EFFECT OF TRANSFERRING DUCKS GROWTH HORMONE GENE AND TOTAL DNA ON GROWTH HORMONE LEVEL, PROTEIN AND DNA OF JAPANESE QUAILS AT THREE GENERATIONS.

BY

Abdel-Sabour, M.S.*; Hassan, A. M.*; Samia A. EI-Feky; Bekhit, M.M.ML * and Ahmed, M.M.M. ****

* Fac. of Agric. Moshtohor, Zagazig Univ. Egypt.

** National Research Center (N.R.C) Egypt.

ABSTRACT

The effect of ducks growth hormone GH gene and total DNA on Japanese quails was studied. The level of GH was higher through ducks GH gene injection in air sac, embryo and combined effect than through DNA and control, but the increase level of GH was within the normal range of GH for birds.

The determination of total protein percentage was higher through ducks GH gene effect than through ducks DNA and control. The results were stable from F_t to F_3. The SDS-PAGE protein howed dditional ew f hree ands n the treated birds than control and these were stable from F_t to F_3.

The agarose gel electrophoresis of total DNA with restricted enzyme Eco RI showed a new band in the treated F,, F_2, F_3 and donor. *In* addition it showed a smeared Pattern intensity appeared on the gel in donor, treatments, F,, F_2 and F_3 than control and was stable from FI to F_2 to F_3.

INTRODUCTION

Genetic engineering is a new tool for genetically improving poultry. It emerged in the early 1980s as a result of discoveries in Molecular Genetics and the understanding of DNA Structure physically and chemically (Shuman, 1991).

The ability to transfer gene into the germ line of birds has created new and revolutionary opportunities for both basic research and the poultry industry. Currently, there is much interest in using gene transfer technology to examine gene expression and to evaluate genetic control elements. Many investigators are transferring genes that may provide commercial birds with growth rates, improved feed efficiency and resistance to diseases. The development of gene transfer technology for avian species, however, has not progressed as rapidly as mammalian gene transfer technology. Progress in producing transgenic birds has been hampered, in part, by the avian reproduction and embryonic developmental system. The newly fertilized avian ovum is very large, fragile and yolk filled (Shuman, 1991).

This study aimed to know the effect of gene and whole DNA transfer on GH level, protein percentage, protein and total DNA in Quails

MATERIALS AND METHODS

I- Materials: -Strains

a)- Donor (birds): -

Ducks growth hormone gene and DNA was isolated from pituitary gland of Baladi ducks (Native ducks) *Anos plotyrhynchos* (2N=80).

b) - Recipient (birds):

Japanese quails fertilized eggs *(Coturnix coturnixjaponca)(2N=l%).*

IL Methods: -1-DNA isolation and purification: -

High molecular weight DNA of ducks and quails was extracted, by Miller *et ai,* (1988) and Miller *et at.,* (1990)

2. Determination of DNA concentration: -by Charles, (1970) **method. ,**

3-Growth hormone gene isolation: - '

The growth hormone gene of ducks was isolated by procedures described by Smith and Flavell (1974) and Ahmed (1995) using DNA/DNA hybridization for gene isolation.

4- Ducks growth hormone gene and DNA from pituitary gland injection in air sac and embryo of eggs.

The microinjection of ducks growth hormone gene and whole DNA into Japanese quails in air sac and embryos were achieved by the method of Salter *et al. (1986)* Ahmed Nagwa *et al.,* (1994) and Ali (1995).

5- Ducks GH gene and DNA from pituitary gland introduced through the aid of 0.3% **lithium acetate and electroporation for;** 2.5 **and 5 minutes at** 220V. by the method of,(Hsu 1992 and Hafez 1994, and Wllmut *et al.*, 1997).

6-The measurement of GH level in blood plasma: -

Plasma was separated from blood added heparin of duck; Japanese quail (control and treatments) three birds for each group from F1, F2andF3 at 6 weeks by radioimmunoassay (RIA) according to Bosselman *et ai,* (1989a and b).

7-The determination of total protein using spectrophotometer: -

Plasma was separated from blood donor, control and treatments, five birds for each group from F1, F2 and F3 at 6 weeks according to Steignere/ *ai,* (1992).

Read on spectrophotometer at wave length 545nm. (UV-2401 PC, UV-Vis Recording spectrophotometer, Shimadzu).

