



**GENOMIC ANALYSIS OF TRANSFORMING GROWTH FACTOR $\beta 2$ GENE
POLYMORPHISM ASSOCIATED WITH PRODUCTIVE PERFORMANCE IN
GIZA M-2 CHICKEN LINE**

Farid S. Nassar

Dep. of Anim. Prod., Fac. of Agric., Cairo Uni., Giza 12613, Egypt.

Corresponding author: Farid S. Nassar; Email: fidsaber_nassar@agr.cu.edu.eg

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ABSTRACT: Transforming growth factor- β (TGF- β) genes considered as one of the large family of multifunctional growth factors that participate in the regulation of a broad range of biological processes in chicken. The current study was conducted to determine genetic polymorphisms of TGF- $\beta 2$ gene by using PCR-RFLP method in Giza M-2 line which selected for high 6- week live body weight (LBW) for seven generations compared to random bred control (RBC) line. Also, the association between TGF- $\beta 2$ with productive performance was evaluated for each line. Results indicated that, Giza M-2 line had higher LBW at different ages, LBW at 6 week of age (LBW6), and carcass parts weights and percentages compared to RBC line. In addition, Giza M-2 line had high TGF- $\beta 2$ -BB genotype frequency value (0.80) which explains the positive effects of TGF- $\beta 2$ gene on LBW and carcass traits in Giza M-2 line compared to RBC line. The current result conducts the broad significant effects of TGF- $\beta 2$ gene on productive performance of chickens. In addition, the association between these valuable productive traits and TGF $\beta 2$ gene will effectively develop the selection process by using Marker-Assisted Selection (MAS) in Giza M-2 line breeding program in the near future.

Keywords: chicken, carcass parts, PCR, selection, transforming growth factor

INTRODUCTION

In broiler production, live body weight (LBW) and carcass traits are considered as the most valuable traits in broiler breeding programs and were continuously under intensive selection which is strongly associated with critical improvement in muscle characteristics in meat type chickens (Baéza *et al.*, 2012 and Nassar, 2021). Moreover, hypertrophy and hyperplasia of myofibers are the process that responsible for the maximum muscle mass (Rehfeldt *et al.*, 2000). However, poultry growth rate and muscling were depending on hypertrophy rather than hyperplasia (Velleman, 2007). Also, growth factors are important inhibitors or stimulators differentiation and proliferation of livestock cells, such as transforming growth factor beta (TGF- β) had been affects hyperplasia and hypertrophy process in poultry cells (Velleman, 2007). The chicken TGF- β is a superfamily protein which consists of four identified members: TGF- β 1, TGF- β 2, TGF- β 3, and TGF- β 4 (Burt and Law, 1994 and Groenen *et al.*, 2000). Many studies were indicates the biological effects of TGF- β isoforms in biological processes in poultry cells which were broad, including effects on immune response, extracellular matrix formation, cell growth, cell proliferation, and cell differentiation (Kubiczkova *et al.*, 2012; Pardali and Ten Dijke, 2012) and associated with feed conversion ratio (Rasal *et al.*, 2015).

In broiler breeding program, there were significant relationships between quantitative loci of the economic traits with genetic markers which led to direct selection on genotype (Lamont *et al.*, 1996 and Sandercock *et al.*, 2009). Moreover, the evolution of polymorphism of markers within the chromosome zone

in chicken genome was affected by the selection process for these economic traits (Loywyck *et al.*, 2008). Thus, the combination between modern molecular methods with traditional genetic selection process may be the better option for breeding chickens for improving productive performance (Li *et al.*, 2003 and Nassar, 2021). In addition, the recent knowledge in molecular genetics has opened new areas to develop productive performance of specific broiler and breeder crosses (Li *et al.*, 2003 and Toosi *et al.*, 2010)

The objectives of the current study were to determine genetic polymorphisms of TGF- β 2 gene by using PCR-RFLP method in Giza M-2 line which selected for high 6-week LBW for \forall generations compared to random bred control (RBC) line, and evaluate associations between TGF- β 2 polymorphisms and growth performance and carcass traits in the studies.

