Genetic Improvement for Disease Resistance and Heat Tolerance in Sheep

By

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ABSTRACT

GENETIC IMPROVEMENT FOR DISEASE RESISTANCE AND HEAT TOLERENCE IN SHEEP

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The genetic improvement of disease resistance and heat tolerance could contribute to the enhancement of health and welfare in the sheep industry. This thesis focuses on gastrointestinal parasite resistance, genetic resistance to scrapie, and heat tolerance in sheep.

There are different methods to measure Fecal egg count (FEC), which may be inconsistent. Not accounting for inconsistencies is problematic when integrating data from different FEC methods for genetic evaluation. Data from two FEC methods, the "Modified McMaster" and the "Triple Chamber McMaster", were compared. The differences in FEC means and variances were significant, making the methods incompatible for direct integration as a single trait. Analysis of FEC from the two methods as separate traits showed high genetic correlation. Thus, FEC data from the two methods were integrated, after adjustment for the means and variances, prior to multivariate analysis with indicator traits, which included FAMACHA©, body condition score, and bodyweight. The genetic correlations between FEC and the indicator traits were low,

suggesting that including those traits with FEC in a multiple-trait model would not offer considerable advantage.

Selection based on scrapie genotype could improve scrapie resistance in sheep. However, few animals are genotyped, resulting in limited gain. Numeric values for scrapie resistance (SR) genotypes were defined from the most vulnerable (0) to the most resistant (4) and then, adjusted to a non-additive genetic effect. Subsequently, an animal model was used to predict SR in non-genotyped animals. The accuracy for prediction ranged from moderate to high, with selection increasing SR of non-genotyped animals. Therefore, the animal model makes better use of available information that could be useful for breeding programs for scrapie resistance in sheep.

Heat stress can negatively affect the growth performance of lambs. Temperature-Humidity Index (THI) above comfort zone was used as the environmental gradient for heat stress to examine genotype by environment interaction in growth traits, using a reaction norm model. A genetic antagonism between growth traits and heat tolerance was found and it should be accounted for in breeding programs. Variation in heritability estimates across the heat stress gradient provides opportunity for selection for growth traits within specific environments.

DEDICATION

This thesis is dedicated to the loving memory of the spirit of my father, **Naser M. Boareki**, and to beloved spirits of whom I was not able to bid my final goodbye before they passed away:

Virginia Bartolome Agcaoili (R.I.P in 2018)

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LIST OF ABBREVIATIONS

GIN: Gastrointestinal nematode.

FEC: Fecal egg count.

LMMR: Fecal egg count from the “Modified McMaster” procedure in natural logarithm scale.

LTCM: Fecal egg count from the “Triple Chamber McMaster” procedure in natural logarithm scale.

LFEC: Integrated fecal egg count from LMMR and LTCM of natural logarithm scale.

BCS: Body condition score.

WT: Body weight.

TSE: Transmissible spongiform encephalopathy.

SR: Genetic resistance to scrapie, scaled from 0 to 4, from the most susceptible to the most resistant genotypes, respectively.

H: Haplotype gene content as the number of haplotype allele in loci (0, 1, or 2).

W50: 50-days adjusted weaning weight.

G50: 50-days adjusted post-weaning gain.

THI: Temperature-Humidity index

G×E: Genotype by environment interaction

CHAPTER 1

GENERAL INTRODUCTION

1.1. The Importance of Sheep

Domestication of the wild ancestors of sheep took place around 9000 B.C in a region located between the modern-day Iran and Iraq, making sheep one of the earliest domesticated species in history (Zygoyiannis, 2006). Since the time of domestication, sheep have been utilized as an important source of meat, fiber, hide, and milk (Zygoyiannis, 2006; Morris, 2009; Hegde, 2019). One advantage for the keeping of sheep over cattle is that they can flourish in conditions where cattle perform poorly, that is, sheep are more efficient and thrifty in harsh environments (Morris, 2009). Therefore, in harsher conditions, the production of sheep is more efficient and economically viable than cattle production. In the Canadian sheep industry, lamb and mutton are the primary products, with an average annual per capita consumption of 0.99 kg per person in 2019 (Agriculture-Canada, 2021a). The total sheep population in 2019 was 827,800 heads from 9,390 farms which are mostly concentrated in the provinces of Ontario, Quebec, and Alberta, which produced a total of 16,870 tons of lambs and mutton. The net cash receipt for sheep in Canada was $186 million in 2019 (Agriculture-Canada, 2021b). As result of immigrations to Canada, the ethnic market contributed to the demand for lambs and mutton (Menzies, 2006).

1.2. Genetic Improvement of Sheep

The advancements of statistical and computational techniques and better understanding of inheritance have allowed for systematic and accurate selection and improvement of desired characteristics of livestock animals. This has been done by using the best linear unbiased predictor (BLUP), which utilizes pedigree information and phenotypic measurements, and accounts for the environmental effects to predict the estimated breeding value (EBV) of a trait for each individual animal. The application of this procedure for genetic evaluation and selection has resulted in permanent and cumulative genetic improvements in many traits including those related to growth, reproduction, and health. For example, in New Zealand from 1987 to 2008, lambing percentage increased from 98% to 125% and carcass weight increased from 14 to 17 kg (Morris, 2009). Although traditional methods of genetic evaluation have proven to be successful, genotyping and genomic selection may accelerate genetic gains. This would be achieved by reducing the generation interval and by increasing accuracy of prediction and selection intensity. Genomic selection is carried out by estimating the genomic breeding value (GEBV), which is the sum of estimated allelic effect for the trait of the animal (Hayes et al., 2009). Genomic selection has been implemented in sheep in Australia, New Zealand, and France (Rupp et al., 2016). In addition, there are major genes in sheep that are known to be associated with economically important traits. Major genes have been used to improve such traits in sheep (Rupp et al., 2016). For example, the Booroola gene was used to improve litter size in Awassi sheep (Gootwine et al., 2001), and prion protein (PrP) gene was used in breeding programs to improve scrapie resistance (Gáspárdy et al., 2018).

1.3. Disease Resistance and Heat Tolerance

The presence of environmental stressors including, but not limited to, disease infection and heat stress could lead to reduced production performance (e.g., growth, reproduction, and survival), increased morbidity and mortality, and greater costs associated with management and medication, which consequently lead to economic loss for livestock producers. Breeding programs aimed at improving health and heat tolerance can provide long term solutions to counteract the negative economic impacts of these environmental challenges. This thesis focuses on genetic improvement for parasite resistance, genetic resistance for scrapie, and heat tolerance in sheep.

1.3.1. Parasite Resistance

The predominant parasites found in Ontario grazing sheep are *Teladorsagia*, *Trichostrongylus* and *Haemonchus contortus* (*H. contortus*). Among them, *H. contortus* is considered the most pathogenic, attaching to the abomasum and ingesting blood, causing anemia, edema, anorexia and death (Westers et al., 2016). High parasite loads of *Haemonchus contortus* can result in economic losses to producers through the increase of morbidity and mortality in sheep, as well as an increase in cost of treatment and preventative measures. Frequent use of medication to combat parasite infection can lead to the development of anthelmintic resistant (AR) parasites, contributing to the ineffectiveness of such medication and leaving producers with no method to maintain the health of their flock (Jan A Van Wyk and Bath, 2002; Ejlertsen et al., 2006; Dobson et al., 2012; Emery et al., 2016). The problem of the evolution of medication-resistant parasites could be counteracted by 1) targeted selective treatment of sheep with high infection levels and/or evidence of significant clinical disease; and 2) the genetic selection for parasite resistant sheep.

However, both short-term (i.e., restriction of medication) and long-term (i.e., genetic selection) approaches require the measurement of parasite infection in individual animals.

The indicative measurements for parasite infection can include fecal egg count (FEC), FAMACHA© score, and body condition score. FEC is one important quantitative measurement for parasite load, in which the GIN parasite eggs in the processed fecal sample are counted under microscope to estimate the animal's state of GIN infection. However, there are different procedures to measure FEC, which can vary in sample weight, floatation solution, number of chambers, and the accuracy of egg detection (eg., Cebra and Stang, 2008; Vadlejch et al., 2011; Paras et al., 2018). The differences among FEC procedures could lead to differences in measurement and, therefore, estimations using different methods may not be consistent (eg. Cringoli et al., 2004; Cebra and Stang, 2008; Paras et al., 2018). The consistency of phenotypic measurements is essential for successful genetic evaluation, and not accounting for inconsistencies can be problematic when integrating FEC data from different methods.

FAMACHA© score is a method for qualitatively estimate the level of anemia in small ruminants by judging the redness of the conjunctiva in relation to a standardized FAMACHA© card. FAMACHA© uses a scale from 1 to 5, with 1 being the least anemic and 5 being the most anemic, and the animal's resulting score is assumed to be proportional to the level of haemonchosis (Kaplan, 2004; Reynecke et al., 2009). The body condition score (BCS) is a score of muscle and fat covering the back and the ribs of the animal, ranging from 1 to 5; 1 being the lowest and 5 the highest covering. Animals with the lowest scores are presumed to be the most severely unhealthy (Burke et al., 2007).

Both FAMACHA© and BCS offer a clear advantage to farmers compared to FEC for measuring GIN infection level, as they could be easily conducted at the farm on many animals, and they do not require specific laboratory equipment and are, therefore, attractive alternatives to FEC. The issue of inconsistency between different FEC procedures and the genetic components for parasite resistance traits are addressed in study in Chapter 2.

1.3.2. Scrapie in Sheep

Scrapie is a transmissible spongiform encephalopathy (TSE) disease that affect the central nervous system of small ruminants (Monleón et al., 2005; Lacroux et al., 2008; Fediaevsky et al., 2010). This disease is caused by the presence of an infectious misfolded protein called prion (Clouscard et al., 1995; Lacroux et al., 2008; Goldmann, 2018). Signs of scrapie infection include neurological and behavioral changes, in which infected animals scratch excessively leading to skin lesions, hence the name "scrapie". The progression of neurological damage will lead to the inevitable death of the animal (Moynihan, 1960; Goldmann, 2018).

In the typical form of scrapie, the risk of infection can be determined by the variation of amino acid sequence encoded in the prion protein (PrP) gene (Hunter and Cairns, 1998; Monleón et al., 2005; Molina et al., 2006; Lacroux et al., 2008). Polymorphisms occur on codons 136, 154, and 171 and result in five major haplotypes (ARR, AHQ, ARH, ARQ, and VRQ), which are associated with the risk of infection. Each haplotype is associated with different level of risk, in which the haplotype "ARR" is associated with the lowest risk and "VRQ" is associated with the highest risk (Álvarez et al., 2007; Sherman, 2011; Gáspárdy et al., 2018; Hagenaars et al., 2018; Ptacek and Ducháček, 2019). The haplotypes can result in 15 possible genotype combinations,

which can be grouped into 5 risk groups (R1, R2, R3, R4, and R5), in which the R1 genotype has the lowest risk and R5 genotype has the highest risk (Molina et al., 2006; Álvarez et al., 2007; Stepanek and Horin, 2017). More information regarding risk genotypes and their respective risk group is included in Table 3.1 in Chapter 3. Because of the association between the genotypes and risk of scrapie infection, genotyping for the purpose of breeding and scrapie eradication programs were implemented in European countries (Gama et al., 2006; Melchior et al., 2010; Ortiz-Pelaez and Bianchini, 2011; Gáspárdy et al., 2018). However, in practice, not all animals are genotyped, limiting the genetic gain for scrapie resistance, in the whole population. Therefore, the problems of few available scrapie genotype records and the potential exploitation of available information to predict the genetic resistance of ungenotyped sheep in population were addressed in Chapter 3.

1.3.3. Heat Stress

Heat stress is an important challenge in livestock industry due to climate change (Rojas-Downing et al., 2017; Wreford and Topp, 2020). As result of response to elevated heat exposure in sheep, changes in biological parameters, such as rectal temperature, respiration rate, and other physiological functions can be observed (Ames et al., 1971; Lowe et al., 2001; Sevi et al., 2001; Srikandakumar et al., 2003). Those changes could be associated with reduced feed intake and utilization and, therefore, muscle catabolism. This is consequently reflected in reduced productive performance such as growth, milk production, and reproduction (Marai et al., 2007). Therefore, the reduction in productive performance from heat stress can result in negative economic impacts in the livestock industry (eg. Mader, 2003; St-Pierre et al., 2003).

The genetic improvement for heat tolerance is a biological solution for addressing heat stress which is permanent and cumulative. This can be done by identifying and selecting individuals that perform well in heat challenging conditions. There is experimental evidence that indicates differences in performance under heat stress among different breeds (genotypes) in sheep (eg. Srikandakumar et al., 2003) cattle (eg. Gaughan et al., 2010) and poultry (eg. Eberhart and Washburn, 1993a; Eberhart and Washburn, 1993b; Yalçin et al., 1997; Cahaner et al., 2008). Different genotypes can perform significantly differently in diverse environments, which means that there is a genotype by environment interaction (G×E). The G×E can be accounted for within population (breed or genotype) by treating the trait in different environments as separate and potentially correlated traits, or with the use of random regression reaction norm model (Hayes et al., 2016). The random regression reaction norm model requires a continuous environmental gradient. The most practical environmental gradient for heat stress is the temperature-humidity index (THI), which combines meteorological measurements for temperature and humidity into one value. Ravagnolo et al (2000) developed a heat stress function for use in dairy cattle by combining phenotypic data with the THI calculated from public metrological data from the nearest weather station.

Evidence for G×E for heat stress were observed in dairy sheep (Finocchiaro et al., 2005) dairy cattle (Boonkum and Duangjinda, 2015; Santana et al., 2015; Bohlouli et al., 2019), beef cattle (Bradford et al., 2016; Santana et al., 2016) and pigs (Zumbach et al., 2008a), indicating genetic antagonism between production traits and heat tolerance. Thus, not accounting for heat stress in breeding programs could lead to the increase in heat sensitivity. To our best knowledge, there are no studies available that address the genetic component for heat stress for growth traits in sheep.

Therefore, the consequences of heat stress and its genetic effect on both direct and maternal components in sheep's growth traits are addressed in Chapter 4.

1.4. Research Objective

The general focus of this thesis was on genetic improvement for disease resistance and heat tolerance in sheep. The specific objectives were:

1- Comparing two fecal egg counting (FEC) methods (the Modified McMaster and the Triple Chamber McMaster), integrating FEC data from different methods, and estimating genetic parameters for parasite resistance traits (CHAPTER 2);

2- Presenting a practical method for the prediction of genetic resistance for scrapie in sheep for animals that lack genotype information (CHAPTER 3);

3- Estimate the genetic parameters for growth traits in sheep as a function of heat stress (CHAPTER 4).

CHAPTER 2

Comparison between methods for measuring fecal egg count and estimating genetic parameters for gastrointestinal parasite resistance traits in sheep

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2.1. Abstract

Fecal egg count (FEC) is an indicative measurement for parasite infection in sheep. Different FEC methods may show inconsistent results. Not accounting for inconsistencies can be problematic for genetic evaluation when integrating measurements from different FEC methods. The objectives of

this study were to evaluate the difference in means and variances between two fecal egg counting methods used in sheep, the Modified McMaster (LMMR) and the Triple Chamber McMaster (LTCM); to estimate variance components for the two FEC methods, treating them as two different traits; and to integrate FEC data from the two different methods and estimate genetic parameters for FEC and other gastrointestinal parasite resistance traits. Fecal samples were collected in a commercial sheep farm in Ontario from adult purebred Rideau-Arcott sheep of both sexes. Fecal egg counting was performed using both Modified McMaster and the Triple Chamber McMaster methods. Other parasite resistance trait records were collected from the same farm including FAMACHA© score, body condition score (BCS), and body weight (WT). The two FEC methods were highly genetically (0.94) and phenotypically (0.88) correlated. However, both the mean and variance between the two FEC methods were significantly different ($P < 0.0001$). Therefore, re-scaling is required prior to integrating data from the different methods. For the multiple trait analysis, data from the two fecal egg counting methods were integrated (LFEC) by using records for the LMMR when available and replacing missing records with re-standardized LTCM records converted to the same mean and variance of LMMR. Heritability estimates were 0.12 ± 0.04, 0.07 ± 0.05 , 0.17 ± 0.06, and 0.24 ± 0.07 for LFEC egg count, FAMACHA©, BCS, and WT, respectively. The estimated genetic correlations between LFEC and the other parasite resistance traits were low and not significant ($P>0.05$) for FAMACHA© ($r= 0.24 \pm 0.32$) and WT ($r= 0.22 \pm 0.19$), and essentially zero for BCS ($r= -0.03 \pm 0.25$), suggesting little to no benefit of using such traits as indicators for LFEC.

Key words: FAMACHA©, fecal egg count, fecal egg counting methods, gastrointestinal parasite resistance, sheep

2.2. Introduction

Gastrointestinal nematode (GIN) infection is considered the most important disease of grazing sheep, causing a high economic impact in sheep industry. Economic losses due to GIN are associated to drug use, animal management costs and production losses associated with decreased live carcass and fleece weight, inefficient feed conversion rates, and reduced lamb survival (Venturina et al., 2013). Moreover, some producers avoid allowing their sheep to graze, due to the risk of infection, constituting a welfare issue for sheep industry (Falzon et al., 2013). In Ontario, the predominant parasites found in grazing sheep are *Teladorsagia*, *Trichostrongylus* and *Haemonchus contortus* (*H. contortus*). Among them, *H. contortus* is considered the most pathogenic, attaching to the abomasum and ingesting blood, causing anemia, edema, anorexia and death (Westers et al., 2016). Thus, alternate methods to control *H. contortus* infection are needed for sheep producers to keep their flocks healthy and productive. There is evidence that some populations of sheep are genetically resistant to GIN infection (Baker, 1998), so resistance to GIN has become a target for selective breeding decisions; such selected sheep populations will become resistant to GIN infections over time and drug administration may no longer be necessary or will be minimized. Fecal egg count (FEC) is a well-used tool in the diagnosis of GIN infection and is

usually considered surrogate measure for parasite load. However, there are some limitations for using FEC as an indicator of the parasite burden of the host, which are often overlooked, such as the fact that FEC is a ratio (eggs per gram of feces) in which any factor that changes the volume of feces (e.g. dry matter, food quality, etc.) can affect the measure (Greer and Sykes, 2012). In mixed infections, the interpretation of FEC should consider the female fecundity of the considered species, for instance, female *Haemonchus contortus* are able to lay thousands of eggs per day in comparison to *Trichostrongylus spp*, which only produces a few hundred (Coyne et al., 1991). In addition, there are resilient animals showing high/moderate FEC values, for which the production performance is not compromised. In this case, FEC would not be a direct measure of the cost of the infection for the host (Greer and Sykes, 2012). Nevertheless, FEC has been widely used as an indicator for host resistance to GIN in sheep showing to be a heritable trait, with a moderate heritability of 0.27 as reviewed by Safari et al. (2005). Therefore, genetic improvement for parasite resistance is possible by using FEC as a phenotype for genetic selection.

