Toxicologic Studies with Lambs Fed Jojoba Meal Supplemented Rations

Charles G. Manos, Patrick J. Schrynemeeckers, Douglas E. Hogue, John N. Telford, Gilbert S. Stoewsand, Donald H. Beerman, John G. Babish, Julia T. Blue, Barbara S. Shane, and Donald J. Lisk*

Jojoba (*Simmondsia chinensis*) meal was incorporated as 5% and 10% of the ration and fed to wether and ewe lambs. Residues of simmondsin and simmondsin 2′-ferulate as determined by high-pressure liquid chromatography were not detected in kidney, liver, muscle, or blood of sheep fed jojoba meal. No direct-acting mutagens or promutagens were found in the jojoba meal or rations containing it. Liver protein and aminopyrene *N*-demethylase activity were significantly lower in the rams fed the 10% jojoba meal ration than in the corresponding controls. Organ function tests showed a significantly decreased BUN and a significantly increased GGTP in the ewes fed the jojoba rations compared to the respective controls. No changes in tissue ultrastructure were observed when examined by electron microscopy for any of the dietary treatment groups. Ensiling jojoba meal with green chopped corn appears to improve its palatability for lambs.

Jojoba (*Simmondsia chinensis*) is a shrub indigenous to arid land in the Western United States. The plant produces nuts that contain a liquid wax (about 50% by weight) that is the only known natural substitute for high-temperature industrial lubrication presently performed by sperm whale oil. It is, therefore, considered an important commodity by the federal government. The liquid wax also has applications in cosmetics, pharmaceuticals, and numerous other products. Since demand for this product currently exceeds supply, large-scale cultivation of jojoba has begun.

The meal remaining after the wax has been extracted has received relatively little attention. It contains a high protein content (Shah and Stegemann, 1983; Yermanos, 1975) and therefore should be of interest to livestock producers as a feed or feed supplement. However, the meal contains toxic constituents, namely simmondsin (Booth et al., 1974) and as many as three other related (cyanomethylene)cyclohexyl glycosides (Elliger et al., 1973; Verbiscar and Banigan, 1978; Verbiscar et al., 1980) including simmondsin 2′-ferulate, 5-desmethylsimmondsin, and 4,5-didesmethylsimmondsin (Verbiscar and Banigan 1983). Williams (1980) reported that mice dosed with purified simmondsin showed elevated blood concentrations of cyanide and thiocyanate, the former apparently resulting from release of cyanide during metabolism of simmondsin. Various approaches to the detoxication of jojoba meal have been published. These include hydration of the cyano compounds to amides using ammonical hydrogen peroxide (Verbiscar et al., 1980) or detoxification using ammonia (Elliger et al., 1976) or lactobacilli (Verbiscar et al., 1981). Microorganisms in the rumen of sheep also detoxify simmondsin in jojoba meal (Verbiscar et al., 1980).

Feeding studies with diets supplemented with jojoba meal have been conducted with broiler chicks (Ngoupayou et al., 1982), rabbits (Ngoupayou et al., 1985), and lambs (Verbiscar et al., 1980, 1981), but these have addressed mainly ration palatability, feed efficiency, and growth. In the work reported here, lambs were fed two levels of jojoba meal in their rations for up to 80 days following weaning. Organ function tests were performed, and hepatic microsomal mixed-function oxidase activity was determined. Direct-acting mutagens and promutagens in the jojoba meal and complete animal rations were measured, and electron microscopic analysis of tissues was performed. Residues of simmondsin and simmondsin 2′-ferulate were determined in tissues and blood, and animal performance and carcass quality were judged.

Table I. Composition[a] of the Complete Lamb Diets

constituent	wt, %	wt, %	wt, %
jojoba meal	0	5.0	10.0
soybean meal	12.5	10.0	7.5
corn meal	68.9	66.4	63.9
hay	15.0	15.0	15.0
corn oil	1.0	1.0	1.0
limestone	2.0	2.0	2.0
salt	0.6	0.6	0.6

[a] Vitamins A and D also added.

