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Fermentation of Cottonseed and Other Feedstuffs in Cattle Rumen Fluid

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Bovine rumen fluid was fermented anaerobically over 48 h with cottonseed, corn, alfalfa, or a mixture of these substrates in anaerobic mineral buffer. Samples taken at different incubation times were derivatized with *n*-butanol and subjected to gas chromatography and mass spectroscopy. No unusual fermentation end-products from the cottonseed substrate were detected. Cottonseed supported rumen fermentation at levels comparable to those of the other substrates. Major components were usually found in the decreasing order of acetate, propionate, butyrate, and valerate, although acetate and propionate concentrations decreased late in the alfalfa and mixed-feed fermentations, eventually allowing butyrate concentrations to exceed those of propionate. As expected, lactate was produced in high concentrations when corn was fermented. The minor components 2-methylpropionate, 2- and 3-methylbutyrate, phenylacetate, phenylpropionate, and caproate also accumulated, with their relative concentrations varying with the substrate. Succinate was produced in substantial amounts only when corn and alfalfa were fermented; it did not accumulate when cottonseed was the substrate. Samples containing cottonseed were derivatized and subjected to reversed-phase high-performance liquid chromatography, revealing that gossypol concentrations did not change during fermentation.

KEYWORDS: Alfalfa; branched-chain fatty acids; composition; corn; cottonseed; gossypol; rumen; volatile fatty acids

INTRODUCTION

The use of permanent rumen fistulae has prompted research focused on understanding the complexity of the rumen ecosystem. This has led to studies of both pure cultures of rumen microbes as well as of the rumen fermentation in toto. Individual species of ruminal bacteria have been cultured to understand their nutritional requirements, metabolic pathways, degradation and biosynthetic patterns, and end-products, as these are not necessarily revealed in whole rumen cultures. Much has been gained from pure culture studies (*1*−*3*).

However, the rumen is such a complex system that population shifts and realistic intermediate and end-product concentrations can sometimes only be determined with fermentations of whole rumen contents. Studies on such material have been conducted to understand degradation patterns as well as microbial responses to various additives such as volatile fatty acids, long-chain fatty acids, or phenolic acids (*4*, *5*). Many other in vitro rumen fermentation projects have covered cellulose digestion, carbon dioxide and methane production, pH regulation, buffering capacity, and fatty acid production (*6*−*11*).

Rumen organisms act in a symbiotic fashion to produce chemical compounds that supply the ruminant with energy. For instance, branched-chain fatty acids produced by amino acid- or peptide-degrading bacteria are supplied to cellulolytic bacteria as amino acid precursors (*1*, *3*, *12*, *13*). These cellulolytic bacteria in turn break down fiber taken in by the animal for energy, forming volatile fatty acids such as acetate, propionate, and butyrate. The ruminant absorbs most of the volatile fatty acids, those with odd-numbered chains primarily for gluconeogenesis and those with even-numbered chains mainly for ketone body formation (*14*). Methanogens use branched-chain fatty acids, as well as amino acids, as nutrients and cofactors for growth, but methanogenesis competes with the more useful pathway leading to propionate synthesis (*11*). Lactate-producing bacteria are particularly stimulated by high-starch feeds. Although some starch promotes efficiency of the rumen fermentation, large amounts of starch are detrimental to an unadapted animal, because the poorly controlled starch fermentation causes a decrease in rumen pH, which can lead to acidosis and even death of the animal (*15*, *16*).

Whole cottonseed is commonly used as a ruminant feedstuff (*17*). It has particular value because of its high fat and protein content. It is also a desirable feedstuff for dairy cattle because of its highly digestible fiber content (*18*). Its use in livestock feeds must be limited, however, to avoid overconsumption of

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gossypol, a toxic polyphenolic plant pigment (*19*). Ruminants can tolerate greater levels of gossypol than monogastric animals because the rumen fermentation appears to inactivate and detoxify substantial amounts of this plant pigment (*20*). Gossypol also has antimicrobial effects toward some gram-positive bacteria like *Lactobacillus* (*21*). This antimicrobial activity may in part be the result of its ability to inhibit lactate dehydrogenase (*22, 23*).

These observations led us to consider whether whole cottonseed and its constituent gossypol had any inhibitory effects upon rumen fermentation when compared to other common feedstuffs such as corn and alfalfa. In particular, we wished to determine if cottonseed would alter rumen fermentation and generate unusual end products not typical of rumen fermentation. To achieve this, concentrations of different acids at different incubation times were measured by gas chromatography (GC) to understand the relationship between feed compositions and resulting organic acid concentrations. Gossypol was measured by reversed-phase high-performance liquid chromatography (HPLC) after being derivatized. Although flow of material out of the rumen and diffusion through the rumen wall are not replicated by this experimental system, the time-varying acid composition data gathered here can lead to powerful inferences about the dynamics of the microbial population and the end products it produces in the rumen.

