Evaluation of Plant-waxes to Estimate Forage Intake in Grazing Cattle

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Summary with Implications

Although key to the efficiency of a cattle operation, feed intake is challenging to evaluate in a grazing setting. However, even within forage-based systems, plant-wax markers may be used to predict dietary choices and feed intake. Plant-waxes are a complex mixture of lipids found on the surface of plants. When sufficiently unique among plants, the composition of diets can be determined from the pattern of these compounds in the forages ingested. These markers were used to delineate the parts of the corn plant and, separately, 8 western rangeland grasses and legumes. Using plant waxes, the components of the corn plant were clearly distinguished. This technique therefore could be useful in a monoculture, such as a corn residue field, to determine the plant parts predominating in the diet. Delineating plants in a complex sward was more difficult, particularly among like species. The use of more markers may help to more explicitly distinguish plants within diverse pastures, such as western rangelands.

Introduction

Within the beef and dairy industry, it has become increasingly important to determine the factors that affect animal intake. One approach for doing so is based on plant-wax markers. Plants contain a complex mixture of aliphatic lipid compounds on their external surface that are essentially inert within the digestive system. Of particular interest are the *n*-alkanes (**ALK**; saturated straight-chain hydrocarbons) and long-chain alcohols (**LCOH**). The concentrations of these compounds can differ greatly among plant species, and even among plant parts, often providing a unique marker profile or signature of a plant. When these profiles are distinctive enough, the composition of cattle diets can be predicted from the pattern of these compounds in the forages ingested. The number of plants that can be delineated depends on the number and profiles of ALK and LCOH measured in the individual plants or plant-parts. As the complexity of a sward increases, such as within mixed species grassland, the number of markers needed to distinguish plants increases. The objective of this study was to assess the ability to delineate the plant composition of corn residue and of a diverse western rangeland.

Procedure

Corn plant

Cob, stalk, husk and leaf samples were taken from a 98.8 acre irrigated corn field located at the Eastern Nebraska Research and Extension Center located near Mead, NE. Ears and leaf blade were removed on site prior to transport to prevent loss. Stalks were cut at the top of the crown roots and bundled. Leaves and stalks were stored to air dry in an open air barn. Ears were husked and separated. Samples were bagged and left open inside a climate controlled building to allow the plant parts to dry. Stalk, cob and leaf samples were all chopped using the Ohio Mill, and then through a Wiley Mill using 1 mm screen. Samples were then composited by plant part.

Western rangeland

Forage samples were collected at the West Central Research and Extension Center (WCREC) in North Platte, NE. Collection sites were primarily native mixed-grass rangeland within the rolling plains and breaks of Major Land Resource Area 73. Ecological sites included loamy upland, loamy lowland and loess breaks. The forages were 3 cool-season (C3) grasses (cheatgrass (*Bromus tectorum*); needle-and-thread (*Hesperostipa comata*); western wheatgrass (*Pascopyrum smithii*)), 3 warm-season (C4) grasses (blue grama (*Bouteloua gracilis*); little bluestem (*Schizachyrium scoparium*); sideoats grama (*Bouteloua curtipendula*)), and 2 legumes (leadplant (*Amorpha canescens*); sweet clover (*Melilotus officinalis*)). Forage samples were collected at peak vegetative and mature states between late-April and late-August 2015. Peak vegetative stage of growth was defined as just before stem elongation for the grasses and before flowering for the legumes. At the mature stage, grasses were fully headed and beginning seed ripening. Legumes were past flowering and in seed development.

Plants were clipped at ground level, shipped overnight on ice to the Ruminant Nutrition Laboratory at the University of Nebraska-Lincoln. Half of the sample was separated by hand into leaf and stem. Depending on the stage at which the plant was collected, the reproductive portion (flower, seed) was also separated. All plant parts and whole plant samples were placed in a 60° C forced air oven for 48 h to determine dry matter. After 48 h, all samples were removed from the oven and ground through a Wiley Mill using a 1 mm screen.

Laboratory Analysis

Extractions were performed in duplicate with 0.200–0.204 g of ground sample. Docosane (C_{22}) and tetratriacontane (C_{34}) were added by weight at a concentration of 0.3 mg / g to serve as internal alkane standards. An internal alcohol standard, n-heptacosanol (1-C_{27}-ol) at a concentration of 1.5 mg / g was added by weight. Samples were extracted overnight using 1M KOH. Hydrocarbons were collected by solid phase extraction using heptane.