There are many methods used to measure FEC in sheep (e.g., the Modified McMaster method and the Triple Chamber McMaster method, among others) that vary in terms of sample weight, floatation solution, centrifugation, number of McMaster chambers, and the precision of egg detection (e.g. Cebra and Stang, 2008; Vadlejch et al., 2011; Paras et al., 2018). The Modified McMaster method is the most common method used to quantify the GIN infection in sheep, which uses a two-chamber slide to count the eggs per gram and can detect as low as 50 eggs/g. However, this methodology has been shown to have lower sensitivity than Mini-FLOTAV for FEC lower than < 500 eggs/g (Amadesi et al., 2020). On the other hand, the Triple Chamber McMaster method uses three-chamber slide to count the eggs per gram and can detect as low as 8 eggs/g. Thus, the

major difference in the two FEC methods is the number of chambers and the detection limit. Variation in FEC methodology may lead to different estimates of parasite load, even within the same sample. Thus, assessment of FEC trends over time or across populations becomes difficult or impossible if these differences in method are not accounted for. In FECs conducted for new world camelid species, Cebra and Stang (2008) compared six quantitative methods: centrifugation-sucrose flotation after 10 minutes, centrifugation-sucrose flotation after 60 minutes, saturated saline McMaster for 15 minutes, saturated saline McMaster for 60 minutes, sucrose McMaster for 15 minutes, and sucrose McMaster for 60 minutes. Their results indicated that some methods provide similar observations for eggs/g, while others were different by factor of up to 8-fold. Paras et al. (2018) compared three fecal egg counting methods: the Modified-Wisconsin, the 3-chamber McMaster, and the Mini-FLOTAC in sheep, cattle, horses, and llamas. They reported an increase in number of observed eggs in sheep by 116.5%, 223.3%, and 49.4% when comparing Mini-FLOTAC to McMaster, Mini-FLOTAC to Wisconsin, and McMaster to Wisconsin, respectively. Cringoli et al (2004) reported significant influence of the type of flotation solution, sample dilution, and McMaster counting area on the observations for FEC in sheep for both gastrointestinal strongyles and *Dicrocoelium dendriticum*. The consistency for phenotypic measurements is essential for successful genetic evaluation, thus, it is important records to be consistent and comparable across populations.

In addition to FEC, there are alternative measurements that can be used to indicate the level of parasite infection in small ruminants such as FAMACHA© score and body condition score (BCS). The FAMACHA© eye score is a method that assesses the level of redness of the mucus membrane of the animals' eye, which indicates animal's state of anemia on a scale of 1-5 (Kaplan

et al., 2004; Reynecke et al., 2009). The body condition score is a measurement of the fat and the muscle covering the back and the loin of the animals. Animals are given a score from 1-5 indicating their level of body condition. Animals in good body condition are presumed to be under little stress from GIN infection. A study conducted in Ontario and Quebec in Canada by Mederos et al. (2014) found that FEC was weakly associated with BCS (r = -0.232) and FAMACHA© (r = 0.178). In addition, previous studies indicate that such traits were genetically associated with parasite resistance and showed low to moderate heritabilities, ranged between 0.08 and 0.49, suggesting that they might be useful for selective breeding decisions (Riley and Van Wyk, 2009; Cloete et al., 2016; Álvarez et al., 2018; Snyman and Fisher, 2019).

The traits FAMACHA© and BCS, despite of the disadvantage of being subjectively measured, offer a clear advantage to farmers compared to FEC as they can be conducted on-farm and do not require specific laboratorial equipment. Thus, these traits could be an attractive alternative for producers to be used as phenotypes for genetic selection for parasite resistance.

The overall objective of this study was to estimate genetic parameters for FEC using data from two different laboratory methodologies for FEC detection, the "modified McMaster" and the "triple chamber McMaster", including genetic correlations with other parasite resistance traits, which were FAMACHA©, BCS and body weight. The specific steps carried out to achieve this overall objective were to: 1) evaluate the differences in mean and variance between the FEC records using two different methods: the “Modified McMaster” and the “Triple Chamber McMaster”; 2) estimate the variance components for the two FEC methods, treating them as two different traits; and 3) integrate FEC data from the two different methods and estimate the genetic

parameters, including genetic correlation with other parasite resistance traits, i.e. FAMACHA©, BCS, and body weight.

2.3. Materials and Methods

Data used in this study were provided by a commercial sheep farm located in Ontario based on routine recordings of animals, which followed the 2013 National Farm Animal Care Council's Code of Practice for the Care and Handling of Sheep (https://www.nfacc.ca/sheep-code). Therefore, no approval was required for this study from the Institutional Animal Care and Use Committee (IACUC) or its equivalent.

2.3.1. Collection of Fecal Samples and Phenotypic Records

Fecal samples (between the years 2012 and 2019) and phenotypic records (between the years 2016 and 2019) for WT, BCS and FAMACHA © were collected during the grazing season (from May to October) from adult pure-bred Rideau-Arcott sheep from a commercial sheep farm in Ontario, Canada. The rams and ewes were managed separately during the grazing season each year. Hence, the management group definition was based on sex and year. Animals were sampled and recorded at different dates of the grazing season, with some repeated records for the same animals. The date of measurement and the management group were later combined to form a group of measurement. The FAMACHA© score was taken by assessing the redness of the mucus membrane of the eyes, indicating the anemic level from scale between 1 (red and normal) and 5 (white and severely anemic) (Mahieu et al., 2007). The BCS was taken by assessing the fat and flesh covering the back and the loin of each animal with scores ranging between 1 (extremely thin)

to 5 (extremely fat). Fecal samples were collected from the rectum of each animal and stored using sealable plastic bags. Samples were immediately placed in a cooler with ice packs to be transported to the laboratories, where they were refrigerated at 4 °C to be analyzed the next day. The FEC was performed on the fecal samples using two different methods: 1) the Modified McMaster (MMR), with lower detection limit of 50 eggs/gram; and 2) the Triple Chamber McMaster (TCM), with lower detection limit of 8 eggs/gram.

Between the years 2012 and 2019, fecal samples were sent to the Animal Health Lab at the Ontario Veterinary College to perform fecal egg counting using the Modified McMaster method. The method was described by Zajac et al. (2012). Following Zajac et al. (2012) protocol, fecal sample of 4 g were mixed well with 56 ml of flotation solution. The mixture was then strained and put in the two-chamber McMaster slide and allowed to sit for 5 minutes before counting the eggs under microscope.

In most recent years (2018 and 2019), additional fecal egg counting was performed at the Department of Animal Biosciences at the University of Guelph using the Triple Chamber McMaster method, in which a 4-gram fecal sample is mixed and homogenized with 26 mL of floating solution (300 g L^{-1} NaCl). The mixture is then strained and put into Triple Chamber McMaster slides and left to sit no less than 5 minutes before the initiation of egg counting under the microscope.

Records with zero fecal eggs count were adjusted to half of the lower detection limit to account for differences in FEC methods. Therefore, the minimum value for FEC adjusted observations were 25 and 4 eggs/gram for the MMR and TCM methods, respectively. These

adjusted minimum values took into account the sensitivity of each of the two methods used to determine FEC, i.e. 50 eggs/gram for MMR (Pereckiene et al., 2007) and 8 eggs/gram for TCM (Westers et al., 2016). FEC values from both methods were transformed by taking the natural logarithm (ln) of egg counts (LMMR and LTCM, respectively) in an attempt to normalize their distributions for the analyses. The total number of records for each trait phenotype and their basic descriptive statistics are presented in Table 2.1.

2.3.2. Evaluation and Comparison between Different Methods to Measure Fecal Egg Count

Whenever the phenotypic records were available for both FEC methods, the differences were evaluated at each year using paired *t*-test. The number of ln FEC records available for both methods in the years 2018 and 2019 were 115 and 87, respectively, with 202 records available in total. In addition, using all records, the means were compared using Welch *t*-test, and the homogeneity of variance for the ln FEC records from the two different FEC methods was tested using Levens's test. All statistical tests were performed using R (R Core team, 2015).

2.3.3. Estimating Genetic Parameters for the Two FEC methods as Two Separate Traits

The variance components for LMMR and LTCM were estimated by restricted maximum likelihood using ASREML (Gilmour et al., 2015) using a bivariate model, as follow:

$$y_{tijklm} = Y_{ti} + M_{tj} + a_{tk} + pe_{tk} + C_{tl} + e_{tijklm}$$

where; y_{tijklm}: phenotypic record for trait t; Y_{ti}: fixed effect of the year; M_{tj}: fixed effect of the month; a_{tk}: random animal additive genetic effect; pe_{tk}: random animal permanent environmental effect; C_{tl}: random group measurement effect, which was formed on based on management group (year-sex) and the date of measurement; and e_{tijklm}: random residual error.

The variance and covariance matrix for the bivariate analysis was:

$$\begin{bmatrix} A\sigma_{a1}^2 & A\sigma_{a12} & 0 & 0 & 0 & 0 & 0 & 0 \\ A\sigma_{a21} & A\sigma_{a2}^2 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & I\sigma_{pe1}^2 & I\sigma_{pe12} & 0 & 0 & 0 & 0 \\ 0 & 0 & I\sigma_{pe21} & I\sigma_{pe2}^2 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & I\sigma_{c1}^2 & I\sigma_{c12} & 0 & 0 \\ 0 & 0 & 0 & 0 & I\sigma_{c21} & I\sigma_{c2}^2 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & I\sigma_{e1}^2 & I\sigma_{e12} \\ 0 & 0 & 0 & 0 & 0 & 0 & I\sigma_{e21} & I\sigma_{e2}^2 \end{bmatrix}$$

Where: the variances are on the diagonal, and the covariances are off of the diagonal; the subscripts a , pe, c, and e: indicate the additive genetic, permanent environmental, group measurement, and residual effects, respectively, and subscripts 1 and 2 refers to the traits; A is the numerator additive relationship matrix; and I is an identity matrix.

The repeatability for FEC methods was calculated as follows:

$$r = \frac{\sigma_a^2 + \sigma_{pe}^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_c^2 + \sigma_e^2}$$

Where: r is the repeatability for the trait and $\sigma_a^2, \sigma_{pe}^2, \sigma_c^2, and\ \sigma_e^2$ are the additive genetic, permanent environmental, group of measurement, and residual effect variances, respectively.

2.3.4. Integrating FEC Data from Different Methods

Whenever records using the LMMR methods were available, they were used. Missing values for LMMR were replaced by adjusted LTCM by shifting and scaling the observations to the LMMR records' as follows (for mean and variance see Table 2.1):

$$LTCM_i^* = \left(\frac{LTCM_i - \overline{LTCM}}{s_{LTCM}}\right) s_{LMMR} + \overline{LMMR}$$

Where: $LTCM_i^*$ is the re-scaled ln fecal egg count record i; $LTCM_i$ is ln fecal egg count record using TCM method; $\overline{LTCM}$ and $\overline{LMMR}$ are the means for ln fecal egg count using TCM and MMR, respectively; and s_{LTCM} and s_{LMMR} are the standard deviations for ln fecal egg count records using TCM and MMR, respectively.

The integrated fecal egg count data (LMMR + LTCM*) will be called LFEC hereafter in this paper.

2.3.5. Estimating Genetic Parameters Among Parasite Resistance Traits

The covariance components were estimated by restricted maximum likelihood using ASREML (Gilmour et al., 2015), using 4-trait model for LFEC, FAMACHA©, BCS, and WT, as follow:

$$y_{tijklm} = Y_{ti} + M_{tj} + a_{tk} + pe_{tk} + C_{tl} + e_{tijklm}$$

where; y_{tijklm}: phenotypic record for trait t; Y_{ti}: fixed year effect; M_{tj}: fixed month effect; a_{tk}: random animal additive genetic effect; pe_{tk}: random animal permanent environmental effect; C_{tl}: random group of measurement effect, which was formed on based on management group (year-sex) and date of measurement; and e_{tijklm}: random residual error.

The variance and covariance matrix for the multivariate analysis was:

$$\begin{bmatrix} A\sigma_{a1}^2 & \cdots & A\sigma_{a14} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ \vdots & \ddots & \vdots & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ A\sigma_{a41} & \cdots & A\sigma_{a4}^2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & I\sigma_{pe1}^2 & \cdots & I\sigma_{pe14} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & \vdots & \ddots & \vdots & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & I\sigma_{pe41} & \cdots & I\sigma_{pe4}^2 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & I\sigma_{c1}^2 & \cdots & I\sigma_{c14} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & \vdots & \ddots & \vdots & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & I\sigma_{c41} & \cdots & I\sigma_{c4}^2 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & I\sigma_{e1}^2 & \cdots & I\sigma_{e14} \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \vdots & \ddots & \vdots \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & I\sigma_{e41} & \cdots & I\sigma_{e4}^2 \end{bmatrix}$$

Where: the variances are on the diagonal, and the covariances are off of the diagonal; the subscripts a , pe, c, and e indicate the additive genetic, permanent environmental, group of measurement, and residual effects, respectively, and subscripts 1 to 4 refers to the traits; A is the numerator additive relationship matrix; and I is an identity matrix.

2.4. Results and Discussions

2.4.1. Descriptive Statistics

The basic descriptive statistics for LMMR, LTCM, LFEC, FAMACHA©, BCS, and WT are shown in Table 2.1. Figure 2.1 shows the number of phenotypic records for the traits over the different years. LMMR and LTCM are measures of the same phenotype (i.e. ln fecal egg count) using different methods. The mean LMMR (mean = 5.86 ln egg/g) was greater than mean LTCM (mean = 4.37 ln egg/g), while the standard deviation for LMMR (s.d = 1.54 ln egg/g) was smaller than LTCM (s.d = 2.14 ln egg/g). This results in greater coefficient of variation for LTCM compared to LMMR, 49.26% and 26.34%, respectively. The LFEC is the integrated ln fecal egg count from both LMMR and LTCM methods, prioritizing the LMMR records over LTCM. Whenever LMMR records were missing, they were replaced by the re-scaled LTCM records.

2.4.2. Differences Between FEC Observation Using Two Different Methods

Results of the comparisons between LMMR and LTCM using a paired *t*-test are presented in Table 2.3. In 2018 the mean difference between the two ln FEC methods was 0.87, significantly different from zero ($P < 0.0001$). In 2019, the mean difference between the measurements was 0.08 and not significantly different from zero ($P = 0.3858$). Thus, the results were not consistent across the two years. This is because the two methods are different in their lower detection limit, causing large disparities between measurements in animals with low infection levels. Figure 2.2 shows a scatter plot for the two FEC methods (LMMR against LTCM) for FEC data from the two

years. Figure 2.3 presents a scatter plot of the difference between methods relative to the observation against the infection level as measured by LTCM (i.e. |LMMR - LTCM| /LTCM against LTCM) for FEC data from the two years. The association between the two FEC methods is positive and increases as the infection levels increases (Figure 2.2). The relative absolute difference between the two FEC methods decreases rapidly with the increase of infection levels from LTCM =1 to 4 (low infection levels), then it decreases gradually, approaching zero in high infection levels (LTCM~10; Figure 2.3). Indeed, the infection levels in the year 2019 were higher than 2018, which can be seen in Figures 2.2 and 2.3 and in their corresponding supplementary figures of untransformed data (Figures 2.S1 and 2.S2, respectively). According to Cringoli et al. (2004), the type of the flotation solution significantly influenced the observation for FEC in sheep for both gastrointestinal strongyles and *D. dendriticum*. Different egg recovery rates were also reported when using different McMaster methods for detecting *Teladorsagia circumcincta*, including with differences due to sample weight, floatation solution, centrifugation, number of McMaster chambers, and multiplication factor (Vadlejch et al., 2011). Paras et al. (2018) compared three different fecal egg counting methods: the Modified-Wisconsin, the 3-chamber McMaster, and the Mini-FLOTAC in different host species including in sheep, cattle, horses, llamas, and spiked sample. When comparing Mini-FLOTAC to McMaster they found the percent increase in number of eggs observed of 166.5%, 27.2% - 53.6%, 4.8%, 1.5%, and 28.8%, in sheep, cattle, horse, llama, and spiked sample, respectively. Also, when comparing Mini-FLOTAC to Wisconsin they found the percent increase in number of eggs observed of 223.3%, 116.5% - 139.8%, 102.6%, 129.0%, and 130.9%, in sheep, cattle, horse, llama, and spiked sample, respectively. When comparing McMaster to Wisconsin they found the percent increase in number of eggs observed of

49.4%, 40.9% - 88.5%, 93.4%, 98.8%, and 79.3%, in sheep, cattle, horse, llama, and spiked sample, respectively. Therefore, inconsistencies among methods used for FEC can be problematic for genetic evaluation, especially when such inconsistencies are not accounted for. This reflects a potential practical problem for commercial sheep producers, where consistency may be difficult to maintain. Thus, information about the FEC method used has important considerations in the integration of data for genetic evaluation.

Results for testing the equality of mean and variance between LMMR and LTCM are shown in Table 2.4. Both means and variances were significantly different ($P < 0.0001$). Therefore, the integrating records from the two FEC methods should be carefully done. It must be noted that the "Triple Chamber McMaster" method is more sensitive than the "Modified McMaster". However, when FEC data were integrated (LFEC), the LMMR records were preferred over LTCM when both were available for an animal. This was done because the majority of the FEC data were performed using the LMMR (see Table 2.1).

2.4.3. Genetic Parameters for the Two FEC Methods as Two Different Traits

The genetic parameters estimated from bivariate analysis treating ln FEC from different methods as two separate traits (LMMR and LTCM) are given in Table 2.5. The estimated heritability for both methods was low, 0.10 (± 0.04) and 0.07 (± 0.05), for LMMR and LTCM, respectively. The heritability estimate for FEC ranges between 0.00 and 0.37 (Snyman and Fisher, 2019) and averaged 0.27 in Safari et al. (2005). Heritability estimates reported in this study were similar to those in Burkina Faso and South Africa, which ranged from 0.06 to 0.17 (Álvarez et al.,

2018), and 0.06 to 0.16 (Matebesi-Ranthimo et al., 2014; Mpetile et al., 2015; Cloete et al., 2016), respectively.

