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Genetic Expression of Some Mitochondrial and Growth-Related Genes and its Impact on Productive Traits in Broilers during Pre- and Post-hatch

Doaa R. Saleh., Shabaan A. Hemeda., Abeer F. El-Nahas and Shymaa A. Khatab

Department of Animal Husbandry and Animal Wealth Development-Genetic Laboratory. Faculty of Veterinary Medicine, Alexandria University. Egypt.

Abstract

THIS study was carried out to assess the Cobb broiler productive traits and the expression of energy metabolism (*avANT*, *ATP5G1* and *ATP5E*) and mitochondrial biogenesis-related genes (*PPARγ* and *avPGC-1α*). Also, the expression of some growth-related genes (*MyoD*, *MyoG* and *MSTN*) in breast muscle during pre-hatch and post hatch. The pre-hatching stage on days 12, 15, and 19 and the post-hatching stage on days 21 and 35. The birds were individually weighted at 0, 7, 14, 21, 28, and 35 days. The growth performance indices like the TBWG, FC, FE, and FCR, weekly feed consumption and total feed intake were evaluated. Different expression levels of *avANT* and *avPGC-1α* genes at the different embryonic stages were observed. The expression level of *ATP5G1*, *ATP5E*, and *PPARγ* were not detected at pre-hatch. Meanwhile, *ATP5G1* was higher in small body weight unlike *ATP5E* that was significantly lower in small BW birds. *avPGC-1α* and *PPARγ* on 21st day showed significant up-regulation in small BW, while at 35th day, no significant difference in their expression in small and large BW broilers. Little expression of growth-related genes was observed at pre-hatch. At post-hatch, *MyoD* was higher in large BW compared to small BW broilers. *MSTN* was up-regulated in small BW compared to large one.

We concluded that the *avANT, PGC-1α, and MyoD* genes have major role at pre-hatch and post hatch-till 21th day. However*, MyoG*, *ATPG1,* and ATP*5E* play strong roles in skeletal muscle growth at the marketing age which could be useful for the genetic improvement in broiler.

Keywords: Cobb broilers, gene expression, mitochondrial genes, pre- and post-hatching stage

Introduction

Modern commercial broiler lines are chosen for efficient productive features such as high feed conversion, rapid development, better meat yield, lower mortality, and cost [1]. Broilers are bred to reach their peak productivity in 35 to 42 days [2]. However, growth is a complex process that is governed by several neuroendocrine pathways and governed by multiple genes [3]. Since the 1950s, chicken breeders have been aiming for highly muscled broilers through artificial selection. Body weight is a polygenic characteristic, and selection to generate modern broilers has resulted in chickens with enhanced muscle mass and quick growth rates [4].

Since the primary role of mitochondria is to provide cellular energy to the living systems, the efficient conversion of food into body mass has been linked to changes in the expression of several genes that are involved in energy metabolism such as avian adenine nucleotide trans locator (*avANT*), *ATP5E* and *ATP5G1* and mitochondrial biogenesis such as avian *PPAR-γ* coactivator-1α (*avPGC-1α*) and Peroxisome proliferator-activated receptor-γ (*PPARγ*) [5]. *avANT* gene protein is found on the mitochondrial inner membrane, plays an important role in maintaining *ATP/ADP* ratio and contributes to the energy-supplying function of mitochondria [6]. *ATP5E* gene encodes the mitochondrial F0F1 ATP synthase subunit epsilon, while *ATP5G1* is a mitochondrial ATP synthase subunit that catalyzes ATP production and involved in the biological process of oxidative phosphorylation and may be linked to oxidative stress [7].

AvPGC-1-α is the most dominant regulatory protein in mitochondrial biogenesis; the expression of nuclear and mitochondrial genes that code for mitochondrial proteins is up regulated as a result of the expression of *PGC-1-α* [8]. *PPARγ* is a

*Corresponding authors Abeer F. El-Nahas, E-mail: abeer.elnahas@alexu.edu.eg. Tel.: +201225043567
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transcription factor that modulates lipid and glucose metabolism [9]. Few data are available about the relationship between the expression of mitochondrial genes among different body weight broiler classes.

Since skeletal muscle development follows a specific pattern of periodic gene expression at the embryonic and postnatal growth [10]. Some of the myogenic regulatory factors (*MRFs*) as *MyoD* and *MyoG* have been proposed to modulate muscle development and carcass performance. The function of this family is to control the myogenic cell lineage and differentiation of myoblasts in all muscle forming regions of chicken embryo and play a role in adult muscle differentiation [11]. On the other hand, myostatin (*MSTN*) is a member of transforming growth factor-β (*TGF-β*) super family and is a negative regulator of skeletal muscle [12].

The aim of this study was to assess the expression of some genes which are implicated in energy metabolism and mitochondrial biogenesis as well as some growth-related genes in breast muscle of Cobb broiler males during pre-hatch and post hatch till the marketing age. Also, evaluating the relationship between the expression of these genes and some productive traits.

Materials and Methods

Ethical approval

All experimental procedures were carried out according to the NIH general guidelines for the care and use of laboratory animals and as recommended and approved by the Ethics of Animal Use in Research Committee (IACUC), Faculty of Veterinary Medicine, Alexandria University, Egypt (Serial Number: 0304596).

Experimental design.

pre-hatch study.

A total of 30 fertile eggs from Cobb broiler breeder were obtained from certified distributers in Egypt for pre hatching mitochondrial gene expression. The eggs were classified into three groups: small, medium, and large according to their weights. Samples from Cobb embryonated chicks including muscle and other developing organs were collected at 12nd, 15thand 19th day of incubation (n=9 per day) and stored at -80°C for posterior use.

Post-hatch study

One hundred- and fifty-day-old male broilers (Cobb 500) chicks were used in this study. At one old day, the chicks were divided into 3 groups according to their body weight: large group (fifty-five), medium group (thirty-nine) and small group (twenty-seven) birds. Samples from pectoral muscle of small, medium, and large groups were collected (n=5 per group) at 21st and 35th day post-hatching and stored at -80°C until used.

Bird management

The chicks were raised on floor. The chicks were given unlimited amounts of feed and water and received all vaccines (Newcastle (B1)+IB, Newcastle (Lasota), Gambaro IBD2, and Newcastle (Lasota) at days 1, 9, 15, and 20). Birds were individually weighed at 0, 7, 14, 21, 28 days, and at the end of the rearing period (35 days). Also weekly feed consumption (FC), total feed intake (TFI) to determine performance indices such as the total body weight gain (TBWG), feed consumption (FC), feed efficiency (FE) and feed conversion ratio (FCR):

BWG (g) = Final BW (g) at the end period – Initial BW (g) at start.