Crude alcohol extractions were obtained by solid phase extraction using heptane/ethyl acetate followed by sterol/stanols separation and derivatization with pyridine and acetice anhydride. *n*-Alkane elutes and LCOH fractions were evaporated to dryness, and re-dissolved in *n*-dodecane for chromatographic analysis.

Table 1. Mean *n*-alkane and long-chain alcohol concentrations (mg • kg^{-1} DM) for corn plant parts.

	n-alkane				Long-chain alcohol		
Plant part	C_{27}	C_{29}	C_{31}	C_{33}	$C_{26}OH$	$C_{28}OH$	$C_{30}OH$
Cob	4.61	11.1	7.42	5.42	19.7	3.23	3.87
Husk	4.25	17.1	19.5	9.80	70.3	25.7	30.9
Leaf	9.29	30.1	56.5	45.6	54.9	57.2	74.2
Stalk	2.59	3.21	3.65	4.77	20.1	5.10	5.52
Grain	3.00	2.28	2.29	3.31	18.3	2.24	4.34

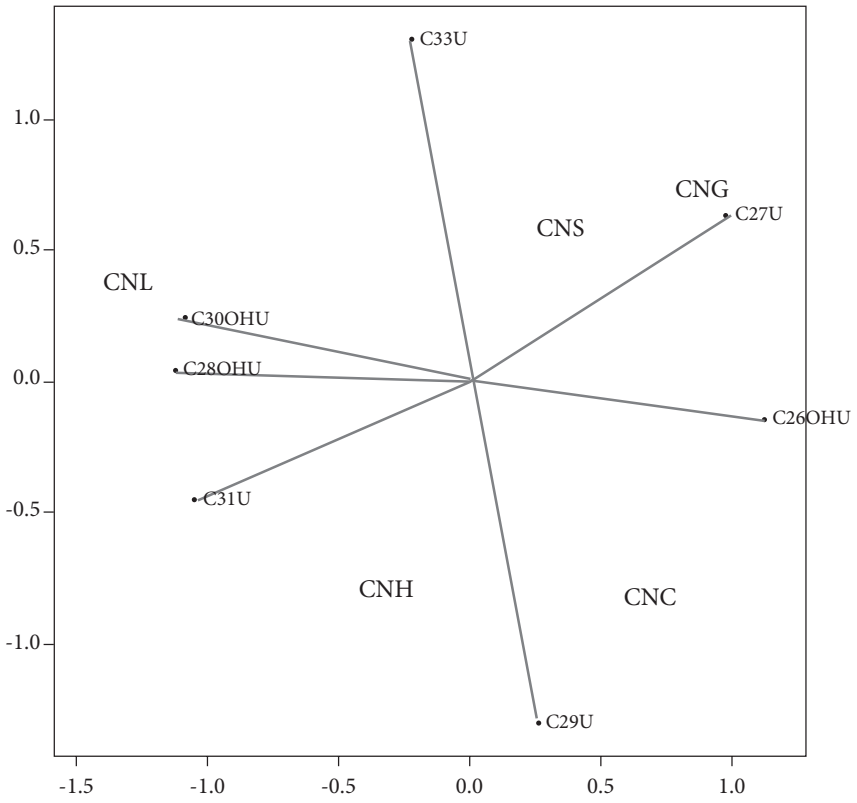

Figure 1. Biplot showing the 5 corn plant parts in a 2-dimensional space derived from principal component analysis based on concentrations of 4 *n*-alkanes (C27U, C29U, C31U and C33U) and 3 long-chain alcohols (C26OHU, C28OHU and C30OHU) once normalized to a unit scaled. The corn plant parts were Corn Plant Cob (CNC), Corn Plant Leaf (CNL), Corn Plant Husk (CNH), Corn Plant Stalk (CNS) and Corn Plant Grain (CNG).

Quantification of ALK and LCOH was carried out by gas chromatography (**GC**), using an Agilent 7820A GC. Samples of an ALK and LCOH standard solution mixture (C_{21} to C_{36}; $C_{20}OH$ to $C_{30}OH$) were included in the GC analyses to determine peak identification and standard response factors. Peak areas were determined with auto-integration and manual review of chromatograms. The ALK and LCOH concentrations were calculated relative to known amounts of the internal standards (C_{22}, C_{34} and $C_{27}OH$).

