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FORAGE VOLATILES AS CUES FOR RUMINANTS

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Interpretative Summary

Are preference and dry matter intake by ruminants dependent upon volatile compounds coming from the forage? If animals respond to such cues then it would be helpful to identify the effective chemical compounds from the many present in most plants. Plant breeders could select plants based on altered concentrations of such compounds and animal nutritionists might add them directly to rations to affect dry matter intake.

Abstract

Forage volatiles may provide cues that affect animal food choices. Collecting and identifying these compounds is challenging. The authors briefly describe the methodology and report several volatile compounds that relate to animal preference.

Introduction

Forage preference studies using sheep (Rumbaugh et al., 1993) and later cattle (Shewmaker et al.1997) provided visual evidence that these ruminants might be using senses of touch and smell when choosing which plants to eat or not eat. Test animals moved across experimental pastures with their muzzles in the plant canopy. This exploratory exercise resulted in animals moving passed some plants, or taking a bite and then moving on, or grazing for a longer period on that plant type. Thus, plant volatiles were considered as one of several likely cues, both physical and chemical, that ruminants might be using during grazing or even when choosing among several hays. Several earlier studies identified incomplete profiles of volatiles or major groups of compounds in forage, but none related animal preference to these compounds. This is a summary of work published by Mayland et al. (1997) plus other ongoing studies by this group.

Instrumentation methods

Analytical techniques (Mayland et al., 1997) require 1) collecting volatiles, 2) adsorbing the volatile compounds, 3) desorbing and separating compounds, 4) detecting and identifying various structural groups from each compound, and 5) identifying and quantifying the volatile compounds. Step 1 may be completed by the non chemist whereas the remaining steps are more specialized. Steps 2, 3, 4, and 5 are conducted using trapping materials, desorbing and cryofocusing instrumentation and capillary gas chromatography (GC). Volatiles are next pulled into a flame ionization detector (FID) or a mass spectrometer (MS) for characterization. Output data are compared with experimental retention indices (RI) and component mass spectra from standard reference mixtures and reference library information. Chemists responsible for steps 2, 3, 4 and 5 must have GC/MS instrumentation skills, must be familiar with the expected volatiles' profile, and must be able to verify presence and identity of each volatile. Similar approaches have been used in insect pheromone or host plant studies (Buttery, and Kamm, 1980) to hopefully develop attractants for insect control.

Headspace (Volatile) Collection

Headspace compounds or volatiles simply refer to the gases rising to the top of a given sample. Some studies enhance gas collection by heating the sample with water or other solvent and flushing volatiles upwards in the column to be collected as a distillate. However, animal and forage scientists are generally interested in those volatiles that emanate from the plant under 'natural conditions'. These are the compounds that animals would detect or be exposed to when searching for a meal. In our studies sample chambers were constructed of borosilicate glass, stainless steel, Viton o-rings and Teflon-FEP tubing. Purified air (large activated charcoal filter) was pumped into the bottom of the chamber, using a stainless steel bellows pump throttled at the exit port with a needle valve. The clean air was pumped through the sample, which was restrained with fine stainless steel mesh, and through a sealable trap containing an adsorbent like Tenax-TA. It was very important that equipment was clean and that no gas leaks existed in the system. A quality control (QC) sample was periodically obtained by exposing a tenax trap to the same sampling system minus the forage sample.

Forage Sampling

Sampling aspects of the study requires carefully consideration. Grazing animals are confronted with a 'bouquet' of volatiles from the forage, and even the soil, and surrounding air. Samples collected from this type of environment also contain volatiles from the environment and artificial field chamber or enclosure. We considered that type of collection would result in a hodgepodge of volatiles precluding interpretation. Clipping and removing forage samples from the field may avoid some contaminants, nevertheless care must be exerted to avoid collecting volatiles from sample bags, storage areas, etc. Plastics and paper products are rich sources of volatiles that will confound results. Glass bottles or fluoropolymer film bags are 'clean' and were chosen in our study. Samples are weighed, placed in the sample chamber described above, and purified air passed through the sample to collect volatiles on the adsorbent. The same procedure is used when evaluating air-dried hay. Several examples follow in which volatiles were collected from forage samples, characterized, and concentrations related to animal eating behavior.