FC (g/bird) = (Feed offered – Feed residue)/No. of bird.

FE (g/g) = BWG ÷ FC.

FCR (g feed/g gain) = Total Feed consumed ÷ Live Body Weight.

Extraction of Total RNA and cDNA synthesis

Total RNA was isolated from embryonic breast muscle (pectoral muscle) according to manufacture instructions, the quality of RNA was checked on 2% agarose. TOP scriptT^{TM} RT Dry MIX (dt 18/Dn6plus) cDNA Synthesis Kit was used to make cDNA from each group's extracted RNA (enzynomics, south Korea) according to manufacturer instruction protocol and the obtained cDNA stored at -20°C until further use. The cDNA was tested using PCR for the housekeeping gene (18S), and the product was tested on a 2% agarose gel.

Expression of mitochondrial and growth-related genes by qPCR

RT-PCR reaction was carried out in Stratagene MX300P real-time PCR system (Agilent Technologies) using SensiFast SYBR Lo-Rox kit (Bioline). In a total volume of 10 µl, consisting of 5 µl of Syber green (enzynomics, South Korea), 1 µl of each primer, 1 µl cDNA, and 3 µl of RNase free water, with an initial heating at 95°C for 10 min, followed by 40 cycles of 95°C for 30 sec and annealing temperature of the primers (Table. 1). The relative expression of the genes was calculated using comparative threshold cycle method $2^{-\Delta\Delta CT}$ [13] and the results reported as fold change differences relative to the control group. 18 S gene was used as endogenous control for normalizer.

Data analysis

To detect the significant changes in the means among the different treatments and the control group; one-way ANOVA followed by Tukey's multiple comparisons test was performed using GraphPad Prism version 9.00 for Windows, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com". Each value represents mean± SE (P>0.05).

Results

Productive traits during pre- and post-hatch periods

On day 12th of pre-hatch, the egg weight of high group was significantly higher than the medium and low groups. Day 15 and 19 showed a significant difference between the high weight group and the medium group, which in turn showed a substantial difference from the low group (table 2).

Growth performance data of broilers used in this study are presented in Tables 3 and 4. There were significant differences among high, medium, and low body weight groups at all weeks from the first week to the last week (5th) as revealed in table 3. Concerning the FE (gain/g of feed consumed) at third and fifth weak; FE was significantly different among high, medium, and low body weight groups; it was significantly higher for the high body weight group (table 4). Feed intake was changed among FE broilers; high FE broilers gained more weight. As predictable, feed conversion ratios (total feed consumed/g life body weight) were also significantly different between high and low FE groups, it was significantly lower for high body weight group than medium group than low body weight group (table 4).

Expression levels of selected genes

Mitochondrial protein gene expression at different stages (pre-hatch, post-hatch)

The *av-ANT* gene was expressed significantly with variable levels during embryogenesis. The highest expression was recorded at E-15 (13.63±0.45) compared with its expression at E-12 (2.58±0.53) and E-19 (5.6±2.3) (Fig (1A). The post hatching expression of *av-ANT* was higher in small BW (6.2±5.7) than large BW (4.4±6) on day 21st. Meanwhile, its expression was very low at day 35th in both small (0.21±0.17) and large body weight birds (0.02±0.019) (Fig 1B). The expression level of *ATPG1* at embryonic stages was very weak or almost undetected at different stages (Fig 2A). At post-hatch, its expression was not detected in both large and small BW at day 21st (0.012±0.012 and 0.003±0.003) and at day 35th it was slightly higher in small body weight (2.3±2.5) compared with the large body weight (0.7±0.9) (Fig 2B).

ATP5E has no significant values at different embryonic periods (Fig 3A). As well as there was no significant difference between small and large BW at day 21st post hatching (0.13±0.09 and 0.013±0.009). On day 35th it was significantly lower in small BW (1.25±1.6) than large BW (2.33±1.33) as shown in Fig (3B).

avPGC-1α was expressed significantly during embryogenesis (Fig 4A). The highest expression of *avPGC-1α* expression was observed at E-12 (16.23 ±13.47) and E-15 (24.53± 8.2) with no significant difference between them. At E-19, it significantly decreased to 5.46±1.6. At post-hatch, *avPGC-1α* revealed significant up regulation in small BW (14.53±12.9) compared to the large BW (1.33±0.6) at day 21st, while at day 35th the expression of the gene is almost undetected in both small and large BW birds (0.66±0.63 and 0.33±0.3) (Fig (4B).

No expression was detected in *PPAR* gene at different embryonic stages as shown in Fig (5A). At day 21st post-hatch, the expression was significantly higher in small BW (45.7±30.4) than large BW (2.5±2.4). There was no variation in *PPAR* gene expression between small and large BW at day 35th post hatching (Fig (5B).

The expression level of *MyoD* was very little (1.25±1.1 at E-12, it decreased to 0.73±0.27 at E-15 and to 0.09±0.9 at E-19) (Fig (6A). *MyoD* was significantly higher in large BW (8.8±0.6) compared to the small BW (4.4±3.5) on day 21st. On day 35th it was slightly higher in large BW (1.0±0.4) than small BW (0.22±0.22) (Fig (6B).

Expression of growth related at different stages (pre-hatch, post-hatch)

The expression of *MyoG* gene was observed E-12th only pre-hatch (Fig 7A). However, at post-hatch, the expression of the gene was not detected at 21th day in both small and large body weight. On day 35th, little difference was observed between small and large BW (Fig 7B).

No expression was detected in *MSTN* gene at E-12, 15, and 19 (Fig 8 A). The expression level of *MSTN* at day 21st post-hatch was significantly higher in small BW (10.6±16.7) compared to the large BW (0.42±0.42). On day 35th little expression was observed in small BW (1.2±0.4) and no expression in the large BW (0.5±0.5) (Fig (8B).

Discussion

A significant difference in egg weight was detected among the three size groups of eggs at the 12th, 15thand 19th day of incubation. Mortola and Al Awam [14] proposed that different-sized eggs result in hatchlings of various sizes. They claim that this change is caused by incorporating different egg yolk remnants, which could reflect a genetic relationship between the size of the egg and the rate of embryonic growth.

