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WHAT IS THE VALUE OF CORN RESIDUE TO GRAZING CATTLE?

BY

EMILY ANN PETZEL

A thesis submitted in partial fulfillment of the requirements for the

Master of Science

Major in Animal Science

South Dakota State University

2018

WHAT IS THE VALUE OF CORN RESIDUE TO GRAZING CATTLE?

EMILY PETZEL

This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science in Animal Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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CONTENTS

LIST OF TABLES.....	vii
LIST OF FIGURES.....	viii
ABSTRACT.....	ix
CHAPTER 1: LITERATURE REVIEW.....	1
INTRODUCTION.....	2
ESTIMATES OF GRAZING SELECTION.....	3
CORN RESIDUES.....	6
UTILIZATION OF RESIDUES.....	8
ENERGY METABOLISM.....	9
CONCLUSION.....	14
LITERATURE CITED.....	15
CHAPTER 2:.....	19
ABSTRACT.....	20
INTRODUCTION.....	22
MATERIALS AND METHODS.....	23
EXP 1: Validation Study.....	23
Animal Husbandry and Sample Collection.....	23
Laboratory Analyses.....	25
NIRS.....	26
Nucleic acids and 16SrRNA gene.....	27
Calculations.....	28
Statistical Analysis.....	29

EXP 2: Field Study	30
Animal Husbandry and Sample Collection.....	30
NIRS.....	32
Calculations.....	32
Statistical Analysis.....	33
RESULTS AND DISCUSSION.....	33
EXP 1.....	33
Effect of rinsing.....	38
EXP 2.....	43
LITERATURE CITED.....	47
CHAPTER 3:.....	60
ABSTRACT.....	61
INTRODUCTION.....	63
MATERIALS AND METHODS.....	64
Experiment 1: In Situ.....	64
Calculations and Statistical Analysis.....	65
Experiment 2: Nutrient Balance.....	66
Animal Husbandry and Sample Collection.....	66
Digestion Collections.....	67
Indirect Respiratory Collection System.....	68
Sample Analysis.....	60
Calculations.....	70
Statistical Analysis.....	71

RESULTS AND DISCUSSION.....	71
Experiment 1.....	71
Digestibility.....	74
Nitrogen Balance.....	76
Gas Exchange.....	77
Energy Balance.....	78
LITERATURE CITED.....	81

LIST OF TABLES

Table 1.1: Nutrient composition of cornstalk and LH residues.....	52
Table 1.2: Effects of rinsing on chemical composition in diet samples in Exp. 1.....	53
Table 1.3: Effect of rinsing on nucleic acid and 16S rRNA gene in diet samples in Exp. 1.....	54
Table 4: Evaluation of the diet composition prediction models in Exp. 1.....	55
Table 5: Estimates of diet selection in cattle grazing corn residues in Exp. 2.....	56
Table 2.1: Chemical composition of feedstuffs fed to cattle	85
Table 2.2: Degradation kinetics from in situ disappearance of different botanical parts of corn residue.....	87
Table 2.3: Total tract digestibility of different botanical parts of corn residue from cattle fed to 90% maintenance energy requirement.....	88
Table 2.4: Influence of different botanical parts of corn residue on nitrogen balance of cattle fed to 90% of maintenance energy requirement.....	89
Table 2.5: Daily O ₂ consumption and CH ₄ and CO ₂ emissions from cattle fed different botanical parts of corn residue at 90% of maintenance and at fast.....	90
Table 2.6: Daily energy losses from cattle fed different botanical parts of corn residue at 90% of maintenance energy requirements.....	91

LIST OF FIGURES

Figure 1.1: Residual plot of the linear regression of the coefficient of variation of chemical components in cornstalk and LH residues and the coefficient of determination of the predicted ratio of cornstalk:LH residues in diet samples.....	57
Figure 1.2: Fitted broken-line plot of coefficient of determination as a function of the coefficient of variation between cornstalk and LH residues.....	58
Figure 2.1: Dry matter disappearance of corn steep liquor from in situ estimates.....	86

ABSTRACT

WHAT IS THE VALUE OF CORN RESIDUE TO GRAZING CATTLE?

EMILY ANN PETZEL

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The United States produces over 370,840,000,000 kg of corn grain and concomitantly more than 303,410,000,000 kg DM of non-grain corn residues (i.e., leaves, husks and stalk) from grain production annually. Although there is an abundance of available corn residue, only 12% of land planted to corn is grazed after harvest (Schmer, 2017), and based on current estimates of nutrient composition (NASEM, 2016), while 30% of available corn residues could maintain the entire United States cow herd. Grazing cattle often select diets with greater nutrient density and digestibility in comparison to the overall biomass available; however, most estimates of nutrient density in corn residues are based on analyses of mechanically harvested residues (i.e., the overall biomass available for grazing). More accurate measurements of selection of botanical parts by grazing cattle and subsequent nutrient intake can allow for improved estimates of performance of cattle grazing corn residues and for development of management strategies that can optimize forage utilization. The first experiment used 6 ruminally cannulated cows to evaluate predictions of diet selection based on chemical components and post-sampling processing techniques in diet samples collected through ruminal evacuation. Predictions of diet composition were improved by increasing differences in concentration of chemical components between cornstalk and leaf and husk (LH) residues up to a coefficient of variation of $22.6 \pm 5.4\%$. Acid detergent insoluble ash, acid detergent lignin, and near infrared reflectance spectroscopy provided the most accurate

estimates of composition of the diet. A second experiment was conducted with 6 ruminally cannulated cows to estimate the caloric value and digestibility of corn husk, leaf and stalk. Cattle were fed to near to their maintenance energy requirements with either corn leaves, husk or stalk and corn steep liquor. Net energy available for maintenance value from corn leaves, husks and stalks were 1.80 Mcal/kg DM, 1.15 Mcal/kg DM and 0.83 Mcal/kg DM, respectively. Differences in energy available for maintenance were largely described by differences in methane emissions; cattle fed leaf residue had 116 and 66% less energy losses from methane than cattle fed husk and stalk, respectively. Clearly, selection of different botanical parts by cattle grazing corn residues can change nutrient and energy intake and diet digestibility. Estimates of nutrient composition of corn residues based on total harvestable biomass are unlikely to accurately reflect diets selected by grazing cows. Development of more accurate estimates of diet selection among grazing cattle and feed values of each botanical part in corn residue will allow more accurate estimates of cattle performance. Further, an improved understanding of the feed value of each botanical part in corn residue to cattle may allow for a better evaluation of different management strategies that influence intake of different botanical parts in corn residues by grazing cattle.

CHAPTER 1:
LITERATURE REVIEW

INTRODUCTION

Currently the United States produces nearly 371,000,000,000 kg of corn residues (i.e., leaves, husks and stalks) that are available for grazing or mechanical harvest.

Amounts of corn residue produced is linearly related to amounts of corn grain produced and corn residues account for 45% to 55% of the total biomass of senesced corn plants (Gallagher and Baumes, 2012). Improvements in agronomic practices and long-term trends in agricultural economies have resulted a 250% increase in corn grain production across the last 60 years. Thus, it is reasonable that availability of corn residues will continue to increase.

Mammals do not produce large amounts of enzymes capable of hydrolyzing complex carbohydrates in forages. However, pregastric fermentation of forages by ruminal microflora allows for utilization of β -linked carbohydrates and produces volatile fatty acids and microbial protein. Thus, ruminants are able to use energy and nutrients from forages to a greater extent than other animals, and can allow for production of products useful to humans (e.g., meat, milk, wool, leather) from land not suitable for growing crops. Unfortunately, rates and extent of ruminal fermentation of corn residues are often limited (Chesson, 1984), in part, because corn residues contain relatively large amounts of lignin and small amounts of ruminally available nitrogen. However, grazing cattle often select diets with greater nutrient density and digestibility in comparison to the available biomass. Indeed, cattle grazing corn residue selectively consume different botanical parts and amounts of lignin and ruminally available N differ in each botanical part (i.e., leaves, husks, and stalks of corn residues). Accurate estimates of diet selection

among cattle grazing corn residues can allow for optimized management strategies and improved estimates of performance among cattle grazing corn residues.

ESTIMATES OF GRAZING SELECTION

Grazed forages can allow for a more economical production system compared to feedlot systems using stored forages. Extension of the grazing season can be accomplished by utilizing crop residues (soybean, wheat, corn, etc.) and cover crops planted after grain removal (radishes, turnips, grasses, etc.). Grazing mature plants and cover crops allows for reduced feeding costs by extending the grazing season. Dry matter intake and thus production from these residues is impacted by several mechanisms. Cattle will consume feed until energy requirements are met (Van Soest, 1994; NASEM, 2016). Cattle consuming residues may not meet energy requirements because of DMI limitations created by bulk fill of the rumen based on gastrointestinal volume (Van Soest, 1994). Several authors have proposed mechanisms that cause bulk fill limitations. Mertens (1987) used neutral detergent fiber (NDF) content and the digestibility of the feedstuffs to estimate voluntary dry matter intake. Greater fiber content increases retention time of the feedstuffs due to the increased fermentation need to digest feed particles to allow for ruminal passage. Mertens estimated that cattle can eat 1.1% of BW in NDF before intake is hindered. Corn residues, or other poor forage sources, contain high fiber content, thus cattle will consume less DM than expected Church (1988) proposed that the mechanism behind bulk fill limitations is based on physical distension of the rumen and neural signals. This follows Mertens' mechanism as greater fiber increase time and total material in the rumen. Previous grazing experience or perceived palatability influence the diet selection of grazing ruminants (Lobato et al., 1980). Non grazing acclimated cattle

will initially select a more diverse diet and will shift consumption patterns over time to a more homogeneous array of the more nutritionally beneficial plants. Cattle will avoid plants that hinder digestion and energy intake (Provenza and Balch, 1987). Some of these hinderances include high lignin content, toxins and glucosinolates among others. As consumption of plant parts or species shifts, nutrient balance of the grazing cattle will change. In chemically diverse pastures, estimates of nutrient consumption may be incorrect if diet selectivity is not accounted for. Increase accuracy of estimates of diet selection will allow for more precise nutrient management of grazing cattle.

Most existing methods for estimating botanical composition of diets of herbivores lack precision and accuracy (Ali et al., 2005). Each method has advantages and disadvantages which should be evaluated prior to use to allow for accurate estimates of diet selection. There are two broad categories for estimating compositional intake; indirect and direct measures.

Indirect measures do not directly quantify the consumption of the animals but merely work to generalize the selection of the animal. One method for indirect estimates is using quadrat biomass samples. A representative sample of the grazing field is collected prior to grazing while another is collected post grazing. The difference in botanical composition between pre and post grazing samples is used to quantify dietary consumption of the animal (Van Soest, 1994). Exclusion cages work similarly but help to account for weathering of plant species or parts. Subsections of the grazing plot are fenced off and serve as the basis for available forage to the animal. After grazing, a sample is taken to quantify plant diversity and abundance from within the cage and from outside of the cage (Van Soest, 1994). More accurate estimates of diet selection can be

obtained through visual observation of the grazing animal and clipping forage similar to intake of the animal. The observer follows behind the grazing ruminant and clips plants similar to what is being ingested (Holechek et al, 1982). This method requires a tame animal in order to be in close proximity without altering grazing behavior. Several other factors may influence the accuracy of this technique; physiological condition, degree of hunger, topography, other animals, and past grazing experience (Krueger et al., 1974). Observer error from this method can occur in brushy or complex pastures due to visual hinderances (Holechek et al., 1982). Another indirect method of estimating diet selection is through transect surveys in which lines are drawn and plants are collected from these lines to get a description of available biomass. These samples work well for short grazing periods and can only allow for intake of groups of animals (Meijs et al., 1982; Reeves et al., 1996).

Direct methods of estimating diet composition and selectivity more accurately reflect intake of the animal but are prone to endogenous contamination. One direct estimate is microhistological classification of masticate or feces based on visual plant structure. Microhistological classification requires a high degree of training and it is difficult to identify closely shaped plants (Holechek et al., 1982). Several other factors can affect the accuracy of this method including differential digestibility of plant parts, presence of woody materials, observer error, calculations and sample preparations (Alipayo et al., 1992). Another direct method to estimate diet composition is quantification of plant waxes in feces. Dove and Mayes. (1991) showed that plant waxes contain different chemical components, one being long-chain hydrocarbons (C₂₅-C₃₅, n-alkanes and alkenes). N-alkanes are found in the cuticular wax and different plants

have varying patterns of individual alkanes which allow for estimates of diet composition (Dove and Mayes, 2005). Estimated reliability of the n-alkane technique decreases as the plant diversity increases due to greater residual error. Evaluation of the n-alkane chain lengths in feces compared to available biomass allow for calculations of diet composition. Accuracy of estimates based on n-alkanes is reduced by the inherent diurnal variation in the concentration of the alkanes in feces (Lewis et al., 200) as well as incomplete recovery of the marker in feces (Ferreira et al., 2015). Another direct method for estimation of diet composition is through collection of diet contents through esophageal or rumen collections. Digesta collections allow for diet nutrient composition estimates but these values may be contaminated by either microbial or salivary contaminations (Holechek and Gross, 1982). Using a washing protocol such as used in in-situ studies (Vanzant et al., 1998) can alleviate these contaminants.

Direct and indirect methods can allow for estimates of diet composition and selectivity of grazing animals. Quantifying intakes of grazing animals can allow for accurate estimates of nutrient status based on consumption of each plant species or part.

CORN RESIDUES

Grazing corn residues is a predominate nutritional management practice in the western Corn Belt (Sulc and Franzluebbers, 2014). There are several advantages to utilizing corn residues for both the soil and animal; managing residue quantity in high-production fields, supplemental revenue and a cost-effective method for integrating crop and livestock systems (Schmer et al., 2017). The most economical use of corn residues is by grazing due to added cost of transportation, harvesting, storing and feeding baled residue (Klopfenstein et al., 1987). Corn residues are abundant in biomass with yields

near to amounts of grain harvested (Owen, 1976). Corn residue is predominantly stalk residue followed by leaf, husk and cob (Gutierrez-Ornelas, 1991; Stalker et al., 2015). Very little corn grain remains after grain harvest and the available grain is rapidly consumed (Fernandez-Rivera, 1989). Stalker et al. (2015) showed that if all residue was utilized, there would be 10 metric tons of corn residue for each beef cow in the United States herd. Current utilization is less than this with the 19 corn belt states grazing 4.87 million ha (Schmer et al., 2017). Corn residue is abundant in availability and with proper management, can be a core feedstuff in cattle production systems in the Midwest.

Botanical parts of corn residue vary widely in nutrient composition. Crude protein (CP) content of harvest corn residue is commonly low (4.5%, Leask and Daynard, 1973). Leaf residue has the greatest CP content (6.5%, Stalker et al., 2015), while husk and stalk are less CP (4.0% and 3.0% respectively, Gutierrez-Ornelas and Klopfenstein, 1991). Corn residue is a fibrous forage containing around 85% neutral detergent fiber (Fernandez-Rivera and Klopfenstein, 1989). Digestibility of each botanical part of corn residue varies. Husk is the most digestible with in-vitro organic matter digestibility (IVOMD) at 64% while stem or stalk is reported at 44% (Stalker et al., 2015). Similarly, Gardine et al. (2016) found husk to be most digestible (DOM, 55.6%) followed by leaf (40.7%) and stem (38.6%). Digestibility of residue botanical parts decreases during the grazing period (Lamm and Ward, 1981) due to weathering and decomposition. Total residue IVOMD dropped from 72.0% to 59.2% in the spring. There is an even greater decrease in digestibility for botanical parts consumed by cattle (38% for leaves and husks).

