44      | 0.44      | 0.44      | 0.44      | 0.41      | 0.38      |
| Sodium, %           | 0.19      | 0.19      | 0.19      | 0.19      | 0.18      | 0.17      |
| Arginine, %         | 1.04      | 0.95      | 0.79      | 1.04      | 1.11      | 1.12      |
| Lysine, %           | 0.83      | 0.76      | 0.64      | 0.83      | 0.89      | 0.88      |
| Methionine+Cystine, % | 0.68    | 0.6      | 0.53      | 0.68      | 0.68      | 0.68      |

*Provided per kg of diet: vitamin A, 9000 IU; vitamin D₃, 2000 IU; vitamin E, 11 IU; vitamin K₃, 2 mg; thiamine, 1.775 mg; riboflavin, 6.6 mg; vitamin B₃, 9.8 mg; vitamin B₆, 29.7 mg; vitamin B₉, 1.176 mg; vitamin B₁₂, 1 mg; vitamin H₂, 0.015 mg; vitamin H₂O, 0.1 mg; choline chloride, 500 mg; Mn, 76 mg; Zn, 66 mg; Fe, 40 mg; Cu, 4 mg; I, 0.04 mg; Se, 0.2 mg.

Table 4. Experiment 1 treatments.

| Treatment          | 1 (control) | 2      | 3      |
|--------------------|-------------|--------|--------|
| 0-21 d of age      |             |        |        |
| Energy level, Kcal ME kg⁻¹ | 3100      | 3100   | 3100   |
| Protein level, %   | 22.3        | 20.3   | 18.3   |
| 21-42 d of age     |             |        |        |
| Energy level, Kcal ME kg⁻¹ | 3100      | 3100   | 3100   |
| Protein level, %   | 19.4        | 17.4   | 15.4   |
| 42-56 d of age     |             |        |        |
| Energy level, Kcal ME kg⁻¹ | 3100      | 3100   | 3100   |
| Protein level, %   | 17.4        | 15.4   | 13.4   |

Table 5. Experiment 2 treatments.

| Treatment          | 1 (control) | 2      | 3      |
|--------------------|-------------|--------|--------|
| 0-21 d of age      |             |        |        |
| Energy level, Kcal ME kg⁻¹ | 3100      | 2900   | 2700   |
| Protein level, %   | 22.3        | 22.3   | 22.3   |
| 21-42 d of age     |             |        |        |
| Energy level, Kcal ME kg⁻¹ | 3100      | 2900   | 2700   |
| Protein level, %   | 19.4        | 19.4   | 19.4   |
| 42-56 d of age     |             |        |        |
| Energy level, Kcal ME kg⁻¹ | 3100      | 2900   | 2700   |
| Protein level, %   | 17.4        | 17.4   | 17.4   |
0.21 (P<0.05), 21-42 (P<0.001) and 42-56 days of age (P<0.01). Lowering dietary protein with fixed level of energy also impaired FCR during different periods but it had a significant effect only at 22-42 days of age (P<0.0001).

In experiment 2, decreasing dietary energy (3100, 2900 and 2700 kcal ME kg⁻¹) with fixed level of protein increased feed intake during different periods but it had a significant effect only at 0-21 days of age (P<0.0001) and body weight gain at 0-21 days of age (P<0.001). Lowering dietary energy with fixed level of protein impaired FCR at 22-42 days of age (P<0.0001).

Results of this study are in agreements with finding of Plavnik and Hurwitz (1990) reported that birds fed low protein diet in an experiment gained least body weight and did not recover the lost body weight as measured at 56 days of age. Leeson et al (1996) fed broilers in the finishing period with 2700, 2900, 3100 and 3300 kcal ME kg⁻¹ and found a decrease in feed intake and an improvement in feed conversion with increasing energy levels (from 2600 to 3200 kcal ME kg⁻¹) with fixed level of energy also impaired FCR during different periods but it had a significant effect only at 0-21 days of age (P<0.0001). Lowering dietary energy with fixed level of protein impaired FCR at 22-42 days of age (P<0.0001).

Table 7. Effect of different dietary energy levels on growth performance, growth hormone and ghrelin gene expression in broiler chickens in different growth periods.

Table 6. Effect of different dietary protein levels on growth performance, growth hormone and ghrelin gene expression in broiler chickens in different growth periods.

Table 8. Effect of different dietary energy levels on growth performance, growth hormone and ghrelin gene expression in broiler chickens in different growth periods.

Table 9. Effect of different dietary energy levels on growth performance, growth hormone and ghrelin gene expression in broiler chickens in different growth periods.

Table 10. Effect of different dietary energy levels on growth performance, growth hormone and ghrelin gene expression in broiler chickens in different growth periods.

Table 11. Effect of different dietary energy levels on growth performance, growth hormone and ghrelin gene expression in broiler chickens in different growth periods.
Table 8. Effect of different dietary protein levels on relative carcass characteristics of broiler chickens in different growth periods.

| Treatment | 0-21 days of age | 21-42 days of age | 42-56 days of age |
|-----------|------------------|------------------|------------------|
|           | Carcass, %       | Carcass, %       | Carcass, %       |
|           | Breast, %        | Thigh, %         | Breast, %        |
|           | Thigh, %         | Fat, %           | Thigh, %         |
|           | Fat, %           | Fat, %           | Fat, %           |
| 1 (control) | 77.75 \(a\) | 19.3 \(a\) | 22.7 | 78.2 | 22 | 25.7 | 2.8 | 76.4 | 23.6 \(a\) | 26.9 | 3.03 |
| 2 | 76.8 \(b\) | 16.5 \(b\) | 23.3 | 74.8 | 19.5 | 25.6 | 2.4 | 77.2 | 21.1 \(b\) | 21.3 | 3.25 |
| 3 | 74.9 \(b\) | 15.4 \(b\) | 23.2 | 75.4 | 18.3 | 24.7 | 2.9 | 75.8 | 19.9 \(b\) | 27.8 | 3.96 |
| ±SEM | 0.39 | 0.97 | 0.5 | 1.76 | 1.27 | 0.65 | 0.2 | 1.6 | 0.7 | 3.6 | 0.33 |
| P | 0.003 <0.05 0.75 | 0.37 0.17 0.5 | 0.23 | 0.8 | 0.01 | 0.41 | 0.18 |

\(a,b,c\)Means within column having different superscripts are significantly different (\(P<0.05\)).

