



**GENETICALLY IMPROVEMENT OF SOME PERFORMANCE TRAITS OF EGYPTIAN LOCAL CHICKENS BY TRANSFERRING GROWTH HORMONE GENE FROM BROILER**

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**ABSTRACT:** The present study was aimed to investigate isolation, cloning and sequencing of chicken Growth hormone gene (cGH) from chicken (Cobb 500 broilers) then transfer chicken Growth hormone gene by two methods the first gene transfer method by using sperm-mediated gene transfer (SMGT) technique and the second gene transfer method using bioresonance (Bio) to local chicken strain (Bandarah). This study was investigated for two generations. The body weight at hatch and 4 weeks of age increased by 3.32 and 8.09 g for SMGT method and by 1.27 and 5.22 g for Bio method respectively. The SMGT method was increased the body weight at 12 wk of age by 60.17 and 7.96 g for males and females respectively. The growth rate during 0-4 weeks of age was 131.64, 130.68 and 132.35%, for SMGT, Bio and control respectively. In conclusion cGH Gene successfully isolated, molecular cloning from Cobb 500 and transferring by two methods SMGT and Bio to produce transgenic chickens of a local strain in Egypt. SMGT is an efficient method that will hopefully facilitate the implementation of strategies for securing the benefits that can be expected to arise from the introduction of transgenic chicken, Bio opens important new perspectives in the field of animal transgenics would be more rapid, with quick and effective delivery of genes to target tissues. Chicken cGH gene was effective in all growth traits and moved from the first generation to the second generation with the same shape and increased the effect. The second generation gave higher body weight at 0, 4, 8 and 12 weeks of age.

**Key words:** Local chicken, cGH gene, Gene transfer, SMGT, Growth traits

## INTRODUCTION

In recent years, the most advanced research in the world has been the genetically modified technology. The manipulation of animal genome has offered a powerful bioreactor for the production of pharmaceutical and industrial proteins (Li and Lu, 2010).

Development of simple and readily adoptable methods to mediate gremlin engineering of the chicken genome will have many applications in research, agriculture and industrial biotechnology (Challagulla *et al.*, 2020). Specific developmental characteristics of the chicken make it an attractive model for the generation of transgenic organisms. Several transgenic chickens have been generated during the last two decades via viral and non-viral transfection.

The new approaches and technologies can be applied to generate a transgenic chicken with regards to recombinant protein productions (Bahrami *et al.*, 2020). Growth trait is very important economic trait in broiler production, and is controlled by complex genes. Chicken growth hormone (cGH) consists of 4,101 base pairs, having five exons and four introns (Kansaku *et al.*, 2008), and containing 191 amino acid residues (Hrabia *et al.*, 2008). Hormones such as the growth hormone, IGF, thyroid hormones and insulin, play important and diverse roles in animal growth (Zhou *et al.*, 2005). Most of the functions of the growth hormone in chickens are mediated by insulin-like growth factors (IGF) which stimulate amino acid uptake, glucose metabolism, DNA synthesis (McMurtry, 1998), protein synthesis, and the proliferation of different cell types (McMurtry *et al.*, 1997). Recombinant DNA technology is another major DNA-

based tool that has gained popular attention in the past decade. This technology allows scientists to find individual genes, cut them out, and insert them into the genome of another organism. The gene transfer technology has been applied by different methods that includes Microinjection, Sperm-mediated cell, Retroviral vectors, Primordial germ cells and Plasmid as vectors. A logical alternative strategy to generate transgenic animals theoretically consists of the introduction of foreign DNA into male gametes before the fertilization process (Spadafora, 2002). As a result of Bioresonance Therapy, a weakened organ receives energy needed for normal functioning, which is redistributed on a wide scale, restoring the workability of affected organs. Thousands of people have been able to cut down on their dosages of medicines, and in some cases have entirely stopped taking them and have regained excellent health. (WHO 2015). Poultry production is an important and diverse component of agriculture all over the world. Meat from native chickens is preferred by more Thai consumers than commercial broilers due to their superior taste and meat texture. However, the native chickens are inferior in production due to their low growth rates, as compared with commercial breed production. (Promwatee, *et al.*, 2013).

The objective of this study was genetic improvement of some performance traits of local chicken (Bandarah) strain by isolate, molecular cloning, sequencing and transferring of GH Gene from Cobb 500, by two methods of gene transfer the first one gene transfer using Sperm Mediated Gene Transfer (SMGT) and the second method gene transfer using Bioresonance (Bio).

**MATERIALS AND METHODS**

The present study was carried out at Faculty of Agriculture Damanhour University Animal and Poultry Production Department and El-Sabahia Poultry Research Station Alexandria, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture. With the cooperation of Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, Egypt and BioMedTec, University of Lübeck, Germany from 2017 to 2019. Bandarah strain was used in this experimental. Bandarah was develop chicken strain could be utilized as foundation stock for meat production (Mahmoud *et al.*, 1989).

**Experimental birds and treatments:**

A total 125 hens and 25 cocks at 8 month of age of Bandrah chicken strain were used to start this study to produce generation one table (1). The birds were assigned in individual cages; feed and water provided *ad-libitum* and divided randomly into 3 groups. Group A used method cGH gene transfer using sperm-mediated cell technique contain 50 hens and 10 cocks, group B used method cGH gene transfer using bioresonance contain 50 hens and 10 cocks, group C control group used classic artificial insemination contain 25 hens and 5 cocks. Second generation was done by classic artificial insemination between cocks and hens from each group. First group SMCT taking 2 mL of mixed solution contains 1 mL of semen 10µg dose of cGH gene, Lipofectin reagent and saline, second group taking 2 mL of mixed solution contains 1 mL of semen which treat by Bioresonance method, Lipofectin reagent and saline and third group taking 2 mL of mixed solution contains 1 mL of semen without any

gene treatment, Lipofectin reagent and saline used in dilution.

