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Studying Some Genes Affecting Growth Traits and Milk Production In Egyptian Barki Sheep Breed

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Submitted by MSc. Ibrahim Abousoliman

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Reviewers:

1. **Prof. Dr. Klaus Wimmers**
   Professor of animal breeding and genetics, Faculty of Agricultural and Environmental Sciences, University of Rostock
   Research Institute for Farm Animal Biology, Dummerstorf

2. **Prof. Dr. Jens Tetens**
   Professor of Functional Breeding, Department of livestock sciences, Georg-August-University Göttingen

3. **Prof. Dr. Gesine Lühken**
   Professor of Pet and pathogenetics, Institute of Animal Breeding and Pet Genetics, Justus-Liebig- University Giessen

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List of abbreviations

ADG……..average daily gain

BW……birth weight

Bp……base pairs

Chr……chromosome

DNA……..deoxyribonucleic acid

F……fat

F_{ST} ……fixation index

GS……genomic selection

GWAS……..genome wide association study

He……heterozygosity

Ho……homozygosity

HWE.....Hardy–Weinberg equilibrium

IBD……...identity by descent

L……lactose

MAF……minor allele frequency

MQ……milk quality

MY……milk yield

P……protein

PC……principal component

PIC……polymorphic information content

SE……standard error

SNP ……single nucleotide polymorphism

TS……total solids

WW……weaning weight

1. General introduction

1.1. Motivation

Egypt is about one million square kilometers and about 95% of this area are desert. Because of the difficult environmental conditions, such as lack of forage resources, shortage of water and fluctuating temperatures, camels, sheep and goats dominate in these areas as they are more adapted compared to other livestock species. Livestock production accounts for approximately 30% of the country's total agricultural income (Elshazly & Youngs 2019). Sheep are an essential component of the Egyptian agriculture sector. Barki sheep is considered one of the main sheep breeds in Egypt and the most adapted breed to the desert conditions. It spreads along the northwestern coast of Egypt. Alongside with their high adaptability to the desert conditions, the breed has the ability to efficiently convert the low quality pastures in this region into different animal products such as meat, milk and wool. Furthermore, it has the ability to cover large distances during the grazing process (El-Wakil *et al.* 2008). Additionally, Barki sheep contribute significantly to livelihood of people in these regions (Sallam *et al.* 2012), as their meat is the main source of daily dietary protein, in addition to milk, which is used to feed the newborn lambs as well. Generally, Barki produces lower milk compared with the other Egyptian sheep breeds (Elshazly & Youngs 2019). Low milk production causes some problems for the newborn lambs such as lambs' starvation, low weaning weights as a result of low average daily gain and eventually death of lambs. Genetic improvement of growth traits such as birth weight, weaning weight and average daily gain will produce heavier lambs, which is positively correlated with mutton production (Sallam *et al.* 2019). Hence, this increases the per capita share of animal protein and the profitability of the animal production enterprise (Wei 2014). Likewise, improvement of milk traits such as milk yield and milk composition leads to increase the lamb's viability and the lambs weaning weights. Accordingly, genetic improvement programs should be established aiming to facilitate the selection of breeding animals, which will actually improve these traits. The following sections provide a detailed introduction to the relevant topics of the thesis, specifically: Economic value and distribution of sheep worldwide, sheep production in Egypt, and sheep breeding approaches.

1.2. State of the art

Sheep represent one of the most important livestock species worldwide. Sheep and goat were the first livestock species domesticated by humans for agricultural purposes and production of meat, milk and wool, which happened approximately 10,000 years ago (Alberto *et al.* 2018). Sheep has high economic and social values in most of the developing countries and considered an essential source of income for the breeders. Therefore, as with other livestock species, a continuous genetic improvement for

economically important traits such as growth and milk performance traits is a key factor in sheep breeding.

1.2.1. World-wide distribution of sheep breeds

The total worldwide population of sheep has increased from 1099 million head in 2010 reaching about 1239 million head in 2019 (FAOSTAT 2019c). Table 1 shows the distribution, the total numbers of sheep and the top 10 sheep producing countries in 2019 around the world, and specifically for Africa, Asia, the Americas and Europe. For Oceania, Australia and New Zealand owns about 65.8 and 26.8 million head of sheep.

Table 1. Sheep distribution and top 10 countries in sheep population around the world (FAOSTAT 2019c)

Worldwide		**Africa**		**Asia**		**Americas**		**Europe**	
Country	**Head in million**	**Country**	**Head in million**	**Country**	**Head in million**	**Country**	**Head in million**	**Country**	**Head in million**
China	163.5	Nigeria	46.9	China	163.5	Brazil	19.7	Russia	21.1
India	74.3	Sudan	40.9	India	74.3	Argentina	14.8	Romania	10.4
Australia	65.8	Chad	35.9	Iran	41.3	Peru	11.3	Greece	8.4
Nigeria	46.9	Ethiopia	31.8	Turkey	35.2	Mexica	8.7	France	7.1
Iran	41.3	Algeria	29.4	Mongolia	32.3	Bolivia	7.5	Italy	7.0
Sudan	40.9	Kenya	27.4	Pakistan	30.9	Uruguay	6.6	Ireland	5.1
Chad	35.9	South Africa	22.1	Uzbekistan	18.2	U.S.A	5.2	Norway	2.3
Turkey	35.2	Morocco	21.6	Indonesia	17.8	Chile	2.2	Portugal	2.1
Mongolia	32.3	Niger	13.2	Kazakhstan	16.9	Cuba	1.5	Germany	1.6
Ethiopia	31.8	Somalia	11.7	Syria	14.6	Colombia	1.5	Spain	1.6
Total	**1239**	**Total**	**408**	**Total**	**527**	**Total**	**89**	**Total**	**128**

These statistics revealed that most of sheep populations are raised in Asia and Africa. Moreover, most of the top 10 countries in sheep production are developing countries as a reflection of the effect of the environmental and geographical reasons. These may reflect the high economic and social values of sheep especially in such countries and the low management processes needed for sheep breeding compared to cattle. In Egypt, the total sheep population reached about 5.7 million animals in 2019 (Sallam 2020).

1.2.2. Social and economic value of sheep worldwide

Sheep farming activity is one of the pillars of worldwide agricultural development, especially in the developing countries and some developed countries such as New Zealand and United Kingdom. It provides the human beings with different sources of products such as meat, milk and wool. Compared to cows and buffalos, sheep breeding enterprises have many economic and social advantages (Zygoyiannis 2006). Sheep breeding in general is characterized by some features that have been reported as follows:

- Low invested capital due to the low price of the unit (sheep) compared to the prices of other established farm animals, including cows and buffaloes (Nix 1988).
- Speed of the capital cycle where lambs can be marketed about six months after their birth. So, the annual return rate may reach 30% of the exploited capital in the production process (Nix 1988).
- Sheep are characterized by the diversity of their production, as they give meat, milk, wool, and hides. These products used in the establishment of some important manufacturing industries, such as processing and canning industry for meat and processed meat, milk products industry and textile and leather industries, which increases the return that the breeder gets throughout the year (Popovic 2018).
- High nutritional value of sheep meat and milk as main products, as it is used in many industries, as well as wool as a by-product (Popovic 2018).
- Sheep reproductive efficiency is high and have the potential to produce twins (Petrovic et al 2012).
- Sheep are considered one of the most suitable agricultural animals for grazing in dry and arid regions, versus other farm animals. This may be due to their high ability to graze and adapt to the environmental conditions prevailing in these areas (El-Wakil et al. 2008).
- It is considered a method of using agricultural crop residues after harvesting; as sheep are sweeping animals and their ability to graze at a low level and thus integrate with cows and do not compete with them. Moreover, sheep surpass cattle in their ability to benefit from the coarse feedstuffs (Morris 2009).
- Works to raise soil fertility; as the resulting manure has a high fertilizer value for many agricultural crops as organic fertilizer, especially on newly reclaimed lands. In addition, it is rapidly dissolving in the soil, and in the case of grazing, its droppings are distributed regularly on the pasture land due to the large movement of sheep from one place to another.
- The possibility of implementing sheep breeding projects in areas that may not be suitable for other farm animals, such as deserts. Also, these projects do not need shelters and equipped barns, and simple shades in safe places suffice (El-Wakil et al. 2008).
- Low investment costs, as they do not require high construction costs, in addition to low feed costs, and their lack of high technical means in production, which lowers their

requirements for technical labor; sheep are taken care of collectively, not individually, and require less labor and time than other animals (Morris 2009) .

- The price of lamb meat is constantly increasing, and the demand for it increases on special occasions and holidays. Therefore, sheep raising is associated with some social events in some countries, especially Islamic countries

1.2.3. Economic exploitation and ecological added value of sheep

Globally, sheep are valuable livestock species because of their ability to live and produce under diverse environmental conditions with high conversion efficiency of forages with low nutritive value into meat, milk and wool.

According to the statistics of the Food and Agriculture Organization of the United Nations (FAO) in 2019, sheep contribute to the global production of meat and milk with significant proportions. The total world production of meat reached 337 million tons, while the global production of sheep meat was 9.9 million tons, equivalent to about 3% of the global production of meat. For milk, the global production of sheep milk was 10.1 million tons, which is more than 1% of the global production, which is about 883 million tons across all livestock species. Likewise, for wool, the global production was about 1.72 million tons around the world.

In 2019, Egypt produced about 2.36 and 4.65 million tons of meat and milk, respectively. Of these, Egyptian sheep produced about 58,336 and 92,478 tons, respectively which considered relatively low. Likewise, the total production of wool in Egypt was about 11,217 tons.

Small ruminants can play a vital role in the management of natural resources and enhancing the environment. Sheep and goats have been demonstrated as an effective tool to Improve rangeland and riparian and watershed management, reducing the incidence of wildfire, controlling noxious weeds, improving wildlife habitat and enhancing tree plantations (Chapman & Reid 2004).

1.3. Sheep breeds in Egypt

Small ruminants play an important role in the Egyptian agriculture sector as a good supplier for meat and meat products. Sheep production serves as a valuable source of income to farmers and as an important source of meat and milk. The contribution of sheep is about 6.4% of the total red meat production (Sallam *et al.* 2012). The sheep population in Egypt has increased rapidly during the last five years, reaching about 5.7 million animals in 2019 (FAOSTAT 2019c). The most common sheep breeds are Barki, Ossimi and Rahmani (Galal *et al.* 2005). The Barki sheep breed is one of the most important breeds in Egypt and it spreads along the North Western Coastal Zone (NWCZ) of Egypt from west of Alexandria to the eastern provinces in Libya representing about 10% of the total Egyptian

sheep population. In Egypt, Barki sheep are raised under three breeding systems comprising intensive, semi-intensive and transhumance system. However, most of Barki sheep flocks are raised under the transhumant system, grazing grasses, little bushes, and plants that grow during the period between June and August as a result of the rainfall of this Mediterranean area. The origin of the breed is North Africa in the coastal Mediterranean zone in Barka city in Lybia. The breed is named after the Libyan province Barka. The breed is well adapted to the arid zone which characterised by high temperatures, hard intense solar radiation, low precipitation and shortage of food and water almost throughout the year. Among all the Egyptian sheep breeds, Barki is characterized by tolerating high temperature with obvious ability to live and produce under hard conditions (Haider 1982; El-Wakil *et al.* 2008).

Figure 1. The geographical distribution of Barki Sheep breed in Egypt. (The yellow rectangle represent the main habitat (Sallam 2019a).

As shown in figure (2), Barki sheep have a relatively small black or brown head, long neck and legs, and a short narrow white body. Fat is stored in the upper part of a triangular tail, while the lower part is thin and reaches to the hock. The fleece is of carpet-wool type and includes some kemp. Prolificacy of Barki ewes is relatively low compared to other Egyptian breeds, which limits productivity (Fahmy *et al.* 1969). Ossimi sheep are white in color with brown or dark brown head and represent about 15% of the total Egyptian sheep population. The ear is semi-pendulous, males are horned but horns are absent

in females. Ossimi sheep have a long body with short neck and round fatty tail. Rahmani sheep are reddish brown in color, have a large head with curved nose and the ear is pendulous. Males are horned while horns are small or absent in females. The body is long with a short neck and an oval fatty tail. Rahmani sheep represent about 12% of the total Egyptian sheep population.

Table 2 shows some productive parameters among the main Egyptian sheep breeds (Elshazly & Youngs 2019).

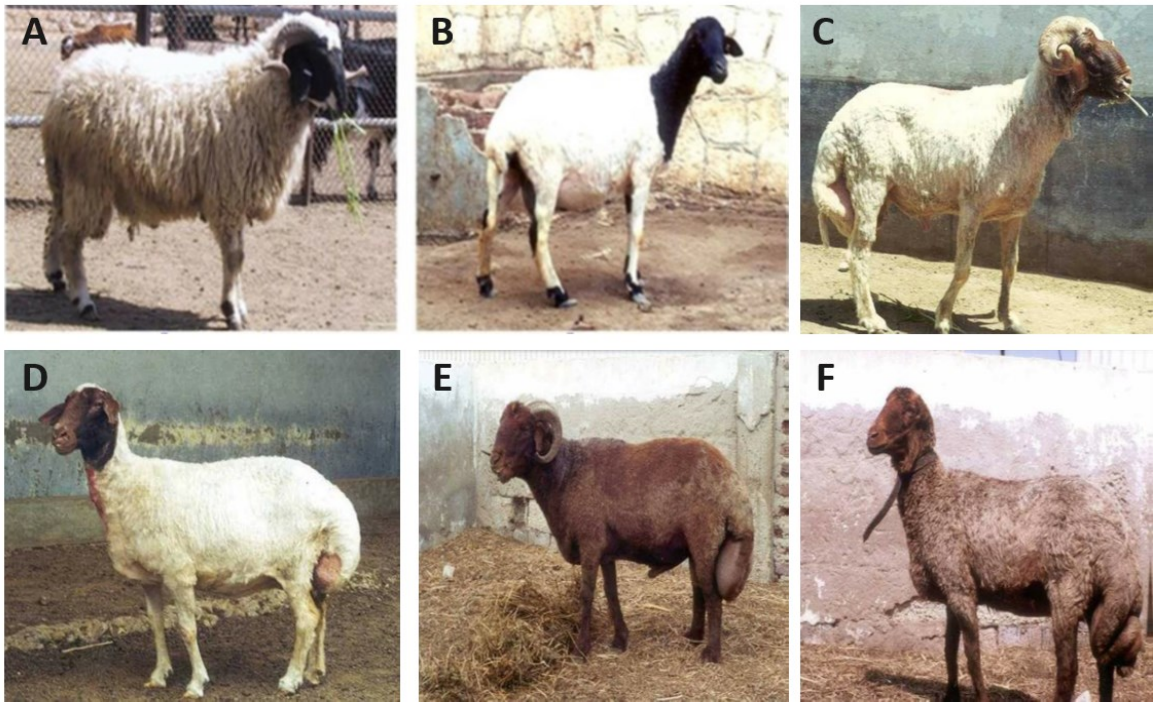

Figure 2. Morphological characteristics of the main sheep breeds in Egypt. (A) Barki ram (B) Barki ewe (C) Ossimi ram (D) Ossimi ewe (E) Rahmani ram (F) Rahmani ewe (Elshazly & Youngs 2019).

Table 2. Productive parameters of the main Egyptian sheep breeds Ossimi, Rahmani and Barki.

Production traits	**Ossimi**	**Rahmani**	**Barki**
Birth weight (kg)	3.06-3.64	3.60-4.00	2.3-3.5
Weaning weight at 3 months (kg)	14	15	12
Average daily gain from birth to weaning (g)	115-135	140-170	120-150
Ram mature body weight (kg)	60-65	65-75	50-60
Ewe mature body weight (kg)	45-50	50-55	40-45
Prolificacy rate (%)	115	125-130	103-105
Lambs mortality rate from birth to weaning (%)	11.8	14.6	16.6
Fleece weight (kg)	1.25	1.50	1.1

1.3.1. Productive traits in sheep

1.3.1.1. Growth traits

There is a positive correlation between meat production and growth traits as the higher growth performance will produce heavier lambs and increase the mutton yield at slaughter age. Accordingly, growth performance has been a common target for genetic improvement in sheep for a long time. It is well-known that growth traits are quantitative traits, which are controlled by both genetic and environmental factors. In addition to the genetic potentiality of the lambs for growth, growth traits are also influenced by different environmental factors such as, nutrition, health condition, sex, litter size, month or season of lambing, age of dam and year of lamb birth (Kuchtík & Dobea 2018). Body weight is one of the important growth and development indices for meat type animals that can be measured at birth or at other animal life stages and regulate incomes from sheep meat production. It influences meat, wool production and fertility of sheep (Wei 2014). Body weight is used for various reproduction, nutrition and preventive management decisions (Buzanskas *et al.* 2014). The growth performance of the individual lamb can be measured in terms of several traits such as birth, weaning and yearling weights. Birth weight is the earliest indicator with considerable impact on growth performance traits (Ptáček *et al.* 2017). The heritability of birth weight was estimated and ranges between 0.19 to 0.39 in some sheep breeds such as Lori-Bakhtiari and Barki, respectively (Zamani & Mohammadi 2008; Ghasemi *et al.* 2019; Sallam *et al.* 2019). Weaning weight is considered one of the most important growth traits as indicator for the pre-weaning growth of the lambs. Estimates of weaning weight heritability were 0.2 in Barki sheep and 0.25 in Iranian Mehraban sheep (Zamani & Mohammadi 2008). The growth rate or average daily gain is an economic trait of interest and used as a criteria for the selection programs of growth traits (Lalit. *et al.* 2016). Heritability of average daily gain from birth to weaning was 0.15 in Afshari sheep (Ghafouri-Kesbi & Eskandarinasab 2018) and 0.24 in Iranian Mehraban sheep and 0.18 in Barki sheep breed. In Barki sheep breed, some parameters were estimated to indicate the productive life of this breed. The average of productive age is 6.6 years, lambing number per ewe is 4.4, the total number of lambs born per ewe are 4.7 lamb, the total weight of lambs born per ewe is 16.2 kg, the total number of lambs weaned per ewe are 4 lambs and the total weight of lambs weaned per ewe is 82.3 kg (Ibrahim & Alsheik 2016).

1.3.1.2. Milk performance traits

Milk is commonly defined as the secretion of the mammary gland and it is used to feed the new born progeny during the early stage of their life, i.e., from birth until weaning. Sheep have been raised for milk for thousands of years, which is much longer than cow milk production (Zervas & Tsiplakou 2011). Sheep milk and its products are widely consumed in many countries, especially the Mediterranean. It has a high degree of similarity with human milk in the total fatty acid composition, which makes it a

good raw material for infant formula production (Martin *et al.* 2016). Sheep milk production and composition are influenced by genetic and environmental factors. Estimates of heritability for milk yield, fat content and protein content in sheep are 0.38, 0.48 and 0.51, respectively (Milan *et al.* 2005). Milk components such as fat, protein and lactose are considered as indicator for milk quality and the efficiency of milk production. Furthermore, it affects the newborn lamb's growth, and increases the immunity of the newborn lambs through feeding on colostrum, which contains considerable amounts of albumin and globulin, especially in the first three days after birth. Ovine milk has on average 7.2 %, 4.6%, 4.8 % and 0.9 % for fat, protein, lactose and minerals, respectively. The milk protein fraction consists of six milk proteins. Those six proteins divided into four caseins proteins (*α*S1-, *β*-, *α*S2- and *κ*-casein) and two whey proteins (*α*-lacto albumin and *β*-lacto globulin) (Tetens *et al.* 2014). Milk protein also is a source for amino acids for the new lambs and it is the main factor in cheese industry. Milk fat is an essential component for healthy lambs feeding as a source of different fatty acids, energy besides milk lactose and it is important in the production of some milk products like butter and yoghurt (Osorio *et al.* 2016).

1.3.1.3. Wool traits

Wool is the natural fiber of domesticated sheep, it is also used as the name of hair from different livestock species such as goat, camel, vicuna, alpaca, angora, rabbit and yak. Wool is widely used to produce clothing, bedding, carpets and textiles around the world. Wool quality traits such as fiber diameter, yellowness, brightness, staple strength, staple length, yield, greasy fleece weight and clean fleece weight are very important in wool industry (Doyle *et al.* 2021). The wool type varies from coarse to fine wool (M-L *et al.* 2007). Estimates for the heritability of most wool traits are generally high (h^2 = 0.3 - 0.6), indicating that wool traits are under genetic control (Benavides & Maher 2000). Many reports identified the association between wool traits and some candidate genes. These studies identified some candidate genes such as *KAP1.1, KAP1.3, K33, KAP21-1* and *KRTAP6-3* genes in different sheep breeds (Itenge *et al.* 2010; Li *et al.* 2019b; Ullah *et al.* 2020). In Barki sheep, wool production is lower than other Egyptian breeds and the fleece is of carpet-wool type including some kemp and usually used in carpets industry (Elshazly & Youngs 2019). Keratin-associated protein 6-1 (*KAP6-1*) gene was identified as a candidate gene for some wool traits in Barki sheep (Sallam *et al.* 2020).

1.3.2. Future of native sheep breeds

Sheep breeds around the world are divided into 3 categories; breeds not at risk, breeds at risk and breeds with unknown risk status. Most of these breeds are common in the developing countries, especially in arid and semi-arid regions. In Africa, sheep breeds are categorized into 88 breeds not at risk, 39 breeds at risk and 690 with unknown risk status (FAOSTAT 2019b). In Egypt, there are 12 sheep breeds that are listed in the Domestic Animal Diversity Information System (DAD-IS) database

(FAOSTAT 2019a). Information about the Egyptian sheep breeds are often lacking. It is important to take care of preserving the diversity of sheep breeds in Egypt. The extinction of well-adapted local breeds will obstruct future sheep farming improvement strategies. There were several attempts of crossbreeding with exotic breeds to improve the productive and reproductive performance of the Egyptian sheep breeds. For example, the Egyptian Ministry of Agriculture performed the Egyptian Finnsheep breeding project aimed improving the productivity of some Egyptian breeds through crossing with the highly prolific Finnsheep (Elshazly & Youngs 2019). Likewise, crossbreeding with Awassi sheep was reported for the same purpose (Shehata 2006). These experiments did not provide the expected results due to the non-adaptation of these exotic breeds to the environmental conditions in Egypt.

Recently, the Egyptian sheep researchers with collaboration with the Egyptian ministry of Agriculture suggested some recommendation to improve the performance and to maintain the diversity of sheep breeds in Egypt such as:

- Crossbreeding among the improved three local fat-tailed breeds (Rahmani, Ossimi, Barki) aiming to get a hybrid for the commercial and fattening purposes (Marai et al. 2009).
- Crossbreeding with the well adapted exotic breeds to the Egyptian environment conditions.
- Using of assisted reproductive technologies such as artificial insemination and embryo transfer. These technologies will help in rapid genetic improvement.
- Genetic evaluation of these breeds for the purpose of applying genetic improvement techniques like marker-assisted selection or genomic selection.

Around the world there are some principles recommended by FAO/UNEP (FAO/UNEP) for genetic improvement of indigenous animals in the tropic regions like:

- Monitoring animal performances and avoidance of inbreeding.
- Selection of outstanding individuals by population screening.
- Use of nucleus herds.
- Establishment of societies for recording pedigree and performance.
- Use of artificial insemination and embryo transfer.
- Using some new approaches for improving the reproductive performance in sheep such as using of hormones for synchronization of estrus, superovulation and induction of early sexual maturity.

- Applying of selection on body weight gain, which is heritable in sheep meat production.

1.4. Molecular marker techniques

The development of molecular genetics and associated technology has facilitated the understanding of the genetic basis of important traits in livestock. Different approaches are widely used to identify the quantitative trait loci (QTLs) underlying economic traits in various livestock species; such as candidate gene association approach, genome-wide scan and selection signatures. Candidate gene approach identify genes that are thought to be responsible for the phenotype variance of the trait of interest based on known function or position in the genome. Similarly, genome-wide association analyses and selection signature studies screens out the genome to identify putative genes or chromosomal region of interest that influence the desired trait across the whole genome. (Rothschild & Sölkner 1997).

1.4.1. Candidate gene approach

The candidate gene technique is an effective way to study the association between phenotypic data of the traits and genotypes of the candidate gene that may affect the physiological pathways of these traits (Andersson 2001)**.** SNP-trait association analysis to detect the candidate genes consists of the following five steps: (step1) detection of a resource population, (step 2) collection of phenotypic data of the trait(s) of interest, (step 3) selection of the candidate genes that may affect the traits, (step 4) genotyping of the individuals of the resource population to identify the genetic polymorphisms in these genes, (step 5) statistical analysis to study the association between the phenotypic and genotypic data (Da 2003). Detection of genes affecting growth traits is important to understand the genetic and physiological pathways affecting this trait. Candidate genes affecting body weight in farm animals such as *GH, IGF-I, LEP, MSTN* and *ADRB3* have been detected (Forrest *et al.* 2007; Gholibeikifard *et al.* 2013) and some QTL studies have been conducted in sheep (Margawati *et al.* 2006)**.** Many researchers also used candidate gene technique to study the association between some candidate genes and milk performance traits in various sheep breeds (Moioli *et al.* 2007; Orford *et al.* 2010)**.** Several of candidate genes like *CSN3, CSN1S, DGAT1, GHR, LEP* and *PRL* have been used as marker genes for milk traits in sheep (Staiger *et al.* 2010)**.** Recently, some genetic markers are available and can be used commercially in industry breeding programs of different livestock species such as cattle, sheep and pigs. Table 3 shows the most common genes which are intensively used in the breeding programs for different economic traits in different species (Dekkers 2004).

Table 3. Most common candidate genes identified in different livestock species and used in breeding programs.

Species	Trait	Candidate genes	References
Dairy cattle	Milk quality	*κ-casein* *β-lacto globulin*	(Medrano & Aguilar-Cordova 1990; Rincón & Medrano 2003)
	Milk yield and composition	*DGAT* *GHR* *FMO3*	(Grisart *et al.* 2002; Lundén *et al.* 2002; Blott *et al.* 2003)
Beef cattle	Appearance	*MC1R / MSHR*	(Klungland *et al.* 1995; Seitz *et al.* 1999)
	Growth and body composition	*MSTN*	(Grobet *et al.* 1998)
Sheep	Body weights	*MEF2B* *TRHDE* *CAPN*	(Mahrous *et al.* 2016b; Zhang *et al.* 2016)
	Growth traits	*MSTN*	(Broad *et al.* 2000; Sahu *et al.* 2017)
	Carcass traits	*UCP1* *LEP* *CAST*	(Barzehkar *et al.* 2009; Yang *et al.* 2014; Aali *et al.* 2017)
	Milk traits	*POU1F1* *LALBA* *PALMD*	(García-Gámez *et al.* 2012b; Moioli *et al.* 2013; Ozmen *et al.* 2014)
Pigs	Meat quality	*RYR* *RN/PRKAG3*	(Fujii *et al.* 1991; Milan *et al.* 2000)
	Growth and feed intake	*MC4R* *IGF-2*	(Kim *et al.* 2000)

1.4.2. Genome Wide Association Study (GWAS)

GWAS is a method for the study of associations between a genome-wide set of single-nucleotide polymorphisms (SNPs) and desired phenotypic traits. Development of SNP genotyping technologies led to the use of GWASs to detect candidate genes for quantitative traits, which increase the accuracy of selection to improve these traits. Currently, GWAS is used for identification of major QTLs for

economic traits in various species of farm animals (Al-Mamun *et al.* 2015). GWAS compares the frequency of alleles or genotypes of many genetic markers between different phenotypes (Hirschhorn & Daly 2005). It is used to screen the whole genome for target genes that correlate with phenotypic traits, using SNPs as genetic markers. They have become an important method for identifying genomic regions harboring candidate genes for important economic traits in livestock. This approach has become feasible because of the large number of single nucleotide polymorphisms (SNPs) discovered by genome sequencing to genotype large number of SNPs (Goddard & Hayes 2007). Compared to linkage based QTL mapping by microsatellites, GWAS has a greater capability to detect causal mutations due to the higher marker density (Koopaee et al 2014). Two main platforms used to perform the GWAS, the Illumina platform (San Diego, California, USA) and the Affymetrix platform (Santa Clara, California, USA) (Hirschhorn & Daly 2005)**.** Genome markers can be tested for association with most of productive traits faster and cheaper than before through using of the developed SNP chip genotyping method. SNP chips enables researchers to genotype thousands of markers with low costs (Fan *et al.* 2010). GWAS depends on studying the correlation between the genotypes and the phenotypic data, so the design of GWAS based on the kind of the phenotypic data. If the phenotypes are categorical, then the GWAS is a case/control study. If the phenotypes are quantitative then the GWAS is a quantitative study. The quantitative design might seem more powerful but the case/control design has also resulted in many successful results (Bush & Moore 2012).

In recent years, GWAS have been carried out for different productive traits in most of livestock species like pigs, cattle, sheep, and chickens, and have identified many genes or molecular markers that could regulate important economic traits in livestock (Gao *et al.* 2019; Ghasemi *et al.* 2019; Li *et al.* 2019a; Liu *et al.* 2019)**.** Genomes of several livestock species have been partially or completely sequenced (Bai *et al.* 2012). Chicken were the first species to be sequenced (Burt 2005) followed by pig (Archibald *et al.* 2010a), cow (Zimin *et al.* 2009), horse (Wade *et al.* 2009) and sheep (Archibald *et al.* 2010b).

The sheep genome information was generated by sequencing the DNA of a single Texel ewe and a single Texel ram using Illumina technology. The latest assembly of the sheep genome (Oar_v3.1) is based on the dataset of the Texel ewe (Jiang *et al.* 2014). Before the sequencing of the sheep genome, there were about 700 genes known in sheep (Zhang *et al.* 2013) but the genes reached about 20,921 after the sequencing of the sheep genome (Flicek *et al.* 2014). In sheep, some GWASs were performed for some growth traits such as birth weight in Lori-Bakhtiari sheep (Ghasemi *et al.* 2019), weaning weight in Australian Merino sheep (Al-Mamun *et al.* 2015), body weight traits in Chinese Fine-Wool Sheep (Lu *et al.* 2020), meat and carcass traits in Scottish Blackface lambs (Zhang *et al.* 2013; Matika *et al.* 2016), milk performance in Lacune sheep (Yuan *et al.* 2019) and some reproductive traits in Rahmani sheep in Egypt (El-Halawany *et al.* 2016).

1.4.3. Selection signatures studies

Selection signatures used to analyze the genomic differentiation among the livestock breeds or diversified populations. The detection of selection signatures is important to characterize livestock genetic resources and to determine the genomic regions and the candidate genes which are responsible for the variation in economic traits (Cesarani *et al.* 2018). They may be used to detect the candidate genes that are associated with some traits such as adaptation to the environment which is difficult in measuring (Yurchenko *et al.* 2018). Detection of selection signatures is important to deeply understand the population origin and genetic processes that causes the phenotypic differentiation in livestock breeds. Understanding the selection effect on particular population may help us to perform better breeding programs to improve the important economic traits in different livestock species (Gurgul *et al.* 2018). The selection signature detection can be used to identify the candidate genes under selection with diversified populations (Chen *et al.* 2016). Selection signature detection is usually carried out with a "top-down" approach, from genotype to phenotype, followed by statistical analysis of the genomic SNP data to identify selection signatures (Bomba *et al.* 2015). Sometimes selection signature studies can be an alternative approach to GWAS in detection of significant SNPs associated with genes or genomic regions which are related with particular traits such as tolerance to high temperatures, adaptation to the environmental conditions and disease resistance (Maiorano *et al.* 2018).