MATERIALS AND METHODS

Rumen Fluid Sampling. Strained rumen fluid samples were taken from a ten-year-old nonlactating fistulated Jersey cow at the National Animal Disease Center. One set (Set 1) was withdrawn before the cow was fed, during a period when her daily ration was 700 g of cracked corn, 700 g of cottonseed, and alfalfa hay ad libitum. The proximate analysis of the rumen fluid, from Woodson-Tenent Laboratories (Des Moines, IA), gave 98.08% water by forced-draft oven evaporation, 0.36% protein, <0.2% crude fiber, 0.85% ash, 0.12% crude fat by acid hydrolysis, and 0.59% carbohydrate by difference. A second set (Set 2) was also collected in the morning, 1.75 h after the cow was fed approximately 2.5 kg alfalfa, when her daily ration was 700 g/day cracked corn, no cottonseed, and alfalfa hay ad libitum.

Fermentation Conditions. Each rumen fluid sample was divided into 10 25-mL portions and added to flasks already containing 25 mL of anaerobic dilution buffer, pH 6.8 (*24*). A stock solution of the latter consisted of 75 mL of 0.6% K_2HPO_4, 75 mL of salt solution, 1 mL of 0.1% resazurin, and 800 mL of water, prepared under a CO_2 atmosphere with addition of 50 mL of 8% Na_2CO_3. The salt solution was composed of 0.6% KH_2PO_4, 1.2% $(NH_4)_2SO_4$, 1.2% NaCl, 0.12% $MgSO_4 \cdot 7H_2O$, and 0.12% $CaCl_2 \cdot 6H_2O$. After autoclaving, 20 mL of 2.5% cysteine sulfide was added as a reducing agent. Each pair of flasks then received 2.5 g each of either alfalfa, corn, cottonseed, or a mixture of 80% alfalfa, 15% corn, and 5% cottonseed, all ground in a Waring blender, with the fifth pair having only buffer. The flasks were flushed with CO_2 before adding rumen fluid and, after mixing, 6 mL was withdrawn from each flask and frozen for analysis of initial composition. Each flask was closed with a butyl rubber stopper vented with a 20-gauge needle, and incubated with periodic mixing at 37 °C. Samples were taken at 4, 8, 12, 24, 32, or 36 h, and 48 h, and frozen until analysis.

Sample Centrifugation and Filtration. Incubation samples were rapidly thawed and thoroughly mixed. Portions of 1 g were withdrawn using a pipet with a disposable tip which had been widened with a razor for better access to a representative volume. The samples were centrifuged in 1.5-mL vials at 10,000 rpm for 10 min. Each sample was filtered with a disposable 0.22-μm cellulose acetate syringe-tip filter, and a 500-μL portion from each was added to a 4-mL glass screw-top vial with 100 μL of 8% (w/v) NaOH and sealed with an open-top septum-covered cap. These samples were then frozen until derivatization.

Sample Derivatization for GC. *n*-Butyl esters of fatty acids in the fermented and centrifuged samples were formed by the method of Lambert and Moss (*25*). Vials containing the samples were thawed, and 300 μL of butylation fluid, consisting of 80% (v/v) *n*-butanol and 20% (v/v) H_2SO_4, was added. This was followed by addition of 750 μL of chloroform and 50 μL of a 7.78 g/L heptanoic acid in chloroform solution, the latter serving as an internal standard. Solutions in capped vials were heated for 2 h at 80 °C and allowed to cool to room temperature before 300 μL of trifluoroacetic acid was added to convert the remaining *n*-butanol to *n*-butyl trifluoroacetate. After 1 h, the mixtures were extracted with three 1-mL portions of deionized water, the aqueous layers being removed with a 1-mL disposable syringe after each extraction. After the third extraction, the organic extracts were transferred to clean vials.

GC Sample Replication. All samples were processed in a standard fashion. Starting with fermentations with identical feeds, two of the four groups of samples, one each from Sets 1 and 2 and each containing samples from all seven incubation times, were prepared for derivatization at one time, with three or four of the samples derivatized together. After derivatization, each sample was divided into two replicates to be chromatographed nonconsecutively. Thus, each concentration for a given incubation time in either Set 1 or Set 2 was an average of four points, derived from two analyses each of two samples of the same feed incubated separately. Calibration plots of all acids were essentially linear. Peak areas were divided by the heptanoic acid peak area in each chromatogram and multiplied by the mean heptanoic acid peak area of all chromatograms.