**Molecular isolation and cloning Growth hormone gene:**

**Chicken liver samples:**

Liver samples were immediately excised from chicken fast growing (cobb500 broilers) as a high producing exotic broiler strain at 3 weeks age. The collected liver tissue was rapidly dissected into small pieces using sterile scalpel, immediately stored at -80°C until RNA extraction.

**Total RNA extraction and cDNAsynthesis :**

Total RNA was extracted from the liver sample using RNA-spin™ Total RNA Extraction Kits (iNtRON Biotechnology, Inc) following manufacturer's recommendations. RNA was quantified using Nano Drop technology with the Epoch Multi-Volume Spectrophotometer System (Biotech, Winooski, VT, USA).

**Amplification of chicken growth hormone (cGH) cDNA by reverse transcriptase polymerase chain reaction (RT-PCR):**

One microgram of each total RNA was reverse transcribed using Superscript II reverse transcriptase (Life Technologies, Inc., Grand Island, NY) according to the manufacturer's directions. A 0.1-ml aliquot of the reaction was used in each PCR, using specific primers for GH. The forward primers located in exons 1 (PE1F) and 4 (PE4F) of the chicken GH (cGH) gene were TCAAGCAACAC-CTGAGCAACTC and TTTTGGCAC-CTCAGACAGAGTG, respectively, and the reverse GH primer located in exon 5 (PE5R) was CTGTGGGTTTATTCCTCGTGT. PCR was carried out using TAKARA TaqDNA polymerase (TAKARA, Otsu, Japan) and a thermal cycler (Gene Amp

PCR System 9700, PE Applied Biosystems, Foster City, CA). The temperature cycling conditions for the PCR were 35 cycles of reactions including denaturation for 30 sec at 95 °C and extension for 1 min at 60 °C, followed by additional extension for 10 min at 60 °C. A 10 µL aliquot of each resulting reaction was electrophoresed on a 2.0% agarose gel, stained with ethidium bromide, and photographed under UV illumination. The amplified cDNA fragments were then subcloned into a pGEM3Zf+ plasmid and subjected to sequencing. DNA sequencing was performed using fluorescent primers and an automated DNA sequence (PE Applied Biosystems 373A).

**cGH gene transfer methods:**

**The first method was using sperm-mediated gene transfer:**

The foreign GH gene was transferred using sperm-mediated gene transfer. The role of the spermatozoa during fertilization is to transfer a haploid genome to the resultant zygote. This capacity has been exploited as an innovative strategy for the delivery of exogenous DNA for the production of transgenic animals (Lauria and Gandolfi., 1993 and Kim *et al.*, 1997).

**The second method was using Bioresonance:**

The broiler cobb 500 blood sample insert inside a magnetic coil targeting the specific gene growth hormone (GH), mRNA with a special software to be stored as a magnetic pattern same like sampling a sound file on a disk the transmitting this pattern to the Bandarah chicken semen with the same technique. (Mohammad *et al.* 2015).

**Birds and their management:**

Chicks hatched from different experimental groups of artificial insemination experiments were kept under similar hygienic and environmental conditions.

Hatched chicks Vaccination and medication were done according to the used program in the station, as follows: Marick disease at hatch ,Hitchener B1 + (IB) ( Spraying at 7 day, Gumboro at 12 day, Lasota at 18 day, Gumboro at 24 day, Avian influenza Injection at 32 day, Lasota + IB (Spraying) at 40 day, Fowl Pox at 50 day and Lasota at 70 day.

Experimental groups were reared under similar managerial and hygienic conditions. Fresh water was automatically available at all time by stainless steel nipples for each cage. The experimental diets were offered to the chicks *ad libitum* in mash form . At hatching day, the chicks were wing- banded, weighed and brooded on floor brooders with electric heaters were used for brooding chicks, at 32°C during the 1<sup>st</sup> week and 3°C was decreased each week thereafter till it reached 22-24°C. Wheat straw of 10 cm depth was used in brooding house. The wet litter was changed with dry one. Chicks were fed *ad libitum* on starting (23% protein and 2900 Kcal/kg) from 0-8 weeks and growing (18.5% protein and 2850 Kcal/kg) from 8-12 weeks.

**Studied traits:**

Chicks were individually weighed. Body weight was recorded at hatch, 4, 8 and 12 weeks of age to the nearest g.

**Growth rate:**

Growth rate was estimated biweekly according to the equation of (Brody, 1945) during the period of hatch up to 12 weeks of age as (0- 4, 4 - 8 and 8- 12 wks).

**Gene Transferring response (R):**

Gene Transferring responses due to gene transfer in the second generation compared to the first generation

$$R_t = (S_t - S_{t-1}) - (C_t - C_{t-1}).$$

Where: R<sub>t</sub>: realized gain due to transferring methods in t<sup>th</sup> generation.

## Local chicken, cGH gene, Gene transfer, SMGT, Growth traits

S and C: average performance of the transferring methods and control populations (Becker, 1985).

### 3.9. Genetic analyses:

At the age 8 week of the experiment, 9 birds from each group in every generation were randomly chosen for collection of blood samples to genetic analyses.

