GENETIC STUDIES ON GROWTH HORMONE AS A PHYSIOLOGICAL INDICATOR IN RABBITS

By

SARA MOHAMED ABD ELGHANY
B.Sc. Agric. Sci. (Genetics), Fac. Agric., Cairo Univ., 2007

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APPROVAL SHEET

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SUPERVISION SHEET

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Title of Thesis: Genetic Studies on Growth Hormone as a Physiological Indicator in Rabbits

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ABSTRACT

The aim of the present study was to evaluate the effects of GH gene polymorphisms on reproduction and growth traits and identify its variability in the rabbits.

A total of 218 blood samples collected from rabbits were used to evaluate the allele frequency distribution for GH gene polymorphisms and its association with reproductive and growth traits in rabbits. The samples included 16 rabbits of the different breeds (Middle-Egypt native breed, Gabali Sinai, Baladi Black, Baladi Red and New Zealand White) and 202 rabbits (118 ♀ and 84 ♂) from APRI rabbits. The traits tested were: (1) body weight (BW) at 5, 6, 8,10 and 12 weeks from birth, (2) daily bodyweight gain (DBG), (3) reproductive traits included age at puberty (AP), Kindling interval (KI), litter size at birth (LS), litter weight (LW) at birth, Litter size at weaning (LSW) and litter weaning (LWW), (4) milk yield. For this purpose, DNA was extracted from rabbit blood samples and used in PCR amplification. The c.-78C >T SNP was genotyped by PCR-RFLP using the digestion by restriction enzyme ***Bsh1236I (BstUI).***

Association analysis between the GH C >T SNP and body weight, growth and reproductive traits was tested in the rabbits population using SAS program. The heterozygous genotype was associated with heavy weight of rabbits in different ages during the growth period. This increase in weight was significant (P < 0.05) at 8 weeks of age. DBG through 5-8 week interval was significantly associated (P < 0.05) with the T/C genotype. The heterozygous genotype (T/C) exhibited higher values in the DG compared to C/C and T/T genotypes. The estimated dominant genetic effect (d) was significant (P < 0.05) in 8 weeks. The C/C genotype showed significant value (P < 0.05) associated with early age of puberty. The estimated additive genetic effect (a) and estimated dominant genetic effect (d) in a population was insignificantly associated (P < 0.05) within all the investigated reproductive traits in rabbits. Polymorphism of heterozygous genotype T/C was associated with milk yield traits of rabbits during the first two weeks in suckling period. Estimated additive genetic effect (a) in a population was significant (P<0.05) within milk yield at the second week of suckling period of rabbits. Estimated dominant genetic effect (d) was significant (P < 0.05) within milk yield at the first week of suckling period of rabbits. In conclusion, the polymorphism of growth hormone (GH) gene in rabbits may has over dominance at the locus c.-78C >T. Positive effects of the heterozygous genotype were recorded compared to both homozygous genotypes on body weight, body gain and milk yield at the first two week. The effect of the C allele of GH gene decreased the age of puberty in rabbits. Effects of the heterozygous genotype in c.-78C >T of GH polymorphisms on the tested traits in current study and on the finishing weight in previous study could be selected as a favorable genotype in rabbits and may be used in Marker-assisted selection (MAS) programs to improve growth performances rabbits.

Key words: Rabbit, GH , association study SNP , reproductive, growth traits.

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INTRODUCTION

Rabbits have been used as a food by a variety of people across the world since the ancient times. Currently, needs and trends related to the health concerns, environment protection and food supply for the low income families are of concern. The rabbits meat qualities, such as quick-breeding source of low-fat, high-protein meat, good taste, interest in low fat diets and healthy eating and ongoing pursuit of chefs and foodies of novel and locally produced foods make rabbits meat popular. However, rabbit meat gives answers to health, environmental, economical concerns (Petrescu *et al*., 2013).

Currently, research in molecular biology has led to the generation of techniques and knowledge that assist and complement the traditional system of genetic improvement. Intensifying research on the occurrence of the different types of molecular markers in the livestock genome was carried out in order to provide more information to assist studies on the quantitative characteristics of zoo technical interest (Regitano, 2005 and Garcia, 2006). However, the state of the art of genomic tools and information available for the rabbits genome is presented in a very low amount compared to other livestock species. The direct application of molecular techniques is a candidate gene approach that can represent a quite effective approach to identify DNA markers associated with production traits in livestock (Rothschild and Soller, 1997). Only a few studies have investigated candidate genes for reproduction (Peiro *et al*., 2008; Merchan *et al*., 2009 and Garcia *et al*., 2010) for meat deposition and growth traits in rabbits (Fontanesi *et al*., 2011 and 2012). Specific regions of the growth hormone (GH) genes were analyzed in order to assist breeding programs by providing additional formation in several animals. Growth hormone (GH) is produced and secreted from the somatotroph cells of the anterior pituitary. Its secretion is tightly regulated by a number of hypothalamic factors, such as growth hormone releasing hormone (GHRH) and somatostatin or growth hormone inhibiting hormone (GHIH). However, GHRH increase GH synthesis and secretions while GHIH inhibits GH release (Devesa *et al*., 1992 and Tannenbaum *et al*., 1984).