MATERIAL AND METHODS

Experimental populations and management

Giza M-2 line is local broiler male line specialized for meat production and produced at the Agriculture Experimental Station, Faculty of Agriculture Cairo University, Egypt (Nassar, 2017). In our study, selected males and females, from the seventh selected generation of Giza M-2 line were mated to produce the eighth generation. Also, males and females from the 15 generation of the RBC line (Nassar, 2017) were mated to produce the RBC chicks as a control group for the Giza M-2 line. Also, five hundred chicks from Giza M-2 line and five hundred chicks from RBC line were wing banded and sexed at hatch, using the vent method. All chicks were reared intermingled, 10 birds/m², in an open

house, deep litter system. Birds were provided with a commercial broiler starter (23% CP and 3,050 kcal ME/kg) and a broiler grower (21% CP and 3,100 kcal ME/kg) diets from 1 to 14 days and from 15 days to 6 weeks of age, respectively. Water and feed were provided *ad libitum* from hatch until 6 weeks of age. Light was provided 24 hours per day. At hatch, chicks were vaccinated against avian influenza virus by using (S/C) injection of H5N2 inactivated vaccine at one week of age. Chicks were also vaccinated against Newcastle disease at 7 days (eye drop, Hitchner, Nobilis®), at 10 days (S/C injection with Newcastle inactivated vaccine, Nobilis®), and at 21 days (eye drop, La Sota strain, Nobilis®). Chicks were also vaccinated against infectious bursal disease at 14 and 24 days (eye drop) using Gumboro D78 strain (Nobilis®).

Experimental measurements for body weight and carcass traits

For all lines, LBW at hatch, 14, 28, and 42 days were obtained individually by using a digital scale. At 42 days of age, 50 males and 50 females from each line were chosen at random. Birds were weighed and the weights were recorded as LBW₆, and then slaughtered after 8 hours of fasting (Papa, 1991). Birds were slaughtered by slitting the throat, cutting the carotid arteries, jugular veins, esophagus and trachea without severing the head (Salwani et al., 2016). After slaughtering, each bird was hanged in a bleeding funnel for 3 minutes, weighed, then scalded in a 68°C water bath for 30 seconds, and the feathers were removed by an automatic circular feather plucker. Birds were eviscerated and weighed; the head and shanks were removed and weighed; lastly, the carcasses were

weighed then chilled. Each chilled carcass was weighed to obtain the carcass weight (CW). The wings with bones were then removed from the front parts and weighed and recorded as wings with bones weight (WBW). Also, the skinless pectoralis major and minor muscles were removed to obtain breast muscles weight (BMW). The bones from the thighs and drumsticks were removed then the skinless leg muscles were weighed as leg meat weight (LMW). Abdominal fat pad were removed and then weighed to obtain abdominal fat pad weight. All traits were also expressed as percentage of LBW at 6 week of age.

Blood samples and DNA isolation

Individual genomic DNA was isolated from venous blood collected in anti-coagulate buffer from 25 males and 25 females from each line at 42 days of age. The extraction was carried out according to the method described by Bailes *et al.* (2007). A polymerase chain reaction (PCR) was carried out with 50 ng genomic DNA from the two lines to determine polymorphism according to the method described by Liu *et al.* (2003).

Development of PCR-RFLP Assays for TGF-β 2.

Genotyping analyses were done by using primers according to the method described by Li *et al.* (2003) as follows:

The PCR primers (5'GCC ATA GGT TCA GTG CAA G 3'; 5' TGA CAG AAG CTC TCA AGC C 3'). The 25-μL reaction volume included 50 ng of template, 1 × reaction buffer, 5 pmol of each primer, 0.16mM dNTP, 1.5mM MgCl₂, and 1 U Taq polymerase. The reaction conditions were 94°C for 3 min; 35 cycles of 94°C, 1 min; 52°C, 1 min; 72°C, 1 min, and an extension at 72°C for 10 min. A single nucleotide polymorphism (SNP) of the TGF-β₂ gene

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was detected by digesting 10 µl of the 284-bp PCR product with 6 U RsaI13 at 37°C overnight. The restriction digests were electrophoresed for 1.5 h at 100 V on a 2.0% agarose gel. Finally, the individual PCR-RFLP fragment sizes for each gene were determined by visualizing the banding pattern staining with ethidium bromide under ultraviolet light.

Statistical analysis

Data were analyzed as a two-way analysis of variance using the SAS software, general linear model (SAS Institute, 2009). The main effects were line and sex. Traits analyzed were: live body weight (LBW) at hatch, 14, 28, and 42 days of age, live body weights at 6 week of age (LBW6), carcass weight (CW), breast muscle weight (BMW), Leg muscle weight including drumstick and thigh (LMW), wings with bones (WBW), and abdominal fat pad weight (AFW), as well as percentages of LBW6 for both males and females Giza M-2 and the RBC lines. All data were reported as least square means (LSM) ± standard errors (SE). Mean values were separated, when significance existed, using Duncan's multiple range test (Duncan's, 1955). Significance level was set at 5%. The following model was used:

$$Y_{ijk} = \mu + L_i + S_j + LS_{ij} + e_{ijk}$$

Where, Y_{ijk} : The K^{th} observation of the j^{th} sex within the i^{th} line, μ : The overall mean, L_i : The effect of the i^{th} line, S_j : The effect of the j^{th} sex, LS_{ij} : The interaction between the i^{th} line and the j^{th} sex, e_{ijk} : Random error.

RESULTS AND DISCUSSION

Live body weight from hatch until 42 days and carcass traits in Giza M-2 and RBC lines

The current results indicated that the Giza M-2 line had a significant higher LBW

compared to RBC line at hatch, 14, 28, and 42 days of age (Table 1). In addition, Giza M-2 line had significantly higher LBW6 compared to RBC line as shown in Table 1. Moreover, LBW from hatch until 42 days of age was influenced by sex for each line. On the other hand, males from each line had significantly higher LBW from hatch, 14, 28, and 42 days of age compared to females as shown in Table 1. As expected, Giza M-2 line produced higher LBW6 than RBC line because LBW at hatch significantly affected broiler performance; thus, birds with heavy weight at day old produced higher LBW at market age. These results agree with the results previously reported by Mendes *et al.* (2011).

Moreover, using selection process for high LBW in commercial broiler lines resulted to reduce the improvement in sexual dimorphism. Giza M-2 females weighted, on the average, about 91% of their males' counterparts at 6 weeks of age. However, the RBC females weighted, on the average, about 96 % of their males' counterparts. This would indicate that selection for increased LBW6 increased the sexual dimorphism by almost 6 % (Table 1). The percentage of the difference between the selected Giza M-2 line and the RBC line decrease by the progress of selection compared to previous results stated by Nassar (2017). These results disagree with the results previously reported by Nassar (2017) and agree with the results previously reported by Mignon-Grasteau *et al.* (2000).

Carcass Traits and Meat yield at 42 days of age in Giza M-2 and RBC lines

Our results indicated that Giza M-2 line had significantly higher LBW6, CW, BMW, LMW, WBW, and AFW than the RBC line at 42 days of age (Table 2). As expected, the males, of both lines, had

significantly higher LBW, CW, BMW, LMW, WBW, and AFW weights compared with the females (Table 2). In addition, Giza M-2 line had significantly higher CW%, BMW%, LMW%, WBW%, and AFW% than the RBC line at 42 days of age (Table 3). However, the RBC line had significantly higher %WBW compared with the Giza M-2 line at 42 days of age (Table 3). Also, the Giza M-2 line males had higher %CW, %BMW, %LMW, and %AFW compared to the males of the RBC line at 42 days of age. However, the Giza M-2 line females had significantly higher WBW% than the males (Table 3). Similar trends were observed in the Giza M-2 line females compared with the females of the RBC line at 42 days of age. However, the RBC females had significantly higher WBW%. On the other hand, for most studied traits, males from Giza M-2 line had higher LBW6 and carcass traits than females of the same line indicating the presence of sexual dimorphism. These results are in accordance with the results previously reported by Mignon-Grasteau *et al.* (2000).

