



Comparative Study for FABP7, TPD52L1, NCOA7, GJA1, and ASF1A mRNA Expressions in Four Chicken Breeds and Their Relationship with Carcass Traits



CrossMark

Dalia M. Aboelhassan^{1*}, Inas S. Ghaly¹, Hasnaa A. Radwan¹, Hesham Abozaid², Eman R. Hassan³ and Ibrahim M. Farag¹

¹Department of Cell Biology, Biotechnology Research Institute, National Research Centre, Giza, Egypt.

²Department of Animal Production, Agricultural and Biology Institute, National Research Centre, Giza, Egypt.

³Department of Poultry Disease, Veterinary Research Institute, National Research Centre, Giza, Egypt.

IN chickens, the breast and thigh weights are the most essential meat traits that are needed for production efficiency and economic benefits by affecting the appearance of the carcass. It is necessary to identify the specific genetic markers associated with such carcass traits. The use of gene expression is a desirable and reliable process for this purpose. The present study aimed to evaluate the effects of expression levels of five genes, FABP7, TPD52L1, NCOA7, GJA1, and ASF1A on breast and thigh weights in native Dokki-4 breed, hybrid Mamourah and Inshas breeds and imported Leghorn breed. Compared to the native breed Dokki-4, the results indicated that the high expression of GJA1 and ASF1A, genes in Mamourah breed as well as the up-regulation of ASF1A gene expression in Inshas and Leghorn breeds were pronounced to be associated with high breast weight. Moreover, the overexpression of FABP7 and GJA1 genes in Mamourah breed and high expression of GJA1 in Inshas and Leghorn breeds were found to be correlated with increasing thigh weight. Whereas, in the native Dokki-4 breed, the high expression of TPD52L1 and NCOA7 genes in breast muscle and ASF1A gene in thigh muscle were accompanied by reducing the weights of such muscles as compared to hybrid and imported chicken breeds.

In conclusion, the present findings confirmed that the expressions of these genes can be useful genetic markers in chicken breed selection for improving carcass traits through increasing the breast and thigh weights.

Keywords: Chickens, Breast and thigh weights, mRNA expression, Genes.

Introduction

The improvement of carcass traits in chickens was revealed to be important for production efficiency and economic benefits for animal production Farms and breeders. This improvement has become very necessary for meeting human needs and the development of food industries in Egypt and several countries of the world [1, 2]. The utilization of traditional chicken breeding programs for the enhancement of favorable

carcass traits takes more time and a big effort because these traits are complex and controlled by multiple genes [3]. So, it is necessary to understand the genetic basis that affects carcass traits to identify the specific genetic parameters or markers that are considered major agents for rapid improvement. Gene expression analyses were found to be an important one of such parameters [4-6]. The use of gene expression is a desirable and reliable procedure that allows the detection

*Corresponding author: Dalia M. Aboelhassan, E-mail: dalia_hemdan@yahoo.com, Tel.: 01003250518

ORCID: 0000-0003-4340-1157

(Received 30/03/2023, accepted 14/05/2023)

DOI: 10.21608/EJVS.2023.202993.1475

©2023 National Information and Documentation Center (NIDOC)

of the favorable genes that are correlated with the determination of the interest of economic carcass traits [5-7]. The assaying of expression levels of the valuable genes had been found to exhibit the best levels that judge the essential chicken traits [3,8,9].

The most important genes whose expressions were implemented to be linked with improving the chicken carcass traits were those associated with encoding and generating protein profiles, such as FABP7, GJA1, ASF1, NCOA7, and TPD52L1. FABP7 (Fatty acid-binding protein7) gene, belongs to the FABP gene family that was demonstrated to encode the cluster of intercellular lipid-binding proteins and transport them to body organs for lipid metabolism or storage. These proteins were clarified to play various roles in metabolism regulation and can protect the different body tissues against peroxide formation as antioxidant-type behavior [6,10].

GJA1 (gap junction protein alpha 1) gene, was revealed to encode and generate one of the most abundant connexin proteins (Connexin 43) that have 43 kDa molecular weight. These proteins were found to be a major factor that affects different muscle development and growth traits in chickens [11].

ASF1 (anti-silencing function 1) gene, is known as a well-conserved protein from yeast to humans. The protein of the vertebrate ASF1 gene was confirmed to act as a histone chaperone that participates in different chromatin-mediated cellular processes and is necessary for cell proliferation [12]. Also, Liu *et al.* [7] reported in chickens that the protein of the ASF1 gene was revealed to be essential for breast muscle weight.

NCOA7 (The nuclear receptor coactivator 7) gene was shown to encode the coactivator proteins that are used for the reduction of oxidative lesions on DNA inside the nucleus. These proteins were found to contain domains that have similar functions to those identified in the mitochondrial OXR-1 protein. The expression of the NCOA7 gene was revealed to be predominantly in breast tissues and neuroblastoma cells [13].

TPD52L1 (Tumor protein D52-like1 protein) gene was established to be responsible for encoding a member of the protein family that includes coiled-coil domains forming hetero- or homomers. These proteins are involved in calcium signaling and cell proliferation [7]. So, the goal of the present study was conducted to

exhibit the effects of expression levels of FABP7, GJA1, ASF1, NCOA7, and TPD52L1 genes on carcass traits, especially breast and thigh muscle weights, to develop molecular parameters for improving such favorable types of interest economical meat production of different chicken breeds in Egypt.