There are many different approaches commonly used for the detection of selection signatures in livestock populations. Fixation Index (F_{ST}) of Weir and Cockerham (1984) is one of the most popular methods to detect selection signatures between diversified populations, groups or breeds. The F_{ST} approach measures genomic differentiation depending on allele frequency between populations and can detect highly differentiated alleles undergoing selection among populations (Weir & Cockerham 1984; McRae *et al.* 2014). F_{ST} value ranges from 0 to 1. High F_{ST} values revealed positive selection, whereas low F_{ST} values suggest negative selection (Zhao *et al.* 2015). Several genomic studies were performed for the detection of selection signatures associated with different economic traits in sheep. For example, a genomic scan was performed for selection signatures using different approaches in six sheep breeds and identified the *NPR2* gene on chromosome 2 to be associated with muscle formation, weight gain, body size and skeletal morphology (Kijas *et al.* 2012; Purfield *et al.* 2017; Manzari *et al.* 2019). Other studies were performed to detect selection signatures associated with adaptation traits in sheep and identified some candidate genes such as *NPAS2* gene for regulation of body temperature and blood pressure (Mastrangelo *et al.* 2017), *RELN* and *TRHDE* genes for cold adaptation (Edea *et al.*

2019) and *LDLRAD4, LRP11, CFI,* and *VLDLR* genes for metabolic response to stress and thermotolerance (Álvarez *et al.* 2020).

1.5. Application in animal breeding

1.5.1. Marker Assisted Selection (MAS)

Marker-assisted selection refers to a modern breeding process where selection of targeted traits is based on specific molecular markers, such as single nucleotide polymorphism (SNPs). Marker assisted selection (MAS) was the first breeding tool to apply molecular markers for the genetic improvement of livestock species. It involves the selection of animals carrying specific alleles indicating genomic regions or genes that are involved in the expression of traits of interest through molecular markers. Molecular markers or genetic markers are small sequences of DNA that reveal polymorphism in genes (Tanksley 1983). Instead of selecting for a trait, the breeder can select for a marker that can be detected very easily and early in selection scheme. Identification of DNA polymorphism and the genes associated with body weight traits provides additional information for MAS and gene-based selection (Goddard & Hayes 2009). The use of DNA markers has provided opportunities to enhance response to selection, in particular for traits that are difficult to improve by traditional selection (low heritability or traits for which measurement of phenotype is difficult such as for disease resistance genes, expensive, and only possible late in life) (Wakchaure *et al.* 2015). With the development and availability of the SNP-chip of molecular markers, MAS has become possible for traits controlled by major genes as well as quantitative trait loci (QTLs). It has become a promising and potent approach for integrating biotechnology with conventional and traditional breeding. MAS is used as a tool to reduce generation interval through early selection. Many genetic markers linked with QTL affecting economic traits in livestock, comprising milk production, growth and health have been verified (Van Tassell *et al.* 2000).The success of MAS is influenced by the relationship between markers and gene of interest (Dekkers 2004), in which three kind of relationships are addressed:

1. The molecular marker is located within gene of interest. This is most favorable situation for MAS and it could be ideally referred to as gene assisted selection.
2. The marker is in linkage disequilibrium (LD) with gene of interest throughout the population. Population wide LD can be found when marker and gene of interest are physically very close to each other.
3. The marker is in linkage equilibrium (LE) with gene of interest throughout whole population

1.5.2. Genomic Selection (GS)

The new advances in genomic technologies and the development of genome SNP-chip give the researchers the ability to implement the genomic selection (GS). Genomic selection is a form of MAS in which genetic markers covering the whole genome (Ibtisham *et al.* 2017) and breeding values of animals can be accurately estimated using dense maker map of chromosomes without information about their phenotype and gene location (Rabier *et al.* 2016). GS relies on the expectation that some QTLs will be in strong linkage disequilibrium (LD) with at least one marker (Schulz-Streeck *et al.* 2012). The genomic selection is based on the analysis of large number of SNPs which increase the accuracy of prediction (Hayes *et al.* 2009) and the rate of genetic gain by decreasing the generation interval (Meuwissen & Goddard 2010). Animals' selection can be performed at very early age, it can even be applied on embryonic stage. The efficiency of genomic selection for sex-limited traits such as milk performance, or low heritable traits (fertility) is high compared to traditional selections (Hiendleder *et al.* 2005). The development of genomic selection simplified the selection process and decreased its cost due to the reduction in generation interval, improving the yearly genetic trend. Further in GS progeny testing is no longer necessary, selection can be done for a much larger number of animals and it may limit inbreeding trends (Henryon *et al.* 2014). Genomic selection has been implemented in different livestock species. In dairy cattle, several studies have been performed to identify genomic regions associated with milk production, health, cow conformation and fertility traits (Wiggans *et al.* 2009; Bolormaa *et al.* 2010; Jiang *et al.* 2010). The reliability of genomic prediction in dairy cattle exceeds 0.7 to 0.8 for production and fertility traits (Lund *et al.* 2011; Wiggans *et al.* 2011). In pigs, genomic selection improved some economic traits such as litter size, post-weaning mortality and number of teats (Tusell *et al.* 2013; Rohrer & Nonneman 2017). The genetic gain was increased up to 50% for post-weaning mortality and teats number in pigs using genomic selection (Knol *et al.* 2016; Lopes *et al.* 2017). In sheep and goats, some studies have been done to improve growth, milk and fertility traits using genomic data (Al-Mamun *et al.* 2015; El-Halawany *et al.* 2016; Matika *et al.* 2016; Ghasemi *et al.* 2019; Yuan *et al.* 2019; Lu *et al.* 2020).

1.6. Aims of the research

In Egypt, sheep are reared mainly for mutton with milk as a minor interest and wool as a by-product. Improving growth traits such as birth weight, weaning weight and average daily gain and milk performance traits like milk yield and milk quality in sheep using new molecular techniques such as the candidate gene approach and the genome-wide scan approach is very important to increase the total meat yield of weaned lambs and decrease the mortality rate of newborn lambs due to starvation. Therefore, in this thesis phenotypic and genotypic data from a Barki ewe's population and their lambs from one breeding season were used. The effect of polymorphisms in some candidate genes on growth and milk performance traits was analyzed. Also, the genomic differences between high and low groups of growth and milk performance traits using genome wide scan were studied. In this thesis, three main objectives were focused:

The goal of the **first study** was to identify segregating polymorphisms of major candidate genes described in recent literature such as *LEP, IGF-1, DGAT1, STAT5A, PRL, CSN1S2, GHR* and *GHRHR* for growth (birth weight, weaning weight and average daily gain) and milk traits (milk yield and milk composition) in a Barki population of ewes and lambs. Subsequently, investigation of the association between the genotypes and phenotypes, which might be used to improve these traits.

The goal of the **second study** was to identify the genomic differences for growth traits in a Barki lamb population using a genome-wide scan to detect the genomic regions and candidate genes that may cause the variability between high and low groups of early growth-related traits.

The aim of the **third study** was to explore the genomic differences between high and low groups for milk performance traits in Barki ewe population to investigate the genomic regions and candidate genes related to milk performance traits.

2. Experimental studies

2.1. Analysis of Candidate Genes for Growth and Milk Performance Traits in the Egyptian Barki Sheep

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Ibrahim Abousoliman designed and performed the experiment, analyzed the data, and wrote the manuscript with the support of and in agreement with his supervisor Prof. Klaus Wimmers and the co-authors of this manuscript.

Analysis of Candidate Genes for Growth and Milk Performance Traits in the Egyptian Barki Sheep

Ibrahim Abousoliman [1, 2], Henry Reyer [1], Michael Oster [1], Eduard Muráni [1], Mosaad Mourad [3], Mohamed Abdel-Salam Rashed [3], Ismail Mohamed [2] and Klaus Wimmers [1, 4,]*

[1] Institute of Genome Biology, Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany

[2] Department of Animal and Poultry Breeding, Desert Research Center, Cairo, Egypt

[3] Faculty of Agriculture, Ain Shams University, Cairo, Egypt

[4] Faculty of Agricultural and Environmental Sciences, University of Rostock, Rostock, Germany

* Corresponding author

Simple Summary

The Barki sheep breed is one of the main sheep breeds in Egypt, and it is well adapted to the harsh desert conditions in the Mediterranean zone. Growth performance has an important role regarding the supply of red meat for human consumption. In addition, milk production is very important to adequately feed newborn lambs and prevent them from starvation. In this study, segregating single nucleotide polymorphisms (SNP) were identified in the coding regions of eight candidate genes for growth and milk traits by direct sequencing. Subsequently, a population of Barki ewes and lambs was screened for these SNPs, and associations between genotypes and traits of interest were assessed. Out of the candidate genes, SNPs of *LEP, STAT5A, PRL*, and *GHRHR* were significantly associated with phenotypes. This study provides first insights into the genetics of milk and growth traits in the Barki sheep. The findings concluded that *LEP, STAT5A, PRL*, and *GHRHR* might be regarded as candidate genes to improve the Egyptian Barki sheep breed.

Abstract

The most common sheep breeds of Egypt are Ossimi, Rahmani, and Barki breeds. The latter one is well adapted to the challenging desert environment, characterized by food shortage and a high temperature fluctuation. Growth performance of Barki sheep has an important economic value in terms of minimizing the shortage of mutton meat in Egypt. Further, milk production is of great importance for feeding newborn lambs. Eight candidate genes, recently associated with production traits in different breeds, were used to study the effect of genotype on lamb growth and ewe milk

traits. The examined genes were *LEP, IGF1, DGAT1, STAT5A, PRL, CSN1S2, GHR*, and *GHRHR*, of which one representative single nucleotide polymorphism (SNP) located in the coding region was selected for genotyping. Data from 251 Barki sheep were used in this study. Association analysis between SNPs and lamb growth traits identified rs420693815 of the *LEP* gene to be significantly associated with weaning weight and average daily gain. In ewes, significant effects on milk yield and composition have been estimated for *LEP* (rs420693815), *STAT5A* (rs161082816), *PRL* (rs422713690), and *GHRHR* (rs414991449). The results indicated that these genes might be considered as interesting candidates for further investigations to improve growth and milk performance in Barki sheep.

Keywords: Barki sheep; growth traits; milk performance; single nucleotide polymorphism; association analysis

1. Introduction

Small ruminants play an important role in supplying meat and meat products in arid regions. Regarding the Egyptian agriculture sector, sheep production serves as a valuable source of income to farmers and as an important source of meat and milk [1]. In Egypt, the contribution of sheep is about 6.4% of the total red meat production. The most common indigenous sheep breeds in Egypt are Ossimi, Rahmani, and Barki [2]. The sheep population in Egypt has increased rapidly during the last five years, reaching about 5.7 million animals in 2017 [3]. Barki sheep spread along the northwestern coastal zone (NWCZ) of Egypt with a population of 470,000 heads (8.5% of the total Egyptian sheep population). Barki sheep are raised under the transhumant system, grazing grasses, little bushes, and plants that grow during the period between August and June as a result of the rainfall of this Mediterranean area. Barki sheep are well adapted to harsh desert conditions, such as feed shortage and high ambient temperatures and have the ability to produce a considerable amount of meat, wool, and milk under these conditions [4]. However, the newborn lambs suffer from the starvation because of the scrawny milk production of their dams. This problem augments lamb mortality particularly in the early ages. Hence, there is a great interest in understanding the genomic architecture of growth and milk traits of these animals in order to improve both meat and milk characteristics. Improvement programs depending on genetic information should be established aiming to facilitate the selection of breeding animals, which will actually improve these important traits. In various sheep breeds, researchers have genotyped such molecular markers in order to study the association of candidate genes and milk performance traits (e.g., some milk traits in Sfakia sheep [5] and carcass traits in Iranian Mehraban sheep [6]). Highly important candidate genes, which showed association with milk and growth traits, are prolactin (*PRL*), leptin (*LEP*), insulin-like growth factor hormone 1 (*IGF1*), diacylglycerol O-acyltransferase 1 (*DGAT1*), signal transducer and activator of transcription 5 (*STAT5*), alpha (α)s2-casein (*CSN1S2*), growth hormone receptor (*GHR*), and growth hormone-releasing hormone receptor (*GHRHR*) [7–9]. Prolactin

is essential for lactation and plays an important role in milk production [10]. Polymorphisms in PRL can be used as a candidate marker associated with milk yield and milk composition traits [7]. Leptin is a non-glycosylated protein, which plays an important role in animal growth and metabolism. It regulates feed intake, energy metabolism, and fat distribution in the body [11]. It has been shown that leptin influences milk performance in cattle [12] and in Murrah buffaloes [13]. As a member of the *IGF* family, *IGF1* is considered an important factor associated with cell differentiation, embryogenesis, metabolism [14, 15], reproduction, and fetal development [16, 17]. Therefore, it is a major candidate gene for most of the productive and economic traits in sheep. *DGAT* plays an important role in triacylglycerol biosynthesis as well as milk and growth traits [18]. The *STAT* family has seven members (*STAT1-4, STAT5A, STAT5B*, and *STAT6*), and *STAT5* is known to play a central role in signal transduction from prolactin to milk protein genes [19]. Caseins represent about 80% of proteins in ruminant milk [20]. The casein family consists of four genes as a cluster (alpha (α)s1-casein, alpha (α)s2-casein, beta (β)-casein, and kappa (κ)-casein) [21,22]. *CSN1S2* alleles are associated with a normal (α) s2-casein synthesis level [23]. The growth hormone receptor initiates several signaling processes regulating body growth. As such, *GHR* and *GHRHR* are valuable candidate genes. Polymorphisms in *GHR* are associated with traits related to growth performance, body size, and meat quality in cattle [24–27]. The genetic analysis of the Barki sheep has so far been limited mainly to the assessment of some candidate genes for wool traits [28]. Concerning growth characteristics, there are only analyses available which examine the effects of FABP4 and calpastatin on some carcass traits of Barki lambs [29]. Heritabilities of some growth traits in Barki sheep are 0.19, 0.20, and 0.18 for birth weight, weaning weight, and average daily gain, respectively [30]. Therefore, the current study aims to identify segregating polymorphisms of major candidate genes for growth and milk traits in a Barki population of ewes and lambs. Subsequently, a SNP trait association analysis was performed to investigate the connection between genotype and production phenotypes as a prerequisite to improve performance parameters in Barki sheep.

2. Materials and Methods

2.1. *Animals and Management*

Samples and data were collected from the farm of the Matrouh Resources Project (location 1) and the Maryout research station (location 2) that belongs to the Desert Research Centre (DRC), Ministry of Agriculture, Egypt. The experiment was carried out according to all ethics and animal rights (DRC) considering all regulations in conformity with the European Union Directive for the protection of experimental animals (2010/63/EU).

Phenotypic and genotypic data from 111 Barki ewes and 140 of their lambs (44 ewes and 66 lambs from location 1 and 67 ewes and 74 lambs from location 2) from one breeding season were used in

this study. Ewes were randomly mated with certain rams of the same breed according to the normal farm practice. No records were available for the rams. Animals were kept under an intensive production system and were housed in semi-open yards throughout the experimental period. The lambs were kept all day with their dams for suckling until weaning at three months of age, respecting the natural ewe-lamb relationship. Some of the examined lambs are not the offspring of the ewes under study. Furthermore, some lambs have no data for their mother's milk. Ewes in the two locations were daily fed on a feed concentrate (0.75 kg), and clover hay (0.5kg) per head during the experimental period, and lambs were daily fed only on their dam's milk from birth to weaning age at 3 months of age. Fresh water was available to sheep ad libitum.

2.2. *Phenotypic Data*

Live body weight for each lamb was recorded at birth and weaning by electronic balance. Average daily gain was calculated for every lamb. Milk yield was recorded biweekly from the time of parturition till weaning using hand milking technique. Lambs were separated from their dams 12 hour before milking. The ewe was milked in the morning; another milking was carried out in the evening by the same approach. Milk yield per day was measured in milliliter by summation the morning and evening milking. Total milk yield was calculated by summation of the daily milk yields for 90 days. Milk samples were stored at -20°C and chemically analyzed to determine the percentages of fat, protein, lactose and total solids using milko-scan (130 A/SN. Foss Electric, Denmark).

2.3. *Blood samples and DNA Extraction*

Blood samples were collected from the jugular vein using test tubes containing disodium ethylene diamine tetra acetic acid (EDTA-Na2). The whole blood samples were stored at -80°C until DNA extraction. DNA was extracted by using a commercially available kit according to manufacturer's instructions (G-spin™ Total DNA Extraction kit; intron Biotechnology, Korea).

2.4. *Detection of Polymorphisms and Genotyping*

Pooled DNA samples were prepared from 5 lamb samples and 5 ewes samples from different locations. The pooled samples were subjected to a polymerase chain reaction (PCR) to amplify specific regions of the candidate genes and to identify segregating SNPs. The PCR assay was performed using respective primer sets in a total volume of 20µl according to the manufacture instructions (SupraTherm Taq). Gene-specific primers were designed with Primer3 software (v.0.4.0) (http://bioinfo.ut.ee/primer3-0.4.0/) according to the latest sheep genome information (Ensembl Oar_v3.1, Build v96). The Primer pairs used to detect SNPs are shown in Table (1). PCR products were separated on a 2% agarose gel and visualized under UV light. For all SNPs investigated, primer pair combinations resulting in only one specific amplification signal were selected. The PCR products were purified using beads purification

method (Agencourt AMPure XP, Beckman Coulter) and sequenced on an ABI 3500 Genetic analyzer (Applied Biosystems, Foster City, USA). Sequencing results were aligned and the SNPs were detected using Bio Edit software (V 7.0.5.3). Subsequently, all animals with phenotypic data were genotyped by Kompetitive Allele Specific PCR (KASP, LGC Genomics, Teddington, Middlesex, UK). KASP assays were developed for corresponding SNPs, validated in a subset of samples and applied to the entire sample set. The PCR mixture consisted of 10 µL according to the manufacture instructions. The PCR products were amplified and analyzed using the Light Cycler 480 machine (Roche, Mannheim, Germany) to identify genotype clusters.

2.5. *Statistical analysis*

The Hardy–Weinberg equilibrium (HWE), polymorphic information content (PIC), heterozygosity (He) and homozygosity (Ho) were tested for all alleles by using Cervus (V3.0.7.0) program [31]. The association analysis between the SNPs of the candidate genes and phenotypes of the studied sheep traits was carried out using the general linear model (GLM) of the analysis of variance (ANOVA) by SPSS V20 (IBM, New York, NY, USA). The statistical model for ewes milk traits used was as follows: $Y_{ijk}= \mu + G_i+ L_j+ e_{ijk}$, where Y_{ijk} is the analyzed trait, μ is the overall mean, G_i is the effect of genotype (i=3 levels, except rs409119650 of *DGAT1* gene where i=2 levels), L_j is the effect of location (j=2 levels) and e_{ijk} is the error effect. Another model was used to detect the effect of genotype on the lamb growth traits as follows: $Y_{ijkl}= \mu + G_i+ L_j+ S_K+ e_{ijkl}$, where Y_{ijkl} is the analyzed trait, μ is the overall mean, G_i is the effect of genotype (i=3 levels), L_j is the effect of location (j=2 levels), S_k is the effect of lamb sex (k= 2 levels) and e_{ijkl} is the error effect. $P < 0.05$ was considered significant. $P < 0.1$ was considered a tendency for significance.

Table 1. Primer pairs of the studied candidate genes

Gene name	Gene ID	Allele[2]	Primer sequence	Product size(bp)	Annealing temperature(°C)
LEP[1]	ENSOARG00000002407	T/G(V181L)	F:AGGAAGCACCTCTACACTC R:CTTCAAGGCTTCAGCACC	471	53
IGF1	ENSOARG00000015856	G/A	F:GTTCTGGAATGGCAGGTTTG R:GCCACTGTCTTTGGATTTTCTC	570	60
DGAT1	ENSOARG00000014070	T/C	F:ACTGTGCTTCAGGGTGTCGG R:GAGTGATGGACTCTAGGAGGAAGG	429	60
PRL	ENSOARG00000009137	A/G	F:TGGAATTTAGATGACAAGCAACTG R:AATTGGTGGCTCAAGTGGTG	745	63
CSN1S2	ENSOARG00000010683	A/C	F:CCCTGAAGGAATCTGCTGAAG R:AGCCAAGCAAAATGATATAGAAGC	855	63
GHR	ENSOARG00000008837	C/T(P448S)	F:TGATGACCCTGATGAGAAGACTG R:TTTTGTTCAGTTGGTCTGTGCTC	857	63
GHRHR	ENSOARG00000007636	C/T	F:TTGTTCTTGGAGGTGAGGACTG R:AACACGGGTGGCTCTCTTG	759	63

STAT5A	ENSOARG00000000809	G/A	F:GGGTGCATACAGGACAGTGC R:CCAGTCTCTGGCTTTCCCAA	446	60

[1] primer design according to [32].

[2] If present, consequences at the protein level are shown.

3. Results

3.1. Phenotypic Data of Growth and Milk Traits

Descriptive statistics of total milk yield in three months and percentages of milk components including fat, protein, lactose, and total solids are shown in Table 2. An overview of growth traits comprising birth weight, weaning weight, and average daily gain of Barki lamb is shown in Table 3.

Table 2. Descriptive statistics of ewe milk traits.

Trait	N	Mean	Standard Deviation	Minimum	Maximum
Total Milk Yield (kg)	111	28.95	12.64	9.90	77.40
Fat (%)	111	4.30	1.74	1.00	9.60
Protein (%)	111	5.11	1.35	2.70	9.50
Lactose (%)	111	6.34	1.42	0.81	9.90
Total Solids (%)	111	18.72	5.38	12.14	34.70

Table 3. Descriptive statistics of lamb growth traits.

Trait	N	Mean	Standard Deviation	Minimum	Maximum
Birth Weight (kg)	140	3.71	0.58	2.42	5.04
Weaning Weight (kg)	140	13.83	3.89	5.15	28.80
Average Daily Gain (kg/day)	140	0.112	0.04	0.02	0.27

3.2. Genetic Parameters

Sequencing results of DNA pooled samples from ewes and lambs revealed segregating SNPs in the studied genes. One SNP was selected from every gene for subsequent genotyping. The selected SNPs were rs420693815 in exon 3 of *LEP*, rs400398060 in exon 3 of *IGF1*, rs409119650 in exon 9 of *DGAT*1, rs161082816 in exon 11 of *STAT5A*, rs422713690 in exon 3 of *PRL*, rs420391387 in exon 8 of *CSN1S2*, rs413776054 in exon 10 of *GHR*, and rs414991449 in exon 13 of *GHRHR*. Results of Hardy–Weinberg equilibrium for all selected SNPs are shown in Table 4. All selected SNPs were in Hardy–Weinberg equilibrium status ($p > 0.05$). rs409119650 and rs161082816 were in low polymorphic information content status (PIC < 0.25), while rs420693815, rs400398060, rs422713690, rs420391387, rs413776054, and rs414991449 were in moderate polymorphic information content status (0.25 < PIC < 0.50). The homozygosity of all loci sites was higher than the heterozygosity, except for rs420391387 and rs422713690, where the homozygosity was equal to the heterozygosity. In lambs, the selected

SNPs for all candidate genes were in Hardy–Weinberg equilibrium status ($p > 0.05$; Table 5). rs409119650 and rs413776054 were in low polymorphic information content status (PIC < 0.25), while rs420693815, rs400398060, rs161082816, rs422713690, rs420391387, and rs414991449 were in moderate polymorphic information content status (0.25 < PIC < 0.50). The homozygosity of all loci was higher than the heterozygosity.

Table 4. Genetic parameters of the SNP markers of the studied candidate genes in the Barki ewe population.

Gene	SNP locus	Genotype	Genotypic frequency	Allele	Allelic frequency	He	Ho	PIC	HWE test (*p* Value)
LEP	rs420693815	TT	0.15	T	0.41	0.48	0.52	0.37	0.35
		GT	0.53	G	0.59				
		GG	0.32						
IGF1	rs400398060	GG	0.47	G	0.69	0.43	0.57	0.34	0.93
		AG	0.43	A	0.31				
		AA	0.10						
DGAT1	rs409119650	TT	0.00	T	0.10	0.18	0.82	0.16	0.49
		CT	0.19	C	0.90				
		CC	0.81						
STAT5A	rs161082816	GG	0.03	G	0.14	0.24	0.76	0.21	0.34
		AG	0.22	A	0.86				
		AA	0.75						
PRL	rs422713690	GG	0.31	G	0.53	0.50	0.50	0.37	0.26
		AG	0.44	A	0.47				
		AA	0.25						
CSN1S2	rs420391387	CC	0.22	C	0.50	0.50	0.50	0.38	0.20
		AC	0.56	A	0.50				
		AA	0.22						
GHR	rs413776054	TT	0.05	T	0.21	0.33	0.67	0.28	0.70
		CT	0.32	C	0.79				
		CC	0.63						
GHRHR	rs414991449	TT	0.43	T	0.66	0.45	0.55	0.35	0.39
		CT	0.47	C	0.34				
		CC	0.10						

Table 5. Genetic parameters describing the SNP markers of the investigated candidate genes in the Barki lamb population.

Gene	SNP locus	Genotype	Genotypic frequency	Allele	Allelic frequency	He	Ho	PIC	HWE test (*p* value)
LEP	rs420693815	TT	0.34	T	0.56	0.49	0.51	0.37	0.13
		GT	0.43	G	0.44				
		GG	0.23						
IGF1	rs400398060	GG	0.51	G	0.70	0.42	0.58	0.33	0.54
		AG	0.39	A	0.30				
		AA	0.10						
DGAT1	rs409119650	TT	0.01	T	0.07	0.13	0.87	0.12	0.68
		CT	0.13	C	0.93				
		CC	0.86						
STAT5A	rs161082816	GG	0.04	G	0.22	0.34	0.66	0.28	0.44
		AG	0.37	A	0.78				
		AA	0.59						
PRL	rs422713690	GG	0.39	G	0.61	0.48	0.52	0.36	0.56
		AG	0.45	A	0.39				
		AA	0.16						
CSN1S2	rs420391387	CC	0.38	C	0.60	0.48	0.52	0.37	0.36
		AC	0.44	A	0.40				
		AA	0.18						
GHR	rs413776054	TT	0.01	T	0.17	0.28	0.72	0.24	0.13
		CT	0.33	C	0.83				
		CC	0.66						
GHRHR	rs414991449	TT	0.49	T	0.70	0.42	0.58	0.33	0.74
		CT	0.43	C	0.30				
		CC	0.08						

He Heterozygosity, **Ho** Homozygosity, **PIC** polymorphic information content, **HWE** Hardy-Weinberg Equilibrium

3.3. Association of SNPs with Milk Traits of Barki Ewes

Table 6 shows the results of the association analysis of SNP with milk traits of ewes. rs420693815 of *LEP* showed a trend for milk yield and fat percentage ($p \leq 0.1$). Ewes with TT genotype had a higher milk yield than ewes with GT and GG genotypes. Ewes carrying the GT genotype had a higher fat percentage than ewes with GG and TT genotypes. For rs161082816 of *STAT5A*, the milk of ewes with AA genotype were significantly ($p \leq 0.05$) higher in lactose percentage compared to ewes with GG and AG genotypes. For rs422713690 of *PRL*, a significant association with milk yield ($p \leq 0.1$) was observed. Ewes with GG genotypes had a higher milk yield than ewes with AG and AA genotypes. rs414991449 of *GHRHR* was significantly associated with total solids percentage ($p \leq 0.05$) and with protein percentage ($p \leq 0.1$). Ewes having TT genotypes had higher total solids and protein percentages than ewes with CT and CC genotypes.

Table 6. Association of SNPs of studied genes with milk traits in Barki ewes (Mean ± SE).

Gene	SNP locus	Genotype	Milk Yield (kg)	Fat %	Protein %	Lactose %	Total Solids %
LEP	rs420693815	TT(15)	35.55±4.18	3.81±0.32	5.33±0.30	6.73±0.24	19.17±1.27
		GT(55)	26.67±1.27	4.56±0.26	5.24±0.20	6.36±0.22	19.05±0.79
		GG(33)	29.77±2.66	3.92±0.20	4.67±0.17	6.23±0.17	17.24±0.66
		p Value	0.053	0.085	0.105	0.397	0.185
IGF1	rs400398060	GG(48)	29.59±1.93	4.22±0.22	5.06±0.19	6.61±0.19	18.93±0.76
		AG(44)	28.46±2.07	4.22±0.26	5.14±0.20	6.22±0.21	18.39±0.76
		AA(10)	28.08±1.83	3.90±0.39	4.35±0.25	5.71±0.18	14.56±0.46
		p Value	0.940	0.969	0.467	0.583	0.572
DGAT1	rs409119650	TT(0)	--	--	--	--	--
		CT(20)	26.25±2.39	4.44±0.28	4.94±0.30	6.18±0.33	18.43±1.05
		CC(84)	29.56±1.45	4.20±0.19	5.04±0.14	6.38±0.14	18.26±0.56
		p Value	0.287	0.607	0.643	0.282	0.807
STAT5A	rs161082816	GG(3)	34.97±4.02	3.45±0.55	4.84±0.81	4.19±1.30	19.28±3.41
		AG(21)	30.39±2.83	3.84±0.31	4.58±0.22	6.38±0.23	17.01±0.86
		AA(73)	28.62±1.52	4.37±0.19	5.21±0.16	6.52±0.15	18.98±0.60
		p Value	0.638	0.297	0.149	0.001	0.199
PRL	rs422713690	GG(33)	32.59±2.98	4.21±0.24	5.09±0.23	6.61±0.22	18.38±0.92
		AG(48)	25.90±1.23	3.95±0.24	4.93±0.18	6.20±0.20	18.02±0.71
		AA(27)	30.62±2.14	4.79±0.35	5.40±0.28	6.18±0.30	19.96±1.07
		p Value	0.052	0.125	0.411	0.152	0.322
CSN1S2	rs420391387	CC(23)	31.44±3.57	4.02±0.32	5.05±0.22	6.31±0.25	17.56±0.72
		AC(58)	28.61±1.37	4.53±0.25	5.12±0.18	6.34±0.19	19.56±0.71
		AA(22)	25.33±2.01	4.06±0.30	5.09±0.34	6.27±0.31	17.47±1.20
		p Value	0.264	0.562	0.886	0.578	0.637
GHR	rs413776054	TT(5)	20.72±2.77	4.93±0.78	5.31±0.37	6.51±0.47	19.52±2.53
		CT(32)	31.10±2.57	4.25±0.25	5.12±0.21	5.98±0.23	18.03±0.86
		CC(64)	29.07±1.50	4.20±0.22	5.09±0.19	6.49±0.18	19.05±0.68
		p Value	0.249	0.556	0.791	0.450	0.667
GHRHR	rs414991449	TT(43)	28.61±1.95	4.49±0.26	5.32±0.22	6.17±0.23	19.26±0.84
		CT(48)	29.71±1.90	4.09±0.25	4.96±0.19	6.57±0.19	18.71±0.72
		CC(10)	26.44±1.86	4.39±0.35	5.12±0.44	5.91±0.44	16.76±1.74
		p Value	0.794	0.215	0.081	0.612	0.035

3.4. Association of SNPs with Growth Traits of Barki Lambs

The results of the association analysis of SNPs with lamb growth traits are summarized in Table 7. The analysis revealed rs420693815 of *LEP* as significantly associated with weaning weight and average daily gain ($p \leq 0.1$). Lambs with GT and GG genotypes had a higher weaning weight and average daily gain than lambs with TT genotype. The other selected SNPs in the candidate genes showed a non-significant association with growth traits ($p \geq 0.1$).

