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Effect of G1705A SNP in Growth Hormone Gene on the Productive and Physiological Performance in Broiler Chicken

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Abstract: In this study two commercial broiler hybrids were used (Cobb500 and Hubbard-F15) to detect the G1705A SNP in the third intron of chicken GH gene and investigate its effect on the productive and physiological performance in broiler chicken. PCR- RFLP method was used to identify this SNP, three genotypes were found when using of Eco RV restriction enzyme wild genotype GG, heterozygous GA and homozygous AA.
Highly significant difference ($p<0.01$) was found between the distribution of the different genotypes, the genotype GG had the highest percentage followed by GA then AA and allele G had the superiority over allele A in both broiler breed, no significant effect of the various genotypes on the productive traits in both broiler breeds of study, significant effect ($p<0.05$) of the various genotypes were found on the serum total protein and triglycerides concentrations of Cobb500 at 14 days of age and the genotypes GG and GA were gave highest mean followed by the genotype AA.

Keywords: GH gene, PCR-RFLP, *Eco* RV, G1705A SNP.

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Introduction

Poultry industry one of the important resources of white meats in the country, which has its crucial position in the national economy, that contributes to the achievement of self-sufficiency and reduce the cost of imported white meat. The global poultry industry have witnessed a major development as many companies have the very high performance in comparison with other livestock and aimed to create different broiler chicken strains and hybrids that because the low costs of white meat as compared with red meat. On the other hand, white meat is nutritional higher in value than red meat because of the high content of protein and low levels of fats and cholesterol which cause Arteriosclerosis disease, obesity, immunosuppression and leg problems Kadlec *et. al.* (1). Nie *et. al*. (2) pointed that somatotropic axis genes plays an important role in chicken growth and development and this axis consists of essential components like growth hormone (GH), insulin-like growth factors (IGF–I and II) and their associated carrier proteins and receptors and other hormones such as insulin, leptin and thyroid hormones. Some results from previous studies had revealed that SNPs of the somatotropic

axis genes affect growth and physiological traits Lei *et. al*. (3).

The chicken growth hormone (GH) gene is one of the effective genes that influence the chicken productive and physiological performance because it plays a crucial role in growth and metabolism, it was located at the end of the long arm of chromosome 27, which consists of 5 exons and 4 introns and a length of 4.1 kilo base .The gene encodes a 191–amino acid mature protein and a 25–amino acid signal peptide and the chicken GH gene is similar to mammalian gene, but the gene in chickens to be the biggest. Studies of polymorphisms of GH gene were carried out by new molecular technology such as Restriction Fragment Length Polymorphism (RFLP) technique and DNA sequencing, molecular studies have shown that substitution, deletion or insertion of a single nucleotide due to changes in a gene, called single nucleotide polymorphisms (SNPs), it is a type of DNA polymorphism which is bi-allelic but extensively distributed along the chicken genome and recent investigations of the whole chicken genome identified over 2.8 million SNPs Beuzen *et. al.*,Yan *et. al.*,Enayati and Rahimi (4, 5 , 6).

Materials and Methods

This study was conducted at the poultry farm of animal resources department – College of Agriculture University of Baghdad. Two hundred one day-old chicks were randomly housed in pens measuring 300 cm wide x 350 cm x 250 cm high (Coob500 and Hubbard separately) and marked with wing tags. Birds were kept indoor, on sawdust litter, in accordance with standard production technology. Commercial diet was provided *ad libitum*. While fresh drinking water was made available at all times. At one day of age water supplied with sugar 50gm/litter, vitamin C 0.5gm/litter was provided. Newcastle B1 Spraying at day one of age and then *Lacota* strain was used in drinking water at (10 , 20 , 30 , 37) days of age , and the chicks were vaccinated in drinking water with Gambaro (*Locard* strain) at 12 and 22 days of age .

