

DISSERTATION

CAPABILITIES OF RAPID EVAPORATIVE IONIZATION MASS SPECTROMETRY TO  
PREDICT LAMB FLAVOR AND OVERVIEW OF FEEDING GENETICALLY MODIFIED  
GRAIN TO LIVESTOCK

Submitted by

Cody Lynn Gifford

Department of Animal Sciences

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Summer 2019

Doctoral Committee:

Advisor: Dale Woerner

Keith Belk

Terry Engle

Jessica Prenni

Adam Heuberger

Copyright by Cody Gifford 2019

All Rights Reserved

## ABSTRACT

### CAPABILITIES OF RAPID EVAPORATIVE IONIZATION MASS SPECTROMETRY TO PREDICT SHEEP MEAT FLAVOR AND AN ASSESSMENT OF FEEDING GENETICALLY MODIFIED GRAINS TO LIVESTOCK

The objective of experiment 1 was to evaluate the ability of rapid evaporative ionization mass spectrometry (REIMS) to predict characteristics of cooked sheep meat flavor using metabolomic data from raw samples. Boneless leg samples were obtained from 150 carcasses of sheep representing three age classifications (n=50 per age classification), at three USDA inspected harvest facilities located in Colorado and California, between October 2017 to June 2018. A trained descriptive panel rated seven flavor attributes. Metabolomic data from fat, lean and ground patties from legs of sheep carcasses were captured through the REIMS platform. Principal component analysis factor scores were used in hierarchical cluster analysis to assess two-level and three-level sensory clusters. Partial least squares (PLS) was used to reduce dimensionality of data before the linear discriminant analysis (LDA) model was built. Eighty percent of the samples were randomly selected to train models and the remaining 20% were used to test prediction accuracy. Mutton carcasses were identified with 88.9% sensitivity and 80.0% precision using external fat of the leg and with 100% sensitivity and 90.9% precision using ground patties. Yearling carcasses were identified with 85.7% precision using lean and lambs were predicted with 70% precision using lean and fat tissue. Greater than 80% accuracy (overall and balanced), sensitivity and precision was achieved in models using lean and ground patties to identify production background (whether the live animal that produced the lean or ground patties

was grain-finished or grass-finished). Prediction accuracies of age classification, production background and two-level flavor performance categories were 68% or higher with various machine learning algorithms coupled with data dimension reduction approaches. Further work is warranted to validate use of this technology in an on-line production setting and additional datasets could be used to further refine or create additional prediction models with better understanding of data processing characteristics.

The review was conducted to assess the scientific literature for evidence of altered health effects in livestock species that have been fed genetically modified grain and any health effects discussed in reference to human consumption of meat products from those animals. Public concern still exists for feeding genetically modified (GM) or genetically engineered (GE) corn to animals that produce animal protein foods. In the U.S., 90% of all corn acres planted in 2013 were from single herbicide or insect resistance GE corn varieties. Regulation of GE crops is mandatory in the U.S. and consists of review and approval by three different Federal agencies. Substantial equivalence is a principle used in evaluating the safety of GE crops to establish that transgenic (GE or GM) varieties are nutritionally similar and as safe as non-transgenic crops. Animal feeding trials can provide further information to establish the safety of GE crops for human and animal consumption. No publications were found that had reported human metabolic effects from consuming beef cattle fed genetically modified grains. No consistent conclusions have been made that feeding GE corn to mice or rats, beef or dairy cattle, swine, or poultry causes any adverse effects to health. Parameters regarding sample size, diet treatments and specified controls exist to guide researchers in designing animal feeding trials with GE crops, but many criticisms of the scientific literature still exist. Additionally, published feeding trials conducted with transgenic corn grain and silage in beef cattle are limited.

## ACKNOWLEDGEMENTS

I would like to thank several individuals for their support during my graduate degree programs at Colorado State University. I am grateful to my parents who initiated my love and passion for agriculture, but more importantly, have supported me in selflessly in life. I have been lucky to have constant support from my wife, Megan. Her encouragement has never faded during the past five years. I cannot count the number of weekends, evenings or late nights that she has accompanied me to complete research or teaching tasks. Personally, and in faith, I have grown with her over the past seven years. I am blessed to have her support me. Anything that I have accomplished in life has been by God's plan and through a strong faith.

Thank you to Dr. Dale Woerner for taking a chance on me as a graduate student, for his unwavering support and encouragement during the past five years. The opportunities that he made me a part of changed the trajectory of my career, shaped the experiences that I have been blessed with and ultimately molded me into a more confident professional.

Dr. Keith Belk has provided endless support in all areas of research, teaching, outreach and personal growth. You taught me how to think critically and challenged me that developing clarity of thought is an ongoing, essential quality. He taught me how to think objectively and how to apply the scientific method to objective questions of interest. I progressed in my program easier knowing that with a simple message or hallway discussion, he would support my endeavors every time.

I was very fortunate to learn from Dr. J. Daryl Tatum. I have continued to learn how to write scientifically from reading his publications and have always enjoyed visiting with him

about any topic. He is one of the greatest teachers and researchers that I have attempted to model myself after.

Dr. Terry Engle has served as one of my greatest mentors during my time at Colorado State University. No matter how busy he may be, he has always helped and supported my research and teaching ability. His remarkable attitude and work ethic in the face of heavy workloads has always been inspiring. I am thankful for each conversation, his willingness to mentor me and all that I have learned from him.

I will be forever grateful to Drs. Jessica Prenni and Adam Heuberger. Not only have they both taught and mentored me in the field of metabolomics but have encouraged me every step along the way. I have really appreciated their willingness to have conversations about any topic. I have benefited tremendously from their wealth of knowledge and experience. Thank you for working with and allowing me to learn from you both. I have been able to learn about a completely new field from both of you.

Thank you to Dr. Mahesh Nair for his continuous support and mentorship during the past year. He never hesitates to offer suggestions or help in any way possible. I have appreciated learning from his experience and expertise. I am grateful for the opportunity I have had to learn from him.

While I will certainly fall short in recognizing everyone that has supported me during my degree programs, the following are a few individuals that I would like to acknowledge: Dr. Rebecca Acheson, Dr. Devin Gredell, Dr. KatieRose McCullough, Blake Foraker, Luke Fuerniss, Clay Carlson, Brenna Klauer, Tanner Adams, Dr. Gina Geonaras, Joanna Swenson, Bailey Schilling, Scott Langley, Erin Karney, Dr. Santiago Luzardo, Dr. Xiang Yang, Dan Sewald, Karissa Isaccs, Dr. Kevin Pond, Dr. Maggie Weinroth, Alexa Strait, Ally Fanning, Dr.

Laura Bellows, Dr. Leslie Cunningham-Sabo, Dr. Mary Harris, Christine Rock, Dr. Corey Broeckling and numerous other individuals that contributed to my research and experience.

## TABLE OF CONTENTS

ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	iv
LIST OF TABLES.....	ix
LIST OF FIGURES.....	x
CHAPTER I.....	1
INTRODUCTION.....	1
CHAPTER II.....	4
REVIEW OF LITERATURE.....	4
Overview of Ovine Carcass Grading.....	4
Ovine Grading Instruments.....	7
Inadequacies of the Current Sheep Grading System.....	8
Overview of Flavor Detection.....	9
Meat Flavor.....	11
Rapid Evaporative Ionization Mass Spectrometry (REIMS).....	14
Predictive Modeling.....	16
Principle component analysis and partial least squares.....	17
Linear Discriminant Analysis.....	19
Machine Learning Algorithms.....	19
Partial least squares discriminant analysis (PLSDA).....	20
Support vector machine (SVM).....	20
Random forest (RF).....	21
XGBoost.....	21
LogitBoost.....	22
LITERATURE CITED.....	23
CHAPTER III.....	29
ASSESSMENT OF EVAPORATIVE IONIZATION MASS SPECTROMETRY (REIMS) TO CHARACTERIZE LAMB FLAVOR.....	29
Introduction.....	29
Materials and Methods.....	31
Sample Collection.....	31
Trained Sensory Analysis.....	32
Rapid Evaporative Ionization Mass Spectrometry (REIMS).....	34
Chemical Analysis.....	34
Statistical Analysis.....	35
Sensory Evaluation and Carcass Attributes.....	35
Predictive Models using Partial Least Squares-Linear Discriminant Analysis.....	36

Predictive Capabilities using Machine Learning Algorithms .....	37
Results and Discussion .....	38
Sheep Carcass Characteristics.....	38
Trained Sensory Ratings .....	39
Predictive Classification Models .....	40
Conclusion .....	44
LITERATURE CITED .....	66
CHAPTER IV .....	68
REVIEW OF LITERATURE – PART II: OVERVIEW OF FEEDING GENETICALLY MODIFIED GRAIN TO LIVESTOCK.....	68
Introduction.....	68
Overview of Safety Assessment .....	69
Animal Feeding Trials .....	71
Transgenic Maize Effects on Rodents (90-Day Trials) .....	71
Animal Studies.....	72
Digestion Process in Ruminants and Non-ruminants .....	73
Transgenic Corn in Beef Cattle Diets .....	74
Transgenic Corn in Diets of Dairy Cattle .....	78
Transgenic Corn in Diets Fed to Poultry .....	80
Detection Methods .....	81
Issues with Study Designs .....	82
Conclusion .....	83
LITERATURE CITED .....	85
APPENDIX.....	91
ADDITIONAL DOCTORAL DEGREE WORK.....	92
Perham, C. C., Gifford, C. L., Woerner, D. R., Engle, T. E., Sellins, K. S., Acheson, R. J., Douglass, L. W., Tatum, J. D., Delmore, R. J., Cifelli, A., McNeill, S. H., and Belk, K. E. 2019. Special-Fed Veal: Separable components, proximate composition, and nutrient analysis of selected raw and cooked, wholesale and retail cuts. <i>Meat Science</i> , 148, 19-31..92	
McNeill, S. H., Belk, K. E., Campbell, W. W. , and Gifford, C. L. 2017. Coming to terms: meat’s role in a healthful diet. <i>Animal Frontiers</i> , 7(4), 34-42. ....	93
Gifford, C. L., O’Connor, L. E., Campbell, W. W., Woerner, D. R., and Belk, K. E. 2017. Broad and inconsistent muscle food classification is problematic for dietary guidance in the U.S. <i>Nutrients</i> , 9(9), 1027.....	94
O’Connor, L. E., Gifford, C. L., Woerner, D. R., Sharp, J. L., Belk, K. E., and Campbell, W. W. 2019. Dietary meat categories and descriptions in chronic disease research are substantively different within and between experimental and observational studies: a systematic review and landscape analysis. Submitted to <i>Advances in Nutrition</i> . ....	95

## LIST OF TABLES

Table 1. Description and reference standard intensities used during sensory panel training for evaluation of sheep descriptive sensory attributes on a continuous line scale from 0 to 100.....	45
Table 2. Least squares means of sheep carcass traits among three age groups.....	46
Table 3. Least squares means of objective color scores among three age groups .....	47
Table 4. Least squares means and SEM of trained sensory ratings for ground sheep samples of varying production characteristics .....	48
Table 5. Least squares means and ranges of percent crude fat and dry matter from ground sheep samples produced from legs of sheep carcasses.....	49
Table 6. Misclassification matrix <sup>1</sup> of 3 age categories predicted <sup>2</sup> by Partial Least Squares-Linear Discriminant Analysis using molecular profiles of samples <sup>3</sup> from sheep carcasses collected using rapid evaporative ionization mass spectrometry (REIMS).....	50
Table 7. Misclassification matrix <sup>1</sup> of 2 production background categories predicted <sup>2</sup> by Partial Least Squares-Linear Discriminant Analysis using molecular profiles of samples <sup>3</sup> from sheep carcasses collected using rapid evaporative ionization mass spectrometry (REIMS). .....	51
Table 8. Misclassification matrix <sup>1</sup> of 3 overall flavor categories predicted <sup>2</sup> by Partial Least Squares-Linear Discriminant Analysis using molecular profiles of samples <sup>3</sup> from sheep carcasses collected using rapid evaporative ionization mass spectrometry (REIMS).....	52
Table 9. Misclassification matrix <sup>1</sup> of 2 overall flavor categories predicted <sup>2</sup> by Partial Least Squares-Linear Discriminant Analysis using molecular profiles of samples <sup>3</sup> from sheep carcasses collected using rapid evaporative ionization mass spectrometry (REIMS).....	53

## LIST OF FIGURES

Figure 1. Projection of partial least squares (PLS) scores and linear discriminant (LDA; LDA developed using factor scores) scores from rapid evaporative ionization mass spectrometry (REIMS) mass bins collected from external fat of legs from sheep carcasses to predict sheep age groups using training and test models.....	54
Figure 2. Projection of partial least squares (PLS) scores and linear discriminant (LDA; LDA developed using factor scores) scores from rapid evaporative ionization mass spectrometry (REIMS) mass bins collected from lean of legs from sheep carcasses to predict sheep age groups using training and test models.....	55
Figure 3. Projection of partial least squares (PLS) scores and linear discriminant (LDA; LDA developed using factor scores) scores from rapid evaporative ionization mass spectrometry (REIMS) mass bins collected from ground meat produced from legs of sheep carcasses to predict sheep age groups using training and test models.....	56
Figure 4. Projection of principal component scores derived from trained sensory ratings for descriptive sensory attributes with each large point representing treatment means. Contribution of sensory attributes to factor scores represented in the loadings plot (bottom).....	57
Figure 5. Projection of principal component scores derived from trained sensory ratings for descriptive sensory attributes colored by two-level sensory classification (positive or negative) determined by hierarchical cluster analysis.....	58
Figure 6. Projection of principal component scores derived from trained sensory ratings for descriptive sensory attributes colored by three-level sensory classification (positive, neutral or negative) determined by hierarchical cluster analysis.....	59
Figure 7. Projection of partial least squares (PLS) scores (top) and linear discriminant (LDA; LDA developed using factor scores) scores (bottom) from rapid evaporative ionization mass spectrometry (REIMS) mass bins collected from lean of legs from sheep carcasses to predict three overall sensory classifications of sheep using training and test models.....	60
Figure 8. Projection of partial least squares (PLS) scores (top) and linear discriminant (LDA; LDA developed using factor scores) scores (bottom) from rapid evaporative ionization mass spectrometry (REIMS) mass bins collected from lean of legs from sheep carcasses to predict three overall sensory classifications of sheep using training and test models.....	61
Figure 9. Prediction accuracies of sheep age category (lamb, yearling or mutton) using rapid evaporative ionization mass spectrometry data collected from lean tissue of ovine carcass legs and 10-fold cross validation for eight machine learning algorithms applied to feature selection (FS; top), principle component analysis (PCA; middle), and PCA followed by FS (bottom) data reduction approaches.....	62

Figure 10. Prediction accuracies of production background category (grain-finished or grass-finished) using rapid evaporative ionization mass spectrometry data collected from lean tissue of ovine carcass legs and 10-fold cross validation for eight machine learning algorithms applied to feature selection (FS; top), principle component analysis (PCA; middle), and PCA followed by FS (bottom) data reduction approaches.....63

Figure 11. Prediction accuracies of overall flavor classification (positive or negative determined from hierarchical cluster analysis of principle components from trained sensory attributes) using rapid evaporative ionization mass spectrometry data collected from lean tissue of ovine carcass legs and 10-fold cross validation for eight machine learning algorithms applied to feature selection (FS; top), principle component analysis (PCA; middle), and PCA followed by FS (bottom) data reduction approaches.....64

## CHAPTER I.

### INTRODUCTION

The United States Department of Agriculture Economic Research Service reported 1.1 pounds (approximately 0.5 kg) of sheep meat per capita consumption within the United States between January to December 2018 (USDA-ERS, 2019). Estimates reported by USDA-ERS (2019) in 1970 for per capita consumption of sheep meat were 2.9 pounds and have continued to decline since that time. Additionally, competitive protein markets and lack of differentiation between U.S. produced sheep compared to imported lamb products have further complicated the issue of low consumption of sheep meat (Jones, 2004). Consumer dissatisfaction with strong flavor profiles of sheep meat may be one possible explanation of low consumption and demand. The 2016 National Lamb Quality Audit identified “eating satisfaction” as the most important quality trait for sheep meat (Hoffman, Dissertation, 2015). Further, this study indicated that 71% of consumers would be willing to pay additional premiums for eating satisfaction characteristics in sheep meat, supporting the need to understand flavor profiles.

Rapid evaporative ionization mass spectrometry (REIMS) is emerging in many areas of science, including human medicine (Balog et al., 2010) and biological sciences. Several scientists recently used REIMS to predict meat quality characteristics and identify animal attributes associated with food fraud. Balog et al. (2016) used REIMS to predict species and breeds and to determine if the technology could have implications for preventing food fraud. Species and breeds were predicted with 100% and 97% accuracy, respectively. In another study using REIMS, fish species were predicted with nearly 99% accuracy (Black et al., 2017). Guitton et al. (2018) was able to identify and predict ractopamine among various pork muscles with accuracy over 95%. Verplanken et al. (2017) reported very high accuracy in segregating samples

with and without boar taint. Comparatively, REIMS allows for quick extraction and ionization capability using a “iKnife” sampling tool coupled with a mass spectrometer (Waters Corporation, 2019).

Maneotis (Thesis, 2017) established a proof of concept for utilizing a tissue sample from sheep carcasses to characterize metabolites driving flavor profiles in sheep meat. In order to evaluate whether flavor profiles of sheep meat could be evaluated with an instrument, it was necessary to conduct a study to identify capabilities of metabolites in driving flavor profiles. The objective of this study was to identify the capabilities of REIMS as a novel method to characterize flavor profiles of various tissues types by generating metabolomic data and evaluating the ability of REIMS to predict characteristics of sheep carcasses.

In general, crops produced from plants whose genetic make-up have been altered via engineering techniques such as recombinant DNA methods are considered genetically modified (GM) or genetically engineered (GE) plants. Many researchers use GM (Snell et al., 2012; Zeljenkova et al., 2014) while others use GE (Fernandez-Cornejo, Wechsler, Livingston, & Mitchell, 2014; Van Eenennaam & Young, 2017) to describe these crops, thus using these acronyms interchangeably. Use of GE crops has increased in the U.S. substantially over the past few decades. Descriptions of traits observed in GE crops can be classified into three generations as follows: generation one includes traits such as herbicide tolerance, resistance to insects and resistance to environmental stress; generation two includes traits such as nutrient enhancement or other value-adding characteristics; and generation three includes traits that offer products beyond the scope of traditional food (Fernandez-Cornejo et al., 2014). However, most of the acres planted in the U.S. utilize crops with traits of herbicide or insect resistance. Fernandez-Cornejo

et al. (2014) reported that, in 2013, approximately 90 percent of acres in the U.S. were planted with GE varieties of corn (Table 1).

Regulation of GE crops in the U.S. consists of regulatory approval by the Environmental Protection Agency (EPA), Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA) (Fernandez-Cornejo et al., 2014; Scientists, 2011). According to the Federal Insecticide, Fungicide, and Rodenticide Act (1972), pesticides such as Bt toxins (an insect resistant protein introduced from *Bacillus thuringiensis*), including a GE plant modified with a Bt gene, must be regulated by the EPA. The safety of GM crops is regulated by the FDA, regardless of whether the crops are consumed by either humans or animals. The Plant Protection Act (2000) requires the Animal and Plant Health Inspection Services (APHIS) Agency of USDA to regulate organisms that modify plant or plant products such as with the use of *Agrobacterium* spp. in gene transfer for development of GE plants.

Since 1996, multiple varieties have been quickly adopted for planting among many other plant species. Between 1996 and 2016 there were 174 GE cultivated crop events from 20 plant species approved in the U.S. (James, 2016a) including crops consumed by livestock such as corn (maize), sugar beets, alfalfa, soybean and others (James, 2016b; Van Eenennaam & Young, 2014). Of those, 41 were GE maize (corn) events approved for use by animal feed and for cultivation in 2016 (ISAAA, 2017). The objective of this review was to conduct a literature search to determine if there is any evidence available among the scientifically published literature to determine whether corn grain from genetically engineered (GE) plant varieties alters metabolism in livestock consuming these GE crops, and any metabolic effects from consuming beef cattle fed genetically modified crops.

## CHAPTER II.

### REVIEW OF LITERATURE

#### *Overview of Ovine Carcass Grading*

The voluntary carcass grading service conducted by the United States Department of Agriculture - Agricultural Marketing Service (USDA-AMS) for lamb, yearling mutton and mutton carcasses became effective on February 16, 1931 (USDA, 1992). The official standards for Quality Grades included fat streaking of the flank, rib feathering, and firmness of the lean and fat. Additional amendments to the grade standards occurred in 1951, 1957, 1960, and 1969 (USDA, 1992). The most recently revised grade standards for lamb and sheep meat became effective July 6, 1992, and required that carcasses be identified with both palatability-indicating characteristics and yield-indicating characteristics when grades are assigned to a carcass (USDA, 1992).

The following ovine grade description summarizes current sheep carcass standards (USDA, 1992). Subjective visual assessment of lean quality and conformation characteristics among three maturity classes has been the primary tool used to assign Quality Grades to ovine carcasses. Ovine carcasses are identified for degree of maturity by evaluating front cannon bones or trotters, shape and color of rib bones, and texture and color of lean tissue. Ossification of epiphyseal cartilage at the distal end of the front cannon or metacarpal bones results in the formation of a spool joint in carcasses from more mature sheep; ovine carcasses from animals with epiphyseal cartilage will present a perfect break joint during the dressing process of animal harvest. Carcasses classified as 'mutton' always have spool joints at the distal end of both front cannon bones. Perfect break joints at the distal end of both front cannon bones will be classified as 'lamb'. Ovine carcasses with one break joint and an imperfect break joint or spool joint can be

classified as yearling mutton, unless other maturity characteristics are typical of 'lamb'. In addition, ovine carcasses classified as lamb typically have rib bones that are slightly wide and moderately flat with lean that is light red in color and finely textured. Carcasses classified as mutton typically have wide, flat rib bones with lean that is dark red colored and coarsely textured. Yearling mutton typically have rib bones and lean that is intermediate to characteristics of lamb and mutton.

In the current grade standards, carcasses can qualify for five Quality Grades: Prime, Choice, Good, Utility and Cull. However, lamb and yearling mutton carcasses are eligible for Prime, Choice, Good and Utility Quality Grades while mutton carcasses are only eligible for Choice, Good, Utility and Cull Quality Grades. Quality Grades are applied via visual assessment of lean quality and conformation traits. Conformation refers to the thickness and fullness of the carcass referencing the proportion of edible tissue available from the carcass weight by focusing on the development of skeletal muscles, although external fat influences conformation scores. Lean quality refers to the texture, firmness and marbling. Unlike beef grading, ovine carcasses usually do not have the *Longissimus dorsi* muscle exposed prior to applying a quality grade. As a result, fat streaking abundance of the flank is used to estimate lean quality. An overall quality grade is determined by balancing lean quality and conformation scores among maturity classifications. However, a few exceptions exist when determining an overall quality grade. As maturity increases, requirements for fat streaking increase within each quality grade. A carcass with superior conformation and inferior lean quality is not eligible for the prime quality grade. Grade standards are applied to ovine carcasses regardless of sex, unless characteristics common to uncastrated males are evident. The extent of these characteristics can result in a reduced overall quality grade by up to two full grades.

Yield grades are based on estimates of the percentage of closely trimmed, semi-boneless or boneless, retail cuts from leg, loin, rack and shoulder of ovine carcasses. Yield grades range from 1 to 5 in numerical designation. In ovine. Since the *Longissimus dorsi* muscle is usually not exposed before grading, fat thickness on the surface of the carcass is used as the basis for assigning a numeric yield grade. Estimated fat thickness can be adjusted to reflect variable deposition across differing parts of the carcass. Yield grades are applied based on the following adjusted fat thickness ranges reported by USDA (1992): Yield Grade 1 = adjusted fat thickness of 0.00 to 0.15 inch; Yield Grade 2 = 0.16 to 0.25 inch; Yield Grade 3 = adjusted fat thickness of 0.26 to 0.35 inch; Yield Grade 4 = adjusted fat thickness of 0.36 to 0.45 inch; and Yield Grade 5 = 0.46 inch or greater.

Grades for ovine carcasses largely relies on subjective visual assessment by trained human graders employed by USDA's Agriculture Marketing Service. Although ovine quality and Yield Grades are primarily assigned by human graders, grading instruments were approved recently for use in estimating yield and quality attributes in the U.S. (discussed further below). Ovine carcasses are marketed using combinations of both USDA Yield and Quality grading carcass characteristics, receiving small premiums or discounts based on the combination of these grades that are assigned. Companies in the U.S. have developed a few programs that involve marketing claims that are based on additional characteristics, such as diet and live animal husbandry practices, to further segregate sheep carcasses into groups meeting specifications for attributes beyond those evaluated by the USDA grading system. While the beef industry has successfully adopted numerous branded beef programs, the sheep industry has one branded program aside from specific programs monitored by individual companies (USDA, 2018). Branded ovine programs may have the capability of successfully being implemented, but

demand for sheep meat would most likely need to grow.

### ***Ovine Grading Instruments***

In the U.S., ovine camera grading instruments were not fully approved until recently in 2018. Research efforts have investigated use of camera grading systems for several years, but full use and implantation was not approved by the USDA-AMS until February 2018. Although approved use of camera grading systems for ovine carcasses is relatively new in the U.S., several researchers investigated instrument predictive capabilities approximately two decades ago and predictive capabilities were first investigated approximately forty years ago in the beef industry. Initial research that evaluated capabilities of video image analysis (VIA) systems to predict lean muscle reported potential for this system to be utilized across the beef industry (Cross et al., 1983; Wassenberg, Allen, & Kemp, 1986). Since that time, several studies have followed that evaluated capabilities of VIA to predict numerous beef quality and yield attributes (Cannell et al., 2002; Cannell et al., 1999; Moore et al., 2010; Shackelford, Wheeler, & Koohmaraie, 1998, 2003; Steiner et al., 2003; Vote et al., 2003). Following considerable research efforts to demonstrate predictive capabilities of VIA camera grading instrument effectiveness, the first beef grading instrument was approved for use in 2001 with adopted performance standards by the USDA-AMS (Woerner and Belk, 2008).