The repeatability estimates for LMMR and LTCM were 0.18 and 0.25, respectively. The expected heritability for the mean of n records (h_n^2) can be calculated by $h_n^2 = n \times h^2/[1+(n-1)\times r]$ (Falconer and Mackay, 1996), where: n is the number of records; h^2 is the estimated heritability for a single record; and r is the estimated repeatability. The expected heritabilities for the mean of three records for LMMR and LTCM were 0.22 and 0.14, respectively, which increased by factor of 2.2 and 2.0 relative to one record, respectively. Therefore, LMMR method benefits more from repeated records than LTCM method. In the current data, the average number of records per animal was 1.30 and 1.80 for LMMR and LTCM, respectively.

In this study, estimates for correlations between LMMR and LTCM (Table 2.5) were very high for genetic (r_g = 0.94), group measurement (r_c = 0.99), and permanent environmental effect (r_{pe} = 0.93), and high for residual (r_e = 0.82) effect, leading to high phenotypic correlation (r_{ph}= 0.88). This was expected since both LMMR and LTCM try to measure the same phenotype. These results support the integration of these tow FEC measures into a single measure as done in this study (i.e. LFEC).

2.4.4. Estimating Genetic Parameters for All Parasite Resistance Traits

Estimates of genetic parameters from multi-trait analysis for parasite resistance traits (including: LFEC, FAMACHA©, BCS, and WT) are shown in Table 2.6. The heritability estimates were low, ranging from 0.07 to 0.24 (Table 2.6). The heritability estimate for LFEC was 0.12 (± 0.04), which was slightly higher than estimates for LMMR and LTCM from the bivariate analysis.

The heritability estimate for FAMACHA© 0.07 (± 0.05) was low with a large standard error (Table 2.6). Snyman and Fisher (2019) reported heritability estimates for FAMACHA© from 0.08 to 0.49. Riley and Van Wyk (2011) reported similar heritability estimates in South African Merinos under moderate GIN challenge (0.08 ± 0.04) and higher heritability in severe GIN challenge (0.17 ± 0.05). In the same study, different heritability estimates were reported depending on adjustment methods made to the treated animals under moderate and severe GIN challenges (range from 0.07 to 0.16, and 0.17 to 0.23, respectively). In another study of the same South African Merino population, Riley and Van Wyk (2009) reported heritability of 0.13 in low GIN challenge and heritability estimates ranging from 0.08 to 0.11 and from 0.20 to 0.24 in moderate and severe GIN challenges, respectively. Cloete et al (2016) reported heritability of 0.13 in Mediterranean Region of South Africa. In Burkina Faso, heritability reported ranged from 0.21 to 0.34 (Álvarez et al., 2018). Higher heritability estimates for FAMACHA© score (0.33 to 0.41) were reported in Dorper Sheep in South Africa (Ngere et al., 2017). The sheep in this current study did not have large parasite loads and did not show disease symptoms. This is likely the main reason why the heritability estimate was similar to the studies with moderate GIN challenge.

In this study, the heritability estimated for BCS was 0.17 ± 0.05 (Table 2.6). Riley and Van Wyk (2009) reported heritability of 0.17, 0.26 to 27, and 0.29 to 0.33, in low, moderate, and severe GIN challenges, respectively. The heritability estimate (h^2 = 0.24 ± 0.07) of WT was the highest in this study (Table 2.6). Riley and Van Wyk (2009) reported heritability of 0.19, 0.30 to 35, and 0.29 to 0.32, in low, moderate, and severe GIN challenges, respectively for WT of lambs of similar age to the ones used in the present study.

The estimates for genetic and phenotypic correlations, are presented in Table 2.7. The genetic correlation between LFEC and FAMACHA© (Table 2.7) was positive and low with large standard error (0.24 ± 0.32). Higher genetic correlations were reported in literature. Álvarez et al (2018) reported genetic correlation between 0.55 and 0.78. Riley and Van Wyk (2009) reported genetic correlation between FAMACHA© and FEC, ranging from 0.73 to 0.85. Van Wyk and Bath (2002) cited perfect correlation of 1.00. The different estimates for genetic correlations reported in the literature could be due to: 1) the level of infection, where higher worm environmental challenge leads to an increase in the heritability and genetic correlation estimates for FAMACHA© and FEC traits; 2) data handling, specifically in relation with subjective clinical scoring for FAMACHA© or the number of McMaster chambers counted; and 3) the different transformation methods of the raw FEC data in the different studies (e.g. Box-cox transformation, log transformations, ln transformations) (Riley and Van Wyk, 2009; Silva et al., 2012; Balconi Marques et al., 2020). Based on the current results of estimated genetic correlations between LFEC and FAMACHA©, there is no clear indication for usefulness for genetic selection for FAMACHA© to genetically reduce the FEC in sheep. However, previous studies with Corriedale sheep found moderate genetic correlation estimates between Log_e (FEC + 100) and FAMACHA© scores (0.55 ± 0.12) suggesting these two traits could be used together for selection towards more resilient or resistant sheep (Balconi Marques et al., 2020). In this study, the low genetic correlation between FAMACHA© and LFEC could be due to the possible contribution from other non-hematophagous GIN species to the FEC records. Despite the low heritability of FAMACHA© scores, collecting its records is less expensive and time consuming than individual FECs. Thus, large number of FAMACHA© records could be generated rapidly and used for selection decisions. In addition,

recording FAMACHA© can be practical for managing anthelmintic resistance to drugs (in GIN populations) by reducing the number of treated animals in the flock (Ejlertsen et al., 2006; Burke et al., 2007; Reynecke et al., 2009; Maia et al., 2015). Therefore, FAMACHA© is an important trait to record. Increasing the number of FAMACHA© records would reduce the observed standard errors and increase the accuracy of estimation of genetic parameters, allowing to assess better its utility for breeding decisions. All other genetic correlations with LFEC were also low with large standard errors (Table 2.7). Therefore, based on these results, none of the traits in this study could be used as good genetic indicator for FEC. However, they may be used by producers as an indication of general health or parasite resilience of the sheep. The only moderate genetic correlation ($r = 0.43 \pm 0.17$) was between BCS and WT (Table 2.7). Riley and Van Wyk (2009) reported genetic and phenotypic correlations between BCS and WT of 0.47 and 0.59, respectively.

Higher estimates of correlation between traits were found due to permanent environmental (-0.57 to 0.54) and group of measurement (-0.67 to 0.89) effects (Tables 2.S1 and 2.S2). For residual effect, the correlations were lower (-0.10 to 0.29) (Table 2.S3), while the estimated phenotypic correlations varied between (-0.13 and 0.56) (Table 2.7).

The genetic parameters estimated in this study were based on records from a single sheep flock, so they may not represent the diversity of flocks in Ontario and Canada. More representative estimates of genetic parameters will be calculated when additional data and potentially from different flocks become available.

2.5. Implications

The two FEC methods used, the Modified McMaster and the Triple Chamber McMaster, are highly correlated. However, they have different scales (means and variances), which should be taken into account when integrating their records. Heritability estimates for FEC and related indicator traits, such as FAMACHA©, BCS, and body weight were low to moderate, indicating that genetic progress for these traits is possible, but it will be achieved as a long-term goal. Genetic correlations between FEC and all indicator traits were low, indicating little to no benefit in using these traits as indicators for FEC. However, they may be used as indicators for general health and resilience to parasite load. The genetic parameter estimates may change when additional records from different sheep flocks become available.

2.6. Tables

Table 2.1. Basic descriptive statistics for the studied parasite resistance traits.

Trait	No. Records	Range	Mean ± s.d.	CV (%)
LMMR	998	3.22 – 9.77	5.86 ± 1.54	26.34
LTCM	678	1.39 – 9.66	4.34 ± 2.14	49.26
LFEC	1,474	3.22 – 9.77	5.79 ± 1.55	26.80
FAMACHA©	1,048	1 – 5	2.70 ± 0.78	28.96
BCS	1,054	1 – 5	2.83 ± 0.62	21.96
WT	1,103	23.00 – 96.00	56.23 ± 12.17	21.65
MMR	998	25 – 17,500	959.44 ± 1,699.65	177.15
TCM	678	4 – 15,644	581.87 ± 1,681.76	289.03
FEC	1,474	25 – 17,500	959.40 ± 1,758.96	183.34

LMMR: natural log transformed fecal egg count using the Modified McMaster method; LTCM: natural log transformed fecal egg count using the triple Chamber McMaster method; LFEC: integrated ln fecal egg counting records from LMMR and LTCM by prioritizing available LMMR records and fill missing records with re-scaled LTCM; FAMACHA©: score system assessing the anemia using color of palpebral conjunctiva, ranging from 1 (red and normal) to 5 (white and anemic); BCS: body condition score; and WT: body weight (kg). MMR, TCM, and FEC: original fecal counts before the ln transformation for LMMR, LTCM, and LFEC, respectively.

Table 2.2. **Descriptive statistics of the pedigree used in the analyses**

Longest Ancestral Path	Number of occurrences
0	35
1	18
2	17
3	26
4	56
5	66
6	53
7	77
8	86
9	107
10	231
11	265
12	213
13	126
Total no. of individuals	1,376
No. of sires	94
No. of dams	561
No. of founders	35
Average number of discrete generation	5.16

Table 2.3. **Comparison of the two fecal egg counting methods (LMMR and LTCM) between two different years of recording.**

year	n	Mean difference (95% CI)	P – value
2018	115	0.87 (0.71 – 1.03)	< 0.0001
2019	87	0.08 (-0.10 – 0.25)	0.3858
Both years	202	0.52 (0.40 – 0.65)	< 0.0001

Table 2.4. **Test for equality of variance (Levens's test) and mean (Welch *t*-test) for alternate fecal egg counting method**

Groups compared		Levens's test		Welch t-test		
Method	**Mean ± s.d**	**F**	**P - value**	**t**	**df**	**P - value**
LMMR	5.86 ± 1.54	118.98	< 0.0001	15.864	1,143.2	< 0.0001
LTCM	4.34 ± 2.137					

LMMR: natural log transformed fecal egg count using the Modified McMaster method; and LTCM: natural log transformed fecal egg count using the triple Chamber McMaster method.

Table 2.5. **Estimates for genetic parameters (± SE) for the two fecal egg counting methods (LMMR and LTCM)**

Estimate	Trait	
	LMMR	**LTCM**
σ_a^2	0.30 ± 0.11	0.25 ± 0.17
σ_c^2	0.90 ± 0.29	1.09 ± 0.36
σ_{pe}^2	0.22 ± 0.12	0.64 ± 0.18
σ_e^2	1.43 ± 0.11	1.65 ± 0.12
h^2	0.10 ± 0.04	0.07 ± 0.05
r^2	0.18 ± 0.04	0.25 ± 0.04
	Correlation	
r_g	0.94 ± 0.17	
r_c	0.99 ± 0.02	
r_{pe}	0.93 ± 0.17	
r_e	0.82 ± 0.03	
r_{ph}	0.88 ± 0.02	

LMMR: natural log transformed fecal egg count using the Modified McMaster method; and LTCM: natural log transformed fecal egg count using the triple Chamber McMaster method; σ_a^2, σ_c^2, σ_{pe}^2, σ_e^2: are genetic, group of measurement, permanent environmental, residual variances; h^2 and r^2: are the heritability and repeatability; and r_g , r_c , r_{pe} , r_e, and r_{ph}: are genetic, group of measurement, permanent environmental, residual, and total phenotypic correlations, respectively.

Table 2.6. **Estimates of variance components and genetic parameters for fecal egg count and other parasite resistance traits**

Estimate	LFEC	FAMACHA©	BCS	WT
$\boldsymbol{\sigma_a^2}$	0.31 ± 0.10	0.04 ± 0.03	0.07 ± 0.03	41.33 ± 8.15
$\boldsymbol{\sigma_c^2}$	0.71 ± 0.21	0.09 ± 0.04	0.11 ± 0.05	103.14 ± 38.92
$\boldsymbol{\sigma_{pe}^2}$	0.28 ± 0.09	0.08 ± 0.03	0.06 ± 0.02	16.03 ± 4.89
$\boldsymbol{\sigma_e^2}$	1.20 ± 0.07	0.39 ± 0.02	0.17 ± 0.01	12.41 ± 0.78
$\mathbf{h^2}$	0.12 ± 0.04	0.07 ± 0.05	0.17 ± 0.06	0.24 ± 0.07
$\mathbf{r^2}$	0.23 ± 0.04	0.20 ± 0.04	0.30 ± 0.05	0.33 ± 0.08

LFEC: integrated natural log transformed fecal egg counting records from LMMR and LTCM by prioritizing available LMMR records and fill missing records with re-scaled LTCM; FAMACHA©: score system assessing the anemia using color of palpebral conjunctiva, ranging from 1 (red and normal) to 5 (white and anemic); BCS: body condition score; and WT: body weight (kg); σ_a^2, σ_c^2, σ_{pe}^2, σ_e^2: are genetic, group of measurement, permanent environmental, residual variances; h^2 and r^2: are the heritability and repeatability.

Table 2.7. **Estimates of genetic correlations ± SE (below diagonal) and phenotypic correlations (above diagonal) among parasite resistance traits**

	LFEC	FAMACHA©	BCS	WT
LFEC	-	0.18	-0.13	-0.07
FAMACHA©	0.24 ± 0.32	-	-0.25	-0.14
BCS	-0.03 ± 0.25	-0.02 ± 0.37	-	0.56
WT	0.22 ± 0.19	-0.01 ± 0.30	0.43 ± 0.17	-

LFEC: integrated natural log transformed fecal egg counting records from LMMR and LTCM by prioritizing available LMMR records and fill missing records with re-scaled LTCM; FAMACHA©: score system assessing the anemia using color of palpebral conjunctiva, ranging from 1 (red and normal) to 5 (white and anemic); BCS: body condition score; and WT: body weight (kg).

Table 2.S1. **Estimates for permanent environmental effect correlations ± SE (below diagonal) among parasite resistance traits.**

	LFEC	FAMACHA©	BCS	WT
LFEC	-			
FAMACHA©	0.29 ± 0.26	-		
BCS	-0.56 ± 0.26	-0.46 ± 0.25	-	
WT	-0.57 ± 0.26	-0.13 ± 0.26	0.54 ± 0.20	-

LFEC: integrated natural log transformed fecal egg counting records from LMMR and LTCM by prioritizing available LMMR records and fill missing records with re-scaled LTCM; FAMACHA©: score system assessing the anemia using color of palpebral conjunctiva, ranging from 1 (red and normal) to 5 (white and anemic); BCS: body condition score; and WT: body weight (kg).

Table 2.S2. **Estimates of group of measurement correlations ± SE (below diagonal) among parasite resistance traits.**

	LFEC	FAMACHA©	BCS	WT
LFEC	-			
FAMACHA©	0.30 ± 0.26	-		
BCS	-0.14 ± 0.25	-0.67 ± 0.19	-	
WT	-0.11 ± 0.24	-0.36 ±0.27	0.89 ± 0.07	-

LFEC: integrated natural log transformed fecal egg counting records from LMMR and LTCM by prioritizing available LMMR records and fill missing records with re-scaled LTCM; FAMACHA©: score system assessing the anemia using color of palpebral conjunctiva, ranging from 1 (red and normal) to 5 (white and anemic); BCS: body condition score; and WT: body weight (kg).

Table 2.S3. **Estimates for residual correlations ± SE (below diagonal) among parasite resistance traits.**

	LFEC	FAMACHA©	BCS	WT
LFEC	-			
FAMACHA©	0.10 ± 0.05	-		
BCS	-0.04 ± 0.05	-0.10 ± 0.04	-	
WT	-0.05 ± 0.05	-0.09 ± 0.04	0.29 ± 0.04	-

LFEC: integrated natural log transformed fecal egg counting records from LMMR and LTCM by prioritizing available LMMR records and fill missing records with re-scaled LTCM; FAMACHA©: score system assessing the anemia using color of palpebral conjunctiva, ranging from 1 (red and normal) to 5 (white and anemic); BCS: body condition score; and WT: body weight (kg).

2.7. Figures

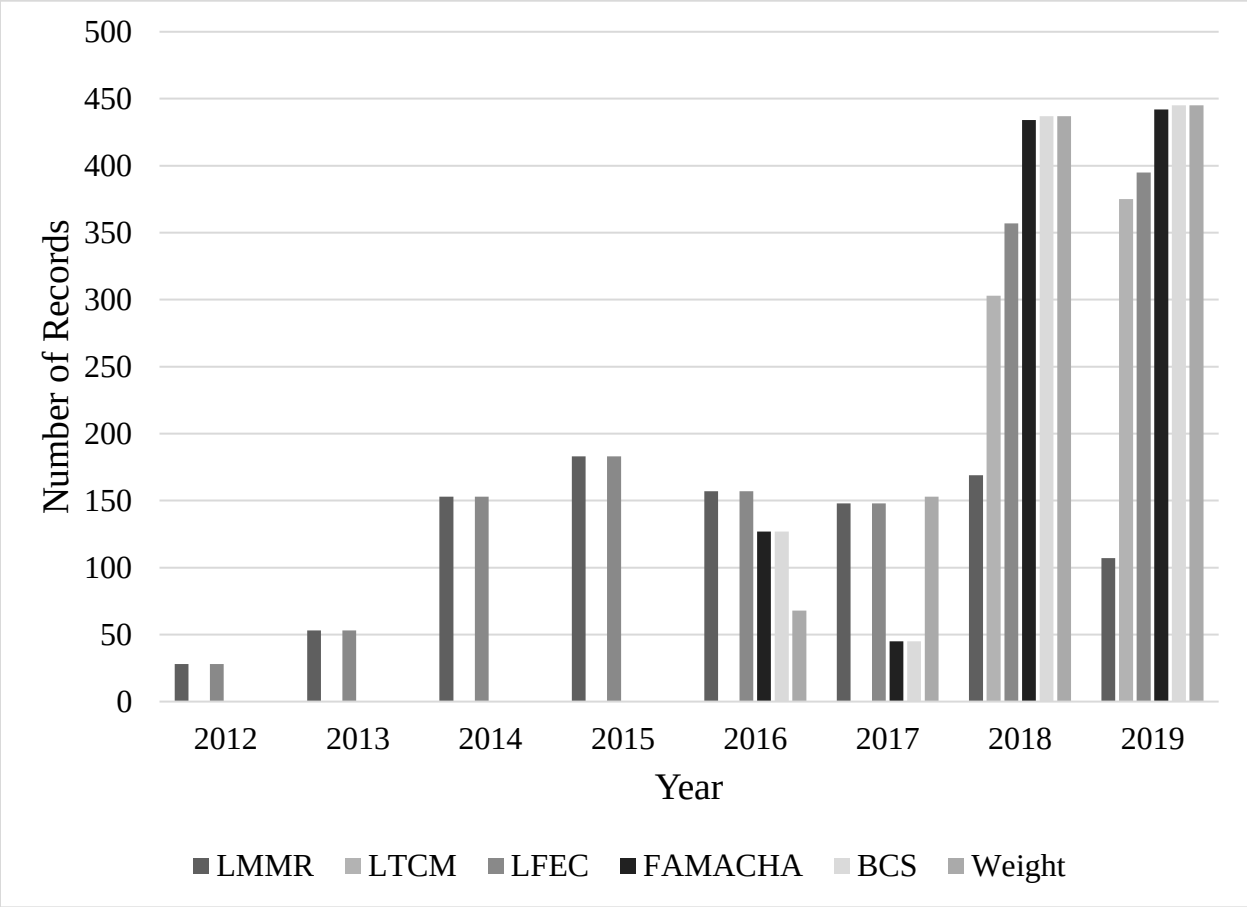

Figure 2.1. Distribution of number of phenotypic records per year.

