CONCLUSION

The results of this study revealed that the solubility of benomyl is low in the pH range of 3–10, being less than 4 ppm. At pH 1, its solubility is substantially higher, and at pH 13 no benomyl can be found because it completely converts to STB.

MBC was present in all the samples tested and its quantity increased as acidity and alkalinity increased. Accordingly, the percentage of benomyl in the solution, as part of other solutes, MBC, STB, and BBU, is very low. The highest value obtained under the experimental conditions tested was 70.7% at pH 7, but this value declines as benomyl converts to MBC or STB.

In practice, benomyl is being used widely for different purposes. In the citrus industry in Florida, formulated 50% WP is suspended at the pH range of 7.6–10.5 for variable periods up to 6 weeks or longer (Hall, 1980). Researchers prepare their benomyl test solutions in water with the aid of organic solvents (Koller et al. 1982; Peterson and Edgington, 1969). Under these conditions actual solute compositions will be widely different. Results obtained in this study can be utilized to help understand probable compositions of solutes when benomyl is prepared as a solution or suspension in water.

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Registry No. STB, 41136-38-3; MBC, 10605-21-7; BBU, 24374-77-4; benomyl, 17804-35-2.

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Effects of Dietary Monensin on Bovine Fatty Acid Profiles

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Variations in fatty acid profiles of bovine tissue were evaluated as a function of the incorporation of the antibiotic monensin into a forage regimen. Fatty acid profiles were obtained for the separate neutral lipid and polar lipid fractions from longissimus muscle and for the total lipid content of subcutaneous adipose tissue. Statistical analysis showed no significant differences in lipid content and only few such differences in the content of most individual fatty acids when comparing control tissue to tissue from animals fed with monensin. Decreased amounts of total saturated fatty acid observed in tissue from monensin-fed animals suggested reduced biohydrogenation in the rumen, consistent with the inhibition of rumen bacterial growth. Increased amounts of odd chain length and branched fatty acids in the same tissue were consistent with increased rumen production of propionate over acetate, with subsequent de novo synthesis of the fatty acids from propionate.

The ionophore antibiotic monensin often is added to cattle feed to increase efficiency of feed utilization. Monensin alters the growth and metabolic activity of Gram-positive rumen bacteria (results of studies of protozoal activity are equivocal), causing a shift in fermentation products toward increased production of propionate and reduced production of acetate and methane (Richardson et al., 1976; Bergen and Bates, 1984). Although many nonlipid metabolic changes associated with the presence of monensin in the bovine diet have been elucidated (Richardson et al., 1976; Potter et al., 1976; Raun et al., 1976; Schelling, 1984), there have been no reports on the effects of monensin on bovine lipid composition, despite the role played by rumen microflora (Christie, 1978) on bovine lipid patterns. The present work was done to detect such effects on bovine lipid patterns, as measured by alterations in tissue content and in fatty acid composition. The experimental protocol was similar to that of a prior study, which uncovered numerous instances of significant differences in specific fatty acid content as a function of bovine dietary regimen (forage vs. grain; Marmer et al., 1984). In both studies, the examination of the fatty acids of separate neutral and polar lipid fractions

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Table I. Lipid Content[a]

	feeding regimen	
	control	monensin fed
	LD Muscle	
%[b] neutral lipid	1.47 ± 0.62	1.32 ± 0.42
% polar lipid	0.87 ± 0.12	0.80 ± 0.06
% phospholipid[c]	0.59 ± 0.04	0.60 ± 0.06
	SQ Adipose	
% total lipid	88.44 ± 3.15	88.31 ± 2.60

[a] For each comparison, mean values (10 animals per feeding regimen) are statistically identical ($p < 0.05$). [b] Weight percent of wet tissue. [c] 25.0 × percent phosphorus.

enabled the detection of differences in those minor fatty acids associated with the cellular and subcellular membranes.