### Random amplified cDNA ends (RACE) analysis of GH mRNA:

The 5'RACE analysis was performed using a 5'/3'RACE kit (Roche, Sandhofer Strasse, Mannheim, Germany) according to the manufacturer's directions. Total RNA was prepared from blood using a GLASS MAX RNA Micro-isolation Spin Cartridge System (Life Technologies, Inc.), and 2 µg of each total RNA were reverse transcribed using PE5R as reverse gene-specific primer and two forward gene-specific primers were used in subsequent amplification of cGH cDNA.

PCR amplification was conducted under the following conditions: 95°C for five minutes, followed by 30 to 35 cycles at 95°C for 45 s, 58°C to 68°C for 30 to 45 s, and 72°C for 30 to 45 s; followed by a final extension at 72°C for five minutes.

A 10 µl aliquot of each PCR reaction was electrophoresed on a 2.0% agarose gel, stained with ethidium bromide, and photographed under UV illumination. The amplified cDNA fragments were sub-cloned into a pGEM3Zf+ plasmid and subjected to sequencing. Sequence analysis was carried out using GENETYX software.

### 3.10. Statistical analysis:

Data were analyzed using SAS, 2004 software (SAS, 2004) by using two ways ANOVA to (methods of transferring and generation). The other symbols are as described previously. Differences were considered to be statistically significant at

$P < 0.05$ . The difference among treatment were tested using Duncan's multiple range test at  $P < 0.05$

Model 1: ( BW.0 ,BW.4wks ,GR. 0-4 and GR. 4-8wks)

$$X_{ijk} = \mu + M_i + G_j + MG_{ij} + e_{ijk}$$

$X_{ijk}$  = the observation of  $ijk$ .

$\mu$  = overall mean.

$M_i$  = effect of the  $i^{\text{th}}$  methods of transferring cGH gene.

$G_j$  = effect of the  $j^{\text{th}}$  generation.

$MG_{ij}$  = the interaction between  $M_i$  methods of transferring and  $G_j$  generation

$e_{ijk}$  = the experimental random error

Model 2: ( BW.8 ,BW.12wks. and GR. 8-12wks)

$$X_{ijkl} = \mu + M_i + G_j + S_k + MG_{ij} + MS_{ik} + GS_{jk} + MGS_{ijk} + e_{ijkl}$$

$X_{ijkl}$  = the observation of  $ijkl$ .

$\mu$  = overall mean.

$M_i$  = effect of the  $i^{\text{th}}$  methods of transferring cGH gene.

$G_j$  = effect of the  $j^{\text{th}}$  generation.

$S_k$  = effect of the  $k^{\text{th}}$  sex.

$MG_{ij}$  = the interaction between  $i^{\text{th}}$  methods of transferring and  $j^{\text{th}}$  generation

$MS_{ik}$  = the interaction between  $i^{\text{th}}$  methods of transferring and  $k^{\text{th}}$  sex.

$GS_{jk}$  = the interaction between  $j^{\text{th}}$  generation and  $k^{\text{th}}$  sex.

$MGS_{ijk}$  = the interaction among  $i^{\text{th}}$  methods of transferring and  $j^{\text{th}}$  generation as well as  $k^{\text{th}}$  sex.

$e_{ijkl}$  = the experimental random error

## RESULTS AND DISCUSSIONS

### Isolation, cloning and sequencing of (cGH) gene:

Total RNA was extracted from chicken liver tissue and the cDNA was successfully prepared. PCR amplification with cGH specific primers generated 429bp fragment .

The amplified cDNA fragments were then sub cloned into pGEM3Zf+ plasmid. Plasmid purification and perform stand-

ard PCR shows a fragment of about 429bp when using specific cGH primers and the same fragment size was generated by double digestion of recombinant plasmid (Fig.1).

Finally the recombinant cDNA with the Gen Bank reference sequence accession number: LC441152.1 (Fig.2). The nucleotide and deduced amino acids were aligned and compared with reference sequence which showed about 99% matching due to heterozygous of the extracted cDNA. The result of this study is suggested an easy method to isolate and cloning of targeted varieties of chicken genes which may be useful to improve the local breed. Molecular biologists exploit the replicative ability of cultured cells to clone genes. Gene cloning also enables scientists to manipulate and study genes in isolation from the organism they came from. This allows researchers to conduct many experiments that would be impossible without cloned genes.

#### **Genetic analyses of GH mRNA**

The amplified cDNA fragments from blood sample of first and second generation were sub cloned into a pGEM3Zf+ plasmid and subjected to sequencing. Sequence analysis was carried out using GENETYX software. Showed that the PCR product size from mRNA was about 798 bp from Bandarah chicken control without any gene treatment, PCR Products Bandarah Growth Hormone GH, mRNA normal Length 800bp for Bandarah chicken by Bio method and PCR Products Bandarah Growth Hormone GH, mRNA Bandarah chicken by SMGT method. Figure 3,4 and 5 showed that the changed in gene GH sequence between methods of gene transfer approved that gene GH which isolated from Cobb 500 was successfully transferred by two methods SMGT and BIO to Bandara

chicken strain. The same sequence in each group in first and second generation showed that changed in GH gene as result of gene transferred by two methods SMGT and BIO was bass from parents to progeny

#### **Performance traits:**

##### **Body weight at hatch (Bw 0) and four weeks (Bw 4) of age:**

Means  $\pm$  SE for Bw 0 and Bw 4 of age as affected by methods and generations are shown in table (2). Body weight at hatch in the generation two was higher significantly than the first one. On the other hand, body weight at hatch for SMGT method had highest significant difference  $P < 0.01$  weight when compared with that Bio and control one. The highest significant ( $P < 0.01$ ) value of Bw 4 was found in SMGT method 176.47 g followed by the control one 170.64 g and the lowest value was found in bio method 168.82 g, while there were no significant difference between the two generation for (Bw 4) 177.77 g and 172.64 g, respectively. The body weight at hatch and at 4 weeks of age increased by 3.32 g and 8.09 for SMGT method and by 1.27 and 5.22 for Bio method, respectively, Table (4).