Material and Methods

Chicken breeds

Four chicken breeds, including the Egyptian native strain (Dokki-4), one imported strain (White Leghorn), and two hybrid strains (Mamourah and Inshas) were selected. The Mamourah strain is an inferred strain as a result of the intermarriage of males of the Alexandria strain (Egyptian native) with females of Dokki-4 strain (Egyptian native). Inshas strain had been developed as a result of mating between Sinai (Egyptian native) and White Plymouth Rock (imported) strains. These chicken breeds were raised under the same environment and management. The broilers were fed yellow maize, soybean cake, full fat extruded soybean, guar Korma cape, calcium phosphate, mixed vitamins, and minerals hydroxymethionine, analog calcium, sodium bicarbonate, calcium carbonate table salt. The nutritional analysis of the above ingredients clarified that the crude protein was not less than 23%; crude fat was not less than 5.76%; crude fiber was not more than 4.2% and representative energy of not less than 3000 kcal/kg diet. Chickens were slaughtered at 30 days and the carcass was eviscerated, weighed, and dissected. Breast (boneless, skinless breast fillet) and thigh, were isolated and weighed. 140 samples of chicken were distributed as 25 samples of breast and 10 samples of the thigh were collected from each breed. Tissues including breast and thigh were rapidly removed, snap-frozen in liquid nitrogen, and stored at -80°C until use.

Gene expression analysis

RNA Extraction

Total RNA was extracted from the tissues using TRIzol® reagent kit (Invitrogen, Germany) according to the manufacturer's protocol of the kit. Briefly, 50 mg of tissues had been separately homogenized in 750 µl of TRIzol® reagent in an autoclaved mortar and incubated for 5 min at room temperature. After incubation, 140 µl chloroform was added to the homogenized samples; the mixture was shaken vigorously, and centrifuged at 12,000xg for

15 min at 4°C. Then, the RNA in the aqueous phase was carefully separated, precipitated by adding 600 µl of 100% isopropyl alcohol, and centrifuged at 12,000xg for 10 min at 4°C. The RNA pellet was washed with 70% DEPC ethanol and dissolved in RNase-free water at 60°C. A nanodrop-1000 spectrophotometer (Thermo Scientific, Rockford, IL, USA) was used to measure RNA concentration and determine its quality. The ratio of absorbance at 260/280 nm was determined between 1.8 and 2.1 for the samples. All samples of RNA were treated with DNase I according to the manufacturer protocol (Grisp, Portugal) to degrade any contamination with genomic DNA, then stored at -80°C until use.

Complementary DNA (cDNA) synthesis

To synthesize the complementary DNA, isolated total RNA was reverse transcribed into cDNA using oligo (dT)₁₅ primer Maxime™ RT PreMix Kit (iNtRON Biotechnology, Korea, Cat. No.25081/96 tubes). The reaction volume was carried out in 20 µl and prepared according to the kit instruction. The reverse transcription (RT) reaction was performed for 60 min at 45°C, RTase inactivation step was terminated for 5 min at 95°C. PCR products containing the cDNA were kept at -20°C until used for DNA amplification [5, 6, 14]. Primers for the genes FABP7, TPD52L1, NCOA7, GJA1, ASF1, and β-actin gene (housekeeping gene) were designed according to Liu et al. [7] (Table 1).

Relative quantification of mRNA expression

Real-Time PCR was performed using TOPreal™ qPCR 2X PreMIX (SYBR Green with low ROX) (enzynomics Korea) to study the relative mRNA expression of FABP7, GJA1, ASF1, NCOA7, and TPD52L1 genes. The final concentration of the primers was 10 pmol. The total volume of the reaction consisted of 20 µl [1 µl of cDNA, 1 µl of each primer set, 10 µl of SYBR green master mix, and 7 µl water].

A Step One Plus instrument (Agilent Stratagene mx3000p) was used, and the enzyme was activated at 95°C for 15 min. denaturation was done at 95°C for 10 sec. and annealing/extension was carried out at 60°C for 15sec. and elongation at 72°C for 30 sec. with forty amplification cycles. Melting curve analysis was also carried out for each target marker. The endogenous control β-actin was used to normalize the Ct values, where $\Delta Ct = (Ct \text{ gene of interest} - Ct \text{ of reference gene})$. The evaluation of the expression of mRNA of each sample was determined by using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

All data including weights of carcass, breast, and thigh as well as gene expression assay were analyzed using General Linear Models (GLM) procedure of the Statistical Analysis System (SAS) [15] followed by Scheffe-test to assess significant differences between groups. The values are expressed as mean±SEM. All statements of significance were based on the probability of $P < 0.05$.

