GENOMIC, MICROBIAL, AND LINEAR RELATIONSHIPS BETWEEN RESIDUAL FEED INTAKE, DRY MATTER INTAKE, AVERAGE DAILY GAIN, AND POST WEANING WEIGHTS IN RANGE SHEEP

by

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DEDICATION

To Mom and Dad.

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ABSTRACT

Over forty years, United States sheep producers have selectively bred range sheep for larger post-weaning weights using estimated breeding values. However, this has led to increased feed intake, prompting this study's objective to quantify the relationship between lamb growth estimated breeding values, feed intake, and feed efficiency. The study involved 40 Targhee and 40 Rambouillet ewe lambs weaned at four months and put on a Growsafe^{TM} feeding system for a 56-day experimental period, with ewes weighed weekly and at the start and end of the trial. Residual feed intake was calculated using body weights, average daily gain, and daily feed intake measurements. The ewe lambs' post-weaning weight estimated breeding value was associated to their phenotypic traits. These procedures were repeated when the same ewes were yearlings and at maintenance. The study found that while post-weaning weight estimated breeding values predicted dry matter intake ($P<0.05$), they lacked association with residual feed intake ($P>0.05$) in both experimental periods. However, the ewe's post-weaning weight estimated breeding value predicted mid-trial body weight in both periods ($P<0.05$). Thus, while selecting higher post-weaning weight animals may increase feed usage due to larger body size, it does not affect feed efficiency. Comparing the residual feed intake between the two experimental periods, growth residual feed intake measurements did not predict residual feed intake in maintenance. Therefore, caution should be used when utilizing residual feed intake from the growth period as an indicator of feed efficiency in maintenance. The study also examined rumen microbial profiles in sheep between the two experimental periods. Methanogenic archaea were relatively increased within the growth experimental period, where a pelleted diet was used, and cellulolytic bacteria were increased in the maintenance experimental period, where hay was provided. Since methanogens are associated with decreased feed efficiency, diet may play a role in changing a sheep's microbiome towards more feed efficient bacteria. Finally, a genome-wide association study found the *Dmrt2* gene associated with DMI and RFI in maintenance. The *Dmrt2* gene may regulate muscle and bone development in the early embryo, affecting feed efficiency in sheep later in life.

CHAPTER ONE

INTRODUCTION

Estimated breeding values are a standard tool used in genetic selection within the United States sheep industry. Using these estimated breeding values, producers can directly select the additive genetics of their flocks, making permanent improvements on economically important traits (Wei et al., 2020). Selection trends by western U.S. producers have focused on faster-growing lambs by selecting for larger weaning weights. For example, from 1978 to 2005, weaning weight increased from 28.6kg to 37.2kg in the Rambouillet sheep breed (Leeds and Lewis, 2006). Weaning weight has a strong positive genetic correlation with pre-weaning growth rate and an intermediate positive correlation with post-weaning growth rate in sheep (Mavrogenis and Constantinou, 1990). Therefore, selection towards larger post-weaning weights has produced lambs that grow faster and are larger at the same age as intended. Faster-growing lambs are important to producers as they can be sold earlier by reaching market weight younger. Producers are therefore selecting increased sheep growth rates to reduce costs associated with keeping lambs on the farm.

However, a greater understanding of how such selection may impact the sheep's overall profitability must be understood. As producers increase the weaning weight of their lambs, they also indirectly increase the size of their mature sheep, given the strong positive genetic correlation between weaning weight and mature weight (Mavrogenis and Constantinou, 1990). Larger sheep eat more feed than smaller sheep to meet their increased energy needs (NRC, 2007). Producers may have higher maintenance feed costs through indirect selection for larger sheep. Increased feed costs are especially harmful to the profitability of sheep operations, as feed costs already make up most overall costs. For growing lambs, feedstuff costs and land lease for grazing makeup 63.17% of the total cost (Raineri et al., 2015). On farms that rely heavily on concentrate diets for their flocks, concentrate feeds can make up to 64% of operational costs alone (Benoit et al., 2019).

Furthermore, limited studies have been reported on the relationship between growth and feed efficiency. Increasing the growth rate of animals increases the energy and protein requirements needed within a shorter period (NRC, 2007). This shortened period of increased feed requirements may lead to lower feed utilization and decreased feed efficiency. If feed efficiency is reduced through increasing growth rates, sheep producers could face increased feed costs for their ewes kept on farm and the market lambs. If nutritional requirements increase, it will require increased usage of natural resources, which could be crippling to sheep producers in rangeland areas where resources are already limited. Moreover, with increased nutritional requirements, adult sheep on range operations may not receive enough nutrients to meet their requirements, leading to lower reproductive success and increased risk of metabolic deficiency. Therefore, it has become increasingly important to research the relationship between feed efficiency and growth-estimated breeding values and build EBVs that allow sheep producers to select directly on feed efficiency.

Selection criteria for feed efficiency have yet to be created primarily due to the expensive nature of collecting feed efficiency data. However, researching associations between feed intake, average daily gain, body weight, and feed efficiency may allow sheep producers to indirectly select feed efficiency by selecting for or against more easily measured traits. In addition, producers may also use genetic and microbial associations to select directly toward feed efficiency. Understanding genetic and microbial associations is also vital for creating genomic selection methods for feed efficiency in the future, as they can increase the overall accuracy of the genomic tools (Zhang et al., 2014; Ross et al., 2020a).

This research aims to characterize feed intake, feed efficiency, and microbial communities during both growth and maintenance production periods, quantify the relationship between lamb growth estimated breeding values, feed intake, and feed efficiency, and build a foundation for genetic and genomic evaluation of feed efficiency specifically within Targhee and Rambouillet breeds of sheep.

Literature Cited

Benoit, M., R. Sabatier, J. Lasseur, P. Creighton, and B. Dumont. 2019. Optimising economic and environmental performances of sheep-meat farms does not fully fit with the meat industry demands. Agronomy for sustainable development 39(4):40.

Leeds, T., and G. Lewis. 2006. Breed comparisons and trends for ewe productivity and lamb growth traits in sheep managed as contemporaries in a western range system. In: PROCEEDINGS-AMERICAN SOCIETY OF ANIMAL SCIENCE WESTERN SECTION. p 86.

Mavrogenis, A., and A. Constantinou. 1990. Relationships between pre-weaning growth, post-weaning growth and mature body size in Chios sheep. Animal Science 50(2):271-275.

NRC. 2007. Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids. Committee on Nutrient Requirements of Small Ruminants.

Raineri, C., T. S. S. Stivari, and A. H. Gameiro. 2015. Lamb production costs: Analyses of composition and elasticities analysis of lamb production costs. Asian-Australasian Journal of Animal Sciences 28(8):1209.

Ross, E. M., B. J. Hayes, D. Tucker, J. Bond, S. E. Denman, and V. H. Oddy. 2020. Genomic predictions for enteric methane production are improved by metabolome and microbiome data in sheep (Ovis aries). Journal of Animal Science 98(10):skaa262.

Wei, C., H. Luo, B. Zhao, K. Tian, X. Huang, Y. Wang, X. Fu, Y. Tian, J. Di, and X. Xu. 2020. The effect of integrating genomic information into genetic evaluations of Chinese Merino sheep. Animals 10(4):569.

Zhang, Z., U. Ober, M. Erbe, H. Zhang, N. Gao, J. He, J. Li, and H. Simianer. 2014. Improving the accuracy of whole genome prediction for complex traits using the results of genome wide association studies. PloS one 9(3):e93017.

CHAPTER TWO

LITERATURE REVIEW

Introduction

Range sheep production is an important sector of the United States sheep industry. The United States sheep industry has a market size of $623,200,000, and 67% of lamb and wool is produced in rangeland states (USDA, 2021). Range sheep operations produce both lamb and wool. Therefore, the practices of and quality of lambs grown from range operations impact the productivity of the sheep industry. Thus, improving range sheep operations' profitability is crucial to helping the United States sheep industry become more competitive in the world market.

Feed costs are among the highest expenses producers face, making up 70% of total costs (Alqaisi et al., 2017). Improving feed efficiency has been one of the top priorities of many animal industries to reduce feed costs. However, despite the economic importance, minimal research has investigated improving feed efficiency in the sheep industry. Residual feed intake (RFI) has been used to represent feed efficiency and is commonly used in other industries to improve feed efficiency, such as in beef cattle (Lamb, 2009). Residual feed intake effectively identifies sheep that consume less feed without affecting performance (Redden et al., 2011). However, RFI is not widely used in the sheep industry because it is expensive to measure.

Instead of selection for feed efficiency, current selection within the sheep industry has selected faster-growing sheep that meet market weights faster. The National Sheep Improvement Program has developed estimated breeding values (EBVs) for weight traits that allow enrolled flocks to select for higher weaning weight EBVs (Notter, 1998). However, the relationship between weight EBVs and feed efficiency has not been investigated. If the relationship between the two measurements is negative, sheep producers could harm their profitability by indirectly selecting less feed-efficient sheep. In addition, decreased feed efficiency in range sheep flocks would be economically destructive. Such operations often face limited feed resources, which may cause lower reproductive success and increased metabolic deficiencies (Hoffman et al., 2017). Therefore, quantifying the relationship between lamb weight EBVs, feed intake, and feed efficiency is crucial for improving the sheep industry's profitability.

Selection using EBVs is a way sheep producers improve flock performance. Building a framework in which EBVs for feed efficiency can be developed is needed to make it easier for producers to select more feed-efficient animals. Furthermore, incorporating such EBVs into selection indexes will give producers a more accurate selection tool to improve the profitability of their operations. Studies indicate that feed efficiency ranges from low to moderately heritable with reported narrow sense heritabilities of 0.11 (Cammack et al., 2005), 0.26 (Snowder and Van Vleck, 2003), 0.46 (Johnson P. L., 2018), and 0.45 (Tortereau et al., 2020). If feed efficiency has a low heritability, it will be more difficult and slower to improve without genetic and genomic tools. Residual feed intake is associated with other traits that are moderately heritable, such as daily feed intake and event feed time, h^2=0.25 and h^2=0.33, respectively (Cammack et al., 2005). Therefore, selection using an index with a feed efficiency EBV and knowing its genetic associations with other important traits would further improve selection accuracy.

Genetic, genomic, and microbiome evaluations are helpful beyond the phenotypic data needed to create a feed-efficiency EBV in sheep. Single Nucleotide Polymorphism (SNP) data can improve the accuracy of EBVs, allowing faster improvement of the trait, especially when using an accurate training data set (Brown et al., 2018). Therefore, identifying associations between feed efficiency and genetic markers in a range sheep training data set is essential to building a direct selection method for feed efficiency in range sheep. Rumen microbial profiling allows for quantifying microbial species that play critical roles in digesting foodstuff in ruminants. Microbial profile and SNP data have become increasingly relevant for the genomic evaluation of phenotypic traits as they can increase the accuracy of selection and thereby increase the genetic gain (Ross et al., 2020b).

Quantitative Genetics and EBVs

Most economically important traits in livestock have large numbers of loci that impact the phenotype. Differences in allele pairing and interactions between various loci can impact and differentiate phenotypes. Therefore, genomic selection for most economically important traits should be on a genome-wide level rather than single marker selection. The genome-wide selection allows more of the genetic variance to be quantified and, therefore, increases selection accuracy.

Genomic selection strategies are used to select multiple specific alleles of the entire genome for a particular trait. Selection strategies may include tandem, independent culling levels, and index selection. Genomic selection strategies make use of best linear unbiased predictions to genetically evaluate livestock. Most genetic selection strategies are based on the following genetic model:

$$P = u + G + E$$

where P is the phenotypic observation, u is the mean phenotype for the given trait, G is the genetic effect of the trait, and E is the environmental effect of the trait. The genetic effect of the trait can further be broken down into two parts; the additive genetic value and the gene combination value. The additive genetic effect is often referred to as the breeding value of an animal, as it is the genetic portion of phenotypic variation that is inherited. Whereas the gene combination effect results from dominance and epistatic interactions of genes and likely will not be inherited.

A common way to encompass a large portion of the additive genetic variance of a trait is to use estimated breeding values. Estimated breeding values are the estimation of the genetic merit of an animal. The best linear unbiased prediction (BLUP) method of calculating EBVs is through a sire evaluation method for a single trait. This estimate is modeled:

$$Y = XB + Zp + e$$

where Y is the vector of the number of records, B is the vector number of levels for fixed effects times one vector, p is the number of levels for random effects, e is the vector of random residual effects of the number of records, X is the design matrix of order number of records times number of levels for fixed effects, and Z is design matrix of order number of records times number of levels for random effects, (Henderson, 1973; Mrode, 2014). X and Z matrixes relate records to fixed and random effects, respectively.

Using the sire BLUP model, EBVs are calculated using matrix notation. The first matrix created is the design matrix X which depicts the sex of the animal. The X matrix’s transpose should be set up as:

$$X' = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 1 \end{bmatrix}$$

where 1s in the first row indicate the animal is male and 1s in the second row indicate female animals (Mrode, 2014). The second design matrix Z indicates how many records each animal has. Z's transpose should be set up as:

$$Z' = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 1 & 1 \end{bmatrix}$$

where animals with one record would have a one on the bottom row, and as the number of records increases, the one is placed higher on the corresponding row (Mrode, 2014). The third matrix that should be formed is matrix y which is the vector of phenotypic observations. Next, a least square equation matrix can be performed as

$$\text{LSE}=\begin{bmatrix} X * X' & X' * Z \\ Z * X' & Z' * Z \end{bmatrix} \begin{bmatrix} b \\ a \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \end{bmatrix}$$

(Mrode, 2014). Furthermore, relationship matrix A needs to be created including attribute relatedness of the animals. The matrix $\text{A}' = (\text{T}^{-1})'\text{D}^{-1}\text{T}^{-1}$. T^{-1} is equal to i – M, where i is the identity matrix, and M is a matrix containing the contribution of the gametes (Mrode, 2014). Both the M and i matrixes are set up with animal ids lining the x and y-axis. On the M matrix, a 0.5 is placed anywhere a known parent and offspring intersect on the matrix grid indicating the 50% of genes that the parent gave to its offspring. Matrix i has a one diagonally down the matrix where each animal id intersects itself on the x and y plane, indicating that the animal is 100% related to itself. D is the variance and covariance matrix for Mendelian sampling and can be calculated by the equation

$$d = (1 - (.25 * (Fs + Fd)))\sigma^2\text{u}$$

where Fs and Fd is the inbreeding coefficient of the sire and dam respectively, and σ^2u is the additive variance of the trait. d is the value for each animal respectively in the diagonal matrix D

(Mrode, 2014). $A^{-1}\alpha$ can then be calculated by multiplying every element of A’ by 2. Adding $A^{-1}\alpha$ to Z’Z in the LSE yields the Mixed Model Equation (MME) (Mrode, 2014). The MME can then be solved through the inversion of the coefficient matrix, which calculates the EBV values for each animal.

EBVs can often include genetic marker data to create genomic enhanced estimated breeding values (GEBVS). Genomic-enhanced estimated breeding values incorporate high-density SNP panels and genomic information to create a more accurate selection tool (Rolf et al., 2010).

Of the selection methods, selection indexes are the most efficient way to select for or against multiple traits. Selection indexes combine the available information about an animal and its relatives' phenotypes, the economic importance of each trait, and genetic correlations between traits to best predict the animal’s overall value. The fundamental estimation for selection indexes is:

$$I = b_1X_1 + b_2X_2 + b_nX_n$$

where I is the index value for the animal, x is a single item for phenotypic information – the average performance record of the group, b is the weight given to each trait based upon economic and genetic correlation data, and n is the total number of items of phenotypic information.

Genome-Wide Association Studies

A genome-wide association study (GWAS) is a standard tool for identifying genomic associations with economically important traits. Genome-wide association studies often use SNP chips to identify associations with specific locations across the genome. The Ovine 50k SNP chip is a common SNP chip used in genotyping sheep. In the GWAS, SNPs act as markers for genetic areas of the genome. Areas closer in linkage disequilibrium to associated SNPs are likely candidate regions for trait-associated genes.

Genome-Wide Association Studies perform genetic to phenotypic associations using either a linear regression or mixed model. The basic linear model for a GWAS is

$$Y \sim Wa + XsBs + g + e$$

where Y is the vector of phenotype values, W is the matrix of covariates, a is the corresponding vector of effect size, Xs is a vector of genotype values for all individuals at SNPs, Bs is the corresponding fixed effect size of genetic variants, g is a random effect that captures the polygenic effect of other SNPs and e is a random effect of residual errors (Uffelmann et al., 2021). The g in the model can be estimated through the following model:

$$g \sim N(0, Va * U)$$

where Va is the additive genetic variation of the phenotype and U is the standard genetic relationship matrix (Uffelmann et al., 2021). Similarly, in the linear GWAS model, e can be estimated through the following model:

$$e \sim N(0, Ve * I)$$

Ve is the residual variance, and I is the identity matrix (Uffelmann et al., 2021). Mixed model GWAS takes the linear model and can include fixed effects to correct for covariates and kinship matrixes to control for random effects. We can then estimate how much variation of the trait can be attributed to each SNP marker through this modeling.

Microbial Profiling

16s rRNA gene sequencing is a standard tool used for microbial profiling. The 16s rRNA gene coding region codes for the RNA portion of the prokaryotic ribosome 30S subunit and is present in all prokaryotic genomes. The genomic 16s rRNA gene is highly conserved with nine interspersed variable regions wherein small nucleotide differences have accumulated at a slow but consistent rate over time sufficient to distinguish microbial taxa. Most commonly variable region four is used in amplificated as it closely mirrors the variability in the 16s rRNA. Variable region three is also commonly amplified to help further distinguish between different microbial species. These region sequences can then be used to identify operational taxonomic unit (OTU) prevalence in samples by the standard of 97% uniqueness of DNA amplicons. Operational taxonomic units are useful approximations of species and therefore can be used to make approximations of the species abundances in a sample.

Many different methods have been deployed to determine the 16s rRNA region of microbial species. Such methods include sequencing amplified ribosomal DNA restriction analysis, automated ribosomal intergenic spacer analysis, terminal restriction fragment length polymorphism, Denaturing Gradient Gel Electrophoresis, and Temperature Gradient Gel Electrophoresis, single-strand conformation polymorphism, and denaturing high-performance liquid chromatography (Nocker et al., 2007). Current methodology, however, has been to use massively parallel sequencing aka next generation sequencing (NGS) (Moorthie et al., 2011).