Additionally, Vleck and Vleck [15]; Prinzinger and Dietz [16] proposed that the oxygen intake of precocial birds grows dramatically during development until it stabilizes just before hatching, as embryos reach their hatching mass. The rise in oxidative metabolic capability throughout embryogenesis is aided by increased mitochondrial density and the enzymatic activities in an oxidative manner [17]. As a result, one of our aims was to investigate the expression level of some of mitochondrial genes including (*avANT*, *ATP5E*,

ATP5G1, *avPGC-1α* and *PPARγ*) pre and post hatching. We found that the most prominent mitochondrial genes to be expressed significantly at different embryonic stages were *avANT* and *avPGC-1α*. In ducklings, Speake et al. [18] suggested that, during embryonic development, there are great variations in fatty acid profile, with an increase in polyunsaturated fatty acid in various tissues making embryonic tissues, particularly vulnerable to free radicals. As a result, *avANT* may contribute to the stress of reestablishing effective oxygen supply via air breathing at the time of hatching after relative oxygen restriction at the end of the incubation period and serve antioxidant activities by helping to the adaptive responses of birds exposed to substantial fluctuations in oxygen availability in the environment [19].

Av-PGC1α is significantly raised in pectoral muscle and liver during embryonic development of chickens contrasted with growing chicken [20]. As a result, *avPGC1α* upregulation in muscle during prenatal development may be associated with regulating mitochondrial biogenesis and fiber-type determination [21-23]. As well as *avPGC1α* governs organisms' energetic responses to changes in the environment by promoting mitochondrial biogenesis, boosting cellular respiration rates, and enabling energy substrate absorption and use [24, 25]. So, the hypothesis that the development of metabolism during embryogenesis in birds is controlled mainly by *avPGC1α* gene that is upregulated in liver and breast muscle at E-18 and E-20 can be proved [20,26]. *PPARγ* gene is a transcription factor that is recognized to be implicated in many metabolic regulatory pathways, mainly in lipid and glucose metabolism [27]. As a result, in a study to measure *PPARγ* gene expression during myogenesis in liver and breast muscle of birds by Walter and Seebacher [20], they found that *PPARγ* gene expression in muscle is consistently raised during embryogenesis, this contrasts with our finding that *PPARγ* expression was not prominent at prehatch. That might be because various transcription factors as well as coactivators are transcribed at various times, and protein concentrations can be varied by translational control, processing, and posttranslational modifications or activation [28]. mRNA levels of the catalytic α1 subunit associated with the activity of the enzyme, so that Na+/K+-ATPase activity is transcriptionally well-ordered by the catalytic α1 subunit excluding the glycosylated β1 subunit [26]. This agrees with our finding that ATP5E and ATPG1 that are nuclear encoded were not expressed pre-embryonic.

On the other hand, the pattern of expression of mitochondrial genes post-hatching was different than prehatching gene expression. Vives-Bauza et al. [29] reported that *ATPG1* was a subunit of mitochondrial ATP synthase that catalyzes ATP production, is a crucial component of complex V of the oxidative phosphorylation cycle. Huang et al. [30] demonstrated that ATP synthase epsilon subunit (*ATP5E*) gene was realized to encode the mitochondrial F0F1 ATP synthase subunit epsilon. The expression level of *ATPG1*and *ATP5E* were not detected in large and small body weight at day 21, while *ATPG1* upregulated in small body weight at day 35 in contrast to *ATP5E* that upregulated in large body weight at day 35. Bottje et al. [31] reported that as broilers with low FE display increased ROS when compared with age-matched high FE broilers from the same genetic line, the mechanism(s) involved in low FE may be like that of aging and certain diseases. We found that *ATPG1* was higher in low body weight with low FE that usually have higher oxidative stress if compared to high body weight with high FE which agrees with Hu et al. [32] also demonstrated that *ATP5G1* relates to oxidative stress and is implicated in the biological process of oxidative phosphorylation.

Massari et al. [10] reported that *avANT1* had been shown to exert a fundamental metabolic control on mitochondrial energy production, increased expression of *avANT1* in tissue would be expected to indicate increased energy needs or output by the animal. In this study a significant upregulation of *avANT* gene expression in low body weight as compared with high body weight at day 21 and 35 was observed. This is agreed with Iqbal et al. [33] who reported that the expression level of *avANT* gene was higher in the breast muscle of broilers with low FE analogized with high FE, the increased levels of *ANT1* expression in low FE birds compared to high FE birds seem to be part of a comprehensive attempt to retain cellular homeostasis and optimum energy production under a comparatively greater oxidative environmental stress. Ojano-Dirain et al. [34] found that LFE broilers had lower amounts of *avANT* mRNA in the breast muscle. This contradicts prior findings of higher *avANT1* expression in LFE breast muscle [33].

Some of the variables implicated in the mitochondrial biogenesis include *PPAR-* γ and *av-PGC-1α* [6,[33]). *PPAR- γ* is essential for fat deposition in chicken [9]. Wu et al. [8] reported that the *PGC-1α* is the most dominant regulatory protein in mitochondrial biogenesis. Our finding revealed that the expression level of *PPAR- γ* and *avPGC-1α*at day 21 were significantly upregulated in small BW if compared with large BW, while their expression at day 35 exhibited no difference between small BW and large BW broiler breast muscle, this agreed with a study made by Ojano-Dirain et al. [5], who revealed that *PPAR-γ* and *avPGC-1α* mRNA expression exhibited no differences in *PPAR-γ* mRNA expression in breast muscle of high and low FE male broiler at 6 to 7- wk.

In addition to the study of mitochondrial gene expression, we investigate some of growth-related genes including *MyoD*, *MyoG* and *MSTN* that exhibited a different way than mitochondrial genes, in their expression, *MSTN* gene expression was not detected pre-hatching, on the other hand, it was upregulated in low FE birds relative to high FE at day 21 and 35 post hatching. Dushyanth et al. [36] studied the expression level of *MSTN* in three lines of chicken at different pre- and post-hatching stages and found that *MSTN* gene expression was higher at early embryonic stages than late stages, while post hatching the degree of expression was initially minimal, but it steadily rose until the age of 28 days and after then, the expression was again lower than in the earlier chick stage. The gene's fluctuating expression might be implicated in the maturation of many organs during the growing period.