TABLE 1

Gene	Sequence	Product size (bp)	Accession number
FABP7	F: 5'-CGTGATCAGGACTCAGAGCA-3' R: 5'-TCTCTTTGCCATCCCATTC-3'	158	NM_205308.2
TPD52L1	F: 5'-TCAGCGTACAAGAAGACGCA-3' R: 5'-GGCATGCTTATGGAATGGCG-3'	152	NM_204215.1
NCOA7	F: 5'-CAATTGTTCCAGGCCAGATT-3' R: 5'-TCTTGCCAAATCAGCATCAG-3'	137	NM_001012878.1
GJA1	F: 5'-CATCAGCAGCGCCAATATC-3' R: 5'-TTCATCTCCCAAGCAGACT-3'	171	NM_204586.2
ASF1A	F: 5'-GACCTGTGCGAAGATTGGA-3' R: 5'-GGAATAAGCCCTGGGTTAGG-3'	158	NM_001044690.1
β-actin	F: 5'-GAGAAATTGTGCGTGACATCA-3' R: 5'-CCTGAACCTCTCATTGCCA-3'	152	NM_205518

*F: Forward

*R: Reverse

Results

The results of carcass, breast, and thigh weights of four different chicken breeds were recorded in Table 2. After normalization and statistical analysis of five genes (FABP7, TPD52L1, NCOA7, GJA1, and ASF1A), mRNA expression levels were identified in breast and thigh muscle tissues of the studied chicken breeds (Tables 3, 4 and Figures 1, 2).

Breast weight and gene expression of native Dokki-4 breed and hybrid Mamourah and Inshas breeds

The results showed a significant increase ($P < 0.05$) in the breast weight of Mamourah breed (57.9 ± 3.24) compared to Dokki-4 breed (23.1 ± 3.2). Mamourah breed showed high expressions of GJA1 (0.39 ± 0.07) and ASF1A (0.35 ± 0.11) genes, and low expression of TPD52L1 (0.17 ± 0.07) and NCOA7 (0.14 ± 0.06) genes as corresponding to GJA1 (0.17 ± 0.09), ASF1A (0.15 ± 0.05), TPD52L1 (0.29 ± 0.12) and NCOA7 (0.22 ± 0.08) genes of Dokki-4 breed, in contrast to the expression of FABP7 gene that possessed no significant difference between Mamourah breed (0.47 ± 0.13) and Dokki-4 breed (0.48 ± 0.15). Also, Inshas breed possessed

a significant increase ($P < 0.01$) in the breast weight (90.8 ± 17.6) compared to Dokki-4 breed (23.1 ± 3.2). Inshas breed was discriminated with higher expression of ASF1A gene (0.17 ± 0.05) and lower expressions of FABP7 (0.14 ± 0.02), TPD52L1 (0.02 ± 0.01), NCOA7 (0.09 ± 0.02) and GJA1 (0.12 ± 0.03) genes than those observed in ASF1A (0.15 ± 0.05), FABP7 (0.48 ± 0.15) and TPD52L1 (0.29 ± 0.12) genes in Dokki-4 breed (Tables 2, 3 and Fig. 1).

Breast weight and gene expression of imported Leghorn breed

The results clarified a significant increase ($P < 0.05$) in the breast weight of Leghorn breed (52.48 ± 5.92) compared to that identified of Dokki-4 breed (23.1 ± 3.2). The Leghorn breed was characterized by overexpression of ASF1A gene (0.31 ± 0.13) and low expression of FABP7 (0.19 ± 0.04) and NCOA7 (0.05 ± 0.02) genes concerning those genes (ASF1A (0.15 ± 0.05), FABP7 (0.48 ± 0.15) and NCOA7 (0.22 ± 0.1)) that detected in Dokki-4 breed. On the other hand, there was no significant difference in the expression of TPD52L1 (0.05 ± 0.01) and GJA1 (0.14 ± 0.04) genes of Leghorn breed and TPD52L1 (0.29 ± 0.12) and GJA1 (0.17 ± 0.09) genes of Dokki-4 breed (Tables 2, 3 and Fig. 1).

TABLE 2. Carcass, breast and thigh weights of different chicken breeds at 30 days old.

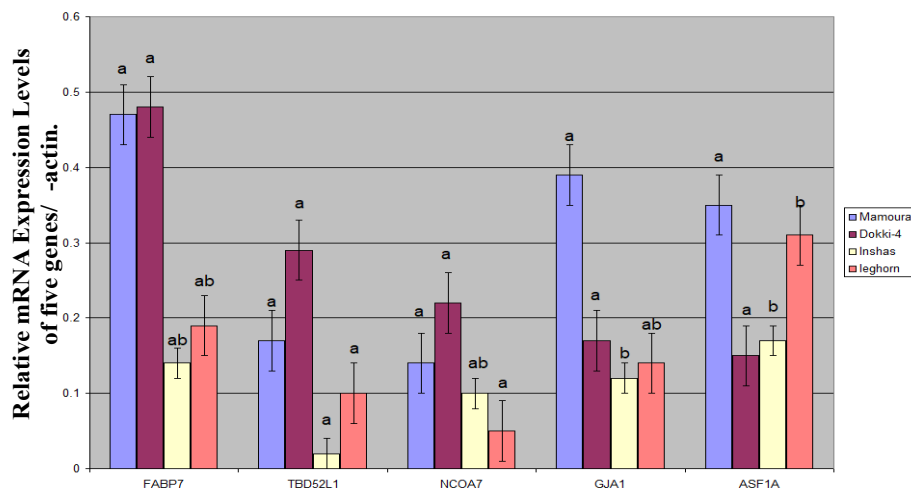
Breed	Carcass wt.	Breast wt.	Thigh wt.
Mamourah	266.2 ± 5.39^b	27.9 ± 3.24^b	25.8 ± 1.7^b
Dokki-4	108.8 ± 7.0^a	23.1 ± 3.2^a	15.46 ± 1.3^a
Inshas	391 ± 16.27^c	90.8 ± 17.6^c	41.2 ± 2.9^c
leghorn	299.2 ± 15.7^b	52.48 ± 5.92^b	30.52 ± 2.8^b

*wt.= weight. Breast fillet= boneless, skinless breast fillet. All data are expressed as mean \pm SEM; a, b, c, means with different letters are significantly different ($P \leq 0.05$).