All methods, however, start with isolating the DNA molecule, and most use polymerase chain reaction amplification (PCR) to amplify the 16s rRNA DNA sequence of the sample. In older gel electrophoresis methods restriction enzymes have been utilized to cut the 16s rRNA

DNA of the microbiomes at the correct place to only get one segment of variable region that codes for a functional gene (Nocker et al., 2007). Gel electrophoresis has been used in these methods to separate DNA fragments by length. As DNA goes through the gel electrophoresis longer fragments will move more slowly than shorter DNA fragments. Using a reference kilobase ladder difference sized DNA fragments can be separated. Different sizes of DNA for different regions of the 16s rRNA DNA have given insight to what species may be in that sample.

Next Generation sequencing can be done through two main methods reversible termination and pyrosequencing (Moorthie et al., 2011). Reversable termination is performed by first hybridization of DNA fragments to probes. Clonal amplification occurs to those probed DNA through surface PCR to create dense clusters of identical DNAs across. A universal sequencing primer is added onto DNA. Then DNA polymerase can add a complementary nucleotide terminator, each labelled with a different fluorescent dye (Moorthie et al., 2011). The fluorescent dye gives a temporal series of color images which can be converted into sequence reads. The terminating groups are chemically cleaved to prepare the templates for the next round of incorporation and imaging. Pyrosequencing occurs by first hybridizing DNA fragments into special beads. These template beads are individually put into a microscopic well and smaller beads are added that contain the necessary components for the sequencing reaction. Nucleotides are added in turn A, then C, then g and then T. If nucleotides are added onto a template bead a phosphate ion is released resulting in a flash of light (Moorthie et al., 2011). The light can be detected by a camera and the incorporating nucleotide sequence can be inferred.

Using the sequence information, scientists can cluster microbes by sequence similarity, typically 97%, across the length of the sequenced 16s rRNA variable region into OTUs (Chiarello et al., 2022) or into unique amplicon sequence variants (ASVs). Both OTUs and ASVs can be compared to sequences of known microbial taxa to help identify their taxonomic placement.

Microbial Diversity

Once OTUs or ASVs are defined within a sample the microbial community can then be compared to other samples' communities using diversity measurements. Two common diversity measures used to analyze microbial communities are alpha and beta diversity. Alpha diversity is the mean species diversity within a site at a local scale, whereas beta diversity is the ratio between regional and local species diversity. Diversity is based upon two principles: species richness and species evenness. Richness is the number of unique entities (species, OTUs, or ASVs) present, while evenness is a measure of how evenly (or skewed) each entity is within the sample.

Simpson and Shannon's indexes are two common alpha-diversity indexes that estimate alpha diversity within a sample. The Shannon index is calculated through the equation:

$$H = -\sum_{i=1}^{s} pi \ln pi$$

(Morris et al., 2014), whereas Simpson's dominance index can be calculated through the equation:

$$\mathrm{D}= \frac{1}{\sum_{i=1}^{s} pi^2}$$

where pi is the proportion of individuals of one species divided by the total number of individuals (Morris et al., 2014). The Shannon index is more responsive as it assumes all species are represented and are randomly sampled. This gives high sensitivity to rarer species found (Morris et al., 2014). The Simpson index, conversely, is a dominance index in that it gives less sensitivity to rare species compared to those more common (Morris et al., 2014).

Whittaker's basic diversity is the most basic beta diversity measurement and is equal to the total number of species divided by the average species richness. Beta diversity measurements for each sample allow for the utilization of Principal Coordinate Analysis (PCoA) to characterize different enterotypes of host microbiomes. Comparing enterotype results between hosts of different phenotypes can allow for the association of unique microbiome structures to said phenotypes.

Microbiome Community Development

Different microbial communities may be associated with more beneficial microbial community compositions that enhance economically important phenotypes, determining how microbial communities change is important in selecting for said traits. Few studies have examined how microbial community compositions change in response to host and environmental changes within sheep.

Zhang et al., 2022, found differences in microbial species abundances in fecal samples between seasons. *Lachnospiraceae* genera were found to be abundant in sheep in harsher conditions during the summer and winter (Zhang et al., 2022). *Lachnospiraceae* acts to improve the digestion of polysaccharides and produce more energy which may be beneficial in using energy more efficiently in cold months (Zhang et al., 2022). Thereby adaptive changes in microbe abundance can occur in response to cold stress and weather within sheep.

Wang et al., 2019, investigated the differences in the microbiome in 27 Tibetan lambs at various time points from 0 to 360 days of life. They found that differences in the ratio of *bacteroidetes* to *proteobacteria* increased as the lambs aged (Wang et al., 2019). This indicates that age is also a contributing factor in shaping microbiomes. Yin et al., 2021, further indicate that specific genera were age discriminatory. Bacteria that increased with age included *Veillonellaceae UCG-001, Prevotella 1, Anaeroplasma, Anaerovibrio, Ruminococcaceae NK4A214 group, Ruminococcus 2, Quinella, Acetitomaculum, Lachnospiraceae ND3007 group, Ruminococcaceae UCG-014, Pseudoramibacter, and Desulfobulbus* (Yin et al., 2021). However, while these specific genera did increase no clear age-related changes occurred at the phyla level after 20 days, and total microbiome structure stabilized after 60 days of age (Yin et al., 2021). Moreover, a sheep's microbial community's maturation speeds up as solid feed is introduced (Yin et al., 2021).

Residual Feed Intake and Feed Efficiency

Five physiological processes are crucial in determining feed efficiency in livestock: feed intake, digestion, metabolism, physical activity, and thermoregulation (Herd and Arthur, 2009). Of these, 73% of all variation can be attributed to heat production from metabolic processes, body composition, and physical activity (Herd and Arthur, 2009). Animals differ in their expression of these physiological processes, leading to differences in feed efficiency. Genetic variation in feed efficiency has been linked to differences in feeding behavior, digestion, methane emission, rumen microbiome, protein turnover, lipid metabolism, and immunity (Cantalapiedra-Hijar et al., 2018).

Feed efficiency is measured in many ways in livestock. The predominant way to measure feed efficiency is by using residual feed intake (RFI). Another less common measurement is the feed conversion ratio. The feed conversion ratio takes an amount of feed and divides it by the amount of weight the animal gained during a specific period. However, the feed conversion ratio is a less-than-ideal feed efficiency measurement. The feed conversion ratio does not consider that animals with similar body sizes differ in their intakes, and feed intake varies by the animal's size and growth. The Kleiber ratio is another feed efficiency measurement that measures growth efficiency independent of body size, similar to the RFI measurement (Rezende et al., 2022). However, the Kleiber ratio differs from RFI as it is purely the ratio of ADG to metabolic BW and does not incorporate feed intake data into its calculation (Rezende et al., 2022). To adjust for differences in variation in size and growth between animals, Koch et al. proposed RFI as an alternative to the feed conversion ratio (Koch et al., 1963). Residual feed intake estimates the difference between actual feed intake and expected intake for a given weight. Koch modeled RFI based on the model:

$$E = X + Ms + As + e$$

where E is the estimated feed intake, X is the regression intercept, Ms is the metabolic midweight regression coefficient times metabolic midweight, As is the average daily gain regression coefficient times average daily gain, and e is the residual error. The modeling considers differences in body weight and rate of gain that the feed conversion ratio does not account for. Furthermore, using a linear index in RFI modeling gives a more accurate selection response by accounting for additional biological variances (Arthur et al., 2001). While RFI is one of the best measurements for feed efficiency, it has several limitations. First, as discussed, there is limited information on the predictiveness of RFI between animal life stages. Secondly, RFI may not be applicable in maintenance periods where body weight change is limited. Finally, it is unknown how pre-test environments affect RFI rankings.

Residual feed intake positively correlates with dry matter intake in sheep during many life stages, including post-weaning, hogget, and adult (Muir et al., 2020). It also correlates with actual feed intake in lambs (Cammack et al., 2005). Residual feed intake seems not to be correlated with average daily gain in growing lambs, suggesting that selection towards lower RFI sheep should not impact weight gain (Zhang et al., 2017). Despite these positive correlations, residual feed intake has been considered a non-repeatable measurement of feed efficiency between life stages within sheep (Redden et al., 2011). Interestingly, in steers RFI was determined to be repeatable between a grass silage plus concentrate diet and a grazed grass or high concentrate diet between a finishing and growing period, but not between grazed grass diet and the high concentrate diet (Lahart et al., 2020). Therefore, there is a need to characterize RFI over different physiological stages further to quantify the utility of RFI for selecting more feed-efficient sheep.

The only negative correlation found in the literature was described by Redden et al. 2010, who suggested that high-feed efficient ewes may produce less offspring than lowly efficient ewes, indicating an inverse relationship between RFI and reproduction expected progeny differences (EPDs). Reproductive efficiency is crucial to sheep producers, so further study into the biological impact of being more feed efficient on reproductive traits is needed.

Feed Efficiency Genomic and Genetic Associations

Cockrum et al., 2012 were one of the first studies to look at the associations of feed efficiency in sheep through a genome-wide association study. The study found that ovine chromosomes 1, 2, 3, and 18 had the greatest number of significantly associated markers with the feed efficiency (Cockrum et al., 2012).

Pasandideh et al., 2018, describe an analysis of feed efficiency using the Kleiber ratio as a standard of feed efficiency within sheep using an OAR50K SNP chip. Pasandideh calculated the Kleiber ratio for multiple age groups, including between ages 0-3 months, 3-6 months, 6-9 months, and 9-12 months, as well as between each age group. Genes associated with a lower Kleiber ratio from ages 0-6 months were *PSAP*, *SCGN*, and *HDGF* (Pasandideh et al., 2018). All the associated genes deal with nutrient usage or growth. For example, *SCGN* is associated with pathways that make secretagogin, a protein that deals with calcium flux and stimulation of the cell proliferation (Pasandideh et al., 2018).

Zhang et al., 2019, characterized gene expression using feed conversion ratio and RNA sequencing to find associations with feed efficiency using six liver transcriptomes of 180-day-old lamb rams. As the previous study discussed, genes associated with metabolic pathways were influential in whether a sheep is feed efficient. Specifically, up and down-regulation of *ADRA2A* and *RYR2* were associated with the feed efficiency (Zhang et al., 2019). In addition, both *ADRA2A* and *RYR2* are suspected to regulate energy through the adrenaline pathways (Zhang et al., 2019). Therefore, differences in the adrenal pathway could lead to energy utilization differences and thus could change the animal's feed efficiency.

Zhang et al., 2021, describe the genetic association with feed efficiency using RFI to measure, screening two specific genes, *ME1* and *CA1*, that are key players in metabolic pathways. Zhang et al. specifically utilized reverse transcriptase poly chain reactions (qRT-PCR) to indicate differences in the expression of feed efficiency. Real-Time Quantitative Reverse Transcription PCR indicates levels of expression of specific target genes through the fluorescence (Kralik and Ricchi, 2017). Through computation, the transcriptome amount is identified through differences in the fluorescence intensity (Kralik and Ricchi, 2017). As transcriptome levels increase or decrease, levels of fluorescence differ increase or decrease, respectively. The differing fluorescence signals allow the user to identify the upregulation and downregulation of transcriptions through computational detection of higher and lower output fluorescence (Kralik and Ricchi, 2017). The *ME1* gene codes for the cytosolic NADP+, which interacts with malate to make pyruvate in NADPH production (Chang et al., 2011).

The *CA1* gene codes for carbonic anhydrase one, which helps improve gastrointestinal health, digestion, and utilization of nutrients (Awad et al., 2017). They found that *ME1* and *CA1* genes had higher expression in low RFI sheep (Zhang et al., 2021a). The difference in expression between different feed efficiency sheep indicates that feed efficiency is partly due to more actively expressed metabolic pathways. Furthermore, synonymous mutations affecting gene function at MEI g.453 when cytosine is replaced with thymine were associated with a lower RFI and increased feed efficiency (Zhang et al., 2021a). Similarly, lower feed conversion ratios were associated with synonymous mutations at *CA1* g.199 where cytosine is replaced with thymine (Zhang et al., 2021a).

Giraldez et al., 2021, described the underlying feed efficiency mechanisms in fattening lambs. The study looked at liver and ileal tissue gene expression and discovered that less feed efficient lambs had a lower expression of *CYP2C19, FADS1,* and *GSTA1* (Giráldez et al., 2021). The *CYP2C19* gene is involved in the metabolism of cholesterol and steroid hormone, indicating that low levels of *CYP2C19* expression negatively affect the ability of less feed-efficient lambs to effectively metabolize important lipid molecules in some circumstances (Giráldez et al., 2021). Furthermore, Cyp2c19 is involved in xenobiotic metabolism and protein processing and transport. The enzyme promotion of xenobiotic metabolism is crucial to arachidonic acid metabolism to form other epoxyeicosatrienoic acids (Giráldez et al., 2021). Therefore, reduced expression and subsequent lowered production of Cyp2c19 may lead to increased arachidonic acid build-up, causing a hepatic pro-inflammatory reaction in low-feed efficiency lambs. This causes energy to be redirected towards inflammatory responses, which is theorized to cause a reduction in the efficiency (Giráldez et al., 2021).

Jackson et al., 1997, took 311 lambs and classified them into two groups: lambs expressing the callipyge gene and lambs not expressing the callipyge gene. They found that animals expressing the callipyge gene were more feed efficient (Jackson et al., 1997). The study further implies that expressed genes that result in abnormally higher meat yields can produce more feed-efficient animals by increasing the total meat yield. While the callipyge gene is excessively able to add muscle to animal carcasses, similar functioning genes with more negligible effects may also play similar roles in increasing feed efficiency in sheep.

Only a few studies have discussed the genetic association of feed efficiency in sheep; however, associations from cattle can be used to pinpoint types of genes that may impact feed efficiency in sheep.

No work has been reported on gene associations with feed efficiency that impact oxidative stress in sheep; however, it has been studied in cattle. The Fibulin 5 protein which acts to protect endothelial dysfunction, was critical in increasing feed efficiency in steers (De Lima et al., 2020). Similarly, the S*ERPINF1* was also associated with feed efficiency in steers (De Lima et al., 2020). *Serpin family F member 1* encodes for the PDF protein, inhibiting endothelial cells and suppressing NAPDH oxidase-mediated ROS production in cells (De Lima et al., 2020). The serpin peptidase inhibitor, clade F, member 1 gene acts as a potent anti-inflammatory, antioxidant, anti-angiogenic, and anti-thrombotic. The association of *SERPINF1* to feed efficiency indicates that genes with antioxidant effects help improve feed efficiency by producing animals that may more easily handle more oxidative stress. These animals demand less energy expenditure towards coping with said stress and therefore are more feed efficient than those who cannot (De Lima et al., 2020).

Feed Efficiency Microbial Association

Differing proportions and amounts of microbial species in the cattle rumen are correlated with differences in the cow's overall feed efficiency (Myer et al., 2015). However, there is minimal published work investigating rumen microbial profiles and feed efficiency in sheep. Perea et al., 2017, looked at what intestinal changes in lamb microbiota were associated with differing feed efficiency phenotypes. Alpha diversity between sheep of different feed efficiency phenotypes did not differ; 13 OTUs had a greater relative abundance in either the inefficient or efficient sheep (Perea et al., 2017). Of these 13 OTUs, six differed in their relative abundance in rumen samples collected between differing efficiency sheep. Efficient sheep tended to have higher numbers of three OTUs, including one from the Ruminococcaceae family, and two OTUs that each most closely resembled *Saccharofermentans acetigens*, whereas inefficient sheep had greater abundances of three OTUs from the Lachnospiraceae family, the *succinivibrio* genus and an unclassified order, UCT N177 (Perea et al., 2017). Ruminococcaceae is a bacterial family to have known fibrolytic members, including cellulolytic species *R. albus* and *R. flavefaciens* (Perea et al., 2017). The Ruminococcaceae family may lead to higher energy release and thus a higher growth rate with the same amount of food.

Ellison et al. 2017, describes the interaction between feed efficiency and diet on microbial species abundance. Important species found in high RFI lambs included the *Ruminococcus brommi* species and 16 further species in the *Prevotella* family (Ellison et al., 2017). *Methanobrevibacter smithii* was found to be more associated with low RFI sheep (Ellison et al., 2017). The association between *methanobrevibacter smithii* and higher feed efficiency was a novel finding as methanogens typically are associated with microbiomes of low feed efficient animals. In lower feed efficient animals, bacteria species *Sharpea azabuensis, Ruminococcus flavefaciens, and Dialister invisus* were all in higher abundance. In contrast, *Mannheim haemolytica* was of greater abundance in higher feed-efficient animals (Ellison et al., 2017). They found that OTUs with greater abundance in higher feed-efficient animals on one diet may not be associated with higher feed efficiency in another diet (Ellison et al., 2017). The central bacterial family they found that had this interaction was *Prevotella*.

Zhang et al., 2021, further characterized the rumen microbiota associated with residual feed intake. They indicate that a healthier rumen microbiota is characterized by the dominance of anaerobic bacteria such as Bacteroidetes and firmicutes (Zhang et al., 2021b). The ratio of firmicutes: to bacteroides may impact the ability of the lamb to absorb more energy. They further found that Prevotellaceae positively correlated with RFI (Zhang et al., 2021b). Prevotellaceae is known to break down proteins and carbohydrates in feed to absorb nutrients (Zhang et al., 2021b). Selenomadales were positively associated with RFI, possibly due to their theorized function of determining gram-negative cell wall type (Zhang et al., 2021b).

McLoughlin et al., 2020, discuss the divergent rumen microbial composition of sheep in feed efficiency over the solid and liquid ruminal fractions. Overall, they found no difference in diversity measurements between the solid or liquid fractions of the rumen (McLoughlin et al., 2020). The lack of differences between fractions indicates that separating fractions in the rumen may not be necessary. They found that while methanogen abundance did not differ between high and low feed-efficient sheep, methanogen species ASV differed based on high or low feed efficiency (McLoughlin et al., 2020). *Methanobrevibacter olleyae* and *Methanobrevibacter millerae* was increased in the low feed efficiency cohort, whereas the *Methanobrevibacter wolinii* was increased in the high feed efficiency cohort (McLoughlin et al., 2020). The differences in methane production between ASVs of *Methanobrevibacter* may result in a more significant loss of dietary energy for the host through differences in methane production, thus decreasing feed efficiency (McLoughlin et al., 2020). The *Prevotella, Fibrobacter, Ruminococcus, Coprococcus, Pseudoramibacter*, and *Pyramidobacter* bacterial families were positively associated with FCR and negatively associated with ADG (McLoughlin et al., 2020).

Fibrobacter and *Ruminococcus* are fiber-digesting bacteria that break down fiber for cellulose which they depend on (McLoughlin et al., 2020). Both may become redundant in high concentrate diets as less cellulose degradation is needed.

Patil et al., 2018 describe how poor feed efficiency may be linked to microbial community structural abnormalities. Low-RFI animals contain a microbiome that contains reactions significantly closer to that of the host's body than the microbiome of high-RFI animals (Patil et al., 2018). The close relation of host body metabolism and microbiome metabolism could indicate that more efficient animals have microbial community metabolic networks that could interface more easily with the metabolic pathways of the hosts. Furthermore, the efficient animals showed more Shannon entropy than the less efficient animals, indicating a higher diversity of microbial species (Patil et al., 2018).

To better understand how differing rumen microbiomes impact sheep feed efficiency, there must be further quantification and comparison of rumen microbiomes between sheep of differing efficiencies.

Rationale for Research

While there are studies on the genomic and microbial associations of feed efficiency in sheep, the literature is incomplete. Genomic analysis of economically important traits has been extensively performed in other livestock species, including cattle, pigs, and chickens. In sheep, the literature is sparse on genomic associations of economically important traits and is even more sparse in the case of microbial associations. Incorporating SNP chips having equal to or greater than 50,000 SNPs has only been minimally used to find associations with feed efficiency. SNP genotyping is crucial in narrowing the amount of candidate genomic regions and genes associated with feed efficiency. As candidate regions and genes are further described, they can be incorporated further into marker-assisted selection. Genomic-enhanced EBVs can be created using the associated genetic data, creating a more accurate breeding value for the traits producers select. Therefore, work is needed using 50k and greater ovine SNP chips and incorporating feed efficiency phenotype data in sheep to help identify more associated genomic regions and improve selection accuracy.

Microbial profiling and analysis for feed efficiency is a relatively new technology in all livestock species. However, it has become increasingly important information in researching feed efficiency mechanisms and their viability to be incorporated into selection criteria. Limited studies have investigated the relationship between feed efficiency, microbial profiles, and diversity within sheep. Therefore, there are significant gaps in our understanding of what microbial populations inhabit the rumens of more feed-efficient animals. Similarly, disputes over some specific bacteria and their relative associations with high and low feed efficiency exist, and further study into the microbiome of sheep will resolve these disputes.

Only one study looked at the feed efficiency of a group of sheep between two life stages (Redden et al., 2011). If feed efficiency changes over time and between production cycles, then feed efficiency associations, both microbial and genomic, may differ depending on the time the phenotypic data is collected. This may lead to inaccurate measurements of feed efficiency being used for selection. This could eventually cause producers to select for feed efficiency in less economically advantageous life stages and would decrease selection accuracy. Therefore, further longitudinal studies over multiple life stages are essential to describe how feed efficiency is regulated and changes throughout the production cycle.

Genomic selection is often based on phenotypic data standardized to an animal's cohort. This can improve selection accuracy, and thus genetic progress as phenotypic standardization can limit the differences in environmental attributes. Due to the economic importance of range sheep to the entire United States sheep industry, selection for economic traits in these sheep should be based upon a cohort of sheep of a range production system to improve accuracy. However, research in both genomic and microbiome associations of feed efficiency has not been limited to a certain type of production system. Limiting the type of production system to range operations would allow for an accurate description of the range type sheep within those range operations. Therefore, there is a need to investigate further genetic and microbial components of range sheep feed efficiency.

Lastly, there has been no attempt in the literature to determine the associations between sheep growth EBVs and feed efficiency. As discussed, the current selection criteria producers use is selection for larger post-weaning weights using post-weaning weight EBV. Further research into how EBVs relate to feeding efficiency would inform producers about the economic gains and costs of selecting fast-growing lambs.

Literature Cited

Alqaisi, O., O. A. Ndambi, and R. B. Williams. 2017. Time series livestock diet optimization: cost-effective broiler feed substitution using the commodity price spread approach. Agricultural and Food Economics 5(1):1-19.

Arthur, P., G. Renand, and D. Krauss. 2001. Genetic and phenotypic relationships among different measures of growth and feed efficiency in young Charolais bulls. Livestock Production Science 68(2-3):131-139.

Awad, W. A., C. Hess, and M. Hess. 2017. Enteric pathogens and their toxin-induced disruption of the intestinal barrier through alteration of tight junctions in chickens. Toxins 9(2):60.

Brown, D., A. Swan, V. Boerner, L. Li, P. Gurman, A. McMillan, J. Van der Werf, H. Chandler, B. Tier, and R. Banks. 2018. Single-step genetic evaluations in the Australian sheep industry. In: Proceedings of the world congress on genetics applied to livestock production. p 460.

Cammack, K., K. A. Leymaster, T. Jenkins, and M. K. Nielsen. 2005. Estimates of genetic parameters for feed intake, feeding behavior, and daily gain in composite ram lambs. Journal of Animal Science 83(4):777-785.

Cantalapiedra-Hijar, G., M. Abo-Ismail, G. Carstens, L. Guan, R. Hegarty, D. A. Kenny, M. Mcgee, G. Plastow, A. Relling, and I. Ortigues-Marty. 2018. Biological determinants of between-animal variation in feed efficiency of growing beef cattle. Animal 12(s2):s321-s335.

Chang, G. G., and L. Tong. 2011. Structure and function of malic enzymes, a new class of oxidative decarboxylases. Biochemistry 42:12721–12733.

Chiarello, M., M. McCauley, S. Villéger, and C. R. Jackson. 2022. Ranking the biases: The choice of OTUs vs. ASVs in 16S rRNA amplicon data analysis has stronger effects on diversity measures than rarefaction and OTU identity threshold. PLoS One 17(2):e0264443.

Cockrum, R., N. Pickering, R. Anderson, D. Hyndman, M. Bixley, K. Dodds, R. Stobart, J. McEwan, and K. Cammack. 2012. Identification of single nucleotide polymorphisms associated with feed efficiency in rams. In: Proc West Sect Am Soc Anim Sci. p 79-83.

De Lima, A. O., J. E. Koltes, W. J. Diniz, P. S. De Oliveira, A. S. Cesar, P. C. Tizioto, J. Afonso, M. M. de Souza, J. Petrini, and M. I. Rocha. 2020. Potential biomarkers for feed efficiency-related traits in nelore cattle identified by co-expression network and integrative genomics analyses. Frontiers in Genetics 11:189.

Ellison, M., G. Conant, W. Lamberson, R. Cockrum, K. Austin, D. Rule, and K. Cammack. 2017. Diet and feed efficiency status affect rumen microbial profiles of sheep. Small Ruminant Research 156:12-19.

Giráldez, F. J., N. Santos, A. Santos, C. Valdés, S. López, and S. Andrés. 2021. Fattening lambs with divergent residual feed intakes and weight gains: Unravelling mechanisms driving feed efficiency. Animal feed science and technology 273:114821. doi: 10.1016/j.anifeedsci.2021.114821

Henderson, C. R. 1973. Sire evaluation and genetic trends. Journal of Animal Science 1973(Symposium):10-41.

Herd, R., and P. Arthur. 2009. Physiological basis for residual feed intake. Journal of animal science 87(suppl_14):E64-E71.

Hoffman, M., S. Reed, S. Pillai, A. Jones, K. McFadden, S. Zinn, and K. Govoni. 2017. Physiology and endocrinology symposium: the effects of poor maternal nutrition during gestation on offspring postnatal growth and metabolism. Journal of Animal Science 95(5):2222-2232.

Jackson, S., R. Green, and M. Miller. 1997. Phenotypic characterization of Rambouillet sheep expressing the callipyge gene: I. Inheritance of the condition and production characteristics. Journal of Animal Science 75(1):14-18.

Johnson P. L., K. K., Wing J, Hickey S, Johnstone P. 2018. Preliminary estimates of genetic parameters for residual feed intake in New Zealand maternal sheep. In: 11th World Congress on Genetics Applied to Livestock Production

Koch, R. M., L. A. Swiger, D. Chambers, and K. E. Gregory. 1963. Efficiency of feed use in beef cattle. Journal of animal science 22(2):486-494.

Kralik, P., and M. Ricchi. 2017. A basic guide to real time PCR in microbial diagnostics: definitions, parameters, and everything. Frontiers in microbiology 8:108.

Lahart, B., R. Prendiville, F. Buckley, E. Kennedy, S. Conroy, T. Boland, and M. McGee. 2020. The repeatability of feed intake and feed efficiency in beef cattle offered high-concentrate, grass silage and pasture-based diets. animal 14(11):2288-2297.

Lamb, G. C., Maddock, T. 2009. Feed Efficiency in Cows. In: N. F. R. a. E. Center (ed.). p 35-42, Florida beef cattle short course.

McLoughlin, S., C. Spillane, N. Claffey, P. E. Smith, T. O'Rourke, M. G. Diskin, and S. M. Waters. 2020. Rumen microbiome composition is altered in sheep divergent in feed efficiency. Frontiers in microbiology 11:1981.

Moorthie, S., C. J. Mattocks, and C. F. Wright. 2011. Review of massively parallel DNA sequencing technologies. The HUGO journal 5:1-12.

Morris, E. K., T. Caruso, F. Buscot, M. Fischer, C. Hancock, T. S. Maier, T. Meiners, C. Müller, E. Obermaier, and D. Prati. 2014. Choosing and using diversity indices: insights for ecological applications from the German Biodiversity Exploratories. Ecology and evolution 4(18):3514-3524.

Mrode, R. A. 2014. Linear models for the prediction of animal breeding values. Cabi.

Muir, S., N. Linden, A. Kennedy, M. Knight, B. Paganoni, G. Kearney, A. Thompson, and R. Behrendt. 2020. Correlations between feed intake, residual feed intake and methane emissions in maternal composite ewes at post weaning, hogget and adult ages. Small Ruminant Research 192:106241.

Myer, P. R., T. P. Smith, J. E. Wells, L. A. Kuehn, and H. C. Freetly. 2015. Rumen microbiome from steers differing in feed efficiency. PloS one 10(6):e0129174.

Nocker, A., M. Burr, and A. K. Camper. 2007. Genotypic microbial community profiling: a critical technical review. Microbial ecology 54:276-289.

Notter, D. R. 1998. The US National Sheep Improvement Program: across-flock genetic evaluations and new trait development. Journal of animal science 76(9):2324-2330.

Pasandideh, M., G. Rahimi-Mianji, and M. Gholizadeh. 2018. A genome scan for quantitative trait loci affecting average daily gain and Kleiber ratio in Baluchi Sheep. Journal of genetics 97:493-503.

Patil, R. D., M. J. Ellison, S. M. Wolff, C. Shearer, A. M. Wright, R. R. Cockrum, K. J. Austin, W. R. Lamberson, K. M. Cammack, and G. C. Conant. 2018. Poor feed efficiency in sheep is associated with several structural abnormalities in the community metabolic network of their ruminal microbes. Journal of Animal Science 96(6):2113-2124.

Perea, K., K. Perz, S. K. Olivo, A. Williams, M. Lachman, S. L. Ishaq, J. Thomson, and C. J. Yeoman. 2017. Feed efficiency phenotypes in lambs involve changes in ruminal, colonic, and small-intestine-located microbiota. Journal of Animal Science 95(6):2585-2592.

Redden, R., L. Surber, B. Roeder, B. Nichols, J. Paterson, and R. Kott. 2011. Residual feed efficiency established in a post-weaning growth test may not result in more efficient ewes on the range. Small Ruminant Research 96(2-3):155-159.

Rezende, M., C. Malhado, S. Biffani, J. Carrillo-Tabakman, M. Fabbri, A. Crovetti, P. Carneiro, and R. Bozzi. 2022. Heritability and genetic correlation of body weight and Kleiber ratio in Limousin and Charolais beef cattle breeds. Animal 16(5):100528.

Ross, E. M., B. J. Hayes, D. Tucker, J. Bond, S. E. Denman, and V. H. Oddy. 2020. Genomic predictions for enteric methane production are improved by metabolome and microbiome data in sheep (Ovis aries). Journal of animal science 98(10):1-14. doi: 10.1093/jas/skaa262

Snowder, G., and L. D. Van Vleck. 2003. Estimates of genetic parameters and selection strategies to improve the economic efficiency of postweaning growth in lambs. Journal of animal science 81(11):2704-2713.

Tortereau, F., C. Marie-Etancelin, J. L. Weisbecker, D. Marcon, F. Bouvier, C. Moreno-Romieux, and D. Francois. 2020. Genetic parameters for feed efficiency in Romane rams and responses to single-generation selection. Animal (Cambridge, England) 14(4):681-687. doi: 10.1017/S1751731119002544

Uffelmann, E., Q. Q. Huang, N. S. Munung, J. de Vries, Y. Okada, A. R. Martin, H. C. Martin, T. Lappalainen, and D. Posthuma. 2021. Genome-wide association studies. Nature Reviews Methods Primers 1(1):59. doi: 10.1038/s43586-021-00056-9

USDA. 2021. Sheep and Goats (January 2021). In: N. A. S. Service (ed.).

Wang, L., K. Zhang, C. Zhang, Y. Feng, X. Zhang, X. Wang, and G. Wu. 2019. Dynamics and stabilization of the rumen microbiome in yearling Tibetan sheep. Scientific Reports 9(1):1-9.

Yin, X., S. Ji, C. Duan, P. Tian, S. Ju, H. Yan, Y. Zhang, and Y. Liu. 2021. Age-related changes in the ruminal microbiota and their relationship with rumen fermentation in lambs. Frontiers in Microbiology 12:679135.

Zhang, D., X. Zhang, F. Li, C. Li, Y. La, F. Mo, G. Li, Y. Zhang, X. Li, and Q. Song. 2019. Transcriptome analysis identifies candidate genes and pathways associated with feed efficiency in hu sheep. Frontiers in Genetics 10:1183.

Zhang, D., X. Zhang, F. Li, L. Yuan, Y. Zhang, X. Li, Y. Zhao, Q. Song, G. Li, and W. Wang. 2021a. Polymorphisms in ovine ME1 and CA1 genes and their association with feed efficiency in Hu sheep. Journal of Animal Breeding and Genetics 138(5):589-599.

Zhang, X., C. Li, K. Shahzad, M. Han, Y. Guo, X. Huang, T. Wu, L. Wang, Y. Zhang, and H. Tang. 2022. Seasonal Differences in Fecal Microbial Community Structure and Metabolism of House-Feeding Chinese Merino Fine-Wool Sheep. Frontiers in Veterinary Science 9

Zhang, X., W. Wang, F. Mo, Y. La, C. Li, and F. Li. 2017. Association of residual feed intake with growth and slaughtering performance, blood metabolism, and body composition in growing lambs. Scientific Reports 7(1):1-11.

Zhang, Y., X. Zhang, F. Li, C. Li, G. Li, D. Zhang, Q. Song, X. Li, Y. Zhao, and W. Wang. 2021b. Characterization of the rumen microbiota and its relationship with residual feed intake in sheep. Animal 15(3):100161.

CHAPTER THREE

METHODS

All procedures and protocols utilizing these animals were approved by Montana State University Animal Care and Use Committee (Protocol #2020-AA13).

Study Population

Purebred and NSIP-enrolled Targhee (n=40) and Rambouillet (n=41) ewe lambs born in 2021 were enrolled from the Montana Agricultural Experiment Station flock. All procedures were performed at Montana State University's Fort Ellis research farm in Bozeman, Montana. Lambs were selected to exhibit variation in each breed's post-weaning weight (PWWT) EBVs in our study population. Tissue sample units were taken from the ear of selected ewe lambs at birth and sent to (Neogen Geneseek, Lincoln, NE) for genotyping on the Ovine 50k SNP chip once enrolled in the study.

Two experimental periods were conducted to determine daily feed intake and average weight gain of the ewe lambs at different physiological stages. Each experimental period used the GrowSafe^{TM} feed-bunk system. The GrowSafe^{TM} system weighs feed in the bunk and records the RFID tags of the lambs that enter the head gate. It then takes the weight of feed that leaves the feed bunk in that bout of eating and assigns it to the ewe. Elevated platforms were constructed to allow sheep access to GrowSafe^{TM} beef cattle head feed gates. Feed bars were placed in the feed trough gate to limit the entrance so that only one animal could access the feed bunk at any given time. Platforms were also placed into the grow safe bunks to create an artificial elevated bunk floor so that the ewes could reach feed.

Experimental Period 1

On August 30th, 2021, a 56-day feeding experimental period began consisting of a 14-day adaption and a 42-day data collection period. Forty-two days is a standard benchmark for measuring feed efficiency in sheep (Cammack et al., 2005). Ewe lambs (121 ± 5 days old) were separated into four pens stratified by breed and initial body weight. Each pen contained two GrowSafe™ feed bunks. Ewe lambs were given ad libitum access to an alfalfa-based pellet and water (Table 1). During the adaptation period, six ewe lambs were removed from the study due to poor adaptation of the GrowSafe™ system, leaving 74 ewes left in study. Poor adaption was indicated by loss of body weight or failure to eat from the GrowSafe™ feed bunks for more than two consecutive days. Ewe lambs were weighed at the beginning and end of the experimental period, as well as weekly throughout the experimental period. Feed samples were taken to a commercial laboratory (Dairy One, Ithaca, NY) for nutrient analysis. Rumen samples were additionally taken five days before the end of the experimental period via an esophageal tube, placed into plastic containers, and frozen at –20° C until DNA extraction.

Experimental Period 2

On April 15th, 2022, a second feeding experimental period began with 70 ewes from the same set of animals from experimental period one, consisting of a 10-day adaption period and a 42-day data collection period. A 10-day adaption period was determined to be enough time as the ewes have already experienced the GrowSafe™ system. Four ewes were removed from study between experimental periods due to illness and inability to retrieve them on range. Yearling ewes (355 ± 5 days old) were separated into two pens stratified by breed and initial body weight.

Each pen contained four GrowSafe^{TM} feed bunks. Ewes were given ad libitum access to an alfalfa hay-based maintenance diet and water (Table 1). Yearling ewes were weighed at the beginning, end, and weekly throughout the experimental period. Feed samples were taken to a commercial laboratory (Dairy One, Ithaca, NY) for nutrient analysis. Rumen samples were additionally taken five days before the end of the experimental period via an esophagus tube, placed into plastic containers, and frozen at –20° C.

Diet Compositions

The chemical compositions of alfalfa pellet and hay diets are listed in Table 1.

Feed Intake and Weight Analysis

Daily feed intakes for ewes were exported by using the Process Intakes and Export Behavior Data routine of the GrowSafe^{TM} Data Acquisition software. The average daily gain of individual ewes was calculated by calculating the slope of a linear regression of weight recorded over the experimental period. Regression coefficients of this linear regression were then used to calculate mid-test body weight (MTBW). Mid-trial metabolic body weight (MBW) was then calculated using $MBW = (MTBW)^{.75}$. Raw feed intake data was transformed into a dry matter intake (DMI) basis. Daily DMI estimates were then modeled via linear regression of each ewe's dry matter feed intake over the days of the experimental period. All feed intake data analysis was performed using R 4.2.1 and R-Studio Build 461 (R Core Team, 2017; R Studio Team, 2023).