UTILIZATION OF RESIDUES

Cattle grazing corn residues are highly selective in the plant parts that are consumed. Fernandez-Rivera (1989) found 70% of the dry matter intake (DMI) of grazing cattle consisted of leaves and husks. Available grain is consumed rapidly, within the first 30 d of grazing (Gutierrez-Ornelas and Klopfenstein, 1991). While the energy content of each botanical part is currently unknown, husk is more digestible (in-vitro dry matter digestibility = 67%, NDF disappearance = 91.6%) than other corn residue parts while leaves provide more protein (7.8%, Fernandez-Rivera and Klopfenstein, 1989). Selective consumption of these botanical parts based on digestibility and optimal N conditions follows the bulk fill mechanism where cattle consume diets that contribute less to ruminal fill (Mertens, 1986; Church, 1988).

Russell et al. (1993) showed that cattle gain weight when grazing allowance is large (1.46 ha/cow), most likely attributed to a greater availability of more nutritious fractions (i.e., husk and leaf). Additionally, strip-grazing cattle increased utilization of the residue (index of 1.48 versus 1.27 for conventional grazing) as cattle consumed greater amounts of residue to meet energy demands. Corn residues are typically used as a winter feedstuff for mid-gestation cattle due to lower nutrient demands at this stage of production. While corn residue is of lower feeding value than other stored forages, impacts on pregnancy of the grazing cow are minimal. Several authors (Morrison et al., 1999; Freetly et al., 2000) have observed no differences in pregnancy rate of cows experiencing change in body condition score (BCS) during the final trimester of pregnancy. Depending on grazing allowance, cows may lose body weight and BCS

during this period (Shinners et al., 2007) but this loss is not expected to have an impact on production.

In addition to grazing cows, corn residues are being incorporated into stocker programs. In steers grazing corn residues, low average daily gain (ADG) is achieved during the grazing period but the steers have compensatory gain while in the feedlot, reaching heavier final weights than control calves (Anderson et al., 2005). Additionally, when the calves are sold on a live weight basis, corn residue grazing is more economical (Anderson et al., 2005). Cox O'Neill et al. (2017) also found that there is reduced return during the grazing season due to lower ADG but cattle compensate during the finishing period. Furthermore, heifers that graze corn residues during backgrounding achieve higher ADG post-grazing compared to control fed heifers (0.33 versus 0.30 kg/d, Larson et al., 2011). Like the grazing mature cow, there are no decreases in pregnancy rate in the heifers grazing corn residue (Larson et al., 2011). Funston and Larson (2010) documented this as reduced body weights of grazing heifers did not affect pregnancy rates.

ENERGY METABOLISM

Greater utilization of corn residues and diet selection of grazing cattle increase the need for more accurate estimates of the energy value of each botanical part. Beef cattle rations are generally formulated on the basis of net energy for maintenance (NEM) and growth (NEg). Current estimates of energy value of corn residue are based on total digestible nutrients (TDN) estimates of baled corn residue and the corresponding NEM is derived from TDN. This energy value represents the composite of corn residue parts which may not accurately reflect the diet selected by the grazing animal. Additionally,

TDN values do not account for different animal functions (breed, age, activity, etc.) and different losses from fermentation, digestibility, and efficiencies of nutrient utilization. Estimates of NEm should be obtained for each botanical part cattle consume (i.e., husk, leaf and stalk) through energetic studies. There are three main reasons for performing animal energy research as described by Johnson et al. (2003); 1) to measure relationships between heat production and gas exchange, 2) evaluate feeding values for feeds that contribute to energy requirements and expenditure, and 3) to predict sources of energy expenditure. To accomplish this all energy expenditures need to be accounted using the net energy system. Gross energy (GE) is calculated by combusting feedstuffs in a standardized (i.e., benzoic acid) adiabatic bomb and GE intakes are estimated by multiplying GE of the feedstuff by animal dry matter intake. Removal of fecal energy outputs from GE corresponds to estimates of digestible energy (DE). Removal of urinary and methane energy results in metabolizable energy (ME). Feed energy available for maintenance (NEm) is calculated by removing heat increment estimates from the ME value.

Measures of fecal, urine and methane energy are simple to obtain. Estimating heat production requires measurement of the increase in heat production over fasting heat production following consumption of food. Calculated heat production includes the heat of fermentation and the heat of nutrient metabolism (Flatt et al., 1969). More precisely, heat increment includes, the work associated with the digestion and metabolism of food, the work of excretion by the kidney and the increased muscular activity of various organs due to metabolism of nutrients (Bondi, 1987).

Heat production can either be calculated from indirect or direct calorimetry. Calorimetry is simply the measurement of heat. These methods of estimating heat differ by what they measure; indirect calorimetry measures heat production and direct calorimetry measures heat loss (Nienaber et al., 2009).

Indirect calorimetry quantifies the substrates of oxidation allowing for calculated estimates of heat production. Several techniques can be implemented to establish the oxidation of substrates. One technique is using a respiration chamber or head box to determine the amount of oxygen (O₂) consumed and carbon dioxide (CO₂) and methane (CH₄) produced. Heat produced for each liter of O₂ consumed is nearly constant regardless of metabolic substrate. The production of CH₄ decreases the energy efficiency of the feedstuff due to the exhalation of energy into the atmosphere. Head boxes do not allow for estimates of CH₄ production from hind gut fermentation which can be a potential drawback as the headbox only surrounds the head versus the whole body (Johnson and Johnson, 2005). Boadi and Wittenberg (2002) showed that 89% of hindgut methane is absorbed by blood and expired through the lungs which the headbox would collect. Whole body calorimeters capture methane losses from flatulence but may lead to decreased accuracy due to bodily fluids remaining on the floor during collection periods (Johnson and Johnson, 2005). In order to estimate consumption and production of the various gases, accurate and precise air flow rates are needed as well as measurements of humidity and temperature to correct gas values. A negative pressure within either the headbox or whole body calorimeter is crucial to prevent error from potential leaks. Estimates of heat production are calculated from the Brouwer (1965) equation:

$$HP \text{ (kcal)} = 3.866 (O_2) + 1.2 (CO_2) - 0.518(CH_4) - 1.431 (N)$$

where O_2 , CO_2 and CH_4 are measured in liters per day and N in grams per day of urinary nitrogen. This method when compared to direct calorimetry allows for testing in various environmental conditions (Nienaber et al., 2009). Based on O_2 consumed and CO_2 produced the respiratory quotient (RQ) can be calculated. The RQ allows for estimates of what substrate is being utilized for energy. A RQ near to 1.0 represents carbohydrates being oxidized for fuel. Utilization of fats will equal a RQ near 0.71 with variation dependent on chain length of fats being metabolized. The RQ for protein utilization is near 0.81 and also varies depending on amino acids present (Brody, 1945).

Another technique of indirect calorimetry is serial slaughter. This was first performed by Hawes and Gilbert in 1861 where they showed that carbohydrates contributed to carcass fat deposition. Lofgreen and Garrett (1968) described using serial slaughter to obtain net energy requirements which were the basis for several beef NRC recommendations. This technique requires larger number of animals as each individual can only be used once. A subset of the population is slaughtered prior to experimentation to get a baseline carcass energy value. Following the conclusion of the trial, another group is slaughtered to get a final energy content. Energy content of the carcasses or body tissue samples is obtained through the use of bomb calorimetry. Energy retention is calculated as the difference in the estimated energy content of the animals before and after the trial. From this energy retention, heat production can be calculated based on the difference between ME and retained energy.

Energy retention can also be estimated from carbon and nitrogen balance. Retained quantities of protein and fat can be estimated from the balance of nitrogen (N) and carbon (C). To achieve the retained energy value, the quantities of elements retained

are multiplied by their corresponding energy value. Nitrogen excretion is obtained through feces and urine while C is excreted in CH₄ and CO₂ additionally. This method tends to overestimate the retained energy due to collection inefficiencies.

All measures estimated through indirect approaches have inherent errors due to the disregard for spatial configurations of molecules and the manner in which energetics are calculated (Blaxter, 1962). While these methods have errors, these have small impacts on estimates of energy retention. Strong correlations between the respiration exchange and the C-N balance studies in terms of heat production have been shown. Using a closed circuit instrument, the Hannah Institute showed a near perfect correlation between the two methods (Blaxter, 1962).

Heat increment can also be measure through means of direct calorimetry. Direct calorimetry works similarly to an adiabatic bomb by quantifying the heat given off by the animal through changes in temperature. Direct calorimetry measures the sensible and evaporative heat losses of the animals (Nienaber et al., 2009). The first such calorimeter was used by Lavoisier and Laplace in the 18th century and the heat released by the guinea pig was used to melt ice surrounding the animal. Further analysis showed that the amount of ice melted corresponds to a definite amount of CO₂ exhaled (Brody, 1945). Further models used the same technique as the bomb calorimeter with heat being transferred to a surrounding mass of water. Newer designs were completed in the 1900's but these apparatuses are cumbersome to use, have high hydrothermal equivalent and are expensive to run. Other designs of direct calorimeters utilize the gradient layer effect which measures temperature changes in insulated air spaces surrounding the animal.

CONCLUSION

Diet selection preferences of grazing cattle can impact the energy status of the animal based on nutrient intake. Thus, it is important to be able to accurately estimate botanical consumption of the animal. Diet selection of both grazing and confined cattle introduces variability in nutrient intake. Currently energy estimates are based on composite samples of available forages which does not reflect animal intake. Furthermore, energy values are calculated from nutrient composition and not nutrient balance studies which fails to reflect differences in fermentation and metabolic utilization of feedstuffs. Consequently, it is important to elucidate energy values for each botanical part consumed by cattle.

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CHAPTER 2:**Estimates of diet selection in cattle grazing cornstalk residues by measurement of chemical composition and near infrared reflectance spectroscopy of diet samples collected by ruminal evacuation^{1,2}**

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ABSTRACT

Six ruminally cannulated cows (570 ± 73 kg) fed corn residues were placed in a 6×6 Latin square to evaluate predictions of diet composition from ruminally collected diet samples. After complete ruminal evacuation, cows were fed 1-kg meals (DM-basis) containing different combinations of cornstalk and leaf and husk (LH) residues in ratios of 0:100, 20:80, 40:60, 60:40, 80:20, and 100:0. Diet samples from each meal were collected by removal of ruminal contents after 1-h and were either unrinsed, hand-rinsed or machine-rinsed to evaluate effects of endogenous compounds on predictions of diet composition. Diet samples were analyzed for NDF, ADF, acid detergent insoluble ash (ADIA), ADL, CP and near-infrared reflectance spectroscopy (NIRS) to calculate diet composition. Rinsing type increased NDF and ADF content and decreased ADIA and CP content of diet samples ($P < 0.01$). Rinsing tended to increase ($P < 0.06$) ADL content of diet samples. Differences in concentration between cornstalk and LH residues within each chemical component were standardized by calculating a coefficient of variation (CV). Accuracy and precision of estimates of diet composition were analyzed by regressing predicted diet composition and known diet composition. Predictions of diet composition were improved by increasing differences in concentration of chemical components between cornstalk and LH residues up to a CV of $22.6 \pm 5.4\%$. Predictions of diet composition from unrinsed ADIA and machine-rinsed NIRS had the greatest accuracy (slope = 0.98 and 0.95, respectively) and large coefficients of determination ($r^2 = 0.86$ and 0.74 , respectively). Subsequently, a field study (Exp. 2) was performed to evaluate predictions of diet composition in cattle (646 ± 89 kg) grazing corn residue. Five cows were placed in 1 of 10 paddocks and allowed to graze continuously or to strip-graze

corn residues. Predictions of diet composition from ADIA, ADL and NIRS did not differ ($P = 0.99$), and estimates of cornstalk intake tended to be greater ($P = 0.09$) in strip-grazed compared to continuously grazed cows. These data indicate that diet composition can be predicted by chemical components or NIRS by ruminal collection of diet samples among cattle grazing corn residues.

KEY WORDS: Cattle, Corn residues, Chemical components, Diet Selection, Near infrared reflectance spectroscopy

INTRODUCTION

Diet selection among cattle grazing forage can have large impacts on animal performance and efficient conversion of plant tissues to body mass when bulk-fill mechanisms limit caloric intake (National Academies of Sciences, Engineering and Medicine, 2016). Annual forages (e.g., corn residues, simple mixtures of cover crops) often have large differences in nutrient content between different plant components and composition of diets selected by grazing cattle may have large impacts on nutrient digestion (Brunsvig et al., 2017). Selection of plant tissues that facilitate optimal rates and extent of digestion among grazing cattle can ameliorate limits in DMI. Unfortunately, accurate measures of plant tissues selected by grazing cattle are difficult to obtain in comparison to cattle that are housed individually and fed in confinement. As a result, a number of indirect methods (e.g., clipping forage before and after grazing, transect surveys of plant populations) have been developed to estimate diet selection among grazing cattle; however, indirect methods of diet selection have inherent amounts of inaccuracy because indirect methods of diet selection fail to account for grazing by indigenous herbivores (e.g., non-domesticated ruminants, insects, rodents) and diet selection estimates include forage that decomposes between measurements (Van Soest, 1994) or is removed from the grazing area by wind. Conversely, measures of diet selection from cattle fitted with ruminal or esophageal cannulas allow direct measures of diet selection in grazing cattle. Direct identification of masticated diet samples via histological measures of ingested plant tissue collected from cannulated cattle is difficult and can be imprecise without proper training (Slater and Jones, 1971; Holechek et al., 1982; Alipayo et al., 1992). Several authors (Dove and Mays 1996; Kelman et al., 2003;

Boland et al., 2012) have suggested that plant waxes in feces may facilitate accurate estimates of diet selection in grazing cattle; however, attempts to estimate diet selection from fecal recoveries of plant waxes are largely variable (Heublein et al., 2017) and it is unclear if fecal recoveries of plant waxes are complete (Dove and Mays, 2005). Diet samples collected from ruminal evacuation are collected before any appreciable amounts of fermentation of ingested plant tissues can occur (Towne et al., 1986; Wells and Russell, 1996). Thus, it is likely that few nutrients are lost from diet samples collected via ruminal evacuation. Yet, diet samples collected by ruminal evacuation contain nutrients from endogenous sources (e.g., saliva, sloughed epithelia; Van Soest, 1994). Methods that mitigate contamination of diet samples collected from ruminal evacuation may allow for accurate estimates of nutrient composition of diets and increase precision of estimates of diet selection based on chemical composition of diet samples. An increased understanding of diet selection among cattle grazing corn residues and other simple mixtures of annual forages could allow for development of nutritional and management strategies that optimize utilization of these plant tissues by cattle.

MATERIALS AND METHODS

Exp. 1: Validation Study

Animal Husbandry and Sample Collection. All procedures that involved the use of animals in this project were approved by the South Dakota State University Institutional Animal Care and Use Committee (protocol approval No. 17-040A).

Beginning 16 d prior to experimentation, 2 ruminally cannulated Angus cows (531 ± 44 kg; 2.5 ± 0.5 yr) and 4 ruminally cannulated Angus x Simmental cows (615 ± 35 kg; 2.25 ± 0.4 yr) housed in a common drylot pen (0.4 ha) were fed long-stem mechanically harvested corn residue (84.2% DM, 3.6% CP, and 72.3% NDF) to ad

libitum and allowed free choice access to pressed mineral and vitamin (Prairie Pride 4% Mineral Block, Ridley Inc, Mankato, MN; 20% Ca, 12% NaCl, 4% P, 1,000 ppm Zn, 100 ppm Cu, 36 ppm I, 36 ppm Se, 143,300 IU/kg Vitamin A, and 35,932 IU/kg Vitamin D₃) and water. Subsequently, cattle were moved to individual pens (1.65 x 2.54 m) in a temperature (19°C) and light-controlled (16 h of light daily) room at the South Dakota State University Ruminant Metabolism Facility and fed the same long-stem corn residues at 0800 h and 2000 h daily with free choice access to the same pressed mineral and vitamin and water. Cows were housed individually for 2 d and then placed in a 6 × 6 Latin square to evaluate predictions of diet intake of cornstalk and leaf and husk (LH) residues via measures of several innate chemical components in diet samples.

