Delineating Complex Forage Mixtures Using Plant-Wax Markers

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Summary with Implications

Plant waxes provide a marker profile of individual plants that, when sufficiently distinct, can be used to estimate the diet composition of grazing cattle. They also may provide a tool for reliably predicting feed intake. The traditional method (nonnegative least squares) to use these markers to predict diet composition has limitations. A newer statistical approach (Bayesian linear unmixing) deemed more efficient was tested with simulation. Samples from 8 forage species in Nebraska were analyzed for their plant-wax marker contents. Those concentrations were used to simulate 1000 diets for 4 mixtures containing 2, 3, 5 or 8 plants. The efficiency of the two methods to predict diet composition was compared. The newer approach outperformed the traditional one in all of the mixtures considered. However, predictions were considerably worse when the number of plants in the mixture was 5 or 8. When forage mixtures are complex, additional steps will be needed to predict diet composition, and thereby feed intake, in grazing cattle.

Introduction

Estimating diet composition and feed intake under grazing conditions is challenging but necessary to determine feed efficiency. Plant-wax markers, particularly *n*-alkanes (**ALK**) and long-chain alcohols (**LCOH**), have been used successfully to estimate the botanical composition of the diet in grazing conditions. The ability of ALK and LCOH to delineate the plants in the diet depends on their marker profiles being sufficiently different among the plants on offer. Importantly, to estimate feed intake using the plant-wax marker technique, the botanical makeup of the diet needs to be estimated first. Given that botanical mixtures are compositional in nature (i.e., are positive and sum to one), constraints have to be imposed during estimation. Traditional methods, do not address some of these constraints. Conversely, more recent statistical methods do and have successfully been used to estimate the composition of mixtures in, for instance, image analysis and geochemistry but, as of yet, not in animal diets. The proposed approach would directly account for such constraints. However, validating the methodology is necessary to make sure it performs (at least) as well as the traditional technique. This validation was performed through a simulation study.

Procedure

As described elsewhere (2017 Nebraska Beef Report, pp. 69–72), 8 plant species commonly found in Nebraska were collected. These 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*)).

Plant-wax marker concentrations (mg / kg) were measured using gas chromatography at two development stages (at peak vegetative and maturity). Four mixtures were simulated using i) sweet clover and cheatgrass, ii) sweet clover, cheatgrass and blue grama, iii) sweet clover, cheatgrass, blue grama, sideoats grama and western wheatgrass, and iv) all 8 species. For all mixtures, 1000 diets were simulated. Analyses were performed using both the traditional and the newer method.

The ability (efficiency) to delineate the mixtures for each of the methods was assessed based on 4 statistics. Firstly, the discrepancy between the true (simulated) values and their estimates, or the estimation error (**EE**), was assessed. Lower EE correspond with improved efficiency. Secondly, the proportion of times that the estimates were within a fixed distance of the true values was determined (**FD**). For reliable predictions, FD should be between 0.93 and 0.97. Thirdly, the importance (significance) of a particular plant-wax marker to separate plants into distinct categories was evaluated. Lastly, procedures that graphically cluster plants depending on similarities in their plant-wax marker contents were used.

Table 1. Efficiency of a newer (Bayesian linear hierarchical unmixing) and a traditional (non-negative least squares) method to estimate diet composition.

Statistic[1]	Plant mixture	Newer	Traditional
EE	2	0.018	0.033
	3	0.034	0.052
	5	0.094	0.175
	8	0.216	0.485
FD	2	0.907	0.698
	3	0.923	0.818
	5	0.652	0.518
	8	0.669	0.491

[1] Statistics: estimation error (EE) and the proportion of times that the estimates were within a fixed distance of the true values (FD).