Expected feed intake was modeled for each ewe through the following equation:

$$Expected\ Feed\ Intake = DMI\ regression\ intercept + B1 * MBW + B2 * ADG + error.$$

Linear regressions on DMI over ADG and MTBW were then calculated to determine coefficients

B1 and B2, respectively. Ewe residual feed intake (RFI) was then calculated for each ewe by taking the difference between their actual feed intake and expected feed intake.

Linear regressions were made between ADG, RFI, DMI, MTBW, and PWWT EBVs. Linear regressions were made based on the model $Y = \beta X$ where Y is a dependent phenotype, X is a different explanatory phenotype, and β is the slope coefficient between the explanatory phenotype and the dependent phenotype. Linear regressions were performed repeatedly to make all combinations between ADG, RFI, DMI, MTBW, and PWWT EBV phenotypes. Additional linear regressions were made between the 1st and 2nd experimental period's ADG, RFI, DMI, and MTBW. The breed was not incorporated into the model as initial T-tests between breeds showed no differences in ADG, RFI, DMI, or MTBW ($P > 0.05$). Associations with $P < 0.05$ were found to be statistically significant.

Genomic Analysis

Sixty-seven animals were genotyped on the Neogen Geneseek 50k Ovine GGP SNP chip at Geneseek (Lincoln, NE). Genetic data retrieved by Neogen Geneseek was then uploaded into Golden Helix SNP and Variation Suite software (Bozeman, MT) for processing and analysis. Quality controls were applied, and SNPs were removed if they had a call rate of less than 90%, a minor allele frequency of less than 0.05, and had not more than two alleles per SNP. These thresholds removed 7,033 SNPs leaving 44,855 SNPs available for subsequent analysis.

Dry matter intake, ADG, RFI and MTBW phenotypes from both experimental periods were added to the remaining genotypic data creating a data set for analysis. A genomic kinship

Table 1. Chemical Composition of Diets used in Experimental Period One and Two.

Components[1]	Period One: Alfalfa Pellets	Period Two: Alfalfa Hay
% Dry Matter	90.6	89.7
% Crude Protein	17.7	17.4
% Available Protein	16.3	15.9
% Adjusted Crude Protein	17.3	16.9
Soluble Protein % CP	44	45
Degradable Protein % CP	78	76
% NDICP	4.1	2.9
% ADF	32.7	31.1
% aNDF	41.7	37.2
% Lignin	7.2	6.8
% NFC	31.3	33.4
% Starch	1.6	1.5
% WSC (Water Sol. Carbs.)	10.0	10.6
% ESC (Simple Sugars)	7.9	8.4
% Crude Fat	1.8	2.2
% Total Fatty Acids	1.2	1.51
% RUFAL	0.45	0.59
% Ash	7.50	9.87
% TDN	61	63
Mcal DE /Kg, 1x	2.77	2.76
Mcal ME /Kg, 1x	2.35	2.34
Mcal NEL /kg, 3x	1.33	1.33
Mcal NEM /kg, 3x	1.40	1.4
Mcal NEG /Kg, 3x	0.82	0.82
Relative Feed Value	142	162
% Calcium	1.62	1.35
% Phosphorus	0.26	0.26
% Magnesium	0.33	0.22
% Potassium	1.31	2.55
% Sulfur	0.25	0.28
% Chloride Ion	0.67	0.61
IVTD 30hr, % of DM	72	77
NDFD 30hr, % of NDF	32	37
kd, % /hr	2.82	3.72
% Lysine	0.90	0.88
% Methionine	0.28	0.27

[1] *Components are reported on a Dry Matter basis*

matrix was also computed using EMMAX incorporated as a random effect in Genome-Wide Association Study (GWAS). A Bonferroni corrected P-value of less than 0.05 was used to denote genome-wide significance for any trait. The UCSC genome browser database was used for the functional annotation of significant SNPs. SNPs that were within an annotated known gene were considered to be candidate genes.

Microbial Analysis

Once microbial samples from both experimental periods were collected, DNA was extracted from rumen fluid samples using Qiagen's QIAamp Power Fecal Pro DNA Kit following the manufacturer's recommendations. DNA quality was assessed using a Generation 5 Biotek scanner and the Epoch software. DNA extraction was repeated if DNA concentration was less than 20 ng/µl or if the 260/280 ratio was not within 1.6-2. 114 DNA samples, 57 and 57 from experimental periods one and two, were subsequently shipped to Novagene for 16s rRNA sequencing of the V3-V4 region. The sequenced DNA was then compared to known microbial species 16s rRNA molecules. If an amplified DNA strand matched a known bacterial species' 16s rRNA region by 97%, the template DNA was determined to be from the same species.

Average species profiles, observed species number, Simpson, Shannon, and calculated Unifrac distance were compared between experimental periods one and two through two-tailed T tests. T-test differences were found significant at $P<0.05$. A PCoA, PCA, and NMDS were also performed between periods to show the distance between microbial samples. Anosim, MRPP, Andonis, and Amova tests were also performed between periods to detect differences in the experimental periods' microbial community compositions.

Literature Cited

Cammack, K., K. A. Leymaster, T. Jenkins, and M. K. Nielsen. 2005. Estimates of genetic parameters for feed intake, feeding behavior, and daily gain in composite ram lambs. Journal of Animal Science 83(4):777-785.

R Core Team. 2017. R: A language and environment for statiscal computing. R Foundation for Statical Computing, Vienna, Austria. URL https://www.R-project.org/.

R Studio Team. 2023. RStudio: Integrated Development for R. RStudio, PBC, Boston, MA.

CHAPTER FOUR

RESULTS

Trait Associations

The median age for the ewes was 121 ± 5 and 355 ± 5 days at the start of the experimental period for trials one and two, respectively. Mid Trial Body Weight, MBW, and ADG differed significantly between the two experimental periods (*P*<0.05, table 2). Sheep in experimental period two had an average MTBW 7.4kg higher than experimental period one. Differences in production measurements are listed in Table 1. Ewes in trial one were growing with 0.26 ± 0.12 kg gain per day compared to trial two, which had a -0.02 ± 0.16 kg per day gain indicating they were neither growing nor losing weight, indicative of a maintenance physiological state. Linear associations were present between experimental period one and experimental period two DMI, MTBW, and MBW of the sheep (*P*<0.05). Still, they were not found between trial one and two RFI nor ADG (*P*>0.05).

Table 2. Production Trait Measurements

Trait	Period One	Period Two	P Value[1]
Age (Days)	121 ± 5	355 ± 5	>0.05
MTBW kg	27.8 ± 4.39	35.2 ± 8.02	<0.05
MBW kg	14.5 ± 1.70	35.2 ± 8.02	<0.05
ADG kg/day	0.26 ± 0.12	-0.02 ± 0.16	<0.05
DMI kg/day	2.01 ± 0.38	2.18 ± 1.62	>0.05
RFI kg/day	0.00 ± 0.24	0.00 ± 1.34	>0.05

[1] P Values of >0.05 indicate significant differences between experimental period one and experimental period two traits using a two-tailed t-test.

Residual feed intake was not associated with PWWT EBVs nor ADG in either trial one or two (P>0.05). The ADG was not associated with DMI in either trial one or two (P<0.05). Post-weaning weight estimated breeding values were not associated with ADG in either trial one or two (P>0.05) but were associated with DMI, MTBW, and MBW (P<0.05). Dry matter intake and MTBW were also associated in trials one and two (P<0.05). The results of linear associations between different traits are summarized in Table 3.

Table 3. Linear Associations Between Traits

Experimental period	Trait 1	Trait 2	P Value[1]	R^2 [2]
Period One	PWWT EBV	RFI	> 0.05	
	ADG	RFI	> 0.05	
	MTBW	RFI	> 0.05	
	DMI	ADG	> 0.05	
	PWWT EBV	ADG	> 0.05	
	PWWT EBV	MTBW	< 0.05	.19
	PWWT EBV	MBW	< 0.05	.19
	PWWT EBV	DMI	< 0.05	.07
	MTBW	DMI	< 0.05	.31
	RFI	DMI	< 0.05	.38
Period Two	PWWT EBV	RFI	> 0.05	
	ADG	RFI	> 0.05	
	DMI	ADG	> 0.05	
	MTBW	RFI	> 0.05	
	PWWT EBV	ADG	> 0.05	
	PWWT EBV	MTBW	< 0.05	.14
	PWWT EBV	MBW	< 0.05	.19
	PWWT EBV	DMI	< 0.05	.08
	MTBW	DMI	< 0.05	.31
	RFI	DMI	< 0.05	.67

[1] P values <0.05 indicate a linear association between trait 1 and trait 2.

[2] R^2 values are given to show the strength of the relationship between linear model and dependent variables within the established linear associations.

Genome-Wide Association Study

A single SNP association was found with the second trial RFI and DMI at Oar2:68812505 when MTBW was a covariable (Bonferroni adjusted $P < 0.05$, Fig 1 and 2). The associated SNP is located in DNA that has homologous DNA in non-sheep reference genomes that has been annotated to contain the Doublesex and Mab-3 Related Transcription Factor 2 (*DMRT2*) gene. Doublesex and Mab-3 Related Transcription Factor 2 enables identical protein binding activity and sequence-specific DNA binding activity and can regulate gene expression of other genes (NCBI, 2023). The *DMRT2* gene has not been associated with DMI or RFI in sheep before. In Bos indicus cattle, *DMRT2* has been associated with residual body weight gain; however, this is *DMRT2* only known association with the feed efficiency (Santana and GOMES, 2015). No direct associations between *DMRT2* and DMI have been established, however, *Dmrt2* has been associated with embryonic development of rib cage and sternum which could have impacts on DMI (Ono et al., 2021). Importantly, limitations on study size likely made this GWAS underpowered. A larger sample size GWAS will be needed to confirm the current associations and possibly detect other markers related to our phenotypes.

Microbial Associations

Sheep in trial two had a higher overall observed species number than sheep in trial one. Alpha diversity differed between the two periods, with maintenance having a higher Simpson index than growth ($P < 0.05$). Beta diversity also differed between trials and was increased in the rumen microbiomes of sheep in growth, comparing both weighted and unweighted unifrac distances ($P < 0.05$). Principal Coordinate Analysis between groups clustered based upon the physiological state of sheep and appear to have population structure based upon the physiological state (Figure 3, Amova *P*<0.05).

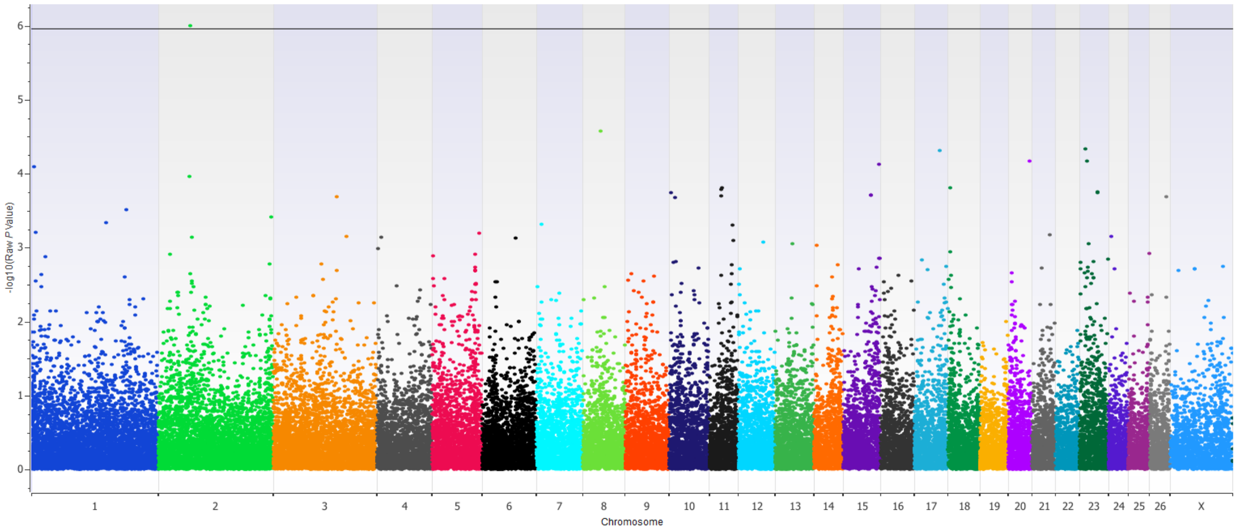

Figure 1. Manhattan Plot of -log$_{10}$(p-values) of the association with experimental period two RFI with MTBW as a covariate. The horizontal black line indicates the Bonferroni corrected threshold of 0.05.

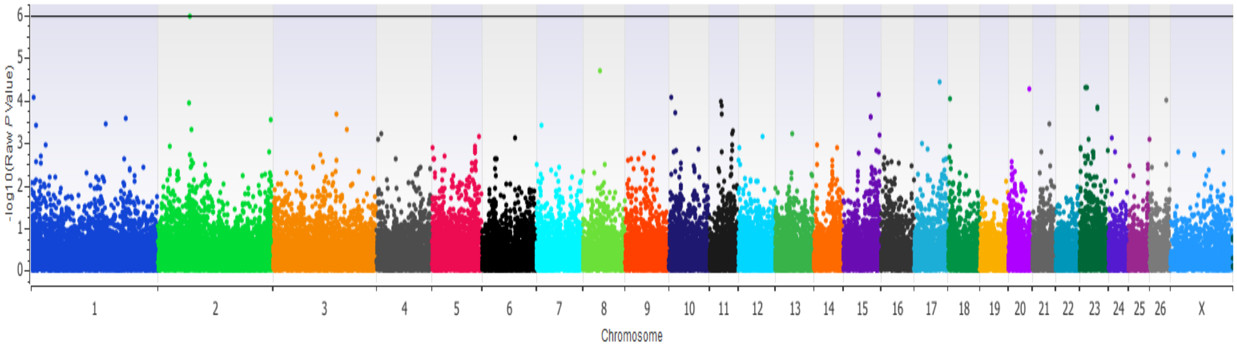

Figure 2. Manhattan Plot of -log$_{10}$(p-values) of the association with experimental period two DMI with MTBW as a covariate. The horizontal black line indicates the Bonferroni corrected threshold of 0.05.

Physiological states appear to have distinct microbial communities, with dissimilarities between growth and maintenance being larger than those within growth and maintenance (Anosim $R^2=.88$, $P<0.05$).

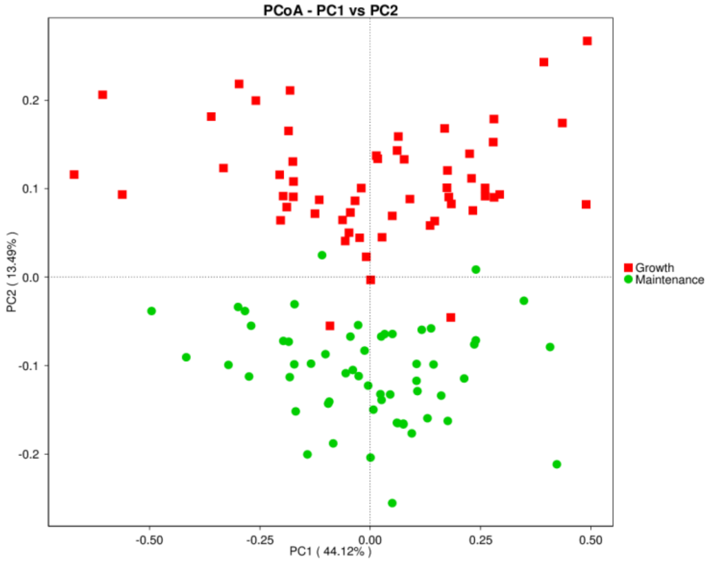

Figure 3: PCoA of Microbiomes. The microbiomes cluster by physiological state with those sampled during growth (red) differentiating from those sampled during maintenance (green)

Specific phyla, classes, orders, families, genera, and species differed between the maintenance and growth physiological states of microbiomes. Phylum, class, order, family, genus, and species taxonomic levels had 17, 34, 73, 110, 251, and 131 differently abundant taxa, respectively. Importantly greater than 90% relative abundance of individual species were put in the “Other” category likely because their sequences were not characterized to a known species. This limited the amount of divergently abundant species found compared to other taxon with less high requirements such as genera. The top 15 relatively divergent microbes of each taxon are recorded in Table 3.

Important phyla that were relatively increased in the maintenance group included Fibrobacterota and Thermoplasmatota (Table 4, $P<0.05$). In contrast, the only phyla that increased in growth were Euryarchaeota (Table 4, $P<0.05$).

Classes that relatively increased during maintenance include Bacilli, Fibrobacteria, and Thermoplasmata (Table 4, $P<0.05$). In addition, a class that increased during growth included Methanobacteria (Table 4, $P<0.05$).

Orders with relative abundances increased in maintenance microbiomes include Bacillales and Oscillospirales (Table 4, $P<0.05$). In addition, orders that increased in growth include Lachnospirales and Methanobacteriales (Table 4, $P<0.05$).

Families increased in maintenance include Bacillaceae, Bacteroidales F082, and Oscillospirales UCG-010 (Table 4, $P<0.05$). Families with increased relative abundance in growth microbiomes include Bacteroidales p-251-o5, Methanobacteriaceae, and Lachnospiraceae (Table 4, $P<0.05$).

Genera that increased abundance when sheep were in maintenance were *Butyrivibrio, Bacillus, Rikenellaceae RC9 gut group, Bacteroidales F082 F082, Anaeroplasma, Oscillospiraceae UCG-002, Staphylococcus, Ruminococcus,* and *Lachnospiraceae XPB1014* group (Table 4, $P<0.05$). Genera that were increased in abundance when sheep were in growth include *Oscillospiraceae NK4A214 group, Bacteroidales p-251-o5 p-251-o5, Methanosphaera*, and *Lachnospiraceae NK3A20 group* (Table 4, $P<0.05$).

Species that were higher in relative abundance in maintenance included *Treponema bryantii, Lachnospiraceae bacterium, Treponema ruminis, Bacteroidales bacterium, Thermoactinomyces khenchelensis, Bacillus thermolactis, Bacillus clausii, Butyrivibrio sp.* and

Prevotella ruminicola (Table 4, $P<0.05$). The species that was more abundant in growth was *bacterium WCE3006* (Table 4, $P<0.05$).