GC. Derivatized organic samples were analyzed using a J&W (Folsom, CA) 30-m × 0.25-mm i.d. DB-5 silica capillary column with a 1-m × 0.25-mm i.d. fused silica guard column in a Hewlett-Packard (Palo Alto, CA) 5890A gas chromatograph linked to a Hewlett-Packard 3396 Series III integrator. The column temperature remained at 50 °C for 10 min, followed by a 2.5 °C/min increase to 150 °C, and remaining at that temperature for 10 min. After each run the column was rapidly taken to 300 °C to expel any remaining high-boiling material. The flow rate of the helium carrier gas and split ratio were 1.2 mL/min and 74.3, respectively, and the injector and flame ionization detector temperatures were 265 °C. Derivatized samples of 3 μL were injected with a 10-μL glass syringe, flushed with acetone, and completely washed with the new analyte before each injection.

High-Temperature GC. Samples containing long-chain fatty acids were analyzed with a J&W 15-m × 0.25-mm i.d. DB-5ht silica capillary column installed in a Hewlett-Packard 6890 gas chromatograph linked to a Gateway (North Sioux City, SD) 2000 486/33C computer running Hewlett-Packard 3365 Series II ChemStation data analysis software. After 10 min at 150 °C, the temperature increased to 175 °C at 2.5 °C/min, and then increased to 300 °C at 5 °C/min, remaining there for 10 min. The helium carrier gas flow rate and split ratio were 1.9 mL/min and 100, respectively, and the injector and flame ionization detector temperatures were 330 °C and 350 °C.

GC Peak Identification. Peaks were identified by GC-electron ionization mass spectroscopy (EIMS) and GC-chemical ionization mass spectroscopy (CIMS), the latter with ammonia, using the same types of GC columns and temperature programs as before and a Finnigan (San Jose, CA) TSQ 700 mass spectrometer. Identifications were confirmed by comparison of GC retention times of tentatively identified peaks with GC retention times of authentic acids derivatized and analyzed in the same manner as the rumen samples. Small peaks sometimes identified as ethyl esters of fatty acids by EIMS were further identified by matching their GC retention times with those of a mixture of fatty acid standards subjected to esterification as before, but with an ethanol−H_2SO_4 solution.

HPLC of Standard Samples. Samples of the cottonseed that was used for feed and added to the rumen fluid, as well as rumen fluid from Set 2 containing cottonseed that had been fermented for 12 and 32 h, were sent to Dr. Millard Calhoun, Texas Agricultural Experiment Station of the Texas A&M University System, San Angelo, TX. The cottonseed was decorticated before analysis. Samples were then weighed, freeze-dried for 24 h at −70 °C, and then transferred to screw-top borosilicate glass tubes. Total and (+)- and (−)-gossypol concentrations were determined essentially as described by Hron et al. (*26*).

Sample Derivatization for HPLC. Samples containing either cottonseed or a mixture of alfalfa, corn, and cottonseed were thawed and shaken, and 0.4-mL portions were withdrawn under a nitrogen

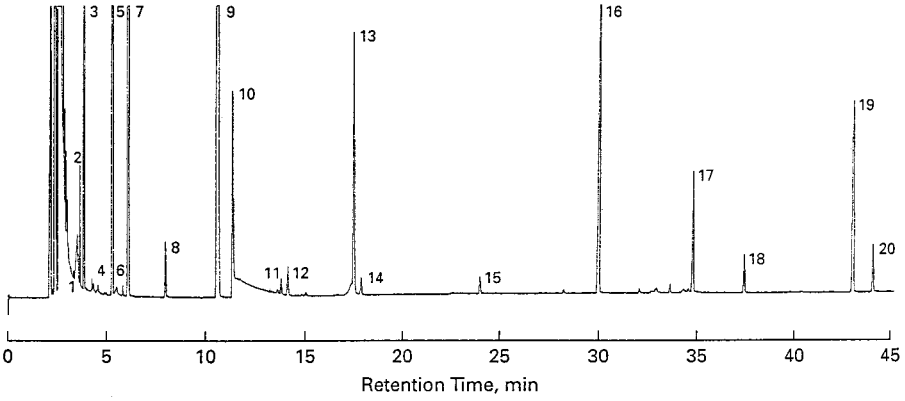

Figure 1. Gas chromatogram from 50 to 150 °C of an *n*-butylated sample of rumen fluid after 24 h of fermentation with alfalfa, corn, cottonseed, and buffer added. Peak identities (acids are *n*-butyl esters unless otherwise noted): (1) ethyl 2-methylpropionate, (2) trifluoroacetic acid, (3) acetate, (4) ethyl 3-methylbutyrate, (5) di-*n*-butyl ether, (6) ethyl valerate, (7) propionate, (8) 2-methylpropionate, (9) butyrate, (10) lactate, (11) 2-methylbutyrate, (12) 3-methylbutyrate, (13) valerate, (14) ethyl heptanoate, (15) caproate, (16) heptanoate standard, (17) unidentified derivatization product, (18) phenylacetate, (19) phenylpropionate, and (20) succinate.

stream. To each was added 4 mL of a solution of 2% (v/v) (*R*)-(−)-2-amino-1-propanol (D-alaninol), 10% glacial acetic acid, and 88% dimethylformamide (*27*, *28*). Each sample was held at 75−90 °C for 90 min and then cooled to room temperature in 30 min. To each was added 16 mL of 78% (v/v) acetonitrile−22% 10 mM aqueous KH_2PO_4 adjusted to pH 3 with H_3PO_4, the same solution used as the eluant.

HPLC. Filtered samples were chromatographed less than 1.5 h after derivatization using an apparatus consisting of an ISCO 2350 pump, an ISCO 2360 gradient programmer, a 10-mm × 4.6-mm i.d. precolumn and a 250-mm × 4.6-mm i.d. column packed with 5-μm diameter Nucleosil C18 beads, both from Sigma-Aldrich (St. Louis, MO), and a Beckman (Berkeley, CA) 165 variable wavelength detector set at 254 nm. Peaks were processed with a Gateway E-4200 computer using a Dionex (Sunnyvale, CA) PeakNet computer interface. Fused peaks were separated by vertical boundaries upon integration. Eluant was fed at room temperature and 1.2 mL/min. Gossypol [2,2′-bis(8-formyl-1,6,7-trihydroxy-5-isopropyl-3-methylnaphthalene)] standards of 25 μL were chromatographed three times each at different concentrations, giving equal areas of (+)- and (−)-gossypol. Regular samples of 250 μL were chromatographed once each, giving a large compound peak containing at least five unidentified components at 2.93 min, a (+)-gossypol peak at 3.90 min, a small unidentified peak at 4.55 min, a (−)-gossypol peak at 4.95 min, and two small unidentified peaks at 7.03 and 8.50 min, the second larger than the first.

RESULTS

Qualitative Composition of Rumen Fluid. A typical 50−150 °C chromatogram of an *n*-butylated sample, this one containing a mixture of cottonseed, corn, and alfalfa added to rumen fluid derived from Set 2 and fermented for 24 h, is shown as **Figure 1**. Peaks corresponding to the *n*-butyl esters of the five C_2−C_7 straight-chain fatty acids, the last one being the internal standard, and of 2-methylpropionate (isobutyrate), 2-methylbutyrate, 3-methylbutyrate (isovalerate), phenylacetate, phenylpropionate (hydrocinnamate), lactate, and succinate were identified, as were di-*n*-butyl ether and trifluoroacetic acid. Also identified were ethyl 2-methylpropionate, ethyl 2-methylbutyrate, ethyl valerate, and ethyl heptanoate. Ethyl propionate and ethyl butyrate sometimes separated themselves from the initial peak. None of the ethyl esters were of sufficient concentration to contribute significantly to the final concentrations of the acids calculated from the *n*-butyl ester peaks. The remaining peaks were not identified, although it appears that the 13.6-min peak is most likely associated with lactate, because it was found only when lactate was present. We do not think that this is the trifluoroacetic ester of *n*-butyl lactate because Salanitro and Muirhead (*29*) found that the retention times and response factors of this derivative and those of *n*-butyl lactate were identical. The 34.9-min peak appears to be a byproduct of *n*-butyl derivatization, and was tentatively identified as a hydrocarbon by EIMS.

High-temperature GC led to the identification by EIMS and CIMS of small peaks of *n*-butyl myristate, palmitate, linoleate, oleate, and stearate, the third averaging 65% (w/w) of the total long-chain fatty acid concentration, slightly higher than expected (*30*), and being 3.7 mM (Set 1) and 0.65 mM (Set 2) in the initial samples of the cottonseed fermentations. They were found in no other fermentation and they rapidly disappeared, being completely absent after 4 h.