8- The determination of protein bands using SDS polyacrylamide gel electrophoresis (SDS-PAGE): a- Plasma extraction, which isolated from donor, control and treatments was determined by Steignere/a/.((1992). **b-Proceduer SDS- PAGE** was carried out according to the method of Laemmli (1970) and Saker(1995)

9-The determination of DNA bands agarose gel electrophoresis : -

a) DNA isolation and purification from control, treated Japanese quail Fl, F2, F_3 and donor was carried out according to Miller *et at.,* (1988) and Miller^ *al.,* (1990).

b) Treatment with restriction endonucleases: -

The extracted DNA was treated with E co RJ restriction enzyme (type II) and high buffer incubated at 37C° for 1 hour or overnight. It digested DNA between guanine and adenine according to Tsai *el al.,* (1993).

c) **Agarose gel electrophoresis: -**

Agarose (gel electrophoresis 1% apparatus GNA-100 , Max, 500v and Polaroid Direct Screen Instant Camera DS 34) was carried out according to the method of Sambrook *et al.,* (1989) and Tsai *et al.,* (1993).

RESULTS

I. Measurements:

1-Growth hormone measurements: -

After six weeks the blood plasma was taken from three birds for each group and from all treatments using raidoimmunassay Table (1) gives the GH level ng/ml in birds injected in air sac, *F*\, $F_2$and F_3 with ducks GH gene and DNA for both doses, lOug and 20 ug, respectively. The table also showed that the level of GH was higher after 20 ug GH gene injection in air sac, embryo and combined effect than in the case of DNA injection and control (56.4, 56.2, 56.1 and51.2) respectively, but the increase was within normal range of GH level for birds. The level of GH was higher through 20ug/egg GH gene injection in air sac, embryo and combined effect than DNA and control in F2 and F3 (55.9, 55.4, 55.0, 56.4, 56.1, 56.1 and 51.0 ng/ml, respectively). Fig. (1), (2) and (3) showed the growth hormone (GH) concentration at different three generations in case of injection in air sac. embryo and combined effect at six age weeks.

2- Determination of total protein: -

After six weeks the blood plasma was taken from three birds for each group and also all treatment using spectrophotometer.

Table (2) gives the total protein percentage/ml in birds injected in air sac, embryo and combined effect (L+ and electroporation) at 2.5 and 5.0 min in FI, F_2 and F_3 with ducks GH gene and DNA for both doses; 10ugand20ug, respectively. The table showed that the total protein percentage is higher through 20ug GH gene injection in air sac, embryo and combined effect than DNA

injection and control (68.4, 67.9, 68.5 and 55.2%), respectively. The total protein % was higher through 20 ng/egg GH gene injection in air sac, embryo and combined effect than

DNA and control in F2 and F3 (67.9, 67.5, 68.2, 68.0, 67.1, 68.7 and 56.0, respectively). These results showed stable inheritance from F, to

Fig (4), (5) and (6) showed the protein (%) in birds injected in air sac, embryo and combined effect in FI, F_2 and F_3.

II- Gel electrophoresis: -

1-SDS polyacrylamide gel electrophoresis (SDS- **PAGE) of total protein: -**

After six weeks the blood plasma was taken from three birds for each group and all treatment FI, F_2 and F_3 using SDS-PAGE protein determined..

Plate (1) gives the electrophoresis analysis (SDS-PAGE) for the total protein of the five samples, donor, control, treatments from FI , F_2 and F_3. Results were obtained after the samples run. New three bands were detected in lanes 2, 3 and 4 as treatment samples, which obtained from the F,, F_2 and F_3. (Plate, 1) also these three bands were shown in the plasma (lane 6). The control Oane 5) did not show these additional new bands.