##### **Body weight at 8 weeks of age (Bw 8):**

Table (3) shows the means  $\pm$ SE for body weight at eight weeks of age for males, females and combined sexes by methods and generations. The averages of the methods for these traits of the males were 386.98 g and 439.95 g for the first and second generation, respectively. The corresponding figures for the females were 326.76 g and 323.56 g, respectively, while, the chick in the second generation had higher significantly ( $P < 0.01$ ) value 388.69 g than the first generation 351.29 g. Moreover, the highest insignificant body weight was found in SMGT method 372.05 g. The differences between the

## Local chicken, cGH gene, Gene transfer, SMGT, Growth traits

overall mean of SMGT method and the control line were 56.72 g for males and -12.94 g for females, Table (4), while, the responses of Bio method were -29.03 g, and -101.13 g for male and female, respectively.

### Body weight at 12 wks of age:

Means  $\pm$  SE of body weight at 12 weeks of age for males, females and combined sexes by methods and generations are presented in table (3).

The results show that, there were highly significant differences ( $P < 0.01$ ) between methods and generations for Bw at 12 wks of age. The second generation values were higher ( $P < 0.01$ ) than the first generation for males 814.31 g and 645.16 g, respectively, followed by females 631.91 g and 533.72 g, respectively. However, the SMGT method was the highest ( $P < 0.01$ ) weight 682.74 followed by Bio method 652.27 g. the method of SMGT was increased the body weight at 12 wk of age by 60.17 g and 7.96 g for males and females, respectively. Table (3) While, Bio method decreased body weight at 12 wks of age by 46.18g, and 71.01g for males, females respectively table (4).

These results agree with (Al-Kelabil J. K. *et al.*, 2019), Who found that cGH genes expression as for body weights, it has a favorable effect during the period of first four weeks, while its effect is limited after 6 weeks old. (Vasilatos-Younken *et al.* 1997) found that the chicken growth hormone (cGH) gene is considered one of the most candidate genes that can influence chicken performance traits because of its crucial function in growth.

The chicken growth hormone (cGH) gene plays a crucial role in controlling growth and metabolism, leading to potential correlations between cGH polymorphisms and economic traits. (Nie Q. *et al.*, 2005).

(Yokoyama and Asahara 2011; Mohamed. 2009 and El-Tahawy. 2005) who all found that introducing foreign DNA into chicken embryonic cells significantly increased body weight of transgenic chicks.

### Growth rate from day at hatch to four weeks of age:

Means and  $\pm$  SE of growth rate during (0-4) weeks of age by methods and generations are presented in table (5). The difference among methods was significant ( $P < 0.05$ ), and it was highly significant ( $P < 0.01$ ) between generations. However, the rate of growth for SMGT, Bio and control has 131.64%, 130.68 and 132.35%, respectively. The growth rate during (0-4) week of age decreased by 3.33% and 1.07% for SMGT and bio methods Table (4).

### Growth rate during (4-8) weeks of age

Table (5) shows the means  $\pm$  SE of growth rate during (4-8) weeks of age by methods and generations. The chicks of the second generation had significantly higher value 76.62% than the first generation 68.51. While, it was no significant difference among methods. The Bio method had the highest growth rate at this period and the lowest one was found in control line. The growth rate during (4-8) week of age increased by 5.85% and 0.86 % for SMGT and bio methods Table (4).

### Growth rate during (8-12) weeks of age

Table (6) presented the mean  $\pm$  SE of the growth rate during 8-12 weeks of age for males and females as affected by methods and generations. The second generation had higher growth rate at this period (64.65%) than the first one (49.94%). The difference among methods and generations were highly significant ( $P < 0.01$ ). For the methods, SMGT and Bio had approximately the same values, which were

the highest growth rate (61.93 and 60.60%), respectively. The SMGT and Bio methods increased growth rate during (8-12) weeks of age by 5.03% and 10.72% for combined sex, respectively table (4).

These results agree that average growth rate increased by introducing foreign DNA the rate of increase was higher as foreign DNA level increased up to the level of 25 µg. However it decreased by increasing the level of foreign DNA to 30µg (El-Garhy 2004; Deeb and Lamont 2002) stated that insulin-like growth factors, as molecular markers for growth rate in native breed. (Elokil, 2015) found that IGF-1 gene doses were highly significant effect ( $P<0.001$ ) on growth rate % from hatch to 4, GR from 4 to 8, GR from 8 to 12, GR from 12 to 16 and GR from hatch to 16 weeks of age. 10µg IGF-1 gene was best dose in GR from hatch to 16 weeks with Mandarah (207.88%) and 15µg IGF-1 gene with Silver Montazah strain (201.54%) compared to zero doses (194.81%), respectively. (El-Garhy, 2011), Average growth rate increased by introducing foreign DNA the rate of increase was higher as foreign DNA level increased up to the level of 25 µg. However it decreased by increasing the level of foreign DNA to 30µg.

