T.C.
ERCİYES UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF AGRICULTRAL SCIENCE AND TECHNOLOGIES

GENETIC ANALYSIS OF GASTROINTESTINAL PARASITE RESISTANCE AND BLOOD COMPOSITION TRAITS IN AKKARAMAN LAMBS

By
Yunus ARZIK

Supervisor
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Co-supervisor
Dr. Stephen N. WHITE

PhD Thesis

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KAYSERİ

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This study was funded by Scientific Research Projects Department of Erciyes University with Project ID: FDK-2020-10807

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SCIENTIFIC ETHICS CONFORMITY

I declare that all information in this study has been obtained in accordance with academic and ethical rules. At the same time, as required by these rules and behaviors, I declare that I have fully cited and referenced all materials and results that are not at the core of this study.

Yunus ARZIK

signature

SUITABILITY FOR INSTRUCTION GUIDE

The thesis named **"Genetic analysis of gastrointestinal parasite resistance and blood composition traits in Akkaraman lambs"** has been prepared in accordance with the Erciyes University Graduate School of Natural and Applied Sciences Thesis Preparation and Writing Guide.

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September 2022, KAYSERİ

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ABSTRACT

Genome-wide association studies (GWAS) have been used as an effective tool to understand the genetics of complex health and adaptation traits such as gastrointestinal parasite (GIP) resistance and blood parameters with the advancement of genotyping technologies. The knowledge obtained from the GWAS can be applied to the field of animal breeding for the genetic improvement of these complex traits. The aim of this study was understanding genetics of gastrointestinal parasite (nematodes, *Moniezia spp*., *Eimeria spp*.) resistance and blood parameters in Akkaraman sheep by implementing specific objectives: i) estimating genomic heritability of the traits, ii) examining the genetic diversity for GIP resistance, iii) investigating genomic regions responsible for GIP resistance and blood parameters by conducting GWAS. The samples from 478 animals were genotyped using the Axiom 50K Ovine Genotyping Array. All statistical analysis was performed using various packages of R statistical environment. Genomic heritability estimates were ranged from 0.00 to 0.34 for parasite resistance traits and 0.00 to 0.55 for blood parameters. These results indicated that measured phenotypes have a low to moderate heritability estimates. The analysis of observed (H_E), expected (H_O) heterozygosity and F_{ST} (0.005-0.023) showed no genetic diversity in terms of parasite resistance of animals. A total of 2 genome- and 10 chromosome-wide significant SNPs for parasite resistance traits and a total of 7 genome- and 13 chromosome-wide significant SNPs for blood parameters were detected as a result of GWAS. Accordingly, *NELL1*, *TENM3*, *ST6GALNAC3*, *ATRNL1*, *HIPK1*, *SYT1*, *ALK, ZNF596*, *TMCO5A*, *PTH2R*, *LARGE1* and *SCG2* gens were subjected as candidate for parasite resistance traits and *SCN7A*, *SCN9A*, *MYADM-like*,

CCDC67, *ITGA9*, *MGAT5*, *SLC19A1, AMPH*, *NTRK2*, *MSRA, SLC35F3, HDAC4*, *SIRT6*, *CREB3L3*, *OSBPL8*, *ZDHHC17*, *CSRP2, NAV3* and *E2F7* genes as well as three undefined regions (*LOC101117887*, *LOC106991526* and *LOC105608461*) were suggested as candidates for blood parameters. Majority of these candidate genes were involved in several basic biological processes that are essential and important for immune system function and cellular growth; specific functions include inflammatory response, erythropoiesis, cellular transport, cell apoptosis, cell differentiation, histone deacetylation and endocytosis. Two most significant SNP (i.e., *rs424122039* and *rs402657016*) for HCT, MCH, and MCHC was found within a genomic region mainly populated by the *MYADM-like* gene family. This region was previously suggested to be under historical selection pressure in many sheep breeds from various parts of the world. These results have implications on animal breeding program studies due to the effect that the genetic background has on parasite resistance and blood parameters, which underlying many productive, health and wellness related traits.

Keywords: Akkaraman sheep, parasite resistance, blood parameters, GWAS, heritability

AKKARAMAN KUZULARDA PARAZİT DİRENCİ VE KAN BİLEŞENLERİ ÖZELLİKLERİNİN GENETİK ANALİZİ

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Erciyes Üniversitesi, Fen Bilimleri Enstitüsü

Doktora Tezi, Eylül 2022

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ÖZET

Genotipleme teknolojilerinin gelişimiyle birlikte, genom-boyu ilişkilendirme çalışmaları (GWAS) parazit direnci ve kan parametreleri gibi kompleks adaptasyon özelliklerinin genetiğinin anlaşılmasında etkin bir araç olarak kullanılmaktadır. GWAS çalışmalarından elde edilen bilgilerin hayvan ıslahında uygulanmasıyla bu özelliklerin genetik iyileştirmelerini mümkün kılmaktadır. Bu çalışmanın amacı, Akkaraman koyunlarında parazit direnci ve kan parametrelerinin genetiğini araştırmaktır. Bu amaçla, 6 parazit direnci ve 18 kan parametresi özellikleri için genomik kalıtım derecesi tahmini ve genom-boyu ilişkilendirme analizi yapılmıştır. Toplamda 478 hayvan Axiom 50K Ovine Genotyping Array kullanılarak genotiplendirilmiştir. İstatistiksel analizler R programında çeşitli paketler kullanılarak yapılmıştır. Parazit direnci özellikleri için genomik kalıtım derecesi tahminleri 0,00 ile 0,34 arasında değişirken, kan parametreleri için ise 0,00 ile 0,55 arasında bulunmuştur. Bu değerler özelliklerin düşük ve orta derecede bir kalıtım derecesine sahip olduğunu göstermektedir. Gözlenen (H_O), beklenen (H_E) heterozigotluk ve F_{ST} (0.005-0.023) analizleri parazit direnci yönünden popülasyonda genetik çeşitlilik olmadığını göstermiştir. GWAS sonucunda parazit direnç özellikleri için toplam 2 genom ve 10 kromozom-boyu önemli SNP ve kan parametreleri için toplam 7 genom ve 13 kromozom-boyu önemli SNP tespit edilmiştir. Yapılan fonksiyonel anatasyonlar sonucunda bu SNP'lerin parazit direnci özellikleri için *NELL1, TENM3, ST6GALNAC3, ATRNL1, HIPK1, SYT1, ALK, ZNF596, TMCO5A, PTH2R, LARGE1* ve *SCG2* genleri için aday olarak gösterilmiştir. Kan parametreleri için ise, *SCN7A, SCN9A, MYADM-like, CCDC67, ITGA9, SLC19AT1,*

AMPH, NTRK2, MSRA, SLC35F3, HDAC4, SIRT6, CREB3L3, OSBPL8, ZDHHC17, CSRP2, NAV3 ve *E2F7* genlerinin ile üç tane tanımlanmamış bölge (*LOC101117887, LOC106991526* ve *LOC105608461*) aday olarak önerilmiştir. Tanımlanan genlerin çoğu, inflamatuar yanıt, immun yanıt, hücre apoptozu, hücresel taşıma, histon deasetilasyonu, hücre farklılaşması, eritropoez ve endositoz gibi bağışıklık sistemi ve hücresel büyüme için gerekli olan temel biyolojik süreçlerde yer aldığı görülmüştür. HCT, MCH ve MCHC için en önemli olan SNP'ler (*rs424122039* ve *rs402657016*), çoğunlukla *MYADM-like* gen ailesine ait genlerin bulunduğu bir genomik bölgede tespit edilmiştir. Bu bölgenin daha önce dünyanın çeşitli bölgelerinden gelen birçok koyun ırkında seçilim altında olduğu öne sürülmüştür. Bu sonuçlar, çiftlik hayvanlarında üreme, sağlık ve adaptasyon gibi birçok ekonomik özellikler için gösterge özelliklerinden olan parazit direnci ve kan parametreleri özelliklerinin genetik arka planın anlamasına katkı sağlayacaktır. Dolayısı ile bu özelliklerin çiftlik hayvanlarında uygulanan ıslah programlarında daha etkin kullanılmasına katkı sunacaktır.

Anahtar Kelimeler: GWAS, Akkaraman, parazit direnci, kan parametreleri, kalıtım derecesi

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A	Numeric relationship matrix
ANOVA	Analysis of variances
CBC	Complete blood counts
CocOPG3	Fecal oocyst counts of *Eimaria spp*. at 3-month age
CocOPG6	Fecal oocyst counts of *Eimaria spp*. at 6-month age
EBV	Estimated breeding value
FAO	Food and Agriculture Organization of the United Nations
FEC	Fecal egg counts
FOC	Fecal oocyst count
F_{ST}	Fixation index (inbreeding coefficient of sub-population)
G	Genomic relationship matrix
GEBV	Genomic estimated breeding value
GIP	Gastrointestinal parasites
GIS	Gastrointestinal system
GPCRs	G-Protein-Coupled Receptors
GWAS	Genome-wide association study
HWE	Hardy Weinberg equilibrium
H_O	Observed heterozygosity
H_E	Expected heterozygosity
LD	Linkage disequilibrium
LMM	Linear mixed model
MonFEC3	Fecal eggs counts of *Moniezia spp*. at 3-month age
MonFEC6	Fecal eggs counts of *Moniezia spp*. at 6-month age
NemFEC3	Fecal eggs counts of nematodes. at 3-month age
NemFEC6	Fecal eggs counts of nematodes. at 6-month age
REML	Restricted maximum likelihood
SNP	Single nucleotide polymorphism
QC	Quality control
QTL	Quantitative trait loci

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INTRODUCTION

Among the domesticated farm animals, sheep (*Ovis aries*) were one of the earliest species (Wild, 1984). It has spread to many different geographical settings and have dispersed to many different continents. Sheep are also one of the most preferred livestock animals by humans as it is a source of mutton, milk and animal fiber for textile industry. It is estimated that the total number of sheep in the world is around 1 billion (Morris, 2009) and sheep production contributes 3% of the total red meat production and 1.5% of the textile fiber marketing in the world. The average per individual meat consumption in the world, which is 41.6 kg, is met by approximately 2.5 kg of mutton. (FAO, 2022). According to TUIK 2020 reports, there are approximately 45 million sheep, 41 million of which are domestic breeds, in Türkiye, and about 20% of the total red meat (about 1.9 million tons) is obtained from sheep production (TUIK, 2020).

Türkiye is a very rich country in terms of animal genetic resources, especially different sheep breeds. According to the 2017 report of the Food and Agriculture Organization-Domestic Animal Diversity (DAD-IS FAO), there are 40 different sheep breeds registered in Türkiye (DAD-IS, 2017). Genetic diversity in farm animals has emerged as a result of adaptation to their region and different breeding practice by humans for long history. Native sheep breeds are important gene resources since they have been well adapted to their environments for centuries and have the best gene-environment interaction (Pilling and Hoffmann, 2011). They are be able to pass on this valuable genetic resource to future generations in a sustainable manner (Hoffmann, 2011). Their significant adaptive capacities built up for thousand years, they have an efficient gene-environment interaction regarding adaptation ability (Hoffmann, 2010).

Among domestic sheep breeds in Türkiye, the Akkaraman (White Karaman) sheep is one of the most well-known and widely used fat-tailed breeds (Yalcin, 1986; Ünal, 2002). It is extensively raised in the Central Anatolia and neighboring regions, and constitutes approximately 45% of the total number of domestic sheep (Ozmen et al., 2020). Additionally, it is one of the most popular and well-known farm animals, by reason of its advantages that make it well-suited to harsh conditions and the utilization of poor pastures. In particular, the fat-tail of Akkaraman sheep provides high survival potential especially during poor feeding periods (Behrem, 2021; Yilmaz and Wilson, 2012). Many abilities that it has gained over the centuries, like many domestic sheep breeds, enables Akkarman sheep to gain easy tolerance and resistance against changing environmental conditions (i.e., drought and harsh climate conditions that may occur as a result of global warming) or new pathogens and parasites that may arise.

Gastrointestinal parasite (GIP) infections are among the most important health problems, particularly in pasture-based sheep and goats production systems (Coles et al., 2005). Tapeworm (*Moniezia spp.*), coccidian (*Eimeria spp.*) and nematode (*Trichostrongylus spp., Teladorsagia circumcincta, Haemonchus contortus, Cooperia spp., Oesophagostomum spp., Chabertia ovina, Bunostomum trigonocephalum* and *Nematodirus spp.*) parasites are among the most common parasites in sheep in Türkiye. GIP infections, together with many clinical symptoms (i.e., weight loss, depression), adversely affect productivity and quality of welfare in animals. Especially in young animals at the early growth stage, GIP causes serious damage to both the farm economy and the national economy, with the effects such as growth retardation. However, developments in the field of molecular genetics have added a new method to parasitic control strategies. More resistant herds can be formed by identifying parasite resistance markers, providing information about their genetics and applying this information to animal breeding (Heringstad, 2010). Simulation studies have shown that the desired genetic improvement for parasite resistance can be achieved by selecting animals with low faecal egg counts in 20 years (Kemper et al., 2013).

On the other hands, the examination of blood parameters is widely used practice to obtain information on the general health condition of animals by performing complete

blood count (CBC) analyses. The increase and decrease in the number of blood cells (erythrocyte, white blood cells, etc.) provide diagnostic advantages, therefore, it is one of the most common methods to investigate of many diseases by veterinarians (Kelly, 1984). Furthermore, blood cells have many essential roles in animals such as survival, performance and resistance to diseases (Müller and Brem, 1991).

Finally, considering the economic losses caused by GIP infections, the efforts against parasitic diseases will be among the main problems in sustainable small ruminant production systems in the future. More specifically, parasitic resistance to anti-parasite drugs, the threat posed by the use of more chemical drugs on public health, and the risks that come with global warming will keep parasite control on the agenda in the small sheep production. On the other hand, the developments in the field of molecular genetics and the possibilities offered to animal breeding area have enabled animal production industry to be seen as an alternative method in the control of parasites. Therefore, more studies on host genetic resistance to the parasite will accelerate the application of these traits in animal breeding programs.

CHAPTER 1

BACKGROUND

1.1. BACKGROUND

1.1.1. Problem Statement

The rapid increase in the human population of the world subsequently the globally increase of food demand encourages agricultural and animal production systems to be sustainable and profitable, as in many production sectors (Bell et al., 2014; DESA, 2022). In this context, it is aimed to produce high quality products with less costs in animal production. Gastrointestinal nematode, tapeworm and coccidian infections are among the most important health problems that cause economic damage, especially in small ruminants raised under pasture conditions (Coles et al., 2005). Tapeworm (*Moniezia spp*.), coccidian (*Eimeria spp*.) and nematode (*Trichostrongylus spp., Teladorsagia circumcincta, Haemonchus contortus, Cooperia spp., Oesophagostomum spp., Chabertia ovina, Bunostomum trigonocephalum* and *Nematodirus spp*.) parasites are the leading types of GIP in sheep. The GIP infection caused by these parasites result in many dysfunctions and function which deteriorated the growth and welfare of animals in the early growth stage of small ruminants.

Although farmers spend a huge effort for the control of parasite infection in sheep in our country, the parasitic diseases and its economic and welfare losses are not fully understood. Furthermore, there is no sufficient awareness among the breeders and authorities in this regard. Parasitic diseases cause many economic losses in animals such as growth retardation, weight loss, animal deaths, treatment costs and labour costs. In our country, there is not yet a sufficient study that clearly shows the economic impact caused by parasitic diseases to the national economy. In a study conducted in Australia, where ovine breeding is much more intense, annual economic damage due to these diseases is reported to be around $436 million (Lane et al., 2015).

In the near future, parasitic diseases will be higher risk for sheep breeding, especially under pasture-based conditions. One of these risks is the genetic resistance mechanisms of parasites against anti-parasitic drugs. In the report published by the European Health Agency in 2017, it is reported that many parasite species have been developed resistance mechanisms against many anti-helminthic drug groups (i.e., benzimidazoles, tetrahydropyrimidines, imidazothiazoles and macrocyclic lactones) (CVMP, 2017). Resistance to anti-parasitic drugs is defined as "the ability of a parasite to tolerate doses of an anthelmintic that would have otherwise killed the same species under normal conditions" (Geary et al., 2012). Drug resistance genes in parasites are formed as a result of a rare mutation. However, these resistance genes can be transferred to new generations, thereby increasing the frequency? of these genes in the current parasite population every generations (Dargatz et al., 2000). In addition to the negative impact of the resistance against anti-parasitic drugs, a second issue is challenge of global warming (i.e., increase in annual temperature averages, modified precipitation regimes, disease outbreaks) which is the result of climate changes. In the near future, the climate change will raise catastrophic effect in the climate zone, which includes our geography. The change of precipitation regimes and temperatures intensify the occurrence of new gastrointestinal parasites infections that are not seen frequently in our country. Furthermore, parasitic infection periods and the frequency of infection during the year may be changed. Considering the projection of the FAO, global warming will increase dramatically in 2050 and according to 2017 FAO report, it is inevitable that animal breeding will be seriously affected in this respect (FAO, 2017).

Additionally, in recent years, the use of intensive chemical agents in parasite control has made the sector dependent on anti-parasitic drugs and it has been increased year by year (Al Kalaldeh et al., 2019a). Both the economic burden of using more drugs and the risk of anti-parasitic drug resistance which parasites have been developed more and more every year, have led the industry to use more innovative and sustainable control methods such as the selection of resistant animal. Beside to anti-parasite drug resistance, the use of intensive anti parasitic drugs creates serious residues on animal foods and indirectly threaten human health (Waltner-Toews and McEwen, 1994). All in all, the damage of GIP and the difficulties on control strategies show the importance of

genetic approaches as a sophisticated and alternative mitigation methods in small ruminant production.