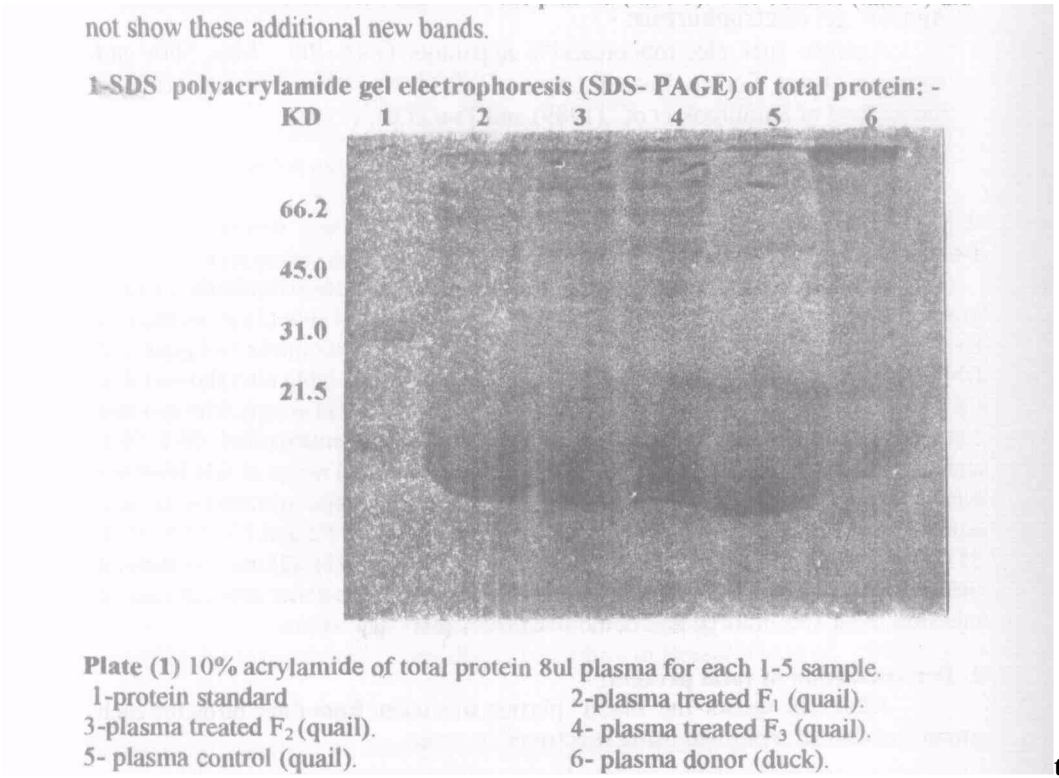

Plate (1) 10% acrylamide of total protein 8ul plasma for each 1-5 sample.
1-protein standard
2-plasma treated F_1 (quail).
3-plasma treated F_2 (quail).
4- plasma treated F_3 (quail).
5- plasma control (quail).
6- plasma donor (duck).

t-SDS polyacrylamide gel electrophoresis (SDS- **PAGE) of total protein: -KD**

Plate (1) 10% acrylamide of total protein 8ul plasma for each 1-5 sample. 1-protein standard 2-plasma treated F, (quail). 3-plasma treated F_2 (quail). , *4*- plasma treated F_3 (quail). 5- plasma control (quail). 6- plasma donor (duck).

2- Agarose gel electrophoresis (total DNA): -

At six weeks of age, the DNA was isolated from the pituitary gland for the donor, control and treated birds from F_u F_2 and F_3 using agarose gel electrophoresis analysis which was used to show the difference in DNA.

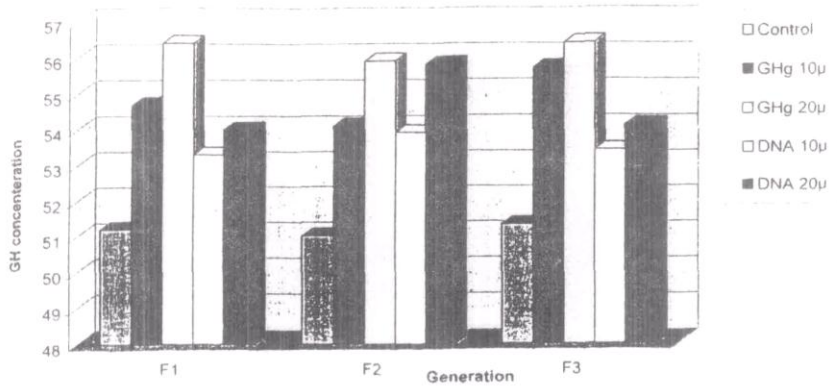

Fig. (1): Histogram showing the relation between GH concentration of control and treated (air sac) at six weeks in F_1, F_2 and F_3 generations.