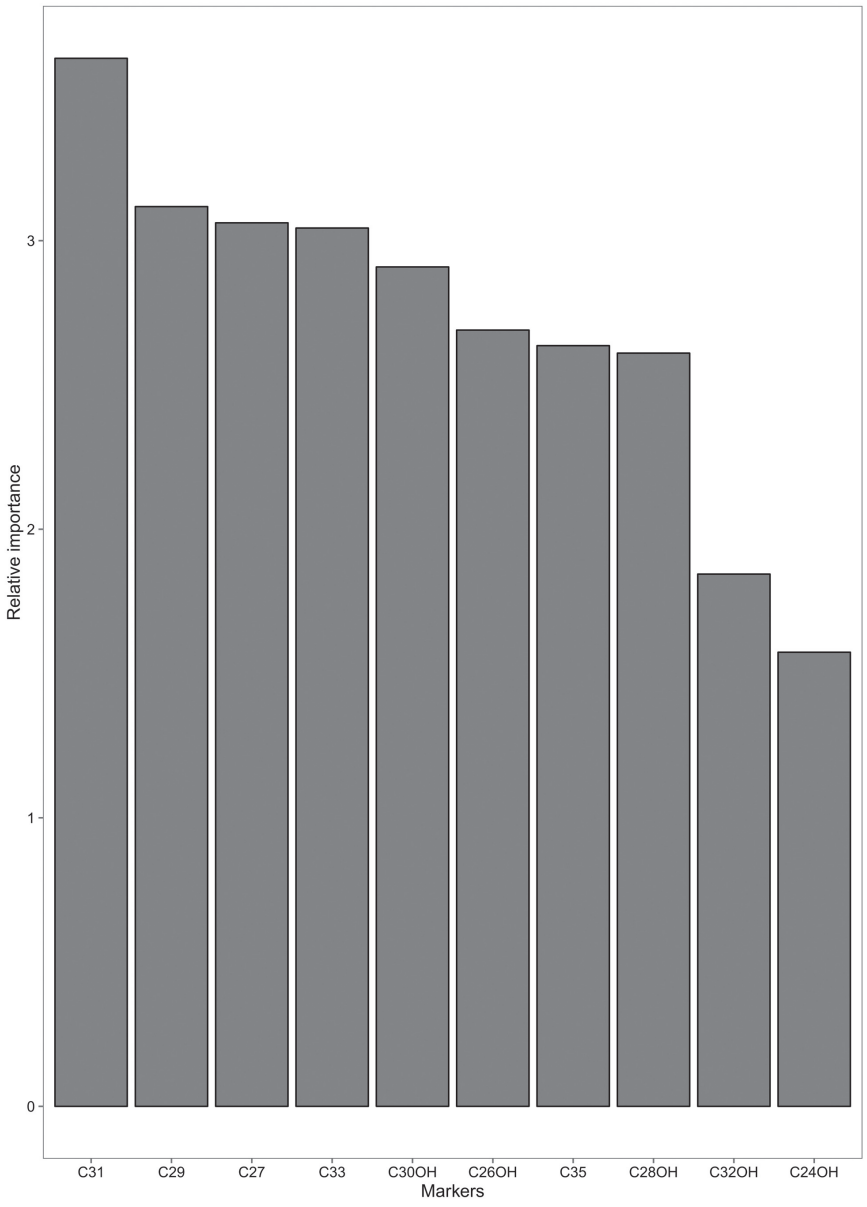

Figure 1. Plant-wax marker importance for 8 plant species at their early (vegetative) stage of growth) based on concentrations of 5 *n*-alkanes (C_{27}, C_{29}, C_{31}, C_{33} and C_{35}) and 5 long-chain alcohols (C_{24}OH, C_{26}OH, C_{28}OH, C_{30}OH and C_{32}OH).

Results

Mean ALK and LCOH concentrations for the 8 plant species were presented in 2017 Nebraska Beef Report, pp. 69–72. In general, ALK concentrations are lower than those of LCOH, which is in accordance with other studies.

With regards to the efficiency of delineating forage mixtures, for the 2-plant mixture the newer method was clearly more efficient, with EE of 0.018 compared to that of traditional method of 0.033 (Table 1). That represented a 50% improvement in efficiency. The FD also was better with the new method, although somewhat lower than the expected range. For the 3-plant mixture similar results were obtained although the reduction in EE was smaller, 0.034 and 0.052 for the new and traditional method, respectively. Still the difference between the two methods represented a 35% improvement in efficiency. In addition, the FD for the new method was close to the range expected, while that for the traditional method was well below.

For the 5-plant mixtures, the EE were 0.094 and 0.175 for the new and traditional method, respectively; however, FD was considerably reduced for both methods to 0.652 (new) and 0.518 (traditional). A similar result was obtained for the 8-plant mixture, with lower efficiencies for both methods: the estimation errors increased substantially and the proportion of times true and estimated values coincided decreased considerably. However, the new method was still around 50% more efficient than the traditional. One likely reason for this significant decline in ability to determine diet composition may be insufficient differences in the ALK and LCOH profiles among the plants when more and similar species were combined.

With this particular set of plant species, delineating relatively simple mixtures (2 or 3 plants) using the new method is quite feasible. However, increasing the number of plants in the mixture dramatically reduced the efficiency of the both methods. As shown in Figure 1, the C_{32}OH and C_{24}OH help the least in species delineation, possibly due to many of the plant species not having these two markers present. When evaluating the similarities in plant-waxes profiles for the different plants, C_{31}, C_{33}, C_{35} and C_{28}OH were common to the cool-

and warm-season grasses, while $C_{26}OH$ was important to distinguish the legumes. Although the $C_{32}OH$ was less important than many other markers to delineate plant species generally, in was unique to C4 grasses; therefore, it facilitated separating the cool- and warm-season grasses.

One possible way to increase the efficiency of the plant-wax methodology, when considering mixtures of more plants, is to add other plant markers such as long-chain fatty acids and alkenes. Additionally, plants could be grouped based on their taxonomy or function to simplify the complexity of the sward being characterized. Such an approach may be particularly valuable when the primary aim is to predict feed intake.

Conclusion

In conclusion, the reliability of predictions of diet composition was improved by using a more sophisticated statistical approach in the evaluation. However, further developments in the application of the plant-wax marker technique are still needed to determine the dietary choices and feed intakes of cattle grazing complex swards typical to western rangelands.

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