The application of VIA systems in the sheep industry have been investigated by several researchers (Brady et al., 2003; Cunha et al., 2004; Einarsson et al., 2014; Hopkins et al., 2004; Rius-Vilarrasa et al., 2009). Brady et al. (2003) evaluated the capability of the lamb vision system (LVS), a type of VIA system, to predict sheep carcass fabrication yield. These researchers reported regression models of LVS carcass measurement output variables + HCW (hot carcass weight) that accounted for 87, 70, 65, and 77% of the variation in weights of

boneless leg, loin, rack, and shoulder primals and 86, 75, 72, and 85% of the variation in weights of bone-in leg, loin, rack, and should primals, respectively (Brady et al., 2003). In a subsequent study conducted by Cunha et al. (2004), equations developed by Brady et al. (2003) were evaluated and newly developed equations were evaluated using data from Brady et al. (2003). Cunha et al. (2004) reported that similar results were observed when using equations developed by Brady et al. (2003), but newly developed equations utilizing USDA Yield Grades explained a greater amount of sheep carcass cutability variation. These authors concluded that the LVS system was able to explain a greater proportion of variation in yield of bone-in leg, loin, rack and shoulder compared to on-line USDA Yield Grades (Cunha et al., 2004).

Similarly, Hopkins et al. (2004) investigated capabilities of the Australian VIAScan system designed to capture 60 linear and area measurements in addition to carcass color measurements. This work further demonstrated the application of VIA systems providing higher levels of accuracy in predicting lean meat yield compared to subjective and probe methods. Additionally, an E + V VIA system used in the United Kingdom resulted in improved prediction of sheep carcass primal weights compared to the Meat and Livestock Commission's EUROP subjective classification system (Rius-Vilarrasa et al., 2009). These studies demonstrated the capability of VIA systems to predict carcass cutability characteristics. Camera-based grading systems have now been implemented in U.S. large commercial sheep harvest facilities. Carcass characteristics that are being measured include those necessary to compute Yield Grades, Quality Grades, ovine cutability calculation, digital images of two views of each carcass, and weights of the leg, loin, rack, shoulder, breast, trotters and neck of the carcass.

### ***Inadequacies of the Current Sheep Grading System***

Quality grades were intended to predict palatability characteristics of ovine carcasses.

However, voluntary grade standards for ovine carcasses are currently only applied to part of the U.S. sheep supply. During fiscal year 2018, only 59.3% of all federally inspected ‘lambs’ were graded. Of these graded ovine carcasses, 91.0% and 9.0%, respectively, were assigned Choice and Prime Quality Grades (USDA-AMS, 2019). While USDA-AMS utilizes maturity characteristics to classify ovine carcasses as lamb, yearling mutton or mutton, the Food Safety Inspection Service (FSIS) branch of USDA responsible for overseeing safety and labeling activities does not have specific criteria that differentiate ovine age categories on product labels, except for designating ‘spring lamb’ (USDA-FSIS, 2019). This leads to continued confusion regarding mislabeling or misrepresentation without a strict definition for ‘lamb’. Ungraded ovine carcasses classified as yearling mutton, mutton or lower quality lamb can be marketed as ‘lamb’, further complicating the sheep grading and marketing systems. Purchasing recommendations from USDA-FSIS suggest selecting graded lamb (Choice or Prime Quality Grades) since age can be associated with variable quality (USDA, 1992). As a result, the current grading system is not being used to segregate ovine carcasses into more specific groups based on quality or yield characteristics.

### ***Overview of Flavor Detection***

Perception of flavor is an incredibly complex interaction that includes olfactory and gustatory sensations. Basic tastes include sweet, salty, bitter, and sour. Some discrepancy exists among the scientific community regarding whether umami is or should be considered a basic taste (Dashdorj, Amna, & Hwang, 2015). Much of the physiological taste perception research has been conducted in knock-out type rats and mice for specific types of receptors. Oral columnar structures comprised of at least 100 polarized neuroepithelial cells are commonly referred to as taste buds. Within a taste bud, numerous receptor cells are present that can transmit

signals from water soluble compounds pertaining to the different basic tastes. Scientific disagreement regarding the concept of tongue mapping has occurred since several studies were conducted in the late 1990's (Lindemann, 2001). However, regional sensitivity differences across the tongue are still considered (Chaudhari & Roper, 2010), particularly for bitter and sour sensitivity.

Three types of taste receptor cells (TRC) are involved in taste detection. Type I TRC is a glial-like cell that may be involved in detection of the salty taste. The exact mechanism of how salty is detected is not well understood. Some research has suggested that detection of the salty taste could be related to the ROMK (potassium channel) on some Type I cells that transduces signals, whereas other research has suggested that there may be direct permeation of sodium ions into interstitial spaces in unknown cell types (Chaudhari & Roper, 2010; Lee & Owyang, 2017). Type II cells are involved in detecting sweet, bitter and umami tastes through G-protein coupled receptors (GPCR) that utilize calcium signaling to induce a signal. The type I receptor has an extracellular heterodimer involved sweet and umami tastes by detecting sugars, synthetic sweeteners, sweet-tasting proteins, L-glutamate and GMP/IMP combinations; the type II receptor does not appear to have an extracellular component leading to some confusion as to the mechanism by which the taste bitter is detected (Chaudhari & Roper, 2010; Lee & Owyang, 2017). The proposed mechanism of Type III cells involves organic acids permeating plasma membranes leading to signaling effects of blocked potassium channels and resulting in activated calcium channels that cause downstream signaling via cytoplasmic calcium (Chaudhari & Roper, 2010; Lee & Owyang, 2017). Activated receptor cells for sweet, umami and bitter can release ATP that initiate an action potential on ATP-receptors on sensory nerve fibers. Detection of the sour note appears to not be fully understood in research, but seems to directly activate

presynaptic cells that respond with nerve cells. Other types of gustatory receptors have been discussed in the context of flavor. Capsaicin compounds found in spicy foods, such as chilis, activate pain receptors (Chaudhari & Roper, 2010). Since flavor is considered a perception, others have suggested that other somatosensory properties such as texture and visual appearance could modulate the perception of flavor – although this specific concept was based on a prospective psychological model by Small and Prescott in 2005. Additionally, work conducted in the 2000's suggested that detecting fat in food may have gustatory effects separate from the basic tastes (Chaudhari & Roper, 2010).

One of the largest contributors to overall flavor is the olfactory system. Aromatic compounds are detected by the olfactory epithelium located in the roof of the nasal cavity. The olfactory epithelium area includes specialized bipolar neurons that interact with the olfactory bulb to transmit signals directly to the brain. Turbulent airflow is required to carry an aromatic compound to the olfactory epithelium. Intentionally smelling via inhaling directly through the anterior end of the nose is considered the orthonasal olfaction route. Detecting aromatic compounds during mastication occurs by progressing towards the nasopharynx and reach the olfactory epithelium from the posterior end of the nose is considered the retronasal olfaction route (Masaoka, Satoh, Akai, & Homma, 2010). Olfactory receptors have numerous seven-transmembrane GPCRs that are used to detect varying structures of volatiles. There seems to be a discrepancy as to the number of different olfactory receptors resulting from a large multigene family (Malnic, Godfrey, & Buck, 2004; Maßberg & Hatt, 2018); hundreds of olfactory receptor genes have been discussed in the scientific literature. Ultimately, flavor is a complex interaction of gustatory and olfactory mechanisms.

### ***Meat Flavor***

Flavor of ovine meat has been characterized with a species distinctive flavor profile and aroma. Mutton flavor or mutton-like flavor has been associated with the intense, offensive flavor of sheep meat originating from animals of any age (Sink & Caporaso, 1977). Branched chain fatty acids (BCFA) comprised of 8 to 10 carbon molecules have been established as compounds responsible for mutton flavor. Specifically, 4-methyloctanoic acid (MOA), 4-methylnonanoic acid (MNA), and 4-ethyloctanoic acid (EOA) are compounds generally associated with the more intense, mutton flavor found in meat from sheep. Tatum et al. described in a 2014 review that previous research has determined that concentration of BCFA are generally greater in ovine adipose tissue from older animals (Watkins et al., 2010).

Two separate studies have demonstrated that the concentration of BCFA increased in wethers and rams as the age of the animal increased, but BCFA concentration was greater in rams compared to wethers after reaching sexual maturity implying that intact rams are prone to increased concentration of BCFA that could be contributing to mutton-flavor (Sutherland & Ames, 1996; Young et al., 2006). Interestingly though, increased propionate production from feeding grain to lambs resulted in greater concentration of BCFA in adipose tissue (Tatum, Zerby, & Belk, 2014; Young et al., 2003), yet sheep meat from grain-fed lambs can have milder flavor attributes and aroma compared to pasture-fed lambs (Young et al., 2003). Additionally, Tatum et al. (2014) described several studies that agreed that meat from lambs fed white clover or alfalfa, grazed on rape, or that were pasture-fed in general, had stronger flavor and aroma than meat from lambs that were grass-fed. Indole (3-methylindole) and methylphenol (4-methylphenol) compounds have been implicated in pastoral flavor development in meat from pasture-fed lambs (Watkins et al., 2013), including off-flavors. Watkins et al. (2013) suggested that seasonal effects on pasture chemical composition as well as pasture higher in total nitrogen

and protein concentration could result in greater pastoral flavor in meat from sheep grazing these forages. Volatile compounds in the form of terpenes and diterpenoids were identified in cooked meat from pasture-fed sheep (Priolo et al., 2004; Young et al., 1997). In a separate study, researchers suggested that 2, 3-octanedione could be a marker in products produced by pasture-fed sheep (Sivadier, Ratel, & Engel, 2010). Both compounds, indole and alkyl phenols, are thought to be produced by rumen microbial metabolism of tryptophan and tyrosine (Watkins et al., 2013). These compounds have been associated with pastoral flavor in sheep meat (Priolo et al., 2001) producing strong, offensive flavor beyond characteristic sheep meat flavor driven by branched chain fatty acids (Young et al., 1997).

Other flavor attributes have been investigated in ovine meat. Higher lamb flavor has been associated to meat from grain-fed lambs compared to higher liver-like flavor being associated to meat from grass-fed animals (Priolo et al., 2001; Watkins et al., 2013). Grassy or green-like flavors in meat may be due to higher levels of polyunsaturated fatty acid content (PUFA) and thermal oxidation of PUFA during cooking (Stelzleni & Johnson, 2008). Watkins et al. (2013) speculated that unsaturated aldehydes and other compounds were probably the result of thermal oxidation of PUFA during cooking.

Numerous compounds contribute to meat flavor and aroma. A large proportion of the sensory experience is affected by inherent compounds that change or develop during storage or upon cooking, which provides insight to the complexity of meat flavor (Calkins & Hodgen, 2007). Specifically, flavor compound development due to the non-enzymatic browning Maillard reaction and lipid oxidation have large effects on the sensory experience (Calkins & Hodgen, 2007; Khan, Jo, & Tariq, 2015). A general description of non-enzymatic browning is the reaction of a free reducing sugar, such as ribose or deoxyribose from deoxyribonucleic acid (DNA) or

ribonucleic acid (RNA), and amino acids. This condensation reaction forms glycosylamine which can produce additional compounds (Calkins & Hodgen, 2007). The combination of a specific amino acid and reducing sugar can generate many different compounds including furans, furanones, aldehydes, ketones, thiazoles, pyrroles, pyrazines, pyridines, thiophenes and several others (Dashdorj et al., 2015; Kosowska et al., 2017). Dashdorj et al. (2015) described hundreds of volatile compounds contributing to sensory perception that resulted from the reaction of a specific amino acid and a reducing sugar during the Maillard reaction. Additionally, sulfur containing compounds formed during the Maillard reaction contribute to flavor profiles and thiophenol is thought to contribute to aroma in sheep meat (Ha & Lindsay, 1991).

Dashdorj et al. (2015) further described cooked meat flavor being driven by lipid oxidation, the interaction between products of the Maillard and lipid oxidation reactions, and the degradation of thiamine during cooking. Lipid oxidation produces volatiles such as nonanal, 2,3-octanedione, pentanal, butanoic acid, and hexanoic acid (Kosowska et al., 2017; Stetzer et al., 2008). Dashdorj et al. (2015) described Maillard reaction and lipid oxidative interaction products are important in positive beef flavor attributes, but independently lipid oxidation products can lead to negative flavor attributes including objectionable flavor and aroma associated with rancidity common to storage conditions prior to cooking (Amaral et al., 2018). Additionally, a major drive of flavor is triacylglycerides and fatty acids in meat products (Dashdorj et al., 2015). These authors described that nearly 70 thermal degradation products have been associated with thiamine degradation and flavor development (Dashdorj et al., 2015).

### ***Rapid Evaporative Ionization Mass Spectrometry (REIMS)***

Rapid evaporative ionization mass spectrometry (REIMS) is a technology platform for analytical mass spectrometry. Although originally developed to differentiate cancerous from

non-cancerous tissue for medical research (Balog et al., 2010; Golf et al., 2015), REIMS has become a promising technology in food and agricultural applications (Black et al., 2017). Compared to other analytical approaches, REIMS has many advantages, including the lack of sample preparation required. Analysis of food samples traditionally has required the sample to be physically prepared (e.g., homogenized, extraction, etc.) before mass spectral data capture. A more traditional analytical method can take a substantially longer amount of time to analyze each sample, particularly when analyzing large sample sizes. In addition to restricting the number of samples that can be analyzed in a timeframe, sample preparation steps using other analytical methods can introduce error.

Comparatively, REIMS requires very little preparation time (Waters Corporation, 2019). The main advantage of REIMS is the quick extraction and ionization capability using the ‘iKnife’ sampling tool coupled with a mass spectrometer. The ‘iKnife’ sampling tool is comprised of a handheld mobile device with flexible plastic tubing attached to a mass spectrometer. Ionization occurs via the ‘iKnife’ sampling tool and electrode creating charged aerosolized droplets as tissue evaporates (Balog et al., 2010). The current heats the metal blade on the ‘iKnife’ sampling tool, cauterizing the surface of the sample. The aerosolized droplets travel through the plastic tubing under vacuum and to the inlet of the mass spectrometer before reaching a heated impactor (Golf et al., 2015). A TOF (time-of-flight) analyzer detects charged ions and mass spectra are available through the REIMS imaging platform (Golf et al., (2015).

The speed of sample collection and mass spectral output allows for faster and greater sample sizes to be analyzed using the REIMS approach, ultimately reducing the amount of time for total analysis. The lack of need for sample preparation using this method compared to other analytical methods also reduces the chance of introducing technical error. An accurate molecular

profile is available within seconds after use of the handheld sampling tool when the technology is paired with a time-of-flight mass spectrometer (Waters Corporation, 2019). Since multiple samples can be analyzed quickly, the use of software platforms paired with multivariate statistical analyses can be used to identify similar and dissimilar mass spectra for differentiation among samples. Highly accurate models have been developed to predict and classify tissues using mass spectra data captured by REIMS (Balog et al., 2010). Further statistical techniques can be used to develop predictive algorithms in research using mass spectral data collected.

Although REIMS is a relatively new technology, it has been used to predict several characteristics of meat products. Balog et al. (2016) used REIMS to predict the species and breeds from which products were derived to determine if the technology could have implications for preventing food fraud. Species and breeds were predicted with 100% and 97% accuracy, respectively. In another study using REIMS, fish species were predicted with nearly 99% accuracy (Black et al., 2017). Guitton et al. (2018) was able to identify and predict ractopamine among various pork muscles with accuracy over 95%. Verplanken et al. (2017) reported very high accuracy in segregating samples with and without boar taint. Other studies have utilized REIMS to identify and predict classification of other foods and applications. These research applications suggest that this technology has large potential to improve and predict quality characteristics of various foods including meat products.

### ***Predictive Modeling***

Kuhn and Johnson (2016) described predictive modeling in reference to forming accurate predictions. Predictive modeling has multiple applications including many business and information technology applications; however, it is also utilized heavily in analysis of ‘omics’ data (Kim & Tagkopoulos, 2018; Kuhn & Johnson, 2013). Correct use of predictive models

expands analysis abilities of multiple types of data including large, biological datasets. While predictive modeling approaches can identify prediction of a quantitative or qualitative outcome, considerations need to be made during model development. Kuhn and Johnson (2016) stated that predictive models often fail for one of four reasons, including: “(1) inadequate pre-processing of the data, (2) inadequate model validation, (3) unjustified extrapolation (e.g., application of the model to data that reside in a space which the model has never seen), or, most importantly, (4) over-fitting the model to the existing data” (Kuhn and Johnson, 2016). Other issues that Kuhn and Johnson (2016) discuss are distinguishing in prediction accuracy compared to interpretation. These authors describe that predictive modeling is focused on the accuracy of whether the event will happen or not rather than why an event happens or not.

### ***Principle component analysis and partial least squares***

Advancements in technology have improved to develop methods that allow large amounts of data to be collected (Bunte et al., 2012). High-dimensional data is described as a greater number of unknown parameters, or variables, than the sample size (Bühlmann & Van De Geer, 2011; James et al., 2013). These large datasets tend to be extremely complex containing enormous amounts of information that can be difficult to conceptualize. Due to the large, complexity of these datasets, dimension reduction techniques have been developed to maintain relevant features while aiding in visualization of the data (Bunte et al., 2012). ‘Noise’ from irrelevant features in a dataset can overpower relevant features, creating more difficulty when attempting to fit a model to a large dataset (Ghatak, 2017a, b).

Two common dimension reduction techniques for large datasets are principal component analysis (PCA) and partial least squares (PLS). Both PCA and PLS find linear combinations among the predictors that best explain the variation within the dataset (James et al., 2013). Such

linear constructs are considered a new set of latent variables, referred to as factor scores (James et al., 2013). Loadings values refer to correlation between the original variables and each component and explain the weight or contribution of each variable to the principle components (Zelterman, 2015). Both factor scores and loadings provide valuable information, particularly when plotted to visualize these relationships.

The PCA method does not include the response variable when determining sources of variation, but PLS does use the response to parse out sources of variation. Due to this difference, PCA is considered unsupervised compared to PLS that is considered supervised (James et al., 2013). Specifically, PCA identifies sources of variation without considering the response variable (James et al., 2013). Eigenvalues indicate how much variance is explained by each principle component. The first component explains the greatest amount of variation followed by additional components that explain gradually less variation. Principal component analysis does remove collinearity for use in other analysis models, often considered a data pre-processing step.

Conversely, PLS uses the response when identifying variance that best explains the response from the predictors (Kuhn and Johnson, 2016). The partial least squares approach identifies components that maximize the variation of the predictors, dimension reduction and maximum correlation between predictors and the response (Kuhn and Johnson, 2016).

In general, pre-processing methods add, delete or transform data used for predictive model training (Kuhn and Johnson, 2016). Data pre-processing or data preparation can directly affect the predictive ability of a model. Kuhn and Johnson (2016) suggested that the pre-processing method depends on the characteristics of the dataset and there does not seem to be a single, correct pre-processing method that the researcher needs to use. Rather, the factors comprising the dataset can lead the researcher to select a pre-processing method. Methods that

reduce collinearity which feature components that explain a large percentage of the data, such as PCA or PLS, can be considered a pre-processing method (Kuhn & Johnson, 2013). This scenario would imply that a group of predictors associated with the first principle component represent similar information in the model. Kuhn and Johnson (2016) described that data transformation can improve model performance by reducing skewness or outlier impact while combinations of predictors can often have better impacts on model performance. Ultimately, several pre-processing methods exist; consideration of how a method affects the data characteristics is necessary.

### ***Linear Discriminant Analysis***

Linear discriminant analysis (LDA) is an approach that can be used to find linear combinations of variables to classify observations into clear groups (Zelterman, 2015). Similar to PLS and PCA, LDA utilizes latent variables to characterize the relationship to the original predictor variables. However, in this supervised method, the number of latent variables is limited to one less than the number of classification categories and the final number of predictors must be less than the number of observations (Kuhn and Johnson, 2016). Due to these rules, dimension reduction techniques need to be performed before LDA. Additionally, variance of the predictor variables is maximized while maximizing distance between classification boundaries in LDA (Kuhn and Johnson, 2016). Multiple studies utilizing REIMS have utilized LDA after also using a dimension reduction method (Balog et al., 2016; Black et al., 2017; Bodai et al., 2018; Guitton et al., 2018; Phelps et al., 2016; Phelps et al., 2018).

### ***Machine Learning Algorithms***

Machine learning refers to in-depth discovery of complex data patterns and trends. Machine learning algorithms assist in predicting likelihood of relationships between response

variables and predictors (Ghatak, 2017b; Ramasubramanian & Singh, 2017). Supervised machine learning utilizes data with known classifications referred to as the training dataset to aid in model development in order to evaluate model capabilities to classify test data (Swan, Mobasher, Allaway, Liddell, & Bacardit, 2013). Machine learning algorithms assist by evaluating complex patterns and relationships within a dataset. In these algorithms, the model is optimized to predict or classify an outcome of interest. Although specific machine learning algorithms are outlined below, many others exist. Additionally, LDA (discussed previously) is a machine learning algorithm that can be utilized with omics data.

#### ***Partial least squares discriminant analysis (PLSDA)***

Partial least squares discriminant analysis (PLSDA) is a dimension reduction technique that transforms large data sets into partial least squares components in order to reduce dimensionality of the data and reduce misclassification of observations. This modeling technique has been considered a common method for chemometric data such as mass spectrometry (Gredell et al., 2019; Gromski et al., 2015; Gromski et al., 2014; Pérez-Enciso & Tenenhaus, 2003). The PLSDA algorithm can be prone to misinterpretation and overfitting (Gredell et al., 2019; Gromski et al., 2014), but also has the ability to handle multicollinear variables. This model can identify PLS components and classify observations into specific classifications. In the context of machine learning, the focus of PLSDA is on classification rather than on dimension reduction.

#### ***Support vector machine (SVM)***

Support vector machine (SVM) is an algorithm based on predicting separability between classifications (Swan et al., 2013). The optimal hyperplane dimension is determined in order to differentiate between classes within data for SVM (Gredell et al., 2019; Ramasubramanian &

Singh, 2017). Specifically, the separation occurs by margin maximization between the closest points of the classes. In linear-SVM, a minimal set of training occurrences to identify the optimal linear classifier are considered in order to determine the boundaries of the margin (Swan et al., 2013). Other parameters that can be used with SVM include linear, radial and polynomial to evaluate various accuracies in model prediction (Gredell et al., 2019).

### ***Random forest (RF)***

Random forest (RF) is a common method that predicts classifications using decision trees (Gredell et al., 2019; Ramasubramanian & Singh, 2017; Swan et al., 2013). This decision tree model works by randomly utilizing a subset of variables in the dataset ( $m$  variables) to build  $n$  decision trees as large as possible. The RF algorithm will operate at an  $m$  of any value less than or equal to the number of predictors, but an  $m$  value selected to be equal to the square root of the number of predictors is typical (Gredell et al., 2019). Swan et al. (2013) simplified this process by describing that each of the decision trees is limited to a random subset of data and the majority class is determined by the vote or decision of each individual tree. Further, trees are decorrelated when  $m$  predictors are used at each decision and different predictors are used for each tree.

### ***XGBoost***

The XGBoost algorithm is a gradient-boosted decision tree in which a gradient reduces the loss as additional models are added. This is a supervised learning approach that has low computational time regardless of whether the data set is large. The XGBoost algorithm can utilize regression, ranking and classification prediction components. Known for its computational efficiency, XGBoost tends to have high model performance capabilities (Chen, He, Benesty, Khotilovich, & Tang, 2015).

### ***LogitBoost***

The LogitBoost algorithm operates as an additive logistic regression model (Gredell et al., 2019). This function uses one node decision trees with weak classifiers applied to each observation and the votes from weak classifiers are used to assign classifications (Tuszynski, 2012). As votes of weak classifiers are combined, a dominant classifier will be identified. The LogitBoost model minimizes loss of the function due to the sequential, additive approach.

## LITERATURE CITED

- Amaral, A. B., SILVA, M. V. d., & LANNES, S. C. d. S. (2018). Lipid oxidation in meat: mechanisms and protective factors—a review. *Food Science and Technology*, 38, 1-15.
- Balog, J., Perenyi, D., Guallar-Hoyas, C., Egri, A., Pringle, S. D., Stead, S., . . . Takats, Z. (2016). Identification of the species of origin for meat products by rapid evaporative ionization mass spectrometry. *Journal of agricultural and food chemistry*, 64(23), 4793-4800.
- Balog, J., Szaniszló, T., Schaefer, K.-C., Denes, J., Lopata, A., Godorhazy, L., . . . Toth, M. (2010). Identification of biological tissues by rapid evaporative ionization mass spectrometry. *Analytical chemistry*, 82(17), 7343-7350.
- Black, C., Chevallier, O. P., Haughey, S. A., Balog, J., Stead, S., Pringle, S. D., . . . Morris, M. (2017). A real time metabolomic profiling approach to detecting fish fraud using rapid evaporative ionisation mass spectrometry. *Metabolomics*, 13(12), 153.
- Bodai, Z., Cameron, S., Bolt, F., Simon, D., Schaffer, R., Karancsi, T., . . . Hardiman, K. (2018). Effect of Electrode Geometry on the Classification Performance of Rapid Evaporative Ionization Mass Spectrometric (REIMS) Bacterial Identification. *Journal of The American Society for Mass Spectrometry*, 29(1), 26-33.
- Brady, A. S., Belk, K. E., LeValley, S. B., Dalsted, N. L., Scanga, J. A., Tatum, J. D., & Smith, G. C. (2003). An evaluation of the lamb vision system as a predictor of lamb carcass red meat yield percentage. *Journal of Animal Science*, 81(6), 1488-1498.
- Bühlmann, P., & Van De Geer, S. (2011). *Statistics for high-dimensional data: methods, theory and applications*: Springer Science & Business Media.
- Bunte, K., Biehl, M., & Hammer, B. (2012). A general framework for dimensionality-reducing data visualization mapping. *Neural Computation*, 24(3), 771-804.
- Calkins, C. R., & Hodgen, J. M. (2007). A fresh look at meat flavor. *Meat science*, 77(1), 63-80.
- Cannell, R. C., Belk, K. E., Tatum, J. D., Wise, J. W., Chapman, P. L., Scanga, J. A., & Smith, G. C. (2002). Online evaluation of a commercial video image analysis system (Computer Vision System) to predict beef carcass red meat yield and for augmenting the assignment of USDA yield grades. *Journal of Animal Science*, 80(5), 1195-1201.
- Cannell, R. C., Tatum, J. D., Belk, K. E., Wise, J. W., Clayton, R. P., & Smith, G. C. (1999). Dual-component video image analysis system (VIASCAN) as a predictor of beef carcass red meat yield percentage and for augmenting application of USDA yield grades. *Journal of Animal Science*, 77(11), 2942-2950.