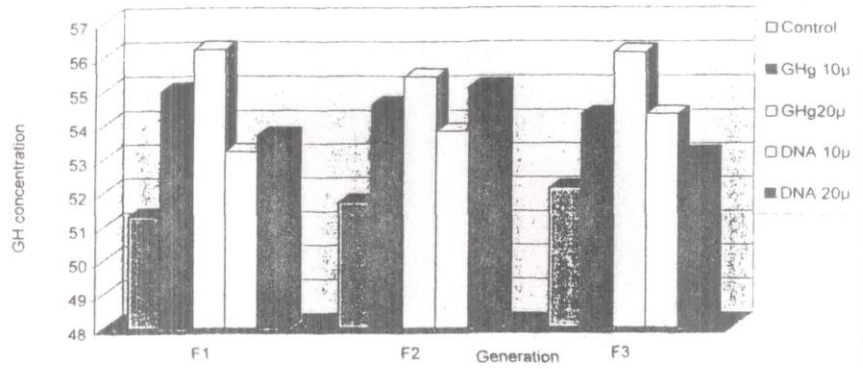

Fig. (2): Histogram showing the relation between GH concentration of control and treated (embryo) at six weeks in F_1, F_2 and F_3 generations.

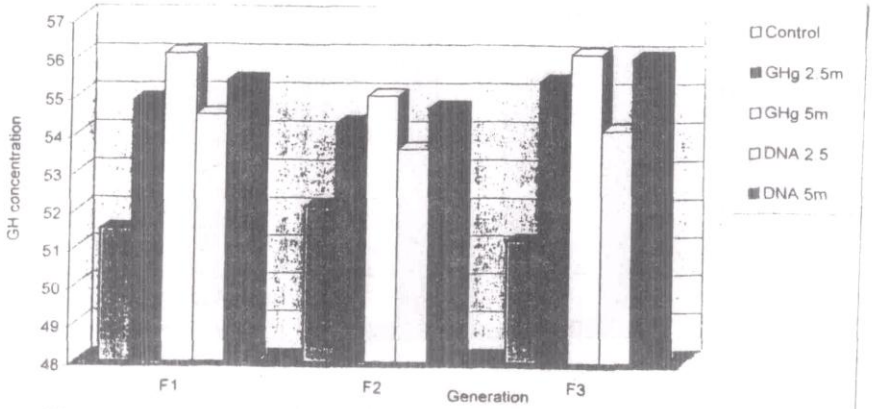

Fig. (3): Histogram showing the relation between GH concentration of control and treated (combined effect) at six weeks in F_1, F_2 and F_3 generations.

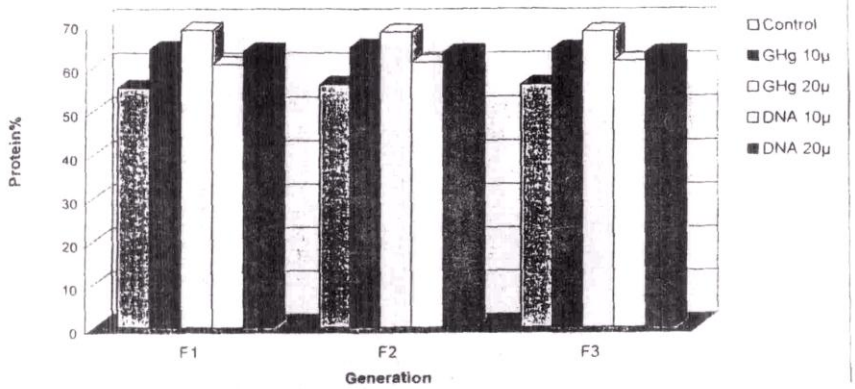

Fig. (4): Histogram showing the relation between protein % of control and treated (air sac) at six weeks in F_1, F_2 and F_3 generations.

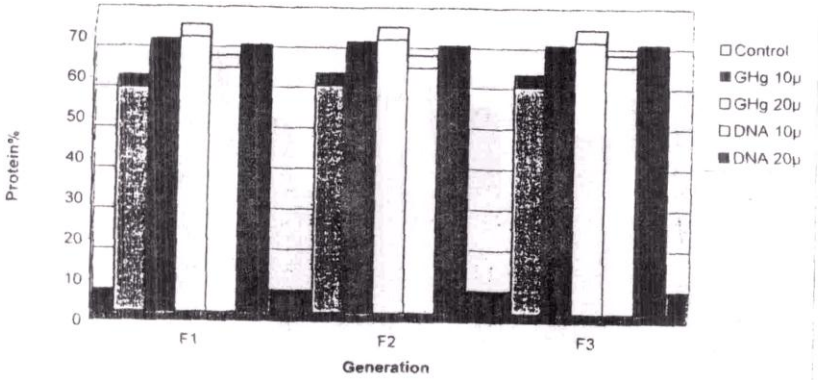

Fig. (5): Histogram showing the relation between protein % of control and treated (embryo) at six weeks in F_1, F_2 and F_3 generations.