EXPERIMENTAL PROCEDURE

Feed Preparation and Analyses. Hexane-extracted jojoba meal was provided by the Jojoba Growers and Processors, Inc., Apache Junction, Az. It was incorporated in diets for lambs. The ingredient composition of these diets is given in Table I. The proximate composition of the diets was determined. Ash and fat were determined in the diets by the procedures cited in the *Official Methods of Analysis* (AOAC, 1975). Protein was determined as Kjeldahl nitrogen × 6.25 and energy with a Paar bomb calorimeter. The amino acid composition of the protein-insoluble fraction was determined by the methods of Krishnamoorthy et al. (1982) and Muscato et al. (1983). The mineral composition of the jojoba meal was determined by atomic absorption except for sodium that was determined by atomic emission spectrometry. Boron and phosphorous were determined colorimetrically (Greweling, 1976). Selenium was determined fluorimetrically (Olson, 1969).

Animal Feeding. Four wether and 12 ewe lambs, balanced for body weight, were allotted to each treatment. The 48 lambs were 3 months old, of the Dorset breed. The

U.S. Department of Energy, Morgantown Energy Technology Center, Morgantown, West Virginia 26507-0880 (C.G.M.), Radian Corporation, Austin, Texas 78766 (P.J.S.), Department of Animal Science (D.E.H., D.H.B.), Section of Biochemistry (J.N.T.), and Toxic Chemicals Laboratory (D.J.L.), New York State College of Agriculture and Life Sciences, Cornell University, Ithaca, New York 14853, Department of Food Science and Technology, New York State Agricultural Experiment Station, Geneva, New York 14456 (G.S.S.), Department of Preventive Medicine (J.G.B.) and Department of Veterinary Pathology (J.T.B.), New York State College of Veterinary Medicine, Cornell University, Ithaca, New York 14853, and Institute for Environmental Studies, Louisiana State University, Baton Rouge, Louisiana 70803 (B.S.S.).

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animals were housed in elevated expanded steel pens, 1.2 m square, with a pair of lambs randomly assigned to each pen. Pens were equipped with external feeders and waterers. Wethers were fed for 80 days and ewe lambs for 48 days. Forty-eight days after feeding began, blood samples were taken from all animals for organ function tests. Feeding of the ewes was terminated at this point, but the wethers were fed for an additional 32 days at which time they had attained market weight and were sacrificed by exsanguination according to common procedures and under federal inspection.

Samples of kidney, liver, muscle (diaphragm), and blood were taken from the wethers at the time of slaughter for analysis of simmondsin residues, measurement of hepatic microsomal mixed-function oxidase (MFO) activity, and histopathologic examination. Jojoba meal and each of the diets were also sampled for measurement of possible direct-acting mutagens and promutagens.

Toxicologic Analyses. Organ function tests included measurement of packed cell volume (PCV) by the microhematocrit method (Wintrobe et al., 1974) and hemoglobin (HB) by the cyanomethemoglobin method (Crosby et al., 1954) while total protein, urea nitrogen (BUN), γ-glutamyl transpeptidase (GGTP), and aspartate transaminase (AST) were performed on a Rotochem CFA 2000 automated chemistry analyzer (Travenol Laboratories, Inc., Deerfield IL) using methods cited in Worthington Diagnostics (1984).

Hepatic MFO activity in the animals was measured since this can be increased by numerous natural or synthetic organic compounds. Liver from each lamb was perfused in situ with a cold 0.9% NaCl solution, weighed, sliced, and homogenized in 4 volumes of ice-cold 1.15% KCl containing 20 mM Tris-HCl buffer, pH 7.4, with a Potter-Elvehjem Teflon-glass homogenizer fitted to a mechanical drill. Enzyme activity was estimated by measuring product formation of two incubated substrates with the 12000*g* supernatent liver fraction in an NADPH-generating system (Conney, 1967) as follows: *p*-nitroanisole *O*-demethylase activity was measured by determining the *p*-nitrophenol produced (Kato and Gillette, 1965); aminopyrene *N*-demethylase activity was determined by measurement of the production of formaldehyde (Nash, 1953); and microsomal protein was analyzed by a modified Lowry procedure (Sutherland et al., 1949).