EXPERIMENTAL SECTION

Animals. Twenty Brangus × Angus × Hereford steers were randomly allotted to two treatments: (1) control—sorghum–sudan forage pasture supplemented with concentrate fed at 1% of body weight—or (2) monensin—the same treatment as above, plus monensin (Eli Lilly and Co., Indianapolis) fed at 150 mg day^{-1}. All animals were slaughtered at the same time. Steers fed monensin showed heavier carcass weight ($p < 0.05$) than those not fed monensin, as well as increased ($p < 0.05$) daily gain (+104 g day^{-1}), rib eye area, fat thickness, and quality grade but slightly lower yield grade. Full details on feed composition, performance data, and carcass characteristics have been reported elsewhere (Ostilie et al., 1981).

Lipid Isolation and Analysis. Approximately 200-g samples of two tissues were separated from each carcass: longissimus muscle (LD) (from between the 12th and 13th ribs) and subcutaneous adipose tissue (SQ). The SQ samples were obtained from the back fat attached to LD samples but included fat from between the 11th and 12th ribs if insufficient sample was attached to the LD tissue sample. Excised samples were sealed into plastic bags and shipped over dry ice from the Oklahoma State University to the Eastern Regional Research Center. Upon receipt, the protocol of Maxwell and Marmer (1983) was followed to systematically comminute the tissues, sample them, and extract them of their lipids. The extraction technique (Marmer and Maxwell, 1981) permitted the isolation of separate neutral and polar lipid fractions from muscle tissue. Percentages (Table I) of neutral and polar lipid and phospholipid of LD and total lipid of SQ are means and standard deviations of values determined for tissue from each of the 10 animals per dietary regimen group. Values for each tissue sample are the averages of duplicate determinations. Percent phospholipid figures are similarly calculated means based on phosphorus analysis of lipid fractions, with a factor of 25.0 (Marmer, 1984) used to convert percent phosphorus to percent phospholipid. Differences between percent phospholipid and percent polar lipid were due to the presence of glycolipid and neutral lipid in the polar lipid fraction.

The isolated lipids were derivatized into fatty acid methyl esters (FAME), and amounts of specific FAME were determined by automated capillary column gas chromatography (GC). GC analysis was performed on a Hewlett-Packard Model 5880A level 4 gas chromatograph (reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned) equipped with a flame-ionization detector and an automatic injector. Separations were accomplished on a Quadrex 100 m × 0.25 mm i.d. SP2340 glass capillary column. [Full details of the GC technique are given by Maxwell and Marmer (1983) along with sample chromatograms.] Then, the normalized data (weight percent of total FAME) from the individual GC runs were consolidated by the protocol of Marmer et al. (1983) and subjected to an analysis of variance and Bonferroni mean separation techniques (Miller, 1981) to discern statistically significant difference ($p < 0.05$ or $p < 0.10$) in FAME profiles effected by addition of monensin to the bovine diet. In addition, by use of an internal standard during GC analysis, data also were tabulated in weight units per tissue portion ("gravimetric", mg of fatty acid/100 g of tissue). The gravimetric profiles, generally favored by nutritionists, give quantities of fatty acids per tissue portion; differences in these profiles may be obscured by differences in the tissue fattiness. Normalized profiles, on the other hand, are not biased by tissue fattiness; the data are related to the concentration of fatty acids in the fat. Complete normalized and gravimetric tabulations are given in the supplementary material (see paragraph at end of paper regarding supplementary material). Summaries of these tabulations are given for muscle in Table II and for adipose in Table III. Because Tables II and III and the supplementary Tables IV and V contain statistical comparisons (control vs. monensin-fed tissue) within six separately studied groups (neutral lipid and polar lipid from muscle, total lipid from adipose, each as normalized and gravimetric reports), independent sets of superscript symbols were used to denote significant differences (capital letters: $p < 0.05$; lower-case letters, $p < 0.10$). These symbols are defined in the footnotes of the tables. Each line in the tables provides comparisons independent of the comparisons of the other lines. Less prevalent fatty acids were not encountered in tissue from each animal of the set in amounts ≥0.10% total fatty acid. For those fatty acid comparisons, statistical analyses were deemed valid if at least 3 degrees of freedom were guaranteed. Therefore, in the supplementary tabulations, tabular entries for certain fatty acids appear without superscripts; these were not analyzed due to insufficient data. Only those comparisons of specific fatty acid content that did show statistically valid differences ($p < 0.10$, $p < 0.05$) are given in Tables II and III. These tables also provide summations of fatty acid content by class (e.g., total saturated fatty acid). The following specific fatty acids, grouped below and in the tables by class, were studied (abbreviations: X:Y = carbon number X:double bond number Y; *i* = iso; ai = anteiso; ω = first double bond position from hydrophobic end; *c* = cis; *t* = trans): normal saturated, 14:0, 15:0, 16:0, 17:0, 18:0, and 20:0; branched saturated, *i*15:0, ai15:0, *i*16:0, ai17:0, and ai19:0; trans monoene, 16:1ω7*t* and 18:1*t* (mixture of positional isomers); cis monoene, 14:1ω5*c*, 16:1ω7*c*, 17:1ω8*c*, 18:1ω9*c*, 18:1ω7*c*, and 20:1ω9*c*; diene, 18:2ω6*c* and 20:2ω6*c*; nondienoic polyene, 18:3ω3*c*, 20:3ω6*c*, 20:4ω6*c*, 20:5ω3*c*, 22:4ω6*c*, 22:5ω3*c*, and 22:6ω3*c*.