Change of Rumen Fluid Components with Time. Concentrations of acids calculated from their *n*-butyl ester peaks are found in **Figures 2** and **3**. Except as noted later, there were no significant differences between Sets 1 and 2; therefore the two figures are from the latter set only.

Figure 2 shows changes of concentration with incubation time of the five acids (acetate, propionate, butyrate, valerate, and lactate) that were found in high amounts. In general, concentrations of the first four are in inverse order of their molecular weights at all incubation times. In the corn and cottonseed fermentations, all four monotonically reached steady-state concentrations by the end of the incubations. In the alfalfa and mixed-feed fermentations, on the other hand, acetate and propionate concentrations reached maxima near 12 h and then decreased, while concentrations of butyrate and valerate increased throughout, causing butyrate concentrations in some cases to exceed those of propionate by 36 h. Lactate appeared only in the corn and mixed-feed fermentations. In the former it was absent until after 12 h, after which it increased steadily. In the latter it appeared only after 12 h, decreasing to zero by 36 h. In the control fermentation, where no substrate except buffer was added to the rumen fluid, there were no significant changes in concentrations of the five acids with time, and lactate never appeared.

The differences between Sets 1 and 2 were fairly minor: (1) initial acetate, propionate, butyrate, and valerate concentrations in Set 1 were 30, 5, 5, and <1 mM, respectively, whereas in Set 2 they were 40, 12, 5, and 1 mM; (2) in Set 1 but not in Set 2, lactate was initially present in the corn, alfalfa, and mixed-feed fermentations, disappearing by 8 h but then reappearing by 12 h in the corn fermentation but not again in the other two fermentations; (3) there were no noticeable maxima in acetate