Statistical analyses were based on principal component analysis (**PCA**) conducted using GenStat for Windows 17th Edition. The PCA technique is used to explain the variation found in a set of observations by drawing out their strongest or most dominant patterns. It is often used to make data easier to visualize. In this study, PCA was used to generate 2-dimesional plots (biplots) based on the ALK and LCOH concentrations of plants or plant-parts. Through these biplots, it was possible to see whether the plant-wax profiles of the individual plant and plant-parts were distinct enough to separate them.

For the parts of the corn plant, 4 ALK (C_{27}, C_{29}, C_{31} and C_{33}) and 3 LCOH ($C_{26}OH$, $C_{28}OH$ and $C_{30}OH$) concentrations were considered. For the plant species in the western rangelands, an additional ALK (C_{35}) and 2 additional LCOH were used ($C_{24}OH$ and $C_{32}OH$) as markers given the greater complexity of the plant mixture. The concentrations of $C_{24}OH$ and $C_{32}OH$ compound were estimated from nearby standard response factors. Because the concentrations of ALK and LCOH differed appreciably among the plant species, the concentrations were normalized to a unit scale within ALK and within LCOH by dividing individual concentrations by their respective sum.

Results

Corn Plant

The ALK and LCOH concentration of the 5 corn plant parts are provided in Table 1. There was large variation in the plant-wax contents of the plant parts. The concentration of C_{27} was relatively low in all plant components. The $C_{26}OH$ compound was predominant in the husk compared to all other parts. The concentrations of all compounds were consistently higher in the leaf of the plant with the exception of $C_{26}OH$. Grain, stalks and cobs seemed to have the lowest overall concentrations of all compounds.

Based on the PCA, 79.9% of the variation in the plant-wax concentrations among plant parts was described along the first or x-axis, while a further 18.1% of the variation was defined along the second or y-axis (Figure 1). With effectively all variation (98%) being explained in just these 2-dimensions, the plant-wax profiles of the various parts of a corn plant allowed them to be clearly distinguished. Leaf had greater concentrations of $C_{30}OH$ and $C_{24}OH$ making its cluster very distinct. Husk and cob clusters were also discernable because of the higher concentrations in C_{29} in both, yet still distinct from each other due to their differing C_{31} and $C_{26}OH$ concentrations,

Table 2. Mean *n*-alkane and long-chain alcohol concentrations (mg • kg^{-1} DM) for 8 forage species at peak vegetative and mature (shown in parentheses) stages of growth.

		n-alkane					Long-chain alcohol				
Class[1]	Specie	C_{27}	C_{29}	C_{31}	C_{33}	C_{35}	$C_{24}OH$	$C_{26}OH$	$C_{28}OH$	$C_{30}OH$	$C_{32}OH$
C3	Cheatgrass	47.2 (42.2)	57.7 (152.4)	39.6 (94.1)	39.9 (22.3)	3.2 (1.7)	351.2 (77.9)	74.0 (144.4)	1308.5 (5066.2)	58.5 (72.0)	0 (0)
	Needle-and-thread	30.7 (30.7)	84.9 (92.1)	89.8 (113.7)	28.5 (22.4)	29.8 (15.5)	0 (75.9)	84.8 (411.6)	4126.4 (8962.5)	180.3 (252.9)	0 (54.1)
	Western wheatgrass	9.6 (50.9)	31.1 (56.6)	59.1 (34.5)	25.5 (6.3)	1.4 (0.8)	0 (0)	40.9 (28.7)	560.1 (39.3)	29.5 (21.6)	0 (0)
C4	Blue grama	13.8 (12.4)	49.0 (47.0)	179.4 (148.7)	121.2 (75.1)	21.1 (18.8)	0 (28.6)	0 (0)	727.2 (111.9)	141.2 (125.4)	2703.3 (11252.9)
	Little bluestem	18.2 (57.7)	28.0 (44.8)	50.5 (58.8)	8.4 (18.9)	1.2 (4.4)	0 (68.9)	44.2 (140.5)	337.0 (134.7)	196.2 (120.4)	5871.8 (13200.2)
	Sideoats grama	29.1 (35.5)	28.6 (45.5)	21.2 (35.6)	15.7 (19.4)	7.0 (3.7)	0 (26.1)	77.7 (324.5)	1268.9 (1624.2)	157.7 (262.3)	900.5 (1244.9)
Leg.	Leadplant	93.4 (48.8)	143.8 (273.4)	38.3 (181.4)	5.5 (16.5)	0.4 (0.1)	193.0 (234.8)	658.2 (757.4)	2856.0 (2057.4)	1076.6 (537.6)	0 (94.9)
	Sweet clover	38.8 (37.0)	268.3 (440.0)	53.0 (51.3)	22.2 (7.5)	1.4 (1.2)	188.6 (219.1)	2464.0 (689.7)	159.8 (108.1)	522.6 (116.0)	0 (0)

[1]Specie classifications were cool-season (C3) and warm-season (C4) grass, and legume (Leg.).