TABLE 3. FABP7, TPD52L1, NCOA7, GJA1 and ASF1A gene expression in breast muscle of different chicken breeds at 30 days old.

Breed	Gene Expression in breast muscle				
	FABP7	TPD52L1	NCOA7	GJA1	ASF1A
Mamourah	0.47 ± 0.13^a	0.17 ± 0.07^a	0.14 ± 0.06^a	0.39 ± 0.17^a	0.35 ± 0.11^a
Dokki-4	0.48 ± 0.15^a	0.29 ± 0.12^a	0.22 ± 0.08^a	0.17 ± 0.09^a	0.15 ± 0.05^a
Inshas	0.14 ± 0.02^b	0.02 ± 0.01^a	0.09 ± 0.02^{ab}	0.12 ± 0.03^b	0.17 ± 0.05^b
Leghorn	0.19 ± 0.04^{ab}	0.05 ± 0.01^{ab}	0.05 ± 0.02^a	0.14 ± 0.04^{ab}	0.31 ± 0.13^b

*All data are expressed as mean \pm SEM; a, b, means with different letters are significantly different ($P \leq 0.05$).



Inshas and Leghorn) at 30 days old.

Thigh weight and gene expression of native Dokki-4 and hybrid Mamourah and Inshas breeds

The present results (Tables 2, 4 and Figure 2) identified a significant increase ($P < 0.05$) in the thigh weight of Mamourah breed (25.8 ± 1.7) compared to that found in Dokki-4 breed (15.46 ± 1.3). Mamourah breed showed up-regulation in the expression of FABP7 (0.76 ± 0.01) and GJA1 (0.25 ± 0.01) genes and down-regulation in the expression of ASF1A (0.55 ± 0.01) gene compared with those observed (FABP (0.48 ± 0.01), GJA1 (0.03 ± 0.02) and ASF1A (0.7 ± 0.07)) in Dokki-4 breed. Also, the results confirmed a high value with a highly significant increase ($P < 0.01$) in the thigh weight of Inshas breed (41.2 ± 2.9) than that recorded in Dokki-4 breed (15.46 ± 1.3). Inshas breed was discriminated with the increased rate of GJA1 gene expression (0.09 ± 0.01) and down-regulation in the expression of ASF1A (0.01 ± 0.004) and FABP7 (0.18 ± 0.03) genes as compared to those determined (GJA1 (0.03 ± 0.02), ASF1A (0.7 ± 0.7) and FABP7 (0.48 ± 0.01), respectively) in Dokki-4 breed. In contrary to the expression of TPD52L1 and NCOA7 genes of Mamourah and Inshas that demonstrated no significant differences compared to Dokki-4 breed.

Thigh weight and gene expression of imported Leghorn breed

The results (Tables 2, 4 and Fig. 2) observed significant increases ($P < 0.05$) in the thigh weight (30.52 ± 2.8) of Leghorn breed to that established of Dokki-4 breed (15.46 ± 1.3). Leghorn breed was discriminated with up-regulation of GJA1 gene expression (0.3 ± 0.1) and down-regulation

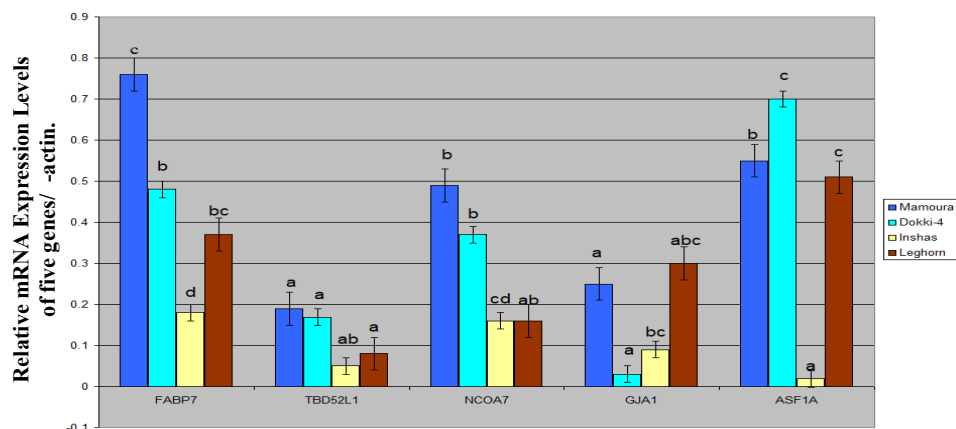
in the expression of TPD52L1 (0.08 ± 0.02) and NCOA7 (0.16 ± 0.1) genes in comparison with GJA1: 0.03 ± 0.02 , TPD52L1 (0.17 ± 0.02) and NCOA7 (0.37 ± 0.09) in Dokki-4 breed, whereas the expression of FABP (0.37 ± 0.1) and ASF1A (0.51 ± 0.15) genes did not show any significant differences compared to Dokki-4 breed (0.48 ± 0.01 and 0.7 ± 0.07 , respectively).

