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Comparative evaluation of growth performance, carcass characteristics and timed series gene expression profile of *GH* and *IGF-1* in two Egyptian indigenous chicken breeds versus Rhode Island Red

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Abstract

Indigenous chicken breeds in developing countries have diverse benefits to rural economy as a source of high-quality animal protein. However, there are few reports on the evaluation of economic traits in Egyptian indigenous breeds. Hence, this study aimed to investigate growth performance, carcass characteristics, body measurements and meat quality traits in two indigenous breeds of chickens (Benha line and Golden Montazah) versus Rhode Island Red as a reference worldwide breed. Besides, a time series expression profile of somatotropic axis genes including *GH* and *IGF-1* and their plasma level concentrations were investigated. Benha line chickens (BL) revealed the highest improved estimates of growth performance, carcass characteristics and meat quality traits. In the same manner, it displayed the highest levels of hepatic *GH* and *IGF-1* and muscle *IGF-1* gene expression compared to Rhode Island Red (RIR) and Golden Montazah (GM) chickens. Accordingly, BL exhibited the highest levels of plasma *IGF-1* and the lowest levels of plasma *GH*. This result suggests the direct association between growth performance, carcass characteristics and levels of *IGF-1* gene expression in the selected chicken breeds. BL is a superior Egyptian genotype with candidate productive traits and competing characteristics, it could be used widely as a proven ancestor of commercial hybrid breeds.

KEYWORDS

carcass characteristics, gene expression, *GH/IGF-1*, growth performance, indigenous breeds, meat quality

1 | INTRODUCTION

The human population is expected to increase to about 9.15 billion by 2050, with the highest predicted rise in developing countries (FAO, 2017). Chickens are the most popular and economic source of good quality protein for human being particularly in rural communities, where poultry farming constitutes the largest portion of animal protein supply chains (El Nagar & Ibrahim, 2007). In such communities, indigenous breeds of chickens participate imperative roles comprising as much as 90% of the poultry population maintaining a balanced farming system (Pym, 2013). The tolerant immune system of indigenous breeds supports their adaptation with harsh environmental conditions and poor husbandry practices (Padhi, 2016).

White meat of indigenous chickens is a preferred food in many cultures around the world (Zamans et al., 2004). As it has superior health aspects and consumer preferability specifications including taste, flavour, high levels of vitamins and minerals, relatively low fat and cholesterol contents, plus the cuts are easier to handle and cheaper compared to red meat (Liu et al., 2012).

Growth and carcass traits are among the most important economic traits that are subject to a significant amount of genetic control (Xu et al., 2013). The genetic regulation of these traits is monitored by the breed genotype and the pathway of the somatotropic axis genes, growth hormone (GH) and insulin-like growth factor-I (IGF-I), (Brown-Borg, 2009). Despite the pathway of GH/IGF-1 is a key regulator of muscle development, growth rate, body size and metabolism in birds, its association with carcass characteristics and growth performance in chicken populations is not fully understood (Brahmkhatri et al., 2015; Jia et al., 2018).

Egypt possesses versatile varieties of indigenous chickens displaying a wide variety of phenotypes, including differential growth rate, body weight and muscularity.

These breeds retain many desirable characteristics such as great adaptation to the harsh environmental conditions (El Nagar & Ibrahim, 2007), resistance to diseases, marvellous meat flavour and taste (Melesse, 2000; Fanatico et al., 2005). For instance, Golden Montazah (GM) and Benha line (BL) are indigenous Egyptian dual-purpose breeds originated consuming breeding programmes that started with crossbreeding, coupled with selection and followed by intensive genetic selection for many selected generations (Mahmoud et al., 1974; Iraqi et al., 2012). These breeds showed enhanced growth and carcass performance compared to other exotic breeds such as Rhode Island Red (RIR), which is an American dual-purpose breed (Ruggieri, 2017). Despite, the genetic uniqueness of Egyptian native breeds, their ability to produce high-quality meat and the great potentiality to adapt with harsh environmental conditions, to date, no accessible information available about growth and carcass characterization of these breeds and very little information is available on the genetic and molecular components of those Egyptian native chickens.

Therefore, we hypothesized that the native genotypes of BL and GM could reveal developed unique features in growth and carcass performance as opposed to RIR which is a common worldwide breed. For this, the three breeds were compared in terms of growth performance, carcass characteristics and meat quality traits. Additionally, a time series gene expression profile of *GH* and *IGF-1* were considered, with their plasma level concentrations in liver and muscle tissues were investigated.

2 | MATERIALS AND METHODS

2.1 | Ethical statement

All experimental procedures of this study were approved by the institutional animal care and use committee (IACUC) of Benha University. Birds were handled with care during the experiment.

2.2 | Population structure

Two indigenous breeds of chickens (BL, GM) along with RIR as an exotic breed were used in the current study. BL chickens are a synthetic line originated from a crossbreeding programme between the local strain of Golden Montazah (GM) and a White Leghorn (WL) (Iraqi et al., 2012). It was subjected to an intensive genetic selection programme depending on BLUP estimates for six generations revealing developed dual-purpose breed with high-performance characteristics in egg production and growth traits (El-Attrouny, 2017).

The GM is synthetic strain developed from a crossbreeding programme between the Rhode Island Red (RIR) and Dokki-4 chickens, using breeding system coupled with selection, for five generations to develop a dual-purpose local breed (Mahmoud et al., 1974).