Table 7. Association of SNPs of studied genes with growth traits in Barki lambs (Mean ± SE).

Gene	SNP locus	Genotype	Birth Weight (kg)	Weaning Weight (kg)	Average Daily Gain (g)
LEP	rs420693815	TT(46)	3.67±0.09	13.25±0.60	106.0±6.0
		GT(58)	3.75±0.07	14.40±0.52	118.0±5.0
		GG(31)	3.82±0.10	14.32±0.61	117.0±6.0
		p Value	0.589	0.075	0.076
IGF1	rs400398060	GG(67)	3.75±0.06	14.35±0.42	118.0±4.0
		AG(52)	3.73±0.09	13.74±0.60	111.0±6.0
		AA(13)	3.63±0.18	12.06±0.92	93.0±10.0
		p Value	0.442	0.354	0.416
DGAT1	rs409119650	TT(1)[@]	4.29	14.00	108.0
		CT(17)	3.86±0.14	14.92±0.62	123.0±7.0
		CC(114)	3.71±0.05	13.73±0.38	111.0±4.0
		p Value	0.519	0.170	0.163
STAT5A	rs161082816	GG(4)	3.15±0.28	11.23±1.76	85.8±19.0
		AG(41)	3.81±0.11	14.67±0.67	120.7±7.0
		AA(66)	3.71±0.07	13.86±0.42	112.8±4.0
		p Value	0.631	0.255	0.273
PRL	rs422713690	GG(48)	3.75±0.07	13.52±0.45	109.0±9.0
		AG(53)	3.74±0.09	14.31±0.59	117.0±6.0
		AA(19)	3.70±0.11	14.16±0.82	116.0±5.0
		p Value	0.965	0.593	0.567
CSN1S2	rs420391387	CC(35)	3.79±0.12	15.07±0.75	125.3±8.0
		AC(40)	3.86±0.08	14.34±0.48	116.5±5.0
		AA(17)	3.72±0.13	14.17±0.63	116.1±7.0
		p Value	0.939	0.335	0.278
GHR	rs413776054	TT(1)[@]	3.5	20	183.3
		CT(35)	3.74±0.11	14.77±0.49	122.5±5.0
		CC(70)	3.76±0.07	14.31±0.49	117.3±5.0
		p Value	0.608	0.446	0.309
GHRHR	rs414991449	TT(49)	3.72±0.08	14.83±0.58	123.4±6.0
		CT(43)	3.79±0.11	13.91±0.58	112.4±6.0
		CC(8)	3.73±0.22	14.92±0.81	124.4±9.0
		p Value	0.856	0.146	0.107

[@] Only one individual of the population carries the TT genotype.

4. Discussion

In this study, a representative SNP of each of the selected functional candidate genes was associated with growth and milk production traits obtained from Barki lambs and ewes. Genotyping results showed that none of the selected SNPs deviates from HWE. These results indicated for the absence of strong selection pressures, probably due to the coherent housing environment and the lack of artificial selection. These facts might contribute to a stability of allelic and genotypic frequency for a long time. Results of the polymorphic information content state and homozygosity to heterozygosity relationships confirmed that an inbreeding scheme was applied at the different locations creating a high genetic variation between populations and lower genetic variation between individuals in the same population. These results suggest that an application of selection employing genomic information will be effective in the respective population. However, the relatively low sample size, due to the lack of management with breeding programs and routine sampling in the Barki sheep,

represents a certain limitation for the genetic evaluation in this study. The results of association of SNPs with lamb growth traits showed that rs420693815 of *LEP* were significantly associated with weaning weight and average daily gain. Interestingly, rs420693815 had also a significant effect on milk traits comprising milk yield and fat percentage in the Barki ewes. The results indicated the inverse relationship between milk yield and fat percentage. Ewes with the highest milk yield had the lowest fat percentage [33]. Accordingly, lambs whose mothers had the highest fat content in their milk had a higher weaning weight and a higher average daily gain. LEP is considered as one of the candidate genes affecting body fat content [11]. Through signaling to the hypothalamus, leptin mediates the balance between feed intake and energy expenditure [34, 35]. Due to its lipolytic effect and the regulation of fat stores, genetic variants of *LEP* might be of relevance in mobilizing lipids for, e.g., milk production with possible implication on the offspring's body weight. In agreement, genetic variants of leptin have been shown to influence milk performance in cattle [36]. A *LEP* polymorphism was found to be significantly associated with milk yield in Najdi ewes of Saudi Arabia [37]. Moreover, several studies indicated the role of *LEP* in growth traits [38, 39]. With respect to the results of the current study, it is questionable if *LEP* (rs420693815) is causative for the effects or acts as tagging SNP in linkage disequilibrium with the causal one. However, *LEP* as candidate gene might be further considered as a locus for improving performance and production traits in the breeding programs of Barki sheep. Furthermore, analyzed SNPs in *STAT5A, PRL,* and *GHRHR* revealed a significant association with milk production traits in the Barki ewes. For *PRL* (rs422713690), animals with the heterozygous AG genotype showed lower milk yields than homozygous animals. *PRL* is a hormone released from the anterior pituitary gland and acts to initiate and maintain lactation [40]. The *PRL* gene is located on the ovine chromosome 20 where putative quantitative trait loci for milk yield, fat, and protein percentage are located [41, 42]. Indeed, a polymorphism in *PRL* has been shown to affect all these traits in Serra da Estrella sheep [43] and milk yield in East Friesian sheep [8]. Consequently, *PRL* might act as a marker gene for milk production traits also in the Barki sheep. Regarding the investigated SNP in *STAT5A* (rs161082816), Barki ewes which carried A alleles showed higher milk lactose percentages compared to animals exhibiting G alleles. *STAT5A* is a key player in mammary gland development [44]. In particular, *STAT5A* is known to mediate *PRL* and *GH* signals via transcriptional stimulation of gene expression in milk-secreting mammary epithelial cells. Due to its prominent role in milk production traits, *STAT5A* has been previously investigated in cattle and goat and genetic variants have been associated with milk fatty acid profiles and milk yield [45–47]. The significant association with milk lactose percentage emphasizes *STAT5A* as a promising candidate gene for further analyses of milk traits in Barki sheep. The SNP located in *GHRHR* (rs414991449) was significantly associated with the percentages of total milk solids and milk protein, whereby the appearance of the T allele prompted the highest values. In fact, *GHRHR* mediates effects of its ligand growth hormone-releasing hormone

(*GHRH*) to regulate growth hormone (*GH*) synthesis and secretion [48–50]. Genetic variants in the functional candidate *GHRHR* might therefore impact on *GH* axis signaling as it has been shown for body growth in humans and mice [51, 52]. In studies on sheep, the *GH* locus has been associated with milk traits such as milk fat percentage and milk yield [53, 54]. Corresponding effects might be mediated via *GHRHR* on *GH* signaling affecting milk production and composition. Results did not support any significant associations of sequence variants of *IGF1, DGAT1, CSN1S2*, and *GHR* with growth or milk traits in the studied Barki population. This might also be related to the fact that for some of the SNPs, a low representation of alternative homozygotes was found in the studied population. However, associations of segregating SNPs in *IGF1* and *DGAT1* with growth, milk, and wool performance traits have been described in various sheep breeds such as Makeoi, Baluchi, Hu, Sarda, and Mehraban [55–62]. These breed differences might be due to artificial selection pressures or housing due to geographical conditions. Clearly, comprehensive approaches including a holistic genomic evaluation are needed to elucidate the genetics and to improve milk and performance traits of Barki sheep.

5. Conclusions

A SNP-trait association analysis was performed to study the effect of genotype on growth and milk performance traits in Egyptian Barki sheep. Results concluded that the selected polymorphisms in *LEP, STAT5A, PRL*, and *GHRHR* were significantly associated with lamb growth and ewe milk traits, while *IGF1, DGAT1, CSN1S2*, and *GHR* genes showed no significant associations. *LEP, STAT5A, PRL*, and *GHRHR* might be considered as interesting candidate genes for further investigations to improve growth and milk performance in the Barki sheep.

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References

1. Sallam, A.M.; Galal, S.; Rashed, M.A.; Alsheikh, S.M. Genetic diversity in Barki sheep breed in its native tract in Egypt. *Egyp. J. Anim. Prod.* **2012**, 49, 19–28.
2. Galal, S.; Abdel-Rasoul, F.; Anous, M.R.; Shaat, I. On-Station Characterization of Small Ruminant Breeds in Egypt. In Characterization of Small Ruminant Breeds in West Asia and North Africa; *Iniguez, L.C., Ed.; ICARDA: Aleppo, Syria*, 2005; Volume 2, pp. 141–193.
3. FAOSTAT. Available online: http://www.fao.org/faostat/ar/#data/QA (accessed on 25 September 2019).
4. El-Wakil, S.I.; Shemeis, A.R.; Ahmed, A.M.; Abdallah, O.Y. Genetic and phenotypic relationships involving body weight, degree of maturity and measurer of gain rate of Barki sheep without having recourse to fitting growth curves. *J. Agric.Sci. Mansoura Univ.* **2008**, 33, 4835–4848.
5. Ibrahim, A.H.M.; Tzanidakis, N.; Sotiraki, S.; Zhou, H.; Hickford, J.G.H. Identification of the association between FABP4 gene polymorphisms and milk production traits in Sfakia sheep. *Arch. Anim. Breed.* **2019**, 62, 413–422.
6. Behzadi, S.; Miraei-Ashtiani, S.R.; Sadeghi, M.; Zamani, P.; Abdoli, R. Association of IGF- I gene polymorphisms with carcass traits in Iranian Mehraban sheep using SSCP analysis. *Iran. J. Appl. Anim. Sci.* **2015**, 5, 121–126.
7. Orford, M.; Tzamaloukas, O.; Papachristoforou, C.; Miltiadou, D. Technical note: A simplified PCR based assay for the characterization of two prolactin variants that affect milk traits in sheep breeds. *J. Dairy. Sci.* **2010**, 93, 5996–5999.
8. Moioli, B.; D'Andrea, M.; Pilla, F. Candidate genes a_ecting sheep and goat milk quality. *Small Ruminant Res.* **2007**, 68, 179–192.
9. Staiger, E.A.; Thonney, M.L.; Buchanan, J.W.; Rogers, E.R.; Oltenacu, P.A.; Mateescu, R.G. Effect of prolactin _-lactoglobulin and _-casein genotype on milk yield in East Friesian sheep. *J. Dairy. Sci.* **2010**, 93, 1736–1742
10. Knight, C.H. Overview of prolactin's role in farm animal lactation. Livest. *Prod. Sci.* **2001**, 70, 87–93.
11. Choudhary, V.; Kumar, P.; Bhattacharya, T.K.; Bhushan, B.; Sharma, A. DNA polymorphism of leptin gene in Bos indicus and Bos Taurus cattle. *Genet. Mol. Biol.* **2005**, 28, 740–742.
12. Nassiry, M.R.; Heravi, M.A.; Alashawkany, A.R.; Ghovati, S. Leptin Gene Polymorphism in Iranian Native Golpayegani and Taleshi Cows. Pak. *J. Biol. Sci.* **2007**, 10, 3738–3741.
13. Jamuna, V.; Gupta, A.K.; Chakravarty, A.K.; Singh, A.; Patil, C.S.; Kumar, M.; Vohra, V. Leptin gene polymorphism in association with Lactation milk yield in Murrah Bu_aloes. *Indian J Anim Sci.* **2016**, 86, 95–97.
14. Estany, J. Association of CA repeat polymorphism at intron 1 of insulin-like growth factor (IGF-I) gene with circulating IGF-I concentration, growth, and fatness in swine. *Physiol. Genomics* **2007**, 31, 236–243.
15. Honarvar, M. Study of polymorphisms in the 5 flanking region of the ovine IGF-I gene in zel sheep. *World Appl Sci J.* **2012**, 16, 726–728.
16. Adam, C.L.; Gadd, T.S.; Findlay, P.A.; Wathes, D.C. IGF-I stimulation of luteinizing hormone secretion, IGF-binding proteins (IGFBPs) and expression of mRNAs for IGFs, IGF receptors and IGFBPs in the ovine pituitary gland. *J. Endocrinol.* **2000**, 166, 247–254.
17. Shen, W.; Wisniowski, P.; Ahmed, L.; Boyle, D.W.; Denne, S.C. Protein anabolic effects of insulin and IGF-I in the ovine fetus. *Am. J.Physiol. Endocrinol. Metab.* **2003**, 284, 48–56.

18. Chen, H.C.; Smith, S.J.; Ladha, Z.; Jensen, D.R.; Ferreira, L.D.; Pulawa, L.K. Increased insulin and leptin sensitivity in mice lacking acyl CoA:diacylglycerol acyltransferase 1. *J. Clin. Investig.* **2002**, 109, 1049–1055.
19. Bole-Feysot, C.; Goffn, V.; Edery, M.; Binart, N.; Kelly, P.A. Prolactin (PRL) and its receptor: Actions signal transduction pathways and phenotypes observed in PRL receptor knockout mice. *Endocr. Rev.* **1998**, 19, 225–268.
20. Haug, A.; Høstmark, A.T.; Harstad, O.M. Bovine milk in human nutrition—a review. *Lipids Health Dis.* **2007**, 6, 1–16.
21. Caroli, A.; Chiatti, F.; Chessa, S.; Rignanese, D.; Bolla, P.; Pagnacco, G. Focusing on the goat casein complex. *J. Dairy Sci.* **2006**, 89, 3178–3187.
22. Selvaggi, M.; Laudadio, V.; Dario, C.; Tufarelli, V. Major proteins in goat milk: An updated overview on genetic variability. *Mol. Biol. Rep.* **2014**, 41, 1035–1048.
23. Ramunno, L.; Cosenza, G.; Pappalardo, M.; Longobardi, E.; Gallo, D.; Pastore, N.; Di Gregorio, P.; Rando, A. Characterization of two new alleles at the goat CSN1S2 locus. *Anim. Genet.* **2001**, 32, 264–268.
24. Garrett, A.; Rincon, G.; Medrano, J.; Elzo, M.; Silver, G.; Thomas, M. Promoter region of the bovine growth hormone receptor gene: Single nucleotide polymorphism discovery in cattle and association with performance in Brangus bulls. *J. Anim. Sci.* **2008**, 86, 3315–3323
25. Waters, S.; McCabe, M.; Howard, D.; Giblin, L.; Magee, D.; MacHugh, D.; Berry, D. Associations between newly discovered polymorphisms in the Bos Taurus growth hormone receptor gene and performance traits in Holstein–Friesian dairy cattle. *Anim. Genet.* **2001**, 42, 39–49.
26. Di Stasio, L.; Destefanis, G.; Brugiapaglia, A.; Albera, A.; Rolando, A. polymorphism of the GHR gene in cattle and relationships with meat production and quality. *Anim. Genet.* **2005**, 36, 138–140.
27. Reardon, W.; Mullen, A.; Sweeney, T.; Hamill, R. Association of polymorphisms in candidate genes with colour, water-holding capacity, and composition traits in bovine M. longissimus and M. semimembranosus. *Meat. Sci.* **2010**, 86, 270–275.
28. Ibrahim, A.H.M.; El-Betar, E.M. Effect of variation in the adrenergic receptor beta 3 (adrb3) gene on wool traits in Barki sheep. *Egypt. J. Genet. Cytol*. **2015**, 44, 61–73.
29. Shehata, M.F.; Ismail, I.M.; Ibrahim, A.H.M. Variation in exon 10 of the ovine calpain3 gene and its association with growth and carcass traits in Egyptian Barki lambs. *Egypt. J. Genet. Cytol.* **2014**, 43, 231–240.
30. Sallam, A.M.; Ibrahim, A.H.; Alsheikh, S.M. Estimation of genetic parameters and variance components of pre-weaning growth traits in Barki lambs. *Small Ruminant Res.* **2019**, 173, 94–100.
31. Kalinowski, S.T.; Taper, M.L.; Marshall, T.C. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol Ecol.* **2007**, 5, 1099–1106.
32. Hashemi, A.; Mardani, K.; Farhadian, M.; Ashrafi, I.; Ranjbari, M. Allelic polymorphism of Makoei sheep leptin gene Identified by polymerase chain reaction and single Strand conformation Polymorphism. *Afr. J. Biotechnol*. **2011**, 10, 17903–17906.
33. Oravcová, M.; Margetín, M.; Peskovicová, D.; Dano, J.; Milerski, M.; Hetenyi, L.J.; Polak, P. Factors affecting ewe's milk fat and protein content and relationships between milk yield and milk components. *Czech J.Anim. Sci*. **2007**, 52, 189–198.
34. Zhou, H.; Hickford, J.G.H.; Gong, H. Identification of Allelic Polymorphism in the Ovine Leptin Gene. *Mol. Biotechnol*. **2009**, 41, 22–25.

35. Friedman, J.M.; Halaas, J.L. Leptin and the regulation of body weight in mammals. *Nature* **1998**, 395, 763–770.
36. Almeida, S.E.; Allmeida, E.A.; Moraes, J.C.F.;Weimer, T. Molecular marker in the LEP gene and reproductive performance of beef cattle. *J Anim Breed Genet*. **2003**, 120, 106–113.
37. Mahmoud, A.; Saleh, A.; Almealamah, N.; Ayadi, M.; Matar, A.; Abou- Tarboush, F.; Aljumaah, R.; Abouheif, M. Polymorphism of leptin gene and its association with milk traits in Najdi sheep. *J Appl Microbiol*. **2014**, 8, 2953–2959.
38. Yang, D.; Chen, H.; Wang, X.; Tian, Z.; Tang, L.; Zhang, Z. Association of polymorphisms of leptin gene with body weight and body sizes indexes in Chinese indigenous cattle. *J Genet Genomics.* **2007**, 34, 400–405.
39. Tahmoorespur, M.; Taheri, A.; Valeh, M.V.; Saghi, D.A.; Ansary, M. Assessment relationship between leptin and ghrelin genes polymorphisms and estimated breeding values (EBVs) of growth traits in Baluchi sheep. *J. Anim. Vet. Adv.* **2010**, 9, 2460–2465.
40. Le Provost, F.; Leroux, C.; Martin, P.; Gaye, P.; Djiane, J. Prolactin Gene Expression in Ovine and Caprine Mammary Gland. *Neuroendocrinology.* **1994**, 60, 305–313.
41. Gutierrez-Gil, B.; El-Zarei, M.F.; Alvarez, L. Quantitative trait loci underlying milk production traits in sheep. *Anim. Genet.* **2009**, 40, 423–434.
42. Barillet, F.; Arranz, J.J.; Carta, A. Mapping quantitative trait loci for milk production and genetic polymorphisms of milk proteins in dairy sheep. *Genet. Sel. Evol.* **2005**, 37, 109–123.
43. Ramos, A.M.; Matos, C.A.P.; Russo-Almeida, P.A.; Bettencourt, C.M.V.; Matos, J.; Martins, A.; Pinheiro, C.; Rangel-Figueiredo, T. Candidate genes for milk production traits in Portuguese dairy sheep. *Small Ruminant Res*. **2009**, 82, 117–121.
44. Cui, Y.; Riedlinger, G.; Miyoshi, K.; Tang,W.; Li, C.; Deng, C.X.; Hennighausen, L. Inactivation of Stat5 in mouse mammary epithelium during pregnancy reveals distinct functions in cell proliferation, survival, and differentiation. *Mol. Cell. Biol.* **2004**, 24, 8037–8047.
45. Brym, P.; Kaminski, S.; Rusc, A. New SSCP polymorphism within bovine STAT5A gene and its associations with milk performance traits in Black-and-White and Jersey cattle. *J. Appl. Genet.* **2004**, 45, 445–452.
46. Schennink, A.; Bovenhuis, H.; Léon-Kloosterziel, K.M.; Van Arendonk, J.A.; Visker, M.H. Effect of polymorphisms in the FASN, OLR1, PPARGC1A, PRL and STAT5A genes on bovine milk-fat composition. *Anim. Genet*. **2009**, 40, 909–916.
47. An, X.P.; Hou, J.X.; Zhao, H.B.; Bai, L.; Peng, J.Y.; Zhu, C.M.; Yan, Q.M.; Song, Y.X.; Wang, J.G.; Cao, B.Y. Polymorphism identification in goat DGAT1 and STAT5A genes and association with milk production traits. *Czech J. Anim. Sci*. **2013**, 58, 321–327.
48. Mayo, K.E. Molecular cloning and expression of a pituitary-specific receptor for growth hormone-releasing hormone. *Mol. Endoc.* **1992**, 6, 1734–1744.
49. Pang, A.L.P.; Chan,W.Y. Molecular basis of diseases of the endocrine system. In Essential Concepts in Molecular Pathology; Coleman,W.B., Tsongalis, G.J., Eds.; *Academic Press: San Diego, CA, USA*, 2010; pp. 289–307.
50. Andrea, G.; Johannes, D.V. Pathophysiology of the Neuroregulation of Growth Hormone Secretion in Experimental Animals and the Human. *Endocr. Rev.* **1998**, 19, 717–797.
51. Godfrey, P.; Rahal, J.; Beamer,W.GHRHreceptor of little mice contains a missense mutation in the extracellular domain that disrupts receptor function. *Nat. Genet.* **1993**, 4, 227–232.
52. Gaylinn, B.D.; Harrison, J.K.; Zysk, J.R.; Lyons, C.E.; Lynch, K.R.; Thorner, M.O. Molecular cloning and expression of a human anterior pituitary receptor for growth hormone-releasing hormone. Mol. *Endocrinol.* **1993**, 7, 77–84.

53. Dettori, M.; Pazzola, M.; Pira, E.; Stocco, G.; Vacca, G. Association between the GHR, GHRHR and IGF1 gene polymorphisms and milk coagulation properties in Sarda sheep. *J. Dairy Res.* **2019**, 86, 331–336.
54. Vacca, G.M.; Dettori, M.L.; Balia, F.; Luridiana, S.; Mura, M.C.; Carcangiu, V. Sequence polymorphisms at the growth hormone GH1/GH2-N and GH2-Z gene copies and their relationship with dairy traits in domestic sheep (Ovis aries). *Mol. Biol. Rep.* **2013**, 40, 5285–5294.
55. Hajihosseinlo, A. E_ect of GH gene polymorphisms on biometric traits in Makeoi sheep. *Ann. Biol. Res*. **2013**, 4, 351–355.
56. Tahmoorespur, M.; Valeh, M.V.; Nassiry, M.R.; Moussavi, A.H.; Ansary, M. Association of the polymorphism in the 50 flanking region of the ovine IGF-I gene with growth traits in the Baluchi sheep. *S. Afr. J. Anim. Sci*. **2009**, 39, 97–101.
57. Negahdary, M.; Hajihosseinlo, A.; Ajdary, M. PCR-SSCP variation of IGF1 and PIT1 genes and their association with estimated breeding values of growth traits in Makeoi Sheep. *Genet. Res. Int.* **2013**, 6, 272346.
58. Gholibeikifard, A.; Aminafshar, M.; Hosseinpour, M.M. Polymorphism of IGF-I and ADRB3 Genes and Their Association with Growth Traits in the Iranian Baluchi Sheep. *J. Agric. Sci. Technol*. **2013**, 15, 1153–1162.
59. Su, R.; Sun, W.; Li, D.; Wang, Q.Z.; Lv, X.Y.; Musa, H.H.; Chen, L.; Zhang, Y.F.; Wu, W.Z. Association between DLK1 and IGF-I gene expression and meat quality in sheep. *Genet. Mol. Res.* **2014**, 13, 10308–10319.
60. Negahdary, M.; Majdi, S.; Hajihosseinlo, A. Genetic e_ect of IGF1, PIT1 and Leptin genes on wool weights in Makeoi sheep. Electron. *J. Biol*. **2014**, 10, 46–51.
61. Scata, M.C.; Napolitano, F.; Casu, S.; Carta, A.; De Matteis, G. Ovine acyl CoA: Diacylglycerol acyltransferase 1-molecular characterization, polymorphisms and association with milk traits. *Anim. Genet*. **2009**, 40, 737–742.
62. Xu, Q.L.; Chen, Y.L.; Ma, R.X.; Xu, P. Polymorphism of DGAT1 associated with intramuscular fat-mediated tenderness in sheep. *J. Sci. Food Agric.* **2009**, 89, 232–237.

2.2. Genome-wide analysis for early growth-related traits of the locally adapted Egyptian Barki Sheep

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Genome-wide analysis for early growth-related traits of the locally adapted Egyptian Barki Sheep

Ibrahim Abousoliman [1, 2], Henry Reyer [1], Michael Oster [1], Eduard Muráni [1], Ismail Mohamed [2] and Klaus Wimmers [1, 3,*]

[1] Institute of Genome Biology, Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany

[2] Department of Animal and Poultry Breeding, Desert Research Center, Cairo, Egypt

[3] Faculty of Agricultural and Environmental Sciences, University of Rostock, Rostock, Germany

* Corresponding author

Simple Summary

The Barki sheep breed spread throughout the northwestern coastal region of Egypt. It is well adapted to the harsh conditions and considered the main source of meat for people in that region. Growth performance traits such as birth and weaning weights are very important in sheep breeding and affect the breeder's profit. In this study, phenotypic data of early-growth performance traits and genomic information of Barki sheep were used to detect genomic regions and candidate genes that could elucidate the variability of these traits in the studied population of Barki lambs. Genome-wide analysis revealed genomic regions covering promising candidate genes, including *CPXM2, EYA2, GDF2, GDF10, LRIG3, MEF2B, SLC16A7, TBX15, TFAP2B* and *TNNC2*, which are involved in the development during the embryonic stage and after birth. The findings present valuable information for a better understanding of the genetics factors influencing early growth-related traits in the Egyptian Barki sheep breed.

Abstract

Sheep play an important role in the livestock sector in Egypt. For sheep meat production, growth traits such as birth and weaning weights are very important and determine the supply and income of local farmers. The Barki sheep originates from the northeastern coastal zone of Africa and due to its good adaptation to the harsh environmental conditions it contributes significantly to the meat production in these semi-arid regions. The objective of this study was to use a genome-wide SNP panel to identify genomic regions that are diversified between groups of individuals of Egyptian Barki sheep with high and low growth performance traits. In this context, from a phenotyped population

of in total 140 lambs of Barki sheep, 69 lambs were considered for a genome-wide scan with the Illumina OvineSNP50 V2 BeadChip. The selected lambs were grouped into divergent subsets with significantly different performance for birth weight and weaning weight. After quality control, 63 animals and 40,383 SNPs were used for analyses. The fixation index (F_{ST}) for each SNP was calculated between the groups. The results verified genomic regions harboring some previously proposed candidate genes for traits related to body growth, i.e., *EYA2, GDF2, GDF10, MEF2B, SLC16A7, TBX15, TFAP2B* and *TNNC2*. Moreover, novel candidate genes were proposed with known functional implications on growth processes such as *CPXM2* and *LRIG3*. Subsequent association analysis showed significant effects of the considered SNPs on birth and weaning weights. Results highlight the genetic diversity associated with performance traits and thus the potential to improve growth traits in the Barki sheep breed.

Keywords: Barki sheep; growth; birth weight; weaning weight; indigenous sheep; lamb; SNP chip.

1. Introduction

Breeding is one of the main drivers affecting allele frequencies, leading to genomic regions with genetic differentiation, thus contributing to the genetic diversity of livestock species [1]. Many studies analyzing the genetic architecture of complex traits using genome-wide SNP data have been conducted in different livestock species in the last decade. In ruminants, these studies have detected candidate genes related to growth, muscle conformation, adaptation and reproduction traits in sheep [2-6]; milk production, reproduction, body constitution, muscle development, coat color and thermotolerance in cattle [7, 8]; and adaptation, coat color, milk composition and growth traits in goats [9, 10]. To identify the corresponding genomic regions, several methods are available that are capable of analyzing genetic variation within a population and between populations or groups of individuals. The calculation of fixation index (F_{ST}) of Weir and Cockerham (1984) is one of the most popular methods to analyze population structure in this framework. The F_{ST}-approach measures genomic differentiation between populations depending on allele frequency and is thus able to identify highly differentiated alleles that undergo selection, while being unbiased for sample size [7, 11].

Growth traits are very important in sheep breeding and considerably affect the resource efficiency and breeder's profit. Growth traits, like other quantitative traits, are controlled by the complex genetic background of the animal as well as environmental factors such as feed and herd management. Gain in body weight is a highly heritable trait and is one of the main indices of selection especially for meat type breeds. However, it also influences wool production and the reproduction performance of sheep [12]. Body weight gain can be monitored at birth or at other animal life stages and largely determines the amount of income from sheep meat production. Weight measurement at birth represents the earliest indicator of growth performance and related traits [13]. Various genetic but also non-genetic

factors affect the birth weight, such as dam's weight, age and nutrient supply during pregnancy [14]. The heritability of birth weight was estimated to be 0.39 and 0.31 in Iranian Mehraban and Lori-Bakhtiari sheep breeds. For weaning weight, heritabilities were estimated to be 0.25 and 0.24 for weaning weight in Iranian Mehraban sheep [5, 14]. Estimates of heritability for birth weight and weaning weight in Barki sheep are 0.19 and 0.20, respectively [15]. For the Barki sheep breeding in particular, high weight at birth is not a primary goal; more importance is given to achieve higher weaning weights [15]. This is also related to the fact that problems for the dam or the newborn lamb should be avoided during the birth process. In fact, Barki sheep show large differences in growth traits, ranging from 2.4 to 5.0 kg for birth weight with an average of 3.7 kg and 5.2 to 28.8 kg for weaning weight with an average of 13.8 kg [16]. In general, although Barki sheep are well adapted to the harsh environmental conditions of northeast Africa and have a high value for farmers in this region, it remains difficult to obtain a large number of genotyped and comprehensively phenotyped animals due to the smallholder sheep farming systems in Egypt [17]. Improving growth traits in sheep using genetics is promising and is being implemented in different sheep populations, but would further benefit from an understanding of the underlying biological processes and functions. The aim of the current study was to gain insight into diversified genomic regions for growth traits in the Barki sheep breed and to derive potential candidate genes that are functionally related to the traits of interest.

2. Materials and Methods

2.1. Animals and Phenotypes

In this study, a population of 140 male and female lambs from single births of the Egyptian Barki sheep were considered for detection of the genomic regions and candidate genes for growth traits comprising birth weight and weaning weight. Animals were raised in the farms of the Desert Research Centre (DRC), Ministry of Agriculture, Egypt. They were kept in an intensive system in semi-open yards. Lambs were from one breeding season and were offspring of 10 rams. Birth weight within 12 hours after parturition and weaning weight after a lactation period of 3 months (90 days) were recorded for every lamb using an electronic balance. From birth to weaning age, the lambs were suckled only their mother's milk daily. Fresh water was offered two times daily to lambs ad libitum. The experiment was conducted according to all ethics and animal rights (DRC) considering all regulations in conformity with the European Union Directive for the protection of experimental animals (2010/63/EU).