Mason *et al.* (2020) reported that genetic selections for increasing productive performance in broilers have been highly successful in improving LBW and carcass traits at market age. Thus, LBW6 and carcass traits of Giza M-2 were significantly improved as results of the intensive selection that had been done for seven generations. Thus, the Giza M-2 line had higher LBW6, BMW, and carcass parts than the RBC line. Moreover, the highest BMW% in Giza M-2 line explains by the highest LBW6 of this line because birds with higher LBW produced the greatest breast portions as reported by Schmidt *et al.* (2009). In the current study, Giza M-2

line had less WBW% compared to the RBC line. This may be occurred by increasing in breast meat portion that caused decreases in the other carcass parts on relative as studies by Fletcher and Carpenter (1993) and Nassar *et al.* (2019).

Association of TGF- β 2 gene with Productive performance in Giza M-2 and RBC lines

The polymorphism of TGF- β 2 was detected by digestion the PCR products by using the restriction enzyme *RsaI*. The following fragments sizing patterns were observed by agarose gel electrophoresis: wild type AA: There was no cleavage of the whole 284 bp segment by *RsaI*; Homozygous BB: *RsaI* cut the sequence to two fragments (184 bp and 100 bp); Heterozygous AB: *RsaI* cut the sequence to three fragments (284 bp, 184 bp and 100 bp) as shown in Figure 1. The current results are in agreement with the results previously reported by Li *et al.* (2003) and Sahib *et al.* (2021). On the other hand, Alleles and genotypes frequencies of TGF- β 2 gene observed for the Giza M-2 and RBC lines in the analyzed samples are reported in Table 4.

The BB homozygous genotype frequency of TGF- β 2 was dominantly with high value in Giza M-2 line (0.80) compared to RBC line (0). However, the frequency of AA homozygous genotype has the highest value of TGF- β 2 locus in RBC line (0.60) whereas; BB genotype had the lowest frequency value (0) in same line (Table 4). In addition, the allele B was the highest frequent allele (0.90) in Giza M-2 line compared to the value of B allele frequency (0.20) in the RBC line. Also, the allele A was the highest frequent allele (0.80) in RBC compared to the value of B allele frequency (0.10) in the Giza M-2 line (Table 4).

Li *et al.* (2006) stated that TGF- β 2 might play a critical role in muscle formation by controlling growth regulation chicken. Moreover, TGF- β 2 alleles or bands can cause phenotypic variation associated with productive performance in chicken (Bennett *et al.*, 2007 and Sahib *et al.*, 2021). Moreover, Li *et al.* (2003) and Sahib *et al.* (2021) stated that, the allele B of the TGF- β 2 gene is dominant in chicken selected for meat production. In addition, Bennett *et al.* (2007) found that, there were significant associations between the TGF- β 2-BB genotype and all LBW from 1 to 6 week of age in chicken. Thus, the current results indicated that, Giza M-2 line had high TGF- β 2-BB genotype frequency value (0.80) that explains the positive effects of TGF- β 2 on LBW at hatch, 14, 28, and 42 days of age in Giza M-2 line compared to RBC line. Our results are in agreement with the results previously reported by Bennett *et al.* (2007)

TGF- β 2 might play a critical role in chicken muscle formation by controlling of cell migration and growth regulation (Li *et al.*, 2006). Also, Chen *et al.* (2013) mentioned that, the TGF- β 2-BB genotype had significant effects on chicken myofiber diameter and Polymorphisms in TGF- β 2 gene are associated with myofiber characteristics in chickens. Thus, the current results indicated that, Giza M-2 line had high TGF- β 2-BB genotype frequency value (0.80) that explains the positive effects of TGF- β 2 on LBW6 and relative carcass traits in Giza M-2 line compared to RBC line. Our results are in accordance with the results

previously reported by Li *et al.* (2006), Chen *et al.* (2013) and Sahib *et al.* (2021).