Tall Fescue Forage

Tall fescue forage was clipped in the field, 100 g subsample (23% dry matter) inserted into the collection chamber, and gas collection continued for 5 minutes. This was repeated for a number of cultivars such that time from clipping to collection was less than 1 hour.

Fifty of 52 compounds emitted from fresh forage were identified yielding 12 to 32 ug/L . (Z)- 3-Hexenyl acetate made up 82 % (mass basis) of total emissions from fresh forage. Green-leaf odor compounds made up 11% in fresh forage. The 'green odor' of freshly cut leaves is derived from eight volatile compounds including four alcohols and four aldehydes (Mayland et al.1997). Quantitative cattle preference scores were available for the eight tall fescues sampled (Mayland et al., 1997). Preference scores were regressed on the concentrations of identified compounds. Preference was positively related to concentrations of 6-methyl-5-hepten-2-one ($R2 = 0.77$, Prob. > 0.04). In a stepwise, multiple regression, preference was positively related to 6-methyl-5-hepten-2-one and negatively related to (Z)-3-hexenyl propionate and acetic acid. This 3-factor multiple regression had an $R2 = 0.97$ and Prob. > 0.002.

Tall Fescue Hay

Tall fescue forage was cut in the field, allowed to air dry, and 140 g subsample (93% dry matter) placed in the collection chamber and sampling time lengthened to 15 minutes. Ninety-nine of 103 detected compounds emitted from air dried hay were identified yielding 0.2 to 0.5 ug/L. The esters that were so predominant in fresh forage were in only trace concentrations in dry forage indicating the nearly complete loss of or oxidation of compounds like (Z)-3-Hexenyl acetate. Overall there was an approximate 100 fold yield decrease in emissions from dry hay compared with fresh tissue.

Livestock grazing preferences for fresh grass were regressed on the volatiles emanating from the dry forage. This application is stretched, but provides some first approximation information. In a 3-factor multiple regression test, preference scores were negatively related to 1-octen-3-one, and positively related (E,Z)-2,4-heptadienal and (E)-2-nonenal having an $R2 = 0.93$ and $Prob >= 0.01$

PM-/AM- Harvested Alfalfa Hay

We have shown that cutting grass and alfalfa during late afternoon, compared with cutting it in the morning, will produce higher quality hay that can be preserved through baling and feeding. Ruminants prefer the PM-cut and will eat more of it (Fisher et al., 1999, Mayland et al., 1998). Time-lapse video filming of feeding episodes suggests that animals provided paired samples of PM and AM hays in all possible combinations identified the PM hay as they walk up to the troughs. This suggests an ability to smell something in the PM or AM hay that they like or dislike, or simply recognized the hay from previous meals. This cue is then used in making their initial choice. Sheaves of alfalfa from hay grown in Idaho and fed in North Carolina were later submitted for collection of volatiles. While the collection, trapping, and GC/MS work is just underway, it appears that there are no gross differences in the volatile species' profile in the PM vs AM cut hay. Instead there appears to be a greater mass of volatiles in the PM than AM-cut hay.

Further Testing and Verification

Mayland et al. (1997) reported the relationship between animal preference and various volatiles in grass pasture or hay. These reports were based only on regression analyses and do not necessarily imply a cause and effect. The report however, provides a starting point for further testing.

Future work might include a simple study using two tubs having screen bottoms. A stream of air is blown through one forage in the bottom tub through a second forage in the upper tub that is accessible to test animals. Dry matter intake of the top hay might then be influenced by aromas circulating through it from the bottom hay. Effect of aromas might be identified, but specific chemical compound(s) would not be known.

Another approach would use specific compounds or groups of compounds that would test the effects of olfaction on feed preference. Corley et al. (1998) proposed such a method, suggesting that at least six animals be used in such studies.

Application

Volatile compounds appear to be involved in animal cueing of forage properties and serve as attractants or detractants to ruminants. Understanding these forage attributes may allow exploitation of the cueing volatiles to increase or limit dry matter intake. Presence of these compounds could be used by plant breeders as criteria to develop germplasm for specific applications. Such knowledge would also help us understand why ruminants select given forage plants. There is much to learn about the role of volatile compounds as cues in forage preference and intake studies. Answers are only a sniff away.

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