Growth hormone plays key roles in postnatal growth promoting and regulating many biological and metabolic functions involved or related to muscle mass deposition, lipid metabolism and bone growth, among many others. Growth hormone modulates carbohydrate, protein and lipid metabolism and promotes postnatal growth in mammals through direct and indirect effects on many tissues. The predominant GH responsive tissues are the liver, bone, muscle and adipose tissues. In the liver, GH promotes the synthesis of the hormone insulin-like growth factor-1 (IGF-1) which is subsequently released into the systemic circulation (Daughaday *et al*., 1972 and Klapper *et al*., 1983). In addition, GH can also promote local IGF-1 production in extra-hepatic tissues like the kidney, muscle and white adipose tissues (Lowe *et al*., 1988 and Lowe *et al*., 1987).

At the present time single nucleotide polymorphisms (SNPs) represent the most innovative molecular markers in genotyping studies. On the other hand, recent advances in high-throughput DNA sequencing, computer software and bioinformatics have facilitated and improved the identification of SNP. Single nucleotide polymorphisms (SNPs) represent one of the most interesting approaches in the animal identification because they are abundant in the genome, genetically stable and amenable to high-throughput automated analysis (Syvänen, 2001). SNPs have been already employed in animal identification and paternity analysis in American and European beef and dairy breeds (Heaton *et al*., 2002 and Werner *et al*., 2004) and in analysis on genetic distance (Werner *et al*., 2002). Single nucleotide polymorphisms (SNPs) are bi-allelic genetic markers and they are easy to evaluate and interpret and are widely distributed within genomes. With proper coverage and density over the whole-genome, SNPs can capture the linkage disequilibrium (LD) information embedded in the genome, which can be used to pinpoint genes underlying human diseases. For domestic animals, these tools can contribute to: i) better understanding of species' evolution, domestication and breed formation, and therefore developing new theories of population genetics; ii) dissecting the genetic mechanisms of complex agricultural traits and iii) improving selection methods for genetic improvement of animal production. High-density SNP arrays were built for important farm animals, firstly for those with reference genomes and then recently for others without reference genomes. This was facilitated by the advent and application of the massive parallel sequencing technologies. The preparation and utilization of SNP arrays are of considerable impacts on the theory and practice of animal breeding and genetics, which will play important roles in the years coming (Fan *et al*., 2010). The restriction fragment length polymorphism (RFLP) of the GH gene have been associated with many production traits, including growth rate, feed efficiency, muscle mass and fat deposition and reproduction traits in different livestock species (Van *et al*., 2003). For example, polymorphisms in this gene have been associated with milk production traits in dairy cattle (Mullen *et al*., 2010), birth weight, carcass traits in beef cattle (Gill *et al*., 2010), growth performances in sheep, fatness, and carcass traits in pigs (de Faria *et al*., 2006). Recently, identified polymorphism in the GH gene that was evaluated their association with only finishing weight (Fontanesi *et al*., 2012).

The present study has been conducted to address the following objectives

1. To identify variability of the GH gene polymorphisms in Egyptian rabbits.

2. To evaluate effects of the GH gene polymorphisms on body weight, growth and reproduction.

REVIEW OF LITERATURE

1. RABBITS

Rabbits (*Oryctolagus cuniculus*) have a number of characteristics that would make them particularly suitable as meat-producing animals especially when compared with other herbivorous animals. Rabbits could contribute significantly in solving the problem of meat shortage (Lebas, 1983). Meat of rabbits has a low cholesterol level, high protein/energy ratio and is relatively rich in essential fatty acids. In recent years, domestic rabbits have been considered as a good alternative source of animal protein for the increasing human population in developing countries (Lukefahr and Cheeke, 1992).

The rabbits industry in Egypt is not widely spread as that for broiler or egg production industries. Consumers still prefer red meat and broilers and that causes the very low consumption of rabbits meat. Local demand of rabbits meat is dependent on small flocks in holders and farmers. They usually experience high mortality rate and low level performance of the local rabbits. Several studies were carried out to investigate the productive potentialities of the native and exotic breeds of rabbits under the Egyptian conditions.Till now, there is a need to obtain more information about the genetic, environmental and managerial aspects of rabbits production to create a profitable industry. Gabali rabbits raised under the Egyptian conditions and introduced from Sinai desert are characterized by their ability to afford environmental conditions and resistance to many diseases. Gabali rabbits are members of a local breed living in north Sinai under the

Egyptian semi-arid conditions. But, this breed was not well enough studied to be acquainted with its genetic aspects completely in earlier research work. The genetic parameters are very important in the progress of genetic improvement of different breeds and in designing its breeding programs that allow the genetic evaluation of such a breed and the study of its genetic properties. Performance comparisons between different breeds and their crosses are justified because genetic differences among strains are relatively large to genetic variation within breeds. These differences are important potential sources of genetic improvement in the efficiency of human food production from rabbits, through gains in the performance from 18 complementary breed effects and heterosis in crossbreeding. Nowadays, the single-trait animal model is widely used for the evaluation of rabbits breeding programs and facilitates obtaining good estimates of variance components (Lukefahr *et al*., 1996 and Iraqi *et al*., 2006). Also, using single-trait animal model is the best model as it increases the accuracy of genetic evaluation and selection when the genetic and environmental correlations between traits as well as other relevant information are included (Ferraz and Eler, 1996). Genetic engineering is a group of techniques used to identify, replicate, modify and transfer the genetic material of cells, tissues or complete organisms. Important applications of genetic engineering in molecular animal breeding are: 1) Marker-assisted selection (MAS). The objective of this technology is to increase disease resistance, productivity and product quality in economically important animals by adding information of DNA markers to phenotypes and genealogies for selection decisions.