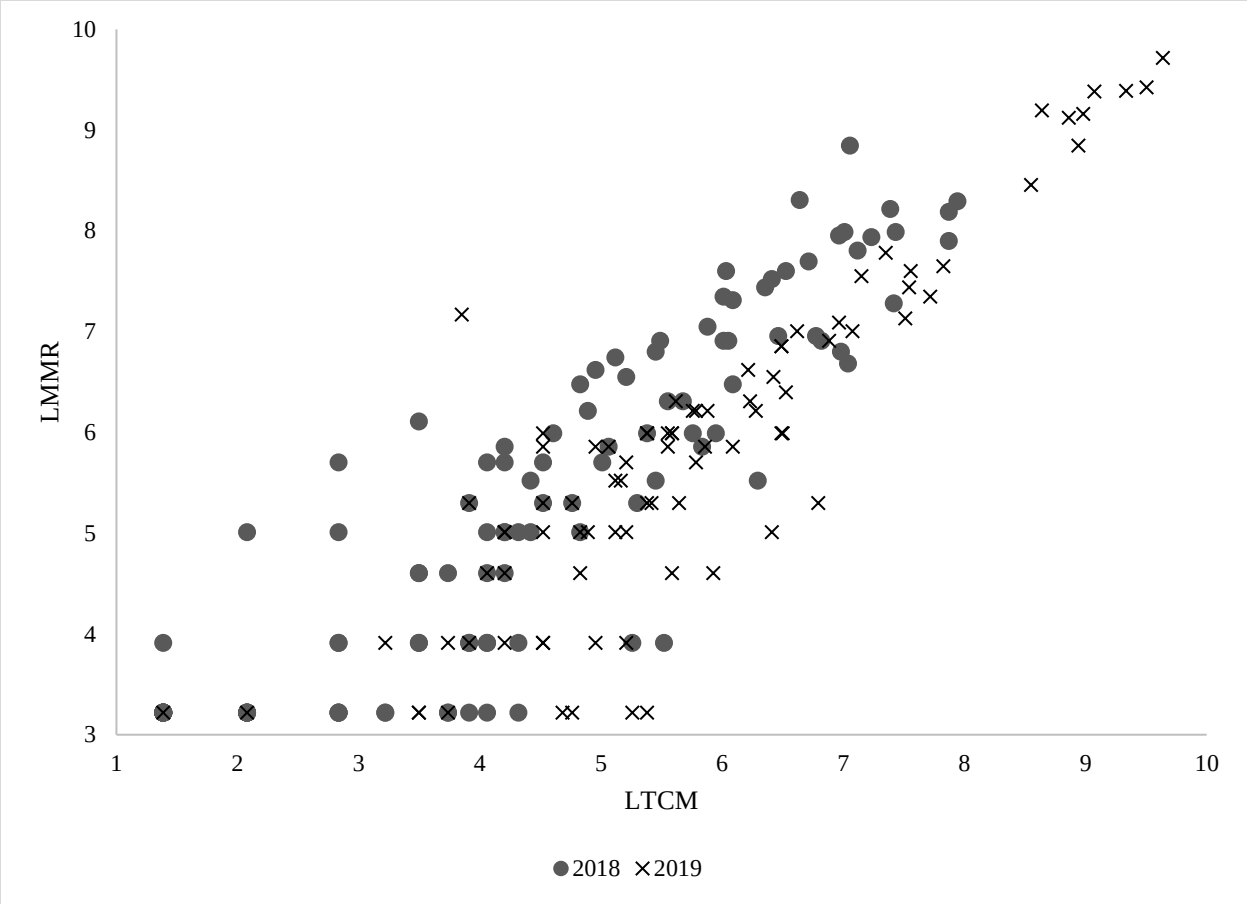

Figure 2.2. **Scatter plot of LMMR values against LTCM values for the recording years 2018 and 2019.**

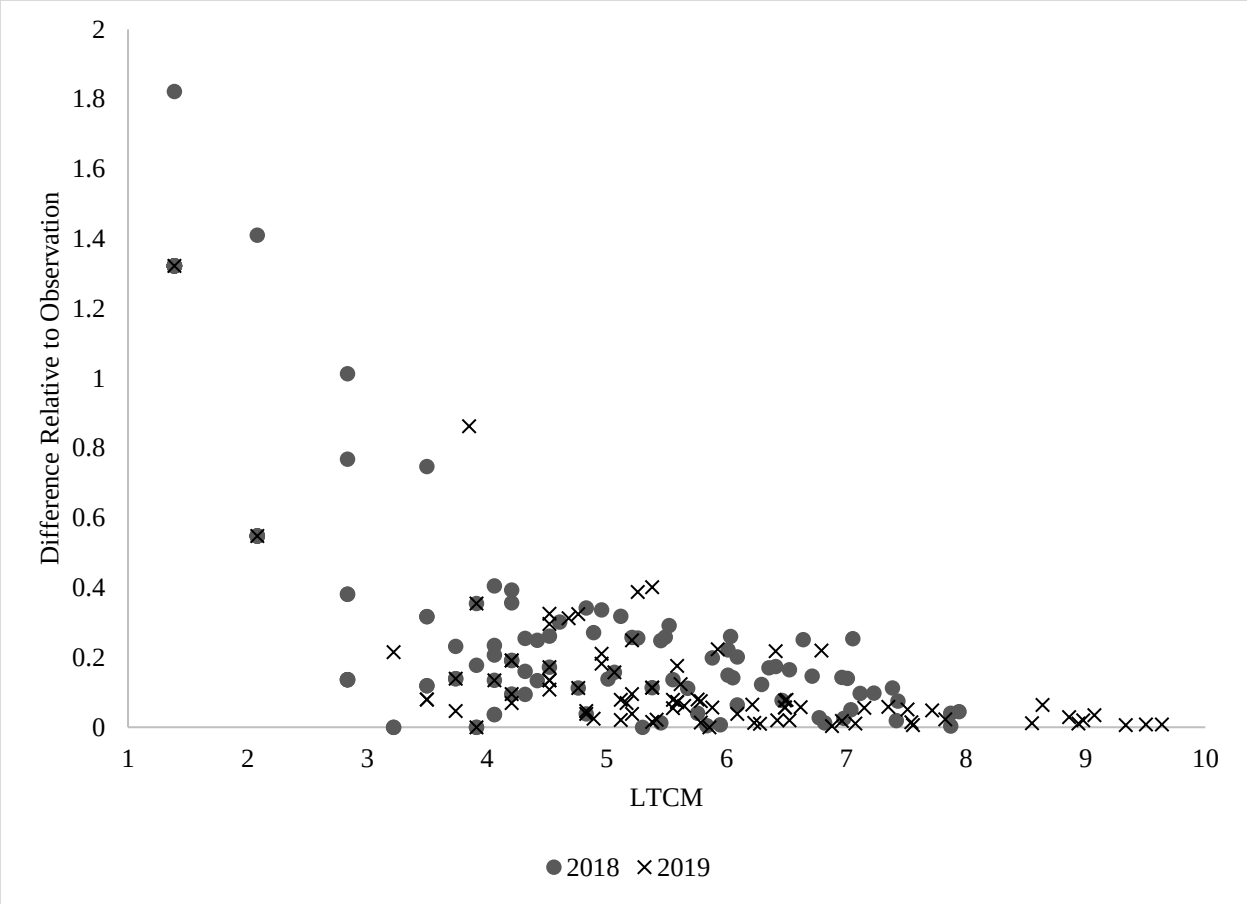

Figure 2.3. **Scatter plot of the absolute difference between FEC methods divided by LTCM (i.e. |LMMR – LTCM| / LTCM), against LTCM for the recording years 2018 and 2019.**

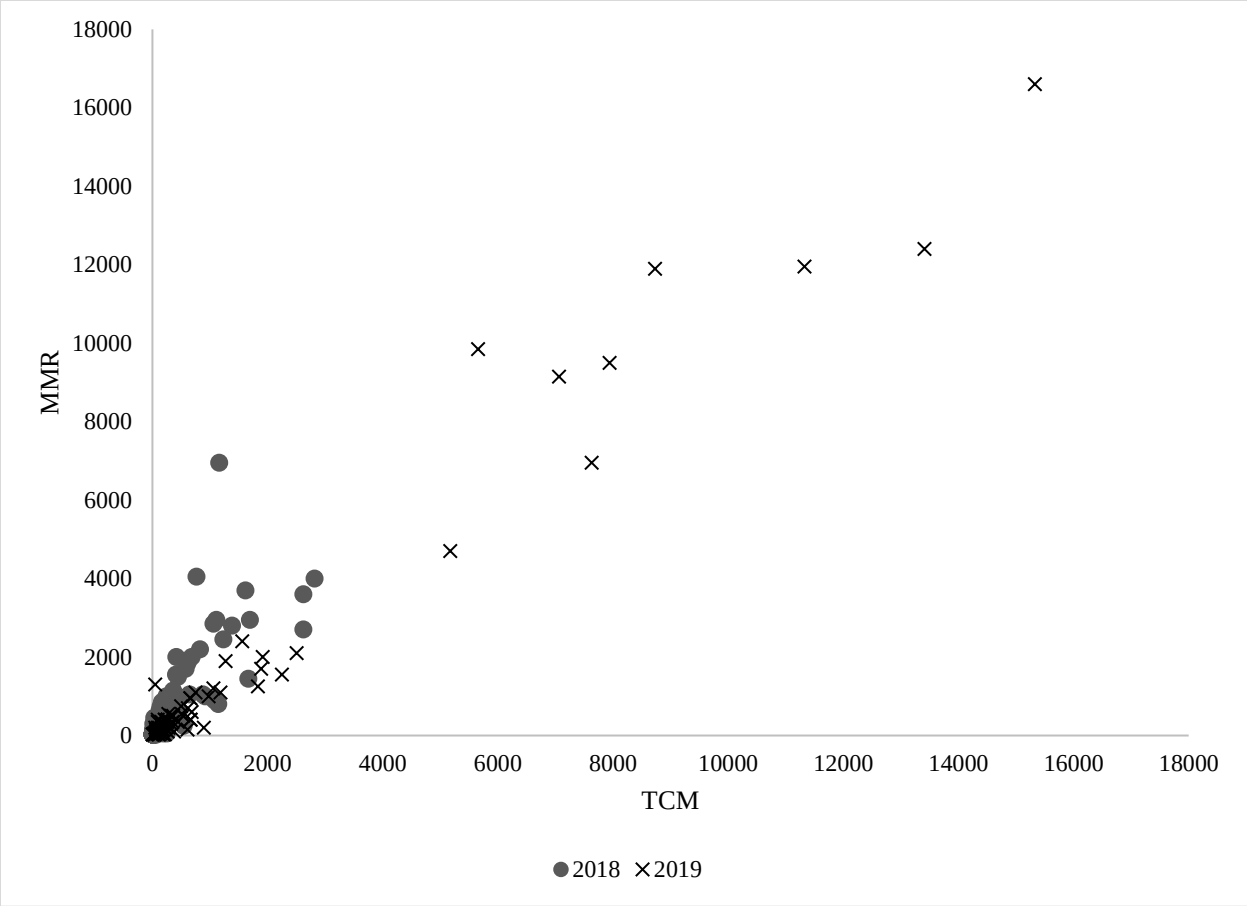

Figure 2.S1. **Scatter plot of untransformed data for MMR values against TCM values for the recording years 2018 and 2019.**

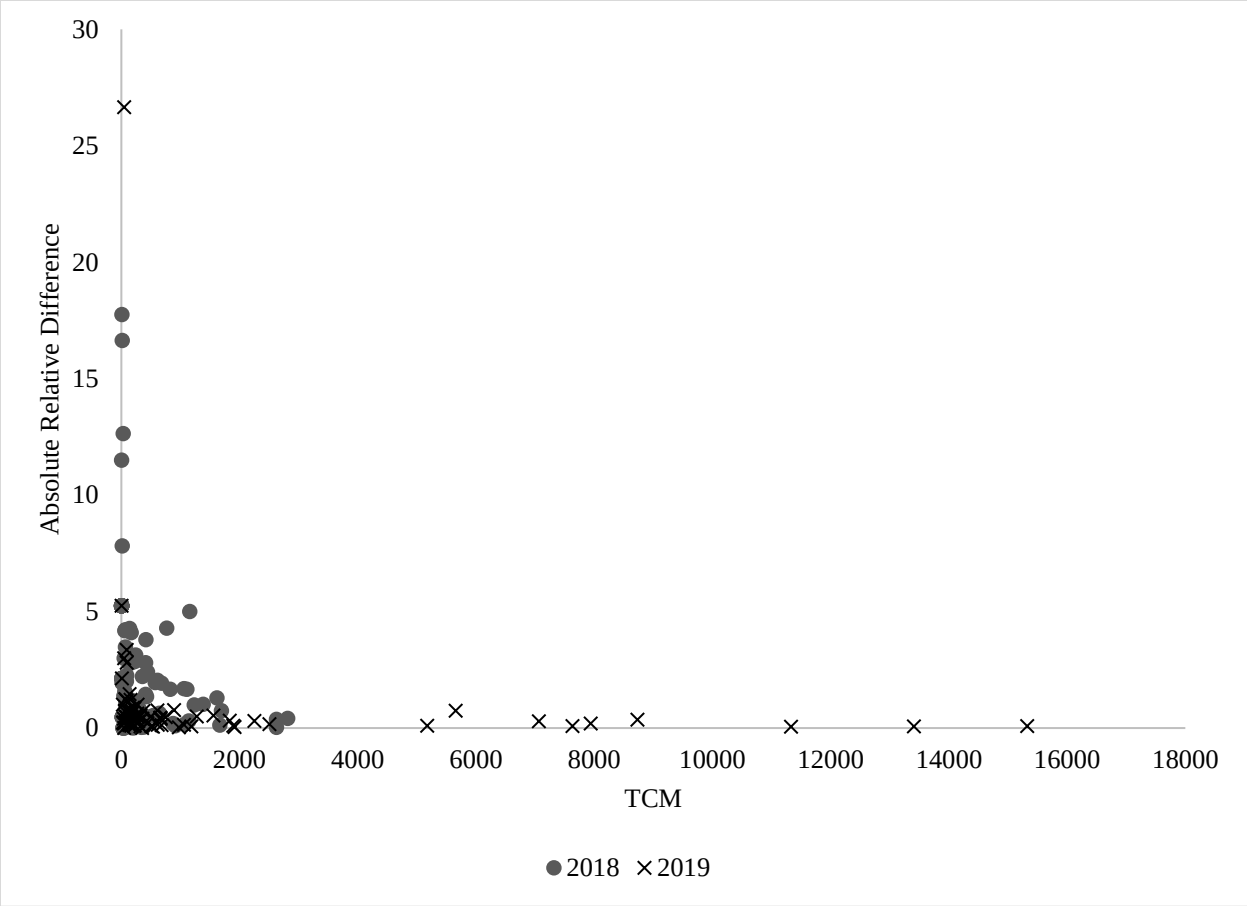

Figure 2.S2. **Scatter plot of untransformed data of the absolute difference between FEC methods divided by TCM (i.e. |MMR – TCM| / TCM) against TCM for the recording years 2018 and 2019.**

CHAPTER 3

Prediction of Genetic Resistance for Scrapie in Ungenotyped Sheep Using a Linear Animal Model

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3.1. Abstract

Selection based on scrapie genotypes could improve the genetic resistance for scrapie in sheep. However, in practice, few animals are genotyped. The objectives were to define numerical values of scrapie resistance genotypes and adjust for their non-additive genetic effect; evaluate prediction accuracy of ungenotyped animals using linear animal model; and predict and assess selection response based on estimated breeding values (EBV) of ungenotyped animals. The scrapie resistance (SR) was defined by ranking scrapie genotypes from low (0) to high (4) resistance based on genotype risk groups, and was also adjusted for non-additive genetic effect of the haplotypes. Genotypes were simulated for 1,671,890 animals from pedigree. The simulated alleles were

assigned to scrapie haplotypes in two scenarios of high (SR_h) and low (SR_l) resistance populations. A sample of 20,000 genotyped animals were used to predict ungenotyped using animal model. Prediction accuracies for ungenotyped animals for SR_h and SR_l were 0.60 and 0.54, and for allele content were from 0.41 to 0.71, respectively. Response to selection on SR_h and SR_l increased SR by 0.52 and 0.28, and on allele content from 0.13 to 0.50, respectively. In addition, the selected animals had large proportion of homozygous for the favorable haplotypes. Thus, pre-selection prior to genotyping could reduce genotyping costs for breeding programs. Using a linear animal model to predict SR makes better use of available information for the breeding programs.