Discussion

The five candidate genes, NCOA7, TPD52L1, FABP7, GJA1, and ASF1A were detected to be located near or they were clustered within the important region (61/83Mb-68.57Mb) on chicken (*Gallus gallus*, GGA3) chromosome 3, this GGA3 region was revealed to be associated with breast muscle weight (BrW) and breast muscle percentage (BrP) in the genome-wide association studies (GWAS) [7].

Previously, several studies used GWAS, in the animal breeding field, and successfully confirmed that the loci or narrow regions were found to affect complex traits such as growth rate, milk production, and fertility in cattle [16-18], meat color, intramuscular fat content and body composition in pigs [19,20], and rates of growth and quality of eggs in chicken [21].

In chickens, breast and thigh muscle yields were demonstrated to be the most important meat traits, because they were revealed to be crucial for the efficiency of the production and economic benefit through their affecting the appearance of the carcass [3].



and Leghorn) at 30 days old.

TABLE 1

Breed/Genes	FABP7	TPD52L1	NCOA7	GJA1	ASF1A
Mamourah	0.76±0.01 ^c	0.19±0.06 ^a	0.49±0.05 ^b	0.25±0.01 ^a	0.55±0.11 ^b
Dokki-4	0.48±0.01 ^b	0.17±0.02 ^a	0.37±0.09 ^b	0.03±0.02 ^a	0.7±0.07 ^c
Inshas	0.18±0.03 ^d	0.05±0.02 ^{ab}	0.16±0.03 ^{cd}	0.09±0.01 ^{bc}	0.01±0.004 ^a
leghorn	0.37±0.1 ^{bc}	0.08±0.02 ^a	0.16±0.1 ^{ab}	0.3±0.05 ^{abc}	0.51±0.15 ^c

*All data are expressed as mean ± SEM; a, b, c, d, means with different letters are significantly different (P≤0.05).

So, the present work studied the effect of expression levels of five candidate genes, NCOA7, TPD52L1, FABP7, GJA1, and ASF1A, on carcass traits, especially breast and thigh weights in four chicken breeds including Mamourah, Dokki-4, Inshas, and Leghorn strains. The expression levels of the mentioned five genes differed between chicken strains. Some gene expressions were up-regulations and others were down-regulations. These gene expressions were found to affect breast and thigh weights.

Our findings were similar to that reported in another study by Tan et al. [3] who compared the gene expressions between two lines of Jingxing yellow chicken breed involving one original (without human-driven selection) line and the other selected one (selected according to increase intramuscular fat content). Their results clarified the expression levels of the IGF2BP1 and some HOXB2 family genes had differed between the two lines. Where, 2424 differentially expressed genes were defined, involving 482 down-regulations and 2012 up-regulations. The up-regulation of the IGF2BP1 gene was associated

with high breast weight in chickens. Interestingly, the down-regulation of HOXB2 gene expressions was revealed to be positively associated with breast muscle weight. Additionally, Kang et al. [9] used large-scale transcription sequencing and analyzed by three regularizing linear regression models (LMM, LASSO, and EN) to identify the expressions of candidate genes affecting breast muscle weight in Tiannong partridge chickens. LMM (Linear mixed model), LASSO (the least absolute shrinkage and selection operator), and EN (elastic net) methods exhibited 43 candidate genes with different expression levels. Some gene expressions had positive effects and others had negative effects on breast muscle weights. By using LMM method, only FOXO3 gene expression was found to have a significant association with the breast muscles, while by using LASSO method, the expressions of candidate genes were revealed to possess a positive effect on breast muscle weights, except PABPC1, AMY1A, and SERPINB6L gene expressions. However, by using EN method, the levels of 43 gene expressions were observed to have negative effects on breast muscle weights.

Concerning the association between high gene expressions and high weights of a chicken body, our results indicated that the high expression, especially of GJA1 and ASF1A in Mamourah breed as well as the up-regulation of ASF1A gene expressions in each of Inshas and Leghorn breeds had been associated with high breast weight. On the other hand, the overexpression of FABP7 and GJA1 gene in Mamourah birds and high expression of GJA1 gene in both Inshas and Leghorn breeds were correlated with increasing thigh weight. These results were supported by Liu et al. [7] who studied in Beijing-You chicken breed (Chinese chickens) the expressions of five candidate genes (NCOA7, TPD52L1, FABP7, GJA1, and ASF1A) and their relationships with improving breast muscle weight. Their results showed only a highly significant ($P < 0.001$) association between overexpression of GJA1 gene and elevation of breast muscle weights, these authors suggested that the GJA1 gene expression has a possibility for the development of breast muscle in the chickens. Also, in Korean chicken breeds (ROSS-308), Park et al. [22] found by Real-time PCR that FABP7 gene expressions were down-regulated in the low body weight group as compared to those observed in the high body weight group, and these authors postulated that the increased expression of FABP7 gene might be associated with the increasing of chicken body weight.

