GENETIC IMPROVEMENT OF SOME PRODUCTIVE TRAITS OF LOCAL CHICKEN STRAIN BY TRANSFERRING GROWTH HORMONE GENE FROM BROILER

A THESIS

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

5. SUMMARY AND CONCLUSION

The present study was carried out at Faculty of Agriculture Damanhour University Animal and Poultry Production Department, El-Sabahia Poultry Research Station in 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. The experimental was aimed to investigate isolation, cloning and sequencing of chicken Growth hormone gene (cGH) as an important gene from chicken fast growing (cobb 500 broilers) as a high producing exotic broiler strain, then transfer chicken Growth hormone gene by two methods the first one gene transfer using sperm-mediated gene transfer (SMGT) technique and the second method gene transfer using bioresonance.to Bandarah develop chicken strains.

A total 125 hens and 25 cocks at 8 month of age from Bandrah a local chicken strain were used to start this study to produce generation one. The birds were assigned in individual cages; and divided randomly into 3 groups. The first (SMGT) used method cGH gene transfer using sperm-mediated gene transfer technique contain 50 hens and 10 cocks, second group (Bio) used method cGH gene transfer using Bioresonance contain 50 hens and 10 cocks, third group without any gene treatment (control) used classic artificial insemination contain 25 hens and 5 cocks. Second generation done by classic artificial insemination between cocks and chicken from each group.

The present work was carried out to study:

- Applicability of the modern biological technology for molecular genetics and genetic engineering to isolation, cloning and sequencing of chicken chicken growth hormone gene. c(GH) gene isolation and cloning from chicken (cobb 500 broilers) as a high producing exotic strain in growth traits and sequencing analysis compared to sequence reference which published in NCBI to transfer it to Bandrah a local chicken strain.
- The effect of methods using Sperm Mediated Gene Transfer and Bioresonance in gene transfer to Bandarah developed chicken strains to produce transgenic chickens of native Egypt breed.
- Produced second generation by classic artificial insemination between cocks and chicken from each group first generation.

Studied traits in each generation:

1- Fertility and hatchability of fertile and total eggs percentage.
2- Chicks were individually weighed Body weight was recorded at hatch to the nearest 0.1 g, and at 4, 8 , 12 and 16 weeks of age to the nearest g.
3- Growth rate was estimated biweekly during periods 0:4, 4:8, 8:12, and 12:16 weeks.
4- The average feed consumption and Feed conversion ratio of each bird was calculated in each group during this period 8:10, 10:12, 12:14, 14:16 and 8:16 weeks.
5- Slaughtering parameters and Shin bone length and Sternum length cm were measured

6- Chicken meat quality was measured (pH value, Water holding capacity, Bound water % of moisture content, Shearing force, Colour intensity), antioxidative status (Determination of Malondialdehyde (MDA) in tissue, Glutathione peroxidase and Superoxide dismutase activity) and Microbiological Study
7- Age and body weight at sexual maturity was record for each hen.
8- Egg number during up to 90 days of the first egg lay.
9- Average egg weight up to 90 days of the first egg lay.
10- Egg mass during the first 90 days of laying were estimated.
11- Genetic analyses to test cGH mRNA gene in chicken groups for first and second generation.

Results obtained could be summarized as follows:

- 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 standard 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.
- The recombinant cDNA with the Gen Bank reference sequence accession number: LC441152.1.
- 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.
- There were no significant difference between methods or generations, the averages of fertility percentage were 89.84%, 88.84% and 91.27 % for SMGT, Bio and control, respectively.
- The highest insignificant values were found for hatchability of fertile and total eggs percentage in the control line at the first generation 95.64% and 88.49%, 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.
- The highest insignificant body weight was found in SMGT method 372.05 g and the lowest weight was found in Bio method 365.76 g for the combined sexes of the body weight at 8 weeks of age.
- The differences between the overall mean of SMGT method and the control line were 56.72 g for males and 12.94 g for females and 43.63 g for combined sex, while, the responses of Bio method were -29.03 g, -101.13 g and 11.78 g for male, female and combined sex, respectively.
- There were highly significant differences ($P<0.01$) between methods and generations for body weight at 12 wks of age.
- The SMGT method was increased the body weight at 12 wk of age by 60.17 g, 7.96 g and 20.02 g for males, females and combined sex respectively.
- The BW at 16 weeks of combined sex was increased by 69.31g for SMGT methods while, decreased by 27.61g for Bio methods.