Gu et al. [37] investigated the expression of *MSTN* in pekin duck pectoral muscle embryos at E11, E14, E17, E20, E23, and E26. They found that *MSTN* decreased gradually until it reached its lowest level at E19, after which it slowly increased, implying that E19 and E20 are the fastest points of pectoral muscle development and a vital transition for pekin duck pectoral muscle building during embryonic stages. Additionally, Fergany *et al.* [38] reported that the expression level of *MSTN* gene was significantly high at E7 relative to E16, while the level of *MSTN* at day 35 (marketing age) was higher in low BW relative to high BW broiler.

Considering *MyoD* and *MyoG* gene expression prehatching, it was found that the expression level of *MyoD* gene was higher at E-12 than E-15 relative to E-19 while *MyoG* mostly prominent at E-12 if compered to E-15 and E-19. Fergany *et al.,* [38] who studied *MyoD* and *MyoG* at E-7, E-13 and E-17 found that *MyoD* gene expression was considerably higher at E7 and peaked at E16, whereas *MyoG* gene expression was significantly higher at E7 and peaked at E16 relative to E13. Also, in a previous study to compare between layers and broilers. On the other hand, the expression level of *MyoD* in breast muscle was significantly higher in large BW relative to small BW broilers at day 21 and 35 posthatching, unlike the expression level of *MyoG* gene that has no significant differences between high and low BW broilers either at day 21, but higher in large BW at day 35. Yin et al. [39] stated that, the relative expression level of *MyoG* and *MyoD* in pectorals muscle were superior in high weight selection than low weight selection at 28 day and 56d of mature broiler, Bentzinger et al. [9] explained the reason for that due to *IGF-I*; it is an endocrine hormone that is released by the liver and acts as an autocrine and paracrine hormone; on day 28 high, BW had greater *IGF-I* mRNA expression in the liver than low BW, *MyoD* as well as *MyoG* expression are positively correlate with *IGF-I*.

Kang et al [40] used the genome-wide association studies and gene expression analyses to identify candidate genes for broiler breast muscle weight and intramuscular fat in genetic improvement programs. They demonstrated 43 candidate genes that play roles in the development and metabolism of skeletal muscle or adipocyte. Additionally, they found that a large number of potential genes were hosted by gene co-expression modules, which also showed a strong correlation with the desired traits of interest.

Conclusion

We concluded that the *avANT, PGC-1α, and MyoD* genes major role at pre-hatch and post hatch-till 21th day. *However, MyoG*, *ATPG1,* and ATP*5E* genes play strong roles in skeletal muscle growth at the marketing age which could be useful for the genetic improvement in broiler chickens. Also, we suggested that gene *ATPG1,* and *ATP5E* co-expression showed a strong correlation with the desired trait.

Consent for publication

Not applicable

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicting Interests

The authors declare no potential conflicts of interests with respect to the research, authorship, and/or publication of this article.

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Authors' Contributions

Investigation, Methodology, and Writing – original draft, by D. S.; Supervision by Sh.H., A. E, and S.K. revised and edited manuscript, by Sh.H., A. E, and S.K.

TABLE 1. Primers sequences used in qRT-PCR.

Gene	Sense primer (5′-3′)	Antisense primer (5′-3′)	Annealing (°C)
AvANT	TGTGGCTGGTGTGGTTTCCTA	GCGTCCTGACTGCATCATCA	60
PPARγ	TGGAATGTCACATAATGCCATCA	TCTGCCAAGAGCTTCTCCTTCT	60
avPGC-1α	CCAAAGGACACGCTCTAGATCA	TCTCGATCGGGAATATGGAGAA	60
TP5E	TTGTTGGGAGTGGAGTGT	TCACCATTCACAGAGCAGT	60
ATPG1	GGACACGGCAAGTAATAGG	CATCAAACAGAAGAGACCCA	58
MSTN	GCAAAAGCTAGCAGTCTATG	TCCGTCTTTTTCAGCGTTCT	59
MYOD	GATTTCCACAGACAACTCCACAT	GAATCTGGGCTCCACTGTCACT	60
MYOG	GTGGGATGGTGATGCTGGAA	TTGGAGAGGAGTGGGAAAGGA	60
18s	CGAAAGCATTTGCCAAGAAT	GGCATCGTTTATGGTCGG	60

TABLE 2. Egg weights at different embryonic days (E-12, 15 and 19).

Group	E-12	E-15	E-19
large	11.333±0.882^{a}	19.000±0.000^{a}	43.333±0.333^{a}
Medium	8.667±0.0.333^{b}	17.667±0.000^{b}	42.000±0.000^{b}
Low	7.000±0.000^{b}	16.000±0.567^{c}	40.000±0.000^{c}
P Value	0.0039	<0.0001	<0.0001

Each value represents mean± SE
Values with different letters within the same column are statistically significant.

TABLE 3. Body weight at different weeks post hatch.

Group	D0	1ST W	2nd W	3rd W	4th W	5th W
High	50.868±0.692	148.208±3.821^{a}	322.264±8.408^{a}	617.547±10.931^{a}	1054.340±20.494^{a}	1843.208±24.936^{a}
Medium	48.205±0.807	122.179±4.455^{b}	218.718±9.802^{b}	419.231±12.743^{b}	816.410±23.890^{b}	1318.205±29.069^{b}
Low	48.741±0.970	103.704±5.353^{c}	160.370±11.781^{c}	296.667±15.316^{c}	626.666±28.714^{c}	1028.148±34.936^{c}
P Value	0.0320	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

D0: day zero (hatching day). Each value represents mean± SE
Values with different letters within the same column are statistically significant.

TABLE 4. Growth traits (FC, BG, FE and FCR) at third and fifth weeks.

Group Measurement	High	Medium	Low	P-Value
FC3	490.000	455.000	375.000	
FC5	1300.000	1100.000	1000.000	
BG3	295.283±9.562^{a}	200.513±11.147^{b}	136.296±13.397^{c}	<0.0001
BG5	788.868±22.909^{a}	501.794±26.706^{b}	401.481±32.097^{c}	<0.0001
FE3	0.587±0.022^{a}	0.427±0.026^{b}	0.354±0.031^{b}	<0.001
FE5	0.607±0.019^{a}	0.456±0.022^{b}	0.401±0.026^{b}	<0.001
FCR3	0.816+0.019^{c}	1.097+0.022^{b}	1.284+0.026^{a}	<0.001
FCR5	0.714+0.015^{c}	0.848+0.017^{b}	0.992+0.021^{a}	<0.001

FC: feed consumption. 3 and 5: 3rd and 5th week. BG: body gain. FE: feed efficiency.
FCR: feed conversion ratio. Each value represents mean± SE.
Values with different letters within the same column are statistically significant.