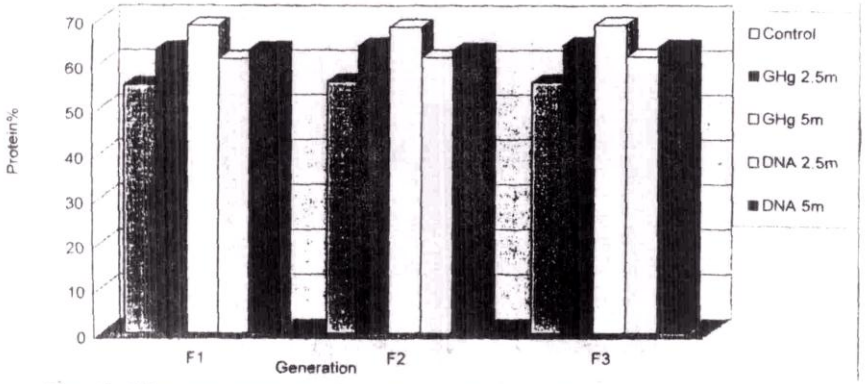

Fig. (6): Histogram showing the relation between protein % of control and treated (combined effect) at six weeks in F_1, F_2 and F_3 generations.

Plate (2) shows the electrophoresis analysis for the totaJ DNA isolated from the birds. Lane 1 marker, lane 2 and 4 donor and recipient (control) without enzyme and 3. 5. 6. 7 and 8 for

donor, control, and treated Japanese quail from F,. F; and F_3 with enzyme Eco RJ. respectively

Plate (2) lane 1 of DNA marker, lane 2 and 4 showed one band and lane 3. 5. 6. 7 and 8 with E co RI enzyme showed a new band in treatments. F-. F; and F, and in donor. The new band appears to have a molecular weight of about 20kb In addition, it showed a smeared pattern appeared on the gel in donor, treatments i e. Fl. F; and F_3 than control and stable trend from F, to *Ft.*

***2*- Agarose gel electrophoresis (total DNA): -**

i. e. F1. F_2 and F_3 than control and stable trend from F_1 to F_3.

2- Agarose gel electrophoresis (total DNA): -

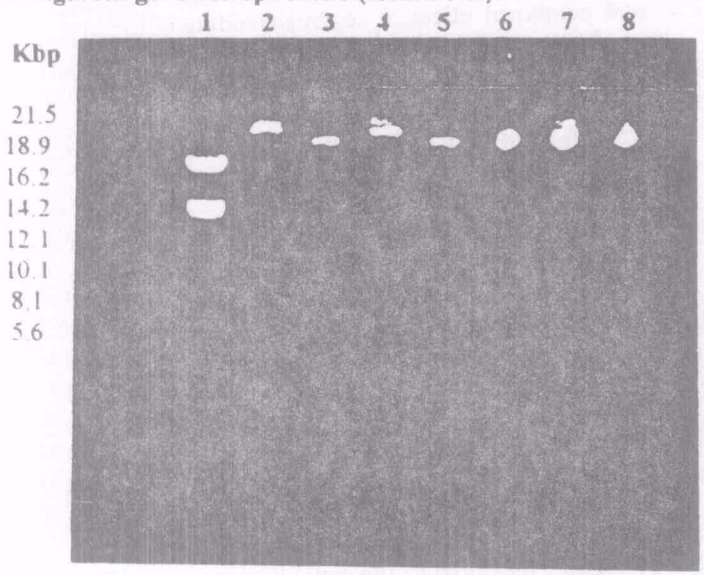

Plate (2) At % agarose gel plate of total DNA 10 µg DNA from all samples and restricted enzyme E co RI.

1-DNA Marker (λ. DNA).
2-Donor without enzyme (duck).
3-Donor with enzyme (duck).
4-Control without enzyme (quail).
5-Control with enzyme (quail).
6-Treatment F (quail).
7-Treatment F_2 (quail)
8-Treatment F_3 (quail).

The previous results showed that.

1- The level of GH was higher through ducks GH gene injection in air sac. embryo and combined effect than through DNA and control, but the increase level of GH was within the normal range of GH for birds.

2- The determination of total protein percentage was higher through ducks GH gene effect than through ducks DNA and control, but the results showed stable trend from F, to F>.

3-The SDS-PAGE of protein showed an increase of new three bands after treatments than control and with stable trend from F. to F-..