## **IN CONCLUSION**

cGH Gene successfully isolate, molecular cloning from Cobb 500, and transferring by two methods SMGT and Bio to production transgenic chickens of a local strain in Egypt Bandarah. The SMGT is an efficient method that will hopefully facilitate the implementation of strategies for securing the benefits that can be expected to arise from the introduction of transgenic chicken, Bio open important new perspectives in the field of animal transgenic would be more rapid, with quick and effective delivery of genes to target tissues. Chicken cGH gene was effect in growth performance and moved from the first generation to the second with the same shape and increased the effect

**Local chicken, cGH gene, Gene transfer, SMGT, Growth traits**

**Table (1):** offspring number at hatch, 4, 8 and 12 weeks of age of methods and two generations.

Generation	Methods of gene transferring	Offspring Number					
		One day	4 wk	8 wk		12 wk	
				male	female	male	Female
One	SMGT	147	131	78	49	76	47
	Bio	139	135	54	78	53	75
	Control	67	65	40	23	39	22
Two	SMGT	264	261	108	116	100	112
	Bio	253	250	102	118	95	110
	Control	108	100	50	36	47	34

**Table (2):** Means  $\pm$  stander errors (SE) of body weight at hatch and four weeks of age by two methods of transferring techniques and generations of Bandarah strain

Generation	Methods of gene transferring	Body weight	
		Bw 0	Bw 4
One	SMGT	34.77 $\pm$ 0.24	174.23 $\pm$ 3.51
	Bio	35.17 $\pm$ 0.30	168.45 $\pm$ 2.92
	Control	35.10 $\pm$ 0.42	173.37 $\pm$ 5.52
Overall gen mean		34.99 $\pm$ 0.17 <sup>B</sup>	171.77 $\pm$ 2.14
Two	SMGT	36.68 $\pm$ 0.18	177.68 $\pm$ 0.19
	Bio	35.03 $\pm$ 0.19	169.03 $\pm$ 0.19
	Control	33.69 $\pm$ 0.30	168.73 $\pm$ 0.32
Overall gen mean		35.49 $\pm$ 0.13 <sup>A</sup>	172.64 $\pm$ 0.22
Overall mth mean	SMGT	36.00 $\pm$ 0.15 <sup>a</sup>	176.47 $\pm$ 1.24 <sup>a</sup>
	Bio	35.08 $\pm$ 0.16 <sup>b</sup>	168.82 $\pm$ 1.05 <sup>b</sup>
	Control	34.23 $\pm$ 0.25 <sup>c</sup>	170.64 $\pm$ 2.27 <sup>b</sup>
<b>Factors</b>		<b>Significant</b>	
Generation (gen)		*	NS
Methods (mth)		**	**
GenXmth		**	NS

SMGT: sperm mediated gene transfer, Bio: Bioresonance method, gen: generation, mth: method, BW 0 body weight at hatch, Bw 4 body weghit at four weeks of age, NS: non-significant, \* Significant at  $\leq 0.05$ , \*\* Significant at  $\leq 0.001$ , <sup>a, b, c</sup> Means within the same column in the same trait with different superscripts are significantly different ( $P \leq 0.05$ ).

**Table (3):** Means  $\pm$ stander errors (SE) of body weight at eight and twelve weeks of age for male and female by two methods of transferring techniques and generations of Bandarh strain.

Generation	Methods of gene transferring	Body weight at 8 wk			Body weight at 12 wk		
		Male	Female	Combined sex	Male	Female	Combined sex
One	SMGT	368.34 $\pm$ 12.72	328.11 $\pm$ 8.08	343.87 $\pm$ 7.16	645.61 $\pm$ 12.76	567.77 $\pm$ 10.62	598.90 $\pm$ 8.78
	Bio	379.32 $\pm$ 10.52	332.42 $\pm$ 7.78	352.99 $\pm$ 6.69	660.71 $\pm$ 14.67	524.67 $\pm$ 11.43	586.86 $\pm$ 11.26
	Control	441.85 $\pm$ 16.79	315.05 $\pm$ 16.02	364.25 $\pm$ 13.95	615.54 $\pm$ 21.50	477.68 $\pm$ 16.81	531.98 $\pm$ 15.57
Overall gen mean		386.98 $\pm$ 7.80 <sup>B</sup>	326.76 $\pm$ 5.63	351.29 $\pm$ 4.89 <sup>B</sup>	645.16 $\pm$ 8.90	533.72 $\pm$ 7.59	580.34 $\pm$ 6.57 <sup>B</sup>
Two	SMGT	480.79 $\pm$ 10.41	336.83 $\pm$ 6.99	401.04 $\pm$ 8.45	873.13 $\pm$ 10.69	678.82 $\pm$ 7.63	743.59 $\pm$ 9.15
	Bio	406.02 $\pm$ 7.34	252.95 $\pm$ 9.76	378.31 $\pm$ 8.32	781.88 $\pm$ 9.89	556.75 $\pm$ 9.85	697.46 $\pm$ 11.41
	Control	497.58 $\pm$ 14.19	336.71 $\pm$ 10.01	377.79 $\pm$ 13.21	782.89 $\pm$ 24.21	580.77 $\pm$ 19.15	656.56 $\pm$ 20.60
Overall gen mean		439.95 $\pm$ 6.42 <sup>A</sup>	323.56 $\pm$ 5.49	388.69 $\pm$ 5.46 <sup>A</sup>	814.31 $\pm$ 7.73	631.91 $\pm$ 7.03	714.60 $\pm$ 6.96 <sup>A</sup>
Overall mean	SMGT	427.42 $\pm$ 9.63	332.12 $\pm$ 5.25	372.05 $\pm$ 5.77	767.22 $\pm$ 13.39	634.94 $\pm$ 7.29	682.74 $\pm$ 7.59 <sup>a</sup>
	Bio	396.81 $\pm$ 6.10	312.79 $\pm$ 7.33	365.76 $\pm$ 5.40	741.21 $\pm$ 9.49	540.71 $\pm$ 7.66	652.27 $\pm$ 8.83 <sup>b</sup>
	Control	459.45 $\pm$ 12.94	325.03 $\pm$ 9.82	369.83 $\pm$ 9.82	684.00 $\pm$ 20.88	521.86 $\pm$ 13.96	584.44 $\pm$ 13.73 <sup>c</sup>
	Sex	416.72 $\pm$ 5.19 <sup>A</sup>	325.45 $\pm$ 4.01 <sup>B</sup>		742.86 $\pm$ 7.55 <sup>A</sup>	586.98 $\pm$ 5.72 <sup>B</sup>	
<b>Factors</b>		<b>Significant</b>					
Generation (gen)		**			**		
Methods (mth)		NS			**		
Sex		**			**		
GenXmth		*			NS		
GenXsex		**			NS		
mthXsex		**			**		
GenXmthXsex		**			**		