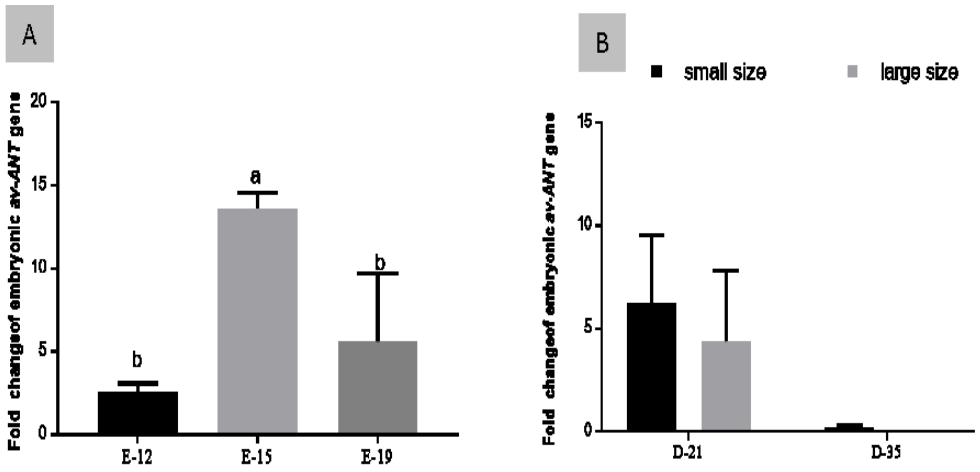

Fig. 1. Fold change of *av-ANT* at (A) prehatch at E-12, 15, and 19, (B) post-hatch at 21st and 35th in small and large size cobb broiler.

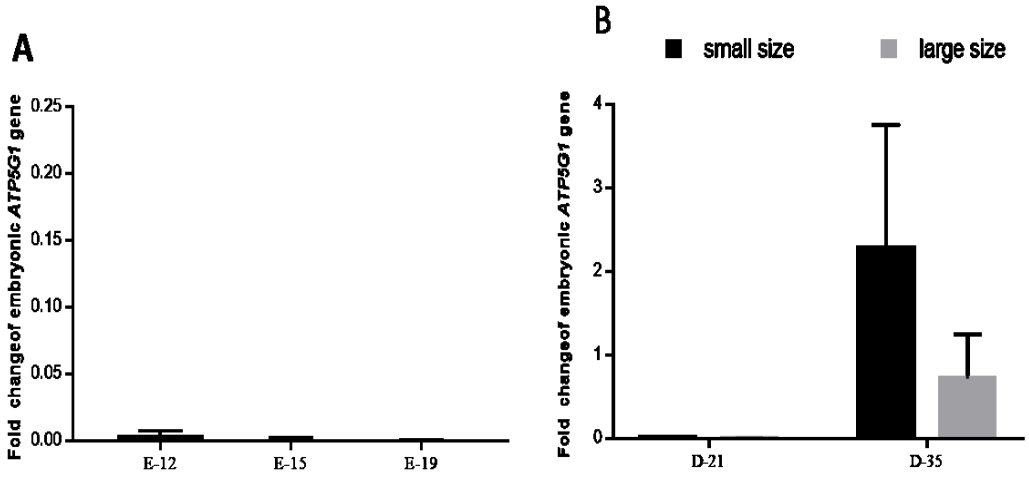

Fig. 2. Fold change of *ATPG1* gene at (A) prehatch at E-12, 15, and 19, (B) post-hatch at 21st and 35th in small and large size cobb broiler.

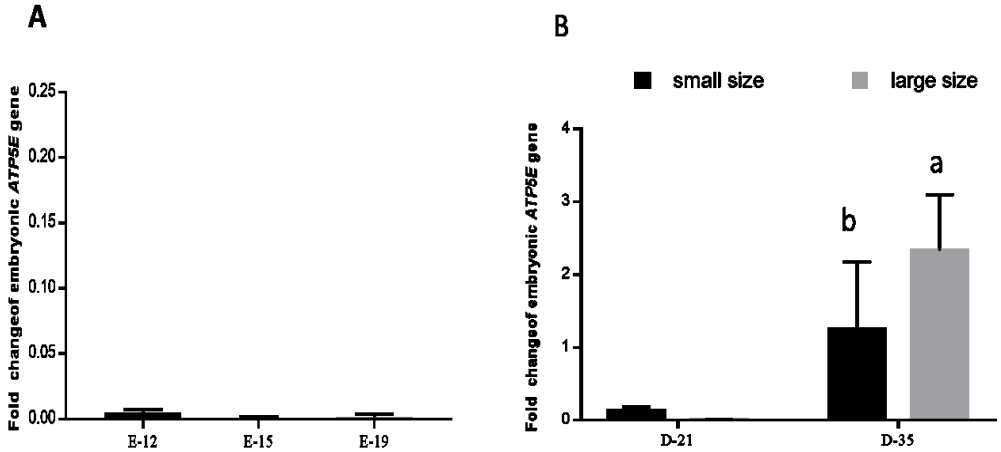

Fig. 3. Fold change of *ATP5E* gene at (A) prehatch at E-12, 15, and 19, (B) post-hatch at 21st and 35th in small and large size cobb broiler.

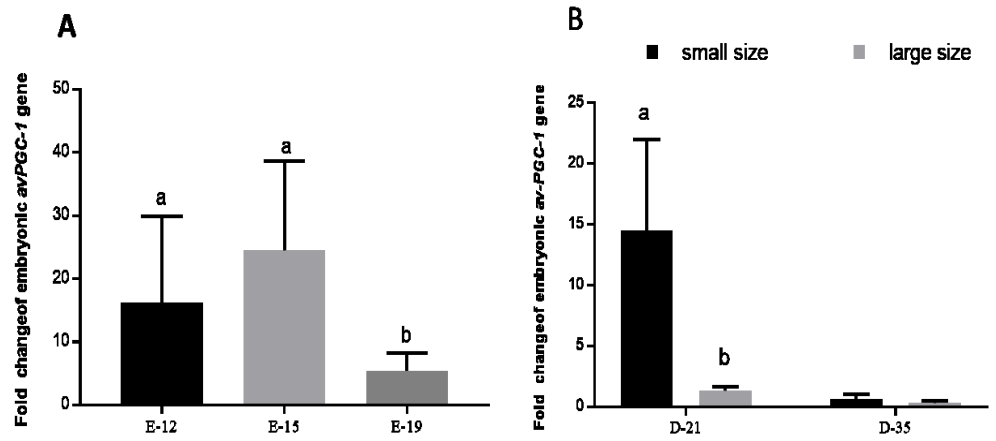

Fig. 4. Fold change of *avPGC-1*gene at (A) prehatch at E-12, 15, and 19, (B) post-hatch at 21st and 35th in small and large size cobb broiler.