The RIR is exotic American dual-purpose breed that was adapted to the Egyptian environmental condition for many years (Hosny, 2006). It was developed in Massachusetts in the late nineteenth century, by crossbreeding birds of Oriental origin such as the Malay with brown Leghorn birds from Italy. It is probably a common ancestor of many commercial hybrid breeds, due to egg-laying abilities and high yields of rich-flavoured meat (Ruggieri, 2017).

2.3 | Chicken, diet and housing

Five hundred eggs from each breed were incubated (total of 1,500 fertile eggs) and hatched in poultry husbandry at the faculty of Agriculture, Benha University. After hatching, a total of nine hundred birds were used in this experiment ($n = 300$ bird/ population group). The total experimental period was 16 weeks. All breeds were raised and housed in the same building with free access to feed and water. Diets were formulated to meet or exceed nutrient requirement according to NRC 1994 with a starter diet (21% crude protein) from hatching to 3 weeks of age, a grower diet (19% crude protein) from 3 to 6 weeks, and a finisher diet (17% crude protein) from week 6 to the end of the experiment. Chickens were kept in light 14 hr/ day and ambient temperature of 25°C to 35°C.

2.4 | Growth performance

Individual body weight (BW) was recorded bi-weekly in a special box, which was placed on a tarred digital scale (0.01g-10kg; Mettler Toledo; Columbus). Average daily body weight gain (ADG) was calculated according to Abdelatty et al., (2020a). Feed intake (FI) was recorded weekly to calculate the average feed conversion ratio (FCR) (Jia et al., 2018). The feed intakes were measured as group mean for each breed during the whole experimental period.

2.5 | Samplings

Fifteen birds from each population were euthanized each two weeks; liver and muscle samples were collected and snap-frozen in liquid nitrogen for gene expression assessment to investigate the changes in gene expression over time.

By the end of the experiment, thirty birds were euthanized from each population and used for collection of blood sample (used for plasma separation according to Jia et al., 2018) and carcass cuts including giblet and carcass weight. Right pectoral muscle was collected and stored in –20°C for further meat quality and amino acid profile assessment similar to (Abdelatty, 2020a).

2.6 | Plasma hormone determination

Plasma levels of *GH* and *IGF-1* were measured using an ELISA kit (Cat # MBS266317, MBS262361, MyBioSource, USA) and were read on ELISA Reader (Thermo Fisher, Varioskan Flash, USA) following the manufacturers' instructions. Measurements were done in technical triplicate. Purified chicken *GH* and *IGF-1* antibodies were used to coat micro-titre plate wells.

2.7 | Carcass characteristics

For determination of carcass characteristics, ninety birds were selected based on the group average from each breed at 16 weeks of age. Birds were euthanized by cervical dislocation and scalded in hot water (60°C for 45 s) and the feathers removed mechanically. The body measurements for shank length, keel length and body circumference were investigated (Abdelatty et al., 2020b). Further, the weights of carcass, giblets (liver, heart, gizzard) and offal (feather, blood, head, legs, viscera and organs) were determined and expressed as a percentage of live body weight.

2.8 | Meat quality

After 24 hr of cooling at 5°C, the ultimate pH (pHU) of breast muscle fillet was measured using pH-meter electrodes (Jenway 3,510 pH-meter, Cole-Parmer, Staffordshire, United Kingdom). Water-holding capacity (WHC) was determined from 10g breast fillet by low-speed centrifugation technique at 10,000× *g* and 5°C for 20 min (Honikel & Hamm, 1994). The drip loss (24 hr and 48 hr postmortem) and cooking loss percentages were evaluated according to the methodology of Honikel (1998). Approximately 31 min was required to get the internal cooking temperature of 75°C. Cooked breast fillet samples were then used to measure the Warner-Bratzler Shear Force (WBSF) by 3,343 Universal Test System Mono columns (Instron, USA).

Breast meat colour was also assessed, lightness (*L**), redness (*a**), and yellowness (*b**) were measured using Chroma Meter CR-410 (Konica Minolta Sensing INC., Osaka, Japan). These colour values were then used for the calculation of colour intensity (chroma) as C = (a*^2 + b*^2) ^0.5, and colour saturation (Hue angle) as h° = arctg b*/a*. Moisture and ash contents of the breast muscles were determined according to (Horowitz, 2000).

2.9 | Muscle amino acids profile and antioxidant activity

Chemical analysis of muscle amino acid profiles was assessed using HPLC (Agilent HP 1200 series; USA). The utilized analytical column was Supelcosil C18 (5 μm particle and 80 Ao pore size). Samples and amino acid standards were injected into the column for separation by HPLC. Amino acids contents in the breast muscle were determined as described by Salah et al. (2019).

For determination of oxidative stress biomarkers, muscle homogenates were used to evaluate malondialdehyde (MDA) by HPLC according to Karatas et al. (2002), while the endogenous antioxidant enzyme (SOD) was analysed by spectrophotometer according to Badr et al. (2019). Protein carbonyl assay was performed based on the method of Patsoukis et al. (2004) as a biomarker of oxidative stress.