- Chaudhari, N., & Roper, S. D. (2010). The cell biology of taste. *The Journal of cell biology*, 190(3), 285-296.
- Chen, T., He, T., Benesty, M., Khotilovich, V., & Tang, Y. (2015). Xgboost: extreme gradient boosting. R package version 0.4-2, 1-4.
- Cross, H. R., Gilliland, D. A., Durland, P. R., & Seideman, S. (1983). Beef Carcass Evaluation by Use of a Video Image-Analysis System. *Journal of Animal Science*, 57(4), 908-917.
- Cunha, B. C. N., Belk, K. E., Scanga, J. A., LeValley, S. B., Tatum, J. D., & Smith, G. C. (2004). Development and validation of equations utilizing lamb vision system output to predict lamb carcass fabrication yields. *Journal of Animal Science*, 82(7), 2069-2076.
- Dashdorj, D., Amna, T., & Hwang, I. (2015). Influence of specific taste-active components on meat flavor as affected by intrinsic and extrinsic factors: an overview. *European Food Research and Technology*, 241(2), 157-171.
- Einarsson, E., Eythorsdottir, E., Smith, C. R., & Jonmundsson, J. V. (2014). The ability of video image analysis to predict lean meat yield and EUROP score of lamb carcasses. [Article]. *Animal*, 8(7), 1170-1177. doi: 10.1017/s1751731114000962.
- Ghatak, A. (2017a). Introduction to machine learning *Machine Learning with R* (pp. 57-78): Springer.
- Ghatak, A. (2017b). *Machine learning with R*: Springer, Singapore.
- Golf, O., Strittmatter, N., Karancsi, T., Pringle, S. D., Speller, A. V., Mroz, A., . . . Takats, Z. (2015). Rapid evaporative ionization mass spectrometry imaging platform for direct mapping from bulk tissue and bacterial growth media. *Analytical chemistry*, 87(5), 2527-2534.
- Gredell, D. A., Schroeder, A. R., Belk, K. E., Broeckling, C. D., Heuberger, A. L., Kim, S. Y., . . . Prenni, J. E. (2019). Comparison of Machine Learning Algorithms for Predictive Modeling of Beef Attributes Using Rapid Evaporative Ionization Mass Spectrometry (REIMS) Data. *Sci Rep*, 9(1), 5721. doi: 10.1038/s41598-019-40927-6
- Gromski, P. S., Muhamadali, H., Ellis, D. I., Xu, Y., Correa, E., Turner, M. L., & Goodacre, R. (2015). A tutorial review: Metabolomics and partial least squares-discriminant analysis--a marriage of convenience or a shotgun wedding. *Anal Chim Acta*, 879, 10-23. doi: 10.1016/j.aca.2015.02.012
- Gromski, P. S., Xu, Y., Correa, E., Ellis, D. I., Turner, M. L., & Goodacre, R. (2014). A comparative investigation of modern feature selection and classification approaches for the analysis of mass spectrometry data. *Anal Chim Acta*, 829, 1-8. doi: 10.1016/j.aca.2014.03.039

- Guitton, Y., Dervilly-Pinel, G., Jandova, R., Stead, S., Takats, Z., & Le Bizec, B. (2018). Rapid evaporative ionisation mass spectrometry and chemometrics for high-throughput screening of growth promoters in meat producing animals. *Food Additives & Contaminants: Part A*, 35(5), 900-910.
- Ha, J. K., & Lindsay, R. (1991). Volatile alkylphenols and thiophenol in species-related characterizing flavors of red meats. *Journal of food science*, 56(5), 1197-1202.
- Hopkins, D. L., Safari, E., Thompson, J. M., & Smith, C. R. (2004). Video image analysis in the Australian meat industry - precision and accuracy of predicting lean meat yield in lamb carcasses. *Meat Science*, 67(2), 269-274. doi: 10.1016/j.meatsci.2003.10.015
- James, G., Witten, D., Hastie, T., & Tibshirani, R. (2013). *An introduction to statistical learning (Vol. 112)*: Springer.
- Khan, M. I., Jo, C., & Tariq, M. R. (2015). Meat flavor precursors and factors influencing flavor precursors—a systematic review. *Meat science*, 110, 278-284.
- Kim, M., & Tagkopoulos, I. (2018). Data integration and predictive modeling methods for multi-omics datasets. *Molecular omics*, 14(1), 8-25.
- Kosowska, M., A MAJCHER, M., & Fortuna, T. (2017). Volatile compounds in meat and meat products. *Food Science and Technology*, 37(1), 1-7.
- Kuhn, M., & Johnson, K. (2013). *Applied predictive modeling (Vol. 26)*: Springer, New York, NY.
- Lee, A., & Owyang, C. (2017). Sugars, sweet taste receptors, and brain responses. *Nutrients*, 9(7), 653.
- Lindemann, B. (2001). Receptors and transduction in taste. *Nature*, 413(6852), 219.
- Malnic, B., Godfrey, P. A., & Buck, L. B. (2004). The human olfactory receptor gene family. *Proceedings of the National Academy of Sciences*, 101(8), 2584-2589.
- Maneotis, K. A. 2017. A proof-of-concept to differentiate among differences in flavor of American lamb using volatile flavor compound analysis. Master's Thesis, Colorado State University, Fort Collins, CO.
- Masaoka, Y., Satoh, H., Akai, L., & Homma, I. (2010). Expiration: the moment we experience retronasal olfaction in flavor. *Neuroscience letters*, 473(2), 92-96.
- Maßberg, D., & Hatt, H. (2018). Human olfactory receptors: Novel cellular functions outside of the nose. *Physiological reviews*, 98(3), 1739-1763.

- Moore, C. B., Bass, P. D., Green, M. D., Chapman, P. L., O'Connor, M. E., Yates, L. D., . . . Belk, K. E. (2010). Establishing an appropriate mode of comparison for measuring the performance of marbling score output from video image analysis beef carcass grading systems. *Journal of Animal Science*, 88(7), 2464-2475. doi: 10.2527/jas.2009-2593
- Pérez-Enciso, M., & Tenenhaus, M. (2003). Prediction of clinical outcome with microarray data: a partial least squares discriminant analysis (PLS-DA) approach. *Human genetics*, 112(5-6), 581-592.
- Phelps, D. L., Balog, J., El-Bahrawy, M., Speller, A., Brown, R., Takats, Z., & Ghaem-Maghamsi, S. (2016). Diagnosis of borderline ovarian tumours by rapid evaporative ionisation mass spectrometry (REIMS) using the surgical intelligent knife ('iKnife').
- Phelps, D. L., Balog, J., Gildea, L. F., Bodai, Z., Savage, A., El-Bahrawy, M. A., . . . McKenzie, J. S. (2018). The surgical intelligent knife distinguishes normal, borderline and malignant gynaecological tissues using rapid evaporative ionisation mass spectrometry (REIMS). *British journal of cancer*, 118(10), 1349.
- Priolo, A., Cornu, A., Prache, S., Krogmann, M., Kondjoyan, N., Micol, D., & Berdagué, J.-L. (2004). Fat volatiles tracers of grass feeding in sheep. *Meat Science*, 66(2), 475-481.
- Priolo, A., Micol, D., & Agabriel, J. (2001). Effects of grass feeding systems on ruminant meat colour and flavour. A review. *Animal Research*, 50(3), 185-200.
- Ramasubramanian, K., & Singh, A. (2017). *Machine learning using R*: Springer.
- Rius-Vilarrasa, E., Bungler, L., Brotherstone, S., Matthews, K. R., Haresign, W., Macfarlane, J. M., . . . Roehe, R. (2009). Genetic parameters for carcass composition and performance data in crossbred lambs measured by Video Image Analysis. *Meat Science*, 81(4), 619-625. doi: 10.1016/j.meatsci.2008.10.020
- Rius-Vilarrasa, E., Bungler, L., Maltin, C., Matthews, K. R., & Roehe, R. (2009). Evaluation of Video Image Analysis (VIA) technology to predict meat yield of sheep carcasses on-line under UK abattoir conditions. [Article]. *Meat Science*, 82(1), 94-100. doi: 10.1016/j.meatsci.2008.12.009
- Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (1998). Coupling of image analysis and tenderness classification to simultaneously evaluate carcass cutability, longissimus area, subprimal cut weights, and tenderness of beef. *Journal of Animal Science*, 76(10), 2631-2640.
- Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2003). On-line prediction of yield grade, longissimus muscle area, preliminary yield grade, adjusted preliminary yield grade, and marbling score using the MARC beef carcass image analysis system. *Journal of Animal Science*, 81(1), 150-155.

- Sink, J. D., & Caporaso, F. (1977). Lamb and mutton flavour: Contributing factors and chemical aspects. *Meat Sci*, 1(2), 119-127. doi: 10.1016/0309-1740(77)90013-4
- Sivadier, G., Ratel, J., & Engel, E. (2010). Persistence of pasture feeding volatile biomarkers in lamb fats. *Food Chemistry*, 118(2), 418-425.
- Steiner, R., Wyle, A. M., Vote, D. J., Belk, K. E., Scanga, J. A., Wise, J. W., . . . Smith, G. C. (2003). Real-time augmentation of USDA yield grade application to beef carcasses using video image analysis. *Journal of Animal Science*, 81(9), 2239-2246.
- Stelzleni, A., & Johnson, D. (2008). Effect of days on concentrate feed on sensory off-flavor score, off-flavor descriptor and fatty acid profiles for selected muscles from cull beef cows. *Meat science*, 79(2), 382-393.
- Stetzer, A., Cadwallader, K., Singh, T., Mckeith, F., & Brewer, M. (2008). Effect of enhancement and ageing on flavor and volatile compounds in various beef muscles. *Meat Science*, 79(1), 13-19.
- Sutherland, M. M., & Ames, J. M. (1996). Free fatty acid composition of the adipose tissue of intact and castrated lambs slaughtered at 12 and 30 weeks of age. *Journal of Agricultural and Food Chemistry*, 44(10), 3113-3116. doi: DOI 10.1021/jf960049h
- Swan, A. L., Mobasheri, A., Allaway, D., Liddell, S., & Bacardit, J. (2013). Application of machine learning to proteomics data: classification and biomarker identification in postgenomics biology. *Omics: a journal of integrative biology*, 17(12), 595-610.
- Tatum, J. D., Zerby, H. N., & Belk, K. E. (2014). A brief overview of factors affecting lamb flavor. A white paper. Lamb Industry Roadmap Product Characteristics Team.
- Tuszynski, J. (2012). caTools: Tools: moving window statistics, GIF, Base64, ROC AUC, etc., r package version 1.17. 1. URL [http://CRAN.R-project.org/package= caTools](http://CRAN.R-project.org/package=caTools). [accessed 01 April 2014].
- USDA. (1992). United States standards for grades of lamb, yearling mutton, and mutton carcasses: USDA, Agricultural Marketing Service, Livestock and Seed Division Washington, DC.
- USDA-AMS. 2019. National summary of meats graded. USDA, Agricultural Marketing Service. Accessed April 6, 2019. Accessible at: [https://www.ams.usda.gov/sites/default/files /media/CY2018GradeVolumeReport.pdf](https://www.ams.usda.gov/sites/default/files/media/CY2018GradeVolumeReport.pdf).
- USDA-ERS. 2019. Supply and disappearance and per capita disappearance. United States Department of Agriculture – Economic Research Service. Accessed March 4, 2019. Accessible at: <https://www.ers.usda.gov/topics/animal-products/sheep-lamb-mutton/#periodic>.

- USDA-FSIS. (2019). Title 9 Code of Federal Regulation False or misleading labeling or practices generally; specific prohibitions and requirements for labels and containers, 317.8. <https://www.law.cornell.edu/cfr/text/9/317.8>. (Accessed April 21, 2019).
- Vote, D. J., Belk, K. E., Tatum, J. D., Scanga, J. A., & Smith, G. C. (2003). Online prediction of beef tenderness using a computer vision system equipped with a BeefCam module. *Journal of Animal Science*, 81(2), 457-465.
- Wassenberg, R. L., Allen, D. M., & Kemp, K. E. (1986). Video Image-Analysis Prediction of Total Kilograms and Percent Primal Lean and Fat Yield of Beef Carcasses. *Journal of Animal Science*, 62(6), 1609-1616.
- Waters Corporation. 2019. REIMS research system with 'iKnife' sampling device. Accessed March 4, 2019. Available at [http://www.waters.com/waters/en\\_US/REIMS-Research-System-with-'iKnife'-Sampling-Device/nav.htm?cid=134846529&locale=en\\_US](http://www.waters.com/waters/en_US/REIMS-Research-System-with-'iKnife'-Sampling-Device/nav.htm?cid=134846529&locale=en_US).
- Watkins, P. J., Frank, D., Singh, T. K., Young, O. A., & Warner, R. D. (2013). Sheepmeat flavor and the effect of different feeding systems: A review. *Journal of Agricultural and Food Chemistry*, 61(15), 3561-3579.
- Watkins, P. J., Rose, G., Salvatore, L., Allen, D., Tucman, D., Warner, R. D., . . . Pethick, D. W. (2010). Age and nutrition influence the concentrations of three branched chain fatty acids in sheep fat from Australian abattoirs. *Meat Sci*, 86(3), 594-599. doi: 10.1016/j.meatsci.2010.04.009
- Young, O., Berdagué, J.-L., Viallon, C., Rousset-Akrim, S., & Theriez, M. (1997). Fat-borne volatiles and sheepmeat odour. *Meat science*, 45(2), 183-200.
- Young, O. A., Lane, G. A., Podmore, C., Fraser, K., Agnew, M. J., Cummings, T. L., & Cox, N. R. (2006). Changes in composition and quality characteristics of ovine meat and fat from castrates and rams aged to 2 years. *New Zealand Journal of Agricultural Research*, 49(4), 419-430. doi: Doi 10.1080/00288233.2006.9513733
- Young, O. A., Lane, G. A., Priolo, A., & Fraser, K. (2003). Pastoral and species flavour in lambs raised on pasture, lucerne or maize. *Journal of the Science of Food and Agriculture*, 83(2), 93-104.
- Zelterman, D. 2015. *Applied Multivariate Statistics with R*. In: Springer International Publishing, Switzerland.

## CHAPTER III.

### ASSESSMENT OF EVAPORATIVE IONIZATION MASS SPECTROMETRY (REIMS) TO CHARACTERIZE LAMB FLAVOR

#### *Introduction*

The U.S. supply of sheep has declined over several decades due to reduced consumption of lamb meat. The United States Department of Agriculture Economic Research Service reported 1.1 pounds (approximately 0.5 kg) of sheep meat was consumed per capita within the United States between January to December 2018 (USDA-ERS, 2019). However, this is based on disappearance data from production through disappearance of sheep meat products and does not account for actual consumption. These data indicate that consumption of sheep meat has continued to decline since 1970 (USDA-ERS, 2019). Additionally, competitive protein markets and lack of differentiation between U.S. produced sheep compared to imported lamb products have further complicated the low consumption of sheep meat (Jones, 2004).

Ultimately, lower demand for sheep meat has created challenges in the U.S. sheep industry. Imported sheep meat, particularly from New Zealand and Australia, occupies valuable space in retail meat cases forcing American sheep meat to compete with imported product. Consumer dissatisfaction with flavor profiles of sheep meat has been suggested as one possible explanation of low consumption and demand. The 2016 National Lamb Quality Audit identified “eating satisfaction” as the most important quality trait for sheep meat (Hoffman, Dissertation, 2015). Further, this study indicated that 71% of consumers would be willing to pay additional premiums for eating satisfaction among sheep meat, supporting the need to understand flavor differences among sheep meat.

Rapid evaporative ionization mass spectrometry (REIMS) is a relatively new technology

platform to sample and collect mass spectrometry data. This new platform is emerging in many areas of science, including human medicine (Balog et al., 2010) and biological sciences. Several scientists have recently used REIMS to predict meat quality characteristics and identify animal attributes associated with food fraud. Balog et al. (2016) used REIMS to predict species and breeds from which product was derived to determine if the technology could have implications for preventing food fraud. Species and breeds were predicted with 100% and 97% accuracy, respectively. In another study using REIMS, fish species were predicted with nearly 99% accuracy (Black et al., 2017). Guitton et al. (2018) was able to identify and predict ractopamine among various pork muscles with accuracy over 95%. Verplanken et al. (2017) reported very high accuracy in segregating samples with and without boar taint.

The REIMS platform requires essentially no preparation time (Waters Corporation, 2019). The main advantage of REIMS is the quick extraction and ionization capability using the ‘iKnife’ sampling tool coupled with a mass spectrometer. The ‘iKnife’ sampling tool is comprised of a handheld mobile device with flexible plastic tubing attached to a mass spectrometer. This technology involves ionization to occur via the ‘iKnife’ sampling tool. The electrical current heats the metal blade on the ‘iKnife’ sampling tool, cauterizing the surface of the sample to create aerosolized molecules that travels through the plastic tubing and is transported to the mass spectrometer. The cauterizing action only occurs a few millimeters deep from the surface of the selected tissue.

Previous work conducted by Maneotis (Thesis, 2017) established proof-of-concept for utilizing a tissue sample from sheep carcasses to characterize metabolites driving flavor profiles in sheep meat. In order to evaluate whether flavor profiles of sheep meat could be evaluated with an instrument, it was necessary to conduct a study to identify capabilities of metabolites in

driving flavor profiles. The objective of this study was to identify the capabilities of REIMS as a novel method to characterize flavor profiles of various tissues types by generating molecular data and evaluating the ability of REIMS to predict characteristics of sheep carcasses.

### ***Materials and Methods***

Institutional Animal Care and Use Committee approval was unnecessary since no live animals were used in this experiment.

#### ***Sample Collection***

Boneless leg samples (Institutional Meat Purchase Specifications #234, 2014) were obtained from 150 sheep carcasses representing three age classifications (n=50 per age classification) at three USDA inspected harvest facilities located in Colorado and California from October 2017 to June 2018. Both legs of the animal were collected. The following dentition criteria were used to determine age classification: 1) 0 permanent incisors (lamb); 2) 2 permanent incisors (yearling); 3) >2 permanent incisors (mutton). Within each age classification, other factors, including production background (conventionally grain-finished and grass-finished), breed-type (white-faced, black-faced, hair-type, crossbred or speckle-faced), and sex (wethers, ewes, rams) were used to select animals producing samples used in this experiment.

Immediately after exsanguination, traits (lot number, origin, breed-type, sex, production background) of selected animals were recorded and a unique carcass tag was applied after pelt removal to maintain identification of each carcass. After 24 h of carcass chill time, objective color (L\*, a\*, b\*) measurements were obtained from the external surface of the leg, surface of the primary flank, subcutaneous or external fat of the 12<sup>th</sup> rib interface and of the exposed *longissimus dorsi* (LD) at the 12<sup>th</sup> rib interface (Hunter Lab Miniscan, Model 45/O-S; Hunter Associates Laboratory Inc., Reston, VA). The exposed LD was allowed to rest for 20 min. prior

to obtaining objective color measurements. Carcass information collected included the following: ribeye area, 12<sup>th</sup> rib external fat thickness (measured at 50% of the length of the LD), adjusted (modified by  $\pm 0.05$ " of 12<sup>th</sup> rib fat thickness) fat thickness, body wall thickness (measured approximately two inches ventral from the LD, hot carcass weight, leg score, carcass confirmation, flank streaking amount, USDA Quality Grade and Yield Grade (if applicable).

Identification of leg samples were maintained during fabrication at each harvest facility. A 10 x 10 cm area consisting of external fat surface and underlying lean were immediately excised, sealed in a vacuum package and placed in dry ice to stop metabolic activity. Remaining leg samples were vacuum sealed, and all samples were transported to the Colorado State University Meat Laboratory. Upon arrival, frozen external samples were placed in -80°C and remaining leg samples were frozen at -20°C until further analysis upon inspection of vacuum package seal integrity.

Before grinding and forming patties for trained sensory panel evaluation, leg samples were thawed at refrigerated temperatures (between 0 to 4°C) for 48 h. External surface of each leg was trimmed to 0 cm subcutaneous fat and large amounts of intermuscular fat were removed, including lymph nodes. Lean of legs with any accompanying intramuscular fat were designated for trained sensory panel sampling. Lean of legs were course ground with a 1/2" grinder plate followed by fine grinding with a 1/8" grinder plate. Ground lamb was immediately formed into 28 mm wide patties weighing approximately 1.5 oz each. Equipment used to grind and form patties were rinsed and dried between preparation of product from each carcass to prevent flavor cross contamination. Patties were crust frozen, sealed in vacuum packaged bags and frozen at -20°C until further analysis.

### ***Trained Sensory Analysis***

Before conducting trained sensory analysis, all panelists attended 14 intensive training sessions to evaluate fat-like, green/hay-like, livery, sour and oxidized attributes using the beef flavor lexicon references described by Adhikari et al. (2011) and additional ground sheep samples from the present study were used to train for lamb flavor identity and mutton-like flavors. References of attributes are presented in Table 1. Frozen ground patties (~42.5 g) were thawed at 0 to 4°C for 12 h prior to cooking. Four patties per animal were cooked in a pre-heated commercial convection oven (Model SCC WE 61 E; Rational, Landsberg Lech, Germany) at 204°C and 0% humidity for 5.5 min. Temperature of patties was monitored during and after cooking using a thermocouple (Type J or T Digi-Sense, Cole Parmer, Vernon Hills, IL).

A warm-up sample originating from extra samples in the study was served to keep panelists calibrated by requiring all individuals to verbally agree on the intensity of each descriptive sensory attribute prior to evaluating additional samples. Cooked patties were divided in half prior to serving to trained sensory panelists for evaluation of descriptive sheep flavor attributes. All trained panelists evaluated lamb flavor ID, fat-like, mutton-like off-flavor, green/hay-like, livery, sour and oxidized attributes on a 100 mm unstructured line scale verbally anchored at 0 (barely detectable) and 100 (extremely intense). The same attributes and references described previously in training were used by trained panelists to evaluate sensory attributes of patties produced from sheep carcasses. Following the warm-up sample, 10 to 12 individual samples were randomly assigned to a six-person trained panel for evaluation of descriptive sensory attributes. Panelists were limited to serving on a maximum of two panels per day with at least five hours between panels over 14 total sensory sessions. Unsalted saltines and distilled water were used before and after sample evaluation for the purpose of neutralizing

and cleansing the palette of each panelist.

### ***Rapid Evaporative Ionization Mass Spectrometry (REIMS)***

Rapid evaporative ionization mass spectrometry (REIMS) was used to capture metabolomic fingerprints of leg samples consisting of external fat from the leg, lean just beneath the external fat surface, and formed patties produced from trimmed legs of sheep carcasses. Vacuum sealed external fat and lean samples (previously stored at -80°C) and vacuum sealed ground sheep patties (previously stored at -20°C) were allowed to thaw for 12 h before REIMS profile capture. An 'iKnife' sampling instrument (Waters Corporation, Milford, MA) coupled with a Synapi G2 Si Q-ToF and REIMS ionization source was used to capture metabolic data. Using the sampling instrument, five burns on the surface of each tissue were collected powered by an Erbotom ICC 300 electrosurgical generator (Erbe Elektromedizin GmbH, Tübingen, Germany). The dry cut option at power of 40 W was used to collect molecular data on lean and ground patties and the cauterizing option at power of 20 W was used to collect molecular data on fat samples. Data were collected in negative ion mode. Leucine-enkephalin (2 ng/mL) was added directly to the REIMS source at a continual flow of 200 µL/min. Burns occurred adjacent to each other in a representative, randomly selected 2.54 x 2.54 cm-square location on each sample. Data were collected at frequencies ranging from 50 to 1,200  $m/z$  and were preprocessed by summing bins of all five burns resulting in one value per sample. Preprocessing included lock mass (leucine enkephalin at 554.25  $m/z$ ) correction, background subtraction and applied normalization of total ion current to create bins of metabolomic data at intervals of 0.5  $m/z$  ranging from 50 to 1200  $m/z$  resulting in 2,300 variables using abstract model builder software (AMX version 1.0.1581.0, Waters Corporation, Budapest, Hungary).

### ***Chemical Analysis***

Chemical analysis occurred at Colorado State University (CSU). Percent moisture and crude fat content were determined among ground meat samples originating from legs of sheep carcasses collected in this study. Ground samples used in chemical analysis were prepared the same as ground patties for trained sensory panel by producing ground meat from legs trimmed to 0 cm subcutaneous fat and removing large portions of intermuscular fat.

#### *Moisture Analysis*

Moisture content was determined using the oven drying method described in AOAC 950.46 and 934.01 (AOAC, 1995). Approximately 1 g of sample was weighted into aluminum tins prior to placing the tins into a forced air drying oven for 24 h at 100° C. Percent moisture content was determined from the formula:  $\%MC = [(initial\ weight - dry\ weight) / initial\ weight] \times 100$ . Moisture content was analyzed at Colorado State University.

#### *Fat Analysis*

Fat content was determined using the chloroform:methanol method described by Folch, Lees, and Stanley (1957). Approximately 1 g of sample was homogenized in chloroform:methanol solution. Solution was placed into an orbital shaker at room temperature for 20 min and filtered through ashless filter paper. Four ml of 0.9% NaCL was added before being refrigerated for 24 h. Upon phase separation of the filtrate, aspirated content was placed into a pre-weighed scintillation vial and dried under N<sub>2</sub> gas followed by vial air drying under a hood for 2 h. Vials were placed into a forced air drying oven for 12 h at 100° C. Percent total fat was calculated from the formula:  $\%TF = [(Total\ volume\ of\ chloroform:methanol) / 10] \times (final\ weight / initial\ weight) \times 100$ .

#### *Statistical Analysis*

##### *Sensory Evaluation and Carcass Attributes*

Trained sensory panelist ratings were averaged to obtain one value per sensory attribute for each sample. The lmer function of the lme4 package in R statistical software (R Core Team, 2018) was used to fit sensory data to a linear mixed model with evaluation order and sensory session included as random effects to evaluate sensory attribute differences among age group, breed-type, production background and predicted sensory classes (described below). The emmeans function of R statistical software (R Core Team, 2018) was used to further characterize and compare carcass characteristics, objective color scores ( $L^*$ ,  $a^*$ ,  $b^*$ ), and chemical analysis (percent crude fat and dry matter) by age classification.

To assess the ability of using metabolomic data to predict sensory characteristics, the PCA function in the FactoMineR package in R (R Core Team, 2018) was used to conduct PCA to calculate overall sensory scores from uncorrelated linear relationship driven latent variables or principle components. The first principle component explains the greatest amount of variation followed by remaining components explaining subsequent decreasing amounts of variation. Factor scores were developed from coefficients assigned to each principle component. Using PCA factor scores, hierarchical cluster analysis (HCA) was performed using the first two principle components (which explain the greatest amount of variation) to group samples based on similar or dissimilar sensory characteristics into two-level (overall positive and negative) and three-level (overall positive, neutral or negative) sensory classifications. Scores plots for each HCA (three-level and two-level sensory classification) were clustered into 2 groups (two-level sensory classification representing positive and negative) or 3 groups (three-level sensory classification representing positive, neutral and negative).