The determination of the presence of possible direct-acting mutagens or promutagens in the jojoba meal and complete animal rations was conducted by a modified method of Ames (Yamasaki and Ames, 1977; Ames et al., 1975) as described by Batzinger et al. (1978) using *Salmonella typhimurium* TA100 with and without the S-9 liver fraction. The modified method has been shown to be less sensitive to exogenous histidine than the original procedure (Batzinger et al., 1978).

Electron microscopic analysis of tissue was performed as previously described (Telford et al., 1982). An imbedding medium using Epon 812 as described by Hayat (1970) was used. Examination of tissue sections was made with a Zeiss Model 10 C transmission electron microscope operated at an accelerating voltage of 60 kV.

Whole kidney, liver, muscle, and blood of one control and one jojoba-fed lamb were taken for analysis of residues of simmondsin and simmondsin 2′-ferulate. They were freeze-dried, milled to a powdery consistency, and mixed. The jojoba meal was also finely ground. From 0.1 to 0.3 g of the control samples was transferred to test tubes, fortified with increasing concentrations of simmondsin hydrate and simmondsin 2′-ferulate standards (Anver Bioscience Design, Inc., Sierra Madre, CA) dissolved in 200–300 μL of 30% acetonitrile (CH_3CN) in water, and allowed to stand overnight. Unfortified samples were similarly allowed to stand overnight in the 30% CH_3CN solution. Eight milliliters of 30% CH_3CN was then added to each test tube; the contents were sonicated for 75 s and again allowed to equilibrate overnight. The mixture was then centrigued and the supernatant analyzed by high-pressure liquid chromatography (HPLC). Reagent blanks and blanks fortified with the two standards were also analyzed.

Table II. HPLC Operating Conditions

parameter	simmondsin hydrate	simmondsin 2′-ferulate
column	IBM C-18 reversed phase	
mobile phase	Waters Model 680 binary grad syst: (a) 0–5.5 min, 15% CH_3CN in water; (b) 5.5–6.5 min, lin grad from 15 to 35% CH_3CN in water; (c) 6.5–17 min (end of run), 35% CH_3CN in water; (d) 5-min equil period at 15% CH_3CN between samples	30% CH_3CN in water
flow, mL/min	1	1
pumps	Waters Model 510	Waters Model M6000
injector	Wisp Model 710B autosampler, 20 μL inj vol	Rheodyne fixed loop, 20 μL
detector	Perkin-Elmer Model LC55 var-wavelength, 220 nm; range 0.05; resp 1 s	
integrator	Hewlett-Packard Model 3390 A	
retention time, min	3.44	4.40

The HPLC analysis was conducted with the operating parameters given in Table II. Quantitation was accomplished by the method of standard additions. The limits of detection of the method for simmondsin and simmondsin 2′-ferulate in the various tissues in parts per million fresh weight were as follows: kidney, 80 and 34; liver, 72 and 8; muscle 242 and 7; blood 106 and 60. The estimated limit of detection for simmondsin was based on the smallest resolved peak area that was calculated by the integrator.

Following slaughter, lamb carcasses were chilled for 24 h and evaluated for USDA quality and yield grade by criteria outlined in the USDA grading standards (USDA, 1969). The quality grade is based upon the conformation of the carcass and the estimated palatability of the lean. The numerical scores range from 13, equivalent to prime plus, to 2, which equals utility minus for lamb and yearling mutton. The yield grade is based on the amount of subcutaneous carcass fat, the quantity of kidney and pelvic fat, and the leg conformation score (estimate of degree of muscling). The numerical scores range from 1.0, which is equivalent to the highest yield of trimmed retail cuts, to 5.9, which equals the lowest yield of trimmed retail cuts. An adjusted fat thickness measurement using the mean of two measures over the longissimus and two measures over the lower rib was employed. Statistical analysis was conducted by using analysis of covariance procedures for a balanced design and using live weight as the covariate (SAS, 1982).

Comparison of means in the tables was accomplished by Duncan"s multiple-range test as described by Steel and Torrie (1960).

RESULTS AND DISCUSSION

Proximate composition of the diets is given in Table III. The protein content of the jojoba meal is in the range

reported by Ngoupayou et al. (1982) and Shah and Stegermann (1983): 24–32%. Shaw and Stegermann (1983) also characterized the proteins electrophoretically. They reported that about 80% of total proteins in solvent-extracted jojoba meal is water soluble. Ngoupayou et al. (1982) reported the fat and ash contents for a series of deoiled jojoba meals to range from 0.74 to 4.4 and 1.9 to 4.9%, respectively. Therefore, although the content of ash found in the jojoba meal used in this study falls in the above range, fat content was about twice that of the highest reported by Ngoupayou et al. (1982). It is possible that hexane extraction of the oil from meal in this study was somewhat less efficient. This may be reflected in the notably higher energy value for our meal (5403 cal/g) compared to that reported for jojoba meal by Utz et al. (1982) (3300 cal/g). Table IV lists the amino acid composition of the protein-insoluble fraction of the jojoba meal. Its composition is not greatly different from that reported by Verbiscar and Banigan (1978). Similarly, mineral content of jojoba meal reported by Utz et al. (1982) resembles that of the meal fed in this study (Table V).

Results of the organ function tests are given in Table VI. Blood urea nitrogen was significantly lower ($p < 0.01$) in ewes fed 10% jojoba meal than in the corresponding controls. γ-Glutamyl transpeptidase was significantly higher in ewes fed the 5% ($p < 0.05$) and 10% ($p < 0.01$) jojoba meal than in the controls. Although there were other noticeable numerical differences among wethers in the values for BUN, GGTP, and AST, they were not significant, perhaps owing to the fewer number of replicated animals.

Table VII lists liver protein, hepatic MFO activity, and liver/body weight percent. Lambs fed the 10% jojoba meal ration had significantly lower ($p < 0.05$) liver protein than those of the other two dietary treatment groups. Aminopyrene *N*-demethylase activity was significantly lower ($p < 0.1$) in lambs fed the 10% jojoba meal ration than control animals. No mutagenic activity, either direct or with metabolic activation (+S-9), was found in the extracts of the jojoba meal or the control or jojoba meal containing lamb rations. No observable changes were detected in kidney, liver, and muscle tissue ultrastructure in any of the ration treatment groups. No specific organ lesions were found in mice dosed with purified simmondsin (Williams 1980) nor in lambs fed deoiled but nondetoxified jojoba meal rations (Trei et al., 1979).

Residues of simmondsin and simmondsin 2′-ferulate were not detected in the tissue or blood. The concentrations of simmondsin and simmondsin 2′-ferulate in the deoiled jojoba meal removed by extraction with acetonitrile were, respectively, 0.08 and 0.41%. Their absence in tissues and blood may indicate that they were insufficiently lipid soluble to be stored in fat deposits. They may have been rapidly excreted intact or as metabolites after contact with *Lactobacillus* or other microorganisms in the rumen.

The average rates of weight gain of the wethers and ewes fed the three diets are given in Table VIII Weight gains

Table III. Proximate Analysis[a] of the Complete Lamb Diets

diet	protein, %	fat, %	ash, %	energy, cal/g
control	16.76	4.24	6.31	4514
5% jojoba meal	15.48	4.48	5.99	4568
10% jojoba meal	15.70	4.37	6.81	4572
jojoba meal alone	25.60	8.63	3.76	5403

[a] Dry-weight basis.

Table IV. Amino Acid Composition of the Protein-Insoluble Fraction[a] of the Hexane-Extracted Jojoba Meal Fed to Lambs

amino acid	% dry wt basis	% total AA in insol protein fractn
alanine	0.72	4.9
arginine	1.02	6.9
aspartic acid	1.65	11.2
cystine (half)	0.27	1.8
glutamic acid	1.94	13.1
glycine	1.24	8.4
histidine	0.35	2.4
isoleucine	0.65	4.4
leucine	1.35	9.1
lysine	0.39	2.6
methionine	0.13	0.9
phenylalanine	0.84	5.7
proline	0.91	6.2
serine	0.76	5.1
threonine	0.90	6.1
tyrosine	0.71	4.8
valine	0.94	6.4
total	14.77	100.0

[a] The insoluble fraction represented 62.5% (dry weight) of the jojoba meal.