2.10 | Differential expression of the somatotropic axis genes (GH/IGF-1)

The liver is the main organ regulating energy metabolism in the body, thus providing energy necessary for cell division and growth, and the muscle mass is the largest protein synthesis organ in the body thus directly related to body weight gain;

therefore, these two organs were targeted in the current manuscript. The liver and breast muscle tissue samples were grinded by Tissue Lyser (LT apparatus, Qiagen, USA) followed by total RNA extraction from the suspension of cells using SV total RNA isolation kit (Promega Corporation, Madison, WI) according to the manufacturer's protocol. Gel electrophoresis was used to check for RNA quality, complementary DNA (cDNA) was synthesized from RNA using High Capacity cDNA reverse transcriptase kit (Thermo Fisher Scientific, USA).

RT-qPCR was performed for each sample in technical triplicates. qPCR consisted of 500 ng/reaction of cDNA (except for NTC and cDNA control), 12.5 µL Maxima SYBR Green qPCR Master Mix (Maxima SYBR Green qPCR, Thermo Fisher Scientific), 0.3 µM of each forward and reverse primer, 10 nM/ 100 Nm ROX Solution, nucleases-free water to a final volume of 25 µl.

Reactions were analysed on an AriaMx Real-Time PCR System (Agilent technologies), two-step cycling protocol, under the following conditions: 95°C for 10 min and 40 cycles of 95°C for 15 s followed by 60°C for 60 s. The relative standard curve method used for quantification of *GH* and *IGF-1* expression.

The quantification of target genes was normalized to housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH), GAPDH is known to be a stable reference gene in chicken related research (Liu et al., 2010; Borowska et al., 2016, 2019; Boo et al., 2020), the names and oligonucleotide sequences of the primers are listed in Table S1. The amount of mRNA of target and reference genes was measured from their corresponding standard curves. The mRNA relative expression level was expressed as the copy concentration of target genes divided by that of the endogenous reference (target gene/ GAPDH) (Lu et al., 2008).

2.11 | Statistical analysis

Data were analysed for each measured trait, plasma hormone concentration and gene expression of GH and IGF-1 in different tissues using (SAS, 2004). Differences were considered significant at $p \leq .05$ and trending where at $(0.05 > p \leq .1)$. Significant differences between means were tested by Duncan's multiple range test (Duncan, 1955).

3 | RESULTS

3.1 | Growth performance and carcass characteristics

As shown in Table 1, there was a significant influence of genotype on growth performance traits of birds (BW, ADG, FCR). Chicks of BL breed exhibited the highest body weight, daily gain and feed intake values ($p < .05$) through the entire experimental period compared to RIR and GM. Interestingly, it revealed a 253 g increase in body weight at week 16 opposed to RIR. While feed conversion ratio followed diverse pattern, as the lowest FCR recorded in BL breed at weeks 4, 12 and 16.

TABLE 1 Least-square means of body weights, daily gain, feed intake and feed conversion ratio (FCR) in GM, RIR and BL[1]

Trait	GM	RIR	BL	*SEM*
Body weights (g)				
At hatching	32^{c}	33^{b}	34^{a}	0.11
Week 4weweeksBW4	203^{c}	227^{b}	254^{a}	1.4
Week 8	603^{c}	630^{b}	665^{a}	3.6
Week 12	952^{c}	980^{b}	1122^{a}	4.8
Week 16	1304^{c}	1409^{b}	1662^{a}	12.9
Daily gain (g)				
Hatching to 4 wks.	6.04^{c}	6.27^{b}	7.62^{a}	0.14
4 to 8 wks.	14.17^{b}	14.43^{b}	14.81^{a}	0.23
8 to 12 wks.	12.58^{b}	12.71^{b}	16.12^{a}	0.26
12 to 16 wks.	12.49^{c}	15.56^{b}	19.04^{a}	0.28
FI(g)[2]				
Week 4	25.7^{c}	27.25^{b}	29.21^{a}	1.53
Week 8	57.62^{c}	62.24^{b}	65.10^{a}	2.41
Week 12	76.2^{c}	82.14^{b}	85.26^{a}	3.13
Week 16	98.30^{c}	107.5^{b}	111.2^{a}	3.68
FCR (g feed/g gain)				
FCR4	4.25^{a}	4.34^{a}	3.83^{b}	0.04
FCR8	4.06^{b}	4.31^{a}	4.39^{a}	0.06
FCR12	6.05^{b}	6.46^{a}	5.28^{c}	0.04
FCR16	7.87^{a}	6.90^{b}	5.94^{c}	0.07

Letters in the same row indicate significant differences at $p < .05$.

[1]Golden Montazah (GM), Rhode Island Red (RIR), Benha line (BL).

[2]FI = Feed intake; FCR = Feed conversion ratio.

BL breed revealed the highest significant value of body measurements followed by RIR and GM breeds, respectively ($p < .05$). Through the comparison between three breeds, it could be noticed that the BL breed surpassed the other two breeds by 8.84%, 16.87%, and 27.45% for shank length, keel length, and body circumference, respectively.

The influence of the genotype was statistically significant for dressing percentage between compared populations. BL breed revealed the highest significant value of dressing percentage ($p < .05$) compared to other breeds Table 2. Concerning the percentage of the liver weight, there were significant differences ($p < .01$) among genotypes but the percentage of gizzard and heart weight exhibited non-significant differences ($p > .05$) among genotypes. Chickens of the BL and RIR had a significantly larger percentage of the giblets than GM breed, where BL chickens had a significantly lower percentage of offal in live weight compared to RIR and GM chickens ($p < .05$).