- Effect of SMGT and Bio methods for body weight at different age observed that the SMGT method increased body weight more than Bio method, but this increase was not significant.
- The growth rate of SMGT, Bio and control has 131.64%, 130.68 and 132.35%, respectively, during (0-4) weeks of age.
- The growth rate during (4-8) week of age increased by 5.85% and 0.86 % for SMGT and bio methods.
- The SMGT and Bio methods increased growth rate during (8-12) weeks of age by 5.03% and 10.72% for combined sex, respectively.
- Using the SMGT method, the response of growth rate during (12-16) week of age was positive, since the estimates were (8.99, 2.33 and 4.72%) for males, females and combined sex, respectively, for Bio method, the growth rate during the same periods increased by (1.91, 2.33 and 0.03) for males and combined sex, respectively.
- The feed consumption increased at different periods of growth for SMGT and Bio methods except at 8-10 and 14-16 wk. of age decreased by 2.05 and 0.04g for SMGT method, respectively.
- For SMGT method the feed conversion increased at different periods of growth except at 14-16 wk. of age it was decreased by 1.58. While, for Bio method, the feed conversion decreased by 1.69 and 0.57 at (8-10 and 14-16 wk. of age).
- The heaviest carcass weight was found in SMGT method 1016.0g, while, the lightest weight was found in control line (841.22). For carcass percentage, Bio method had the heaviest percentage 67.03% when compared with the other methods.
- The shin bone and Sternum bone length were shortened significantly (≤ 0.01) in the second generation compared with the first one (10.5 vs 13.50) and (12.31 vs 17.22), respectively.
- The same trend (low pH in SMGT and Bio methods and high pH in control were observed in first and second generation.
- SMGT and Bio methods reduced WHC and shearing force compared with control, and the opposite trend was observed in the meat optical density.
- The methods SMGT and Bio showed property of good values of pH, optical density, water holding capacity and shearing force than control.
- The methods SMGT and Bio significantly decreased lipid peroxidation as indicated by the levels of MDA and significantly enhanced the tissue antioxidative status as indicated with the levels of GSH-Px and SOD.
- The two method of transferring were decreased the ASM at the second generation by 35.01 d and 21.65 d on SMGT method and Bio method, respectively.
- The responses of BWSM were negative (-81.47) and (-124.42) as a result of SMGT method and Bio method, respectively.
- Egg number which produced during the first 90 days from SMGT method and Bio methods pullets significantly increased compared with the control.
- There were highly significant difference between methods found, the SMGT method had the heaviest egg weight 49.20 g followed by control one 48.22g and the lightest egg weight was found in the Bio method 47.44g.
- The SMGT and Bio method improved egg mass during the first 90 days of laying by 512.62 g and 272.15g, respectively.

- 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.
- PCR Products cGH, mRNA normal Length 798bp for Bandarah chicken control without any gene treatment, the same result in first and second generation.
- PCR Products GH, mRNA normal Length 800bp for transgenic chicken Bandarah chicken which treat by Bio method, the same result in first and second generation.
- PCR Products GH, mRNA normal Length 810bp for transgenic chicken Bandarah chicken which treat by SMGT method, the same result in first and second generation.

In conclusion:

cGH Gene successfully isolate, molecular cloning of from Cobb 500, as an important gene from high producing chicken breed and transferring by two methods SMGT and Bio to production transgenic chickens of a local strain in Egypt Bandarah.

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 transgenesis would be more rapid, with quick and effective delivery of genes to target tissues.

Chicken cGH gene was effect in all productive performance and moved from the first generation to the second with the same shape and increased the effect.