1.1.2. Justification

Animal breeding is commonly used method for mitigating gastrointestinal parasite infections, as in many diseases, by including indicator phenotypes associated with parasite resistance in breeding programs in sheep (Jackson et al., 2009; Venturina et al., 2013). In small ruminants, animal which have genotypes commonly associated with high parasite resistance are selected for mating (Zvinorova et al., 2016). In many studies, it has been reported that there is a genetic variation in terms of individual parasite resistance in sheep. In studies, heritability estimates of phenotypes directly related to parasite resistance in sheep such as the faecal egg count (FEC), faecal oocyst count (FOC), were close to moderate (ranged from 0.03 to 0.16) which show genetic of animal relatively contribute the parasite load (A Pacheco et al., 2021; Sechi et al., 2009).

On the other hand, the recent technological developments have enabled to use molecular genetics effectively in animal breeding. In particular, a more detailed mapping of the sheep genome has led to the development of Genome-Wide Association Analysis (GWAS) methods based on the association of recorded phenotypes with high density polymorphisms (Single Nucleotide Polymorphism-SNP) that spread entire the genome. These methods allow the discovery of genomic regions that are responsible for health-related traits even with low in heritability. Genomic regions associated with production and wellness traits such as parasite resistance, blood parameters, weaning weight were determined in GWAS studies (Al Kalaldeh et al., 2019b; Atlija et al., 2016; Kijas et al., 2012; Pickering et al., 2015; Yilmaz et al., 2022). In addition to genomic region discovery study, with the technological and molecular advancements have enabled the integration of health-related traits which are controlled by the small effects of a large number of (multigenic) genes in breeding programs. Therefore, it has attempted to be used as a selection criterion in breeding programs beside the production traits (Heringstad, 2010).

As a result, the heritability estimates of parasite resistance-associated phenotypes and blood parameters suggest that genetic improvement of these traits is possible by animal breeding methods. Moreover, by detecting the genetic variations associated with these traits, it makes it possible to select resistant animals against diseases by tracking the whole genome or specific regions (QTL) in the genome.

1.1.3. Objectives

In this study, it is aimed to investigate genetics of health and adaptation traits (i.e., parasite resistance and blood parameters) in Akkaraman sheep by implementing genomic heritability estimates and GWAS analysis. The specific motivations for the emergence of this study were the requirement of alternative mitigation strategies for parasite infection will be required in the near future in Akkaraman. Besides that, the need to achieve the necessary knowledge to play a role in filling the existing gap in order to popularize the genome-based selection methods (Marker-Assisted Selection-MAS, Genomic Selection-GS etc.) that production systems of our country strongly needs. Lastly, the need to genetically improve the economically important traits (make more resistant, adaptive, productive and competitive breed) of Akkaraman sheep which are able to efficiently utilize the arid pasture and constitute a significant part of our country's sheep population.

With this purpose, the main objectives of the study were:

1) Estimation of genomic heritabilities for parasite resistance traits and blood parameters.
2) Implementing genome-wide association studies (GWAS) to uncover underlying genetic background of parasite resistance phenotypes and blood parameters in Akkaraman sheep.

1.2. LITERATURE REVIEW

1.2.1. Akkaraman sheep as an indigenous breed

It is known that sheep were domesticated in the Fertile Crescent region about 11,000 years ago (Zeder, 2008). Since the domestication of sheep, it has been an important farm animal that primarily benefited from its meat and then its fleece and milk. With domestication, sheep spread to different geographies with humans. As a result of adaptation to different geographies, genetic isolation, and different selection strategies applied by humans, many breeds of sheep have emerged (Kijas et al., 2012). In this context, Türkiye is a very rich country in terms of genetic diversity of domestic sheep as a result of its cultural and geographical diversity. According to DAD-IS records, more than 40 domestic sheep breeds are reported (DAD-IS, 2017). Akkaraman sheep is known as the domestic sheep breed with the highest number of breeds, especially in the Central Anatolian region. The main characteristics of the breed are that having fat tail, resistant to arid climatic conditions and benefiting from relatively arid pastures at a higher level than other breeds.

Native breeds have adapted to the conditions of the region (i.e., climate, geography, local pathogen) in the best way for centuries. The internal and external factors that they have been exposed for a long time have given them the best genetic combination, and with the physiological adaptation revealed by this genetic structure. Therefore, they have maximized their productivity and survival characteristics. This genetic structure has given them superiority such as higher survival and adaptation capacity and they have continued to exist for centuries. Classical breeding and one-way selection attempts in the 20th century put pressure on the genetic diversity of native breeds. However, today, the identification of reference genomes in many species and breeds, especially sheep, and the detection of functional variants in the genome of farm animals have popularized modern breeding methods and provided more sophisticated approaches in breeding programs. In this way, it has been included in breeding programs for the traits such as adaptation ability and resistance to diseases in native breeds.

1.2.2. Gastro-intestinal parasite resistance in sheep

1.2.2.1. The host immunity to GIP infection in sheep

The immune response developed against GIP infections is a complex mechanism involving various biochemical and cellular components. The immune response that develops against the GIP infections is determined by the animal's genetic structure as well as the type and density of the parasite to which it is exposed. For example, the immunity for the infections of *Nematodirus* is faster than the immune response to the infections of *Teledorsagia* species (Smith et al., 1985; Taylor and Thomas, 1986). The immune response to parasite infections in lambs begins at 2 - 3 months of age and continues until 10 - 12 months of age as long as exposure to the parasite continues. Also regular exposure to the parasite in adult animals maintains immunity. (Seaton et al., 1989; Stear et al., 1996). In GIP infections, the immune response begins with the entry of the parasite into the body, the first defense is to prevent the parasite establishment to the body, then to suppress the increase of the parasite number in the body, and finally to expel the parasite from gastrointestinal system - GIS (Seaton et al., 1989; Stear et al., 1996, 1995). As it is known, the immune response in vertebrates is divided into two as the innate and adaptive immune response.

The innate immune response is the first line of defense in parasite infections as well as in many infections (i.e., bacterial, viral). The mucus layer and enteric smooth muscle contractions in the GIS are the first physical barriers of the innate immune system. In addition to mucin, bioactive molecules such as defensin and trefoil factor are secreted from neck and epithelial goblet cells in the stomach and intestinal mucosa, thereby suppressing larval development and stimulating inflammation (Kim and Khan, 2013; McGuckin et al., 2011). In a study conducted in lambs divided into two groups as resistant and susceptible to nematode infection, it was observed that the expression level of genes associated with the formation and functions of enteric smooth muscle were increased (Diez-Tascón et al., 2005). Pattern recognition receptors (PRRs), such as C-type lectine receptors (CLRs) and Toll-like (TLRs) receptors and G-protein-Coupled Receptors (GPCRs) have key roles in recognizing pathogens like parasites and parasite antigens and activating the immune system (Geijtenbeek and Gringhuis, 2009; Glass, 2012; Wang et al., 2019). On the other hand, cytotoxic and pro-inflammatory cells such

as connective tissue and mucosal mast cells stimulate immune cells (i.e., eosinophils) by secreting histamine and increase vascular permeability, forming another line of defense against parasite infections (Urb and Sheppard, 2012; Voehringer, 2013).

When exposed to an antigen, antigen-presenting cells (APCs) (i.e., activated dendritic cells and macrophages) tend to move to local lymph nodes and introduce the antigens to their cognate T-cell receptor via MHC class I or II carrier molecules. The activation of the T cell by APCs activates the adaptive immune response and leads the secretion of cytokines, which leads to T-cell differentiation as well as the proliferation of additional T cells (McRae et al., 2015). Many genes directly or indirectly involved in the synthesis, expression and production of bioactive molecules, receptors and many cellular components involved in both the innate immune response and the adaptive immune response are the main contributor of individual differences in parasite resistance in sheep. Furthermore, many studies are being done from genome to phenome to understand the immune genetics of resistance against GIP.

1.2.2.2. Economic loses of GIP infection in sheep

Gastro-intestinal parasite infections are the most important health problems, particularly in free-range sheep and goats (Coles et al., 2005). Tapeworm (*Moniezia spp.*), coccidian parasite (*Eimeria spp.*) and nematodes (i.e., *Trichostrongylus spp., Teladorsagia circumcincta, Haemonchus contortus, Cooperia spp., Oesophagostomum spp., Chabertia ovina, Bunostomum trigonocephalum* and *Nematodirus spp.*) are among the most common parasites in sheep. In gastro-intestinal parasite infections, clinical symptoms and economic losses in animals are directly related to the parasite load that the animal is exposed to.

The economic damage caused by the parasitic diseases is twofold: the most direct one are the losses of productivity caused by infection, such as growth retardation, weight loss, and deaths in especially juvenile lambs, and the more indirect one is the cost of parasitic controls which consist of the cost of chemical drugs and the veterinary service costs. It has been reported that the annual economic loss due to parasitic diseases in Australia, where sheep and goat farming is very intensive, is around $436 million

(Lane, et al., 2015). Similarly, the economic cost of parasitic diseases in England in 2005 is estimated to be around £84 million (Nieuwhof and Bishop, 2005). In a study conducted in the UK, the weight loss in lambs during the growth period from infection is accounted for two-thirds of this cost. Moreover, a 10% decrease in live weight gain of lambs in growth period costs £4.40 per lamb (Vickers and Wright, 2013). Considering this situation, economic losses due to parasitic infections should not be ignored in a sustainable production in sheep industry.

1.2.2.3. Genetic parameter estimates of parasite resistance in sheep

Control of parasitic diseases in small ruminant production has been a very important challenge like many other diseases. In herd health management, many strategies and practices are applied to mitigate parasite infection. In line with the concept of preventive veterinary medicine and creating healthy herds, breeding for more resistance animal has been started to be used effectively in farm animals (Hu et al., 2020). Intensive selection studies on production traits were carried out in breeding studies until the end of the 20th century (Fariello et al., 2014). Therefore, it was observed that there was a depression on the adaptation and wellness traits in farm animals due to the production sided intensive selection (Strandén et al., 2019). However, in recent years, it has been aimed in breeding programs to obtain more resistant herds by including these complex disease resistance traits in selection indexes, and is among the commonly used strategies in disease control strategy of many countries (Windon et al., 1987; Egger et al., 2013). Moreover, the model simulation studies have shown that the desired genetic improvement for parasite resistance can be achieved by selecting animals with low fecal egg counts in a short period (Kemper et al., 2013). In order to achieve a rapid and effective genetic progress for the production (i.e., milk and meat yield etc.) and wellness (i.e., heat stress, disease resistance etc.) traits in a population in a breeding program carried out in farm animals, knowing the genetic parameters (i.e., genetic variance, heritability and genetic covariance) of these traits is very important. Moreover, to get more accurate estimated breeding value (EBV) and accordingly give an accurate selection decision for a population, it is essential to know genetic parameters (Fogarty, 1995; Safari et al., 2007).

Heritability are estimated by using the animal mixed model in populations with deep and comprehensive pedigrees, by including the level of inbreeding in the population into the model (Henderson, 1950). In this classical approach, relationship is calculated among the animals over the numeric relationship (A) matrix obtained from pedigree information, and the effect arising from the genetic background of animals. Achieving deep and comprehensive pedigree in farm animals is a long and labor-intensive process. Therefore, it is very difficult to create pedigrees with the life-time traits such as disease resistance, especially in domestic breeds keep in small herds. This situation makes it difficult to adequately understand the genetic behavior of economically important traits and to implement an effective breeding program in these breeds. However, the genomic relationship (G) matrix obtained by using genomic data through SNP panels representing the whole genome has enabled the estimation of genetic parameters for traits. Using G matrix instead of the A matrix obtained by pedigree for many years is very feasible cost effective by regarding long-term evaluation (de Los Campos et al., 2015; Lynch and Ritland, 1999; Ritland, 1996; Thompson, 1975).

Indicator traits used for gastro-intestinal parasite resistance are complex traits as in many disease resistance phenotypes in sheep. The main reason for this complexity is that many physiological and immunological process are involved in the biological mechanisms such as entry and invasion of parasite in the body, and the formation of the host-parasite interactions (Behnke et al., 2003). The disadvantages of these complex traits such as measuring difficulties, limits fully understanding of the mechanism for resistance of the disease. Indicator phenotypes used in the determination of parasite resistance in sheep are evaluated in 3 groups including parasitological (i.e., fecal egg/oocyte count, number of adult larvae), immunological (i.e., IgA, IgE and IgG levels) and pathological (plasma pepsinogen, albumin, fructosamine concentrations, dag score, fecal consistency and packed cell volume) (Dominik, 2005). In terms of cost, feasibility and reliability, fecal egg/oocyst count is the commonly used indicator phenotype in the parasite resistance studies (Atlija et al., 2016; Huisman et al., 2005; Mortimer et al., 2017).

Studies on genetic parameter estimation of gastro-intestinal parasite resistance indicator phenotypes have mostly focused on nematode species due to their high economic damage. However, studies on coccidian and tapeworm infections are rather limited compared to nematode studies. In studies conducted with different nematode species and different breeds, it is seen that gastrointestinal nematode resistance has a heritability varying from low to moderate (Berton et al., 2019; Bishop et al., 1996; Gowane et al., 2019; Gutiérrez-Gil et al., 2010; Huisman et al., 2008; Mpetile et al., 2015). In studies conducted for *Haemonchus contortus* FEC in Santa Ines sheep raised in the tropical region, heritability was reported to be around 0.10 (Berton et al., 2019). In another study, it was reported that the heritability of fecal egg counts for nematodes in Scottish Blackface lambs 1-6 months old ranged between 0.01 and 0.22 (Bishop et al., 1996). Furthermore, Gowane et al., (2019) found the heritability of FEC phenotypes performed in the pre- and post-drench period in Avikalin and Malpura sheep as 0.21 - 0.03 and 0.19 - 0.12, respectively. In another study conducted in Spanish Churra dairy sheep, the heritability of the fecal egg count traits for *Teladorsagia circumcincta* were found to be between 0.09 and 0.12 (Gutiérrez-Gil et al., 2010). On the other hand, in the studies conducted by Huisman et al., (2008) and Mpetile et al., (2015) on merinos, which is the world's common sheep breed, the heritability for the FEC trait was reported as 0.18 and 0.10, respectively. Additionally, in a study conducted in Texel, Ile de France, Morada Nova sheep and crossbreds of these breeds, it was reported that tapeworm (*Moniezia expansa*) egg and coccidian (*Eimeria spp*.) oocyst numbers varied in different genotypes (breeds) (McManus et al., 2009). Finally, in a study by A. Pacheco et al., (2021) in Scottish Blackface sheep, the heritability estimates of fecal *Strongyles spp*. , *Nematodirus spp*. eggs and Coccidian (*Eimeria spp.)* oocysts were found to be 0.17, 0.09 and 0.09, respectively. In general, the heritability of the phenotypes associated with GIP resistance range from low to moderate. This shows that these traits are complex and multi-genic traits. Fortunately, the fact that these traits have such high heritability indicates that breeding is possible in sheep for these traits and, therefore, more resistant herds against parasitic infections can be obtained by implementing breeding practice.

1.2.2.4. Application of genomics in parasite resistance

Recent technological developments have enabled the effective use of molecular genetics in animal husbandry (Al Kalaldeh et al., 2019a; Arzik et al., 2022; Berton et al., 2017;

Kizilaslan et al., 2021; Meuwissen et al., 2001; Yilmaz et al., 2022). A more detailed mapping of the sheep reference genome (OAR_v4.1) has led to the development of Genome-Wide Association Analysis (GWAS) methods based on the association of recorded phenotypes with high-density polymorphisms (Single Nucleotide Polymorphism-SNP) that span the whole genome. These methods enable the identification of genomic regions that are responsible for complex traits which have low heritability that are influenced by the small effects of many (multi-genic) genes such as production and health-related traits (Mucha et al., 2018; White et al., 2012; Yilmaz et al., 2022).

According to recent research, sheep and goats have individual resistance and resilience to parasitic infections (Morris et al., 2005; Nagamine et al., 2012). Many genomic regions, SNPs and QTLs associated with gastro-intestinal parasite resistance related traits have been reported in many studies in different sheep breeds using SNP panels obtained by microarray technologies (Ahbara et al., 2021; Al Kalaldeh et al., 2019b; Álvarez et al., 2019; Atlija et al., 2016; Benavides et al., 2015; Periasamy et al., 2014; Pickering et al., 2016). Genome-wide association studies in sheep have reported that genes associated with acquired immune response, mucus secretion and hemostasis are directly associated with parasite resistance (Benavides et al., 2015). For instance, the QTL (Quantitative Traits Loci) explaining high variance in chromosome 2 was discovered in GWAS with 600,000 SNP for fecal egg count in a study with large number of sheep in Australia (Al Kalaldeh et al., 2019a). In the association studies with microsatellites and genome-wide SNP panel performed in Churra sheep aged 4 to 11 years, FEC and IgA-related QTL regions were detected on OAR6 (Atlija et al., 2016; Gutiérrez-Gil et al., 2009). In the association analysis for parasite resistance in Katahdin sheep by Becker et al., (2020), many statistically related genes and SNPs were found on OAR 1, 2, 3, 16, 23 and 24. Statistically significant regions on chromosome 5 and 26 were also reported in the multi-breed genome-wide association analysis for nematode resistance phenotypes (FEC, DAG) in Texel, Coopworth, Romney and Perendale sheep (Pickering et al., 2016). In Tunisian sheep selection signature analysis, many genes involved in pathways of intestinal wound healing (i.e., *CXCR1*, *CXCR2*, *IL-4*, *VIL1*), gastro-intestinal expulsion (i.e., *IL-4*, *IL-13*) and innate immune response (*IL-4*, *IL-13*, *SLC22A4*, *SLC22A5*) have been reported to be associated with GIP resistance (Ahbara

et al., 2021). In a study conducted by Yaman et al., (2021) in Turkish native breeds namely Karacabey Merino, Kivircik, Imroz (Gokceada), Chios, Cine capari and Karakacan sheep, a total of 14 variants on the *TLR4* gene were found to be associated with coccidian oocyst count. Lastly, no previous study related to Tapeworm (*Moniezia spp.)* resistance in sheep has been found. It is one of the first studies to examine the genetic structure of resistance of tapeworm parasite. Association analyzes with SNP panel representing the sheep genome on the whole genome scale and many other genetic variants partially help to understand the genetic background of GIP resistance. Therefore, it is strongly show that selection studies for gastro-intestinal parasite resistance in sheep by following relevant markers on the genome (marker-assisted selection) will accelerate genetic progress in terms of these traits.