TABLE 2 Least-square means of body measurement, carcass traits in GM, RIR and BL[1]

Trait	GM	RIR	BL	*SEM*
Body measurement				
Shank length (cm)	7.94[b]	8.19[c]	8.84[a]	0.04
Keel length (cm)	15.21[b]	15.72[c]	16.87[a]	0.06
Body circumference (cm)	26.04[b]	26.17 [c]	27.45[a]	0.10
Carcass traits				
Dressing, (%)	59.87[b]	61.41[c]	63.58[a]	0.15
Liver, (%)	2.37[b]	2.42[c]	2.64[a]	0.05
Gizzard, (%)	2.14[a]	2.15[a]	2.16[a]	0.05
Heart, (%)	0.80[a]	0.80[a]	0.81[a]	0.04
Giblets, (%)	5.22[b]	5.34[a]	5.35[a]	0.03
Offal, (%)	32.05[b]	31.28[a]	29.94[c]	0.24

[1]Golden Montazah (GM), Rhode Island Red (RIR), Benha line (BL); [2]FI = Feed intake; FCR = Feed conversion ratio. Letters in the same row indicate significant differences at $p < .05$.

TABLE 3 Least-square means of meat quality traits in GM, RIR and BL[1]

Traits	GM	RIR	BL	*SEM*
PH_U	5.83[a]	5.85[a]	5.83[a]	1.26
WHC%	82.40[a]	85.45[a]	83.91[a]	2.0
Drip loss % (24h)	2.69[a]	1.83[a]	1.92[a]	0.38
Drip loss % (48h)	4.23[a]	3.55[b]	2.92[c]	0.73
Cooking loss%	16.14[a]	10.46[b]	11.94[ab]	1.66
Colour				
L*	54.27[b]	55.25[b]	56.54[a]	0.47
a*	9.24[a]	8.46[a]	8.75[a]	0.38
b*	7.53[a]	8.35[a]	7.83[a]	0.27
Colour intensity	11.92[b]	11.83[a]	11.76[a]	0.30
Colour saturation	39.10[b]	40.70[ab]	41.90[a]	1.80
WBSF	3.06[a]	2.83[a]	2.77[a]	0.14
Final moisture	73.19[a]	73.67[a]	72.33[a]	0.75
Ash%	0.85[a]	0.82[b]	0.77[b]	0.06

[1]Golden Montazah (GM), Rhode Island Red (RIR), Benha line (BL); [2]FI = Feed intake; FCR = Feed conversion ratio. Letters in the same row indicate significant differences at $p < .05$

3.2 | Meat quality traits

As indicated in Table 3, the findings of most meat quality attributes investigated in the present research, including PHU, WHC, drip loss (24 hr) percentage, colour redness (a*) and yellowness (b*), WBSF, moisture and ash contents, showed no important distinctions between the different breeds ($p < .05$). However, the lowest drip loss (48h) percentage was generated by BL, while the proportion of cooking losses produced by the GM line was higher than RIR and BL ($p > .05$). In addition, the lightness (L *) of breast meat in the Benha line (BL) was greater than the other lines ($p > .05$). Also, lower colour intensity and higher colour saturation were investigated in BL meat followed by RIR line, and GM line ($p < .05$), respectively.

3.3 | Amino acids profile and antioxidant activity

As regards the analysis of amino acids (Table 4), higher concentrations of arginine, glutamine and histidine were found in BL breed followed by RIR and GM breed ($p < .05$). Furthermore, BL line manifested higher contents of lysine, leucine, isoleucine, valine, methionine and aspartic acid than GM ($p < .05$) but was comparable to the values of RIR line ($p > .05$). On the other hand, there were no important variations between the remaining essential and non-essential amino acid profiles of the three chicken lines ($p > .05$). In comparison to GM breed, BL and RIR breast meat generated lower MDA and PC contents revealing similar values with no significant differences between the two breeds. However, BL exhibited the highest significant SOD content as opposed to the other breeds (Figure 1).

TABLE 4 Least-square means of amino acid composition in muscles of GM, RIR and BL[1]

Concentration g/100g	GM	RIR	BL	*SEM*
Essential amino acid				
Lysine	7.20[b]	8.15[a]	8.35[a]	0.24
Leucine	8.31[a]	8.42[a]	8.54[a]	0.15
Isoleucine	4.57[b]	4.96[ab]	5.36[a]	0.13
Valine	5.27[b]	5.77[a]	5.96[a]	0.14
Methionine	2.23[b]	2. 52[a]	2.56[a]	0.07
Histidine	3.65[b]	3.75[b]	4.04[a]	0.08
Non-essential amino acid				
Glycine	4.73[a]	4.76[a]	5.19[a]	0.12
Tyrosine	2.77[a]	2.79[a]	2.92[a]	0.06
Serine	3.25[a]	3.10[a]	3.02[a]	0.09
Aspartic	9.05[b]	9. 84[a]	10.08[a]	0.20
Glutamic	15.06[a]	16.19[a]	17.09[a]	0.79
Alanine	5.52[a]	5.72[a]	5.92[a]	0.14
Arginine	4.55[b]	4.75[b]	5.03[a]	0.10

[1]Golden Montazah (GM), Rhode Island Red (RIR), Benha line (BL); [2]FI = Feed intake; FCR = Feed conversion ratio. Letters in the same row indicate significant differences at $p < .05$

3.4 | Plasma levels of GH and IGF-1

At each time point, the three genotypes showed significant differences in GH and IGF-1 plasma concentrations ($p < .05$). The highest plasma GH concentrations were observed at week 4, followed by a clear reduction in GH levels with the increasing age. Although BL exhibited the lowest levels of plasma GH concentration, GM revealed the highest levels of it (Figure 2a).