### ***Predictive Models using Partial Least Squares-Linear Discriminant Analysis***

Preprocessed REIMS data from fat, lean and ground patty tissues from legs of sheep

carcasses were used to develop predictive models for several animal and sensory characteristics. Each model was developed similarly using PLS-LDA. Variables from raw mass spectra output with correlation coefficients  $\geq 0.90$  were identified and total number of mass bins of each tissue type were reduced. Identified samples from HCA were paired with molecular data from each tissue type to predict sensory classification using molecular compound data. Using the `pls.lda` function in R under the `plsgenomics` package (R Core Team, 2018), PLS output determined optimal number of PLS components to use for the model. Scores values from optimal number of PLS components were used for subsequent LDA predictability. Eighty percent of the pre-processed data from each model were randomly selected for model training and 100 iterations of the remaining 20% of the data were used to test the prediction accuracy of each model developed PLS-LDA model.

As part of PLS-LDA, the 20% test data were used in a confusion matrix to test predicted classifications against true classifications. Overall prediction accuracy and balanced prediction accuracy were calculated to assess predictive capabilities of each PLS-LDA model. Overall prediction accuracy was considered the number of true positives divided by the total number of samples among all classifications. To prevent bias of dominant classes, balanced prediction accuracy was calculated as the average accuracy of each class. Sensitivity and precision were calculated for each class. Sensitivity was calculated as the number of true positives divided by the sum of true positives and false negatives. Precision was calculated as the number of true positives divided by the sum of the number of true positives and false positives. Prediction models were assessed with mass bins that were mean centered or mean centered and pareto scaled.

### ***Predictive Capabilities using Machine Learning Algorithms***

Preprocessed REIMS data from lean tissue from legs of sheep carcasses were used to develop predictive models for several animal and sensory characteristics using machine learning algorithms. Before machine learning prediction algorithms were assessed, feature selection (FS), PCA and FS performed on PCA data were performed as dimension reduction approaches. The PCA in the FactoMineR package and rfe function in the caret package of R (R Core Group, 2018) were used to conduct PCA and FS, respectively. Dimension reduction using PCA was performed with unit variance scaling. Feature selection considers variation in the predictor variables and separation of classifications. Following dimension reduction approaches, eight machine learning algorithms were used to compare predictive accuracies. Machine learning algorithms included LDA, LogitBoost, PLSDA, XGBoost, SVM (linear, poly and radial), and random forest. All machine learning algorithm assessment used 10-fold cross validation by removing 10% of the data for use as a test set. The remaining data was used as the training set and the procedure was repeated 10 times resulting in the average prediction accuracy.

## ***Results and Discussion***

### ***Sheep Carcass Characteristics***

Results presented in Tables 2 and 3 characterize the sample of lamb carcasses used in this study. Hot carcass weight, adjusted 12<sup>th</sup> rib fat thickness, body wall thickness, ribeye area, mean marbling level and calculated yield grade differed ( $P<0.0001$ ) among all age categories of sheep carcasses (Table 2). Notably, all carcass characteristics were greatest from yearling sheep and lowest for mutton. Weights and carcass measurements of yearling sheep were most likely greater due to longer periods of intensive feeding. Manetotis (2016) observed similar findings for carcass performance of yearling sheep. Objective color measurements differed ( $P<0.05$ ) among all tissue types (LM, flank, external surface of the leg) across each age category except for a\*

and b\* measurements among the LD. Mutton carcasses produced higher a\* ( $P<0.05$ ) and b\* ( $P<0.05$ ) values from the flank and external surface of the leg. Physiological effects typically cause an increase in redness as an animal matures. In a separate study, increased a\* values were observed in ewes similar to the present study (Jaborek et al., 2018).

### ***Trained Sensory Ratings***

Trained sensory ratings of ground sheep samples among varying age groups, breed-types, production backgrounds, 3-level sensory classifications and 2-level sensory classifications are presented in Table 4 to characterize the sample used to develop flavor prediction models. Fat-like and oxidized sensory ratings were numerically similar among all models. Lamb flavor ID ratings were lower ( $P<0.05$ ) for the ‘positive’ classification of both 3-level and 2-level sensory models (Table 4). Although no statistical difference was observed, lamb flavor ID ratings were numerically higher in these data compared to sensory panel ratings conducted by Maneotis (Thesis, 2017). Mutton-like and green/hay-like off-flavor intensities were higher ( $P<0.05$ ) among black-face and crossbred breed types and were highest for the ‘negative’ sensory group among both 3-level and 2-level overall sensory classifications (described in more detail later). Mutton-like off-flavor was numerically higher among samples from lamb carcasses. Livery, sour and oxidized ratings were rated numerically lower compared to all other sensory attributes (Table 4). Livery was also different ( $P<0.05$ ) among all models (Table 4). Ground patties produced from sheep carcasses had the highest ( $P<0.05$ ) percent crude fat and dry matter among yearlings and lowest ( $P<0.05$ ) among mutton carcasses (Table 5). Since large amounts of external fat and seam fat were removed prior to grinding, differences in crude fat are more likely related to amount of marbling deposited in muscles of the leg. Observationally, several heavy weight carcasses produced from yearlings appeared to have visually greater levels of marbling

when tissue samples were extracted from the surface of the leg. Jaborek et al. (2018) described long-fed lambs that remained in feedlots gaining fat tissue. Remaining on feed for more days compared to younger, lighter lambs is most likely associated with increased levels of intramuscular fat, provided individual animals have genetic potential to deposit these increased levels of marbling.

### *Predictive Classification Models*

Balanced prediction accuracy was 64.2%, 73.3%, and 72.3% for external fat from the leg, lean of the leg, and ground patties, respectively, when predicting lamb (0 permanent incisors), yearling (2 permanent incisors) and mutton ( $\geq 2$  permanent incisors) age classifications (Table 6). Mutton age classification was predicted with 88.9% sensitivity and 80.0% precision using external fat of the leg and with 100% sensitivity and 90.9% precision using ground patties. Yearling age classification were predicted with 85.7% precision using lean and lambs were predicted with 70% precision using lean and fat tissue (Table 6). Sensitivity of lambs and yearlings using fat and ground patty tissues ranged from 50 to 62.5% (Table 6). When lambs and yearlings were combined into a single classification, overall model prediction increased to nearly 90% accuracy in distinguishing lean of mutton samples from younger animals (lambs and yearlings). Projection of PLS-LDA scores for age classification by tissue type are shown in Figures 1 through 3. Each age classification was distinctly separated visually on PLS-LDA plots using metabolite data from all tissue types (Figures 1-3).

Greater than 80% accuracy (overall and balanced), sensitivity and precision was achieved in models using lean and ground patties to predict production feed background (Table 7). Fat samples were less accurate (60%) in determining whether a carcass was produced by an animal fed a grain-based or grass-based diet (Table 7). Prediction models were tested for sex-

classification (ewes, wethers and rams) and breed-type (black-face, white-face, hair-type, and crossbred), but analysis predetermined that only 1 or 2 components was optimal for these models across all tissue types resulting in very low predictability. The objective of this study did not include comparing groups of sex-classes or breed-types; therefore, equal sample sizes of each sex-class and breed-type were not collected. Although Tatum et al. (2014) summarized studies that described differences in molecular compounds between sex-classes and breed-types, particularly with rams and finewool breeds of sheep, the proportion of samples originating from animals with these characteristics may not have been represented in great enough quantity to predict strong accuracy for these model attributes. Specifically, rams only comprised 4% of the total sample size. Final models evaluating sex-class and breed-type were not presented.

Since mutton flavor has been characterized as intense, strong flavors (Tatum et al., 2014; Watkins et al., 2010; Young et al., 1997), sensory panelists were trained to quantify the mutton-like attribute as the objectionable flavor, if present, in samples. Figure 4 shows the contribution of sensory attributes (from trained sensory evaluation) to PCA factor scores. Mutton-like intensity contributed the largest influence on factor scores in component 1, followed by lamb flavor ID. 46.8%

Using hierarchical clustering of principal component factor scores from the trained sensory ratings, a two-level sensory class (positive and negative overall sensory classification) and a three-level sensory class (positive, neutral and negative overall sensory classification) were developed. Projection of principal component scores from trained sensory ratings to develop a colored two-level sensory classification (positive and negative) is shown in Figure 5. Cluster assignment using the hierarchical cluster analysis was used to evaluate if PLS-LDA approaches could predict overall sensory classifications based on sensory ratings and predict those sensory

classifications using metabolite data from each tissue type. Similarly, Figure 6 shows the projection of principal component scores from trained sensory ratings colored as a three-level sensory classification (positive and negative). Notably, the samples clustering in the ‘negative’ sensory classification of the three-level system in Figure 6 were from lambs that had numerically higher ratings of mutton-like off-flavors among trained sensory evaluation. Figures 7 and 8 show the projection of PLS-LDA with separation occurring for each classification. However, the misclassification matrix for the three-level sensory classification model resulted in mid to low accuracies. Overall prediction accuracy was 34.5% for lean tissue and 58.6% for ground patties (Table 8). Balanced prediction accuracies improved for the two-level overall sensory model (positive and negative classes; 58.9% accuracy for lean and 57.2% accuracy for ground patties; Table 9).

Predictive accuracy results from PLS-LDA using these data were variable across models. While some classes received higher accuracy within models, such as in prediction of mutton age class with 88.2% or greater sensitivity, other models had much lower predictive capability. Use of pareto scaling on REIMS bins from lean tissue of legs from ovine carcasses resulted in reduced accuracy. Correct prediction of grain-finished or grass-finished background was predicted with 80% accuracy using mean centered mass bins, but was only 60% when using mean centered and pareto scaled mass bins to predict feeding type. Similar reductions in predictive accuracy were observed with mass bins from lean of legs from ovine carcasses to predict age classification and two-level sensory performance.

Balanced prediction accuracies reported by Gredell (Dissertation, 2018) ranged from 55.6 to 83.8%. Studies that have used REIMS data to predict food quality or food fraud prevention attributes reported higher accuracies. For example, Balog et al. (2016) reported predictive

capability of 100% to identify species and 97% accuracy to identify different breeds. In another study using REIMS, fish species were predicted with nearly 99% accuracy (Black et al., 2017). Guitton et al. (2018) was able to identify and predict ractopamine among various pork muscles with accuracy over 95%. Verplanken et al. (2017) reported very high accuracy in segregating samples with and without boar taint. These studies did utilize well defined groups that seem inherently different.

Using REIMS data collected from lean tissue from legs of ovine carcasses, eight machine learning algorithms were used to evaluate accuracy of predicting age classification (lamb, yearling or mutton classification developed by dentition parameters), production background (grain-finished or grass-finished) or overall sensory performance (positive or negative developed via hierarchical cluster analysis of PCA from trained sensory ratings). For each prediction outcome, a combination of eight machine learning algorithms and dimension reduction combinations were evaluated. Visualization of predictive accuracies are shown in Figures 9-11. In general, predictive accuracies were higher among machine learning algorithms that used FS as a dimension reduction approach. Specifically, REIMS data that was subjected to FS had a 67.3% accuracy of correctly predicting age classification when the random forest algorithm was used (Figure 9). Similarly, FS applied to REIMS data resulted in 86.7% accuracy of correctly predicting samples that were grain-finished or grass-finished when the support vector machine polynomial or random forest algorithms were used (Figure 10). Correct prediction of positive or negative flavor classification was similar between FS and PCA-FS reduced data (Figure 11). The XGBoost algorithm was 70% accurate in correctly predicting two-level flavor groups using the FS approach. Similarly, the PLSDA and XGBoost algorithms were 68.7% and 68.6% accurate in predicting two-level flavor performance using a PCA-FS approach, respectively. Similarly,

several models assessed by Gredell et al. (2019) using REIMS data from beef samples had high predictive accuracies with machine learning algorithms after FS and PCA-FS approaches.

### ***Conclusion***

Use of REIMS is a unique platform to capture high resolution metabolic profiles faster and without lengthy sample preparation compared to other analytical approaches. More importantly, REIMS is able to provide metabolite information in real-time, indicating high potential for its use in harvest facilities at production speed. Work conducted by Balog et al. (2010) and Verplanken et al. (2017) showed higher prediction accuracies for meat characteristics, but this may be due to better defined groups for comparison.

The present study evaluated a variety of factors including age classification, production background, sex-class and breed-type. This work provided an evaluation of REIMS in determining and predicting various sheep carcass characteristics among three tissue types. Multiple models were evaluated for prediction accuracy. Lamb flavor ID was not statistically different between lamb, yearling and mutton carcasses. Mutton-like and green/hay-like off-flavor intensities were higher for 'negative' sensory classifications among both 3-level and 2-level sensory groups. Higher balanced prediction accuracies were observed from 3-age classifications (Lamb, Yearling, and Mutton) and production background (Grain-finished or Grass-finished) models. The 2-level sensory classification model had higher prediction accuracy compared to the 3-level sensory classification model. Use of machine learning algorithms showed potential as other analysis tools to further evaluate predictive accuracies. This approach used 10-fold cross validation, and higher cross validation may result in different accuracies. These data support the need for additional analysis work to be completed to identify which types of scaling or models are optimal for assessing metabolomic data. Lower prediction accuracies with these data could

also be due to the mixed live animal attributes comprising each age group, although this was a nationally representative sample.

This experiment added insight regarding the difficulty in using the electrode ionization source to capture metabolite data from pure fat samples. Future work adapting the REIMS technology or sample management could prove to help better generate molecular data from pure fat samples. Annotation and identification of specific compounds among REIMS metabolic data could improve understanding of flavor profiles that are being influenced by specific sheep flavor attributes. Further research is warranted to validate use of this technology in production settings and additional datasets could be used to further refine or create additional prediction models. Further work predicting sensory characteristics in sheep meat from the U.S. supply could help establish branded programs based on flavor. This technology shows promise to potentially segregate carcasses of more desirable from less desirable sensory characteristics. However, additional research will be needed to validate additional models and an on-line application for use. Further, consumer ratings of flavor are needed to expand predictive model capability. Expanded models utilizing consumer responses could further add to the ability to use molecular data to predict overall liking, flavor intensity or off-flavor presence in future work.

**Table 1.** Description and reference standard intensities used during sensory panel training for evaluation of sheep descriptive sensory attributes on a continuous line scale from 0 to 100.

Attribute	Description	Reference
Lamb Flavor ID	Amount of lamb flavor identity in the sample.	Additional ground sheep samples from this study were identified and selected to use for training.
Fat-Like <sup>1</sup>	The aromatics associated with cooked animal fat. <sup>1</sup>	Hillshire Farms lit'l Beef Smokies = 45 <sup>1</sup> Additional ground sheep samples from this study used were identified and selected to use for training.
Mutton-like	The offensive aromatics and flavor associated with mutton.	Additional ground sheep samples from this study were identified and selected to use for training.
Sour <sup>1</sup>	The fundamental taste factor associated with citric acid. <sup>1</sup>	0.015% Citric Acid solution = 10 <sup>1</sup> 0.050% Citric Acid solutions = 25 <sup>1</sup>
Livery <sup>1</sup>	The aromatics associated with cooked organ meat/liver. <sup>1</sup>	Beef Liver = 50 <sup>1</sup>
Green/Hay-Like <sup>1</sup>	Brown/green dusty aromatics associated with dry grasses, hay, dry parsley, and tea leaves. <sup>1</sup>	Dry parsley = 40 <sup>1</sup> Whole frenched rib roast, grass-fed, roasted to 62.8°C = 30
Oxidized/Rancid <sup>1</sup>	The aromatics commonly associated with oxidized fat and oils. These aromatics may include cardboard, painty, varnish, and fishy. <sup>1</sup>	Ground beef fully cooked, chilled, allowed to remain uncovered under refrigeration for 6 h and microwaved to 82.2°C = 40

<sup>1</sup>Adapted from Adhikari et al. (2011)

**Table 2.** Least squares means of sheep carcass traits among three age groups<sup>2</sup>.

Treatment Group	<i>n</i>	Hot Carcass Weight (kg)	Adjusted 12 <sup>th</sup> Rib Fat Thickness (cm)	Body Wall Thickness (cm)	Ribeye Area (cm <sup>2</sup> )	Marbling <sup>1</sup> at 12 <sup>th</sup> Rib Interface	Calculated Yield Grade
<i>P</i> -value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Age <sup>2</sup>							
Lamb	50	31.3 <sup>b</sup>	0.64 <sup>b</sup>	2.13 <sup>b</sup>	17.15 <sup>b</sup>	457.6 <sup>b</sup>	2.9 <sup>a</sup>
Yearling	50	40.5 <sup>c</sup>	0.88 <sup>c</sup>	2.64 <sup>c</sup>	18.82 <sup>c</sup>	518.0 <sup>c</sup>	3.9 <sup>b</sup>
Mutton	50	27.5 <sup>a</sup>	0.22 <sup>a</sup>	1.03 <sup>a</sup>	13.16 <sup>a</sup>	303.1 <sup>a</sup>	1.3 <sup>c</sup>
SEM <sup>3</sup>		1.33	0.05	0.11	0.51	20.1	0.21

<sup>abc</sup>Least square means in the same column without a common superscript differ ( $P < 0.05$ ) due to treatment.

<sup>1</sup>Marbling scores were recorded and assessed as: Practically devoid = 100, Traces = 200, Slight = 300, Small = 400, Modest = 500, Moderate = 600, Slightly abundant = 700, Moderately abundant = 800.

<sup>2</sup>Age group defined as: Lamb = 0 Permanent Incisors, Yearling = 2 Permanent Incisors, Mutton = >2 Permanent Incisors.

<sup>3</sup>Standard error (largest) of the least squares means.

**Table 3.** Least squares means of objective color scores<sup>1</sup> among three age groups<sup>2</sup>.

Treatment Group	n	LD <sup>3</sup>			Flank			Leg		
		L*	a*	b*	L*	a*	b*	L*	a*	b*
P-value		0.0077	0.2611	0.2576	0.0058	0.0003	0.0075	<0.0001	<0.0001	<0.0001
Age <sup>2</sup>										
Lamb	50	41.77 <sup>a</sup>	16.73	11.96	43.65 <sup>b</sup>	12.47 <sup>a</sup>	4.25 <sup>a</sup>	69.97 <sup>a</sup>	2.75 <sup>a</sup>	4.85 <sup>a</sup>
Yearling	50	39.68 <sup>b</sup>	16.05	11.33	40.87 <sup>a</sup>	13.20 <sup>a</sup>	3.89 <sup>a</sup>	66.36 <sup>a</sup>	2.42 <sup>a</sup>	4.46 <sup>a</sup>
Mutton	50	38.75 <sup>b</sup>	16.61	11.64	44.64 <sup>b</sup>	14.93 <sup>c</sup>	6.46 <sup>b</sup>	60.68 <sup>b</sup>	7.45 <sup>b</sup>	8.88 <sup>b</sup>
SEM <sup>4</sup>		0.69	0.32	0.27	0.86	0.43	0.62	1.50	0.57	0.47

<sup>abc</sup>Least square means in the same column without a common superscript differ ( $P < 0.05$ ) due to treatment.

<sup>1</sup>L\*: 0 = black, 100 = white; a\*: negative number = green, positive number = red; b\*: negative number = blue, positive number = yellow.

<sup>2</sup>Age group defined as: Lamb = 0 Permanent Incisors, Yearling = 2 Permanent Incisors, Mutton = >2 Permanent Incisors.

<sup>3</sup>LD=*longissimus dorsi* muscle.

<sup>4</sup>Standard error (largest) of the least squares means.

**Table 4.** Least squares means and SEM of trained sensory ratings<sup>1</sup> for ground sheep samples of varying production characteristics.

Treatment Group	<i>n</i>	Lamb Flavor ID	Fat-Like	Mutton-Like	Green/Hay -Like	Livery	Sour	Oxidized
Age <sup>2</sup>		<i>P</i> =0.2352	<i>P</i> =0.1566	<i>P</i> =0.0632	<i>P</i> =0.7759	<i>P</i> =0.0002	<i>P</i> =0.1931	<i>P</i> =0.2996
Lamb	50	29.28	15.81	15.12	13.96	6.00 <sup>a</sup>	2.90	3.61
Yearling	50	30.00	17.04	12.93	14.08	5.16 <sup>a</sup>	3.83	3.49
Mutton	50	28.47	16.87	11.84	14.51	3.23 <sup>b</sup>	3.24	4.37
SEM <sup>7</sup>		0.63	0.49	1.00	0.58	0.47	0.37	0.44
Breed-type <sup>3</sup>		<i>P</i> =0.3182	<i>P</i> =0.6365	<i>P</i> =0.0129	<i>P</i> =0.3316	<i>P</i> =0.0054	<i>P</i> =0.4104	<i>P</i> =0.0717
White-face	63	28.85	16.92	13.42 <sup>ab</sup>	13.78	4.95 <sup>b</sup>	3.50	4.34
Black-face	26	30.52	16.70	15.58 <sup>a</sup>	14.16	6.68 <sup>a</sup>	3.40	3.41
Crossbred	50	28.86	16.62	17.16 <sup>a</sup>	14.25	3.74 <sup>b</sup>	2.88	3.09
Hair-type	11	30.28	16.07	11.11 <sup>b</sup>	16.24	4.29 <sup>ab</sup>	4.15	5.12
SEM <sup>7</sup>		1.35	1.05	2.10	1.23	1.03	0.79	0.92
Production Background <sup>4</sup>		<i>P</i> =0.0925	<i>P</i> =0.5786	<i>P</i> =0.0569	<i>P</i> =0.0026	<i>P</i> =0.0191	<i>P</i> =0.1546	<i>P</i> =0.5997
Grain-finished	75	29.87	16.73	14.41	13.19 <sup>b</sup>	5.47 <sup>a</sup>	3.02	3.95
Grass-finished	75	28.63	16.42	12.19	15.17 <sup>a</sup>	4.13 <sup>b</sup>	3.63	3.69
SEM <sup>7</sup>		0.51	0.40	0.82	0.46	0.40	0.30	0.36
3-Level Sensory Class <sup>5</sup>		<i>P</i> <0.0001	<i>P</i> =0.7883	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> =0.0371	<i>P</i> =0.1136
Positive	86	27.54 <sup>b</sup>	16.74	8.83 <sup>c</sup>	12.70 <sup>b</sup>	4.12 <sup>b</sup>	2.86 <sup>a</sup>	3.60
Neutral	55	31.57 <sup>a</sup>	16.35	17.14 <sup>b</sup>	15.98 <sup>a</sup>	4.84 <sup>b</sup>	4.01 <sup>b</sup>	3.84
Negative	9	31.45 <sup>a</sup>	16.31	32.50 <sup>a</sup>	17.31 <sup>a</sup>	11.03 <sup>a</sup>	3.52 <sup>ab</sup>	5.85
SEM <sup>7</sup>		30.49	1.2	1.15	1.24	1.05	0.85	1.02
2-Level Sensory Class <sup>6</sup>		<i>P</i> <0.0001	<i>P</i> =0.1897	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> =0.0001	<i>P</i> =0.0007	<i>P</i> =0.0502
Positive	54	27.95 <sup>b</sup>	16.85	9.25 <sup>b</sup>	12.94 <sup>a</sup>	3.99 <sup>b</sup>	2.79 <sup>b</sup>	3.45
Negative	96	31.56 <sup>a</sup>	16.08	20.50 <sup>a</sup>	16.38 <sup>b</sup>	6.23 <sup>a</sup>	4.27 <sup>a</sup>	4.48
SEM <sup>7</sup>		0.56	0.47	0.63	0.51	0.46	0.34	0.42

<sup>abc</sup>Least square means in the same column without a common superscript differ (*P* < 0.05) due to treatment.

<sup>1</sup>Attributes were scored using an unstructured line scale anchored at both ends: 0 = barely detectable; 100 = extremely intense

<sup>2</sup>Age group defined as: Lamb = 0 Permanent Incisors, Yearling = 2 Permanent Incisors, Mutton = >2 Permanent Incisors.

<sup>3</sup>Breed-type defined as: Black-face = black-face breed types such as Hampshire or Suffolk breeds, Crossbred = smut or speckled faced, Hair-type = Dorper breed characteristics with no complete body wool, White-face=white-face breed types such as Rambouillet, Colombia, Dorset or Polypay.

<sup>4</sup>Production background defined as: Grain-finished = animal was fed primarily a grain-based diet during the stage leading up to harvest, Grass-finished = animal was fed primarily a grass- or pasture-based diet during the stage leading up to harvest. <sup>5</sup>Three reference classes assigned using hierarchical clustering of principal components of PCA data. Positive = cluster 1, Neutral = cluster 2, Negative = cluster 3.

<sup>6</sup>Two reference classes assigned using hierarchical clustering of principal components of PCA data. Positive = cluster 1, Negative = cluster 2.

<sup>7</sup>Standard error (largest) of the least squares means.

**Table 5.** Least squares means and ranges of percent crude fat and dry matter from ground sheep samples produced from legs of sheep carcasses.

Treatment	<i>n</i>	% Crude Fat		% Dry Matter	
		LS Mean	Range	LS Mean	Range
Age Group		<i>P</i> <0.0001		<i>P</i> <0.0001	
Lamb	50	5.34 <sup>b</sup>	2.71-7.91	27.73 <sup>b</sup>	25.40-30.20
Yearling	50	6.33 <sup>a</sup>	4.02-9.56	28.59 <sup>a</sup>	25.83-33.17
Mutton	50	4.42 <sup>c</sup>	1.30-7.98	26.30 <sup>c</sup>	20.30-31.40
SEM <sup>1</sup>		0.21		0.28	

<sup>abc</sup>Least square means in the same row without a common superscript differ (*P* < 0.05) due to treatment.

<sup>1</sup>Standard error (largest) of the least squares means.

**Table 6.** Misclassification matrix<sup>1</sup> of 3 age categories predicted<sup>2</sup> by Partial Least Squares-Linear Discriminant Analysis using molecular profiles of samples<sup>3</sup> from sheep carcasses collected using rapid evaporative ionization mass spectrometry (REIMS).

<b>External Fat derived from legs of sheep carcasses</b>						
Reference Class <sup>4</sup>	Predicted Class			Total	Sensitivity	Precision
	Lamb	Yearling	Mutton			
Lamb	7	5	1	13	53.8%	70.0%
Yearling	3	4	1	8	50.0%	40.0%
Mutton	0	1	8	9	88.9%	80.0%
Total	10	10	10	30		

Overall Prediction Accuracy 73.3%  
 Balanced Prediction Accuracy 64.2%  
 No. of PLS Components 17

<b>Lean derived from legs of sheep carcasses</b>						
Reference Class <sup>4</sup>	Predicted Class			Total	Sensitivity	Precision
	Lamb	Yearling	Mutton			
Lamb	7	0	3	10	70.0%	70.0%
Yearling	3	6	1	10	60.0%	85.7%
Mutton	0	1	9	10	90.0%	69.2%
Total	10	7	13	30		

Overall Prediction Accuracy 73.3%  
 Balanced Prediction Accuracy 73.3%  
 No. of PLS Components 16

<b>Ground sheep meat derived from legs of sheep carcasses</b>						
Reference Class <sup>4</sup>	Predicted Class			Total	Sensitivity	Precision
	Lamb	Yearling	Mutton			
Lamb	6	5	0	11	54.5%	60.0%
Yearling	3	5	0	8	62.5%	50.0%
Mutton	1	0	10	11	90.9%	100.0%
Total	10	10	10	30		

Overall Prediction Accuracy 70.0%  
 Balanced Prediction Accuracy 72.3%  
 No. of PLS Components 10

<sup>1</sup>Samples derived from sheep carcasses include external fat from the leg, lean from the leg or ground patties from legs trimmed to 0 cm external fat and trimmed seam fat.

<sup>2</sup>Number of samples falling into each respective classification category after prediction.

<sup>3</sup>Models were built using 80% of the original data and tested using the remaining 20%.

<sup>4</sup>Reference class assigned using animal characteristics. Lamb = 0 Permanent Incisors, Yearling = 2 Permanent Incisors, Mutton = >2 Permanent Incisors.

**Table 7.** Misclassification matrix<sup>1</sup> of 2 production background categories predicted<sup>2</sup> by Partial Least Squares-Linear Discriminant Analysis using molecular profiles of samples<sup>3</sup> from sheep carcasses collected using rapid evaporative ionization mass spectrometry (REIMS).

<b>External Fat derived from legs of sheep carcasses</b>					
Reference Class <sup>4</sup>	Predicted Class		Total	Sensitivity	Precision
	Grain-finished	Grass-finished			
Grain-finished	9	6	15	60.0%	60.0%
Grass-finished	6	9	15	60.0%	60.0%
Total	15	15	30		
Overall Prediction Accuracy	60.0%				
Balanced Prediction Accuracy	60.0%				
No. of PLS Components	3				
<b>Lean derived from legs of sheep carcasses</b>					
Reference Class <sup>4</sup>	Predicted Class		Total	Sensitivity	Precision
	Grain-finished	Grass-finished			
Grain-finished	12	3	15	80.0%	80.0%
Grass-finished	3	12	15	80.0%	80.0%
Total	15	15	30		
Overall Prediction Accuracy	80.0%				
Balanced Prediction Accuracy	80.0%				
No. of PLS Components	7				
<b>Ground sheep meat derived from legs of sheep carcasses</b>					
Reference Class <sup>4</sup>	Predicted Class		Total	Sensitivity	Precision
	Grain-finished	Grass-finished			
Grain-finished	13	3	16	81.3%	86.7%
Grass-finished	2	12	14	85.7%	80.0%
Total	15	15	30		
Overall Prediction Accuracy	83.3%				
Balanced Prediction Accuracy	83.5%				
No. of PLS Components	13				

<sup>1</sup>Samples derived from sheep carcasses include external fat from the leg, lean from the leg or ground patties from legs trimmed to 0 cm external fat and trimmed seam fat.

<sup>2</sup>Number of samples falling into each respective classification category after prediction.

<sup>3</sup>Models were built using 80% of the original data and tested using the remaining 20%.

<sup>4</sup>Reference class assigned using animal characteristics. Grain-finished = animal was fed primarily a grain-based diet during the stage leading up to harvest, Grass-finished = animal was fed primarily a grass- or pasture-based diet during the stage leading up to harvest.

**Table 8.** Misclassification matrix<sup>1</sup> of 3 overall flavor categories predicted<sup>2</sup> by Partial Least Squares-Linear Discriminant Analysis using molecular profiles of samples<sup>3</sup> from sheep carcasses collected using rapid evaporative ionization mass spectrometry (REIMS).

<b>Lean derived from legs of sheep carcasses</b>						
Reference Class <sup>4</sup>	Predicted Class			Total	Sensitivity	Precision
	Positive	Neutral	Negative			
Positive	8	9	1	18	44.4%	47.1%
Neutral	9	2	0	11	18.2%	18.2%
Negative	0	0	0	0	0%	0%
Total	17	11	1	29		
Overall Prediction Accuracy		34.5%				
Balanced Prediction Accuracy		20.9%				
No. of PLS Components		11				
<b>Ground sheep meat derived from legs of sheep carcasses</b>						
Reference Class <sup>4</sup>	Predicted Class			Total	Sensitivity	Precision
	Positive	Neutral	Negative			
Positive	14	7	1	22	63.6%	82.4%
Neutral	3	3	0	6	50.0%	27.3%
Negative	0	1	0	1	0%	0%
Total	17	11	1	29		
Overall Prediction Accuracy		58.6%				
Balanced Prediction Accuracy		37.9%				
No. of PLS Components		11				

<sup>1</sup>Samples derived from sheep carcasses include lean from the leg or ground patties from legs trimmed to 0 cm external fat and trimmed seam fat.

<sup>2</sup>Number of samples falling into each respective classification category after prediction.

<sup>3</sup>Models were built using 80% of the original data and tested using the remaining 20%. Reference class assigned using hierarchical clustering of principal components of PCA data. Positive = cluster 1, Neutral = cluster 2, Negative = cluster 3.

**Table 9.** Misclassification matrix<sup>1</sup> of 2 overall flavor categories predicted<sup>2</sup> by Partial Least Squares-Linear Discriminant Analysis using molecular profiles of samples<sup>3</sup> from sheep carcasses collected using rapid evaporative ionization mass spectrometry (REIMS).

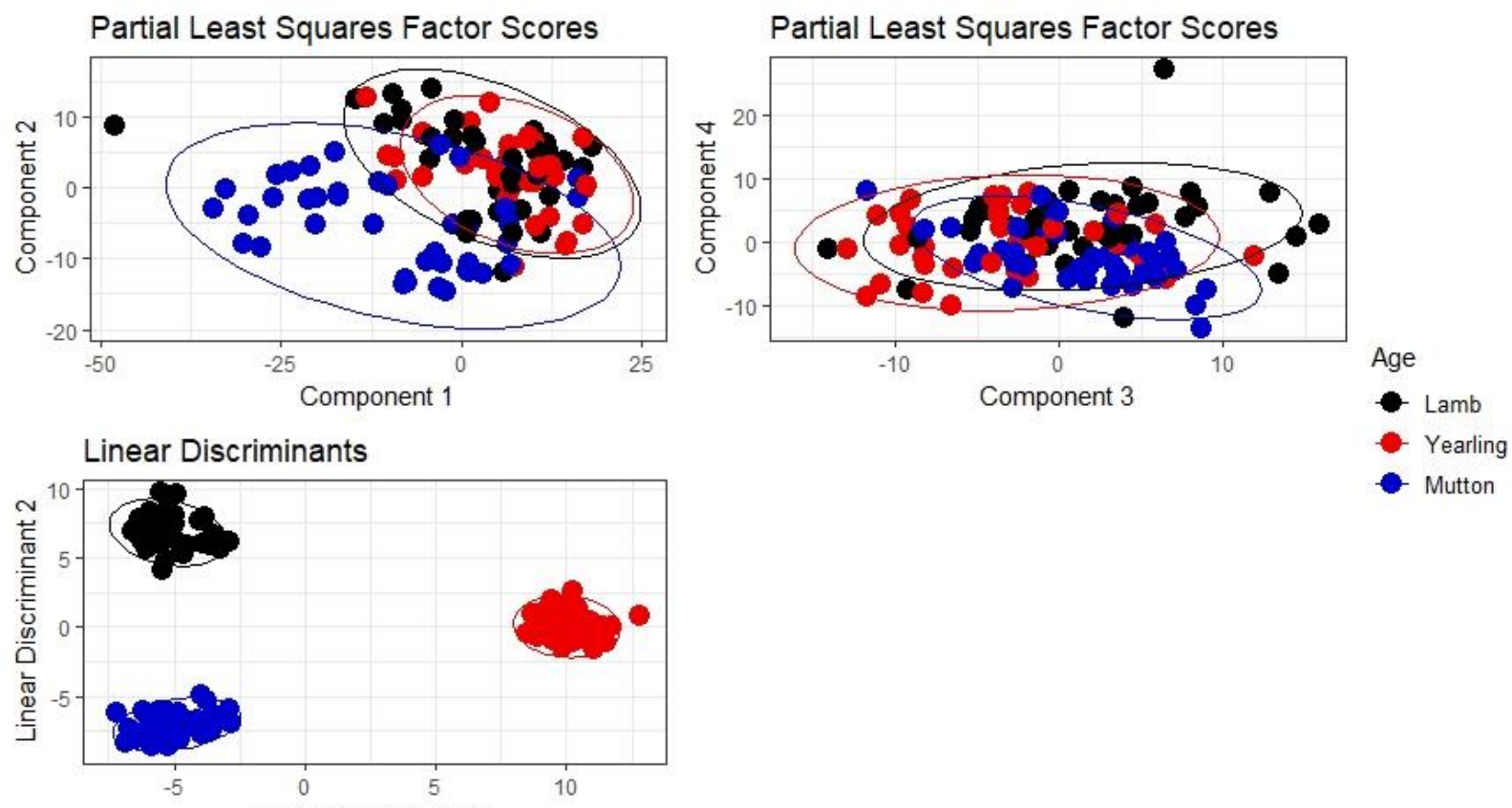
<b>Lean derived from legs of sheep carcasses</b>					
Reference Class <sup>4</sup>	Predicted Class		Total	Sensitivity	Precision
	Positive	Negative			
Positive	13	5	18	72.2%	68.4%
Negative	6	5	11	45.5%	50.0%
Total	19	10	29		
Overall Prediction Accuracy		62.1%			
Balanced Prediction Accuracy		58.9%			
No. of PLS Components		2			
<b>Ground sheep meat derived from legs of sheep carcasses</b>					
Reference Class <sup>4</sup>	Predicted Class		Total	Sensitivity	Precision
	Positive	Negative			
Positive	14	6	20	70.0%	73.7%
Negative	5	4	9	44.4%	40.0%
Total	19	10	29		
Overall Prediction Accuracy		62.1%			
Balanced Prediction Accuracy		57.2%			
No. of PLS Components		10			

<sup>1</sup>Samples derived from sheep carcasses include lean from the leg or ground patties from legs trimmed to 0 cm external fat and trimmed seam fat.

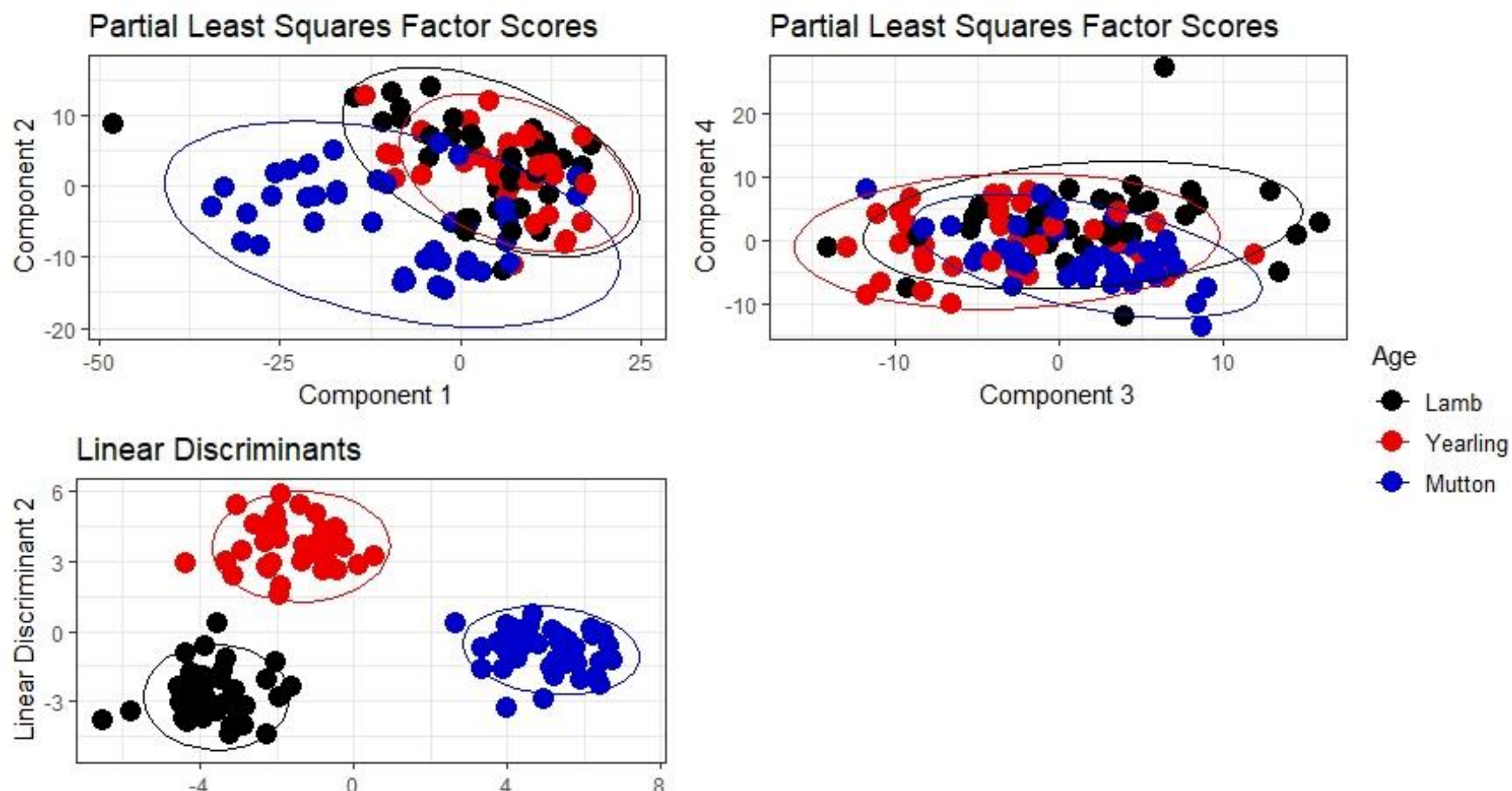
<sup>2</sup>Number of samples falling into each respective classification category after prediction.

<sup>3</sup>Models were built using 80% of the original data and tested using the remaining 20%.

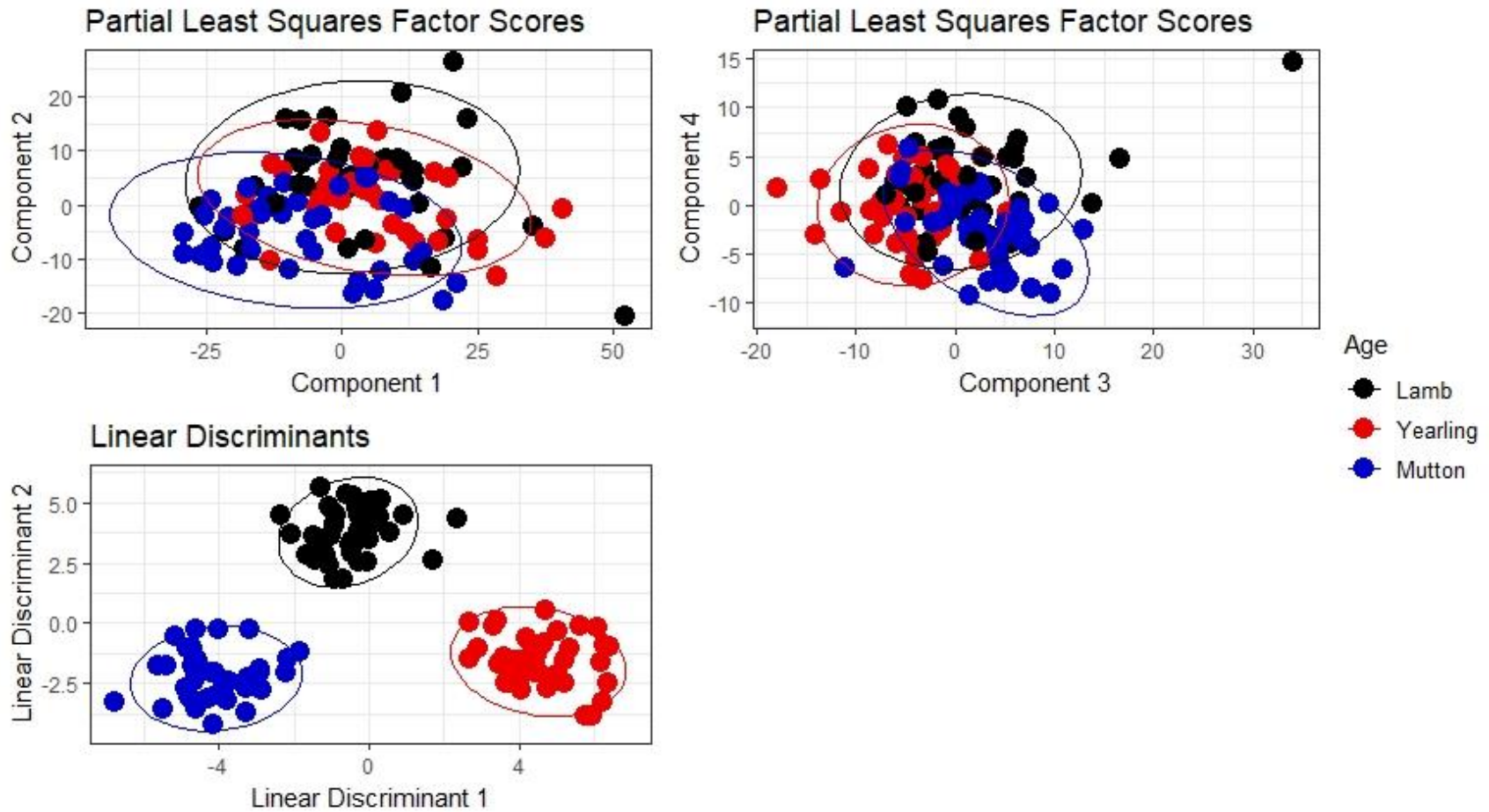
<sup>4</sup>Reference class assigned using hierarchical clustering of principal components of PCA data. Positive = cluster 1, Negative = cluster 2.



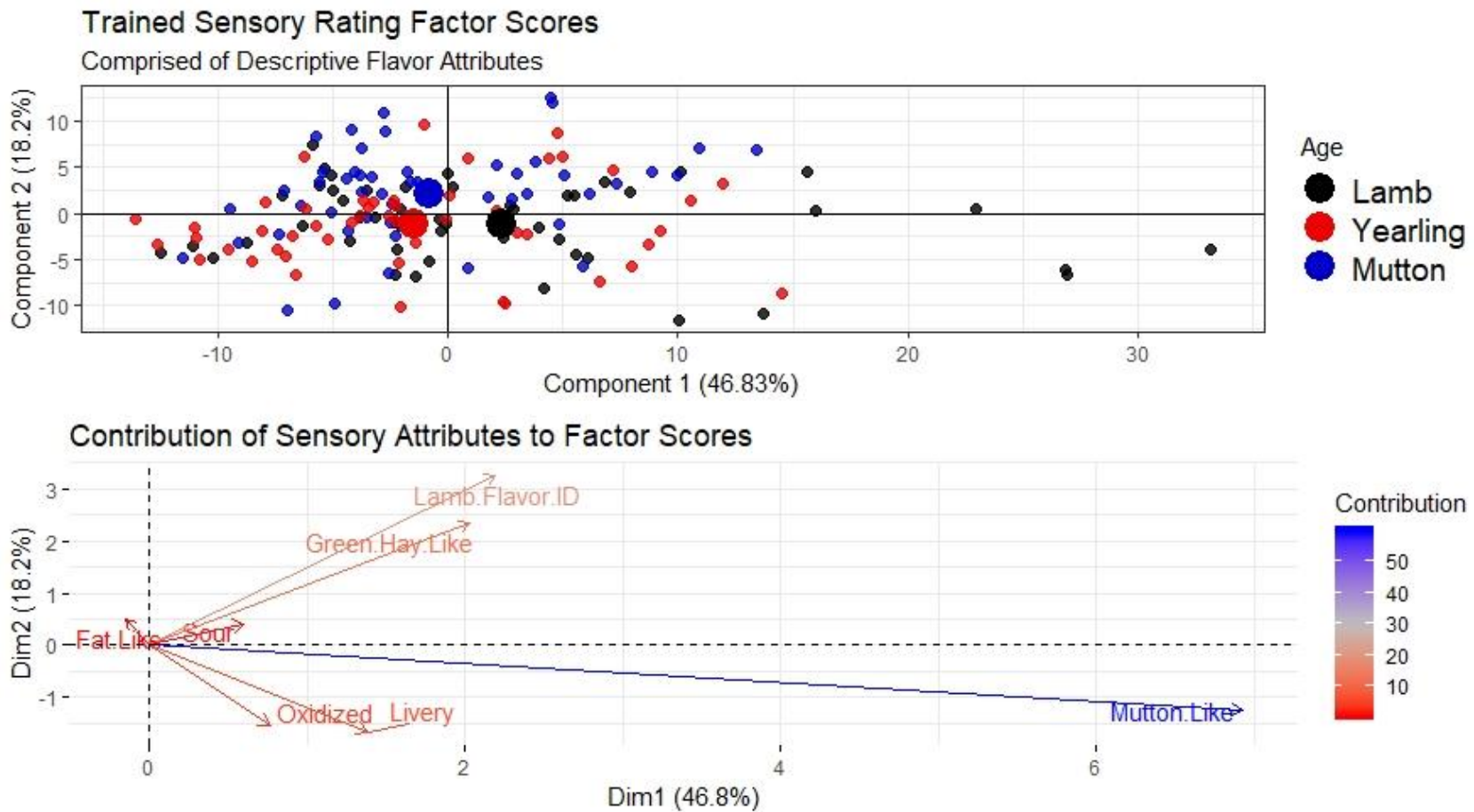
**Figure 1.** Projection of partial least squares (PLS) scores (top) and linear discriminant (LDA; LDA developed using factor scores) scores (bottom) from rapid evaporative ionization mass spectrometry (REIMS) mass bins collected from external fat of legs from sheep carcasses to predict sheep age groups using training and test models.



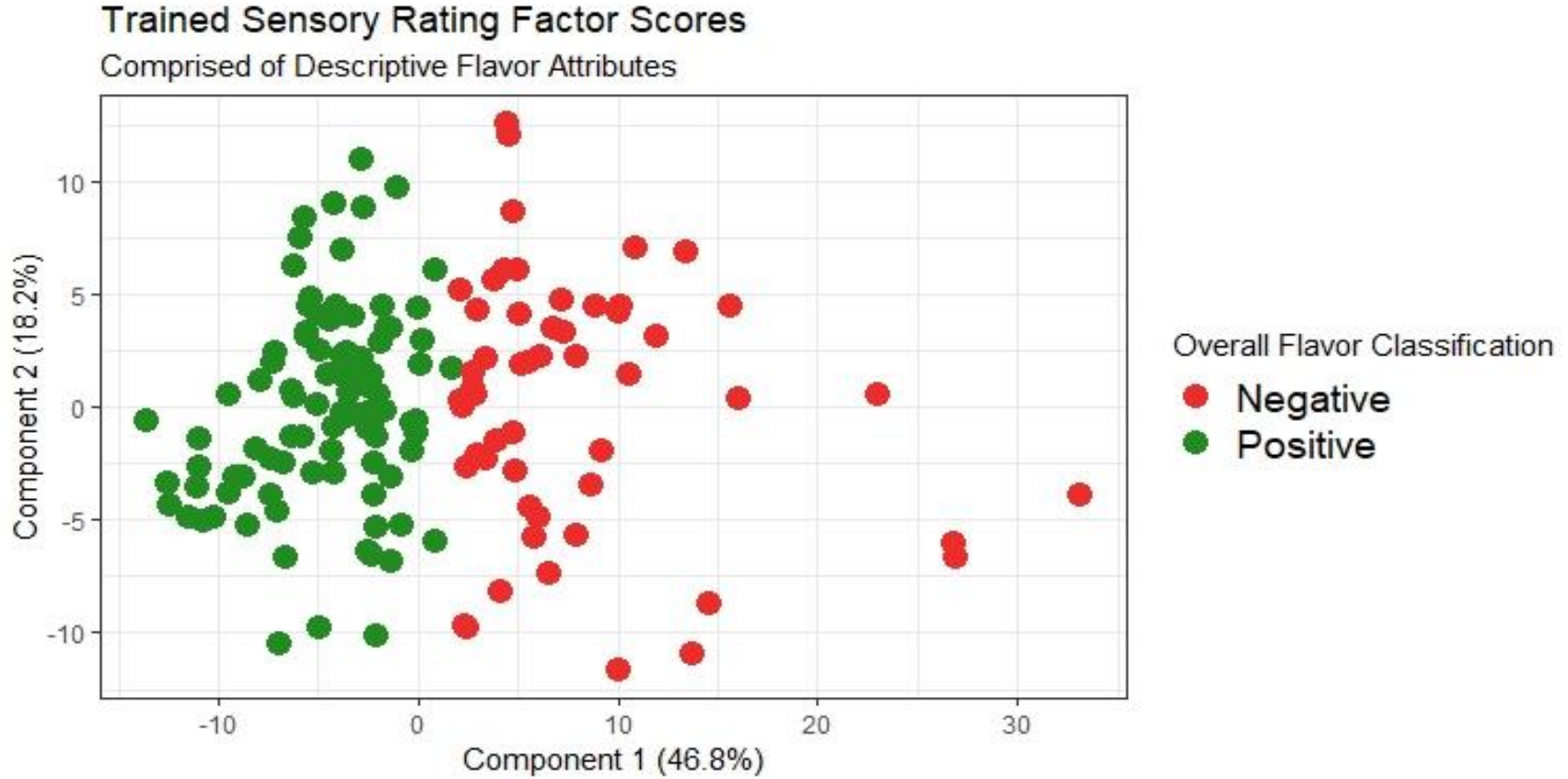
**Figure 2.** Projection of partial least squares (PLS) scores (top) and linear discriminant (LDA; LDA developed using factor scores) scores (bottom) from rapid evaporative ionization mass spectrometry (REIMS) mass bins collected from lean of legs from sheep carcasses to predict sheep age groups using training and test models.



**Figure 3.** Projection of partial least squares (PLS) scores (top) and linear discriminant (LDA; LDA developed using factor scores) scores (bottom) from rapid evaporative ionization mass spectrometry (REIMS) mass bins collected from ground meat produced from legs of sheep carcasses to predict sheep age groups using training and test models.



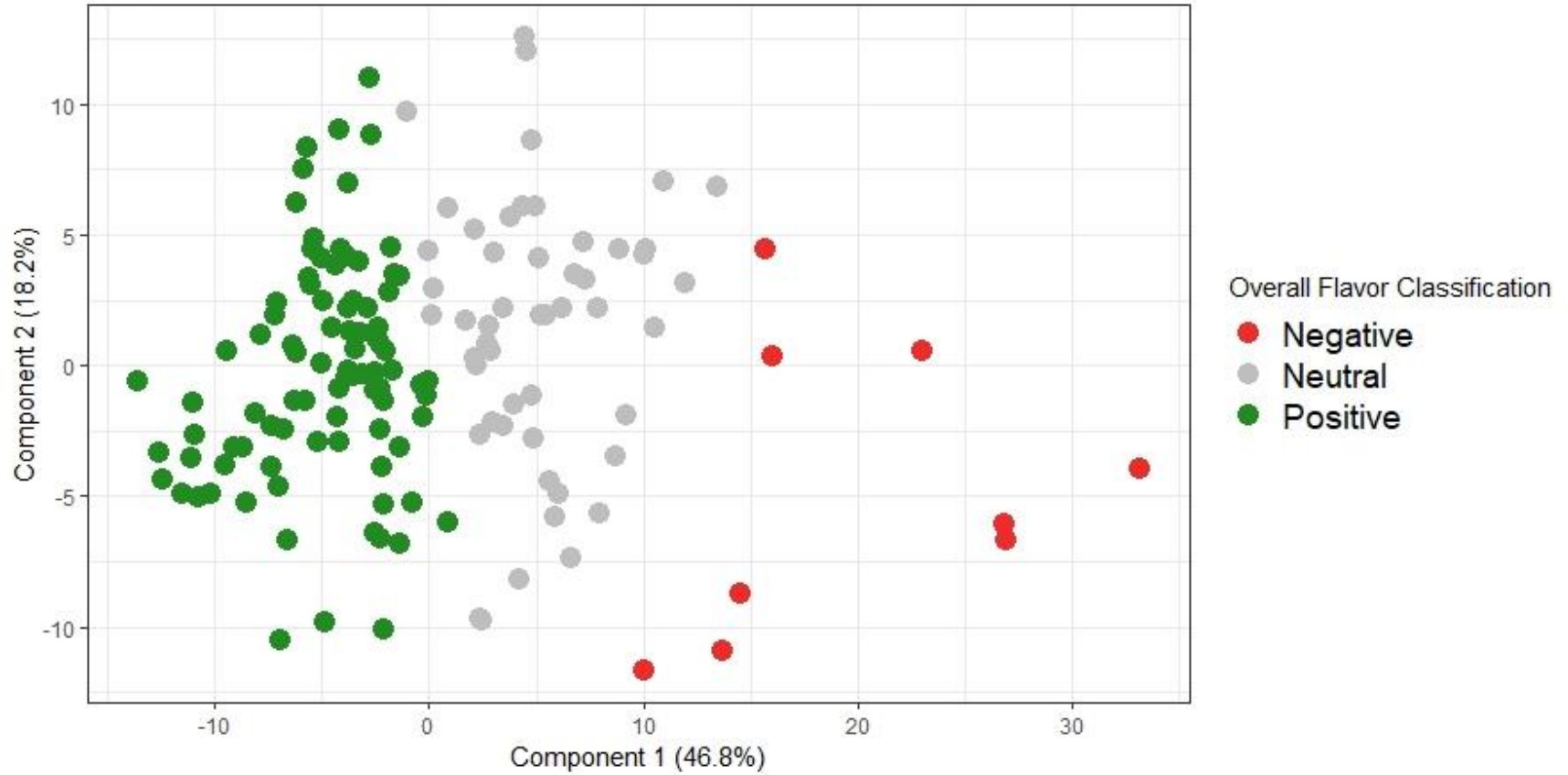
**Figure 4.** Projection of principal component scores derived from trained sensory ratings for descriptive sensory attributes with each large point representing treatment means. Contribution of sensory attributes to factor scores represented in the loadings plot (bottom).



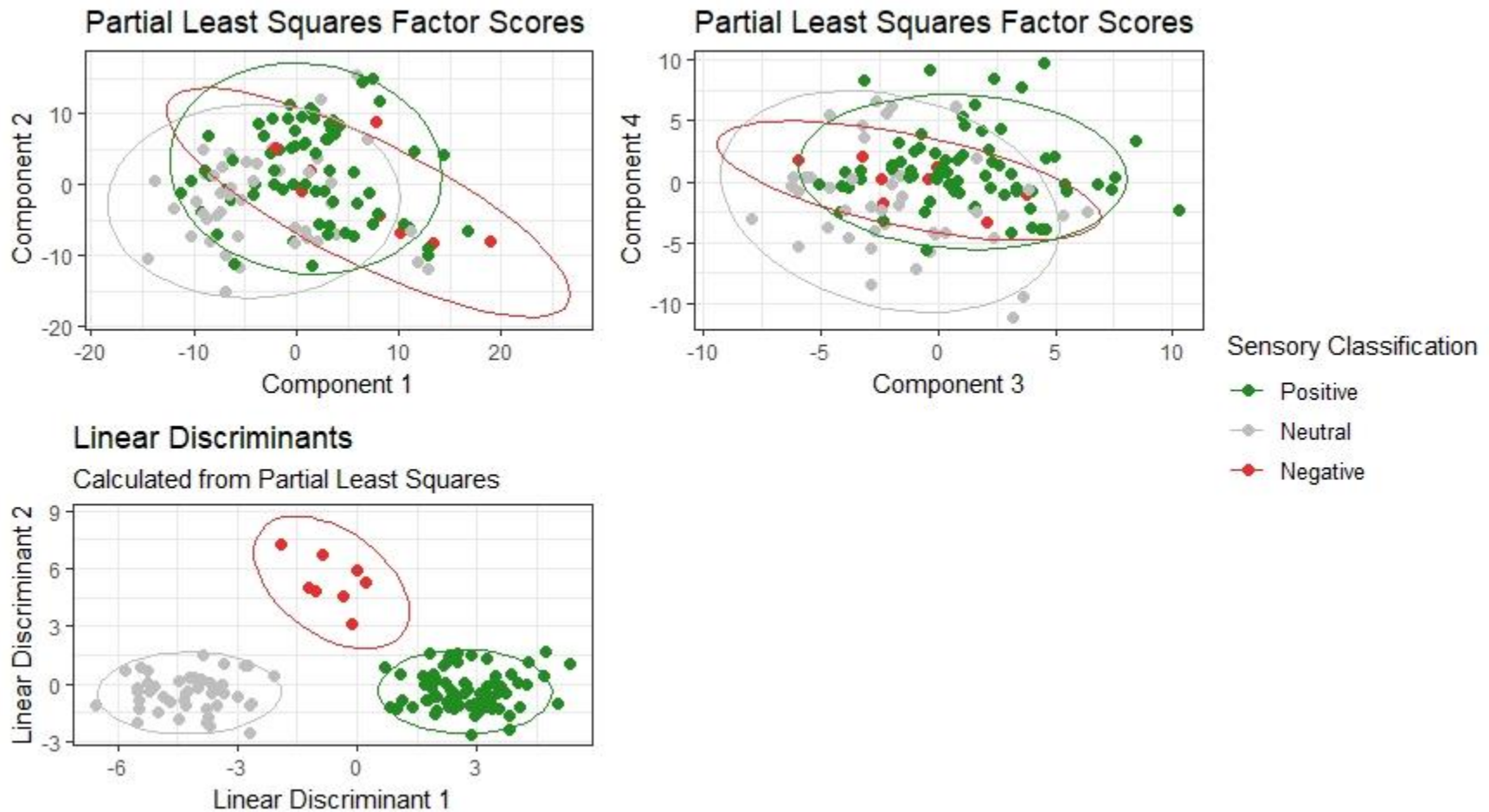
**Figure 5.** Projection of principal component scores derived from trained sensory ratings for descriptive sensory attributes colored by two-level sensory classification (positive or negative) determined by hierarchical cluster analysis.

### Trained Sensory Rating Factor Scores

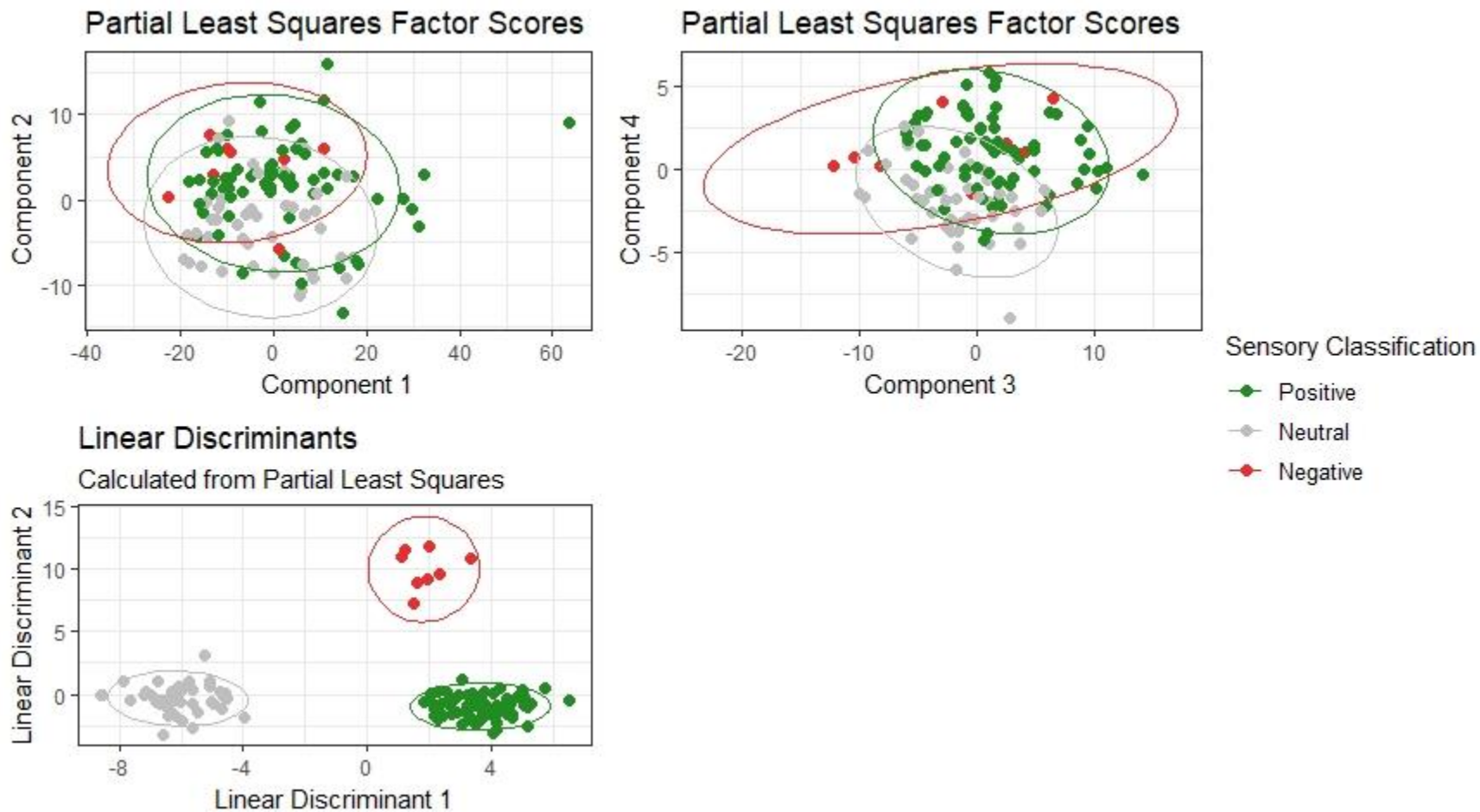
Comprised of Descriptive Flavor Attributes



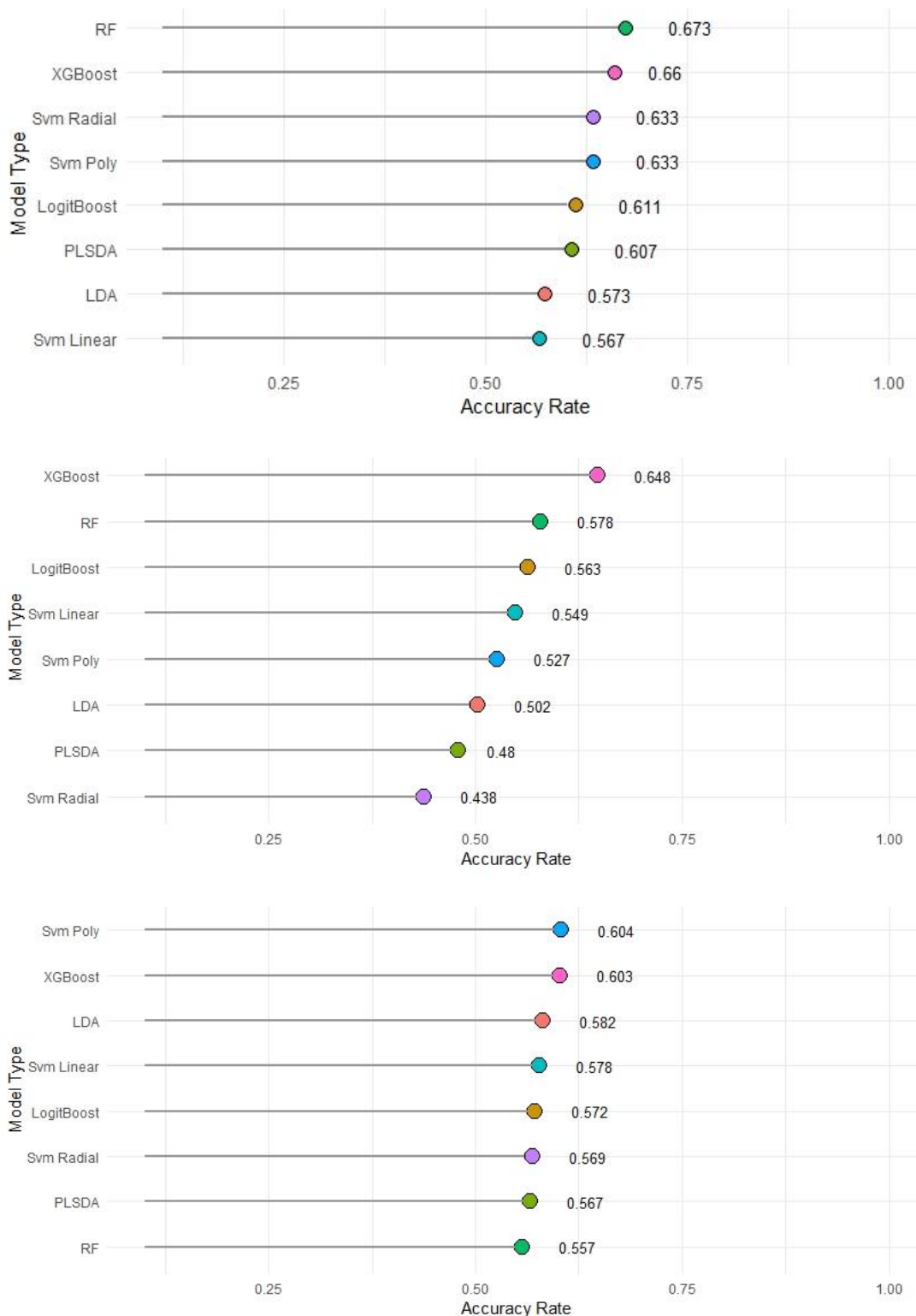
**Figure 6.** Projection of principal component scores derived from trained sensory ratings for descriptive sensory attributes colored by three-level sensory classification (positive, neutral or negative) determined by hierarchical cluster analysis.



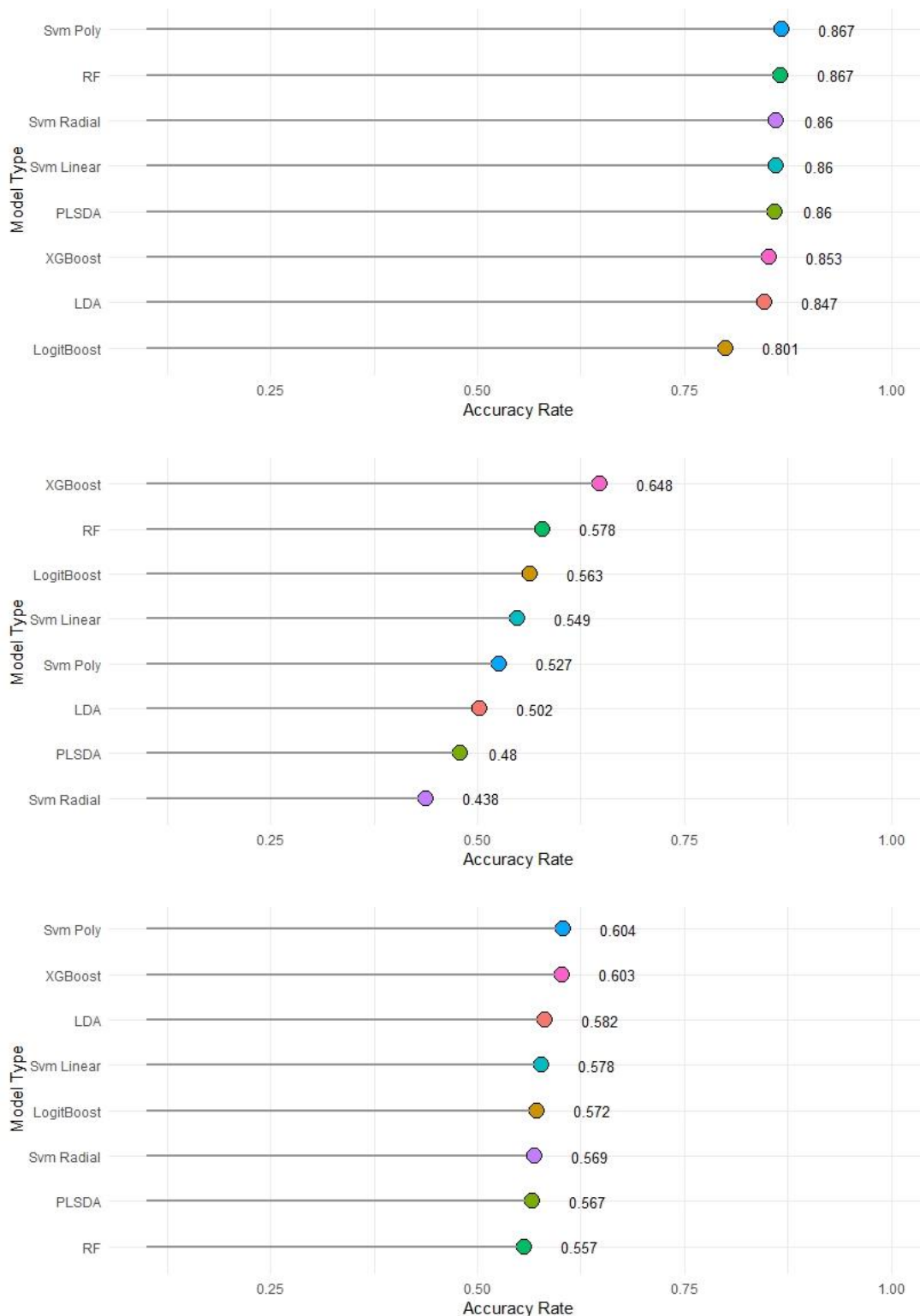
**Figure 7.** Projection of partial least squares (PLS) scores (top) and linear discriminant (LDA; LDA developed using factor scores) scores (bottom) from rapid evaporative ionization mass spectrometry (REIMS) mass bins collected from lean of legs from sheep carcasses to predict three overall sensory classifications of sheep using training and test models.



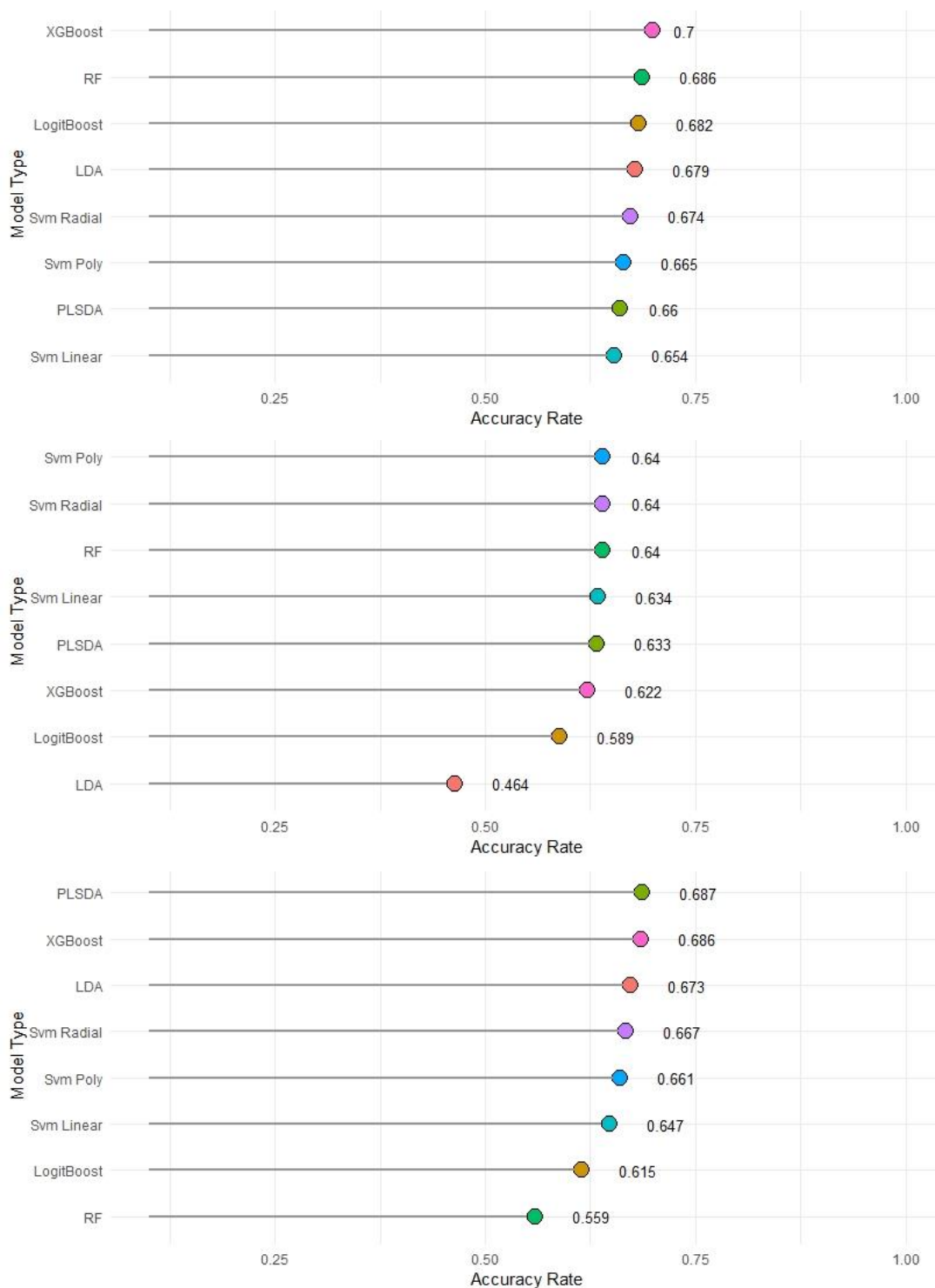
**Figure 8.** Projection of partial least squares (PLS) scores (top) and linear discriminant (LDA; LDA developed using factor scores) scores (bottom) from rapid evaporative ionization mass spectrometry (REIMS) mass bins collected from lean of legs from sheep carcasses to predict three overall sensory classifications of sheep using training and test models.



**Figure 9.** Prediction accuracies of sheep age category (lamb, yearling or mutton) using rapid evaporative ionization mass spectrometry data collected from lean tissue of ovine carcass legs and 10-fold cross validation for eight machine learning algorithms applied to feature selection (FS; top), principle component analysis (PCA; middle), and PCA followed by FS (bottom) data reduction approaches.



**Figure 10.** Prediction accuracies of production background category (grain-finished or grass-finished) using rapid evaporative ionization mass spectrometry data collected from lean tissue of ovine carcass legs and 10-fold cross validation for eight machine learning algorithms applied to feature selection (FS; top), principle component analysis (PCA; middle), and PCA followed by FS (bottom) data reduction approaches.



**Figure 11.** Prediction accuracies of overall flavor classification (positive or negative determined from hierarchical cluster analysis of principle components from trained sensory attributes) using rapid evaporative ionization mass spectrometry data collected from lean tissue of ovine carcass legs and 10-fold cross validation for eight machine learning algorithms applied to feature selection (FS; top), principle component analysis (PCA; middle), and PCA followed by FS (bottom) data reduction approaches.

## LITERATURE CITED

- Adhikari, K., E. Chambers IV, R. Miller, L. Vázquez-Araújo, N. Bhumiratana, and C. Philip. 2011. Development of a lexicon for beef flavor in intact muscle. *J. Sens. Stud.* 26:413–420.
- Balog, J., Szaniszlo, T., Schaefer, K.-C., Denes, J., Lopata, A., Godorhazy, L., . . . Toth, M. (2010). Identification of biological tissues by rapid evaporative ionization mass spectrometry. *Analytical chemistry*, 82(17), 7343-7350.
- Balog, J., D. Perenyi, C. Guallar-Hoyas, A. Egri, S. D. Pringle, S. Stead, O. P. Chevallier, C. T. Elliott, and Z. Takats. 2016. Identification of the Species of Origin for Meat Products by Rapid Evaporative Ionization Mass Spectrometry. *J. Agric. Food Chem.* 64:4793–4800.
- Black, C., Olivier, P. Chevallier, S. A. Haughey, J. Balog, S. Stead, S. D. Pringle, M. V Riina, F. Martucci, P. L. Acutis, M. Morris, D. S. Nikolopoulos, Z. Takats, and C. T. Elliott. 2017. A real time metabolomic profiling approach to detecting fish fraud using rapid evaporative ionisation mass spectrometry. *Metabolomics* 13.
- Gredell, D. 2018. Assessment of rapid evaporative ionization mass spectrometry (REIMS) to characterize beef quality and the impact of oven temperature and relative humidity on beef. Ph.D. Dissertation, Colorado State University, Fort Collins, CO.
- Gredell, D. A., Schroeder, A. R., Belk, K. E., Broeckling, C. D., Heuberger, A. L., Kim, S. Y., . . . Prenni, J. E. (2019). Comparison of Machine Learning Algorithms for Predictive Modeling of Beef Attributes Using Rapid Evaporative Ionization Mass Spectrometry (REIMS) Data. *Sci Rep*, 9(1), 5721. doi: 10.1038/s41598-019-40927-6
- Hoffman, T. W. 2015. Benchmark of lamb quality in U.S. retail and foodservice markets. Ph.D. Dissertation, Colorado State University, Fort Collins, CO.
- Maneotis, K. A. 2017. A proof-of-concept to differentiate among differences in flavor of American lamb using volatile flavor compound analysis. Master's Thesis, Colorado State University, Fort Collins, CO.
- Jaborek, J., Zerby, H., Moeller, S., Wick, M., Fluharty, F., Garza III, H., . . . England, E. (2018). Effect of energy source and level, and animal age and sex on meat characteristics of sheep. *Small Ruminant Research*, 166, 53-60.
- Jones, K. G. 2004. Trends in the US sheep industry. USDA Economic Research Service Washington, DC.
- R Core Team. 2016. R: A Language and Environment for Statistical Computing. Vienna, Austria.

- Tatum, J. D., Zerby, H. N., & Belk, K. E. (2014). A brief overview of factors affecting lamb flavor. A white paper. Lamb Industry Roadmap Product Characteristics Team.
- USDA-ERS. 2019. Supply and disappearance and per capita disappearance. United States Department of Agriculture – Economic Research Service. Accessed March 4, 2019. Accessible at: <https://www.ers.usda.gov/topics/animal-products/sheep-lamb-mutton/#periodic>.
- Van Berkel, G. J. 2003. An overview of some recent developments in ionization methods for mass spectrometry. *European Journal of Mass Spectrometry* 9(6):539-562.
- Verplanken, K., S. Stead, R. Jandova, C. Van Poucke, J. Claereboudt, J. Vanden Bussche, S. De Saeger, Z. Takats, J. Wauters, and L. Vanhaecke. 2017. Rapid evaporative ionization mass spectrometry for high-throughput screening in food analysis: The case of boar taint. *Talanta* 169:30–36.
- Waters Corporation. 2019. REIMS research system with 'iKnife' sampling device. Accessed April 6, 2019. Available at [http://www.waters.com/waters/en\\_US/REIMS-Research-System-with-'iKnife'-Sampling-Device/nav.htm?cid=134846529&locale=en\\_US](http://www.waters.com/waters/en_US/REIMS-Research-System-with-'iKnife'-Sampling-Device/nav.htm?cid=134846529&locale=en_US).
- Watkins, P. J., Rose, G., Salvatore, L., Allen, D., Tucman, D., Warner, R. D., . . . Pethick, D. W. (2010). Age and nutrition influence the concentrations of three branched chain fatty acids in sheep fat from Australian abattoirs. *Meat Sci*, 86(3), 594-599. doi: 10.1016/j.meatsci.2010.04.009
- Young, O., Berdagué, J.-L., Viallon, C., Rousset-Akrim, S., & Theriez, M. (1997). Fat-borne volatiles and sheepmeat odour. *Meat science*, 45(2), 183-200.

## CHAPTER IV.

### REVIEW OF LITERATURE – PART II: OVERVIEW OF FEEDING GENETICALLY MODIFIED GRAIN TO LIVESTOCK

#### *Introduction*

In general, crops produced from plants whose genome have been altered via engineering techniques, such as use of recombinant DNA methods, are considered genetically modified (GM) or genetically engineered (GE) plants. Many researchers use GM (Snell et al., 2012; Zeljenkova et al., 2014) while others use GE (Fernandez-Cornejo, Wechsler, Livingston, & Mitchell, 2014; Van Eenennaam & Young, 2017) to describe these crops, thereby using these acronyms interchangeably. Use of GE crops has increased in the U.S. substantially over the past few decades. Descriptions of traits observed in GE crops can be classified into three generations as follows: generation one includes traits such as herbicide tolerance, resistance to insects and resistance to environmental stress; generation two includes traits such as nutrient enhancement or other value-added traits; and generation three includes traits that offer products beyond the scope of traditional food (Fernandez-Cornejo et al., 2014). However, most of the acres planted in the U.S. utilize crops with traits of herbicide or insect resistance. Fernandez-Cornejo et al. (2014) reported that in 2013, approximately 90 percent of acres in the U.S. were planted with GE varieties of corn.

Regulatory requirements for use of GE crops in the U.S. call for regulatory approval by the Environmental Protection Agency (EPA), Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA) (Fernandez-Cornejo et al., 2014; Scientists, 2011). According to the Federal Insecticide, Fungicide, and Rodenticide Act (1972), pesticides such as Bt toxins (an insect resistant protein introduced from *Bacillus thuringiensis*), including a GE plant modified with a Bt gene, must be regulated by the EPA. The safety of GM crops is

regulated by the FDA, regardless whether consumed by either humans or animals. The Plant Protection Act (2000) requires the Animal and Plant Health Inspection Services (APHIS) branch of USDA regulate organisms that modify plant or plant products such as with the use of *Agrobacterium* spp. in gene transfer for development of GE plants.

A genetically modified event refers to the insertion of DNA into a plant genome (Pilacinski et al., 2011). Since 1996, multiple varieties have been quickly adopted among many other plant species. Between 1996 and 2016 there were 174 GE cultivated crop events from 20 plant species approved in the US (James, 2016a) including crops consumed by livestock such as corn (maize), sugar beets, alfalfa, soybean and others (James, 2016b; Van Eenennaam & Young, 2014). Of those, 41 were GE maize (corn) events approved for use by animal feed and for cultivation in 2016 (ISAAA, 2017). The objective of this review was to conduct a literature search to determine if there is any evidence available among the scientifically published literature to determine whether corn grain from genetically engineered (GE) plant varieties alter metabolism in livestock consuming these GE crops, and any metabolic effects from consuming beef cattle fed genetically modified crops.

### ***Overview of Safety Assessment***

Public controversy on the safety of genetically engineered (GE) plant crops for animal and human consumption has continued since the first GE corn variety was approved and released in 1996 (Swiatkiewicz, et al., 2014; Van Eenennaam & Young, 2014, 2017). Concerns that have continued during this time mainly include the risk of GE crops to the environment and to human health (Domingo & Gine Bordonaba, 2011). A principle long used in the evaluation of the safety of GM or GE foods by the FDA is the concept of substantial equivalence. This concept is based on “a comparison of the composition and/or characteristics of food derived from the GE plant

variety to that of food derived from the parental variety or other commonly consumed varieties with special emphasis on important nutrients, anti-nutrients, and toxicants that occur naturally in the food” (U.S. Food and Drug Administration, 2015) to assure that the GE or GM food variety is as safe and nutritionally similar as food from non-GE plants (Domingo & Gine Bordonaba, 2011; Van Eenennaam, 2013).

Animal feeding trials can provide additional information to support safety and nutritional assessments. Safety of GM food and feed assessed by the European Food Safety Authority (EFSA) have published guidelines to demonstrate equivalence include molecular, compositional, phenotypic, agronomic and other analyses (European Food Safety, 2011, 2013). The expert’s panel of EFSA recommended that animal trials using rodents should be conducted by feeding GM feed for a period of 90 days (European Food Safety, 2011, 2013; Snell et al., 2012; Van Eenennaam, 2013). Snell et al. (2012) concluded that no evidence was available to suggest that a 90-day feeding period was insufficient to assess the safety of GM feed in rodents. Snell et al. (2012) further indicated that feeding trials lasting longer than 90 days were unnecessary, unless a 90-day feeding period still caused doubt about the safety of the specific GM feed. However, the 90-day rodent feeding trial method was designed to detect the majority of changes that could occur (Snell et al., 2012), but longer feeding trials may be necessary to evaluate potential “effects on reproductive or endocrine tissues, effects on development, chronic toxicity, carcinogenicity, etc.” (European Food Safety, 2013). Snell et al. (2012) also described that the protocol recommends including at least n=10 animals per sex and group along with 3 different doses of the test material, and a control group. In this same review, only 6 of 24 studies (studies included soybean, rice, maize, potato, and triticale) met the sample size recommendations in long-term trials (greater than 90-day feeding periods).

## *Animal Feeding Trials*

### *Transgenic Maize Effects on Rodents (90-Day Trials)*

The five studies included (Hammond, Dudek, Lemen, & Nemeth, 2004; Hammond et al., 2006; Hammond et al., 2006; MacKenzie et al., 2007; Malley et al., 2007) in the review by Snell et al. (2012), in which researchers fed rats for 90-days, concluded that there were no differences between GM maize and conventional non-transgenic diets. Similarly, there were no effects observed between rats consuming diets containing varying doses of the GM maize diet and the non-transgenic diet (Liu et al., 2012). No diet-related significant differences were observed for body weight, hematology, serum chemistry, serum hormone concentration, reproductive system parameters, necropsy, or histopathology results in a 90-day feeding trial of male rats fed transgenic and non-transgenic maize (Guo et al., 2015).

A separate 90-day study reported no significant differences in body weight, feed utilization, necropsy and histopathology of internal organs, and serum chemistry including cholesterol, triglycerides and multiple liver enzymes from rats consuming transgenic and non-transgenic maize (Han, Zou, He, Huang, & Mei, 2016). Similarly, in a 90-day feeding study with male and female Wistar Han RCC rats (N=160) varying dosed treatment diets of transgenic maize or non-transgenic maize, no significant differences were observed among results of hematology, clinical biochemistry analyses, necropsy or histopathology of internal organs (Zeljenkova et al., 2014). Researchers reported minor histopathological and biochemical effects in rats fed transgenic Bt corn in a three-generation feeding trial, but the same researchers concluded that severe health effects were not observed (Kilic & Akay, 2008). Additionally, researchers that conducted a study resulting in differences in metabolites from kidney and liver samples of rats fed a Roundup-tolerant GM maize or non-GM maize for 2 years and concluded

that because differences between animals in the treatment group were greater than the effects of diet, no definitive conclusion could be made regarding safety or pathology of transgenic diets (Mesnage, Arno, Séralini, & Antoniou, 2017).

Finamore et al. (2008) fed diets consisting of transgenic, a parent line control, and non-transgenic maize that was raised in neighboring fields for 30 or 90 days to determine immune responses to diets fed to two age groups (21 days of age and 18-19 months of age). These researchers concluded that alterations were observed in intestinal and peripheral immune responses. They also concluded that, although these results did not confirm significant immune dysfunctions, immune responses should be considered in GM safety assessments (Finamore et al., 2008). However, it is unclear how many rats were assigned to each treatment. In contrast, researchers concluded no immunogenic, allergenic or adjuvant capacity resulted from a study using female C3H/JeJ mice (n=11 to 13 per treatment group) aged 5 weeks of age administered 250 µL of TypCry1Ab toxin alone or with lupin extract along with a positive cholera toxin control by gavage for 35 days (Andreassen et al., 2016).

### *Animal Studies*

Plants, including those that comprise the feed consumed by animals, contain deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The public continues to fear GE foods, however, all foods contain DNA and DNA from food has not posed a risk to human health prior to the development of GE foods (Van den Eede et al., 2004). The DNA from foods begins to degrade immediately after harvest (Van Eenennaam & Young, 2017). Further degradation occurs during digestion, heating and food processing (Van Eenennaam & Young, 2017). Along with protein digestion, DNA and RNA from feed sources are broken down into nucleotides. Researchers have suggested that plant derived DNA is degraded during normal digestion

processes (Beever & Phipps, 2001; Jonas et al., 2001; Van Eenennaam & Young, 2017).

### ***Digestion Process in Ruminants and Non-ruminants***

Normal digestion in both ruminants and non-ruminants results in processes that break down feed – mechanically and enzymatically – throughout the digestive tract to allow simpler compounds to be absorbed and metabolized. Ruminant animals (i.e., beef cattle, dairy cattle, sheep, goats) undergo fermentation and enzymatic processes as part of normal digestion (The National Academies of Sciences, 2016). Ruminants contain a unique, characteristic four compartmentalized stomach (rumen, reticulum, omasum, abomasum) that serves as the site of anaerobic microbial (including bacteria, protozoa, and fungi) fermentation yielding methane (CH<sub>4</sub>), microbial crude protein and cells, volatile fatty acids (such as acetate, propionate and butyrate), carbon dioxide (CO<sub>2</sub>), nitrogen (NH<sub>3</sub>) and hydrogen gas (H<sub>2</sub>) (The National Academies of Sciences, 2016). Volatile fatty acids serve as the major energy source for ruminant animals (The National Academies of Sciences, 2016).

While the rumen, reticulum and omasum are described as the site of fermentation and feed storage, the abomasum has been described as the glandular compartment that connects to the proximal duodenum of the small intestine (The National Academies of Sciences, 2016). Microbial and non-digested protein from feed are further digested by gastric digestion in the abomasum to form peptide fragments that ultimately result in free or peptide bound amino acids; these stimulate hormone release of pancreatic enzymes. Enzymatic secretion from the pancreas occurs in the duodenum to further break down nutrients into smaller compounds. proteolytic enzymes (such as trypsin, chymotrypsin and elastase that later elicit further digestion by a variety of peptidases) from the pancreas in a solution containing other enzymes, electrolytes, bicarbonate and other components required for digestion. Researchers have concluded that digestibility of

protein from corn and other protein supplements is greater compared to other forages in cattle.

The jejunum of the small intestine serves as the site where most of absorption occurs. Transporters move peptide bound and free amino acids across the epithelia in intestinal tissue for physiologic utilization. Research suggests that 65 to 80% of total amino acids that progressed to the duodenum are absorbed, but other studies indicated that there is variation in the amount of individual amino acids that are absorbed in cattle. Some microbial protein and protein from feed are not digested or absorbed, leading to excretion.

Non-ruminant animals contain a single chambered stomach that results in similar gastric digestion processes, such as the release of hydrochloric acid (HCl) and pepsin from chief and parietal cells. In contrast, non-ruminant animals do not rely on anaerobic microbial fermentation compared to ruminant animals. Poultry also have a single chambered stomach, although they have additional organs that aid in mechanical digestion. Although non-ruminant animals and poultry have vastly different GI tract systems, similar digestion and absorption mechanisms that occur in the portions of the small intestine in ruminants also occur at the site of the small intestine in non-ruminants and poultry including hormonal release of pancreatic enzymes that further digest protein and transporters that move peptide bound and free amino acids across the epithelia (Van Eenennaam & Young, 2017).

### ***Transgenic Corn in Beef Cattle Diets***

Most of the scientific literature describes feeding trials conducted with dairy cattle, rodents and other animal models. Scientific literature reporting studies of the impact of transgenic corn in beef cattle diets are limited, but a few studies that have been conducted have revealed no adverse effects to beef cattle metabolism from feeding GE corn. Researchers investigating the effect of transgenic Bt and non-transgenic corn silage, grain and residue (stalks)

in Holstein dairy cows (n=16) and beef steers (n=67 and n=128) in three separate experiments reported no consistent effect on cattle performance based on treatment diet (Folmer, Grant, Milton, & Beck, 2002). In an experiment by Folmer et al., evaluating beef steers grazing transgenic and non-transgenic corn residue, daily gain of steers was not influenced by diet ( $P=0.12$ ). Two treatment pastures were used to test grazing preference. Results of grazing preference indicated that an average of 47.5% of steers were observed grazing Bt residue, an average of 52.5% of steers were observed grazing non-Bt residue, and a similar distribution of steers grazing Bt and non-Bt residue was observed ( $P=0.51$ ). In the corn silage experiment evaluating beef steers fed Bt corn silage and near isogenic non-Bt corn silage, the Bt corn silage diet resulted in greater DMI ( $P=0.02$ ). However, these researchers concluded that there was no consistent effect of cattle performance, but rather, they suggested that the background genetics of the corn hybrids seemed to have a greater impact on performance than the presence of the Bt trait (Folmer et al., 2002).

In a separate feeding trial that evaluated the effects of Bt-Cesar silage on Deutsche Holstein cattle (n=40) fed to 550 kg, these researchers concluded that a small amount of small fragmented plant DNA could be transferred to beef lymphocytes from transgenic silage that has not fully degraded DNA and has increased absorption time in the animal (Einspanier et al., 2001). Further, they concluded that almost no DNA was transferred to milk in a second experiment that they conducted on fistulated dairy cows.

Some individuals have proposed that DNA from transgenic plants could potentially be transferred to commensal and gastrointestinal bacteria in cattle, further altering metabolic function via the microbiome. Further speculation has suggested that transgenic plant DNA fragments could be absorbed through portions of the small intestine during normal digestion and

absorption. However, in 2009 a study published in the World Journal of Microbiological Biotechnology (Shedova et al., 2009) concluded that several factors affect whether transgenic DNA can be absorbed into circulation via normal digestive processes. The size of the plant DNA fragment and the stage of degradation that the transgenic DNA is undergoing are crucial to any biological effect that could potentially occur. These researchers concluded that the probability of recombinant DNA transfer to bacteria involved in cattle digestion seems to be low due to the potential for DNA degradation occurring from rumen fluid, and initially degraded if the DNA is derived from an ensiled feed (Shedova et al., 2009).

Effects of feeding transgenic corn on the microbiome also has been investigated in swine. Since the physiological system of swine is more similar to that of humans, researchers conducted a long term feeding study with male landrace crossbred pigs weaned at 28 days of age, given a 12 day adaptation period and assigned by blocking to one of four diet treatments for 110 days (Buzoianu et al., 2012). Treatments consisted of an isogenic diet, Bt-maize diet, isogenic diet for 30 days and Bt-maize diet for 80 days or Bt-maize diet for 30 days and isogenic diet for 80 days. These researchers concluded that diet did not contribute to differences in fecal *Enterobacteriaceae spp.*, *Lactobacillus spp.* or total anaerobes. This study had one significant difference observed in cecal *Holdemaniania spp.* abundance ( $P = 0.05$ ) in treatment 3 (isogenic followed by Bt-maize), but these researchers reported that this difference was most likely due to the change in source of corn from the isogenic line to the transgenic line at day 30 rather than from the Bt-maize alone since no effects were observed in the treatment consisting of only transgenic corn.

Previous studies evaluating effects of feeding transgenic corn have demonstrated no effects on carcass traits. In a randomized block design that evaluated 108 pigs fed from 37 to 127

kg of body weight by consuming a sole source of corn from a transgenic line, commercial hybrid line or a near-isoline control, no significant differences were observed for final BW, ADG, gain to feed ratio, hot carcass weight, LM area or depth, 10th-rib fat, marbling score or color score among animals across all treatments (Stein et al., 2009). Similarly, another publication describing evaluating effects of feeding a separate transgenic line, a similar non-transgenic line and two conventional non-transgenic lines of corn to barrows (n=72) and gilts (n=72) housed separately by gender in blocks in an open-fronted building and to barrows (n=80) and gilts (n=80) housed separately by gender in blocks in an environmentally controlled finishing building found no differences in growth or carcass characteristics in pigs fed transgenic corn (Hyun et al., 2004). A follow-up study, designed almost identical to that conducted by Hyun et al. (2004) using a different transgenic line of corn and different breed types of pigs housed in an open-fronted building (n=72 barrows and n=72 gilts), and in an environmentally controlled finishing building (n=80 barrows and n=80 gilts), also found no differences in growth or carcass characteristics from pigs fed transgenic corn (Hyun et al., 2005).

In contrast, Walsh et al. (2012a) evaluated crossbred landrace male weanling pigs (n=32) that were blocked by weight and litter at 28 days of age and fed either a transgenic maize or non-transgenic maize diet for 31 days found that pigs fed transgenic maize consumed significantly more feed ( $P<0.05$ ), were less efficient in feed to gain ( $P<0.05$ ) and had decreased goblet cells per  $\mu\text{m}$  of duodenal villus ( $P<0.10$ ). Higher kidney weights ( $P<0.10$ ) was also observed in pigs fed transgenic corn, however, no other differences were observed in liver enzymes (alanine aminotransferase, aspartate aminotransferase, and gamma glutamyl transferase) or in histopathology (villus height and crypt depth of small intestine, or goblet cells per  $\mu\text{m}$  of the jejunum and ileum) of these pigs.

### *Transgenic Corn in Diets of Dairy Cattle*

Donkin et al. (2003) evaluated effects of including transgenic corn in diets consumed by dairy cattle on milk production traits. Three experiments were conducted by researchers comparing diets consisting of silage from GE and non-GE varieties fed to multiparous dairy cattle (Donkin et al., 2003). Experiment 1 consisted of 12 dairy cows stratified by milk production randomly assigned to one of two treatment groups with three 21-day treatment periods consisting of a 14-day adaptation period fed Bt-MON810 transgenic or isogenic control corn silage and grain. Experiment 2 consisted of 16 multiparous Holstein cattle randomly assigned to one of two treatment groups with three 28-day treatment periods consisting of a 14-day adaptation period consisting of the same diets as experiment 1. No differences were observed between Bt-MON810 and control diets for milk production among experiments 1 and 2. However, cows fed the treatment diet had slightly greater ( $P=0.06$ ) dry matter intake (DMI) than cows fed the control diet. Additionally, greater milk lactose and SNF resulted ( $P<0.05$ ) from cows fed the treatment diet in experiment 1, but no differences were observed when both experiments were analyzed together. Experiment 3 consisted of similar adjustment time and sample size, except 4 of the 16 dairy cows were cannulated and diets consisted of corn silage and grain from RR-GA21 or control. No differences existed for DMI or digestibility between cows fed either diet.

Similarly, no effects were observed in (DMI), total milk production, fat-adjusted milk production, body weight (BW), or body condition score (BCS) in mid-lactation multiparous Holstein dairy cows in a randomized block design crossover trial with repeated measures fed either a control diet or treatment diet containing 45% corn silage from a hybrid GE corn variety containing mepsps and cry1ab genes (Calsamiglia et al., 2007). However, only 8 cows were

evaluated in this study. Both diets were analyzed for presence or absence of Cry1Ab protein to validate that no cross contamination occurred, and chemical analysis resulted in no major nutritional composition differences between either diet. Similar to these authors' previous work, Donkin et al. (2013) found a slightly greater percent of milk protein, lactose and SNF ( $P < 0.05$ ) were found in milk from cows fed the transgenic diet, but no DNA or protein encoded for transgenic genes were detected in milk from cows fed the transgenic diet.

Steinke et al. (2010) fed Bt-MON810 transgenic or isogenic control corn diets for 25 months to 36 Simmental dairy cows. The transgenic diet did not influence DMI, milk yield, or energy intake. Cows fed the transgenic diet had greater milk fat, milk protein and milk urea ( $P < 0.05$ ) during the first lactation. Although cows fed the control diet had significantly ( $P < 0.05$ ) lower lactose concentrations with greater ( $P < 0.05$ ) body weight, BCS and backfat, these researchers attributed these differences from cows fed both diets to biological variation. Similarly in a 2 x 2 Latin square study evaluating 26 Holstein dairy cows being fed diets including either transgenic or isogenic soybean meal and hulls, researchers concluded that no differences were observed between BW, BCS, DMI, milk composition or overall acceptability in sensory evaluation that a diet inclusive of transgenic soybeans is equivalent to control diets (Weiss, Simons, & Ekmay, 2015).

Steinke et al. (2010) identified lower concentration of the Cry1ab protein in silage and corn kernels compared to that of whole cobs but did not measure concentration in fresh corn. However, researchers have reported that the ensiling process may be one factor responsible for degrading the Cry1Ab protein in silage from bt-corn (Lutz, Wiedemann, & Albrecht, 2006; Steinke et al., 2010). Data reported by Lutz et al. (2006) revealed that the Cry1Ab protein degrades substantially over time. Steinke et al. (2010) also reported, using enzyme-linked

immunosorbent assay (ELISA) methods, that only full Cry1Ab protein could be detected during the first 8 days of ensiling. Additionally, the concentration of transgenic protein differs between corn plant tissues such as the kernel, leaves, stem or root.

Castillo-Lopez et al. (2014) utilized a 4 by 4 latin square design to test for effects of feeding 16 multiparous dairy cows a diet consisting of silage from a non-transgenic control (DKC63-78), Bt resistant hybrid (DKC63-78) or two reference hybrids (DKC61-42 and DK62-30) concluded that the Bt resistant variety of silage resulted in significantly higher DMI, but found no other effects to milk production traits, milk composition, BW or BCS. Additionally, three studies published by researchers found that sheep and dairy cattle fed Bt corn silage had growth performance similar to animals fed control diets (Barriere et al., 2001a; Barriere et al., 2001b; Barriere et al., 2001c). A meta-analysis that identified and evaluated 48 peer-reviewed publications suggested that genetically modified corn hybrids do not affect lactation performance of dairy cows or nutrient composition of whole-plant corn silage compared to other non-transgenic varieties used in corn silage.

#### ***Transgenic Corn in Diets Fed to Poultry***

Some researchers have evaluated feeding transgenic corn to poultry rather than ruminants. Researchers evaluating poultry (n=12 male broilers and n=12 laying hens) following a diet consisting of 50% of either conventional or Bt-Cesar maize reported amplifying and detecting plant DNA fragments in muscle, liver, spleen and kidney samples. However, none of the detected plant DNA contained Bt-maize gene fragments (Einspanier et al., 2001).

McNaughton et al. (2007) evaluated a transgenic line, a near isoline line, and three non-transgenic lines of maize to broilers (n=120 per treatment) housed in pens containing 50% females and 50% males. These researchers observed no differences in mortality, total body

weight gain, feed to gain ratio, or carcass yield characteristics including pre-chill kidney weight, liver measured as weight per total chilled carcass weight, chilled carcass weight, and weights of breast, thigh, wing, leg and abdominal weight. Similarly, feeding a transgenic line, a transgenic line with an herbicide application, an isogenic hybrid line or a non-transgenic control line of corn to broilers (N = 1,600) blocked by gender into pens (n = 25 per pen) resulted in no differences of final body weight, dressing percentage, or yield percentage of fat pad, drums, thighs, wings, *Pectoralis major* or *Pectoralis minor* from broilers across all treatment diets (Brake, Faust, & Stein, 2003).

### ***Detection Methods***

Several detection methods that have been used previously to detect GM material in plants are largely either protein or DNA based methods (Fraiture et al., 2015; Grohmann, 2010). However, other reviews have described transcriptomic and metabolomic analysis as important methods for detecting and classifying molecular characteristics of GM crops (Li et al., 2017). One review also described bioassay methods, such as applying herbicide to a GM plant, as a testing method for determining if crops contain GM genes (Holst-Jensen, 2009). Immunoassay technologies are the main protein-based methods that are described in recent publications (Fraiture et al., 2015; Grohmann, 2010; Holst-Jensen, 2009). The enzyme-linked immunosorbent assay (ELISA) is the most common protein-based method, but also requires specific antibody development for each protein to be effective (Grohmann, 2010). Protein based methods tend to be easy, rapid methods to execute, but since these methods are affected by the expression of each protein, these methods can pose challenges (Fraiture et al., 2015; Grohmann, 2010; Holst-Jensen, 2009). Currently the most common methods used to detect GM crops are polymerase chain reaction (PCR) technologies (Fraiture et al., 2015; Grohmann, 2010; Holst-Jensen, 2009; Querci,

Van den Bulcke et al., 2010), however, Fraiture et al. (2015) suggested that quantitative-PCR (qPCR) is the most common method to detect, identify, and quantify GM material using SYBR Green or TaqMan chemistry.