1.2.2. Genetics of blood parameters in sheep

1.2.2.1. Blood parameters

Examination of blood variables is widely used practice to obtain information on the general health condition of mammals by performing complete blood count (CBC) analyses. Knowing the increase and decrease in the number of blood cells (erythrocyte, white blood cells, etc.) helps to understand the health condition of an animal, therefore, it is used as the most common methods to investigate of many diseases by veterinarians (Kelly, 1984). Furthermore, blood cells have many essential roles in animals such as survival, performance and resistance to diseases (Müller and Brem, 1991). Basically, blood cells consist of erythrocytes (red blood cell), leukocytes (white blood cells) and platelets (Kickler, 2005).

Red blood cells represent about 30% to 50% of the total blood volume and the main function of them is to provide oxygen and carbon dioxide transfer between the lungs and tissues (Ganesh et al., 2009). With this basic function, erythrocytes are an important factor that directly affects performance and growth in animals. The oxygen carrying capacity of erythrocytes depends on many factors. The morphology of the erythrocyte and the amount of hemoglobin are important criteria used to determine the oxygen carrying capacity of the erythrocyte. Therefore, while evaluating red blood cells, many features related to erythrocytes are evaluated in addition to the number of erythrocytes. These are the ratio of erythrocytes to circulating blood volume (HCT), mean

corpuscular volume (MCV), total hemoglobin count (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and actual measurement of the width of the red cell standard deviation (RDW-SD) (Eklom and Lill, 2006; Tripette et al., 2009; von Tempelhoff et al., 2016).

White blood cells are a component of an animal's innate and adaptive immune system, and their primary function is to protect the organism from antigens such as bacteria, viruses, and parasites. Lymphocytes, neutrophils, monocytes, basophils, and eosinophils are all types of white blood cells (leukocytes). The percentage of these cells is also used in the examination of white blood cells, in addition to the total white blood cell count (WBC). It is also known that white blood cells are directly related to resistance to stress as well as immunity in the body (Naylor et al., 2021). On the other hand, the ability of the mammalian blood platelet to adhere to subjected sub-endothelial collagen and help regulate platelet aggregation contributes to hemostasis (Weiss, 1999). The most commonly used traits to monitor platelet changes are platelet count (PLT), mean platelet volume (MPV), and platelet count to total blood volume (PCT).

1.2.2.1. Genetic parameters of blood parameters

Many natural factors, such as selection, genetic mutation, genetic drift, and migration, affect additive genetic variance of traits and, as a result, heritability. For example, the different populations exposed to the different natural and artificial factors for hundreds of years show variation in heritability estimates for the same trait. Despite the fact that different breeds were studied, the heritability estimates for many traits were quite similar to the results of previous studies. The previous study clarifies that the heritability estimates of many blood parameters in different sheep breeds have been concordantly? moderate. This situation may imply that no systematic selection was applied to these traits. Thus, genetic improvement of these traits through selection strategies is entirely feasible in terms of adaption and wellness trait such as blood compositions.

There have been few studies on the heritability estimates for health and adaptation traits such as blood traits (white blood cell, red blood cell, platelets etc.) in sheep. The studies conducted previously show that generally the blood traits have low to moderate heritability. For instance, in a study of Santa Inês sheep, heritability estimates using the

genomic relationship matrix derived from SNP genotyping data were 0.18, 0.17, 0.22, 0.20, and 0.16 for the RBC, PLT, WBC, HCT, and HGB traits, respectively (Berton et al., 2019). In another study conducted on some blood traits of Santa Inês sheep, the heritability estimate for HCT was found to be 0.18 (Rodrigues et al., 2021).

1.2.2.2. Genome-wide association studies for blood parameters

Environmental factors such as health, nutrition, and altitude have a significant impact on blood parameters in sheep (Smith and Sherman, 1994). Aside from environmental influences, another factors that influence blood parameters is the animal's genetic architecture. However, there haven't been enough studies on the understanding of the genetic mechanisms of blood parameters in sheep. Blood parameters in sheep, like so many other quantitative traits, can be inherited and are controlled by specific QTL or genes on genome (Gonzalez et al., 2013; Zhu et al., 2020). The sheep reference genome was discovered as a result of genome sequencing efforts as in many species. This reference genome enables researchers to investigate the genetic mechanisms underlying many traits at whole-genome level (Davoli et al., 2016; Gonzalez et al., 2013; Yilmaz et al., 2022; Zhu et al., 2020).

A genome-wide association analysis of 7 red blood cell phenotypes was performed in a study with three different domestic sheep breeds (Columbia, Polypay, and Rambouillet) by Gonzalez (2013). In the study, SNPs on chromosome 18 associated with increased MCHC and decreased MCH were found to be significant. The ovine HapMap project discovered that the same genomic region associated with production, survival, and reproduction traits is also related to heavy selective pressure (Kijas et al., 2012). In another study, 6 potential genes associated with these traits were identified in genome-wide association analyses for erythrocyte phenotypes in Alpine merino sheep (Zhu et al., 2020).

CHAPTER 2

2.MATERIAL AND METHODS

2.1. Animal materials and sample collection

The study was implemented in Ankara, Turkey (39°41' N, 33°01' E). The location is recognized for having a continental climate with severe, snowy winters and dry summers. The average annual rainfall is 389 millimeters, and the average annual temperature is 11.7 °C. The average altitude of the region is 938 meters, and the majority of pasture is low-quality pastures. 480 Akkaraman lambs (186 males and 294 females) were used in the study. They were randomly chosen from 3 separate farms that are members of the National Community-based Small Ruminant Breeding Program. Lambs were born in January and February of 2021 and weaned at an average age of three months. After weaning, 372 of them were grazed on pasture without any additional feeding during the summer, while 114 of them were applied to a 90-day finishing period with 750-1,000 g concentrate feed/day. For the studied population, the selection decisions were made during the mating season (e.g., August-September) according to the performance of pre-weaning growth traits.

The studied animals were followed from birth till the sample collection, which was around 6-months of age. The other records including environmental factors (sex, herd, birth type, feeding type, location) were recorded. Fecal samples were collected from 129 animals at 3-months of age (May in 2021) and 478 animals at 6-months of age (August in 2021). During sample collection in August, approximately 6 ml (in vacuum EDTA tubes) blood sample were taken from the *V. jugularis* of the animals. In terms of both ethics statement and the objectivity of the study results, animals were examined to see if they show clinical signs of any disease, and suspected animals were excluded from the study. Moreover, it was ensured that at least 60 days had passed since the last anti-parasite treatment of animals. 20 – 30 g fecal samples were taken directly from the

rectum of animals to minimize contamination by using exam gloves. The samples were transferred laboratory in cold chain transport and were stored at +4 °C until fecal examination.

For CBC, the samples were transferred and analyzed immediately (within 6 hours) for 18 blood parameters, to protect the blood samples from hemolysis, at ZETA Laboratories (Ankara, TÜRKİYE). 8 of these phenotypes were erythrocyte phenotypes namely total red blood cell count (RBC, 10^{12}/dl), hemoglobin (g/dl), hematocrit (%), mean corpuscular volume (fl), mean corpuscular hemoglobin (pg), mean corpuscular hemoglobin concentration (g/dl), coefficient of variation of RBC volume distribution width and standard deviation of RBC volume distribution width. Leukocyte related traits were total white blood cell count (10^9/dl), neutrophils (%), lymphocytes (%), monocytes (%), eosinophils (%) and basophils (%). Lastly, platelet (PLT) related parameters were total platelet count (10^9/dl), mean platelet volume (fl), procalcitonin (ng/ml) and platelet distribution width (fl). Preceding further analyses, outliers were identified and observations exceeding three standard deviations ± mean for each trait were excluded from the data. Descriptive statistics of these parameters were presented in Table 1.

2.2. Phenotypic measurements

GIP resistance phenotypes in sheep were estimated based on the fecal egg counts from three different parasite species. The phenotypes for nematode egg counts at 3-month age and at 6-month age were NemFEC3 and NemFEC6 respectively. The phenotypes for *Moniezia sp.* at 3-month age and at 6-month age were MonFEC3 and MonFEC6 respectively. Lastly, the phenotypes for fecal oocyst counts from coccidian parasites were CocFOC3 at 3-month age and CocFOC6 at 6-month age. The number of eggs for nematode, tapeworm and oocyst for coccidian parasite were counted by using McMaster technique (MAFF, 1986). Accordingly, 2±0.1 g feces were mixed with 28 ml flotation solution (saturated saline water) and coarse particles were removed by sieving with a tea strainer. Two chamber of the slide (0.15 ml each) were counted with light microscope with 10X objective at 10X magnification for each animal. The results were multiplied by 50 to obtain egg and oocyst count per gram. The worm (nematode) eggs were found

very rarely in animals during the egg counts. The consultation with veterinary parasitologists, it was concluded that the low precipitation amount and arid climatic conditions in the spring period affected the life cycle of the parasites and did not cause an intense infection.

The blood samples were taken from the *V. jugulars* to 6 ml EDTA-coated vacutainer and transferred to laboratory in cooling box. To avoid hemolysis of blood within 6 hours the samples were analyzed for 18 parameters. Eight of these phenotypes were Red Blood Cell phenotypes including red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), RBC volume distribution width coefficient of variation (RDW-CV) and RBC volume distribution width standard deviation (RDW-SD). White Blood Count phenotypes were White blood cell (WBC), neutrophils (NEU), lymphocytes (LYM), monocytes (MON), eosinophils (EOS) and basophils (BAS). Additionally, the ratio of neutrophils to lymphocytes (NEU/LYM) was calculated and analyzed as an additional phenotype. Lastly, Platelet phenotypes were Platelets (PLT), mean platelets volume (MPV), procalcitonin (PCT) and platelets distribution width (PDW). The details of blood parameters were presented in Table 1.

2.3. DNA Extraction, genotyping and quality control (QC)

After CBC, the samples were transferred to International Center for Livestock Research and Training (ICLRT), Genetic Laboratory for extraction of DNA. To avoid from contamination risk, DNA extraction was implemented with the Qiacube HT automated device using a commercial kit (Qiagen Blood kit, Hilden, Germany). Quality control of the extracted DNA was performed before further analysis. Samples that passed the quality criteria (A260/280>1.8, A260/230>1.5, >70 ng/µl) stored at -20°C until genotyping.

Genotyping was performed using the Axiom 50K Ovine Genotyping Array (Thermo Fisher Sci, Waltham, MA, USA) with the GeneTitane MultiChanel instrument at the ICLRT Genetics Laboratory. Axiom 2.0 Assay 96 Array Format Manual Workflow protocols were applied in genotyping (Arzik et al., 2022). These procedures are as follows: 1) DNA Amplification 2) Fragmentation and Precipitation 3) Centrifugation, Drying, Resuspension 4) Quality control 5) Denaturation and Hybridization 6) Staining

and Stabilization 7) Genotype reading. The first 4 steps of the processes were performed manually and the last 3 steps were performed on the genotyping instrument. Genotyping quality control (QC) step was done with Axiom Analysis Suite program. Genotypes were obtained as homozygous ('AA' and 'BB') or heterozygous ('AB') alleles for each sample and SNP to be used in bioinformatics analysis and saved as a '.txt' file.

The step of QC for raw genotypic data is critical in order to eliminate genotyping errors and increase statistical power as well as to increase the reliability and accuracy of the genotypes during the genome-wide association studies. Therefore, in addition to the Axiom Analysis Suit program, one more quality control step was applied for the data by using the R GenABEL package (Aulchenko et al., 2007; R Development Core Team, 2016). The criteria accepted by many researchers and used in many studies were applied (McCarthy et al., 2008; The Wellcome Trust Case Control Consortium, 2007; Weale, 2010). The most important issue to be aware of at this stage is to know that the data consist of genetically isolated populations from each other. Thus, when performing quality control, the data of the populations was evaluated within themselves, and the data sets were reassembled to include the common SNP regions before proceeding to the analysis step. For this purpose, the criteria to be applied with "check.marker" function of the GenABEL package are as follows:

1) The SNPs below the 0.05 (5%) minor allele frequency (Minor Allele Frequency, MAF) were subtracted from the data set.
2) SNPs associated with the X chromosome were removed from the analysis.
3) For each SNP region, animals were genotyped at a minimum of 95% and the SNPs below this value were removed from the data set (SNP Call Rate).
4) For each sample, a minimum of 90% of the SNP regions were genotyped and samples below this value were removed from the data set (Sample Call Rate).
5) The samples showing the genotypic similarity over 95%, IBS (Identity by State), were removed.
6) The SNPs deviating from HWE (Hardy-Weinberg Equilibrium) were removed from the analyses.

2.4. Missing SNP imputation and genomic relationship matrix (G) calculation

Before proceeding to the genetic diversity, genomic heritability estimation and genome-wide association analyzes in which genomic information is used, imputation was applied to the quality controlled genomic data. SNPs that are not genotyped with sufficient quality after QC are imputed according to the current status of this SNP in the population. The main motivation for the imputation of missing genotypes is the reassignment of SNPs that cannot be read during genotyping and cannot meet the criteria during QC. This technique is a routine procedure applied to increase statistical power further analysis in genotyping studies. Two basic approaches are used to assign missing genotypes, the first of which is the imputation of the mean marker in the population, rather than the unknown SNP. Another technique is to estimate the unknown marker based on the multivariate normal distribution based on the Expectation Maximization-EM algorithm (Chen and Abecasis, 2007). EM algorithm was used in the study and the process was carried out by R GenABEL packages (Aulchenko et al., 2007).

Using the population structure (PS) in GWAS models by performing principal component (PC) analysis cannot completely solve the problem of incorrect SNP association. In other words, the population structure cannot clearly reveal the kinship situation between animals. As a result, the genomic relationship matrix (G), as described by Astle and Balding (2009), was calculated and used to estimate genetic parameters and to be used in GWAS (Astle and Balding, 2009; VanRaden, 2008).

2.5. Genetic diversity analyses

One of the most widely used methods for measuring genetic differentiation between two populations is of fixation index (F_{ST}) test. In the F_{ST} test, it is tested whether the difference in allele frequencies between the two populations is statistically different. In the study, two subpopulations were formed from studied animals (For CocOPG3 and CocOPG6, if oocyst numbers were lower than 1000, animals were assigned in uninfected group and rest of them were assigned in infected group. For NemFEC3, MonFEC3, NemFEC6 and MonFEC6, if eggs were not detected in feces animals were assigned in uninfected group, rest of them were assigned in infected group). The F_{ST} calculation for these two subpopulations is as follows:

$$F_{ST} = (H_T - H_S) / H_T$$

H_T is the mean of the heterozygous allele of the whole population, H_S is the mean of the heterozygous allele of the subpopulation. The degree of the F_{ST} value shows whether there is a differentiation in terms of this feature in both subpopulations. In the study, F_{ST} values of populations will be calculated over approximately 50,000 SNPs (Weir and Cockerham, 1984). Additionally, expected and observed heterozygosity for subpopulation (infected and uninfected) were calculated. Analyzes were made using the "cgmisc" package in R (Kierczak et al., 2015).

2.6. Genomic heritability estimates

Total phenotypic variance ($\boldsymbol{\sigma_p^2}$), additive genetic variance ($\boldsymbol{\sigma_u^2}$) and random residual variance ($\boldsymbol{\sigma_e^2}$) were estimated by using the univariate mixed analysis approach based on the Animal Mixed Model and 'Restricted Maximum Likelihood' (REML) approach by using 'sommer' package of R (Covarrubias-Pazaran, 2016) before genomic heritability estimation. The environmental factors taken are included in the model as a fixed effect, whereas the genomic relationship matrix was included in the model to capture the covariance between individuals in terms of the phenotypes of interest. The animal mixed model is given below;

$$\mathbf{y = \mu + X\beta + Zu + e}$$

$$\boldsymbol{V} = \begin{bmatrix} \boldsymbol{Z_i G \sigma^2_{u_i} Z'_i + I \sigma^2_{e_i}} & \cdots & \boldsymbol{Z_i G \sigma_{u_{i,j}} Z'_j + I \sigma_{e_{i,j}}} \\ \vdots & \ddots & \vdots \\ \boldsymbol{Z_i G \sigma_{u_{i,j}} Z'_j + I \sigma_{e_{i,j}}} & \cdots & \boldsymbol{Z_j G \sigma^2_{u_j} Z'_j + I \sigma^2_{e_j}} \end{bmatrix}$$

where **y** is the vector of individual observations of each trait focused, **μ** is the population mean regarding the trait of interest, **β** is the vector of fixed environmental effects (sex, birth type, birth month etc.), **u** is the polygenic background effect obtained from MVN ($u \sim 0, \sigma_u^2$), **e** is the vector of random residual errors obtained from MVN ($e \sim 0, \sigma_e^2$) while **X** and **Z** are the design matrices mapping fixed effects and polygenic background effects to each observation, respectively. Furthermore, σ_u^2 and σ_e^2 are the additive genetic variance and random residual variances for the particular trait, respectively, where i and 'j' represent different traits to be examined in bivariate analyses and **I** is the

identity matrix. At last, G represents the genomic relationship matrix derived by VanRaden (2008).

$$\boldsymbol{G} = \frac{\boldsymbol{ZZ'}}{\boldsymbol{2\sum p_i(1-p_i)}}$$

After estimating variance component of each trait, the narrow sense heritability estimates of each trait were calculated by using the model given below;

$$\boldsymbol{h^2} = \frac{\boldsymbol{\sigma_u^2}}{\boldsymbol{\sigma_p^2(\sigma_u^2 + \sigma_e^2)}}$$

where $\boldsymbol{h^2}$ is the heritability estimate of each trait, $\boldsymbol{\sigma_u^2}$ is the estimate of genetic variance for each trait, $\boldsymbol{\sigma_p^2}$ is total phenotypic variance and $\boldsymbol{\sigma_e^2}$ is the error variance. The environmental factors taken are included in the model as a fixed effect, whereas the genomic relationship matrix was included in the model to capture the covariance between individuals in terms of the phenotypes of interest.