All chicken populations showed a linear increase in plasma IGF-1 concentrations with increasing age. BL displayed the highest plasma IGF-1 concentrations throughout the experimental period. While GM chickens revealed the lowest plasma IGF-1 concentrations (Figure 2b).

3.5 | Gene expression level of *GH* and *IGF-1* in liver and breast muscle

In the studied genotypes, at different time points, hepatic gene expression levels of *GH* and *IGF-1* showed a dramatic up-regulation with advancing in age. BL exhibited the highest significant expression of hepatic-somatotropic axis genes *(GH*, *IGF-1)* compared to RIR and GM (Figure 3 and Figure 4). However, at 0 and 4 weeks of age, BL and RIR breeds revelled similar *IGF-1* expression pattern with no significant differences ($p > .05$) followed by a sharp significant increase at weeks 8, 12 and 16.

Regarding breast muscle tissues, the highest *GH* expression observed in GM chicken at week 0, the lowest expression noticed in BL at week 4. Despite the gene expression levels of *GH* in BL were lower than GM chickens at 0, 4 and 8 weeks of age, *GH* levels of BL raised significantly at weeks 12 and 16 to be higher than those of GM and RIR (Figure 3). On the other side, at week 0 the *IGF-1* mRNA levels revealed a similar low expression pattern with non-significant differences between BL and RIR. Beginning from week 4 BL displayed highly elevated expression levels compared to RIR and GM breeds (Figure 4).

4 | DISCUSSION

Selection and crossbreeding are two major approaches in poultry breeding to increase birds' performance in the production system. BL and GM breeds are Egyptian indigenous dual-purpose breeds that subjected to long term breeding programmes applying crossbreeding and selection methods (Iraqi et al., 2012; Mahmoud et al., 1974). Hence, we assumed the superiority of these breeds and conducted a comparative evaluation of growth performance, carcass and meat quality traits of these breeds as opposed to RIR which is a common worldwide breed. Additionally, a time series gene expression profile of *GH* and *IGF-1* were considered with their plasma level concentrations as key regulatory genes of growth and carcass traits.

Our results revealed that the genotype markedly affected growth performance traits. Benha chickens showed the highest improved values of BW, ADG, FI, FCR, body measurements (shank length, keel length and body circumference) and most of the carcass traits (dressing and giblets percentage), when compared to RIR and GM breeds. These results could be attributed to the genetic makeup of BL that originated from crossbreeding programme and subjected to intensive genetic selection for more than six generation. Studies of Havenstein et al. (2003) and Dou et al. (2017) reported the potentiality of selection programmes to increase growth rates and muscularity in chickens by more than 300%. Other investigations confirmed the ability of genetic selection programme to improve breast muscle size, and weight concomitantly (Marks, 1993; Le Bihan-Duval et al., 1998; Scheuermann et al., 2003; Jia et al., 2018).

The pHU values of breast meat were close among the studied breeds ($p > .05$) and of intermediate-range comparable to the experiments of Choo et al., (2014); Mikulski et al. (2011) and Yin et al. (2013) that conducted to evaluate the effect of genotypes on meat quality traits. The majority of meat quality attributes, including WHC, cooking loss, colour, and tenderness, are usually influenced by pH value. Hence, the physicochemical characteristics of breast meat from the three genotypes were statistically comparable, but BL and RIR positively yielded lower Drip loss % (48h) and cooking loss percentages than the GM line. This high cooking loss percentage of GM line was not related to the low pH and WHC values as previously reported by Choo et al. (2014) but could be entirely attributed to the genetic influence. This influence was also evident in BL, which had the highest value of breast meat *L**. In the earlier report of Petracci et al. (2004) the paler breast meat is generated from lower pH and inferior water-holding capacity (WHC), while darker breast meat is associated with higher pHU and cooking yield. However, the *L* value*, WHC, drip loss, and cooking loss values exhibited from BL, in addition to the insignificant difference between the three-lines for *a** value, *b** value, colour intensity and colour saturation, classify BL and RIR within the normal quality meat (Carvalho et al., 2017; Kralik et al., 2018).

Furthermore, BL breast meat is somewhat tender than the other lines according to the WBSF data. The moisture content potentially influences the overall acceptability and palatability of the meat by contributing to its tenderness (Warriss, 2010). With respect to moisture and ash content, there was no significant difference between BL, RIR and GM ($p > .05$). Similarly, Fanatico et al. (2005) and Jung et al. (2014) noted that the genotype had limited influence on both moisture and ash contents. The results of the present research are compatible with the results of Yin et al. (2013) that