Measures of diet selection were accomplished by completely evacuating ruminal contents from cows at 1900 h daily for 6 d. Immediately after evacuation of ruminal contents (Reid, 1965), cows were fed meals (1 kg DM) composed of various combinations of cornstalk and LH residues (Table 1.1). Cornstalk and LH residues were previously ground (Thomas Wiley Mill Model 4; Thomas Scientific USA, Swedesboro, NJ) to pass a 6 mm screen and each meal was offered in 250 g (DM basis) aliquots to eliminate sorting of each component from meals by cows. Meals fed to cows consisted of different combinations of cornstalk and LH residues in ratios of 0:100, 20:80, 40:60, 60:40, 80:20, and 100:0. Additionally, LH residues were provided as a mixture of leaves (78.7%) and husk (21.3%) in amounts identical to the composition of individual corn plants measured in Exp. 2 and were similar to previous reports of relative amounts of LH commonly observed in corn residues (Stalker et al., 2015). Samples (50 g DM) of each meal were collected daily, composited and ground to pass a 1 mm screen (Thomas Wiley

Mill Model 4) for determination of DM, OM, NDF, ADF, acid detergent insoluble ash (ADIA), ADL, and N. Replicate measurements of mechanically harvested biomass were determined by quadrat sampling ($n = 10, 0.25 \text{ m}^2$) after physically unrolling a cornstalk bale. Within the quadrat, plant biomass was collected and subsequently separated into stalk, leaf, husk, and cob prior to DM determination. Corn grain was not included in measures of harvested biomass in cornstalk bales because no corn grain was recovered.

Diet samples from each meal were collected by removal of ruminal contents after 1 h (Harris et al., 1967; Gelvin et al., 2004; Kirch et al., 2007) and ruminal contents removed prior to diet sampling were replaced. Diet samples were weighed and an aliquot (250 g) was dried (55°C for 36 h). Concurrently, another aliquot (250 g) was hand-rinsed by wrapping diet samples in 4 layers of cheesecloth and slowly vortexing samples by hand for a total of 30 revolutions in 4 L of clean tap water prior to drying (55°C for 36 h). After each aliquot of diet samples were partially dried, diet samples were ground to pass a 1 mm screen (Thomas Wiley Mill Model 4; Thomas Scientific USA) and 4 g of each hand-rinsed diet sample was then placed into polyethylene bags (10 x 20 cm, pore size = $50 \pm 10 \mu\text{m}$; Dacron, Ankom Technology, Fairport, NY) and machine-rinsed 5-times in a commercial washer (Fabric-Matic, Model A511S, Maytag, Newton, IA); each rinse consisted of a 1 min. rinse followed by a 2 min. spin cycle (Vanzant et al., 1998). After machine-rinsing, samples were dried at 55°C for 24 h.

Laboratory Analyses

Samples of unrinsed, hand-rinsed and machine-rinsed diet samples were analyzed in duplicate for DM, OM, N, NDF, ADF, ADIA and ADL. Dry matter was measured by drying at 105°C for 16 h, and OM was determined by combustion (500°C for 16 h).

Additionally, N content was analyzed by the Dumas procedure (method no. 968.06; AOAC, 2016; rapid Max N exceed; Elementar, Mt. Laurel, NJ). Neutral detergent fiber was measured as described by Van Soest et al. (1991) and included additions of α -amylase and sodium sulfite; ADF was measured nonsequential to NDF (Van Soest et al., 1991), and ADIA was calculated by combustion (500°C for 16 h) of ADF residue. Acid-detergent lignin was measured after thoroughly soaking ADF residue in 72% (wt/wt) sulfuric acid for 3 h and agitating ADF residue in acid each 30 min (Van Soest and Robertson, 1980). Measures of NDF, ADF, and ADL were corrected for ash content which was measured by combustion (500°C for 8 h).

NIRS

Near infrared reflectance spectroscopy (NIRS) was recorded at 2-nm intervals from 1,100 to 2,498 nm with a Foss NIRS 5000 (Foss Inc., Eden Prairie, MN). Predictions of ratios of cornstalk:LH residues were calculated from standards (n = 50) that ranged from 100:0 to 0:100 cornstalk:LH residues; each intermediate standard was created by replacing 2% of cornstalk with LH residues. Each fifth standard was excluded from development of the predictive model to subsequently evaluate accuracy and precision of predictions. A predictive model was developed from the full spectrum using WINISI II (version 1.02a; FOSS INC, Eden Prairie, MN) by regression of modified partial least squares means. Subsequently, NIRS predictions of ratios of cornstalk:LH residues were corrected for particle size using standard normal variate (SNV) and Detrend (WINSI II; FOSS INC). Curve fitting math treatments were analyzed with the math treatment 1, 4, 4, 1 (derivative, gap, smooth, and smooth 2), which generated the most robust equation (SE of calibration equation = 0.40, $R^2 = 0.99$, SE of the cross

validation = 1.82, coefficient of determination in the cross-validation = 0.99). The predictive model was validated by regression of NIRS predictions of ratios of cornstalk:LH residues with known amounts of cornstalk:LH residues in the 10 cornstalk:LH residue standards removed from model development (slope = 1.01, bias = 0.036, SE of prediction = 2.5, $r^2 = 0.99$). Predictions of ratios of cornstalk:LH residues in diet samples were obtained by scanning each diet sample and comparing the spectra to the reference values.

Nucleic acids and 16S rRNA gene

Nucleic acids were isolated from samples using the repeated bead beating plus column method, as previously described by Yu and Morrison (2004). Briefly, 86.46 ± 11.86 mg of dried, ground diet sample were lysed by bead beating with 0.4 g of zirconium beads at 3,000 rpm (3 min) in lysis buffer (0.5 M NaCl, 50 mM Tris-HCl, 50 mM EDTA, 4% SDS), followed by heat treatment at 70°C (15 min). Lysate was recovered by centrifugation ($16,000 \times g$, 5 min, 4°C). Impurities and SDS were removed by ammonium acetate precipitation (10 M, 20% volume). Deoxyribonucleic acid was recovered from the lysate using isopropanol precipitation, then further purified using column filtration (QIAamp DNA Stool Kit, QIAGEN GmbH, Hilden, Germany). Nucleic acid concentration was determined using the NanoDrop 2000 Spectrophotometer (ThermoScientific, Waltham, MA).

Quantitative polymerase chain reactions were carried out with the Stratagene Mx3005P Thermocycler (Agilent Technologies, Santa Clara, CA) in a 20 μ l volume containing the following reagents: 10 μ l SYBR green 1 Step 2xiTaq (BioRad, Hercules, CA), 2 μ l of each primer (40 μ M 1114F and 1275F; Denman and McSweeney, 2006), 1 μ l

template DNA (at 10ng/μl concentration), and 5 μl H₂O. Polymerase chain reaction amplification was initiated by a hot start at 95°C for 3 min, followed by 40 cycles of 95°C for 10 s and 60°C for 30 s. Cycles to threshold (Ct) were determined by the MxPro-Mx3005 software (V4.10).

Calculations

Dry matter was calculated as partial DM (55°C for 36 h) multiplied by DM measured after drying at 105°C for 16 h. Composition of mechanically harvested bales of corn residues was calculated (DM-basis) as the quotient of each corn residue and total biomass. Differences in concentration of each chemical component between cornstalk and LH residue were standardized by calculating a coefficient of variation to compare effects of greater differences in concentration of chemical components between cornstalk and LH residues on predicted diet composition. The average concentration of each chemical component was calculated across cornstalk and LH residues, and a standard deviation was calculated between the concentration of a chemical component in cornstalk or LH residue. Coefficients of variation were calculated as the quotient of the standard deviation and the average of the nutrient concentration within each chemical component (NDF, ADF, ADIA, ADL, or CP). The absolute value of differences in concentrations of NDF, ADF, ADIA, ADL or CP in diet samples and cornstalk ($\Delta_{\text{cornstalk}}$) or LH (Δ_{LH}) residues were calculated, and proportional intakes of cornstalk and LH residue were calculated as:

$$\text{Proportional cornstalk intake} = \frac{\Delta_{\text{LH}}}{\Delta_{\text{cornstalk}} + \Delta_{\text{LH}}}$$

$$\text{Proportional LH intake} = \frac{\Delta_{\text{cornstalk}}}{\Delta_{\text{cornstalk}} + \Delta_{\text{LH}}}$$

The ratio of cornstalk:LH was calculated as the quotient of proportional cornstalk intake and proportional LH intake using data from treatments containing 20:80, 40:60, 60:40, and 80:20 cornstalk:LH residue. Predicted ratios of cornstalk:LH were regressed on known ratios of cornstalk:LH using the MIXED procedures of SAS (SAS Inst. Inc., Cary, NC). The correlation coefficient of regressions were calculated using the MIXED procedures of SAS as one minus the quotient of the sum of the squared error in the full model and the sum of the squared error in the reduced model (Kuehl, 2000). Initially, effects of greater differences between chemical components on accuracy of predictions of diet composition were evaluated by linearly regressing the correlation coefficient and coefficients of variation. Subsequently, effects of greater differences between chemical components on accuracy of predictions of diet composition were evaluated with a 2-slope straight broken-line model (Robbins et al., 1979) using the NLIN procedure of SAS because the plot of correlation coefficients and coefficients of variation appeared to be asymptotic. Cycles to threshold from each diet samples were calculated relative to amounts of 16S rRNA gene provided from plant chloroplasts by calculating the absolute value of the difference between the diet sample Ct value and the Ct value from plant chloroplasts in each meal (ΔCt). Relative 16S rRNA gene values were calculated as $2^{\Delta Ct}$ times the proportional DNA concentration.

Statistical Analysis

Data from 1 cow in a single period was removed from statistical analysis of measures of nucleic acids and rRNA gene data because it did not appear to be a component of a normal population (studentized residual = 5.7).

Data were analyzed for a Latin square using the MIXED procedure of SAS with the following model:

$$Y_{ijkl} = \mu + R_i + M_j + RM_{ij} + C_k + P_l + \varepsilon_{ijkl}$$

where Y_{ijkl} = the dependent variable, μ = the overall mean, R_i = the fixed effect of rinse type ($i = 1, 2, 3$), M_j = fixed effect of the ratio of cornstalk:LH residues in meal ($j = 1, \dots, 6$), RM_{ij} = fixed effect of the interaction of rinse type and meal, C_k = the random effect of cow ($k = 1, \dots, 6$), P_l = the random effect of period ($l = 1, \dots, 6$); and ε_{ijkl} = residual error. Denominator degrees of freedom were calculated by the Kenward and Roger adjustment (Kenward and Roger, 1997). Treatment means were calculated using the LSMEANS option. Effects of different combinations of cornstalk and LH residues in each meal were determined with linear and quadratic contrasts. Effect of rinse type were evaluated with the F -statistic. When the F -statistic was significant ($P \leq 0.05$) means were separated using the Student's t -test with the PDIF option of SAS.

Exp. 2: Field Study

Animal Husbandry and Sample Collection. All procedures that involved the use of animals in this project were approved by the South Dakota State University Institutional Animal Care and Use Committee (protocol approval No. 16-083A).

Beginning November 29, 2016 (64 d after grain harvest), a trial was conducted among cows grazing corn residues 6.9-km northeast of Brookings, SD (44°22'23.63" N 96°45'46.46" W) to evaluate predictions of ratios of cornstalk:LH residue intake with ADIA, ADL or NIRS. After grain harvest, a corn residue field was divided into 10 paddocks (2.02 ha). Temperature and precipitation data were obtained from a weather station located 4.82 km south of the grazing site. Chemical composition of grazed corn

residues (Table 1) was determined by analysis of biomass from 10 quadrats (0.25 m^2) stratified across the field and collected 18 d prior to grazing. Quadrat biomass was stored at 60°C and separated into cornstalk, leaf and husk prior to laboratory analysis. All corn residue samples were ground to pass a 1 mm screen (Thomas Wiley Mill Model 4; Thomas Scientific USA). Cornstalk and LH residues were analyzed for DM, OM, NDF, ADF, ADIA, ADL and CP as described in Exp. 1.

Grazing treatments consisted of continuous (3.5 animal unit days/ha) or strip-grazing (169 animal unit days/ha). Treatments were designed to achieve an identical stocking rate (5.6 animal unit month/ha) in a 48 d grazing period, but allowed comparison of a nearly 50-times greater stocking density in strip-grazing paddocks compared with continuous grazing paddocks. Twenty-one Angus (705 ± 61.3 kg, 5 ± 2 yr) and 19 Angus x Simmental (654 ± 41.1 kg, 5 ± 2 yr) cows were blocked by BW. Subsequently, 4 cows within each BW block were randomly assigned to paddocks. An additional 6 Angus (483 ± 23.2 kg, 2 yr) and 4 Angus x Simmental (544 ± 24.6 kg, 2 yr) cows previously fitted with ruminal cannulas were randomly assigned to each paddock to allow concurrent measures of diet selection. Thus, each paddock contained a total of 5 cows. Strip-grazed cattle were given access to additional corn residues at 0700 h and 1900 h daily, and all cows were individually fed 358 g DM of a common protein supplement that contained (DM-basis) 88.9% soybean meal, 4.5% molasses, 4.5% urea, 2.1% Cr_2O_3 at 0700 h daily and was designed to meet RDP requirements (NRC, 1996). After 7 d, diet samples were collected once, beginning at 0700 h by total ruminal evacuation (Reid, 1965). After removal of rumen contents, cows were allowed to graze for 45 min and rumen contents were removed, weighed and a sample was frozen (-20°C) prior to analysis of DM, ADIA

and ADL. Rumen contents removed prior to diet sampling were replaced before cows were returned to paddocks.

Unrinsed diet samples were dried at 55°C for 36 h and then ground to pass a 1 mm screen (Thomas Wiley Mill Model 4; Thomas Scientific USA). An aliquot of unrinsed diet sample was machine-rinsed as previously described in Exp. 1 and subsequently dried at 55°C for 36 h. Measures of ADIA content in unrinsed diet samples and ADL content and NIR spectra were quantified in machine-rinsed diet samples as described in Exp. 1.

NIRS

A NIRS model was developed to predict ratios of cornstalk:LH residues using procedures described in Exp. 1, but the predictive model was developed from cornstalk and LH residues collected from quadrat samples in Exp. 2. The NIRS predictive model used the 2, 10, 10, 1 math treatment with SNV and Detrend (SE of calibration = 1.98, $r^2 = 1.00$, SE of cross validation = 2.16, coefficient of determination in the cross-validation = 0.99). Identical to Exp. 1, the NIRS predictive model was validated (slope = 1.01, bias = -0.62, SEP = 2.81, $r^2 = 0.99$) by regression of NIRS predictions of ratios of cornstalk:LH residues with known amounts of cornstalk:LH residues in 10 standards removed from development of the predictive model.

Calculations

Dry matter was calculated as partial DM (55°C for 36 h) multiplied by DM measured after drying at 105°C for 16 h. Relative amounts of each corn residue in quadrat samples were calculated on a DM basis as the quotient of amounts of each

residue and total biomass. Diet composition of ratios of cornstalk:LH residues were calculated as described in Exp. 1.

Statistical Analysis

Diet samples from 1 cow in a continuous grazing paddock were removed from predictions of diet composition because this cow was observed grazing outside of its paddock and predictions of diet composition did not appear to be representative of a normal population (studentized residual = 5.1). Data from each paddock were analyzed as a randomized block design using the MIXED procedure of SAS with the following model:

$$Y_{ijk} = \mu + T_i + A_j + TA_{ij} + B_k + \varepsilon_{ijk}$$

Where Y_{ijk} = the dependent variable, μ = the overall mean, T_i = the fixed effect of grazing management treatment ($i= 1$ or 2), A_j = the fixed effect of chemical component and NIRS ($j= 1, 2$, or 3), TA_{ij} = the fixed effect of the interaction of treatment and effect of chemical component and NIRS, B_k = the random effect of block ($k = 1, \dots, 5$); and ε_{ijk} = residual error. Denominator degrees of freedom were calculated by the Kenward and Roger adjustment (Kenward and Roger, 1997). When the F -statistic was significant ($P \leq 0.05$) means were separated using the Student's t -test with the PDIFF option of SAS.