2.7. Genome-wide association studies (GWAS)

Linear Mixed Models were used to detect significant SNPs associated with phenotypes. In the model, the additive effect calculated with the genomic association matrix (SNP-based identity-by-state) was included to account for the fixed genotypic effect of SNPs, population structure, and kinship background. The linear model to be used in the analysis is as follows:

$$y = \mu + X\beta + Zu + e$$

A widely used graphical method, the Manhattan Plot, was used to visualize the p-values showing the significance of the SNP effects. The p-values of the SNP effects calculated as a result of the association analyzes were created by ordering the position information of the SNPs on each chromosome on the -log10 scale. Statistical significance threshold value (threshold) was used to avoid Type 1 Error (probability of rejection if the null hypothesis is true) accumulated in multiple SNP tests.

The approach of Benferoni correction for multiple tests was used to determine genome-wide significant and suggestive thresholds for the traits (Devlin and Roeder, 1999). The Genome-wide significant threshold was 1.23e-06 (0.05/40,463) and the chromosome-wide significant threshold was 3.21e-05 (1/40,463). The quantile-quantile plots of the observed test statistics for each SNP were compared with the test statistics expected under the null hypothesis of "no association". A Q-Q plot was obtained for each phenotype. Basically, the nature of deviations from the "no association" line was evaluated by visualizing two probability distributions (y-axis observed, x-axis expected test statistics) against each other. Total SNP deviations from expected test values indicate familial associations, systematic errors such as genotyping errors and cryptic population construction.

2.8. Functional annotaion of significant SNPs

Using the Oar_v4.0 genome assembly, we managed to get positional information for associated SNPs and candidate genes from the NCBI Genome Data Viewer. (Rangwala et al., 2021). As candidates, genes that are overlapping with SNPs were proposed. Furthermore, if the founded SNP is not located on a gene, the region of ± 250 Kbp around the detected significant SNP is searched for a candidate gene. DAVID Bioinformatics Resources 2021 was used to obtain biological information on the identified candidate genes. (Huang et al., 2009). Additionally, previously identified QTL related to sheep parasite resistance and blood parameters were checked through Animal QTL Database to see if there is any overlap with the SNPs identified in this study (Hu et al., 2022). In case of insufficient annotation in the sheep genome reference, orthology between species was used, Moreover, the annotation of specific genes from cattle, goats, mice, and humans were evaluated for shared biological function. Finally, the metabolic pathways involving the genes of candidate genes were depicted by their Gene Ontology terms, which can be explored further on the QuickGo website. (Binns et al., 2009).

CHAPTER 3

3. RESULTS

3.1. Descriptive statistics

Fecal egg counts for nematodes and tapeworms, and fecal oocyst counts for coccidian parasites were performed in gram faces of 129 animals at 3-month age and 475 animals at 6-month age. Descriptive statistics and prevalence are given in Table 1. According to Table 1, the maximum egg count in gram feces were 485, 14,383, 271 and 141,834 for NemFEC3, MonFEC3, NemFEC6 and MonFEC6 respectively. The oocyst count in gram feces of coccidian parasites were found to be 38,203 and 23,139 for CocFOC3 and CocFOC6 respectively. No nematode eggs were detected in 93.90% of the animals, and no eggs belonging to *Moniezia sp.* were detected in 68.63% of the animals. Finally, no oocyst was found in only 5.68% of the animals in the oocyst count of coccidian parasites.

Table 1. Descriptive statistics and prevalence (%) of gastrointestinal infection in sheep.

Trait	N	Mean	Minimum	Maximum	% 0 values	SD
NemFEC3	129	36.51	0	485	68.59	68
MonFEC3	129	1,410	0	14,383	38.01	2,528
CocFOC3	129	1,698	0	38,203	4.95	3,607
NemFEC6	475	6.68	0	271	93.90	28
MonFEC6	475	1,173	0	141,834	68.63	8,066
CocFOC6	475	2,627	0	23,139	5.68	3,071

The outliers for each trait were excluded from the data for further analyses. The mean values for RBC, HGB, HCT, MCV, MCH, MCHC, RDW-CV and RDW-SD were 5.43 ± 0.12, 9.51 ± 0.05, 23.25 ± 0.45, 45, 19 ± 0.24, 24.16 ± 6.90, 50.86 ± 1.22, 14.30 ± 0.31 and 23.32 ± 0.50 respectively. WBC, NEU, LYM, MON, EOS and BAS mean values were 9.68 ± 0.18, 33.65 ± 0.85, 61.32 ± 0.72, 0.94 ± 0.03, 0, 63 ± 0.02, and 0.42 ± 0.01, respectively. Finally, the mean values were 455.19 ± 8.96, 12.24 ± 0.29, 0.29 ± 0.006 and 15.37 ± 0.05 for PLT, MPV, PCT and PDW, respectively. Table 2 contains more information of the phenotype data.

Table 2. Descriptive statistics of blood parameters.

Parameters	**Abr.**	**N**	**Mean ± SE***	**Min.**	**Max.**
Red blood cell (10^{12}/L)	RBC	478	5.43 ± 0.12	0.31	10.70
Hemoglobin (g/dL)	HGB	468	9.51 ± 0.05	6.60	12.20
Hematocrit (%)	HCT	478	23.25 ± 0.45	4.20	44.20
Mean corpuscular volume (fL)	MCV	476	45.19 ± 0.24	35.70	58.40
Mean corpuscular hemoglobin (pg)	MCH	475	24.16 ± 6.90	1.60	71.50
Mean corpuscular hemoglobin concentration (g/dL)	MCHC	477	50.86 ± 1.22	3.60	139.70
RBC volume distribution width coefficient of variation	RDW-CV	476	14.30 ± 0.31	2.00	35.10
RBC volume distribution width standard deviation	RDW-SD	477	23.32 ± 0.50	0.00	57.00
White blood cell (10^{9}/L)	WBC	477	9.68 ± 0.18	0.85	18.28
Neutrophils (%)	NEU	477	33.65 ± 0.85	4.80	85.60
Lymphocytes (%)	LYM	474	61.32 ± 0.72	21.10	92.30
Monocytes (%)	MON	382	0.94 ± 0.03	0.00	4.60
Eosinophils (%)	EOS	457	0.63 ± 0.02	0.00	2.20
Basophils (%)	BAS	466	0.42 ± 0.01	0.00	1.20
Platelets (10^{9}/L)	PLT	214	455.19 ± 8.96	105.00	819.00

Mean platelets volume (fL)	MPV	425	12.24 ± 0.29	4.70	21.10
Procalcitonin (ng/mL)	PCT	209	0.29 ± 0.006	0.07	0.51
Platelets distribution width (fL)	PDW	419	15.37 ± 0.05	12.50	18.60

*SE= Standard Error

3.2. SNP marker summary

The number of animals used in the analyzes for each trait varies due to the removal of outliers from the data in the quality control of the phenotypic data. The number of samples for the relevant traits were given in the descriptive statistics tables (See Table 1 and 2). Although the number of SNPs for each trait changes after the SNP QC analysis, there were 49,931 SNPs in the raw genotype data, while this number decreased to a minimum of 40,463 SNPs after applying the QC criteria mentioned in the material method section. During genotyping, only one animal could not pass as a result of the analyzes made in the Axiom Suit Analyzer program, which is the quality control program of the genotyping platform, due to its poor quality values. In the QC analyzes performed with GenABEL package, all samples passed the analysis, but approximately 9,000 SNPs did not pass the SNP criteria. This shows that the protocol was fully implemented during genotyping and a relatively successful genotyping was performed.

3.3. Genetic diversity analyses for parasite resistance

The animals used in the study were divided into two groups, infected and uninfected. H_E and H_O analyzes were performed to see if there is a genetic differentiation between these two populations. In general, the average estimates for H_E and H_O are presented in Table 3. The average estimate of H_E and H_O did not differ statistically (p-value > 0.05) between the two populations.

Table 3. Estimates of genetic diversity parameters for the infected and non-infected cohorts of Akkaraman sheep.

Traits/Groups (N)	Expected heterozygosity (H_E) Mean ± Sd	Observed heterozygosity (H_O) Mean ± Sd	F_{ST} Pairwise Mean ± Sd
NemEPG3			0.0019 ± 0.002
Infected (43)	0.3804 ± 0.117	0.3870 ± 0.135	
Uninfected (86)	0.3828 ± 0.114	0.3832 ± 0.123	
MonEPG3			0.0018 ± 0.002
Infected (81)	0.3828 ± 114	0.3840 ± 0.123	
Uninfected (48)	0.3809 ± 0.116	0.3853 ± 0.132	
CocOPG3			0.0023 ± 0.032
Infected (60)	0.3821 ± 0.115	0.3862 ± 0.128	
Uninfected (69)	0.3813 ± 0.115	0.3830 ± 0.126	
NemEPG6			0.0005 ± 0.00
Infected (29)	0.3784 ± 0.119	0.3882 ± 0.143	
Uninfected (446)	0.3841 ± 0.112	0.3834 ± 0.114	
MonEPG6			0.0008 ± 0.001
Infected (149)	0.3826 ± 0.114	0.3826 ± 0.119	
Uninfected (326)	0.3840 ± 0.112	0.3842 ± 0.115	
CocOPG6			0.0010 ± 0.001
Infected (298)	0.3835 ± 0.113	0.3839 ± 0.116	
Uninfected (177)	0.3831 ± 0.114	0.3833 ± 0.118	
Total (479)	0.3842 ± 0.112	0.3837 ± 0.114	

The average F_{ST} estimates were found to be 0.0019, 0.0018, 0.0023, 0.0005, 0.0008 and 0.0010 for NemEPG3, MonEPG3, CocOPG3, NemEPG6, MonEPG6 and CocOPG6, respectively. The results did not show the differences between the genetics of infected and non-infected lambs.

3.4. Genomic heritability estimates

3.4.1. Parasite resistance

Heritability estimates for parasite resistance phenotypes range from low to moderate in Akkaraman sheep. As seen in Table 3, the lowest heritability was calculated for MonFEC3 (0.00) and NemFEC6 (0.009). Heritability estimates for the NemFEC3, CocFOC3, MonFEC6 and CocFOC6 traits were found to be moderate as 0.34, 0.11, 0.30 and 0.25, respectively. When the margins of error of the heritability estimations are

examined, the errors of the heritability estimations of the traits collected in 3 months were found to be higher than those of the traits obtained in the 6-month period (See Table 3).

Table 4. Genomic heritability (h^2) estimates for parasite resistance traits.

Trait	h^2	[1]SE
NemFEC3	0.34	0.28
MonFEC3	0.00	0.20
CocFOC3	0.11	0.24
NemFEC6	0.01	0.05
MonFEC6	0.30	0.11
CocFOC6	0.25	0.15

[1] standard error

3.4.2. Blood parameters

The estimations of heritability for blood parameters were ranged from 0.00 to 0.55, which indicates those phenotypes to have low to moderate heritabilities in Akkaraman sheep. In general, erythrocyte traits have heritability estimates ranging from 0.07 to 0.23, while for the leukocyte related traits this range is slightly higher with 0.02 for EOS (%) and 0.55 for WBC (10^9/L). On the other hand, heritability estimates for platelet parameters were ranging between 0.00 and 0.13, where the former was found for PCT (ng/mL) and the later for PLT (10^9/L), respectively. Table 2 shows the estimations of genomic heritability, along with their standard errors.

Table 5. Genomic heritability (h^2) estimates for the traits.

Parameters	**h^2**	**[1]SE**
RBC	0.20	0.10
HGB	0.12	0.08
HCT	0.21	0.10
MCV	0.14	0.09
MCH	0.20	0.10
MCHC	0.23	0.10
RDW-CV	0.10	0.08
RDW-SD	0.07	0.07
WBC	0.55	0.13
NEU	0.15	0.09
LYM	0.14	0.09
NEU/LYM	0.20	0.10
MON	0.22	0.10
EOS	0.02	0.06
BAS	0.27	0.01
PLT	0.13	0.10
MPV	0.11	0.09
PCT	0.00	0.12
PDW	0.04	0.07

[1] standard error

3.5. Genome-wide association studies

Linear Mixed Model (LMM) was used to identify important SNPs associated with phenotypes. The greatest advantage of LMM in GWAS analysis is that it can eliminate the false positive results from population stratification and animal relationships. The genotypic effect of SNPs and the random genetic effect (additive effect) of the animal calculated by the SNP-based genomic relationship matrix (G). The genotype in each SNP marker will be coded 0, 1 and 2 according to the number of copies of the alleles, and each SNP genotype was added as a constant effect to the model and the process was

repeat for each SNP. Animals were applied to the model as a random effect in the model for the parameterization of genetic effect. The dependent variable in the LMM equation was directly the measured phenotypes. For all animals, all other fixed effects except SNP effects are included in the model in the presence of a calculated polygenic background effect, and variance components (i.e., additive genetic variance and error variance) are calculated by the maximum likelihood method.

The observed test statistics for each SNP were compared to the test statistics expected under the null hypothesis using quantile-quantile (Q-Q) plots (Figure 3 and 5). A Q-Q plot and the estimated inflation factor lambda (λ) were attained for each phenotype which was set to 1 with genomic control applied to p-values as defined by (Devlin and Roeder, 1999). Manhattan plots were used to visualize the –log10 (p-values) of all SNPs respective to their position at the relevant chromosome. Genome- and chromosome-wide significance thresholds defined by using Bonferroni correction were implemented to avoid increasing Type 1 Error rate due to multiple testing of SNPs (Benjamini and Hochberg, 1995). Accordingly, the genome-wide significant threshold was 1.23e-06 (0.05/40,463) and the chromosome-wide significant threshold was 3.21e-05 (1/40,463).

3.5.1. Parasite resistance

Genome-wide association analysis was performed for 6 different traits (namely NemFEC3, MonFEC3, CocFOC3, NemFEC6, MonFEC6 and CocFOC6) belonging to three different parasite groups (i.e., nematodes, tapeworm and coccidian parasite) associated with GIP resistance. A LMM model was used to calculate SNP effects and relevant p-values in genome-wide association analyses. SNP effects and environmental factors were included in the model as fixed effects, whereas the individual effects of animals and the effect arising from genetic background of animal were included in the model as random effects. Genetic effect was taken account by using the genomic association matrix (G) in the model. The effect of sampling age from environmental factors was found to be significant for all traits associated with parasite resistance, except for the MonFEC6 trait. The herd effect was found to be significant for only MonFEC3 and NemFEC3 traits. While the sex effect was found to be effective only in MonFEC3 trait, no environmental factor was found to be effective on the MonFEC6 trait. Finally, for the CocFOC3 trait, there was an interaction effect between the feed

type and sex, while for the NemFEC6, a 3-way interaction was found between the herd, sex and feed type.

Table 6. Significant SNP associated with parasite resistance phenotypes.

Traits	SNP name[1]	Chr.	Position (bp)[2]	P-value	Associated genes	
					Name	Distance (bp)
NemFEC3	*rs415401096*	1	275345749	$1.44x10^{-05}$	*ZNF596*	near~5kb
NemFEC3	*rs408499938*	21	23300938	$1.90x10^{-05}$	*NELL1*	within
NemFEC6	*rs421027634*	26	12363158	$1.08x10^{-07}$	*TENM3*	within
NemFEC6	*rs413573397*	1	51891269	$5.48x10^{-06}$	*ST6GALNAC3*	within
NemFEC6	*rs403250421*	7	30603665	$1.09\ x10^{-05}$	*TMCO5A*	near~35kb
MonFEC3	*rs409854037*	2	209775617	$1.01x10^{-05}$	*PTH2R*	near~200kb
MonFEC6	*rs401554073*	22	34528500	$6.92x10^{-07}$	*ATRNL1*	within
CocFOC3	*rs428814111*	3	177167791	$5.63\ x10^{-06}$	*LARGE1*	near~200kb
CocFOC3	*rs406580275*	1	90278893	$1.79x10^{-05}$	*HIPK1*	within
CocFOC3	*rs405203400*	3	114958514	$2.02x10^{-05}$	*SYT1*	within
CocFOC6	*rs405331699*	2	224516770	$3.05\ x10^{-06}$	*SCG2*	near~200kb
CocFOC6	*rs412952616*	3	36311950	$9.18\ x10^{-06}$	*ALK*	within

[1]SNP position based on OAR_v4.0; [2]additive genetic variance explained by each SNP

Overall, a total of 12 SNPs were found to be statistically significant as a result of the GWAS of the traits associated with GIP resistance. 2 of these SNPs on OAR 22 and 26, for NemFEC6 and MonFEC6 traits, were found to be genome-wide significant, while the other 10 SNPs found as chromosome-wide. These important 12 SNPs were located on 6 different sheep chromosomes (OAR1, 2, 3, 7, 21, 22, and 26.). On the other hand, all these SNPs are located on or near (± 200 kb) the genes defined on the sheep genome.