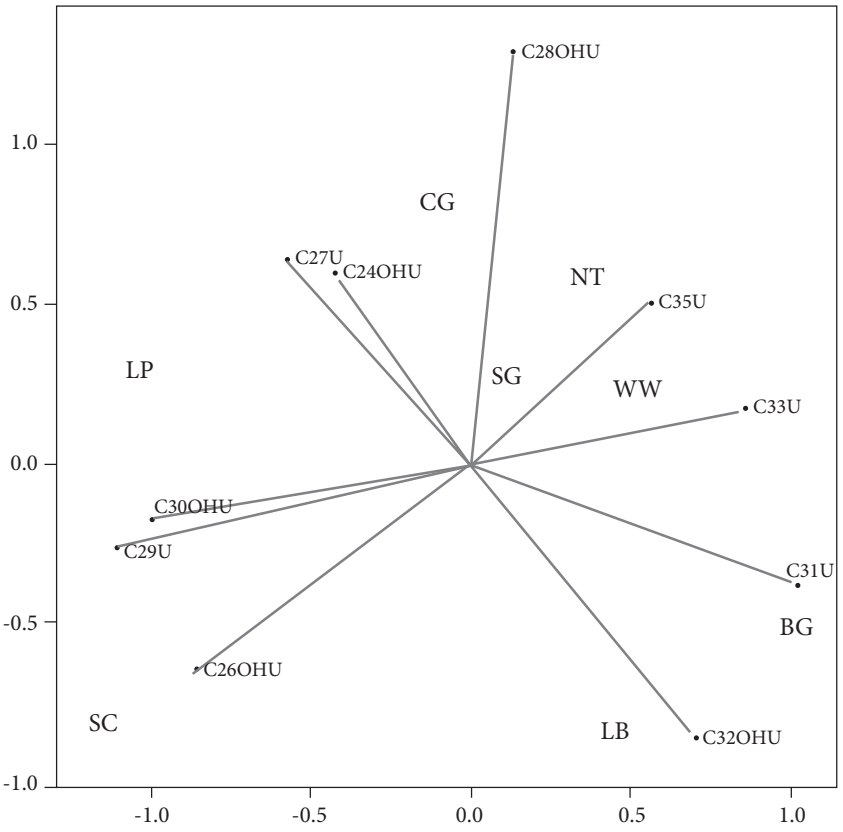

Figure 2. Biplot showing the 8 forage species at their peak vegetative state in a 2-dimensional space derived from principal component analyses based on concentrations of 5 *n*-alkanes (C27U, C29U, C31U, C33U and C35U) and 5 long-chain alcohols (C24OHU, C26OHU, C28OHU, C30OHU and C32OHU) once normalized to a unit scaled. The forage species were Sweet Clover (SC), Leadplant (LP), Cheatgrass (CG), Sideoats grama (SG), Needle- and-thread (NT), Western Wheatgrass (WW), Blue Grama (BG) and Little Bluestem (LB).

respectively. Stalk and grain were the most closely related with higher concentrations in C_{27}. However, stalks contained more C_{33} allowing it to appear separate from grain.

Western Rangeland

The ALK and LCOH concentrations of the 8 plant species found in western rangelands are provided in Table 2. There was large variation in the plant-wax content of plants within and across growth stages. Leadplant and sweet clover contained higher concentrations of C_{29} during both vegetative and mature states. Blue grama had higher concentrations of C_{33} when compared to other plants. All plants had low concentrations of C_{35}. The LCOH amounts, when present, were considerably higher than ALK concentrations. The concentration of $C_{28}OH$ was highest in cheatgrass and needle- and-thread at maturity. The compound $C_{32}OH$ only appeared at extremely high concentrations in mature warm-season grasses (blue grama, little bluestem and sideoats grama).