Other studies clarified the functions of the FABP gene expressions in the chickens that were found to be essential for the inactivation of lipid peroxidation in the cell membrane, participation in the absorption of long-chain fatty acids after digestion in the small intestine into the cells of intestinal epithelial and transport these fatty acids to the organisms for synthesis of triglyceride [10, 23]. So, the down-regulation of FABP gene expressions might lead to a decrease in the concentration of plasma triglyceride and consequently affect the body weight including various muscle weights [10, 24].

Moreover, in another study, the correlation between the highly significant expressions of LCORL and MAPT genes and the large breast muscle of the chickens had been established by Liu et al. [8]. Also, Kubota et al. [25] demonstrated significant linkages between the increased expressions of MC4R, CAPN1, and ADSL genes and high body weights (including breast and thigh weights) in Korat chickens of Thailand. Furthermore, D'Andre et al. [26] observed high

significant expression of LPL (Lipoprotein lipase) gene in fast-growing chickens (White Recessive Rock) than those found in slow-growing chickens (Xinghua). This elevation of LPL gene expression was demonstrated to have a positive effect on lipid metabolism and transport it into different body organs affecting body weight.

Regarding the correlation between high expressions, of TPD52L1 and NCOA7 genes and reduction in breast weights observed in the native Dokki-4 breed in this study. These results were supported by the study of Ka et al. [27] who clarified overexpression with the high significance of MC4R gene in low body weights of some chicken breeds, as compared to that shown in high body weights in other breeds. Also, Piorkowska et al. [28] detected different expressions of the CAPN1 gene in two lines of chickens, including fast- and slow-growing birds. A highly significant increase in CAPN1 gene expression was observed in low-weight breast muscles in fast-growing chickens, as compared to that found in the high-weight breast muscles in slow-growing broilers.

Regarding to the low gene expressions and their association with high weights of breast or thigh muscles, the present study noted that the down-regulation of the expressions of TPD52L1 and NCOA7 genes was accompanied by high breast muscle weight in Mamourah, Inshas, and Leghorn breeds. In addition, down-regulation in the expression of TPD52L1 gene (in Mamourah breed), TPD52L1 and ASF1A genes (in Inshas breed), and TPD52L1 and NCOA7 (in Leghorn breed) was associated with high-weight muscles of the thigh. This corresponds with Li et al. [29] study who found a down-regulation of expression levels of calpain and calpastatin genes through neonatal pigs' development, which correlated with high-protein accumulation in animal body organs.

In conclusion, the present results proved that the expressions of GJA1, ASF1A FABP7, TPD52L1, and NCOA7 genes could be important genetic markers in chicken breed selection for the improvement of carcass traits through increasing the breast and thigh weights.

Acknowledgments

We wish to thank National Research Centre, Egypt for providing research facilities during the whole research. This research was supported by National Research Centre, Egypt (Grant No. 12050413).

Conflict of interest

The authors declare that they have no conflict of interest.

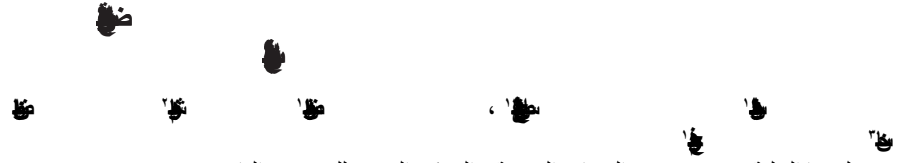
Funding statement

This work was financially supported by the National Research Centre, Egypt (Grant No. 12050413). Author Dalia M. Aboelhassan has received research support from National Research Centre.