RESULTS AND DISCUSSION

Separate profiles of muscle tissue are given for neutral lipid fractions—which approximate the contribution of contiguous adipose tissue—and polar lipid fractions—which emphasize the contribution from the cellular and subcellular membrane lipids.

In the complete tabulations of fatty acid data (Tables IV and V of the supplementary material), separate data are presented for all identified fatty acids, which include over 96% of the total GC peak area. For brevity, data for individual unidentified peaks are not presented, though the sums are given for unidentified saturated fatty acids

Table II. Fatty Acid Composition of Muscle[a]

	%				mg/100 g of tissue			
	neutral lipid		polar lipid		neutral lipid		polar lipid	
fatty acid	control	monensin	control	monensin	control	monensin	control	monensin
saturated								
normal	50.79	50.30	36.90	36.47	578.3	513.7	148.9	139.6
17:0	0.98[B]	1.12[A]						
20:0			0.29[c]	0.22[d]				
branched	1.42	1.43	0.54	0.69	16.0	14.7	2.9	5.1
unidentified	0.98	0.98	0.67	0.51	10.9	10.1	2.3	2.2
total	53.19	52.71	38.11	37.67	605.2	538.5	154.1	146.9
unsaturated								
trans monoene	1.58	1.74	0.56	0.65	17.3	17.9	2.3	2.6
cis monoene	42.33	42.71	31.59	31.57	483.5	435.9	127.4	122.6
17:1ω8*c*	0.55[b]	0.67[a]						
20:1ω9*c*			0.13[c]	0.10[d]				
total monoene	43.91	44.45	32.15	32.22	500.8	453.8	129.7	125.2
diene	1.29	1.26	16.24	16.38	14.2	12.6	69.6	63.1
other polyene	0.56	0.40	10.88	11.31	4.8	4.0	50.7	48.5
total ω6	1.57	1.38	23.06	23.43	16.0	13.7	101.5	93.4
total ω3	0.28	0.28	4.07	4.26	3.1	2.9	18.6	18.2
unidentified	1.05	1.18	2.63	2.43	11.8	12.4	11.9	11.1
total unsaturated	46.81	47.29	61.91	62.34	531.7	482.8	261.7	248.0
total fatty acid	100.00	100.00	100.00	100.00	1136.9	1021.4	415.9	394.9

[a] Data for individual fatty acids are given only for those instances in which statistically valid differences were observed. Detailed fatty acid profiles are given in supplementary material. Bonferroni *t* tests were used to make specific comparisons between the fatty acid contents of monensin-supplemented tissue vs. control tissue. The following comparisons and associated separation symbols are used above: normalized data (%), neutral lipid, a and b ($p < 0.10$) and A and B ($p < 0.05$); polar lipid, c and d ($p < 0.10$). Comparisons for a given row are independent of comparisons for other rows. Fatty acid abbreviations: 17:0, heptadecanoic; 20:0, icosanoic; 17:1ω8*c*, *cis*-9-heptadecenoic; 20:1ω9*c*, *cis*-11-icosenoic.