4- The agarose gel clcctrophoresis of totaJ DNA with restricted enzyme *(E* co RJ) showed a new band in treatments F,. F;. F? and donor In addition it showed a smeared pattern intensity appeared on the gel in donor, treatments. *F*\. F; and Fi than control or other low molecular weight DNA (after restricted digested) appeared on 1% gel as smear and stable trend from F; to F_3

DISCUSSION

II- Measurements: -

1- Growth hormone measurements: -

Measurements of gro\\th hormone in transgenic quails showed thai the level of GH ng/ml plasma is higher after 20ug/egg GH gene injection in air sac (564%). embryo (56.2%) and combined effect, i. e.. by the aiding of Li-and elcctroporalion (56.1%) than DNA injection as reached (54.0. 53.7and55.4). respective!} and than control (51.2%). This increase is within the normal range of GH level for birds This result agreed with analysis of plasma of GH chicken The plasma GH of birds range per ml is from 18 to 254 ng/ml. Bosslman *ei al.* (1989 a and b)

In conclusion, the transgenic quail obtained from this investigation has no lia/ardous effects. The growth hormone gene or DNA was isolated form safe organism, i. e.. ducks that are edible organisms Tins process was earned out without the aid of any virus neither pathogenic nor attenuated The *level of* GH although increased in some instances but still in the range of these ducks Therefore the products of this investigation is quite safe for consumption b> human-being Maclean (1994) stated Uiat it is safe to eat transgenic animal (mamalam and birds). He added tliat food from transgenic animal therefore cam no conceivable new direat 10 health

2-Total protein measurements: -

The total protein percentage measured by the method of Steigner *ei al.*. (1992) was increased following injection with 20ug GH gene in air sac (68.4%). embryo (67.9%). combined effect (68.5%) and DNA injection (63.6%) increased with control which reached (55.2) These results were stable in the successful generation: Fi to F,. Because injection of ducks GH gene or DNA was in fertilized eggs and due to increasing of homozygotes during segregating generations of F_2 and F_3 These results were in contrast with injection before fertilization These results were similar to Bosselman *et al.*. (1989 a and b).

III- Gel electrophones!*: -1 *SDS -PAGE total protein: -

SDS PAGE total protein of the treatments form: F,. F: and F_3. showed new additional three bands (14.53, 23.10 and 21.5 KD). These bands were absent in the control. This could indicate that the GH gene of ducks was transferred from ducks to quails. The results showed stability from F, to F:toF_3. These results agree with that of Souza <?/o/...(1984): Langley *et al.*. (1987.aand b); Tsai and Tseng (1992): Tsai *et al.*. *(1*993) and Kuo and Tsai (1993)

Table (1): **Effect of ducks GH gene and DNA injection egg in air sac, embryo, combined effect on GH level in F_1, F_2 and F_3.**

Injection	Air sac	Embryo Means	Combined effect	
Treatments	plasma GH	Plasma GH	Treatments	Plasma GH
Fi	F,	F,	F,	F,
Control: - Non-drilled	51.2	51.2	Control: - Eggs only	51.2
Ducks GH gene doses lOug/egg	54.7	55.0	Eggs + Li+ elec. GH gene ,2.5 min	54.9
20u/egg	56.4	56.2	GH gene ,5.0 min	56.1
Ducks DNA doses: - lOug/egg	53.3	53.2	Eggs + Li+ elec.. DNA , 2.5 min	54.5
20u/egg	54.0	53.7	DNA , 5.0 min	55.4

The

F,	F_z	F_z	¥2	Fj
Control: - Non-drilled	51.0	51.7	Control: - Eggs only	52.1
DucksGH gene doses lOug/egg	54.1	54.6	Eggs + Li+ elec. GH gene , 2.5 min	54.3
20u/egg	55.9	55.4	GH gene, 5.0 min	55.0
Ducks DNA doses: - lOug/egg	53.9	53.8	Eggs + Li + elec., DNA , 2.5 min	53.6
20u/egg	55.8	55.1	GH gene, 5.0 min	54.7
F_3	F_3	F_3	F,	F_3
Control Non-drilled	51.3	52.1	Control: - Eggs only	51.2
DucksGH gene doses lOug/egg	55.2	54.3	Eggs + Li +elec., GH gene ,2.5 min	55.4
20u/egg	56.4	56.1	GH gene, 5.0 min	56.1
Ducks DNA doses: - lOug/egg	53.4	54.3	Eggs +Li+ elec.. DNA , 2.5 min	54.1
20u/egg	54.1	53.1	DNA , 5.0 min	56.0

range of GH level in birds = (18- 254 n g /ml).