Five ml of blood were collected from the brachial vein of all chicks under the study. These samples were collected in EDTA tubes and kept in freezer (-18 ºC) for DNA extraction by using DNA extraction kit (Promega, USA. From blood collected at 14 and 42 day of age, serum was obtained by putting the blood samples in a clean dry plain plastic tube and then was allowed to clot at 37 ºC for 30 minutes before being centrifuged. The tubes were centrifuged at 6000 rpm for 5 minutes; then the serum was collected and kept in freezer (-18 ºC) until used for serum biochemical analysis, before DNA extraction blood volume was reduced to 20 microliters and cell lysis buffer increased to 500 microliters because all the blood cells of chicken are nucleated and contained DNA and proteins levels in chicken blood higher than in mammals blood . The primers was supplied from Alpha DNA/Canada, as lyophilized powder of different picomols concentrations F-**5′** TCCCAGGCTGCGTTTTGTTACTC **3′** and R-**5′** ACGGGGGTGAGCCAGGACTG **3′** gene bank (AY461843) according to Lei *et. al.* (3).

PCR Reaction

The PCR reaction was performed in 0.2ml tubes by mixing master mix reagents in final volume of 20 µl. The amplification was performed in a TECHNE (T-C 5000) thermal cycler and the reaction mixture was prepared according to the procedure that suggested by the manufacture company (BIONEER, Korea) using 75-90 ng/ µl of DNA and 0.8 µl of primers and then complete the PCR reaction volume to 20 µl by distilled water finally reaction mixture vortexes thoroughly. PCR mixture without DNA template was used as a negative control. Thermal cycle with the following profile: Initial denaturation at 94 ^{o}C for 4 minutes, 35 cycles of 94 ^{o}C for 30 seconds,54 ^{o}C for 30 seconds,72 ^{o}C for 30 seconds and a final elongation at 72 ^{o}C for 5 minutes
PCR products (8µ) were digested with 3 units of *Eco*RV restriction enzyme at 37 ^{o}C overnight. Restriction pattern were visualized in a 1.5% agarose gel electrophoresis stained with Ethidium bromide.

Productive Traits

Weekly live body weights (grams) were individually recorded for each chick during the six week experimental period and then weekly body weight gains (grams) were calculated for each chick under study. At 42 days of age, 15 males and 15 females for each hybrid were randomly chosen then weighed and slaughtered after 12 hours of starvation, immersed in 53°C water for 2 minutes, and plucked in a rotary drum, chickens heads and legs were removed. The liver, gizzard, heart were removed, weighed, then calculated as a percentage of carcass weight and dressed carcass were weighed and calculated as percentage of live body weight. Each carcass was cut into breast muscle, back, thigh muscle, drumstick, wings and neck. All weights were recorded and calculated as a percentage of live body weight Toghyani *et. al.* (7).

Serum Biochemical Parameters

Serum GH concentration was measured by using ELISA Kit provided from CUSABIO Company and the rest physiological parameters (total protein, cholesterol, triglycerides and glucose) concentrations were measured by an automatic biochemical analyzer (Accent 200-Poland) following the instructions of the corresponding reagent kit.

Statistical Analysis

Data were analyzed using Statistical Analysis System (SAS) (8) to study the effect of growth hormone (GH) gene polymorphism on various characteristics (productive and physiological traits). Duncan multiple range test was used to compare the average means, Duncan (9).

Results

PCR-RFLP Analysis

Polymerase Chain Reaction (PCR) amplified regions, which showed a molecular weight of 429 bp, represents the region of the growth hormone gene. This technique was used with primers for growth hormone gene according to Nie *et. al*. (2). To detect the PCR product, DNA ladder (100-1000) bp was used and the gel was photographed by a digital camera. The same PCR product size was obtained by (2, 3) then

the PCR products which underwent restriction digestion with *Eco* RV enzyme (GAT/ATC) to detect G1705A SNP in the third intron of growth hormone gene and it was able to cut at this position only when SNP is present (when G convert to A). The following fragment sizing patterns were observed by agarose gel electrophoresis (Figure 1). 1. Wild type GG: No cleavage of the whole 429 bp segment by *Eco* RV (lane 1, 3, 4,6,8,11,13 and 14).

2. Heterozygous GA: *Eco* RV was cut the sequence to show three fragments in agarose gel electrophoresis (429 bp , 312 bp and 117 bp) (Lane 2,5,9,12 and 15).

3. Homozygote AA: *Eco* RV was cut the sequence to show two fragments in agarose gel electrophoresis (312 bp and 117 bp). (Lane 7,10).

The results of present study are similar with previous study of Nie *et. al*. and Lei *et. al.* (2,3) on the Chines chicken.