RESULTS AND DISCUSSION

Exp. 1

Corn residues remaining after grain harvest are a heterogeneous mixture of stalks, leaves, husks and cobs. Stalker et al. (2015) reported that corn residues are comprised predominately (DM basis) of stalks and leaves (40.5% and 35.1%, respectively) and that the remainder is husks (9.6%) and cobs (14.8%). A similar composition of harvested corn

residues fed in Exp. 1 (stalk = $50 \pm 1.8\%$, leaves = $27 \pm 1.0\%$, husk = $15 \pm 1.0\%$, cob = $9 \pm 1.3\%$) and in corn residues grazed in Exp. 2 (stalk = $35 \pm 3.2\%$, leaves = $47 \pm 2.3\%$, husk = $11 \pm 0.7\%$, cob = $7 \pm 2.4\%$) was observed compared to the measures of Stalker et al. (2015).

Generally, diets of cows grazing corn residues are comprised of cornstalk and LH residues because grain and cobs do not contribute measurable amounts to the diet (Lamm and Ward, 1981; Gutierrez-Ornealas and Klopfenstein, 1991; Stalker et al., 2015).

Perhaps it is not surprising that only small amounts (e.g., $<1\%$; Humberg et al, 2009) of grain remain in corn residue after grain harvest because harvest of grain from corn is often the primary incentive to corn production. Stalker et al. (2015) reported that grain is only a minor component of total corn residues available to grazing cattle, and Gutierrez-Ornealas and Klopfenstein (1991) determined that corn grain remaining in corn residues is rapidly consumed by grazing cattle. Subsequently, these authors (Gutierrez-Ornealas and Klopfenstein, 1991) concluded that corn grain is unlikely to provide measurable amounts of nutrients to cattle grazing corn residues. Similar to grain, cobs apparently do not contribute to diets of cattle grazing corn residues (Lamm and Ward, 1981; Stalker et al., 2015). Cobs contain large amounts of lignin (12%; Pointner et al., 2014), small amounts of CP (2.6% CP; Fernandez-Rivera and Klopfenstein, 1989), and in vitro estimates of DM digestion are small (36%; Fernandez-Rivera and Klopfenstein, 1989). Thus, it is not surprising that cattle grazing corn residue avoid intake of cobs (Stalker et al., 2015; Lamm and Ward, 1981), and that several authors (Lamm and Ward, 1981; Fernandez-Rivera and Klopfenstein, 1989) have limited estimates of diet selection among cows grazing corn residues to that of LH and cornstalk residue alone.

Greater differences in concentrations of a chemical component between each constituent in a binary mixture of plants or plant components may allow for improved predictions of diet composition from diet samples collected from ruminally cannulated cattle. Improvements in direct measures of diet selection from ruminally cannulated cattle may be useful in estimating diet selection among cattle grazing annual forages other than corn. It is reasonable that greater differences in concentrations of a chemical component should reduce impacts of residual variance in analysis on predictions of diet composition from a binary mixture if residual variance in analysis is similar across measures of each chemical component. Differences in concentration of each chemical component (i.e., NDF, ADF, ADL, ADIA and CP) between cornstalk and LH residues were standardized by calculating a coefficient of variation (CV) within each chemical component. Correlation coefficients were calculated by regression of the predicted cornstalk:LH ratio from each combination of rinsing and chemical component and diet samples with known ratios of cornstalk:LH. Subsequently, the correlation coefficient and differences in concentration of each chemical component between cornstalk and LH residues (differences were standardized across each chemical component by calculating CV) were regressed to evaluate effects of increased differences in concentrations of each chemical component on predictions of diet composition.

The relationship between correlation coefficients and differences in concentration of chemical components did not follow a linear model. Linear regression of correlation coefficients and standardized differences in chemical components between cornstalk and LH residue described only a modest amount of variation ($r^2 = 0.51$). Furthermore, when linear estimates accurately describe observations then residuals should be evenly

dispersed about each estimate. Residuals from linear regression of the correlation coefficients calculated from predictions of diet samples compared to the known diet composition on CV between concentration of each chemical component were plotted (Fig. 1.1). Residuals from the linear model formed an uneven distribution (i.e., U-shaped; Fig. 1.1) and failed the lack-of-fit test ($P = 0.03$). Therefore, a 2-slope nonlinear approach (Robbins et al., 1979) was used to evaluate improvements in coefficients of determination among estimates of diet composition in response to greater differences in concentration of chemical components in cornstalk and LH residues. The nonlinear model contributed to a nearly 55% improvement in the estimates of variance (pseudo- $r^2 = 0.79$) than linear estimates ($r^2 = 0.51$).

Predictions of diet composition from chemical components with greater differences in concentration between cornstalk and LH residues improved linearly up to a CV of $22.6 \pm 5.4\%$ (Fig. 1.2), but there was no improvement in predictions of diet composition by use of chemical components that had greater differences in concentration between cornstalk and LH residues. Nonetheless, these data indicate that much of the variation between predictions of diet composition and known diet composition ($r^2 = 0.86 \pm 0.11$) can be explained when diet composition is estimated from chemical components that have large differences ($CV \geq 22.6\%$) in concentration between cornstalk and LH residues. It is likely that predictions of diet composition were improved from chemical components with larger differences in concentration, because large differences in concentration between cornstalk and LH residues likely diminished effects of residuals associated with laboratory analyses on predictions of diet composition. However, factors other than residuals from laboratory analyses probably limited further improvements in

predictions of diet composition (e.g., variation related to sample collection). It is important to note that the discontinuous first derivative utilized in the 2-slope nonlinear method only provides an approximation of the data. Therefore, caution is suggested when predicting diet composition from chemical components that differ in concentrations near to the point that has been calculated to provide the optimal correlation between predicted and known diet composition unless experimental methods and conditions closely match those presented herein.

Nonetheless, these data provide strong evidence that diet composition can be predicted from different chemical components in diet samples from ruminally cannulated cows grazing binary mixtures of forage when there is a sufficient difference between the compositions of the 2 parts in the binary mixture. Brunsvig et al. (2017) previously measured effects of stocking density on diet selection of heifers grazing binary mixtures of cool-season annual forages from NDF content of diet samples. Diet composition predicted from NDF was poorly correlated to known diet composition in this study; however, differences in NDF between cornstalk and LH residues were small (CV = 5.6%) whereas differences in NDF between brassica and grass in the study of Brunsvig et al. (2017) were more than 2-times the amount of difference predicted in this study to allow optimal accuracy in prediction of diet samples among cattle grazing binary mixtures of forage. In this study, ADIA and ADL were the only chemical components that had large enough differences (CV = 75.8% and CV = 46.5%, respectively) in concentration between cornstalk and LH residues to allow for close correlations between predicted and known diet composition.

Effect of Rinsing

A large number of studies investigating nutrient disappearance from ruminally incubated polyester bags have used various rinsing techniques to remove endogenous nutrients and to improve estimates of nutrient disappearance from feed (Balch and Johnson, 1950; Coblenz et al., 1997; Vanzant et al., 1998). Chemical composition of diet samples that were hand-rinsed, machine-rinsed or unrinsed were measured to determine impacts of endogenous contributions from cattle on chemical composition of diet samples. There was no interaction of rinsing and meal composition (Table 1.2). Concentration of NDF and ADF increased ($P < 0.01$; Table 1.2) with greater rinsing, and chemical composition of ruminally evacuated diet samples more nearly matched chemical composition of feed offered. Similarly, ADL concentration of diet samples (Table 1.2) tended to increase ($P = 0.06$) and more nearly matched concentrations of ADL in feed offered after greater rinsing. However, CP concentration in diet samples decreased ($P < 0.01$; Table 1.2) with greater amounts of rinsing, and ADIA content was less after machine-rinsing but hand-rinsing had lesser impacts on loss of ADIA from diet samples. Cattle saliva contains a myriad of soluble proteins and mucopolysaccharides (Bailey and Balch, 1961; Boda et al. 1965; Bartley, 1976). Additionally, many epithelia lining the ruminal and esophageal lumen and resident ruminal microbiota contain protein. It is likely that greater amounts of rinsing removed soluble proteins and endogenous components that were loosely (e.g., saliva, epithelia, ruminal microbiota) associated with diet samples (Nocek, 1985; DeBoer et al., 1987; McAllister et al., 1994). Indeed, measures of nucleic acids were 88% and 85% less in machine-rinsed diet samples in comparison to measures of nucleic acids in hand-rinsed and unrinsed diet samples (Table

1.3). Amounts of 16S rRNA gene relative to amounts for 16S rRNA gene from plant chloroplasts in machine-rinsed diet samples were 98% and 96% less than ($P = 0.02$) than relative amounts of 16S rRNA gene in unrinsed and hand-rinsed diet samples, respectively (Table 1.3). Overall, these data suggest that machine-rinsing was able to remove most proteinaceous contribution from both epithelia and ruminal bacteria.

Endogenous contributions of cattle to ruminally evacuated diet samples do not contain fiber components (i.e., NDF, ADF, ADL, ADIA). Rinsing increased concentrations of NDF, ADF and ADL in diet samples, but rinsing decreased concentrations of ADIA. Rinsing with aqueous solutions containing neutral soaps results in losses of silica from plant materials and decreases measures of ADF because silica is not dissolved in solutions that contain acidic soaps (Van Soest, 1994). Perhaps, greater amounts of rinsing prior to measures of ADIA resulted in more extensive losses of silica from diet samples and subsequent under-estimates of ADIA. Measures of ADIA include rinsing of diet samples with acidic soaps prior to measures of ash. It is likely that acidic soaps remove endogenous contributions from cattle to diet samples but do not dissolve silica. Therefore, rinsing prior to measures of ADIA could have contributed to decreased measures of ADIA in diet samples and differences in concentration of ADIA between ruminally evacuated diet samples and ADIA concentration in feed offered were greater with greater amounts of rinsing. Measures of NDF and ADF do not include rinsing with soaps prior to analysis. Greater removal of proteinaceous endogenous contributions from cattle to diet samples prior to analysis by greater amounts of rinsing could then allow for greater concentrations in measures of NDF and ADF that more nearly match concentrations of NDF and ADF in feed offered. Several authors have suggested that

mucopolysaccharides in bovine saliva contribute to spurious measures of ADL (Lascano et al., 1970; Theurer, 1970; Olson, 1991). Greater removal of mucopolysaccharides from diet samples by greater amounts of rinsing should have decreased measures of ADL. Indeed, greater amounts of rinsing decreased measures of ADL but to a lesser extent compared to increases in NDF and ADF in response to greater amounts of rinsing. It is possible that endogenous proteinaceous contributions to diet samples from cattle were greater than contributions of mucopolysaccharides.

Analyses of samples with a known composition across a range of applicable concentrations allows for development of predictive equations that account for any variance associated with analytical procedures (i.e., variance estimates for the slope; Youden, 1951; Mandel and Linnig, 1957) and for any constant error in analysis (i.e., variance estimates for the ordinate; Youden, 1951; Mandel and Linnig, 1957) by linear regression. When linear models ideally predict concentrations of analytes then regression of known sample amounts and analyzed amounts results in unity, an ordinate that proceeds through the origin and a model that describes all variances in observations (Youden, 1951). Ratios of cornstalk:LH calculated from NDF, ADF, ADIA, ADL, CP, and NIRS analysis of unrinsed, hand-rinsed and machine-rinsed diet samples were regressed with ratios of known composition (Table 1.4). Linear models developed from NDF, ADF, CP and ADL were poor in accuracy (i.e., slope), precision (i.e., SE of the slope), and failed to account for much of the variation between estimates of diet composition and known diet composition. Concentration of NDF and ADF between cornstalk and LH residues were similar. Poor accuracy of the models developed from NDF and ADF were likely because differences in concentrations of NDF and ADF

between cornstalk and LH residues were less than amounts needed for optimal sensitivity of analysis of diet composition. Differences in CP between cornstalk and LH residues were greater than differences in NDF and ADF between cornstalk and LH residues. However, estimates of diet composition from analysis of CP in diet samples were poor.

Unrinsed diet samples likely contained large amounts of endogenous protein components (e.g., salivary protein, sloughed epithelium, microbial cells). Greater amounts of rinsing did not increase the accuracy of the estimates from CP. It is possible that greater amounts of rinsing removed proteinaceous components of endogenous origin, but that soluble proteins in cornstalk and LH residues were also removed during rinsing. Additionally, estimates of diet composition from analysis of NDF, ADF and CP only accounted for small amounts of variation between estimates of diet composition and known diet composition. Differences in ADL between cornstalk and LH were greater than NDF, ADF and CP, but estimates of diet composition from analysis of ADL were poor. Several authors have reported that salivary mucopolysaccharides and other endogenous components can contribute to measures of artifact lignin (Lascano et al., 1970; Theurer, 1970; Olson, 1991). It is reasonable that greater amounts of rinsing would increase accuracy and precision of estimates of diet samples from analysis of ADL if endogenous components obviated accurate estimates of diet composition. Regression of estimates of diet composition from machine-rinsed diet samples and known diet composition resulted in a slope (0.68) nearer to unity and accounted for a greater amount of variation ($r^2 = 0.89$) in comparison to unrinsed ($r^2 = 0.84$) and hand-rinsed ($r^2 = 0.76$) diet samples. Nonetheless, estimates of diet composition from ADL in machine-rinsed diet samples resulted in lesser accuracy compared to estimates of diet composition from

analysis of ADIA in unrinsed diet samples. Generally, measures of ADL have large inherent variation (Porter and Singleton, 1971; Sunvold and Cochran; 1991, Moore and Jung, 2001), and variation in analysis may have limited accuracy of estimates of diet composition from ADL even though differences in concentration of ADL between cornstalk and LH residues were large (CV = 46.5%). Estimates of diet composition from ADIA content in unrinsed diet samples were near to unity (i.e., 0.98) and the ordinate did not differ from the origin ($P = 0.91$). Regression of estimated diet composition and known diet composition resulted in a large coefficient of determination ($r^2 = 0.86$). Analysis of ADIA involves a washing step with an acidic detergent that may have removed any endogenous contaminants and allowed accurate estimates of meal composition from unrinsed ADIA. Furthermore, endogenous components (e.g., salivary protein, sloughed epithelium, microbial cells) do not contain ADIA. Greater amounts of rinsing decreased the accuracy of estimates of diet composition from ADIA. It is possible that greater amounts of rinsing contributed to greater loss of ADIA from cornstalk and LH residues. Overall, these data indicate that measures of chemical components in diet samples can provide accurate estimates of diet composition among cows fed binary mixtures of forage when endogenous contamination is minimal and when differences in concentrations of chemical components are sufficient to allow for adequate sensitivity of analyses.

Near infrared reflectance spectroscopy can allow for rapid, less costly measures of analytes in comparison to measures via wet chemistry and is non-destructive (Stuth et al., 2003). However, NIRS measures reflectance of radiation limited to the near infrared spectra (Shenk and Westerhaus, 1996). Incomplete measures of spectral reflectance fail

to separate all unique chemical compounds because multiple molecular species can have identical measures of reflectance in a limited spectral range. Nonetheless, measures of reflectance of radiation across the near infrared spectrum can produce predictive models with high amounts of accuracy and precision when reflectance of near infrared radiation by unique sample matrices are accounted for by development of predictive models based on measures of near infrared reflectance within a unique matrix. Further, comparison of predictions from spectrochemical models (Shenk and Westerhaus, 1996) from known amounts of analyte across a range of concentrations allows for estimates of standard error of prediction to evaluate suitability of predictive models (Stuth et al., 2003). Regression of estimates of diet composition from analysis of machine-rinsed samples by NIRS resulted in a slope near to unity (slope = 0.95) and linear estimates described much of the variation ($r^2 = 0.74$) between estimates of diet composition and known diet composition. Estimates of diet composition by NIRS analysis of hand-rinsed (slope = 2.69; intercept = 3.14) and unrinsed (slope = -1.69; intercept = 5.78) diet samples were inaccurate. It is possible that contamination of diet samples by endogenous components reduced accuracy and precision of predictive models by NIRS because endogenous components were not represented in the sample matrix during development of predictive models (Stuth et al., 2003).