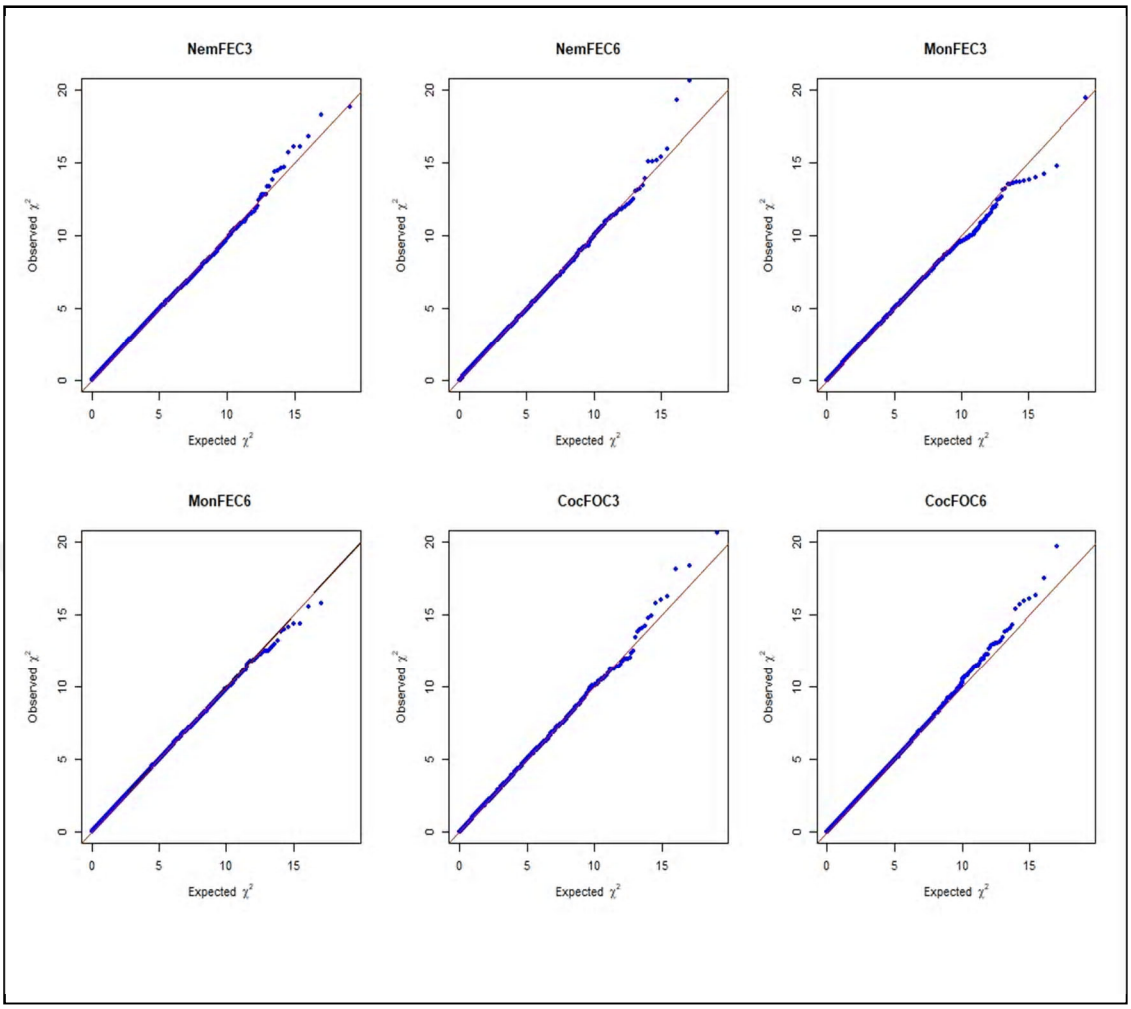


Figure 1. Quantile-quantile (Q–Q) plots of genome-wide association studies (GWAS) for the parasite resistance traits.

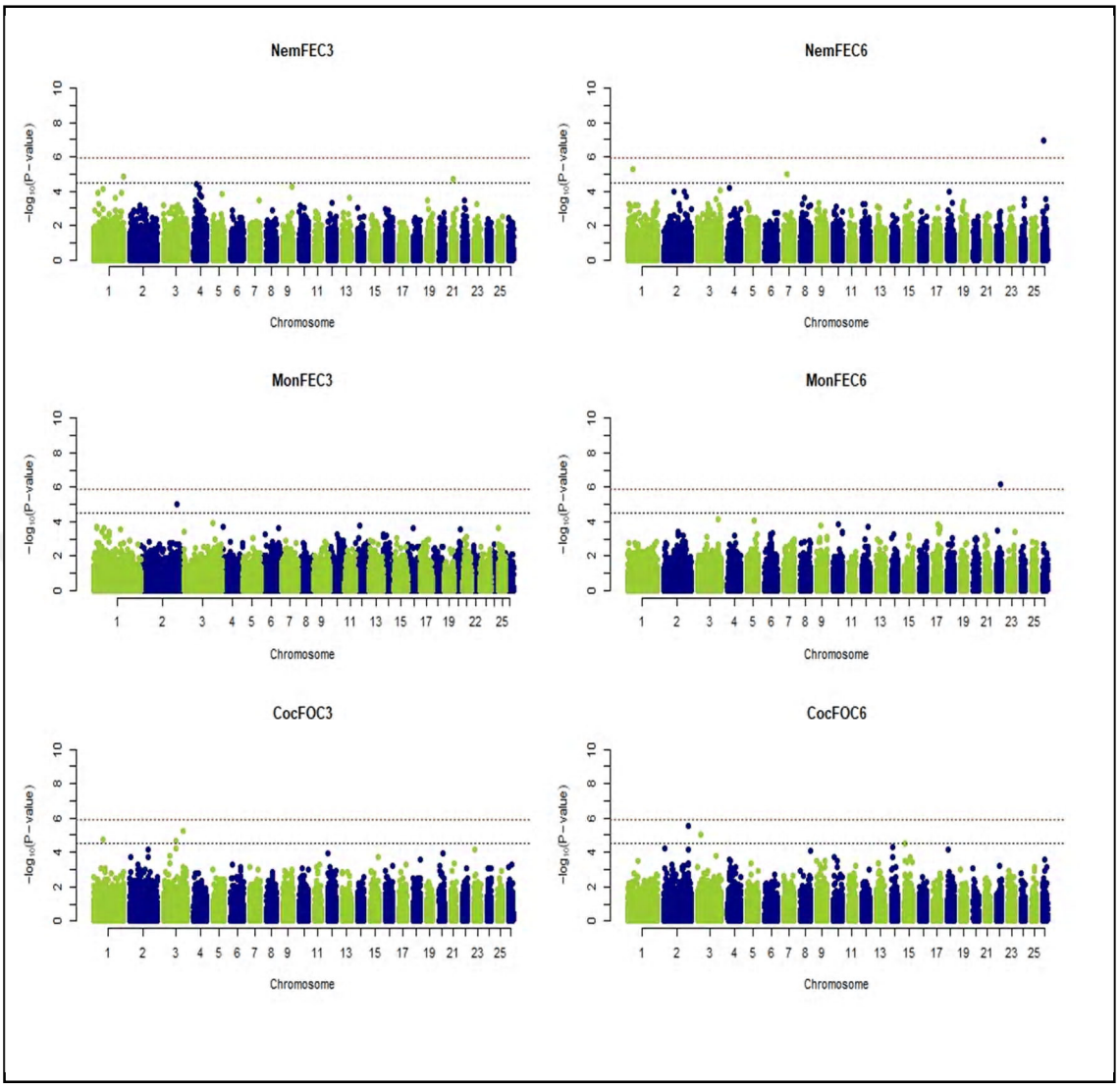


Figure 2. Manhattan plots of six parasite resistance traits. Red dash line genome-wide significance level and black dash line chromosome-wide significance level. Nematode egg count at 3-month age (NemFEC3), Nematode egg count at 6-month age (NemFEC6), Tape worm egg count at 3-month age (MonFEC3), Tape worm egg count at 6-month age (MonFEC6), *Eimeria spp*. oocyst count at 3-month age (CocFOC3), *Eimeria spp*. oocyst count at 6-month age (CocFOC6).

3.5.2. Blood parameters

Before progressing the GWAS, a linear animal mixed models were implemented to estimate the environmental effects (sex, birth type, birth month etc.) for each traits. As a result, sex, herd, and age (in days) were found to have statistically significant effects on WBC and RDW CV traits, whereas, NEU, LYM, EOS, and MCH live weight was found to be statistically significant. Eventually, interactions between herd and feeding type, as well as sex and feeding type, were discovered to be important for WBC and LYM, and were incorporated into the GWAS models.

Table 7. Significant SNP associated with the blood phenotypes.

Traits	SNP name	Chr.	[1]Position (bp)	P-value	[2]Vg (%)	Other associated phenotypes	Associated genes	
							Name	Distance (bp)
RBC	*rs418188401*	2	142026337	$4.08x10^{-06}$	0.42	RDW_CV	*SCN7A*	Within
RBC	*rs423050506*	1	1879393	$8.81x10^{-06}$	0.87	-	*HDAC4*	Near
RBC	*rs415321135*	2	142135301	$1.30x10^{-05}$	0.48	-	*SCN9A*	Within
HCT	*rs402657016*	18	19382508	$9.59x10^{-08}$	1.12	MCH, MCHC	-	-
HCT	*rs424122039*	18	19330647	$1.61x10^{-07}$	1.06	MCH, MCHC	*MYADM-like*	Within
MCH	*rs424122039*	18	19330647	$3.00x10^{-08}$	2.96	HCT, MCHC	*MYADM-like*	Within
MCH	*rs402657016*	18	19382508	$5.84x10^{-07}$	2.20	HCT, MCHC	-	-
MCHC	*rs424122039*	18	19330647	$4.83x10^{-11}$	2.15	HCT, MCH	*MYADM-like*	Within
MCHC	*rs402657016*	18	19382508	$2.07x10^{-09}$	1.82	HCT, MCH	-	-
MCV	*rs427024436*	5	17364739	$3.03x10^{-06}$	0.57	-	*SIRT6, CREB3L3*	Near
RDW_CV	*rs415321135*	2	142135301	$3.66x10^{-06}$	0.67		*SCN9A*	Within
RDW_CV	*rs424460878*	21	10141822	$1.36x10^{-05}$	0.67	-	*CCDC67*	Within
RDW_CV	*rs409151807*	2	142204729	$1.48x10^{-05}$	0.68	-	*SCN9A*	Within
RDW_CV	*rs418188401*	2	142026337	$2.20x10^{-05}$	0.40	RBC, HCT	*SCN7A*	Within
WBC	*rs423900300*	19	11192604	$1.19x10^{-06}$	0.74	-	*ITGA9*	Within
NEU	*rs419009933*	20	45260345	$8.94x10^{-07}$	4.35	LYM	*LOC101117887*	Within
NEU	*rs422235744*	13	43611954	$1.75x10^{-05}$	1.57	-	*LOC106991526*	Within
LYM	*rs419009933*	20	45260345	$3.19x10^{-06}$	3.07	NEU	*LOC101117887*	Within
NEU/LYM	*rs416257223*	2	175533494	$1.81x10^{-05}$	1.51		*MGAT5*	Within

EOS	*rs429955018*	1	263341417	$3.05x10^{-07}$	18.32	-	*SLC19A1*	Within
EOS	*rs428421565*	3	112164752	$2.27x10^{-05}$	4.66	-	*OSBPL8, ZDHHC17, CSRP2*	Near
EOS	*rs424356478*	3	112927310	$3.32x10^{-05}$	1.98	-	*NAV3, E2F7*	Near
BAS	*rs417370910*	4	82433190	$6.26x10^{-06}$	1.00	-	*AMPH*	Within
PLT	*rs419857573*	2	99213936	$1.76x10^{-07}$	2.73	PCT	*LOC105608461*	Within
PLT	*rs410481204*	2	34556977	$1.20 x10^{-05}$	1.83	-	*NTRK2*	Within
MPV	*rs424459233*	2	102985180	$1.51x10^{-06}$	5.24	-	*MSRA*	Within
PCT	*rs419857573*	2	99213936	$8.10 x10^{-07}$	7.58	PLT	*LOC105608461*	Within
PCT	*rs426696259*	25	6748890	$8.73x10^{-06}$	4.40	-	*SLC35F3*	Within

[1]SNP position based on OAR_v4.0; [2]additive genetic variance explained by each SNP

The adjusted p-values were obtained as a result of the GWAS for each traits and were illustrated on –log10 scale in the Manhattan plots given in Figures 2, 3 with the genome- and chromosome-wide thresholds (i.e., dashed lines). As a result, a total of 7 genome-wide significant SNPs and 13 chromosome-wide significant SNPs were subjected by the GWAS implemented on 15 blood traits. However, any important or suggestive SNPs were not to be found for 4 of the relevant phenotypes, namely HGB, RDW_SD, MON and PDV. Table 3 contains additional information on the significant SNPs.

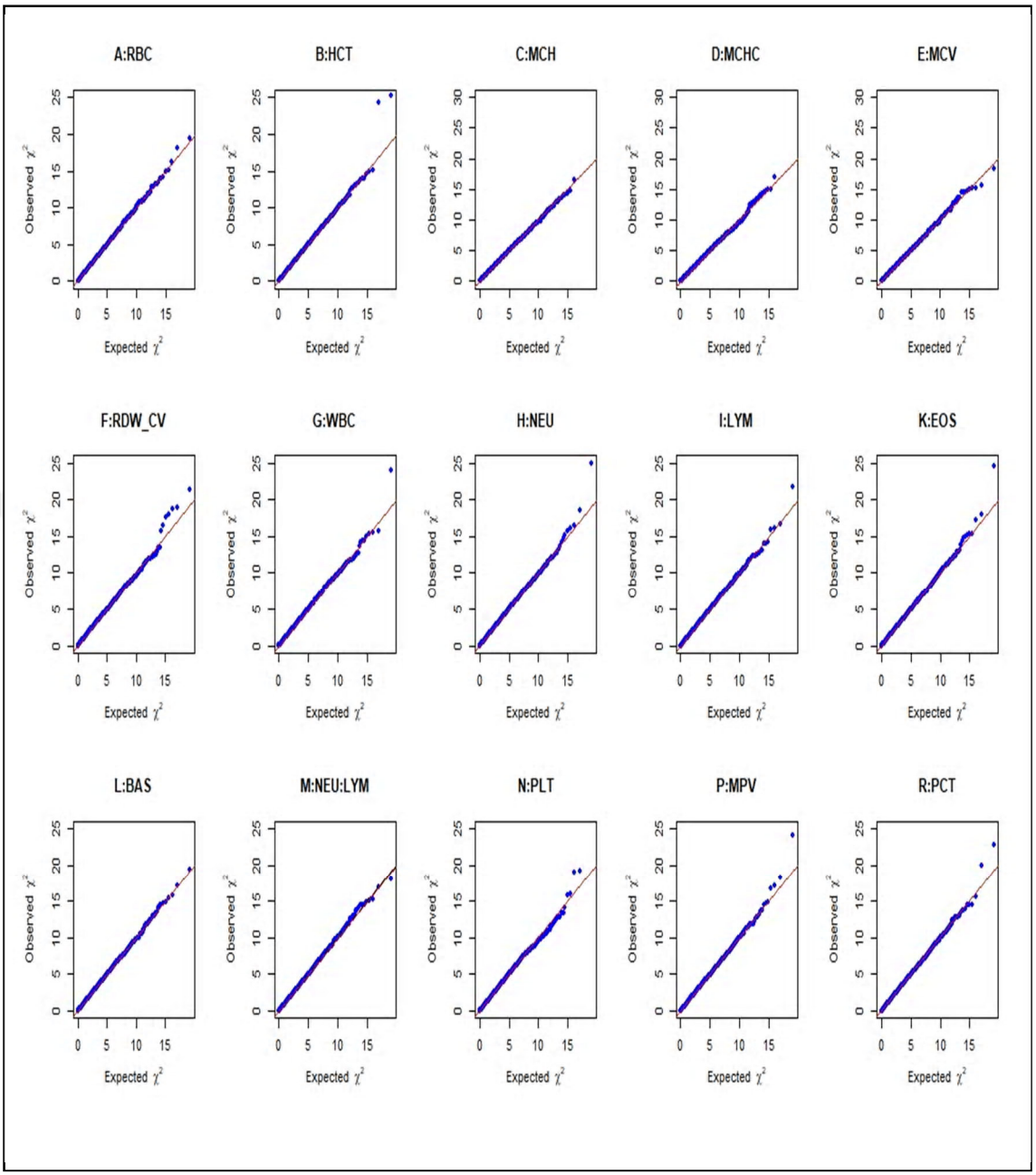


Figure 3. Quantile-quantile (Q–Q) plots of genome-wide association studies (GWAS) for the blood parameters.

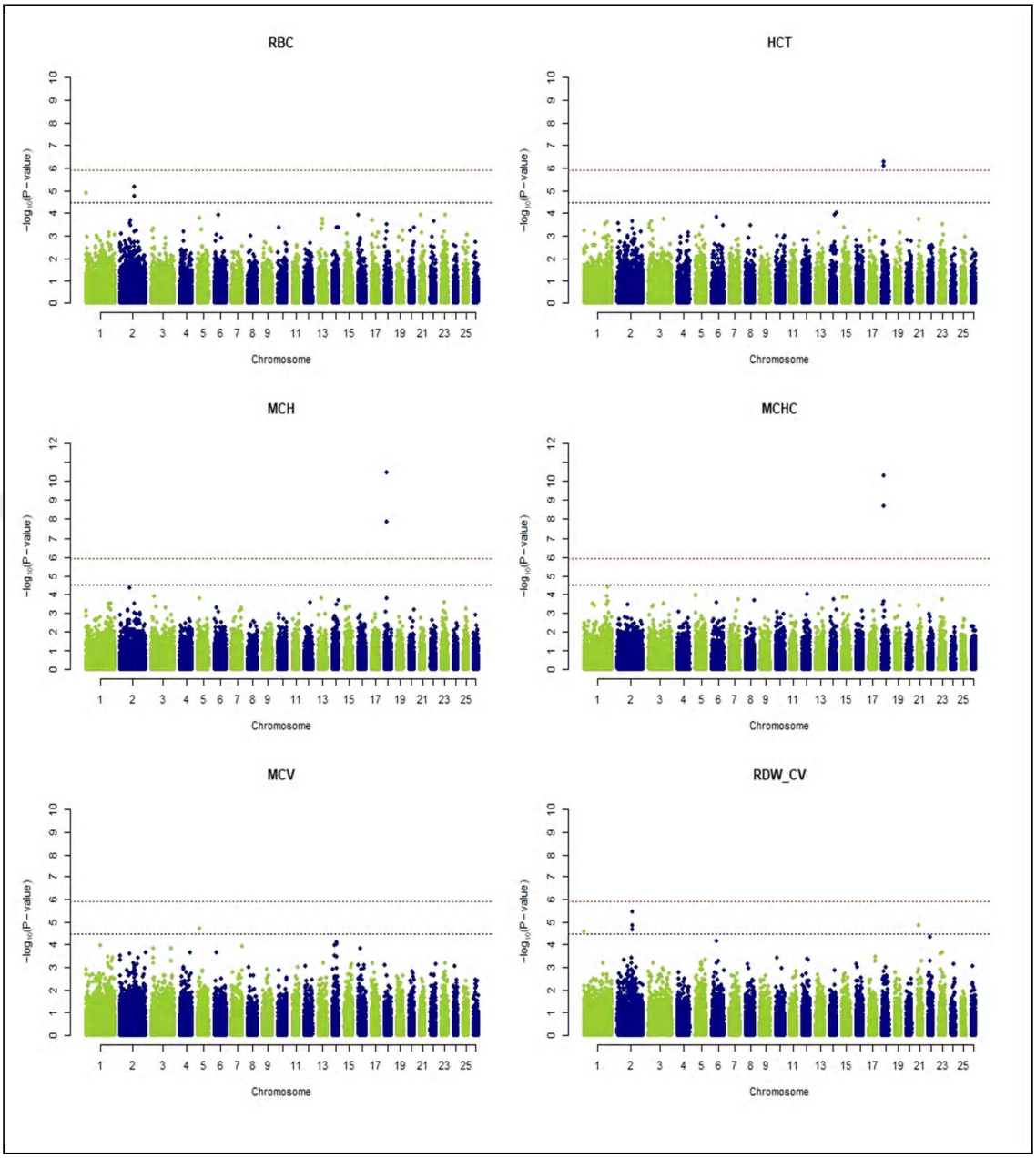


Figure 4. Manhattan plots of GWAS for six erythrocyte traits. Red dashed horizontal line (i.e., upper horizontal line) represent Bonferroni-corrected genome-wide significance level, while black dashed horizontal line (i.e., lower horizontal line) is for Bonferroni-corrected chromosome-wide significance level. The six traits were: red blood cell count (RBC), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV) and RBC volume distribution width coefficient of variation (RDW_CV).

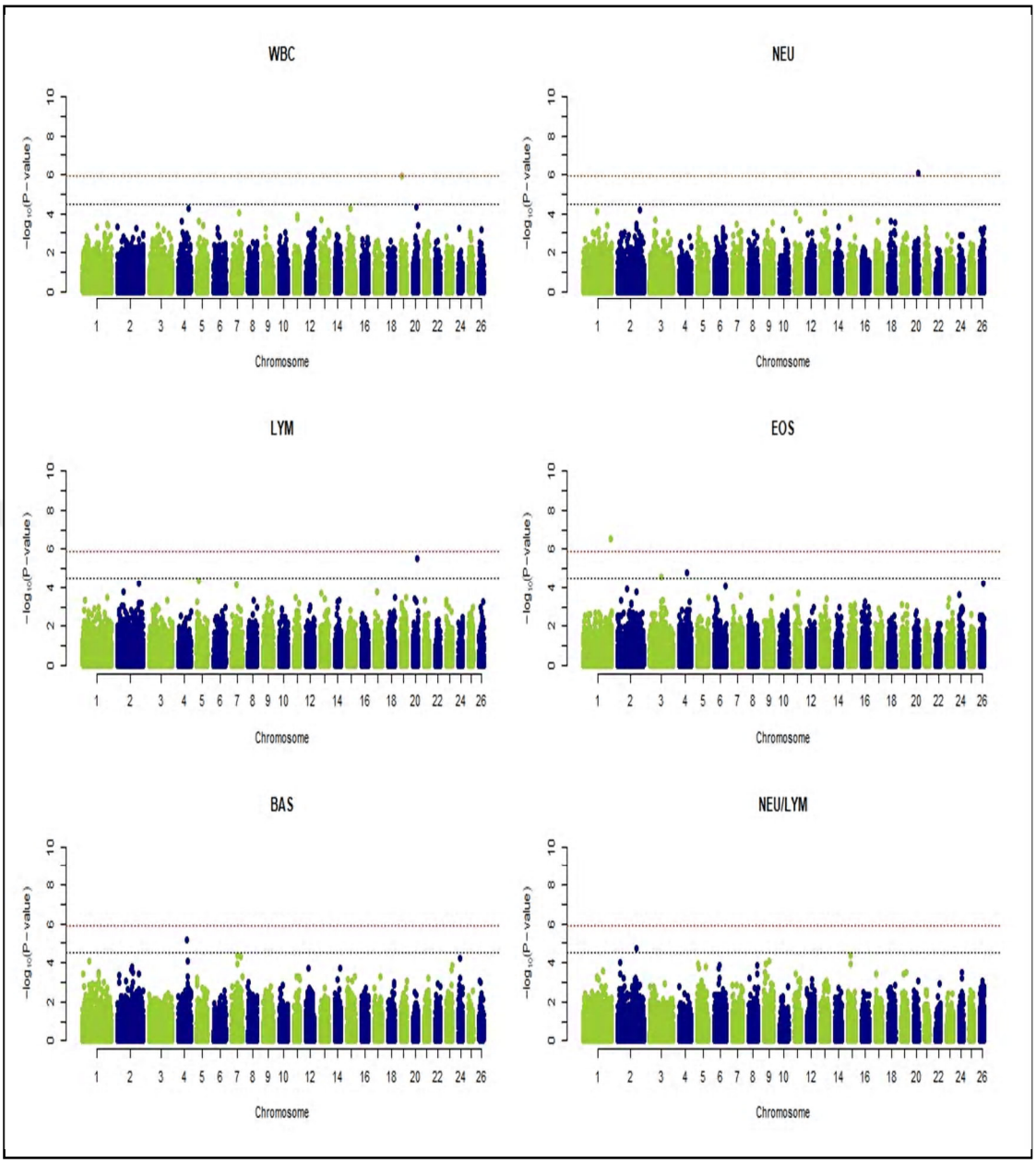


Figure 5. Manhattan plots of GWAS for leucocytes related traits. Red dashed horizontal line (i.e., upper horizontal line) represent Bonferroni-corrected genome-wide significance level, while black dashed horizontal line (i.e., lower horizontal line) is for Bonferroni-corrected chromosome-wide significance level. White blood cell count (WBC), neutrophils (NEU), lymphocytes (LYM), eosinophils (EOS), basophils (BAS) and neutrophils: lymphocytes (NEU/LYM).

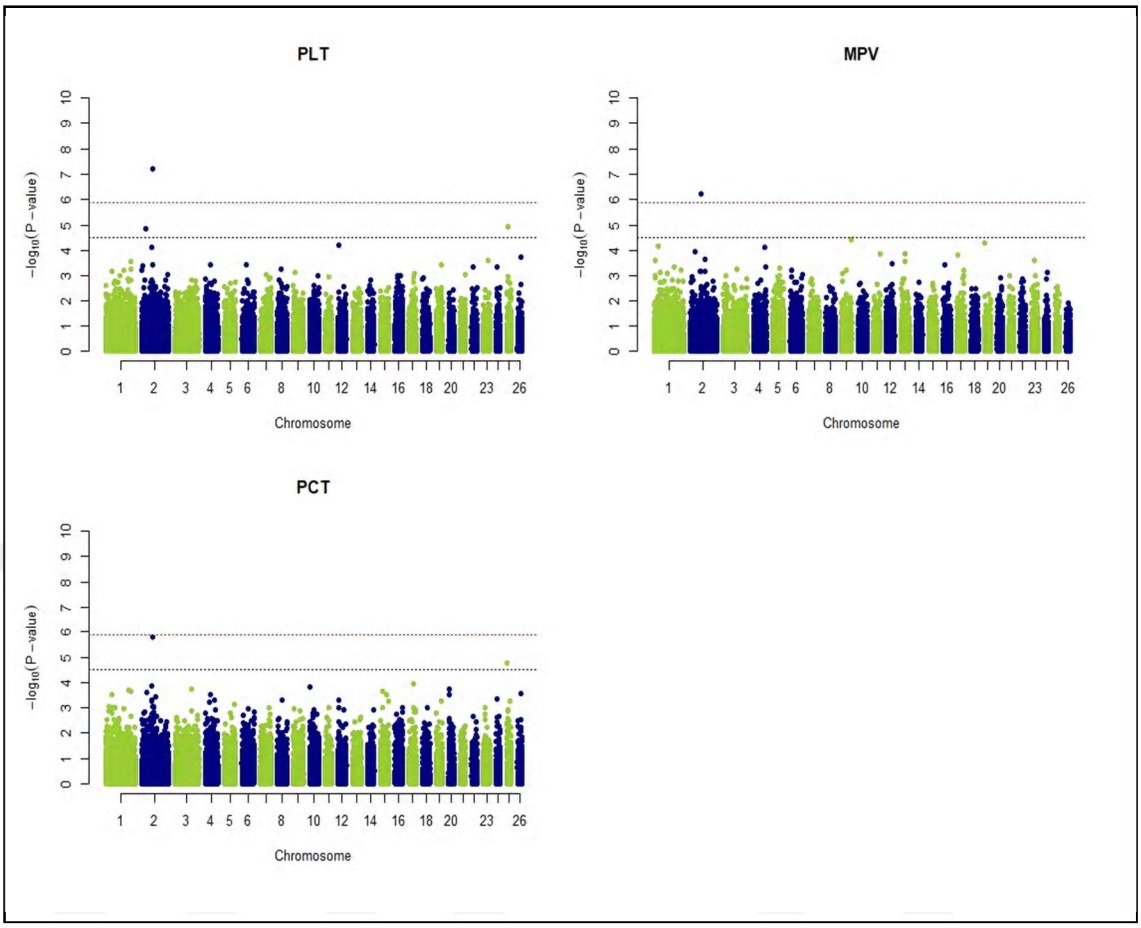


Figure 6. Manhattan plots of GWAS for thrombocyte traits. Red dash horizontal line Bonferroni genome-wide significance level and black dash horizontal line chromosome-wide significance level. Platelets count (PLT), mean platelet volume (MPV), procalcitonin (PCT).

3.6. Candidate genes and QTLs

Using the Oar_v4.0 genome assembly, we managed to get positional information for associated SNPs and candidate genes from the NCBI Genome Data Viewer. (Rangwala et al., 2021). As candidates, genes that are overlapping with SNPs were proposed. As a result of the GWAS studies for the traits associated with GIP resistance, a total of 12 different SNPs were detected on 6 different chromosomes. Seven of these SNPs were located on a specific gene namely Neural Epidermal Growth Factor-Like 1 (*NELL1*), Teneurin Transmembrane Protein 3 (*TENM3*), ST6 N-Acetylgalactosaminide Alpha-2,6-Sialyltransferase 3 (*ST6GALNAC3*), Attractin Like 1 (*ATRNL1*), Homeodomain Interacting Protein Kinase 1 (*HIPK1*), Synaptotagmin 1 (*SYT1*) and ALK Receptor Tyrosine Kinase (*ALK*) in the genome. The other 5 of these SNPs are located in regions

near (up to 200 kb) by specific genes namely Zinc Finger Protein 596 (*ZNF596*), Transmembrane and Coiled-Coil Domains 5A (*TMCO5A*), Parathyroid Hormone 2 Receptor (*PTH2R*), LARGE Xylosyl- And Glucuronyltransferase 1 (*LARGE1*) and Secretogranin II (*SCG2*) on the genome.

As a result of GWAS for blood traits, 11 genome- and chromosome-wide significant SNPs were found to be located on particular genes, namely Sodium Voltage-Gated Channel Alpha Subunit 7 (*SCN7A*), Sodium Voltage-Gated Channel Alpha Subunit 9 (*SCN9A*), Myeloid-associated Differentiation Marker-like (*MYADM-like*), Coiled-Coil Domain-Containing Protein 67 (*CCDC67*), Integrin Subunit Alpha 9 (*ITGA9*), Alpha-1, 6-Mannosylglycoprotein 6-Beta-N-Acetylglucosaminyltransferase (*MGAT5*), Solute Carrier Family 19 Member 1 (*SLC19A1*), Amphiphysin (*AMPH*), Neurotrophic Receptor Tyrosine Kinase 2 (*NTRK2*), Methionine Sulfoxide Reductase A (*MSRA*) and Solute Carrier Family 35 Member F3 (*SLC35F3*) on OAR1, 2, 4, 5, 19, 21 and 25. 8 of the significant SNPs were in close vicinity with the identified genes that are Histone Deacetylase 4 (*HDAC4*), Sirtuin 6 (*SIRT6*), CAMP Responsive Element Binding Protein 3 Like 3 (*CREB3L3*), Oxysterol Binding Protein Like 8 (*OSBPL8*), Zinc Finger DHHC-Type Palmitoyltransferase 17 (*ZDHHC17*), Cysteine and Glycine Rich Protein 2 (*CSRP2*), Neuron Navigator 3 (*NAV3*) and E2F Transcription Factor 7 (*E2F7*) on OAR1, 3 and 5. Also 3 of the important SNPs were located in unidentified region namely *LOC101117887*, *LOC106991526* and *LOC105608461*) on OAR2, 13 and 20.

CHAPTER 4

4. DISCUSSION

Parasitic diseases and economic losses due to these diseases have always been a problem that needs to be solved in small ruminant breeding (especially pasture-based small ruminant production systems). Global warming and the accompanying climate change have increased the frequency of parasite infection and the diversity of parasites in a region. On the other hand, the resistance developed by parasites against anti-parasite agents is increasing day by day. When all these situations come together, it will be more difficult to reduce parasitic infections in farm animals in the future and alternative mitigation strategies are needed.

The increase of technological advancements has paved a way for genome sequencing technology and consequently, the reference genomes of economically important farm animal species were obtained. With the knowledge of reference genomes, functional variants (i.e., SNP, CNV) in the genome for each population/breed were determined and their commercial use was provided with designed panels. Furthermore, the genetic of economically important traits such as parasite resistance, hematological traits, milk and meat yield in livestock has been investigated by associating phenotype and genotype data with a LMM based analysis, which is the mainly used method in GWAS (Gonzalez et al., 2013; Mucha et al., 2018; White et al., 2012; Yilmaz et al., 2022; Zamani et al., 2021). Moreover, this information obtained from the genome has turned into application in animal breeding by including complex traits in selection indexes in many national breeding programs of the countries. Consequently, breeding of animals that are more resistant to diseases and have higher adaptation capacity has been started.

In this study, the main objective was to understand the genetic background of health traits (i.e., parasite resistance and blood traits) in Akkaraman sheep, a widespread indigenous sheep breed in Türkiye. For this purpose, the genomic heritability was estimated for 6 parasite resistance-related traits and 19 blood traits, and the genome-wide association analyses led to the proposed 12 specific genes to parasite resistance traits and 19 particular genes and three unidentified locations for blood phenotypes (Table 5 and 6) for those traits were implemented.

4.1. Parasite resistance

There are not enough parasite resistance studies in Akkaraman sheep due to natural infection in pasture. However, studies conducted in other sheep breeds around the world reported a higher parasite load than the current study (Gowane et al., 2019; Mpetile et al., 2015; A Pacheco et al., 2021). In a study with Scottish Blackface breeds, the mean fecal egg count of *Strongles* and *Nematodarius* species in naturally infected animals in the pasture was reported as 714 and 226, respectively. In the same study, the average number of fecal oocysts belonging to *Eimeria* species was reported as 30,631 (A. Pacheco et al., 2021). In the present study, the mean of total nematode egg count was reported as 36.5 and 6.68 in 3- and 6-month old lambs, respectively. Furthermore, in the present study, mean fecal oocyst counts for coccidian parasites were found to be 1,698 and 2,627 in 3- and 6-months old lambs, respectively. There are many reasons why the parasite load in Scottish Blackface sheep was higher than the current study. The most important of these is the difference in precipitation between the regions. In humid areas, the high contamination due to the parasite's life cycle directly affect the parasite load in animals. In addition, the infection of the pasture is among the reasons for the difference between the two studies.