2.2. Genotyping and Quality Control

For each lamb, blood was sampled from the jugular vein in EDTA-containing tubes and stored at - 80 ^{o}C until DNA extraction. DNA extraction was performed according to the manufacturer's instructions with the G-spin Total DNA Extraction kit (iNtRON Biotechnology, Seoul, Korea). Out of the entire

population of 140 animals, 69 lambs with considerable differences in the respective growth trait were genotyped using the Illumina OvineSNP50 V2 BeadChip (Illumina, San Diego, USA).The relative identity-by-descent (IBD) was calculated for all pairs of lambs. The genetic relatedness average was 0.15. The raw signal intensities of the 53,516 SNPs on the chip were imaged using the iScan Reader (Illumina) and converted into genotype calls with the GenomeStudio software (version 2.0). The samples with call rates < 90% were removed from further analysis. The SNPs with genotype call rates < 98%, minor allele frequencies (MAF) < 0.05, in high linkage disequilibrium ($r^2 > 0.5$) within windows of 50 SNPs and significant deviation from Hardy-Weinberg equilibrium at $P < 10^{-6}$ were removed from the analysis. JMP Genomics software (version 9) was used for the quality control. A total of 63 animals and 40,383 SNPs remained and passed the quality control. Base pair positions and names of SNP markers were updated to the latest version of the ovine genome (Oar_v3.1 accessed on 6 July 2020). SNPs not located on autosomes and lacking reference SNP (rs) identifiers were excluded.

2.3. F_{ST} analysis and screening for candidate genes

For each of the traits birth weight (BW) and weaning weight (WW), the genotyped animals were divided into two subgroups, each representing the extreme phenotypes for the respective trait with significant differences between the groups. Student's t-test was used to compute the differences between the means of high and low groups. The fixation index (F_{ST}) for each SNP was calculated between the groups low BW (LBW) – high BW (HBW) and low WW – high WW (HWW) by the SNPRelate R package (version 1.24.0) using Weir & Cockerham method [18, 19]. F_{ST} values were Z-transformed using the following equation $Z(F_{ST}) = (F_{ST} - \mu F_{ST}) / \sigma F_{ST}$ where μF_{ST} is the overall mean of F_{ST} values and σF_{ST} is the standard deviation of F_{ST} values. Only the SNPs having the top 0.05% of $Z(F_{ST})$ values were selected for further analysis. Manhattan plots of $Z(F_{ST})$ value for each SNP were constructed using qqman package (version 0.1.4) in R software. To identify the candidate genes, Ensembl database was used to select the genes within 2-Mb windows around high $Z(F_{ST})$ SNPs. Genes harboring a highlighted $Z(F_{ST})$ SNP were considered as positional candidate genes. Functional candidate genes in the respective genomic region were identified according to their functional relation with the phenotypes employing available gene annotations from GeneCards (http://www.genecards.org; accessed on 19 May 2021) and Uniprot (http://www.uniprot.org; accessed on 19 May 2021) databases.

2.4. Marker-trait association analysis

The association analysis was carried out to test the effect of the genotypes of the selected SNPs on the phenotypes of birth and weaning weight by SPSS V20 (IBM, New York, USA) using the general linear model (GLM) of the analysis of variance (ANOVA) (Supplementary Table S1 and S2). The statistical model was: $Y_{ijk} = \mu + G_i + S_j + e_{ijk}$, where Y_{ijk} is the analyzed trait, μ is the overall mean, G_i is the effect of genotype

(i = 3 levels), S_j is the effect of lamb sex (j = 2 levels), and e_{ijk} is the error effect. $P \leq 0.05$ was considered significant, $P \leq 0.01$ highly significant, and $P > 0.05$ not significant.

3. Results

3.1. Phenotypic data of growth traits

Descriptive statistics of birth weight and weaning weight of Barki lambs with divergent performance in the respective trait are shown in Table 1.

Table 1. Descriptive statistics of lamb growth traits.

Trait	Abbreviation	Group*	Mean	SD	Min	Max	*P* value**
Birth weight (kg)	BW	HBW	4.3	0.3	4.0	5.0	$P < 0.001$
		LBW	3.2	0.3	2.6	3.5	
Weaning weight (kg)	WW	HWW	18.1	2.5	15.0	28.8	$P < 0.001$
		LWW	11.2	1.7	7.4	13.3	

*HBW= High birth weight, LBW = Low birth weight, HWW = High weaning weight, LWW = Low weaning weight. ** = *P* value computed using t-test, SD = Standard deviation.

3.2. Detection of Genomic Regions and Candidate Genes

For the investigation of genomic regions and candidate genes of growth traits, subgroups with distinct differences in the respective traits were analyzed. In particular, Z(F_{ST}) values were calculated to explore genomic differences between groups using genome-wide distributed SNPs. Figure 1 shows a Manhattan plot of SNP-specific Z(F_{ST}) values for BW and highlighted genomic regions and SNPs with highest Z(F_{ST}) values. Obtained SNPs and regions were distributed on chromosomes 1, 2, 3, 5, 6, 8, 13, 14, 16, 17, 19, 22, 23, 24 and 25 (cut-off Z(F_{ST}) ≥ 7.68, Table2). Genomic regions highlighted by the selected SNPs were mined for positional and functional candidate genes (Table 2). This analysis yielded 8 positional candidate genes comprising *PCKS5, WDR35, LAMA5, HYDIN, FAM160A1, GPR26, PTPRM* and *DNAH3*. Moreover, a total of 13 functional candidate genes in the indicated genomic regions were identified, which are known to affect BW or are involved in the development during the embryonic stage and after birth. These comprise *TBX15, SLC16A7, LRIG3, MATN3, OSR1, CITED2, LAMA5, URI1, SUCLG2, CPXM2, LAMA1, GDF2* and *GDF10*. Among the vital processes to which these genes contribute are the development of body organs such as limbs, liver, lungs, brain, skeletal system and muscles. Except for the SNPs indicating the genomic regions harboring BRINP1, *CITED2, CPXM2, GDF2* and *GDF10*, the marker-trait association analysis confirmed a significant relationship of the candidate loci with BW in the Barki lamb population (Table 2).

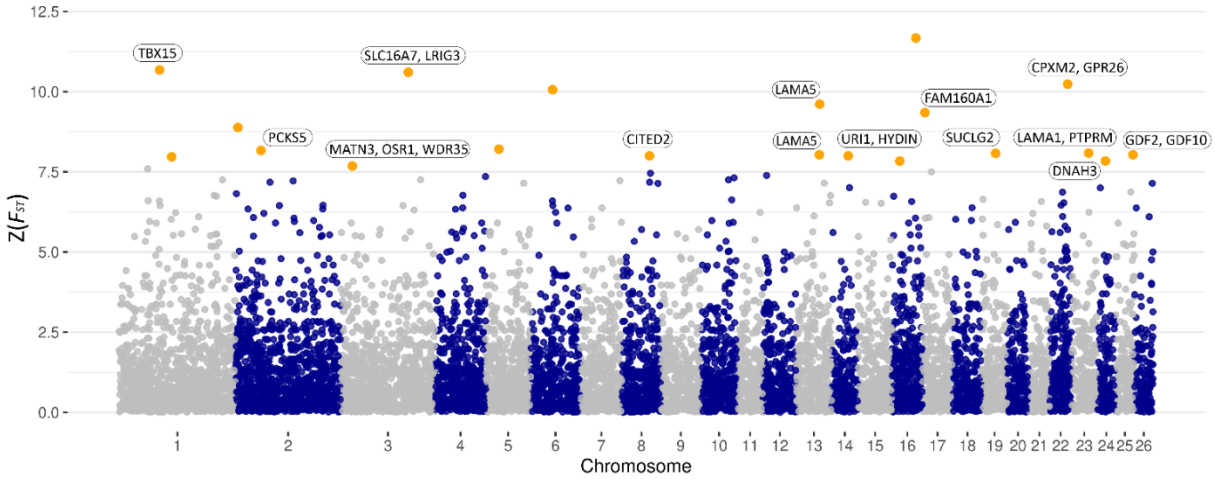

Figure 1. Manhattan plot of the Z(F_{ST}) values for each single nucleotide polymorphism (SNP) between Barki sheep groups divergent in birth weight (LBW – HBW). Orange dots represent SNPs that passed the cut-off threshold at 99.95% of the percentile distribution (Z(F_{ST}) ≥ 7.68).

Table 2. Genomic regions positions and candidate genes within 2 Mb windows around SNPs that passed the cut-off threshold (Z(F_{ST}) ≥ 7.68) for divergence in birth weight (BW).

Rs identifier	Chr	Position	MAF	Z(F_{ST})	*P* value[1]	Candidate genes[2]
rs426652102	1	95369397	0.335	10.68	0.015	***TBX15,*** *SPAG17*
rs409727057	1	124117118	0.220	7.97	0.041	*CLDN17, GRIK1*
rs423222301	2	5472326	0.301	8.88	0.568	*BRINP1, TLR4*
rs419514091	2	60044110	0.369	8.17	0.044	*PCKS5, RFK, ENSOARG00000012533*
rs428614465	3	27732721	0.492	7.68	0.001	***MATN3, OSR1,*** *WDR35, TTC32*
rs417060060	3	160252310	0.446	10.61	< 0.0001	***SLC16A7, LRIG3***
rs413547561	5	31477570	0.481	8.21	0.026	*PRR16, FAM170A*
rs413364871	6	51650705	0.301	10.06	0.023	--
rs427385309	8	64075259	0.276	8.00	0.534	***CITED2,*** *U5, NMBR*
rs406649973	13	54940415	0.444	9.61	0.047	***LAMA5,*** *CDH28, CDH4*
rs419112095	13	54219758	0.295	8.03	0.301	***LAMA5,*** *RPS21, ADRM1*
rs410323459	14	39415649	0.328	8.00	< 0.0001	***URI1,*** *HYDIN, CMTR2, POP4*
rs423237115	16	56144068	0.412	11.67	0.002	*MYO10*
rs413049228	16	18186560	0.134	7.84	0.010	*ZSWIM6, SMIM15*
rs413966946	17	6026379	0.465	9.34	0.001	*FAM160A1, GATB, SH3D19*
rs430430903	19	33088813	0.289	8.08	0.005	***SUCLG2,*** *ENSOARG00000010424*
rs417719085	22	42323039	0.444	10.23	0.113	***CPXM2,*** *GPR26, ACADSB*
rs412781362	23	41177245	0.127	8.07	0.003	***LAMA1,*** *PTPRM, RAB12*
rs421209784	24	18748352	0.086	7.84	0.011	*DNAH3, LYRM1, TMEM159*
rs429736586	25	41911462	0.348	8.03	0.182	***GDF2, GDF10,*** *PTPN20*

[1] *P* value resulting from association analysis of the respective SNP with BW; [2] Gene names in bold = functional candidate genes, underlined = positional candidate genes, only italic = closest up- and downstream located genes within 2 Mb window, Chr = chromosome, MAF = minor allele frequency, Z(F_{ST}) = SNP-specific Z-transformed fixation index.

For WW, 20 SNPs, which reached the threshold Z(F_{ST}) value at 99.95% of the percentile distribution were identified (cut-off Z(F_{ST}) ≥ 6.99, Table 3). These SNPs indicated 13 genomic regions distributed on chromosomes 1, 2, 3, 5, 6, 10, 12, 13, 15, 18, 20, 22 and 25 as illustrated in Figure 2. Considering these regions, 7 positional candidate genes, directly tagged by one of the identified SNPs, were detected. These genes are *ASB3, SOX5, TP53RK, DGKZ, FBLN5, PCDH15* and *GLUD1*. Moreover, 19 functional candidate genes were identified in the respective genomic regions for WW including *MEGF9, PTPRU, FABP3, MEF2B, HAPLN4, NCAN, WNT9A, WNT3A, POSTN, EYA2, MMP9, TNNC2, ACP2, LRP4, FBLN5, TFAP2B, BMPR1A, GDF2* and *GDF10*. Considering the SNPs indicative for these candidate genes, all SNPs except rs405054059 appeared to be significantly associated with WW (Table 3).

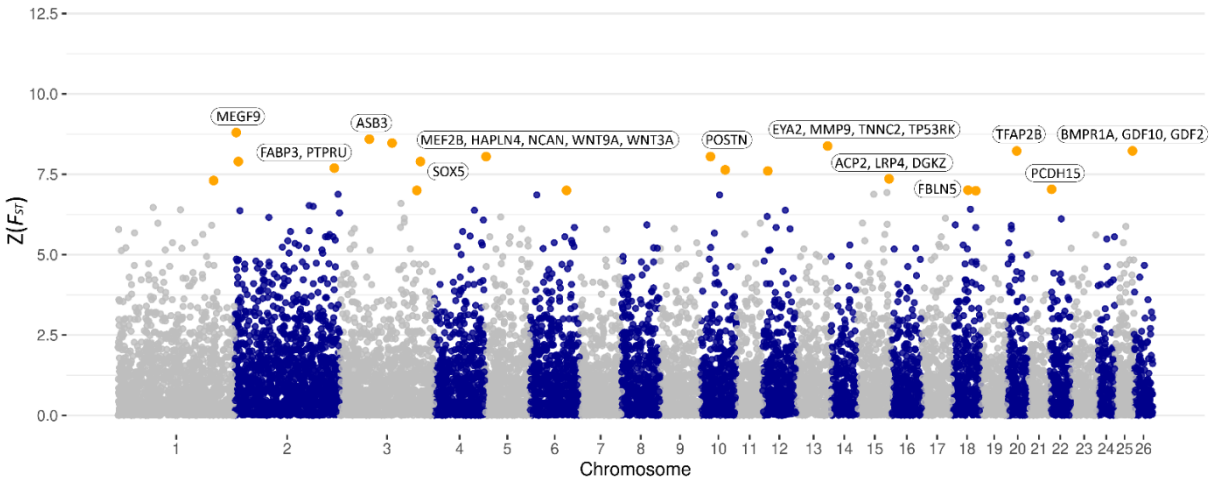

Figure 2. Manhattan plot of the Z(F_{ST}) values for each single nucleotide polymorphism (SNP) of weaning weight. Orange dots represent SNPs that passed the cut-off threshold at 99.95% of the percentile distribution (Z(F_{ST}) ≥ 6.99).

Table 3. Genomic regions positions and candidate genes within 2 Mb around SNPs that passed the cut-off threshold (Z(F_{ST}) ≥ 6.99) for divergence in weaning weight (WW).

Rs name	Chr	Position	MAF	Z(F_{ST})	*P* value[1]	Candidate genes[2]
rs401497638	1	226047403	0.274	7.30	0.166	*IL12A, IQCJ*
rs402362274	2	4014386	0.194	8.80	0.005	***MEGF9**, CDK5RAP2, BRINP1*
rs427650461	2	8985061	0.259	7.90	0.024	*TNFSF15, TMEM268*
rs405054059	2	236085906	0.354	7.69	0.249	***FABP3**, MATN1, **PTPRU***
rs420573745	3	70227376	0.500	8.59	0.008	*ASB3, CHAC2*
rs422502823	3	124010853	0.408	8.47	< 0.0001	*MGAT4C, C12orf50*

rs425747978	3	191139722	0.269	7.90	0.006	*SOX5, BCAT1, ETNK1*
rs410754805	3	182669294	0.341	7.00	0.063	*RESF1, AMN1*
rs429678680	5	3076397	0.224	8.10	0.023	***MEF2B, HAPLN4, NCAN, WNT9A, WNT3A**, PRSS38, SNAP47*
rs418926568	6	86505675	0.381	7.00	0.010	*SLC4A4, GC*
rs401888979	10	25115607	0.219	8.10	0.010	***POSTN**, RFXAP, SERTM1*
rs428497629	10	59974520	0.263	7.64	0.078	*SLITRK1, SLITRK6*
rs426943634	12	12266633	0.436	7.60	0.001	*RGS18, BRINP3*
rs413169429	13	74924747	0.303	8.38	0.003	***EYA2, MMP9, TNNC2**, TP53RK, SLC13A3, SLC2A10*
rs411451096	15	74597812	0.399	7.36	0.002	***ACP2, LRP4**, DGKZ, CREB3L1, MDK*
rs430684800	18	36936501	0.322	7.01	0.118	*NOVA1, FOXG1*
rs426036565	18	55537048	0.414	6.99	0.05	***FBLN5**, TC2N, TRIP11*
rs410079568	20	23394498	0.470	8.23	0.004	***TFAP2B**, PKHD1*
rs421690996	22	5090223	0.345	7.03	0.050	*PCDH15, U3, SYCE1*
rs400432841	25	41241866	0.459	8.23	0.046	***BMPR1A, GDF10, GDF2**, GLUD1, U6, SHLD2*

[1] *P* value resulting from association analysis of the respective SNP with WW; [2] Gene names in bold = functional candidate genes, underlined = positional candidate genes, only *italics* = closest up- and downstream located genes within 2 Mb window, Chr = chromosome, MAF = minor allele frequency, Z(F_{ST}) = SNP-specific Z-transformed fixation index.

4. Discussion

In this study, we used the Ovine SNP50 V2 BeadChip to investigate the genomic differences between groups of Barki sheep that differed significantly in growth traits (birth and weaning weight). The average BW of Barki lambs was similar to those of lambs of the native Egyptian breeds such as Rahmani and Ossimi breeds with 3.73 and 3.9 kg. However, in terms of WW, Rahmani and Ossimi lambs are slightly heavier than Barki lambs with average weights of 17.63 and 14.05 kg, respectively [20, 21]. Also BW (5.08 kg) and WW (29.8 kg) of Romney lambs, as one of the worldwide economically important meat type breeds, were reported to be higher than the weights of Barki sheep [22]. In order to elucidate genetic contributions, genetic differentiations between the subgroups were investigated by the calculation of SNP-specific Z(F_{ST}) values. A total of 15 genomic regions were detected to exhibit divergent allele frequencies for BW in Barki lambs. Regions on chromosomes 1, 3 and 16 are proposed to be the most promising regions, since they enclose four SNPs with the highest Z(F_{ST}) values. Within the genomic region on chromosome 1 at 95.3 Mb, T-box 15 (*Tbx15*) appears to be the most promising functional candidate gene. *Tbx15* gene is a member of T-box gene family, which encodes for transcription factors. T-box genes play a critical role in the development of different tissues and organs

in vertebrates and invertebrates such as the skeletal system during the embryonic stage [23]. The expression of Tbx15 was reported in the development of forelimb and hind limb buds in chick and mouse [24, 25]. Within chromosome 3, the solute carrier family 16 member 7(*SLC16A7*) gene at 159.6 Mb and the leucine-rich repeats and immunoglobulin-like domains protein 3 (*LRIG3*) gene at 160.4 Mb were proposed as additional promising candidates for BW. *SLC16A7* was reported to have a role in skeletal muscle as a lactate cotransporter [26]. *LRIG3* is known to be involved in placenta and embryonic development in pigs [27]. Moreover, the carboxypeptidase-like protein X2 encoding gene (*CPXM2*) located on chromosome 22 at 42.4 Mb plays an important role in growth processes. It was reported to be one of the genes that regulate backfat thickness at different life stages in pigs [28]. However, no significant marker-trait association was found between the corresponding SNP and BW in the investigated Barki lamb population (P = 0.113).

Within the genomic region on chromosome 5 at 3.1 Mb, the myocyte enhancer factor-2B (*MEF2B*) gene was proposed as a functional gene for WW. This gene encodes a member of the myocyte enhancer factor-2 family (*MEF2A, MEF2B, MEF2C* and *MEF2D*), which play an important and critical role in cell development, embryonic development, muscle tissues growth and development processes [29, 30]. Polymorphism in the 3'-UTR of the *MEF2B* gene showed a significant correlation with some growth traits in New Ujumqin Sheep such as body weight and chest girth at 4 and 6 months [31]. Previous studies revealed a significant association between *MEF2B* and skeletal muscle satellite cell and reproductive traits in pigs [32] as well as diameter of muscle fibers in goats [33]. The SNP indicating the *MEF2B* region was found to be significantly associated with WW (P = 0.023). Within chromosome 13, the eyes absent homolog 2 (*EYA2*) and the fast skeletal muscle troponin C (*TNNC2*) genes were revealed as candidates for weaning weight. In Ethiopian sheep, EYA2 was proposed as a candidate for embryonic development of tendons, bones and cartilages [34]. *TNNC2* plays a critical role in skeletal muscle contraction and modulates the Ca2+-activation characteristics of muscle fibers [35] and is highly expressed during the myoblast differentiation and skeletal muscle development [36]. Previous studies on *TNNC2* reported a significant association with growth traits in porcine skeletal muscles [37] and with carcass weight, marbling score in three native sheep breeds [38].

Within the region on chromosome 20 at 23.4 Mb, the transcription factor AP-2B (*TFAP2B*) gene was proposed as a functional candidate for weaning weight. *TFAP2B* was documented in vertebrates and invertebrates and has a critical role during embryonic development [39]. The function of *TFAP2B* in the development of craniofacial structures, limb formation, kidney and skin development was reported in mice [40].

Based on the indicated genomic regions of BW and WW, some functional candidate genes were proposed such as growth differentiation factors 2 and 10 genes (*GDF2, GDF10*) on chromosome 25. *GDF2* and *GDF10* are members of the transforming growth factor-beta (TGF- β) super family and the

bone morphogenetic protein family (BMP), also known as bone morphogenetic protein 9 and 3B (*BMP9, BMP3B*) [41]. Previous studies reported a significant correlation between *GDF2* and *GDF10* genes and weaning weight in New Zealand dual-purpose sheep [42]. *GDF10* regulates cell growth and differentiation in embryonic and adult tissues. Genetic polymorphism in the bovine *GDF10* gene showed a significant effect on some body measurements in Chinese indigenous cattle [43]. Results of the marker-trait association analysis confirmed the significant effect of the majority of selected SNPs on both BW and WW and reflected the contribution of these SNPs to the phenotypic differences between high and low groups of these traits. However, the robustness of association analysis is a matter of sample size [44], whereas the F_{ST}-approach is expected to be less affected by the sample size [45].

5. Conclusions

The genome-wide SNPs analysis revealed a number of genomic regions containing putative QTL for growth traits including birth weight and weaning weight. The QTL regions cover a number of promising functional candidate genes like *CPXM2, EYA2, GDF2, GDF10, LRIG3, MEF2B, SLC16A7, TBX15, TFAP2B* and *TNNC2*, which deserve further investigation, due to their relation to biological processes, including metabolism, body growth, organ morphogenesis, skeletal muscle development, and cell proliferation and differentiation. Moreover, the marker-trait association analysis revealed a significant relationship of the considered SNPs to the studied traits. Our findings provide valuable information for a better understanding of the genetics of early growth-related traits and might contribute to the improvement of these traits in the Barki sheep breed.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1. Table S1: Association of selected SNP genotypes with birth weight in Egyptian Barki lambs (Mean ± SE), Table S2: Association of selected SNP genotypes with weaning weight in Egyptian Barki lambs (Mean ± SE).

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Institutional Review Board Statement: All animal handling procedures and samples collection are done with the approval of the Department of Animal Health (DRC) Committee, do not require an animal experimentation permit according to the regulations of the Desert Research Center (DCR)

Ethics Board, and complained in their implementation with the European Union Directive on the Protection of the Experimental Animals (2010/63/EU).

Data Availability Statement: Genotypic and phenotypic information have been deposited at Open Science Framework (https://osf.io/qj29r/)

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References

1. Smith, J.M.; Haigh, J. The hitch-hiking effect of a favourable gene. *Genet. Res*. **2007**, 89(5-6), 391-403. DOI: 10.1017/S0016672308009579
2. Kijas, J.W.; Lenstra, J.; Hayes, B.; Dalrymple, B. Genome-wide analysis of the world's sheep breeds reveals high levels of historic mixture and strong recent selection. *PLoS Biol*. **2012**, 10(2), e1001258. DOI: 10.1371/journal.pbio.1001258
3. Purfield, D.C., McParland, S.; Wall, E.; Berry, d. The distribution of runs of homozygosity and selection signatures in six commercial meat sheep breeds. *PLoS One.* **2017**, 12(5), e0176780. DOI: 10.1371/journal.pone.0176780
4. Rochus, C.M., Tortereau, F.; Plisson-Petit, F.; Servin, B. Revealing the selection history of adaptive loci using genome-wide scans for selection: an example from domestic sheep. *BMC Genom.* **2018**, 19(1), 71. DOI: 10.1186/s12864-018-4447-x
5. Ghasemi, M.; Zamani, P.; Vatankhah, M.; Abdoli, R. Genome-wide association study of birth weight in sheep. *Animal*. **2019**, 13(9), 1797-1803. DOI: 10.1017/S1751731118003610
6. Yurchenko, A.A., Deniskova, T.; Yudin, N.; Reyer, H.; Wimmers, K.; Larkin, D. High-density genotyping reveals signatures of selection related to acclimation and economically important traits in 15 local sheep breeds from Russia. *BMC Genom*. **2019**, 20(3), 294. DOI: 10.1186/s12864-019-5537-0
7. Zhao, F., McParland, S.; Du, L.; Berry, D. Detection of selection signatures in dairy and beef cattle using high-density genomic information. *Genet. Sel. Evol*. **2015**, 47(1), 49. DOI: 10.1186/s12711-015-0127-3
8. Taye, M., Lee, W.; Dessie, T.; Cho, S.; Kim, H. Whole genome detection of signature of positive selection in African cattle reveals selection for thermotolerance. *Anim. Sci. J*. **2017**, 88(12), 1889-1901. DOI: 10.1111/asj.12851
9. Burren, A., Neuditschko, M.; Signer-Hasaler, H.; Flury, C. Genetic diversity analyses reveal first insights into breed-specific selection signatures within Swiss goat breeds. *Anim. Genet*. **2016**, 47(6), 727-739. DOI: 10.1111/age.12476
10. Kim, E.S., Elbeltagy, A.; Aboul-Naga, A.; Rothschild, M. Multiple genomic signatures of selection in goats and sheep indigenous to a hot arid environment. *Hered*. **2016**, 116(3), 255-264. DOI: 10.1038/hdy.2015.94
11. McRae, K.M., McEwan, J.; Dodds, K.; Gemmell, N. Signatures of selection in sheep bred for resistance or susceptibility to gastrointestinal nematodes. *BMC Genom*. **2014**, 15(1), 637. DOI: 10.1186/1471-2164-15-637
12. Wei, C.H.L., C.S., Encyclopedia of modern sheep production technology. *Agriculture Press, Beijing, China*. **2014**, 70 -77.

13. Ptáček, M., Ducháček, J.; Stádník, L.; Hakl, J.; Fantová, M. Analysis of multivariate relations among birth weight, survivability traits, growth performance, and some important factors in Suffolk lambs. *Arch Anim Breed*. **2017**, 60(2), 43-50. DOI: 10.5194/aab-60-43-2017
14. Zamani, P.; Mohammadi, H. Comparison of different models for estimation of genetic parameters of early growth traits in the Mehraban sheep. *J Anim Breed Genet*. **2008**, 125(1), 29-34. DOI: 10.1111/j.1439-0388.2007.00676.x
15. Sallam, A.M.; Ibrahim, A.H.; Alsheikh, S.M. Estimation of genetic parameters and variance components of pre-weaning growth traits in Barki lambs. Small Ruminant Res. **2019**, 173, 94-100. DOI: 10.1016/j.smallrumres.2018.11.027
16. Abousoliman, I., Reyer, H.; Oster, M.; Murani, E.; Wimmers, K. Analysis of candidate genes for growth and milk performance traits in the Egyptian Barki sheep. *Animals*. **2020**, 10(2), 197. DOI: 10.3390/ani10020197
17. Sallam, A.M. A missense mutation in the coding region of the toll-like receptor 4 (TLR4) gene affects milk traits in Barki sheep. *Anim Biosci.* **2021**, 34(4), 489-498. DOI: 10.5713/ajas.19.0989
18. Weir, B.S.; Cockerham, C.C. Estimating f-statistics for the analysis of population structure. *Evolution*. **1984,** 38(6), 1358-1370. DOI: 10.1111/j.1558-5646.1984.tb05657.x
19. Zheng, X., Levine, D.; Shen, J.; Laurie, C.; Weir, B. A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinform*. **2012**, 28(24), 3326-3328. DOI: 10.1093/bioinformatics/bts606
20. Marai, I.F.M.; Daader, A.H.; Bahgat, L.B. Performance traits of purebred Ossimi and Rahmani lambs and their crosses with Finnsheep born under two accelerated mating systems. *Arch Anim Breed*. **2009**, 52(5), 497-511. DOI: 10.5194/aab-52-497-2009
21. Abd-Allah, M.; Abass, S.; Allam, F. Reproductive performance of Rahmani and Chios sheep and their lambs under Upper Egypt conditions. *Online J. Anim. Feed Sci*. **2011**, 1, 121-129.
22. Thomson, B.C.; Muir, P.D.; Smith, N.B. Litter size, lamb survival, birth and twelve week weight in lambs born to cross-bred ewes. *Journal of New Zealand Grasslands*. **2004**, 66, 233-237. DOI: 10.33584/jnzg.2004.66.2532
23. Papaioannou, V.E. T-box genes in development: from hydra to humans. *Int. Rev. Cytol*. **2001**, 207, 1-70. DOI: 10.1016/s0074-7696(01)07002-4
24. Gibson-Brown, J.J.; Agulnik, S.; Chapman, D.; Garvey, N.; Papaioannou, V. Evidence of a role for T-box genes in the evolution of limb morphogenesis and the specification of forelimb/hindlimb identity. *Mech. Dev*. **1996**, 56(1-2), 93-101. DOI: 10.1016/0925-4773(96)00514-x
25. Candille, S.I.; Chen, C.; Russ, A.; Barsh, G. Dorsoventral Patterning of the Mouse Coat by Tbx15. *PLOS Biol*. **2004**, 2(1), e3. DOI: 10.1371/journal.pbio.0020003
26. Carraro, L.; Ferrarasso, S.; Cardazzo, B.; Bargelloni, L. Expression profiling of skeletal muscle in young bulls treated with steroidal growth promoters. *Physiol Genomics*. **2009**, 38(2), 138-148. DOI: 10.1152/physiolgenomics.00014.2009
27. Metodiev, S.; Young, J.; Onteru, S.; Rothschild, M.; Dekkers, J. A whole-genome association study for litter size and litter weight traits in pigs. *Livest Sci*. **2018**, 211, 87-97. DOI: 10.1016/j.livsci.2018.03.004
28. Pérez-Montarelo, D.; Madsen, O.; Alves, E.; Fernandez, A. Identification of genes regulating growth and fatness traits in pig through hypothalamic transcriptome analysis. *Physiol Genomics*. **2014**, 46(6), 195-206. DOI: 10.1152/physiolgenomics.00151.2013
29. Firulli, A.B. Miano, J.; Bi, W.; Johnson, A.; Schwarz, J. Myocyte enhancer binding factor-2 expression and activity in vascular smooth muscle cells. Association with the activated phenotype. *Circ. Res*. **1996**, 78(2), 196-204. DOI: 10.1161/01.res.78.2.196