Exp. 2

Biomass of corn residues remaining after harvest was 7,230 kg/ha, and cornstalk and LH residue comprised 35% and 65% of corn residue (DM-basis), respectively. Average temperature during the grazing period was $-2.0 \pm 3.6^\circ\text{C}$, and wind speeds

averaged 18.5 ± 8.0 km/h. It is likely that no biomass accumulated during the grazing period because of low temperatures and a short photoperiod.

Predictions of diet composition (Table 1.5) were limited to measures of ADIA in unrinsed diet samples and ADL and NIRS in machine-rinsed diet samples. Chemical components used for predictions of diet composition in this study were selected because ADIA and ADL were the only 2 chemical components with adequate differences in concentration between cornstalk and LH residues to allow optimal accuracy in predictions of diet composition (Fig. 1.2). Additionally, regression of predicted diet composition from ADIA in unrinsed diet samples and ADL or NIRS in machine-rinsed diet samples with known diet composition resulted in slopes nearest to unity in Exp. 1. There was no interaction ($P = 0.16$; Table 1.5) between grazing treatment and analyte (ADIA, ADL or NIRS) used for prediction of diet composition. Nonetheless, estimates of cornstalk:LH ratios in diet samples collected from cows that strip-grazed corn residues were numerically greater than cows that continuously grazed corn residues when diet composition was predicted by ADIA and NIRS; however, predictions of diet cornstalk:LH ratios from measures of ADL were numerically less among strip-grazed cows than continuously grazed cows. Differences between measures of ADL in cornstalk and LH residues were 48% less in Exp. 2 than in Exp. 1 (Exp. 1 CV = 46.5% vs. Exp. 2 CV = 22.2%). Further, differences between measures of ADL in cornstalk and LH residues in Exp. 2 were less than the amount of difference determined in Exp. 1 to allow optimal predictions of diet composition. Additionally, measures of ADL within cornstalk and LH residues were more variable in Exp. 2 compared to Exp. 1 (Table 1.1). Endogenous mucopolysaccharides can impact measures of ADL from diet samples

collected by ruminal evacuation (Van Soest, 1994). Measures of concentration of ADL in machine-rinsed diet samples could have been impacted by small losses of soluble proteins from corn residues. Analyses of ADIA include rinsing in acidic soap prior to gravimetric measures of ADIA. Thus, losses of soluble proteins from corn residues likely had no impact on measures of ADIA in diet samples. Small losses of soluble proteins from corn residues could have impacted NIRS measures of machine-rinsed diet samples; however, it is likely that measures of NIRS in diet samples are a composite of several chemical components in corn residues and these composite measures could have diluted effects of small losses of innate soluble components in corn residues.

Overall, estimates of cornstalk:LH ratios were 2-times greater among strip-grazed cows compared to continuously grazed cows ($P = 0.02$). Several authors (Fernandez-Rivera and Klopfenstein, 1989; Gutierrez-Ornealas and Klopfenstein, 1991) have measured biomass before and after cattle grazed corn residue and concluded that cattle grazing corn residue often select diets with greater apparent digestibility in comparison to the average of all biomass available for grazing. Typically, strip-grazed cattle select diets with less apparent digestibility compared to cattle allowed to continuously graze (Blount et al., 1991). Estimates of total-tract digestibility of diets were not determined in this study, but estimates of cornstalk:LH ratio seem to suggest that strip-grazed cows selected diets with less apparent digestibility (i.e., greater cornstalk:LH ratio) compared to cattle that continuously grazed. Further, estimates of cornstalk:LH ratio indicate a greater intake of cornstalk residue in comparison to indirect measures of diet selection in cattle that continuously grazed corn residue (Fernandez-Rivera and Klopfenstein, 1989; Gutierrez-Ornealas and Klopfenstein, 1991). Indirect measures of diet composition are

often unable to account for grazing of plant residues by wild herbivores, and plant residues that decompose during the grazing period (Van Soest, 1994). Additionally, indirect measures of diet selection among cattle grazing corn residues would likely overestimate intake of LH residues removed by wind.

Overall, diet composition among cattle grazing cornstalk and LH residues can be accurately predicted from chemical components of diet samples collected by ruminal evacuation. Chemical components with greater differences in concentration between each constituent in a binary mixture of forage and that are not found endogenously in cattle are likely to allow improved estimates of diet composition. These data indicate that ADIA from unrinsed diet samples allowed for greater accuracy and precision in estimates of cornstalk:LH ratios among cattle fed corn residues. Additionally, NIRS analyses of diet samples that were machine-rinsed allowed accurate and precise estimates of diet composition. However, caution should be used when applying predictive models from NIRS to other experiments, because NIRS only measures a limited spectral range and likely reflects a composite of chemical components in diet samples. Therefore, predictions of diet composition from NIRS should be calculated from calibration equations developed from corn residues directly available to cattle for grazing. Nonetheless, direct measures of diet samples collected by ruminal evacuation may allow large opportunity for an increased understanding of cattle grazing binary mixtures of annual forage.

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Table 1.1. Nutrient composition of cornstalk and LH residues

Item	OM	NDF	ADF	ADIA ¹	ADL	CP
Exp. 1						
Cornstalk	97.09 ± 0.03	78.06 ± 0.95	54.55 ± 0.50	2.39 ± 0.23	9.40 ± 0.11	3.30 ± 0.04
LH	94.65 ± 0.59	72.13 ± 0.27	43.01 ± 0.71	7.91 ± 0.31	4.75 ± 0.59	3.72 ± 0.10
Exp. 2						
Cornstalk	94.96 ± 0.78	73.76 ± 5.46	49.66 ± 4.58	2.31 ± 0.70	8.63 ± 0.96	3.08 ± 0.28
LH	87.54 ± 3.14	67.88 ± 1.98	40.19 ± 1.88	8.81 ± 2.78	6.29 ± 2.58	4.82 ± 0.52

¹Acid detergent insoluble ash

Table 1.2. Effects of rinsing on chemical composition in diet samples in Exp. 1

Item, % of DM	Cornstalk:LH ratio						SEM	P-value			
	0:100	20:80	40:60	60:40	80:20	100:0		Rinse	Linear	Quadratic	Interaction ²
NDF ^A								<0.01	<0.01	0.78	0.79
Unrinsed	68.5	68.7	70.0	71.8	71.9	73.6	1.04				
Hand-rinsed	69.6	70.0	72.9	75.2	74.1	77.1	1.04				
Machine-rinsed	76.0	77.4	79.1	80.3	81.1	82.4	1.04				
ADF ^A								<0.01	<0.01	0.46	0.95
Unrinsed	43.7	45.9	47.8	49.2	53.0	54.2	1.05				
Hand-rinsed	43.6	47.3	49.0	51.7	53.3	55.6	1.05				
Machine-rinsed	45.8	48.3	51.0	52.8	55.0	56.9	1.05				
ADIA ^{1,B}								<0.01	<0.01	0.12	0.47
Unrinsed	7.6	6.7	6.0	4.7	3.6	2.8	0.29				
Hand-rinsed	7.4	6.8	5.7	4.9	4.0	2.8	0.29				
Machine-rinsed	5.4	4.8	4.1	3.4	2.3	1.5	0.29				
ADL ^C								0.06	<0.01	0.85	0.70
Unrinsed	4.9	5.7	6.5	6.5	7.7	8.6	0.26				
Hand-rinsed	4.9	5.6	6.6	6.9	7.5	8.2	0.26				
Machine-rinsed	5.0	5.9	6.6	7.3	8.1	8.6	0.26				
CP ^D								<0.01	0.01	0.27	0.84
Unrinsed	4.3	4.2	4.2	3.9	4.1	3.9	0.15				
Hand-rinsed	3.8	3.9	3.7	3.4	3.8	3.6	0.15				
Machine-rinsed	2.8	2.6	2.5	2.5	2.5	2.3	0.15				

¹Acid detergent insoluble ash²Rinse × cornstalk:LH ratio^AUnrinsed < hand-rinsed < machine-rinsed^BUnrinsed = hand-rinsed > machine-rinsed^CUnrinsed = hand-rinsed < machine-rinsed^DUnrinsed > hand-rinsed > machine-rinsed

Table 1.3. Effect of rinsing on nucleic acid and 16S rRNA gene in diet samples in Exp. 1

	Cornstalk:LH ratio				SEM	<i>P</i> -value		
	20:80	40:60	60:40	80:20		<i>Rinse</i>	<i>Linear</i>	<i>Quadratic</i>
DNA Concentration (ng/mg) ^A						<0.01	0.11	0.07
Unrinsed	3.4	2.7	2.6	2.9	0.45			
Hand-rinsed	5.1	3.4	3.7	3.5	0.45			
Machine-rinsed	0.6	0.2	0.4	0.6	0.45			
Ct Value ¹						0.16	0.81	0.18
Unrinsed	16.0	15.2	15.2	16.4	1.3			
Hand-rinsed	15.3	13.0	13.5	14.7	1.3			
Machine-rinsed	14.8	14.1	13.9	14.0	1.3			
Relative 16S rRNA gene ^{2,B}						0.01	0.60	0.48
Unrinsed	53.0	131.1	78.6	77.7	41.9			
Hand-rinsed	97.0	34.0	29.8	146.3	41.9			
Machine-rinsed	3.6	1.6	-0.3	6.8	41.9			

¹Cycles to threshold

²Amount of 16S rRNA gene relative to amounts of 16S rRNA gene provided from chloroplasts in each meal

^AHand-rinsed > unrinsed > machine-rinsed

^BUnrinsed = hand-rinsed > machine-rinsed

Table 1.4. Evaluation of the diet composition prediction models in Exp. 1

Item	Slope	Intercept	SE _{slope}	SE _{intercept}	r ²	P-value	
						<i>Slope</i>	<i>Intercept</i>
NDF							
Unrinsed	-0.05	0.28	0.021	0.043	0.54	0.03	0.09
Hand-rinsed	0.10	0.83	0.192	0.371	0.37	0.64	0.27
Machine-rinsed	-0.41	4.58	0.348	0.666	0.42	0.26	<0.01
ADF							
Unrinsed	2.62	0.88	1.313	4.419	0.71	0.07	0.85
Hand-rinsed	1.41	1.15	0.488	0.961	0.58	0.01	0.26
Machine-rinsed	2.61	1.07	0.341	0.810	0.86	<0.01	0.21
ADIA¹							
Unrinsed	0.98	0.05	0.129	0.332	0.86	<0.01	0.91
Hand-rinsed	0.61	0.27	0.078	0.183	0.86	<0.01	0.27
Machine-rinsed	28.86	-18.09	9.024	22.554	0.66	<0.01	0.45
ADL							
Unrinsed	2.29	-1.11	1.163	2.602	0.84	0.07	0.68
Hand-rinsed	0.33	0.42	0.081	0.274	0.76	<0.01	0.23
Machine-rinsed	0.68	0.21	0.078	0.234	0.89	<0.01	0.40
CP							
Unrinsed	-0.06	1.51	0.698	1.503	0.44	0.93	0.34
Hand-rinsed	0.16	1.75	0.565	1.481	0.56	0.79	0.26
Machine-rinsed	-0.02	1.61	0.032	0.069	0.41	0.46	<0.01
NIRS							
Unrinsed	-1.69	5.78	1.940	4.134	0.46	0.40	0.22
Hand-rinsed	2.69	3.14	2.343	6.736	0.59	0.29	0.68
Machine-rinsed	0.95	0.19	0.192	0.442	0.74	<0.01	0.69

¹Acid detergent insoluble ash

Table 1.5. Estimates of diet selection in cattle grazing corn residues in Exp. 2

Item	Continuous	Strip	SEM	<i>P</i> -value		
				<i>Treatment</i>	<i>Chemical component</i>	<i>Treatment x chemical component</i>
Ratio cornstalk:LH				0.02	0.71	0.16
ADIA ¹	0.21	0.56	0.21			
ADL	0.39	0.37	0.21			
NIRS	0.11	0.49	0.21			

