STUDYING SOME GENES AFFECTING MILK PRODUCTIVITY IN EGYPTIAN BUFFALOES

By

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B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2007

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Department: Genetics **Approval:** **/ / 2019**

ABSTRACT

The future bio-economy of dairy industry relies on the identification of an affordable approach for increasing milk production and its constituents. Egyptian buffalo *(Bubalus bubalis)* contributes by about 50% of total milk production in Egypt, therefore it is considered as an essential dairy animal. This study aimed to differentiate between high and low milk producer buffaloes (*Bubalus bubalis*) using cDNA-AFLP, MC4R gene, microsatellite and SCoT markers. The results of cDNA-AFLP showed a great difference between high and low milk producers, which clarify that difference in gene expression results in different productivety. MCR4 gene primer resulted in a band of 800bp, that didn’t show any SNPs consequently, no difference between high and low milk producers. The six microsatellite primers showed bands of 350, 377, 496, 247, 262 and 280 bp, where three of which were digested by restriction enzyme. SCoT results of bulked samples showed unique bands that were sequenced and aligned to *Bubalus bubalis* (taxid: 89462) sequences. Alignment results showed similarity to the following encoding genes, Class V myosin, Ubiquitin-conjugating enzyme E2 D4 and gacyl-phosphatase 2 (ACYP2), which play a crucial role in organelle trafficking and many pathways, that may affect milk production traits. These results showed that cDNA-AFLP and SCoT markers were better than the other methods in clarifying the difference between high and low milk producing traits in Egyptian buffalo (*Bubalus bubalis).*

Keywords: *Bubalus bubalis,* SCoT, microsatellite markers, cDNA-AFLP, MC4R.

DEDICATION

I dedicate my thesis to whom my heartfelt thanks, to my beloved Husband Mahmoud who supported me through my long journey and always encouraged me, to my sweet little angels Judy, Talia and Salma for bearing me through all this, encouraging and praying for me and to my Mum Magda and Dad Muhammad who have been always my source of inspiration and backbone. Special thanks to my dear sisters Ghadeer and Nuhair who have never left my side. Sincere appreciation to all my family for encouraging and always praying for me.

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ABBREVIATIONS

APS	**Ammonium Per Sulphate**
AFLP	**Amplified Fragment Length Polymorphism**
β-LG	**Beta-Lactoglobuline**
BSA	**Bovine Serum Albumine**
B.V	**Breeding Value**
BM	**Buffalo Milk**
cDNA	**Complementary DNA**
CM	**Cow Milk**
EDTA	**Ethylene Di-amine Tetra Acetic acid**
H	**High milk producing samples**
CSN3	**Kappa-Casien gene**
L	**Low milk producing samples**
M	**Marker**
MC4R	**Melanocortine-4 receptor**
Pbs	**Phosphate buffer saline**
PCR-RFLP	**Polymerase Chain Reaction-Restriction Fragment Length**
QTL	**Quantitative Trait Loci**
R.E	**Restrection Enzyme**
SSR	**Simple Sequence Repeat**
SNP	**Single Sequence Repeat**
SSCP	**Single Strand Conformation Polymorphism**
SCoT	**Start Codon Targeted**
TEMED	**Tetramethylethylenediamine**
TAE	**Tris Acetic EDTA**
TBE	**Tris Boric EDTA**

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INTRODUCTION

The world population is increasing at a rapid rate and is expected to reach 8–9 billion by the end of 2030 (Mehra, 2001 and Pasha, 2013). Consequently advancement in scientific and technological fields linked to animal production and related biotechnologies are a must (Deb *et al*., 2016).

In Egypt, buffaloes are fed on poor quality nutritional resources, like crop-residues and industrial by-products containing high fibrous materials, this is because of their ability to utilize poor value feed resources. This is why water buffalo (*Bubalus bubalis*) is considered an efficient converter of poor quality resources into high quality milk and meat, in addition to power, fuel, and by-products such as hides, hoof, and bones, as well as manure to be used as fertilizer. Buffaloes are also more resistant to most of the diseases than domestic livestock. This feature helps buffalo to survive in hot humid regions. However, an improper feeding regimen and low food availability and quality certainly have an impact on reproductive and productive performances, by increasing mortality rates, longer calving interval, and reduction of growth rates (Tiwari *et al*., 2007; Sarwar *et al*., 2009; Pasha and Khan, 2010; Marai *et al*, 2010 and Deb *et al*., 2016).

Buffalo population is distributed worldwide, even though the majority (around 97 %) present in Asia, and 3,717 million are in Africa, mostly in Egypt (2.24 %); 3.3 million. Buffaloes are the second largest source of milk worldwide, in Egypt it contributes by about 50 % of total milk production – 1041533 heads- with Lactation duration of 210-280 days, and Milk yield of 2034030 tones (http://www.fao.org.).

Buffalo milk is more preferred by the consumer because of its white color and rich nutrition, as it contains higher content of fat (6.5-7.0 %), lactose, casein, whey proteins, and minerals than cow milk, which is responsible for its high energy and nutritive value making it preferred in several dairy industries as cheese, yogurt and ice cream (Hamad *et al.*, 2014 and Khedkar *et al.*, 2016). Egypt suffers from a massive gap in milk and meat production, detected through annual imports (http://www.fao.org.). This is why awareness must be paid to the genetic improvement of domestic buffalo breeds to increase their role in the agricultural production system.

Molecular markers are important because they are used to clarify any phenotypic difference that is genetically controlled, also used to discriminate groups of individuals. (Reinosa and Abad, 2012). One of the important molecular markers are microsatellite markers, which are repetitive regions of DNA where short nucleotide sequences (ranging in length from 1–6 or more bp) are repeated 5–50 times, where the number of repeats differs between individuals of the same species. They have high mutation rates than other areas of DNA, which results in high genetic diversity (Richard *et al.*, 2008 and Gulcher, 2012). One of the markers is cDNA-AFLP, it is a technique that allows genome-wide expression analysis in any species without the need for prior sequence knowledge. The cDNA-AFLP method involves reverse transcription of mRNA into double-stranded cDNA, followed by restriction digestion, ligation of specific adapters and fractionation of this mixture of cDNA fragments into smaller subsets by selective PCR amplification, which is separated on high-resolution gels, for the cDNA-AFLP fingerprints visualization and analysis (Vuylsteke *et al.*, 2007).

There are different milk genes that affect milk production traits, milk quality and content, such as: the caseins, the whey proteins, lactalbumin, lactoglobulin, leptine, and Melanocortin-4 receptor (Bawden *et al*., 1994; Deng *et al.*, 2016).

Melanocortin-4 receptor (*MC4R*) is associated with feed intake, growth, fatness, and carcass composition in many domestic animals. There is single nucleotide polymorphism (SNPs) in *MC4R* which is associated with milk production traits in water buffalo (Deng *et al*, 2016). Start codon targeted polymorphism (SCoT), gene expression analysis is extremely important in studying genetic difference; it is developed based on the short conserved region flanking the ATG start codon in plant genes. This method uses single primers of 18-mer in single primer polymerase chain (Wu *et al.*, 2013).

The objectives of this study were: