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Characterization of volatile organic compounds and odors by in-vivo sampling of beef cattle rumen gas, by solid-phase microextraction, and gas chromatography–mass spectrometry–olfactometry

Abstract

Volatile organic compounds (VOCs) and odors in cattle rumen gas have been characterized by in-vivo headspace sampling by solid-phase microextraction (SPME) and analysis by gas chromatography–mass spectrometry–olfactometry (GC–MS–O). A novel device enabling headspace SPME (HS-SPME) sampling through a cannula was designed, refined, and used to collect rumen gas samples from steers. A Carboxen–polydimethylsiloxane (PDMS) fiber (85 μm) was used for SPME sampling. Fifty VOCs from ten chemical groups were identified in the rumen headspace. The VOCs identified had a wide range of molecular weight (MW) (34 to 184), boiling point (-63.3 to 292 $^{\circ}\text{C}$), vapor pressure (1.05×10^{-5} to 1.17×10^2 Pa), and water solubility (0.66 to 1×10^6 mg L^{-1}). Twenty-two of the compounds have a published odor detection thresholds (ODT) of less than 1 ppm. More than half of the compounds identified are reactive and have an estimated atmospheric lifetime of <24 h. The amounts of $>$ VFAs, sulfide compounds, phenolic compounds, and skatole, and the odor intensity of VFAs and sulfide compounds in the rumen gas were all higher after feeding than before feeding. These results indicate that rumen gases can be an important potential source of aerial emissions of reactive VOCs and odor. In-vivo sampling by SPME then GC–MS–O analysis can be a useful tool for qualitative characterization of rumen gases, digestion, and its relationship to odor and VOC formation.

Keywords

Rumen gas, Odor, In vivo sampling, SPME, GC-MS-O

Disciplines

Agriculture | Animal Sciences | Bioresource and Agricultural Engineering | Environmental Sciences

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Characterization of volatile organic compounds and odors by *in vivo* sampling of beef cattle rumen gas using solid phase microextraction and gas chromatography-mass spectrometry-olfactometry

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18 **Abstract** Volatile organic compounds (VOCs) and odors in cattle rumen gas were
19 characterized using *in vivo* headspace sampling with solid phase microextraction (SPME)
20 coupled with gas chromatography-mass spectrometer-olfactometry (GC-MS-O) analysis.
21 A novel device allowing for headspace SPME (HS-SPME) sampling through the cannula
22 was designed, refined, and used to collect rumen gas samples from steers.
23 Carboxen/polydimethylsiloxane (PDMS) fiber (85 μm) was used in the SPME sampling.
24 Fifty VOCs belonging to 10 chemical functional groups were identified in the rumen
25 headspace. The identified VOCs had a wide range of molecular weight (MW) (34 to 184),
26 boiling point (-63.3 to 292 $^{\circ}\text{C}$), vapor pressure (1.05×10^{-5} to 1.17×10^2 Pa), and water
27 solubility (0.66 to 1×10^6 mg/L). Twenty two compounds have a published odor detection
28 threshold (ODT) of less than 1 ppm. More than half of the identified compounds are
29 reactive and have an estimated atmospheric lifetime of < 24 hr. The amounts of VFAs,
30 sulfide compounds, phenolics and skatole, and odor intensity of VFAs and sulfide
31 compounds in the rumen gas were all higher after feeding than those before feeding.
32 These results indicate that rumen gases can be an important potential source of aerial
33 emissions of reactive VOCs and odor. *In vivo* sampling via SPME coupled with GC-MS-
34 O analysis can be a useful tool for qualitative characterization of rumen gases, digestion,
35 and its relation to odor and VOC formation.

36

37 **Keywords** Rumen gas · Odor · *in vivo* sampling · SPME · GC-MS-O

38

39 **Introduction**

40 Rumen headspace is saturated with compounds produced during digestion.
41 Degradation of feed in the rumen is the processes of fermentation resulting from the
42 physical and microbiological activities that digest feed under anaerobic conditions. These
43 products could be useful (VFAs, microbial proteins, B-vitamins), useless (CH₄, CO₂), or
44 even harmful (ammonia, nitrates) for the host animal [1]. Composition of ruminal fluid
45 has implications on the digestion processes. Thus, chemical composition of ruminal fluid
46 is important for nutritional studies. Feed utilization and feed additives can have an impact
47 on odor and gas emissions from manure. Cattle production is associated with aerial
48 emissions of odor, VOCs and other gases originating mainly from manure and the
49 animals themselves. Chemical compositions of rumen liquid and gas can have
50 implications on air quality. Rumen gas can be released to atmosphere via eructations and
51 exhaled breath. Digested products from rumen can be also released with manure and
52 therefore be a source of aerial emissions of VOCs and odor.

53 Numerous methods are available for the collection of rumen fluid for analysis.
54 Rumen fluid can easily be collected through a cannula that is surgically placed in the
55 rumen [2]. Other approaches with animals without rumen cannula either involved the use
56 of stomach tubes [3] or percutaneous needle aspiration (rumenocentesis) [4]. Both
57 techniques are stressful to the animals. Samples taken by stomach tube are often
58 contaminated with saliva [3] and rumenocentesis has led to infections in some cases [4].

59 Characterization of fermentation products is used in assessing the extent and
60 nature of the microbial fermentations [5]. Several methods are used for the quantification
61 of these products. High-performance liquid chromatography was used to quantify ethanol,
62 n-butanol and VFAs in the early 1980s [6]. Gas chromatography has been commonly
63 employed to quantify VFAs and alcohols in rumen fluid [7, 8, 9, 10, 11, 12] since the
64 1960s. Most of these methods involve time-consuming sample preparation procedures.
65 Solvent extraction with ether [12] or methylene chloride [10] and pre-injection
66 derivatizations of acids [7, 13, 14] are often used. Comparison of sampling and analytical
67 method between the present and previous studies to determine the rumen fermentation
68 products in rumen fluid is presented in Table 1 [2, 9, 10, 11, 15, 16, 17].

69 To date, nearly all studies focused on the characterization of ruminal fluid itself.
70 Relatively little is known about the composition of rumen gas and its implication for
71 gaseous emissions. Sampling of gas instead of liquid is more challenging from the
72 analytical standpoint. However, one benefit is a minimization of multiphase liquid-solid
73 sample matrix which requires extensive sample preparation. Measurement of gases
74 produced by rumen microbes could be very useful in evaluating diets, animal health
75 status, feed additives, dietary amendments, and rumen fermentation [15].

76 Only one study reported sampling and analysis of rumen gas to gain information
77 about rumen processes [15]. Dewhurst et al. [15] investigated certain gases in rumen
78 headspace using active gas collection to 2L food-grade polyethylene terephthalate (PET,
79 also referred to as Melinex) bottles with rubber stoppers for on-site and next day analyses
80 in laboratory using selected-ion-flow-tube mass spectrometer. A total of 14 gases,
81 including several alcohols, ammonia, five VFAs (from acetic to hexanoic), acetone,
82 acetaldehyde, and H₂S and other sulfides were reported [15]. However, potential sample
83 recovery problems and uncertainties associated with quantitative analysis of gas samples
84 in rumen still exist with this approach and similar conventional sampling methods. This is
85 due to the porous nature of polymeric materials used for sampling containers, adsorption
86 to walls, condensation and partitioning to water, reactivity of gases and reactions between
87 gases inside the sample container, and false positives caused by gases emitted by
88 sampling containers.

89 Poor sample recoveries for VFAs were reported when PET sampling bags were
90 used [18, 19, 20]. Mean gas sample recoveries were 66.1% for 7 VFAs from acetic to
91 hexanoic acid, respectively, after 24 hr storage time at room temperature in PET bags
92 [18]. These recoveries were 27.6%, 61.4%, 73.9, 51.1%, and 38.2% for acetic, propanoic,
93 butanoic, pentanoic, and hexanoic acids, respectively [18]. The PET bags were also not
94 recommended for the collection and 24 hr storage of H₂S or ammonia [20]. Alcohols,
95 VFAs, ammonia can also readily partition to water in air samples. Acetaldehyde is a
96 reactive gas and is typically sampled via derivatization [21].

97 No olfactometry analyses were reported on rumen liquid or gas in previous
98 studies. Odor analysis could provide additional insight to the specific makeup of gas,
99 particularly in some cases where human nose is more sensitive than conventional

100 analytical detectors. Many compounds well known to be in rumen liquid are also known
101 to be offensive odorants and are emitted from manure. Thus, the link between specific
102 diet, rumen gases and livestock odor warrants research.

103 Solid phase microextraction eliminates the use of sampling containers and it
104 combines sampling and sampling preparation into one step. Air sampling with SPME
105 presents many advantages over conventional sampling methods [22, 23, 24, 25] due to its
106 simplicity, reusability, very good sample recovery [18] and hydrophobic behavior of
107 SPME coatings. Koziel et al [18] reported average 105% ($\pm 11.4\%$) recoveries of gaseous
108 VFAs (from acetic to hexanoic acid) at room temperature and 24 hrs storage time from
109 the 75 μm Carboxen/PDMS SPME fiber coatings [18]. The variability (measured as
110 standard deviation) for recoveries of VFAs were as low as 2.0%, 3.6%, 9.7%, and 5.6%
111 for propanoic, butanoic, pentanoic, and hexanoic acids, respectively. Spinhirne and
112 Koziel used SPME to sample the headspace gases of closed *in vitro* cultures to evaluate
113 ruminal fluid and ruminal fluid with feed containing a feed additive using GC-MS [16].
114 Spinhirne et al. [26] reported the use of SPME for on-site breath sampling of steers and
115 characterization of 21 VOCs [26].

116 This study was conducted to characterize volatile organic compounds (VOCs) and
117 odors in cattle rumen gas through *in vivo* sampling of the rumen gas. In this research, a
118 novel device allowing for headspace SPME (HS-SPME) sampling through the cannula
119 was designed, refined, and used to collect rumen gas samples from steers. Rumen gas
120 samples were analyzed using a GC-MS-Olfactometry system allowing for simultaneous
121 VOCs/odor qualitative characterization [27, 28, 29]. To our knowledge, this is the first of
122 this kind of investigation to conduct *in vivo* SPME and evaluating rumen gas odor.

123

124 **Experimental**

125

126 **Rumen Gas Sampling Device**

127 A novel device (Figure 1a,b,c) allowing for headspace (HS) SPME sampling through
128 the cannula was designed, refined and used to collect rumen gas samples from three
129 steers for three days. This device uses a cannula stopper modified with a sealed septum
130 port for insertion of SPME fibers. The objective was to (1) modify a typical cannula plug

131 with common, low cost materials, (2) **make** the modified plug easily removable for
132 replacement with a regular plug, (3) make the modified plug safe to animal while
133 sampling, (4) make the separation of rumen gas and fluid possible, (5) to provide a means
134 of SPME insertion into the sealed rumen headspace, and (6) to protect the fragile SPME
135 fiber assembly from possible damage by floating undigested and partially digested feed
136 and rumen fluid. A PVC ‘snorkel’ was constructed internally to protect the SPME fibers
137 from contact with the rumen fluid/forage mixture while allowing the fibers to interact
138 with the headspace gases. The main tubing and screen for the device were made from 6
139 cm dia PVC tubing and bushings purchased at a local hardware store (Lowe’s, Ames, IA).
140 The ‘snorkel’ was fixed to the cannula plugs with bushings. A 3 mm dia (1/8”) bulkhead
141 fitting (Swagelok, Kansas City, KS) was mounted in the center of the plug. A
142 thermogreen half-hole septum (Supelco, Bellefonte, PA) was inserted into the bulkhead
143 fitting and held tight with the Swagelok nut to seal and to guide the SPME needle into the
144 rumen headspace. All dimensions are provided in Figure 1c.

145

146 Sampling of Rumen Headspace Gas with SPME

147

148 Carboxen/PDMS SPME fiber (85 μm) (Supelco, Bellefonte, PA, USA) was used
149 for rumen headspace gas sampling. Carboxen/PDMS fiber coating has proven to be very
150 effective in extracting VFAs and sulfides [30, 31], i.e., the types of compounds known to
151 be in rumen gas [15]. Carboxen has small diameter (10A on average) pores which are
152 suitable to adsorb molecules in the C₂-C₁₂ range [24]. Fibers were conditioned
153 according to the manufacturer’s instructions. Fiber assemblies had their tensioning spring
154 removed and samples were collected manually, i.e. without SPME holder. Before
155 sampling, fiber was desorbed for 5 min at 260 °C, then wrapped in clean aluminum foil.
156 Tight wrapping of SPME assemblies in aluminum foil sealed the fibers from the ambient
157 environment. The operator wore nitrile gloves and avoided direct contact with the SPME
158 needle to minimize interferences. SPME fibers were transported to and from the
159 laboratory enfolded in aluminum foil, placed inside a clean jar with tight cover and
160 placed in an ice cooler immediately after sampling.

161 Three rumen cannulated (101 mm I.D.) Angus steers (868 ± 49 kg body weight) were
162 fed 27.2 kg rations of Fescue grass hay twice daily (8:00 hr and 16:00 hr) in individual
163 (3.7 m \times 12.6 m) pens. The feed were weighed before each feeding. Water was available
164 *ad libitum*. All sampling was conducted on October 9th – 11th, 2005 at the Iowa State
165 University Beef Nutrition Center, Ames, Iowa. The steers were individually restrained in
166 a hydraulic chute during the SPME sampling.

167 Rumen gas samples were collected before morning feeding (9:00 am) and 2 hours
168 after feeding (1.00 pm). For each animal, the cannula stopper was replaced with the
169 modified sampling device. For each cattle, sampling device was fitted in rumen cannula
170 for 5 min before SPME sampling to allow rumen gases to reach equilibrium inside the
171 headspace of sampling device. During SPME extraction the septum fitted in the sampler
172 was pierced using the SPME needle and exposed the SPME fiber to the headspace for 5
173 min. These sampling times were selected based on animal wellbeing considerations and
174 on previous experience with restraining steers in hydraulic chutes [26]. After SPME
175 sampling was complete, SPME fiber was enfolded in aluminum foil to be transferred to
176 the Atmospheric Air Quality Laboratory at Iowa State University to be analyzed. The
177 desorption time of SPME fiber was 40 min at 260 °C. The same sampling device, i.e., the
178 modified cannula plug was used for all 3 steers. Thorough rinsing with hot water and air
179 drying was used to clean the device between applications. HS-SPME extraction of blank
180 device did not result in significant amounts of target analytes selected for analyses.

181

182 Analysis of Rumen Gases

183

184 Multidimensional GC-MS-O (Microanalytics, Round Rock, TX, USA) was used
185 for all analyses [27, 28, 29]. The system integrates GC-O with conventional GC-MS
186 (Agilent 6890N GC / 5973 MS, Wilmington, DE, USA) as the base platform with the
187 addition of an olfactory port and flame ionization detector (FID). The system was
188 equipped with a non-polar precolumn and polar analytical column in series as well as
189 system automation and data acquisition software (MultiTrax™ V. 6.00 and AromaTrax™
190 V. 6.61, Microanalytics and ChemStation™, Agilent). The general run parameters used
191 were as follows: injector, 260 °C; FID, 280 °C, column, 40 °C initial, 3 min hold, 7 °C

192 /min, 220 °C final, 10 min hold; carrier gas, He. Mass to charge ratio (m/z) range was set
193 between 33 and 280. Spectra were collected at 6 scans/sec and electron multiplier voltage
194 was set to 1200 V. The MS detector was auto-tuned weekly.

195 The identity of compounds was verified using (a) reference standards (Sigma-
196 Aldrich, Fisher, Fluka) and matching their retention time on multidimensional GC
197 capillary column and mass spectrums; (b) matching mass spectrums of unknown
198 compounds with BenchTop/PBM (Palisade Mass Spectrometry, Ithaca, NY, USA) MS
199 library search system and spectrums of pure compounds, and (c) by matching the
200 description of odor character. Rumen gas was analyzed only qualitatively. Rumen gas
201 abundance was measured using area counts under peaks of characteristic single ions for
202 separated gases. The peak area counts were reported for comparisons only. **One human**
203 panelist was used to sniff separated compounds simultaneously with chemical analyses.
204 Odor caused by separated gases was evaluated with a 64-descriptor panel and intensity
205 scale in Aromatrx software [27, 28, 29]. Odor evaluations consisted of comparisons of
206 (a) the number of odor events and (b) the total odor measured as the product of odor
207 intensity and odor event time length recorded in an aromagram. Aromagrams were
208 recorded by panelists utilizing the human nose as a detector. Odor events resulting from
209 separated analytes eluting from the column were characterized for odor descriptor and
210 odor intensity.

211

212 **Results and Discussion**

213

214 **Effects of SPME extraction time on VOCs and odor of rumen Gas**

215

216 The effects of SPME extraction time 1 min, 5 min (triplicate) and 10 min,
217 respectively, at the fixed temperature of 39 °C inside the headspace of rumen for selected
218 compounds is presented in Figure 2. These selected compounds were the main rumen
219 fermentation products and also compounds which significantly contributed to the
220 offensive odor of rumen gas. Odorous gases included most of the well known gases
221 emitted from cattle and swine operations [28], e.g., VFAs, volatile sulfur compounds
222 (VSCs), phenolics and indolics. The amount of each selected compound extracted by

223 SPME fiber increased with the sampling time except for H₂S. This could be due to the
224 Carboxen-PDMS coating's limited number of adsorption sites and possible competitive
225 adsorption and displacement. Higher MW compounds, e.g., semi-VOCs, can displace
226 lower MW compounds as a consequence of competition for active sites on the fiber [25],
227 particularly for complex matrices. This can be minimized when shorter extraction times
228 are used [23, 32]. In this study, 5 min was selected for all target compounds. This
229 sampling time was selected due to the feasibility of restraining the steer for a limited time
230 of sampling.

231 The repeatability of *in vivo* rumen gas sampling using the modified
232 cannula/SPME port sampler was evaluated by comparing 3 replicate rumen gas samples.
233 Average RSD of selected compounds except propanoic acid was 26 %. The RSDs for
234 octene isomers, phenolics, indole, alcohols were less than 20%. Both the VSCs and VFAs
235 had much greater RSD ($\geq 30\%$). Compounds, H₂S, 3-methyl thiophene, 4- methyl phenol
236 and indole had RSDs of 19%, 13%, 18% and 16%, respectively. This is likely because of
237 the relatively short sampling time and the dynamic nature of the rumen headspace. The
238 dynamic nature of rumen gas was also implicated as a possible source of uncertainties by
239 Dewhurst et al. [15].

240 Effect of SPME extraction time on total odor and the total number of odor events
241 for the series of aromagrams of rumen gas is shown in Figure 3. The total odor was
242 estimated as the sum of products of odor duration and odor intensity for all odor events in
243 all time series samples of rumen headspace. As can be seen in Figure 3, longer extraction
244 time resulted in a significant increase in the total odor and total number of odor events.

245 Sixteen characteristic odors that were most frequently present in rumen gas were
246 selected for further evaluation of the effects of SPME sampling time on odor (Figure 4).
247 These characteristic odors were correlated with corresponding compounds (Table 2).
248 Data presented in Figure 4 indicates that the odor intensity of most of those characteristic
249 odors increased with longer sampling time. Particularly noteworthy were
250 'mushroom/moldy (1-octen-3-one)' and 'taco shell (2'-aminoacetophenone)'. The
251 presence of these odor-causing compounds could be easily overlooked in a conventional
252 analysis of MS chromatograms. This is because they were present in rumen gas at very
253 low concentrations and the resulting MS detector responses were not within the

254 background signal. Only the use of more sensitive detector (i.e., human nose) and
255 matching of aromagrams with total ion chromatogram (TIC), that is possible with the
256 GC-MS-O approach, allowed us to identify those compounds. We were able to identify
257 them only because of their significant odor intensity and the characteristic odor perceived
258 by olfactometry panelist. Both 1-octen-3-one and 2'-aminoacetophenone are very potent
259 odorants. The odor detection threshold in air (ODT_{air}) for 1-octen-3-one is 0.03-1.12 ng/L
260 [33]. The ODT_{air} of 2-aminoacetophenone is not published. However, its ODT in water
261 (ODT_{water}) is 0.2 μ g/L [33].

262

263 Identification of VOCs in rumen headspace

264

265 Rumen gas samples were analyzed on a multidimensional GC-MS-O system
266 allowing for the simultaneous identification and analysis of chemicals and corresponding
267 odors and the collection of a chromatogram and aromagram. Comparison of a typical
268 chromatogram (lower, red line) and aromagram (upper, black line) of rumen gas after
269 feeding is shown in Figure 5. A variety of compounds with wide range of odor
270 characteristics were found. Total ion chromatogram typically showed a complex
271 peak/compound pattern. The 50 compounds with most prominent peaks are listed in
272 Table 2. These compounds are typical rumen fermentation products such as VFAs and
273 VSCs have been reported in the literature [15]. The aromagram (upper, black line in
274 Figure 2) recorded as many as 38 distinct odors in rumen gas. The majority of odors
275 detected in rumen gas were perceived as offensive (Table 2).

276 Fifty VOCs belonging to 10 chemical function groups were identified in rumen
277 gas, i.e., sulfides and thiols (8), VFAs (7), ketones (4), alkanes (17), alcohols (2),
278 phenolics (4), benzenes (3), nitrogen heterocycles (3), aldehydes (1), and monoterpenes
279 (4), of which 37 have never been reported in previous studies of rumen fluid and gases [9,
280 10, 11,12,15,16,17, 33] (Table 2). It is interesting to mention that chemical compound
281 groups identified in this study were similar to those found previously in ambient air at a
282 dairy farm [34] except for VSCs. One new chemical group found in rumen gas in this
283 study was monoterpenes. Four monoterpenes including α -pinene, camphene, sabinene
284 and limonene were identified in rumen gas. Sunesson et al. [34] reported that

285 monoterpenes were found in ambient air in dairy farm and attributed the sawdust used for
286 bedding as being the main source. Wood is well known source of monoterpenes. In this
287 study, other sources (e.g., *Fescue* grass in the feed) could be the source of monoterpenes
288 [35]. Thus, eructated rumen gas could be another potential source of monoterpenes in
289 ambient air in and around cattle feedlots and possibly dairies. However, more research is
290 needed to confirm this hypothesis. Rabaud et al. [36] reported that the vast majority of
291 compounds emitted from a commercial dairy such as VFAs, alcohols, aldehydes and
292 ketones resulted from carbohydrate oxidation and fermentation during and after digestion.

293 Thirty four out of 50 compounds identified in this study were confirmed with the
294 retention time and spectrums of authentic standard compounds (Table 2). The remaining
295 15 were identified with BenchTop/PBM mass spectrometry library search system (match
296 above 70%) and by matching their known odor character [37]. Identified VOCs had a
297 wide range of molecular weight (MW) (34 to 184), boiling point (-63.3 to 292 °C), vapor
298 pressure (1.05×10^{-5} to 1.17×10^2 Pa) and water solubility (0.66 to 1×10^6 mg/L). As many
299 as 22 compounds had a published ODT less than 1 ppm [33]. Four compounds including
300 2-butanone, toluene, phenol and p-cresol are classified as HAPs [38]. As many as 54% of
301 compounds had the estimated atmospheric lifetime < 24 hours based on the reaction with
302 OH radicals. Estimating actual emissions of reactive organic compounds from rumen
303 gases could be useful in emission inventories of in protected airsheds with large cattle
304 population.

305

306 Comparison of rumen gases between before and after feeding

307 The feasibility of rapid testing of rumen gas with SPME to elucidate useful
308 information related to digestion was demonstrated by sampling before and after feeding.
309 Twelve odorous gases were then selected for further comparisons. Odorous gases
310 included the well known gases emitted from manure, e.g., VFAs, VSCs, phenolics and
311 skatole. A qualitative comparison of the 12 rumen gas compounds before and after
312 feeding for three animals and 3 days is shown in Figure 6. Carbon 2-C6 short-chain fatty
313 acids were identified, including acetic acid, propanoic acid, butanoic acid, 3-methyl
314 butanoic acid, pentanoic acid and hexanoic acid. Volatile fatty acids are the main
315 products of bacterial fermentation in the rumen and are absorbed through the rumen wall

316 into blood stream and form the primary energy source for the host animal [39]. Low
317 molecular weight VFAs had been used to determine the energetic efficiency of microbial
318 fermentation in the ruminant [16]. Many researchers believe that C2 to C9 VFAs are the
319 most important odor indicators compared to all other volatile organic compounds (VOCs)
320 found in agricultural air [40, 41]. All VFAs had higher relative abundance in rumen gas
321 after feeding compared with their abundance before feeding. This could be due to the pH
322 of the rumen fluid which is very responsive to meals and chewing behavior; ruminal pH
323 decreases rapidly following meals and increases rapidly during rumination [42]. At lower
324 ruminal pH after feeding, a greater fraction of the VFAs was previously observed in the
325 associated form in rumen fluid, so the concentration of VFAs in rumen gas would likely
326 increase. Dewhurst et al. [15] observed that the relative concentrations of VFAs in rumen
327 gas and rumen fluid decreased with increasing chain length. In addition, the overall molar
328 proportions of VFAs were very similar between rumen fluid and rumen gas [15].

329 The apparent amounts of dimethyl sulfide, dimethyl disulfide and dimethyl
330 trisulfide in rumen gas after feeding were much higher than those before feeding.
331 Dimethyl sulfide after feeding was more than 10-fold higher than that before feeding.
332 These observations were consistent with the significant levels of production of dimethyl
333 sulfide by the rumen reported previously [43, 44] and consistent with the higher levels of
334 dimethyl sulfide production instantly after feeding [45]. It is noteworthy that dimethyl
335 sulfide (DMS) is an inverse 'greenhouse effect' gas [45, 46] and may be released to
336 atmosphere by cattle eructation. Besides VFAs and VSCs, phenol, 4-ethyl phenol and
337 skatole was also present in higher amounts in after feeding rumen gas. While these
338 preliminary observations are generally consistent with literature had only limited
339 statistical significance due to relatively small number of replications (3 steers and 3 days).
340 Only phenol showed significant difference between before and after feeding (p-
341 value=0.0391).

342 Comparison of the difference of total odor and characteristic odors associated
343 with the rumen gas samples collected before and after feeding of three animals and 3
344 days is shown in Figure 7. There were no significant differences in the total odor
345 measured with GC-O approach of rumen gas between before and after feeding (p-
346 value=0.9293). However, the comparison of the characteristic odor was consistent with

347 the specific compounds discussed above. The odor intensity in rumen gas caused by
348 VFAs and VSCs after feeding was higher than that before feeding. One of the
349 characteristic odors, i.e., 'onion, foul' (caused by dimethyl sulfide) showed significant
350 difference (p-value=0.0049) which is consistent the increase of dimethyl sulfide in the
351 rumen headspace (p-value=0.0867).

352 More research is needed to quantify gases in the rumens headspace and to
353 determine the rumen liquid-gas correlations related to digestion. Rumen gases reflect the
354 processes of fermentation within the entire reticulo-rumen system. Thus, *in vivo* sampling
355 of rumen gases may overcome some of the sampling challenges related to
356 compartmentalization and variation across the rumen [3]. The information about rumen
357 gases can be potentially used to assess the extent of digestion of feed in the rumen [2, 15]
358 and to diagnose disease or rumen disfunction.

359

360 **Conclusions**

361 Several conclusions can be made from this study:

- 362 1. New device proved useful for *in vivo* rumen gas collection with SPME in field
363 conditions. Sampling times as long as 10 min were practical. Longer extraction
364 times may be possible with free-ranging steers, if the septum port is protected.
- 365 2. SPME-GC-MS-O can be a useful technique to monitor feed digestion *in vivo* and
366 to observe the relation between feed and odor/VOC emissions from beef cattle
367 operations.
- 368 3. Rumen gas contains at least 50 VOCs belonging to 10 chemical **functional** groups.
369 In this research, 34 were confirmed with pure standards. Identified compounds
370 had a wide range of MW, boiling point, vapor pressure and water solubility. New
371 chemical group found in rumen gas were monoterpenes.
- 372 4. Many of the most offensive and characteristic odorants associated with livestock
373 production were found in rumen gas. Odorous gases included those emitted from
374 manure such as VFAs, VSCs, phenolics, and indolics. As many as 22 compounds
375 had an ODT < 1 ppm. These results indicate that rumen gases could be a source of
376 aerial emissions and odor. **Amendments to the** rumen environment could
377 potentially have implication to odor control.

378 5. More than half the rumen gas compounds identified in this research are reactive
379 and have an estimated atmospheric lifetime of < 24 hours. At least one gas
380 (dimethyl sulfide) is suggested as an inverse 'greenhouse effect' gas. More
381 research is warranted to determine actual concentrations and emissions of these
382 rumen gases to atmosphere as they may be important odor sources in areas with
383 large cattle populations.

384

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388

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- 462

FIGURE CAPTIONS

463

464 Figure 1. Schematic of device for *in vivo* sampling rumen gas with SPME. Part A: cattle
465 rumen with modified cannula and SPME sampling device. Part B: cross section
466 of rumen. Part C: Modified cannula with SPME in rumen headspace.

467 Figure 2. Effect of time on HS-SPME extraction of 16 compounds from rumen gases
468 before feeding at fixed temperature 39 °C inside cattle rumen with 85 µm
469 Carboxen/PDMS fiber. Extraction time = 1 min, 5 min (n = 3), and 10 min.
470 Error bars signify the standard deviation of the mean. Number in parentheses is
471 the single ion of each compound used for peak area count integration.

472 Figure 3. Effect of time on HS-SPME extraction of total odor from rumen gases before
473 feeding at fixed temperature 39 °C inside cattle rumen with 85 µm
474 Carboxen/PDMS fiber. Extraction time = 1 min, 5 min (n = 3), and 10 min.
475 Error bars signify the standard deviation of the mean. Number signifies total
476 odor events in a sample.

477 Figure 4. Effect of time on HS-SPME extraction of 19 characteristic odors from rumen
478 gases before feeding at fixed temperature 39 °C inside cattle rumen with 85 µm
479 Carboxen/PDMS fiber. Extraction time = 1 min, 5 min (n = 3), and 10 min.
480 Error bars signify the standard deviation above the mean.

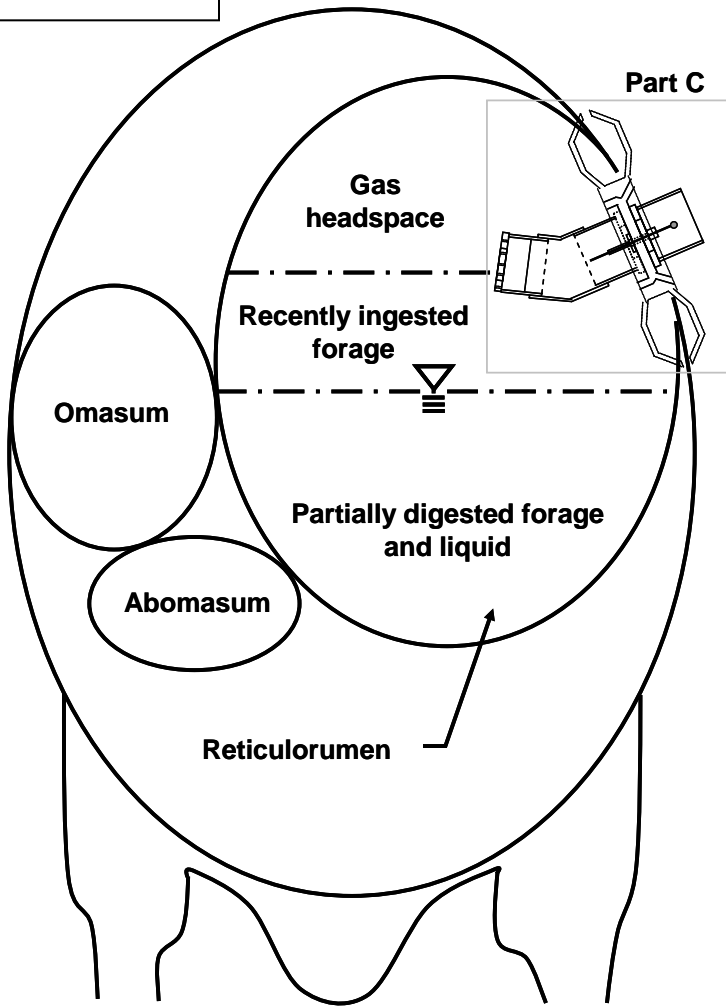
481 Figure 5. Total ion chromatogram (TIC) (lower, red line) and aromagram (upper, black
482 line) of rumen gas after feeding. Samples were collected using Carboxen/PDMS
483 85 µm SPME fiber and 5 min *in vivo* rumen sampling time. Numbers signify
484 odor events (Table 2).

485 Figure 6. Comparison of peak area count of 12 characteristic compounds in rumen gas for
486 before and after feeding of three steers with 85 µm Carboxen/PDMS SPME
487 fiber. Extraction time = 5 min. Error bars signify the standard deviation above
488 the mean. Number in parentheses is the single ion of each compound used for
489 peak area count integration. * = significant difference between before and after
490 feeding.

491 Figure 7. Comparison of the difference of total odor and characteristic odors between
492 rumen gases before and after feeding of three steers. Error bars signify the plus
493 standard deviation of the mean.

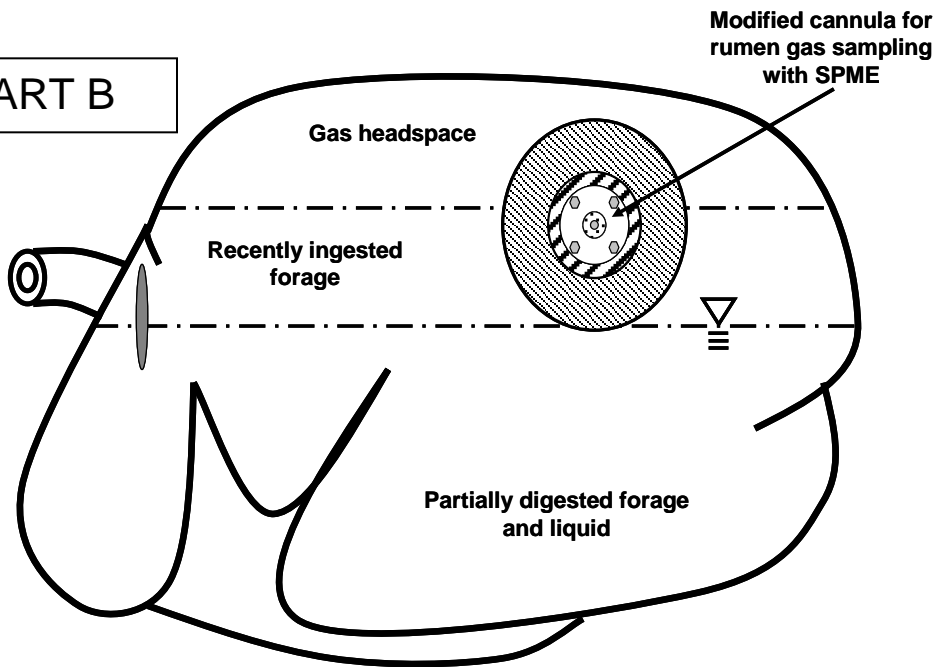
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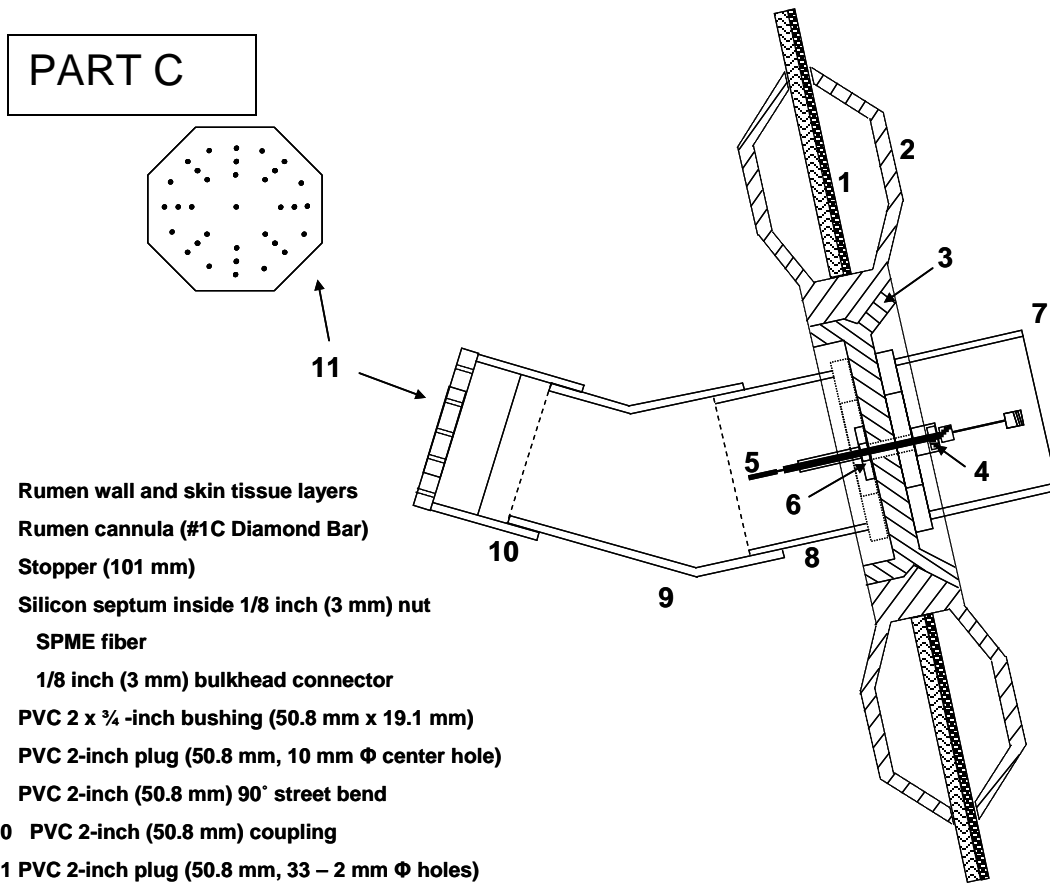