Keywords: Sheep, scrapie resistance, BLUP, selection response, prediction accuracy

3.2. Introduction

In the typical form of scrapie, the risk of infection is determined by variation in amino acid sequence encoded in the prion protein (PrP) gene (Monleón et al., 2005; Molina et al., 2006; Lacroux et al., 2008; Goldmann, 2018). Polymorphisms occur on codons 136, 154, and 171 and result in five common haplotypes (ARR, AHQ, ARH, ARQ, and VRQ) associated with the scrapie risk of infection, in which the haplotype ARR is associated with lowest risk, and the haplotype VRQ is associated with highest risk of scrapie infection (Gáspárdy et al., 2018; Goldmann, 2018; Hagenaars et al., 2018; Ptacek and Ducháček, 2019). Other additional haplotypes have been observed in sheep, but due to their extreme rarity were not considered important for breeding programs (Stepanek and Horin, 2017; Goldmann, 2018). A total of 15 common possible genotype

combinations are associated with 5 risk groups (R1, R2, R3, R4, and R5), in which R1 genotypes are associated with low risk for scrapie infection (i.e. the most favorable genotypes) and R5 are associated with highest risk of infection (Molina et al., 2006; Álvarez et al., 2007; Stepanek and Horin, 2017). Due to the association of genotypes with the risk of scrapie, the use of genotyping for breeding programs is appealing for scrapie eradication programs (Kao et al., 2001; Ortiz-Pelaez and Bianchini, 2011; Stepanek and Horin, 2017; Gáspárdy et al., 2018). However, in practice, not all animals are being genotyped with breeding rams, which are more likely to be genotyped than ewes. Thus, genotypic information is limited, as only a small fraction of the total sheep population is genotyped. Gengler et al. (2007) proposed the use of a practical method to predict the allele content of bi-allelic locus in ungenotyped animals by using the Best Linear Unbiased Predictor (BLUP), in which the number of observed alleles in the genotype (0, 1, or 2) are used as a response variable, assuming complete heritability.

Selection based on risk group of the genotypes could be practiced (Molina et al., 2006; Álvarez et al., 2007; Stepanek and Horin, 2017; Gáspárdy et al., 2018). However, the genotypes corresponding to the risk groups do not act additively. For animal breeding purposes, the additive genetic effect is important since offspring inherit the alleles, rather than the genotype. There is no study that accounts for the non-additivity of risk groups corresponding to the genotypes. Therefore, adjusting for the non-additive effect is needed to account for the differences in contribution of the 5 different haplotypes alleles to scrapie resistance in sheep.

The objectives of this research were to: 1) define numeric values of scrapie resistance genotypes and adjust them for their non-additive genetic effect; 2) evaluate the accuracy of using BLUP for prediction of scrapie resistance and allele content of ungenotyped animals; and 3)

predict selection response and assess the change of genetic merit of selected ungenotyped animals based on estimated breeding value. The hypothesis of this research was that it is possible use a linear animal model for genetic evaluation and selection of ungenotyped sheep for scrapie resistance based on a small proportion of genotyped animals.

3.3. Materials and Methods

3.3.1 Adjusting the genetic resistance to scrapie for non-additive genetic effect

The numeric value of scrapie resistance genotypes was defined by ranking the scrapie genotypes from 0 (most susceptible) to 4 (most resistant), which were based on risk levels previously presented in literature (Molina et al., 2006; Álvarez et al., 2007; Stepanek and Horin, 2017) (see Table 3.1). The additive genetic effect of a haplotype was calculated as half of the homozygous resistance value relative to the most susceptible haplotype (VRQ), which was set to zero. The assumed additive genetic effect for scrapie resistance (SR) for each haplotype is shown in Table 3.1, along with the adjusted scrapie resistance (SR) genotypes for the non-additive genetic effects for all possible 15 genotypes, which were created by summing the additive genetic effects of the haplotypes. This step is important, as non-additive genetic effects were present. For example, the most susceptible haplotype VRQ is completely dominant over the haplotypes ARH and ARQ. Thus, genotypes VRQ/VRQ, ARH/VRQ, and ARQ/VRQ, have the same scrapie resistance level equal to 0 and are in the same risk group of R5, which is different from the risk group for the homozygous haplotypes ARH and ARQ. For animal breeding and genetic

improvement purposes, additive genetic effects are more important than the non-additive genetic effects, as are transmitted to the next generation. Therefore, SR needs to be adjusted for non-additive genetic effects.

3.3.2. Simulated Data

The pedigree containing 1,671,890 sheep from the GenOvis database (www.genovis.ca; Guelph, ON, Canada) was used to simulate genotypes at a single locus with five alleles resulting in 15 possible common genotypes. The genotype frequencies resulting from the simulation are shown in Table 3.2. The simulated alleles were assigned to scrapie haplotypes to create two population, high-SR or low-SR, assuming high and low frequencies for the most resistant haplotype (ARR), respectively (Table 3.3). A total of 20,000 animals were randomly chosen (out of 1,671,890 individuals) to have genotype records in the study with all other animals assumed to be ungenotyped. Basic descriptive statistics of SR (0 - 4) in the two populations of high and low SR (SR_h and SR_l, respectively) and the allele content (0, 1, or 2) for the haplotypes (Hc_1, Hc_2, Hc_3, Hc_4, and Hc_5) are presented in Tables 3.4 and 3.5, for whole population (n = 1,671,890) and the randomly chosen animals (n = 20,000) that had their genotypic information available (tested animals), respectively.

3.3.3. Prediction of scrapie resistance and haplotype gene content using the animal model

The scrapie resistance phenotypes (SR_h and SR_l) and haplotype allele contents (Hc_1, Hc_2, Hc_3, Hc_4, and Hc_5) where predicted for the ungenotyped animals using the observed records of the

20,000 genotyped animal using ASREML (Gilmour et al., 2015) and the following univariate linear animal model:

$$y_i = \mu + a_i + e_i$$

Where, y_i: record of the ith animal for the trait being analyzed (SR_h, SR_l, Hc_1, Hc_2, Hc_3, Hc_4, or Hc_5); μ: Overall mean trait ; a_i: additive genetic effect of the ith animal; and e_i: residual error.

The variance and covariance matrix for the univariate analysis was:

$$\begin{bmatrix} \boldsymbol{A}\sigma_a^2 & 0 \\ 0 & \boldsymbol{I}\sigma_e^2 \end{bmatrix}$$

Where, σ_a^2: is the additive genetic variance for the analyzed trait; σ_e^2: is the residual variance; $\boldsymbol{A}$ is the additive relationship matrix; and $\boldsymbol{I}$ is an identity matrix.

The heritability was assumed to be almost complete ($h^2 = 0.99$), thus assuming a negligible residual. In order to estimate breeding values (EBV) for all animals (including the ungenotyped with no genotyped relatives) in the pedigree, unknown parents were assigned to genetic group based on sex and breed.

Accuracy of prediction for the ungenotyped individuals was evaluated using Pearson's correlation between the EBV for SR or haplotype allele content and the true genetic value for all ungenotyped animals.

3.3.4. Selection response

The responses to selection were predicted for ungenotyped animals by assuming that animals with breeding values ≥ mean would be selected, which makes the selection intensity equal to 0.798 (Falconer and Mackay, 1996), as follows:

$$R_T = 0.798 \times r_T \times \sigma_{aT}$$

Where, R_T is the predicted selection response for trait T (i.e. SR_h, SR_l or haplotype allele contents (Hc_1, Hc_2, Hc_3, Hc_4, and Hc_5)); $r_{t,T}$ is thecorrelation between the predicted breeding value for the ungenotyped animals for trait T and the true genetic values for trait T; and σ_{aT} is the additive genetic standard deviation for trait T, which is essentially equal to standard deviation of trait T, assuming a trait h^2= 0.99 (see Table 3.4).

The difference in genetic merit between the original population and selected animals that had EBV ≥ mean EBV was calculated for the ungenotyped animals as:

$$D_T = \mu_{Ts} - \mu_{Tn}$$

Where, D_T is the difference in true genetic merit for trait T (i.e. SR_h, SR_l or haplotype allele contents (Hc_1, Hc_2, Hc_3, Hc_4, and Hc_5)) between selected (μ_{Ts}) and unselected (μ_{Tn}) animals.

In addition, selection based on EBV was performed at different selection truncation points (-1.282, -0.842, -0.525, -0.253, 0.000, +0.253, +0.525, +0.842, and +1.282) to assess: 1) the proportion of animals selected at different truncation point; 2) the proportion of recovered homozygous from ungenotyped animals among the selected animals (i.e. number of homozygous

among the selected animals / number of homozygous among unselected animals) at different truncation point; 3) allele frequencies at different selection truncation points; and 4) homozygous genotype frequencies at different selection truncation points.

3.4. Results.

3.4.1. Accuracies for prediction of scrapie resistance and haplotype gene content using the animal model

The Pearson's correlation coefficients between EBV and the trait true genetic value are shown in Table 3.6 for ungenotyped animals. The correlation between the EBV and the trait true genetic values (on diagonal) represents the accuracies of direct selection. Accuracies of prediction ranged from 0.41 to 0.71. Accuracy of predicting SR_h for ungenotyped individuals was high 0.60. In addition, the predicted SR_h was positively correlated (r = 0.58) with the most favorable haplotype in this analysis ARR (H_1), and negatively correlated with the other haplotypes (H_2, H_3, H_4, and H_5 corresponding to AHQ, ARH, ARQ, and VRQ, respectively). The correlation between predicted allele content for ARR (Hc_1) with its true content and SR_h was 0.71 and 0.47, respectively. This means that selection based on SR_h is expected to increase SR and ARR allele content, while decreasing the allele content for other haplotypes (i.e., AHQ, ARH, ARQ, and VRQ). On the other hand, prediction of SR_l was less accurate when predicting the true SR_l (r = 0.54) compared to SR_h, and was negatively associated with the least favorable allele in this scenario, i.e. H_1 (VRQ), while positively correlated with the other haplotypes (i.e., ARQ, ARH, AHQ, and ARR). This means that selection for SR_l will increase SR by replacing ARR by the other haplotypes in the population.

3.4.2. Selection response

3.4.2.1. Predicted selection response

Table 3.7 presents the predicted selection response per generation for scrapie resistance based on selecting animals with EBV ≥ mean EBV for SR_h, SR_l and the haplotype allele contents (Hc_1, Hc_2, Hc_3, Hc_4, and Hc_5) for ungenotyped animals (i.e., a selection intensity = 0.798). Selection of SR_h EBV was predicted to increase scrapie resistance by 0.46 and ARR (Hc_1) allele content by 0.36, and decrease the other haplotype allele contents (i.e., for AHQ, ARH, ARQ, VRQ). Selection for Hc_1 EBV was predicted to increase the SR_h by 0.36 and ARR (Hc_1) allele content by 0.44, and decrease the other haplotypes allele contents. On the other hand, in the low resistance population (low-SR), direct selection for SR_l EBV was predicted to increase SR by 0.33 and increase the allele content of haplotypes ARQ, ARH, AHQ, and ARR, while decreasing the allele content of the most unfavorable haplotype VRQ. Compared to VRQ, selection for the other haplotypes was predicted to be slower, reflecting their lower accuracies of prediction (Table 3.6) and lower standard deviations (Table 3.5), which are dependent on the allele frequencies.

3.4.2.2. Difference in genetic merit between selected and unselected animals

Table 3.8 presents the difference in true genetic merit when animals were selected if their EBV was ≥ mean (selection intensity = 0.798). The true response was slightly different from the

predicted response (Table 3.7). This shows that genetic improvement for SR is possible for ungenotyped animals using predictions from a linear animal model and the achieved improvement is similar to the predicted selection response. The difference in genetic merit when all animals were genotyped and selected based on true values (SR or allele contents) ≥ mean are presented on Table 3.9. In high-SR_h population, selection increased SR_h by 0.64 and increased the allele content of ARR (Hc_1) and AHQ (Hc_2) by 0.29 and 0.16, respectively. On the other hand, in a low-SR population, selection increased SR_l by 0.71 accompanied of a decrease in allele content of VRQ (Hc_1) by 0.74, while increasing haplotype allele contents for ARQ (Hc_2), ARH (Hc_3), AHQ (Hc_4), and ARR (Hc_5) by 0.33, 0.13, 0.15, and 0.13, respectively. As expected, when all animals were genotyped, there is a higher gain than when only a fraction of the animals was genotyped for scrapie (Table 3.8). Table 3.10 presents the relative gain from having a fraction of genotyped animals compared to having all animals genotyped. The relative gain ranged between 13.2% and 95.0%, which is higher for higher allele frequency haplotypes than for low allele frequency haplotypes and higher for high-SR population compared low-SR population. Likewise, in the case of haplotype allele content, the relative gain was higher for high allele frequencies than for low allele frequencies. In practice, not all animals are being genotyped. Therefore, using an animal model can be practical and beneficial by adding extra information in an eradication program for scrapie in sheep. In addition, even considering the lowest relative gain of 13.2% (Table 3.10), it still would be beneficial, as the number of animals genotyped were a fraction of the whole population (i.e. 20,000 out of 1,671,890 animals).

3.4.2.3. Effect of selection at different selection truncation points

As expected, as the selection truncation point increased, the proportion of selected (Figure 3.1) and the recovered homozygous from the ungenotyped population (Figure 3.2) for the targeted haplotype allele decreased. On the contrary, as the selection truncation point increased, the allele frequency (Figure 3.3) and the homozygous frequency (Figure 3.4) for the targeted haplotype allele increased. Selecting animals based on their EBV being ≥ mean EBV resulted in selecting between 33.6% and 60.0% individuals (Figure 3.1) and recovering between 75.8 to 99.2% of homozygous genotypes (Figure 3.2). Assuming H_1 as the favorable haplotype allele (i.e. ARR), selection based on Hc_1 EBV ≥ mean EBV resulted in selecting 50.7 % from the population and recovering a total of 87.7% of the target homozygous genotype (ARR/ARR). This means that by pre-selecting 50.7% of individuals based on EBV for allele content for genotyping, 87.7% of homozygous genotypes could be captured from the original population. Thus, this would reduce the number of animals required for genotyping. In the case of low ARR allele frequency (i.e. H_5), when pre-selecting based on EBV for allele content ≥ mean EBV, the number of individuals required for genotype validation was reduced to 37.8% (Figure 3.1), while recovering 98.3 % (Figure 3.2) of homozygous genotype (ARR/ARR) from the original population. Thus, the genotyping cost could be reduced even more.

3.5. Discussion.

The accuracy for prediction of haplotype allele contents ranged between 0.41 and 0.71 (Table 3.6). Gengler el al. (2007), who first proposed the allele content model, used it for the myostatin gene in Belgium blue cattle, with prediction accuracies between 0.47 and 0.50. In

another study in Canadian Holstein cattle, the prediction accuracy for allele content was as high as 0.93 (Gengler et al., 2008). Legarra and Vitezica (2015) reported accuracies between 0.52 and 0.56 using the allele content model. The allele content model can be applicable for prediction in bi-allelic major genes, such as for maedi -visna (Leymaster et al., 2013) and for litter size (Davis, 2005). However, it must be acknowledged that some alleles in major gene may not act additively. Applying allele content model to scrapie as first proposed by Gengler el al. (2007) is possible when only considering the number ARR haplotypes and disregarding the importance of the other haplotypes. However, the PrP gene underlying scrapie phenotypes is multi-allelic with different contributions from the different haplotypes to the level of scrapie resistance in sheep (Molina et al., 2006; Álvarez et al., 2007; Stepanek and Horin, 2017). In this study, the different contributions of haplotypes in the SR genotypes were considered by defining numeric values for SR and adjusting them to non-additive genetic effects prior to their use in the linear animal model. The accuracy for prediction of SR in high and low SR populations were 0.60 and 0.54, respectively. There were no previous studies that considered the same approach to construct the SR values to compare to. In the current study, a random sample of 20,000 animals with genotyped records across both sexes was used to predict the genetic effect of the ungenotyped animals. However, in practice, rams are more likely to be genotyped than ewes due their higher impact in the herds. In this case, the accuracy of prediction of SR or allele content could be expected to be even higher, as rams have more close relationships with the ungenotyped population than ewes.

Selection response on SR and allele content was predicted for ungenotyped sheep (Table 3.7). The selection response depends on selection intensity, accuracy, and the additive genetic standard deviation (Falconer and Mackay, 1996). The differences between the trait prediction

accuracies (Table 3.6) and the additive genetic standard deviations (Table 3.4) explain the differences in predicted responses. Selecting ungenotyped animals with EBV ≥ mean resulted in increase in genetic merit for SR and allele content (Table 3.8), and increased favorable haplotype allele frequency (Figure 3.3). Breeding programs for genetic improvement for SR involve selection based on scrapie genotypes. Such breeding programs were successful in increasing ARR frequency and SR in Czech Republic (Stepanek and Horin, 2017), Netherland (Hagenaars et al., 2010; Melchior et al., 2010), Belguim (Dobly et al., 2013), and Hungary (Gáspárdy et al., 2018). However, in all previous studies, the genetic change was limited to the animals being genotyped. In this research, the genetic improvement for SR was possible for the ungenotyped animals when using predictions from a linear animal model.

Selecting ungenotyped animals with EBV ≥ mean resulted in reduction of number of animals compare to unselected population (Figure 3.1), while capturing large proportion of homozygous genotypes from the unselected population (Figure 3.2). The increase of selection truncation point resulted in increased frequency for homozygous genotypes (Figure 3.4), while decreasing the proportion of animals selected from the population (Figure 3.1). In the breeding program in Netherlands, genotyping for selection of ARR/ARR rams was compulsory between the year 2004 and 2007 (Hagenaars et al., 2010). Genotyping to identify homozygous (ARR/ARR) rams is important for breeding purposes, as they are 100% guaranteed to transmit the ARR allele to their progeny. The use of linear animal model provides another tool to reduce the number of genotyped animal by pre-selecting based on EBV prior to genotyping. The pre-selected animals with EBV ≥ mean include a large proportion of ARR/ARR genotypes from the unselected population (Figure 3.2). Without pre-selection, 100% of the animals in the population must be

genotyped in order to identify all the ARR/ARR animals in the population. When animals with EBV ≥ mean were pre-selected, smaller proportion (34% - 60%) would need to be genotyped in order to identify a large proportion (76% - 99%) of ARR/ARR animals (Figures 3.1 and 3.2). Thus, genotyping cost for identifying most of the ARR/ARR animals in the population could be reduced. As pre-selection truncation point increases, the proportion of animal selected decreases (Figure 3.1), but the frequency of ARR/ARR animals among the selected animals increases (Figure 3.4). Thus, pre-selecting at higher truncation point would identify large proportion of ARR/ARR among the selected animals, thus saving genotyping cost to confirm the homozygous ARR/ARR status of the animals.

Breeding programs can contribute to the reduction of prevalence of the typical form of scrapie in sheep. Arnold and Rajamyagam (2020) estimated an annual reduction of 28% in scrapie prevalence cases between 2005 and 2019 in Great Britain. Hagenaars et al. (2010) reported a trend for reduction of scrapie prevalence in active scrapie surveillance in Netherland between the years 2002 and 2008. They reported 0% typical scrapie cases in genotypes with risk levels R1 and R2. The current study showed that selecting ungenotyped sheep based on EBV could increase SR (Tables 3.7 and 3.8) and ARR allele frequency and its homozygous genotype frequency (Figures 3.3 and 3.4), what could reduce typical scrapie prevalence in sheep. However, the atypical form of scrapie (Nor98) could occur in sheep that are resistant to typical scrapie (Cassmann et al., 2021). However, Nor98 scrapie type is believed to be a spontaneous disease and it is unlike to be naturally contagious among sheep and, thus, its prevalence is low (Cassmann and Greenlee, 2020; Acín et al., 2021). Therefore, a well-established breeding program for the typical scrapie can contribute to the reduction of scrapie prevalence.