The PCA for vegetative plants showed 55.3% of the variation between plant parts was described on first or x-axis (Figure 2). An additional 35.2% was defined along the second or y-axis, for a total of 90.5% of variation being defined in these 2-dimsensions. For mature plants, 65.8% of

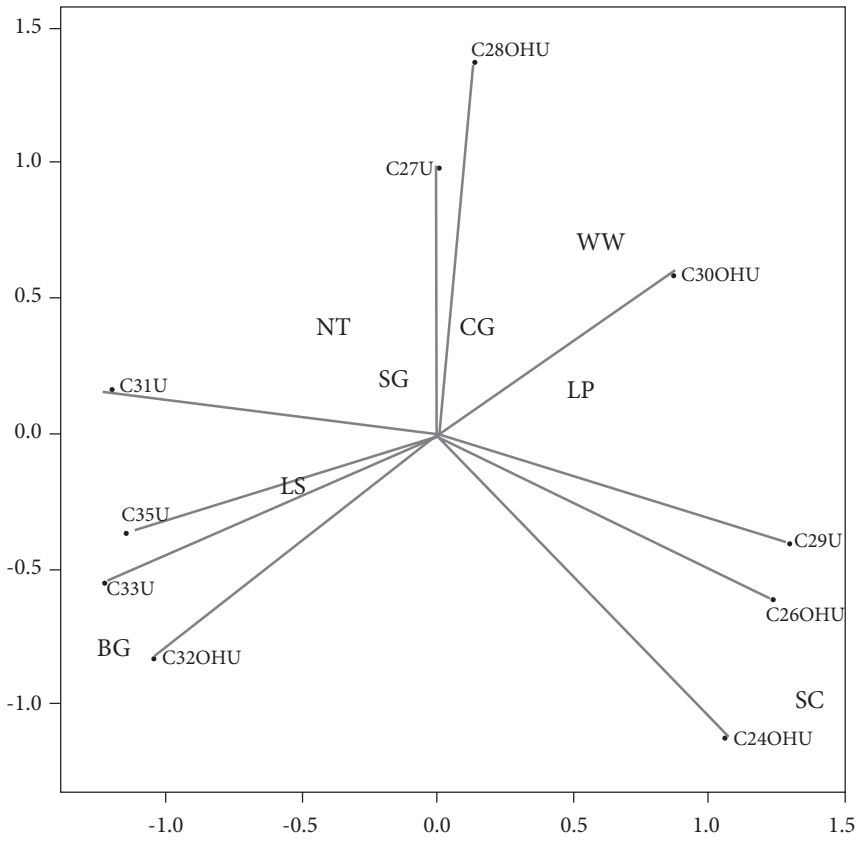

Figure 3. Biplot showing the 8 forage species at their mature state in a 2-dimensional space derived from principal component analyses based on concentrations of 5 *n*-alkanes (C27U, C29U, C31U, C33U and C35U) and 5 long-chain alcohols (C24OHU, C26OHU, C28OHU, C30OHU and C32OHU) once normalized to a unit scaled. The forage species were Sweet Clover (SC), Leadplant (LP), Cheatgrass (CG), Sideoats grama (SG), Needle- and-thread (NT), Western Wheatgrass (WW), Blue Grama (BG) and Little Bluestem (LB).

the variation was described along the first axis, followed by 27.4% explained along the second axis, totaling 93.2% of the variation being defined along the first two axes (Figure 3).

The spread of the 8 forage species in the biplots clearly showed the ability to discriminate legumes from grasses. The greater concentrations of C_{29}, $C_{24}OH$ and $C_{26}OH$ in legumes resulted in their clustering. High concentrations of $C_{32}OH$ made C4 grasses stand out, particularly blue grama that also had high concentrations of C_{33}. Stronger separation of the grasses was captured along the y-axis, but they still could not be unequivocally differentiated. Cheatgrass, western wheatgrass, little bluestem and sideoats grama clustered together and were not separable based on their ALK and LCOH profiles alone.

Conclusions

Using ALK and LCOH concentrations, the parts of the corn plant could be clearly delineated. However, the specie-specific profiles of the plant-wax markers were not distinct enough to distinguish among plants comprising a complex western rangeland. That issue is explored further elsewhere (2017 Nebraska Beef Report, pp. 73–75). The plant-wax characteristics appear useful for assessing dietary choices in cattle grazing a monoculture like corn residue. Such information may benefit management decisions, including deciding when animals might be moved to alternative grazing areas. However, to delineate choices in a complex sward such as western rangelands, additional plant markers will be needed to more clearly distinguish plant species.

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