References

1. Agostini, P.S., Santos, R.R., Khan, D.R., Siebert, D. and Van der Aar, P. The optimum valine: lysine ratios on performance and carcass traits of male broilers based on different regression approaches. *Poultry Science*, **98**(3), 1310-20 (2019). <https://doi.org/10.3382/ps/pey454>.
2. Araujo, R.G., Polycarpo, G.V., Barbieri, A., Silva, K.M., Ventura, G. and Polycarpo, V.C. Performance and economic viability of broiler chickens fed with probiotic and organic acids in an attempt to replace growth-promoting antibiotics. *Brazilian Journal of Poultry Science*, **21**(2), 1-7 (2019). <https://doi.org/10.1590/1806-9061-2018-0912>.
3. Tan, X., Liu, R., Li, W., Zheng, M., Zhu, D., Liu, D., Feng, F., Li, Q., Liu, L., Wen, J. and Zhao, G. Assessment the effect of genomic selection and detection of selective signature in broilers. *Poultry Science*, **101**(6), 101856 (2022). <https://doi.org/10.1016/j.psj.2022.101856>.
4. Erbilgin, A., Civelek, M., Romanoski, C.E., Pan, C., Hagopian, R., Berliner, J.A. and Lusic, A.J. Identification of CAD candidate genes in GWAS loci and their expression in vascular cells [S]. *Journal of Lipid Research*, **54**(7), 1894-905 (2013). <https://doi.org/10.1194/jlr.M037085>.
5. Chen, R., Wen, C., Cheng, Y., Chen, Y., Zhuang, S. and Zhou, Y. Effects of dietary supplementation with betaine on muscle growth, muscle amino acid contents and meat quality in Cherry Valley ducks. *the Journal of Animal Physiology and Animal Nutrition*, **103**(4), 1050-105(2019). <https://doi.org/10.1111/jpn.13083>.
6. Wang, X., Khan, R., Raza, S.H., Li, A., Zhang, Y., Liang, C., Yang, W., Wu, S. and Zan, L. Molecular characterization of ABHD5 gene promoter in intramuscular preadipocytes of Qinchuan cattle: Roles of Evi1 and C/EBP α . *Gene*, **690**, 38-47 (2019). <https://doi.org/10.1016/j.gene.2018.12.030>.
7. Liu, R., Sun, Y., Zhao, G., Wang, F., Wu, D., Zheng, M., Chen, J., Zhang, L., Hu, Y. and Wen, J. Genome-wide association study identifies loci and candidate genes for body composition and meat quality traits in Beijing-You chickens. *PLoS one*, **8**(4), e61172 (2013). <https://doi.org/10.1371/journal.pone.0061172>.
8. Liu, Z.H., Lu, L., Wang, R.L., Lei, H.L., Li, S.F., Zhang, L.Y. and Luo, X.G. Effects of supplemental zinc source and level on antioxidant ability and fat metabolism-related enzymes of broilers. *Poultry Science*, **94**(11), 2686-2694 (2015). <https://doi.org/10.3382/ps/pev251>.
9. Kang, H., Zhao, D., Xiang, H., Li, J., Zhao, G. and Li, H. Large-scale transcriptome sequencing in broiler chickens to identify candidate genes for breast muscle weight and intramuscular fat content. *Genetics Selection Evolution*, **53**(1), 1-23 (2021). <https://doi.org/10.1186/s12711-021-00656-9>.
10. Goel, A., Ncho, C.M. and Choi, Y.H. Regulation of gene expression in chickens by heat stress. *Journal of Animal Science and Biotechnology*, **12**(1), 1-13 (2021). <https://doi.org/10.1186/s40104-020-00523-5>.
11. Shahjahan, M., Liu, R.R., Zhao, G.P., Zhang, J.J., Zheng, M.Q., Li, Q.H. and Wen, J. Polymorphisms in GJA1 and their association with growth traits in chicken. *Genetics and Molecular Research*, **14**(4), 18839-18850 (2015). <https://doi.org/10.4238/2015>.
12. Sanematsu, F., Takami, Y., Barman, H.K., Fukagawa, T., Ono, T., Shibahara, K.I. and Nakayama, T. Asf1 is required for viability and chromatin assembly during DNA replication in vertebrate cells. *Journal of Biological Chemistry*, **281**(19), 13817-27 (2006).
13. Söllner, J., Lattrich, C., Häring, J., Görse, R., Ortman, O. and Treeck, O. A polymorphism in the nuclear receptor coactivator 7 gene and breast cancer susceptibility. *Oncology Letters*, **3**(1), 131-134 (2012).
14. Kasimanickam, V.R. and Kasimanickam, R.K. Sertoli, Leydig, and Spermatogonial Cells' Specific Gene and Protein Expressions as Dog Testes Evolve from Immature into Mature States. *Animals*, **12**(3), 271 (2022). <https://doi.org/10.3390/ani12030271>.