SMGT: sperm mediated gene transfer, Bio: Bioresonance method, gen: generation, mth: method, NS: non-significant, \* Significant at  $\leq 0.05$ , \*\* Significant at  $\leq 0.001$ , <sup>a, b, c</sup> Means within the same column in the same trait with different superscripts are significantly different ( $P \leq 0.05$ ).

**Local chicken, cGH gene, Gene transfer, SMGT, Growth traits**

**Table (4):** Response of two methods of gene transferring for body weight at hatch, four week, eight week, twelve week of age and growth rate during the period( 0:4, 4:8, 8:12 weeks) of age.

Methods	SEX	SMGT	Bio
Body weight at hatch		3.32	1.27
Body weight at four weeks		8.09	5.22
Body weight at eight weeks	Male	56.72	-29.03
	Female	-12.94	-101.13
	combined sexes	43.63	11.78
Body weight at twelve weeks	Male	60.17	-46.18
	Female	7.96	-71.01
	combined sexes	20.11	-13.98
Growth rate 0: 4		-3.33	-1.07
Growth rate 4: 8		5.85	0.86
Growth rate 8 : 12	Male	-9.67	-0.84
	Female	16.79	34.93
	combined sexes	5.03	10.72

SMGT: sperm mediated gene transfer, Bio: Bioresonance method

**Table (5):** Means  $\pm$  stander errors (SE) of growth rate from day of hatch to four weeks of age and from four to eight weeks of age by two methods of transferring techniques and generations for Bandarah strain

Generation	Methods of gene transferring	Growth rate	
		0:4	4:8
One	SMGT	131.62 $\pm$ 1.04	66.29 $\pm$ 1.16
	Bio	129.16 $\pm$ 1.11	70.89 $\pm$ 1.65
	Control	130.27 $\pm$ 1.33	69.25 $\pm$ 0.93
Overall gen mean		130.35 $\pm$ 0.66 <sup>B</sup>	68.51 $\pm$ 0.77 <sup>B</sup>
Two	SMGT	131.65 $\pm$ 0.22	77.25 $\pm$ 1.48
	Bio	131.45 $\pm$ 0.24	76.86 $\pm$ 1.47
	Control	133.63 $\pm$ 0.40	74.36 $\pm$ 2.71
Overall gen mean		131.91 $\pm$ 0.16 <sup>A</sup>	76.62 $\pm$ 0.98 <sup>A</sup>
over all mean	SMGT	131.64 $\pm$ 0.37 <sup>ab</sup>	71.93 $\pm$ 1.01
	Bio	130.68 $\pm$ 0.41 <sup>b</sup>	74.14 $\pm$ 1.12
	Control	132.35 $\pm$ 0.57 <sup>a</sup>	71.30 $\pm$ 1.24
factors		<b>Significant</b>	
Generation (gen)		**	**
Methods (mth)		*	NS
GenXmth		*	NS