Table 4. Relative Abundances of Bacterial and Archaeal Taxa in Maintenance or Growth State Sheep

Taxa	Growth Relative Abundance	sd	Maintenance Relative Abundance	sd	*P* Value
Phylum					
Euryarchaeota	5.31%	3.61%	3.31%	2.54%	0.000870709
Proteobacteria	0.67%	0.76%	1.84%	3.49%	0.016262492
Actinobacteriota	2.23%	1.41%	3.07%	2.05%	0.012419831
Cyanobacteria	0.20%	0.38%	0.71%	0.83%	6.28E-05
Spirochaetota	0.46%	0.41%	0.82%	0.53%	8.46E-05
Desulfobacterota	0.18%	0.10%	0.40%	0.15%	5.05E-14
Patescibacteria	0.07%	0.04%	0.22%	0.12%	1.44E-12
Synergistota	0.07%	0.10%	0.19%	0.13%	8.90E-08
Fibrobacterota	0.15%	0.21%	0.26%	0.22%	0.009229816
Thermoplasmatota	0.04%	0.07%	0.12%	0.10%	2.16E-05
Verrucomicrobiota	0.02%	0.02%	0.09%	0.07%	3.22E-09
Elusimicrobiota	0.01%	0.01%	0.05%	0.03%	7.41E-14
Bdellovibrionota	0.00%	0.01%	0.01%	0.02%	0.002567902
Nitrospirota	0.00%	0.00%	0.00%	0.01%	0.031230902
Planctomycetota	0.00%	0.01%	0.01%	0.01%	0.042039066
Class					

Table 4 Continued

Methanobacteria	5.31%	3.61%	3.31%	2.54%	0.000871
Clostridia	48.60%	10.49%	43.20%	7.06%	0.001704
Bacilli	2.72%	1.16%	6.38%	2.91%	3.47E-13
Vampirivibrionia	0.12%	0.16%	0.68%	0.83%	3.75E-06
Actinobacteria	0.36%	0.57%	0.92%	0.97%	0.000306
Spirochaetia	0.45%	0.41%	0.80%	0.53%	0.00018
Alphaproteobacteria	0.25%	0.46%	0.52%	0.76%	0.025873
Desulfovibrionia	0.17%	0.10%	0.37%	0.14%	1.53E-14
Synergistia	0.07%	0.10%	0.19%	0.13%	8.90E-08
Fibrobacteria	0.15%	0.21%	0.26%	0.22%	0.00923
Acidimicrobiia	0.03%	0.05%	0.11%	0.26%	0.019584
Gracilibacteria	0.03%	0.03%	0.11%	0.10%	4.09E-07
Saccharimonadia	0.04%	0.03%	0.11%	0.06%	6.62E-13
Thermoplasmata	0.04%	0.07%	0.12%	0.10%	2.16E-05
Kiritimatiellae	0.01%	0.02%	0.06%	0.07%	8.50E-07
Order					
Burkholderiales	0.16%	0.21%	0.32%	0.27%	0.000319766
Thermoactinomycetales	0.02%	0.01%	0.21%	0.14%	2.44E-13
Desulfovibrionales	0.17%	0.10%	0.37%	0.14%	1.53E-14
Pseudonocardiales	0.03%	0.02%	0.25%	0.18%	3.02E-13
Aeromonadales	0.09%	0.13%	0.32%	0.40%	0.0000701
Clostridia	0.32%	0.13%	0.63%	0.20%	1.18E-16

Table 4 Continued

Spirochaetales	0.45%	0.41%	0.80%	0.53%	0.00017972
Gastranaerophilales	0.12%	0.16%	0.68%	0.83%	0.00000375
Staphylococcales	0.05%	0.03%	0.77%	0.76%	1.28E-09
Acholeplasmatales	0.66%	0.64%	1.54%	1.04%	0.000000436
Bacillales	0.20%	0.21%	2.14%	1.84%	8.52E-11
Oscillospirales	9.58%	2.59%	11.66%	2.43%	0.0000234
Lachnospirales	26.05%	7.03%	17.06%	4.03%	6.93E-13
Methanobacteriales	5.31%	3.61%	3.31%	2.54%	0.000870709
Erysipelotrichales	1.41%	0.60%	0.99%	0.27%	0.00000777
Family					
Erysipelotrichaceae	1.10%	0.48%	0.72%	0.22%	7.44E-07
Bacteroidales p-251-o5	1.51%	1.58%	0.79%	0.53%	0.001739055
Methanobacteriaceae	5.31%	3.61%	3.30%	2.54%	0.000871306
Lachnospiraceae	25.87%	6.99%	16.92%	4.01%	6.64E-13
Bacillaceae	0.19%	0.19%	2.10%	1.81%	1.06E-10
Ruminococcaceae	1.77%	0.85%	2.81%	0.96%	1.09E-08
Rikenellaceae	4.35%	2.19%	5.39%	2.29%	0.014997848
Bacteroidales F082	1.18%	0.74%	2.08%	0.76%	3.29E-09
Acholeplasmataceae	0.66%	0.64%	1.54%	1.04%	4.36E-07
Staphylococcaceae	0.05%	0.03%	0.77%	0.76%	1.28E-09
Gastranaerophilales	0.12%	0.16%	0.68%	0.83%	3.75E-06

Table 4 Continued

Spirochaetaceae	0.45%	0.41%	0.80%	0.53%	0.00017972
Oscillospirales UCG-010	0.25%	0.10%	0.57%	0.20%	3.49E-17
Hungateiclostridiaceae	0.32%	0.13%	0.63%	0.20%	7.98E-17
Bacteroidales BS11 gut group	0.02%	0.02%	0.27%	0.33%	3.94E-07
Genera					
Oscillospiraceae NK4A214 group	6.04%	2.02%	5.35%	1.56%	0.042468695
Bacteroidales p-251-o5 p-251-o5	1.51%	1.58%	0.79%	0.53%	0.001739055
Methanosphaera	1.74%	1.27%	0.51%	0.38%	1.70E-09
Blautia	1.71%	0.67%	0.45%	0.27%	3.67E-21
Acetitomaculum	4.03%	1.84%	1.47%	0.76%	7.73E-15
Lachnospiraceae NK3A20 group	9.75%	4.15%	2.52%	1.08%	3.78E-19
Butyrivibrio	1.24%	0.70%	2.78%	1.34%	2.70E-11
Bacillus	0.16%	0.19%	1.69%	1.42%	5.76E-11
Rikenellaceae RC9 gut group	4.31%	2.19%	5.35%	2.28%	0.01450155
Bacteroidales F082 F082	1.18%	0.74%	2.08%	0.76%	3.29E-09
Anaeroplasma	0.57%	0.57%	1.36%	0.92%	0.000000334
Oscillospiraceae UCG-002	0.17%	0.09%	0.90%	0.28%	1.37E-28
Staphylococcus	0.04%	0.02%	0.68%	0.62%	1.61E-10
Ruminococcus	1.30%	0.63%	1.87%	0.69%	0.00000911
Lachnospiraceae XPB1014 group	0.38%	0.18%	0.95%	0.46%	5.25E-13
Species					

Table 4 Continued

Others	96.58%	1.85%	91.21%	2.11%	3.58E-27
Bacterium WCE3006	0.09%	0.07%	0.01%	0.01%	4.79E-15
Treponema bryantii	0.05%	0.06%	0.12%	0.09%	0.033817508
Lachnospiraceae bacterium	0.09%	0.05%	0.16%	0.06%	0.037781582
Treponema ruminis	0.02%	0.02%	0.11%	0.12%	0.038730396
Bacteroidales bacterium	0.01%	0.01%	0.11%	0.10%	0.042587757
Saccharopolyspora rectivirgula	0.02%	0.01%	0.15%	0.11%	0.043792372
Thermoactinomyces khenchelensis	0.01%	0.01%	0.15%	0.11%	0.044472583
Staphylococcus equorum	0.01%	0.01%	0.16%	0.18%	0.044472583
Bacterium MC2003	0.04%	0.03%	0.24%	0.21%	0.044472583
Bacterium AC2043	0.25%	0.29%	0.48%	0.13%	0.044472583
Bacillus thermolactis	0.03%	0.02%	0.42%	0.38%	0.045781003
Bacillus clausii	0.03%	0.01%	0.44%	0.40%	0.048007197
Butyrivibrio sp.	0.75%	0.48%	1.41%	0.76%	0.048007197
Prevotella ruminicola	0.26%	0.45%	1.98%	1.36%	0.049256778

Literature Cited

NCBI. 2023. Dmrtc2 doublesex and mab-3 related transcription factor like family C2 [Mus musculus (house mouse)]. In: N. I. o. Health (ed.).

Ono, K., K. Hata, E. Nakamura, S. Ishihara, S. Kobayashi, M. Nakanishi, M. Yoshida, Y. Takahata, T. Murakami, and S. Takenoshita. 2021. Dmrt2 promotes transition of endochondral bone formation by linking Sox9 and Runx2. Communications Biology 4(1):326.

Santana, M. H. d. A., and R. d. C. GOMES. 2015. Genome-wide association with residual body weight gain in Bos indicus cattle.

CHAPTER FIVE

DISCUSSION

Trait Associations

The lack of an association between post-weaning weight estimated breeding values (PWWT EBV) and residual feed intake (RFI) indicates that producers are not selecting on feed efficiency when they are selecting towards higher weaning weight lambs. This result is further supported by the lack of association between RFI and mid trial body weight (MTBW). Moreover, metabolic body weight (MBW) has not been associated with RFI in sheep in the past (Zhang et al., 2017; Zhang et al., 2021). Selection towards larger lambs at weaning does not disproportionately increase the metabolic need compared to the body size increase.

While the PWWT EBVs are positively associated with MTBW, they are not associated with ADG. There are strong phenotypic and genetic correlations between weaning weight and ADG, 0.94 and 0.55 (Rashidi et al., 2008; Malik et al., 2016). Environmental factors may have created enough variance in the current study's sheep's MTBW and ADG to where PWWT EBVs were not associated with ADG. However, since weaning weight is correlated with mature size and body weight is associated with ADG, when producers use PWWT EBVs to make selection decisions, they should expect to see larger, faster growing lambs that reach higher weights at weaning (Herd et al., 1993). Moreover, due to the correlation between mature size and weaning weight selection using PWWT EBVs will lead to larger ewes in their flock (Herd et al., 1993). As there is a positive relationship between dry matter intake (DMI) and MTBW, the larger ewes produced by this selection will have higher feed intakes. The positive relationship between

PWWT EBVs and DMI further supports that increases in feed intakes are related to larger body weight. Borg 2004 suggested that dry matter requirements increased 3.34 kg per base ewe bred from selection to improve weaning weight (Borg, 2004). Increasing DMI in sheep for producers who live in forage-limited areas resulted in a reduction of flock size to meet nutrient requirements, which limits revenue by reducing future lamb crop sizes (Borg, 2004). Selection towards higher extremes in PWWT EBVs may limit the herd size in operations in resource limited areas. However, Borg 2004 suggests that increasing preweaning growth is economically advantageous due to an increased lamb value at market (Borg, 2004). In Borg's simulation, most negative economic effects from increasing weaning weight were a consequence of larger ewes, due to the positive correlation between yearling weight and weaning weight. The economic value of weaning weight selection was approximately $0.80 higher in non-discounted market and the impact of yearling weight was further changed depending upon resource availability and forage price (Borg, 2004). Selection towards weaning weight may be more advantageous when forage cost is low, feed resources are not limited, and markets do not discount heavy weight lambs. The use of PWWT EBVs for selection alone may not be advantageous to resource limited producers due to the higher DMI of feedstuff as mature ewes. However, selection using PWWT EBVs may be advantageous for some producers if incorporating it into a selection index with a large negative weight for yearling weight.

R^2 values of linear models between traits remained roughly the same between experimental periods, apart from the association between RFI and DMI, where R^2 values increased from .38 in experimental period one to .67 in experimental period 2. The increase in association strength may be due to the lack of ADG in experimental period 2. Suppose feed intake is increased in one sheep compared to another sheep while they both are not gaining weight. In that case, it is easy to identify the one who is eating less feed efficiently, associated in our study with a higher RFI. When two sheep eat different amounts but gain weight independently, it is harder to model the difference in feed efficiency using DMI as the single variable. The difference in association strength when modeling feed efficiency between maintenance and growth indicates that while ADG is not directly associated with RFI, ADG likely does impact feed efficiency in growing animals. Therefore, the lack of association between RFI and ADG is likely an artifact of how RFI is calculated.

The lack of association of RFI and DMI between experimental periods indicates that RFI and DMI are not repeatable between growth and maintenance physiological states. Redden et al., 2011 found similar results where RFI and DMI were not correlated between sheep in post-weaning growth and at yearling age (Redden et al., 2011). In cattle, however, RFI was shown to be repeatable between growing and finishing phases, where they were fed a high concentrate or a grass silage plus concentrate diet (Lahart et al., 2020). Forage similarity was suggested to cause similar intake and digestive characteristics between the two diets, leading to correlated RFI (Lahart et al., 2020). In our study, the chemical composition and forage source was similar suggesting that the difference in RFI between experimental periods is independent of diet. The reranking of RFI has been shown as animals mature (Lahart et al., 2020). Since ewes in the second experimental period were at yearling age, they were more mature than ewes in the first experimental period, which may have played a part in reranking the RFI of the ewes. More work is needed to understand how the biological mechanisms that affect RFI change between growth and maintenance physiological periods in sheep.

Mid-trial body weight, MTBW, ADG, and MBW are associated between growth and maintenance, indicating that these measurements are predictable in the two physiological states. This relationship is important as MTBW, ADG, and MBW only depict one of the five physiological processes crucial in determining livestock feed efficiency, namely metabolism (Herd and Arthur, 2009). More work must be done to indicate the relationship between digestion of feed, physical activity, and thermoregulation between life stages to determine the overall predictiveness of feed efficiency between time points. As DMI was not predictive between life stages, it suggests that at least one of the five major physiological processes associated with feed efficiency is not predictable in sheep, indicating there likely will always be some inaccuracy when using feed efficiency measurements created from data in one physiological state later in another physiological state. Furthermore, the lack of association of RFI between experimental periods indicates a lack of fitness of RFI to compare feed efficiency between multiple time points. Therefore, if producers select feed efficiency using RFI in the future, they should consider RFI during growth as a different trait than RFI while at maintenance.

Doublesex and Mab-3 Related Transcription Factor 2 Gene

The function of the Doublesex and Mab-3 Related Transcription Factor 2 (*Dmrt2*) gene has yet to be explored within sheep. *Dmrt2*'s function has been studied primarily in mice. Doublesex and Mab-3 Related Transcription Factor 2 is part of the *Dmrt* gene family known to regulate myogenesis and skeletal development during embryogenesis (Bellefroid et al., 2013).

In mice myogenesis, the *Dmrt2* gene regulates the *Pax3-Dmrt2-Myf5* regulatory cascade (Sato et al., 2010). *Dmrt2* regulates myogenesis in the myotomes of embryos by activating the *Myf5* gene (Sato et al., 2010). Expression of the *Dmrt2* gene creates the Dmrt2 protein, which binds to and promotes the expression of *Myf5*, which in turn initiates myogenesis by forming the early epaxial myotome (Sato et al., 2010). Likewise, a lack of *Dmrt2* expression has been shown to disrupt the early formation of the myotome and the subsequent formation of skeletal muscle (Sato et al., 2010). Differences in *Dmrt2* expression have resulted in the diffusion of myogenic markers in the myotome and correlate with a lack of laminin, a required ligand in the formation of the myotome (Kwang Won et al., 2006). In sheep, the role of the *Myf5* gene is like that in mice and cattle in that it promotes and regulates skeletal muscle growth (Wang et al., 2017). If the mouse-derived mechanism occurs between *DMRTt2* and *Myf5* in sheep, *DMRTt2* may promote muscle fiber development in sheep. The hypothesized increased development of muscle fiber may allow for more accessible and less costly muscle growth and maintenance of that growth, increasing sheep's feed efficiency. Furthermore, if *DMRTt2* promotes the maintenance of muscle fibers, less metabolic energy may be needed by the sheep, which in turn may decrease the feed intake of the sheep.

During the skeletal development of mice, the *Dmrt2* gene works by regulation and functional collaboration of the *Runx2* gene in the *Sox9-Dmrt2-Runx2* cascade in pre-hypertrophic chondrocytes (Ono et al., 2021). *Runx2* regulates the Indian hedgehog signaling molecule (*Ihh*) gene in mice responsible for chondrocyte hypertrophy (Yoshida et al., 2004). Overexpression of *Dmrt2* induced a greater expression of *Ihh*, thereby promoting bone growth in the mice (Ono et al., 2021). *Dmrt2* expression is a promoter of *Runx2* expression and can therefore regulate *Ihh* expression. Using long bovine bones, hedgehog signaling was found to play a role in significantly reducing bone destruction, indicating its importance in keeping bones healthy and intact (Zhang et al., 2020). In sheep, hedgehog signaling pathways govern epithelial structure and disruptions to said pathways can cause midline abnormalities (Chen, 2016; Shaomei et al., 2020); they may also play a role in skeletal bone development and maintenance, like in mice and cattle. Since Hedgehog signaling pathways likely play a role in skeletal growth in sheep, the *DMRT2* gene may play a similar regulatory role to that in mice. This mechanism may partially explain the association between RFI and *DMRT2*. As *DMRT2* promotes easier maintenance of bones, increases in *DMRT2* would make maintaining bones in sheep easier, and overall, the sheep likely would not have to expend as much energy. Alternatively, *DMRT2*'s association with the embryonic development of mice's sternum and ribcage likely could impact DMI by increasing body size (Ono et al., 2021). In mice with *Dmrt2* knockouts, mice exhibited dwarf phenotypes with small sternums and ribcages, indicating that greater amounts of *DMRT2* expression may lead to larger mice (Ono et al., 2021). If higher expression of *DMRT2* increases the body size of sheep, it would also increase the energy requirements of the sheep and therefore may increase DMI. Further work is needed to fully understand the role of *DMRT2* in impacting DMI in sheep.

Maintenance and Growth Microbial Associations

The uniqueness of the microbiomes associated with either growth or maintenance was shown in how they differed and clustered independently (Figure 1). The anosim test suggests there are much larger differences between microbiomes associated with either physiological state than within each physiological state's microbiome, supporting the idea that the two experimental periods' microbiomes have unique microbial community compositions. Moreover, the two microbial communities were characterized by differing alpha and beta diversities. Increased alpha diversity within the maintenance sheep is likely due to the higher species count found in those sheep's microbiomes. Increased microbial diversity in older ewes has been previously shown (Yin et al., 2021). Increased beta diversity in growth experimental period ewes compared to maintenance experimental period ewes indicates that the rumen microbiomes are becoming more like each other as the animal ages. Yin et al., 2021, also concluded that within-group similarity increases between sheep microbiomes age-dependently (Yin et al., 2021). Changing diets, especially at weaning, as a sheep grows appears to be a strong promoter of increased microbial diversity and microbial community maturity (Wang et al., 2019; Yin et al., 2021). Since our lambs were weaned at the start of the first experimental period, their microbiomes were likely less mature, which led to a lessened alpha diversity and a more deviating microbiome community composition. As the sheep were introduced to hay and range graze diet between experimental periods and then an alfalfa hay diet during the second experimental period, their microbiomes had more time to be introduced to forages and mature by selection towards fiber digestion. These differences in rumen microbiome maturity led to the uniqueness of microbiomes between the two experimental periods. The relative differences in the high-level taxon, such as phylum and class, further support the uniqueness of different microbiomes between growth and maintenance sheep.