Table (2): **Effect of ducks GH gene and DNA injection egg in air sac embryo, combined effect on protein % in F_1, F_2 and F_3.**

	Combined effect	Embryo	air sac	Injection
Protein %	Treatments	Protein%	Protein %	Treatments
F,	F,		F,	F,
55.2	Control: -Eggs only	55.2	55.2	Control: - Non-drilled
63.2	Eggs + Li+ elec. GH gene ,2.5 min	643	64.1	Ducks GH gene doses lOug/egg
68.5	GH gene 5.0 min	67.9	68.4	20u/egg

61.1	Eggs + Li+elec., DNA , 2.5 min	60.2	60.5	Ducks DNA doses: lOug/egg
63.2	DNA 5.0 min	62.9	63.6	20n/egg
Fz	F_2	F_2	F,	F_z
56.0	Control: -Eggs only	56.0	56.0	Control: - Non-drilled
64.1	Eggs+ Li+ elec. GH gene, 2.5 min	63.9	64.5	Ducks GH gene doses lOug/egg
68.2	GHgene 5.0 min	67.5	67.9	20n/egg
61.4	Eggs+ Li+elec., DNA , 2.5 min	605	60.9	Ducks DNA doses: 10ng/egg
63.0	DNA 5.0 min	63.0	63.2	20n/egg
F,	F,		F,	F_3
55.9	Control: -Eggs only	55.9	55.9	Control: - Non-drilled
64.3	Eggs +Li + elec. GH gene ,2.5 min	53.2	64.0	Ducks GH gene doses lOug/egg
68.7	GH gene, 5.0 min	67.1	68.0	20u/egg
61.6	Eggs +Li+ elec., DNA , 2.5 min	60.9	61.2	Ducks DNA doses: lOug/egg
63.7	DNA 5.0 min	63.4	63.0	20n/egg

2- Agarose gel electrophoresis (total DNA)

Agarose gel electrophoresis for the total DNA with restricted enzyme (Eco RI) of treatment of; $F_{(}$, F_2 and F_3 donor and recipient (control.), showed new additional one band. This band was absent in the control. It means that the GH gene of ducks was transferred from ducks to quails. In addition it showed a smeared pattern intensity appeared on the gel in donor, treatments: F,, F_2 and F_3 than control. These results agree with analysis of total DNA from yeast by Tsai *et ai,* (1993). However, the lines of the treated samples showed some indication of the existence of some foreign DNA acquried following injections. These results showed stability from; FI to F_3 and were similar to Bosselman *et al.*. (1989 a and b).

From the previous results, we can conclude that, the higher body weight birds which have a new DNA band in its DNA could be a transgenic bird and the DNA, introduced might be carrying the gene sequence of GH gene(s).

REFERENCES

Ahmed Nagwa, A.; Riad, S.A.; Ali, A.M.M.; El-Wardany, A.E. and Ah, B.A. (1994): Effect of introducing duck and turkey DNA on embryonic

development of fayoumi chicks. Egyptian J. Anim. Prod Supplement
31:587-599. Ahmed, M.M.M. (1995): Isolation of growth hormone gene from ducks. M.Sc. Thesis. Dept. Animal Breeding, Faculty of Agr.Cairo, Univ. Ali, B.A. (1995): Effect of foreign DNA on genetical and
cytologecal chickenembryocharacters.M.Sc., Thesis. Animal Prod.
Dept., Fac. Agric., Cairo, Univ., Bosselman, R.A; Hsu, R.Y.; Boggs, T.; Hu, S.; Bruszewski, J. S.; Kozar, L.;
Martin, F.; Green, C; Jacobsen. F; Nicholson, M; Schultz, J.A; Semon.
K.M.; Rishell, W.; and Stewart, R.G. (1989b): Replication-defective
vectors of reticuloendotheliosis virus transducer exogenous genes into
somatic cells of the unincubated chicken embryo. J. Virology, 63 (6):
2680-2688. Bosselman, R.A; Hsu, R.Y.; Boggs. T., Hu, S; Bruszewski, J.S.; Kozar, L.;
Martin, F.; Green, C.; Jacobsen, F.; Nicholson, M; Schultz. J.A.:
Semon K.M.; Rishell. W.; and Stewart, R.G; (1989a): Germline
transmission of exogenous genes in chickens. Science. 243: 533-535 Carlstrom, A. and Johansson (1983): Agarose gel electrophoresis-
Immunofixation scand. *).* Immunol. 17 Suppl 10, 23-32. Charles, P. (1970): Isolation and characterization of deoxyribonucleic acids.
(ledox,L; ed) uptake of informative molecules by I cells North Holl
and Publishing Company, Amsterdam- London, pp 16-23. Hafez, P.M. (1994): Improved transformation efficiency of dextran genes in
Saccharomyces cerevisae using the combined effect of electroporation
andalklion. AI-Azhar J. Agric. Res., 20: 147-155. Hsu, T.T. (1992): Electrofusion of mouse, pig and goat blastomeres at various
stages during embryonic development. Taiwan Livestock Res. 25 (1)
11-24. Kuo, J.L. and Tsai, H.J. (1993): Electrophoretic analysis and recombinant fish
growth hormone secreted from *Saccharomyces cerevisiae* by using factor- leader sequence J. Fish. Soc. Taiwan. 20(1): 51-58. Laemmli, U.K. (1970): Cleavage of structural proteins during the assembly of the
head of bacteriophage T4.Nature, 227(15) 680-685 Langley, K.E; Berg, T.F.; Lai, P.H.; Wypych, J.; Everett, R.R .;Berg,T.F.;
Krabill, L.F., Davis, J.M.; and Souza, L.M. (1987 b): Recombinant
DNA-derived bovine growth hormone from *E coli.* 2.Biochemical,
biophysical, immunological and biological comparison with pituitary
hormone. Eur. J. Biochem 163: 323- 330.