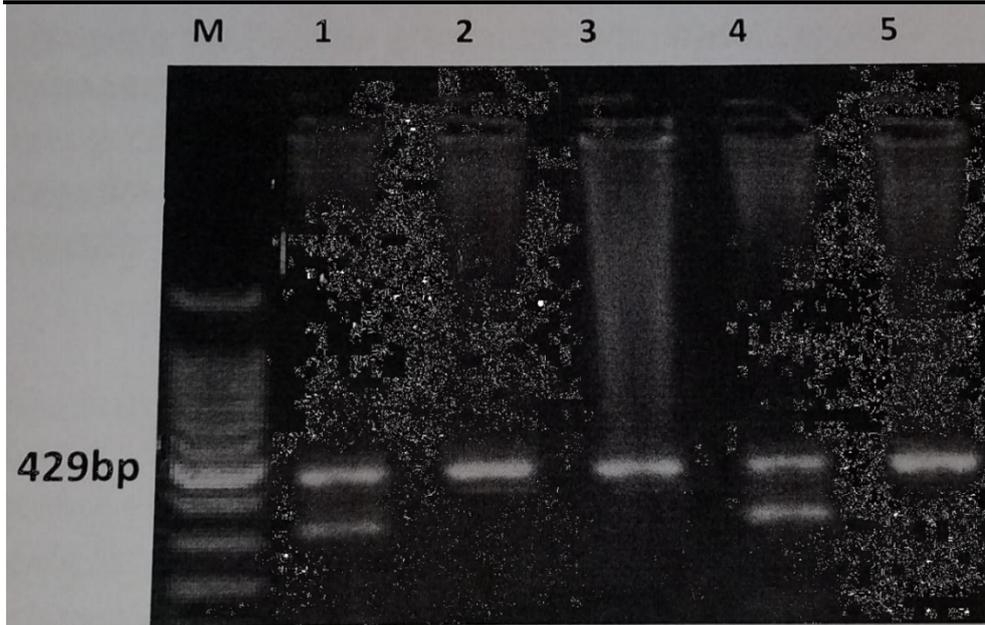
SMGT: sperm mediated gene transfer, Bio: Bioresonance method, gen: generation, mth: method, NS: non-significant,\* Significant at  $\leq 0.05$ , \*\* Significant at  $\leq 0.001$ , <sup>a,b,c</sup> Means within the same column in the same trait with different superscripts are significantly different (P $\leq 0.05$ ).

**Table (6):** Means  $\pm$  stander errors (SE) of growth rate during 8:12 weeks of age for male and female by two methods of transferring techniques and generations of Bandarah strain

Generation	Methods of gene transferring	Growth rate 8:12 wk		Combined sex
		Male	Female	
one	SMGT	55.10 $\pm$ 2.86	55.61 $\pm$ 2.04	55.40 $\pm$ 1.67
	Bio	52.28 $\pm$ 2.93	47.25 $\pm$ 2.02	49.80 $\pm$ 1.80
	Control	32.99 $\pm$ 0.83	44.23 $\pm$ 2.45	39.58 $\pm$ 1.64
Overall gen mean		49.17 $\pm$ 1.80	50.54 $\pm$ 1.34	49.94 $\pm$ 1.09 <sup>B</sup>
two	SMGT	57.92 $\pm$ 0.93	75.40 $\pm$ 1.07	67.69 $\pm$ 1.06
	Bio	63.93 $\pm$ 0.53	85.18 $\pm$ 2.61	67.78 $\pm$ 0.99
	Control	45.48 $\pm$ 1.74	47.23 $\pm$ 1.49	46.84 $\pm$ 1.17
Overall gen mean		60.54 $\pm$ 0.60	70.15 $\pm$ 1.50	64.65 $\pm$ 0.78 <sup>A</sup>
Overall	SMGT	56.66 $\pm$ 1.37	65.79 $\pm$ 1.41	61.93 $\pm$ 1.04 <sup>a</sup>
	Bio	60.54 $\pm$ 1.03	60.75 $\pm$ 2.86	60.60 $\pm$ 1.13 <sup>a</sup>
	Control	37.27 $\pm$ 1.29	45.64 $\pm$ 1.48	42.63 $\pm$ 1.13 <sup>b</sup>
	Sex	56.00 $\pm$ 0.87 <sup>B</sup>	59.71 $\pm$ 1.17 <sup>A</sup>	
<b>factors</b>	<b>Significant</b>			
Generation (gen)	**			
Methods (mth)	**			
Sex	**			
GenXmth	NS			
GenXsex	**			
mthXsex	**			
GenXmthXsex	**			

SMGT: sperm mediated gene transfer, Bio: Bioresonance method, gen :generation, mth: methods, NS: non-significant,\* Significant at  $\leq 0.05$ , \*\* Significant at  $\leq 0.001$ , <sup>a,b,c</sup> Means within the same column in the same trait with different superscripts are significantly different ( $P \leq 0.05$ ).

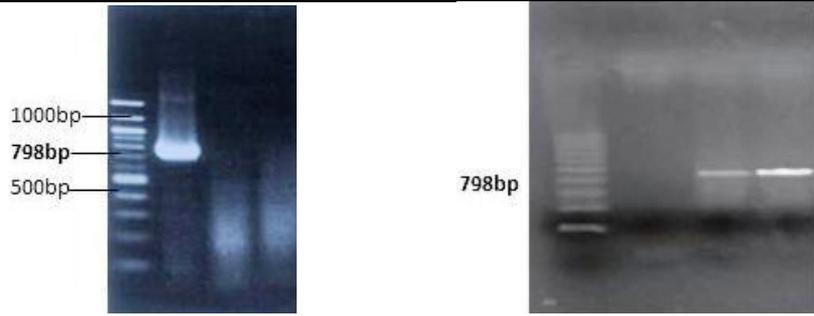
Local chicken, cGH gene, Gene transfer, SMGT, Growth traits