15. Roberts, D.M. and Saxe, J.E. Validity of a statistics attitude survey: A follow-up study. *Educational and Psychological Measurement*, **42**(3), 907-912 (1982). <https://doi.org/10.1177/001316448204200326>.
16. Jiang, L., Liu, J., Sun, D., Ma, P., Ding, X., Yu, Y. and Zhang, Q. Genome wide association studies for milk production traits in Chinese Holstein population. *PLoS one*, **5**(10), e13661 (2010). <https://doi.org/10.1371/journal.pone.0013661>.
17. Sahana, G., Gulbrandsen, B., Bendixen, C. and Lund, M.S. Genome-wide association mapping for female fertility traits in Danish and Swedish Holstein cattle. *Animal Genetics*, **41**(6), 579-88 (2010). <https://doi.org/10.1111/j.1365-2052.2010.02064.x>.
18. Bolormaa, S., Hayes, B.J., Savin, K., Hawken, R., Barendse, W., Arthur, P.F., Herd, R.M. and Goddard, M.E. Genome-wide association studies for feedlot and growth traits in cattle. *Journal of Animal Science*, **89**(6), 1684-97 (2011). <https://doi.org/10.2527/jas.2010-3079>.
19. Fan, B., Onteru, S.K., Du, Z.Q., Garrick, D.J., Stalder, K.J. and Rothschild, M.F. Genome-wide association study identifies loci for body composition and structural soundness traits in pigs. *PLoS one*, **6**(2), e14726 (2011). <https://doi.org/10.1371/journal.pone.0014726>.
20. Luo, W., Cheng, D., Chen, S., Wang, L., Li, Y., Ma, X., Song, X., Liu, X., Li, W., Liang, J. and Yan, H. Genome-wide association analysis of meat quality traits in a porcine Large White× Minzhu intercross population. *International Journal of Biological Sciences*, **8**(4), 580 (2012). <https://doi.org/10.7150/ijbs.3614>.
21. Xie, L., Luo, C., Zhang, C., Zhang, R., Tang, J., Nie, Q., Ma, L., Hu, X., Li, N., Da, Y. and Zhang, X. Genome-wide association study identified a narrow chromosome 1 region associated with chicken growth traits. *PLoS one*, **7**(2), e30910 (2012). <https://doi.org/10.1371/journal.pone.0030910>.
22. Park, J.S. and Shim, K.S. Proteomic changes in broiler liver by body weight differences under chronic heat stress. *Poultry Science*, **101**(5), 101794 (2002). <https://doi.org/10.1016/j.psj.2022.101794>.
23. Mujahid, A., Akiba, Y., Warden, C.H. and Toyomizu, M. Sequential changes in superoxide production, anion carriers and substrate oxidation in skeletal muscle mitochondria of heat-stressed chickens. *FEBS Letters*, **581**(18), 3461-3467 (2007). <https://doi.org/10.1016/j.febslet.2007.06.051>.
24. Xie, J., Tang, L., Lu, L., Zhang, L., Lin, X., Liu, H.C., Odle, J. and Luo, X. Effects of acute and chronic heat stress on plasma metabolites, hormones and oxidant status in restrictedly fed broiler breeders. *Poultry Science*, **94**(7), 1635-1644 (2015). <https://doi.org/10.3382/ps/pev105>.
25. Kubota, S., Vandee, A., Keawnakient, P., Molee, W., Yongsawatdikul, J. and Molee, A. Effects of the MC4R, CAPN1, and ADSL genes on body weight and purine content in slow-growing chickens. *Poultry Science*, **98**(10), 4327-4337 (2019). <https://doi.org/10.3382/ps/pez262>.
26. D'Andre, H.C., Paul, W., Shen, X., Jia, X., Zhang, R., Sun, L. and Zhang, X. Identification and characterization of genes that control fat deposition in chickens. *Journal of Animal Science and Biotechnology*, **4**(43), 1-16 (2013).
27. Ka, S., Kerje, S., Bornold, L., Liljegren, U., Siegel, P.B., Andersson, L. and Hallböök, F. Proviral integrations and expression of endogenous avian leucosis virus during long term selection for high and low body weight in two chicken lines. *Retrovirology*, **6**(1), 1-3 (2009). <https://doi.org/10.1186/1742-4690-6-68>.
28. Piórkowska, K., Nowak, J. and Połtowicz, K. The normalisation of CAPN gene expression in M. pectoralis superficialis in broiler lines differing in growth rate and their relationship to breast muscle tenderness. *British Poultry Science*, **56**(4), 452-458 (2015). <https://doi.org/10.1080/00071668.2015.1058918>.
29. Li, Z., Cao, B., Zhao, B., Yang, X., Fan, M.Z. and Yang, J. Decreased expression of calpain and calpastatin mRNA during development is highly correlated with muscle protein accumulation in neonatal pigs. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **152**(4), 498-503 (2009). <https://doi.org/10.1016/j.cbpa.2008.12.004>.



^١ قسم بيولوجيا الخلية - معهد بحوث التقنيات الحيوية - المركز القومي للبحوث - القاهرة - مصر.
^٢ قسم الانتاج الحيواني - معهد البحوث الزراعية والبيولوجية - المركز القومي للبحوث - القاهرة - مصر.
^٣ قسم امراض الدواجن - معهد البحوث البيطرية - المركز القومي للبحوث - القاهرة - مصر.

في الدجاج ، تعتبر أوزان الصدر والفخذ من أهم سمات اللحوم اللازمة لكفاءة الإنتاج والفوائد الاقتصادية من خلال التأثير على مظهر الذبيحة. ومن الضروري تحديد العلامات الجينية المحددة المرتبطة بصفات الذبيحة. ويعد استخدام التعبير الجيني عملية مرغوبة وموثوقة لهذا الغرض. هدفت الدراسة الحالية إلى تقييم تأثيرات مستويات التعبير لخمسة جينات ، FABP7 ، TPD52L1 ، NCOA7 ، GJA1 ، و ASF1A على أوزان الصدر والفخذ في سلالة الدقي ٤ الأصلية وسلالات المعمورة الهجينة وأنشاص وسلالة الليغورن المستوردة. مقارنة بالسلالة المحلية الدقي -٤ ، أشارت النتائج إلى أن التعبير العالي لـ GJA1 و ASF1A ، والجينات في سلالة المعمورة وكذلك التنظيم الأعلى للتعبير الجيني ASF1A في سلالات انشاص وليجهورن قد لوحظ ارتباطه بارتفاع وزن الصدر. علاوة على ذلك ، وجد أن الإفراط في التعبير عن جينات FABP7 و GJA1 في سلالة المعمورة والتعبير العالي لـ GJA1 في سلالاتي Inshas و Leghorn يرتبطان بزيادة وزن الفخذ. بينما ، في سلالة Dokki-4 الأصلية ، كان التعبير العالي لجينات TPD52L1 و NCOA7 في عضلة الصدر وجين ASF1A في عضلة الفخذ مصحوبًا بتقليل أوزان هذه العضلات مقارنة بسلالات الدجاج الهجين والمستوردة. في الختام ، أكدت النتائج الحالية أن تعبيرات هذه الجينات يمكن أن تكون علامات وراثية مفيدة في اختيار سلالات الدجاج لتحسين سمات الذبيحة من خلال زيادة أوزان الصدر والفخذ.

نقطة: الدجاج وأوزان الصدر والفخذ ، تعبير الرنا الرسول ، الجينات.