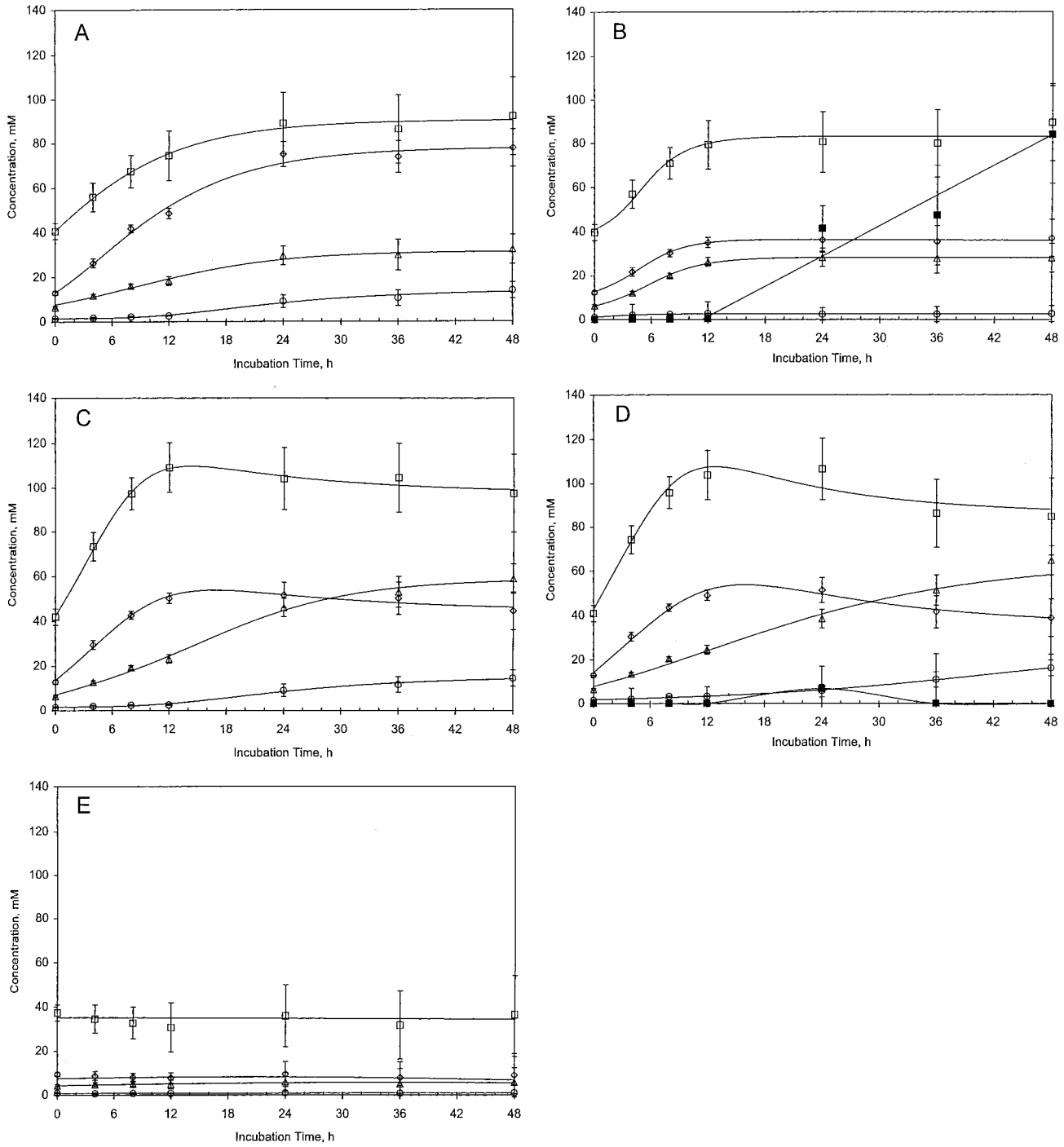

Figure 2. Change of concentration with incubation time of acetate (□), propionate (◇), butyrate (△), valerate (○), and lactate (■). Added ingredients in addition to buffer: (A) cottonseed; (B) corn; (C) alfalfa; (D) alfalfa, corn, and cottonseed; (E) none. Ranges are the standard deviations over four analyses.

and propionate concentrations in the alfalfa fermentation in Set 1; their maxima in the mixed-feed fermentation of Set 1 occurred at the same time as that in Set 2 but at slightly lower concentrations; (4) the final valerate concentration in the alfalfa fermentation of Set 2 (20 mM) is much higher than that of Set 1 (5 mM).

Concentrations varying with incubation times of the minor components 2-methylpropionate, 2- and 3-methylbutyrate, caproate, phenylacetate, phenylpropionate, and succinate are shown in **Figure 3**. The last was quite variable with time and with added feed, never appearing in the cottonseed or control fermentations, but appearing after 12 h in the other three and continuing to increase through 48 h with the alfalfa and corn fermentations, becoming the highest of the minor components. In general, however, except for the mixed-feed fermentation the concentrations of most minor components did not increase as fast at 48 h as they did earlier.

Phenylpropionate was always found in higher concentrations than phenylacetate, except in the cottonseed fermentation, when the latter became higher after 16 h. Of the three branched-chain fatty acids, 2-methylpropionate was always highest, followed by 2- and 3-methylbutyrate in that order. Highest concentrations of the minor components were found in the cottonseed fermentation, followed in order by the alfalfa, mixed-feed, corn, and control fermentations. Caproate concentrations were usually fairly low.