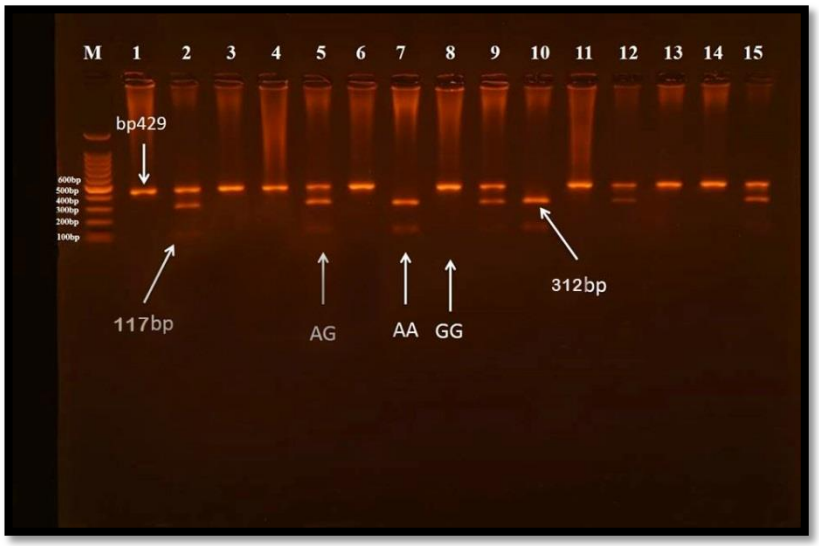

Figure (1): PCR product digested with *Eco* RV electrophoresis on 1.5%. Lane M: DNA ladder (100-1000). Lane1,3,4,6,8,11,13 and 14 : Wild type GG genotype 429 bp, Lane2,5,9,12 and 15: heterozygote GA genotype 429, 312 and 117bp, Lane 7 and 10: Homozygote AA genotype 312 and 117 bp .The (RFLP) products were agarose gel at 5 volt/cm^2 for 1hour. Visualized under U.V light after stain with Ethidium Bromide

Genotype Distribution

It is obvious from table (1) that there were high significant differences ($p<0.01$) in the genotype GG of growth hormone gene followed by GA and then AA amounted 47.06, 45.88 and 7.06 % in Cobb500 and in Hubbard 61.05, 36.84 and 2.11% respectively. The results of the present study are similar to previous results which were obtained from Nie *et. al.*, Lei *et.al.* (2,3) who used *Eco* RV endonuclease enzyme to detect the G1705A SNP in the third intron of GH gene in the Chinese chicken.

Table (1): Distribution of growth hormone gene genotypes (No. and %) of Cobb500 and Hubbard F-15 broiler chicken

Genotype	Cobb 500	Hubbard F-15)
	No. (%)	No. (%)
GG	40 (47.06)	58 (61.05)
GA	39 (45.88)	35 (36.84)
AA	6 (7.06)	2 (2.11)
Total	85 (100)	95 (100)
Chi-square value (χ^2)	10.359 **	12.071 **
** (P<0.01)		

Allele Frequency

According to Hardy–Weinberg low the results of table (2) showed superiority of allele G over allele A amounted 0.70 and 0.30 for Cobb500 respectively and in Hubbard (0.79 and 0.21) respectively. The results of this study are agreed with previous studies of Nie *et. al*., Lei *et. al*. (2,3) which refers to the dominance of allele G over allele A of the SNP G1705A in the third intron of GH gene in the Chines chicken.

Table (2): Allele frequency of Growth hormone gene in Cobb500 and Hubbard F-15 broiler chickens

Genotype	Cobb 500	HubbardF-15
G	0.70	0.79
A	0.30	0.21
Total	100%	100%

The Association of G1705A SNP with Weekly Live Body Weight

Table (3) showed non-significant differences between the various genotypes (GG, GA and AA) of the growth hormone gene in the mean of weekly live body weight for Cobb500 and Hubbard during the experiment period.