¹Acid detergent insoluble ash

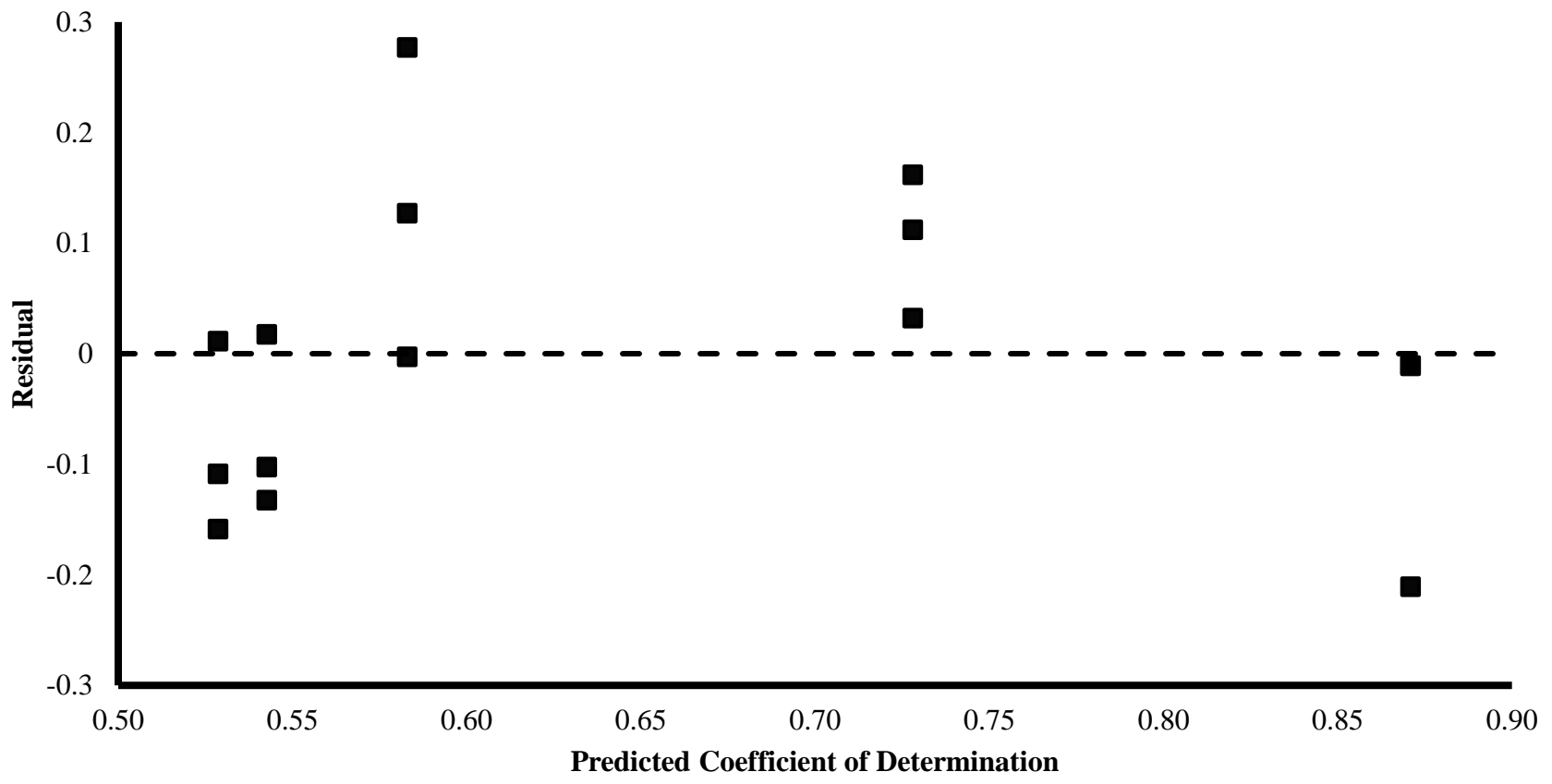


Figure 1.1. Petzel

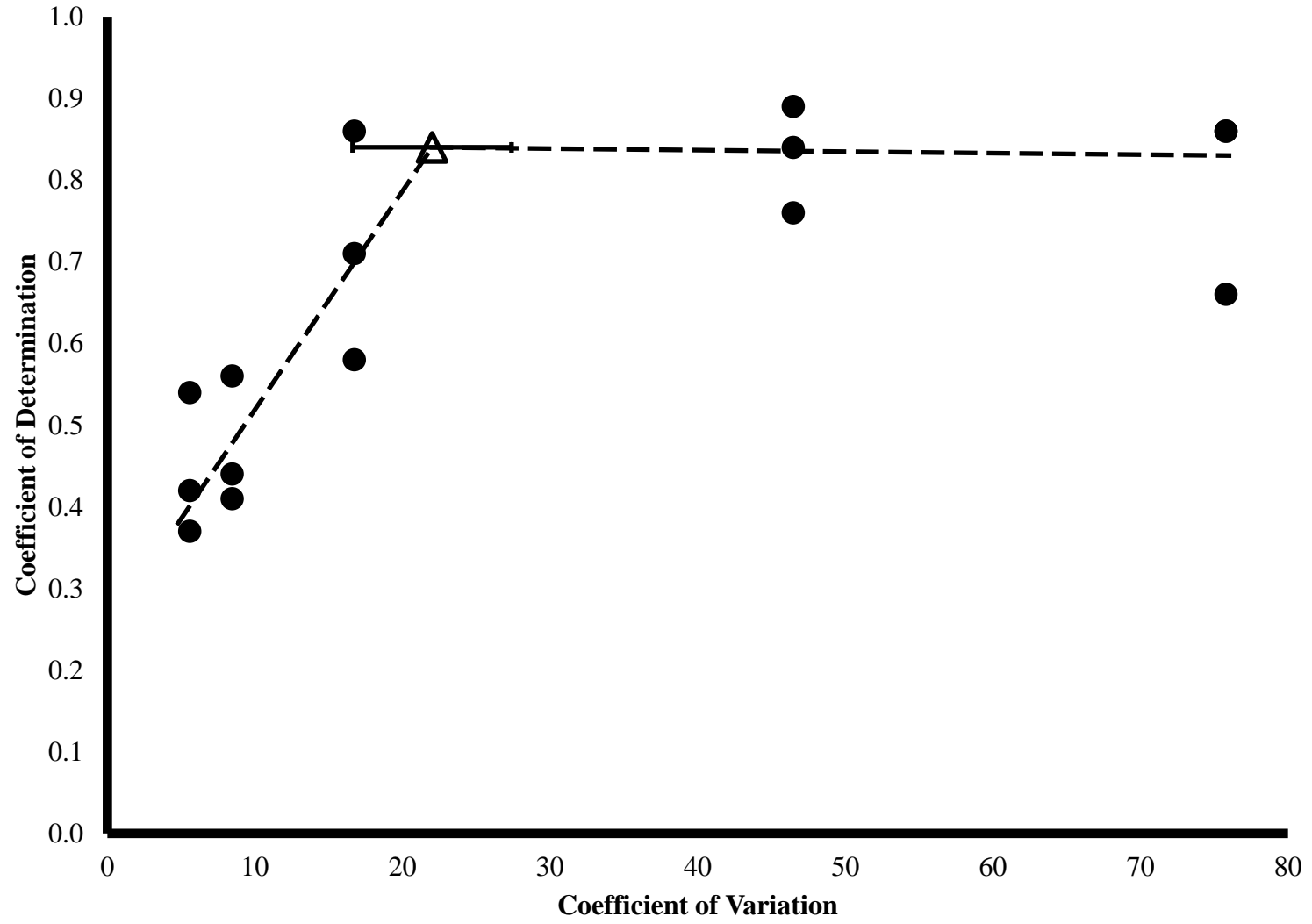


Figure 1.2. Petzel.

Figure captions

Figure 1.1. Residual plot of the linear regression of the coefficient of variation of chemical components in cornstalk and LH residues and the coefficient of determination of the predicted ratio of cornstalk:LH residues in diet samples

Figure 1.2. Fitted broken-line plot of coefficient of determination as a function of the coefficient of variation between cornstalk and LH residues.

CHAPTER 3:**An evaluation of digestibility and caloric value of different botanical parts of corn residue fed to cattle.**

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ABSTRACT

Currently most tabular values of energy content in corn residues available to cattle are based on model estimates from measures of concentrations of chemical components derived from samples of mechanically harvested corn residues. Grazing cattle often select diets with greater digestibility and presumably greater energy concentration in comparison to the average of the overall biomass available for grazing. Thus, estimates of diet energy and nutrient concentration from tabular values or samples of mechanically harvested corn residue are unlikely to be strongly correlated to diets selected by cattle grazing corn residues. Unfortunately, even if diets selected by cattle grazing corn residues are known few data are available on the energy content and nutrient digestion among each botanical part of corn residues (i.e., leaves, husks, stalks). Thus, predictions of performance dependent on energy available to cattle from corn residues will be limited without accurate measures of energy concentration and nutrient digestibility from each component of corn residues. We used 6 ruminally cannulated cows (mean 559.4 ± 50.9 kg) in a replicated 3×3 Latin square experiment to determine energy metabolism, apparent nutrient digestibility and N balance of cattle fed diets that consisted of individual components of corn residues (i.e., leaves, husk or stalk). As a percent of digestible energy, metabolizable energy was greater for leaf residue which can be attributed to a less ($P < 0.02$) production of CH_4 . Methane energy production, as a percent of gross energy or digestible energy, were less ($P < 0.01$) for leaf residue in comparison to husk and stalk. Heat production (Mcal/d) was not different between leaves, husks or stalk; however, when amounts of heat production were corrected for differences in gross energy or digestible amounts of heat produced was less ($P \leq 0.01$) among cows

fed leaves in comparison to stalk or husks. Subsequently, net energy available from maintenance was greater ($P < 0.01$) for leaf (1.80 Mcal/kg DMI) than husk (1.15 Mcal/kg DMI) or stalk (0.83 Mcal/kg DMI). Despite differences in energy available from different botanical parts of corn residue, total tract digestion of neutral detergent fiber and acid detergent fiber was greater ($P < 0.01$) for husks and leaves compared to stalks. Similarly, husk had greater DM and OM digestibility in comparison to both leaves and stalks. Results from this trial indicate that leaves have the greatest energy value to cattle even though total tract digestion of husk was greater.

INTRODUCTION

Diets selected among grazing cattle are often influenced by a relatively capricious behavior that seems to be related to a myriad of factors (e.g., forage quality, forage availability, environmental factors, and competition for feed). Thus, it is perhaps unsurprising that cattle that graze corn residues appear to select a diet dissimilar to the average of the available biomass (Fernandez-Rivera and Klopfenstein, 1989, Stalker et al., 2015, Petzel et al., 2018). A precise explanation for why cattle graze certain botanical parts remains elusive, but diet selection among grazing cattle appears to be related to differences in nutrient density, plant toxins, and stimulation of olfactory and gustatory senses (Provenza, 1995). Ultimately, diet selection among grazing cattle impacts intake of energy available to support maintenance and other productive physiological functions. Current estimates of energy available to from (National Academies of Sciences, Engineering, and Medicine, 2016) corn residue are reflective of the total available biomass after harvest of corn grain. Current estimates of performance are based on the predicted diet energy content (National Academies of Sciences, Engineering, and Medicine, 2016). An improved understanding of the energy available from each botanical part of corn residue together with previous reports of diet selection may allow for improved accuracy and precision in predictions of performance among cattle grazing corn residues.

MATERIALS AND METHODS

All procedures that involved the use of animals in this project were approved by the South Dakota State University Institutional Animal Care and Use Committee (protocol approval No. 17-092A)

Experiment 1: In situ disappearance

Animal Husbandry and Sample Collection

To estimate rates of ruminal digestion of each botanical part of corn residue and corn steep liquor we conducted an in situ study. Corn leaves, husks and stalks were harvested beginning on November 10, 2017 for measures of nutrient and energy (i.e., Exp. 2). Measures of in situ disappearance were conducted on an aliquot of a composited sample the same corn leaves, husks, stalk and steep liquor used in Exp. 2 after samples were partially dried (55°C) and ground to pass a 2-mm screen in a Wiley Mill (Thomas Wiley Mill Model 4; Thomas Scientific USA, Swedesboro, NJ). Four grams of ground sample (DM basis) were placed in a polyester bag (Dacron, 10 x 20 cm, 50 ± 10 µm pore size; R1020, Ankom Technology, Macedon, NY, USA) in triplicate and then suspended in the rumen of two cannulated Simmental x Angus (562 ± 3kg BW) cows fed to ad libitum amounts of long-stem corn residue for 14 d prior to measures of in situ disappearance. During in situ incubations, cows were allowed ad libitum access to long-stem corn residue and provided corn steep liquor (1-kg) twice daily at 0700 h and 1900 h. Bags were incubated for either 4, 8, 12, 24, 36, 48, 72 or 96 h. After incubation bags were mechanically rinsed 5-times in a commercial washer (Fabric-Matic, Model A511S, Maytag, Newton, IA); each rinse consisted of a 1 min rinse followed by a 2 min spin

cycle (Vanzant et al., 1998). Estimates of 0 h disappearance were achieved by rinsing polyester bags identically to bags previously ruminally incubated. After mechanical rinsing, all samples were dried at 55°C for 24 h, and analyzed for DM, OM and NDF.

Calculations and Statistical Analysis

Nearly, all corn steep liquor was removed from bags used to estimate 0 h disappearance ($89.8 \pm 3.2\%$ DM) and no corn steep liquor remained in polyester bags after 4 h of incubation. Thus, corn steep liquor was considered to rapidly disassociate in the rumen, and estimates of rates of corn steep liquor disappearance were not calculated, because it was unlikely that measures of disappearance used in the trial would accurately characterize rates of ruminal disappearance of corn steep liquor. Rates of DM, OM and NDF disappearance from corn leaves, husks and stalks were calculated following the model described by Orskov and McDonald (1979):

$$\text{Disappearance (\%)} = \text{ID} + \text{SD} * (1 - e^{-\text{kd} * t})$$

where ID is the proportion of chemical fraction that immediately disassociates from corn residue parts, SD is the proportion of chemical fraction that is potentially dissociable, Kd is the rate of disassociation (h^{-1}) and t is the time of exposure in the rumen. The equation was fitted using the Marquardt method for iterative, nonlinear, least squares estimation in SAS. Pool sizes (ID, SD and ND) and Kd were analyzed as a completely randomized design using the mixed procedures of SAS. The model contained effects of botanical part (i.e., corn leaves, husk, and stalk) and effect of cow was random.

Experiment 2: Nutrient and energy balance

Animal Husbandry and Sample Collection

Beginning 7 d prior to experimentation, 6 ruminally cannulated cows (3 Angus, 563 ± 72 kg initial body weight (BW); 3 Simmental x Angus, 576 ± 59 kg initial BW) were housed in a common drylot pen (0.4 ha) and fed long-stem corn residue to ad libitum. Subsequently, cattle were moved to stanchions in a temperature (23°C) and light-controlled (16 h of light daily) room at the South Dakota State University Ruminant Metabolism Facility 1 d prior to experimentation. Cows were then allocated to one of two replicated 3 x 3 Latin squares with 19 d periods (7 days for adaptation, 8 days for collection and 4 days of rest) to determine the energy and nutrient availability from corn stalk, leaf or husk. Cows were fed diets each 12 h at 0700 and 1900h daily in individual forage feeders that were predominately comprised of either corn stalks, leaves or husks. Cows had ad libitum access to water and a pressed mineral block (Trace Mineralized Salt, American Stockman, Overland Park, KS; 96.5% NaCl, 4,000 ppm Zn, 1,600 ppm Fe, 1,200 Mn, 260 ppm Cu, 100 ppm I, and 40 ppm Co). Corn steep liquor (CSL) was added to diets in amounts designed to meet or exceed the needs for ruminally available N and to provide identical amounts of energy ($23 \text{ kcal NE}_m/\text{kg BW}^{0.75}$). Cows were fed to 90% of the predicted maintenance energy requirement (Mcal/d; NASEM, 2016) and amounts of feed offered were calculated from measures of BW collected at the beginning of each period.

Corn leaves, husks and stalks were harvested beginning on November 10, 2017 and after the dry matter (DM) of grain had reached 84%. Standing plants were cut with a

sickle mower (Ford 501, Ford Motor Company, Troy, MI) placed at ground level. Subsequently, plants were gathered and separated by hand into stalk, leaf, and husk. After harvest, stalks were ground through a wood chipper (3" Chipper Shredder, DR Power Equipment, Vergennes, VT) to allow a bulk density nearer to that of leaves and husks and allow for a similar frequency of feed delivery to cows during the experiment. Feed provided (Table 2.1) was adjusted every three days for dry matter content of the corn residue parts to maintain the level of feeding. Daily grab samples (200 g) of corn leaves, husks and stalks were composited within period and botanical part for analysis of chemical composition. A single lot of corn steep liquor was obtained from a commercial manufacturer (Archer Daniel Midland, Marshall, MN), sampled every third day (20g) and composited for analysis of chemical composition. Diets were mixed daily by hand immediately prior to feeding.

Digestion Collections

At 0700 and 1900 h from d 4 to 11 a 10 g bolus of Cr_2O_3 in a gelatin capsule (Size 07, Torpac, Fairfield, NJ) was orally administered to allow estimates of fecal output. Fecal spot samples (100 g) were collected thrice daily on d 8 to 11 by manual stimulation and composited (300 ± 8.6 g/d) by cow within each period. Fecal samples were collected each 4 h beginning at 0700 h on d 8 and sampling time was delayed 1 h each day so that composite samples reflected each hour in a 12-h period.

Cows were fitted with a Foley catheter (24 French, 75 cc Foley catheter, C. R. Bard Inc., Covington, GA) on d 7 to allow total collections of urine from d 8 to 11. Catheters were attached (0.8 cm ID, Fisherbrand, Pittsburgh, PA) plastic collection vessel

(20 L, Model:RFS22, Cambro Manufacturing Company, Huntington Beach, CA) containing 900 mL of 10% (wt/wt) H₂SO₄. Total urine collections were measured daily and an aliquot (1% of daily output) was composited by cow within each period and frozen at -20°C.

Indirect Respiration Calorimetry

Measures of respired gas and methane produced during the fed-state were achieved by placing each cows head and neck into an open-circuit respiration calorimeter (76 x 76 x 180 cm) on days 12 and 13. Air flow from each calorimeter was measured by individual mass flow meters (Dresser MicroSeries ptz+Log, GE Oil and Gas, Houston, TX) and set to a flow rate of 780 L/min. Calorimeters were run prior to gas collection (20 volumes of each calorimeter) to allow sampled air to be reflective of cows gas production. Continuous sampling of incoming and outgoing air was diverted to collection bags (61 x 61 cm Laminate, PMC, Oak Park, IL) using glass rotameters (SHO-RATE, Brooks Instrument, Hatfield, PA). Measures of air flow from each calorimeter were corrected for temperature, relative humidity (TRH-100, Pace Scientific, Mooresville, NC) and barometric pressure (P350-D-0inch, Pace Scientific, Mooresville, NC) which was measured every min (XR5-SE Data logger, Pace Scientific, Mooresville, NC). Ethanol recoveries were $99.08 \pm 3.75\%$ for O₂ and $90.25 \pm 2.32\%$ for CO₂. Measures of respired air and methane were collected each 12 h. Concentrations of carbon dioxide and methane were measured using near infra-red reflectance spectroscopy and oxygen was measured via paramagnetic detection (Emerson X-Stream XE, Emerson Process Management,

Solon, OH) after calibration to a standard gas containing 19.68% O₂, 1.01% CO₂, 0.1% CH₄).