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PART B



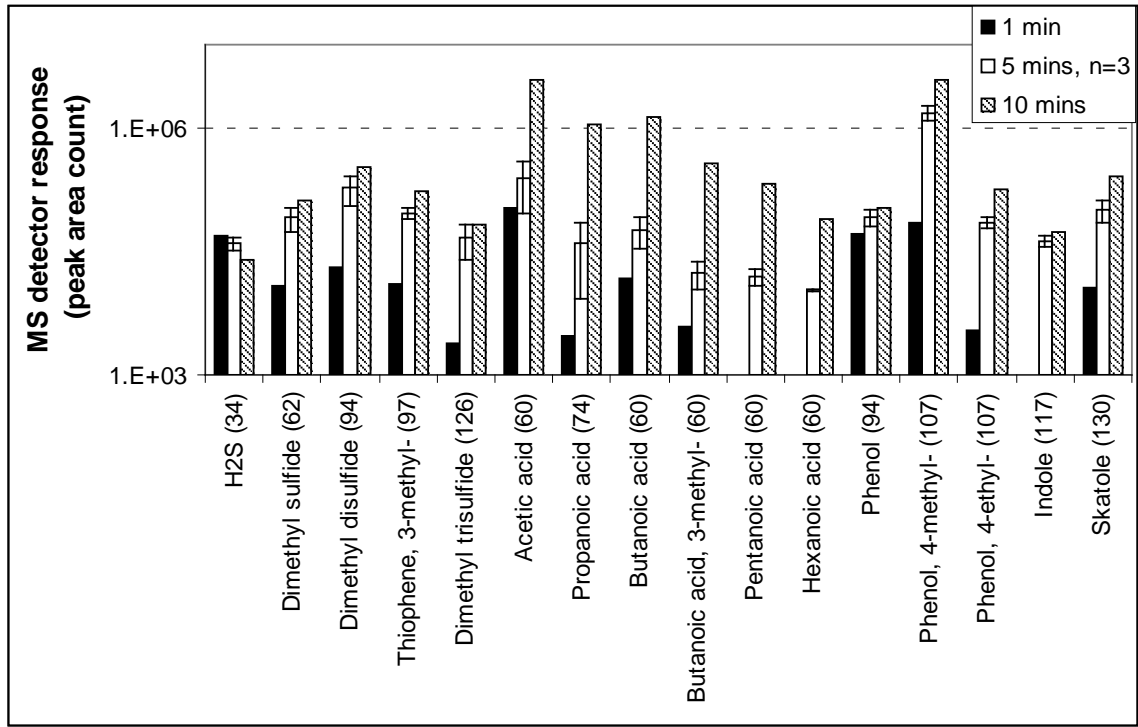
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497

498 **Figure 1 (Parts A, B and C)**

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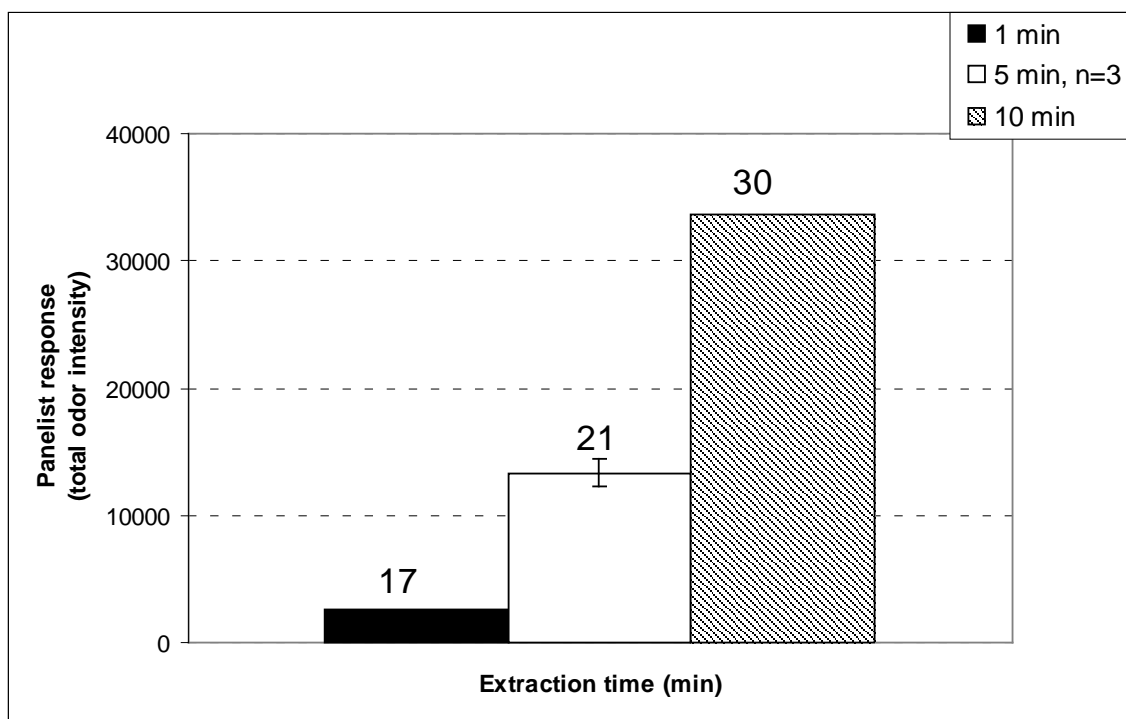


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501 **Figure 2**

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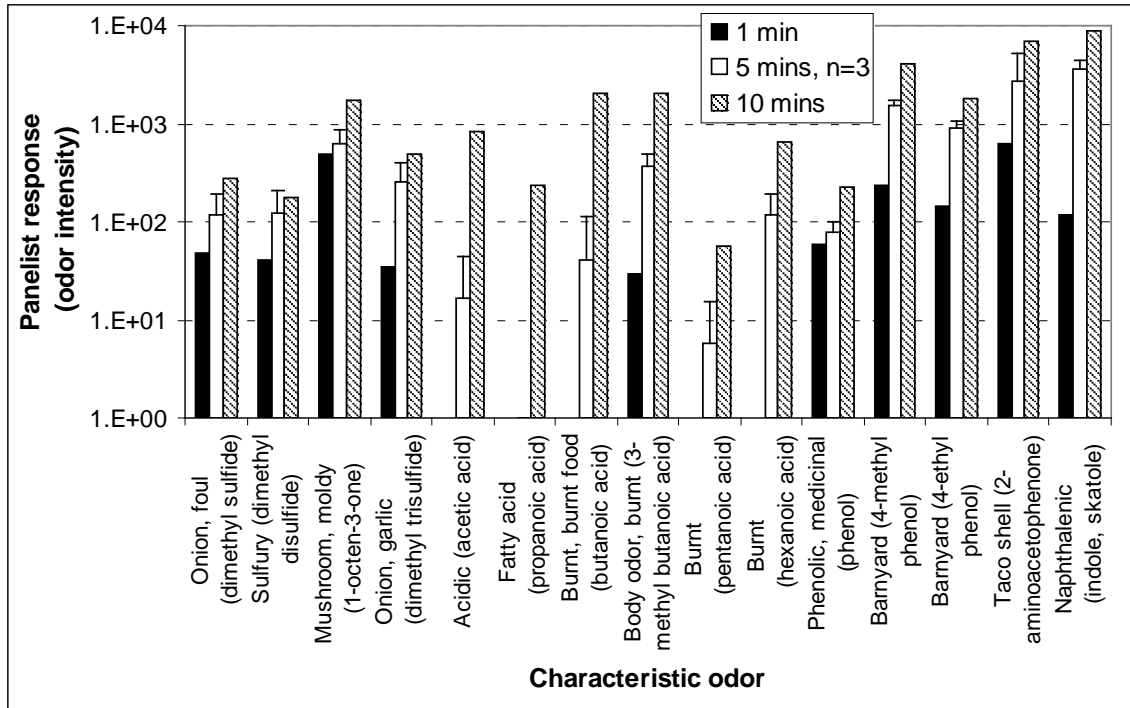
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505 **Figure 3**