Moreover, the current results indicated that, the strong pressure of artificial selection for high LBW at 6 weeks of age that occurred in the Giza M-2 line for seven generations had positive effects on the assessment of TGF- β 2 gene polymorphism in Giza M-2 line genome which is associated with growth performance and muscles formation in chickens, as mentioned by Fonteque *et al.* (2014) and Sahib *et al.* (2021). In addition, the genetic diversity of breeds or lines can be evaluated by the proportion of polymorphic loci, genotype and allelic frequencies, and by observed and expected heterozigosity (Fonteque *et al.*, 2014).

CONCLUSION

The strong pressure of artificial selection processes occurred in Giza M-2 after seven generations of selection for high LBW at 6 weeks of age had positive effects on LBW, at hatch, 14, 28, and 42 days of age, LBW6, and carcass traits. Also, the Giza M-2 line had high TGF- β 2-BB genotype frequency value (0.80) that explains the positive effects of TGF- β 2 on LBW at hatch, 14, 28, and 42 days of age with increasing in meat production in Giza M-2 line compared to RBC line. Also, the current study identifies TGF- β 2 as a candidate gene of quantitative trait loci (QTL) for growth and carcass traits which may be useful to improve productive performance in Giza M-2 line in their selection strategies.

Chicken, carcass parts, PCR, selection, transforming growth factor

Table (1): LSM and SE of live body weight (gm) at different ages in Giza M-2 line compared to RBC line at 42 days of age.

Line	Traits ¹	Live Body Weight (gm)			
		At Hatch	14 days	28 days	42 days
Giza M-2		39.93 ^{a*}	279.48 ^a	529.05 ^a	1019.04 ^a
RBC		36.22 ^b	152.72 ^b	344.76 ^b	611.09 ^b
SE		0.11	1.92	3.36	6.14
Sex					
Male		38.14 ^a	224.44 ^a	451.05 ^a	846.09 ^a
Female		38.01 ^a	207.76 ^b	422.76 ^b	784.04 ^b
SE		0.14	2.02	4.15	9.13
Line*Sex					
Giza M-2 ♂		39.99 ^a	289.23 ^a	542.45 ^a	1068.86 ^a
Giza M-2 ♀		39.86 ^a	269.73 ^b	515.65 ^b	972.21 ^b
RBC ♂		36.28 ^b	159.64 ^c	359.65 ^c	626.32 ^c
RBC ♀		36.15 ^b	145.79 ^d	329.87 ^d	595.86 ^d
SE		0.14	2.98	6.64	11.46
Probability					
Line		0.0001	0.0001	0.0001	0.0001
sex		0.0001	0.0001	0.0001	0.0001
Line*Sex		0.0001	0.0001	0.0001	0.0001

*a-b Means between line or sex or the interaction, within age, followed by different superscripts, differ significantly ($p \leq 0.05$).