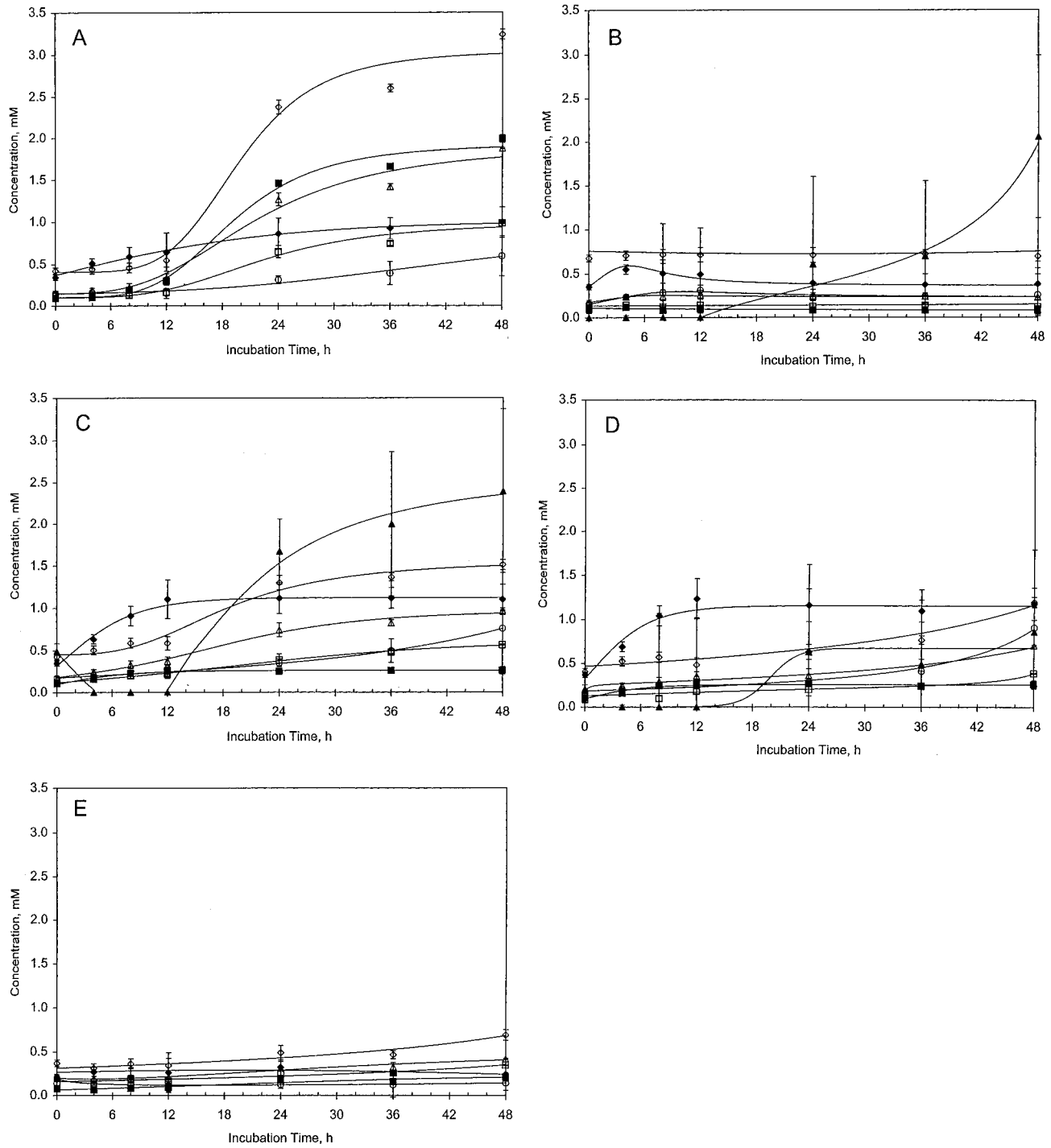

Figure 3. Change of concentration with incubation time of 2-methylpropionate (◇), 2-methylbutyrate (△), 3-methylbutyrate (□), caproate (○), phenylacetate (■), phenylpropionate (◆), and succinate (▲). Panels and ranges are as defined in **Figure 2**.

Set 1 differs from Set 2 in the following ways: (1) succinate is found initially in all but the control fermentation of Set 1, but only in the alfalfa fermentation of Set 2; (2) succinate is produced much earlier in the corn fermentation but much later in the alfalfa fermentation in Set 1 than in Set 2; (3) phenylacetate concentration exceeds that of phenylpropionate not only in the cottonseed fermentation of Set 1 but in the control fermentation also; (4) minor component concentrations are higher thoughout the control fermentation in Set 1 than in Set 2, even though the cow had not been fed before the Set 1 sample was withdrawn.

The cottonseed used in feed and in fermentations had 0.73% total gossypol, consisting of 0.41% (+)-gossypol and 0.32% (−)-gossypol, using a factor of 0.55 to multiply concentrations in decorticated cottonseed. The absence of either methylgossypol isomer and the excess of (+)- over (−)-gossypol indicates that the sample came from Upland cotton (*Gossypium hirsutum*).

Both (+)- and (−)-gossypol were detected in all samples from the cottonseed fermentations but not from any of the mixed fermentation samples, in which cottonseed is only 5% of the total feed. Although there is some variability in their concentrations from samples taken at 4, 8, and 12 h, there is clearly no change in any concentration with time. In addition, total concentrations from Sets 1 and 2 are the same, being about 0.35 g/L, of which 53% is (+)-gossypol and 47% is (−)-gossypol.

DISCUSSION

Although the general organic composition of the rumen has been known for years, this project differs in having followed the changes of acids during rumen fluid fermentations over time, with common feeds such as corn and alfalfa and an unusual substrate like cottonseed. As such, this information can extend our knowledge of how the rumen microbiota forms its most important products. It also presents us with more information about the fermentation of cottonseed. No unusual or unanticipated end-products were detected with the fermentation of these substrates but a number of interesting relationships were observed.

All of the identified compounds had previously been found in rumen fluids (*14*, *31*). In addition to those expected from microbial processes occurring in the rumen, di-*n*-butyl ether was generated after protonation of the *n*-butanol oxygen atom during derivatization under acidic conditions.