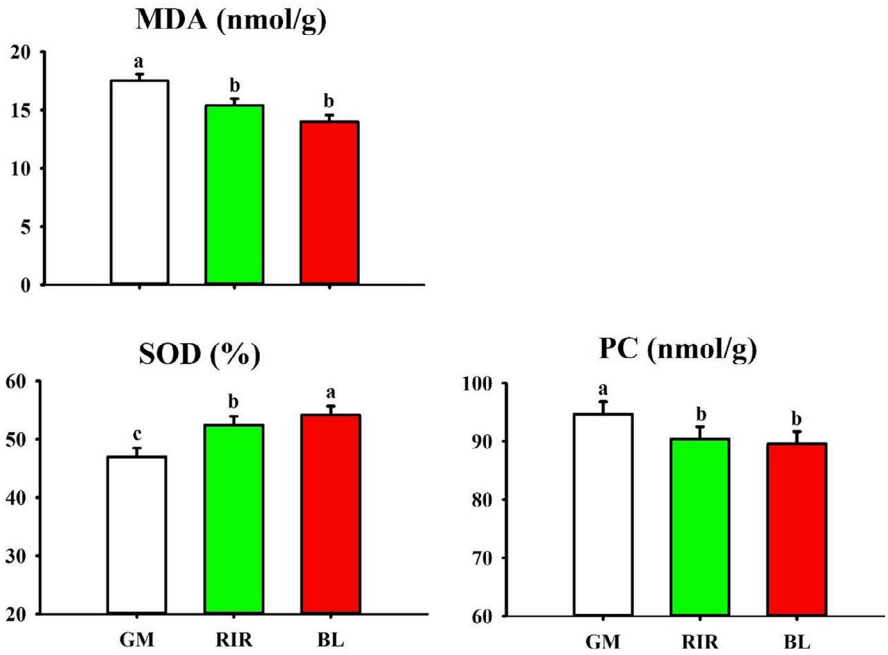

FIGURE 1 Oxidative stress markers of MDA, SOD and PC in BL, RIR and GM. [a, b, c]Estimates with the same letters are not significantly different ($p < .05$) [Colour figure can be viewed at wileyonlinelibrary.com]

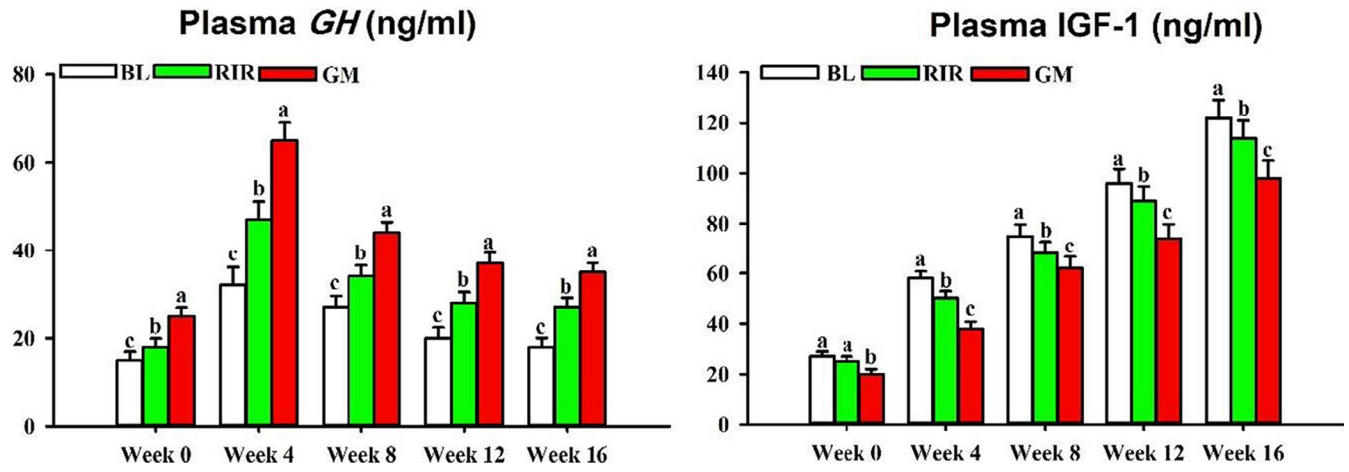

FIGURE 2 Plasma hormone concentrations of somatotropic axis genes, growth hormone (GH) and insulin-like growth factor-1 (IGF-1), of BL, RIR and GM at weeks 0,4,8,12,16. [a, b, c]Estimates with the same letters are not significantly different ($p < .05$) [Colour figure can be viewed at wileyonlinelibrary.com]

reported most meat quality characteristics of crossbreed lines were similar comparable to pure lines. Nonetheless, earlier studies have shown that the variation in the quality characteristics of chicken meat is mainly correlated with the genotype (Tang et al., 2009), but also others have detected no influence (Mikulski et al., 2011).

In the current study, BL had high levels of most evaluated amino acids compared to RIR and GM. However, studies of Yin et al. (2013) and Zhao et al. (2011) recorded no important distinction in amino acid content between different genotypes. In contrast to the published research of Jia et al. (2009) who observed that pure line goats contained more individual amino acids than hybrid goats. It also confirms that distinct amino acid compositions are slightly attributed to the genetic makeup (Jung et al., 2014).

The activity of the antioxidant enzyme, SOD, in BL was greater RIR and GM, assuming superior lipid oxidative stability than the other breeds. However, the MDA concentrations expressed by the three genotypes were significantly smaller than the perceived limit for raw meat rancidity similar to (Demirhan & Candoğan, 2017). In the present research, carbonyl groups were used to evaluate protein oxidation resulting from oxidative deamination of alkaline amino acids (Zakrys-Waliwander et al., 2012). The oxidative stability of BL breast meat protein was higher than GM Line but similar to RIR. The protective impact of the SOD enzyme against lipid oxidation and protein carbonylation was obviously seen in BL and RIR, consistent with prior published findings (Delles et al., 2014).