30. Iida, K.; Hidaka, K.; Takeuchi, M.; Yutani, C.; Morisaki, T. Expression of MEF2 genes during human cardiac development. *Tohoku J. Exp. Med*. **1999**, 187(1), 15-23. DOI: 10.1620/tjem.187.15
31. Zhang, L.; Ma, X.; Xuan, J.; Wang, H.; Wu, M.; Du, L. Identification of MEF2B and TRHDE gene polymorphisms related to growth traits in a new Ujumqin sheep population. *PLoS One*. **2016**, 11(7), e0159504. DOI: 10.1371/journal.pone.0159504
32. HE Bo, Z.R.; Yuanzhu, X.; HU, C. Culture, Identification and biological characteristics of skeletal muscle satellite cells of the Neonatal pig. *Acta veterinaria et Zootechnica sinica*. **2006**, 6, 555-559.
33. Chen, L.; Cheng, B.; Li, L.; Zhan, S.; Zhong, T.; Chen, Y.; Zhang, H. The molecular characterization and temporal-spatial expression of myocyte enhancer factor 2 genes in the goat and their association with myofiber traits. *Gene*. **2015**, 555(2), 223-230. DOI: 10.1016/j.gene.2014.11.007
34. Ahbara, A.; Bahbahani, H.; Almathen, F.; Agoub, M.; Mwacharo, J. Genome-wide variation, candidate regions and genes associated with fat deposition and tail morphology in Ethiopian indigenous sheep. *Front. Genet*. **2019**, 9(699), 1-21. DOI: 10.3389/fgene.2018.00699
35. Farah, C.S.; Reinach, F.C. The troponin complex and regulation of muscle contraction. *FASEB J*. **1995**, 9(9), 755-767. DOI: 10.1096/fasebj.9.9.7601340
36. Bucher, E.A.; Maisonpierre, P.; Konieczny, S.; Emerson, C. Expression of the troponin complex genes: transcriptional coactivation during myoblast differentiation and independent control in heart and skeletal muscles. *Mol. cel. biol*. **1988**, 8(10), 4134-4142. DOI: 10.1128/mcb.8.10.4134-4142.1988
37. Li, Y.; Chen, Y.; Li, J.; Wang, C.; Liu, X.; Ling, F.; Zhong, W. Molecular characterization, expression profile and polymorphisms of the porcine TNNC2 gene. *Hereditas*. **2008**, 145(6), 274-282. DOI: 10.1111/j.1601-5223.2008.02083.x
38. Xu, Q.; Kang, K.; Yan, F.; An, J.; Chen, Y. Characterization of the fast skeletal troponin C (TNNC2) gene in three Chinese native sheep breeds. *Arch Anim Breed*. **200**8, 51, 572-581. DOI: 10.5194/aab-51-572-2008
39. Zhao, F.; Satoda, M.; Licht, J.; Hayashizaki, Y.; Gelb, B. Cloning and characterization of a novel mouse AP-2 transcription factor, AP-2delta, with unique DNA binding and transactivation properties. *J. boil. chem*. **2001**, 276(44), 40755-40760. DOI: 10.1074/jbc.M106284200
40. Moser, M.; Pscherer, A.; Roth, C.; Becker, J.; Fassler, R. Enhanced apoptotic cell death of renal epithelial cells in mice lacking transcription factor AP-2beta. *Genes Dev*. **1997**, 11(15), 1938-1948. DOI: 10.1101/gad.11.15.1938
41. Tian-Ke, L.; Chun-Nian, L.; Xia, L.; Jie, P.; Xiao-Yun, W.; Min, C.; Wen, Q.; Ping, Y. GDF-10 Gene Polymorphism and Its Association with Production Traits in Gannan Yak. *Scientia Agricultura Sinica*. **2014**, 47(1), 161-169. DOI: 10.3864/j.issn.0578-1752.2014.01.017
42. Pickering, N.K. Genetics of flystrike, dagginess and associated traits in New Zealand dual-purpose sheep : a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Animal Science at Massey University, Palmerston North, New Zealand. **2013**, Massey University.
43. Adoligbe, C.; Zan, L.; Farougou, S.; Wang, H.; Ujjan, J. Bovine GDF10 gene polymorphism analysis and its association with body measurement traits in Chinese indigenous cattle. *Mol Biol Rep*. **2012**, 39(4), 4067-4075. DOI: 10.1007/s11033-011-1188-1
44. Hong, E.P.; Park, J.W. Sample Size and Statistical Power Calculation in Genetic Association Studies. *Genomics Inform*. **2012**, 10(2), 117-122. DOI: 10.5808/GI.2012.10.2.117
45. Porto-Neto, L.R.; Lee, S.; Lee, H.; Gondro, C. Detection of signatures of selection using Fst. *Methods Mol Biol*. **2013**, 1019, 423-436. DOI: 10.1007/978-1-62703-447-0_19

2.3. Genome-Wide SNP Analysis for Milk Performance Traits in Indigenous Sheep: A Case Study in the Egyptian Barki Sheep

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Genome-Wide SNP Analysis for Milk Performance Traits in Indigenous Sheep: A Case Study in the Egyptian Barki Sheep

Ibrahim Abousoliman [1, 2], Henry Reyer [1], Michael Oster [1], Eduard Muráni [1], Ismail Mohamed [2] and Klaus Wimmers [1, 3,]*

[1] Institute of Genome Biology, Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany

[2] Department of Animal and Poultry Breeding, Desert Research Center, Cairo, Egypt

[3] Faculty of Agricultural and Environmental Sciences, University of Rostock, Rostock, Germany

* Corresponding author

Simple Summary

The Barki sheep is one of the three main breeds in Egypt, which is spread mainly throughout the northwestern coastal zone, which has harsh conditions. Considering the harsh, semi-arid habitat of this breed, milk performance traits such as milk yield and milk composition have a very important role in the feeding of newborn lambs and affect their growth during the early stage of life. In this study, rare milk performance data and genomic information of Barki sheep were used to uncover diversified genomic regions that could explain the variability of milk yield and milk quality traits in the studied population of Barki ewes. Genome-wide analysis identified genomic regions harboring interesting candidate genes such as *SLC5A8, NUB1, TBC1D1, KLF3* and ABHD5 for milk yield and *PPARA* and *FBLN1* genes for milk quality traits. The findings offer valuable information for obtaining a better understanding of the genetics of milk performance traits and contribute to the genetic improvement of these traits in Barki sheep.

Abstract

Sheep milk yield and milk composition traits play an important role in supplying newborn lambs with essential components such as amino acids, energy, vitamins and immune antibodies and are also of interest in terms of the nutritional value of the milk for human consumption. The aim of this study was to identify genomic regions and candidate genes for milk yield and milk composition traits through genome-wide SNP analyses between high and low performing ewes of the Egyptian Barki sheep breed, which is well adapted to the harsh conditions of North-East Africa.

Therefore, out of a herd of 111 ewes of the Egyptian Barki sheep breed (IBD = 0.08), ewes representing extremes in milk yield and milk quality traits (*n* = 25 for each group of animals) were genotyped using the Illumina OvineSNP50 V2 BeadChip. The fixation index (F_{ST}) for each SNP was calculated between the diversified groups. F_{ST} values were Z-transformed and used to identify putative SNPs for further analysis ($Z(F_{ST}) > 10$). Genome-wide SNP analysis revealed genomic regions covering promising candidate genes related to milk performance traits such as *SLC5A8, NUB1, TBC1D1, KLF3* and *ABHD5* for milk yield and *PPARA* and *FBLN1* genes for milk quality trait. The results of this study may contribute to the genetic improvement of milk performance traits in Barki sheep breed and to the general understanding of the genetic contribution to variability in milk yield and quality traits

Keywords: Barki sheep; milk performance; genome wide SNPs; genomic regions; candidate genes.

1. Introduction

Sheep have been raised for milk production for thousands of years, before most other mammalian species [1]. In many countries around the world, especially in the Mediterranean region, sheep milk and its products are widely consumed by humans and are considered an important food resource. Sheep contribute about 5% of the total annual milk production in Egypt, whereas cows and buffaloes are the major suppliers of milk. This is a reflection of the management system of sheep milk production, which is characterized by subsistence and smallholder farming systems [2]. Sheep milk is highly similar to human milk in fatty acids composition, which makes it a suitable raw material for infant formula production [3]. Moreover, milk is the most important feed resource for newborn lambs during the early stage of their lifetime, from birth to weaning age (90 days), providing energy and proteins for growth and antibodies against infections and diseases [4]. Milk components such as fat, protein and lactose are important indicators of milk quality, which affects the growth and healthy feeding of the newborn lambs. Sheep milk production and composition are influenced by genetic and environmental factors. Estimates of heritability for milk yield, fat content and protein content in some sheep breeds such as Churra ewes were 0.32, 0.29 and 0.41, respectively [5,6]. The Barki sheep is one of the three most important breeds in Egypt, as it has adapted well to the harsh environmental conditions of Egypt's northwestern coastal zone, where it is raised for meat, as its main product, and milk, as its by-product. The total population of Barki sheep is about 470,000 heads, which are owned by small holders [7]. The current Barki sheep breeding is characterized by a phenotypic selection approach considering mainly the number of offspring and the growth performance of lambs. In addition, the general health status is included, which enables ewes and lambs to cope with the harsh environmental conditions. Neither a structured breeding program nor a genetic selection program is applied. The amount of milk produced by Barki sheep in particular is low compared to the other native Egyptian breeds or worldwide breeds, possibly due to the absence of any attempts to perform

phenotypic or genomic selection of milk performance traits in this breed. This low production affects lambs' growth and viability and increases the percentage of the lambs lost due to inanition. It is also noticeable that the production of milk and its composition varies greatly between individuals in the Barki sheep breed, which is attributed to both genetic and environmental factors [8]. Therefore, it is feasible to study the differences between high and low productive individuals. The development of high-density SNP arrays and bioinformatics tools enables researchers to detect genomic regions that contribute to phenotypic variation in different livestock species, using different approaches based on linkage disequilibrium, allele frequency or haplotype characteristics [9]. To gain further knowledge about the genetic architecture, the fixation index (F_{ST}) approach of Weir and Cockerham is a suitable method, also for small data sets, to uncover genomic differences between experimental populations or groups and detect genomic regions with divergent allelic frequencies indicating putative candidate genes [10,11]. In this context, several studies were performed using genome-wide SNP data and revealed some candidate genes for milk traits in dairy cattle [12–14], sheep [15, 16] and goats [17, 18]. Previously, F_{ST} approach was conducted to detect some candidate genes for productive and reproductive traits such as fertility in Egyptian native Rahmani sheep breed [19]. The aim of the current study is to explore genomic differences of Barki ewes divergent in milk performance traits, thereby identifying genomic regions and candidate genes related to milk yield and milk composition.

2. Materials and Methods

2.1. Animals and phenotypes

The experiment was conducted in accordance with all ethical and animal welfare standards of the Desert Research Center, taking into account all regulations in compliance with the European Union Directive for the Protection of Experimental Animals (2010/63/EU). A population of 111 Egyptian Barki ewes aged between 4 and 5 years was kept in the farms of Desert Research Centre, Ministry of Agriculture, Egypt under an intensive system and housed in semi-open yards for one breeding season. All ewes in the study were sired by 10 rams. Throughout the experimental period, ewes were fed daily on a feed concentrate (0.75 kg per head) and clover hay (0.5 kg per head). Fresh water was available to sheep ad libitum. Ewes were in the same parity and lactation period. Milk yield was recorded from parturition for a period of 3 months by hand milking in the morning and evening. Daily milk yield was measured by summation of the morning and evening milking. Total milk yield was calculated by summation of the daily milk yields for 90 days. Milk was sampled and stored at -20 C. Milk from mixed samples of morning and afternoon milk were analyzed for percentages of fat, protein, lactose, and total solids using milko-scan (130 A/SN Foss Electric, Hillerod, Denmark). For genetic analysis of milk traits, both milk yield and milk composition served as selection criteria. For milk yield, in total 50 ewes were selected from the two tails of the phenotypic distribution and divided into two subgroups (high

milk yield—HMY represent top 25 animals and low milk yield—LMY represent bottom 25 animals), each representing the extreme phenotypes for the milk yield trait. For milk composition, the measured values for fat, protein, lactose and total solids were used for a principal component analysis to calculate animal-individual eigenvalues. Therefore, the phenotypic correlation matrix was used to compute principal components using R statistical software [20]. The first and second principal components explained about 59.7% and 19.3% of the phenotypic variance of the traits. The first principal component was considered for grouping of animals according to milk quality (Supplementary Figure S1). Ewes having extreme negative loadings on PC1 were considered as high milk quality (HMQ) animals (*n* = 25), whereas individuals with extreme positive loading on PC1 were assigned to the low milk quality (LMQ) group (*n* = 25). Student's t-test was used to compute the differences between the group means using SPSS V20 (IBM, New York, NY, USA). Phenotypic Spearman correlation coefficients among milk performance traits and PC1 were calculated for all animals (*n* = 111).

2.2. Genotyping and quality control

DNA was extracted from blood samples, collected from the jugular vein of all ewes, using the G-spin Total DNA Extraction kit (iNtRON Biotechnology, Seoul, Korea) according to the manufacturer's instructions. Out of the entire population of 111 animals, 71 ewes were genotyped using the Illumina OvineSNP50 V2 BeadChip (Illumina, San Diego, CA, USA). The genetic relatedness of all pairs of ewes was assessed by calculating relative identity–by-descent (IBD) probabilities, which revealed an average relatedness of 0.08. The raw signal intensities of the 53,516 SNPs on the chip were imaged using the iScan Reader (Illumina). The signals were converted into genotype calls using the Genome Studio software (version 2.0). The SNPs with genotype call rates <90%, minor allele frequencies (MAF) < 0.03 [21] and significant deviation from Hardy–Weinberg equilibrium at $p < 10^{-6}$ were removed from analysis using JMP Genomics software (version 9). Base pair positions and names of SNP markers were updated to the latest version of the ovine genome of Texel breed (Oar_v3.1 accessed on 6 July 2020). SNPs not located on autosomes and lacking rs identifiers were excluded. After quality control, 49,184 SNPs were used for analyses.

2.3. Genome Wide F_{ST} calculation

SNPRelate R package was used to calculate the F_{ST} of Weir and Cockerham for each SNP between the subgroups (LMY-HMY and LMQ-HMQ) [22]. The resultant distribution of F_{ST} values were Z-transformed and the extreme tail of the distribution was used to identify putative SNPs for further analysis, using a threshold $Z(F_{ST}) > 10$. In addition, all SNPs that passed the cutoff threshold at $Z(F_{ST}) > 5$ were listed in Supplementary Tables S1 and S2 [23]. Manhattan plots of the genome-wide $Z(F_{ST})$ values were performed using qqman package in R software. Genomic regions with the highest $Z(F_{ST})$ values were considered as region of interest. Genes within 1 megabase (Mb) regions up- and downstream of SNPs

with highest Z(F_{ST}) values were scrutinized based on positional and functional evidences according to the Ensembl database. Genes harboring a highlighted SNP were considered positional candidate genes. Genes within the 2-Mb window were considered functional candidate genes, taking into account their functional relationship to phenotypes using available gene annotations from the GeneCards (http://www.genecards.org (accessed on: 3 February 2021) and Uniprot (http://www.uniprot.org (accessed on: 3 February 2021) databases.

3. Results

3.1. Phenotypic data of milk performance traits

Descriptive statistics of milk yield and milk composition, comprising fat, protein, lactose and total solids percentages and principal component 1 (PC1) for milk quality (MQ), are shown for the Barki subgroups in Table 1. A high MQ is indicated by negative loadings on PC1, whereas a low MQ is represented by positive values.

Table 1. Descriptive statistics of milk performance traits.

Trait	**Group**	**N**	**Mean**	**SE**	**Min**	**Max**	***p* value**
Milk yield MY(kg)	HMY	25	41.97	2.02	31.50	72.00	$p < 0.001$
	LMY	25	17.20	0.71	9.90	23.40	
Milk quality (MQ; PC1)	HMQ	25	-1.11	0.08	-0.63	-2.21	$p < 0.001$
	LMQ	25	2.05	0.29	4.91	0.14	
Milk fat (%)	HMQ	25	6.28	0.26	4.78	9.60	$p < 0.001$
	LMQ	25	2.61	0.13	1.45	3.60	
Milk protein (%)	HMQ	25	6.85	0.25	5.20	9.20	$p < 0.001$
	LMQ	25	4.09	0.10	2.85	4.60	
Milk lactose (%)	HMQ	25	7.99	0.14	7.30	9.90	$p < 0.001$
	LMQ	25	5.14	0.22	1.01	6.10	
Total solids (%)	HMQ	25	25.49	0.87	20.00	33.10	$p < 0.001$
	LMQ	25	14.83	0.18	12.68	16.24	

* HMY = High milk yield, LMY = Low milk yield, HMQ = High milk quality, LMQ = Low milk quality. ** *p* value computed using t-test, SE = Standard error, PC1 = Principal component 1.

Significant correlation coefficients were determined between the milk composition traits (Table 2). The highest correlation coefficient was obtained for TS and P (0.83), followed by the coefficients of F and TS (0.47), and TS and L (0.43). There was no considerable correlation between milk yield and milk

composition traits. The correlation coefficients obtained for PC1 showed that all milk composition traits are correlated to varying degrees by PC1, with protein and total solids having the highest correlation coefficients.

Table 2. Phenotypic Spearman correlation among milk performance traits.

Trait	MY	F	P	L	TS	PC1
MY	1					
Fat	- 0.12	1				
Protein	-0.05	0.38**	1			
Lactose	0.05	0.26**	0.29**	1		
Total solids	0.06	0.47**	0.83**	0.43**	1	
PC1	-0.06	0.69**	0.90**	0.61**	0.94**	1

**highly significant correlation ($p < 0.01$) using t-test for the significance. PC1 = Principal component 1.

3.2. Detection of genomic regions and candidate genes

The animals were divided into two subgroups representing extreme phenotypes for milk yield (HMY and LMY) and milk composition (HMQ and LMQ). $Z(F_{ST})$ values were calculated to investigate the genomic differences between the groups using a genome-wide SNP panel. For milk yield, a number of genomic regions and SNPs were indicated to differentiate between the groups ($Z(F_{ST}) > 10$, Figure 1). These regions and SNPs were distributed on the chromosomes 1, 3, 4, 6, 12, 18 and 19 (Table 3). Within these genomic regions, *OR6C75, ANO4, MCTP2* and *SNRK* were identified as positional candidate genes. Moreover, *SLC5A8, NUB1, TBC1D1, KLF3* and *ABHD5* were proposed as functional candidate genes, which are known to affect lactation, mammary gland development and secretion and fatty acids synthesis and lipids' metabolism.

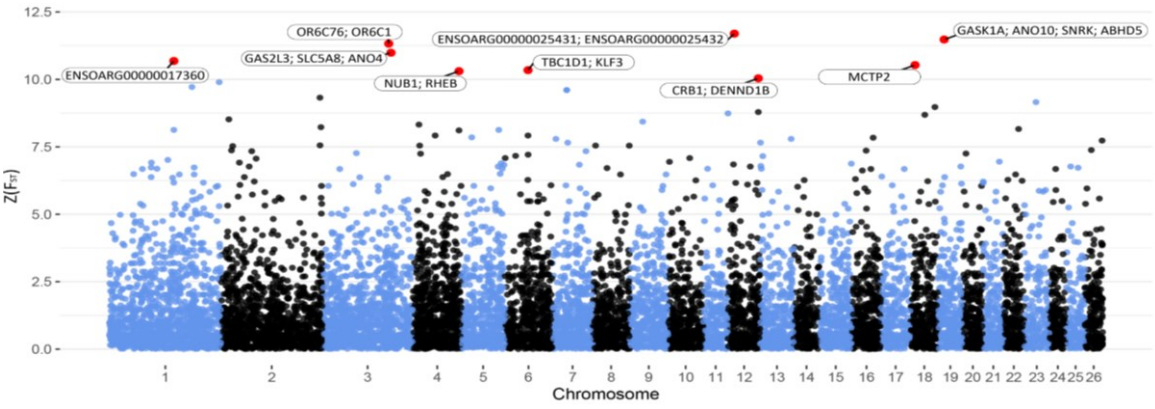

Figure 1. Manhattan plot of Z(F_{ST}) values at each SNP for milk yield. The red dots represent the SNPs that passed the cut-off threshold at Z(F_{ST}) = 10and are labelled with candidate genes within a 2 Mb window.

Table 3. Genomic positions and putative candidate genes derived from SNPs differentiating between ewes divergent in milk yield and milk quality (Z(F_{ST}) > 10).

Trait	Rs name	Chr	Position	MAF	F_{ST}	Z(F_{ST})	Candidate genes*
Milk yield	rs412092721	1	158753375	0.477	0.263	10.68	*ENSOARG00000017360*
	rs428217479	3	164177406	0.429	0.278	11.32	*OR6C76, OR6C1, OR6C75*
	rs430736025	3	169823214	0.374	0.270	10.99	*GAS2L3,* ***SLC5A8****, ANO4*
	rs420351948	4	109338529	0.332	0.319	13.07	*ENSOARG00000001351*
	rs399050266	4	113362135	0.350	0.254	10.30	***NUB1****, RHEB*
	rs418394216	6	57451934	0.201	0.255	10.34	***TBC1D1****,* ***KLF3***
	rs427343726	12	15424350	0.228	0.287	11.70	*ENSOARG00000025431, ENSOARG00000025432*
	rs412626910	12	79966574	0.421	0.247	10.04	*CRB1, DENND1B*
	rs430297634	18	11841541	0.433	0.259	10.53	*MCTP2*
	rs423654488	19	15202234	0.352	0.281	11.48	*GASK1A, ANO10, SNRK,* ***ABHD5***
Milk quality	rs408700818	3	220103217	0.370	0.300	10.14	*ATXN10,* ***FBLN1, PPARA***
	rs414244120	3	220048441	0.485	0.299	10.09	***FBLN1****, ATXN10,* ***PPARA***

* Gene names in bold = functional candidate genes; underlined = positional candidate genes; only *italic* = closest up- and downstream located genes within 1 Mb window; Chr = Chromosome, MAF = Minor allele frequency.

Figure 2 shows the Manhattan plot representation of SNP-specific Z(F_{ST}) values for milk quality. A genomic region and corresponding SNPs located on chromosomes 3 are highlighted to be linked to this trait in the Barki sheep population (Table 3). Positional and functional candidate genes derived by the selected SNPs are indicated in Table 3. Within the genomic region on chromosome 3, *ATXN10* gene was identified as a positional candidate gene. Moreover, *FBLN1* and *PPARA* genes were designated as functional candidate genes in the identified genomic region.

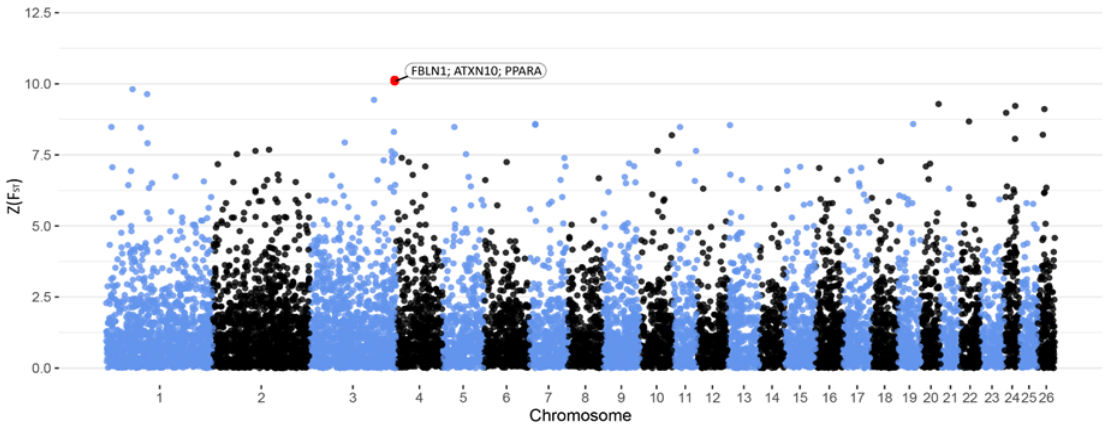

Figure 2. Manhattan plot of $Z(F_{ST})$ values at each SNP comparing LMQ and HMQ Barki ewes. The red dots represent the SNPs that passed the cut-off threshold at $Z(F_{ST}) = 10$ and are labelled with candidate genes within a 2 Mb window..

4. Discussion

The averages of milk yield and milk composition traits (fat, protein, lactose and total solids percentages) in this study were similar to the previously recorded values of Barki ewes with 44.7 kg, 4.17%, 3.34%, 5.01% and 15.88%, respectively [24]. The results of the correlation analysis between the recorded milk traits confirmed the positive correlation among milk composition traits [25]. In contrast to other studies in sheep and cattle, there was no considerable negative correlation between milk yield and milk composition traits, possibly due to the overall low milk production of Barki sheep and the limited breeding efforts on these traits. Furthermore, a positive correlation was revealed between PC1 and milk composition traits as shown in Table 2. For comparison, the average milk yield of the Rahmani breed (70.75 kg), which is another important indigenous Egyptian sheep breed, was reported to be significantly higher [26]. The correlation results among milk yield and milk components were in agreement with those of ewes from the ancient Iberian Churra breed, which also have low average milk performance [6]. The ewes in this study were considered not substantially related according to genetic relatedness (IBD = 0.08) and were suitable for the application of the F_{ST} approach [27]. For milk yield, a total of seven genomic regions were identified to differentiate comparing HMY and LMY animals as shown in Table 3. Scrutiny of the genes in the identified genomic regions revealed functional candidates on chromosomes 3, 4, 6, 18 and 19. Several QTL for milk yield on these chromosomes in different genomic regions in the Sheep Genome were reported previously in various sheep breeds [8, 28–33]. The same genomic region on chromosome 18 was detected to be associated with milk yield in East Friesian and Dorset sheep breeds [32]. In the genomic region on chromosome 3 at 169.8 Mb, Solute Carrier Family 5 (Sodium/Monocarboxylate Cotransporter) Member 8 (*SLC5A8*) was previously reported to be associated with milk yield in Italian Holstein dairy cows [34]. The genomic region on chromosome 4 harbors the Negative Regulator of Ubiquitin-Like Proteins 1 (*NUB1*) gene as one of the

proposed genes affecting milk yield and contributing to the proteasomal degradation pathway. *NUB1* was previously proposed as a strong candidate gene explaining the variation in milk yield in Gir X Holstein (Girolando) crossbreed animals [35]. The QTL on chromosome 6 at 57 Mb includes *TBC1* Domain Family Member 1 (*TBC1D1*) and Kruppel-Like Factor 3 (*KLF3*). Selection signatures study in dairy and beef cattle revealed *TBC1D1* as candidate for milk production [36]. In Holstein cows, a scan for polymorphisms in *TBCID1* yielded two SNPs associated with milk protein yield [37] and another SNP associated with fat and protein percentages [38]. The importance of *KLF3* was suggested in Chinese Holstein cows based on its physiological and biochemical functions in many processes such as cell proliferation, differentiation, homeostasis and apoptosis [39, 40]. Moreover, a SNP in *KLF3* was significantly associated with milk yield and protein yield also in Chinese Holstein [41]. The Abhydrolase Domain Containing 5 (*ABHD5*) gene, which resides on chromosome 19 at 15.5 Mb, represents a prospective functional candidate, based on its important role in lipid metabolism, the energy balance signaling pathway and triglyceride metabolism in dairy cows and Qinchuan cattle [42, 43]. For milk quality, a genomic region on chromosome 3 was shown to be differentiated between HMQ and LMQ ewes, confirming previously reported QTL for milk fat percentage [30,33], protein percentage [44, 45] and lactose percentage [46]. Within this genomic region on chromosome 3, *PPARA* and *FBLN1* genes were proposed as candidates. The Peroxisome Proliferator Activated Receptor Alpha (*PPARA*) gene located at 220.6 Mb is a member of the *PPAR*s family, which has a critical role in the regulation of milk fat synthesis in lactating ruminants [47]. *PPARA* is one of the genes involved in lipid metabolism in mammary gland in dairy cows [48]. In Charolais X German Holstein cross-breed dairy cows, *PPARA* was associated with milk yield and protein synthesis [49]. In line with the results of the Barki study, Fibulin 1 (*FBLN1*) located on chromosome 3 at 220 Mb was reported to be associated with milk protein yield and protein percentage in dairy cattle [25]. In addition, *FBLN1* was reported to play a critical role in the development and cell differentiation of the mammary gland [50]. However, due to the limited sample size available for Barki sheep in the current study, the results deserve further investigation involving a larger number of animals and other indigenous sheep breeds.

5. Conclusions

The results of the genome-wide analysis uncovered some genomic regions contributing to variability in milk performance traits such as milk yield and milk quality in Bakri sheep. These regions harbor some interesting functional candidate genes such as *SLC5A8, NUB1, TBC1D1, KLF3* and *ABHD5* for milk yield, and *PPARA* and *FBLN1* for milk quality traits. These genes deserve further investigation to analyze the association between genetic variations of these genes and their respective milk phenotypes. Given the current absence of structured genetic improvement programs in Barki sheep, the current analysis provides insights into genomic regions that are critical for milk quantity and quality in ruminants. Our

findings offer valuable information for the future improvement of milk performance traits and the associated assurance of offspring supply in the Barki sheep breed.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ani11061671/s1. Table S1: Genomic positions derived from SNPs differentiating between ewes divergent in milk yield ($Z(F_{ST}) > 5$), Table S2: Genomic positions derived from SNPs differentiating between ewes divergent in milk quality ($Z(F_{ST}) > 5$), Figure S1: Eigenvalues from the principle component analysis for milk quality traits. Animals have been assigned to low (red) and high (blue) milk quality.

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References

1. Zervas, G.; Tsiplakou, E. The effect of feeding systems on the characteristics of products from small ruminants. *Small Ruminant Res*. **2011**, *101*, 140–149.
2. Sallam, A. A missense mutation in the coding region of the toll-like receptor 4 gene affects milk traits in Barki sheep. *Anim. Biosci*. **2021**, *34*, 489–498.
3. Martin, C.R.; Ling, P.; Blackburn, G.L. Review of Infant Feeding: Key Features of Breast Milk and Infant Formula. *Nutrients*. **2016**, *8*, 279.
4. Kalyankar, S.D. Sheep: Milk. In Encyclopedia of Food and Health; Caballero, B., Finglas, P.M., Toldrá, F., Eds.; Academic Press: Oxford, UK, **2016**; pp. 758–763.
5. Milan, P.; Mekic, C.; Zujovic, M. Genetic principles relating to improvement of milk yield in sheep and goats. *Biotech. Anim. Husb*. **2005**, *21*, 73–78.