Table III. Fatty Acid Composition of Adipose Tissue[a]

	%		mg/100 g of tissue	
fatty acid	control	monensin	control	monensin
saturated				
normal	52.54	50.96	41046.9	40699.5
17:0	1.15[b]	1.28[a]	905.5[D]	1019.3[C]
branched	2.02	2.11	1592.6	1689.3
ai17:0	0.83[b]	0.90[a]	655.2[d]	718.8[c]
unidentified	1.17	1.31	919.1	1043.8
total	55.43	54.38	43558.6	43432.6
unsaturated				
trans monoene	2.19	2.52	1724.0	2007.6
18:1*t*	2.19[B]	2.52[A]	1724.0[D]	2007.6[C]
cis monoene	39.52	40.03	31112.8	31864.5
17:1ω8*c*	0.63[B]	0.74[A]	492.4[D]	582.4[C]
18:1ω7*c*			857.3[D]	930.4[C]
total monene	41.71	42.55	32836.8	33872.1
diene	1.20	1.11	941.9	882.2
other polyene	0.23	0.24	177.1	189.5
total ω6	1.20	1.11	941.9	882.2
total ω3	0.23	0.24	177.1	189.5
unidentified	1.43	1.72	1129.5	1366.4
total unsaturated	44.57	45.62	35095.3	36310.2
total fatty acid	100.00	100.00	78643.9	79742.9

[a] Analysis of total lipid extract; data for individual fatty acids are given only for those instances in which statistically valid differences were observed. Detailed fatty acid profiles are given in supplementary material. Bonferroni *t* tests were used to make specific comparisons between the fatty acid contents of monensin-supplemented tissue vs. control tissue. The following comparisons and associated separation symbols are used above: normalized data (%), a and b ($p < 0.10$) and A and B ($p < 0.05$); gravimetric data (mg/100 g of tissue), c and d ($p < 0.10$) and C and D ($p < 0.05$). Comparisons for a given row are independent of comparisons for other rows. Fatty acid abbrevations: 17:0, heptadecanoic; ai17:0, 14-methylhexanoic; 18:1*t*, *trans*-octadecenoic mixture; 17:1ω8*c*, *cis*-9-heptadecenoic; 18:1ω7*c*, *cis*-11-octadecenoic.

(9 peaks) and for unidentified unsaturated fatty acids (23 peaks). All unidentified peaks were extremely small and often not seen in individual runs. No significant differences ($p \leq 0.10$) were seen in any comparisons of these individual components. Tables II (muscle) and III (adipose) are condensed from these detailed reports.

The most notable findings must be the similarity of lipid and fatty acid content between that of the control tissue and monensin-fed tissue. The neutral, polar, and phospholipid content of muscle and the total lipid content of adipose were found to be statistically identical ($p > 0.05$; Table I). These findings parallel those of Potter et al. (1976). We now have observed that monensin exerts little influence over the fatty acid composition of the lipid. This is in sharp contrast to the numerous differences ($p < 0.05$) found in a prior study of bovine fatty acid profiles that compared a forage vs. grain regimen (Marmer et al., 1984). The statistically valid differences ($p < 0.05$ or 0.10; Tables II and III) discussed below were, in fact, the only ones detected from examination of 60 individual fatty acids in six comparisons (cf. supplementary material). For discussion purposes, the specific differences may be considered as arising from alteration in the rumen's capacity to biohydrogente, to enlongate a fatty acid chain, and to synthesize fatty acids de novo.