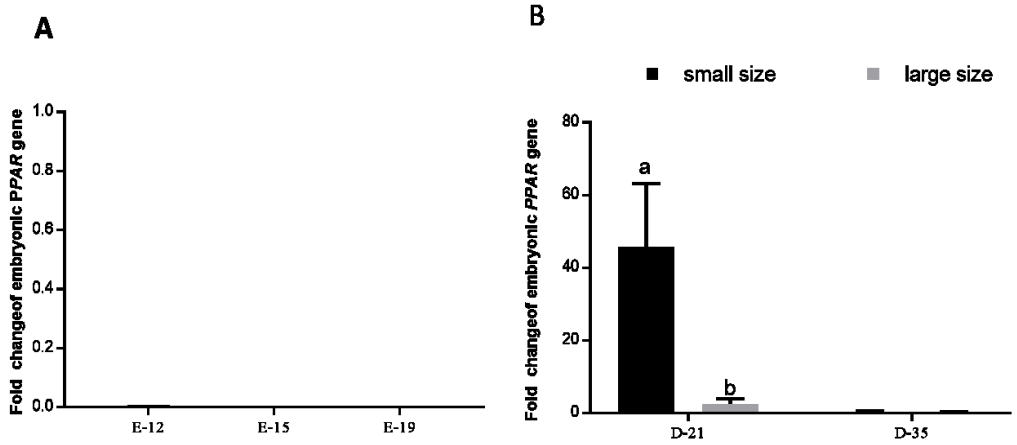

Fig. 5. Fold change of *PPAR$_\gamma$* gene at (A) prehatch at E-12, 15, and 19, (B) post-hatch at 21st and 35th in small and large size cobb broiler.

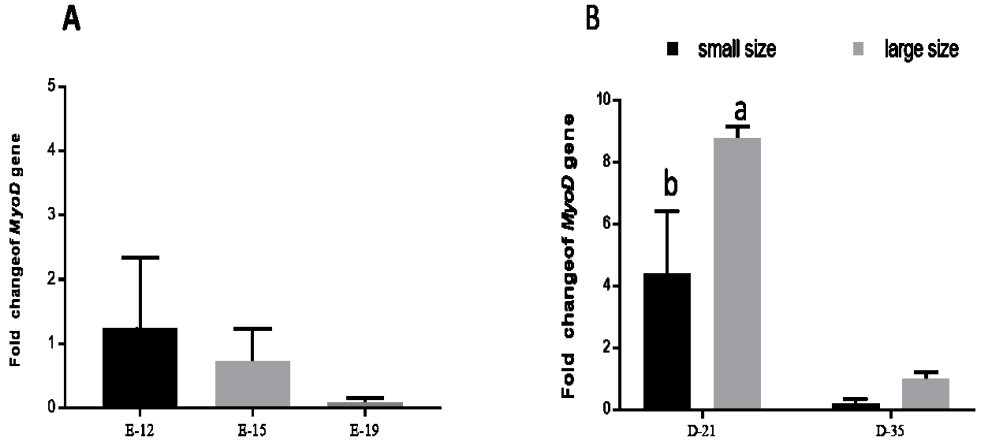

Fig. 6. Fold change of *MyoD* gene at (A) prehatch at E-12, 15, and 19, (B) post-hatch at 21st and 35th in small and large size cobb broiler.

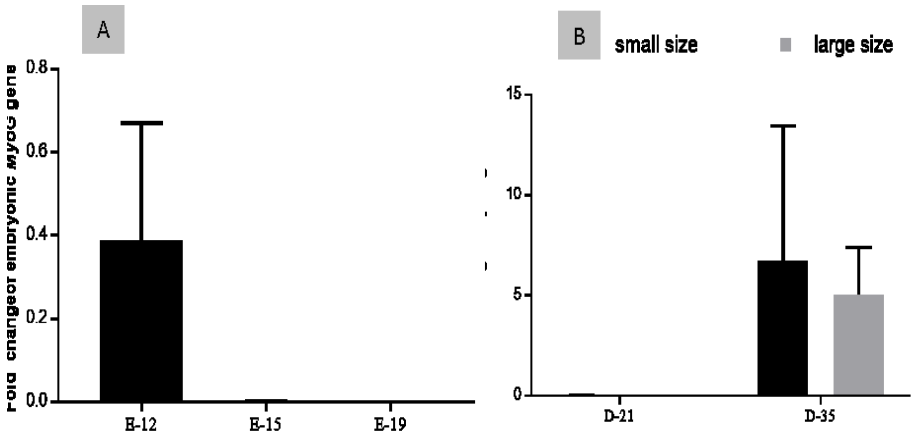

Fig 7. Fold change of *MyoG* gene at (A) prehatch at E-12, 15, and 19, (B) post-hatch at 21st and 35th in small and large size cobb broiler.

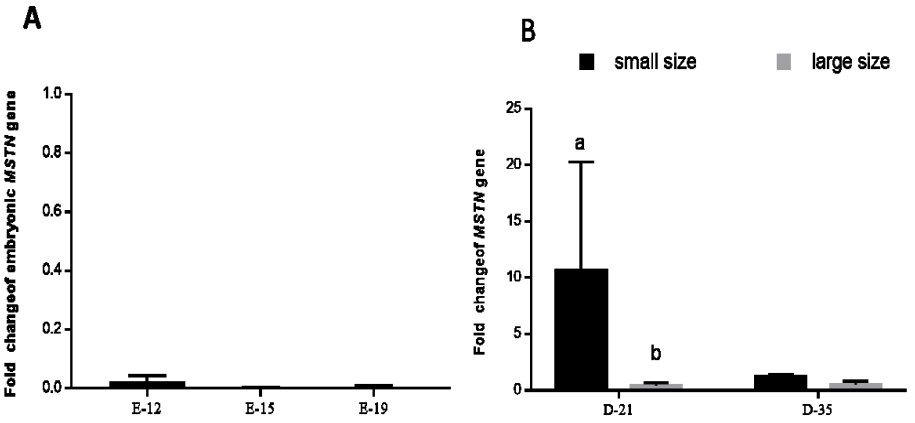

Fig 8. Fold change of *MSTN* gene at (A) prehatch at E-12, 15, and 19, (B) post-hatch at 21st and 35th in small and large size cobb broiler.