Table (3): Effect of Growth hormone gene polymorphism on weekly live body weight (g)

Body Weight (gm/week)	Cobb500			Hubbard F-15		
	GG	GA	AA	GG	GA	AA
1	a 167.25 ± 2.9	a 164.87± 2.1	a 165.83 ± 4.5	a 155.08± 1.9	a 158.71± 2.4	a 147.5 ± 12.5
2	a 420.62 ± 7.0	a 430.89± 6.5	a 417.5 ± 23.3	a 438.96 ± 5.3	a 447.42 ± 6.2	a 425 ± 3.5
3	a 904.62 ± 12.4	a 918.71 ± 13.7	a 896.66± 30.3	a 891.89 ± 10.4	a 906.71 ±11.6	a 877.5 ± 62.5
4	a 1463.75±23.2	a 1486.67±23.7	a 1428.33±37.3	a 1423.41±19.1	a 1432 ± 23.1	a 1287.5 ± 77.5
5	a 1928.0 ± 31.0	a 1962.69±34.4	a 1877.5±62.3	a 1946.64±29.3	a 1994.5±34.2	a 1790.0 ± 4.0
6	a 2569.38±53.1	a 2603.08±48.6	a 2544.17±91.2	a 2596.64±39.3	a 2609.14± 48.1	a 2490.0 ± 12.5

Means with different superscripts within each bread are significantly different at 0.05 level

The Association of G1705A SNP on the Mean of Weekly Weight Aain

Results of table (4) showed non-significant differences between the various genotypes (GG, GA and AA) of the growth hormone gene in the mean of weekly weight gain for Cobb500 and Hubbard during the experiment period.

Table (4): Effect of Growth hormone gene polymorphism on weekly weight gain (g)

Weight gain (gm/week)	Cobb500			HubbardF-15		
	GG	GA	AA	GG	GA	AA
1 *	a 93±2.3	a 89.10±1.9	a 93.33±4.4	a 89.44±1.6	a 92±2.0	a 90±1.0
2	a 253.37±5.6	a 266.02±5.5	a 251.66±21.0	a 283.87±4.1	a 288.7±4.3	a 277.5±22.5
3	a 484±8.4	a 487.82±8.3	a 479.16±11.8	a 452.93±6.2	a 459.28±6.5	a 452.5±27.5
4	a 559.12±13.5	a 567.94±11.6	a 531.66±17.9	a 531.51±10.8	a 525.28±13.2	a 528±1.5
5	a 464.25±18.3	a 476.02±16.1	a 449.16±36.3	a 523.22±16.6	a 562.57±19.1	a 502.5±37.5
6	a 641.37±35.1	a 640.38±33.2	a 666.66±32.8	a 650±20.5	a 614.57±28.7	a 700±8.5

Means with different superscripts within each bread are significantly different at 0.05 level.
***weight gain was measured from age 3 -7 days**

Effect of G1705A SNP on the Live and Carcass Weight, Dressing and Dressing with Giblets

Results of table (5) showed insignificant differences between the different genotypes (GG, GA and AA) of the growth hormone gene in the (live body and carcass weight, dressing and dressing with giblets percentage) for Cobb500 and Hubbard during the experiment period.

Table (5): Effect of Growth hormone gene polymorphism on live and carcass weight, dressing and dressing with giblets

Traits	Cobb500			HubbardF-15		
	GG	GA	AA	GG	GA	AA
Live weight (g)	a 2685.40±94.5	a 2606.7±47.8	a 2700±25.0	a 2751.8±76.1	a 2518±51.3	a 2638.8±99.9
Carcass weight (g)	a 2037.70±69.7	a 1987.33±43.4	a 2053±7.5	a 2201.43±63.8	a 1979.3±44.4	a 2078±82.4
Dressing (%)	a 75.92±0.4	a 76.20±0.5	a 76.03±0.9	a 79.99±0.6	a 78.6±0.2	a 78.72±0.5
Dressing with giblets (%)	a 80.17±0.4	a 80.40±0.5	a 79.64±0.7	a 83.96±0.6	a 82.80±0.3	a 82.90±0.3

Means with different superscripts within each bread are significantly different at 0.05 level

Effect of G1705A) SNP on Relative Weight of Carcass Cuts and Giblets

The results of Table (6) showed insignificant differences between the different genotypes (GG, GA and AA) of growth hormone gene in the relative weights of all studied traits for each hybrids .The high correlation between the relative weight of the cuts and giblets with the insignificant effect of the various genotypes on the live body weight and carcass weight led to insignificant effect on the traits above.