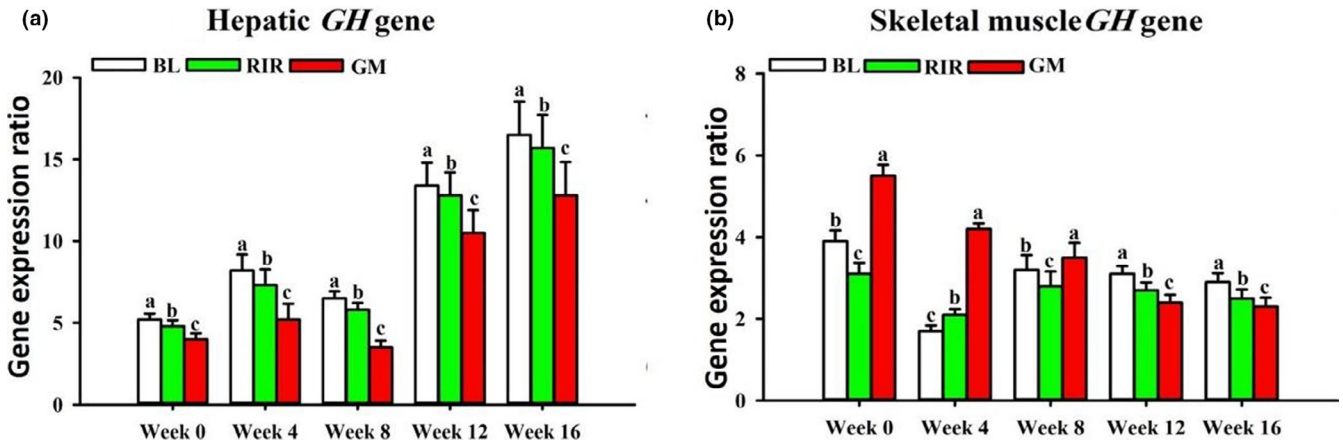

FIGURE 3 Growth hormone (*GH*) gene expression ratio: a) gene expression ratio of *GH* in liver tissues of BL, RIR and GM chickens at 0,4,8,12,16 week of age. b) gene expression ratio of *GH* in muscle tissues of BL, RIR and GM chickens at 0,4,8,12,16 week of age. Changes in gene expression were denoted as relative change (ratio of target gene/reference gene). *GAPDH* was used as reference gene. [a, b, c]Estimates with the same letters are not significantly different ($p < .05$) [Colour figure can be viewed at wileyonlinelibrary.com]

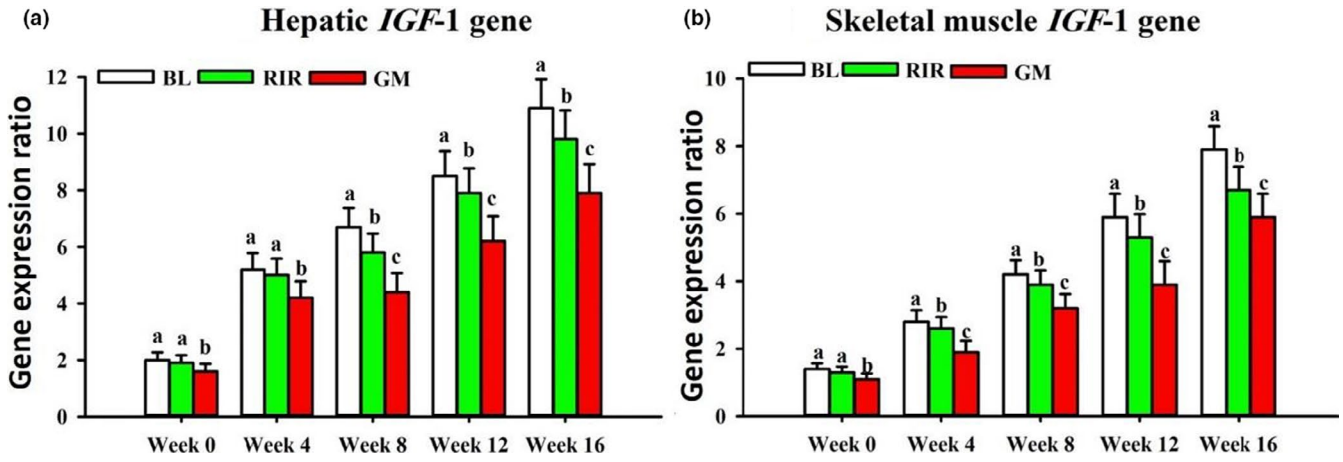

FIGURE 4 Insulin-like growth factor-1 (*IGF-1*) gene expression ratio: a) gene expression ratio of *IGF-1* in liver tissues of BL, RIR and GM chickens at 0,4,8,12,16 week of age. b) gene expression ratio of *IGF-1* in muscle tissues of BL, RIR and GM chickens at 0,4,8,12,16 week of age. Changes in gene expression were denoted as relative change (ratio of target gene/reference gene). GAPDH was used as reference gene. [a, b, c]Estimates with the same letters are not significantly different ($p < .05$) [Colour figure can be viewed at wileyonlinelibrary.com]