Langley, K.E; Berg, T.F.; Strickland, T.W.; Fenton, D.M. ; Boone. T.C.; and
Wypych, J (1987a): I recombinant DNA-derived bovine growth
hormone from *E. coli.* 1. Demonstration that the hormone is
expressed in reduced from, and isolation of the hormone in oxidized,
native from.Eur. J. Biochem. 163: 313- 321. Maclean, N. (1994): Transgenic animals in perspective. In Animals with novel
gene, Maclean, N; (Ed) pp. 17. Miller, S.A.; Dykes, D.D. and Polesky, H.F. (1988): A simple salting out
procedure for extracting DNA from human nucleated cells. Nucl. Acid.
Res. 16(3): 1215. Miller, S.A; Dykes, D.D. and Polesky, H.F. (1990): DNA extraction system.
Oncogene Science, 16: 1-4. Saker, M.M. (1995): Studies on tissue cultures some legumes. Ph.D. Thesis.
Botany (plant Molecular Biology) Dept., Faculty of Science Tanta
Unver. Salter, D.W; Smith, E.J.; Hughes, S.H.; Wright, S.E., Fadly, A.M.; Witter, R.L.;
and Crittenden, L.B. (1986): Gene insertion into the chicken germ line
by retroviruses. Poultry Sci. 65: 1445-1458. Sambrook, J.; Fritsch, E.F.; Maniatis. T. eds(1989): "Molecularcloning"Cold
Spring Harbor laboratory press, New York.

Shuman, R. M. (1991): Production of transgenic birds. Experientia, 47: 897-905. Smith, D.B; and, Flavell. R.B. (1974): The relatedness and evolution of repeated nucleotide sequences in the genomes of some Gravineae species. Biochemical genetics. 12(3): 243-256. Souza. L.M; Boone, T.C.; Murdock, D.; Langiey, K.; Wypych, J.; Fenton, D.; Johnson, S.; Lai, P.H.; Hsu, R.; and Bosselman, R. (1984): Application of recombinant DNA technologies to studies on chicken growth hormone. The J. of Exp. Zool. ,232: 365-373. Steigner, J.W.; Nestor, K.K.; and Lilburn, M.S.; (1992): Growth and development of lines of Japanese quail divergently sleeted for body weight at 4 weeks of age. Comp. Biochem. Physiol. 1021(2): 389-393. Tsai, H. J.; and Tseng, C.F. (1992): Expression of rainbow trout growth hormone cDNA in *E. coli* from vector utilizing Lac promoter.!, fish. Soc. Tawan, 19(1): 45-53. Tsai, H.J; Tseng, C.F.; and Kuo, T.T.; (1993): Expression of rainbowtrout growth hormone cDNA in yeast. Bull. Inst. Zool. Academic Sincia. 32(3): 162-170. Wllmut, J.; Schnieke A.E.; Mcuhir, J.; Kind, A.J. and Campbell, K.H.; (1997): Viable offspring derived from fetal and adult mammalian cells. Nature. 385 (27): 810-813.