Precision Nutrition Tools to Support Extensive Forage-Based Livestock Systems

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ACADEMIC ABSTRACT

Precision nutrition is the future of the livestock nutrition industry and is expected to improve the health and performance of animals through individualized feeding and management. Several developments in precision nutrition exists for ration formulation, feedstuff analysis, and individualized feeding, particularly in intensive livestock systems. Although research in precision animal nutrition exists, there are few practical tools for grazing animals in pasture-based systems. The overarching goal of this work was to develop and evaluate tools to support precision nutrition for extensive, forage-based livestock systems. This goal was addressed through three complementary studies exploring relevant tools.

In a preliminary project, the objective was to assess the accuracy of a handheld spectral sensing device for predicting the dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), and crude protein (CP) composition of hay. A follow-on experiment pilot tested the spectral sensing device for on-animal use to monitor the composition of hay consumed during normal feeding behavior. We explored these objectives through time-series observations of forage sampling of mixed-grass hay, which was scanned with a spectral sensor programmed to measure light reflectance at 18 wavelengths before bench chemistry analysis for each sample. The data collected were split into three parts and used in random forest regressions. We found that the resulting root mean square prediction errors (RMSPE) for each of the four models were promising, especially for the two fiber fractions, with the lowest error rates of 5.85% for NDF and 8.05% for ADF. We investigated the following objective by placing spectral sensors on

halters worn by horses consuming hay and comparing the spectral readings to forage samples collected from where the animal was eating. Although a small dataset, the mounted sensor system showed promising results, with RMSPEs of 8.02% (DM), 5.07% (NDF), 4.52% (ADF), and 23.5% (CP). Further development on the halter system as well as extensive data collection on grazing animals and a variety of forage is necessary for confirming the practicality of this technology.

In the second project, our objective was to perform a quantitative literature review to investigate dietary and feed factors affecting total-tract digestibility of dry matter (DMD), crude protein (CPD), neutral detergent fiber (NDFD), ether extract (EED), non-structural carbohydrates (NSCD), non-fiber carbohydrates (NFCD), and residual organic matter (rOMD). Additionally, we aimed to assess how our equations behaved for estimating digestible energy (DE) compared with existing modeling systems and to evaluate them against independently measured DE from the literature. We explored this objective through a literature review that yielded 54 studies, which were used to develop linear mixed-effect regressions, with five models derived for each nutrient using several explanatory variables. Models were selected based on their ability to explain dataset variation and stability when predicting DE in example rations. Two models were developed for DE estimation: one using measured data from the literature, and another using both measured data and calculated data from reference tables when values were not provided in the literature. We found that the models explained variation well. When evaluated against measured DE from 17 studies, our calculated system provided DE estimations similar to those of existing systems. Overall, this new approach offers an additional, practical tool for estimating energy supplies in equine diets.

In the final study, our objective was to determine whether thermal imaging of body surface temperature could provide an objective means of body condition scoring (BCS) in mature horses of the Quarter Horse (QH) and Thoroughbred (TB) breed types, as well as in multiparous gestating beef cows. We explored this objective by capturing thermal images on one or both sides of each animal's body while five to eight trained scorers assigned BCS. Several covariates were monitored for their influence on assigned BCS, including cloud coverage, animal breed, individual scorer, and scorer's location. Random forest regressions were derived to evaluate the ability of the thermal camera to accurately BCS horses and cows with data split into three parts. After models were created for each body region, we found that the root mean square error (RMSE, % mean) ranged from 7.6% to 10.6% for horses and 6.81% to 13.4% for cows. We also assessed between-scorer variability by calculating the coefficient of variation (CV). The variability among the eight horse scorers ranged from 13% to 14% and 10.8% to 12.1% for the five cow scorers. Using surface temperature obtained through thermal imaging displays promise as an alternative method to objectively BCS horses and beef cows.

Precision Nutrition of Extensive Forage-Based Livestock Systems

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GENERAL AUDIENCE ABSTRACT

Precision nutrition management of livestock emphasizes an understanding of animal individuality and a precise representation of feedstuffs. The goal of this initiative is to improve the health and performance of grazing animals. Although research in this field has expanded, there is a lack of practical tools for grazing livestock in pasture-based systems. The goal of this work was to address this lack of practical tools through three complementary studies. Our first study evaluated the use of a hand-held device designed to determine the nutrient quality of hay. This device is a low-cost approach to monitor forage composition over time. Four hay composition indicators were of focus: moisture (dry matter, DM), two types of fiber (neutral detergent fiber, NDF, and acid detergent fiber, ADF), and protein (crude protein, CP). The spectral sensing device was programmed to measure light reflectance at different wavelengths before using those wavelengths to estimate nutrient content using machine learning algorithms. The system was developed and tested using data collected over four months. Weekly, 10 hay bales were scanned and sampled, with collected samples analyzed in the laboratory for comparison. After model development and evaluation, the device appeared to accurately estimate the DM and fiber (NDF and ADF) contents of hay, although it was less precise for protein. For practical applications, the device was also tested as a wearable device, mounted on horses while the animals consumed hay. Although a small dataset, the technology showed promise for continuous monitoring of forage quality while mounted on grazing livestock. However, more research is necessary to build a bigger dataset and test over a wider variety of forage types to improve its reliability and usefulness for production use.

Our second study reviewed data from 54 published studies to examine how various dietary factors influence the digestibility of several nutrients, including dry matter (DMD), crude protein (CPD), fiber (NDFD), fat (EED), carbohydrates (NSCD, NFCD), and organic matter (rOMD). Using statistical models, we analyzed how feed type and composition impact digestibility and developed equations to estimate digestible energy (DE) in equine diets. Two models were created for each nutrient: one based on measured data from studies and another incorporating calculated reference values when data were not provided in the literature. The models performed well, explaining a high percentage of variation in digestibility, with accuracy levels comparable to existing DE estimation systems. Our system may eventually provide a practical tool to better estimate energy availability in different diets. However, additional research is necessary to assess the systems on more independent datasets before confidently recommending its use for ration balancing.

Body condition scoring (BCS) is a popular method of visually assessing an animal's nutritional status, but scoring relies heavily on human interpretation. The purpose of our third study was to determine if body surface temperature obtained from thermal cameras could provide an objective way to estimate BCS. Horses and cows had thermal images taken while also assigned a body condition score for seven or five specific body regions, respectively, with additional factors like breed, weather, and housing conditions recorded as covariates. Machine learning models were created for each body region to estimate BCS from body surface temperatures. Moderate accuracy was shown in predicting BCS for both species with errors ranging from 7% to 13%. Factors like cloud coverage (%) and differences between individual scorers also appeared to strongly influence the assigned body scores. Overall, thermal imaging for surface temperature may provide accurate body condition evaluations in livestock.

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GENERAL INTRODUCTION

Nutritional management of livestock in extensive grazing systems is essential for both the beef cattle and equine industries. An important aspect of managing these pasture-based systems is ensuring that animals receive the nutrients necessary to grow, remain healthy, and perform optimally for economic and personal satisfaction. Horses and beef cattle, two livestock species commonly managed in primarily extensive systems, have established daily nutrient requirements to meet their respective productive goals (NRC, 2007; NASEM, 2016). In pasture-based management systems commonly used for these animals, forage is the most abundant and the most variable component of the ration. Compared with intensive systems, where animals are mostly housed indoors, the reliance on standing forage in pastures introduces challenges in nutritional management due to variations in forage species, differences in pasture management practices, animal selectivity, and seasonal fluctuations in forage quality.

A strategy to address these known challenges in extensive systems is through the development and adoption of precision technologies. These tools vary in type but can provide information that may be missed in day-to-day livestock management without excessive time or labor costs. The equine industry, which economically contributes to a wide range of sectors including agriculture, tourism, veterinary medicine, and technology, has a value of $177 billion and supports 2.2 million jobs (American Horse Council, 2024). The incorporation of precision technologies in this industry can be used to empower horse owners looking for ways to improve their horses' lives through welfare, nutrition, and overall management. By identifying problems like obesity or related illness, these technologies may also reduce the need for costly veterinary interventions.

Similarly, the beef cattle industry holds a high economic value, with the United States recognized as the world's largest consumer of beef products (USDA, 2025). Additionally, based on the 2022 Census of Agriculture, there are 87.9 million beef cattle in the U.S. on over 700k farms managed over 138 million hectares (USDA, 2024). Because beef cattle operations primarily cover large grasslands, precision technologies that support accurate monitoring of feed and forage composition can significantly improve management decisions and animal productivity. One notable precision technology for more rapid forage composition testing is near-infrared spectroscopy (NIRS), which has been developed into a commercial product for laboratory and in-field applications (Hossain et al., 2024). NIRS was created as a more affordable alternative to traditional wet chemistry and provides producers with an accessible method to monitor the quality of the forage their animals consume (Shenk and Westerhaus, 1994). Presently, there is a need for research development in several areas, including continuous monitoring of forage intake and quality in grazing livestock, modern tools to accurately predict diet utilization in horses, and redefining body condition scoring for more objective assessments to carefully monitor animal feed efficiency.

To develop more tools for the nutritional management of livestock in grazing systems, we targeted three steps of this management process and developed resources to address (1) the composition of forage during consumption (Chapter 2), (2) how the feed is digested for energy in equine diets (Chapter 3), and (3) a new option for evaluating dietary adequacy (Chapter 4). In addition to these original research chapters, Chapter 1 will provide a comprehensive overview and background of existing work in the precision nutrition and technology space, connected to the following three chapters.

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CHAPTER 1: Literature Review

Precision Animal Nutrition: An Emerging Field

Nutrition is central to animal health, productivity, and well-being. Over the past several decades, our understanding of how to meet animal nutrient requirements has dramatically improved. Current work focuses on advancing the emerging science of precision nutrition. Precision nutrition is a science-based system that incorporates known biological processes and technologies to meet the needs of individual animals. Through careful accounting of nutrient supplies and a focus on physiological needs, this system is intended to maximize animal performance, optimize profitability, and promote timely decision-making (González et al., 2018). Reddy and Krishna (2009) discussed that precision nutrition involves the efficient use of available feed resources to optimize the animals' nutrient response. Precision nutrition focuses on addressing individual animal needs rather than managing animals as a group because no two animals are the same. Moreover, it involves meeting the exact nutrient requirements of an animal to maximize performance while being economically and environmentally minded (Litherland et al., 2011).

In contrast, the traditional nutrition approaches that existed prior to the precision nutrition initiatives often relied on managing animals as groups rather than as individuals. These methods were more generalized, based on known physiological processes in the body to predict or estimate factors, such as individual animal water and feed intake (González et al., 2018), assuming animals acted as the average of their management group. Animals were traditionally fed in groups, where individual intake was difficult to monitor, and forage composition testing was a rare occurrence. The goals of group management approaches were to be economical, simple, and efficient; however, group management ignores unique nuances associated with individual animal needs. In group feeding management, it is common practice for dairy farms to collect and submit forage samples for dry matter (DM) testing to control known variation in ingredient DM content and adjust their total-mixed rations (TMR) appropriately (Litherland et al., 2011). However, the samples take time to process and may not be collected frequently enough to address the high variability in ingredient DM content before it can negatively impact performance, leading to animals often being over- or underfed (Litherland et al., 2011).

These delays in processing time are a primary limitation preventing the advancement of precision nutrition. In order to support broader adoption of precision feeding, we need better guidance on feed intake and behavior, which are both necessary to provide individualized care for animals (Kaur et al., 2023). Individualized management is important because animals will respond to diets differently based on their unique genetic make-up. For example, individual cows have unique genetic merits for various production parameters, and their genetic potential cannot be maximized without individual consideration (Kaur et al., 2023). In addition, individual animals within the same species have varying nutrient requirements due to genetic and environmental factors, as well as life stage (Archer et al., 1999). The unique variability among individual animals underscores the importance of precise nutritional management.

Although knowing individual animal needs is important, precise representation of individual feedstuffs is critical to ensure diets are adequately meeting those needs. Feedstuffs vary widely in nutritional value and digestibility due to several factors, including storage, processing, and harvesting (Knudsen et al., 2023). Feed nutrient optimization is most affected by available energy and protein content and imbalances can result in poor animal performance (Knudsen et al., 2023). Reference table values are commonly used to estimate the nutrient composition of diets based on individual ingredient inclusion (Patience et al., 2017); however, use of these reference values can result in inaccurate estimations of dietary nutrient composition because of the differences in nutrient concentration and digestibility among individual feed sources (Knudsen et al., 2023). Given the known variability along with the importance of accurate feed information, there have been efforts towards precise representation of individual feedstuffs. Traditional approaches to determine feed composition were through bench chemistry and feed sampling, which provide the most accurate results but require expensive equipment, can take weeks to get results, and can be cost prohibitive. Near-infrared reflectance spectroscopy is a well-known example of a technology created as an alternative to bench chemistry for affordable and quick feed quality determination (Knudsen et al., 2023). Individual feedstuffs can greatly impact animal performance, and an understanding of those differences is crucial for precise feeding.

Precision Livestock Farming: Intensive vs Extensive Systems

In contrast to intensive livestock systems, advancement in precision nutrition is more challenging in forage-based extensive livestock systems. Technologies established in intensive, indoor systems are more widely published, likely due to the ease of application in indoor settings. For example, feed intake data has been collected by feed weighing systems and devices with identification sensors, like radio-frequency identification (RFID), which have benefitted both the overall health and allowed for early disease detection in dairy cows (Kaur et al., 2023). Additionally, automatic feeders provide individualized feeding in group settings, a process that is simple to organize within intensive, indoor systems compared to extensive, outdoor setups (Kaur et al., 2023). Indoor animal operations are also usually located closer to roads or other infrastructure and have better access to cellular data and internet (Menendez et al., 2022).

As opposed to intensive systems, precision livestock farming (PLF) technologies for extensive production are much more limited in terms of research or adoption (Roxburgh and Pratley, 2015). Extensive systems, unlike intensive ones, do not usually have a centralized area for the operation and are typically more rural with less access to resources (Menendez et al., 2022). Extensive livestock systems, which involve animals grazing on large farms, pose additional challenges for technology applications and tracking individual animal forage consumption. Sensors tested in these systems have struggled with durability and intolerance to variable climate in outdoor environments (Aqeel-ur-Rehman et al., 2014). Other common challenges can include variable terrain, herd size, and dispersity over rangelands that can interfere with wireless connections and satellite positioning systems (Bahlo et al., 2019). Economic value for farmers is also a concern as there is a perceived lack of value in PLF investment, which could be because extensive systems typically require less labor than intensive (Herrero and Thornton, 2013; Bahlo et al., 2019).

Given the known challenges associated with PLF technology development for extensive grazing systems, there is a need for additional precision nutrition tools and support for this sector of the livestock industry. Specifically, tools are necessary for determination of feed composition, assessment of feed utilization, and evaluation of dietary adequacy. To address the uncertainties surrounding feed composition, spectral sensing holds potential as a tool for continuous, on-animal monitoring of feed composition in pasture-based livestock systems. After determining the composition of such feeds, understanding how feedstuffs are digested and utilized to provide animal digestible energy is crucial for individual monitoring. Estimation of each feedstuff through literature-derived models using the chemical composition from the spectral sensing can enable more precise rations and supplementation to maximize animal performance. Even with an informed ration, it is important to monitor how animals utilize the energy from their diets to ensure expectations of animal performance can be continually calibrated to better represent individual circumstances. Dietary adequacy can be observed externally over time, based on animal body condition, and how adipose tissue accumulates along their bodies. For example, excessive body fat could indicate that a diet is overproviding energy. A technology with this opportunity could be thermal imaging for body surface temperature. In turn, this allows for more proactive animal management to prevent obesity or undernourishment-related issues.

Determination of Feed Nutrient Composition

Traditional Techniques. For forage composition determination, there are several existing tools to identify nutrient composition, including traditional benchtop chemistry, near-infrared reflectance spectroscopy (NIRS), and spectral sensing. Benchtop chemistry includes different laboratory-based procedures and methods for the determination of dry matter (DM), organic matter (OM), neutral and acid detergent fibers (NDF and ADF), crude protein (CP), and fat or ether extract (EE). The method for determining the dry matter (DM) content of forage is relatively inexpensive, quick, and straightforward. Dry matter content is determined by measuring the mass after over-drying the sample between 60º and 104ºC for 3 to 48 hours (Weiss and Hall, 2020). Organic matter (OM) is determined by subtracting the ash content from 100%, as ash is the inorganic matter leftover after either ignition or complete oxidation of a sample (Katoch, 2023). The AOAC *Official Method* 942.05 for total Ash of Animal Feed is the official procedure for determination of ash in forage (AOAC, 2000). In brief, crucibles are used to contain an individual sample during the heating process. A muffle furnace with a pyrometric controller is used to ignite the samples to burn off the organic matter before leaving with the residue as ash (Undersander et al., 1993).

Neutral detergent fiber (NDF) and acid detergent fiber (ADF) are two widely utilized fiber fractions important to livestock rations. The detergent system established by Van Soest et al. (1991) is considered the standard for forage fiber and carbohydrate fraction analyses (Weiss and Hall, 2020). The system partitions feeds into fractions to predict the nutritive value of a given feed mechanistically, but it does not divide the feed into chemically pure samples (Van Soest et al., 1991). The AOAC-approved neutral detergent method incorporates amylase and sodium sulfite (Weiss and Hall, 2020). A popular method that is most user-friendly is the ANKOM filter-bag for NDF, ADF, and lignin (ANKOM Technology, Macedon, NY), although there are some concerns about its results compared to those from filter crucible-based methods (Weiss and Hall, 2020). As a fraction of the cell wall, NDF represents the insoluble part left after refluxing in heated neutral detergent (pH 7.0) solution and filtered (NASEM, 2016). On the other hand, ADF is another fraction of the cell wall, containing primarily cellulose and lignin, along with some ash and nitrogen (NASEM, 2016). Within forage classifications, concentration of ADF is highly related to the concentration of NDF (NASEM, 2016). There are several fiber fractions in feedstuffs other than NDF and ADF used for ration balancing, including non-structural carbohydrates (NSC), water-soluble carbohydrates (WSC), starch, and non-fiber carbohydrates (NFC), however NDF and ADF are the two primary fractions.

Crude protein is another macronutrient with high importance for livestock nutrition. For determination of crude protein (CP) value in dried forage, combustion methods are the most common as they are accurate and generate no waste, although they tend to be more expensive than the Kjeldahl analysis, which produces hazardous waste (Weiss and Hall, 2020). In the combustion process, the concentration of nitrogen (N) is multiplied by a conversion factor (usually 6.25) to determine the CP concentration (Weiss and Hall, 2020). Traditional Kjeldahl methods involve digesting samples in sulfuric acid with a copper sulfate or copper-selenium catalyst, then nitrogen (N) is converted to ammonia, before being distilled and titrated (Cherney, 2000). In fresh and ensiled forage, total N may only be 60 to 80% true protein, with the remainder including non-protein N and minimal lignified nitrogen (Van Soest, 1994).

The chemical composition of fat is primarily referred to as fat, crude fat, or ether extract (EE). Ether extract provides the estimate of fat content in feed (Katoch, 2023). The method involves continuously volatizing, condensing and passing ether through a sample, where the ether-soluble materials are extracted into a flask (Katoch, 2023). After that process, the remaining ether is distilled and collected before the crude fat is dried and weighed (Katoch, 2023). Thiex et al. (2019) stated that the Randall modification of the standard Soxhlet extraction method for crude fat determination involves submerging the test portion in boiling solvent to reduce the overall time required for extraction. Bench chemistry is an important tool for precise feedstuff composition determination; however, it can be time-consuming and cost-prohibitive for producers.

Emerging Technologies. Although bench chemistry provides highly accurate results, other technologies such as the near-infrared reflectance spectroscopy (NIRS) have been developed as an affordable alternative. Near-infrared reflectance spectroscopy (NIRS) is a spectrographic analytical technique used to estimate forage quality, as originally described by Norris et al. (1976) (Katoch, 2022). NIRS is a computerized system that analyzes forage quality by measuring the light reflectance of compounds in forages at wavelengths between 750 and 2500 nm, and then the reflected infrared radiation is converted into electrical energy, which is interpreted by a computer (Katoch, 2023). As light radiation passes through a sample, the light is absorbed based on the vibration frequencies of the molecules present, which ultimately produces a spectrum unique to the composition of the sample (Hossain et al., 2024). The original intention was to develop NIRS as an alternative to wet chemistry, which is laborious and expensive (Katoch, 2022). Initially, NIRS was only available in laboratories as a benchtop system, but has since become more affordable and compact, and now various handheld systems are available for in-field assessments (Cherney et al., 2021). NIRS most commonly estimates forage quality indicators like DM, CP, NDF, and ADF (Hossain et al., 2024).

Since its development, NIRS has been widely used in animal nutrition, including to analyze grain, fecal, and forage quality parameters (Lobos et al., 2019; Ikoyi and Younge, 2022; Noel et al., 2022). Beyond animal nutrition, NIRS has also been applied in other agricultural systems, like dairy product analysis and soil testing (Vincent and Dardenne, 2021). In a study on hay and straw samples, NIRS demonstrated the highest accuracy in estimating the dry matter, crude protein and fiber content of the dried forages (Foskolos et al., 2015). NIRS serves as an alternative to wet chemistry for chemical analysis that is non-destructive, affordable, and produces results rapidly. Since the development of the hand-held version, the technology has become widely available, as it allows producers to monitor forage more frequently and gain a better understanding of what they are feeding their animals. Some extension specialists at land-grant universities offer this technology free of charge for local producers, which is a resource to help producers towards improving livestock health and nutrition. A potential downside is the importance of calibration for accurate results (Corson et al., 1999). To calibrate an NIRS device, various statistical methods are deployed to analyze the electromagnetic spectrum against reference data with known physical and chemical compositions (Hossain et al., 2024). That said, the development of robust NIRS calibration models can be tricky with the density of data required but are necessary for accurate results. For example, analyzing grass hay requires a fully developed calibration model to produce reliable chemical composition estimates. Another limitation is the necessary preparation of samples used for NIRS analysis, which involves grinding and drying for consistent and accurate spectra data (Hossain et al., 2024). Studies have displayed the poor performance of NIRS calibration models when sample preparation is insufficient because of the influence of moisture content or particle size (Ikoyi and Younge, 2020). NIRS is a well-known technology that exists in many commercial laboratories but requires very specific and usually expensive equipment so other spectral strategies have been investigated for other applications.

Spectral sensing and similar technologies have been studied as additional options to NIRS. In essence, spectroscopy is the study of light interactions with matter (Knudsen et al., 2023). All types of spectroscopy involve assessing the absorption, emission or scattering interactions of electromagnetic radiation (light) with chemical components (McLaughlin and Glennon, 2011). The idea behind using spectral sensing (spectroscopy) to estimate forage quality is similar to NIRS, where cell wall content, chlorophyll, and plant color are indicative of nutritive plant quality (Hu et al., 2010). This connection enables spectral sensing to collect spectral data from light reflectance on the exterior of forage. Unlike NIRS, spectral systems can measure not only near-infrared spectra, but also across wavelengths within ultraviolet (UV) or visible light spectra (Askari et al., 2019; López-Calderón et al., 2020). Spectral sensing has primarily been applied in remote sensing studies for forage quality determination.

Specifically, these published systems mostly use remote sensing for large-scale monitoring and mapping of grasslands (Wijesingha et al., 2020; Geipel et al., 2021). The remote sensing technologies employed include Unmanned Aerial Vehicle (UAV)-borne and satellite-based systems that are applied to monitor variations in pasture quality components like protein and fiber (Jennewein et al., 2021). One example is a proximal (hand-held) hyperspectral sensor, which uses a canopy pasture probe with a spectral reflectance of 500 to 2,400 nm to measure forage quality over a 0.25^2 m area (Pullanagari et al., 2012). Aerial and space-based remote sensors, such as the satellite “RapidEye”, have been tested because of their ability to monitor detailed changes in vegetation (Wu et al., 2020). Ramoelo et al. (2012) experimented with the potential of the RapidEye sensor’s red-edge band (around 710 nm) to estimate canopy nitrogen concentration in South Africa savanna grass. Another technique utilized to monitor pasture forage quality is UAV-borne imaging spectroscopy (Wijesingha et al., 2020). Wijesingha et al. (2020) tested a UAV with a hyperspectral camera to image fresh forage to assess the crude protein (CP) and acid detergent fiber (ADF) concentrations across a field, comparing the results to wet chemistry values determined from samples collected in multiple subplots. Using wavelengths from 450 to 950 nm at an altitude of about 20 m above the forage, their resulting models tended to predict forage with high CP content less accurately than forage with low CP content but predicted ADF with more consistent accuracy, regardless of whether ADF values were low or high (Wijesingha et al., 2020).

In general, the benefits of spectral sensing are dependent on the application of either hand-held or distant monitoring. Hand-held remote sensing for forage quality is low-cost and efficient. Hand-held sensing has great potential to predict forage quality at a lower cost than wet-chemistry analysis, but, similar to the NIRS, calibration and sample preparation may be necessary for the most accurate assessment (Biewer et al., 2009; Kawamura et al., 2009; Katoch, 2023). For distant remote sensing techniques, like aerial or satellite technologies, the benefits include less labor-intensive forage testing, a higher potential for more accurate estimation of pasture quality, ease of variation monitoring, and efficiency in analysis. That said, the cost, practical application, and limitation to on-site forage serve as disadvantages of these technologies. Although the costs associated may be higher than those of hand-held sensing, the ability to assess larger areas at once makes the cost difference between the two technologies marginal (Katoch, 2023). Additionally, although distant methods can explain some variation in pasture quality, they only evaluate standing forage quality and fail to monitor forage composition as it is grazed by animals (McKeon et al., 2009).