Methanogens, archaea that produce methane under anaerobic conditions, belongs to the phylum Euryarchaeota (Guo et al., 2005). Increasing Euryarchaeota in the physiological growth state of sheep might have resulted from the different diets between the two physiological stages. Decreasing particle size in anaerobic digestion has been associated with increased methane production (Izumi et al., 2010). Since Euryarchaeota encompasses the methanogens that produce methane, decreased Euryarchaeota in the maintenance experimental period might be due to the larger particle size in the alfalfa hay compared to the pellets. Ortolani et al., compared the fermentation of fibrous feedstuff in a ground or a non-processed form and suggested that grinding of fibrous feedstuff may promote higher rates of fermentation, estimated from larger gas production, and increase nutrient availability (Ortolani et al., 2020). Reduction of particle size may increase nutrient availability by increasing the surface area of feedstuff that microorganisms can reach thereby increasing the hydrolysis of cellulose in said feedstuff (Hu et al., 2004). Moreover, accumulated methane levels were increased in the ground feedstuff in the first 24 hours (Ortolani et al., 2020). This suggests that our pelleted diet may have had greater nutrient availability due to smaller particle size, which may increase the relative amounts of methanogens we found in our growth period microbiomes.

Methanobacteria was the most prevalent archaeal class within the sheep microbiomes for either physiological state, making up 99% and 95% of the archaeal community for growth and maintenance. Similarly, in the order below Methanobacteria, Methanobacteriales was also highly prevalent, making up 99% and 96% of archaeal orders in growth and maintenance. Previous results found the prevalence of Methanobacteria within the Archaeal kingdom to be similarly high at 85% and that Methanobacteriales is a typical prevalent archaeal order in ruminants (Huang and Li, 2018; Malik et al., 2022). The prevalence of Methanobacteria and Methanobacteriales within the archaeal grouping indicates they play a prominent role in methanogenesis in the sheep rumen. The ~2% relative decrease of Methanobacteriales shown between growth and maintenance suggests less methanogenesis in the maintenance sheep. Methane production has been linked to lower feed efficiency in sheep and can account for a 16.1% loss of digestible energy (Randhir Singh et al., 2019; Paganoni et al., 2017). Therefore, growing sheep with higher percentages of Archaea, specifically Methanobacteriales, likely have lower energy efficiency than if they were to have more mature microbiomes like at maintenance.

The genera *Methanosphaera* also increased relatively in growth. Higher proportions of *Methanosphaera*, differing with its higher methanogenic taxon, has been negatively correlated with methane production in dairy cattle, beef cattle, and sheep but has been associated as a marker for reduced methane output (Kittelmann et al., 2014; Shi et al., 2014; Danielsson et al., 2017; Smith et al., 2022). *Methanosphaera* differs from other methanogenic archaea because it produces methane by reducing methanol through methylotrophic methanogenesis (Smith et al., 2022). Methylotrophic methanogenesis has lower H_2 requirements than other types of methane synthesis, making it more energetically advantageous and producing lower amounts of methane (Smith et al., 2022). Thus, an increased *Methanosphaera* percent within the methanogens of a rumen microbiome should decrease the methane production and energy loss of the rumen for a given sheep. However, since *Methanosphaera* still creates methane, it is less energy efficient than non-methane producers. The relative increase in abundance in growth physiological stage sheep of *Methanosphaera* indicates that while the growth state sheep have relatively low methane emitting Methanobacteria, they likely are still producing more methane than the maintenance sheep relative to body size, as the maintenance state sheep have lower Methanobacteria proportions.

Thermoplasmata was the only Archaea class that was increased in maintenance and can perform methanogenesis (Bueno de Mesquita et al., 2023). However, Thermoplasmata's slight relative increase during maintenance compared to the larger relative increase of Methanobacteria in growth suggests the increase in Thermoplasmata is merely a result of a more diversified and mature microbiome and not an indication of increased methanogenesis within the rumen.

The order of Oscillospirales is known to contain the *Oscillospiraceae* genera that perform in cellulolytic processes such as the degradation of cellulose and hemicellulose (Yildirim et al., 2021). Increased amounts of Oscillospirales in the maintenance period of sheep indicate a higher need to break down foodstuff through cellulolytic processes. The difference may be due to a difference in processing between feedstuffs. The alfalfa pellets would already have been broken mechanically down, and therefore there is a lessened need for Oscillospirales in the rumen. Likewise, larger particle sizes in hay may support higher amounts of Oscillospirales. Bals et al. proposed that particle size reduction during pelleting improved sugar yields by increasing cellulose accessibility in enzymatic saccharification within pellets used for biofuels (Bals et al., 2014). This occurred with only a slight decline in hemicellulose (Bals et al., 2014). If sugar availability is similarly increased within alfalfa pellets, the need for Oscillospirales within the rumen may be lowered due to the higher already available sugars. Similarly, genera *Oscillospiraceae UCG-002* and family *Oscillospirales UCG-010*, which fall under the Oscillospirales order, were increased in maintenance. These results further indicate the important role of the Oscillospirales taxon in breaking down feedstuff within the sheep rumen.

The *Oscillospiraceae NK4A214 group*, a genus in the Oscillospirales order, conversely is increased in the growth trial sheep. Previously it has been shown that the *Oscillospiraceae NK4A214 group* increases when going from an alfalfa diet to a wheat straw diet in the lower intestines of sheep (Li et al., 2023). Li et al., 2023, suggested that lessened nutrient availability and recalcitrant structure in the wheat straw caused more species to be employed for codegradation, such as the *Oscillospiraceae NK4A214 group* (Li et al., 2023). Our results differ, in that the *Oscillospiraceae NK4A214 group* was increased in sheep fed a pellet diet that contains might have more easily accessible cellulose that may be easier to degrade.

Bacteroides is a microbial phylum with cellulolytic capacity prevalent in the rumen (Naas et al., 2014). The related genera *Bacteroidales p-251-o5* was found to be increased during growth, whereas the genera *Bacteroidales F082* and *species Bacteroidales bacterium* were increased during maintenance. Increased cellulolytic capacity in maintenance would allow for easier digestion of structural carbohydrates from the hay. However, *Bacteroidales p-251-o5's* association with the growth period and the pelleted diet complicates this finding. Bacteroides contain a large group of microbes that are very genetically diverse (Andrade et al., 2020). Differences found between *Bacteroidales* taxa may be due to the genetic diversity of the *Bacteroidales* order. Differences between associations in methane production in the *Bacteroidales F082 group* and another *Bacteroidales BS11 gut group* have been shown, where the *Bacteroidales F082 group* was negatively associated with methane emissions and *Bacteroidales BS11* was positively associated (Andrade et al., 2020). Further study is needed to understand how different genera and families in Bacteroides functionally differ.

The order Lachnospirales are butyrate-producing bacteria critical for rumen fermentation and healthy gut microbiomes (Ma et al., 2020). Previous studies showed that *Lachnospiraceae*, a family under Lachnospirales, began showing higher abundance after 20 days and positively correlated with butyrate in sheep (Yin et al., 2021). The association of Lachnospirales, *Lachnospiraceae*, and the *Lachnospiraceae XPB1014 group* with the growth period sheep indicates an increased utilization of butyrate in growing sheep. Intraruminal production of butyrate has been suggested to promote rumen epithelial tissue growth (Sakata and Tamate, 1978). The rumen epithelium is crucial to the sheep as it regulates the absorption of volatile fatty acids starting when the sheep start digesting solid feed. Therefore, an increased abundance in Lachnospirales may encourage the growth of rumen epithelial tissue and thus be critical in lambs around weaning.

Ruminococcus and Butyrivibrio are fiber digesting bacterial genera that play crucial roles in the breakdown of complex carbohydrates. *Butyrivibrio sp.* can utilize and break down complex polysaccharides, e.g., xylan and pectin (Bryant and Small, 1956; Hungate, 2013). *Ruminococcus* bacteria can produce cellulases and hemicellulases, which break down the structure of plants into simple sugars (Zhang et al., 2018). The increased prevalence of both *Ruminococcus* and *Butyrivibrio* in the maintenance period suggests there is a higher need for fiber degradation in the alfalfa hay compared to the processed pelleted diet.

The *Treponema* genus contained two species associated with the maintenance period, *Treponema bryantii,* and *Treponema ruminis*. Additionally, the phylum Fibrobacterota and the class Fibrobacteria also relatively increased in maintenance. *Treponema* has been shown to share mutual interaction with Fibrobacteria, where *Treponema* breaks down hemicellulose, and Fibrobacteria provides glucose to Treponema (Xie et al., 2018). The interaction of the two bacterial lines provides a mechanism to break down hemicellulose in both the maintenance and growth diets. While both diets had similar hemicellulose, indicated by similar NDF, the pelleting process may have altered the sugar availability of alfalfa in a similar way to that found during pellet processing for biofuels (Bals et al., 2014). If so, the increased sugar availability in a pelleted diet may decrease the amount of bacteria that can fill the niche of hemicellulose and cellulose degradation, limiting the proportions of Fibrobacterota and Treponema.

Thermoactinomyces khenchelensis has been previously found and described within the soil sediment of terrestrial hot springs but has not been previously found in animal hosts (Mokrane et al., 2016). The species isolate grew in fructose, glucose, and maltose media and grew with pH ranges of 7.0- 9.0 and temperature ranges of 37-55 °C (Mokrane et al., 2016). Sheep rumen temperatures average around 40 °C and have rumen pH averages ranging from 6.4 to 6.8 (Jasmin et al., 2011; Vesterdorf et al., 2022). The lower pH in the rumen compared to the soil sediment suggests that some *Thermoactinomyces khenchelensis* strains may be able to live in a lower pH than found previously. Rumen temperatures and the availability of glucose from other microbes within the rumen might be why *Thermoactinomyces khenchelensis* was able to colonize the rumen initially. However, lower than optimum pH is likely limiting the population growth of *Thermoactinomyces khenchelensis* in sheep in both physiological stages. Moreover, previous studies indicate that feeding pelleted rations tends to lower the rumen pH by 0.25 to 0.31 units compared to feeding non-pelleted rations (Li et al., 2021). Therefore, if sheep in a growth physiological stage have a lowered rumen pH due to their pelleted diet, the lower pH could have further limited the species ' growth, explaining *Thermoactinomyces khenchelensis's* lower abundance in the growth experimental period.

Bacillus thermolactis and *Bacillus clausii* belong to the Bacillus genera under the class Bacilli. The taxon under Bacilli all were higher in abundance within maintenance microbiomes. Bacilli species have been previously used as probiotics in adult sheep. Supplementation of *Bacillus licheniformis* and *Bacillus subtilis* in ewe diets increased milk production, and supplementation of *Bacillus amyloliquefaciens H57* altered ewe microbiome communities and was associated with a higher weight gain (Kritas et al., 2006; Schofield et al., 2018). In addition, introducing the *Bacillus amyloliquefaciens H57* was associated with decreases in *Methanobrevibacter* and influenced specific *Prevotella* species prevalence (Schofield et al., 2018). While we did not introduce specific bacillus species, these studies indicate that the increase in the Bacilli class found in maintenance sheep likely affects the abundance of other species found in the rumen, specifically cellulolytic and methanogenic microbes. Our results of decreased Methanobacteria in microbiomes with increased Bacilli further corroborate Schofield et al., 2018's discussion that Bacilli species may alter the amount of methanogenesis within the sheep rumen.

Prevotella ruminicola is a bacterium that can digest various proteins and peptides through the action of peptidases (Wallace et al., 1997). The increased amount of *Prevotella ruminicola* in maintenance sheep suggests increased amounts of degradable protein within the rumen for *Prevotella ruminicola* to utilize. However, in experimental periods one and two, crude protein was similar between the alfalfa pellets and alfalfa hay. Huang et al., 2015, concluded that different conditioning temperatures and times when creating pellets could change the protein profiles of the feed without changing the total protein content (Huang et al., 2015). If this is also true between alfalfa hay and alfalfa pellets, differences in protein and peptide structures could have limited *Prevotella ruminicola*'s ability to utilize the alfalfa pellet's protein during the growth experimental period. Therefore, protein profiles, not just total crude protein, may be needed to understand microbiome differences between two diets fully.

Literature Cited

Andrade, B. G., H. Afli, F. A. Bressani, R. R. Cuadrat, P. S. de Oliveira, G. B. Mourão, L. L. Coutinho, J. M. Reecy, J. E. Koltes, and M. M. de Souza. 2020. Fecal and Ruminal Microbiome Components Associated With Methane Emission in Beef Cattle.

Bals, B. D., C. Gunawan, J. Moore, F. Teymouri, and B. E. Dale. 2014. Enzymatic hydrolysis of pelletized AFEX™-treated corn stover at high solid loadings. Biotechnology and bioengineering 111(2):264-271.

Bellefroid, E. J., L. Leclère, A. Saulnier, M. Keruzore, M. Sirakov, M. Vervoort, and S. De Clercq. 2013. Expanding roles for the evolutionarily conserved Dmrt sex transcriptional regulators during embryogenesis. Cellular and Molecular Life Sciences 70(20):3829-3845. doi: 10.1007/s00018-013-1288-2

Borg, R. C. 2004. Developing breeding objectives for Targhee sheep, Virginia Tech.

Bryant, M. P., and N. Small. 1956. The anaerobic monotrichous butyric acid-producing curved rod-shaped bacteria of the rumen. Journal of Bacteriology 72(1):16-21.

Bueno de Mesquita, C. P., D. Wu, and S. G. Tringe. 2023. Methyl-Based Methanogenesis: an Ecological and Genomic Review. Microbiology and Molecular Biology Reviews:e00024-00022.

Chen, J. K. 2016. I only have eye for ewe: the discovery of cyclopamine and development of Hedgehog pathway-targeting drugs. Natural product reports 33(5):595-601.

Danielsson, R., J. Dicksved, L. Sun, H. Gonda, B. Müller, A. Schnürer, and J. Bertilsson. 2017. Methane production in dairy cows correlates with rumen methanogenic and bacterial community structure. Frontiers in microbiology 8:226.

Guo, Y., W. Hu, and J. Liu. 2005. Methanogens and manipulation of methane production in the rumen. Wei Sheng wu xue bao= Acta Microbiologica Sinica 45(1):145-148.

Herd, R., V. Oddy, and G. Lee. 1993. Effect of divergent selection for weaning weight on liveweight and wool growth responses to feed intake in Merino ewes. Australian Journal of Experimental Agriculture 33(6):699-705.

Herd, R. M., and P. F. Arthur. 2009. Physiological basis for residual feed intake1. Journal of Animal Science 87(suppl_14):E64-E71. doi: 10.2527/jas.2008-1345

Hu, Z.-H., H.-Q. Yu, and R.-F. Zhu. 2005. Influence of particle size and pH on anaerobic degradation of cellulose by ruminal microbes. International Biodeterioration & Biodegradation 55(3):233-238.

Huang, J., and Y. Li. 2018. Rumen methanogen and protozoal communities of Tibetan sheep and Gansu Alpine Finewool sheep grazing on the Qinghai–Tibetan Plateau, China. BMC microbiology 18(1):1-11.

Huang, X., C. Christensen, and P. Yu. 2015. Effects of conditioning temperature and time during the pelleting process on feed molecular structure, pellet durability index, and metabolic features of co-products from bio-oil processing in dairy cows. Journal of dairy science 98(7):4869-4881.

Hungate, R. E. 2013. The rumen and its microbes. Elsevier.

Izumi, K., Y.-k. Okishio, N. Nagao, C. Niwa, S. Yamamoto, and T. Toda. 2010. Effects of particle size on anaerobic digestion of food waste. International biodeterioration & biodegradation 64(7):601-608.

Jasmin, B. H., R. C. Boston, R. B. Modesto, and T. P. Schaer. 2011. Perioperative ruminal pH changes in domestic sheep (Ovis aries) housed in a biomedical research setting. Journal of the American Association for Laboratory Animal Science 50(1):27-32.

Kittelmann, S., C. S. Pinares-Patino, H. Seedorf, M. R. Kirk, S. Ganesh, J. C. McEwan, and P. H. Janssen. 2014. Two different bacterial community types are linked with the low-methane emission trait in sheep. PloS one 9(7):e103171.

Kritas, S., A. Govaris, G. Christodoulopoulos, and A. Burriel. 2006. Effect of Bacillus licheniformis and Bacillus subtilis supplementation of ewe's feed on sheep milk production and young lamb mortality. Journal of Veterinary Medicine Series A 53(4):170-173.

Kwang Won, S., W. Yingdi, K. Hiroki, R. K. Jae, A. Z. David, and L. J. Randy. 2006. Targeted disruption of the DM domain containing transcription factor Dmrt2 reveals an essential role in somite patterning. Developmental Biology 290(1):200-210. doi: https://doi.org/10.1016/j.ydbio.2005.11.027

Lahart, B., R. Prendiville, F. Buckley, E. Kennedy, S. Conroy, T. Boland, and M. McGee. 2020. The repeatability of feed intake and feed efficiency in beef cattle offered high-concentrate, grass silage and pasture-based diets. animal 14(11):2288-2297.

Li, B., X. Sun, Q. Huo, G. Zhang, T. Wu, P. You, Y. He, W. Tian, R. Li, and C. Li. 2021. Pelleting of a total mixed ration affects growth performance of fattening lambs. Frontiers in Veterinary Science 8:629016.

Li, Y., J. Gao, Y. Xue, R. Sun, X. Sun, Z. Sun, S. Liu, Z. Tan, W. Zhu, and Y. Cheng. 2023. Nutrient availability of roughages in isocaloric and isonitrogenous diets alters the bacterial networks in the whole gastrointestinal tract of Hu sheep. BMC Microbiology 23(1):70. doi: 10.1186/s12866-023-02814-z

Ma, T., C. Villot, D. Renaud, A. Skidmore, E. Chevaux, M. Steele, and L. L. Guan. 2020. Linking perturbations to temporal changes in diversity, stability, and compositions of neonatal calf gut microbiota: prediction of diarrhea. The ISME journal 14(9):2223-2235.

Malik, P., S. Trivedi, A. Kolte, V. Sejian, R. Bhatta, and H. Rahman. 2022. Diversity of rumen microbiota using metagenome sequencing and methane yield in Indian sheep fed on straw and concentrate diet. Saudi Journal of Biological Sciences 29(8):103345.

Malik, Z., D. Dalal, S. Dahiya, C. Patil, and R. Dahiya. 2016. Genetic analysis of growth traits in Harnali sheep. Veterinary world 9(2):128.