Chicken growth hormone (*GH*) and Insulin-like growth factor-I (*IGF-I*) are candidate somatotropic axis genes that promote growth performance and carcass quality traits in chickens (Anh et al., 2015; Brahmkhatri et al., 2015). Despite the critical roles of somatotropic axis genes in the genetic regulation of growth performance and muscle development, the molecular genetic mechanism by which they stimulate growth and carcass quality traits in chicken populations is not fully understood (Jia et al., 2018). In the current study, a timed expression profile of *GH* and *IGF-1* was investigated in liver and muscle tissues of the three genotypes. Alongside, investigating the plasma level concentrations of these genes. Accordingly, BL showed the lowest *GH* and the highest *IGF-1* plasma concentrations in agreement with the previous studies of Zhao et al. (2004); Rahimi, (2005) and Jia et al., (2018) that reported the inverse relation between *GH* and *IGF-1* suggesting the fast-growing chickens had lower GH plasma levels than the slow-growing hens. The negative correlation between plasma GH levels and hepatic GH binding activity in chicken breeds could be attributed to the downregulation of hepatic GH receptors by elevated plasma GH levels (Mao et al., 1998). It is therefore likely that the GM and RIR have lower hepatic GH binding activities compared with BL. Thus, the selection for high growth rate in BL chickens might have increased hepatic GH binding activity. Since this mechanism regulates among others the synthesis of IGF-1 –and thereby growth rate–this may be the underlying regulatory mechanism for elevated growth rate in the BL breed.

Further, BL displayed the highest significant expression of hepatic *GH* and *IGF-1* transcripts. These results were in the same line with the results of Jia et al., (2018) who reported that the Avian hybrid exhibited the highest transcript level of hepatic *GH* and *IGF-1* in compared to the Daweishan mini chickens and Wuding chickens. Despite BL showed the highest significant expression of hepatic *GH*, it showed the lowest *GH* expression in skeletal muscle tissues at week 4. Thus, *GH* expression was differently regulated in hepatic and muscle tissues indicating different roles of this hormone. The various roles of *GH* and its interactions with its receptors require more clarification in future studies, especially in recently improved chicken breeds that selected for fast growth or food conversion.

According to Jia et al., (2018), the selection for growth performance and body size has altered the expression profiles of somatotropic axis genes in avian hybrid. Hence, we could ascribe the high expression level of hepatic *GH* and *IGF-1* mRNA in BL to the rigorous genetic selection that participated in the hybrid genetic makeup of BL.

Studies of Jia et al. (2018) and Zhao et al. (2004) clearly confirmed the direct and major role of *IGF-1* expression in the genetic regulation of growth rate and muscularity. Accordingly, the superiority of BL in growth performance and muscle characteristics could be explained by the presence of high transcript levels of *IGF-1*.

5 | CONCLUSION

In conclusion, the indigenous breed of BL chickens showed superior characteristics in terms of growth performance, carcass characteristics, and meat quality traits when compared to GM and RIR. This superiority of BL genotype could be attributed to the genetic regulation of somatotropic axis genes of *GH* and *IGF-1* that might be influenced by the intensive genetic selection programme that previously practised on it. This breeding programme significantly promoted the gene expression profile of somatotropic axis genes compared to RIR and GM. This investigation sheds light on the great importance of traditional breeding programmes that affected positively in molecular genetic mechanisms creating superior genotypes with candidate productive traits and competing characteristics.

6 | STUDY LIMITATION

Due to funding limitation, the pituitary gene expression of GH was not assessed. Additionally, other somatotropic axis genes like *GHR*, *IGF1R* should have been assessed for more understanding to the signalling pathway of *GH/IGF-1* regulating energy and protein synthesis in chicken.

Despite the use of one very stable and well-documented reference gene (GAPDH) in the current study and several former similar studies (Liu et al., 2010; Borowska et al., 2016, 2019; Boo et al., 2020). We recommend using not less than 2 reference genes for normalization of the target genes to have higher stability.

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CONFLICT OF INTERESTS

The authors declare that they have no compact of interests.

AUTHOR CONTRIBUTIONS

Mahmoud M. El-Attrouny, Mahmoud M. Iraqi and Omnia A. Badr designed the experiment, collected and analysed growth and carcass data. Islam I. Sabike worked on the meat quality part. Omnia A. Badr, Alzahraa M. Abdelatty, Mahmoud M. Moustafa investigated the amino acid profile, timed gene expression, plasma concentration and analysed the related data. All authors wrote and drafted the corresponding sections of the manuscript and revised the complete manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The protocol for the conducted animal experiments was approved by the institutional animal care and use committee (IACUC) of Benha university.

CONSENT FOR PUBLICATION

Not applicable.

SOFTWARE AND DATA REPOSITORY RESOURCES

None of the data were deposited in an official repository.

IMPLICATIONS OF THE STUDY

This study investigates the effect of breeding programmes on altering growth performance, carcass characteristics, meat quality and timed gene expression profile of somatotropic axis genes in three indigenous breeds of chickens. BL is a superior Egyptian genotype with candidate productive traits and competing characteristics, it could be used widely as a proven ancestor of commercial hybrid breeds. The breeding programme that practiced on Benha line significantly promoted the gene expression profile of somatotropic axis genes compared to GM and RIR. This investigation shed light on the great importance of traditional breeding programmes that affected positively in molecular

genetic mechanisms creating superior genotypes with candidate productive traits.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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