After measures of gas exchange during the fed-state, cattle were fasted using the washed rumen technique (Kim et al., 2013). Briefly, at 0900 h on d 14, reticulorumen contents were evacuated (Reid, 1965) and maintained at 29 °C. Immediately after removal of ruminal contents, the reticulorumen was rinsed with 10 L of tap water (39°C) that was removed through suction (10 gallon 4-Peak-HP Shop Vacuum, Shop-Vac, Williamsport, PA), and the rinsing procedure was subsequently repeated for a total of 4 rinses. Subsequently, measures of respired gas and methane produced were collected from 1900 h on d 14 to 1900 h on d 15. After measures of gas exchange, 10 kg of pooled rumen contents from 2 Angus steers fed mechanically harvested corn residue was placed in the rumen together with each cows' original rumen contents, and cows were provided a 7 d period of rest before beginning the next experimental period.

Sample Analysis

Feces, diet, and orts samples were partially dried for 48 h at 55°C in a forced-air oven, and ground to pass a 1-mm screen using a Wiley mill (Thomas Wiley Mill Model 4; Thomas Scientific USA, Swedesboro, NJ). Feed, feces and orts were analyzed for DM, organic matter (OM), neutral detergent fiber (NDF), acid detergent fiber (ADF), nitrogen (N) and gross energy (GE). Dry matter was measured by drying at 105°C for 16 h (method no. 930.15, AOAC, 2016), and OM was determined by combustion (500°C for 16 h, method no. 942.05, AOAC, 2016). Neutral detergent fiber was measured as described by Van Soest et al. (1991) and included additions of α -amylase and sodium

sulfite; ADF was measured nonsequential to NDF (Van Soest et al., 1991). Measures of NDF and ADF were corrected for ash content which was measured by combustion (500°C for 8 h). Nitrogen content was analyzed via the Dumas procedure (method no. 968.06; AOAC, 2016; rapid Max N exceed; Elementar, Mt. Laurel, NJ). Urine samples were analyzed for DM, N and GE. Gross energy was determined using an automatic isoperibolic calorimeter (Parr 1261, Parr Instrument Company, Moline, IL). Urine energy was analyzed by lyophilizing 15 mL of urine in small plastic pouches (5.0 x 7.6 cm, 1 mil polypropylene bag, Associated Bag, Milwaukee, WI). Fecal concentrations of Cr₂O₃ was determined via atomic absorption spectroscopy (357.87 nm; AAnalyst 200, PerkinElmer, Waltham, MA) after digestion in potassium bromate and acid manganese sulfate (Williams et al., 1962).

Calculations

Total oxygen (O₂) consumption and carbon dioxide (CO₂) and methane (CH₄) production were calculated by standardized air flow and concentrations in sampled air. Heat production was calculated as described by Brouwer (1965):

$$HP(kcal) = \frac{(16.18(O_2, \frac{L}{d}) + 5.02(CO_2, \frac{L}{d}) - 2.17(CH_4, \frac{L}{d}) - 5.99(Urine, \frac{g}{d}))}{4.183}$$

Dry matter was calculated as partial DM multiplied by DM measured after drying at 105°C. Fecal output was calculated as the quotient of the amount of Cr₂O₃ bolused daily by the Cr₂O₃ concentration in feces. Urinary N output was calculated as the quotient

of daily urine output and urine N concentration. Intake of DM, OM, NDF, ADF, and N was estimated by multiplying dry matter intake (DMI) and concentration of DM, OM, NDF, ADF and N in the diet. Output of DM, OM, NDF and ADF was estimated by multiplying fecal DM output and fecal concentration of nutrients. Nitrogen output was calculated as fecal N output plus urine N output. Digestible, metabolizable and net energy for corn residue were calculated after correcting for energy provided from corn steep liquor (NASEM, 2016).

Statistical Analysis

Data from 1 cow in period 2 was excluded due to small intakes (less than 50% of DM offered) that did not appear to be reflective of a normal population (studentized residual = 3.7). Additionally, another cow was removed from the study in period 3 because of small DMI during the recovery period.

Data were analyzed for a Latin square using the MIXED procedure of SAS with fixed effect of botanical part and random effects of cow, period and square. Denominator degrees of freedom were calculated by the Kenward and Roger adjustment (Kenward and Roger, 1997). Treatment means were calculated using the LSMEANS option. Differences in corn leaves, husks and stalks were evaluated with the F-statistic. When the F-statistic was significant ($P \leq 0.05$) means were separated with the PDIFF option of SAS.

RESULTS AND DISCUSSION

Experiment 1

In situ disappearance of corn husks, leaves and stalks are reported in Table 2.2. Interestingly, amounts of DM that immediately disappeared ($P < 0.01$) were greatest for

corn stalks, intermediate for corn husks and least for corn leaves. Similarly, proportions of OM that immediately disappeared from corn stalks were 40% greater ($P < 0.01$) than amounts of OM that immediately disappeared from leaves and husks. Amounts of DM and OM available to slowly disappear ($P < 0.01$) were greatest for husk, least for stalk and leaves were intermediate. Rates of DM disappearance ($P < 0.01$) were greatest for leaf, intermediate for husk and least for stalk. Similarly, rates of OM disappearance were 74% greater ($P < 0.01$) for leaves compared to husks and stalks, but rate of OM disappearance from husks and stalks were not different ($P = 0.14$). Amounts of neutral detergent fiber that immediately disappeared ($P < 0.01$) was greatest for husks, least for leaves and intermediate for stalks. Yet, amounts of NDF able to slowly disappear ($P < 0.01$) were greatest for leaf, intermediate for husk and least for stalk. Interestingly, however, rates of NDF disappearance ($P < 0.01$) were greatest for stalks, intermediate for leaves and husks was least. Perhaps not surprising, amounts of DM, OM, and NDF that did not disappear ($P < 0.01$) were greatest for stalks, least for husks and intermediate for leaves.

Water soluble nutrients make up between 14 and 27% (DM-basis) of corn residues (Chen et al., 2007). Proportions of OM in stalks that immediately disappeared (28.15%) were 40% greater ($P < 0.01$) than proportions of OM in husks and leaves that immediately disappeared (20.02 and 20.22%, respectively). Similarly, proportions of DM that immediately disappeared from stalks were greater ($P < 0.01$) than from husks and leaves; however it is unclear if small differences in amounts DM or OM that immediately disappeared between stalks, leaves or husks would contribute to any measurable responses in ruminal fermentation. Stalks in many warm-season grasses have various

anatomical features (e.g. primary wall lignification, cell wall middle lamellas, crystalline regions of cellulose (Kerley et al., 1988 & Jung and Deets, 1993) that can limit digestion of soluble nutrients in corn residues (Wilson and Mertens, 1995). Grinding disrupts physical organization of cells in warm-season stalks and can increase fiber digestion when mean ruminal retention times remains the same (Wilson and Mertens, 1995). Our data seem to indicate that corn stalks contained greater amounts of rapidly soluble extracellular or intracellular materials in comparison to husks and leaves. It is likely that physical disruption (e.g., mechanical grinding, chewing) of cells allows access of soluble nutrients in corn residues to ruminal microbes for fermentation.

Fiber is largely comprised of the insoluble cell wall matrix and consists predominately of cellulose, hemicellulose and lignin (Van Soest, 1994). Hemicellulose is more soluble than cellulose and greater concentrations of hemicellulose in plant fiber has been related to greater amounts of potentially fermentable fiber in feeds (Van Soest, 1994). Husks had greater amounts of hemicellulose (35.9% DM; Table 2.1) than leaves (25.3% DM) which had greater amounts of hemicellulose than stalks (19.1% DM).

However, lignin can limit amounts of fiber available to be slowly fermented via cross-linking with hemicellulose (Jung and Deets, 1993). Stalks had greater amounts of lignin (10.8 % DM; Table 2.1) than husks (6.1% DM) and leaves (4.5% DM). Furthermore, stalks often have greater xylem tissue development, greater proportions of cellulose, and less photosynthetic tissues resulting in a greater cell wall concentration that form conjugate bond with lignin than leaves (Jung and Deetz, 1993; Wilson and Kennedy, 1996). Thus, fiber in corn stalks are often less accessible to ruminal fermentation. Indeed, greater amounts of NDF were available to be fermented (i.e.,

slowly disappeared) from husks and leaves compared to stalks. Even though lignin can limit amounts of fiber available to ruminal fermentation, lignin content often has little impact on rate of NDF digestion (Smith et al., 1972). Differences between rates of NDF disappearance between leaves, husks and stalks were small compared to differences in rates of OM and DM disappearance.

Digestibility

Measures of nutrient intake and total tract digestion are reported in table 2.3. Because cows were offered feed in amounts designed to provide isocaloric diets intake of DM, OM, NDF and ADF differed ($P < 0.01$) between husks, leaves and stalks. Fecal excretion of DM and OM were greatest ($P < 0.01$) for leaves, intermediate when cows were fed stalks and least for husks. However, fecal output of NDF and ADF were greatest for stalks, intermediate for leaves and least for husks. Thus, estimates of total tract digestion of DM and OM tended ($P = 0.09$) to be greater for husks than leaves and stalks, and total-tract digestibility of NDF for husks and for leaves were 12 and 9% greater ($P < 0.01$) than measures of total-tract NDF digestibility among cows fed stalks. Similarly, total-tract digestibility of ADF was less for stalks ($P < 0.01$) than husks and leaves which did not differ ($P = 0.06$).

Currently, we are not aware of any published reports of measures of total-tract digestibility of corn husk, leaves or stalks in ruminants; however, several authors have reported in vitro estimates of DM or OM total-tract digestion in corn husks, leaves or stalks. Several authors (Fernandez- Rivera and Klopfenstein, 1989; Gutierrez-Ornelas and Klopfenstein, 1991) reported that in vitro DM disappearance from husks were nearly

50% greater than in vitro DM disappearance from leaves or stalks. Similarly, previous measures (Gutierrez-Ornelas and Klopfenstein, 1991; Stalker et al. 2015) of in vitro OM disappearance between corn husks, leaves and stalks suggest that in vitro OM disappearance from husks is nearly 50% greater than OM disappearance from stalks, but that OM disappearance from husks is only 29% greater than estimates of OM disappearance from leaves. Similar to in vitro estimates of DM and OM disappearance, total-tract DM ($P = 0.09$) and OM digestion were greatest ($P = 0.05$) when cows were fed husks in comparison to leaves or stalks. Interestingly, measures of total-tract DM and OM digestion among each botanical part in corn residues in this study were greater than previous reports of in vitro estimates of DM and OM disappearance. Furthermore, differences between measures of total-tract DM and OM disappearance between husks, leaves and stalks in this study were less than previous reports (Fernandez- Rivera and Klopfenstein, 1989; Gutierrez-Ornelas and Klopfenstein, 1991; Stalker et al. 2015) of in vitro estimates. Nonetheless, measures of NDF and ADF digestion were greater ($P < 0.01$) when cows were fed corn husks and leaves compared to corn stalks.

It is possible that ruminal disappearance of nutrients from corn residues can be limited by inadequate amounts of ruminally available N to support optimal fermentation. In this study, cows were provided isocaloric amounts of corn steep liquor designed to meet or exceed needs of ruminally available N; however, previous in vitro estimates of total-tract nutrient disappearance (Fernandez- Rivera and Klopfenstein, 1989; Gutierrez-Ornelas and Klopfenstein, 1991) did not provide supplemental sources of available N. It is possible that the greater amounts of total-tract DM and OM disappearance measured in vivo in this study were because inclusion of corn steep liquor in diets mitigated

limitations in microbial fermentation of corn residue, and it remains unclear if in vitro estimates of total-tract nutrient disappearance (Tilley and Terry, 1963) are strongly correlated with total-tract nutrient disappearance among cattle fed corn residues. Additionally, lignin content can often limit ruminal fermentation of fiber. Typically, stalks have greater amounts of lignin in comparison to corn leaves and husks (Barten, 2013). Corn stalks have relatively large amounts of guaiacyl lignin that allows for more extensive cross-linking with fiber (Van Soest, 1994) and can reduce fiber digestibility (Jung and Casler, 2006). Nonetheless, it is important to note that cattle in this study were fed to amounts below ad libitum intake to allow for similar caloric intakes and to estimate energy available for maintenance. It is possible that limited intakes may have increased total mean retention time of feed and allowed for increased amounts of total tract nutrient disappearance in comparison to cattle fed to ad libitum.

Nitrogen Balance

As expected, N intake (Table 2.4) was greatest ($P = 0.01$) when cows were fed leaves, least among cows fed husk and stalk was intermediate. Similarly, urine N (g/d) was greatest ($P < 0.01$) among cows fed leaves, intermediate among cows fed stalks and least when cows were fed husk. Additionally, amounts of N excreted in urine as a proportion of N intake were less in cattle fed husk compared to cattle fed stalks or leaves. Castillo et al., (2000) calculated that urine N output was more closely related to N intake ($R^2 = 0.76$) than N excreted in feces (0.48). Furthermore, these same authors reported that urine N output increases exponentially with greater N intake but that fecal N output increases linearly with greater amounts of N intake. Indeed, urine N output seemed to be

related to N intake in this study and N excreted in urine decreased at a greater rate in response to differences in N intake.

Amounts of N excreted as a proportion of total amounts of N output was greatest in urine and least in feces when cows were fed corn stalks compared to husks and leaves. Kebreab et al. (2002) noted that increased fermentable energy increased amounts of N excreted in feces and decreased N excreted in urine. Our data seem to be in agreement with the calculations of Kebreab et al. (2002), because greater husks and leaves provided greater amounts of fermentable substrate (Table 2.3) and greater amounts of total-tract NDF disappearance. Amounts of N retained did not differ between corn husks, leaves or stalks, which may have been expected since cattle were fed similar amounts of energy.

Gas Exchange

There were no differences (Table 2.5) in differences in production of CO₂ or O₂ consumption when cows were fed corn husks, leaves or stalks or when cows were fasted. However, the respiratory quotient tended ($P = 0.07$) to be greater among cattle fed husks compared to leaves or stalks. Interestingly, methane production (L CH₄ / kg DMI) was greatest ($P < 0.01$) when cows were fed corn husks, intermediate when cows were fed stalks and least for cows fed leaves. Differences in methane production subsequently contributed to a nearly 27% numerical increase in daily methane production (L CH₄ / cow) by cows fed husks in comparison to leaves or stalks. It is unclear why daily methane production was greater ($P = 0.04$) from cows previously fed corn stalks in comparison to cows previously fed husks or leaves during fasting ($P < 0.05$).

Energy Balance

Measures of energy balance between cows fed corn husks, leaves or stalks are presented in Table 2.6. Cows were fed amounts designed to provide similar amounts of NE based on a priori estimates of total digestible nutrients (Weiss, 1993) and predictive models of NE content (NASEM, 2016). Thus, as expected, daily gross energy intake (Mcal/d) differed between husks, leaves and stalk. Daily fecal energy losses ($P < 0.01$) were greatest for cows fed leaves, least for husks and stalk was intermediate. However fecal energy losses ($P < 0.01$) were greatest as a proportion of GE intake when cows were fed stalks compared to leaves and husks. Thus, digestible energy (DE) intake was greatest for cattle consuming leaves compared to stalks and husks. Intake of DE as a proportion of DMI ($P < 0.01$) was also greatest when cows were fed corn leaves, but DE intake as a proportion of DMI among cows fed husks was intermediate and least when cows were fed stalks. However, digestible energy intake as a proportion of GE intake was nearly 11% greater ($P < 0.01$) when cattle were fed husks or leaves than when cattle were fed stalks. The NASEM (2016) estimates DE of cornstalks to be 2.32 ± 0.35 Mcal/kg which is greater than all three botanical parts of corn residue evaluated in the present study. Corn residue is primarily stalk and leaf (40.5 and 35.1%) with husks and cobs in smaller proportions (9.6 and 14.8%, Stalker et al., 2015). Therefore, if the composition corn residues consumed by cattle are identical to the composition of corn residues in a field then our data seem to indicate that DE intake would 1.73 Mcal/kg of DMI. However, cattle grazing corn residues often select diets different from the average of the overall biomass available (Lamm and Ward, 1981, Fernandez-Rivera et al., 1989, and Gutierrez-Ornelas et al., 1991; Petzel et al., 2018).