This research proposed the use of a linear animal model as a practical method for genetic evaluation and selection for SR of ungenotyped sheep. Different scrapie eradication strategies used in breeding programs were described in previous studies. For instance, in Great Britain, Arnold et al. (2002) proposed genotyping purebred rams in the nucleus flocks used for cross-breeding and selecting the homozygous ARR/ARR and the carriers (ARR/ARQ, ARR/ARH, and ARR/AHQ) rams at the pure breeding level. Molina et al. (2006) compared different strategies in Spanish Merinos. They concluded that the optimum strategy was to genotype rams and eliminate ARQ/ARQ and VRQ carriers. According to Gáspárdy et al. (2018), in the Hungarian national breeding program, rams are genotyped and only rams at risk groups (R1, R2, and R3) are allowed to breed. In all previous studies, the genetic selection for SR was limited to the animals genotyped. This study has shown that a linear animal model can be used to provide additional information for ungenotyped animals, which will be particularly useful wherever genotyping for scrapie is not intensively practiced. Therefore, the use of an animal model could make better use of the available information to enhance breeding programs for the genetic improvement for SR in sheep.

3.6. Conclusions

Moderate to highly accurate estimated breeding values for scrapie resistance and haplotype allele content for ungenotyped animals can be obtained from a linear animal model using genotype data from only a fraction of the total sheep population. Thus, selecting ungenotyped animals based on EBV could result in effective genetic gains for scrapie resistance and allele content. Individuals with EBV ≥ mean were shown to carry a large proportion of homozygous genotypes. Thus, pre-

selection prior to genotyping could reduce the number of animals needed to be genotyped to identify individuals with the favorable homozygous genotypes in the population and, consequently, reduce the genotyping cost. Therefore, a linear animal model could make better use of the available information for genetic improvement of scrapie resistance in sheep.

3.7. Acknowledgments

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3.8. Tables

Table 3.1. Risk groups, unadjusted and adjusted scrapie resistance genotypes, and additive genetic values for scrapie resistance for each haplotype.

Genotypes	Risk group[1]	Unadjusted SR[2]	Adjusted SR[3]
ARR/ARR	R1	4	4
ARR/AHQ	R2	3	3.5
AHQ/AHQ	R2	3	3
ARQ/AHQ	R3	2	2
AHQ/ARH	R3	2	2
ARR/ARH	R3	2	2.5
ARR/ARQ	R3	2	2.5
AHQ/VRQ	R4	1	1.5
ARR/VRQ	R4	1	2
ARQ/ARQ	R4	1	1
ARQ/ARH	R4	1	1
ARH/ARH	R4	1	1
ARH/VRQ	R5	0	0.5
ARQ/VRQ	R5	0	0.5
VRQ/VRQ	R5	0	0

Haplotype	Additive genetic value[4]
ARR	2
AHQ	1.5
ARH	0.5
ARQ	0.5
VRQ	0

[1]Risk group: genotype for prion protein (PrP) gene at codons 136,154, and 171, and their risk group to scrapie from low (R1) to high (R5) risk; [2]Unadjusted SR: unadjusted numeric values for scrapie resistance genotypes from low (0) to high (4) scrapie resistance; [3]Udjusted SR: adjusted scrapie resistance genotypic value for a non-additive genetic effect, by adding the additive genetic effects of the haplotypes; [4]additive genetic value for haplotypes.

Table 3.2. **Genotype count and frequencies in the simulation**

Genotype[1]	Count	Frequency
H_1/H_1	482,112	0.2884
H_1/H_2	334,271	0.1999
H_1/H_3	144,469	0.0864
H_1/H_4	79,958	0.0478
H_1/H_5	75,690	0.0453
H_2/H_2	221,928	0.1327
H_2/H_3	126,363	0.0758
H_2/H_4	41,457	0.0248
H_2/H_5	48,086	0.0288
H_3/H_3	48,011	0.0287
H_3/H_4	29,460	0.0176
H_3/H_5	11,376	0.0068
H_4/H_4	10,224	0.0061
H_4/H_5	11,188	0.0067
H_5/H_5	7,297	0.0044

[1]Genotypes at single locus resulting from simulation of multi-allelic locus with 5 different haplotypes (H_1 - H_5)

Table 3.3. Genotypes assigned to haplotypes for the scrapie resistance analyses[1]

Haplotype[2]	SR_h	SR_l
H_1	ARR	VRQ
H_2	AHQ	ARQ
H_3	ARH	ARH
H_4	ARQ	AHQ
H_5	VRQ	ARR

[1]Two populations of different levels of scrapie resistance. Where, SR_h has high frequency for ARR haplotype, while SR_l has very low frequency for ARR haplotype.[2]Haplotypes simulated in the study, for which frequencies from Table 2 and adjusted SR values from Table 1 were assigned.

Table 3.4. Descriptive statistics for all the individuals in the population

Trait[1]	Range	Mean ± SD	CV%
SR_h	0 - 4	2.98 ± 0.96	32.10
SR_l	0 - 4	0.78 ± 0.77	99.45
Hc_1	0 - 2	0.96 ± 0.79	82.26
Hc_2	0 - 2	0.59 ± 0.71	119.71
Hc_3	0 - 2	0.24 ± 0.49	201.66
Hc_4	0 - 2	0.11 ± 0.33	303.10
Hc_5	0 - 2	0.10 ± 0.31	321.41

[1]SR_h and SR_l are the scrapie resistance traits (SR) in a high SR population and a low SR population (see Table 3); and Hc_1 – Hc_5 are the haplotype allele contents, i.e. the number of a given scrapie haplotypes observed in the genotype (0, 1, or 2).

Table 3.5. Descriptive statistics for the 20,000 individuals sampled with genotypic records used for analysis

Trait[1]	Range	Mean ± SD	CV%
SR_h	0 – 4	2.98 ± 0.95	32.01
SR_l	0 – 4	0.78 ± 0.77	99.04
Hc_1	0 – 2	0.95 ± 0.79	82.54
Hc_2	0 – 2	0.60 ± 0.71	119.04
Hc_3	0 – 2	0.24 ± 0.49	203.65
Hc_4	0 – 2	0.11 ± 0.33	299.49
H_5	0 – 2	0.10 ± 0.31	318.71

[1]SR_h and SR_l are the scrapie resistance traits (SR) in a high SR population and a low SR population (see Table 3); and Hc_1 – Hc_5 are the haplotype allele contents, i.e. the number of a given scrapie haplotypes observed in the genotype (0, 1, or 2).

Table 3.6. Correlation coefficients[1] (accuracies) between predicted breeding values for SR or for haplotype allele content and their corresponding true values in ungenotyped individuals.

	True						
Predicted	**SR_h**	**SR_l**	**Hc_1**	**Hc_2**	**Hc_3**	**Hc_4**	**Hc_5**
SR_h	**0.602**	-	0.576	-0.167	-0.454	-0.240	-0.102
SR_l	-	**0.537**	-0.552	0.290	0.136	0.264	0.237
Hc_1	0.478	-0.416	**0.707**	-0.537	-0.278	-0.079	-0.036
Hc_2	-0.139	0.217	-0.536	**0.702**	-0.070	-0.128	-0.003
Hc_3	-0.430	0.121	-0.317	-0.081	**0.646**	0.087	-0.129
Hc_4	-0.300	0.307	-0.123	-0.187	0.103	**0.484**	0.060
Hc_5	-0.140	0.300	-0.052	-0.012	-0.198	0.059	**0.412**

[1]The correlation between the prediction on the same trait (SR_h, SR_l, Hc_1, Hc_2, Hc_3, Hc_4, or Hc_5; on diagonal) and between the prediction on the different traits (off diagonal). SR_h and SR_l are the scrapie resistance traits (SR) in a high SR population and a low SR population (see Table 3), and Hc_1 – Hc_5 are the haplotype allele contents, i.e. the number of a given scrapie haplotypes observed in the genotype (0, 1, or 2).

Table 3.7. Predicted selection response[1] from the same trait (on diagonal) and from different traits[2] (off diagonal) in ungenotyped individuals

	Response on						
Selection on	**SR_h**	**SR_l**	**Hc_1**	**Hc_2**	**Hc_3**	**Hc_4**	**Hc_5**
SR_h	**0.459**	-	0.362	-0.095	-0.177	-0.063	-0.025
SR_l	-	**0.331**	-0.347	0.165	0.053	0.070	0.059
Hc_1	0.364	-0.256	**0.444**	-0.305	-0.108	-0.021	-0.009
Hc_2	-0.106	0.133	-0.337	**0.399**	-0.027	-0.034	-0.001
Hc_3	-0.327	0.075	-0.199	-0.046	**0.252**	0.023	-0.032
Hc_4	-0.229	0.189	-0.077	-0.106	0.040	**0.128**	0.015
Hc_5	-0.106	0.185	-0.032	-0.007	-0.077	0.016	**0.102**

[1]Response to selection based on predicted breeding values and assuming intensity = 0.798. [2]Traits are SR_h, SR_l, Hc_1, Hc_2, Hc_3, Hc_4, and Hc_5, where SRh and SR_l are the scrapie resistance traits (SR) in a high SR population and a low SR population (see Table 3), and Hc_1 – Hc_5 are the haplotype allele contents, i.e. the number of a given scrapie haplotypes observed in the genotype (0, 1, or 2).

Table 3.8. True selection response[1] from the same trait (on diagonal) and from different traits[2] (off diagonal) in ungenotyped individuals

	Response on						
Selection on	**SR_h**	**SR_l**	**Hc_1**	**Hc_2**	**Hc_3**	**Hc_4**	**Hc_5**
SR_h	**0.515**	-	0.432	-0.159	-0.145	-0.075	-0.052
SR_l	-	**0.284**	-0.332	0.185	0.048	0.052	0.045
Hc_1	0.397	-0.271	**0.452**	-0.284	-0.126	-0.036	-0.001
Hc_2	-0.180	0.193	-0.450	**0.496**	-0.013	-0.037	0.003
Hc_3	-0.509	0.143	-0.356	-0.001	**0.366**	0.044	-0.052
Hc_4	-0.261	0.246	-0.077	-0.119	0.014	**0.129**	0.053
Hc_5	-0.094	0.232	-0.024	0.005	-0.139	0.031	**0.126**

[1]Response to selection calculated based on estimated breeding values (EBV). [2]Traits are SR_h, SR_l, Hc_1, Hc_2, Hc_3, Hc_4, and Hc_5, where SRh and SR_l are the scrapie resistance traits (SR) in a high SR population and a low SR population (see Table 3), and Hc_1 – Hc_5 are the haplotype allele contents, i.e. the number of a given scrapie haplotypes observed in the genotype (0, 1, or 2).

Table 3.9. True selection response[1] from the same trait (on diagonal) and from different traits[2] (off diagonal) when all animals in the populations are genotyped (n=1,671,890).

	Response on						
Selection on	**SR_h**	**SR_l**	**Hc_1**	**Hc_2**	**Hc_3**	**Hc_4**	**Hc_5**
SR_h	**0.645**	-	0.294	0.155	-0.244	-0.109	-0.096
SR_l	-	**0.711**	-0.737	0.333	0.126	0.148	0.130
Hc_1	0.433	-0.318	**0.476**	-0.295	-0.114	-0.038	-0.028
Hc_2	-0.075	0.155	-0.523	**0.693**	-0.08	-0.055	-0.034
Hc_3	-1.043	0.153	-0.555	-0.243	**0.890**	-0.027	-0.065
Hc_4	-1.076	1.149	-0.492	-0.354	-0.073	**0.950**	-0.031
Hc_5	-1.453	1.622	-0.464	-0.282	-0.170	-0.036	**0.951**

[1]Response to selection calculated based on estimated breeding values (EBV). [2]Traits are SR_h, SR_l, Hc_1, Hc_2, Hc_3, Hc_4, and Hc_5, where SR_h and SR_l are the scrapie resistance traits (SR) in a high SR population and a low SR population (see Table 3), and Hc_1 – Hc_5 are the haplotype allele contents, i.e. the number of a given scrapie haplotypes observed in the genotype (0, 1, or 2).

Table 3.10. Relative gain in the true selection response when a fraction of the population was genotyped for scrapie (n=20,000) relative to the response when the whole population (n=1,671,890) was genotyped.

Trait[1]	Relative gain %
SR_h	80.00
SR_l	39.94
Hc_1	94.96
Hc_2	71.57
Hc_3	41.12
Hc_4	13.58
Hc_5	13.25

[1]Traits are SR_h, SR_l, Hc_1, Hc_2, Hc_3, Hc_4, and Hc_5, where SR_h and SR_l are the scrapie resistance traits (SR) in a high SR population and a low SR population (see Table 3), and Hc_1 – Hc_5 are the haplotype allele contents, i.e. the number of a given scrapie haplotypes observed in the genotype (0, 1, or 2).

3.9. Figures

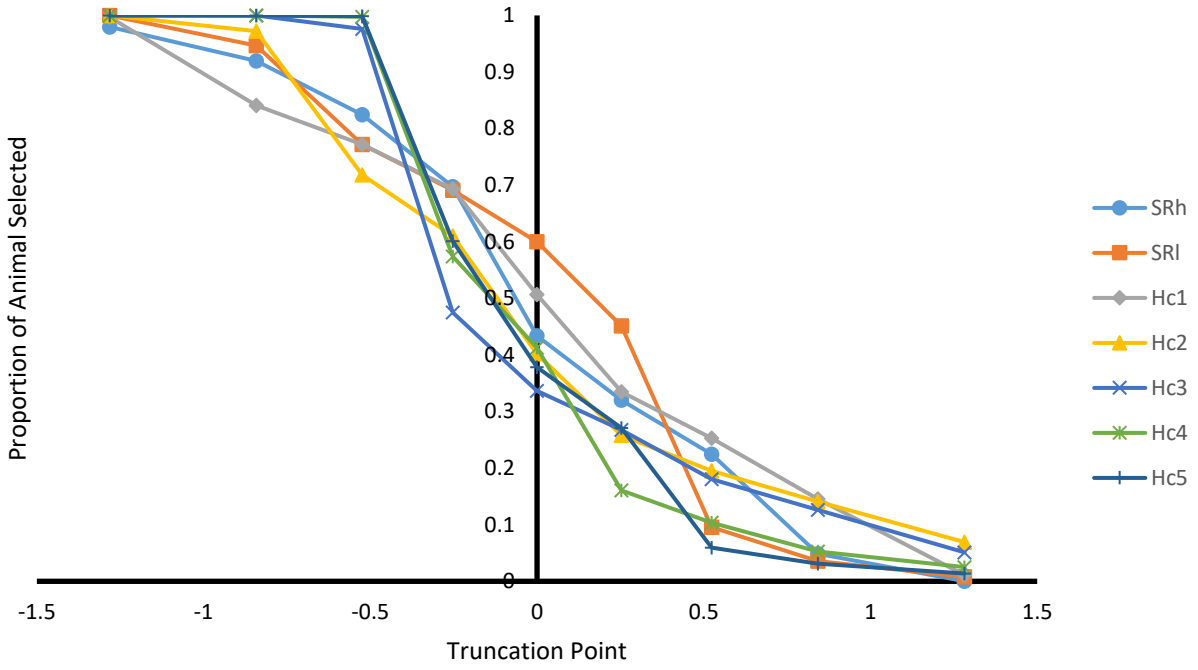

Figure 3.1. Proportion of animals selected from population at different selection truncation points in unit of standard deviations.

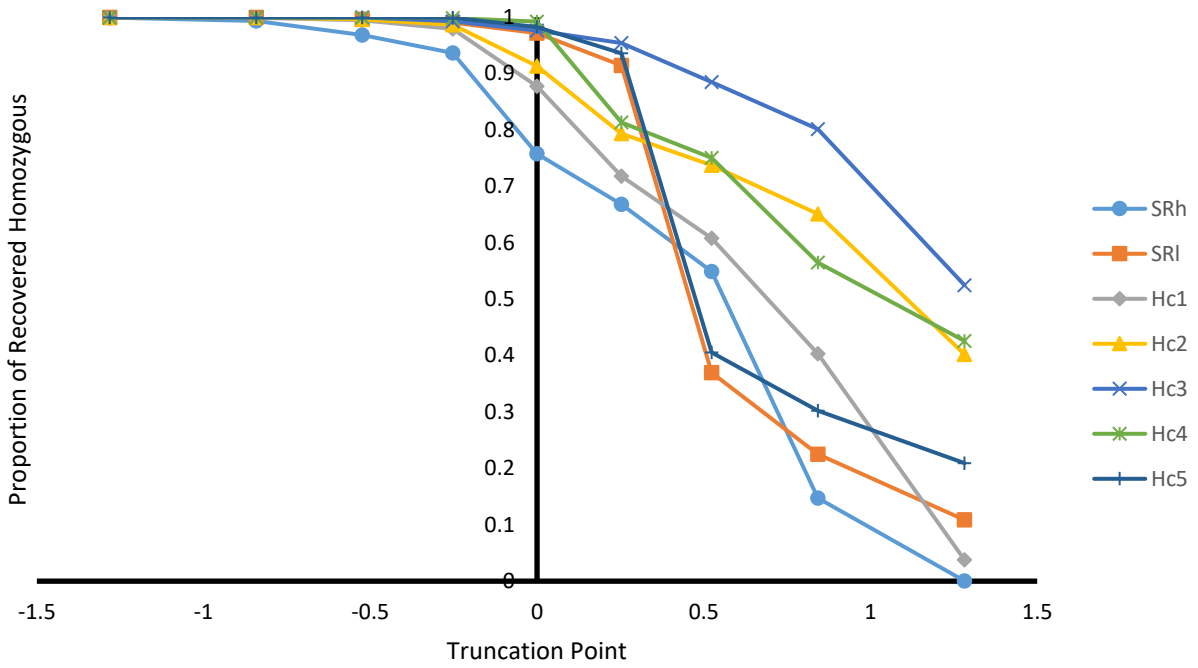

Figure 3.2. Proportion of recovered homozygous genotypes from the unselected population at different selection truncation points in unit of standard deviations.