6. Othmane, M.H.; Carriedo, J.A.; Primitivo, F.S.; De La Fuente, L.F. Genetic parameters for lactation traits of milking ewes: Proteincontent and composition, fat, somatic cells and individual laboratory cheese yield. *Genet. Sel. Evol*. **2002**, *34*, 581–596.
7. Sallam, A.M.; Ibrahim, A.H.; Alsheikh, S.M. Estimation of genetic parameters and variance components of pre-weaning growth traits in Barki lambs. *Small Rumin Res*. **2019**, *173*, 94–100.
8. Abousoliman, I.; Reyer, H.; Oster, M.; Muráni, E.; Mourad, M.; Rashed, M.A.; Mohamed, I.;Wimmers, K. Analysis of candidate genes for growth and milk performance traits in the Egyptian Barki sheep. *Animals* **2020**, *10*, 197.
9. Saravanan, K.A.; Panigrahi, M.; Kumar, H.; Bhushan, B.; Dutt, T.; Mishra, B.P. Selection signatures in livestock genome: A review of concepts, approaches and applications. *Livest. Sci*. **2020**, *241*, 104257.
10. Weir, B.S.; Cockerham, C.C. Estimating f-statistics for the analysis of population structure. *Evolution* **1984**, *38*, 1358–1370.
11. Porto-Neto, L.R.; Lee, S.H.; Gondro, C. Detection of signatures of selection using Fst. *Methods Mol. Biol*. **2013**, *1019*, 423–436.
12. Bouwman, A.C.; Bovenhuis, H.; Visker, M.H.P.W.; Arendonk, J.A.M. Genome-wide association of milk fatty acids in Dutch dairy cattle. *BMC Genet*. **2011**, *12*, 43.
13. Li, C.; Sun, D.; Jiang, L.; Liu, J.; Zhang, Q.; Zhang, Y.; Zhang, S. Advances on genome-wide association study for economically important traits in dairy cattle. *Hereditas* **2012**, *34*, 545–550.
14. Maxa, J.; Neuditschko, M.; Russ, I.; Förster, M.; Medugorac, I. Genome-wide association mapping of milk production traits in Braunvieh cattle. *J. Dairy Sci.* **2012**, *95*, 5357–5364.
15. Saridaki, A.; Antonakos, G.; Theodorides, H.; Zoidis, A.L. Combined haplotype blocks regression and multi-locus mixed model analysis reveals novel candidate genes associated with milk traits in dairy sheep. *Livest. Sci.* **2019**, *220*, 8–16.
16. García-Gámez, E.; Gutiérrez-Gil, B.; Sahana, G.; Arranz, J. GWA analysis for milk production traits in dairy sheep and genetic support for a QTN influencing milk protein percentage in the LALBA gene. *PLoS ONE*. **2012**, *7*, e47782.
17. Scholtens, M.; Jiang, A.; Smith, A.; Littlejohn, M.; Blair, H. Genome-wide association studies of lactation yields of milk, fat, protein and somatic cell score in New Zealand dairy goats. *J. Anim. Sci. Biotechnol.* **2020**, *11*, 55.
18. Mucha, S.; Morde, R.; Coffey, M.; Conington, J. Genome-wide association study of conformation and milk yield in mixed-breed dairy goats. *J. Dairy Sci.* **2018**, *101*, 2213–2225.
19. El-Halawany, N.; Zhou, X.; Al-Tohamy, A.; El-Sayd, Y.; Shawky, A.; Michal, J.; Jiang, Z. Genome-wide screening of candidate genes for improving fertility in Egyptian native Rahmani sheep. *Anim. Genet*. **2016**, *47*, 513.
20. Laenoi,W.; Rangkasenee, N.; Uddin, M.; Cinar, M.;Wimmers, K.; Schellander, K. Association and expression study of MMP3, TGF*b*1 and COL10A1 as candidate genes for leg weakness-related traits in pigs. *Mol. Biol. Rep*. **2012**, *39*, 3893–3901.
21. Zhang, L.; Liu, J.; Zhao, F.; Ren, H.; Xu, L.; Lu, J. Genome-Wide Association Studies for Growth and Meat Production Traits in Sheep. *PLoS ONE.* **2013**, *8*, e66569.
22. Zheng, X.; Levine, D.; Shen, J.; Gogarten, S.M.; Laurie, C.; Weir, B.S. A High-performance Computing Tool set for Relatedness and Principal Component Analysis of SNP Data. *Bioinformatics* **2012**, *28*, 3326–3328.
23. Yaxin, Y.; Zhangyuan, P.; Ran, D. Whole-Genome Sequencing of Bamei Mutton Sheep for Screening the Genes and SNPs Associated with Litter Size under Selection. *Res. Square* **2021**, *1*, 1–17.
24. Farrag, B.; El-Hawy, A.; El-Bassiony, M. Improving Reproductive and Productive Efficiency of Barki Sheep by using GnRHand Selenium. *World's Vet. J.* **2017**, *7*, 128–136.

25. Raven, L.-A.; Cocks, B.; Pryce, J.; Hayes, B. Genes of the RNASE5 pathway contain SNP associated with milk production traits in dairy cattle. *Genet. Sel. Evol*. **2013**, *45*, 25.
26. Abd-Allah, M.; Abass, S.; Allam, F. Factors affecting the milk yield and composition of Rahmani and Chios sheep. *Glob. J. Dairy Farm. Milk Prod*. **2013**, *1*, 53–59.
27. Miller, M.B.; Basu, S.; McGue, M. The Minnesota Center for Twin and Family Research genome-wide association study. *Twin Res. Hum. Genet*. **2012**, *15*, 767–774.
28. Calvo, J.H.; Marcos, S.; Beattie, A.E.; Gonzalez, C.; Jurado, J.J.; Serrano, M. Ovine alpha-amylase genes: Isolation, linkage mapping and association analysis with milk traits. *Anim. Genet*. **2004**, *35*, 329–332.
29. Calvo, J.H.; Martínez-Royo, A.; Beattie, A.E.; Dodds, K.G.; Marcos-Carcavilla, A.; Serrano, M. Fine mapping of genes on sheep chromosome 1 and their association with milk traits. *Anim. Genet*. **2006**, *37*, 205–210.
30. Raadsma, H.W.; Jonas, E.; McGill, D.; Thomosom, P. Mapping quantitative trait loci (QTL) in sheep. II. Meta-assembly and identification of novel QTL for milk production traits in sheep. *Genet. Sel. Evol.* **2009**, *41*, 45.
31. Arnyasi, M.; Komlósi, I.; Lien, S.; Czeglédi, L.; Nagy, S.; Jávor, A. Searching for DNA markers for milk production and composition on chromosome 6 in sheep. *J. Anim. Breed. Genet.* **2009**, *126*, 142–147.
32. Mateescu, R.G.; Thonney, M.L. Genetic mapping of quantitative trait loci for milk production in sheep. *Anim. Genet.* **2010**, *41*, 460–466.
33. Garcia-Gámez, E.; Gutiérrez-Gil, B.; Suarez-Vega, A.; de la Fuente, L.F.; Arranz, J.J. Identification of quantitative trait loci underlying milk traits in Spanish dairy sheep using linkage plus combined linkage disequilibrium and linkage analysis approaches. *J. Dairy Sci.* **2013**, *96*, 6059–6069.
34. Cellesi, M.; Dimauro, C.; Sorbolini, S.; Macciotta, N. Maximum difference analysis: A new empirical method for genome-wide association studies. *Ital. J. Anim. Sci.* **2016**, *15*, 396–406.
35. da Cruz, A.S.; Silva, D.; Minasi, L.; da Cruz, A. Single-Nucleotide Polymorphism variations associated with specific genes putatively identified enhanced genetic predisposition for 305-day milk yield in the Girolando crossbreed. *Front. Genet.* **2021**, *11*, 573344.
36. Zhao, F.; McParland, S.; Kearney, F.; Du, L.; Berry, D. Detection of selection signatures in dairy and beef cattle using high-density genomic information. *Genet. Sel. Evol.* **2015**, *47*, 49.
37. Liu, R.; Sun, D.; Wang, Y.; Yu, Y.; Zhang, Y. Fine mapping QTLs affecting milk production traits on BTA6 in Chinese Holstein with SNP markers. *J. Integr. Agric.* **2013**, *12*, 110–117.
38. Jiang, J.; Liu, L.; Gao, Y.; Shi, L.; Sun, D. Determination of genetic associations between indels in 11 candidate genes and milk composition traits in Chinese Holstein population. *BMC Genet*. **2019**, *20*, 48.
39. Xu, Q.; Mei, G.; Sun, D.; Liu, J. Detection of genetic association and functional polymorphisms of UGDH affecting milk production trait in Chinese Holstein cattle. *BMC Genom*. **2012**, *13*, 590.
40. Eaton, S.A.; Funnell, A.; Sue, N.; Pearson, R.; Crossley, M. A Network of Krüppel-like Factors (Klfs): Klf8 is repressed by Klf3 and activated by Klf1 in vivo. *J. Biol. Chem*. **2008**, *283*, 26937–26947.
41. Zongjun, Y.; Mei, G.; Liu, Y.; Ding, X.; Zhang, Q. Polymorphism Identification and Association with Milk Production Traits of KLF3 Gene in a Chinese Holstein Population. *J. Anim. Vet. Adv*. **2010**, *9*, 2784–2787.
42. Contreras, G.A.; Strieder-Barboza, C.; Raphael, W. Adipose tissue lipolysis and remodeling during the transition period of dairy cows. *J. Anim. Sci. Biotechnol*. **2017**, *8*, 41.

43. Wang, X.; Khan, R.; Raza, S.; Zan, L. Molecular characterization of ABHD5 gene promoter in intramuscular preadipocytes of Qinchuan cattle: Roles of Evi1 and C/EBPα. *Gene* **2019**, *690*, 38–47.
44. Gutiérrez-Gil, B.; Zarei, M.; Alvarez, L.; Bayón, Y.; Arranz, J. Quantitative trait loci underlying milk production traits in sheep. *Anim. Genet*. **2009**, *40*, 423–434.
45. García-Gámez, E.; Gutiérrez-Gil, B.; Sánchez, J.P.; Arranz, J.J. Replication and refinement of a quantitative trait locus influencing milk protein percentage on ovine chromosome 3. *Anim. Genet.* **2012**, *43*, 636–641.
46. Jawasreh, K.; Amareen, A.A.; Aad, P. Effect and Interaction of β-Lactoglobulin, Kappa Casein, and Prolactin Genes on Milk Production and Composition of Awassi Sheep. *Animals* **2019**, *9*, 382.
47. Bionaz, M.; Chen, S.; Khan, M.; Loor, J. Functional Role of PPARs in Ruminants: Potential Targets for Fine-Tuning Metabolism during Growth and Lactation. *PPAR Res*. **2013**, *2013*, 684159.
48. Bernard, L.; Toral, P.G.; Chilliard, Y. Comparison of mammary lipid metabolism in dairy cows and goats fed diets supplemented with starch, plant oil, or fish oil. *J. Dairy Sci.* **2017**, *100*, 9338–9351.
49. Friedrich, J.; Brand, B.; Ponsuksili, S.; Kuehn, C.; Schwerin, M. Detection of genetic variants affecting cattle behaviour and their impact on milk production: A genome-wide association study. *Anim. Genet.* **2016**, *47*, 12–18.
50. Menzies, K.K.; Lefevre, C.; Macmillan, K.; Nicholas, K. Insulin regulates milk protein synthesis at multiple levels in the bovine mammary gland. *Funct. Integr. Genom*. **2009**, *9*, 197–217.

3. General discussion

In this chapter, the possibility of improving the growth traits such as birth weight, weaning weight and average daily gain and milk performance traits like milk yield and milk composition in the Egyptian Barki sheep breed using two different approaches is discussed. The findings of the first study provide insights into the genetics of milk and growth traits in the Barki sheep based on eight candidate genes, recently associated with production traits in different breeds. The second and third study identified genomic regions harboring interesting candidate genes for growth and milk performance traits in a Barki sheep population. These findings provide valuable information for a better understanding of the genetics of these traits and contribute to the genetic improvement in Barki sheep. Finally, the implementation of these results in current Barki sheep breeding schemes by marker-assisted selection or genomic selection provides alternatives to improve these traits. Therefore, advantages, disadvantages and requirements to implement genomic information in Barki sheep breeding will be addressed and discussed.

3.1. Phenotypic data of growth and milk traits in Barki sheep

Barki sheep as one of the three main sheep breeds in Egypt play a critical role in supplying people with meat and milk especially in the arid and semi-arid regions of Egypt (Sallam *et al.* 2012). Growth traits such as birth weight, weaning weight and average daily gain are very important in sheep breeding industry. Milk performance traits such as milk yield and milk quality play a main role in the feeding of the new born lambs especially during the first stage of their life. Genetic improvement of growth traits and milk performance traits is one of the main breeding goals of researchers in Egypt now using different molecular genetic techniques. Growth and milk performance traits like other quantitative traits, are affected by genetic background of the animal and non-genetic or environmental factors. Growth traits considers indicators of the lamb adaptation to the existing environmental factors and extensively used in the selection programs in different sheep breeds (Singh *et al.* 2006; Lalit. *et al.* 2016). Body weights and growth rate during the pre-weaning stage are early indicators of the post weaning growth rates (Hanford *et al.* 2002; Zhang *et al.* 2008). Birth weight and early growth rate of the animal are determined by the genetic potential of the animal and environmental factors (Mandal *et al.* 2006; I *et al.* 2017). Birth weight is the earliest indicator with considerable impact on growth performance traits (Ptáček *et al.* 2017). It ranges between 2.3 to 4.0 kg in the Egyptian sheep breeds (Elshazly & Youngs 2019). Heritability estimation of birth weight is influenced by maternal genetic and environmental effects (Zamani & Mohammadi 2008). High weight at birth in Barki sheep is not favorable to avoid any problems for the dam or the newborn lamb during the birth process such as dystocia; more importance is to achieve higher weaning weights (Sallam *et al.* 2019). Weaning weight

is considered one of the most important growth traits as indicator for the pre-weaning growth of the lambs. Weaning weight is affected by birth weight and the growth rate after birth, also weaning weight is highly correlated with pre-weaning growth rate. Likewise, gain in body weight is a highly heritable trait and is one of the main indices of selection especially for meat type breeds. It also influences most of the productive traits of sheep. Body weight gain can be easily monitored at different life stages and determines incomes from sheep meat production (Wei 2014). The growth rate or average daily gain is an economic trait of interest and may be used as a criteria for the selection programs of growth traits (Lalit. *et al.* 2016). Birth weight, weaning weight and average daily gain from birth to weaning are correlated traits. Correlation between birth weight and weaning weight was 0.48 in Kermani sheep (Bahreini Behzadi *et al.* 2007). Sheep milk production play a very critical role in supplying new born lambs with considerable sufficient amounts of milk to decrease mortality rates due to starvation. Estimates of heritability for milk yield, fat content and protein content in sheep are 0.38, 0.48 and 0.51, respectively (Milan *et al.* 2005).

In general, productive traits in Barki sheep breed are relatively low compared to other different breeds around the world which characterized by high growth and milk performance rates. This may be due to genetic factors as a result of long-term genetic improvement and environmental factors such as feed quality, housing systems and veterinary care. So, improvement of genetic and environmental factors will lead to achieve higher growth rates, higher slaughtering weights, and higher meat yield in lambs. Also lower lambs mortality rates due to ewes' high milk production.

According to the presented results in the first study, phenotypic data of growth traits comprising birth weight, weaning weight and average daily gain were recorded in Barki lambs population and reported to be similar with records of some native Egyptian breeds (Marai *et al.* 2009; Abd-Allah *et al.* 2011). In a comparison with some worldwide breeds, averages were reported to be heavier than those of Barki lambs (Thomson 2004).

Also, averages of milk performance traits in Barki ewes such as milk yield and milk composition showed lower value than other native or worldwide breeds (Othmane *et al.* 2002; Abd-Allah *et al.* 2013). These variations of growth and milk performance traits with other breeds may be due to the effect of the genotype between breeds or other environmental factors. A representative SNP of each of the selected functional candidate genes in the first study was selected for genotyping in both lambs and ewes samples. Genotyping results indicated that all selected SNPs were in Hardy–Weinberg equilibrium status in both lambs and ewes. Results of the genetic parameters confirmed the absence of selection processes and applying of inbreeding system leading to stability of allelic and genotypic frequency, high genetic variation between populations or breeds and lower genetic variation between individuals in the same population or breed. Inbreeding usually causes some changes in the population such as

increasing in inbreeding, reduction of genetic diversity, loss of heterozygosity and stability of allelic frequency (Shikano *et al.* 2001; Curik *et al.* 2014).

Summary at a glance: Phenotypic data of growth and milk traits in Barki sheep

- ✓ Growth traits averages were similar with those of native breeds and lower than those of worldwide sheep breeds.
- ✓ Milk performance traits averages are lower than those of other native or worldwide sheep breeds.

3.2. The candidate gene approach for growth and milk performance traits

During the past few decades, intrinsic advances have been achieved through the application of molecular genetics in the identification of loci or genes that affect the economic productive traits in livestock species (Andersson 2001). These advances enabled researches and breeders to implement marker-assisted selection using these genes to promote genetic improvement programs (Dekkers 2004). The candidate gene approach has been used to study the association between phenotypic data of the traits and genotypes of the candidate gene that may affect the physiological pathways of these traits (Andersson 2001; Bush & Moore 2012). Detection of genetic polymorphisms and the candidate genes associated with these traits provides valuable information for marker-assisted selection (Goddard & Hayes 2009). There are many genetic markers available which can be used commercially in breeding programs of different livestock species. In dairy cattle, the most common genetic markers are κ-casein, β-lacto globulin (Medrano & Aguilar-Cordova 1990; Rincón & Medrano 2003), *FMO3* (Lundén *et al.* 2002), *DGAT* (Grisart *et al.* 2002), and *GHR* (Blott *et al.* 2003) for milk quality, milk yield and composition. In beef cattle, some genetic markers were detected for commercial using such as Myostatin for growth and body composition (Grobet *et al.* 1998), *MC1R* and *MGF* for the appearance (Klungland *et al.* 1995; Seitz *et al.* 1999). In sheep breeding, the most important and common markers are Callipyge for growth and body composition (Freking *et al.* 2002), Booroola, Hanna and Inverdale markers for reproduction traits (Galloway *et al.* 2000; McNatty *et al.* 2001; Wilson *et al.* 2001). In pigs, *MC4R* and *IGF-2* used as genetic markers for growth, body composition and feed intake (Kim *et al.* 2000). Also, *RYR* and *RN/PRKAG3* markers used for meat quality traits (Fujii *et al.* 1991; Milan *et al.* 2000). Previously, many researchers used candidate gene technique to study the association between some candidate genes and milk performance traits in various sheep breeds (Moioli *et al.* 2007; Orford *et al.* 2010; Staiger *et al.* 2010).

Currently in Egypt, candidate gene approach is used extensively to study the effect of genetic polymorphism of candidate genes on most of the productive traits in Egyptian sheep breeds (table1).

Using of these candidate genes information to implement marker-assisted selection especially in Barki sheep will contribute to improve the productive performance and decreases mortality rates. Applying of marker-assisted selection in Barki sheep breeding programs is relatively easy but there are some consequences such as additional costs and the small holder breeding system. Also the necessity to validate the results in larger scale using higher number of animals and different sheep breeds.

Table 1. Candidate genes for different productive traits in some Egyptian sheep breeds.

Breed	Trait	Candidate genes	Reference
Barki	Growth performance and body conformation traits	*BMP4*	(Ibrahim 2019)
	Productive life	*IGF1R*	(Ibrahim & Alsheik 2016)
	Growth and carcass traits	*CAPN3*	(Shehata *et al.* 2014)
	Lifespan traits	*FOXO3*	(Ibrahim & Alsheikh 2014)
	Milk performance traits	*TLR4*	(Sallam 2020)
	Reproductive traits	*BMP2 - GDF9*	(Ibrahim 2021)
	Wool traits	*KAP6-1*	(Sallam *et al.* 2020)
Rahmani and Ossimi	Growth traits	*GH - CAPN4*	(Othman *et al.* 2015; Mahrous *et al.* 2016a)
	Meat related traits	*CAST - MSTN - DGAT1 - IGFBP3 - FecB*	(Mahrous *et al.* 2015)
	Fecundity traits	*GDF9 – BMP15*	(Barakat *et al.* 2017)

These results confirmed that candidate gene technique is an effective approach to study the association between phenotypes of the traits and genotypes of the candidate gene that may functionally share in the physiological pathways of these traits (Andersson 2001).

Some candidate genes comprising *LEP, IGF-1, DGAT1, STAT5A*, *PRL, CSN1S2, GHR* and *GHRHR* have been previously reported to be associated with growth and milk performance traits in different sheep breeds worldwide, and were selected for studying the association between the genetic polymorphisms of these genes and the phenotypes of the Barki sheep analyzed here. The results of association of SNPs of some selected candidate genes with lambs growth traits showed that genetic polymorphism in Leptin gene was significantly associated with weaning weight and average daily gain. Leptin also plays a major role in control of body growth, adoptability, and immune function (Zieba *et al.* 2003) and is involved in the regulation of feed intake and energy balance (Javanmard *et al.* 2008). Leptin can be considered as one of the best biological markers reflecting body fatness in animals (Nassiry *et al.* 2007).

Many studies reported the effect of *LEP* gene on growth traits. Some studies on association between Leptin gene polymorphism and productive traits have been reported in cattle (Buchanan *et al.* 2002; Lagonigro *et al.* 2003; Schenkel *et al.* 2005), sheep and poultry (Boucher *et al.* 2006; Shojaei *et al.* 2010; Sadeghi *et al.* 2014; Hajihosseinlo *et al.* 2015) with promising results.

The results of association analysis showed a significant effect of some of the selected SNPs of candidate genes on some milk traits. Interestingly, genetic polymorphism of *LEP* gene associated significantly with milk yield and milk fat percentage. *LEP* gene was reported to be a candidate gene for milk traits in different species. It has also been shown that Leptin gene influence milk performance in dairy cattle (Almeida *et al.* 2003; Buchanan *et al.* 2003), Buffalos (Jamuna *et al.* 2016) and sheep (Mahmoud *et al.* 2014). Moreover, *PRL, STAT5A* and *GHRHR* genes might be proposed as marker genes due to its significant effect on some milk performance traits. Prolactin (*PRL*) was reported as a candidate marker for milk production in dairy sheep (Knight 2001; Orford *et al.* 2010). Polymorphisms in prolactin gene were reported to be associated with milk traits in some sheep breeds (Ramos *et al.* 2009; Staiger *et al.* 2010). *STAT5* known as mammary gland factor as a mediator of prolactin signalling and can activate transcription of milk protein genes in response to prolactin (Wakao *et al.* 1994; Dario *et al.* 2009). *STAT5A* gene has been investigated in some dairy cattle breeds as a candidate gene affecting milk production and composition (Brym *et al.* 2004; Khatib *et al.* 2008). *GHRHR* plays a critical role in the regulation of growth hormone synthesis and secretion (Mayo 1992; Giustina & Veldhuis 1998).

Summary at a glance: Candidate genes for growth and milk performance traits of Barki sheep

- ✓ *LEP* polymorphism (Val181Leu) were significantly associated with lambs weaning weight and average daily gain.
- ✓ Selected polymorphisms in *LEP, STAT5A, PRL*, and *GHRHR* were significantly associated with milk yield and milk fat percentage, milk lactose percentage, milk yield, and total solids percentage, respectively.
- ✓ *IGF1, DGAT1, CSN1S2*, and *GHR* genes showed no significant associations with the studied traits.
- ✓ *LEP, STAT5A, PRL*, and *GHRHR* genes might be considered as interesting candidates for further investigations to improve growth and milk performance in the Barki sheep.

3.3. Genome-wide SNP analysis revealed genomic regions and candidate genes for growth and milk performance traits

Growth traits and milk performance traits are similar to many quantitative traits that are assumed to be influenced by both genetics and environmental factors (Sallam 2019b). These factors lead to phenotypic differences in these traits among the individuals. Studying these differences using new technologies such as genome wide scan, developed high density SNP arrays, bioinformatics and statistical models enables researchers to detect genomic regions covering candidate genes, which are significantly associated with the studied traits and responsible for phenotypic differences of the traits in different livestock species (Cesarani *et al.* 2018; Gao *et al.* 2019; Saravanan *et al.* 2020). There are many approaches to detect genomic differences between populations and groups of individuals depending on linkage disequilibrium (LD), allele frequency spectrum, reduced local variability, and haplotype characteristics (Qanbari & Simianer 2014). The fixation index (F_{ST}) of Weir and Cockerham (1984) is one of the most popular methods which is suitable for small data sets and can be used to uncover genomic differences between experimental populations or groups and detect genomic regions with divergent allelic frequencies (Weir & Cockerham 1984; Porto-Neto *et al.* 2013).

To complement the attempts to genetically improve the growth and milk performance traits of the Egyptian Barki sheep, the second and third studies were conducted using genome wide SNPs data. Our results of the second study using F_{ST} approach detected genomic regions covering some previously proposed candidate genes associated with the growth traits such as *., EYA2, GDF2, GDF10, MEF2B, SLC16A7, TBX15, TFAP2B* and *TNNC2.* Moreover, novel candidate genes were proposed such as *CPXM2* and *LRIG3*. These genes may cause the phenotypic differences between the two groups with high and low growth trait performance. These genes were reported before to be associated with vital processes such as development of body organs such as skeletal system and limps during the embryonic stage, cell growth and differentiation, muscle tissues growth, body weights at different life stages and carcass weight (Firulli *et al.* 1996; Papaioannou 2001; Adoligbe *et al.* 2012). More recently, the use of genome-wide scan and selection signature data increases the potential to implement genomic selection using genetic markers or SNPs that cover the entire genome (Goddard & Hayes 2007). The derived candidate genes from genome-wide scans can be used in breeding programs for different productive traits in livestock species. Genome wide scans have been carried out to detect the candidate genes for different growth traits in most of sheep breeds. Three genes were reported as candidates for birth weight in Lori-Bakhtiari sheep using GWAS, including *RAB6B* (a member of RAS oncogene family), *Tf* serotransferrin and *GIGYF2* (GRB10 interacting GYF protein 2) (Ghasemi *et al.* 2019). GWAS in Australian Merino sheep verified *NCAPG* and *LCORL* genes for weaning weight (Al-Mamun *et al.* 2015). *AADACL3, VGF, NPC1* and *SERPINA12* genes were detected as candidates for body weight traits in Chinese Fine-Wool Sheep (Lu *et al.* 2020). Also, (Almasi *et al.* 2021) indicated that both *ATP8A2* and

PLXDC2 genes could be considered as candidates for post weaning body weight traits in Lori-Bakhtiari sheep. In beef cattle, genome-wide scan results lead to a detection of myostatin gene (*MSTN*) as a candidate which can be used in breeding programs for growth performance. The mutations on *MSTN* that underlie the phenotypic variation of muscle hypertrophy were identified in different cattle breeds (Andersson & Georges 2004).

In our third study, genome wide SNP analysis was performed to identify genomic regions and candidate genes, which are differentiated between the groups with high and low milk performance traits. Our results revealed genomic regions harboring some interesting candidate genes related to milk performance traits such as *SLC5A8, NUB1, TBC1D1, KLF3* and *ABHD5* for milk yield and *PPARA* and *FBLN1* genes for milk quality trait. These genes were identified to be associated with milk yield, milk protein, milk fat, milk lactose lipid metabolism, the energy balance signaling pathway and triglyceride metabolism and mammary gland development (Menzies *et al.* 2009; Contreras *et al.* 2017; Jiang *et al.* 2019; da Cruz *et al.* 2021).

Genome-wide SNP analysis have been widely applied in the breeding programs for milk traits of different livestock species. In different dairy cattle breeds, some genes were detected as candidates for milk performance traits such as *DGAT1, PLBD1* and *MGST1* in Holstein Cattle (Cruz *et al.* 2019; Liu *et al.* 2020), *CDH2* in dual-purpose Xinjiang Brown cattle (Zhou *et al.* 2019). DGAT1 is one of the most important genes which used in breeding programs for milk performance in different livestock species. The results of genome-wide scan detected DGAT1 as a candidate gene for milk performance traits such as milk yield and milk components (Cruz *et al.* 2019; Liu *et al.* 2020). In Italian water buffalo, *MFSD14A, SLC35A3, PALMD, RGS22* and *VPS13B* genes were detected to be associated with milk yield and milk composition (Liu *et al.* 2018). Furthermore, genome-wide scan studies have identified genes related with milk traits in different sheep breeds including *PALMD* and *RFP145* in Italian Altamurana sheep (Moioli *et al.* 2013), *LALBA* in Spanish Churra sheep (García-Gámez *et al.* 2012a), *GH1* in Serrada Estrela sheep (Vacca *et al.* 2013), *SUCNR1* and *PPARGC1A* genes in Lacune sheep (Yuan *et al.* 2019).

A second way to use the output data from genome wide SNPs analysis in the genetic improvement of many quantitative traits through the genomic selection without knowledge of specific candidates but with using all marker information. Currently, genome wide analysis is an effective tool in selection programs due to the large number of detected SNPs by genome sequencing (Goddard & Hayes 2007). Genome-wide scans were used for identification of major QTLs for economic traits in various species of farm animals throughout screening the whole genome for target genes that correlate with phenotypic traits, using single nucleotide polymorphisms (SNPs) as genetic markers (Al-Mamun *et al.* 2015). Genomic selection is very promising tool in terms of genetic improvement but its application in breeding programs for different livestock species is relatively difficult especially for native breeds due

to its high cost and needs high number of phenotyped and genotyped individuals to increase the statistical power of the analysis. In Barki sheep which characterized by small holder breeding system, it is difficult to implement the genomic selection. Using genome information to identify candidate genes to use in Barki sheep breeding is quite promising but needs validation using an independent population with higher number.

In Egypt, only two studies on Egyptian sheep breeds using genome-wide scan data have been performed. First study identified some genomic regions in Barki sheep and goats to be under selection for adaptation traits. Within these regions, several candidate genes were detected to affect adaptation traits to hot arid environments such as *FGF2, GNAI3, PLCB1, BMP2, BMP4, GJA3, GJB2, MYH, TRHDE* and *ALDH1A3* genes for thermo-tolerance, body size and development, energy and digestive metabolism (Kim *et al.* 2016). Second study in Egyptian native Rahmani sheep detected some promising candidate genes comprising *ROR1, HTR6, BIRC6* and *NCAM1*, which may improve ewes reproductive performance (El-Halawany *et al.* 2016). These studies confirmed the possibility of using genome wide scan analysis to detect the genomic regions and candidate genes for the productive traits and for more understanding the genetics of these traits in the Egyptian sheep breeds.

Summary at a glance: Genome-wide SNPs for growth and milk performance traits

- ✓ Genome-wide SNPs analysis revealed some genomic regions for growth and milk performance traits.
- ✓ Selected genomic regions harbors promising candidate genes for growth traits comprising *EYA2, GDF2, GDF10, MEF2B, SLC16A7, TBX15, TFAP2B, TNNC2, LRIG3 and CPXM2.*
- ✓ Genome-wide SNP analysis revealed genomic regions covering interesting candidate genes related to milk performance traits such as *SLC5A8, NUB1, TBC1D1, KLF3* and *ABHD5* for milk yield and *PPARA* and *FBLN1* genes for milk quality trait.
- ✓ Genome-wide SNPs results needs a validation in an independent population with larger number of animals and other indigenous sheep breeds.

Conclusions

In this thesis, analyses were conducted to study the potentiality of improving growth traits such as birth weight, weaning weight and average daily gain in Barki sheep lambs and milk performance traits such as milk yield and milk composition in Barki sheep ewes. The first study focused on studying the association between genetic polymorphisms of some candidate genes and the phenotypic data of growth and milk performance traits. The analysis results revealed a significant association between polymorphism of *LEP* gene and weaning weight and average daily gain in Barki lambs. Results also concluded a significant association between polymorphisms in *LEP* gene with milk yield and milk fat percentage*, STAT5A* gene with milk lactose percentage*, PRL* gene with milk yield*,* and *GHRHR* gene with milk total solids percentage. Based on the results, the dissection of the genetic variation especially at the LEP locus might deserve further attention. This could aid to develop a genotyping method to validate the results in independent Barki sheep population and to be putatively used in MAS.