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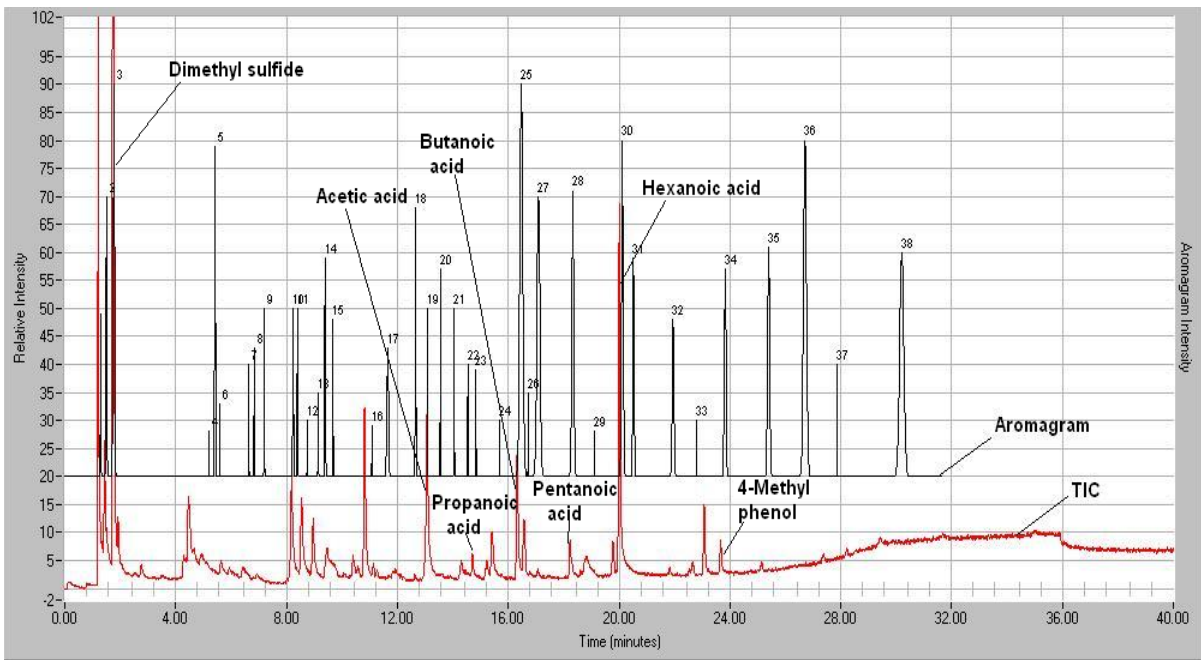


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509 **Figure 4**

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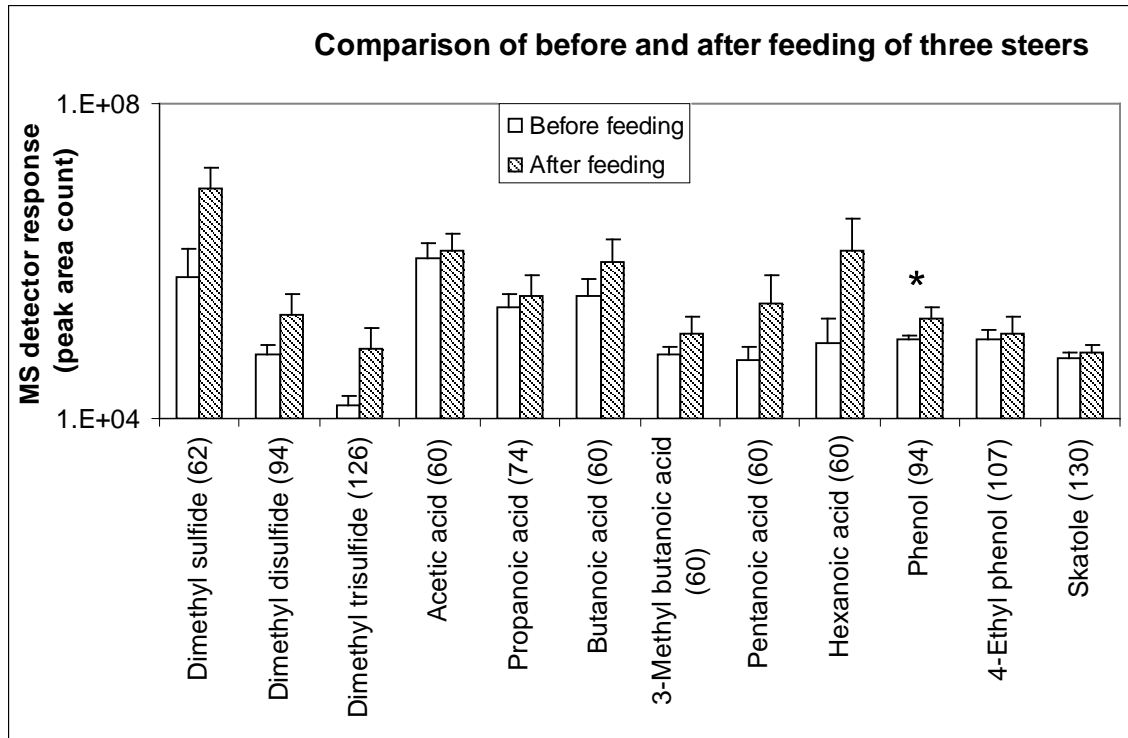
513 **Figure 5**

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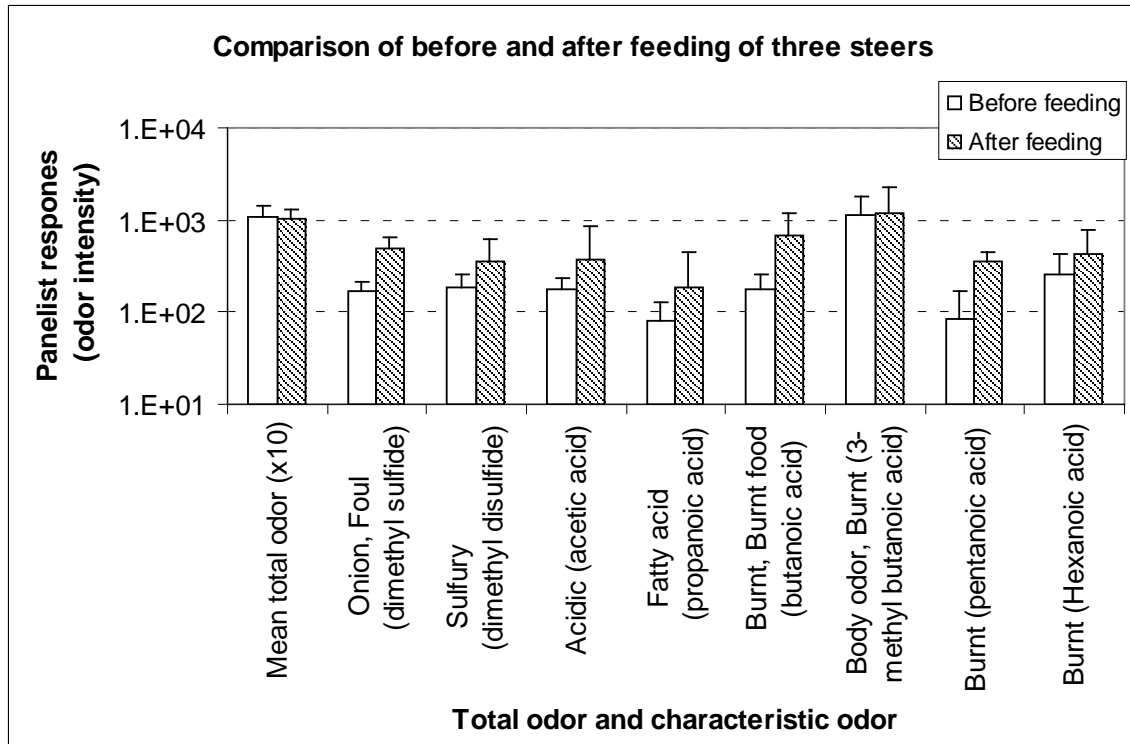