Spectral sensing can be used on individual animals to provide precise representation of individual feedstuffs and varying forage quality. This is possible and necessary because of how weather, geospatial variation, and bale-to-bale variation can influence forage nutrient composition. The weather over a growing season can affect the quality of pasture. For example, the amount of precipitation and corresponding soil moisture can impact plant growth. A plant with a limited amount of soil moisture can become stressed, which typically results in higher protein content and lower fiber levels compared to a non-stressed plant (Mueller and Orloff, 1994). On the other hand, drought stress can trigger an increase plant tissue soluble carbohydrate concentration (Mueller and Orloff, 1994). The weather can also affect the quality of forage after it is cut. When hay is cut and left to cure, rainfall during this period can substantially influence its quality (Mueller and Orloff, 1994). Mueller and Orloff (1994) found that the impact depends on the amount and duration of rain, but leaching of nutrients is usually the primary cause of quality loss, where rain damage decreases digestibility and increases fiber concentration.

Although it can be influenced by weather, geospatial variation pertains to how chemical composition or forage quality varies throughout any given field. For grazing animals, this geospatial variation makes it challenging to determine forage availability across large areas (Zimmer et al., 2021). Producers stock fields with livestock based on this availability to ensure the long-term health of the forage, as pasture is an important economic commodity in extensive systems where it serves as the main source of nutrition for the animals. Additionally, livestock are individuals and do not graze uniformly, with some animals being more selective than others. A partial method of mitigating the variability involves selective field sampling, but the process is time consuming and needs to be performed annually (Karl and Herrick, 2010). Remote sensing has been extensively tested as a solution to this challenge by measuring spatiotemporal variability (Zimmer et al., 2021). Even so, remote sensing, a broad method for assessing geospatial variation, would usually only be performed periodically and does not capture the daily variation in forage quality (Ball et al., 2001).

Similar to geospatial variation within a field, hay harvested and baled from a single field will not have identical chemical composition in every bale made. When testing for hay quality, samples are cored from randomly selected bales and mixed to best represent the nutritional content of the entire lot of hay (Putnam, 2003). There are several factors that can influence forage quality between bales, including moisture content, growth stage at the time of harvest, bale shape, and bale density (Coblentz et al., 2000). Additionally, the nutrient variation between bales was demonstrated by Putnam and Orloff (2002) who compared cored samples from a uniform lot of alfalfa hay. With these factors in mind, other research has investigated the ability of spectral sensing to monitor changes over time in pasture grass.

Previous research has tested the ability of a handheld spectral sensor to predict the chemical composition of cool-season grass pasture (Wright et al., *Submitted*). In particular, the spectral sensor used by Wright et al. (*Submitted*) most confidently estimated the fiber fractions of neutral detergent fiber (NDF) and acid detergent fiber (ADF) in the pasture grass, likely due to the relationship between plant color, cell wall content, and chlorophyll concentration (Hu et al., 2010). The purpose of the study was to assess whether the sensor could accurately estimate forage composition, as a step toward developing an on-animal spectral sensor system for continuous monitoring during grazing. Overall, the spectral sensor demonstrated success and showed promise for future applications in monitoring pasture composition (Wright et al., *Submitted*). Further development of spectral sensing is warranted given the success with pasture grass to investigate ability on dried forage as well as mounted on grazing livestock. Although NIRS is an established method for in-field static forage chemical composition analysis, there are currently no systems available to continuously monitor forage consumed by grazing livestock. Therefore, there is a need to develop such systems to fill this gap and enable producers to make well-informed decisions about feeding their animals.

Identification of Animal Nutrient Requirements

Traditional Tools. Once we have tools for chemical composition determination, we need to understand how animals use the forage towards meeting energy requirements. The primary system in nutrition to account for the energy necessary and available for animal maintenance and production is the net energy system. Specifically, the net energy (NE) system describes the energy content of feed or the energy requirement of an animal for a specific physiological function (Kromann, 1973). The energy metabolism scheme of the NE system consists of four components: gross energy intake (GE), digestible energy (DE), metabolizable energy (ME), and, ultimately, net energy (NE). Gross energy refers to the total energy content of the feed consumed by the animal. A significant portion of GE is lost as fecal energy, with the remaining DE available for metabolic processes. After DE, additional energy is lost through urine and gas. The remaining energy after these losses is called metabolizable energy (ME). The final losses between ME and NE occur as heat, which is required to metabolize the nutrients of ME

(Kromann, 1973). The remaining energy after the heat increment loss is net energy (NE), which is allocated for maintenance of life and production. The four major components of the NE system are measured and calculated based on different factors while building upon each other.

The first component is gross energy, which is the foundation of the NE system. Gross energy (GE) can be determined by combusting the feed consumed in a bomb calorimeter. Digestible energy (DE) is measured from the energy content in feed as GE intake and GE in feces. Metabolizable energy (ME) is determined from DE, where the energy content of urine can be measured in a bomb calorimeter, and gaseous losses can be measured by placing an animal in a respiration chamber. Bomb calorimetry can be used through either a direct or indirect method. The main difference between these methods is that the indirect method determines energy or heat production by measuring oxygen consumption and CO_2 production, whereas the direct method measures heat transfer (Simonson and DeFronzo, 1990). Direct calorimetry measures heat production by assessing the difference in temperature of water layers as heat is produced from the internal environment using a respiration chamber (Simonson and DeFronzo, 1990). In contrast, indirect calorimetry measures gas exchange in the room, as there is a fixed relationship between heat production and gas exchange (Simonson and DeFronzo, 1990). For animal applications, indirect calorimetry accounts for the entirety of an animal, including both respiration and flatulence. Simonson and DeFronzo (1990) emphasized that heat generated during the combustion of a feed sample in a bomb calorimeter is equivalent to the heat generated when that feed sample is oxidized to CO_2 and H_2O in a controlled manner within the body. Of the NE system, digestible energy (DE) system is a common component used for energy consideration in livestock rations.

There are both advantages and disadvantages to using DE systems to determine the energy available in equine rations. DE systems are simpler, user-friendly, and have some practical advantages compared to NE systems, which require more information and are generally more complex. However, despite being more complicated, NE systems have greater potential to accurately determine the ability of feedstuffs to meet true energy requirements due to the partitioning of specific physiological losses (NRC, 2007). In the United States, NE systems have been developed for cattle (NASEM, 2016), but historically, the DE system has always been used for horses (NRC, 1989). Because it is easily measured and represents ration digestibility, DE has some utility in feed evaluation. However, it overlooks energy loss from feed digestion and metabolism, which can be problematic for ruminants (NASEM, 2016). Harris (1997) argued that a DE system would overpredict the value of forage compared to equine diets that include grains or fats (NRC, 2007). The most fully developed NE system for horses is the French horse feed units (UFC) system (Martin-Rosset et al., 1994), but the equine NRC (2007) retained the DE system for horses. This decision was based on the belief that the French system was incomplete at that time and that the reference feedstuff in that system, barley, may cause confusion in the U.S., where it is less commonly used as horse feed (NRC, 2007). Additionally, there is more familiarity with the DE system in the U.S., and more is known about estimating DE from the chemical composition of feeds (NRC, 2007). The committee responsible for the next edition of the equine NRC will need to consider the recent publications since the 6th edition and determine whether there is room for improvement with the DE system or if there is potential to introduce the first U.S. NE system for horses.

Traditionally, the foundation of the NE system is determination of feed and fecal gross energy (GE) through bomb calorimetry, which combusts samples and measures the heat released during that process. Although it would likely be the most accurate, it is unrealistic to use bomb calorimetry to estimate energy utilization for animals on a routine basis outside of research laboratories. Therefore, several studies have used measurements determined in published digestibility studies to derive and produce equations for simple estimation of DE content in feedstuffs. Prior to 1989, the DE value of horse feed was commonly estimated from data compiled in research of other species (NRC, 1978). The NRC (1989) established an equine-specific method due to the variation in digestive processes and DE feed values across species. The current methods for estimating digestible energy (DE) in the equine NRC (2007) are based on the chemical composition of feeds. These calculations, presented in the NRC (1989), were derived from the work of Fonnesbeck (1981), who reviewed data from 108 equine *in-vivo* and *in-vitro* digestibility studies, though no specific characteristics of feedstuffs used were reported. In the NRC (1989), there are two equations provided based on two different feed categories: (1) dry forages and roughages, pasture, range plants, and forages fed fresh, and (2) energy feeds and protein supplements.

Further work by Pagan (1998a) introduced a different equation for estimating DE, based on 30 different diets (120 observations). When compared, the predicted DE content from both the NRC (1989) and Pagan (1998a) equations yielded similar estimations for many feed types (Pagan, 1998a). However, Pagan (1998a) suggested that neither equation could accurately predict the DE content of feeds high in fiber or fat. Although the two equations produced similar results, it is important to note that diet digestibility can be influenced by external factors, such as individuality, exercise, and feed processing (Hintz et al., 1986; Pagan, 1998b). A key characteristic of these models is that they are empirical rather than mechanistic, meaning the chemical composition values used to estimate DE from these equations should fall within the range of compositions from which the data were derived. For example, if an empirical DE model was developed using data with a fat content no higher than 5%, the DE of feedstuffs with more than 5% fat should not be predicted using that model (Tylutki, 2011). In contrast, mechanistic models, like those proposed in the Beef Cattle NASEM (2016), are typically more robust, connect with biological systems, and, once complete, can allow for predictions across a wider range of values (Tylutki, 2011).

Within the beef cattle NRC, both empirical and mechanistic functions are used to estimate the digestible energy content of feeds. As described by Fox et al. (1995), the appropriate application of empirical and mechanistic functions depends on the situation. Empirical functions are best used when there is limited information on feed composition or when users have less experience (NASEM, 2016). In contrast, mechanistic functions, which account for more physiological processes, should be applied in educational settings or when evaluating feed programs that require consideration of performance variation specific to a particular type of production (NASEM, 2016). For example, mechanistic methods for predicting energy content in ruminant diets includes using a mechanistic rumen model to estimate apparent total digestible nutrients (TDN) and other factors for each feed before applying equations to estimate ME and various forms of NE (NASEM, 2016). TDN is calculated by estimating the apparent digestibilities of carbohydrate fractions, protein, and fats, which are mechanistically determined by simulating the digestion processes of feedstuffs within the rumen and small intestine (NASEM, 2016). Particularly for horses, further development of estimation methods that account more for physiology is important for advancement in precision feeding.

Overall, there is a need within the literature for more tools to estimate the DE from nutrient digestibility in horses. The NRC (2007) directly commented that while digestible energy values for some common horse feedstuffs have been determined in feeding trials, there are a limited number of equine-specific studies compared to studies with other species. The empirical and static nature of current equine DE prediction systems limits the ability to model physiological changes in animals over time, unlike the existing mechanistic models used for beef cattle (NASEM, 2016). This limitation may be a result of the clear lack of many robust equine digestibility studies in the literature.

Most commonly, published equine digestibility trials use apparent digestibility methods. The apparent digestibility of nutrients is measured in digestibility trials, which can be determined by total fecal collection (TFC) or with indigestible dietary markers. Some commonly used internal and external markers include acid insoluble ash, chromic oxide, lignin, and *n*-alkanes, among others (Sales, 2012). TFC is considered the most accurate way to measure apparent total tract digestibility, by using the amount of feed consumed with total collection of feces (Schurg, 1981). However, TFC is laborious, time-consuming, and requires animals to be individually confined, which is why dietary markers have been developed to mitigate these challenges. An indigestible marker is used to calculate digestibility by comparing the ratio of the marker added to the ration with the amount found in a smaller, representative sample of feces, rather than the TFC (Sales, 2012). When Sales (2012) assessed the statistical differences between TFC and several types of dietary markers, it appeared that in all-forage diets, the TFC and marker methods produced almost identical measurements of digestibility. However, for diets with both forage and concentrate, the markers differed in results from TFC, with the acid-insoluble ash marker being most reliable, based on the data in their dataset from previous equine digestibility studies (Sales, 2012).

Since the 20th century, equine digestibility studies of varying quality have been conducted to assess the utilization of specific nutrients by the body. However, compared to other species, equine digestibility research is less frequent and has fewer supporting data (NRC, 2007). Many equine studies use apparent total tract digestibility methods, which involve subtracting the nutrient content in feces from the nutrient content of the diet consumed by the animal, without accounting for endogenous losses (NRC, 2007). Within the existing equine digestibility literature, endogenous fecal losses are usually not determined, and most DE values are apparent rather than true DE (NRC, 2007). While studies of true total tract digestibility would provide more accurate results, apparent digestibility still offers insight into the bioavailability of various feedstuffs.

Emerging Needs. For precise ration determination in horses, we do not have the tools to predict nutrient digestibility summarized from the available literature. A major limitation in developing robust models to predict nutrient digestibility in horses is the lack of consistent and reliable literature measuring digestibility, particularly in modern equine diets where cereal grains are less prevalent. In the United States, significantly less funding is allocated for equine research in comparison to food animal research, likely due to the lower profile and priority of equine studies for grant agencies (Topliff, 2002; Jansson and Harris, 2013). In other countries, such as France, there have been numerous studies on equine digestibility that correlate well with the feedstuffs fed in that country, but not necessarily with those fed in the U.S. (NRC, 2007). As a result, few tools exist to accurately predict nutrient digestibility from the available literature and additional development is necessary.

Monitoring Individual Responses to Diet

Traditional Tools. Once we have the tools to estimate dietary digestibility, we need to confirm how well diets work for individuals in grazing settings. This can be determined by assessing energetic and body fat storage because animals receiving an excess of energy will appear over-conditioned and those not receiving enough energy will be under-conditioned. The major physiological source of energy is adenosine triphosphate, or ATP. Adenosine triphosphate (ATP) is the primary source of energy found in cells, with carbohydrates and fats being the largest contributors to ATP under normal circumstances. Any excess energy consumed is stored in the body as glycogen or triglycerides (NRC, 2007). Because energy is required to store and later utilize endogenous energy sources, the body is most efficient when absorbed energy is used immediately (NRC, 2007). The amount of fat stored in horses appears to vary based on individuals, as studies report conflicting results, with fat constituting anywhere from 5% to 10% or more of body weight (McMiken, 1983; Lawrence et al., 1986; Kane et al., 1987). Body fat resulting from energy utilization of livestock is indicative of dietary adequacy and must be monitored in individual animals.

Body reserves and body condition management are crucial to the long-term health and productivity of livestock. For food animals, such as beef cattle, careful management of body energy reserves is important for overall economic success (NASEM, 2016). At either extreme of body energy reserves–too fat or too thin–cows face an increased risk of metabolic disorders, conception challenges, dystocia, and compromised offspring (Meyer et al., 2010; Funston et al., 2012; Long et al., 2012). Overfeeding cattle to the point of obesity is costly and can lead to calving problems as well as reduced dry matter intake (Buskirk et al., 1992). For gestating cows that are too thin, issues with inadequate milk supply may arise, potentially compromising the calves and predisposing them to long-term health and production challenges (NASEM, 2016).

For both horses and beef cattle, the most common method for monitoring of dietary adequacy is through body condition scoring.

There are industry-standard body condition scoring (BCS) systems for horses, based on Henneke et al. (1984), and for beef cattle, as described by Wagner et al. (1988), both using a scale from one (emaciated) to nine (overly obese). The purpose of body condition scoring for any species is to assess the influence of feed intake, energy expenditure, and various internal and external factors on body adipose tissue accumulation. For horses, factors that influence body condition include the availability and amount of feed, weather, exercise intensity, and reproductive status (Shuffitt and TenBroeck, 2003). Henneke et al. (1984) pioneered the system for horses, where a score of one is extremely emaciated, five is the ideal condition, and nine is considered overly obese. Veterinarians commonly include a BCS score in their evaluations during wellness visits to track condition over time. For both species, the evaluation of body condition involves visual observation and physical palpation.

Equine body condition scoring (BCS) involves evaluation of muscle and adipose tissue presence in specific areas of the body known for accumulating most of the noticeable fat coverage. These areas are along the neck or crest, behind the shoulder, along the withers, the rib cage, over the loin, and around the tailhead. Each region is assigned a score from one to nine and then averaged to get an overall score for a horse. Typically, palpation of these various areas is necessary to thoroughly assess the adipose tissue content and assign an accurate score. A similar process is used for beef cattle, with areas such as the brisket, ribs, pins, hooks, spine, and tailhead evaluated to determine body condition (Eversole et al., 2009). Timing is a critical aspect for evaluating body condition of cows, with guidelines emphasizing assessments of condition at weaning, 60 to 90 days prior to calving, and at calving (Eversole et al., 2009). However, although BCS is common, it has both advantages and disadvantages, particularly in terms of subjectivity.

Body condition scoring provides a system for both amateurs and professionals to assess the condition of their animals, which could indicate the need for a dietary change or veterinary intervention. It is a straightforward, highly visual system that is easy to perform. Additionally, in contrast to smaller animals, obtaining accurate body weights for livestock can be challenging for the average person, making body condition scoring a practical substitute. Proper scoring can help prevent animal obesity, that leads to overwhelmingly negative health consequences, such as the development of metabolic disorders and laminitis (Boosman et al., 1991; Thatcher et al., 2012; Daradics et al., 2021). Although usually straightforward, body condition scoring is inherently subjective, as identifying changes in adipose tissue accumulation can be challenging, even for those with proper training (Evans, 1978; Kristensen et al., 2006; Mottet et al., 2009). In horses, the Henneke et al. (1984) system, originally developed using Quarter Horse mares, is now universally applied across different horse breeds. This universal application complicates accurate assessments due to the known variations in body composition among horse breeds (Suagee et al., 2008; Brooks et al., 2010). Beef cattle producers are typically advised to employ experienced body scorers or participate in BCS training to accurately assess their own herds (Roche et al., 2009). Although BCS is an established but beneficial tool for producers and animal owners, it requires refinement and investigation into alternatives to mitigate the subjectivity for precision nutrition initiatives in individual animals.

Emerging Tools. Given the known subjectivity surrounding body condition scoring and the clear necessity for a system to visually rate the condition of livestock, there is an opportunity to develop new technology or systems to address this gap. Specifically, a new system should retain the benefits of BCS while addressing the subjectivity that can result from incorrect condition scores. The use of image-based technologies, like thermal imaging, offer potential for creating more objective and accurate BCS systems for both horses and beef cattle.

Imaged-based BCS systems investigated in the literature include depth imaging, three-dimensional (3D) imaging, and thermal imaging. Several technologies based in machine-learning have been tested for deployment of accurate and automated systems to regularly monitor the body condition of cows. Notably, depth imaging and three-dimensional (3D) imaging have emerged as possible solutions to the subjective nature of body condition scoring cattle (Kojima et al., 2022; Xiong et al., 2023). Xiong et al. (2023) utilized depth imaging of beef cattle to obtain several body measurements in a machine-learning based analysis. Although the depth imaging offers a low-cost agricultural application, the models failed to accurately predict BCS in beef cows with scores less than 4.5 (out of 9) (Xiong et al., 2023). Through use of machine learning algorithms, Kojima et al. (2022) successfully developed models for predicting BCS with three-dimensional imaging in multiparous beef cows. Although research has shown promise with these two image-based methods, they are not without their challenges. Thermal imaging may offer an alternative to depth or 3D imaging for assessing body condition score.

Thermal imaging, or thermography, is a camera-based technology used to measure surface temperature. It is a user-friendly tool that enables the measurement of an animal's surface temperature without requiring physical contact. In simple terms, surface temperature can be determined by analyzing the wavelengths and intensity of electromagnetic radiation emitted by objects (Speakman and Ward, 1998). The term infrared thermography originates from the fact that the electromagnetic radiation measured originates from the infrared region of the light spectrum (Speakman and Ward, 1998). Thermal imaging has yet to be assessed for its capacity to connect body fat reserves with surface temperature in livestock. Existing applications of thermal imaging in livestock are primarily found in veterinary medicine studies, where it is used to develop affordable tools for diagnostics of conditions ranging from orthopedic lameness to chronic infections and even reproduction purposes (Anagnostopoulos et al., 2021; Maśko et al., 2021; Okur et al., 2023). In contrast, human medicine has utilized thermal imaging to manage overweight patients, where the technology has shown potential to detect body fat reserves (Chudecka et al., 2014; Salamunes et al., 2017). Studies have primarily found that higher percentages of abdominal body fat in humans correlates with a lower skin surface temperature (Chudecka et al., 2014; Salamunes et al., 2017). This correlation enables the application of thermal imaging in real circumstances for low-cost body fat detection in humans. Based on this, thermal imaging holds the same potential for detecting adiposity in livestock but does have both advantages and disadvantages in application.

There are both benefits and drawbacks to employment of image-based systems to BCS livestock. The benefits include potential for automation, higher accuracy, and improved objectivity. The disadvantages may include chances for technological malfunctions, high costs, limited accessibility, and difficulty in use. For thermal imaging, it is relatively low cost but can be inaccurate depending on environmental conditions, like cloud cover. Cloud coverage pertains to the amount of direct sunlight, which has a direct influence on surface temperature (Liu et al., 2008). Therefore, the most notable limitation of thermal imaging is the highly influential environmental variables on accurate surface temperature readings, where controlled environments may be required for reliable results. Although thermal imaging has been tested on humans, it has not yet been applied for body fat measurement in livestock. Environmental factors shoulder be considered in evaluation to effectively assess the accuracy and functionality of the technology.

Advancing the Science of Precision Nutrition

Overall, precision nutrition tools and support for extensive livestock grazing systems are essential. Specifically, advancements are necessary for tools to monitor forage quality, estimate digestible energy, and objectively body condition score (BCS) livestock. The first step, where spectral sensing holds potential, is to develop tools for low-cost, in-field forage analysis to monitor the nutrients consumed by grazing animals. Once resources for chemical composition identification are available, it is critical to understand how animals utilize that forage. In general, there is a substantial need to update the tools for equine digestible energy (DE) estimation with nutrient digestibility data from modern equine diets. Presently, there are no tools to predict equine nutrient digestibility summarized from the latest available literature, warranting the need for development of a modeling system through a systematic review. Once tools for estimating digestibility are established, confirmation of how well diets perform for individual animals in grazing settings is required. BCS remains a commonly used method to monitor fat accumulation in livestock. However, this method is inherently subjective and underscores the need for more objective assessment methods. Thermal imaging of body surface temperature could address this gap but requires evaluation for reliability in the field. The development of tools in each of these three areas would greatly benefit extensive livestock grazing systems.

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CHAPTER 2: Spectral Sensing to Monitor Nutrient Concentrations in Mixed-Grass Hay

This chapter was submitted as Alexandra P. Webster, Ryan K. Wright, and Robin R. White. Short Communication: Spectral Sensing to Monitor Nutrient Concentrations in Mixed-Grass Hay, Journal of Animal Science, 2025.

Abstract

This study aimed to evaluate a hand-held spectral sensing device to predict the nutrient composition of mixed-grass hay compared to bench chemistry analysis. Metrics included dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), and crude protein (CP). A secondary objective was to test the sensor for on-animal use to monitor hay quality during consumption. From January to April, the sensor scanned 10 mixed-grass round hay bales weekly, and grab samples were collected immediately after for bench chemistry analysis. The sensor measured reflectance at 18 wavelengths along with distance using Light Detection and Ranging (LiDAR). After averaging the readings collected over 10 seconds at 1 hertz, they were used, along with various weather descriptors, to form the 19 primary features of a random forest regression. Data were split into three parts for analysis: 15% for hyperparameter tuning, 55% for training, and 30% for evaluation. Models produced root mean square prediction errors (RMSPE; % mean) of 9.80% (DM), 5.83% (NDF), 7.86% (ADF), and 22.5% (CP) compared to bench chemistry results. A proof-of-concept evaluation for use of this tool as a wearable sensor was performed by mounting the spectral sensor to halters worn by grazing horses. Forage composition estimated by the wearable sensor was within a similar numeric range as composition derived from wet chemistry of grab samples collected from the area of the bale where the animal was eating. The proof-of-concept demonstrated the sensor could collect data while animals were grazing. Although difficult to interpret given the small sample size, the RMSPE calculated by comparing the sensor estimates to the grab sample chemical composition were 12.9% (DM), 5.23% (NDF), 5.66% (ADF), and 18.5% (CP). Future development of this tool, for hand-held or wearable use, should collect more extensive data across a wider range of farms and forages for robust determination of sensor reliability and accuracy.

Introduction

Forage provides the primary source of nutrients for livestock in grazing systems. Forage quality varies with management and environmental conditions and is the primary driver of operation costs as well as grazing animal body condition, reproductive success, and growth rates (Moore et al., 2020b). Improved understanding of variability in forage quality may enable more specific nutritional management of livestock in grazing systems, thereby helping to reduce costs and enhance productivity (Smith et al., 2021). Specifically, more frequent monitoring of the chemical composition of forages consumed by grazing livestock can help determine the ideal quantity, timing, and composition of supplemental feed. Although traditional forage sampling and bench chemistry analysis could support more frequent monitoring of forage quality, this approach is labor-intensive and expensive to implement (Weiss and Hall, 2020).

Over the past decades, several advances in methodologies for forage quality determination, including near-infrared reflectance spectroscopy (NIRS) and spectral sensing, have emerged to address these limitations. Of those advances, NIRS is one of the most widely used technologies to mitigate the financial and time constraints of traditional bench chemistry methods (Brown and Moore, 1987). Outside of forage analysis, NIRS has been applied in other areas like dairy product analysis and soil testing (Vincent and Dardenne, 2021). Though less prevalent commercially, spectral sensing shows promise in research settings, particularly for monitoring forage composition in large grasslands with remote sensing (Cho et al., 2007; Shorten et al., 2019).