There were no differences ($P > 0.16$) in losses of urinary energy when cows were fed corn husks, leaves or stalks. However, GE and DE lost as methane energy was less ($P < 0.01$) among cows fed leaves compared to when cows were fed husks and stalks. Similarly, energy losses from methane as a proportion of DMI ($P < 0.01$) were least for leaves, intermediate for stalks and greatest when cows were fed husks. These data indicate that diets selected by cattle grazing corn residues may have large impacts on energy lost as methane. It seems likely that differences in methane energy losses may be related to differences in ruminal fermentation. A myriad of factors (e.g., lignin, N concentration, rates and extent of fermentation) can apparently influence methane energy losses from ruminants (Moe and Tyrell, 1979). Johnson and Johnson (1995) concluded that energy lost as methane is less variable when DM digestibility is improved, and DM intake is inversely correlated with methane emission from cattle (Johnson et al., 1993). Yet, greater concentration of lignin and other cell wall fiber components contribute to greater methane emission (Moe and Tyrell, 1979 and Beever et al., 1989). It seems plausible that greater rates of particulate passage and lesser rate and extent of ruminal fermentation of fiber could contribute to reduced energy losses of methane from cattle. Indeed, greater amounts of feed processing reduces amounts of methane produced by cattle (Blaxter, 1989). Corn stalks in this trial were ground, however, it seems unlikely that processing increased ruminal particulate passage rate because cows were not fed to ad libitum. Differences in methane energy losses contributed to differences ($P < 0.01$) in metabolizable energy (ME) derived from corn husk (1.49 Mcal/kg DMI), leaves (1.98 Mcal/kg DMI), and stalks (1.17 Mcal/kg DMI). Furthermore, ME as a proportion of DE was greatest ($P < 0.01$) for leaves compared to husks or stalks. Current ME library

values (NASEM, 2016) indicate that corn residues contain 1.9 Mcal/kg DM. However, data from this study would indicate that ME content of corn residues is less than current library values. Current estimates of the efficiency of DE conversion to ME is 82% (NASEM, 2016). Cows fed husks and stalks converted DE to ME at 81.1% and 78.9%, respectively, but cows fed leaves converted DE to ME at 88.2%. Apparently, the ME:DE ratio can range between 0.82 and 0.93 and is influenced by diet (Vermorel and Bickel, 1980; Hales et al., 2014).

Daily amounts of heat produced (HP) did not differ ($P = 0.81$) when cows were fed corn husks, leaves or stalks; however, amounts of DE lost as HP was less ($P < 0.01$) when cows were fed leaves compared to husks and stalks. Fasting heat production did not differ ($P = 0.96$) when cows were fed husks, leaves or stalks.

Diets were formulated by estimates of NE_m from total digestible nutrients (TDN) values. Total digestible nutrients were calculated from proximate analysis (Weiss, 1993) of corn residue from a separate study (Petzel et al., 2018). Husk and stalk NE_m estimations from TDN were greater than measured NE_m (0.95 and 1.25 versus 0.83 and 1.15 respectively).

The estimated value of net energy for maintenance (NE_m /kg DM) was greater ($P < 0.01$) for leaves (1.80 Mcal/kg DMI) than for husks and stalks (1.15 and 0.83, respectively). Furthermore, predictions of NE_m (NASEM, 2016) from ME in husks, leaves and stalks were less than observed NE_m . Tabular values for NE_m of cornstalks are 1.06 Mcal/kg DMI (NASEM, 2016); however, our data indicate that the NE_m of the overall corn residues available to cattle is nearly 19% greater (i.e., 1.26 Mcal/kg DMI).

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Table 2.1. Chemical composition of feedstuffs fed to cattle

Item, %	Feedstuff			
	Husk	Leaf	Stalk	CSL ¹
DM	81.6	87.1	61.2	48.0
OM	96.8	91.9	95.1	88.6
NDF	76.7	63.1	64.1	3.1
ADF	40.8	37.8	45.0	2.7
ADL	6.1	4.5	10.8	ND
ADIA	1.7	5.7	2.3	0.1
CP	2.4	7.7	3.0	33.5

¹Corn Steep Liquor

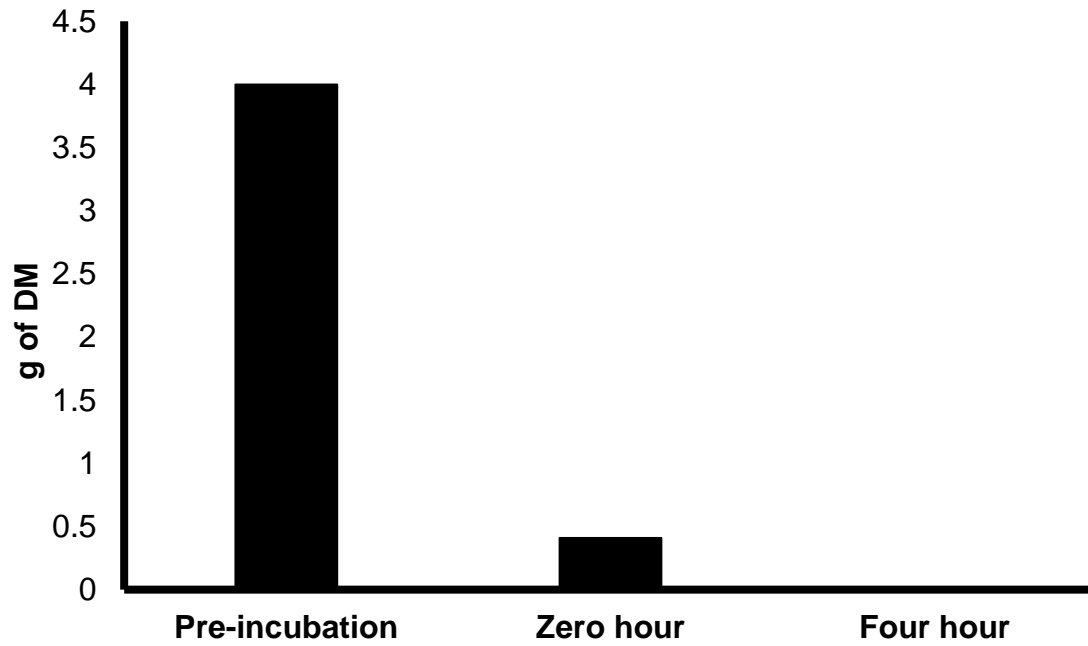


Figure 2.1. Dry matter disappearance of corn steep liquor from in situ estimates

Table 2.2. Degradation kinetics from in situ disappearance of different botanical parts of corn residue.

Item, %	Residue Type			SEM	P-value
	Husk	Leaf	Stalk		
Dry Matter					
ID	16.63 ^B	12.49 ^A	29.32 ^C	0.13	<0.01
SD	69.90 ^C	64.69 ^B	37.35 ^A	0.27	<0.01
ND	13.77 ^A	22.82 ^B	33.33 ^C	0.19	<0.01
Kd, %/h	2.84 ^B	3.98 ^C	2.18 ^A	0.03	<0.01
Organic Matter					
ID ¹	20.02 ^A	20.22 ^A	28.15 ^B	0.11	<0.01
SD ²	73.92 ^C	59.63 ^B	38.53 ^A	1.91	<0.01
ND ³	6.06 ^A	20.15 ^B	33.32 ^C	2.02	<0.01
Kd ⁴ , %/h	2.48 ^A	4.10 ^B	2.22 ^A	0.11	<0.01
Neutral Detergent Fiber					
ID	11.88 ^C	2.43 ^A	9.41 ^B	0.18	<0.01
SD	69.71 ^B	71.70 ^C	44.12 ^A	0.13	<0.01
ND	18.41 ^A	25.87 ^B	46.47 ^C	0.05	<0.01
Kd, %/h	4.25 ^A	4.82 ^B	4.97 ^C	0.04	<0.01

¹ID: Immediately disappeared²SD: Slowly disappeared³ND: Residue that did not disappear⁴Kd: Rate of disappearance

Table 2.3. Total tract digestibility of different botanical parts of corn residue from cattle fed to 90% of maintenance energy requirement.

Item	Residue Type			SEM	P-value
	Husk	Leaf	Stalk		
DM					
Intake, g/d	4755.7 ^C	6547.1 ^A	5168.2 ^B	0.2	<0.01
Fecal Excretion, g/d	1125.46 ^C	1760.76 ^A	1428.68 ^B	63.88	<0.01
Digested, g/d	3630.2 ^B	4786.31 ^A	3788.48 ^B	187.05	<0.01
Digestibility, % of intake	75.84	72.70	73.04	3.71	0.09
OM					
Intake, g/d	4604.1 ^B	6018.34 ^A	4921.54 ^B	203.44	<0.01
Fecal Excretion, g/d	950.85 ^C	1415.43 ^A	1251.13 ^B	54.99	<0.01
Digested, g/d	3653.25 ^B	4602.92 ^A	3715.14 ^B	200.19	<0.01
Digestibility, % of intake	78.72	75.97	75.03	3.36	0.05
NDF					
Intake, g/d	3648.93 ^A	4126.51 ^A	3332.38 ^B	127.15	<0.01
Fecal Excretion, g/d	503.86 ^C	687.04 ^B	815.48 ^A	35.22	<0.01
Digested, g/d	3145.04 ^B	3439.47 ^A	2541.09 ^C	134.17	<0.01
Digestibility, % of intake	86.03 ^A	83.33 ^A	76.49 ^B	1.22	<0.01
ADF					
Intake, g/d	1936.36 ^B	2467.2 ^A	2396.03 ^A	79.67	<0.01
Fecal Excretion, g/d	322.74 ^C	491.81 ^B	590.35 ^A	30.22	<0.01
Digested, g/d	1613.62 ^B	1975.39 ^A	1835.64 ^A	71.8	<0.01
Digestibility, % of intake	83.2 ^A	80.07 ^A	75.76 ^B	1.34	<0.01

Table 2.4. Influence of different botanical parts of corn residue on nitrogen balance of cattle fed to 90% of maintenance energy requirements.

Item	Residue Type			SEM	<i>P</i> -value
	Husk	Leaf	Stalk		
N intake, g/d	102.57 ^B	137.36 ^A	116.16 ^{AB}	8.05	<0.01
N excreted, g/d					
Urine	36.77 ^C	62.77 ^A	50.24 ^B	6.66	<0.01
Feces	22.06 ^B	31.50 ^A	19.13 ^B	12.90	<0.01
Total	58.83 ^B	94.27 ^A	69.29 ^B	6.18	<0.01
N excretion, % of total N excretion					
Urine	62.38 ^B	66.15 ^B	72.46 ^A	2.07	<0.01
Feces	37.62 ^A	33.85 ^A	27.54 ^B	2.07	<0.01
N excretion, % of N intake					
Urine	32.92 ^B	42.13 ^A	40.04 ^{AB}	6.80	0.08
Feces	20.41 ^A	22.31 ^A	16.26 ^B	8.34	<0.01
N retained g/d	46.23	45.58	49.50	13.39	0.93

Table 2.5. Daily O₂ consumption and CH₄ and CO₂ emissions from cattle fed different botanical parts of corn residue at 90% of maintenance and at fast.

Item	Residue Type			SEM	P-value
	Husk	Leaf	Stalk		
O ₂ consumption at maintenance					
L/cow	1901.18	1846.79	1929.96	160.61	0.90
L/kg SBW ^{0.75}	17.10	16.86	16.84	1.06	0.98
CO ₂ production at maintenance					
L/cow	1733.66	1557.88	1637.41	99.29	0.20
L/kg SBW ^{0.75}	15.63	14.23	14.61	0.68	0.17
Maintenance RQ	0.92	0.85	0.88	0.03	0.07
CH ₄ production at maintenance					
L/cow	95.16	74.50	74.96	68.44	0.17
L/kg DMI	18.13 ^B	10.82 ^A	14.64 ^{AB}	6.43	0.02
O ₂ consumption at fast					
L/cow	1416.69	1444.05	1394.62	122.52	0.92
L/kg SBW ^{0.75}	12.37	12.81	11.97	2.88	0.69
CO ₂ production at fast					
L/cow	969.24	964.81	956.80	69.06	0.99
L/kg SBW ^{0.75}	8.74	8.81	8.48	0.48	0.83
Fasting RQ	0.71	0.70	0.71	0.03	0.75
CH ₄ production at fast					
L/cow	5.97 ^{AB}	5.63 ^B	7.80 ^A	0.71	<0.05
L/kg SBW ^{0.75}	0.05	0.05	0.07	0.008	0.17

Table 2.6. Daily energy losses for cattle fed different botanical parts of corn residue at 90% of maintenance energy requirements.

Item	Residue Type			SEM	P-value
	Husk	Leaf	Stalk		
GE ¹ , Mcal/kg of DMI	3.40	3.64	3.27	0.21	0.17
GE, Mcal	16.21 ^B	23.83 ^A	16.56 ^B	1.34	<0.01
Fecal energy, Mcal	4.72 ^C	6.92 ^A	6.00 ^B	0.31	<0.01
Fecal Energy, %GE	26.51 ^B	27.15 ^B	34.18 ^A	2.20	<0.01
DE ² , Mcal/kg of DMI	1.85 ^{A,B}	2.19 ^A	1.46 ^B	0.22	<0.05
DE, Mcal	8.87 ^B	14.30 ^A	7.18 ^B	1.31	<0.01
DE, % of GE	73.49 ^A	72.85 ^A	65.82 ^B	2.20	<0.01
Urinary energy, Mcal	1.40	1.28	1.36	0.25	0.91
Urinary energy, %GE	7.85	5.08	7.79	1.43	0.18
Urinary energy, %DE	10.84	7.00	12.15	2.33	0.16
Urinary energy, Mcal/kgurine	0.11 ^B	0.14 ^A	0.12 ^B	0.01	<0.01
CH ₄ ³ energy, Mcal	0.90	0.70	0.71	0.65	0.16
CH ₄ energy, Mcal/kg of DMI	0.13 ^B	0.06 ^A	0.10 ^{AB}	0.06	<0.02
CH ₄ energy, Mcal % of GE	4.22 ^A	1.74 ^B	3.69 ^A	1.94	<0.01
CH ₄ energy, Mcal % of DE	5.70 ^A	2.45 ^B	6.10 ^A	2.54	<0.01
ME ⁴ , Mcal/kg of DMI	1.49 ^B	1.98 ^A	1.17 ^B	0.22	<0.01
ME, Mcal	7.16 ^B	12.91 ^A	6.26 ^B	1.25	<0.01
ME, % of DE	81.08 ^B	88.18 ^A	78.87 ^B	2.48	<0.01
HP ⁵ , Mcal	9.32	8.88	9.30	0.73	0.81
HP, % DE	70.50 ^A	47.76 ^B	81.10 ^A	5.34	<0.01
Retained energy, Mcal	1.48 ^B	7.65 ^A	0.02 ^B	1.19	<0.01
FHP ⁶ , Mcal	6.59	6.65	6.48	0.55	0.96
FHP, Mcal/kg SBW ^{0.75}	0.059	0.060	0.057	0.014	0.75
NEm ⁷ , Mcal/kg DMI	1.15 ^B	1.80 ^A	0.83 ^B	0.23	<0.01
NEm, %DE	40.98 ^B	62.74 ^A	35.71 ^B	6.75	<0.01

¹Gross Energy²Digestible Energy³Methane⁴Metabolizable Energy⁵Heat production⁶Fasting heat production⁷Net energy for maintenance