Mokrane, S., N. Bouras, A. Meklat, A. Lahoum, A. Zitouni, C. Verheecke, F. Mathieu, P. Schumann, C. Spröer, and N. Sabaou. 2016. Thermoactinomyces khenchelensis sp. nov., a filamentous bacterium isolated from soil sediment of a terrestrial hot spring. Antonie van Leeuwenhoek 109:311-317.

Naas, A. E., A. Mackenzie, J. Mravec, J. Schückel, W. Willats, V. Eijsink, and P. Pope. 2014. Do rumen Bacteroidetes utilize an alternative mechanism for cellulose degradation? MBio 5(4):e01401-01414.

Ono, K., K. Hata, E. Nakamura, S. Ishihara, S. Kobayashi, M. Nakanishi, M. Yoshida, Y. Takahata, T. Murakami, and S. Takenoshita. 2021. Dmrt2 promotes transition of endochondral bone formation by linking Sox9 and Runx2. Communications Biology 4(1):326.

Ortolani, I. R., Z. Amanzougarene, and M. Fondevila. 2020. In vitro estimation of the effect of grinding on rumen fermentation of fibrous feeds. Animals 10(4):732.

Paganoni, B., G. Rose, C. Macleay, C. Jones, D. Brown, G. Kearney, M. Ferguson, and A. Thompson. 2017. More feed efficient sheep produce less methane and carbon dioxide when eating high-quality pellets. Journal of Animal Science 95(9):3839-3850.

Randhir Singh, B., S. Lalit, and S. Artabandhu. 2019. Methane production and microbial protein synthesis in adult sheep fed total mixed ration as mash and as complete feed block. Carbon Management 10(3):241-253 , year = 2019. doi: 10.1080/17583004.2019.1586280

Rashidi, A., M. S. Mokhtari, A. S. Jahanshahi, and M. M. Abadi. 2008. Genetic parameter estimates of pre-weaning growth traits in Kermani sheep. Small Ruminant Research 74(1-3):165-171.

Redden, R. R., L. M. M. Surber, B. L. Roeder, B. M. Nichols, J. A. Paterson, and R. W. Kott. 2011. Residual feed efficiency established in a post-weaning growth test may not result in more efficient ewes on the range. Small Ruminant Research 96(2):155-159. doi: https://doi.org/10.1016/j.smallrumres.2010.12.007

Sakata, T., and H. Tamate. 1978. Rumen epithelial cell proliferation accelerated by rapid increase in intraruminal butyrate. Journal of Dairy Science 61(8):1109-1113.

Santana, M. H. d. A., and R. d. C. GOMES. 2015. Genome-wide association with residual body weight gain in Bos indicus cattle.

Sato, T., D. Rocancourt, L. Marques, S. Thorsteinsdóttir, and M. Buckingham. 2010. A Pax3/Dmrt2/Myf5 regulatory cascade functions at the onset of myogenesis. PLoS genetics 6(4):e1000897.

Schofield, B. J., N. Lachner, O. T. Le, D. M. McNeill, P. Dart, D. Ouwerkerk, P. Hugenholtz, and A. V. Klieve. 2018. Beneficial changes in rumen bacterial community profile in sheep and dairy calves as a result of feeding the probiotic Bacillus amyloliquefaciens H57. Journal of applied microbiology 124(3):855-866.

Shaomei, L., C. Wenshuo, Z. Xinting, L. Zhiwei, Y. Ge, H. Xuewen, and M. Chunyan. 2020. Comparative investigation of coarse and fine wool sheep skin indicates the early regulators for skin and wool diversity. Gene 758:144968. doi: https://doi.org/10.1016/j.gene.2020.144968

Shi, W., C. D. Moon, S. C. Leahy, D. Kang, J. Froula, S. Kittelmann, C. Fan, S. Deutsch, D. Gagic, and H. Seedorf. 2014. Methane yield phenotypes linked to differential gene expression in the sheep rumen microbiome. Genome research 24(9):1517-1525.

Smith, P. E., A. K. Kelly, D. A. Kenny, and S. M. Waters. 2022. Differences in the composition of the rumen microbiota of finishing beef cattle divergently ranked for residual methane emissions. Frontiers in Microbiology 13

Vesterdorf, K., D. T. Beatty, A. Barnes, and S. K. Maloney. 2022. Rumen temperature is a reliable proxy of core body temperature in sheep (Ovis aries). Animal Production Science 62(17):1671-1682.

Wallace, R. J., N. McKain, G. A. Broderick, L. M. Rode, N. D. Walker, C. J. Newbold, and J. Kopecny. 1997. Peptidases of the rumen bacterium, Prevotella ruminicola. Anaerobe 3(1):35-42.

Wang, J., H. Zhou, R. H. Forrest, J. Hu, X. Liu, S. Li, Y. Luo, and J. G. Hickford. 2017. Variation in the ovine MYF5 gene and its effect on carcass lean meat yield in New Zealand Romney sheep. Meat science 131:146-151.

Wang, L., K. Zhang, C. Zhang, Y. Feng, X. Zhang, X. Wang, and G. Wu. 2019. Dynamics and stabilization of the rumen microbiome in yearling Tibetan sheep. Scientific Reports 9(1):1-9.

Xie, X., C. Yang, L. L. Guan, J. Wang, M. Xue, and J. X. Liu. 2018. Persistence of cellulolytic bacteria Fibrobacter and Treponema after short-term corn stover-based dietary intervention reveals the potential to improve rumen fibrolytic function. Frontiers in Microbiology 9:1363.

Yildirim, E., L. Ilina, G. Laptev, V. Filippova, E. Brazhnik, T. Dunyashev, A. Dubrovin, N. Novikova, D. Tiurina, and N. Tarlavin. 2021. The structure and functional profile of ruminal microbiota in young and adult reindeers (Rangifer tarandus) consuming natural winter-spring and summer-autumn seasonal diets. PeerJ 9:e12389.

Yin, X., S. Ji, C. Duan, P. Tian, S. Ju, H. Yan, Y. Zhang, and Y. Liu. 2021. Age-related changes in the ruminal microbiota and their relationship with rumen fermentation in lambs. Frontiers in Microbiology 12:679135.

Yoshida, C. A., H. Yamamoto, T. Fujita, T. Furuichi, K. Ito, K.-i. Inoue, K. Yamana, A. Zanma, K. Takada, and Y. Ito. 2004. Runx2 and Runx3 are essential for chondrocyte maturation, and Runx2 regulates limb growth through induction of Indian hedgehog. Genes & development 18(8):952-963.

Zhang, H., M. Shao, and H. Huang. 2018. The dynamic distribution of small-tail Han sheep microbiota across different intestinal segments. Front Microbiol 2018; 9: 32.

Zhang, L., Y. Yang, Z. Liao, Q. Liu, X. Lei, M. Li, Z. Zhang, D. Hong, M. Zhu, and B. Li. 2020. Genetic and pharmacological activation of Hedgehog signaling inhibits osteoclastogenesis and attenuates titanium particle-induced osteolysis partly through suppressing the JNK/c-Fos-NFATc1 cascade. Theranostics 10(15):6638.

Zhang, X., G. Li, F. Li, D. Zhang, L. Yuan, Y. Zhao, Y. Zhang, X. Li, Q. Song, and W. Wang. 2021. Effect of feed efficiency on growth performance, body composition, and fat deposition in growing Hu lambs. Animal Biotechnology:1-16.

Zhang, X., W. Wang, F. Mo, Y. La, C. Li, F. Li. 2017. Assocation of residual feed intake with growht and slaughtering performance, blood metabolism and body composition in growing lambs. Scientific Reports 7(1):1-11.

CHAPTER SIX

CONCLUSION

Using PWWT EBVs to increase growth rates in sheep to reach market weight faster may not be a profitable strategy. This study found that PWWT EBVs do not associate with ADG, indicating that the selection of higher PWWT EBVs does not increase growth rates. Instead, producers may be increasing the size of their sheep at weaning and, by the indirect selection, increasing their flock's mature body size. Larger ewes have higher DMI than smaller ewes; therefore, indirect selection for higher-weight mature ewes using PWWT EBVs will increase farm feed costs and decrease the overall flock profitability. Increased revenues of larger market lambs may not always recuperate this feed expense increase. In rangelands, due to decreasing forage resources from drought, these effects may have exaggerated impacts on flocks that run larger mature-sized sheep.

The genomic association of *DMRT2* with RFI and DMI in maintenance is the first time this gene has been associated with either trait in sheep. The association of *DMRT2* with these traits indicates that processes early in utero, such as myotome myogenesis and chondrocyte hypertrophy, may play an important role in feed efficiency and feed intake later in life. While selecting a particular allele frequency of one gene is not necessarily practical, including *DMRT2* in marker-assisted selection for DMI and RFI in the future could increase selection accuracy, and further study is warranted.

The differing microbial associations and microbiome structures between growth and maintenance depict two age-diet-related microbial community compositions within range-type sheep. Many of the microbes associated with either the growth or maintenance period differed depending upon the diet’s carbohydrate structure, largely related to the additional processing of the pelleted diet. The alfalfa hay with increased structure increased microbes that degrade cellulose and hemicellulose, whereas the less structured pelleted diet increased methanogenic Archaea. Differences in microbial structure between growth and maintenance suggest that the microbiomes of sheep differ as they age due to being introduced to different feedstuff.

Residual feed intake rankings of the ewes on trial changed between trial periods one and two. Differences in microbiome community compositions and growth rate of the ewes may alter the feed efficiency as the ewes go from a growth to a maintenance physiological state. The difference in RFI between growth and maintenance shows that RFI in growth is not predictive of RFI in maintenance, and therefore they should be considered different traits. Since RFI needs to be considered different traits between growth and maintenance, RFI also may differ between other physiological states, such as lactation or late pregnancy, or even between the same physiological states at different times. Further research needs to be done on the overall predictiveness of RFI between time points before creating any genomic selection tools using the RFI phenotype to ensure that the genomic selection tool encapsulates a single trait.

REFRENCES CITED

Alqaisi, O., O. A. Ndambi, and R. B. Williams. 2017. Time series livestock diet optimization: cost-effective broiler feed substitution using the commodity price spread approach. Agricultural and Food Economics 5(1):1-19.

Andrade, B. G., H. Afli, F. A. Bressani, R. R. Cuadrat, P. S. de Oliveira, G. B. Mourão, L. L. Coutinho, J. M. Reecy, J. E. Koltes, and M. M. de Souza. 2020. Fecal and Ruminal Microbiome Components Associated With Methane Emission in Beef Cattle.

Arthur, P., G. Renand, and D. Krauss. 2001. Genetic and phenotypic relationships among different measures of growth and feed efficiency in young Charolais bulls. Livestock Production Science 68(2-3):131-139.

Awad, W. A., C. Hess, and M. Hess. 2017. Enteric pathogens and their toxin-induced disruption of the intestinal barrier through alteration of tight junctions in chickens. Toxins 9(2):60.

Bellefroid, E. J., L. Leclère, A. Saulnier, M. Keruzore, M. Sirakov, M. Vervoort, and S. De Clercq. 2013. Expanding roles for the evolutionarily conserved Dmrt sex transcriptional regulators during embryogenesis. Cellular and Molecular Life Sciences 70(20):3829-3845. doi: 10.1007/s00018-013-1288-2

Benoit, M., R. Sabatier, J. Lasseur, P. Creighton, and B. Dumont. 2019. Optimising economic and environmental performances of sheep-meat farms does not fully fit with the meat industry demands. Agronomy for sustainable development 39(4):40.

Borg, R. C. 2004. Developing breeding objectives for Targhee sheep, Virginia Tech.

Brown, D., A. Swan, V. Boerner, L. Li, P. Gurman, A. McMillan, J. Van der Werf, H. Chandler, B. Tier, and R. Banks. 2018. Single-step genetic evaluations in the Australian sheep industry. In: Proceedings of the world congress on genetics applied to livestock production. p 460.

Bryant, M. P., and N. Small. 1956. The anaerobic monotrichous butyric acid-producing curved rod-shaped bacteria of the rumen. Journal of Bacteriology 72(1):16-21.

Bueno de Mesquita, C. P., D. Wu, and S. G. Tringe. 2023. Methyl-Based Methanogenesis: an Ecological and Genomic Review. Microbiology and Molecular Biology Reviews:e00024-00022.

Cammack, K., K. A. Leymaster, T. Jenkins, and M. K. Nielsen. 2005. Estimates of genetic parameters for feed intake, feeding behavior, and daily gain in composite ram lambs. Journal of Animal Science 83(4):777-785.

Cantalapiedra-Hijar, G., M. Abo-Ismail, G. Carstens, L. Guan, R. Hegarty, D. A. Kenny, M. Mcgee, G. Plastow, A. Relling, and I. Ortigues-Marty. 2018. Biological determinants of between-animal variation in feed efficiency of growing beef cattle. Animal 12(s2):s321-s335.

Chang, G. G., and L. Tong. 2011. Structure and function of malic enzymes, a new class of oxidative decarboxylases. Biochemistry 42:12721–12733.

Chang, G.-G., and L. Tong. 2003. Structure and function of malic enzymes, a new class of oxidative decarboxylases. Biochemistry 42(44):12721-12733.

Chen, J. K. 2016. I only have eye for ewe: the discovery of cyclopamine and development of Hedgehog pathway-targeting drugs. Natural product reports 33(5):595-601.

Chiarello, M., M. McCauley, S. Villéger, and C. R. Jackson. 2022. Ranking the biases: The choice of OTUs vs. ASVs in 16S rRNA amplicon data analysis has stronger effects on diversity measures than rarefaction and OTU identity threshold. PLoS One 17(2):e0264443.

Cockrum, R., N. Pickering, R. Anderson, D. Hyndman, M. Bixley, K. Dodds, R. Stobart, J. McEwan, and K. Cammack. 2012. Identification of single nucleotide polymorphisms associated with feed efficiency in rams. In: Proc West Sect Am Soc Anim Sci. p 79-83.

Danielsson, R., J. Dicksved, L. Sun, H. Gonda, B. Müller, A. Schnürer, and J. Bertilsson. 2017. Methane production in dairy cows correlates with rumen methanogenic and bacterial community structure. Frontiers in microbiology 8:226.

De Lima, A. O., J. E. Koltes, W. J. Diniz, P. S. De Oliveira, A. S. Cesar, P. C. Tizioto, J. Afonso, M. M. de Souza, J. Petrini, and M. I. Rocha. 2020. Potential biomarkers for feed efficiency-related traits in nelore cattle identified by co-expression network and integrative genomics analyses. Frontiers in Genetics 11:189.

Dietz, F., S. Franken, K. Yoshida, H. Nakamura, J. Kappler, and V. Gieselmann. 2002. The family of hepatoma-derived growth factor proteins: characterization of a new member HRP-4 and classification of its subfamilies. Biochemical Journal 366(2):491-500.

Ellison, M., G. Conant, W. Lamberson, R. Cockrum, K. Austin, D. Rule, and K. Cammack. 2017. Diet and feed efficiency status affect rumen microbial profiles of sheep. Small Ruminant Research 156:12-19.

Giráldez, F. J., N. Santos, A. Santos, C. Valdés, S. López, and S. Andrés. 2021. Fattening lambs with divergent residual feed intakes and weight gains: Unravelling mechanisms driving feed efficiency. Animal feed science and technology 273:114821. doi: 10.1016/j.anifeedsci.2021.114821

Guo, Y., W. Hu, and J. Liu. 2005. Methanogens and manipulation of methane production in the rumen. Wei Sheng wu xue bao= Acta Microbiologica Sinica 45(1):145-148.

Henderson, C. R. 1973. Sire evaluation and genetic trends. Journal of Animal Science 1973(Symposium):10-41.

Herd, R., and P. Arthur. 2009. Physiological basis for residual feed intake. Journal of animal science 87(suppl_14):E64-E71.

Herd, R., V. Oddy, and G. Lee. 1993. Effect of divergent selection for weaning weight on liveweight and wool growth responses to feed intake in Merino ewes. Australian Journal of Experimental Agriculture 33(6):699-705.

Hu, Z.-H., H.-Q. Yu, and R.-F. Zhu. 2005. Influence of particle size and pH on anaerobic degradation of cellulose by ruminal microbes. International Biodeterioration & Biodegradation 55(3):233-238.

Hoffman, M., S. Reed, S. Pillai, A. Jones, K. McFadden, S. Zinn, and K. Govoni. 2017. Physiology and endocrinology symposium: the effects of poor maternal nutrition during gestation on offspring postnatal growth and metabolism. Journal of Animal Science 95(5):2222-2232.

Huang, J., and Y. Li. 2018. Rumen methanogen and protozoal communities of Tibetan sheep and Gansu Alpine Finewool sheep grazing on the Qinghai–Tibetan Plateau, China. BMC microbiology 18(1):1-11.

Huang, X., C. Christensen, and P. Yu. 2015. Effects of conditioning temperature and time during the pelleting process on feed molecular structure, pellet durability index, and metabolic features of co-products from bio-oil processing in dairy cows. Journal of dairy science 98(7):4869-4881.

Hungate, R. E. 2013. The rumen and its microbes. Elsevier.

Izumi, K., Y.-k. Okishio, N. Nagao, C. Niwa, S. Yamamoto, and T. Toda. 2010. Effects of particle size on anaerobic digestion of food waste. International biodeterioration & biodegradation 64(7):601-608.

Jackson, S., R. Green, and M. Miller. 1997. Phenotypic characterization of Rambouillet sheep expressing the callipyge gene: I. Inheritance of the condition and production characteristics. Journal of Animal Science 75(1):14-18.

Jasmin, B. H., R. C. Boston, R. B. Modesto, and T. P. Schaer. 2011. Perioperative ruminal pH changes in domestic sheep (Ovis aries) housed in a biomedical research setting. Journal of the American Association for Laboratory Animal Science 50(1):27-32.

Johnson P. L., K. K., Wing J, Hickey S, Johnstone P. 2018. Preliminary estimates of genetic parameters for residual feed intake in New Zealand maternal sheep. In: 11th World Congress on Genetics Applied to Livestock Production

Kittelmann, S., C. S. Pinares-Patino, H. Seedorf, M. R. Kirk, S. Ganesh, J. C. McEwan, and P. H. Janssen. 2014. Two different bacterial community types are linked with the low-methane emission trait in sheep. PloS one 9(7):e103171.

Koch, R. M., L. A. Swiger, D. Chambers, and K. E. Gregory. 1963. Efficiency of feed use in beef cattle. Journal of animal science 22(2):486-494.

Kralik, P., and M. Ricchi. 2017. A basic guide to real time PCR in microbial diagnostics: definitions, parameters, and everything. Frontiers in microbiology 8:108.