Second and third study focused on using of Genome-wide SNPs data sets to identify genomic regions and candidate genes, which differentiated between low and high producing groups of growth and milk performance traits using the fixation index approach (F_{ST}). Results of the second study revealed genomic regions covering some promising functional candidate genes for growth traits such as *EYA2, GDF2, GDF10, MEF2B, SLC16A7, TBX15, TFAP2B, TNNC2*, *LRIG3* and *CPXM2*. Third study detected genomic regions harboring some candidates like *SLC5A8, NUB1, TBC1D1, KLF3* and *ABHD5* for milk yield and *PPARA* and *FBLN1* genes for milk quality trait. The results underline the polygenic nature of the traits, which would argue for a GS approach to improve the traits. However, the use of the data to set a focus on specific candidate genes is more appropriate such as *TBX15, EYA2*, *ABHD5* and *FBLN1* genes for birth weight, weaning weight, milk yield and milk quality, respectively .

The results of this work showed that using the candidate gene approach and genome-wide SNP analysis, some genes can be discovered as interesting candidates for improving growth and milk yield in Barki sheep. It was shown that there are genomic regions containing promising candidate genes related to these traits that deserve further investigation to better understand the genetic contribution to the variability of growth and milk yield traits in Barki sheep. This would be an important step towards improving quantitative traits in the Egyptian Barki sheep breed.

Summary

Sheep is one of the main livestock species in the agricultural sector in Egypt. The main three sheep breeds in Egypt are Ossimi, Rahmani and Barki. The latter one is considered very important especially in the arid and semi-arid regions of Egypt. Barki sheep spreads along the north western coastal zone of Egypt and it is well adapted to live under the harsh environmental conditions. Moreover, it is the main source of income for people in these regions and a major supplier for meat and milk. Growth traits are very important in sheep farming and it affects the breeders profit through achieving high growth rates and weaning weights to increase the meat yield from lambs. Sheep milk production play a very critical role in supplying new born lambs with considerable sufficient amounts of milk to decrease mortality rates due to starvation. Although the application of molecular genetic techniques are very effective in the genetic improvement of these traits, knowledge about the genetics of these traits in Barki sheep is currently sparse. In this context three genetic studies were carried out using a Barki sheep population comprising growth and milk performance traits

In this thesis, the **first chapter** provides a general introduction into the topic of genetic improvement in sheep. Firstly it started with the distribution, social and economic value of sheep around the world, followed by the sheep situation in Egypt and overview on growth and milk production in sheep. And finally it introduced the using of different approaches for genetic improvement in sheep and the future of native breeds in Egypt.

The **second Chapter** presents three experimental studies as a part of this thesis:

The **first study** presents the analysis of some candidate genes for growth and milk performance traits in the Egyptian Barki sheep. The aim of this study is to perform a SNP trait association analysis to investigate the connection between genotypes and production phenotypes. In conclusion, this study confirmed the using of some candidate genes such as *LEP, PRL, STAT5A* and *GHRHR* as promising candidates to improve growth and milk performance in Barki sheep.

To further address the complex nature of early growth related traits, the **second study** focused on using of genome-wide SNP analysis to detect genomic regions and candidate genes that related to growth traits in Barki lambs. Moreover, this study revealed genomic regions harboring interesting candidate genes, which differentiated between high and low groups of growth traits such as *EYA2, GDF2, GDF10, MEF2B, SLC16A7, TBX15, TFAP2B, TNNC2*, *LRIG3* and *CPXM2.*

The **third study** used the same approach of the second paper aiming to identify candidate genes for milk performance traits. This study investigated genomic regions covering promising candidates like *SLC5A8, NUB1, TBC1D1, KLF3* and *ABHD5* for milk yield and *PPARA* and *FBLN1* genes for milk quality trait in Barki ewes' population.

The **third chapter** presents the general discussion of the experimental studies and their interconnections. This chapter also discussed the possibility of using candidate genes resulted from the experimental studies in breeding programs to improve growth and milk performance traits in the Egyptian Barki sheep breed.

In summary, this work provided insights into the genetics of growth and milk yield traits using the candidate gene approach and genome-wide SNP analysis. Genetic variation was demonstrated for traits of interest that can be used for breeding. Understanding the genetics of these traits will provide valuable information that will help improve growth and milk yield traits in the Egyptian Barki sheep breed.

Zusammenfassung

Schafe sind eine der wichtigsten Nutztierarten in der Landwirtschaft in Ägypten. Die drei wichtigsten Schafsrassen in Ägypten sind Ossimi, Rahmani und Barki. Die letztere wird vor allem in den ariden und semiariden Regionen Ägyptens als sehr wichtig angesehen. Das Barki-Schaf ist entlang der nordwestlichen Küstenzone Ägyptens verbreitet und ist gut an die rauen Umweltbedingungen angepasst. Außerdem ist es die Haupteinnahmequelle für die Menschen in diesen Regionen und ein wichtiger Lieferant für Fleisch und Milch. Wachstumsmerkmale sind in der Schafzucht sehr wichtig und beeinflussen den Gewinn des Züchters durch das Erreichen hoher Wachstumsraten und Absetzgewichte, um den Fleischertrag der Lämmer zu erhöhen. Die Milchproduktion von Schafen spielt eine sehr kritische Rolle bei der Versorgung von neugeborenen Lämmern mit einer ausreichenden Menge an Milch, um die Sterblichkeitsrate durch Verhungern zu verringern. Obwohl die Anwendung molekulargenetischer Techniken sehr effektiv bei der genetischen Verbesserung dieser Merkmale ist, ist das Wissen über die Genetik dieser Merkmale bei Barki-Schafen derzeit spärlich. In diesem Zusammenhang wurden drei genetische Studien mit einer Barki-Schafpopulation durchgeführt, die Wachstums- und Milchleistungsmerkmale umfassen

In dieser Arbeit wird im **ersten Kapitel** eine allgemeine Einführung in das Thema der genetischen Verbesserung bei Schafen gegeben. Es beginnt mit der Verbreitung, dem sozialen und wirtschaftlichen Wert von Schafen auf der ganzen Welt, gefolgt von der Situation der Schafe in Ägypten und einem Überblick über Wachstum und Milchproduktion bei Schafen. Und schließlich werden die verschiedenen Ansätze zur genetischen Verbesserung von Schafen und die Zukunft der seltenen Rassen in Ägypten vorgestellt.

Das **zweite Kapitel** stellt drei experimentelle Studien als Teil dieser Arbeit vor:

Die **erste Studie** präsentiert die Analyse einiger Kandidatengene für Wachstums- und Milchleistungsmerkmale beim ägyptischen Barki-Schaf. Das Ziel dieser Studie ist es, eine SNP-Merkmalsassoziationsanalyse durchzuführen, um den Zusammenhang zwischen Genotypen und Produktionsphänotypen zu untersuchen. Zusammenfassend bestätigt diese Studie die Verwendung einiger Kandidatengene wie *LEP, PRL, STAT5A* und *GHRHR* als vielversprechende Kandidaten zur Verbesserung des Wachstums und der Milchleistung bei Barki-Schafen.

Um die komplexe Natur der mit dem frühen Wachstum verbundenen Merkmale weiter zu erforschen, konzentrierte sich die **zweite Studie** auf die Verwendung einer genomweiten SNP-Analyse, um genomische Regionen und Kandidatengene zu entdecken, die mit den Wachstumsmerkmalen bei Barki-Lämmern zusammenhängen. Darüber hinaus enthüllte diese Studie genomische Regionen, die interessante Kandidatengene beherbergen, die zwischen hohen und niedrigen Gruppen von

Wachstumsmerkmalen unterscheiden, wie *EYA2, GDF2, GDF10, MEF2B, SLC16A7, TBX15, TFAP2B, TNNC2, LRIG3* und *CPXM2*.

Die **dritte Studie** verwendete den gleichen Ansatz wie die zweite Arbeit, um Kandidatengene für Milchleistungsmerkmale zu identifizieren. Diese Studie untersuchte genomische Regionen, die vielversprechende Kandidaten wie *SLC5A8, NUB1, TBC1D1, KLF3* und *ABHD5* für die Milchleistung und *PPARA* und *FBLN1* Gene für das Merkmal Milchqualität in der Population der Barki-Schafe abdecken.

Das **dritte Kapitel** präsentiert die allgemeine Diskussion der experimentellen Studien und deren Zusammenhänge. In diesem Kapitel wird auch die Möglichkeit diskutiert, die aus den experimentellen Studien resultierenden Kandidatengene in Zuchtprogrammen zu verwenden, um die Wachstums- und Milchleistungsmerkmale bei der ägyptischen Barki-Schafrasse zu verbessern.

Zusammenfassend lässt sich sagen, dass diese Arbeit Einblicke in die Genetik von Wachstums- und Milchleistungsmerkmalen unter Verwendung des Kandidatengen-Ansatzes und der genomweiten SNP-Analyse lieferte. Es wurde genetische Variation für Merkmale von Interesse nachgewiesen, die für die Zucht verwendet werden können. Das Verständnis der Genetik dieser Merkmale wird wertvolle Informationen liefern, die zur Verbesserung von Wachstums- und Milchleistungsmerkmalen bei der ägyptischen Barki-Schafrasse beitragen werden.

References

Aali M., Moradi-Shahrbabak H., Moradi-Shahrbabak M., Sadeghi M. & Yousefi A.R. (2017) Association of the calpastatin genotypes, haplotypes, and SNPs with meat quality and fatty acid composition in two Iranian fat- and thin-tailed sheep breeds. *Small Ruminant Research* **149**, 40-51.

Abd-Allah M., Abass S. & Allam F. (2011) Reproductive performance of Rahmani and Chios sheep and their lambs under Upper Egypt conditions. *Online Journal of Animal and Feed Research* **1**, 121-9.

Abd-Allah M., Abass S. & Allam F. (2013) Factors affecting the milk yield and composition of Rahmani and Chios sheep. *Global Journal of Dairy Farming and Milk Production* **1**, 53-9.

Adoligbe C., Zan L., Farougou S., Wang H. & Ujjan J.A. (2012) Bovine GDF10 gene polymorphism analysis and its association with body measurement traits in Chinese indigenous cattle. *Molecular Biology Reports* **39**, 4067-75.

Al-Mamun H.A., Kwan P., Clark S.A., Ferdosi M.H., Tellam R. & Gondro C. (2015) Genome-wide association study of body weight in Australian Merino sheep reveals an orthologous region on OAR6 to human and bovine genomic regions affecting height and weight. *Genetic Selection Evolution* **47**, 66.

Alberto F.J., Boyer F., Orozco-terWengel P., Streeter I., Servin B., de Villemereuil P., Benjelloun B., Librado P., Biscarini F., Colli L., Barbato M., Zamani W., Alberti A., Engelen S., Stella A., Joost S., Ajmone-Marsan P., Negrini R., Orlando L., Rezaei H.R., Naderi S., Clarke L., Flicek P., Wincker P., Coissac E., Kijas J., Tosser-Klopp G., Chikhi A., Bruford M.W., Taberlet P. & Pompanon F. (2018) Convergent genomic signatures of domestication in sheep and goats. *Nature Communications* **9**, 813.

Almasi M., Zamani P., Mirhoseini S.Z. & Moradi M.H. (2021) Genome-wide association study for postweaning weight traits in Lori-Bakhtiari sheep. *Tropical Animal Health Production* **53**, 163.

Almeida S.E.M., Almeida E.A., Moraes J.C.F. & Weimer T.A. (2003) Molecular markers in the LEP gene and reproductive performance of beef cattle. *Journal of Animal Breeding and Genetics* **120**, 106-13.

Álvarez I., Fernández I., Traoré A., Pérez-Pardal L., Menéndez-Arias N.A. & Goyache F. (2020) Genomic scan of selective sweeps in Djallonké (West African Dwarf) sheep shed light on adaptation to harsh environments. *Scientific Reports* **10**, 2824.

Andersson L. (2001) Genetic dissection of phenotypic diversity in farm animals. *Nature Reviews Genetics* **2**, 130-8.

Andersson L. & Georges M. (2004) Domestic-animal genomics: deciphering the genetics of complex traits. *Nature Reviews Genetics* **5**, 202-12.

Archibald A.L., Bolund L., Churcher C., Fredholm M., Groenen M.A., Harlizius B., Lee K.T., Milan D., Rogers J., Rothschild M.F., Uenishi H., Wang J. & Schook L.B. (2010a) Pig genome sequence analysis and publication strategy. *BMC Genomics* **11**, 438.

Archibald A.L., Cockett N.E., Dalrymple B.P., Faraut T., Kijas J.W., Maddox J.F., McEwan J.C., Hutton Oddy V., Raadsma H.W., Wade C., Wang J., Wang W. & Xun X. (2010b) The sheep genome reference sequence: a work in progress. *Animal Genetics* **41**, 449-53.

Bahreini Behzadi M.R., Shahroudi F.E. & Van Vleck L.D. (2007) Estimates of genetic parameters for growth traits in Kermani sheep. *Journal of Animal Breeding and Genetics* **124**, 296-301.

Bai Y., Sartor M. & Cavalcoli J. (2012) Current status and future perspectives for sequencing livestock genomes. *Journal of Animal Science and Biotechnology* **3**, 8.

Barakat I., Salem L.M., Daoud N.M., Khalil W.K.B. & Mahrous K. (2017) Genetic polymorphism of candidate genes for fecundity traits in Egyptian sheep breeds. *Biomedical Research* **28**, 851-7.

Barzehkar R., Salehi A. & Mahjoubi F. (2009) Polymorphisms of the Ovine Leptin Gene and its Association with Growth and Carcass Traits in Three Iranian Sheep Breeds. *Iranian Journal of Biotechnology* **7**, 241-6.

Benavides M.V. & Maher A.P. (2000) Quantitative genetic studies on wool yellowing in Corriedale sheep. II. Clean wool colour and wool production traits: genetic parameter estimates and economic returns. *Australian Journal of Agricultural Research* **51**, 191-6.

Blott S., Kim J.-J., Moisio S., Schmidt-Küntzel A., Cornet A., Berzi P., Cambisano N., Ford C., Grisart B., Johnson D., Karim L., Simon P., Snell R., Spelman R., Wong J., Vilkki J., Georges M., Farnir F. & Coppieters W. (2003) Molecular dissection of a quantitative trait locus: a phenylalanine-to-tyrosine substitution in the transmembrane domain of the bovine growth hormone receptor is associated with a major effect on milk yield and composition. *Genetics* **163**, 253-66.

Bolormaa S., Pryce J.E., Hayes B.J. & Goddard M.E. (2010) Multivariate analysis of a genome-wide association study in dairy cattle. *Journal of Dairy Science* **93**, 3818-33.

Bomba L., Nicolazzi E.L., Milanesi M., Negrini R., Mancini G., Biscarini F., Stella A., Valentini A. & Ajmone-Marsan P. (2015) Relative extended haplotype homozygosity signals across breeds reveal dairy and beef specific signatures of selection. *Genetics Selection Evolution* **47**, 25.

Boucher D., Palin M.F., Castonguay F., Gariépy C. & Pothier F. (2006) Detection of polymorphisms in the ovine leptin (LEP) gene: Association of a single nucleotide polymorphism with muscle growth and meat quality traits. *Canadian Journal of Animal Science* **86**, 31-5.

Broad T.E., Glass B.C., Greer G.J., Robertson T.M., Bain W.E., Lord E.A. & McEwan J.C. (2000) Search for a locus near to myostatin that increases muscling in Texel sheep in New Zealand. *Proceedings of the New Zealand Society of Animal Production* **60**, 110-2.

Brym P., Kamiński S. & Ruść A. (2004) New SSCP polymorphism within bovine STAT5A gene and its associations with milk performance traits in Black-and-White and Jersey cattle. *Journal of Applied Genetics* **45**, 445-52.

Buchanan F.C., Fitzsimmons C.J., Van Kessel A.G., Thue T.D., Winkelman-Sim D.C. & Schmutz S.M. (2002) Association of a missense mutation in the bovine leptin gene with carcass fat content and leptin mRNA levels. *Genetic Selection Evolution* **34**, 105-16.

Buchanan F.C., Van Kessel A.G., Waldner C., Christensen D.A., Laarveld B. & Schmutz S.M. (2003) Hot topic: an association between a leptin single nucleotide polymorphism and milk and protein yield. *Journal of Dairy Science* **86**, 3164-6.

Burt D.W. (2005) Chicken genome: current status and future opportunities. *Genome Research* **15**, 1692-8.

Bush W.S. & Moore J.H. (2012) Chapter 11: Genome-Wide Association Studies. *PLOS Computational Biology* **8**, e1002822.

Buzanskas M.E., Grossi D.A., Ventura R.V., Schenkel F.S., Sargolzaei M., Meirelles S.L.C., Mokry F.B., Higa R.H., Mudadu M.A., da Silva M.V.G.B., Niciura S.C.M., Júnior R.A.A.T., Alencar M.M., Regitano L.C.A. & Munari D.P. (2014) Genome-Wide Association for Growth Traits in Canchim Beef Cattle. *PLoS One* **9**, e94802.

Cesarani A., Sorbolini S., Criscione A., Bordonaro S., Pulina G., Battacone G., Marletta D., Gaspa G. & Macciotta N.P.P. (2018) Genome-wide variability and selection signatures in Italian island cattle breeds. *Animal Genetics* **49**, 371-83.

Chapman C.K. & Reid C. (2004) Sheep and Goats: Ecological Tools for the 21st Century.

Chen M., Pan D., Ren H., Fu J., Li J., Su G., Wang A., Jiang L., Zhang Q. & Liu J.-F. (2016) Identification of selective sweeps reveals divergent selection between Chinese Holstein and Simmental cattle populations. *Genetics Selection Evolution* **48**, 76.

Contreras G.A., Strieder-Barboza C. & Raphael W. (2017) Adipose tissue lipolysis and remodeling during the transition period of dairy cows. *Journal of Animal Science and Biotechnology* **8**, 41.

Cruz V.A.R., Oliveira H.R., Brito L.F., Fleming A., Larmer S., Miglior F. & Schenkel F.S. (2019) Genome-Wide Association Study for Milk Fatty Acids in Holstein Cattle Accounting for the DGAT1 Gene Effect. *Animals(Basel)* **9**, 997.

Curik I., Ferenčaković M. & Sölkner J. (2014) Inbreeding and runs of homozygosity: A possible solution to an old problem. *Livestock Science* **166**, 26-34.

da Cruz A.S., Silva D.C., Minasi L.B., de Farias Teixeira L.K., Rodrigues F.M., da Silva C.C., do Carmo A.S., da Silva M.V.G.B., Utsunomiya Y.T., Garcia J.F. & da Cruz A.D. (2021) Single-Nucleotide Polymorphism Variations Associated With Specific Genes Putatively Identified Enhanced

Genetic Predisposition for 305-Day Milk Yield in the Girolando Crossbreed. *Frontiers in Genetics* **11**, 573344-.

Da Y. (2003) Statistical analysis and experimental design for mapping genes of complex traits in domestic animals. *Acta Genetica Sinica* **30**, 1183-92.

Dario C., Selvaggi M., Carnicella D. & Bufano G. (2009) STAT5A/AvaI polymorphism in Podolica bulls and its effect on growth performance traits. *Livestock Science* **123**, 83-7.

Dekkers J.C. (2004) Commercial application of marker- and gene-assisted selection in livestock: strategies and lessons. *Journal of Animal Science* **82** E-Suppl, E313-28.

Doyle E.K., Preston J.W.V., McGregor B.A. & Hynd P.I. (2021) The science behind the wool industry. The importance and value of wool production from sheep. *Animal Frontiers* **11**, 15-23.

Edea Z., Dadi H., Dessie T. & Kim K.-S. (2019) Genomic signatures of high-altitude adaptation in Ethiopian sheep populations. *Genes & Genomics* **41**, 973-81.

El-Halawany N., Zhou X., Al-Tohamy A.F., El-Sayd Y.A., Shawky A.E., Michal J.J. & Jiang Z. (2016) Genome-wide screening of candidate genes for improving fertility in Egyptian native Rahmani sheep. *Animal Genetics* **47**, 513.

El-Wakil S.I., Shemeis A.R., Ahmed A.M. & Abdallah O.Y. (2008) Genetic and phenotypic relationships involving body weight, degree of maturity and measurer of gain rate of Barki sheep without having recourse to fitting growth curves. *Journal of Agriculture Sciences. Mansura University* **33**, 4835–48.

Elshazly A. & Youngs C. (2019) Feasibility of utilizing advanced reproductive technologies for sheep breeding in Egypt. Part 1. Genetic and nutritional resources. *Egyptian Journal of Sheep and Goat Sciences* **14**, 39-52.

Fahmy M.H., Galal E.S.E., Ghanem Y.S. & Khishin S.S. (1969) Genetic parameters of Barki sheep raised under semi-arid conditions. *Animal Science* **11**, 361-7.

Fan B., Du Z.-Q., Gorbach D.M. & Rothschild M.F. (2010) Development and Application of High-density SNP Arrays in Genomic Studies of Domestic Animals. *Asian-Australasian Journal of Animal Sciences* **23**, 833-47.

FAO/UNEP Recommendations of FAO/UNEP Expert Panel On Animal Genetic Resources. URL www.fao.org/3/ah806e/AH806E03.htm.

FAOSTAT (2019a) Breed data sheep for sheep in Egypt. URL http://www.fao.org/dad-is/browse-by-country-and-species/en/.

FAOSTAT (2019b) Domestic animal diversity information system (DAD-IS). URL http://www.fao.org/dad-is/en/.

FAOSTAT (2019c) http://www.fao.org/faostat/ar/#data/QA.

Firulli A.B., Miano J.M., Bi W., Johnson A.D., Casscells W., Olson E.N. & Schwarz J.J. (1996) Myocyte enhancer binding factor-2 expression and activity in vascular smooth muscle cells. Association with the activated phenotype. *Circulation Research* **78**, 196-204.

Flicek P., Amode M.R., Barrell D., Beal K., Billis K., Brent S., Carvalho-Silva D., Clapham P., Coates G., Fitzgerald S., Gil L., Girón C.G., Gordon L., Hourlier T., Hunt S., Johnson N., Juettemann T., Kähäri A.K., Keenan S., Kulesha E., Martin F.J., Maurel T., McLaren W.M., Murphy D.N., Nag R., Overduin B., Pignatelli M., Pritchard B., Pritchard E., Riat H.S., Ruffier M., Sheppard D., Taylor K., Thormann A., Trevanion S.J., Vullo A., Wilder S.P., Wilson M., Zadissa A., Aken B.L., Birney E., Cunningham F., Harrow J., Herrero J., Hubbard T.J., Kinsella R., Muffato M., Parker A., Spudich G., Yates A., Zerbino D.R. & Searle S.M. (2014) Ensembl 2014. *Nucleic Acids Research* **42**, D749-55.

Forrest R.H., Hickford J.G. & Frampton C.M. (2007) Polymorphism at the ovine beta3-adrenergic receptor locus (ADRB3) and its association with lamb mortality. *Jornal of Animal Science* **85**, 2801-6.

Freking B.A., Murphy S.K., Wylie A.A., Rhodes S.J., Keele J.W., Leymaster K.A., Jirtle R.L. & Smith T.P.L. (2002) Identification of the single base change causing the callipyge muscle hypertrophy phenotype, the only known example of polar overdominance in mammals. *Genome research* **12**, 1496-506.

Fujii J., Otsu K., Zorzato F., de Leon S., Khanna V.K., Weiler J.E., O'Brien P.J. & MacLennan D.H. (1991) Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science* **253**, 448-51.

Galal S., Abdel-Rasoul F., Anous M.R. & Shaat I. (2005) Characterization of Small Ruminant Breeds in West Asia and North Africa. Conference paper, *Iniguez, L.C. (Ed.). 2, ICARDA, Aleppo, Syria* **2**, 141-93.

Galloway S.M., McNatty K.P., Cambridge L.M., Laitinen M.P., Juengel J.L., Jokiranta T.S., McLaren R.J., Luiro K., Dodds K.G., Montgomery G.W., Beattie A.E., Davis G.H. & Ritvos O. (2000) Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. *Nature Genetics* **25**, 279-83.

Gao N., Chen Y., Liu X., Zhao Y., Zhu L., Liu A., Jiang W., Peng X., Zhang C., Tang Z., Li X. & Chen Y. (2019) Weighted single-step GWAS identified candidate genes associated with semen traits in a Duroc boar population. *BMC Genomics* **20**, 797.

García-Gámez E., Gutiérrez-Gil B., Sahana G., Sánchez J.-P., Bayón Y. & Arranz J.-J. (2012a) GWA analysis for milk production traits in dairy sheep and genetic support for a QTN influencing milk protein percentage in the LALBA gene. *PLoS One* **7**, e47782-e.

García-Gámez E., Gutiérrez-Gil B., Sahana G., Sánchez J.-P., Bayón Y. & Arranz J.-J. (2012b) GWA Analysis for Milk Production Traits in Dairy Sheep and Genetic Support for a QTN Influencing Milk Protein Percentage in the LALBA Gene. *PLoS One* **7**, e47782.

Ghafouri-Kesbi F. & Eskandarinasab M. (2018) Heritability of relative growth rate and its relationship with growth-related traits in Afshari sheep. *Gene Reports* **12**, 225-9.

Ghasemi M., Zamani P., Vatankhah M. & Abdoli R. (2019) Genome-wide association study of birth weight in sheep. *Animal* **13**, 1797-803.

Gholibeikifard A., Aminafshar M. & Hosseinpour Mashhadi M. (2013) Polymorphism of IGF-I and ADRB3 Genes And Their Association With Growth Traits In The Iranian Baluchi sheep. *Journal of agricultural science and technology (JAST)* **15,** 1153-62.

Giustina A. & Veldhuis J.D. (1998) Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. *Endocrine Reviews* **19**, 717-97.

Goddard M.E. & Hayes B.J. (2007) Genomic selection. *Journal of Animal Breeding and Genetics* **124**, 323-30.

Goddard M.E. & Hayes B.J. (2009) Mapping genes for complex traits in domestic animals and their use in breeding programmes. *Nature Reviews Genetics* **10**, 381-91.

Grisart B., Coppieters W., Farnir F., Karim L., Ford C., Berzi P., Cambisano N., Mni M., Reid S., Simon P., Spelman R., Georges M. & Snell R. (2002) Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. *Genome Research* **12**, 222-31.

Grobet L., Poncelet D., Royo L.J., Brouwers B., Pirottin D., Michaux C., Ménissier F., Zanotti M., Dunner S. & Georges M. (1998) Molecular definition of an allelic series of mutations disrupting the myostatin function and causing double-muscling in cattle. *Mammalian Genome* **9**, 210-3.

Gurgul A., Jasielczuk I., Ropka-Molik K., Semik-Gurgul E., Pawlina-Tyszko K., Szmatoła T., Szyndler-Nędza M., Bugno-Poniewierska M., Blicharski T., Szulc K., Skrzypczak E. & Krupiński J. (2018) A genome-wide detection of selection signatures in conserved and commercial pig breeds maintained in Poland. *BMC Genetics* **19**, 95.

Haider A.I. (1982) Studies of the performance of some breeds of goats and their crosses under desert condition in Egypt. PhD Thesis, *Faculty of Agriculture*. Alexandria University.

Hajihosseinlo A., Jafari S. & Ajdary M. (2015) The relationship of GH and LEP gene polymorphisms with fat-tail measurements (fat-tail dimensions) in fat-tailed Makooei breed of Iranian sheep. *Advanced Biomedical Research* **4**, 172.

Hanford K.J., Van Vleck L.D. & Snowder G.D. (2002) Estimates of genetic parameters and genetic change for reproduction, weight, and wool characteristics of Columbia sheep. *Journal of Animal Science* **80**, 3086-98.

Hayes B.J., Bowman P.J., Chamberlain A.J. & Goddard M.E. (2009) Invited review: Genomic selection in dairy cattle: Progress and challenges. *Journal of Dairy Science* **92**, 433-43.

Henryon M., Berg P. & Sørensen A.C. (2014) Animal-breeding schemes using genomic information need breeding plans designed to maximise long-term genetic gains. *Livestock Science* **166**, 38-47.

Hiendleder S., Bauersachs S., Boulesteix A., Blum H., Arnold G.J., Fröhlich T. & Wolf E. (2005) Functional genomics: tools for improving farm animal health and welfare. *Scientific and Technical Review* **24**, 355-77.

Hirschhorn J.N. & Daly M.J. (2005) Genome-wide association studies for common diseases and complex traits. *Nature Reviews Genetics* **6**, 95-108.

I S.K., C V.K., G G., Nath S. & A K.T. (2017) Estimates of direct and maternal (co)variance components as well as genetic parameters of growth traits in Nellore sheep. *Tropical Animal Health Production* **49**, 1431-8.

Ibrahim A. & Alsheik S.M. (2016) Exploring polymorphism and effects of the IGFIR gene on productive life in Barki Ewes. *Egyptian Journal of Genetics and Cytology* **44**.

Ibrahim A. & Alsheikh S. (2014) Variation in the Forkhead Box Class O3 (FOXO3) gene and its association with lifespan traits in Barki ewes. *Egyptian Journal of Genetics and Cytology* **43**, 339-52.

Ibrahim A.H.M. (2019) Association of growth performance and body conformational traits with BMP4 gene variation in Barki lambs. *Growth Factors* **37**, 153-63.

Ibrahim A.H.M. (2021) Genetic variants of the BMP2 and GDF9 genes and their associations with reproductive performance traits in Barki ewes. *Small Ruminant Research* **195**, 106302.

Ibtisham F., Zhang L., Xiao M., An L., Ramzan M., Nawab A., Zhao Y., Li G. & Xu Y. (2017) Genomic selection and its application in animal breeding. *Thai Journal of Veterinary Medicine* **47**, 301-10.

Itenge T., Hickford J., Forrest R., McKenzie G.W. & Frampton C. (2010) Association of Variation in the Ovine KAP1.1, KAP1.3 and K33 Genes with Wool Traits. *International Journal of Sheep and Wool Science* **58**(1):1-19.

Jamuna V., Gupta A.K., Chakravarty A.K., A. S., Patil C.S., Kumar M. & Vohra V. (2016) Leptin gene polymorphism in association with Lactation milk yield in Murrah Buffaloes. *Indian Journal of Animal Sciences* **86**, 95-7.

Javanmard A., Mohammadabadi M.R., Zarrigabayi G.E., Gharahedaghi A.A., Nassiry M.R., Javadmansh A. & Asadzadeh N. (2008) Polymorphism within the intron region of the bovine leptin gene in Iranian Sarabi cattle (Iranian Bos taurus). *Russian Journal of Genetics* **44**, 495-7.

Jiang J., Liu L., Gao Y., Shi L., Li Y., Liang W. & Sun D. (2019) Determination of genetic associations between indels in 11 candidate genes and milk composition traits in Chinese Holstein population. *BMC Genetics* **20**, 48.