Table (6): Effect of Growth hormone gene polymorphism on relative weight of carcass cuts and giblets

Traits (%)	Cobb500			Hubbard F-15		
	GG	GA	AA	GG	GA	AA
Thighs	a 14.06±0.30	a 13.67±0.42	a 13.52±0.41	a 14.54±0.44	a 14.27±0.76	a 14.16±0.17
Drum stick	a 11.62±0.33	a 11.87±0.29	a 12.07±0.65	a 13.35±0.27	a 13.04±0.28	a 12.76±0.21
Wings	a 9.35±0.21	a 9.42±0.24	a 10.11±0.64	a 9.72±0.18	a 10.05±0.27	a 9.96±0.11
Neck	a 5.38±0.31	a 5.73±0.41	a 6.33±0.99	a 5.52±0.17	a 5.32±0.24	a 5.29±0.21
Back	a 21.43±0.52	a 21.24±0.65	a 18.63±0.67	a 22.40±0.43	a 22.14±0.60	a 22.42±0.70
Breast	a 36.72±0.76	a 35.73±0.54	a 38.35±3.14	a 32.50±0.55	a 32.38±0.67	a 32.31±0.36
Heart	a 0.47±0.02	a 0.50±0.02	a 0.45±0.04	a 0.51±0.02	a 0.52±0.02	a 0.52±0.03
Liver	a 2.33±0.03	a 2.24±0.10	a 1.91±0.28	a 2.20±0.08	a 2.27±0.05	a 2.36±0.17
Gizzard	a 1.50±0.05	a 1.46±0.04	a 1.33±0.01	a 1.34±0.05	a 1.45±0.05	a 1.41±0.17

Means with different superscripts within each bread are significantly different at 0.05 level

Effect of G1705A SNP on Some Serum Biochemical Parameters of Coob500

Results of table (7) showed significant differences ($p<0.05$) in the total protein concentration of chicken blood serum at 14 days of age where the genotypes GA and GG had higher mean amounted 3.2 g/dl as compared with the genotype AA which recorded 2.6 g/dl. Also had been observed a significant differences ($p<0.05$) in triglycerides concentration at 14 days of age amounted in genotype AA 123 mg/dl and then followed by GA and GG amounted 121.7 mg/dl . While there were no significant effect for various genotypes in the rest traits above at 14 and 42 days of age.

Table (7): Effect of growth hormone gene polymorphism per age in some serum biochemical parameters of Cobb500

Traits	14 days			42 days		
	GG	GA	AA	GG	GA	AA
GH (pg/ml)	a 2166±5.4	a 2173.4±1.1	a 2173±4.0	a 1784.6±40.1	a 1826±1.57	a 1826±3.00
GHR (mg/l)	a 0.97±0.00	a 0.97±0.00	a 0.99±0.01	a 1.28±0.00	a 1.28±0.003	a 1.26±0.01
Total protein (g/dl)	a 3.2±0.06	a 3.2±0.12	b 2.6±0.0	a 3.3±0.8	a 3.3±0.19	a 2.9±0.0
Triglyceride (mg/dl)	b 121.7±0.23	b 121.7±0.23	a 123.0±0.0	a 66.4±0.43	a 65.9±0.56	a 64.50±0.50
Cholesterol (mg/dl)	a 121.4±0.27	a 121.5±0.26	a 121±0.50	a 113.6±1.65	a 112.8±0.26	a 112±0.0
Glucose (mg/dl)	a 229.7±0.19	a 229.9±0.20	a 229.2±0.40	a 228.9±0.16	a 228.6±0.17	a 228.5±0.45

Means with different superscripts within each bread are significantly different at 0.05 level

Effect of G1705A SNP on Some Serum Biochemical Parameters of Hubbard F-15

It was seen from table (8) insignificant differences between the genotypes (GG,GA and AA) of GH gene in their effects on (GH , GHR , total protein , triglycerides , cholesterol and glucose) concentrations in blood serum at 14 and 42 days of age .

Table (8) Effect of growth hormone gene polymorphism per age in some serum biochemical parameters of Hubbard F-15

Traits	14 days			42 days		
	GG	GA	AA	GG	GA	AA
GH (pg/ml)	a 2170.4±1.95	a 1888.6±184.6	a 2176±1.00	a 1825.3±0.72	a 1823.4±1.02	a 1829±9.0
GHR (mg/l)	a 0.97±0.01	a 0.98±0.01	a 0.99±0.01	a 1.28±0.01	a 1.29±0.01	a 1.27±0.01
Total protein (g/dl)	a 3.3±0.09	a 3.4±0.14	a 3.1±0.10	a 3.1±0.09	a 3.5±0.14	a 3.2±0.30
Triglyceride (mg/dl)	a 123±0.19	a 122.9±0.31	a 123±0.0	a 60.4±0.23	a 60.2±0.31	a 60.4±1.4
Cholesterol (mg/dl)	a 124.4±0.53	a 123±0.61	a 119.5±0.45	a 120±0.55	a 120.6±0.61	a 122±0.01
Glucose (mg/dl)	a 230±0.25	a 228.6±0.17	a 229.3±0.40	a 228.8±0.17	a 228.6±0.17	a 228.5±0.45

Means with different superscripts within each bread are significantly different at 0.05 level

Discussion

Johnson *et. al.* (10) found that GH concentrations were not correlated with growth rate. Actually, selection for higher growth rate resulted in lower basal GH concentrations and this conclusion led to conclude the abundance of GHR limits growth especially during the active growth phase of the young chicken and the growth selected chickens, expressed higher levels of GHR. These results were different from the previous studies Nie *et. al.*,Lei *et. al.* (2.3) about the significant effect of the SNP G1705A in intron 3 of GH gene and it had positive correlation with meat quality and live body weight of the different strains of the Chinese chicken where the genotype AA and GA recorded significant increase as compared with the genotype GG at the different ages of the study. The difference in the results of this study with the previous results caused by the difference in the studied sample size as well as the difference in the studied breeds and conditions of the experiment. The non-significant differences in the body weight between the various genotypes of SNP G1705A in the third intron of GH gene led to the absence of differences between the various genotypes in the trait of body weight gain and these results were disagreed with the previous study Nie

et. al., Lei *et. al.* (2,3) in the Chines chickens.

The insignificant differences in the live body weight, carcass weight, dressing and dressing with giblets caused by insignificant effect of various genotypes for each hybrids under study (GG, GA and AA) on the live body weight and body weight gain. The results of this study were disagreed with Ghelghachi *et. al.* (11) who referred to significant effect of the GH gene polymorphism on the productive performance of Arian broiler with increase in live body weight and carcass weight as compared to wild genotype. The difference of present study with the others may be because of the difference of broiler hybrids, number of study birds and experiment conditions like environment.

Bingxue *et. al.*(12) referred to significant effects of mutation G to A in the third intron of GH gene diagnosed by endonuclease enzyme *Eco* RV on the breast muscle weight in the Chines chicken. Also Ghelghachi *et. al.* (11) pointed to the significant effects of GH gene polymorphism on the relative weights of breast muscle, drum stick, wings and lean in Arian broiler.

The environmental factors may be affecting directly or indirectly the growth hormone gene expression which affects the effectiveness and impact on the body or target tissue, led to the difference between the results of this experiment and other results in a different environment.

These differences in blood serum total protein concentrations between the various genotypes caused by the crucial role played by GH in stimulation of protein synthesis through reducing proteolysis and decreasing protein oxidation leading to a net increase in protein accretion Bell *et.al.* (13).The significant effect of GH gene polymorphism in serum triglycerides concentration caused by the important role of GH in lipids metabolism, which is characterized by lipogenic effect. Evidence for a lipogenic effect of GH was presented by Proudman *et.al.* (14) who found that hypophysectomized turkey pullets had lower plasma triglyceride levels compared to that of their intact counterparts, and GH administration to the hypophysectomized animals elevated circulating triglyceride levels. In vitro evidence for a direct lipogenic action of GH could be found in the observation that GH promotes acetate incorporation into liver lipids Cupo and Cartwright (15).

The reason of insignificant effects between the various genotypes in the above traits may be because of the intensive selection programs for the rapid growth leading to a negative correlation or lack of correlation in the effect of growth hormone gene with the (productive, physiological) traits and growth represented by increasing the number and size of body cells and the physiological characteristics represented by the different metabolic processes in the body of bird Decuypere *et. al.* (16). Also Buyse *et. al.* (17) showed the importance of the secretion pattern, time and age of growth hormone in the growth and metabolism of birds.

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