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520 **Figure 6**

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522

523 **Figure 7**

524 Table 1. Comparison of sampling and analytical methods used to characterize VOCs in
 525 rumen gas and rumen fluid.

Reference #	Sampling	Sampling Preparation	Analyses	Odor Analysis	Identified compounds
This work	SPME, cattle with rumen cannula, <i>in vivo</i> sampling	SPME (Carboxen/PDMS) Extraction condition: 39 °C, 5 min	GC-MS-O	Sniff port on GC-MS-O	Cattle rumen headspace 50 compounds
Dewhurst, et al. [15]	cattle with rumen cannula, rumen gas was pumped into evacuated plastic bottles (2L) <i>in vivo</i>	The caps of the bottles containing the rumen gas were punctured with a needle connected directly to the inlet port of the SIFT-MS	Selected-ion-flow-tube Mass (SIFT-MS)	None	Dairy cows rumen headspace 14 compounds
Spinhirne, et al. [16]	Ruminal fluid from cannulated heifer	SPME (DVB/Carboxen/PDMS) Extraction condition: 39 °C, 1 min	GC-MS	None	Heifer rumen fluid headspace 12 compounds
Schneider, et al. [17]	Fistulated cow	Centrifuged and filtered, reacted with NaOH, then derivatized with trifluoroacetic acid and extracted by chloroform	GC-FID	None	Cow rumen fluid 20 compounds
Teunissen, et al. [9]	Fistulated sheep	Filtered on a Whatman GF/C glass microfiber filter and centrifuged. The supernatants were pipetted into 1.5 ml Eppendorf reaction vessels, and then stored at 4-10 °C up to 48 h	GC-FID	None	Sheep rumen liquor 22 compounds
Faichney, et al. [10]	Fistulated sheep and cattle	Distilled and made alkaline with sodium hydroxide, then evaporated on a hot plate and dried. Then dissolved in acetone	GC-FID	None	Sheep and cattle rumen fluid 6 compounds
Williams, et al. [11]	–	Centrifuged and extracted by methylene chloride	GC-FID	None	Goat rumen liquor, plasma, and tissue of ruminants 2 compounds
Calabro, et al. [2]	Rumen cannula.	Centrifuged and diluted with oxalic acid	GC-FID	None	Buffalo and sheep rumen fluid 4 compounds

526

527

528 Table 2. Summary of compounds identified in rumen gas.

529

No	Retention time	Compound	CAS	MW	Odor threshold ^h (ppm)	Odor character
1	* 1.20	H ₂ S	7783-06-4	34.08	0.01778	Sewer
2	1.41	cis-1,2-Dimethyl cyclopropane	930-18-7	70.14	n/a	Sweet
3	1.53	2-methyl-1-butene	563-46-2	70.14	n/a	
4	* 1.68	Ethanethiol	75-08-1	62.13	0.001072	Foul, fecal
5	* 1.70	Dimethyl sulfide ^a	75-18-3	62.13	0.002239	Onion, garlic
6	* 1.80	1-Propanethiol	107-03-9	76.16	0.001259	
7	*1.93	2-Propanone ^a	67-64-1	58.08	14.45	
8	2.15	3-Hexyne	928-49-4	82.15	n/a	
9	*2.71	2-Butanone	78-93-3	72.11	7.762	
10	3.50	2-Nitro pyridine	15009-91-3	124.1	n/a	
11	3.96	2,4-Hexadienal	142-83-6	96.13	0.0005495	
12	* 4.11	2-Pentanone	107-87-9	86.14	1.549	Ketone
13	* 4.36	Octane	111-65-9	114.2	5.754	
14	* 4.76	4-Octene	7642-15-1	112.2	n/a	
15	* 4.56	3-Octene	14919-01-8	112.2	n/a	
16	* 4.48	2-Octene	111-67-1	112.2	0.07586	
17	* 5.66	Methyl benzene ^b	108-88-3	92.14	1.549	Ketone
18	* 5.88	Dimethyl disulfide ^b	624-92-0	94.2	0.0123	Sulfury
19	*6.41	2-Pentanol	6032-29-7	88.15	n/a	
20	* 6.50	3-methyl thiophene	616-44-4	98.17	n/a	Sulfury, skunky
21	* 7.03	Nonane	111-84-2	128.3	1.259	
22	8.08	2,6-dimethyl-1,7-Octadiene	6874-35-7	138.1	n/a	
23	8.21	3-Nonyne	20184-89-8	124.2	n/a	
24	*8.25	Alpha-pinene	80-56-8	136.2	0.6918	Ketone
25	8.63	3,7-Dimethyl-octa-1,6-diene	-	138.1	n/a	Moldy
26	*8.88	Camphene	79-92-5	136.2	n/a	
27	9.03	2,6-Dimethyl-2-octene	4057-42-5	140.3	n/a	Sweet
28	9.71	Sabinene	3387-41-5	136.2	n/a	
29	10.68	3-Ethyl-2,5-dimethyl-1,3-Hexadiene	62338-07-2	138.1	n/a	
30	10.91	1-Methyl- 4-[1-methylethyl] cyclohexene	1195-31-9	138.3	n/a	
31	*11.33	Limonene	138-86-3	136.2	0.4365	

32	11.88	1-Methyl-4-[1-methylethyl] benzene	99-87-6	134.2	n/a	
33	12.01	[2Z]-8-Methyl-2,7-nonadien-4-one	89780-46-1	152.1	n/a	
34	* 12.56	Dimethyl trisulfide	3658-80-8	126	0.00166	Onion, garlic
35	* 13.03	Acetic acid ^{a,b,c,d,e,g}	64-19-7	60.05	0.1445	Acidic
36	13.68	2-Butyl naphthalene	1134-62-9	184.3	n/a	
37	* 14.48	2-Ethyl-1-hexanol	104-76-7	130.2	0.2455	
38	* 14.65	Propanoic acid ^{a,b,c,d,e,g}	79-09-4	74.08	0.03548	Burnt, burnt food
39	* 15.18	Dimethyl propanedioic acid	595-46-0	132.1	n/a	Burnt
40	* 16.28	Butanoic acid ^{a,b,c,d,e,g}	107-92-6	88.11	0.00389	Burnt, body odor
41	* 17.00	3-Methyl butanoic acid ^{b,c,d,e,g}	503-74-2	102.1	0.002455	Burnt, body odor
42	* 18.18	Pentanoic acid ^{a,b,c,d,e}	109-52-4	102.1	0.03715	Burnt, body odor
43	* 19.98	Hexanoic acid ^{a,b,c,d}	142-62-1	116.2	0.01259	Fatty acid
44	* 20.95	Dimethyl sulfone	67-71-0	94.1	n/a	Burnt
45	* 22.51	Phenol	108-95-2	94.11	0.1096	Medicinal, phenolic
46	* 23.63	4-Methyl phenol	106-44-5	108.1	0.1096	Barnyard, urious
47	* 25.01	4-Ethyl phenol	620-17-7	122.2	0.001862	Barnyard, phenolic
48	* 26.28	3-Propyl phenol	621-27-2	136.2	n/a	Phenolic
49	* 28.65	Indole ^f	120-72-9	117.2	0.000032	Barnyard
50	* 29.26	Skatole ^f	83-34-1	131.2	0.000562	Naphthalenic

530

531 ^aDewhurst, et al. [15]; ^bSpinhirne, et al. [16]; ^cSchneider, et al. [17]; ^dTeunissen, et
532 al. [9]; ^eFaichney, et al. [10]; ^fWilliams, et al. [11]; ^gCalabro, et al. [2].

533 ^hDevos, et al. [33].

534 *Confirmed with authentic standards.

535 n/a=not available