4.1.1. Genetic diversity

In the study, it was observed that there was no genetic differentiation between the infected and non-infected group. Statistically, the difference between the H_E and H_O estimates between the two groups was not significant (See Table 3). Moreover, FST test which shows the proportion of the genetic differences between two group, illustrated that these two groups showed no differentiation (ranged from 0.0005 to 0.0023) in terms of genetic diversity. In a study conducted in Tunisian sheep, no difference was found

between the groups in terms of H_E and H_O between the two groups infected and uninfected with *H. contortus* infection (Ahbara et al., 2021). Similarly, another research from Ethiopia reported that there was no genetic differentiation between two groups of Bonga sheep (infected and non-infected) by regarding GIP infection (Dolebo et al., 2019). However, the study conducted on Brazilian Santa Inês sheep, the variability were detected in terms of parasite resistance (Berton et al., 2017).

Although traits associated with parasite resistance have a low to moderate heritability, one of the reasons for the lack of genetic differentiation between infected and uninfected groups may be positive selection of parasite resistance. The animals could be naturally or artificially selected for GIP infections over time. In other words, the existence of Akkaraman sheep in the region for centuries may have enabled them to gain resistance against pathogens such as parasite in the region. Thus, all animals in the sampled populations may have been selected for the putative alleles with parasite resistance. The fact that the slight deviation from the normal distribution of egg and oocyst counts from animals supports this situation. Another reason for the lack of genetic diversity may have been that the animals were not sufficiently exposed to parasites in pasture conditions. They may not show the genetic potential that provides resistance to the actually existing parasite. In other words, it may be that the current genetic potential is not reflected in the phenotype due to this insufficient parasite load, and therefore the phenotype-genotype association cannot reveal the different genetic structure. In order to reveal genetics of resistance against GIP infections and to understand the genetic structure that provides resistance more clearly, it may be recommended that parasite resistance phenotypes have more variation, and studies should be conducted by creating controlled infections.

4.1.2. Genomic heritability

In our study, the heritability of eggs/oocyst counts of Nematodes, Tapeworms and coccidian parasites in Akkarman lambs at 3 and 6 months of age was estimated using LMM. In estimation of heritability, the genomic relationship matrix (G) obtained from the genomic data from a sheep panel containing 50,000 SNPs was used instead of the numerical relationship matrix (A). Heritability estimates of the traits ranged from 0 (MonFEC3) to 0.34 (NemFEC6). In general, the error margin of the heritability

estimates for the phenotypes collected in the 3-month age were found to be higher than the phenotypes formed in the 6-month period. One of the main reasons for this situation may be that animals are not sufficiently exposed to parasites during this period. In addition, the low number of infected animals reduces the statistical power. Another issue may be that the immune system of animals is not sufficiently developed at the age of 3 months. Thus, it can be evaluated that the animal does not adequately reflect the effect of the genetic potential on its immune system.

Studies on genetic parameter estimation of gastro-intestinal parasite resistance indicator phenotypes have mostly focused on nematode species due to their high economic damage. In studies conducted with different nematode species and different breeds, it is seen that gastrointestinal nematode resistance has a heritability varying from low to moderate (Berton et al., 2019; Bishop et al., 1996; Gowane et al., 2019; Gutiérrez-Gil et al., 2010; Huisman et al., 2008; Mpetile et al., 2015). For instance, in studies conducted for *Haemonchus contortus* FEC in Santa Ines sheep raised in the tropics, heritability was reported to be around 0.10 (Berton et al., 2019). In another study, it was reported that the heritability of fecal egg counts for nematodes in Scottish Blackface lambs 1-6 months old ranged between 0.01 and 0.22. (Bishop et al., 1996). Furthermore, Gowane et al., (2019) found the heritability of FEC phenotypes performed in the pre- and post-drench period in Avikalin and Malpura sheep as 0.21 - 0.03 and 0.19 - 0.12, respectively. In another study conducted in Spanish Churra dairy sheep, the heritability of the fecal egg count trait for *Teladorsagia circumcincta* was found to be between 0.09 and 0.12 (Gutiérrez-Gil et al., 2010). On the other hand, in the studies conducted by Huisman et al., (2008) and Mpetile et al., (2015) on merinos, which is the world's common sheep breed, the heritability for the FEC trait was reported as 0.18 and 0.10, respectively. In our study, the heritability of the total nematode egg number was found to be 0.34 and 0.01 in 3- and 6-months old lambs, respectively. It can be said that the results are partially similar when compared to the previous studies and our current study. It is expected that the genetic resistance to these diseases can be different in animals that have obtain the gene-environment relationship for many years in environments where contamination is intense. However, the isolation of the breed/population, different breeding practices, different breeding goals, and different levels of exposure to parasites in their environment provide different genetic advantages

in terms of parasite resistance to different populations and breeds. This is the most important factor explaining the difference in heritability estimations. On the other hand, the high margin of error of heritability estimations in the current study and previous studies shows there were unexplained environmental factors in the models. While this is the case, when the life cycles of nematodes (very dependent on humidity and temperature) are taken into account, environmental factors (especially humidity, warm and rainy climates) constitute the reasons for the differences between populations and breeds.

Studies on coccidian and tapeworm infections are rather sparse compared to nematode studies. In a study conducted in Texel, Ile de France, Morada Nova sheep and crossbreds of these breeds, it was reported that tapeworm (*Moniezia expansa*) egg and coccidian (*Eimeria spp*.) oocyst numbers varied in different genotype (breed) animals (McManus et al., 2009). Finally, in a study by A. Pacheco et al., (2021) in Scottish Blackface sheep, the heritability estimates of fecal *Strongyles spp*. , *Nematodirus spp*. eggs and Coccidian (*Eimeria spp.)* oocysts were found to be 0.17, 0.09 and 0.09, respectively. Considering the age groups used in this study, the heritability estimation for coccidian in 3-month-old lambs was found to be similar to the heritability (0.11) obtained in the current study. One of the most important reasons why coccidian parasite causes transmission and disease in animals at an early age is the hygienic conditions of the existing environment. For this reason, it is expected that the genetic resistance to these diseases will be different in animals that have obtain the gene-environment relationship for many years in environments where contamination is intense.

In general, the heritability of the phenotypes associated with GIP resistance range from low to moderate. This shows that these traits have complexity and multi-genic characteristics. Fortunately, the fact that these traits have low to moderate heritability which indicates that breeding is possible in sheep for these traits and more resistant herds against parasitic infections can be obtained by designing proper breeding program. As a matter of fact, breeding studies applied for parasite resistance in breeding strategies of many countries support this idea of the genetic improvement on parasite resistance.

4.1.3. Genome-wide association studies

As a result of the GWAS, 12 distinct SNPs were found to be statistically significant performed for traits associated with parasite resistance. While 2 of these SNPs were significant at genome-wide level, and 10 of them were found to be chromosome-wide significant. The top SNP (rs421027634) was found to be on OAR26 for NemFEC6 trait with a p-value of 1.08e-07, whereas the SNP (rs401554073) with the second-highest p-value (6.92e-07) for the MonFEC6 trait was found on OAR22. The distribution of important SNPs on the chromosome is 3 SNPs in OAR1, 3 SNPs in OAR3, 2 SNPs in OAR2, and 1 SNP in OAR7, 21, 22 and 26. Considering that the phenotypes belong to 3 different parasite groups acquired at 3 and 6 months, no SNPs with pleiotropic effect associated with these traits were found. This situation may be due to the different genetic backgrounds of the immune defense mechanisms against the parasite burden. Significant SNPs were annotated according to their positions on the reference genome (OAR_v4.0) using the National Center for Biotechnology Information (NCBI) Genome Data Viewer. While 7 of the SNPs were located on a particulate gene defined on the genome, 5 of them were found nearby (± 250 kb) at least one particular gene. Accordingly, the suggested genes as candidate for parasite resistance were namely *NELL1*, *TENM3*, *ST6GALNAC3*, *ATRNL1*, *HIPK1*, *SYT1*, *ALK, ZNF596*, *TMCO5A*, *PTH2R*, *LARGE1, SCG2.*

As a result of the GWAS performed for fecal egg count phenotypes (NemFEC3 and NemFEC6) obtained at 3 and 6 months of age associated with nematode resistance, the significant SNPs were found over OAR1 7, 21 and 26. SNPs important for NemFEC3 are associated with the *ZNF596* and *NELL1* genes, while significant SNPs for NemFEC6 were associated with *TENM3*, *ST6GALNAC3* and *TMCO5A*. *NELL1* is a cytoplasmic protein-coding gene that plays a role in the control of cell growth and differentiation. Proteins encoded by NELL1 play a significant role in many basic functions such as cell growth, cell differentiation, immune development and ion transport by participating in biological processes such as cell differentiation (GO:0030154), regulation of gene expression (GO:0010468), and molecular functions such as protein kinase C binding (GO:0005080), calcium ion binding (GO:0005509) and heparin binding (GO:0008201). *ZNF596* is another associated gene plays a regulatory role in cellular for NemFEC3 trait on OAR1. This gene particularly encodes

protein which involved in biological process such as regulation of transcription, DNA-templated (GO:0006355). *TENM3* gene were found to be associated with SNP rs421027634 and have the highest p-value (in the scale of –log10) as a result of GWAS for NemFEC6 phenotype. Teneurin proteins synthesized by this gene take place in many cell components, especially in the cell membrane (GO:0016020), and take part in many biological functions such as cell-cell adhesion (GO:0098609), cell adhesion (GO:0007155) and signal transduction (GO:0007165). Teneurins (Tens) are a family of highly conserved proteins and are very important in cell-cell adhesion. Studies show that the Wnt signaling system, which moderates the innate immune response and adaptive immune response with the production of inflammatory cytokine, upregulates the Ten-3 protein (Bastías-Candia et al., 2019). It is also might be that these proteins are involved in both wound-repairing and signal transduction in tissue and cell destruction in the alimentary tract by larval and adult parasites in nematode infections. *ST6GALNAC3* gene was the one of the associated gene for NemFEC6 traits on OAR1. This gene is the member of sialyltransferase family, which transfers sialic acids from CMP-sialic acid to carbohydrate group terminal positions in glycoproteins (Lee et al., 1999). This gene plays an active role in the glycoprotein metabolic process (GO:0009100). As is known, glycoproteins (i.e., CD8, CD4) are released from cytotoxic T (Tc) cells that kill virus or other pathogen-infected cells in the adaptive immune system and provide essential signals that activate the maturation of B cells, Tc cells and macrophages (McRae et al., 2015). *TMCO5A* (Transmembrane and Coiled-Coil Domains 5A) is a gene which encode protein particularly integral component of membrane (GO:0016021) and playing role in the protein binding (GO:0005515) molecular function.

After the GWAS analysis, *PTH2R* and *ATRNL1* genes were found to be associated with the fecal egg count phenotypes of *Moniezia spp.* (MonFEC3 and MonFEC6) on OAR2 and 22, respectively. *rs401554073* SNP which was associated with *ATRNL1*gene is the genome-wide significant SNP with the p-value of 6.92e-07. Interestingly, these two genes are directly related to G-Protein-Coupled Receptors (GPCRs). While *PTR2G* gene is a member of the G-protein coupled receptor 2 family, *ATRNL1* gene play an active role upstream of or within G protein-coupled receptor signaling pathway. Particularly, protein coded by *PTR2G* is located in component of cell membrane

(GO:0005886, GO:0005887, GO:0016020 and GO:0016021) and actively involving in cell surface receptor signaling pathway (GO:0007166), G protein-coupled receptor signaling pathway (GO:0007186) and adenylate cyclase-modulating G protein-coupled receptor signaling pathway GO:0007188). Similar to *PTH2R* gene, *ATRNL1* gene codes cell membrane protein which involving in G protein-coupled receptor signaling pathway (GO:0007186). By acting as gate-keepers, these GPCRs play an important role in the induction of cytokines and other signals responsible for the stimulation and manipulation of the adaptive immune system (Pierce et al., 2002). They specifically shape macrophage immune responses to extracellular pathogens as well as injury-related danger molecules (Hohenhaus et al., 2013; Lattin et al., 2008). GPCRs play an important role in recognizing the pathogens and antigens of macrophages, which are part of the innate immune system. Therefore, it can be thought that the recognition of the parasite in tapeworm infections and the formation of immune response against it can be related to the density of these receptors. GPCRs and therefore, *PTH2R*, *ATRNL1* genes were suggested as candidate genes for resistance to *Moniezia spp.* infection in sheep.

As a result of GWAS for CocFOC3 and CocFOC6, 5 genes namely *LARGE1*, *HIPK1*, *SYT1*, *SCG2* and *ALK* were suggested as candidate genes for resistance to coccidian parasite infection. *LARGE1* gene on OAR3 encodes the N-acetylglucosaminyltransferase gene family. Therefore, it encodes a glycosyltransferase that participates in the glycosylation of alpha-dystroglycan. This gene involving in glycoprotein biosynthetic process (GO:0009101) which is the important biomolecules in the innate immune response against parasite infections. Also, this gene acts biological process of muscle cell cellular homeostasis (GO:0046716). Enteric smooth muscle contraction as part of the innate immune response plays an important role expulsion of the parasite from the gastrointestinal system. Therefore, this gene can be suggested as candidate gene for coccidian parasite resistance. In addition, studies have shown that *LARGE* gene are among the genes under the virus-driven selection pressure, and the post-translational modifications of the *LARGE* gene are critical for the binding of different phylogenetic viruses, especially Lassa fever virus and lymphocytic-choriomeningitis virus (Kunz et al., 2005; Rojek et al., 2007). *SYT1* is another gene associated with CocFOC3 phenotype. This gene encodes synoptotagmins which are

integral membrane proteins of synaptic vesicles and involving in many functions about Calcium transport (GO:0017156, GO:0048791) and neurotransmitter secretion (GO:0016192, GO:0016079). It is also acts in biological process of dopamine secretion such as positive regulation of dopamine secretion (GO:0033603) and regulation of dopamine secretion (GO:0014059). Similarly, *ALK* gene was found to be associated for *Eimeria spp*. resistance is protein coding gene related biological process of regulation of dopamine receptor signaling pathway (to GO:0060159). Dopamine is a neurotransmitter actively play a role in the regulation of the immune system (Li et al., 2007). In studies, it has been reported that dopamine levels increase in brain of chickens in cases of infection or heat stress, however, dopamine levels in the brain were decreased in broiler chickens exposed to *Eimeria spp.* (Calefi et al., 2019). Conspicuously, as a result of GWAS for Coccidian parasite resistance traits (CocFOC3 and CocFOC6), *HIPK1, SCG2* and *ALK* genes on OAR1, 2 and 3 were found to be associated. The most obvious common characteristics of these three genes is that they take part in the biological processes of cell apoptosis. In particular, the *HIPK1* gene plays a role in the endothelial cell apoptotic process (GO:0072577) and extrinsic apoptotic signaling pathway (GO:0097191), and the *SCG2* gene in the negative regulation of endothelial cell apoptotic process (GO:2000352) and negative regulation of extrinsic apoptotic signaling pathway (GO:2001237), and finally the *ALK* gene in the regulation of apoptotic process (GO:0045664). During coccidiosis, the sporozoites taken into the body through digestion penetrate the epithelial cells and settle into the cell. Later, these sprozoites divide asexually and multiply and merozoites are formed as a result of division. After the number of generation, their number increases continuously within the cell and they cause death by rupturing the cell they have settled in (Novilla and Carpenter, 2004). In a study, it was reported that intestinal parasites activate intrinsic and extrinsic factors in intestinal epithelial cells, causing the cell to go to apoptosis (Kapczuk et al., 2020). The genes related to cell apoptosis are associated with *Eimeria spp.* resistance may be that the increase of these merozoites in the cell may have activated apoptosis (intrinsic and extirinsic) factors in the cell. On the other hand, *ALK* and *SCG2* genes were found to be associated with mitogen-activated protein kinase (MAPK) signaling pathway, and particularly, they involve in positive regulation of MAPK cascade (GO:0043410) and MAPK cascade (GO:0000165) pathways, respectively. It has been reported that MAPK signal is suppressed in host intestinal epithelial cells in an infection caused by

Cryptosporidium, which is a *protozoan* parasites that infects intestinal epithelial cells like *Eimeria* species (He et al., 2021). It has been revealed that the *HIPK1* gene is associated with chronic inflammatory diseases such as Crohn's disease, primary sclerosing cholangitis and ulcerative colitis (Ellinghaus et al., 2016). *SCG2* gene, which is associated with the coccidian parasite resistance phenotype, plays a very active role in the formation of innate immune response and adaptive immune response and immune defense in the body, as well as taking part in cell apoptosis pathways. In particular, it is involved in many biological processes such as inflammatory response (GO:0006954), eosinophil chemotaxis (GO:0048245), positive chemotaxis (GO:0050918), induction of positive chemotaxis (GO:0050930) and endothelial cell migration (GO:0043542). It is thought to contribute to immune development in parasite infections.

In general, the results of functional annotation revealed that all of the subjected genes namely *NELL1*, *TENM3*, *ST6GALNAC3*, *ATRNL1*, *HIPK1*, *SYT1*, *ALK, ZNF596*, *TMCO5A*, *PTH2R*, *LARGE1* and *SCG2* were found to be related with parasite resistance. Many of these genes involve in many important basic biological functions that are essential for immune system and inflammation such as eosinophil chemotaxis, inflammatory response, cell apoptosis, immune-related protein synthesis, intercellular signaling. Another important point was the genes for the phenotypes in the same parasite group were found in the same or similar process. For instance, *PTH2R* and *ATRNL1* genes found to be related with tapeworm count were involved in the synthesis of G-Protein-Coupled Receptors (GPCRs). Another point is that *HIPK1*, *SCG2* and *ALK* genes were associated with cell apoptic process which may be occurs as a result of coccidiosis in intestinal epithelial cell. Finally, *SCG2* and *ALK* genes found to be for coccidian parasite resistance were actively involving in MAPKs signaling pathway which has significant role for immune response in parasite infection.