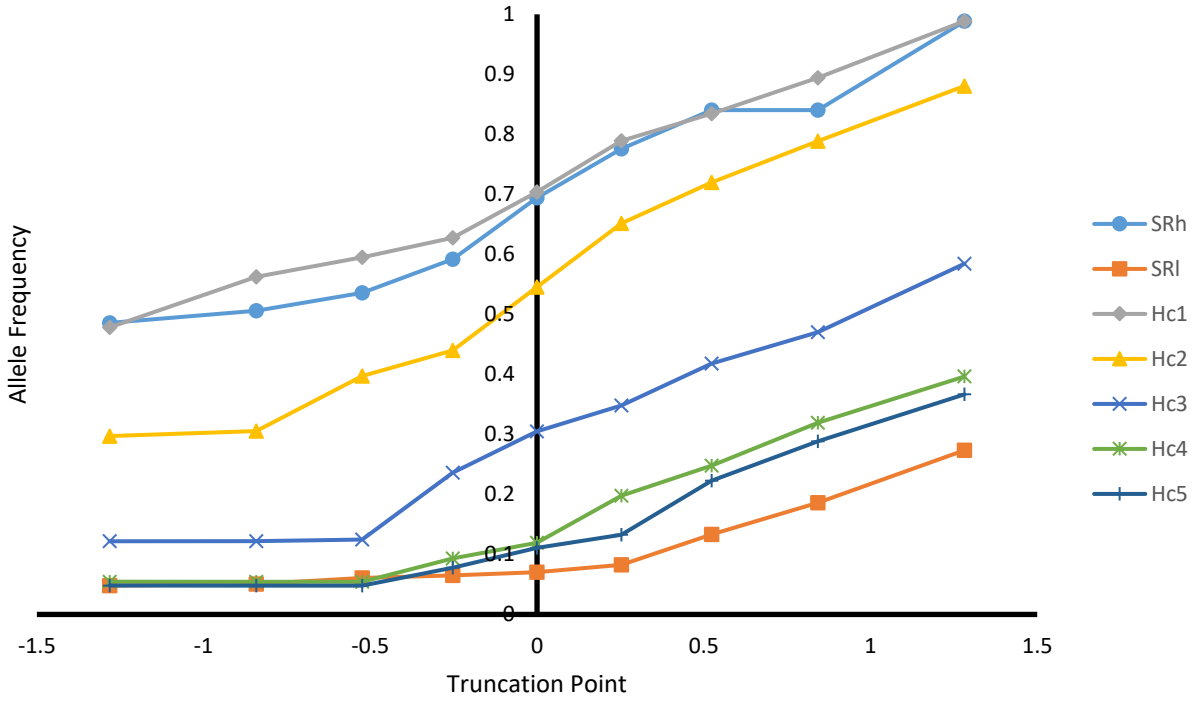

Figure 3.3. Allele frequency at different selection truncation points in unit of standard deviations.

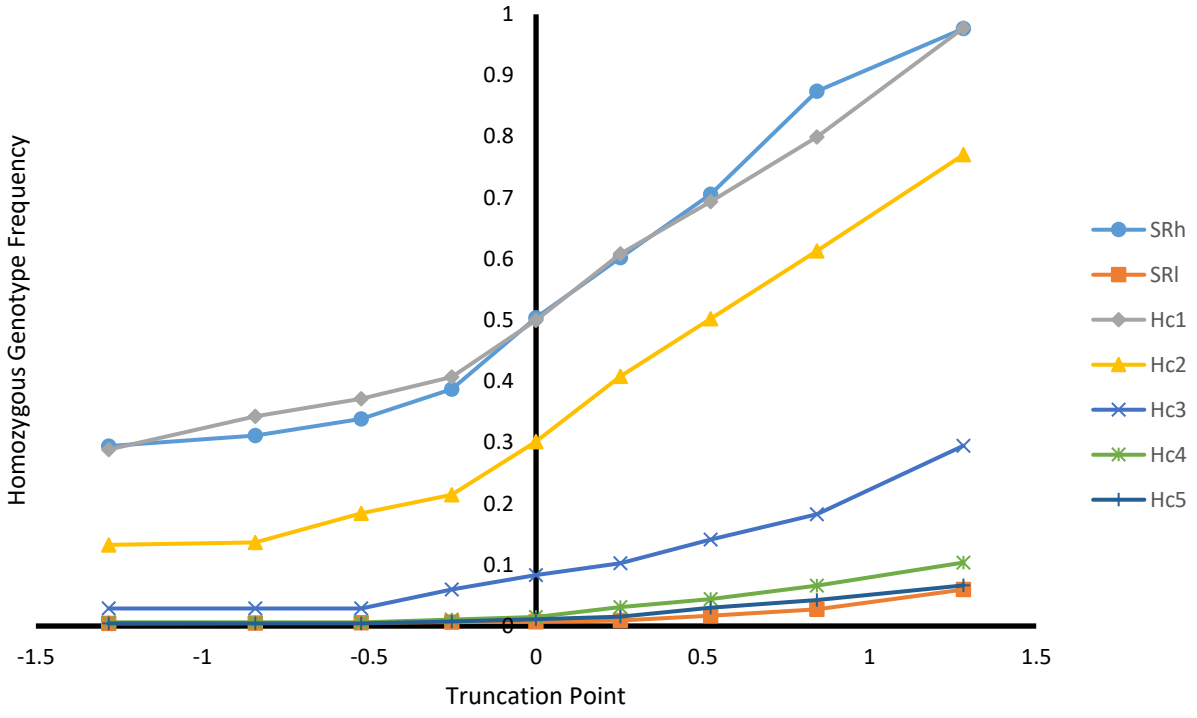

Figure 3.4. Homozygous genotype frequency at different selection truncation points in unit of standard deviations.

CHAPTER 4

Genetic Parameters for Growth Traits in Sheep as a Function of Heat Load

4.1. Abstract

The objective of this study was to estimate genetic parameters for 50-day weaning weight (W50) and 50-day post-weaning gain (G50) in Rideau-Arcott sheep as a function of heat stress. A total of 110,512 and 90,204 phenotypic records for W50 and G50, respectively, were matched to metrological data retrieved from the nearest weather station. The heat load was calculated as average daily temperature-humidity index (THI) that the animals were exposed to until the phenotypic record was measured for each trait. Heat stress thresholds of THI = 54.60 and THI = 55.62 were determined for W50 and G50, respectively. The differences between the THI value above the thresholds and the set thresholds values were used as the environmental gradient for heat stress in a reaction norm model to estimate the genetic parameters. Direct genetic correlation between intercepts ($W50_0$ and $G50_0$) and their corresponding reaction norm slopes ($W50_1$ and $G50_1$) for heat load were -0.32 and -0.68, for W50 and G50, respectively. In addition, a small negative genetic correlation (-0.15) was found between the $W50_0$ and the $G50_1$. The negative genetic correlations indicate the presence of a genetic antagonism between heat tolerance and growth traits. A moderately positive genetic correlation (0.41) between the intercepts of the traits was found. The remaining genetic correlations were small and neglectable. There was no evidence for slope in the maternal component for W50. However, high maternal genetic antagonism

between G50 and heat tolerance was estimated (-0.75). Heritability estimates across 10 units of heat load for W50 ranged from 0.09 to 0.11 and 0.05, for direct and maternal, respectively, and for G50 ranged from 0.12 to 0.19 and from 0.01 to 0.02, for direct and maternal, respectively. Thus, the response to selection changes according to the environment. It can be concluded that in genetic selection for growth traits, heat stress must be accounted for in order to counteract the genetic antagonism with heat tolerance.

Keywords: direct and maternal genetic effects, growth traits, heat stress, and sheep

4.2. Introduction

The exposure to heat stress in sheep leads to changes in biological parameters, such as rectal temperature, respiration rate, and physiological functions (Ames et al., 1971; Lowe et al., 2001; Sevi et al., 2001; Srikandakumar et al., 2003; Marai et al., 2007). This can lead to reduced feed intake and utilization, and eventually muscle catabolism (Marai et al., 2007). As a consequence of heat stress, productive performance in growth, milk production, and reproduction may be negatively affected (Marai et al., 2007). The reduction of productive performance can result in negative economic impacts to the livestock industry (Mader, 2003; St-Pierre et al., 2003).

Genetic improvement for heat tolerance is a biological solution that is permanent and cumulative. Breeding for thermotolerance may be achieved by considering the genotype by environment interaction (G×E) that may exist during the selection process. The presence of G×E

means that the same genotype can perform differently under different environmental conditions (Falconer and Mackay, 1996). There are experimental evidences that indicate differences in thermal adaptation between breeds, as a result of exposure to heat stress in sheep (Srikandakumar et al., 2003), beef cattle (Gaughan et al., 2010), and poultry (Eberhart and Washburn, 1993b; Eberhart and Washburn, 1993a; Yalçin et al., 1997; Cahaner et al., 2008). Additionally, evidence for G×E in livestock has been observed for dairy sheep (Finocchiaro et al., 2005), cattle (Brügemann et al., 2011; Boonkum and Duangjinda, 2015; Santana et al., 2015; Bohlouli et al., 2019), and pigs (Zumbach et al., 2008a; Zumbach et al., 2008b), which indicates genetic antagonism between production traits and heat tolerance. Thus, not accounting for heat stress could result in selection for more heat sensitive individuals. The G×E can be accounted for within a breed by treating phenotypes recorded in different environments as different traits in an animal model, or by using a random regression reaction norm model (Hayes et al., 2016). The random regression reaction norm model requires phenotypes to be collected across a continuous environmental gradient. The temperature-humidity index (THI) is a useful environmental indicator for heat stress, which combines measurements of the temperature and the relative humidity. Ravagnolo et al (2000) developed a heat stress function based on THI derived from metrological data from public weather stations to calculate the environmental gradient, which was used to evaluate phenotypic data of dairy cattle.

Until recently, there was no genetic study done for sheep growth traits, such as the weaning weight and the post-weaning gain that included information of heat stress. Therefore, the objective of this study was to estimate genetic parameters weaning weight and post-weaning gain as a function of heat load in sheep.

4.3. Materials and Method

4.3.1. Data

Phenotypes for growth traits for Rideau-Arcott sheep between the years 1989 and 2016 were obtained from the GenOvis database (www.genovis.ca; Guelph, ON, Canada). Weights, taken between the age of 28 and 80 days, were corrected to 50-day (W50) then pre-adjusted for sex, litter size, and age of the dam. The 100-day weight records, taken between 70 and 135 days, were used to calculate the 50-days gain after weaning (G50), as the difference between the 100-day and 50-day corrected to the number of days postweaning, and then were adjusted for sex, litter size, and the dam age.

Weather parameters were downloaded using *weathercan* package in R (LaZerte, 2020). Measurements for temperature (T) and relative humidity (RH) were obtained from the nearest weather station to the flocks, in which the distance between the flock and the weather station ranged between 0.26 and 85.13 km and averaged 22.96 km, with 98% of the flocks closer than 50 km. In a genetic study for heat stress conducted by Bradford et al. (2016), they used distances as far as 144 km. Also, the study described by Aguilar et al. (2009) used distances between the flock and the weather station ranging from 3 to 137 km and with an average of 61 km. Therefore, the range of distances between the flock and the weather station used in the present study is within the range of the previous studies. The daily temperature humidity index (THI) was calculated using

maximum temperature and minimum relative humidity (Ouellet et al., 2019), using the following formula:

$$THI = (1.8 \times T_{max} + 32) - [(0.55 - 0.005 \times RH_{min}) \times (1.8 \times T_{max} - 26)$$

Where, THI: the temperature humidity index; T_{max}: the maximum daily temperature; and RH_{min}: the minimum daily relative humidity.

The daily THI records were calculated for all dates for which the animal exposed to such THI, between birth and weaning record dates, and between weaning and post-weaning record dates, for W50 and G50, respectively. Then, the average THI for each animal was used as the environmental gradient for the corresponding phenotypic record of each animal. Basic descriptive statistics for the phenotypes and the THI are presented in Table 4.1.

4.3.2. Estimating heat stress threshold

Linear models were used to fit the adjusted phenotypes, using ASREML (Gilmour et al., 2015). The following univariate equation was used for W50:

$$y_{ijkl} = \mu + C_i + L_j + m_k + mpe_k + a_l + e_{tijkl}$$

Where, y_{tijkl}: phenotypic record for W50; μ: fixed effect for the overall mean; C_i: random effect of the contemporary group i; L_j: random effect for litter j; m_k: random maternal genetic effect; mpe_k: random maternal permanent environmental effect; a_l: random direct additive genetic effect l; and e_{tijkl}: residual error.

Another univariate model was used for the adjusted G50. The model equation was the same as for W50, except that the maternal permanent environmental effect (mpe_k) was omitted. The residual terms for the phenotypes were fitted against THI to estimate the breakpoint (threshold) for W50 and G50, using the *segmented* R package (Vito R Muggeo, 2020). The estimates for heat stress thresholds are presented in Table 4.2, which were used in the function of heat stress in the reaction norm model to estimate the genetic parameters.

4.3.3. Estimating Genetic Parameters

Variance components and genetic parameters were estimated for W50 and G50 in a bivariate model using ASREML (Gilmour et al., 2015), using the following equations:

$$y_{1ijkl} = \mu_1 + H_1 + C_{1i} + L_{1j} + m_{1k} + mpe_{1k} + a_{1l} + a_{1l} \times H_1 + e_{1ijkl}$$

$$y_{2ijkl} = \mu_2 + H_2 + C_{2i} + L_{2j} + m_{2k} + m_{2k} \times H_2 + a_{2l} + a_{2l} \times H_2 + e_{2ijkl}$$

Where, y_{tijkl}: phenotypic record for trait t, 1 or 2, corresponding to W50 and G50, respectively ; μ_t: fixed effect for the overall mean corresponding to trait t; H_t: fixed linear regression heat load as a function for heat stress (see text) for trait t ; C_{ti}: random effect of the contemporary group i, corresponding to trait t; L_{tj}: random effect for litter j, corresponding to trait t; m_{tk}: random maternal genetic effect, corresponding to trait t; mpe_{1k}: random maternal permanent environmental effect for the trait W50; a_{tl}: random direct additive genetic effect l, corresponding to trait t; and e_{tijkl}: residual error, corresponding to trait t.

The H_t is a function that describes the effect of the environmental gradient for heat stress on trait t (W50 or G50) above its THI threshold. The covariable H_t c can be described as follow:

$$H_t = \begin{cases} 0 & if\ THI \leq THI_h \\ (THI - THI_h) & if\ THI > THI_h \end{cases}$$

Where, THI: the temperature-humidity index (THI) that animals' phenotype was exposed to during the growth stage for trait t; and THI_h: the THI threshold above which the performance of the trait is affected (see Table 4.2). The variance and covariance matrices for the bivariate analysis were:

$$G = \begin{bmatrix} \sigma^2_{a1} & \sigma_{a1h1} & \sigma_{a1a2} & \sigma_{a1h2} \\ \sigma_{h1a1} & \sigma^2_{h1} & \sigma_{h1a2} & \sigma_{h1h2} \\ \sigma_{a2a1} & \sigma_{a2h1} & \sigma^2_{a2} & \sigma_{a2h2} \\ \sigma_{h2a1} & \sigma_{h2h1} & \sigma_{h2a2} & \sigma^2_{h2} \end{bmatrix} \otimes A;$$

$$M = \begin{bmatrix} \sigma^2_{m1} & \sigma_{m1m2} & \sigma_{m1mh2} \\ \sigma_{m2m1} & \sigma^2_{m2} & \sigma_{m2mh2} \\ \sigma_{mh2m1} & \sigma_{mh2m2} & \sigma^2_{mh2} \end{bmatrix} \otimes A;$$

$$MPE = \begin{bmatrix} \sigma^2_{mpe1} & 0 \\ 0 & 0 \end{bmatrix} \otimes I\ ;$$

$$C = \begin{bmatrix} \sigma^2_{c1} & \sigma_{c12} \\ \sigma_{c21} & \sigma^2_{c2} \end{bmatrix} \otimes I\ ;$$

$$L = \begin{bmatrix} \sigma^2_{l1} & \sigma_{l12} \\ \sigma_{l21} & \sigma^2_{l2} \end{bmatrix} \otimes I\ ;\ \text{and}$$

$$R = \begin{bmatrix} \sigma^2_{e1} & \sigma_{e12} \\ \sigma_{e21} & \sigma^2_{e2} \end{bmatrix} \otimes I$$

Where, $G, M, MPE, C, L,$ and R are covariance matrices with variances (on diagonals) and covariances (off diagonals), for direct genetic, maternal genetic, maternal permanent environment, contemporary group, litter, and residual effects, respectively; σ^2_{a1} and σ^2_{h1} are the additive genetic variances for W50 and its heat tolerance component, respectively; σ^2_{a2} and σ^2_{h2} are the additive genetic variances for G50 and its heat tolerance component, respectively; σ^2_{m1} is the maternal genetic variance for W50; σ^2_{m2} and σ^2_{mh2} are the maternal genetic variance for G50 and its heat tolerance component, respectively; σ^2_{mpe1} is the maternal permanent environmental variances for W50; σ^2_{c1} and σ^2_{c2} are the contemporary group variances for W50 and G50, respectively; σ^2_{l1} and σ^2_{l2} are the litter variances for W50 and G50, respectively; and σ^2_{e1} and σ^2_{e2} are the residual variances for W50 and G50, respectively; $\otimes$: Kroneker product; A: additive relationship matrix; and I: identity matrix.

4.4. Results and Discussion

Estimates of variance components from the bivariate model are presented in Table 4.3. The possible interaction between heat stress and maternal genetic effect on W50 was not examined based on results from preliminary analyses, which indicated no significant heat stress slope ($W50_1$) for W50 maternal genetic effect (results not shown). Bradford et al (2016) did not find evidence for G×E in the maternal components of growth in Angus beef cattle. Another study conducted by Santana et al (2016) in three breeds of beef cattle and found evidence for G×E for maternal component for weaning weight. The current study showed evidence for heat stress slope ($G50_1$) in the maternal component for G50 with negative covariance for maternal intercept($G50_0$) and slope

($G50_1$). The rank correlation between breeding values of comfort zone and at heat 10 unit of heat stress in the maternal genetic components was 0.26. It is commonly accepted that when rank correlation < 0.80, indicates significant G×E. In addition, the direct genetic component in this study for both W50 and G50 negative covariance for the intercepts ($W50_0$ and $G50_0$) and the slopes ($W50_1$ and $G50_1$), with rank correlations of between breeding values of comfort zone and 10 units of heat stress of 0.72 and 0.59, for W50 and G50, respectively. This negative covariance for the intercept and the slope indicates genetic antagonism between those productive growth traits and heat tolerance. Genetic antagonism has been reported for different productive traits such as milk production in dairy cattle (Ravagnolo and Misztal, 2002; Boonkum et al., 2011; Brügemann et al., 2011; Boonkum and Duangjinda, 2015) and dairy sheep (Finocchiaro et al., 2005), and growth traits in beef cattle (Santana et al., 2016) and pigs (Zumbach et al., 2008a). Production traits in livestock require nutritional inputs and utilization of energy for anabolism, and it is likely that the more productive the animal is, the more energy inputs they require (e.g. better nutrition). The exposure to heat stress can disrupt the biological processes required for anabolism, which leads to reduced feed intake, feed utilization, and even catabolism (Marai et al., 2007) causing this reduction in productive performance.