References

1. Wang, B. Y., Chien, L. H. and Roan, S. W. A computer simulation model to evaluate the optimal market age of broilers. *Journal of Animal and Veterinary Advances*, **11**(14), 2493-2502 (2012).
2. O'Sullivan, N., Dunnington, E. A., Larson A. and Siegel, P. Correlated responses in lines of chickens divergently detected for fifty-six-day body weight. 2. Organ growth, deoxyribonucleic acid, ribonucleic acid, and protein content. *Poultry Science*, **71** (4), 598-609 (1992).
3. Ojano-Dirain, C., Toyomizu, M., Wing, T., Cooper, M. and Bottje, W.G. Gene expression in breast muscle and duodenum from low and high feed efficient broilers. *Poultry Science*, **86**, 372-381 (2007).
4. Baik, S.H. and Lee, J. Adenine nucleotide translocase 2: an emerging player in cancer. *Stem Cell Research Medcine*, **1** (10), 15761/JSCRM.1000111 (2016).
5. Wang, Y., Miao, X., Li, H., Su, P., Lin, L., Liu, L. and Li, X. The correlated expression of immune and energy metabolism related genes in the response to Salmonella enterica serovar enteritidis inoculation in chicken. *BMC Veterinary Research*, **16** (1), 257 (2020).
6. Wu, Z., Puigserver, P., Andersson, U., Zhang, C., Adelmant, G., Mootha, V., Troy, A., Cinti, S., Lowell, B., Scarpulla, R.C. and Spiegelman, B.M. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell*, **98**,115–124 (1999).
7. Sato, K., Fukao, K., Seki, Y. and Akiba, Y. Expression of the chicken peroxisome proliferator-activated receptor-gamma gene is influenced by aging, nutrition, and agonist administration. *Poultry Science*, **83**(8), 1342-7 (2004).
8. Bentzinger, C.F., Wang, Y.X. and Rudnicki, M.A. Building muscle molecular regulation of myogenesis. Cold Spring Harb. *Perspective Biology*, **4** (2), a008342 (2012).

9. Massari, M.E. and Murre, C. Helix-loop-Helix proteins regulators of transcription in eukaryotic organisms. *Molecular Cell Biology*, **20** (2), 429-440 (2000).

10. Mcpherron, A.C. and Lee, S.J. Double muscling in cattle due to mutations in the myostatin gene. *The Proceedings of the National Academy of Sciences*, **94** (23), 12457–12461 (1997).

11. Livak, J. K. and Schmittgen, D.T. Analysis of relative gene expression data using real time quantitative PCR and the 2-ΔΔct method. *Methods*, **25**, 402-408 (2001).

12. Mortola. J.P and Al Awam, K. Growth of the chicken embryo: Implications of egg size. *Comparative biochemistry and physiology. Part A, Molecular & Integrative Physiology*, **156** (4), 373-379 (2010).

13. Vleck, C.M. and Vleck, D.B. Metabolism and energetics of avian embryos. The Journal of experimental zoology. Supplement: Published under Auspices of the American Society of Zoologists and the Division of *Comparative Physiology and Biochemistry*, **1**, 111-1125 (1987).

14. Prinzinger, R. and Dietz, V. Qualitative course of embryonic o2 consumption in altricial and precocial birds. *Respiration Physiology*, **100** (3), 289-294 (1995).

15. Hulbert, A. J. and Else, P. L. Mechanisms underlying the cost of living in animals. *Annual Review of Physiology*, **62**(1), 207–235 (2000).

16. Speake, B.K., Murray, A.M. and Noble, R.C. Transport and transformations of yolk lipids during development of the avian embryo. *Progress in Lipid Research*, **37**, 1–32 (1998).

17. Rey, B., Spée, M., Belouze, M., Girard, A., Prost, J., Roussel, D. and Duchamp, C. Oxygen recovery up-regulates avian UCP and ANT in newly hatched ducklings. *Journal of Comparative Physiology* B, **180**(2), 239–246 (2009).

18. Walter, I. and Seebacher, F. Molecular mechanisms underlying the development of endothermy in birds (*Gallus gallus*): a new role of PGC-1 alpha? *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, **293**(6), R2315-22 (2007).

19. Scarpulla, RC. Nuclear activators and coactivators in mammalian mitochondrial biogenesis. *Biochimica et Biophysica Acta,* **1576**, 1–14 (2002).

20. Baar, K., Wende, A.R., Jones, T.E., Marison, M., Nolte, L.A., Chen, M.A.Y., Kelly, D.P. and Holloszy, J.O. Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1. *FASEB Journal*, **16**, 1879 – 1886 (2002).

21. Lin, J., Wu, H., Tarr, P.T., Zhang, C.Y., Wu, Z.D., Boss, O., Michael, L.F., Puigserver, P., Isotani, E., Olson, E.N., Lowell, B.B., Bassel-Duby. R. and Spiegelman, BM. Transcriptional Co-activator PGC-1 drives the formation of slow-twitch muscle fibers. *Nature*. **418**, 797– 801 (2002).

22. Houten, S. M. and Auwerx, J. PGC-1alpha: turbocharging mitochondria. *Cell*, **119**(1), 5–7 (2004).

23. Hood, D.A., Irrcher, I., Ljubicic, V. and Joseph, A.M. Coordination of metabolic plasticity in skeletal muscle. *Journal Experimental Biology*, **209**, 2265–2275 (2006).

24. Walter, Isabel and Seebacher, Frank. Endothermy in birds: underlying molecular mechanisms. *J. Journal Experimental Biology*, **212**, 2328-2336 (2009).

25. Walczak, R. and Tontonoz, P. PPARadigms and PPARadoxes: Expanding roles for PPAR in the control of lipid metabolism. *Journal of Lipid Research*, **43**, 177–186 (2002).

26. Greenbaum, D., Colangelo, C., Williams, K. and Gerstein, M. Comparing protein abundance and mRNA expression levels on a genomic scale. *Genome Biology*, **4**,117 (2003).