4.2. Blood parameters

The wide range of results from the very few studies implemented on blood traits were found in sheep. (Berton et al., 2017; Chirkena et al., 2016; Jiménez-Penago et al., 2021; Zhu et al., 2020). For example, a study implemented on Santa Inês sheep proposed the mean values for RBC, PLT, WBC, HCT, and HGB traits to be 9.97, 383.8, 10.96, 29.05 and 8.43 respectively (Berton et al., 2017). In our study, these values were found to be

similar for HGB (9.51) and WBC (9.68), however, for PLT (455.0) and HCT (23.25), the values were found to be higher and to be lower for RBC (5.43). Additionally, in a study aimed on Alpine Merino sheep, the means for MCH, MCHC and RWD_CV were found to be 13.24, 37.74 and 0.39. The findings of this study were lower than the mean for MCH (24.16), MCHC (50.86) and RWD_CV (14.30) obtained in the current study. Various environmental (i.e., climatological, ecotype, altitude), biological, age, and health conditions, as well as different genetic structures of the genotypes, may explain the probable variations.

4.2.1. Genomic Heritability Estimates

In our study low to moderate (from 0.00 to 0.55) heritability estimates were found for blood phenotypes (Table 2). Studies conducted on the estimation of heritabilities for blood phenotypes of sheep are quite scarce. In a study of Santa Inês sheep, heritability estimates using the genomic relationship matrix were found to be 0.18, 0.17, 0.22, 0.20 and 0.16 for the RBC, PLT, WBC, HCT and HGB traits, respectively (Berton et al., 2019). Results in the current study were similar for RBC (0.20), PLT (0.13), HCT (0.21) and HGB (0.12), but higher for WBC (0.55). Again for Santa Inês sheep, the heritability estimation for HCT (0.18) was found to be compatible with our current study (Rodrigues et al., 2021). Additive genetic variance of the traits and, accordingly, heritability is affected by many natural factors such as selection, mutation, genetic drift and migration as well as artificial selection (Charlesworth, B. and Charlesworth, 2010). Populations exposed to these natural and artificial factors for different periods of time and in various manner can be expected to have differing heritability for the same trait. However, the heritability estimates of many traits in our study were quite similar with the results of previous studies, although different breeds were studied. The concordant moderate heritability estimates of many blood phenotypes in different sheep breeds might lead to suggest that there was not any systematic selection applied to these phenotypes which might alter the share of genetic variance in the phenotypic characteristics of these animals. Thus, the genetic improvement of these phenotypes by selection strategies is quite possible.

4.2.2. Genome-wide association studies

As a result of GWAS conducted in this study, a total of 20 SNPs were found to be significant for 15 different blood phenotypes, with 7 being genome-wide and 13 being chromosome-wide significant. No significant SNPs were found for HGB, RDW_SD, MON and PDW traits. The current study and previous studies show that the number of SNPs in the peaks obtained for complex traits is low in the GWAS analyses performed in sheep (Esmaeili-Fard et al., 2021; Gonzalez et al., 2013; White et al., 2012; Yilmaz et al., 2022; Zhu et al., 2020). The main reasons for that are suggested to be the relatively short LD in the sheep genome and the highly polygenic structure of the traits (Kijas et al., 2012; Zhang et al., 2014).

Erythrocytes are an important factor that directly affects performance and growth as well as the health status of animals (Peng et al., 2017). GWAS of 6 erythrocyte-related traits in our study ended up with 8 associated SNPs, including 2 genome-wide and 6 chromosome-wide significant on OAR1, 2, 5, 18 and 21 (Table 3). Importantly, among those, SNPs with pleotropic effects were observed -namely *rs424122039*, *rs402657016* and *rs418188401*- for RBC, HCT, MCH, MCHC and RDW_CW traits. The top associated SNP regarding all traits was observed for MCHC (p = 4.83e-11), which was *rs424122039* on OAR18 (Figure 1). Additionally, top 2 significant SNPs (i.e., rs424122039, rs402657016), which are adjacent within a ~52 Kb distance discovered in *MYADM-like* gene on OAR18, were also associated with multiple traits that are MCH, MCHC and HCT. Interestingly, the association between the MYADM-like gene family and RBC abnormalities in sheep was previously reported by Gonzalez, (2013). In the multi-breed GWA study, the discovered region and *rs424122039* specifically were found to be associated with MCHC of three sheep breeds namely Columbia, Polypay, and Rambouillet. The region specifically carrying MYADM-like gene family has been reported to be conserved throughout many mammalian species (i.e., cow, pig, goat) (Wang et al., 2013; Zimin et al., 2009). Protein products synthesized by the MYADM gene family have been reported to be involved in membrane formation and regulation process in myeloid cell lines (Dannaeus et al., 2005; Pettersson et al., 2000). In addition to these functions, the increase in MYADM gene expression levels in pluripotent cells involves in the completion of erythropoiesis (Pettersson et al., 2000). In addition to these, the genomic region containing those associated SNPs -nearly 800Kb window-

was found to be also a gene-rich region mainly populated by Myeloid-associated Differentiation Marker-like (*MYADM-like*) protein coding genes, pseudogenes and other specific genes with a high number of SNP variants. The same region was also found to be significant in selection signature analyses of the ovine HapMap project, which was carried out with 74 sheep breeds from many parts of the world (Kijas et al., 2012) and also by Adam et. al., (2021) with thin- and fat-tailed sheep breeds from Sudan and China (Abied et al., 2021). The extreme historical selection pressure of the genomic region emphasizes that this region provides superiority in adaptive and survival capacity of sheep. Although, the top SNP and the region of interest previously associated with MCHC (Gonzalez et al., 2013), this is the first study discovering the association between *rs424122039* and other red blood cell traits (i.e., MCH and HCT) in sheep.

On the other hand, SNPs within *SCN7A* and *SCN9A* genes were found to be associated with RBC and RDW_CV traits with pleiotropic effects. These SCN gene family encode voltage-gated sodium channel proteins which is mainly involved in biological process such as ion transport (GO:0006811) and especially sodium ion transport (GO:0006814) in mammalian cells. Likewise, *HDAC4* gene was found nearby to *rs423050506* SNP which is significant for RBC trait. This gene encodes proteins belonging to class II of the histone deacetylase family. As it is well known, histones play critical role in transcriptional regulation, accordingly, cell cycle progression, and developmental events. This gene involving many biological process like in regulation of striated muscle cell differentiation (GO:0051153).

Three genome-wide and four chromosome-wide significant SNPs were associated with leukocytes related traits on OAR1, 2, 3, 4, 13, 19 and 20 (Figure 2). SNP named *rs423900300* was found to be associated with WBC trait and it is located within *ITGA9* gene on OAR19. This gene encodes an alpha integrin protein which mediate cell-cell and cell-matrix adhesion and involves in many biological processes such as cell adhesion (GO:0007155), integrin-mediated signaling pathway (GO:0007229), extracellular matrix organization (GO:0030198) and neutrophil chemotaxis (GO:0030593). In a study of breast cancer in human, the expression of *ITGA9* was downregulated and lost in majority of breast cancer cell line (Mostovich et al., 2011). On the other hand, SNP *rs419009933* within *LOC101117887* on OAR20 showed pleotropic effect on NEU and LYM traits. Interestingly, this SNP had a negative effect

on NEU with explaining 4.35 % of additive genetic variance, whereas it had positive effect on LYM with explaining 3.07 % of additive genetic variance (Table 3). *MGAT5* gene was found to be related with neutrophils-lymphocytes ratio (NEU/LYM). *MGAT5* encodes a protein belonging to the glycosyltransferase family and involves in many biological processes such as the positive regulation of cell migration (GO:0030335) and positive regulation of STAT signaling pathway (GO:1904894). In a study, it was shown that deficiency of *MGAT5* gene in allergen challenged mice increased neutrophilic inflammation (Bahaie et al., 2011). This gene also was found to be associated with T cells glycosylation and plasma composition of IgG glycome in ulcerative colitis in human (Pereira et al., 2020).

SNP rs429955018 on OAR1 were associated with EOS with relatively high additive genetic variance (18%). This SNP were located within Solute Carrier Family 19 Member 1 (*SLC19A1*) gene. This gene involves in many biological processes such as folic acid transport (GO:0015884), vitamin metabolic process (GO:0006766). It is well known that folic acid and other vitamins have critical role in production of white blood cells particularly granulocytes (Abe et al., 2013). The ~750Kbp long region flanked by SNP *rs428421565* and *rs424356478*, which are associated with EOS trait, on OAR3 includes many genes (i.e., *OSBPL8*, *ZDHHC17*, *CSRP2, NAV3*, *E2F7*). Finally, SNP *rs417370910* within Amphiphysin (*AMPH*) gene on OAR4 was associated with BAS (Figure 4). *AMPH* mainly involves in endocytosis (GO:0006897) biological process in cell. Amphiphysin is one of the adhesion proteins, and used in many cells that perform endocytosis, which is a process regarding immune system cells (i.e., basophiles, macrophages) that is important in destroying the antigens (i.e., soluble antigens) (Sokol et al., 2009).

Two genome-wide and three chromosome-wide significant SNPs were detected for PLT, MPV and PCT on OAR2 and 25. SNP rs419857573 within *LOC105608461* has pleotropic effect on PLT and PCT by explaining 2.73 % and 7.58 % of additive genetic variance in the trait, respectively. Besides that, rs424459233 within Methionine Sulfoxide Reductase A (*MSRA*) gene on OAR2 were associated with MPV with 5.24 % of additive genetic variance.

In general, the results of functional annotation revealed that many of the important genes such as *SCN7A, SCN9A, HDAC4, MYADM-like, ITGA9, MGAT5, SLC19A1, SLC35F3* and *AMPH* involve in many important basic biological functions that are essential for immune system and growth in the body such as cellular transport, histone deacylation, cell differentiation, erythropoiesis, endocytosis (Bahaie et al., 2011; Dannaeus et al., 2005; Mostovich et al., 2011; Pereira et al., 2020; Pettersson et al., 2000; Sokol et al., 2009). Some of these SNPs (i.e., *rs418188401*, *rs402657016*, *rs424122039*, *rs419009933* and *rs419857573*) associated with genes with pleotropic effects on WBC, RDW_CV, HCT, MCH, MCHC, NEU, LYM, PLT and PCT. Another important point in the study is that SNP rs424122039 was found in a gene-rich region mainly populated with MYADM-like genes, which were previously associated with the MCHC trait (Gonzalez et al., 2013). It has also been shown in previous studies that this region is under historical selection pressure (Abied et al., 2021; Kijas et al., 2012). In this context, the MYADM-like gene was suggested by Gonzalez et. al., (2013) as a potential candidate gene associated with erythrocyte related traits (i.e., MCHC), moreover, the results of our current study confirmed this hypothesis. Therefore, our current study is one of the limited number of studies to understand the genetic underlying blood phenotypes in sheep. The current study and this kind of studies will shed light on the genetic improvement of these traits and their application to selection programs.

CHAPTER 5

5. CONCLUSION

As a conclusion, in our study, Genomic heritability for the traits were estimated and heritability estimates were found from low to moderate. Furthermore, as a result of GWAS, it has been illustrated that 2 genome-wide and 10 chromosome-wide significant SNP for parasite resistance traits. Accordingly, these 12 SNP were found to be associated with 12 particular genes namely *NELL1*, *TENM3*, *ST6GALNAC3*, *ATRNL1*, *HIPK1*, *SYT1*, *ALK, ZNF596*, *TMCO5A*, *PTH2R*, *LARGE1* and *SCG2* and these genes were suggested as candidate gene for parasite resistance after functional annotations. On the other hand, 7 genome-wide and 13 chromosome-wide significance SNPs are associated with blood phenotypes. These SNPs were associated with 19 genes namely *SCN7A, HDAC4, SCN9A, MYADM*-like*, SIRT6, REB3L3, CCDC67, ITGA9, MGAT5, SLC19A1, OSBPL8, ZDHHC17, CSRP2, NAV3, E2F7, AMPH, NTRK2, MSRA, SLC35F3,* and three unidentified locations – (*LOC101117887*, *LOC105608461* and *LOC106991526*). The results of this study can be utilized as a selection criterion in sheep breeding approaches.

Considering that parasite resistance and blood cell (i.e., erythrocytes and leukocytes) are directly related to adaptation and survivability of sheep, the resultant breeding approaches may produce more resistant and sustainable animals against disease which have remarkable economic impact. Finally, in order to gain more sophisticated knowledge on the genetic mechanisms of parasite resistance and blood phenotypes in sheep, particularly Akkaraman more studies are required. In this study, we get many questions, possible explanation and meaningful result about the genetics of adaptation traits (i.e., parasite resistance and blood phenotypes). End up the study many genes, SNP and QTL were found, and they were subjected as candidate for the relevant traits. Learning more the knowledge about the genetics of parasite resistance and blood

parameters, and transfer this knowledge to applications, it is strongly recommended that this and similar studies be conducted on larger numbers and in different populations

CHAPTER 6

6. RECOMMENDATIONS AND FUTURE DIRECTIONS

Adaptation and disease-related problems in farm animals are among the most important factors that increase replacement costs in herds. The fact that breeding programs focused only on production traits until the last ten years has caused herds to suffer genetic loss in terms of these traits, which are mostly negatively related to production traits. Health traits such as parasite resistance and blood phenotypes appear to have moderate heritability in our current study and previous studies. However, there is a need to reveal the phenotypes related to GIP resistance, in which the immunogenic mechanisms of resistance can be understood more clearly, and studies in this direction are required. In other words, phenotypes at the molecular level and highly correlated are needed in order to clearly understand the different genetic potential that exists and provides resistance to parasites.

In particular, performing genomic studies in more animals, in different populations, for different diseases will contribute to the reduction of disease-related economic inputs. In addition, many other omics technologies such as transcriptomic, metabolomics, epigenomics and proteomics should be examined with a systems biology approach in order to better understand the genetic background of GIP resistance traits in sheep. On the other hand, the genetic correlations of disease and adaptation traits such as GIP resistance and blood phenotypes with many other production traits such as weight gain, weaning weight should be investigated and used especially in animal breeding. In this way, a more properly and comprehensive selection index can be established, and a sustainable selection index can be constructed in animal breeding, which is the application area of genetics to livestock. Finally, considering the widespread use of CRISPR technologies in parallel with technological developments in the future, a rapid genetic progress in terms of economic traits in farm animals will be possible with

genetic markers that explain the high genetic variation to be obtained as a result of the current study and similar studies.

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low-input low-output farming systems in Zimbabwe. **Small Rumin. Res. 143**, 75–83.

CURRICULUM VITAE

KİŞİSEL BİLGİLER

Adı Soyadı: Yunus ARZIK

Uyruğu: Türkiye (T.C)

EĞİTİM

Derece	Kurum	Mezuniyet Tarihi
Yüksek Lisans	Wageningen Üniversitesi, Zootekni	2017
Lisans	Kafkas Üniversitesi, Veteriner Fakültesi	2012
Lise	Develi Lisesi, Develi	2006

İŞ DENEYİMLERİ

Yıl	Kurum	Görev
2017- Halen	UHAEMM, ISLAH ve Genetik Bölümü	5
2019-2021	UHAEMM, Islah ve Genetik Bölümü, Genetik Laboratuvar Sorumlu Yöneticiliği	2
2013-2014	Çanakkale Ayvacık İlçe Tarım ve Orman Müdürlüğü	2

YABANCI DİL

İngilizce (Yökdil: 88.75/100)

YAYINLAR

1. **Arzik, Y**., Kizilaslan, M., White, S.N., Piel, L.M.W., Cinar, M.U., 2022. Estimates of genomic heritability and genome-wide association studies for blood parameters in Akkaraman sheep. (Submitted to Nature Scientific Reports)

2. Kizilaslan, M., **Arzik, Y**., Cinar, M.U., Konca, Y., 2021. Genome-wise engineering of ruminant nutrition-nutrigenomics: applications, challenges, and future perspectives–a review. Ann. Anim. Sci. https://doi.org/DOI: 10.2478/aoas-2021-0057

3. Yilmaz, O., Kizilaslan, M., **Arzik, Y**., Behrem, S., Ata, N., Karaca, O., Elmaci, C., Cemal, I., 2022. Genome-wide association studies of preweaning growth and in vivo carcass composition traits in Esme sheep. J. Anim. Breed. Genet. 139, 26–39. https://doi.org/10.1111/jbg.12640

4. **Yunus Arzik**, B.J. (Bart) Ducro, Lydia de Haer, Effect of Inbreeding on Milk Traits in Dutch Holstein Dairy Cattle, I. International Livestock Science Congress 31 October - 3 November, Oral presentation, Antalya / Turkiye.

5. Master Thesis, March 2017, Effect of Inbreeding on Milk Traits and Survival of Calves in Dutch Holstein Dairy Cattle, Animal Breeding and Genomics Centre, Wageningen University and Research, The Netherlands.

6. Master Thesis, July 2017, Detection of Genomic Regions for Methane Emission in Dairy Cows by Using Genome-wide Association Studies (GWAS), Animal Breeding and Genomics Centre, Wageningen University and Research, The Netherlands.

7. Book Section, January 2022, Animal Breeding in Farm Animals, Republic of Türkiye Ministry of Agriculture and Forestry, Department of Training and Publication, ISBN NO 978-605-9175-80-7