Jiang L., Liu J., Sun D., Ma P., Ding X., Yu Y. & Zhang Q. (2010) Genome Wide Association Studies for Milk Production Traits in Chinese Holstein Population. *PLoS One* **5**, e13661.

Jiang Y., Xie M., Chen W., Talbot R., Maddox J.F., Faraut T., Wu C., Muzny D.M., Li Y., Zhang W., Stanton J.A., Brauning R., Barris W.C., Hourlier T., Aken B.L., Searle S.M.J., Adelson D.L., Bian C., Cam G.R., Chen Y., Cheng S., DeSilva U., Dixen K., Dong Y., Fan G., Franklin I.R., Fu S., Guan R., Highland M.A., Holder M.E., Huang G., Ingham A.B., Jhangiani S.N., Kalra D., Kovar C.L., Lee S.L., Liu W., Liu X., Lu C., Lv T., Mathew T., McWilliam S., Menzies M., Pan S., Robelin D., Servin B., Townley D., Wang W., Wei B., White S.N., Yang X., Ye C., Yue Y., Zeng P., Zhou Q., Hansen J.B., Kristensen K., Gibbs R.A., Flicek P., Warkup C.C., Jones H.E., Oddy V.H., Nicholas F.W., McEwan J.C., Kijas J., Wang J., Worley K.C., Archibald A.L., Cockett N., Xu X., Wang W. & Dalrymple B.P. (2014) The sheep genome illuminates biology of the rumen and lipid metabolism. *Science* **344**, 1168-73.

Khatib H., Monson R.L., Schutzkus V., Kohl D.M., Rosa G.J. & Rutledge J.J. (2008) Mutations in the STAT5A gene are associated with embryonic survival and milk composition in cattle. *Journal of Dairy Science* **91**, 784-93.

Kijas J.W., Lenstra J.A., Hayes B., Boitard S., Porto Neto L.R., San Cristobal M., Servin B., McCulloch R., Whan V., Gietzen K., Paiva S., Barendse W., Ciani E., Raadsma H., McEwan J. & Dalrymple B. (2012) Genome-wide analysis of the world's sheep breeds reveals high levels of historic mixture and strong recent selection. *PLOS Biology* **10**, e1001258.

Kim E.S., Elbeltagy A.R., Aboul-Naga A.M., Rischkowsky B., Sayre B., Mwacharo J.M. & Rothschild M.F. (2016) Multiple genomic signatures of selection in goats and sheep indigenous to a hot arid environment. *Heredity* **116**, 255-64.

Kim K.S., Larsen N., Short T., Plastow G. & Rothschild M.F. (2000) A missense variant of the porcine melanocortin-4 receptor (MC4R) gene is associated with fatness, growth, and feed intake traits. *Mammalian Genome* **11**, 131-5.

Klungland H., Våge D.I., Gomez-Raya L., Adalsteinsson S. & Lien S. (1995) The role of melanocyte-stimulating hormone (MSH) receptor in bovine coat color determination. *Mammalian Genome* **6**, 636-9.

Knight C.H. (2001) Overview of prolactin's role in farm animal lactation. *Livestock Production Science* **70**, 87-93.

Knol E.F., Nielsen B. & Knap P.W. (2016) Genomic selection in commercial pig breeding. *Animal Frontiers* **6**, 15-22.

Koopaee H.K. & Koshkoiyeh A.E. (2014) SNPs Genotyping Technologies and Their Applications in Farm Animals Breeding Programs: Review. *Brazilian Archives of Biology and Technology* **57**, 87-95.

Kuchtík J. & Dobea I. (2018) Effect of some factors on growth of lambs from crossing between the Improved Wallachian and East Friesian. *Czech Journal of Animal Science* **51**, 54-60.

Lagonigro R., Wiener P., Pilla F., Woolliams J.A. & Williams J.L. (2003) A new mutation in the coding region of the bovine leptin gene associated with feed intake. *Animal Genetics* **34**, 371-4.

Lalit., Malik Z.S., Dalal D.D. & Patil C.S. (2016) Genetics of Growth Traits in Sheep: A Review. *International Journal of Recent Research in Life Sciences* **3**, 12-6.

Li B., Fang L., Null D.J., Hutchison J.L., Connor E.E., VanRaden P.M., VandeHaar M.J., Tempelman R.J., Weigel K.A. & Cole J.B. (2019a) High-density genome-wide association study for residual feed intake in Holstein dairy cattle. *Journal of Dairy Science* **102**, 11067-80.

Li S., Zhou H., Gong H., Zhao F., Wang J., Liu X., Hu J., Luo Y. & Hickford J.G.H. (2019b) Identification of the Ovine Keratin-Associated Protein 21-1 Gene and Its Association with Variation in Wool Traits. *Animals(Basel)* **9**, 450.

Liu J.J., Liang A.X., Campanile G., Plastow G., Zhang C., Wang Z., Salzano A., Gasparrini B., Cassandro M. & Yang L.G. (2018) Genome-wide association studies to identify quantitative trait loci affecting milk production traits in water buffalo. *Journal of Dairy Science* **101**, 433-44.

Liu L., Zhou J., Chen C.J., Zhang J., Wen W., Tian J., Zhang Z. & Gu Y. (2020) GWAS-Based Identification of New Loci for Milk Yield, Fat, and Protein in Holstein Cattle. *Animals (Basel)* **10**.

Liu Z., Yang N., Yan Y., Li G., Liu A., Wu G. & Sun C. (2019) Genome-wide association analysis of egg production performance in chickens across the whole laying period. *BMC Genetics* **20**, 67.

Lopes M.S., Bovenhuis H., van Son M., Nordbø Ø., Grindflek E.H., Knol E.F. & Bastiaansen J.W. (2017) Using markers with large effect in genetic and genomic predictions. *Journal of Animal Science* **95**, 59-71.

Lu Z., Yue Y., Yuan C., Liu J., Chen Z., Niu C., Sun X., Zhu S., Zhao H., Guo T. & Yang B. (2020) Genome-Wide Association Study of Body Weight Traits in Chinese Fine-Wool Sheep. *Animals (Basel)* **10**.

Lund M.S., Roos A.P., Vries A.G., Druet T., Ducrocq V., Fritz S., Guillaume F., Guldbrandtsen B., Liu Z., Reents R., Schrooten C., Seefried F. & Su G. (2011) A common reference population from four European Holstein populations increases reliability of genomic predictions. *Genetic Selection Evolution* **43**, 43.

Lundén A., Marklund S., Gustafsson V. & Andersson L. (2002) A nonsense mutation in the FMO3 gene underlies fishy off-flavor in cow's milk. *Genome Research* **12**, 1885-8.

M-L P., K M. & A N. (2007) Genetic parameters for wool traits in Finnsheep lambs. *Agricultural and Food Science* **16**.

Mahmoud A., Saleh A., Almealamah N., Ayadi M., Matar A., Abou- Tarboush F., Aljumaah R. & Abouheif M. (2014) Polymorphism of leptin gene and its association with milk traits in Najdi sheep. *Journal of Pure and Applied Microbiology* **8**, 2953-9.

Mahrous K.F., Hassanane M.S., Abdel Mordy M., Shafey H.I. & Rushdi H.E. (2015) Polymorphism of some genes associated with meat related traits in Egyptian sheep breeds. *Iranian Journal of Applied Animal Science* **5**, 655-63.

Mahrous K.F., Hassanane M.S., Shafey H.I., Abdel Mordy M. & Rushdi H.E. (2016a) Association between single nucleotide polymorphism in ovine Calpain gene and growth performance in three Egyptian sheep breeds. *Journal of Genetic Engineering and Biotechnology* **14**, 233-40.

Mahrous K.F., Hassanane M.S., Shafey H.I., Abdel Mordy M. & Rushdi H.E. (2016b) Association between single nucleotide polymorphism in ovine Calpain gene and growth performance in three Egyptian sheep breeds. *Journal of Genetic Engineering& Biotechnology* **14**, 233-40.

Maiorano A.M., Lourenco D.L., Tsuruta S., Ospina A.M.T., Stafuzza N.B., Masuda Y., Filho A.E.V., Cyrillo J.N.d.S.G., Curi R.A. & Silva J.A.I.I.d.V. (2018) Assessing genetic architecture and signatures of selection of dual purpose Gir cattle populations using genomic information. *PLoS One* **13**, e0200694.

Mandal A., Neser F.W.C., Rout P.K., Roy R. & Notter D.R. (2006) Estimation of direct and maternal (co)variance components for pre-weaning growth traits in Muzaffarnagari sheep. *Livestock Science* **99**, 79-89.

Manzari Z., Mehrabani-Yeganeh H., Nejati-Javaremi A., Moradi M.H. & Gholizadeh M. (2019) Detecting selection signatures in three Iranian sheep breeds. *Animal Genetics* **50**, 298-302.

Marai I.F.M., Daader A.H. & Bahgat L.B. (2009) Performance traits of purebred Ossimi and Rahmani lambs and their crosses with Finnsheep born under two accelerated mating systems. *Archives in Animal Breeding* **52**, 497-511.

Margawati E.T., Raadsma H.W., Martojo H., Subandriyo & Muladno (2006) Quantitative Trait Loci (QTL) Analysis for Production Traits of Birth Weight and Weight 360 days in Backcross Sheep. *HAYATI Journal of Biosciences* **13**, 31-5.

Martin C.R., Ling P.-R. & Blackburn G.L. (2016) Review of Infant Feeding: Key Features of Breast Milk and Infant Formula. *Nutrients* **8**, 279.

Mastrangelo S., Tolone M., Sardina M.T., Sottile G., Sutera A.M., Di Gerlando R. & Portolano B. (2017) Genome-wide scan for runs of homozygosity identifies potential candidate genes associated with local adaptation in Valle del Belice sheep. *Genetics Selection Evolution* **49**, 84.

Matika O., Riggio V., Anselme-Moizan M., Law A.S., Pong-Wong R., Archibald A.L. & Bishop S.C. (2016) Genome-wide association reveals QTL for growth, bone and in vivo carcass traits as assessed by computed tomography in Scottish Blackface lambs. *Genetics Selection Evolution* **48**, 11.

Mayo K.E. (1992) Molecular cloning and expression of a pituitary-specific receptor for growth hormone-releasing hormone. *Molecular Endocrinology* **6**, 1734-44.

McNatty K.P., Juengel J.L., Wilson T., Galloway S.M. & Davis G.H. (2001) Genetic mutations influencing ovulation rate in sheep. *Reproduction, Fertility and Development* **13**, 549-55.

McRae K.M., McEwan J.C., Dodds K.G. & Gemmell N.J. (2014) Signatures of selection in sheep bred for resistance or susceptibility to gastrointestinal nematodes. *BMC Genomics* **15**, 637.

Medrano J.F. & Aguilar-Cordova E. (1990) Polymerase chain reaction amplification of bovine β-lactoglobulin genomic sequences and identification of genetic variants by RFLP analysis. *Animal Biotechnology* **1**, 73-7.

Menzies K.K., Lefèvre C., Macmillan K.L. & Nicholas K.R. (2009) Insulin regulates milk protein synthesis at multiple levels in the bovine mammary gland. *Functional & Integrative Genomics* **9**, 197-217.

Meuwissen T. & Goddard M. (2010) Accurate Prediction of Genetic Values for Complex Traits by Whole-Genome Resequencing. *Genetics* **185**, 623.

Milan D., Jeon J.T., Looft C., Amarger V., Robic A., Thelander M., Rogel-Gaillard C., Paul S., Iannuccelli N., Rask L., Ronne H., Lundström K., Reinsch N., Gellin J., Kalm E., Roy P.L., Chardon P. & Andersson L. (2000) A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. *Science* **288**, 1248-51.

Milan P., Cvijan M., Dragana R. & Miroslav Ž. (2005) Genetic principles relating to improvement of milk yield in sheep and goats. *Biotechnology in Animal Husbandry* **21**, 73-8.

Moioli B., D'Andrea M. & Pilla F. (2007) Candidate genes affecting sheep and goat milk quality. *Small Ruminant Research* **68**, 179-92.

Moioli B., Scatà M.C., Steri R., Napolitano F. & Catillo G. (2013) Signatures of selection identify loci associated with milk yield in sheep. *BMC Genetics* **14**, 76.

Morris S.T. (2009) Economics of sheep production. *Small Ruminant Research* **86**, 59-62.

Nassiry M.R., Moussavi A.H., Alashawkany A.R. & Ghovati S. (2007) Leptin gene polymorphism in Iranian native Golpayegani and Taleshi cows. *Pakistan journal of biological sciences : PJBS* **10**, 3738-41.

Nix J. (1988) The economics of sheep production. *British Veterinary Journal* **144**, 426-433.

Orford M., Tzamaloukas O., Papachristoforou C. & Miltiadou D. (2010) Technical note: A simplified PCR-based assay for the characterization of two prolactin variants that affect milk traits in sheep breeds. *Journal of Dairy Science* **93**, 5996-9.

Osorio J.S., Lohakare J. & Bionaz M. (2016) Biosynthesis of milk fat, protein, and lactose: roles of transcriptional and posttranscriptional regulation. *Physiological Genomics* **48**, 231-56.

Othman O., Alam S.S., HebaA.M.Abd E. & Abd-El O.M. (2015) Genotyping of Growth Hormone Gene in Egyptian Small Ruminant Breeds. *Biotechnology(faisalabad)* **14**, 136-41.

Othmane M.H., Carriedo J.A., San Primitivo F. & De La Fuente L.F. (2002) Genetic parameters for lactation traits of milking ewes: protein content and composition, fat, somatic cells and individual laboratory cheese yield. *Genetics Selection Evolution* **34**, 581-96.

Ozmen O., Kul S. & Unal E.O. (2014) Polymorphism of sheep POU1F1 gene exon 6 and 3'UTR region and their association with milk production traits. *Iranian Journal of Veterinary Research* **15**, 331-5.

Papaioannou V.E. (2001) T-box genes in development: from hydra to humans. *International Review of Cytology* **207**, 1-70.

Petrovic M.P., Caro Petrovic V., Ruzic Muslic D., Maksimovic N., Ilic Z., Milosevic B. & Stojkovic J. (2012) Some important factors affecting fertility in sheep. *Biotechnology in Animal Husbandry* **28**, 517-528.

Popovic N. (2018) Economic aspects of sheep farming on the family farm models in the hilly-mountain regions of Serbia. *Economics of Agriculture* **65**, 1395-1410.

Porto-Neto L.R., Lee S.H., Lee H.K. & Gondro C. (2013) Detection of signatures of selection using Fst. *Methods in Molecular Biology* **1019**, 423-36.

Ptáček M., Ducháček J., Stádník L., Hakl J. & Fantová M. (2017) Analysis of multivariate relations among birth weight, survivability traits, growth performance, and some important factors in Suffolk lambs. *Archives in Animal Breeding* **60**, 43-50.

Purfield D.C., McParland S., Wall E. & Berry D.P. (2017) The distribution of runs of homozygosity and selection signatures in six commercial meat sheep breeds. *PLoS One* **12**, e0176780.

Qanbari S. & Simianer H. (2014) Mapping signatures of positive selection in the genome of livestock. *Livestock Science* **166**, 133-43.

Rabier C.-E., Barre P., Asp T., Charmet G. & Mangin B. (2016) On the Accuracy of Genomic Selection. *PLoS One* **11**, e0156086-e.

Ramos A.M., Matos C.A.P., Russo-Almeida P.A., Bettencourt C.M.V., Matos J., Martins A., Pinheiro C. & Rangel-Figueiredo T. (2009) Candidate genes for milk production traits in Portuguese dairy sheep. *Small Ruminant Research* **82**, 117-21.

Rincón G. & Medrano J.F. (2003) Single nucleotide polymorphism genotyping of bovine milk protein genes using the tetra-primer ARMS-PCR. *Journal of Animal Breeding and Genetics* **120**, 331-7.

Rohrer G.A. & Nonneman D.J. (2017) Genetic analysis of teat number in pigs reveals some developmental pathways independent of vertebra number and several loci which only affect a specific side. *Genetics Selection Evolution* **49**, 4.

Rothschild M. & Sölkner M. (1997) Candidate gene analysis to detect genes controlling traits of economic importance in domestic live-stock. *Probe* **8**, 13-20.

Sadeghi S., Hajihosseinlo A. & Bohlouli M. (2014) Haplotype association of ovine leptin gene on breeding value of body measurements in Makooei sheep breed. *Biotechnology in Animal Husbandry* **30**, 233-42.

Sahu A.R., Jeichitra V., Rajendran R. & Raja A. (2017) Polymorphism in exon 3 of myostatin (MSTN) gene and its association with growth traits in Indian sheep breeds. *Small Ruminant Research* **149**, 81-4.

Sallam A.M. (2019a) Risk factors and genetic analysis of pre-weaning mortality in Barki lambs. *Livestock Science* **230**, 103818.

Sallam A.M. (2020) A missense mutation in the coding region of the toll-like receptor 4 (TLR4) gene affects milk traits in Barki sheep. *Asian-Australasian Journal of Animal Sciences* **34**, 489-498.

Sallam A.M., Gad-Allah A.A. & Al-Bitar E.M. (2020) Association analysis of the ovine KAP6-1 gene and wool traits in Barki sheep. *Animal Biotechnology*, 1-7.

Sallam A.M., Galal S., Rashed M.A. & Alsheikh S.M. (2012) Genetic diversity in Barki sheep breed in its native tract in Egypt. *Egyptian Journal of Animal Production* **49**, 19-28.

Sallam A.M., Ibrahim A.H. & Alsheikh S.M. (2019) Estimation of genetic parameters and variance components of pre-weaning growth traits in Barki lambs. *Small Ruminant Research* **173**, 94-100.

Sallam A.M.I., A.H.; Alsheikh, S.M. (2019b) Estimation of genetic parameters and variance components of pre-weaning growth traits in Barki lambs. *Small Ruminant Research* **173**, 94 - 100.

Saravanan K.A., Panigrahi M., Kumar H., Bhushan B., Dutt T. & Mishra B.P. (2020) Selection signatures in livestock genome: A review of concepts, approaches and applications. *Livestock Science* **241**, 104257.

Schenkel F.S., Miller S.P., Ye X., Moore S.S., Nkrumah J.D., Li C., Yu J., Mandell I.B., Wilton J.W. & Williams J.L. (2005) Association of single nucleotide polymorphisms in the leptin gene with carcass and meat quality traits of beef cattle. *Journal of Animal Science* **83**, 2009-20.

Schulz-Streeck T., Ogutu J.O., Karaman Z., Knaak C. & Piepho H.P. (2012) Genomic Selection using Multiple Populations. *Crop Science* **52**, 2453-61.

Seitz J.J., Schmutz S.M., Thue T.D. & Buchanan F.C. (1999) A missense mutation in the bovine MGF gene is associated with the roan phenotype in Belgian Blue and Shorthorn cattle. *Mammalian Genome* **10**, 710-2.

Shehata M., Ismail I. & Ibrahim A. (2014) Variation in exon 10 of the ovine Calpain3 gene and its association with growth and carcass traits in Egyptian Barki lambs. *Egyptian Journal of Genetics and Cytology* **43**, 231-40.

Shikano T., Chiyokubo T. & Taniguchi N. (2001) Temporal changes in allele frequency, genetic variation and inbreeding depression in small populations of the guppy, Poecilia reticulata. *Heredity* **86**, 153-60.

Shojaei M., Mohammad Abadi M., Asadi Fozi M., Dayani O., Khezri A. & Akhondi M. (2010) Association of growth trait and Leptin gene polymorphism in Kermani sheep. *Journal of Cell and Molecular Research* **2**, 67-73.

Singh D., Kumar R., Pander B.L., Dhaka S.S. & Singh S. (2006) Genetic Parameters of Growth Traits in Crossbred Sheep. *Asian-Australasian Journal Animal Sciences* **19**, 1390-3.

Staiger E.A., Thonney M.L., Buchanan J.W., Rogers E.R., Oltenacu P.A. & Mateescu R.G. (2010) Effect of prolactin, β-lactoglobulin, and κ-casein genotype on milk yield in East Friesian sheep. *Journal of Dairy Science* **93**, 1736-42.

Tanksley S.D. (1983) Molecular markers in plant breeding. *Plant Molecular Biology Reporter* **1**, 3-8.

Tetens J.L., Drögemüller C., Thaller G. & Tetens J. (2014) DNA-based identification of novel ovine milk protein gene variants. *Small Ruminant Research* **121**, 225-31.

Thomson B.C., P.D. Muir and N.B. Smith (2004) Litter size, lamb survival, birth and twelve week weight in lambs born to cross-bred ewes. *Journal of New Zealand Grasslands* **66**, 233-7.

Tusell L., Pérez-Rodríguez P., Forni S., Wu X.-L. & Gianola D. (2013) Genome-enabled methods for predicting litter size in pigs: a comparison. *Animal : an international journal of animal bioscience* **7 11**, 1739-49.

Ullah F., Jamal S.M., Zhou H. & Hickford J.G.H. (2020) Variation in the KRTAP6-3 gene and its association with wool characteristics in Pakistani sheep breeds and breed-crosses. *Tropical Animal Health and Production* **52**, 3035-43.

Vacca G.M., Dettori M.L., Balia F., Luridiana S., Mura M.C., Carcangiu V. & Pazzola M. (2013) Sequence polymorphisms at the growth hormone GH1/GH2-N and GH2-Z gene copies and their relationship with dairy traits in domestic sheep (Ovis aries). *Molecular Biology Reports* **40**, 5285-94.

Van Tassell C.P., Ashwell M.S. & Sonstegard T.S. (2000) Detection of putative loci affecting milk, health, and conformation traits in a US Holstein population using 105 microsatellite markers. *Journal of Dairy Science* **83**, 1865-72.

Wade C.M., Giulotto E., Sigurdsson S., Zoli M., Gnerre S., Imsland F., Lear T.L., Adelson D.L., Bailey E., Bellone R.R., Blöcker H., Distl O., Edgar R.C., Garber M., Leeb T., Mauceli E., MacLeod J.N., Penedo M.C.T., Raison J.M., Sharpe T., Vogel J., Andersson L., Antczak D.F., Biagi T., Binns M.M., Chowdhary B.P., Coleman S.J., Della Valle G., Fryc S., Guérin G., Hasegawa T., Hill E.W., Jurka J., Kiialainen A., Lindgren G., Liu J., Magnani E., Mickelson J.R., Murray J., Nergadze S.G., Onofrio R., Pedroni S., Piras M.F., Raudsepp T., Rocchi M., Røed K.H., Ryder O.A., Searle S., Skow L., Swinburne J.E., Syvänen A.C., Tozaki T., Valberg S.J., Vaudin M., White J.R., Zody M.C., Broad Institute Genome Sequencing P., Broad Institute Whole Genome Assembly T., Lander E.S. & Lindblad-Toh K. (2009) Genome sequence, comparative analysis, and population genetics of the domestic horse. *Science (New York, N.Y.)* **326**, 865-7.

Wakao H., Gouilleux F. & Groner B. (1994) Mammary gland factor (MGF) is a novel member of the cytokine regulated transcription factor gene family and confers the prolactin response. *Embo journal* **13**, 2182-91.

Wakchaure R., Ganguly S., Praveen P., Kumar A., Sharma S. & Mahajan T. (2015) Marker Assisted Selection (MAS) in Animal Breeding: A Review. *Journal of Drug Metabolism and Toxicology* **6**, 1-4.

Wei C.H.L., C.S. (2014) Encyclopedia of modern sheep production technology. *Agriculture Press: Beijing, China*, 70 -7.

Weir B.S. & Cockerham C.C. (1984) Estimating f-statistics for the analysis of population structure. *Evolution* **38**, 1358-70.

Wiggans G.R., Sonstegard T.S., VanRaden P.M., Matukumalli L.K., Schnabel R.D., Taylor J.F., Schenkel F.S. & Van Tassell C.P. (2009) Selection of single-nucleotide polymorphisms and quality of genotypes used in genomic evaluation of dairy cattle in the United States and Canada. *Journal of Dairy Science* **92**, 3431-6.

Wiggans G.R., VanRaden P.M. & Cooper T.A. (2011) The genomic evaluation system in the United States: Past, present, future. *Journal of Dairy Science* **94**, 3202-11.

Wilson T., Wu X.Y., Juengel J.L., Ross I.K., Lumsden J.M., Lord E.A., Dodds K.G., Walling G.A., McEwan J.C., O'Connell A.R., McNatty K.P. & Montgomery G.W. (2001) Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. *Biology of Reproduction* **64**, 1225-35.

Yang G., Forrest R., Zhou H., Hodge S. & Hickford J. (2014) Genetic variation in the ovine uncoupling protein 1 gene: association with carcass traits in New Zealand (NZ) Romney sheep, but no association with growth traits in either NZ Romney or NZ Suffolk sheep. *Journal of Animal Breeding and Genetics* **131**, 437-44.

Yuan Z., Li W., Li F. & Yue X. (2019) Selection signature analysis reveals genes underlying sheep milking performance. *Archives in Animal Breeding* **62**, 501-8.

Yurchenko A.A., Daetwyler H.D., Yudin N., Schnabel R.D., Vander Jagt C.J., Soloshenko V., Lhasaranov B., Popov R., Taylor J.F. & Larkin D.M. (2018) Scans for signatures of selection in Russian cattle breed genomes reveal new candidate genes for environmental adaptation and acclimation. *Scientific Reports* **8**, 12984.

Zamani P. & Mohammadi H. (2008) Comparison of different models for estimation of genetic parameters of early growth traits in the Mehraban sheep. *Journal of Animal Breeding and Genetics* **125**, 29-34.

Zervas G. & Tsiplakou E. (2011) The effect of feeding systems on the characteristics of products from small ruminants. *Small Ruminant Research* **101**, 140-9.

Zhang C., Yang L. & Shen Z. (2008) Variance components and genetic parameters for weight and size at birth in the Boer goat. *Livestock Science* **115**, 73-9.

Zhang L., Liu J., Zhao F., Ren H., Xu L., Lu J., Zhang S., Zhang X., Wei C., Lu G., Zheng Y. & Du L. (2013) Genome-Wide Association Studies for Growth and Meat Production Traits in Sheep. *PLoS One* **8**, e66569.

Zhang L., Ma X., Xuan J., Wang H., Yuan Z., Wu M., Liu R., Zhu C., Wei C., Zhao F. & Du L. (2016) Identification of MEF2B and TRHDE gene polymorphisms related to growth traits in a new Ujumqin sheep population. *PLoS One* **11**, e0159504.

Zhao F., McParland S., Kearney F., Du L. & Berry D.P. (2015) Detection of selection signatures in dairy and beef cattle using high-density genomic information. *Genetics Selection Evolution* **47**, 49.

Zhou J., Liu L., Chen C.J., Zhang M., Lu X., Zhang Z., Huang X. & Shi Y. (2019) Genome-wide association study of milk and reproductive traits in dual-purpose Xinjiang Brown cattle. *BMC Genomics* **20**, 827.

Zieba D.A., Amstalden M., Morton S., Gallino J.L., Edwards J.F., Harms P.G. & Williams G.L. (2003) Effects of leptin on basal and GHRH-stimulated GH secretion from the bovine adenohypophysis are dependent upon nutritional status. *Journal of Endocrinology* **178**, 83-9.

Zimin A.V., Delcher A.L., Florea L., Kelley D.R., Schatz M.C., Puiu D., Hanrahan F., Pertea G., Van Tassell C.P., Sonstegard T.S., Marçais G., Roberts M., Subramanian P., Yorke J.A. & Salzberg S.L. (2009) A whole-genome assembly of the domestic cow, Bos taurus. *Genome Biology* **10**, R42.

Zygoyiannis D. (2006) Sheep production in the world and in Greece. *Small Ruminant Research* **62**, 143-7.

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Curriculum Vitae

Ibrahim Abousoliman

Date of birth: 22.08.1985

Place of birth: Sharkia governorate, Egypt

Nationality: Egyptian

Education

2019 – 2021	**PhD student**
	Institute of Genome Biology, FBN, Dummerstorf, Germany
	Faculty of Agricultural and Environmental Sciences, University of Rostock
2015 – 2018	**PhD student**
	Faculty of Agriculture, Ain Shams University, Egypt
2012 – 2014	**Master degree** in Agricultural sciences (Animal breeding)
	Faculty of Agriculture, Ain Shams University, Egypt
2003 – 2006	**Bachelor degree** in Agricultural sciences (Animal production)
	Faculty of Agriculture, Zagazig University, Egypt

Work

2015 – 2018	Assistant researcher, Desert Research Center, Egypt
2010 – 2014	Researcher assistant, Desert Research Center, Egypt
2007 – 2009	Animal production Engineer, Desert Research Center, Egypt

Awards

2018	Joint supervision scholarship, Missions sector, Ministry of Higher Education, Egypt

Languages

English: B2 level (IELTS 6.0)

Arabic: native speaker

Training

1. Healthy welfare of desert animals and poultry in the Egyptian deserts, November 30 - December 4, **2014**, Desert Research Center, Cairo, Egypt.

2. Production and husbandry of sheep and camels under desert conditions, January 20-23, **2013**, Desert Research Center, Cairo, Egypt.
3. Conservation of cattle genetic resources, July 8- 18, **2012**, Ain Shams University, Cairo, Egypt & Cordoba University, Spain.
4. Small Ruminant welfare under desert conditions, May, **2008**, Desert Research Center, Cairo, Egypt.

List of Publications and conferences contributions

1. **Abousoliman, I**.; Reyer, H.; Oster, M.; Murani, E.; Mohamed, I.; Wimmers, K. Genome-wide analysis for early growth-related traits of the locally adapted Egyptian Barki Sheep. Genes. In review.
2. **Abousoliman, I**.; Reyer, H.; Oster, M.; Murani, E.; Mohamed, I.; Wimmers, K. Genome-Wide SNP Analysis for Milk Performance Traits in Indigenous Sheep: A Case Study in the Egyptian Barki Sheep. Animals **2021**, 11, 1671.
3. **Abousoliman, I**.; Reyer, H.; Oster, M.; Muráni, E.; Mourad, M.; Rashed, M.A.; Mohamed, I.;Wimmers, K. Analysis of candidate genes for growth and milk performance traits in the Egyptian Barki sheep. Animals **2020**, 10, 197.
4. Ismail, M. Ismail., Mourad, M., Rashed, M.A and **Ibrahim Abousoliman** (**2017**) Myostatin gene sequencing and its association with growth performance of Maghrabi camel breed. Egyptian J. Desert Res., 67, No. 1, 115-124
5. **Ibrahim Abousoliman**., Mourad, M., Rashed, M.A and Ismail, I.M. (**2015**) Molecular characterization of camel growth hormone gene in Maghraby camel breed. Animal Science Reporter, volume 9, issue 2, 50:55.
6. **Abousoliman, I**.; Reyer, H.; Oster, M.; Muráni, E.; Mourad, M.; Rashed, M.A.; Mohamed, I.;Wimmers, K. Analysis of candidate genes for growth and milk performance traits in the Egyptian Barki sheep. The International Congress on the Breeding of Sheep and Goats. 15-16 October 2020. WCCB, Bonn, Germany.
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Declaration:

I hereby, declare under oath that I have completed the work submitted here independently and have composed it without outside assistance. Furthermore, I have not used anything other than the resources and sources stated and where I have taken sections from these works in terms of content or text, I have identified this appropriately.

Dummerstorf, ……

Ibrahim Abousoliman