Table 9. Effect of different dietary energy levels on relative carcass characteristics of broiler chickens in different growth periods.

| Treatment | 0-21 days of age | 21-42 days of age | 42-56 days of age |
|-----------|------------------|------------------|------------------|
|           | Carcass, %       | Carcass, %       | Carcass, %       |
|           | Breast, %        | Thigh, %         | Breast, %        |
|           | Thigh, %         | Fat, %           | Thigh, %         |
|           | Fat, %           | Fat, %           | Fat, %           |
| 1 (control) | 77.75 | 19.3 | 22.7 | 78.2 | 22 | 25.7 \(a\) | 2.8 \(a\) | 76.4 | 23.6 | 26.9 | 3.03 \(a\) |
| 2 | 76.8 | 20.5 | 22.5 | 77.7 | 22.9 | 25.1 \(ab\) | 1.6 \(b\) | 80.2 | 24.2 | 27.8 | 2.66 \(ab\) |
| 3 | 76.2 | 20.3 | 22.6 | 78.3 | 23.3 | 23.7 \(ab\) | 1.7 \(b\) | 78.3 | 24.9 | 26.2 | 2.07 \(b\) |
| ±SEM | 0.67 | 0.74 | 0.7 | 1.1 | 0.82 | 0.5 | 0.22 | 1.43 | 0.5 | 0.93 | 0.26 |
| P | 0.62 | 0.46 | 0.99 | 0.91 | 0.54 | 0.056 | 0.007 | 0.22 | 0.28 | 0.49 | 0.07 |

\(a,b,c\)Means within column having different superscripts are significantly different (\(P<0.05\)).

Plasma growth hormone concentration

Data for growth hormone concentration from experiment are presented in Tables 6 and 7. There was no effect of dietary energy and protein levels on growth hormone concentration during different period but there was a tendency for an increase in growth hormone concentration as dietary energy with fixed level of protein decreased (experiment 2).

Similarly, the current results are in agreements with the finding of Attia et al. (2003), who reported that the low energy diet increased growth hormone concentration at 21 days of age. Plasma growth hormone was found to be increased during the rapid growth period. Then, plasma growth hormone concentration declined during the slow growth period to reach low concentration in adult birds (Darras et al., 2000; Vasilatos-Younken et al., 1999). In these experiments, the data establish a link between plasma growth hormone concentration and different days of age (21, 42 and 56 days of age) in chicks. Growth hormone concentration was high at 21 and 56 days of age and it was decreased at 42 days of age. This results showed rapid growth period was at 0-21 and 42-56 days of age and then growth hormone concentration declined during the slow growth period at 21-42 days of age in broiler chickens (Tables 6 and 7).

Ghrelin mRNA expression

Ghrelin mRNA expression in the broiler chicken proventriculus was detected (Figure 1). The specificity of the amplified cDNA fragment of chicken ghrelin was verified by agarose gel 1% and sequencing PCR product ghrelin fragment (203 bp). Results of the present study are in agreement with Geelissen et al. (2003), who reported that chicken ghrelin in proventriculus tissue and glandular portion of the avian stomach. Ghrelin mRNA expression is mainly observed in the proventriculus (Wada et al., 2003), who indicating that the primary site of ghrelin production is the proventriculus. Richards et al. (2006) showed that proventriculus had the highest ghrelin gene expression in broiler chicken.

Effect of dietary energy and protein levels on ghrelin gene expression

The effect of dietary energy and protein levels on ghrelin gene expression of broiler chicken is presented in Tables 6 and 7. We looked at the effect of dietary energy and protein levels on ghrelin mRNA in the proventriculus in broiler chickens. There was no effect of dietary energy and protein levels on ghrelin gene mRNA abundance in the proventriculus for broiler chucks. Kaiya et al., (2007) demonstrated that the levels of ghrelin increased abdominal fat yield from 0.39% to 0.57% and increasing dietary protein levels linearly reduced abdominal fat in broilers (van Nguyen and Bunchasak, 2005; Smith and Pesti, 1998; Attia et al., 2001). The significant effect of energy levels on abdominal fat is similar to the previous study of Seatton et al., (1978) and Attia et al., (1998) found an increasing carcass fat with elevation of energy levels.
mRNA in proventriculus were altered in response to feeding states in layer chicks. They reported ghrelin mRNA in proventriculus increased in response to fasting. They suggested a role of ghrelin as a hunger signal (Kaiya et al., 2007), but to the best of our knowledge no studies have been performed on the effect of dietary energy and protein levels on ghrelin gene expression in broiler chicken; the experiments of our current study are the first ones.

Conclusions

In summary, we investigated the effects of different energy and protein contents of the diet on performance, growth hormone concentration and ghrelin gene expression in broiler chicken. We have characterized chicken ghrelin cDNA in proventriculus tissue in broiler chicken. We also found that growth hormone concentration and ghrelin gene expression are differently suppressed by diet manipulations, but we did not show significant effect in these experiments. Additional studies are needed to investigate the role of nutrition on plasma growth hormone concentration and ghrelin gene expression in proventriculus tissue in broiler chicken.

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