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Table (2): LSM and SE of LBW6 and carcass parts weights (g) in Giza M-2 line compared to RBC line at 42 days of age

Traits ¹	Weight (gm)					
	LBW6	CW	BMW	LMW	WBW	AFW
Giza M-2	1043.50 ^a	709.57 ^a	160.30 ^a	149.50 ^a	82.93 ^a	19.30 ^a
RBC	642.95 ^b	388.43 ^b	82.83 ^b	64.54 ^b	58.52 ^b	10.62 ^b
SE	14.86	8.79	2.59	3.11	0.67	0.51
Sex						
Male	886.84 ^a	585.47 ^a	129.28 ^a	115.09 ^a	72.64 ^a	15.56 ^a
Female	799.61 ^b	512.53 ^b	113.85 ^b	98.94 ^b	68.81 ^b	14.36 ^b
SE	15.56	7.56	3.69	2.46	0.65	0.11
Line*Sex						
Giza M-2 ♂	1103.36 ^a	751.06 ^a	172.57 ^a	160.21 ^a	86.28 ^a	20.41 ^a
Giza M-2 ♀	983.63 ^b	668.08 ^b	148.04 ^b	138.79 ^b	79.58 ^b	18.20 ^b
RBC ♂	770.32 ^c	419.89 ^c	86.00 ^c	69.98 ^c	58.99 ^c	10.71 ^c
RBC ♀	615.58 ^d	356.97 ^d	79.66 ^d	59.10 ^d	58.05 ^c	10.52 ^c
SE	19.01	11.04	3.49	3.59	1.06	0.53
Probability						
Line	0.0011	0.0001	0.0001	0.0001	0.0001	0.0001
sex	0.0011	0.0001	0.0010	0.0011	0.0007	0.0543
Line*Sex	0.0001	0.0001	0.0013	0.0021	0.0001	0.0001

¹LBW6 = live body weight at 6 week; CW= carcass weight; BMW= breast muscle weight; LMW=drumstick+ thigh weight; WBW, wings with bone weight; AFW = abdominal fat weight.

*a-b Means between line or sex or the interaction, within age, followed by different superscripts, differ significantly ($p \leq 0.05$).

Table (3): LSM and SE of carcass parts percentages in Giza M-2 line compared to RBC line at 42 days of age.

Traits*	%LBW6				
	%CW	%BMW	%LMW	%WBW	%AFW
Line					
Giza M-2	67.99 ^{a**}	15.34 ^a	14.31 ^a	7.95 ^b	1.85 ^a
RBC	60.32 ^b	12.88 ^b	10.02 ^b	9.11 ^a	1.65 ^b
SE	0.64	0.19	0.18	0.17	0.03
Sex					
Male	65.36 ^a	14.23 ^a	12.48 ^a	8.31 ^b	1.71 ^b
Female	62.95 ^b	13.99 ^b	11.8 ^b	8.76 ^a	1.79 ^a
SE	0.69	0.23	0.20	0.12	0.05
Line*Sex					
Giza M-2♂	68.07 ^a	15.64 ^a	14.52 ^a	7.82 ^c	1.85 ^a
Giza M-2♀	67.92 ^a	15.05 ^a	14.11 ^a	8.09 ^c	1.85 ^a
RBC ♂	62.64 ^b	12.83 ^b	10.44 ^b	8.80 ^b	1.57 ^c
RBC ♀	57.99 ^c	12.94 ^b	9.60 ^b	9.43 ^a	1.74 ^b
SE	1.08	0.27	0.29	0.16	0.07
Probability					
Line	0.0109	0.0103	0.009	0.0019	0.0001
sex	0.0001	0.0001	0.0001	0.0001	0.0001
Line*Sex	0.0001	0.0001	0.0001	0.0001	0.0001