27. Vives-Bauza, C., Magrane, J., Andreu, A.L. and Manfredi, G. Novel role of ATPase subunit C targeting peptides beyond mitochondrial protein import. *Molecular Biology of the Cell*, **21**(1), 131–139 (2010).

28. Huang, Y.J., Jan, Y.H., Chang, Y.C., Tsai, H.F. and Hsiao, M. ATP synthase subunit Epsilon overexpression promotes metastasis by modulating AMPK signaling to induce epithelial-to-mesenchymal transition and is a poor prognostic marker in colorectal cancer patients. *Journal of Clinical Medicine*, **8**(7), 1070 (2019).

29. Bottje, W., Iqbal, M., Tang, Z. X., Cawthon, D., Okimoto, R., Wing, T. and Cooper, M. Association of mitochondrial function with feed efficiency within a single genetic line of male broilers. *Poultry Science,* **81**(4), 546–555 (2002).

30. Hu, Y., Chen, X., Lin, H., Hu, Y. and Mu, X. Study on the antiendotoxin action of pulsatillae decoction using an affymetrix rat genome array. *Cell Immunology*, **l**. 257(1–2), 32–37 (2009).

31. Iqbal, M., Pumford, N. R., Tang, Z. X., Lassiter, K., Wing, T., Cooper, M. and Bottje, W. Low feed efficient broilers within a single genetic line exhibit higher oxidative stress and protein expression in breast muscle with lower mitochondrial complex activity. *Poultry Science,* **83** (3), 474–484 (2004).

32. Ojano-Dirain., Pumford C., N. R., Iqbal M., Wing T., Cooper M. and Bottje W. G. Biochemical evaluation of mitochondrial respiratory chain in duodenum of low and high feed efficient broilers. *Poultry Science,* **84**, 1926–1934 (2005).

33. Nisoli, E., Clementi, E., Paolucci, C., Cozzi, V., Tonello, C., Sciorati, C., Bracale, R., Valerio, A., Francolini, M., Moncada, S. and Carruba, MO. Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. *Science*, **299**, 896-899 (2003).

34. Dushyanth, K., Bhattacharya, T. K., Shukla, R., Chatterjee, R. N., Sitaramamma, T., Paswan, C. and Guru Vishnu, P. Gene expression and polymorphism of Myostatin gene and its association with growth traits in chicken. *Animal Biotechnology*, **27**(4), 269–277 (2016).

35. Gu, L.H., Xu, T.S., Huang, W., Xie, M., Shi, W.B., Sun, S.D. and Hou, S.S. Developmental characteristics of pectoralis muscle in Pekin duck embryos. *Genetics and Molecular Research*, **12** (4), 6733-6742 (2013).

36. Fergany, A.M., Hemeda, S.A., El-Nahas, A.F. and Abd, W.S. Polymorphism and expression of some myogenic genes at embryonic stages and 37 days age of Cobb broiler chickens and their impact on the marketing weights. International *Journal of Recent Scientific Research*, **8** (8), 19435-19440 (2017).

37. Yin, H., Zhang, S., Gilbert, E.R., Siegel, P.B., Zhu, Q. and Wong, E.A. Expression profiles of muscle genes in postnatal skeletal muscle in lines of chickens divergently selected for high and low body weight. *Poultry Science,* **93**(1), 147-154 (2013).

التعبير الجيني لبعض الجينات المرتبطة بالميتوكوندريا والنمو وأثرها على الصفات الإنتاجية في دجاج اللحم خلال فترة ما قبل الفقس وما بعد الفقس

دعاء صالح، شعبان حميده، عبير النحاس وشيماء خطاب

قسم رعاية الحيوان وتنمية الثروة الحيوانية – معمل الوراثة – كلية الطب البيطري – جامعة الإسكندرية – مصر.

الملخص

أجريت دراسة HIS لتقييم الصفات الإنتاجية لدجاج التسمين كوب والتعبير عن التمثيل الغذائي للطاقة (avANT وATP5G1 وATP5E) والجينات المرتبطة بالتكوين الحيوي للميتوكوندريا (PPARγ وavPGC-1α). أيضًا، التعبير عن بعض الجينات المرتبطة بالنمو (MyoD وMyoG وMSTN) في عضلات الصدر أثناء ما قبل الفقس وما بعد الفقس. مرحلة ما قبل الفقس في الأيام 12 و15 و19 ومرحلة ما بعد الفقس في الأيام 21 و35. تم وزن الطيور بشكل فردي عند 0 و7 و14 و21 و28 و35 يومًا. تم تقييم مؤشرات أداء النمو مثل TBWG وFC وFE وFCR واستهلاك العلف الأسبوعي وإجمالي تناول العلف. لوحظت مستويات مختلفة من التعبير عن جينات avANT وavPGC-1α في المراحل الجنينية المختلفة. لم يتم الكشف عن مستوى التعبير عن ATP5G1 وATP5E وPPARγ في مرحلة ما قبل الفقس. وفي الوقت نفسه، كان ATP5G1 أعلى في أوزان الجسم الصغيرة على عكس ATP5E الذي كان أقل بشكل ملحوظ في الطيور ذات الوزن الجسمي الصغير. أظهر avPGC-1α وPPARγ في اليوم الحادي والعشرين ارتفاعًا ملحوظًا في أوزان الجسم الصغيرة، بينما في اليوم الخامس والثلاثين، لم يكن هناك فرق كبير في التعبير عنهما في دجاج التسمين ذو الوزن الجسمي الصغير والكبير. لوحظ القليل من التعبير عن الجينات المرتبطة بالنمو في مرحلة ما قبل الفقس. بعد الفقس، كان MyoD أعلى في أوزان الجسم الكبيرة مقارنة بدجاج التسمين ذو الوزن الجسمي الصغير. تم رفع مستوى MSTN في أوزان الجسم الصغيرة مقارنة بالكبيرة.

لقد خلصنا إلى أن الجينات avANT وPGC-1α وMyoD لها دور رئيسي في مرحلة ما قبل الفقس وبعد الفقس حتى اليوم الحادي والعشرين. ومع ذلك، تلعب MyoG وATPG1 وATP5E أدوارًا قوية في نمو العضلات الهيكلية في سن التسويق مما قد يكون مفيدًا للتحسين الوراثي في دجاج التسمين.

الكلمات الدالة: دجاج اللحم كوب، التعبير الجيني، جينات الميتوكوندريا، مرحلة ما قبل الفقس وما بعده.