Kritas, S., A. Govaris, G. Christodoulopoulos, and A. Burriel. 2006. Effect of Bacillus licheniformis and Bacillus subtilis supplementation of ewe's feed on sheep milk production and young lamb mortality. Journal of Veterinary Medicine Series A 53(4):170-173.

Kwang Won, S., W. Yingdi, K. Hiroki, R. K. Jae, A. Z. David, and L. J. Randy. 2006. Targeted disruption of the DM domain containing transcription factor Dmrt2 reveals an essential role in somite patterning. Developmental Biology 290(1):200-210. doi: https://doi.org/10.1016/j.ydbio.2005.11.027

Lamb, G. C., Maddock, T. 2009. Feed Efficiency in Cows. In: N. F. R. a. E. Center (ed.). p 35-42, Florida beef cattle short course.

Lahart, B., R. Prendiville, F. Buckley, E. Kennedy, S. Conroy, T. Boland, and M. McGee. 2020. The repeatability of feed intake and feed efficiency in beef cattle offered high-concentrate, grass silage and pasture-based diets. animal 14(11):2288-2297.

Leeds, T., and G. Lewis. 2006. Breed comparisons and trends for ewe productivity and lamb growth traits in sheep managed as contemporaries in a western range system. In: PROCEEDINGS-AMERICAN SOCIETY OF ANIMAL SCIENCE WESTERN SECTION. p 86.

Li, B., X. Sun, Q. Huo, G. Zhang, T. Wu, P. You, Y. He, W. Tian, R. Li, and C. Li. 2021. Pelleting of a total mixed ration affects growth performance of fattening lambs. Frontiers in Veterinary Science 8:629016.

Li, Y., J. Gao, Y. Xue, R. Sun, X. Sun, Z. Sun, S. Liu, Z. Tan, W. Zhu, and Y. Cheng. 2023. Nutrient availability of roughages in isocaloric and isonitrogenous diets alters the bacterial networks in the whole gastrointestinal tract of Hu sheep. BMC Microbiology 23(1):70. doi: 10.1186/s12866-023-02814-z

Ma, T., C. Villot, D. Renaud, A. Skidmore, E. Chevaux, M. Steele, and L. L. Guan. 2020. Linking perturbations to temporal changes in diversity, stability, and compositions of neonatal calf gut microbiota: prediction of diarrhea. The ISME journal 14(9):2223-2235.

Malik, P., S. Trivedi, A. Kolte, V. Sejian, R. Bhatta, and H. Rahman. 2022. Diversity of rumen microbiota using metagenome sequencing and methane yield in Indian sheep fed on straw and concentrate diet. Saudi Journal of Biological Sciences 29(8):103345.

Malik, Z., D. Dalal, S. Dahiya, C. Patil, and R. Dahiya. 2016. Genetic analysis of growth traits in Harnali sheep. Veterinary world 9(2):128.

Mavrogenis, A., and A. Constantinou. 1990. Relationships between pre-weaning growth, post-weaning growth and mature body size in Chios sheep. Animal Science 50(2):271-275.

McLoughlin, S., C. Spillane, N. Claffey, P. E. Smith, T. O’Rourke, M. G. Diskin, and S. M. Waters. 2020. Rumen microbiome composition is altered in sheep divergent in feed efficiency. Frontiers in microbiology 11:1981.

Mokrane, S., N. Bouras, A. Meklat, A. Lahoum, A. Zitouni, C. Verheecke, F. Mathieu, P. Schumann, C. Spröer, and N. Sabaou. 2016. Thermoactinomyces khenchelensis sp. nov., a filamentous bacterium isolated from soil sediment of a terrestrial hot spring. Antonie van Leeuwenhoek 109:311-317.

Moorthie, S., C. J. Mattocks, and C. F. Wright. 2011. Review of massively parallel DNA sequencing technologies. The HUGO journal 5:1-12.

Morris, E. K., T. Caruso, F. Buscot, M. Fischer, C. Hancock, T. S. Maier, T. Meiners, C. Müller, E. Obermaier, and D. Prati. 2014. Choosing and using diversity indices: insights for ecological applications from the German Biodiversity Exploratories. Ecology and evolution 4(18):3514-3524.

Mrode, R. A. 2014. Linear models for the prediction of animal breeding values. Cabi.

Muir, S., N. Linden, A. Kennedy, M. Knight, B. Paganoni, G. Kearney, A. Thompson, and R. Behrendt. 2020. Correlations between feed intake, residual feed intake and methane emissions in maternal composite ewes at post weaning, hogget and adult ages. Small Ruminant Research 192:106241.

Myer, P. R., T. P. Smith, J. E. Wells, L. A. Kuehn, and H. C. Freetly. 2015. Rumen microbiome from steers differing in feed efficiency. PloS one 10(6):e0129174.

Naas, A. E., A. Mackenzie, J. Mravec, J. Schückel, W. Willats, V. Eijsink, and P. Pope. 2014. Do rumen Bacteroidetes utilize an alternative mechanism for cellulose degradation? MBio 5(4):e01401-01414.

NCBI. 2023. Dmrtc2 doublesex and mab-3 related transcription factor like family C2 [Mus musculus (house mouse)]. In: N. I. o. Health (ed.).

Nocker, A., M. Burr, and A. K. Camper. 2007. Genotypic microbial community profiling: a critical technical review. Microbial ecology 54:276-289.

Notter, D. R. 1998. The US National Sheep Improvement Program: across-flock genetic evaluations and new trait development. Journal of animal science 76(9):2324-2330.

NRC. 2007. Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids. Committee on Nutrient Requirements of Small Ruminants.

Ono, K., K. Hata, E. Nakamura, S. Ishihara, S. Kobayashi, M. Nakanishi, M. Yoshida, Y. Takahata, T. Murakami, and S. Takenoshita. 2021. Dmrt2 promotes transition of endochondral bone formation by linking Sox9 and Runx2. Communications Biology 4(1):326.

Ortolani, I. R., Z. Amanzougarene, and M. Fondevila. 2020. In vitro estimation of the effect of grinding on rumen fermentation of fibrous feeds. Animals 10(4):732.

Paganoni, B., G. Rose, C. Macleay, C. Jones, D. Brown, G. Kearney, M. Ferguson, and A. Thompson. 2017. More feed efficient sheep produce less methane and carbon dioxide when eating high-quality pellets. Journal of Animal Science 95(9):3839-3850.

Pasandideh, M., G. Rahimi-Mianji, and M. Gholizadeh. 2018. A genome scan for quantitative trait loci affecting average daily gain and Kleiber ratio in Baluchi Sheep. Journal of genetics 97:493-503.

Patil, R. D., M. J. Ellison, S. M. Wolff, C. Shearer, A. M. Wright, R. R. Cockrum, K. J. Austin, W. R. Lamberson, K. M. Cammack, and G. C. Conant. 2018. Poor feed efficiency in sheep is associated with several structural abnormalities in the community metabolic network of their ruminal microbes. Journal of Animal Science 96(6):2113-2124.

Perea, K., K. Perz, S. K. Olivo, A. Williams, M. Lachman, S. L. Ishaq, J. Thomson, and C. J. Yeoman. 2017. Feed efficiency phenotypes in lambs involve changes in ruminal, colonic, and small-intestine-located microbiota. Journal of Animal Science 95(6):2585-2592.

Pitta, D., N. Indugu, K. Narayan, and M. Hennessy. 2022. Symposium review: Understanding the role of the rumen microbiome in enteric methane mitigation and productivity in dairy cows. Journal of Dairy Science

R Core Team. 2017. R: A language and environment for statiscal computing. R Foundation for Statical Computing, Vienna, Austria. URL https://www.R-project.org/.

R Studio Team. 2023. RStudio: Integrated Development for R. RStudio, PBC, Boston, MA.

Raineri, C., T. S. S. Stivari, and A. H. Gameiro. 2015. Lamb production costs: Analyses of composition and elasticities analysis of lamb production costs. Asian-Australasian Journal of Animal Sciences 28(8):1209.

Randhir Singh, B., S. Lalit, and S. Artabandhu. 2019. Methane production and microbial protein synthesis in adult sheep fed total mixed ration as mash and as complete feed block. Carbon Management 10(3):241-253 , year = 2019. doi: 10.1080/17583004.2019.1586280

Rashidi, A., M. S. Mokhtari, A. S. Jahanshahi, and M. M. Abadi. 2008. Genetic parameter estimates of pre-weaning growth traits in Kermani sheep. Small Ruminant Research 74(1-3):165-171.

Redden, R., L. Surber, B. Roeder, R. Kott. 2010. Residual feed intake of growing western range ewes American Sheep Industry Association, Nashville, TN.

Redden, R., L. Surber, B. Roeder, B. Nichols, J. Paterson, and R. Kott. 2011a. Residual feed efficiency established in a post-weaning growth test may not result in more efficient ewes on the range. Small Ruminant Research 96(2-3):155-159.

Redden, R. R., L. M. M. Surber, B. L. Roeder, B. M. Nichols, J. A. Paterson, and R. W. Kott. 2011b. Residual feed efficiency established in a post-weaning growth test may not result in more efficient ewes on the range. Small Ruminant Research 96(2):155-159. doi: https://doi.org/10.1016/j.smallrumres.2010.12.007

Rezende, M., C. Malhado, S. Biffani, J. Carrillo-Tabakman, M. Fabbri, A. Crovetti, P. Carneiro, and R. Bozzi. 2022. Heritability and genetic correlation of body weight and Kleiber ratio in Limousin and Charolais beef cattle breeds. Animal 16(5):100528.

Rolf, M. M., J. F. Taylor, R. D. Schnabel, S. D. McKay, M. C. McClure, S. L. Northcutt, M. S. Kerley, and R. L. Weaber. 2010. Use of bovine SNP50 data for feed efficiency selection decisions in Angus cattle. Pg.

Ross, E. M., B. J. Hayes, D. Tucker, J. Bond, S. E. Denman, and V. H. Oddy. 2020a. Genomic predictions for enteric methane production are improved by metabolome and microbiome data in sheep (Ovis aries). Journal of animal science 98(10):1-14. doi: 10.1093/jas/skaa262

Ross, E. M., B. J. Hayes, D. Tucker, J. Bond, S. E. Denman, and V. H. Oddy. 2020b. Genomic predictions for enteric methane production are improved by metabolome and microbiome data in sheep (Ovis aries). Journal of Animal Science 98(10):skaa262.

Sakata, T., and H. Tamate. 1978. Rumen epithelial cell proliferation accelerated by rapid increase in intraruminal butyrate. Journal of Dairy Science 61(8):1109-1113.

Santana, M. H. d. A., and R. d. C. GOMES. 2015. Genome-wide association with residual body weight gain in Bos indicus cattle.

Sato, T., D. Rocancourt, L. Marques, S. Thorsteinsdóttir, and M. Buckingham. 2010. A Pax3/Dmrt2/Myf5 regulatory cascade functions at the onset of myogenesis. PLoS genetics 6(4):e1000897.

Schofield, B. J., N. Lachner, O. T. Le, D. M. McNeill, P. Dart, D. Ouwerkerk, P. Hugenholtz, and A. V. Klieve. 2018. Beneficial changes in rumen bacterial community profile in sheep and dairy calves as a result of feeding the probiotic Bacillus amyloliquefaciens H57. Journal of applied microbiology 124(3):855-866.

Shaomei, L., C. Wenshuo, Z. Xinting, L. Zhiwei, Y. Ge, H. Xuewen, and M. Chunyan. 2020. Comparative investigation of coarse and fine wool sheep skin indicates the early regulators for skin and wool diversity. Gene 758:144968. doi: https://doi.org/10.1016/j.gene.2020.144968

Shi, W., C. D. Moon, S. C. Leahy, D. Kang, J. Froula, S. Kittelmann, C. Fan, S. Deutsch, D. Gagic, and H. Seedorf. 2014. Methane yield phenotypes linked to differential gene expression in the sheep rumen microbiome. Genome research 24(9):1517-1525.

Smith, P. E., A. K. Kelly, D. A. Kenny, and S. M. Waters. 2022. Differences in the composition of the rumen microbiota of finishing beef cattle divergently ranked for residual methane emissions. Frontiers in Microbiology 13

Snowder, G., and L. D. Van Vleck. 2003a. Estimates of genetic parameters and selection strategies to improve the economic efficiency of postweaning growth in lambs. Journal of animal science 81(11):2704-2713.

Snowder, G. D., and L. D. Van Vleck. 2003b. Estimates of genetic parameters and selection strategies to improve the economic efficiency of postweaning growth in lambs. Journal of animal science 81(11):2704-2713. doi: 10.2527/2003.81112704x

Tortereau, F., C. Marie-Etancelin, J. L. Weisbecker, D. Marcon, F. Bouvier, C. Moreno-Romieux, and D. Francois. 2020. Genetic parameters for feed efficiency in Romane rams and responses to single-generation selection. Animal (Cambridge, England) 14(4):681-687. doi: 10.1017/S1751731119002544

Uffelmann, E., Q. Q. Huang, N. S. Munung, J. de Vries, Y. Okada, A. R. Martin, H. C. Martin, T. Lappalainen, and D. Posthuma. 2021. Genome-wide association studies. Nature Reviews Methods Primers 1(1):59. doi: 10.1038/s43586-021-00056-9

USDA. 2021. Sheep and Goats (January 2021). In: N. A. S. Service (ed.).

Vesterdorf, K., D. T. Beatty, A. Barnes, and S. K. Maloney. 2022. Rumen temperature is a reliable proxy of core body temperature in sheep (Ovis aries). Animal Production Science 62(17):1671-1682.

Wallace, R. J., N. McKain, G. A. Broderick, L. M. Rode, N. D. Walker, C. J. Newbold, and J. Kopecny. 1997. Peptidases of the rumen bacterium, Prevotella ruminicola. Anaerobe 3(1):35-42.

Wang, C., C. Zhang, T. Yan, S. Chang, W. Zhu, M. Wanapat, and F. Hou. 2020. Increasing roughage quality by using alfalfa hay as a substitute for concentrate mitigates CH4 emissions and urinary N and ammonia excretion from dry ewes. Journal of animal physiology and animal nutrition 104(1):22-31.

Wang, J., H. Zhou, R. H. Forrest, J. Hu, X. Liu, S. Li, Y. Luo, and J. G. Hickford. 2017. Variation in the ovine MYF5 gene and its effect on carcass lean meat yield in New Zealand Romney sheep. Meat science 131:146-151.

Wang, L., K. Zhang, C. Zhang, Y. Feng, X. Zhang, X. Wang, and G. Wu. 2019. Dynamics and stabilization of the rumen microbiome in yearling Tibetan sheep. Scientific Reports 9(1):1-9.

Wei, C., H. Luo, B. Zhao, K. Tian, X. Huang, Y. Wang, X. Fu, Y. Tian, J. Di, and X. Xu. 2020. The effect of integrating genomic information into genetic evaluations of Chinese Merino sheep. Animals 10(4):569.

Xie, X., C. Yang, L. L. Guan, J. Wang, M. Xue, and J. X. Liu. 2018. Persistence of cellulolytic bacteria Fibrobacter and Treponema after short-term corn stover-based dietary intervention reveals the potential to improve rumen fibrolytic function. Frontiers in Microbiology 9:1363.

Yildirim, E., L. Ilina, G. Laptev, V. Filippova, E. Brazhnik, T. Dunyashev, A. Dubrovin, N. Novikova, D. Tiurina, and N. Tarlavin. 2021. The structure and functional profile of ruminal microbiota in young and adult reindeers (Rangifer tarandus) consuming natural winter-spring and summer-autumn seasonal diets. PeerJ 9:e12389.

Yin, X., S. Ji, C. Duan, P. Tian, S. Ju, H. Yan, Y. Zhang, and Y. Liu. 2021. Age-related changes in the ruminal microbiota and their relationship with rumen fermentation in lambs. Frontiers in Microbiology 12:679135.

Yoshida, C. A., H. Yamamoto, T. Fujita, T. Furuichi, K. Ito, K.-i. Inoue, K. Yamana, A. Zanma, K. Takada, and Y. Ito. 2004. Runx2 and Runx3 are essential for chondrocyte maturation, and Runx2 regulates limb growth through induction of Indian hedgehog. Genes & development 18(8):952-963.

Zhang, D., X. Zhang, F. Li, C. Li, Y. La, F. Mo, G. Li, Y. Zhang, X. Li, and Q. Song. 2019. Transcriptome analysis identifies candidate genes and pathways associated with feed efficiency in hu sheep. Frontiers in Genetics 10:1183.

Zhang, D., X. Zhang, F. Li, L. Yuan, Y. Zhang, X. Li, Y. Zhao, Q. Song, G. Li, and W. Wang. 2021a. Polymorphisms in ovine ME1 and CA1 genes and their association with feed efficiency in Hu sheep. Journal of Animal Breeding and Genetics 138(5):589-599.

Zhang, H., M. Shao, and H. Huang. 2018. The dynamic distribution of small-tail Han sheep microbiota across different intestinal segments. Front Microbiol 2018; 9: 32.

Zhang, L., Y. Yang, Z. Liao, Q. Liu, X. Lei, M. Li, Z. Zhang, D. Hong, M. Zhu, and B. Li. 2020. Genetic and pharmacological activation of Hedgehog signaling inhibits osteoclastogenesis and attenuates titanium particle-induced osteolysis partly through suppressing the JNK/c-Fos-NFATc1 cascade. Theranostics 10(15):6638.

Zhang, X., C. Li, K. Shahzad, M. Han, Y. Guo, X. Huang, T. Wu, L. Wang, Y. Zhang, and H. Tang. 2022. Seasonal Differences in Fecal Microbial Community Structure and Metabolism of House-Feeding Chinese Merino Fine-Wool Sheep. Frontiers in Veterinary Science 9

Zhang, X., G. Li, F. Li, D. Zhang, L. Yuan, Y. Zhao, Y. Zhang, X. Li, Q. Song, and W. Wang. 2021b. Effect of feed efficiency on growth performance, body composition, and fat deposition in growing Hu lambs. Animal Biotechnology:1-16.

Zhang, X., W. Wang, F. Mo, Y. La, C. Li, and F. Li. 2017. Association of residual feed intake with growth and slaughtering performance, blood metabolism, and body composition in growing lambs. Scientific Reports 7(1):1-11.

Zhang, Y., X. Zhang, F. Li, C. Li, G. Li, D. Zhang, Q. Song, X. Li, Y. Zhao, and W. Wang. 2021c. Characterization of the rumen microbiota and its relationship with residual feed intake in sheep. Animal 15(3):100161.

Zhang, Z., U. Ober, M. Erbe, H. Zhang, N. Gao, J. He, J. Li, and H. Simianer. 2014. Improving the accuracy of whole genome prediction for complex traits using the results of genome wide association studies. PloS one 9(3):e93017.