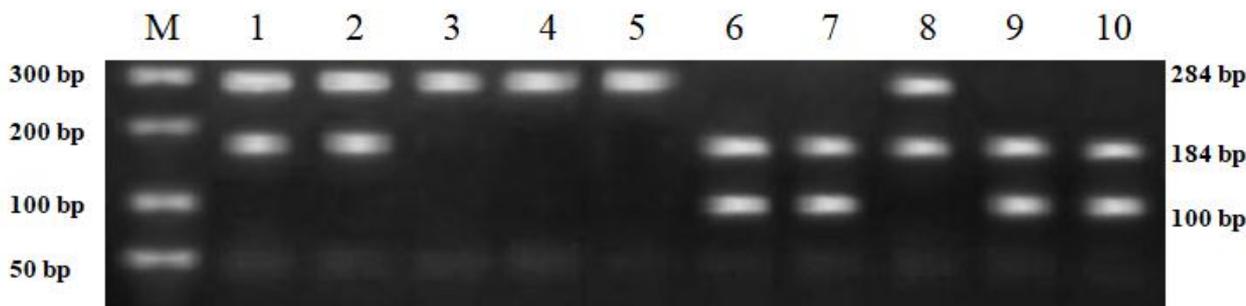
* %CW = carcass weight as percentage of LBW at 6 week of age; %BMW = breast muscle weight as percentage of LBW at 6 week of age; %LMW= drumstick = thigh weight as percentage of LBW at 6 week of age; %WBW= wings with bone weight as percentage of LBW at 6 week of age; %AFW= abdominal fat weight as percentage of LBW at 6 week of age.

**a-b Means between lines or sex or the interaction, within traits, followed by different superscripts, differ significantly ($p \leq 0.05$).

Table (4): Frequencies of alleles and genotypes of TGF- β 2 gene in Giza M-2 and RBC lines.

Lines	Genotype frequencies			Allele frrequencies	
	AA	AB	BB	A	B
Giza M-2	0	0.20	0.80	0.10	0.90
RBC	0.6	0.4	0	0.80	0.20

Figure (1): PCR-RFLP pattern for transforming growth factor- β 2 (TGF- β 2) gene. TGF- β 2 promoter region with *RsaI* digestion. M: DNA marker, Lanes 1-5 represent RBC line; Lanes 6-10 represent Giza M-2 line.



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التحليل الجيني لعامل النمو المحول بيتا ٢ والمرتبب بالاداء الانتاجي فى خط جيزة أم

فريد صابر نصار

قسم الانتاج الحيواني – كلية الزراعة – جامعة القاهرة – جمهورية مصر العربية

تعتبر جينات عوامل النمو المحول بيتا واحدة من عائلة كبيرة من الجينات التى تساهم فى تنظيم العديد من العمليات والأنشطة الحيوية فى الدجاج. وتهدف الدراسة الحالية إلى دراسة تعدد المظاهر الوراثية لجين عامل النمو المحول بيتا ٢ فى خط جيزة أم -٢ بعد ٧ أجيال من الانتخاب لأعلى وزن جسم عند ٦ أسابيع من العمر بالمقارنة مع خط الكنترول وعلاقته مع الصفات الانتاجية. وأظهرت النتائج أن وزن الجسم الحي فى الاعمار المختلفة وكذلك اوزان الأجزاء المختلفة للذبائح كانت أعلى فى خط جيزة أم -٢ بالمقارنة مع خط الكنترول. كما أظهرت النتائج أن التكرار الجيني للتركيب الوراثي BB لعامل النمو المحول بيتا ٢ فى خط جيزة أم -٢ = ٠.٨٠ فى حين كانت قيمته فى خط الكنترول = صفر وهذا يعنى أن هناك تأثير إيجابي لجين عامل النمو المحول بيتا ٢ على زيادة الكفاءة الانتاجية لخط جيزة أم -٢ سواء فى زيادة وزن الجسم وكذلك زيادة أجزاء الذبائح المختلفة مقارنة بخط الكنترول. نستنتج من ذلك أنه يمكن الاستفادة من وجود المظاهر الوراثية لجين عامل النمو المحول بيتا ٢ مثل الاستفادة من المظهر الوراثي BB فى إختيار أفضل الافراد فى برنامج الانتخاب الوراثي فى خط جيزة أم -٢. لزيادة كفاءة برنامج الانتخاب والاسراع من عمليات تحسين الكفاءة الانتاجية فى خط جيزة أم -٢.

الكلمات المفتاحية: الدجاج، أجزاء الذبائح، تفاعل البلمرة المتسلسل، الانتخاب، عامل النمو المحول