Van Eenennaam & Young (2017) concluded that the available data suggests that there are no adverse effects to human health from eating foods of animals that consumed GE crops (Van Eenennaam & Young, 2017). Further, they concluded that trace levels of transgenic DNA from plants cannot be consistently detected in meat, milk or eggs from animals that had consumed GE crops. Other researchers testing milk from Simmental dairy cows (n=18 treatment group, n=18 control group) fed a quantified amount of 6.1 mg/kg of Cry1Ab protein from transgenic corn grain, corn stem pellets and corn silage over a 25-month period (Guertler et al., 2010) were unable to detect whole or fragments of DNA from this protein in milk from these cows.

### *Issues with Study Designs*

Many reviews have criticized the current literature for variation in study designs utilized in animal feeding trials with GE feeds. Snell et al. (2012) concluded that “the major insufficiencies not only include lack of use of near isogenic lines, but also statistical power underestimation, absence of repetitions” and the “over-interpretation of differences, which are often within the normal range of variation, and poor toxicological interpretation of the data.” Other researchers argue that statistically significant differences and biological relevance can become confused, particularly when too few animals are included in a study design where significant differences were detected (Van Eenennaam, 2013). Several studies do not mention the line of GE corn being used in studies. Additionally, Van Eenennaam (2013) argued that an appropriate sample size should be determined to appropriately test for intended effects in animal feeding trials, but with unintended effects, multiple comparisons performed on the same sample

should use an appropriate multiple comparison method such as a Bonferroni adjustment or False Discovery Rate to prevent observing statistical significance by chance.

One of the most notable controversial studies evaluating GE corn in a long-term feeding trial with rats gained attention with scientists and the public. The publication by Seralini et al. (2012) in *Food and Chemical Toxicology* reported increased growth of tumors in female Sprague-Dawley rats fed GM corn. The study faced numerous criticisms from several scientists, eventually leading to the paper being retracted from the journal; but the work was eventually republished in the journal *Environmental Science Europe* in 2014. Many criticisms are rendered about the original publication, including the line of rats used (which are more susceptible to developing tumors), too few animals were used in each treatment, too few controls were used compared to animals that received the diet treatment, and other studies have demonstrated opposite effects.

### ***Conclusion***

No peer-reviewed journal articles were found to have reported human metabolic effects from consuming beef cattle fed genetically modified grains. The available scientific literature regarding feeding transgenic corn to animals has not demonstrated adverse metabolic effects. Feeding trials that have been conducted in animals such as dairy cattle, swine, poultry and rodents are the most extensive. Van Eenennaam & Young (2017) concluded that no repeatable negative metabolic effects have been detected in these animal studies. In a recent review, researchers concluded that “traces of dietary DNA and protein cannot be reliably detected in meat, milk, or eggs” (Van Eenennaam & Young, 2017). Some reviews have concluded that further GM feeding trial research should not be pursued unless future designs can add to the

available data or utilize more comprehensive study designs since no adverse health effects have been detected in feeding trials conducted in the past two decades. However, diagnostic methods have been developed further since GE crops were first approved, and some previous studies that failed to use an isogenic control limited interpretation.

Some researchers have concluded that the wide variability in animals per treatment, number of treatments and use of controls results in difficulty in comparing feeding trial results, particularly in rodent models. To address this, Van Eenennaam and Young (2014) described suggested parameters for designing animal feeding trials. Although these suggested parameters exist to guide researchers in clarifying the context of each design to other publications conducted in the same species, many criticisms of the scientific literature still exist. Additionally, published feeding trials conducted with transgenic corn grain and silage in beef cattle are limited. Further research is still needed in beef cattle.

## LITERATURE CITED

- Andreassen, M., Bøhn, T., Wikmark, O.-G., Bodin, J., Traavik, T., Løvik, M., & Nygaard, U. C. (2016). Investigations of immunogenic, allergenic and adjuvant properties of Cry1Ab protein after intragastric exposure in a food allergy model in mice. *BMC immunology*, 17(1), 10.
- Barriere, Y., Gibelin, C., Argillier, O., & Mechin, V. (2001a). Genetic analysis in recombinant inbred lines of early dent forage maize. I - QTL mapping for yield, earliness, starch and crude protein contents from per se value and top cross experiments. *Maydica*, 46(4), 253-266.
- Barriere, Y., Verite, R., Brunschwig, P., Surault, F., & Emile, J. (2001b). Feeding value of corn silage estimated with sheep and dairy cows is not altered by genetic incorporation of Bt176 resistance to *Ostrinia nubilalis*. *Journal of Dairy Science*, 84(8), 1863-1871.
- Barriere, Y., Verite, R., Brunschwig, P., Surault, F., & Emile, J. C. (2001c). Feeding value of corn silage estimated with sheep and dairy cows is not altered by genetic incorporation of Bt176 resistance to *Ostrinia nubilalis*. *Journal of Dairy Science*, 84(8), 1863-1871.
- Beever, D., & Phipps, R. (2001). The fate of plant DNA and novel proteins in feeds for farm livestock: a United Kingdom perspective. *Journal of Animal Science*, 79(E-Suppl), E290-E295.
- Brake, J., Faust, M., & Stein, J. (2003). Evaluation of transgenic event Bt11 hybrid corn in broiler chickens. *Poultry Science*, 82(4), 551-559.
- Buzoianu, S. G., Walsh, M. C., Rea, M. C., O'Sullivan, O., Crispie, F., Cotter, P. D., . . . Lawlor, P. G. (2012). The effect of feeding Bt MON810 maize to pigs for 110 days on intestinal microbiota. *PLoS One*, 7(5), e33668.
- Calsamiglia, S., Hernandez, B., Hartnell, G. F., & Phipps, R. (2007). Effects of corn silage derived from a genetically modified variety containing two transgenes on feed intake, milk production, and composition, and the absence of detectable transgenic deoxyribonucleic acid in milk in Holstein dairy cows. *J Dairy Sci*, 90(10), 4718-4723. doi: 10.3168/jds.2007-0286
- Castillo-Lopez, E., Clark, K., Paz, H., Ramirez, H. R., Klusmeyer, T., Hartnell, G., & Kononoff, P. (2014). Performance of dairy cows fed silage and grain produced from second-generation insect-protected (*Bacillus thuringiensis*) corn (MON 89034), compared with parental line corn or reference corn. *Journal of dairy science*, 97(6), 3832-3837.
- Domingo, J. L., & Gine Bordonaba, J. (2011). A literature review on the safety assessment of genetically modified plants. *Environ Int*, 37(4), 734-742. doi: 10.1016/j.envint.2011.01.003

- Donkin, S. S., Velez, J. C., Totten, A. K., Stanisiewski, E. P., & Hartnell, G. F. (2003). Effects of feeding silage and grain from glyphosate-tolerant or insect-protected corn hybrids on feed intake, ruminal digestion, and milk production in dairy cattle. *J Dairy Sci*, 86(5), 1780-1788. doi: 10.3168/jds.S0022-0302(03)73763-1
- Einspanier, R., Klotz, A., Kraft, J., Aulrich, K., Poser, R., Schwagele, F., . . . Flachowsky, G. (2001). The fate of forage plant DNA in farm animals: a collaborative case-study investigating cattle and chicken fed recombinant plant material. *European Food Research and Technology*, 212(2), 129-134. doi: DOI 10.1007/s002170000248
- European Food Safety, A. (2011). Guidance for risk assessment of food and feed from genetically modified plants. *EFSA J*, 9, 2150.
- European Food Safety, A. (2013). Considerations on the applicability of OECD TG 453 to whole food/feed testing. *EFSA Journal*, 11(7), 3347-n/a. doi: 10.2903/j.efsa.2013.3347
- Federation of American Scientists. (2011). U.S. Regulation of Genetically Modified Crops. Available online at <https://fas.org/biosecurity/education/dualuse-agriculture/2.-agricultural-biotechnology/us-regulation-of-genetically-engineered-crops.html>. Accessed August 16, 2017.
- Fernandez-Cornejo, J., Wechsler, S., Livingston, M., & Mitchell, L. (2014). Genetically Engineered Crops in the United States. U.S. Department of Agriculture, Economic Research Service(ERR-162).
- Finamore, A., Roselli, M., Britti, S., Monastra, G., Ambra, R., Turrini, A., & Mengheri, E. (2008). Intestinal and peripheral immune response to MON810 maize ingestion in weaning and old mice. *Journal of agricultural and food chemistry*, 56(23), 11533-11539.
- Folmer, J., Grant, R., Milton, C., & Beck, J. (2002). Utilization of Bt corn residues by grazing beef steers and Bt corn silage and grain by growing beef cattle and lactating dairy cows. *Journal of animal science*, 80(5), 1352-1361.
- Fraiture, M.-A., Herman, P., Taverniers, I., De Loose, M., Deforce, D., & Roosens, N. H. (2015). Current and new approaches in GMO detection: challenges and solutions. *BioMed research international*, 2015.
- Grohmann, L. (2010). Detection of genetically modified plants in seeds, food and feed *Genetic Modification of Plants* (pp. 117-136): Springer.
- Guertler, P., Paul, V., Steinke, K., Wiedemann, S., Preissinger, W., Albrecht, C., . . . Meyer, H. H. D. (2010). Long-term feeding of genetically modified corn (MON810) - Fate of cry1Ab DNA and recombinant protein during the metabolism of the dairy cow. *Livestock Science*, 131(2-3), 250-259. doi: 10.1016/j.livsci.2010.04.010

- Guo, Q. Y., He, L. X., Zhu, H., Shang, J. L., Zhu, L. Y., Wang, J. B., & Li, Y. (2015). Effects of 90-Day Feeding of Transgenic Maize BT799 on the Reproductive System in Male Wistar Rats. *Int J Environ Res Public Health*, 12(12), 15309-15320. doi: 10.3390/ijerph121214986
- Hammond, B., Dudek, R., Lemen, J., & Nemeth, M. (2004). Results of a 13 week safety assurance study with rats fed grain from glyphosate tolerant corn. *Food Chem Toxicol*, 42(6), 1003-1014. doi: 10.1016/j.fct.2004.02.013
- Hammond, B., Lemen, J., Dudek, R., Ward, D., Jiang, C., Nemeth, M., & Burns, J. (2006). Results of a 90-day safety assurance study with rats fed grain from corn rootworm-protected corn. *Food Chem Toxicol*, 44(2), 147-160. doi: 10.1016/j.fct.2005.06.008
- Hammond, B. G., Dudek, R., Lemen, J. K., & Nemeth, M. A. (2006). Results of a 90-day safety assurance study with rats fed grain from corn borer-protected corn. *Food Chem Toxicol*, 44(7), 1092-1099. doi: 10.1016/j.fct.2006.01.003
- Han, S., Zou, S., He, X., Huang, K., & Mei, X. (2016). Potential subchronic food safety of the stacked trait transgenic maize GH5112E-117C in Sprague-Dawley rats. *Transgenic Res*, 25(4), 453-463. doi: 10.1007/s11248-016-9944-6
- Holst-Jensen, A. (2009). Testing for genetically modified organisms (GMOs): Past, present and future perspectives. *Biotechnology Advances*, 27(6), 1071-1082.
- Hyun, Y., Bressner, G., Fischer, R., Miller, P., Ellis, M., Peterson, B., . . . Hartnell, G. (2005). Performance of growing-finishing pigs fed diets containing YieldGard Rootworm corn (MON 863), a nontransgenic genetically similar corn, or conventional corn hybrids. *Journal of animal science*, 83(7), 1581-1590.
- Hyun, Y., Bressner, G. E., Ellis, M., Lewis, A. J., Fischer, R., Stanisiewski, E. P., & Hartnell, G. F. (2004). Performance of growing-finishing pigs fed diets containing Roundup Ready corn (event nk603), a nontransgenic genetically similar corn, or conventional corn lines. *J Anim Sci*, 82(2), 571-580.
- International Life Sciences Institute (2003). *Best Practices for the Conduct of Animal Studies to Evaluate Crops Genetically Modified for Input Traits*. Washington, DC: International Life Sciences Institute; 62, 1-60.
- International Life Sciences Institute (2007). *Best Practices for the Conduct of Animal Studies to Evaluate Crops Genetically Modified for Output Traits*. Washington, D.C: International Life Sciences Institute; 202, 1-194.
- ISAAA. (2017). *GM Approval Database*. Accessed August 2, 2017. Available online at <http://www.isaaa.org/gmapproval/database/default.asp>.
- James, C. (2016a). *Global Status of Commercialized Biotech/GM Crops*. ISAAA Brief 52-2016: Executive Summary. ISAAA: Ithaca (NY).

- James, C. (2016b). Global Status of Commercialized Biotech/GM Crops: 2016. ISAAA Brief No. 52. ISAAA: Ithaca (NY).
- Jonas, D., Elmadfa, I., Engel, K.-H., Heller, K., Koziowski, G., König, A., . . . Kleiner, J. (2001). Safety considerations of DNA in food. *Annals of Nutrition and Metabolism*, 45(6), 235-254.
- Kilic, A., & Akay, M. T. (2008). A three-generation study with genetically modified Bt corn in rats: Biochemical and histopathological investigation. *Food and Chemical Toxicology*, 46(3), 1164-1170. doi: 10.1016/j.fct.2007.11.016
- Li, R., Quan, S., Yan, X., Biswas, S., Zhang, D., & Shi, J. (2017). Molecular characterization of genetically-modified crops: Challenges and strategies. *Biotechnology Advances*.
- Liu, P., He, X., Chen, D., Luo, Y., Cao, S., Song, H., . . . Xu, W. (2012). A 90-day subchronic feeding study of genetically modified maize expressing Cry1Ac-M protein in Sprague-Dawley rats. *Food Chem Toxicol*, 50(9), 3215-3221. doi: 10.1016/j.fct.2012.06.009
- Lutz, B., Wiedemann, S., & Albrecht, C. (2006). Degradation of transgenic Cry1Ab DNA and protein in Bt-176 maize during the ensiling process. *Journal of Animal Physiology and Animal Nutrition*, 90(3-4), 116-123. doi: 10.1111/j.1439-0396.2005.00571.x.
- MacKenzie, S. A., Lamb, I., Schmidt, J., Deege, L., Morrissey, M. J., Harper, M., . . . Delaney, B. (2007). Thirteen week feeding study with transgenic maize grain containing event DAS-O1507-1 in Sprague-Dawley rats. *Food Chem Toxicol*, 45(4), 551-562. doi: 10.1016/j.fct.2006.09.016
- Malley, L. A., Everds, N. E., Reynolds, J., Mann, P. C., Lamb, I., Rood, T., . . . Delaney, B. (2007). Subchronic feeding study of DAS-59122-7 maize grain in Sprague-Dawley rats. *Food Chem Toxicol*, 45(7), 1277-1292. doi: 10.1016/j.fct.2007.01.013
- McNaughton, J. L., Roberts, M., Rice, D., Smith, B., Hinds, M., Schmidt, J., . . . Delaney, B. (2007). Feeding performance in broiler chickens fed diets containing DAS-59122-7 maize grain compared to diets containing non-transgenic maize grain. *Animal Feed Science and Technology*, 132(3-4), 227-239. doi: 10.1016/j.anifeedsci.2006.03.021
- Mesnage, R., Arno, M., Séralini, G.-E., & Antoniou, M. N. (2017). Transcriptome and metabolome analysis of liver and kidneys of rats chronically fed NK603 Roundup-tolerant genetically modified maize. *Environmental Sciences Europe*, 29(1), 6.
- Nguyen, H., & Jehle, J. (2007). Quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in transgenic maize Mon810/Quantitative Analyse der saisonalen und gewebespezifischen Expression von Cry1Ab in transgenen Mon810-Mais. *Journal of Plant Diseases and Protection*, 82-87.
- Querci, M., Van den Bulcke, M., Žel, J., Van den Eede, G., & Broll, H. (2010). New approaches in GMO detection. *Analytical and Bioanalytical Chemistry*, 396(6), 1991-2002.

- Pilacinski, W., Crawford, A., Downey, R., Harvey, B., Huber, S., Hunst, P., Lahman, L. K., MacIntosh, S., Pohl, M., Rickard, C., Tagliani, L., & Weber, N. (2011). Plants with genetically modified events combined by conventional breeding: an assessment of the need for additional regulatory data. *Food and Chemical Toxicology*, 49(1), 1-7.
- Shedova, E., Albrecht, C., Zverlov, V. V., & Schwarz, W. H. (2009). Stimulation of bacterial DNA transformation by cattle saliva: implications for using genetically modified plants in animal feed. [Article]. *World Journal of Microbiology & Biotechnology*, 25(3), 457-463. doi: 10.1007/s11274-008-9910-4
- Snell, C., Bernheim, A., Berge, J. B., Kuntz, M., Pascal, G., Paris, A., & Ricroch, A. E. (2012). Assessment of the health impact of GM plant diets in long-term and multigenerational animal feeding trials: A literature review. *Food and Chemical Toxicology*, 50(3-4), 1134-1148. doi: 10.1016/j.fct.2011.11.048
- Stein, H. H., Rice, D., Smith, B., Hinds, M., Sauber, T., Pedersen, C., . . . Peters, D. (2009). Evaluation of corn grain with the genetically modified input trait DAS-59122-7 fed to growing-finishing pigs. *Journal of animal science*, 87(4), 1254-1260.
- Steinke, K., Guertler, P., Paul, V., Wiedemann, S., Ettle, T., Albrecht, C., . . . Schwarz, F. J. (2010). Effects of long-term feeding of genetically modified corn (event MON810) on the performance of lactating dairy cows. *Journal of Animal Physiology and Animal Nutrition*, 94(5), e185-e193. doi: 10.1111/j.1439-0396.2010.01003.x.
- Swiatkiewicz, S., Swiatkiewicz, M., Arczewska-Wlosek, A., & Jozefiak, D. (2014). Genetically modified feeds and their effect on the metabolic parameters of food-producing animals: A review of recent studies. *Animal Feed Science and Technology*, 198, 1-19. doi: 10.1016/j.anifeedsci.2014.09.009
- The National Academies of Sciences, Engineering, and Medicine. (2016). *Nutrient Requirements of Beef Cattle*. Washington, DC: The National Academies Press.
- U.S. Food and Drug Administration. (2015). FDA's regulatory program for foods derived from genetically engineered source. Available online at <https://www.fda.gov/newsevents/testimony/ucm468833.htm>. Accessed August 2, 2017.
- Van den Eede, G., Aarts, H., Buhk, H.-J., Corthier, G., Flint, H. J., Hammes, W., . . . von Wright, A. (2004). The relevance of gene transfer to the safety of food and feed derived from genetically modified (GM) plants. *Food and Chemical Toxicology*, 42(7), 1127-1156.
- Van Eenennaam, A. L. (2013). GMOs in animal agriculture: time to consider both costs and benefits in regulatory evaluations. *Journal of animal science and biotechnology*, 4(1), 37.
- Van Eenennaam, A. L., & Young, A. E. (2014). Prevalence and impacts of genetically engineered feedstuffs on livestock populations. *J Anim Sci*, 92(10), 4255-4278. doi: 10.2527/jas.2014-8124

- Van Eenennaam, A. L., & Young, A. E. (2017). Detection of dietary DNA, protein, and glyphosate in meat, milk, and eggs. *J Anim Sci*, 95(7), 3247-3269. doi: 10.2527/jas.2016.1346
- Walsh, M. C., Buzoianu, S. G., Gardiner, G. E., Rea, M. C., Ross, R. P., Cassidy, J. P., & Lawlor, P. G. (2012). Effects of short-term feeding of Bt MON810 maize on growth performance, organ morphology and function in pigs. *British Journal of Nutrition*, 107(3), 364-371.
- Weiss, W., Simons, C., & Ekmay, R. (2015). Effects of feeding diets based on transgenic soybean meal and soybean hulls to dairy cows on production measures and sensory quality of milk. *Journal of dairy science*, 98(12), 8986-8993.
- Zeljenkova, D., Ambrusova, K., Bartusova, M., Kebis, A., Kovriznych, J., Krivosikova, Z., . . . Steinberg, P. (2014). Ninety-day oral toxicity studies on two genetically modified maize MON810 varieties in Wistar Han RCC rats (EU 7th Framework Programme project GRACE). *Arch Toxicol*, 88(12), 2289-2314. doi: 10.1007/s00204-014-1374-8

## APPENDIX

## ADDITIONAL DOCTORAL DEGREE WORK

Perham, C. C., Gifford, C. L., Woerner, D. R., Engle, T. E., Sellins, K. S., Acheson, R. J., Douglass, L. W., Tatum, J. D., Delmore, R. J., Cifelli, A., McNeill, S. H., and Belk, K. E. 2019. Special-Fed Veal: Separable components, proximate composition, and nutrient analysis of selected raw and cooked, wholesale and retail cuts. *Meat Science*, 148, 19-31.

### Overview of research and important findings:

Nutrition research is essential for aiding in nutrition policy and allowing consumers to make informed purchasing decisions. As livestock and food production systems change over time, it is necessary to have access to nutrient data that are reflective of current production systems. Data from this study were used to update nutrient values on raw and cooked cuts from special-fed veal calves in the USDA National Nutrient Database for Standard Reference (SR) Release 27, now part of the USDA Food Composition Database. Whole loin roasts, center-cut hindshanks, ground veal, osso buco forechanks, loin chops, leg cutlets and shoulder blade chops were collected from six U.S. suppliers, and shipped to the Colorado State University Meat Laboratory. Upon arrival these products were immediately assessed for vacuum package integrity and frozen at -20°C to stop metabolic activity. These products were assigned to cooking assignments. Raw and cooked homogenates from dissected components were used for nutrient analysis. Samples for analysis of proximate, fatty acid, mineral and vitamin composition were combined from composites of lean, external fat (subcutaneous fat), and seam fat (intermuscular fat). Major findings from this study provided updated, current nutrient values, expanded identification of individual fatty acids, and established concentration of choline for veal cuts to be included in USDA SR 27.

McNeill, S. H., Belk, K. E., Campbell, W. W. , and Gifford, C. L. 2017. Coming to terms: meat's role in a healthful diet. *Animal Frontiers*, 7(4), 34-42.

Overview of this work and important findings:

Although meat is a highly nutrient dense food that provides high quality protein and numerous other essential nutrients to human physiological function, implication of meat in human disease development is still a focus in nutrition and health research. This broad overview summarizes key issues associated with inconsistent dietary guidance of meat and muscle foods. As researchers focus on the impact of meat on human health, inconsistent use of terminology and specificity of foods assessed creates confusion in dietary guidance. Examples of how terminology used to define meat is confusing include defining red meat with processed meat products such as bacon or sausage. This is problematic considering the beef and pork industries have improved availability of lean red meat, but definitions used in nutrition research do not consistently reflect lean red meat in assessment of dietary consumption and health outcomes. As dietary guidance and policy are constantly developed nationally and globally, it is essential to have definitions and muscle foods assessed consistently to more accurately interpret data resulting from nutrition research.

Gifford, C. L., O'Connor, L. E., Campbell, W. W., Woerner, D. R., and Belk, K. E. 2017. Broad and inconsistent muscle food classification is problematic for dietary guidance in the U.S. *Nutrients*, 9(9), 1027.

Overview of this work and important findings:

Current dietary intake assessment methods are used to obtain dietary information from consumers to understand consumption trends and evaluate how diets impact human health. Dietary intake information is used as a guide to establish dietary recommendations on the consumption of muscle foods, including red meat, processed meat, poultry, and fish. This work summarized how U.S. intake values for muscle foods are estimated and grouped with three common methods which include food frequency questionnaires; food disappearance data from the U.S. Department of Agriculture Economic Research Service; and dietary recall information from the National Health and Nutrition Examination Survey data. There are discrepancies in these estimated intakes, due to the inconsistent way that muscle foods are classified into groups. Researchers often inconsistently classify muscle foods into broad or overlapping groups with lack of regard to nutrient content. This can lead to implication of scientific conclusions and dietary recommendations. Information included in this review demonstrated the need for using more detail when assessing dietary intake and making conclusions about health outcomes. Addition of muscle food categorization and description could improve interpretation of nutrition research for dietary policy and recommendation development for the public.

O'Connor, L. E., Gifford, C. L., Woerner, D. R., Sharp, J. L., Belk, K. E., and Campbell, W. W. 2019. Dietary meat categories and descriptions in chronic disease research are substantively different within and between experimental and observational studies: a systematic review and landscape analysis. Submitted to *Advances in Nutrition*.

#### Overview of research and important findings:

This systematic review and landscape analysis summarized patterns in dietary meat (skeletal muscle and associated tissues from mammalian, avian, and aquatic species being referred to as muscle foods) categorizations (what names foods are referred to) and descriptions (how foods are described or defined) used throughout nutrition-related chronic disease literature. Variation in terminology of muscle foods was highlighted in the 2015 Scientific Report of the Dietary Guidelines Advisory Committee. A total of 1,020 category names and 776 descriptions from those categories were identified from 369 articles that assessed muscle food consumption and prevention of cardiovascular disease, obesity, type 2 diabetes, or cancer in adults 19 years of age or older. Studies were identified using predetermined search criteria entered into PubMed, Cochrane, and CINAHL databases. Studies with publication dates up to March 2018 were included in initial assessment for inclusion into this systematic review. Specificity (degree of information included) of categories was analyzed on a 1-7 ordinal scale as 1) broad/undescriptive; “fish”, 2) muscle food type; “red meat”, 3) species; “poultry”, 4) broad and one other descriptor; “processed meat”, 5) type/species and one other descriptor; “fresh red meat”, 6) broad/type and two other descriptors; “poached lean fish”, and 7) specific product; “luncheon meat”. Specificity of categories from observational studies was higher in recent articles but categories from randomized controlled trials has been less specific since 2000. Of studies assessed, a description was included for 76% and 82% of observational studies and

randomized controlled trials, respectively. Researchers described processed meat, red meat, and total meat more frequently than poultry or fish. Among processed meat, 31% included a common term used in public regulatory definitions of what processed meat is. Muscle food categories and descriptions are different within and among observational studies and randomized controlled trials. More specific and practical muscle food classification guidelines are needed for future research regarding muscle food consumption and chronic disease risk or development.