Genetic correlations for the direct genetic components are presented in Table 4.4. Negative correlations between intercept and slope within traits were estimated for W50 (-0.32) and were smaller in magnitude than G50 (-0.68). Thus, heat stress can have more negative impact in post-weaning growth (G50) than earlier stage of growth (W50). Santana et al (2016) reported genetic correlation for weaning weight and heat tolerance in three breeds of beef cattle of -0.30, -0.43, and -0.40, in Nelore, Brangus, and Tropical Composite, respectively. Thus, their correlations were

similar to the equivalent trait of W50 in this study. Zumbach et al (2008a) reported genetic correlation of -0.50 in carcass weight of pigs. Numerous studies in dairy cattle (Ravagnolo and Misztal, 2002; Bohmanova et al., 2008; Boonkum et al., 2011; Boonkum and Duangjinda, 2015) reported a negative genetic correlation between numerous production traits and heat tolerance, ranging between -0.04 and -0.95. In the case of dairy sheep, Finocchiaro et al (2005) reported larger genetic correlations ranging between -0.73 and -0.86. Based on the results of this study and other previous studies, it is likely that productive traits recorded at earlier age have lower magnitude of antagonism than traits recorded at later age. This is possibly because younger animals could be managed or raised in more stable environment (i.e., receive more close monitoring and care). Correlation between the intercepts for the traits ($W50_0$ and $G50_0$) was positive and moderate ($r = 0.41$). Boareki et al (2020) reported a genetic correlation between weaning weight and post-weaning weight of 0.82. However, the difference between the studies is that Boareki et al. (2020) used the 100-day weight (post-weaning weight) rather than using the 50-day gain after weaning (G50), as was done in this study. Correlation between the W50 intercept ($W50_0$) and G50 slope ($G50_1$) was small and negative ($r = -0.15$), indicating that the antagonistic effect was also small. Correlations between W50 slope ($W50_1$) and both intercepts and slope for G50 ($G50_0$ and $G50_1$) were small in magnitude and positive (0.05 and 0.03) with large standard error. Thus, such correlations were virtually zero. The use of two-trait model is important for those traits to account for potential culling biases.

Genetic correlations for the maternal genetic components are presented in Table 4.5. Highly negative correlations between intercept and slope within G50 ($G50_0$ and $G50_1$) was estimated (-0.75). Santana et al. (2016) estimated small negative maternal genetic antagonism for

weaning weight with heat stress in three beef cattle breeds of -0.17, -0.25, and -0.37, for Nelore, Brangus, and Tropical Composite, respectively. The maternal genetic components include the maternal environment caused by the genetics of the dam, and how well she raises her lambs. This can include maternal care and the milk nutrient profile that influence the growth of the young animal. The antagonism with heat stress in milk components in dairy sheep was reported to be as high as -0.86 by Finocchiaro et al. (2005). The correlation between intercepts ($W50_0$ and $G50_0$) was positive (r = 0.58). The correlation between W50 intercept ($W50_0$) and G50 slope ($G50_1$) was small and negative (r = -0.14) with large standard error. Thus, this correlation is virtually zero. This study presented genetic parameters for growth traits accounting for heat stress in sheep which include both the direct and maternal genetic parameters. The genetic antagonisms between growth traits and heat tolerance in both direct and maternal genetic components suggest that in presence of heat stress, such antagonism need to be accounted for in the breeding program.

The direct and maternal heritability for W50 and G50 across 10 units of heat load gradient are presented in Figure 4.1 and Figure 4.2, respectively. The direct and maternal heritability for W50 at the absence of heat stress were 0.10 and 0.05, respectively (Figure 4.1). When heat load increases to 10 units of THI, the direct heritability increases to 0.11 and the maternal heritability was stable at 0.05 (Figure 4.1). The direct and maternal heritability for G50 at the absence of heat stress were 0.20 and 0.02, respectively (Figure 4.2). When heat load increases to 10 units of THI, the direct heritability decreases to 0.12 and the maternal heritability decreases to 0.01 (Figure 4.2). The maternal effect has a great contribution in earlier life stage of animals' life and decreases as the animal grows older (e.g. Boareki et al., 2020). Thus, in general production for W50 (younger age trait) the maternal heritability was larger than G50 (older age trait). However, the maternal

genetic heritability at 25 units of heat load was larger in G50 than W50. In a study for beef cattle conducted by Bradford et al (2016), the direct heritability decreased, and the maternal heritability increased with the increase of heat load for both weaning weight and yearling weight. In another study for beef cattle by Santana et al (2016), the direct heritability tend to decrease with the increase of heat load and the maternal heritability had concave shape where the minimum heritability was in intermediate. In dairy cattle, Ravagnolo and Misztal (2000) reported increase in heritability with the increase for heat stress. Also, Boonkum and Duangjinda (2015) reported an increase in both narrow and broad sense heritability in dairy cattle with the increase of heat stress. A study in pigs for carcass weight by Zumbach et al (2008a), showed increase in heritability at higher level of heat load. They also carried out a two-trait model analysis for carcass weight under no heat stress and heat stress. They estimated heritability of 0.14 and 0.28, for no heat stress and heat stress, respectively. Cardoso and Tempelman (2012) showed that the heritability estimate in beef cattle was highest in improved environment. The changes in heritability across environments are due to the changes in variance components. Thus, selection for specific environment will result in specific response.

4.5. Conclusions

This study illustrates negative genetic correlations between heat stress and growth trait in sheep. The antagonism between growth traits and heat tolerance suggests that selection for growth alone will lead to increase sensitivity to heat stress. Thus, such an antagonism needs to be accounted for in breeding programs within environments where heat stress is present. Heritability

estimates for the response to heat stress from this study can provide an opportunity for selection for growth trait in stressful environment, particularly heat stresses associated with global warming.

4.6. Tables

Table 4.1. **Data description for phenotype[1] and environmental[2] measurements**

Data	No. record	Range	Mean ± SD	CV%
W50	110,512	3.35 – 59.35	18.66 ± 4.51	24.15
G50	90,204	0.00 – 86.31	17.46 ± 4.51	32.22
THI_{W50}	110,512	12.09 – 77.19	52.27 ± 12.68	24.26
THI_{G50}	90,204	8.61 – 76.12	58.03 ± 13.77	23.73

[1]W50: 50-day adjusted weaning weight in kg; and G50: 50-day adjusted post-weaning gain in kg

[2]THI_{W50}: average temperature-humidity index for W50; and THI_{G50}: average temperature-humidity index for G50

Table 4.2. Estimated heat stress thresholds at which the growth performance started to reduce

Trait	THI_h ± SD
W50	54.60 ± 1.40
G50	55.62 ± 0.85

W50: 50-day adjusted weaning weight in kg; and G50: 50-day adjusted post-weaning gain in kg.

Table 4.3. **variance component estimated accounting for heat stress**

	Traits	
component	**W50**	**G50**
$\boldsymbol{\sigma_a^2}$	2.079 ± 0.142	6.692 ± 0.295
$\boldsymbol{\sigma_{ah}}$	-0.056 ± 0.010	-0.372 ± 0.023
$\boldsymbol{\sigma_h^2}$	0.015 ± 0.001	0.045 ± 0.003
$\boldsymbol{\sigma_m^2}$	0.957 ± 0.079	0.604 ± 0.111
$\boldsymbol{\sigma_{mmh}}$	-	-0.045 ± 0.010
$\boldsymbol{\sigma_{mh}^2}$	-	0.006 ± 0.001
$\boldsymbol{\sigma_{mpe}^2}$	0.307 ± 0.055	-
$\boldsymbol{\sigma_c^2}$	5.679 ± 0.293	11.120 ± 0.591
$\boldsymbol{\sigma_l^2}$	2.603 ± 0.068	3.653 ± 0.103
$\boldsymbol{\sigma_e^2}$	9.542 ± 0.092	11.922 ± 0.142

W50: 50-day adjusted weaning weight in kg; and G50: 50-day adjusted post-weaning gain in kg; σ_a^2: direct additive genetic variance for intercept; $\sigma_{a,h}$: covariance between slope and intercept at the direct genetic component; σ_h^2: direct additive genetic variance for slope; σ_m^2: maternal genetic variance for the intercept; $\sigma_{m,mh}$: covariance between slope and intercept at the maternal genetic component; σ_{mh}^2: maternal genetic variance for the slope; σ_{mpe}^2: maternal permanent environmental variance; σ_c^2: contemporary group variance; σ_l^2: litter variance; and σ_e^2: residual variance.

Table 4.4. **Genetic correlation for the direct genetic components**

	$W50_0$*	$W50_1$	$G50_0$	$G50_1$
$W50_0$	-			
$W50_1$	-0.319 ± 0.045	-		
$G50_0$	0.414 ± 0.034	0.054 ± 0.049	-	
$G50_1$	-0.151 ± 0.040	0.026 ± 0.050	-0.682 ± 0.018	-

W50: 50-day adjusted weaning weight in kg; and G50: 50-day adjusted post-weaning gain in kg; *the subscripts 0 and 1, indicating estimates for intercept and slope respectively.

Table 4.5. **Genetic correlation for the maternal genetic components**

	$W50_0$*	$G50_0$	$G50_1$
$W50_0$	-		
$G50_0$	0.581 ± 0.075	-	
$G50_1$	-0.142 ± 0.082	-0.748 ± 0.060	-

W50: 50-day adjusted weaning weight in kg; and G50: 50-day adjusted post-weaning gain in kg; *the subscripts 0 and 1, indicating estimates for intercept and slope respectively.

4.7. Figures

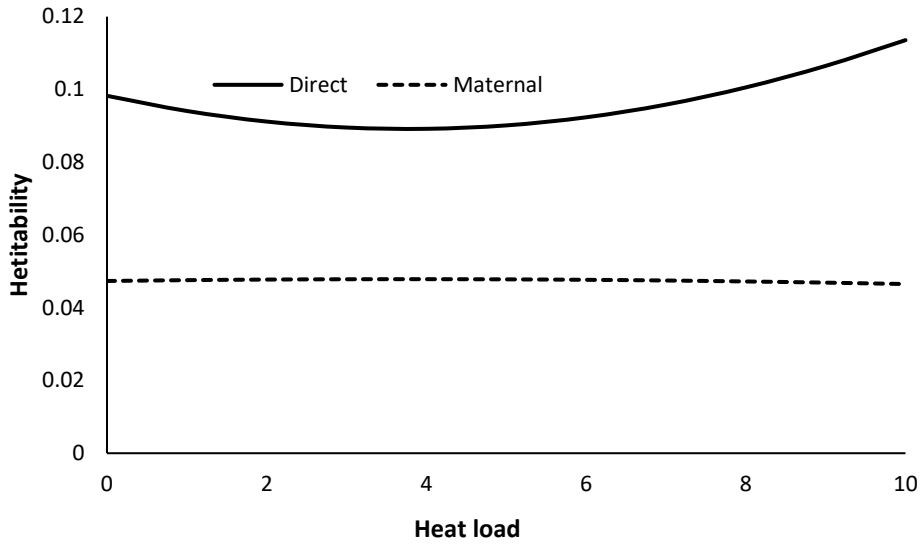

Figure 4.1. Estimate for direct and maternal heritability for W50 across units of THI (heat load).

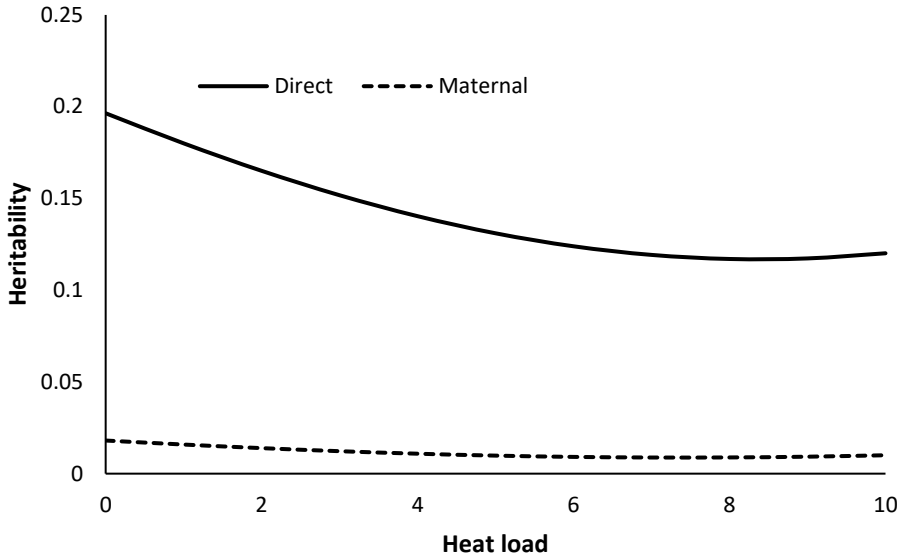

Figure 4.2. **Estimate for direct and maternal heritability for G50 across units of THI (heat load).**

CHAPTER 5

GENERAL DISCUSSION

Disease occurrence and heat stress can negatively affect the economic viability of animal production and the welfare of farmed animals. This thesis focused on gastrointestinal parasite resistance, genetic resistance for scrapie, and heat tolerance in sheep. This thesis addressed problems and solutions for such traits, and their application for genetic improvement in sheep.

Chapter 2 addressed the problem of taking fecal egg count (FEC) measurements using two different procedures: The Modified McMaster and the Triple Chamber McMaster, which have different lower detection limits of 50 and 8 eggs/gram, respectively. The results showed that the differences in means and variances between the FEC methods are significant. Therefore, FEC data measured using the two different procedures cannot be directly integrated for use in genetic evaluations. One way to account for the differences between FEC methods is to treat them as separate traits. The high genetic correlation ($r_g = 0.94$) between the two FEC methods can account for missing records when estimating EBV for FEC. However, when including other parasite resistance indicator traits such as FAMACHA©, BCS, and bodyweight, the model treating the two FEC methods as separate traits failed to converge. Another way to account for the differences between the FEC methods was to pre-adjust FEC method by rescaling to the mean and variance of the other method prior to integration. The multi-trait analysis for the integrated FEC data with the other parasite resistance traits showed a small genetic correlation among all the traits. This indicated little to no benefit of including such traits with FEC in multivariate model. However,

these findings should not be generalized to the Canadian sheep population, since the number of observations was small and limited to a single flock. With the increase of number of records and the flocks, a more decisive generalization could be made about the other parasite resistance traits (FAMACHA©, BCS, and bodyweight). Therefore, the collection of such phenotypes should not be abandoned, as they remain cheaper and less labor-intensive to measure on farm in comparison to FEC, allowing a much larger number of records to be generated compared to FEC. In addition, FAMACHA© and BCS records could be used as cheaper alternative to FEC in management decisions for administrating anti-parasite medication for vulnerable sheep and, thus, slowing down the evolution of drug resistant parasites (Dobson et al., 2012; Emery et al., 2016).

Chapter 3 addressed the problem of limited genotypic information for scrapie by utilizing available genotypic records and pedigree information to predict the genetic resistance in ungenotyped animals and the potential exploitation of the predicted information for the genetic improvement for scrapie resistance in sheep. The original numerical values for scrapie resistance (SR) were adjusted for a non-additive genetic effect, which varied from the least resistance (0) to the most resistance (4) for the 15 possible scrapie genotypes. Such adjustment allowed for differences in genetic effect among all possible genotypes to be accounted for. These numeric values for SR were used as the response variable in an animal model assuming complete heritability in small random sample (1.2% of the total population) of genotyped animals to predict the SR in ungenotyped animals (98.8 % of the total population). In addition, gene content model at single targeted haplotype allele was also used (N. Gengler et al., 2007). The accuracy of prediction was sufficient to allow for substantial genetic change (e.g. between 0.28 and 0.52 increase in SR, and between 0.13 and 0.50 in allele content), resulting from the response to

selection in ungenotyped animals. Identifying homozygous ARR rams for breeding is important, as they are guaranteed to transmit the most resistant haplotype to their progeny. The selected animals with EBV ≥ mean retained a large proportion of homozygous genotypes from the original population and increased the frequency of homozygous genotypes compared to the unselected population. Thus, pre-selection prior to genotyping could reduce the cost of identifying those valuable homozygous animals in the population. The animal linear mixed model could make better use of the available information on scrapie genotypes, which can supplement breeding programs with additional information to improve the genetic resistance to scrapie. In addition, this method can be applied to other traits controlled by major genes, such as reproductive traits (Davis, 2005) and Maedi-Visna susceptibility (Leymaster et al., 2013). Moreover, integrating gene content model with low heritable phenotypes could enhance the accuracy for EBV prediction, as illustrated by Legarra and Vitezica (2015). Therefore, Chapter 3 presented a practical method that could be extended to other applications.

Chapter 4 addressed the impact of heat stress and its interaction with both direct and maternal genetic components for growth traits in sheep. The growth traits in sheep considered in this study were the 50-days adjusted weaning weight (W50) and 50-days adjusted post-weaning gain (G50). To best our knowledge, up until now, there were no studies that estimated genetic parameters for growth traits, as a function of the level of heat stress, in sheep. Some studies estimated genetic parameters for similar growth traits in other livestock species, such as beef cattle (Bradford et al., 2016; Santana et al., 2016) and pigs (Zumbach et al., 2008a). In this study, there was no evidence for genotype by environment interaction (G×E) for heat stress in the maternal genetic component for W50. However, there was evidence for G×E in the direct component for W50 and in both direct

and maternal components for G50, which confirmed the genetic antagonism between growth traits and heat tolerance in sheep. Therefore, this antagonism must be accounted for during genetic selection in order to avoid an unintentional increase in susceptibility to heat stress from selection for production traits. The direct and maternal heritability varied across the heat load gradient. Thus, selection at different points along the heat load gradient will lead to different responses. The results of this study have important implications when considering climate change and the warming of the planet. Additionally, these results can be used when considering genetic exchange (i.e. live animals, semen, and embryos) to regions where greater heat stress is present.

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