Certain rumen bacteria such as *Butyrivibrio fibrisolvens* and *Ruminococcus albus* can produce ethanol, especially under perturbed fermentation conditions such as rumen acidosis (*12*, *32*, *33*), but under normal conditions this compound is quickly absorbed and metabolized by the host animal's tissue (*34*). In the *in vitro* incubations with corn, however, ethanol may have accumulated, and it likely esterified a small portion of the fatty acids during *n*-butyl derivatization, accounting for the identification of ethyl derivatives in some samples. Ethanol itself was not found by GC; either it eluted in the initial peak or it was not extracted by chloroform during derivatization.

Figures 2 and **3** indicate that the rate of acid production followed typical saturation kinetics, with decreasing rates of product formation, especially after 12−18 h of fermentation. This is a reflection of microbial activity and its diminished metabolism after substrates and nutrients are depleted and inhibitory end-products accumulate. The appearance of the major acids reflects their formation from the microbial metabolism of carbohydrates. Acetate, propionate, and butyrate can arise from amino acid deamination (*3*), but the most prevalent source for these acids in the rumen is carbohydrate degradation (*12*). Research has also indicated that propionate formation and methanogenesis are competing metabolic pathways (*11*). It is interesting to note the large increase in lactic acid production when corn was the primary substrate, which reflects the inability of the rumen fermentation to direct starch metabolism exclusively toward propionate rather than lactate. This is a consequence of microbial population shifts and a declining pH that alters starch metabolism (*11*). Because corn is high in starch, the large amount of lactate produced in the corn fermentations is expected. Normally lactate is only a transient intermediate of rumen fermentation, as indicated by the fact that lactate did not appear in significant quantities in the cottonseed and alfalfa fermentations.

Butyrate concentrations were higher than those of propionate by the end of both alfalfa and mixed-feed fermentations. High butyrate production can be attributed to several factors including a possible increase in protozoal activity (*35*). It was accompanied by a decrease in acetate and propionate concentrations.

The minor acids were elevated in fermentations containing cottonseed and alfalfa. These included valeric and caproic acids, which stimulate cellulolytic bacteria (*12*). This elevated level of production can be attributed to metabolism of the amino acids and lipids contained in these high-protein substrates. Valeric acid can be formed from amino acid deamination via Strickland reactions or from a proline ring-opening reaction coupled with deamination (*3*, *36*), as well as from carbohydrate degradation, as is the case with caproic acid. These acids can subsequently be used by many microbial species to synthesize higher-chain acids, alcohols, and aldehydes (*12*).

Succinate was found only in fermentations where corn and/or alfalfa were present, which may reflect fermentation conditions that inhibited its subsequent metabolism. Succinate is generally a precursor for propionate production and is converted by bacteria such as *Selenomonas ruminantium*. As a result, it is usually found only as a transient intermediate (*33*), and this role may help to explain the large variability in its concentrations over different replicates. Succinate did not accumulate in cottonseed fermentations, which may reflect, in part, the diversion of reducing equivalents to the unsaturated fatty acids found in whole cottonseed.

The three branched-chain fatty acids, 2-methylpropionate and 2- and 3-methylbutyrate, are produced by deamination of isoleucine, leucine, and valine, respectively, by amino acid-degrading bacteria like *Peptostreptococcus* (*3*, *13*). They are essential for amino acid synthesis in many species of bacteria.

The aromatic fatty acids phenylacetate and phenylpropionate can be formed from the deamination of phenylalanine (*37*) or tyrosine (*2*) by amino acid-degrading bacteria; however, most phenylpropionate comes from plant-derived lignin precursors and other phenolic compounds (*38*). Phenylpropionate stimulates cellulose digestion (*2*, *39*). The corn and control fermentations have lower phenylpropionic acid concentrations than do fermentations of other feeds.

Gossypol concentrations, regardless of form, did not change during fermentation of cottonseed. This suggests that these forms must be complexed by rumen components during fermentation, reducing their ability to reach the bloodstream of ruminants, and that the complexes must be broken during their derivatization with D-alaninol. This observation extends that of Reiser and Fu (*20*).

CONCLUSIONS

The rumen hosts one of the most complex microbial systems in existence. This project has followed the fermentation of cottonseed, corn, alfalfa, and a mixture of the three, in rumen fluid and buffer over time, extending our knowledge of the dynamics of the rumen. The fermentation of cottonseed yielded no unusual fermentation end-products. Identified products are typical of a high-protein oilseed crop and are related to this feedstuff's nutrient composition. Gossypol is not consumed during rumen fermentation, presumably being complexed.

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