Due to the connection of plant color and chlorophyll on plant nutritive quality, spectral sensing can be used to estimate forage composition based on light reflectance on the exterior of the plant (Ali et al., 2012). Although spectral sensing is similar to NIRS, where it collects near-infrared spectra, it can also sense wavelengths within ultraviolet (UV) or visible light spectra (Askari et al., 2019; Katoch, 2023). Unlike NIRS, which is intended to replace bench chemistry analysis (Shenk and Westerhaus, 1994), spectral sensing has the potential to be applied in a diversity of contexts, including continuous, on-animal monitoring. The small size of spectral sensors allows for the integration into wearable devices, like livestock halters, for real-time, non-invasive monitoring of forage quality as animals consume feed. This is significant because it can help producers make timely adjustments to feeding practices by providing information about the nutrients that cattle are fed. While distant spectral sensing methods can monitor changes in standing pasture quality, these tools fail to monitor forage consumed by animals on a continuous basis, which is necessary for precision feeding in extensive livestock systems.

Our first objective was to assess the accuracy of a hand-held spectral sensing device to predict the dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), and crude protein (CP) composition of hay. Our second objective was to pilot test the spectral sensing device for on-animal use to monitor the composition of hay consumed during normal feeding behavior. We hypothesized that the hand-held spectral sensor would accurately predict the DM, NDF, ADF, and CP composition of mixed-grass hay. Additionally, we hypothesized that the spectral sensor could be mounted on halters worn by horses to collect spectra data of grazed forage.

Materials & Methods

All procedures in this study were reviewed and approved by the Virginia Tech Institutional Animal Care and Use Committee (IACUC Protocol #22-137). To achieve our primary objective, we designed a hand-held spectral sensing system and tested it on mixed-grass hay baled into 1.5 m round bales ("bales") weekly for 12 weeks beginning 19 January 2024 to 1 April 2024. The mixed-grass hay bales were harvested from pastures at the Virginia Tech Kentland Farm and were predominantly comprised of Kentucky 31 tall fescue, though other pastures grass species were also present in baled fields. Each week, 30 forage samples were collected from 10 round bales, with three samples per bale. For each sample, a spectral reading was taken directly over the surface of a round bale in livestock pastures. The corresponding forage sample was then collected by hand from the area scanned and brought back to the laboratory. Once in the laboratory, each sample was scanned again in a controlled, indoor environment. Every forage sample was analyzed through traditional bench chemistry methods for dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), and crude protein (CP), as described below.

Sensor Design

A spectral sensor, which leveraged open-source design, was used to collect readings both in the field and in the laboratory. Initially, the sensor was used to scan selected hay samples in the field prior to sample collection. The samples were then scanned again in the laboratory before chemical analysis. The sensor system was comprised of a SparkFun ESP32 Thing Plus microprocessor, a SparkFun Triad Spectroscopy sensor, a Global Positioning System (GPS) unit, and a LiDAR LED Distance Measurement Sensor (SparkFun Electronics, Niwot, CO). The spectral sensor was programmed in Arduino IDE v 2.0.4 (Arduino Core Team, 2023) to recognize 18 individual light frequencies ranging from 410 nanometers (nm) (Ultraviolet) to 940 nm (Infrared). The LiDAR was included to monitor the distance between the sensor and the bale, ensuring any variation in distance was tracked during use.

The scanning process was conducted at a rate of 1 hertz, or once per second. The in-field sensor was secured to a plastic plate which was held approximately 15 centimeters parallel to each forage sample for every reading of the study. For the laboratory scans, the same sensor system mounted on a plastic plate was used. However, it was placed in a 15.24 cm x 15.24 cm x 15.24 cm cardboard box with the sides removed and a hole at the top to ensure sufficient light exposure. The purpose of the box was to maintain a consistent sensor position and more controlled conditions for each in-laboratory reading. The purpose of re-scanning the samples in the laboratory was to determine if there would be a difference between the spectra produced in the field versus in a controlled environment, as notable differences in these reading environments would affect potential for hand-held or wearable in-field use of this system.

Experimental Design

Round bales fed to pastured livestock housed on the Virginia Tech campus farm in Blacksburg, VA were selected as the target of this experiment. Over 12 consecutive weeks, 10 round bale feeders were used as sampling locations. Following normal farm management protocol, bales within these feeders were replaced based on how quickly they were consumed. Because bales were grazed by varying numbers and different species of livestock, replacement rates and times were unique for each location. Every week for each round bale, three sampling locations were selected from which spectral data and physical grab samples were collected. No formal randomization procedure was used for selecting sampling locations on each round bale. The three sampling sites were chosen arbitrarily to represent a balanced sampling from different heights and positions around the bale’s surface. Spectral data were collected by holding the handheld plate approximately 15 to 20 centimeters away from and parallel to the surface of the hay. The spectral sensor collected data for 10 seconds, which were logged directly to a field computer via a universal serial bus. Cloud coverage (%) at the time of sampling was obtained from the weather forecasting service AccuWeather, Inc. (2024), and a short weather description (e.g., “sunny”, “overcast”, etc.) was noted based on visual observations of weather conditions. Sampling did not occur during active precipitation; however, some sampling times followed recent bouts of precipitation. If hay samples were wet from previous precipitation when collected, it was noted for analysis.

After scanning the location and collecting weather data, a hand-grab sample was collected from the area directly below the sensor. The hand-grab hay sample was then bagged and brought back to the laboratory. In the laboratory, the benchtop spectral sensor was used for repeat scanning. During this procedure, the hay sample was placed within a box holding the benchtop spectral sensor, and another 10 seconds of data were logged. The forage sample was subsequently prepared for chemical analysis as described below.

Due to sensor failure, one week of samples was unavailable for analysis. Furthermore, during the final two weeks of collection, two bale locations were empty in the second-to-last week, and four were empty in the last week, resulting in missing samples. These bale feeders remained empty because farm management was encouraging livestock to return to grazing fresh forage coming up in the pastures. By the end of the experiment, a total of 312 forage samples were collected and analyzed using bench chemistry. Each sample was matched to two sets of spectral readings: one obtained in the field, and the other obtained on the benchtop.

Pilot Application and Technology Evaluation

To better explore how this spectral sensing system could be used to characterize the chemical composition of hay consumed by livestock, a protective casing and halter mount were designed to attach the spectral sensor to a livestock halter. The sensor was tested on six mixed-breed horses for one hour per day during four days of collection in March 2024. The group of horses included two geldings and four mares, between the ages of 9 and 20 years old at the time of the trial. On each testing day, horses wore the halter monitor for one hour while under visual observation. Forage samples were collected twice per hour per horse while the horses were actively grazing on the hay bale offered free choice in their pastures. The forage samples were collected approximately 30 minutes apart, though the exact timing varied based on when the horses were eating. The cloud coverage and ambient temperature were noted at the beginning of each session (AccuWeather, 2024), along with the time of sample collection. Forage samples were bagged and taken back to the laboratory for weighing and subsequent bench chemistry analysis for dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), and crude protein (CP), as described below.

Halter Sensor Design

The spectral sensor technology utilized for the halter monitors was similar to that of the hand-held sensor used in this study. Each halter sensor system consisted of a SparkFun Triad Spectral Sensor, a SparkFun GPS unit, and a nine-degree-freedom inertial motion unit (IMU) sensor and microSD card reader packaged as a M5Stack ESP32 Gray microprocessor (SparkFun Electronics, Niwot, CO; M5Stack, Shenzhen, China). The system also included a rechargeable battery, a generic break-away horse halter, a microSD card, a self-adhesive wrap, and a USB-C power cord (Fig. 1). This system was programmed in Arduino IDE v 2.0.4 (Arduino Core Team, 2024). The halter sensors were powered on at the beginning of each data collection session and powered off at the end. Spectral readings were stored on the microSD cards inserted into each halter sensor. Because spectral readings were collected continuously, the spectral readings that aligned with the recorded time of each sample collection were separated from the rest of the data. However, due to sensor data collection failures, only about 50% of the data could be successfully matched to the corresponding forage sampling times. The final pilot evaluation dataset included spectral data correlating with a total of nine forage samples.

Benchtop Chemistry

Traditional bench chemistry was used to determine the DM, NDF, ADF, and CP content of the forage samples. After drying in an oven at 55ºC for 72 hours, samples were reweighed to determine dry matter content based on AOAC method 930.15 (AOAC, 2000). The samples were then ground down in a Wiley mill and passed through a 1 mm screen. The ANKOM 200 fiber analyzer system (ANKOM Technology, Macedon, NY) was used for NDF and ADF determination in alignment with the methods published by AOAC (2000). The solutions used for NDF and ADF were pre-mixed and sold by ANKOM Technology. The pre-mixed NDF solution contained H_2O, ethylenediaminetetraacetic acid disodium (EDTA), sodium lauryl sulfate, triethylene glycol, sodium borate, and sodium phosphate (ANKOM Technology, Macedon, NY). The pre-mixed ADF solution contained H_2O, sulfuric acid (concentrate), and cetyltrimethylammonium bromide (ANKOM Technology, Macedon, NY). Heat-stable alpha amylase was used for the NDF analysis, and the results were not corrected for ash. The AOAC method 990.03 was followed for CP analysis in the Elementar vario EL cube CHNS elemental analyzer (Elementar Americas Inc., Ronkonkoma, NY) (AOAC, 2000).

Data Analysis

All data preparation and analyses were completed in R v. 4.3.1 (R Core Team, 2024). Following the collection of hand-held spectral data, the data were recorded in individual text files with labels for each round bale location and sample number (out of three). The text file names also specified if the data were obtained in the field or laboratory. The text files were read into R using the readxl package (Wickham and Bryan, 2023) and then the 10 readings (one per second for the 10 seconds of each sample) were averaged into one reading per forage sample.

Random Forest regressions were utilized to develop methods using spectral data to estimate forage DM, CP, NDF, and ADF. After omitting missing values, the data were randomly divided into three sections from the total sample population: 55% for model training, 15% for hyperparameter tuning, and 30% for independent evaluation. The chemical composition (DM, CP, NDF, or ADF) served as the response variable for each regression, and the explanatory variables/features included the 18 wavelengths of spectral data, LiDAR measurements, cloud coverage (%), and weather conditions. For each regression, the tuneRF function (Liaw and Wiener, 2002) was used for the identification of the best hyperparameters using 15% of the dataset. The models were then trained, and parameters were estimated using the 55% of data allocated for training. Random forest regressions were fit using the randomForest package (Liaw and Wiener, 2002). After the training phase, the resultant model was assessed against the remaining 30% of the dataset reserved for independent evaluation. The root mean squared prediction error (RMSPE) of each model was used in the evaluation to measure how well the spectral sensor could predict each chemical composition measure. In addition, the root mean square error standard deviation ratio (RSR) and the concordance correlation coefficient (CCC) (Lin, 1989) were calculated. Although universally applicable standards for interpreting RMSPE and RSR have been proposed (Khosravi et al., 2018; Chen et al., 2025), it is challenging to translate these standards to individual model use cases and individual datasets, as acceptable error rates will be highly context dependent. In this evaluation, we focus on directional changes in RMSPE, RSR, and CCC for comparison among models. Lower RMSPE and RSR are preferrable, while CCC closer to 1 is more ideal. Although interpretation of the RMSPE is more intuitive as it is a percentage, practical interpretation of RSR can be informed by its definition as the ratio of the RMSPE to the standard deviation of the data. Using this definition, a value of 1 or higher reflects that a model has limited advantage compared to predicting values based on the mean and standard deviation of the data. RSR can be a valuable tool in highlighting when model confidence is inflated due to limited variability in the observations.

The derived models were further tested for their ability to predict the chemical composition of the forage samples collected during the on-animal pilot test. Utilizing the models developed from the data collected in the main experiment with the hand-held spectral sensor, the forage chemical composition predicted from the halter sensor data were compared with the composition determined through bench chemistry. The RMSPE, RSR, and CCC were calculated for each model to evaluate how well the halter sensors could predict each of the four composition metrics.

Results

Hand-Held Spectral Sensor

Of the 342 forage samples collected, seven samples were noted as "wet" during the 8th week of data collection. The DM bench chemistry values from those samples resulted in higher moisture content than the rest of the samples, creating somewhat dichotomous data. The DM bench chemistry percentages of the hay averaged 87.7% (as-fed) and ranged from 43.4% to 98.2 % (as-fed) (Table 1). The random forest regression predicting DM content resulted in an RMSPE (% mean) of 9.80%, a CCC of 0.610, and RSR of 0.793 (Table 1).

The system also produced favorable fit statistics when evaluating other bench chemistry values. The random forest regression for NDF generated an RMSPE of 5.83%, a CCC of 0.201, and RSR of 0.969 (Table 1). The measured NDF ranged from 60.6% to 81.6%, with a mean of 71.2% DM. Similarly, ADF random forest regression performed with an 7.86% RMSPE, a CCC of 0.274, and RSR of 0.934 (Table 1). Measured ADF values varied from 31.4% to 53.1% with a mean of 43.7% (% DM). The random forest regression for CP produced an RMSPE of 22.5%, a CCC of 0.355, and RSR of 0.881 (Table 1). The measured CP values ranged from 4.32% to 15.6% and averaged 9.78% (% DM).

Halter Spectral Sensors

The spectral sensor was packaged into a wearable design and applied to horse halters. It was successful in collecting spectral data while mounted on horses that grazed on mixed-grass hay. The RMSPE calculated by comparing the sensor estimated chemical composition of the grazed forage compared to the chemical composition obtained from grab samples of these bales was 12.9% (DM), 5.23% (NDF), 5.66% (ADF), and 18.5% (CP) (Table 1). The RSR and CCC values for this comparison are also included in Table 1.

Discussion

Evaluation of the Spectral Sensor in Predicting Dried Forage Composition

The evaluation of this hand-held spectral sensing system demonstrated its capability to estimate the chemical composition metrics of DM, NDF, ADF, and CP in grass hay (Table 1) from a fairly narrow dataset representing hay during one season, from one farm. Although the standard for reliable and accurate analysis of forage is through benchtop chemistry, benchtop analyses can be impractical for routine use by producers because of high costs and associated labor (Katoch, 2023). The hand-held spectral sensor proposed in this paper is a rapid and affordable complement to bench chemistry, which may eventually produce comparable function to hand-held NIRS systems developed with similar objectives (Prigge et al., 2021). Remote sensing and UAV-borne imaging spectroscopy are other technologies deployed as bench chemistry alternatives with various levels of accuracy and practicality (Pullanagari et al., 2012; Barnetson et al., 2020). Similar to hand-held spectral sensors, they utilized wavelengths ranging anywhere from 450 nm to 2400 nm and display strong capabilities in predicting forage quality (Pullanagari et al., 2012; Barnetson et al., 2020). The cost-effective and readily accessible nature of the hand-held sensor are key attributes supporting it as an on-farm forage quality assessment tool that is complementary to other chemical composition assessment methods and systems. In particular, the small size of the system and the focus on applications for wearable devices to monitor animal intake, highlight an alternative use case for spectral sensing of forage quality compared with previous studies focused on field-scale assessments.

The spectral sensor displayed an RMSPE of 12.9% (% mean), a CCC of 1.30, and RSR of -0.012 when estimating the DM content of hay (Table 1). The focus of the present study was on hay, which is a form of fresh grass, cut at maturity, left to wilt, and then rolled into round bales (Zamudio et al., 2024). The wilting phase is when the cut pasture grass dries to approximately 80% to 85% dry matter and loses the green color commonly associated with fresh grass (Moore et al., 2020a; Zamudio et al., 2024). The fit statistics for evaluating the DM estimates are likely influenced by the moisture content of the hay, which is less variable and less prone to rapid changes. These consistencies in the chemical composition could have contributed to intermediate RMSPE, lower CCC, and high RSR compared with other chemical components tested.

Our NDF and ADF models had lower RMSPE compared with the DM model. The performance of these models is likely due to the complex composition of these fiber fractions, which contain multiple chemical components that interact with the sensor's spectral measurements. In particular, the primary components of ADF are cellulose and lignin, and in NDF, are hemicellulose, cellulose, and lignin. Because this study was conducted on forage that was not actively growing, the NDF and ADF contents of the hay depend on what point in the growing season the grass was cut and baled. The high NDF and ADF bench chemistry values infer that the hay was likely cut post-bloom at a mature growth stage when the cell wall had high cellulose and lignin contents (Lloyd et al., 1961; Krämer et al., 2012). In addition, other studies also had correlating levels of success in estimating NDF and ADF contents of forage using spectroscopy (Starks et al., 2004; Liu et al., 2022).

Although the RMSPE were favorable, the RSR and CCC of the NDF and ADF models suggested less ideal fit compared with the DM model. In particular, the RSR are above (NDF) or close to 1 (ADF), likely due to the narrow range of observations (Table 1). The CCC are also very low (Table 1), suggesting challenges with predictive accuracy and precision. Given the critical role these fiber components play in animal nutrition and feeding, accurate estimation of

NDF and ADF is critical to support livestock management systems (Beauchemin, 1996). The less favorable RSR and CCC compared with the DM model suggests that additional data collection on different types of forage with wider ranges of composition values is necessary before the NDF and ADF estimates produced by this system will be reliable for practical uses.

The CP model returned fit statistics that were intermediate relative to DM and the fiber components (NDF and ADF). Although the CP model had higher RMSPE (22.5%), it also produced intermediate RSR (0.881) and CCC (0.355). Collectively, these fit statistics suggest that the CP was more variable than other chemical components tested, but that the spectral system struggled to explain this variability in a generalizable manner. These performance attributes could reflect differences in spectra reflectance between individual amino acids or light scatter caused by various particle sizes in hay (Shenk et al., 1979). For example, NIRS has been shown to predict CP content of forage quite well in wavelengths majorly above those detected by our spectral sensor in the infrared region (Lippke and Barton, 1988). The fit statistics are also reflective of the variability within the CP measurements, as CP was more variable than other chemical components evaluated within this work.

Protein is an essential nutrient for growth, and providing adequate supplies of protein is necessary for livestock production (Poppi and McLennan, 1995). Routine analysis of forage chemical composition is important to ensure animals are receiving sufficient nutrients. If forage is inadequate in protein, producers must make informed changes to livestock rations to meet the nutrient requirements associated with their production goals. Although the accuracy of the sensor system for estimating CP was limited, definitive conclusions on the suitability of the system are dependent on broader data collection and model evaluation efforts, as the present data preclude definitive assessment of the degree to which model performance is driven by inadequate sensor capacity (lower wavelengths than are typically associated with CP) or by high data variability.

During data analysis, the data was randomly split, and we recognize that this can lead to favorable model evaluation, particularly when known grouping factors are present within the data. In this case, we did not have a clear grouping factor that could be used for data splitting, as all were confounded with time, and sampling across storage times to achieve variability in chemical composition as part of the experimental design. In future evaluations across several farms, we expect that data split randomly across farms would produce more conservation evaluation.

Evaluation of the On-Animal Spectral Sensor in Predicting Hay Composition While Mounted on Horses

This study demonstrated proof-of-concept for the use of this spectral sensor as a wearable forage monitoring system. It is important to recognize that only nine forage samples were able to be aligned with spectral data, and drawing strong conclusions about the accuracy of algorithms in estimating forage chemical composition based on data collected while on animals is impractical with the small dataset. When compared with chemical composition assessed on hand-grab samples obtained from the grazed forages, the RMSPE (% mean) were 12.9% for DM, 5.23% for NDF, 5.66% for ADF, and 18.5% for CP (Table 1). The RSRs were 1.30 for DM, 1.18 for NDF, 0.798 for ADF, and 0.891 for CP. The CCCs were -0.012 for DM, -0.360 for NDF, 0.394 for ADF, and 0.272 for CP. The proof-of-concept was able to demonstrate predictions within a similar numeric range to those observed via wet chemistry, though the accuracy and precision of predictions cannot be reliably assessed from the small sample size.

Conclusions

Overall, this demonstration supports further data collection efforts to enable more comprehensive evaluation of the suitability of this system for use in supporting forage evaluation and relevant on-farm decision making. Specifically, additional testing on larger sample sizes will be necessary to discuss accuracy, consistency, and translatability of this performance in broader hand-held and grazing contexts. Additionally, an emphasis on improving the durability and functionality of the mounted sensors for in-field use will be vital for practical implementation.

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Tables & Figures

Table 2-1: Model performance

	DM	NDF	ADF	CP
Chemical composition of samples determined by bench chemistry				
Mean, % DM	87.7[c]	71.2	43.7	9.78
Minimum, % DM	43.4[c]	60.6	31.4	4.32
Maximum, % DM	98.2[c]	81.6	53.1	15.6
Spectral predictions using hand-held sensor				
n[d]	312	312	312	312
RMSPE[a], % Mean	9.80	5.83	7.86	22.5
Mean Bias, % MSE	0.66	1.29	0.89	0.76
Slope Bias, % MSE	4.19	1.29	0.67	0.070
RSR	0.793	0.969	0.934	0.881
CCC	0.610	0.201	0.274	0.355
Spectral predictions using unseen data from halter sensors mounted on horses				
n[e]	9	9	9	9
RMSPE[b], % Mean	12.9	5.23	5.66	18.5
Mean Bias, % MSE	30.4	0.00	7.97	4.47
Slope Bias, % MSE	17.2	60.8	26.3	35.0
RSR	1.30	1.18	0.798	0.891
CCC	-0.012	-0.360	0.394	0.272

[a] Root mean square prediction error (RMSPE) of each chemical component as predicted on the independent evaluation data

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[b] Root mean square prediction error (RMSPE) between observed and predicted values of each chemical component when predicted on unseen data from halter sensors

[c] % as-fed

[d] Number of samples collected and analyzed by bench chemistry and aligned with spectral readings from hand-held sensor

[e] Number of samples collected that aligned with spectral readings from halter sensors

DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; CP, crude protein; CCC, concordance correlation coefficient; MSE, mean square error; RSR, root mean square error standard deviation ratio.

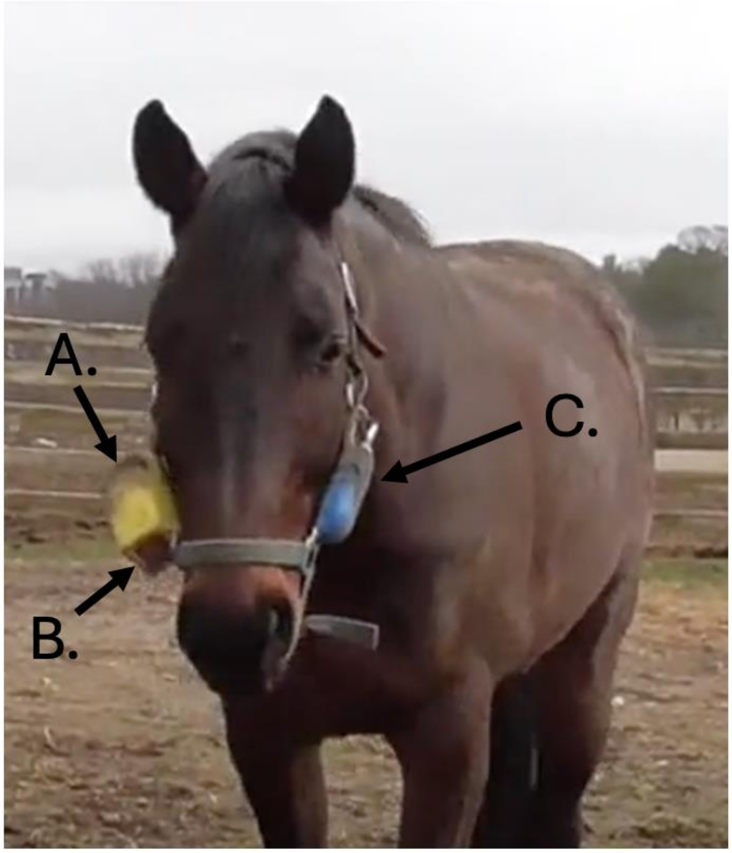

Figure 2-1: Spectral Halter Monitor Set-Up

An image depicting the set-up of the spectral halter sensors on a horse during the data collection phase. Part A. is the homemade sensor protection case. Part B. is the spectral sensor placed behind clear plastic to protect it from debris and damage while allowing for spectral readings. Part C. is the portable and rechargeable battery power source that is connected to the sensor through a USB-C cord attached to the underside of the break-away halter. All components were attached to the halter using self-adherent medical wrap seen in this image in yellow and blue. The set-up allowed the animals to freely move within their paddock space and roll as desired.

CHAPTER 3: Estimating Total-Tract Digestibility of Nutrients and Their Contribution to Digestible Energy Supplies in Equine Diets

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Abstract

Our objectives were to use a quantitative literature review to explore dietary and feed factors influencing apparent total-tract digestibility of dry matter (DMD), crude protein (CPD), neutral detergent fiber (NDFD), ether extract (EED), non-structural carbohydrates (NSCD), non-fiber carbohydrates (NFCD), and residual organic matter (rOMD) in equine diets, and to assess their contributions to digestible energy (DE) supplies. Data from 54 studies were modeled using linear mixed-effect regressions, with publication as a random effect to account for study variability. For each nutrient, five models were derived with explanatory variables including: dry matter intake (DMI; % BW/day) and DM (% as-fed), and dietary components (CP, organic matter, EE, NDF, acid detergent fiber, NSC, starch, and NFC as % of DM), and feed types (forage, non-forage fiber, legumes, cereal, and oil proportions). Model selection was based on concordance correlation coefficient (CCC), sigma hat error, and corrected Akaike Information Criteria, with models chosen based on their performance in simulating digestibility on example diets. Two models, one from measured and one from calculated data, for each nutrient were used to estimate dietary DE content and compared to existing DE estimation methods. Selected models explained variation well, with CCCs ranging from 0.740 to 0.969 (CPD: 0.886; NDFD: 0.775; EED: 0.813; NSCD: 0.969; rOMD: 0.740). The models were evaluated against measured DE from 17 studies. For reference, the DE estimations were also evaluated relative to those from other current DE systems. Overall, this approach offers an additional, practical tool for estimating energy supplies in equine diets.

Introduction

The health and performance of horses typically relies on balancing dietary nutrient composition with estimated individual horse requirements. Within this ration balancing paradigm, an understanding of feed utilization is critical for accurate feeding. Diets balanced using NRC (NRC, 2007) established requirement equations are intended to provide the energy and nutrients that meet or exceed the requirement for body maintenance, growth, exercise, and reproduction (Bergero and Valle, 2007). Yet, the industry standard for equine ration balancing determines digestible energy (DE) from total diet composition and does not consider how the nutrients from different feedstuffs are digested and utilized to fulfill the estimated nutritional requirement.

The reference standard for determining the digestibility of nutrients and energy is through *in-vivo* methods. However, these *in-vivo* methods can be time consuming and laborious, as they involve either total fecal collection or the use of an indigestible marker combined with fecal sampling throughout the day to estimate fecal mass produced (Cichorska et al., 2014). Although these methods are important for experimental work exploring how diets influence nutrient digestibility, assessing digestibility as a part of conventional horse management is impractical. Thus, it becomes critical to have models of nutrient digestibility to support feeding management decisions. Existing feeding management decision support models, like the equine NRC (NRC, 2007) which estimates DE based on estimated DE values for each feed, summed within diets. This approach assumes that individual feeds have a unique and constant DE, which is not affected by broader dietary conditions. In practice, however, there are associative effects that may impact nutrient digestibility and subsequent DE content of diets (Thompson et al., 1984; Palmgren Karlsson et al., 2000; Goachet et al., 2014). For instance, feed processing and level of intake can influence digestibility in horses (Särkijärvi and Saastamoinen, 2006; Clauss et al., 2014), as can dietary nutrient composition (e.g., high fat negatively impacting NDF digestibility). In nutrition models used for other species (NASEM, 2016; National Academies of Sciences and Medicine, 2021), this challenge is addressed through estimating DE based on dietary nutrient digestibility, which is predicted from a variety of dietary and feed factors. To generate a DE model using dietary nutrient digestibility, it is common to leverage quantitative literature summaries (White et al., 2017a; White et al., 2017b).

To date, there are few models published of nutrient digestibility in horses (Pagan, 1998; Zeyner and Kienzle, 2002; Sales et al., 2013; Martin-Rosset et al., 2021), likely due to the limited availability of data within the literature for model derivation. Hansen and Lawrence (Hansen and Lawrence, 2017) developed a system to predict forage digestibility based on macronutrient composition because forage represents the majority of the equine diet. Similarly, there have been several studies (Kienzle and Zeyner, 2010; Martin-Rosset et al., 2021) to predict metabolizable energy (ME) content of both forages and mixed diets as ME provides a more accurate estimate of energy available for productive functions than digestible energy (DE) (Martin-Rosset et al., 2021). Two commonly used but current systems for estimating DE from dietary composition were developed based on data from digestibility trials in the 20th century (NRC, 1989; Pagan, 1998). The feedstuffs utilized in equine diets have changed considerably over the past several decades to incorporate more non-forage fibers and commercial concentrates. As such, derivation of new strategies to estimate DE that are based on dietary nutrient digestibility may be both useful and timely.

Our objective was to conduct a quantitative literature review to investigate dietary and feed factors that affect total-tract digestibility of dry matter (DMD), crude protein (CPD), neutral detergent fiber (NDFD), ether extract (EED), non-structural carbohydrates (NSCD), non-fiber carbohydrates (NFCD), and residual organic matter (rOMD) in horses. We also developed supplementary models and equations for predicting OM, ADF, and starch digestibilities. Additionally, we aimed to assess the behavior of our equations for estimating digestible energy (DE) in comparison to some existing methods and evaluate them against independent measured DE from the literature. We hypothesized that models could be created to represent nutrient digestibility in horses from a combination of dietary descriptors and chemical composition from published literature, and that DE estimated from dietary nutrient digestibility would perform similarly to existing DE systems.

Materials and Methods

Data Collection and Preparation

A literature search was performed to collect a database of available measures from *in-vivo* digestibility studies in horses. Data were collected from published, peer-reviewed papers that reported apparent total tract digestibility measured from equine digestibility trials. Articles were searched for using the keywords of "digestibility," "equine," and "horses". Searches were conducted between 1 September 2023 to 30 November 2023 using Google Scholar (Google, 2024) and Web of Science (Clarivate Analytics, 2024). Every article recovered was screened for references with relevant titles which were then subsequently searched and evaluated for inclusion. The article was not considered if it referred to animals other than horses. Studies were excluded if they failed to report the specific diet(s) fed, diet percent inclusions, average animal body weight (kg), dietary intake amounts (including forage and/or concentrate intake, kg/d), chemical composition of the diet(s) and apparent or true total tract digestibility of crude protein (CPD), crude fat or ether extract (EED), dry matter (DMD), and neutral detergent fiber (NDFD). Studies were also excluded if they were not written or translated in English. Preferably, the studies also provided the apparent total tract digestibility of organic matter (OMD), acid detergent fiber (ADFD), non-structural carbohydrates (NSCD), and non-fiber carbohydrate (NFCD) but none of these were required for a study to be retained in the dataset. For each qualifying paper, animal descriptions were recorded including sample size, and average animal age. This meta-analysis did not use any live animals and therefore no approval was needed through the Virginia Tech Institutional Animal Care and Use Committee.

A total of 181 observations collected from 54 publications comprised the dataset. Data summary statistics are reported in Table 1. Figure 1 outlines the process of selecting studies found in the literature review for this meta-analysis in a "Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Diagram" (Page et al., 2021). The publications were listed in Table S1. The composition of individual feeds was assumed to be the average composition reported in the Dairy One Feed Composition Library (DairyOne, 2023) when specific diet components were not reported. If a specific feed was not provided in Dairy One, Feedipedia (ICA and FAO, 2022) was used as a backup source of chemical composition information. For one commercial feed supplement, online product information was sourced for chemical composition from the Mad Barn Feed Bank (MadBarn, 2023). Using measured feed composition where available, and book values for composition when unavailable, dietary nutrient composition was calculated as individual feed nutrient composition multiplied by the diet inclusion percentage summed within diet. Table 2 reflects the adjustments made between the measured data and the calculated data used to achieve a common sample size of 248 for each dietary composition variable.

In December 2024, an additional literature search was conducted in Google Scholar (2024) using the keywords of "digestibility", "equine", "horse/s", "pony", "ponies", "gelding/s", "mare/s", "nutrient", and "nutrition". The PRISMA diagram was updated with the additional review (Fig. 1). In addition to incorporating more terms, the exclusion parameters were modified to allow any studies that measured at least one apparent total tract digestibility of DM, OM, NDF, ADF, NSC, NFC, CP, or residual organic matter (rOM). After this and the previous review, a total of 181 observations from 54 publications were included in the dataset.

Model Derivation Procedure for Prediction of Digestibility

Models were derived using the lmer (Kuznetsova et al., 2013) and lmerTest packages (Kuznetsova, 2017) in R (version 3.1.0; R Core Team, 2024). For each of the seven response variables (DMD, CPD, NDFD, NSCD, NFCD, rOMD, and EED), five full models were developed based on five different options for explanatory variables. Explanatory variables were categorized as those reflecting dietary composition (DMI, percent of metabolic bodyweight per day, DM, % as-fed, as well as CP, OM, EE, NDF, ADF, Starch, and NFC, % of DM) or those reflecting feed types used within the diet (dietary forage proportion, non-forage fiber (NFF) proportion, legume proportion, cereal grain proportion, and supplemental oil proportion, DM basis). The NFC content of diets was inconsistently reported and therefore was often calculated as OM – CP – NDF – EE. The NSC content of the diets, if not provided, was calculated as the sum of dietary starch and sugar, if starch and sugar were reported individually. If an older publication reported a calculated NSC as 100 – (OM + CP + NDF + EE), it was categorized in our dataset as NFC. The category *Legume* contained feedstuffs and diets of all legume hay, mostly legume hay, alfalfa meal, and/or alfalfa pellets. The category *Forage* contained all types of forage feedstuffs which were legume hay, mostly legume hay, alfalfa pellets, mostly grass hay, grass hay, straw, oat hay, alfalfa meal, grass pasture, and/or Bermuda grass hay. The category *NFF* (non-forage fiber) included beet pulp, linseed meal, and/or soybean hulls. The category *Cereal* represented cereal grains and included oats, barley, steamed flaked corn, sorghum grain, maize grain, broken rice, maize germ meal, maize starch, sweet feed, and maize cobs. The category *Oil* represented supplemental oil added in diets in the form of liquid vegetable oil, canola oil, soybean oil, palm oil, and/or corn oil. Summary statistics for these variables within the dataset are reported in Table 1.

Given that we had to calculate a large number of dietary composition values, we explored two methods of representing dietary variables: using only measured values (MD) or using measured values where available and feed database (i.e., calculated) values otherwise (CD). There are important trade-offs associated with using CD, which are highlighted in the limitations section of this work (Schlageter-Tello et al., 2020). Using this as a base level of organization, we derived five models for each response variable, which were based on explanatory variables including: measured dietary values (MD); calculated dietary values with main effects only (CD); calculated dietary values with key interactions explored among variables (CD-I); calculated dietary values and feed type variables (CD-F); and calculated dietary values and feed type variables with key interactions explored among variables (CD-I-F). Figure 2 defines the explanatory variables available for use in each model type. Apparent total tract digestibility of residual organic matter (rOM) was incorporated in the calculated diets and since no studies directly measured rOM, it was calculated as the difference in apparent digestibility of OMD –

CPD – NDFD – EED – NSCD, to represent the digestibility of nutrients remaining after removing these five parts. Publication was used as a random effect in all models.

Although it is commonplace to weight meta-analytic models based on the inverse of the standard error measurement for the response variable, this study did not apply a weight during model derivation. Models were not weighed because a large proportion of the studies did not report standard errors for their digestibility estimates, and weighting based on number of animals is an imperfect representation of information credibility. If models were derived thorough an approach that weighted observations based on their credibility, a large proportion of the studies would not be used in model derivation due to unreported standard errors and thus their effective weight would be zero. Weighting of data used in meta-analyses provides greater confidence in inference when conclusions are drawn across very different experimental conditions. However, in this case, weighting would have decreased the potential to develop thorough models representing the data available in the literature. Nevertheless, the lack of weighting is a major limitation of this work and may influence the credibility of the models in extrapolating past the context of the derivation data.

For each model, a 4-step derivation process was used as described in our previous works (Roman-Garcia et al., 2016; White et al., 2016). In brief, models were derived using a backward stepwise elimination approach. During the initial step, a full suite of explanatory variables was included, dictated by the combinations(s) of variables to be used for that model. The model was derived, and variables were checked for significance. The variable with the highest *P*-value was identified and removed, after which the model was derived again. This process of sequential elimination of the variable with the highest *P*-value continued until a model was identified where all variables were significant ($P<0.05$) or tending toward significance ($P<0.10$). For models where interactions were considered, non-significant main effects were retained within models when they were a component of a significant interaction term. Because missing values can cause instability in the backward stepwise elimination procedure, we then checked each omitted variable for significance within the final model individually. In cases where an omitted variable was identified as significant and improved the fit of the model, it was retained within the model. After checking omitted variables within the final model, the variance inflation factors (VIF) were calculated for all explanatory variables. We targeted a VIF < 10 for main effects and < 500 for interactions in models with multiple interaction terms (Akinwande et al., 2015).

Evaluating Model Performance

Following the objective of this work, we evaluated models based on their ability to explain the variation inherent within the dataset. The fit statistics most closely evaluated were the adjusted and unadjusted concordance correlation coefficient (CCC) (Lin, 1989), the adjusted and unadjusted root mean square errors (RMSE, % mean), the sigma hat errors ($\hat{\sigma}_e$) and corrected Akaike information criterion (AICc) (Hurvich and Tsai, 1993). Fit statistics referenced as "adjusted" refer to those that include the estimated random study effects when predicting digestibility outcomes. Those referenced as "unadjusted" refer to those that do not include the estimated random study effects when predicting digestibility outcomes. Although the inclusion of the random study effects is important for controlling a known source of variability (i.e., random differences in observations among studies), their use when comparing model estimates and predicted outcomes can lead to false confidence in estimating variability explained by the primary elements of the model.

In addition to evaluating models using the fit statistics, we also evaluated their practical performance by exploring predictions generated by each model across a range of simulated diet conditions. Specifically, we checked that models were able to predict digestibility values that were within the range of those observed within the data as model inputs were shifted to reflect diet composition included in the data. The point of this simulation testing was to identify models that become unstable in some diet combinations. For example, when a model began to predict digestibility values of >100, it would be identified as an unstable model. Typically, this instability can occur due to overfitting or overreliance on study effects in explaining variation within the dataset. In some cases, the model with the best fit statistics was less stable than a model with similar but less ideal fit statistics. In these cases, stability was prioritized, and the model with greater stability but less ideal fit statistics was selected for use in estimating DE.

Estimating DE from Digestibility Equations

In addition to exploring how models differed in their capacity to explain variation within the dataset, we also explored how models of nutrient digestibility could be used to estimate dietary DE concentration. We accomplished this objective by estimating total digestible nutrient (TDN) percentage in the diet in two ways. First, we selected the measured data (M) derived model for each key nutrient (CP, NDF, EE, and NFC) and secondly, we picked one derived model from calculated dietary values (C) for the nutrients of CP, NDF, EE, NSC, and rOM. Following the approach used in previous literature, TDN was then used to estimate dietary DE. Although the derivation of the equations presented in the following text is reported in the results of this work, we included specific equations in text here for clarity of explanation. For use within the broader context of estimating DE using the M models, we selected the CP digestibility (D_{CP}, % of CP) function:

$$D_{CP} = 118 - (0.974 \times NDF) - (0.246 \times Starch)$$

where, *NDF* is dietary neutral detergent fiber percent, and *Starch* is dietary starch percent.

The NDF digestibility (D_{NDF}, % of NDF) function selected was:

$$D_{NDF} = 34.3 + (0.780 \times EE) + (0.508 \times NSC)$$

where, *EE* is dietary ether extract percent and *NSC* is dietary non-structural carbohydrate percent.

The EE digestibility (D_{EE}, % of EE) function:

$$D_{EE} = -198 + (7.59 \times EE) + (16.9 \times DMI) + (0.154 \times DM) + (1.99 \times OM)$$

where, *DMI* is dry matter intake as a percent of metabolic body weight, *DM* is dietary dry matter percent, and *OM* is dietary organic matter percent.

The NFC digestibility (D_{NFC}, % of NFC) function was:

$$D_{NFC} = 3.98 + (0.878 \times ADF) + (2.01 \times NFC)$$

where, *ADF* is dietary acid detergent fiber percent and *NFC* is dietary non-fiber carbohydrate percent.

Using these estimates of nutrient digestibility for the M models, TDN percent within the diet was calculated as:

$$TDN = (CP \times D_{CP} \div 100) + (NDF \times D_{NDF} \div 100) + ((EE \times D_{EE} \div 100) \times 2.25) + (NFC \times D_{NFC} \div 100).$$

For use within the broader context of estimating DE using the C models, we selected the CP digestibility (D_{CP}, % of CP) function:

$$D_{CP} = 60.4 + (1.61 \times CP) + (0.814 \times ADF) - (48.9 \times Forage) - (0.0858 \times CP \times ADF) + (3..20 \times CP \times Forage)$$

where, *Forage* is the proportion of all forage feed stuffs and *CP* is dietary crude protein percent.

The NDF digestibility (D_{NDF}, % of NDF) function selected was:

$$D_{NDF} = 108 - (18.2 \times DMI) - (2.37 \times CP) - (1.36 \times NDF) + (0.0550 \times NDF \times CP) + (0.381 \times NDF \times DMI).$$

The EE digestibility (D_{EE}, % of EE) function:

$$D_{EE} = 42.3 + (0.937 \times EE) + (0.348 \times Starch).$$

The NSC digestibility (D_{NFC}, % of NSC) function was:

$$D_{NSC} = 139 - (0.911 \times NDF) - (15.4 \times Legume)$$

where, *Legume* is the proportion of all legume-based feed stuffs.

The rOM digestibility (D_{NFC}, % of rOM) function was:

$$D_{rOM} = 17.5 + (1.85 \times NFC).$$

Using these estimates of nutrient digestibility for the C models, TDN percent within the diet was calculated as:

$$TDN = (CP \times D_{CP} \div 100) + (NDF \times D_{NDF} \div 100) + ((EE \times D_{EE} \div 100) \times 2.25) + (NSC \times D_{NSC} \div 100) + (rOM \times D_{rOM} \div 100)$$

where, rOM was calculated for each feedstuff as the difference of OM – (CP + NDF + NSC + EE). If feed OM content was not provided, then it was substituted with the difference of 100 – Ash content. If feed NSC content was not provide, then it was substituted with the sum of feed starch + sugar content.

Finally, for both the measured and calculated models to convert from TDN to DE (Mcal/kg), we used the function:

$$DE = \frac{(4.4 \times TDN)}{100}$$

where *TDN* is total digestible nutrient percent.

After developing this system of equations for estimating DE, we compared these approaches to Pagan (Pagan, 1998) and the equine NRC (NRC, 1989) across a variety of example rations.

The example diets utilized for the comparison were: 1) a completely forage based diet; 2) a ration containing 60% forage and 40% beet pulp; 3) a ration containing 60% forage and 40% rolled oats; 4) a ration containing 60% forage and 40% Purina Ultium (Purina Ultium Competition Feed, Land O'Lakes Inc., The Purina Animal Nutrition Center, Gray Summit, Missouri), a well-known and commonly used performance horse feed; and 5) a ration containing 60% forage and 40% Triple Crown Senior (Triple Crown Senior Feed, Triple Crown Nutrition Inc., Wayzata, Minnesota) with the first ingredient as beet-pulp, a non-forage fiber. These diets were specifically selected to compare performance of the DE estimation approaches against rations reflecting historical feeding strategies (diets 1 and 3) compared with more modern feeding strategies (diets 2, 4 and 5).

The equation from Pagan (Pagan, 1998) used to compare with our system is:

$$DE\ x\ \left(\frac{Mcal}{kg}DM\right)$$

$$= \left(2{,}118 + 12.18\ \times\ (CP) - 9.37\ \times\ (ADF) - 3.83\ \times\ (\%hemicellulose) + 47.18\ \times\ (Fat) + 20.35\ \times\ (\%NFC) - 26.3\ \times\ (Ash)\right)\ \div 1000$$

where,

$$Hemicellulose\ (\%) = NDF - ADF$$

and,

$$Non - Fiber\ Carbohydrates\ (NFC) = (100 - \%NDF - \%Fat - \%Ash - \%\ CP)$$

and, if a feed contained greater than 5% ether extract or crude fat content, the estimated DE was increased by 0.044 Mcal/kg for each one percent of fat over 5 percent.

Two equations were utilized for comparison with the NRC (NRC, 1989). The first equation was used for dry forages and roughages, pasture, range plants, and forages fed fresh:

$$DE \times \left(\frac{Mcal}{kg}\right) = 4.22 - 0.11\ \times\ (\%ADF) + 0.0332\ \times\ (\%CP) + 0.00112\ \times\ (\%ADF^2)$$

The second NRC (NRC, 1989) equation was used for energy feed and protein supplements:

$$DE\ \times\ \left(\frac{Mcal}{kg}\right) = 4.07 - 0.055\ \times\ (\%ADF).$$

Independent Evaluation of DE Estimation Equations

For independent evaluation of our two systems for estimating the digestible energy (DE) content of feeds based on chemical composition, we used the DE values reported in the literature within our data set. A total of 63 treatments from 17 studies were included in this evaluation. For each treatment, we used the following values from each publication: average animal bodyweight (BW), dry matter intake (DMI, kg/day), diet percent inclusions (percentage of each feedstuff in the diet), the chemical composition of each feedstuff or diet (% DM), and the measured DE content (Mcal/kg). The energy content of each feed or treatment was included only if the publication explicitly stated that a bomb calorimeter was used for determination of energy content of feed and feces in the materials and methods. Studies providing DE content (Mcal/kg) as an estimation using other equation systems, were excluded. If a study provided the gross energy (GE) intake and GE digestibility of each treatment, these values were used to calculate DE (DE, Mcal/kg = ((GE Intake x GE Digestibility (%))/DMI)). For studies that did not report the chemical composition for all nutrients (DM (% as-fed), CP, EE, NDF, ADF, NSC, NFC, Starch, and OM or Ash, % DM), the average composition values used in the primary analysis were substituted. When Ash was provided rather than OM, OM was calculated as the difference: 100% minus the total Ash content (%). Since residual organic matter (rOM) is not typically reported, it was calculated for each feedstuff as OM – (CP + NDF + EE + NSC).

In addition to comparing DE content of each treatment to our models, we also compared them to the models developed by Pagan (1998) and NRC (1989). To accurately apply the Pagan (1998) system to treatments containing feeds with fat contents higher than 5%, we adjusted the Pagan equation by adding 0.044 Mcal/kg for each one percent fat over five percent. For treatments without feeds exceeding five percent fat content, this adjustment was not necessary. Furthermore, to account for the specific feedstuffs used in each treatment, the proportion of each feedstuff within the total diet was also calculated if it fell into any of the following categories: *Forage, Legume, NFF, Cereal,* and *Oil.* Figure 4 presents four different scatterplots to represent each of the four prediction models compared to the observed or measured DE values from the literature. For each model, the root mean squared prediction error (RMSPE, % mean) was used in the evaluation to measure how well each DE model system could predict the DE content of various experimentally measured feeds. The RMSPE is the percent error compared to the ground truth values of the experimentally measured DE (Hodson, 2022). The supplementary files include table S5, which provides the values used in the plot in a table format, along with the DMI and average BW for each study.

Results and Discussion

Factors Influencing Digestibility of DM (DMD)

Models of DMD were able to represent most of the variation within the literature (Table 3). The five DMD models ranged from a $\hat{\sigma}_e$ of 4.33% to 5.58%, where the model considering feedstuffs and calculated dietary values (CD-I-F) was least successful at explaining variation (0.834 CCC). The models using measured data and calculated data without interactions were most successful at explaining variation in the dataset. The second most successful (CD) model represented a complicated model (0.879 CCC, 5.58% $\hat{\sigma}_e$).

Dietary NDF percent was related to DM digestibility only in the MD model (Table 3). The relationship between NDF percent and DM digestibility identified by this investigation aligns with previous studies that concluded that fiber content was a better predictor of DMD in forages than CP (Edouard et al., 2008). Hansen and Lawrence (Hansen and Lawrence, 2017) determined that although most nutrients were associated with DMD, NDF concentration represented the best predictive factor. Specifically for forages, the more mature the forage, the higher NDF or cell-wall components it will possess, which could make it less appealing to horses. Other studies have established that for ruminant species, NDF content is highly relevant to the DMD of feedstuffs (Duncan et al., 1990; Du et al., 2016; de Souza et al., 2018).

The CD model showed a considerable indirect relationship between dietary ether extract (EE) intake and DMD (Table 3). This relationship was previously identified experimentally by Delobel et al (Delobel et al., 2008) who noted that DM digestibility was much higher in a high fat ration (9.6% EE) than the low-fat diets (2% EE). Both diets included the same low-fat concentrate (2% EE, DM) but the high-fat diet was supplemented with 80 g of linseed oil to achieve an EE content of 9.6% (% DM) (Delobel et al., 2008). The impact of fat in livestock rations is often specifically dissected into supplemental fat (i.e., fat coming from fats and oils added into the ration) and fat in dietary ingredients. Interestingly, Bush et al (Bush et al., 2001) found that additional fat increases the *in-vitro* DMD of alfalfa but overall, the digestibility of DM was not influenced by fat supplementation. Collectively, these results may suggest that total dietary fat, or fat coming primarily from ingredients, may be the primary driver of associations with DMD. In this study, we accounted for supplemental fat as oil and did not derive any models where oil was influential on DMD but, future quantitative exercises may benefit from exploring these differences in more depth given the importance of high fat diets in modern equine nutrition.

Factors Influencing Digestibility of CP (CPD)

Table 4 highlights how models of CPD accounted for notable variation within the literature. The CPD models ranged from an $\hat{\sigma}_e$ of 3.22% to 5.43%, where the model utilizing only measured data was most successful at explaining variation (0.0.925 CCC, 5.03% $\hat{\sigma}_e$). Similarly, the model focused on both interactions and feed variables was also able to explain a large proportion of variation in the data set (0.886 CCC, 3.22% $\hat{\sigma}_e$).

In most models, there was a strong, positive relationship between dietary CP percent and CP digestibility (Table 4). Multiple studies have this link between dietary CP percent (% DM) and CP digestibility (Oliveira et al., 2015; Saastamoinen and Särkijärvi, 2020; Vasco et al., 2021). For example, Oliveira et al. (2015) found increasing the CP content of horse diets improved CPD, up to 11.6% CP in the diet. However, since all the studies in our dataset were apparent digestibility trials, a limitation of our work is the potential influence of endogenous loss on apparent digestibility values. Protein, like fat, is a nutrient known to contribute to endogenous losses in feces. For instance, in one study described by the NRC (2007), endogenous nitrogen (N) loss was estimated to be 5.8 mg N/g DMI, and the true prececal digestibility of forage protein in that study was 37% (Lindberg and Karlsson, 2001). The very limited number of publications assessing true versus apparent equine digestibility which limits the ability to robustly estimate endogenous losses. Further work on true total tract digestibility in horses is necessary to fully address this gap.

Other critical relationships involved indirect associations of NDF and forage on CPD, and interactions of ADF and CP driving shifts in CPD. There is limited experimental work to draw upon within equine literature to explain these associations. However, increasing dietary NDF may reflect more CP coming from forage sources, or bound in forage cell walls, which would contribute to lower CPD. In pigs, dietary fiber both decreased apparent CP digestibility (Zhang et al., 2013) and triggered an increase in endogenous protein losses (Jansman et al., 2002). The negative association between NDF and CPD has also been observed previously in ruminant species (Yang et al., 2018). The interactions between ADF and CP are more challenging to interpret, but may be reflective of pasture versus dry forages, and the impact of forage maturity in those settings on CP digestibility. Although not specifically assessed in this study, Bockisch et al. (2023) suggested that CP digestibility can be predicted using fiber-associated nitrogen, which could be added as a variable in future work. Further investigation on these specific relationships would help refine the confidence around these equations and confirm their utility in estimating CPD in equine rations.

Factors Influencing Digestibility of NDF (NDFD)

Like those derived for other nutrients, models of NDFD were largely able to explain variation within the dataset (Table 5). The NDF digestibility models ranged from a $\hat{\sigma}_e$ of 4.41% to 7.49%, where the model with the calculated diet information and feedstuffs was least successful at explaining variation (0.762 CCC). The model utilizing only measured data displayed the most favorable fit statistics (0.906 CCC, 5.84% $\hat{\sigma}_e$), but this is likely due to the sample size (*n* = 42) differing drastically from the other four models (*n* ranging from 221 to 228).

Two models included a positive and negative relationship between EE and NDF digestibility (Table 5). Jansen et al (Jansen et al., 2002) observed that a high-fat diet with a soybean oil content of 158 g per kg DM depressed apparent fiber digestibility compared to a primarily corn starch and glucose ration. On the other hand, Delobel et al (Delobel et al., 2008) found that a higher fat diet correlated with an increase in NDFD. In an *in-vitro* study, additional dietary fat increased *in-vitro* NDF digestibility of rolled oats, but no other tested feedstuffs were affected by the treatment (Bush et al., 2001). The inconsistency of the association between EE and NDF digestibility is also identified in summaries of rations fed to ruminants (Nawaz and Ali, 2016; Weld and Armentano, 2017). There are several possible reasons why some diets may show higher NDFD when EE is increased, including the basal rate of EE in the ration, the primary fiber sources, and the type of fat fed. For this possibility, we added the feed type of Oil to account for supplementary fat added to equine diets and like dietary EE, displayed a positive relationship with NDFD. More in-depth reporting of fatty acid composition of fats used in equine rations may benefit future modeling exercises designed to further explore how fat sources can support or negatively impact fiber digestibility.

Dietary NDF percent was also identified by several models as a primary driver of NDF digestibility (Table 5). This negative relationship between dietary NDF (%) and NDFD was apparent in the three models including interactions. This result was supported with the literature, where we found publications that exhibited negative influences of dietary NDF content on NDFD. Godwin et al (Godwin et al., 2021) found through an *in*-vitro study that NDFD was negatively related to NDF content of hays in horses. Previously, NDF was preferred to as “cell wall constituents” (CWC) (Rittenhouse, 2014) and Fonnesbeck (Fonnesbeck, 1968) found that as CWC content increased with forage maturity, digestibility of nutrients decreased. Similarly, a relationship has been observed in ruminant species where NDFD was negatively correlated with NDF content (Schulze et al., 2014; Du et al., 2016). The negative relationship between dietary NDF content and NDFD correlates clearly with the general understanding that as forages decline in quality, overall digestion decreases.

Factors Influencing Digestibility of EE (EED)

Table 6 shows that models of EED successfully captured the variability found in the literature. The five EED models ranged from a $\hat{\sigma}_e$ of 6.00% to 14.4%, where the model with calculated diet information and no interactions was least successful at explaining variation in the dataset (0.813 CCC, 14.4% $\hat{\sigma}_e$). The model involving measured dietary values only performed the best at variation explanation (0.955 CCC, 6.00% $\hat{\sigma}_e$). The evidential challenge to explain variation in fat digestibility could pertain to the different methods for fatty acid versus ether extract measurements or fewer studies measuring specifically for EED.

In four of the five models, there was an important relationship between dietary ether extract (EE) content and EE digestibility (Table 6). In the models considering measured values (MD) and calculated values (CD), there was an apparent positive influence of dietary EE on EED, where an increase in dietary EE increased the estimated digestibility of EE. This correlates with the literature where there was evidence of a positive influence on EED by dietary EE. Specifically, Bush et al (Bush et al., 2001) found that fat digestion was highest for their high fat-containing diets of 21.4% EE (DM) from 15% corn oil than their control low-fat diets (0 to 10% corn oil; 5.6 to 15.5% EE, % DM). Additionally, all models align with Lindberg et al (Lindberg and Karlsson, 2001), who found that their diets with the lowest fat content had the lowest corresponding EED compared to other higher fat rations.

Apparent digestibility does not account for the endogenous losses in feces like true digestibility and there are few publications available to provide enough true digestibility data for development of estimation equations. Particularly for fat, as the amount of dietary fat increases, apparent digestibility also increases because endogenous losses remain constant. Fat digestibility is also thought to vary considerably with fat source. To address this, the feed type “Oil” was incorporated to account for supplemental dietary fat separately from basal feed fat content. The addition of supplemental oil in equine diets appears to positively influence EED, as shown in our CD-F model in Table 6. There are several older studies that reported an increase in apparent digestibility of fat after the addition of fat (e.g., oil) to the diet (Kane and Baker, 1977; Kane et al., 1979; Rich et al., 1981; McCann et al., 1987; Scott et al., 1989; Potter et al., 1992; Hughes et al., 1995; Julen et al., 1995). Kronfeld et al. (2004) assessed both the apparent and true digestibility of three diets: forage only, mixed feeds with added fat, and added fat diets. Their findings support our results, where the most apparent digestible diet contained added fats (Kronfeld et al., 2004). For true digestibility, the added fat diets reached almost 100% digestibility, with an endogenous fecal fat loss of 0.22 g/kg BW per day (Kronfeld et al., 2004). A limitation of our study is the inability to account for endogenous fat, which affects the accuracy of the digestibility equations. Future work on high fat diets (fat >5%) in horses should focus on true digestibility rather than apparent digestibility of fat. The significance of dietary EE influence on EED relates to the practice of feeding performance horses with higher energy requirements supplementary fat rather than cereal grains to decrease the risk of digestive ailments associated with high starch content (Lindberg and Karlsson, 2001). The extensively tested effects of EE on EED display that feeding high amounts of fat might not impede the digestion of that supplemental fat, thus be utilized more by the equine body to maximize efficiency and overall animal performance.

Factors Influencing Digestibility of NFC (NFCD) and Residual Organic Matter (rOMD)

The one measured data model (MD) for NFC digestibility and three calculated data models (CD, CD-I, CD-F) for residual organic matter (rOM) digestibility accounted for substantial variability reported in the literature (Table 7). The three rOMD models ranged from an $\hat{\sigma}_e$ of 4.21% to 13.9%, where the model of calculated diet information with no consideration for interactions was least successful at explaining variation (0.740 CCC, 13.9% $\hat{\sigma}_e$). Between the other two rOMD models, the model performed and behaved well to explain dataset variation included calculated diet information and their interactions (0.722 CCC, 4.21% $\hat{\sigma}_e$). Although not a direct comparison, the MD model for NFCD exhibited ideal ability to account for literature variability (0.994 CCC, 1.68% $\hat{\sigma}_e$). It is important to recognize that there was an insufficient amount of data to develop models with interactions and interactions with feedstuffs.

Within the only NFCD model, the dietary factors of ADF and NFC appeared to have the most influence on NFCD. There was a positive influence of dietary ADF (%) and of dietary NFC content on NFCD in the model. In terms of the literature, there is a substantial gap in research such that no studies were found to assess non-fiber carbohydrate digestibility. This may be due to word-type confusion within the industry. Specifically, for many years non-structural carbohydrate (NSC) content was determined by subtracting the amount of NDF, CP, EE, and ash from total DM but more recently, a change was made for NFC to represent that difference instead (NRC, 2007). Therefore, the term NSC became primarily used to describe a chemically analyzed fraction of a feed (Council, 2001; NRC, 2007). That said, there are several studies assessing non-structural carbohydrates and starch digestibility, but those efforts may need to consider assessing the understudied NFC digestibility in conjunction with future NSC trials (Zeyner et al.; Varloud et al., 2004; Jouany et al., 2008).

Factors Influencing Digestibility of NSC (NSCD)

Similar to the other nutrient models derived, the models for NSC digestibility were representative of the variation in the dataset (Table 8). These models displayed an $\hat{\sigma}_e$ from 1.31% to 3.27%, where the model using only measured values was least successful at explaining variation (0.898 CCC, 3.27% $\hat{\sigma}_e$). The model accounting for feedstuffs (CD-F) performed and behaved the best with a CCC of 0.969 and $\hat{\sigma}_e$ of 1.31%. During model derivation, there were several variables that influenced NSCD, but these five models were best for each category, respectively. In our supplementary tables, we shared our models developed for starch digestibility specifically.

In three of the five models, dietary NSC percent was strongly correlated with NSC digestibility (Table 8). The MD model displayed a positive relationship, whereas the CD-I and CD-I-F models showed a negative influence of dietary NSC (%) on NSCD. These contrasting results may be attributed to the analytical procedures used to measure NSC in feed as NSC is typically calculated as the sum of dietary starch and water-soluble carbohydrates (WSC) (NRC, 2007). However, whether it accounts for all NSC is laboratory dependent (NRC, 2007). In general, NSC digestibility is high (>90%) because of the starch component, which is known to be a source of high energy for horses (NRC, 2007). While starch is highly digestible, excessive starch intake can be detrimental in digestive tract health in horses, which could also explain the variation in our results (NRC, 2007). Additionally, the processing method of feed, particularly high-starch feeds, can influence starch (or NSC) digestibility (Julliand et al., 2006; NRC, 2007). Our study did not account for effects of processing on digestibility because studies in our dataset did not consistently report feed processing methods. However, future work should consider incorporating processing methods, especially for estimating the digestibility of NSC and starch.

Use of Digestibility Functions in Estimating Digestible Energy (DE)

Overall, our calculated data (C) equation for estimating DE behaved more similarly to the system created by Pagan (Pagan, 1998) than the NRC (NRC, 1989) equation (Fig. 3). Our measured data (M) equation produced the highest DE estimation for all example rations and may be less reliable than our C equation due to the limited data available for derivation. On the other hand, the NRC (NRC, 1989) system resulted in the lowest DE estimation for all rations, in comparison to the other three systems. The first three example diets (forage only (1), forage plus beet pulp (2), and forage plus oats (3)), which are all at or below 5% in fat content, were on average estimated to have a higher DE content (1: 2.24; 2: 2.41; 3: 2.77 Mcal/kg) than what the Pagan (1998) system predicted. The opposite was true for the diets with Purina Ultium (4) a performance commercial concentrate, and Triple Crown Senior (5), an NFF-based commercial concentrate. Our calculated equation estimated the DE content lower for both diets (4: 2.58; 5: 2.53 Mcal/kg) than Pagan (1998) (4: 2.66; 5: 2.57 Mcal/kg).

The current equations as published in the equine NRC (NRC, 2007) utilized for estimating DE of horse feeds from feed chemical composition were originally published in the NRC 1989 edition. The equations were based on work by Fonnesbeck (Fonnesbeck, 1981), who summarized data from 108 *in-vitro* or *in-vivo* equine digestibility studies pre-1981 and did not report any specific information about the feedstuffs used in the digestibility trials (NRC, 2007). An alternative equation used for estimating DE in horse feed from chemical composition was developed by Pagan (Pagan, 1998) from 30 different diets or 120 observations. The NRC (NRC, 1989) has two equations, one for fresh and dry forages and one for energy feeds and protein supplements while Pagan (Pagan, 1998) utilizes one equation for estimation of all feedstuffs. Pagan (Pagan, 1998) reported that their equation was similar for many feeds to the NRC (NRC,

1989) but noted that neither equation accurately predicted DE content of some high-fiber feeds, and feeds that were high in fat (NRC, 2007). To make up for this, Pagan (Pagan, 1998) recommended that an adjustment in their equation should be made for feeds with more than five percent fat by increasing the DE (as-fed basis) by 0.044 Mcal/kg for each one percent of fat above five percent (NRC, 2007). This adjustment was the reason that the Pagan (1998) equation estimated a higher DE content in diets four and five than our calculated equation. Without that change, the Pagan (1998) equation would have produced a lower DE estimate than ours.

Our calculated equation and the Pagan (1998) equation behaved more similarly than our measured data equation or the NRC (1989) equation, which both estimated DE on opposite ends of the scale. Other modeling systems for estimating DE by other authors have been published (Zeyner and Kienzle, 2002; Martínez Marín et al., 2022) but the systems by Pagan (Pagan, 1998) and the NRC (NRC, 1989) are two of the more well-known ways for estimating DE of equine feeds in the United States (NRC, 2007). Based on this comparison, an independent evaluation using experimentally measured DE was performed before confidently recommending the calculated diet equation system for ration formulation.

Evaluation of Measured (M) and Calculated (C) Equations Using Independent Data

Our two DE equations and the DE estimated by Pagan (1998) and NRC (1989) were compared to the reported DE values available within the literature. The RMSPE values (% mean observed DE) for our measured (M), our calculated (C), the Pagan (1998) and NRC (1989) systems were 0.515, 0.351, 0.339, and 0.343. The comparison of observed and predicted values for these systems is included in Figure 4. Overall, the C, NRC (1989), and Pagan (1998) systems had similar performance when compared to the literature data. All models showed slight mean and slope bias (Figure 4) with models tending to over-predict DE concentrations, with that over-prediction increasing in scale at higher predicted concentrations. The more empirical NRC and Pagan systems produced marginally improved RMSPE compared with the C system, though all systems performed better than the M system. This reflects a trade-off with the M system, although it is based on more reliable nutrient composition data, the smaller number of treatment means going into the equations make the system less able to represent the variation shown by the breadth of diets available for evaluation. More consistent reporting of nutrient composition information data in equine nutrition studies will be essential to minimize this trade off in the future (McNamara et al., 2016).

Limitations

There are a number of data and analysis limitations that should be considered relative to this work. These include, among others, the reliance on feed database nutrient composition data, the inability to account for endogenous losses, and the limited accounting of factors known to influence nutrient digestibility in equine rations.

As detailed in section 2.1, we did not have measured data for every feedstuff and therefore, had to resort to using average feed library values. Although it is likely that the average horse owner would estimate the composition of their horse's feed by using publicly available feed composition databases, there are known challenges associated with using these feed composition tables in meta-analyses (Schlageter-Tello et al., 2020). Feed databases often suffer from feed misclassification of feed samples, incorrect units, and analytical mistakes. Because our models rely on the values coming from these databases, they could be affected by these limitations. Broader reporting of measured feed composition values in future work will be essential to prevent this limitation as new digestibility equations are derived.

Endogenous losses of protein and fat are also likely to influence apparent total tract digestibility measurements. Due to the limited reporting of endogenous losses in equine diets, we were unable to convert the digestibility metrics to a true basis, which would have allowed for more consistent comparisons across nutrient and feed intake levels. The lack of accounting of endogenous losses should be considered when applying the protein and fat digestibility models in external settings.

For our measured models, 40% of the diets with fat chemical composition provided were greater than 5% fat, with the highest fat content at 27.8%. For our calculated models, 12.1% of the diets contained a fat content of 5% or more, with the highest fat content estimated from databases as 63.7%. Due to the limited inclusion of high fat diets in the calculated models, the models may struggle to adequately represent diets with high fat content (greater than 5%). Given the relevance and importance of high fat rations in modern equine diets, this is an area of the equine digestibility literature that needs further development, as our dataset did not include enough studies and treatments using high fat diets.

A final limitation we would like to highlight is the limited capacity to account for factors known to influence digestibility in equine rations. In particular, we did not account for feed processing or feed physical form in our analysis due to limited information consistently available among studies. Future work exploring feed processing or physical form adjustments could help to address this limitation.

Conclusions

From the 54 studies and 181 observations, five models were developed for estimating nutrient digestibility of each response variable of DMD, CPD, NDFD, EED, NSCD, NFCD, and rOMD. Overall, the models involving calculated data appeared to be more reliable and stable than models including only measured data. Our equations to estimate DE produced similar results in example diets to two other established methods of estimating DE used within the North American feeding industry. When compared to experimentally measured DE, the calculated system produced more similar estimations than our measured system, but both produced realistic DE estimations. Still, the results imply that with continued refinement and testing on independent datasets, this approach has the potential to become a dependable method for estimating the DE of feed ingredients.

Supplementary Material

Tables S1, S2, S3, S4, and S5 are included as supplementary data associated with this article.

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Tables

Table 3-1. Summary of data contained in dataset

Variable	n	Mean	SD	Minimum	Maximum
Animal description					
n of Animals	248	7.75	4.54	3.00	24.0
Animal Age, years	235	8.99	3.58	0.801	19.5
DMI, % metabolic BW	246	1.71	0.571	0.422	3.88
BW, kg	248	449	117	113	636
Forage intake, kg/d	231	5.54	2.48	0.678	12.0
Concentrate intake, kg/d	213	2.39	1.76	0.00	8.89
Nutrient composition of total diet, % of DM					
DM (% as-fed)	190	89.2	7.28	0.919	97.4
CP	240	11.3	2.78	5.8	96.0
NDF	239	52.7	9.61	30.7	72.1
NFC	18	22.8	9.75	11.5	38.4
EE	163	3.72	3.89	0.80	27.8
ADF	227	29.0	7.92	3.60	46.3
OM	142	92.0	2.63	83.6	96.0
NSC	66	15.8	8.05	3.10	35.9
Starch	57	13.2	8.66	0.0984	30.6
Ash	131	6.28	1.99	0.63	10.7
Apparent total-tract nutrient digestibility, %					
DM	240	57.2	10.9	31.3	90.1
CP	225	63.6	11.0	30.0	83.6
NDF	230	44.4	11.6	11.3	77.0

NFC	12	77.8	10.6	55.6	97.7
EE	151	49.0	23.6	–38.1	87.7
ADF	202	39.0	12.6	–0.750	76.0
NSC	14	90.1	6.59	78.2	97.6
Starch	79	92.1	10.9	47.8	99.9
OM	78	54.8	8.79	33.4	73.3
rOM	12	66.4	19.6	34.7	98.9
Feed Type Inclusions					
Forage[b]	248[a]	0.725	0.238	0.14	1.33
Legume[c]	248[a]	0.0681	0.220	0.00	1.00
NFF[c]	248[a]	0.00783	0.0356	0.00	0.408
Cereal[e]	248[a]	0.0898	0.140	0.00	0.630
Oil[f]	248[a]	0.0123	0.0674	0.00	0.630

Abbreviations: ADF, acid detergent fiber; CP, crude protein; DMI, dry matter intake; OM, organic matter; NDF, neutral detergent fiber; NFC, non-fiber carbohydrate; EE, ether extract, DM, dry matter; NSC, non-structural carbohydrate; rOM, residual organic matter; BW, body weight; SD, standard deviation; NFF, non-forage fiber; kg, kilogram; d, day.

[a] n indicates number of treatments with feed type reported

[b] Forage diets included diets with legume hay, mostly legume hay, alfalfa pellets, mostly grass hay, grass hay, straw, alfalfa meal, oat hay, grass pasture, and/or Bermuda grass hay.

[c] Diets with legume forages included legume hay, mostly legume hay, alfalfa meal, and/or alfalfa pellets.

[d] Diets with NFF sources included beet pulp, linseed meal, and/or soybean hulls in kg per day.

[e] Diets with cereal grain included oats, barley grain, steam flaked corn, sorghum grain, maize grain, broken rice, maize germ meal, maize starch, sweet feed, and/or maize cobs.

[f] Diets supplemented with additional fat as oil including vegetable oil, canola oil, soybean oil, corn oil, and/or palm oil.

Table 3-2. Mean dietary composition (% of DM) of diets used in studies and included in the meta-analysis, including both (1) as reported and (2) when not reported in the data set but combined with values provided by Dairy One, Feedipedia or Mad Barn Feed Bank.

	As reported only			Reported and combined		
Item	n	Mean	SD	n	Mean	SD
DM (% as-fed)	190	89.2	7.27	248	89.0	8.60
OM	142	92.0	2.63	248	95.0	14.9
CP	240	11.3	2.78	248	11.4	2.73
NDF	239	52.7	9.61	248	53.0	10.6
ADF	227	29.0	7.92	248	28.9	7.57
EE	163	3.72	3.89	248	3.70	4.26
NSC	66	15.8	8.05	248	18.2	7.99
Starch	57	15.8	8.67	248	10.8	7.94
NFC	18	22.8	9.75	248	27.2	17.4

Abbreviations: ADF, acid detergent fiber; CP, crude protein; DMI, dry matter intake; OM, organic matter; NDF, neutral detergent fiber; NFC, non-fiber carbohydrate; EE, ether extract, DM, dry matter; NSC, non-structural carbohydrate; SD, standard deviation.

Table 3-3. Models Explaining Variation in Total Tract Digestibility of Dry Matter (DM) Using Measured Dietary Information (MD), Calculated Diet Information With (CD-I) or Without (CD) Interaction Terms and Feed Variables (CD-F, CD-I-F), Including Slope and Standard Error (SE)

	MD	CD	CD-I	CD-F	CD-I-F
Intercept	86.3 (4.15)	64.3 (7.62)	76.7 (7.55)	66.2 (2.20)	78.0 (7.64)
NDF^a	–0.702 (0.0681)				
DM^c			–0.282 (0.0840)		–0.280 (0.0860)
CP^a		1.13 (0.232)			
EE^a		0.284 (0.110)			
DMI^b	4.78 (1.57)	4.62 (1.59)			
NFC^a		0.491 (0.0733)	0.0644 (0.0315)		
OM^a		–0.473 (0.0836)			
NSC^a		0.145 (0.0675)			
Starch			–1.27 (0.598)		0.454 (0.0904)
Forage				–15.7 (2.58)	
Legume				9.94 (2.66)	
Cereal				16.8 (3.63)	–76.8 (39.8)
DM^c x Starch			0.0190 (0.00693)		
DM x Cereal					0.854 (0.449)
Fit Statistics					

n	225.0	238.0	234.0	234.0	234.0
Observed Mean[a]	57.2	57.2	57.1	57.1	57.1
Predicted Mean[a]	57.2	57.2	57.3	57.3	57.3
RMSE, % mean	8.69	8.94	9.93	9.86	10.1
Unadjusted RMSE, % mean	15.8	15.7	18.0	17.6	18.2
Mean bias, % of MSE	0.00	0.00	0.0627	0.100	0.0655
Slope Bias, % of MSE	1.28	1.40	1.06	1.07	1.10
RSR	0.809	0.814	0.941	0.907	0.949
CCC	0.883	0.879	0.840	0.842	0.834
Unadjusted CCC (uCCC)	0.690	0.684	0.573	0.583	0.555
AICc	1511	1623	1626	1588	1616
Sigma Hat Error	5.52	5.58	4.37	4.33	4.47

Abbreviations: MD, models using only measured dietary information; CD, models using calculated diet information; CD-I, models using calculated diet information and their interactions; CD-F, models using calculated diet information, their interactions, and feed variables; CD-I-F, models using calculated diet information, feed type variables, and all interactions; SE, standard error; CP, crude protein; EE, ether extract; OM, organic matter; NSC, non-structural carbohydrates; NDF, neutral detergent fiber; NFC, non-fiber carbohydrates; DM, dry matter; RMSE, root mean square error; CCC, concordance correlation coefficient; MSE, mean square error; RSR, Root mean standard deviation ratio; AICc, Akaike information criterion.

[a] % of DM

[b] % metabolic BW per day

[c] % of as-fed

Table 3-4. Models Explaining Variation in Total Tract Digestibility of Crude Protein (CP) Using Measured Dietary Information (MD), Calculated Diet Information With (CD-I) or Without (CD) Interaction Terms and Feed Variables (CD-F, CD-I-F), Including Slope and Standard Error (SE)

	MD	CD	CD-I	CD-F	CD-I-F
Intercept	118 (9.52)	68.9 (5.71)	27.0 (7.37)	35.3 (6.74)	60.4 (9.94)
DMI[b]			2.82 (1.58)		
NDF[a]	–0.974 (0.149)	–0.423 (0.0721)			
CP[a]		1.59 (0.248)	4.04 (0.554)	3.83 (0.532)	1.61 (0.807)
Starch[a]	–0.246 (0.136)				
ADF[a]			0.471 (0.230)	0.790 (0.217)	0.814 (0.221)
Forage				–14.8 (3.03)	–48.9 (10.1)
Legume				10.7 (2.57)	
CP[a] x ADF[a]			–0.0789 (0.0189)	–0.0833 (0.0185)	–0.0858 (0.0188)
CP[a] x Forage					3.20 (0.872)
Fit Statistics					
n	49.0	225	221	223	223
Observed Mean[a]	63.3	63.5	63.5	63.5	63.5
Predicted Mean[a]	63.4	63.5	63.8	63.9	63.9
RMSE, % mean	6.95	7.66	7.88	7.74	7.62
Unadjusted RMSE, % mean	19.52	13.8	14.0	12.3	12.5
Mean bias, % of MSE	–0.00	–0.00	0.508	0.517	0.505
Slope Bias, % of MSE	0.834	1.43	1.51	1.43	1.37
RSR	1.06	0.813	0.831	0.726	0.737

CCC	0.925	0.884	0.878	0.881	0.886
Unadjusted CCC (uCCC)	0.243	0.652	0.631	0.705	0.696
AICc	336	1513	1477	1456	1462
Sigma Hat Error	5.03	5.43	3.34	3.4	3.22

Abbreviations: MD, models using only measured dietary information; CD, models using calculated diet information; CD-I, models using calculated diet information and their interactions; CD-F, models using calculated diet information, their interactions, and feed variables; CD-I-F, models using calculated diet information, feed type variables, and all interactions; SE, standard error; CP, crude protein; DMI, dry matter intake; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; DM, dry matter; RMSE, root mean square error; CCC, concordance correlation coefficient; MSE, mean square error; RSR, Root mean standard deviation ratio; AICc, Akaike information criterion.

[a] % of DM

[b] % metabolic BW per day

Table 3-5. Models Explaining Variation in Total Tract Digestibility of Neutral Detergent Fiber (NDF) Using Measured Dietary Information (MD), Calculated Diet Information With (CD-I) or Without (CD) Interaction Terms and Feed Variables (CD-F, CD-I-F), Including Slope and Standard Error (SE)

	MD	CD	CD-I	CD-F	CD-I-F
Intercept	34.3 (4.53)	40.5 (6.94)	108 (17.8)	79 (12.6)	95.6 (19.6)
DMI^b		3.37 (1.98)	–18.2 (7.85)	–15.2 (8.09)	3.40 (1.86)
EE^a	0.780 (0.224)				–14.5 (4.70)
CP^a		0.594 (0.324)	–2.37 (0.988)		–1.88 (1.06)
NDF^a			–1.36 (0.316)	–0.741 (0.226)	–1.08 (0.351)
ADF^a		–0.299 (0.145)			
NSC^a	0.508 (0.176)				
NFC^a					1.12 (0.385)
Starch^a					–0.169 (0.0914)
Legume				–6.57 (187)	–12.2 (3.60)
Oil					2520 (856)
NDF^a x CP^a			0.0550 (0.0184)		0.0489 (0.0191)
NDF^a x EE^a					0.237 (0.0883)
NDF^a x NFC^a					–0.0202 (0.00768)
NDF^a x DMI^b			0.381 (0.137)	0.348 (0.142)	
NDF^a x Oil					–50.5 (20.1)
Fit Statistics					

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n	42.0	228.0	221.0	221.0	221.0
Observed Mean[a]	43.6	44.3	44.2	44.2	44.2
Predicted Mean[a]	43.6	44.3	44.5	44.6	44.5
RMSE, % mean	11.7	15.2	15.4	15.7	15.4
Unadjusted RMSE, % mean	24.0	26.3	25.5	25.5	24.7
Mean bias, % of MSE	0.00	0.00	0.284	0.311	0.186
Slope Bias, % of MSE	1.07	2.95	2.60	2.46	2.59
RSR	0.874	1.01	0.975	0.982	0.943
CCC	0.906	0.776	0.775	0.762	0.775
Unadjusted CCC (uCCC)	0.558	0.367	0.408	0.381	0.440
AICc	297	1668	1585	1579	1578
Sigma Hat Error	5.84	7.49	4.41	4.52	4.40

Abbreviations: MD, models using only measured dietary information; CD, models using calculated diet information; CD-I, models using calculated diet information and their interactions; CD-F, models using calculated diet information, their interactions, and feed variables; CD-I-F, models using calculated diet information, feed type variables, and all interactions; SE, standard error; CP, crude protein; NFC, non-fiber carbohydrates; EE, ether extract; CP, crude protein; DMI, dry matter intake; NSC, non-structural carbohydrates; NDF, neutral detergent fiber; ADF, acid detergent fiber; DM, dry matter; RMSE, root mean square error; CCC, concordance correlation coefficient; MSE, mean square error; RSR, Root mean standard deviation ratio; AICc, Akaike information criterion.

[a] % of DM

[b] % metabolic BW per day

Table 3-6. Models Explaining Variation in Total Tract Digestibility of Ether Extract (EE) Using Measured Dietary Information (MD), Calculated Diet Information With (CD-I) or Without (CD) Interaction Terms and Feed Variables (CD-F, CD-I-F), Including Slope and Standard Error (SE)

	MD	CD	CD-I	CD-F	CD-I-F
Intercept	–198 (91.2)	42.3 (4.48)	105 (33.8)	63.2 (6.76)	89.3 (8.42)
EE[a]	7.59 (1.01)	0.937 (0.275)	–27.1 (13.5)		–2.29 (0.672)
DMI[b]	16.9 (6.12)				
DM[c]	0.154 (0.0754)		–0.673 (0.391)		
OM[a]	1.99 (0.957)				
Starch[a]		0.348 (0.202)			
Forage				–19.2 (8.72)	–81.7 (14.5)
Oil				72.5 (28.5)	
EE[a] x DM[c]			0.320 (0.155)		
EE[a] x Forage					10.4 (2.04)
Fit Statistics					
n	56.0	149.0	149.0	149.0	149.0
Observed Mean[a]	52.6	49.1	49.1	49.1	49.1
Predicted Mean[a]	52.6	49.1	49.9	50.0	50.0
RMSE, % mean	9.63	26.0	25.5	25.5	23.4
Unadjusted RMSE, % mean	29.2	44.6	46.0	43.4	40.8
Mean bias, % of MSE	0.00	0.09	0.390	0.462	0.465
Slope Bias, % of MSE	0.744	3.13	2.98	3.98	3.90
RSR	0.881	0.938	0.974	0.920	0.843
CCC	0.955	0.813	0.815	0.812	0.847
Unadjusted CCC (uCCC)	0.561	0.318	0.302	0.370	0.559

AICc	407	1293	1277	1258	1239
Sigma Hat Error	6.00	14.4	6.73	6.92	6.34

Abbreviations: MD, models using only measured dietary information; CD, models using calculated diet information; CD-I, models using calculated diet information and their interactions; CD-F, models using calculated diet information, their interactions, and feed variables; CD-I-F, models using calculated diet information, feed type variables, and all interactions; SE, standard error; CP, crude protein; OM, organic matter; DMI, dry matter intake; EE, ether extract; DM, dry matter; RMSE, root mean square error; CCC, concordance correlation coefficient; MSE, mean square error; RSR, Root mean standard deviation ratio; AICc, Akaike information criterion.

[a] % of DM

[b] % metabolic bodyweight per day

[c] % of as-fed

Table 3-7. Models Explaining Variation in Total Tract Digestibility of Non-Fiber Carbohydrates (NFC) Using Measured Dietary Information (MD) and Total Tract Digestibility of Residual Organic Matter (rOM) Using Calculated Diet Information With (CD-I) or Without (CD) Interaction Terms and Feed Variables (CD-F, CD-I-F), Including Slope and Standard Error (SE)

	MD	CD	CD-I	CD-F	CD-I-F
Intercept	3.98 (13.3)	17.5 (21.9)	134 (21.1)	49.1 (14.5)	None
ADF[a]	0.878 (0.238)				
NDF[a]			–1.45 (0.389)		
NFC[a]	2.01 (0.198)	1.85 (0.697)			
Legume				40.4 (12.1)	
Fit Statistics					
n	10.0	12.0	12.0	12.0	
Observed Mean[a]	77.1	66.4	66.4	66.4	
Predicted Mean[a]	77.1	66.4	67.3	67.1	
RMSE, % mean	1.55	27.9	17.9	19.2	
Unadjusted RMSE, % mean	9.80	27.9	30.5	34.2	
Mean bias, % of MSE	0.00	0.00	0.488	–0.649	
Slope Bias, % of MSE	0.175	3.33	1.52	0.0854	
RSR	0.632	0.980	1.09	1.20	
CCC	0.994	0.740	0.722	0.678	
Unadjusted CCC (uCCC)	0.789	0.269	0.187	0.158	
AICc	72.1	107	109	103	
Sigma Hat Error	1.68	13.9	4.21	4.46	

Abbreviations: MD, models using only measured dietary information; CD, models using calculated diet information; CD-I, models using calculated diet information and their interactions; CD-F, models using calculated diet information, their interactions, and feed variables; CD-I-F, models using calculated diet information, feed type variables, and all interactions; SE, standard error; NFC, non-fiber carbohydrates; NDF, neutral detergent fiber; ADF, acid detergent fiber; RMSE, root mean square error; CCC, concordance correlation

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coefficient; MSE, mean square error; RSR, Root mean standard deviation ratio; AICc, Akaike information criterion.

[a] % of DM

Table 3-8. Models Explaining Variation in Total Tract Digestibility of Non-Structural Carbohydrates (NSC) Using Measured Dietary Information (MD), Calculated Diet Information With (CD-I) or Without (CD) Interaction Terms and Feed Variables (CD-F, CD-I-F), Including Slope and Standard Error (SE)

	MD	CD	CD-I	CD-F	CD-I-F
Intercept	79.3 (4.61)	126 (5.85)	556 (115)	139 (5.31)	184 (36.8)
NSC[a]	0.730 (0.236)		–19.6 (4.90)		–7.77 (2.33)
NDF[a]				–0.911 (0.0879)	
ADF[a]		–1.22 (0.183)			
DM[c]			–5.19 (1.24)		
Forage					–128 (41.3)
Legume				–15.4 (2.56)	
NSC[a] x DM[c]			0.220 (0.0528)		
NSC[a] x Forage					10.5 (2.73)
Fit Statistics					
n	14.0	14.0	14.0	14.0	14.0
Observed Mean[a]	90.1	90.1	90.1	90.1	90.1
Predicted Mean[a]	90.1	90.1	90.0	90.0	90.1
RMSE, % mean	2.95	1.91	2.10	0.896	2.05
Unadjusted RMSE, % mean	6.93	3.78	4.04	2.11	6.01
Mean bias, % of MSE	0.00	0.00	0.0122	0.896	0.0237
Slope Bias, % of MSE	0.0709	0.629	1.36	0.834	0.276
RSR	0.939	0.513	0.548	0.288	0.811
CCC	0.898	0.961	0.952	0.969	0.956

Unadjusted CCC (uCCC)	0.372	0.817	0.783	0.951	0.622
AICc	90.9	78.4	103	71.3	85.3
Sigma Hat Error	3.27	2.10	2.25	1.31	2.03

Abbreviations: MD, models using only measured dietary information; CD, models using calculated diet information; CD-I, models using calculated diet information and their interactions; CD-F, models using calculated diet information, their interactions, and feed variables; CD-I-F, models using calculated diet information, feed type variables, and all interactions; SE, standard error; NSC, non-structural carbohydrates; NDF, neutral detergent fiber; ADF, acid detergent fiber; DM, dry matter; RMSE, root mean square error; CCC, concordance correlation coefficient; MSE, mean square error; RSR, Root mean standard deviation ratio; AICc, Akaike information criterion.

[a] % of DM

[c] % of as-fed

Figures

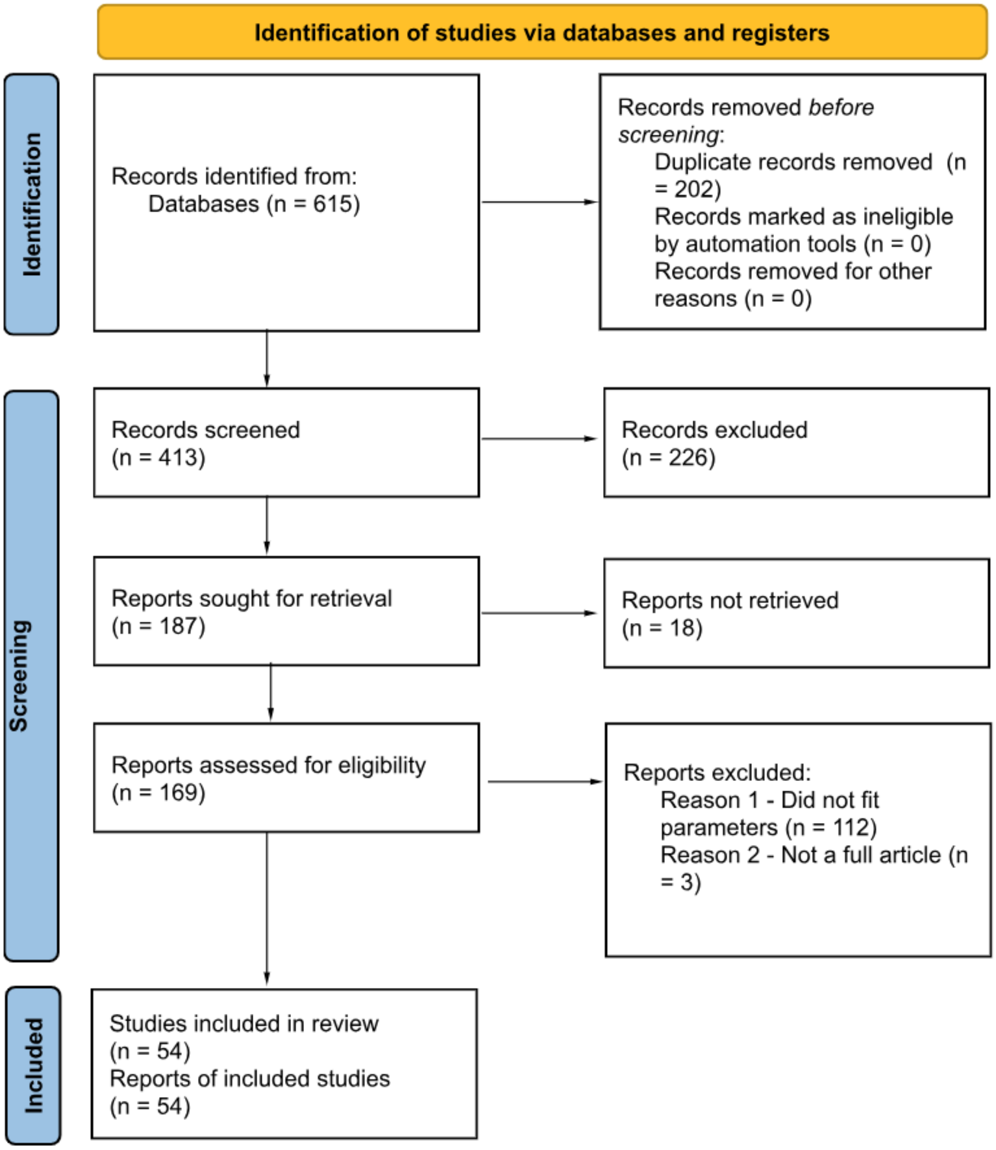


Figure 3-1. "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA) flow diagram displaying the process of the systematic literature review completed for this meta-analysis

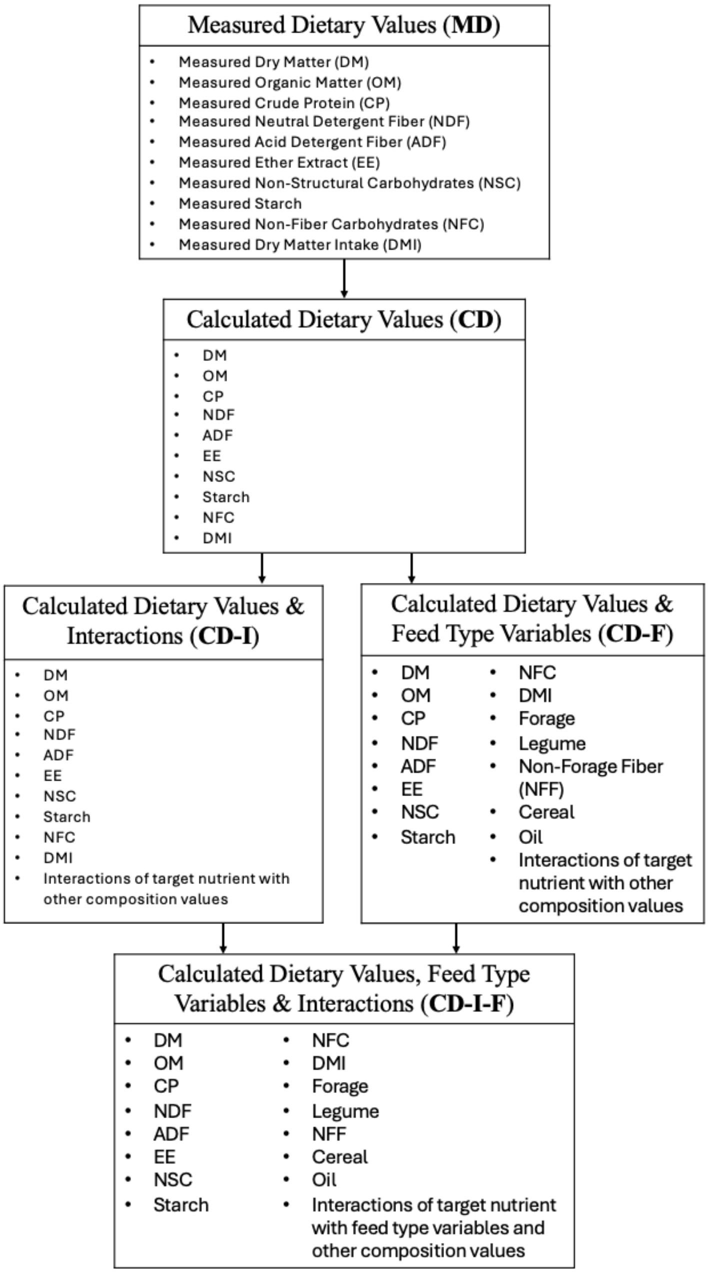


Figure 3-2. Model Types & Explanatory Variables

A visual explanation of the five models produced for each response variable (DMD, CPD, NDFD, EED, NSCD, NFCD, rOMD). The target nutrient represents each response variable tested. Other composition factors include dry matter intake (DMI, % metabolic bodyweight per day) and the nine nutrient composition explanatory variables (DM, CP, OM, NDF, ADF, EE, NSC, starch, and NFC). Feed type variables include the five feedstuff categories of forage, legume, non-forage fiber (NFF), cereal grain, and supplemental oil. The arrows represent the order the models were developed for each response variable.

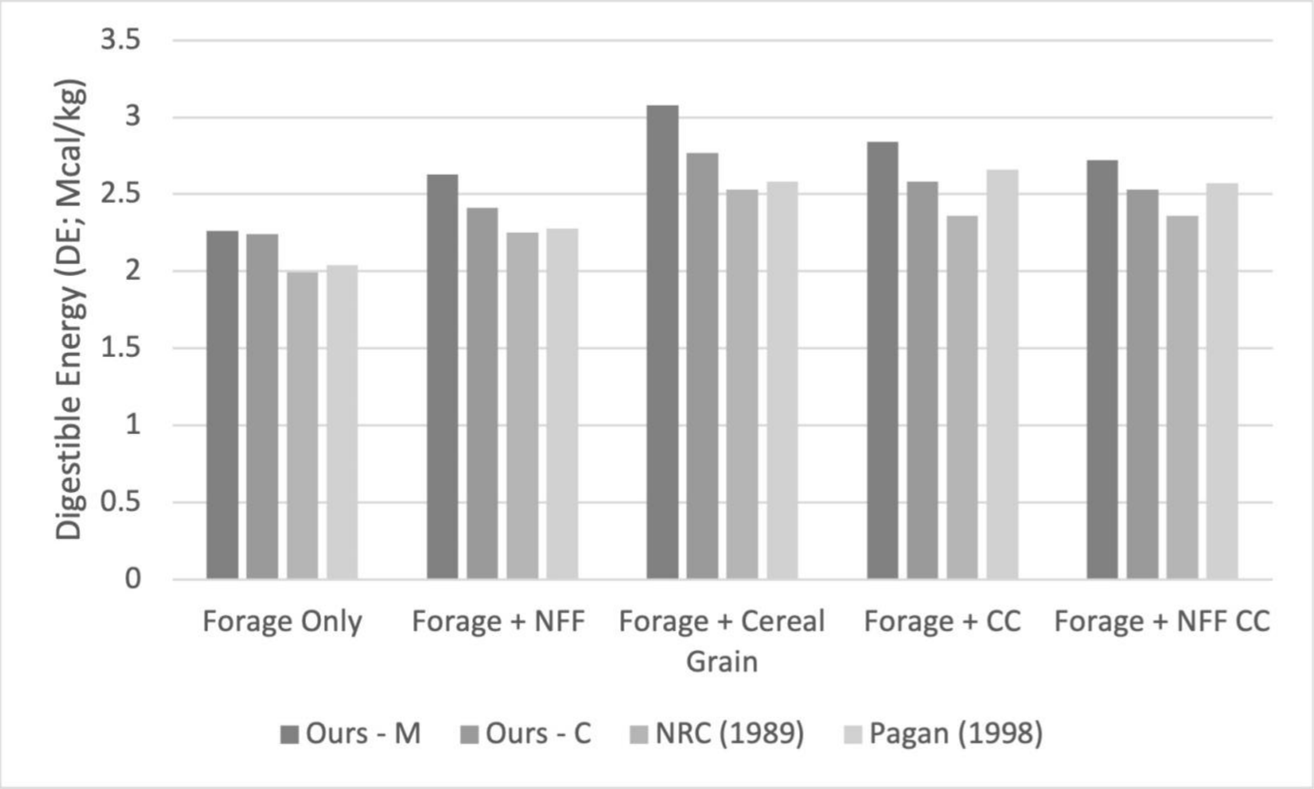
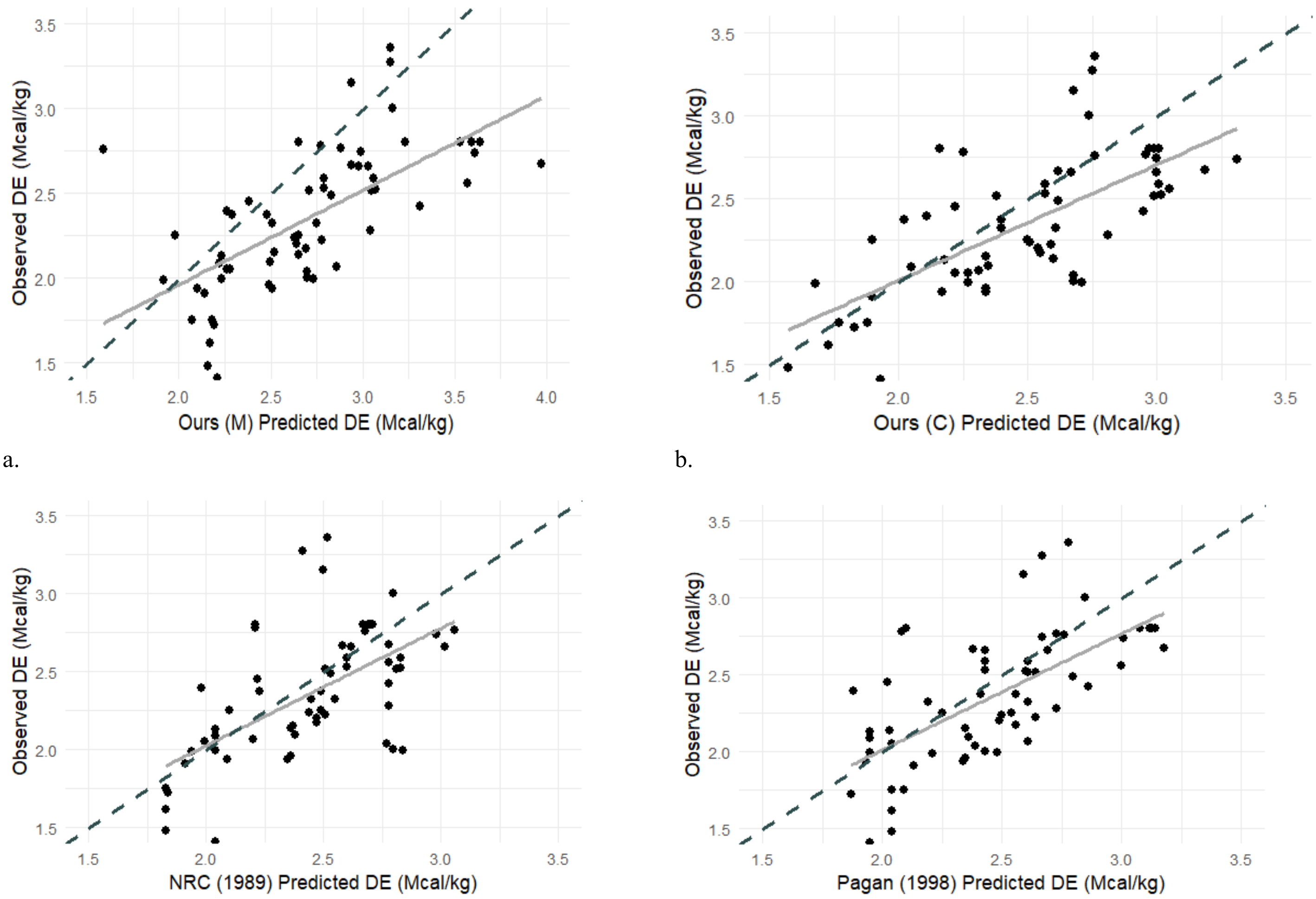


Figure 3-3. Comparison of predicted digestible energy (DE) by four different equation systems in five different examples of modern equine diets

Five different examples of modern equine diets were utilized to compare the results for digestible energy (DE) estimation using four different equation systems. From left to right, the darkest gray bar represents the values from our measured data equation, "Ours – M", the next lighter bar represents the values from our calculated data equation, "Ours – C", the third bar represents the results from the equine NRC [19] equation ,"NRC (1989)", and the lightest colored bar on the right represents the results from the Pagan [16] equation, "Pagan (1998)", for DE estimation. The first diet encompassed a solely forage based diet with 100% mid-maturity grass hay. The second diet included 60% mid-maturity grass hay and 40% beet pulp shreds, a common non-forage fiber (NFF). The third diet included 60% mid-maturity grass hay and 40% rolled oats (cereal grain). The fourth diet included 60% mid-maturity grass hay and 40% a performance horse commercial concentrate (CC). The final diet included 60% mid-maturity grass hay and 40% a NFF based commercial concentrate. The concentrates in diets four and five contained a fat content of more than 5%.



a.

b.

c.

d.

Figure 3-4. Four scatterplots depicting the results after independent evaluation of four equation systems compared to experimentally measured DE from the literature

Four scatterplots depicting the results of the independent evaluation of our two proposed digestible energy (DE) estimation equations, the measured data equation (“Ours (M)”) in plot a, and the calculated data equation (“Ours (C)”) in plot b. The equations were used to estimate the DE content of feedstuffs that were experimentally measured in 63 different treatments across 17 studies for DE content in Mcal per kg (“Observed”). If provided, the chemical composition and average values for each feedstuff was calculated and plugged into each proposed equation.

Additionally, the DE estimations by two existing equations, the NRC [19] (“NRC (1989)”) in plot c and Pagan [16] (“Pagan (1998)”) in plot d, were used for each treatment diet using the chemical composition and/or average feedstuff values from databases. The solid gray line in each plot represents the line of best fit and the dashed dark gray line represents the line of unity.

Supplementary Materials

Table S3-1. Listing of Papers in Dataset

Agazzi, et al. (2011). J. Eq. Vet. Sci. 31:13-18.	Al Jassim. (2006). Ani. Feed Sci. Tech. 125:33-44.	Bachmann, et al. (2019). Livestock Sci. 223:16-23.
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Table S3-2. Models Explaining Variation in Total Tract Digestibility of Organic Matter (OM) Using Measured Dietary Information (MD), Calculated Diet Information With (CD-I) or Without (CD) Interaction Terms and Feed Variables[a] (CD-F, CD-I-F), Including Slope and Standard Error (SE)

	MD	**CD**	**CD-I**	**CD-F**	**CD-I-F**
Intercept	84.4 (5.66)	92.3 (4.53)	168 (45.9)	66.5 (2.15)	118 (142)
DMI[b]	5.39 (1.48)	3.39 (1.38)			
OM[a]			–1.26 (0.497)		–0.230 (1.54)
NDF[a]	–0.666 (0.0858)	–0.727 (0.0699)			6.30 (2.73)
ADF[a]					–9.73 (3.41)
EE[a]	–0.77 (0.372)	–0.81 (0.419)			–0.998 (0.479)
Starch[a]			–7.65 (3.22)		
Forage				–14.9 (2.85)	–86.5 (29.0)
Legume				10.0 (2.68)	150 (82.3)
OM[a] x NDF[a]					–0.0734 (0.0293)
OM[a] x ADF[a]					0.102 (0.0365)
OM[a] x Starch[a]			0.0884 (0.0348)		
OM[a] x Forage					0.0900 (0.309)
OM[a] x Legume					–1.63 (0.900)
Fit Statistics					
n	120.0	181.0	176.0	176.0	176.0
Observed Mean[a]	56.0	56.5	56.4	56.4	56.4
Predicted Mean[a]	56.0	56.5	56.5	56.5	56.6
RMSE, % mean	5.80	7.66	9.15	9.80	7.90
Unadjusted RMSE, % mean	10.9	12.58	13.7	13.1	11.7

Mean bias, % of MSE	0.00	–0.00	0.0561	0.0601	0.142
Slope Bias, % of MSE	1.20	0.0712	2.15	3.03	1.05
RSR	0.785	0.803	0.879	0.835	0.751
CCC	0.903	0.859	0.781	0.736	0.849
Unadjusted CCC (uCCC)	0.677	0.632	0.523	0.536	0.674
AICc	718	1163	1174	1170	1126
Sigma Hat Error	3.66	4.80	4.28	4.70	3.59

[a] % of DM

[b] % metabolic BW per day

Table S3-3. Models Explaining Variation in Total Tract Digestibility of Acid Detergent Fiber (ADF) Using Measured Dietary Information (MD), Calculated Diet Information With (CD-I) or Without (CD) Interaction Terms and Feed Variables[a] (CD-F, CD-I-F), Including Slope and Standard Error (SE)

	MD	**CD**	**CD-I**	**CD-F**	**CD-I-F**
Intercept	32.5 (4.48)	32.5 (4.48)	19.2 (8.26)	19.4 (8.22)	39.8 (12.3)
DMI[b]	3.77 (2.25)	3.77 (2.25)	12.6 (4.27)	12.4 (4.25)	13.3 (4.27)
ADF[a]			0.600 (0.341)	0.590 (0.340)	–0.0542 (0.457)
EE[a]					–8.49 (3.38)
Oil					1094 (438)
NFF				24.5 (14.7)	
ADF[a] x EE[a]					0.236 (0.111)
ADF[a] x DMI[b]			–0.370 (0.174)	–0.367 (0.173)	–0.360 (0.173)
ADF[a] x Oil					–36.5 (18.1)
Fit Statistics					
n	198	198.0	195.0	195.0	195.0
Observed Mean[a]	39.0	39.0	39.0	39.0	39.0
Predicted Mean[a]	39.0	39.0	39.5	39.5	39.5
RMSE, % mean	19.8	19.8	19.9	19.8	19.7
Unadjusted RMSE, % mean	31.9	31.0	32.0	31.7	30.9
Mean bias, % of MSE	0.00	0.00	–0.511	0.443	0.391
Slope Bias, % of MSE	3.27	3.27	0.158	3.05	3.20
RSR	0.987	0.987	0.997	0.989	0.963
CCC	0.750	0.750	0.743	0.746	0.749
Unadjusted CCC (uCCC)	0.389	0.389	0.369	0.380	0.402
AICc	1488	1488	1440	1432	1433
Sigma Hat Error	8.53	8.53	4.88	4.87	4.89

[a] % of DM

[b] % metabolic BW per day

Table S3-4. Models Explaining Variation in Total Tract Digestibility of Starch Using Measured Dietary Information (MD), Calculated Diet Information With (CD-I) or Without (CD) Interaction Terms and Feed Variables[a] (CD-F, CD-I-F), Including Slope and Standard Error (SE)

	MD	**CD**	**CD-I**	**CD-F**	**CD-I-F**
Intercept	126 (6.09)	343	444 (72.5)	93.2 (4.19)	108 (6.34)
Starch[a]		0.221 (0.0723)	–16.2 (3.61)		–0.318 (0.221)
DM[c]		–3.01 (0.790)	–3.96 (0.801)		
NDF[a]	–0.716 (0.0925)				–0.418 (0.102)
CP[a]		1.17 (0.318)			
EE[a]	–0.355 (0.116)				
Forage				–5.11 (2.94)	
Cereal					27.2 (11.0)
Starch[a] x DM[c]			0.182 (0.0399)		
Starch[a] x NDF[a]					0.0155 (0.00480)
Starch[a] x Cereal					–2.42 (0.700)
Fit Statistics					
n	48.0	79.0	74.0	74.0	74.0
Observed Mean[a]	88.5	92.1	91.7	91.7	91.7
Predicted Mean[a]	88.5	92.1	91.9	92.4	91.9
RMSE, % mean	3.64	3.21	3.36	4.87	3.48
Unadjusted RMSE, % mean	11.1	16.6	14.3	12.5	11.1
Mean bias, % of MSE	0.00	0.00	0.279	2.70	0.295
Slope Bias, % of MSE	0.687	0.460	1.50	2.63	3.95
RSR	0.764	1.35	1.13	1.00	0.888
CCC	0.966	0.960	0.958	0.904	0.954

Unadjusted CCC (uCCC)	0.592	0.165	0.259	0.204	0.437
AICc	316	490	440	464	455
Sigma Hat Error	3.80	3.37	2.66	3.54	2.87

[a] % of DM
[c] % of as-fed

Table S3-5. Measured DE (Mcal/kg) from 63 Treatments in 17 Publications Compared to Estimated DE from Chemical Composition by NRC (1989), Pagan (1998), and our two systems, (ME) and (CE).

TID	Actual	ME	CE	NRC (1989)	Pagan (1998)	BW, kg	DMI, kg/d
1*	2.80	3.64	3.01	2.67	3.14	488.5	8
2*	2.80	3.59	2.99	2.69	3.13	482.3	8.3
3*	2.80	3.53	2.99	2.70	3.12	489	8.4
4*	2.80	3.23	2.97	2.71	3.08	493.6	8.4
5	2.59	2.79	2.57	2.60	2.43	596	11.8
6	2.53	2.79	2.57	2.60	2.43	596	11.8
7	1.94	2.10	2.17	2.09	1.93	596	12
8	2.05	2.26	2.22	2.00	2.04	577	12.12
9	2.05	2.28	2.27	2.00	2.04	577	13.85
10	2.28	3.04	2.81	2.78	2.73	549	7.66
11*	2.42	3.31	2.95	2.78	2.86	549	7.6
12*	2.56	3.57	3.05	2.78	3.00	549	7.37
13*	2.67	3.97	3.19	2.78	3.18	549	7.3
14*	1.99	1.92	1.68	1.94	2.21	493	7.6415
15	2.38	2.29	2.02	2.23	2.41	493	7.5429
16	2.52	2.71	2.38	2.51	2.64	493	7.5429
17	3.00	3.16	2.74	2.80	2.85	493	6.4936
18	2.00	2.73	2.71	2.84	2.48	483.3	8.72
19	2.00	2.70	2.68	2.80	2.43	483.3	8.72
20	2.03	2.70	2.68	2.77	2.39	483.3	8.73
21	2.14	2.65	2.60	2.36	2.03	483.3	8.73
22	2.66	2.98	3.00	3.02	2.69	492.5	5.62
23	2.74	2.99	3.00	2.98	2.67	492.5	5.61
24	2.59	3.06	3.01	2.83	2.61	492.5	5.625
25	2.53	3.07	3.02	2.83	2.60	492.5	5.621
26	2.52	3.05	2.99	2.81	2.61	492.5	5.625
27	2.77	2.88	2.96	3.06	2.73	407.5	4.35
28*	2.74	3.61	3.31	2.98	3.01	407.5	3.92
29	2.00	2.23	2.27	2.04	1.95	284	6.43
30	2.13	2.23	2.18	2.04	1.95	284	5.33
31	2.09	2.22	2.05	2.04	1.95	284	3.73
32	1.41	2.21	1.93	2.04	1.95	284	2.14
33	1.91	2.14	1.90	1.91	2.13	284	5.53
34	1.75	2.18	1.88	1.83	2.04	284	5.33
35	1.62	2.17	1.73	1.83	2.04	284	3.87
36	1.48	2.16	1.57	1.83	2.04	284	2.14
37	2.32	2.75	2.61	2.55	2.61	422	6.839
38*	2.49	2.83	2.62	2.53	2.80	422	6.6
39	2.25	2.65	2.50	2.49	2.54	422	6.981
40	2.37	2.48	2.40	2.49	2.56	422	6.635
41	1.72	2.19	1.83	1.84	1.87	543	8.83
42	2.32	2.51	2.40	2.45	2.19	540	8.17
43*	2.07	2.86	2.31	2.20	2.61	542	7.61
44	2.25	1.98	1.90	2.10	2.25	555.6	9.45

45	2.76	1.59	2.76	2.68	2.76	555.6	6.77
46	2.40	2.26	2.11	1.98	1.88	542	10.2
47	2.45	2.38	2.22	2.22	2.02	542	9.51
48	2.66	3.03	2.67	2.62	2.43	542	8.64
49	2.67	2.94	2.62	2.58	2.38	542	8.64
50	1.75	2.07	1.77	1.83	2.09	300	6
51*	3.27	3.15	2.75	2.41	2.67	300	6
52*	3.36	3.15	2.76	2.52	2.78	300	6
53	3.15	2.94	2.68	2.50	2.59	300	6
54	2.78	2.77	2.25	2.21	2.08	613.8	9.207
55	2.80	2.65	2.16	2.21	2.10	613.8	6.23
56	1.96	2.49	2.34	2.36	2.35	483.6	8.18
57	1.94	2.51	2.34	2.35	2.34	483.6	8.08
58	2.10	2.50	2.35	2.38	2.36	483.6	7.94
59	2.15	2.52	2.34	2.37	2.35	483.6	7.83
60	2.20	2.64	2.54	2.47	2.49	484.2	11
61	2.24	2.63	2.51	2.44	2.50	484.2	10.7
62	2.18	2.69	2.55	2.47	2.56	484.2	10.6
63	2.22	2.78	2.59	2.51	2.64	484.2	10.6

Abbreviations: TID, treatment ID; ME, our system from measured data only models; CE, our system from calculated data models; BW, body weight; DMI, dry matter intake; DE, digestible energy.
* Indicates treatments containing five percent or more fat

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CHAPTER 4: Assessment of Thermal Imaging to Objectively Body Condition Score Mature Horses and Multiparous Gestating Beef Cows

This chapter was submitted as Alexandra P. Webster, Ryan K. Wright, Jillian B. Hammond, Naa A. Kotey, Claire B. Gleason and Robin R. White. Assessment of Thermal Imaging to Objectively Body Condition Score Mature Horses and Multiparous Gestating Beef Cows, , 2025.

Abstract

This study explored whether thermal imaging could provide an objective means of body condition scoring (BCS) horses and multiparous, gestating beef cows. This study consisted of two parts: one part assessed BCS in horses of the Quarter Horse or Thoroughbred breed types while the other evaluated BCS of gestating beef cows. In both parts of the study, ground truth BCS were assigned by five to eight trained scorers for each animal. Thermal images were also collected from one or both sides of the body and analyzed for surface temperature. Surface temperature and BCS were evaluated with the whole body, and for five (cows) or seven (horses) specific body regions. Covariates were monitored, including breed, ambient temperature, cloud coverage (%), housing conditions, blanketing status, individual scorer, and scorer's location. To evaluate the ability of the thermal camera to accurately BCS horses and cows, random forest regressions were also derived. The dataset was divided into three portions: 15% for hyperparameter tuning, 55% for model-training, and 30% for independent evaluation. Unique models were created for each body area assigned a BCS for horses (the neck, shoulder, withers, ribs, loin, tailhead, and overall) or beef cows (the brisket, shoulder, ribs and spine, hooks and pins, and overall). The root mean square error (RMSE, % mean) was the primary fit statistic used to evaluate each model's performance during the independent evaluation phase. Across all body regions, the RMSE values (% mean) ranged from 7.6% to 10.6% for horses and 6.81% to 13.4% for cows. Between-scorer variability was assessed for both datasets by calculating the coefficient of variation (CV) among scorers. The variability among the eight horse scorers ranged from 13% to 14% and from 10.8% to 12.1% for the five cow scorers. Overall, thermal imaging shows promise as an objective tool for BCS assignment in both horses and beef cows. However, future refinement of the method is important and should consider conflating factors like environment, housing, and management.

Introduction

Body condition scoring (BCS) is a qualitative method for ranking body fat reserves, typically on a scale of 1 to 9, and serves as a vital tool in assessing animal health and management effectiveness. Obesity is an increasingly significant animal health concern, particularly for horses and cattle, because overweight animals are more prone to health issues like metabolic dysfunction, insulin resistance, dystocia, and laminitis (Boosman et al., 1991; Geor and Harris, 2009; Daradics et al., 2021). Conversely, under- and malnutrition can also have negative consequences such as poor performance, infertility, and an increased risk of disease (Hogan and Phillips, 2016; Jarvis and McKenzie, 2021; Silva et al., 2023).

Although BCS is intended to be a straightforward method for monitoring livestock energy reserves, it has proven to be subjective, necessitating the development of more objective tools (Evans, 1978; Kristensen et al., 2006). Specifically, tracking changes in adipose tissue content over time can be challenging, even with proper training (Mottet et al., 2009). Wyse et al. (2008) reported that BCS is often underestimated by horse owners, particularly by those with overconditioned horses (>50%). The current system for body condition scoring horses, which involves both visual observation and external palpation, was developed by Henneke et al. (1984). Although this system is applied universally across horse breeds and sexes, it was originally designed for Quarter Horse mares (Henneke et al., 1984; Suagee et al., 2008). Given the known variations in adiposity among horse breeds, the Henneke system may be less reliable when assessing breeds of markedly different body types, like Thoroughbred or draft horses (Suagee et al., 2008; Brooks et al., 2010). Similarly, beef cattle are scored on a scale of 1 to 9, as described in Wagner et al. (1988). For the most accurate results, producers are advised to either hire experienced scorers or receive personalized training to evaluate their own cattle (Roche et al., 2009). Overall, the current BCS methods are limited by their reliance on expertise or training for proper evaluations. A new method to score both horses and cows objectively is needed to help reduce the prevalence of obesity and provide accurate assessments of animal health.

Some technologies have been tested to mitigate the subjectivity surrounding body condition scoring livestock with varying degrees of success. In horses, models were developed using morphometric measurements to allow owners to estimate their horses' body weight in a cost-effective manner based on breed type (Martinson et al., 2014; Catalano et al., 2016; Catalano et al., 2019). Although limited by its high cost, a second method tested was ultrasonography of subcutaneous fat in horses, which was shown to potentially serve as a more accurate way to objectively BCS horses (Martin-Gimenez et al., 2016). In cattle, machine learning techniques have been extensively assessed to estimate BCS. In particular, depth imaging and three-dimensional imaging show promise to objectively body condition score cattle (Kojima et al., 2022; Xiong et al., 2023). For example, Xiong et al. (2023) created a system using depth images of cows in chutes to record multiple body measurements and compare these to manually assigned BCS. Although the approach was generally successful, it was unable to accurately estimate BCS in cows with a score of 4.5 or lower (out of 9) (Xiong et al., 2023).

Thermal imaging is a technology that measures surface temperature, and there is increasing interest in using thermal imaging in veterinary medicine research to develop low-cost methods to diagnose ailments like lameness and infections, or for reproduction purposes (Anagnostopoulos et al., 2021; Maśko et al., 2021; Okur et al., 2023). Although thermal imaging has not yet been investigated as a tool to monitor adipose accumulation in livestock species, there are several studies in human medicine that demonstrate the promising use of thermal imaging for body fat monitoring (Chudecka et al., 2014; Salamunes et al., 2017). Both

Salamunes et al. (2017) and Chudecka et al. (2014) found that in the abdominal region of humans, higher percentages of body fat were associated with lower skin surface temperatures, as detected by thermal imaging. Given the success of detecting body fat reserves in humans and the other emerging efforts in technology for body condition scoring livestock, thermal imaging was tested to evaluate its potential for detecting adiposity in horses and cattle.

Our objective was to determine whether thermal imaging could provide an objective means of body condition scoring (BCS) mature horses of the Quarter Horse and Thoroughbred breed-types and in multiparous gestating beef cows. These two species were chosen to assess how adiposity varies among different types of grazing livestock. We hypothesized that thermal imaging would enable BCS assignment in both species, because surface temperature will be affected by body fat reserves, which act as insulation and energy storage. This research could eventually offer a cost-effective, non-invasive tool for horse owners and cattle producers alike to monitor the condition of their animals.

Materials & Methods

All procedures in this study were reviewed and approved by the Virginia Tech Institutional Animal Care and Use Committee (Protocol #22-137). The thermal camera utilized in this study was a FLIR ONE Edge Pro (Teledyne FLIR, LLC, Wilsonville, Oregon). Additionally, a Panasonic video camera camcorder (Panasonic Holdings Corporation, Kadoma, Osaka, Japan) was used to video record each animal for body scorers that may have been unable to attend the in-person scoring session.

Animals & Experimental Design – Horses

Data collection was conducted from February 2024 to June 2024. Due to substantial known variability in equine body and breed types (Brooks et al., 2010), we narrowed our assessment and selected two distinctly different breeds of horses that were most prevalent in southwest Virginia: those that were either full or predominantly Thoroughbred (TB) and those that were either full or predominantly Quarter Horse (QH). The criteria for participation in the study required horses to be older than 3 years of age and considered either the breed of QH or TB. Horses were excluded if they were younger than 3 years of age, pregnant, lactating, or breeding stallions. To achieve a large enough sample size, horses, both privately and university owned, were included in this study. Each privately owned horse owner signed a consent document, and all body condition scorers signed confidentiality agreements before data collection began. The goal was to score and thermal image every horse twice, with at least two months between visits to allow for any potential changes in body condition during the transition into the warmer season. In total, 84 horses were scored in the first phase and 81 in the second phase, with 78 horses participating in both phases. Although the aim was to assess every horse twice, various factors unrelated to the study, including ownership change and owner-elected euthanasia for undisclosed reasons, prevented a small number of horses from being reassessed. Of the two breed categories, 50 QH-types and 34 TB-types were scored in the first phase, and 48 QH-types and 33 TB-types were scored in the second phase. Across both phases, a total of 165 BCS were assigned, and 330 thermal images were collected for analysis (Table 1).

Additional covariates recorded during each visit included ambient temperature (ºC), cloud coverage (%), calendar date, animal coat color, animal sex, whether the animal wore a blanket immediately before imaging, whether thermal images were taken inside or outside, visual determination of coat or fur density, viewing number, and geographical location (city). The owners provided information on the animal's breed and year of birth (YOB). Animal coat colors included appaloosa, black, bay, chestnut, dark bay, red roan, grey, pinto, palomino, grullo, dun, buckskin, and all variations of grey. The sex of the horses was categorized as either mare or gelding. Coat density was visually determined in-person, and horses were assigned to one of four categories: *normal, thick, full clip,* or *partial clip*. *Normal* was assigned to horses with a visibly smooth coat approximately 2.54 cm long. *Thick* was assigned to horses with visibly dense coats equal to or longer than 5.1 cm. *Full clip* was assigned to horses whose owners shaved their hair, whether that included their leg hair or not, with a coat length less than 2.54 cm. *Partial clip* was assigned to horses with partially shaved hair that was less than 2.54 cm long and unshaved areas measured at 2.54 cm or greater in length. Viewing number refers to the first or second time the animal was evaluated and imaged. Geographical location was categorized by the name of the town the horse was in when imaged. The YOB of the horses ranged from the oldest, born in 1995, to the youngest, born in 2020.

The horses were body condition scored based on the widely used method established by Henneke et al (1984), and assigned a score from 1 to 9 for seven body regions. Our only deviation from the original method was that all body scoring was done visually, without any physical palpation of the external body. The evaluated body regions included the neck, shoulder, withers, ribs, loin, tailhead, and an overall body score. On both sides of every horse's body, a video recording and a side-profile thermal image were taken. Thermal images were captured approximately 2 meters away from each horse. A total of eight body condition scorers scored all the horses either in-person or through video. Ideally, each scorer would have attended every in-person imaging and scoring session, but this was logistically impractical. To be consistent, every scorer was required to score every animal at least once in-person and the video recording allowed scorers to participate remotely when unavailable. The body scorers consisted of one post-doctoral researcher, three undergraduate students, and four graduate students at Virginia Tech, whose horse experience varied from less than one year to over ten years. Prior to beginning the study, each body scorer was required to complete an online training course and quiz curated by the first author. The purpose of the training was to standardize the protocol for scoring and ensure that the scorers, regardless of experience level, could visually BCS horses. A passing grade of 90% or higher was necessary to participate as a body scorer.

Animals & Experimental Design – Beef Cows

This study involved two visits to a herd of multiparous, gestating beef cows turned out in approximately 15 hectare grass pastures. The purpose of choosing multiparous beef cows was because they were no longer growing and had already successfully produced at least one offspring. Since the primary function of beef cows is reproduction, this study aimed to assess the changes in body condition score (BCS) during a typical pregnancy. The first visit was in April 2024 and the second in June 2024. During each visit, the objective was to capture thermal images to observe body surface temperatures and to visually BCS 50 cows from a herd of 80 or more without disrupting their normal routine. In April, the cows still had their calves from the previous calving season, but the calves were weaned by farm management before the second visit in June. During the first visit, 50 cows were successfully imaged and body condition scored. During the second visit, due to some technical difficulties, only 47 cows were successfully thermal imaged and body condition scored. The technical difficulties, including accidental repeat images or scoring of the same cow and capturing unusable images or video, were attributed to the large herd of similar-looking cows free-roaming over the 15 ha pasture. Between the two visits, 29 cows were imaged and scored twice, resulting in a total of 97 cows scored with corresponding thermal images collected (Table 1).

On both days, additional covariates included cloud coverage (%), calendar date, viewing number, animal distance from the camera during imaging, days pregnant, sun coverage, the side of the body imaged, year of birth (YOB), and ambient temperature (ºC). The ambient temperature (ºC) and cloud coverage (%) values were obtained from AccuWeather (2024). The cow breeds included pure Angus, half-Simmental and half-Angus, 3/4 Angus and 1/4 Simmental, ¾ Simmental and 1/4 Angus, and 7/8 Angus and 1/8 Simmental. All but one of the cows were black, with the exception being a shade of tan. The YOB of the cows ranged from the oldest, born in 2007, to the youngest, born in 2021. For each cow, the number of days pregnant was determined by subtracting the days between the AI service dates and imaging dates. The gestational stages of the cows ranged from 91 to 112 days in April, and from 158 to 179 days in June, based on artificial insemination (AI) breeding dates. After subsequent pregnancy checking, it was determined that three cows from the first visit and one cow from the second visit were not pregnant. These cows were included in the dataset as “not pregnant”. Thermal images were captured from either the left or right profile view of each cow’s body, depending on which side was more accessible in the pasture. Sun coverage was recorded as “sunny” or “shady” based on the cow’s location when imaged, because cows preferred the shade under trees on hotter days, which could decrease the surface temperature of their body compared to cows imaged in direct sunlight. The distance between the thermal camera and the cow during imaging was categorized into three groups because study equipment did not allow for precise measurements. Rather, distance was estimated based on how much of the cow filled the thermal image. This was a factor to consider because the cows had varying flight zone sizes, and the thermal camera was unable to zoom. The three distance categories were approximately 3 to 5 meters away (“close”), 6 to 25 meters away (“far”), and 26 to 55 meters away (“very far”). The viewing number referred to the first or second visit to the herd.

For the beef cattle iteration, five body condition scorers evaluated the cows either in-person or remotely through video. Each scorer was required to attend at least one of the two visits. The five scorers included one post-doctoral researcher and four graduate-level students, whose experience with beef cattle ranged from less than one year to five or more years. The five scorers, regardless of experience level, were required to complete an online training designed to familiarize them with the protocol for the study and teach them how to body condition score (BCS) beef cows. The training, organized by the first author, required a passing grade (90% or higher) of a post-training quiz to participate in the experiment. The cows were scored on a scale of 1 to 9 (Wagner et al., 1988), with scores assigned to each of the five categories: brisket, shoulder, ribs and spine, hooks and pins, hooks and pins, and an overall body score.

Data Processing

For both parts of this study, body condition scorers were randomly and anonymously assigned a unique ID to distinguish one scorer from another. Each animal was scored, and the scores were submitted through an online form. The online form recorded the scorer ID, whether the scorer completed the form in-person or remotely, the date and timestamp, the animal ID, and the selected body scores for each part of the body in a multiple-choice format. Data from the submitted forms was automatically collected in an Excel file. The scores were manually transferred to a master Excel sheet and organized by animal ID and scorer ID.

Thermal image readings were processed using FLIR Thermal Studio Suite software (Teledyne FLIR, LLC, Wilsonville, Oregon). A circular-shape tool was used to outline the body areas scored in each thermal image to retrieve the average surface temperature. Figure 1 illustrates an example of where each average thermal reading was taken in horses and beef cows.

Statistical Analysis

All data preparation and analyses were conducted in R v 4.3.1 (R Core Team, 2024). Separate analyses were performed for each species with similar procedures. The main Excel file for each species was read into R using the *readxl* package (Wickham and Bryan, 2023). The main Excel file had one sheet with all the BCS data, and another with the surface temperature readings retrieved from the thermal images. Once read into R, the two sheets were merged and aligned into one data frame.

Equine Data Analysis

During analysis, we focused on assessing three aspects: (1) the accuracy of body surface temperatures in predicting BCS, (2) how the additional covariates influenced BCS, and (3) scorer variability by body region.

First, we assessed how well BCS could be predicted using body surface temperatures obtained by the thermal images. Random forest regressions were utilized for training and evaluation, using thermal image readings to predict the body condition scores (on a scale of 1 to 9) for each of the seven body regions. An additional model was created to represent the averages across all seven regions. After omitting any missing values, the data were randomly split into three parts, 55% for model training, 15% for hyperparameter tuning, and 30% for independent evaluation, as described in our previous work (Chen and White, 2024). For each body region regression, the tuneRF function (Liaw and Wiener, 2002) was used to identify the best hyperparameters using 15% of the dataset. Fifty-five percent of the dataset was then used for model training and parameter estimation. Random forest regressions were fitted using the hyperparameters through the randomForest package (Liaw and Wiener, 2002). The features used for estimating BCS included ambient temperature, cloud coverage, time, location (inside or outside), blanketing status, year of birth (YOB), coat color, sex, breed, coat density, viewing number, body scores, and the thermal surface temperature readings from both sides of the body. For the categorical "breed" feature, horses were classified as 100% Thoroughbred (TB), 100% Quarter Horse (QH), QH crossbreed, or TB crossbreed. After training, the final model was assessed against the 30% of the dataset saved for independent evaluation. The root mean square error (RMSE, % mean) for each model was calculated to evaluate how well thermal imaging could predict BCS in each of the seven body regions and across all regions.

To address the second focus, a linear mixed-effects model (Kuznetsova et al., 2013) was used, with fixed effects for surface temperature (ºC) and all covariates. The BCS assigned by individual scorers was the response variable. Individual horse was included as a random effect. The additional covariates included blanketing status, location (inside or outside), cloud coverage (%), ambient temperature (ºC), year of birth (YOB), coat color, viewing number, breed, coat density, scorer, and whether the scorer was in-person or remote. The associations identified between BCS assigned and the various covariates was determined by the P-value, with values of $P < 0.05$ considered as significant, and tendencies toward significance were noted at $P < 0.10$.

Finally, to assess scorer variability, we calculated the mean and standard deviation (SD) of scores assigned by horse, viewing number (first or second time viewing), and body region. These SD and mean values were then used to calculate the coefficient of variation (CV) for each horse, viewing number, and body region ($SD \div Mean \times 100$). The mean CV was calculated for each body region, to reflect the expected variability in human-derived BCS at each site. This served as a baseline reference for interpreting the quality of the models in terms of their ability to estimate BCS with consistency similar to trained human scorers.

Beef Cow Data Analysis

Analysis of the beef cow data was completed the same way as the horse data to address the same three factors: (1) the accuracy of body surface temperatures in predicting BCS, (2) how the additional covariates influenced BCS, and (3) scorer variability by body region. Random forest regressions were used for training and evaluation to predict the BCS (on a scale of 1 to 9) in beef cows based on thermal image surface temperature readings, using a procedure similar to that described above for horses. The covariates differed slightly and included ambient temperature (ºC), cloud coverage (%), coat color, days pregnant, year of birth, breed, side of the body imaged, distance from the camera, sun coverage, body scores, viewing number, and the thermal surface temperature readings. Along with the differences in covariates considered between the species, only five body regions were modeled for cows (brisket, shoulder, ribs and spine, and hooks and pins) rather than the seven for horses.

The influence of additional covariates on BCS was again assessed through a linear mixed-effects model; however, the covariates used differed slightly from the horses and included: breed, coat color, cloud coverage (%), ambient temperature (ºC), year of birth (YOB), days pregnant, side of body imaged, viewing number, distance from the thermal camera during imaging, sun or shade coverage, scorer, and whether the scorer was in-person or remote. Individual cow was included as the random effect. The adjustment in covariates used were based on species differences and environment where the cows were imaged. For example, none of the horses were gestating and these cows were gestating so including the covariate of "days pregnant" was necessary, along with distance from camera during imaging because the cattle were not restrained for imaging like the horses.

Similar to the horse data, variability among the five BCS scorers was assessed by calculating the mean and standard deviation (SD) of scores assigned by cow, viewing number, and body region. The SD and mean values were then used to calculate the coefficient of variation (CV) for each cow, viewing number, and body region ($SD \div Mean \times 100$). The scores obtained from the cow BCS data served as a reference point to evaluate the ability of the models created to consistently estimate BCS in beef cows like trained human scorers.

Results & Discussion

Ability of Thermal Imaging to Accurately Predict BCS in Each Body Region in Horses

In the horse dataset, the accuracy of body surface temperatures obtained by thermal imaging to predict BCS varied across body regions, with values ranging from 7.6% to 10.6% RMSE (% mean) among all regions (Table 2). The shoulder region resulted in a model with the lowest error (7.6% RMSE), and the ribs resulted in a model with the highest error (10.6% RMSE). However, when averaging all the regions, the result was an RMSE of 8.7% (% mean). This suggests that regional differences in thermal properties may influence the accuracy of BCS predictions from surface temperature readings. Although not specifically studied in horses, differences in the thermal properties of body regions have been assessed in obese and non-obese humans (Rashmi and Snekhalatha, 2020). In these studies, areas like the abdomen and neck displayed more thermal variation in non-obese people than obese people (Rashmi and Snekhalatha, 2020). In relation to our study, horses, like humans, have varying amounts of subcutaneous fat in different regions of the body, which could affect thermal surface temperature readings. In horses, subcutaneous fat distribution could impact the surface temperature in each body region. For example, the shoulder region may have resulted in more accurate predictions because it typically holds less fat than areas that resulted in less accurate predictions, like the ribs. Future investigation into the thermal properties of different body regions in horses may determine the source of body region differences and eventually improve the applicability of body surface temperature measurement as a tool to body condition score horses.

Influence of Covariates on Assigned BCS in Horses

Of all the covariates considered for horses, the cloud coverage (%), breed-type, scorer location (in-person or not), and individual scorer influenced assigned BCS the most but varied in significance based on body region (Table 2). Across all regions, both breed-type and scorer were highly significant ($P < 0.001$). The impact of breed-type is likely correlated with the known morphological variations in horses (Brooks et al., 2010). Because horses of different breeds vary in size, and given that the mass of a horse influences heat loss, larger horses (from bigger breeds) with more surface area are likely to lose more heat than horses of smaller breeds (MacCormack and Bruce, 1991). This could explain why breed-type was highly influential on the results, where the differences in breed size and corresponding heat loss may affect surface temperature readings. Cloud coverage (%) influenced the assigned BCS of all body region models, apart from the ribs, where the most influence was observed in the prediction of BCS in the loin and tailhead regions ($P<0.001$). The reason for influence of cloud coverage on assigned BCS is unclear, but could be due to shadowing and poor visibility, particularly for those scoring through video rather than in-person. Additionally, the difference between in-person and remote scoring only impacted the results for the shoulder ($P = 0.003$) and rib regions ($P = 0.0061$). These findings emphasize the importance of considering all potential sources of influence on thermal surface temperature when using thermal imaging for BCS assessment.

Variability Among Scorers in Horses

Scorer variability remains an important factor in assessing the ability of thermal imaging to predict BCS. When evaluating variability in body condition scores among the eight scorers in horses, the values ranged from 13.0 to 14.0% (Table 2). The overall score category, where scorers assign a general single score for the entire body, displayed the lowest variability (12.3%) of all body regions. The variation observed among scorers in this study supports the idea that BCS is inherently subjective and can be challenging to standardize (Mottet et al., 2009; Thatcher et al., 2012). The findings highlight the previously identified need for more objective methods, like thermal imaging to measure body surface temperature, to reduce the subjectivity that can affect the accuracy of body condition scoring.

Ability of Thermal Imaging to Accurately Predict BCS in Each Body Region in Beef Cows

Thermal imaging demonstrated similar accuracy for BCS prediction in gestating beef cows as it did for horses. Across the 6 models using thermal image data to predict BCS in beef cows, RMSE values ranged from 6.81% to 13.4% (% mean; Table 3). The brisket region had the lowest RMSE (6.81%), while the hooks and pins had the highest RMSE (12.2%). Predicting BCS of the whole animal resulted in greater error (RMSE of 13.4%). The high error values associated with most regions, except for the brisket and shoulder, could be associated with challenges in scoring larger surface areas. Although not reliant on thermal imaging, previous research successfully used the rump angle to objectively BCS beef cows, because they found that a higher rump angle correlated with a higher BCS (Pfeifer et al., 2017). This study's model for the hooks and pins region–equivalent to the rump region in other studies–was less successful when compared to the other body regions. This may be due how the hooks and pins were grouped together in a single category. In future work, this hypothesis could be tested by dividing the scoring categories into smaller, more specific body regions (e.g., ribs, spine, hooks, pins) instead of combining them, as was done in this study.

Influence of Covariates on Assigned BCS in Beef Cows

Much like what was observed with the horse data, the influence of covariates on beef cow BCS differed by body regions (Table 3). For all five body region models, individual scorer was highly influential on the BCS results ($P < 0.001$). The impact of whether a scorer assessed each cow in-person or remotely was most significant in the brisket ($P = 0.0021$) and shoulder regions ($P < 0.001$), generally significant in the ribs and spine ($P = 0.010$) as well as the hooks and pins region (*P* = 0.0302), and tended towards significance for the overall score (*P* = 0.0505). Ferguson et al. (2006) found that digital images were sufficient for accurate body condition scoring of dairy cows in comparison to in-person scoring. In spite of that conclusion, their study did not specifically examine the differences in accuracy for each body region and focused only on overall BCS (Ferguson et al., 2006). Interestingly, the covariates appeared to have more impact on the BCS results for the horses than the cattle. For beef cows, cloud coverage (%) and sun coverage were the only other notable variables. Developing robust methods for thermal imaging to assign BCS should account for all potential confounding factors to ensure accuracy and reliability because of the inherent subjectivity in visually body condition scoring livestock.

Variability Among Scorers in Beef Cows

Similar to the findings with horses, scorer variability was notable in the beef cow data. Among the five BCS scorers assessing the cows, variability ranged from 10.8% overall to 12.1% for the hooks and pins region (Table 3). Overall, in connection with the previous section, in-person scoring appeared to have a more substantial effect on the consistency of scores for beef cows compared to the horses. This could be due to fewer scorers involved in the beef cow portion of the trial as well as the fewer animals and opportunities to score the animal in-person, compared to the horse portion of the trial.

An important caveat and limitation to consider when exploring the results of this study, is that the variability in assigning BCS among scorers means that there is no true ground truth. Unlike measurements of body weight scales or even ultrasound-measured fat deposits, it is based on individual interpretation and feel, which can be influenced by proper training. Evans (1978) specifically investigated the subjective nature of BCS in cows and emphasized the importance of proper training to achieve accurate condition scores. Kristensen et al. (2006) later demonstrated that BCS accuracy improved significantly after training veterinarians. In our study, scorers received some prior training through a quiz and post-test, which may have helped limit variability, but additional or more intensive training could have further reduced discrepancies between scorers. Subsequent research on thermal imaging for BCS in horses and cows could test its applicability by using more experienced and well-trained scorers or scorers with similar backgrounds who complete more in-depth BCS training. The results suggest that thermal imaging may offer an objective tool for BCS but, the subjective nature of scoring remains a limiting factor. The use of known objective methods, like ultrasound-measured fat depth, may be essential in redefining how body condition scoring of livestock is described. Aligning these measurements of fat thickness with body surface temperatures obtained through thermal imaging could facilitate a more objective and accessible determination of BCS.

Overall Implications and Future Directions

This study demonstrates the potential of thermal imaging as an objective method for assessing BCS in livestock. Still, further investigation is necessary to evaluate the differences in accuracy between species and the influence of covariates. Future research should include larger, more diverse datasets with different breeds and more controlled environments. The gaps from our dataset that could benefit from expansion of same size include a wider variety of breed types (for both species), ages, and management conditions like housing as well as increasing the training of body scorers or using more experienced industry professionals rather than students to BCS. Environmental factors, like cloud coverage and sun exposure, significantly impacted the results because of their influence on body surface temperature. While minimizing these variables would allow for more precise evaluations of thermal imaging, it is not practical and incorporating ways to account for these factors in practical circumstances may be most effective.

Thermal imaging of body surface temperature could become a practical tool for livestock management if those conflating variables are addressed. It could also be assessed in conjunction with ultrasound-obtained fat density to reconsider how BCS is understood and provide a true ground truth measurement for comparison. Its application may help animal health and welfare by decreasing the prevalence of obesity. Overall, thermal imaging shows promise as an objective method for body condition scoring in horses and beef cows.

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Tables & Figures

Table 4-1. Summary of the Number of Horses and Cows Imaged and Scored in Each Phase

	Phase 1[1]	Phase 2[2]	Both Phases
Horses:			
Total[3]	84	81	165
QH-Types[4]	50	48	98
TB-Types[5]	34	33	67
Overlapped[6]			78
Cows:			
Total[3]	50	47	97
Overlapped[6]			29

[1] Phase 1: the first visit to each farm

[2] Phase 2: the second visit to each farm

[3] Total number of animals imaged and body conditioned score in each phase or over both phases

[4] QH-Types: Number of horses falling into the breed category of "quarter horse" (QH)

[5] TB-Types: Number of horses in the breed category of "thoroughbred" (TB)

[6] Overlapped: The number of animals imaged and scored both in phase 1 and phase 2

Table 4-2. Performance of Models Developed Using Body Surface Temperatures Obtained from a Thermal Camera to Body Condition Score (BCS) Mature Horses

*Covariates Influencing Predicted BCS Scores (P-*value)

Covariates	Neck	Shoulder	Withers	Ribs	Loin	Tail-head	Overall[7]	Across Regions[8]
Cloud Coverage[1] (%)	0.029	0.005	0.0741	0.171	<0.001	<0.001	0.0246	
YOB	0.027	0.042	0.0887	0.147	0.222	0.831	0.0725	
Color[2]	0.0899	0.037	0.0485	0.0398	0.0724	0.157	0.0376	
Breed[3]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Coat Density[4]	0.0718	0.02	0.105	0.0012	0.156	0.486	0.0078	
Scorer[5]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
In-Person[6]	0.387	0.003	0.107	0.0061	0.785	0.782	0.326	
Variability Between BCS Scorers by Body Region								
Scorer Variability (%)	13.5	13.7	14	13.2	13.4	13	12.3	
Accuracy in Predicting BCS by Body Region and Across All Regions								
RMSE, % Mean	8.4	7.6	10.3	10.6	9.1	7.8	9.0	8.7

[1] % Cloud coverage at time of imaging

[2] Color of Animal: Bay, Dark Bay, Grey, Dark Grey, Chestnut, Red Roan, Pinto, Appaloosa, Palomino, Grullo, Dun, Buckskin, Black

[3] Breed of Animal: Thoroughbred (pure), Quarter Horse (pure), Thoroughbred cross, Quarter Horse cross

[4] Visual Determination of Coat Density: Normal, Thick, Partial Clipped, Full Clipped

[5] Individual Scorer

[6] Location of Scorer at time of Scoring either in-person or through video

[7] Overall BCS chosen by Scorers

[8] Average across all body regions (neck, shoulder, withers, ribs, loin, tailhead, & overall)

BCS, body condition score; YOB, year of birth; RMSE, root mean square error

Table 4-3: Performance of Models Developed Using Body Surface Temperatures Obtained from a Thermal Camera to Body Condition Score (BCS) Multiparous Gestating Beef Cows

*Covariates Influencing BCS Scores Assigned by Body Condition Scorers (P-*value)						
Covariates	Brisket	Shoulder	Ribs & Spine	Hooks & Pins	Overall[5]	Across Regions[6]
Cloud Coverage (%)[1]	0.203	0.0138	0.218	0.0296	0.129	
Scorer[2]	<0.001	<0.001	<0.001	<0.001	<0.001	
In-Person[3]	0.0021	<0.001	0.010	0.0302	0.0505	
Sun Coverage[4]	0.912	0.0893	0.0557	0.0972	0.0708	
Variability between BCS Scorers by Body Region						
Scorer Variability (%)	11.1	11.1	11.9	12.1	10.8	
Accuracy in Predicting BCS by Body Region and Across All Regions						
RMSE, % Mean	6.81	7.45	11.2	12.2	10.3	13.4

[1] % Cloud coverage of time of imaging

[2] Individual Scorer

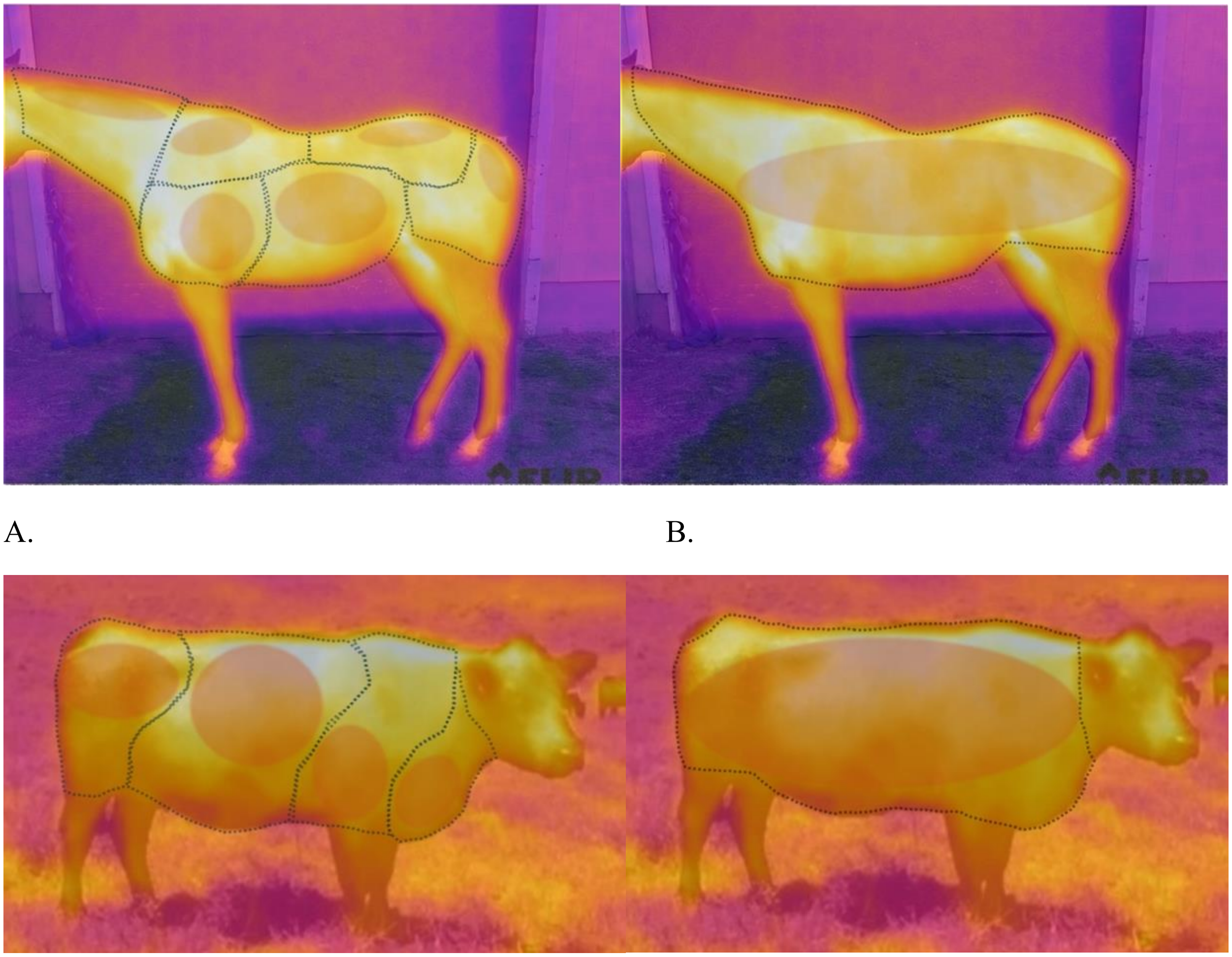
[3] Location of Scorer at time of Scoring: Either in-person or through video

[4] Sun Coverage: If animal was in the shade or in the sun when imaged

[5] Overall BCS chosen by Scorers

[6] Average across all body regions (Brisket, Shoulder, Ribs & Spine, Hooks & Pins, and Overall)

BCS, body condition score; RMSE, root mean square error



A. B.

C. D.

Figure 4-1: Visual Depiction of BCS Regions and Surface Temperature Readings Collected on Horses and Beef Cows

Visual demonstration of areas on each species for body condition scoring of anatomical regions, outlined in dotted lines, and surface temperature measurements, highlighted within each anatomical region. The oval highlights in each region are where the average surface temperature values (ºC) were obtained using the FLIR Thermal Suite Studio Software (Teledyne FLIR, LLC, Wilsonville, Oregon. Image A represents an example of the six regions assigned body scores in horses and image B represents the entire body of a horse assigned a BCS. Image C represents an example of the four body regions assigned BCS in beef cows and image D represents the entire body of a beef cow assigned a BCS.

GENERAL CONCLUSION

Precision technologies have the potential to enhance the nutritional management of livestock in extensive grazing systems. Given the substantial economic contributions of both the beef cattle and equine industries to the United States, additional support is imperative to secure the future of these sectors. The overarching goal of this work was to explore the use of precision technologies in extensive grazing systems to address the challenges in continuous forage composition monitoring, the need for modern methods to estimate digestible energy (DE) in contemporary equine diets, and the development of more objective means to assess dietary adequacy in livestock.

In Chapter 2, we evaluated a hand-held spectral sensing device and the same device when mounted on horses while grazing to predict the chemical composition of mixed-grass hay. The hand-held device accurately estimated the dry matter (DM), neutral detergent fiber (NDF), and acid detergent fiber (ADF) contents of hay, although it was less precise in determining crude protein (CP) contents. Although the pilot study with on-animal sensors was based on a small dataset, the resulting data demonstrated promise for continuous monitoring of forage quality in grazing livestock. Nonetheless, the on-animal sensors require further development in terms of functionality and durability; future work should focus on more extensive data collection from grazing animals consuming forage of variable composition.

In Chapter 3, we conducted a quantitative literature review on the digestibility of nutrients and developed a new set of equations to predict DE in modern equine diets. The models derived for each of the seven response variables explained a considerable portion of the variation reported in the literature, despite nutrient digestibility being influenced by many dietary and feed factors. Our equations, based on calculated diet information, produced DE estimates similar to those from two other established DE estimation systems commonly used in the North American feeding industry. When compared to the experimentally measured DE, our calculated equations were more reliable and realistic than the equations based solely on measured dietary information. The proposed system for estimating DE from the chemical composition of modern equine diets holds promise as a dependable option after further refinement and testing on independent datasets.

In Chapter 4, we assessed the use of thermal imaging to measure body surface temperature as an objective means to body condition score (BCS) horses and beef cattle. Although using surface temperature to determine BCS shows promise for both species, robust methods must address confounding factors such as environmental conditions and management practices. The significant variation observed between body scorers underscores the need to redefine BCS in livestock, as no true ground truth exists. Future studies might incorporate ultrasound-measured fat thickness alongside body surface temperatures to develop a reliable, accessible, and affordable tool for horse owners and producers to accurately monitor dietary adequacy in terms of adiposity.

Overall, the future of this work should focus on expanding data collection, because our algorithms are currently constrained by the available dataset–a limitation common to many precision technologies. In particular, the work presented in Chapter 4 would greatly benefit from employing a different ground truth measurement instead of relying solely on visual body condition scoring, given substantial variation observed even among large groups of scorers. For horses, BCS is widely used by veterinarians, nutritionists, and horse owners, but it is often prone to error due to differences in breed and body type, which affect the distribution of muscle and adipose tissue. Such errors can lead to inappropriate ration adjustments, potentially promoting obesity or malnutrition. Even when using body weight scales, BCS remains subjective, as body weight does not differentiate between muscle and fat–especially for animals with intermediate scores (e.g., BCS 4 to 6), where overly emaciated or obese individuals are more easily identified.