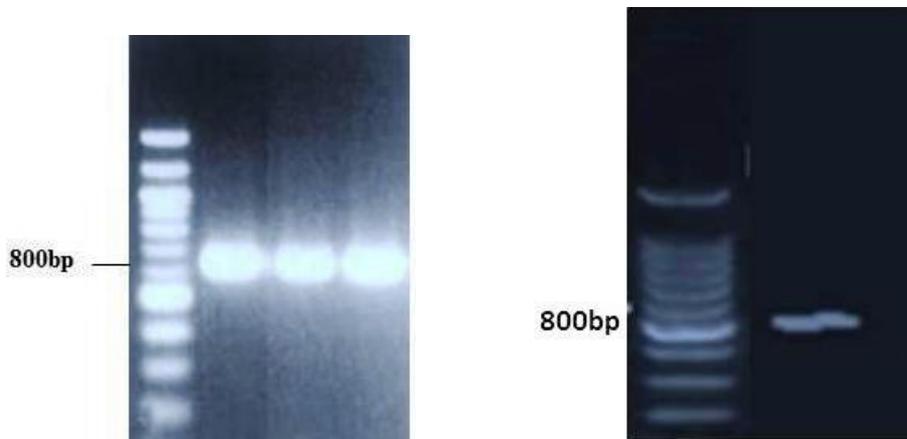
**Fig(1):**Agarose gel electrophoresis (1.5%) of PCR products using GH forward (F) and GH reverse (R) primers using cDNA as a template. M: 100 bp DNA Ladder from Cobb broiler



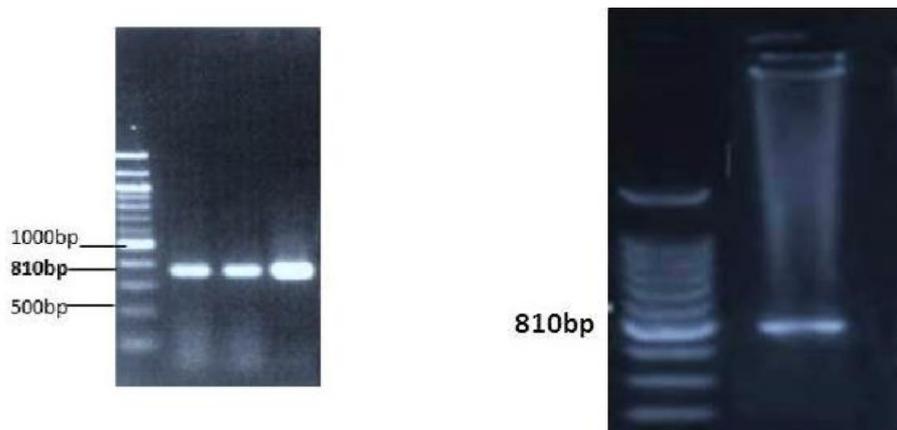
**Fig(2):**Sequencing alignment result of cGH recombinant gene by standard Sanger sequencing method using T7 forward primer.



**Fig (3):** PCR Products Bandarah Growth Hormone GH, mRNA Length 798bp, for Bandarah chicken control without any gene treatment of first and second generation



**Fig (4):** PCR Products Bandarah Growth Hormone GH, mRNA normal Length 800bp, from Bandarah chicken by Bio method of first and second generation



**Fig (5):** PCR Products Bandarah Growth Hormone GH, mRNA Bandarah chicken by SMGT method first and second generation.

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## الملخص العربي

### التحسين الوراثي لبعض الصفات الانتاجية للدجاج المحلي المصري عن طريق نقل جين هرمون النمو من بداري التسمين

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كان الهدف من الدراسة هو عزل واستنساخ ومعرفة تتابع القواعد النيروجينية وتسلسل جين هرمون نمو الدجاج (cGH) المعزول من بداري التسمين (كب ٥٠٠) ثم نقل جين هرمون النمو لسلاله دجاج البندر (احدى سلالات الدجاج المحليه المحسنه) بطريقتين ، الطريقه الاولى نقل الجينات بواسطة الحيوانات المنوية (SMGT) الطريقه الثانيه: نقل الجينات باستخدام الرنين الحيوي (Bio) إلى سلالة الدجاج المحليه (البندرة). هذه الدراسة تمت علي جيلين متتاليين. زاد وزن الجسم عند الفقس وعند ٤ أسابيع من العمر بمقدار ٣.٣٢ جم و ٨.٠٩ بطريقه SMGT و ١.٢٧ و ٥.٢٢ بطريقه (Bio) على التوالي. تم زيادة وزن الجسم بطريقه SMGT عند عمر ١٢ أسبوع بمقدار ٦٠.١٧ جرام و ٧.٩٦ جرام للذكور والإناث على التوالي. كان معدل النمو خلال ١٠ الفتره من عمر يوم حتى عمر ٤ أسابيع من العمر ١٣١.٦٤ ، ١٣٠.٦٨ و ١٣٢.٣٥ % ، SMGT ، Bio و Control على التوالي. الخلاصه تمت عمليه عزل جين هرمون النمو cGH Gene من كتاكت اللحم Cobb 500 ونقله بطريقتين SMGT و Bio لإنتاج دجاج معدّل وراثيًا من سلالة البندر المحليه المصريه . وجد ان نقل الجين باستخدام الحيوانات المنويه SMGT هي طريقه فعاله فى انتاج دجاج مهندس وراثيا ، وكذلك فإن طريقه Bio تفتح آفاقًا جديدة مهمة في مجال انتاج الحيوانات المهندسه وراثيا بشكل سريع وفعال لوصول الجينات المرغوبه إلى الأنسجه المستهدفة. وقد كان نقل جين هرمون النمو ذو تأثير واضح علي صفات النمو مقارنة بالكنترول وكذلك تم انتقال الجين من الجيل الأول إلى الجيل الثاني بنفس الشكل وزاد من انتاجيه الصفات. وكان وزن الجسم عند عمر يوم و ٤ و ٨ و ١٢ اسبوع في الجيل الثاني افضل من الجيل الاول .

الكلمات الداله: الدجاج المحلي ، جين هرمون النمو cGH ، نقل الجينات ، SMGT ، صفات النمو