Biohydrogenation is a bacterial process (rather than a protozoan one; Harfoot, 1978) that occurs in the rumen. Alteration of the rumen's bacterial growth and metabolic activity by monensin may manifest itself in changes specifically in the content of the tissue's unsaturated fatty acids. A relationship between dietary monensin and rumen biohydrogenation is supported in our work by the observation that total saturated fatty acid concentration is less in lipid from monensin-fed tissue than from control tissue. Although a concomitant trend in the ratio of monoene to polyene (i.e., partial hydrogenation to no hydrogenation) would also be expected, no such trend was observed (Tables II and III). The specific saturate 20:0 may arise from biohydrogenation of 20:1ω9*c*, which in turn may arise from chain enlongation of 18:1ω9*c* (Gurr and James, 1975). Both 20:0 and 20:1ω9*c* were found in higher concentration in the polar lipid fraction of control muscle than of monensin-fed tissue ($p < 0.10$; Table II). Increased concentration of trans fatty acids in monensin-fed tissue, specifically 18:1*t* in adipose tissue (Table III), may reflect lesser biohydro-

genation of trans fatty acids of dietary origin (Gurr and James, 1975) or partial hydrogenation of polyunsaturates (Christie, 1978).

The effect of monensin on suppression of chain enlongation, as speculated above regarding 20:1ω9c, may be nonexistent, based on examination of the composition of polyunsaturated fatty acids. No differences were seen between control and monensin-fed tissues either in the concentrations of ω6 fatty acids that arise from enlongation and dehydrogenation of dietary 18:2ω6c or in the concentrations of ω3 fatty acids that arise similarly from dietary 18:3ω3c.

Enhanced concentrations of the 17-carbon acids by monensin (Tables II and III) may arise from de novo fatty acid synthesis from propionate; increased propionate output over acetate, as was discussed in the introduction, is a manifestation of monensin incorporation into bovine feed. The normal saturate 17:0, its desaturation product 17:1ω8c, and the branched ai17:0 all may arise from de novo synthesis from propionate (Vernon, 1980), and concentrations of all these acids are greater in tissue from monensin-fed animals than in control tissue. The same pattern observed for the 17-carbon acids is seen for the sums of all odd-chain normal saturates and branched saturates.

CONCLUSIONS

The sparsity of variations in bovine lipid content and fatty acid composition suggests that monensin has only a minor effect on lipid metabolism in cattle. Those variations in fatty acid composition that were detected may have resulted from alterations in biohydrogenation and de novo fatty acid synthesis.

Registry No. Monensin, 17090-79-8.

Supplementary Material Available: Normalized fatty acid composition (Table IV) and fatty acid composition by weight per tissue portion (Table V) (6 pages). Ordering information is given on any current masthead page.

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Ukwa Seed (*Treculia africana*) Protein. 1. Chemical Evaluation of the Protein Quality

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Amino acid analyses of defatted breadfruit seed (Ukwa) were made. The defatted seed contains 19% protein, which is higher than that for cereals and similar to most pulses. The amino acid content and calculated chemical score indicate that Ukwa seed is adequate in furnishing most essential amino acids in the human diet with sulfur-containing amino acids and tryptophan as the limiting amino acids. Ukwa seed is particularly high in aromatic amino acids. It is a potential source of good protein.

The ever increasing problem of feeding the fast growing population of Nigeria as well as the population of farm livestock has continued to pose a very serious problem. Treculia *africana* (breadfruit seed), locally called "Ukwa", is a popular traditional food among the Igbos of the Southern part of Nigeria. There are many different ways of preparing Ukwa seed as part of the daily menu. The seed can be roasted until the testa becomes brittle for easy removal, and the cotyledon can then be eaten. The seed can also be blanched to ease the removal of the hard testa and then cooked, mashed, and served with yam. It can also be made into Ukwa porridge.

Although the seed is widely consumed only among the Igbos, very little information is available in the literature on its chemical composition in relation to its nutritive value and its possible application in food processing. Recent work on the seed has shown that Treculia seed contains about 4–7% total lipid content (Nwaokorie, 1983). With evidences of gross inadequate availability and consumption of protein foods in Nigeria coupled with both population explosion and urbanization, the nutritional problems resulting from inadequate protein consumption will remain if efforts are not made toward finding other available and cheaper sources of protein. This paper therefore reports

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