Table V. Mineral Composition of Jojoba Meal[a]

element	%	ppm
Ca	0.12	
K	0.86	
Mg	0.26	
Na	0.008	
P	0.39	
Al		68
B		15
Cd		0.05
Cr		3.0
Cu		20
Fe		393
Mn		31
Ni		12.8
Pb		2.3
Se		0.32
Zn		31

[a] Dry-weight basis.

Table VI. Results[a] of Organ Function Tests

animal dietary treatment	PCV, %	HB, g/dL	total protein, g/dL	BUN, mg/dL	GGTP, IU/L	AST, IU/L
			Wethers			
control	38 ± 1.7[x]	13.3 ± 0.8[x]	6.7 ± 0.2[x]	18 ± 3.7[x]	34 ± 2.7[x]	66 ± 4.0[x]
5% jojoba	39 ± 1.0[x]	13.1 ● 0.1[x]	7.3 ± 0.8[x]	15 ± 4.0[x]	40 ± 0.0[x]	51 ± 4.0[x]
10% jojoba	38 ± 0.7[x]	13.3 ± 0.2[x]	7.1 ± 0.2[x]	12 ± 1.3[x]	41 ± 3.9[x]	59 ± 3.0[x]
			Ewes			
control	39 ± 0.8[x]	13.8 ± 0.4[x]	7.4 ± 0.2[x]	21 ± 1.7[x*]	34 ± 1.1[x]	66 ± 2.4[x]
5% jojoba	37 ± 0.8[x]	13.3 ± 0.3[x]	7.2 ± 0.1[x]	18 ± 1.2[x]	43 ± 2.9[y]	63 ± 2.1[x]
10% jojoba	37 ± 0.8[x]	13.4 ± 0.2[x]	7.1 ± 0.1[x]	14 ± 1.1[y]	39 ± 1.4[y*]	66 ± 2.8[x]

[a] Average ± standard error; dissimilar letter superscripts indicate significant differences ($p < 0.05$) between respective treatment means. Asterisks indicate $p < 0.001$.

Table VII. Data[a] for Liver Protein, Hepatic MFO Activity, and Liver/Body Weight Ratios of Lambs Fed Jojoba Meal

animal dietary treatment	mg protein/g liver	*p*-nitroanisole *O*-demethylase, nmol/mg protein per h	aminopyrene *N*-demethylase, nmol/mg protein per h	liver/body wt, %
control	33.92 ± 0.26^{x}	9.28 ± 0.98^{x}	8.24 ± 1.77^{x}	1.93 ± 0.06^{x}
5% jojoba meal	34.72 ± 0.34^{x}	8.06 ± 1.17^{x}	5.36 ± 1.52^{xy}	1.83 ± 0.08^{x}
10% jojoba meal	$31.59 \pm 1.19^{y*}$	6.67 ± 0.85^{x}	3.15 ± 1.01^{y}	1.83 ± 0.13^{x}

[a] Average ± standard error; dissimilar letter superscripts indicate significant differences ($p < 0.10$) between respective treatment means. Asterisks indicate $p < 0.05$.

Table VIII. Rates[a] of Animal Weight Gain

animal dietary treatment	init body wt, kg	rate wt gain, g/day
	Rams	
control	29.1 ± 1.5^{x}	240 ± 10^{x}
5% jojoba meal	27.0 ± 0.8^{x}	190 ± 20^{x}
10% jojoba meal	28.0 ± 0.8^{x}	190 ± 10^{x}
	Ewes	
control	23.2 ± 0.8^{x}	$217 \pm 15^{x*}$
5% jojoba meal	22.9 ± 0.7^{x}	172 ± 23^{x}
10% jojoba meal	23.6 ± 0.9^{x}	119 ± 14^{y}

[a] Average ± standard error; dissimilar letter superscripts indicate significant differences ($p < 0.05$) between respective treatment means. Asterisks indicate $p < 0.01$.

were significantly lower for the ewes fed the 10% jojoba meal compared to the control ($p < 0.01$) or 5% jojoba meal ($p < 0.05$) rations. The ewes fed the 5% jojoba ration showed a weight gain numerically lower than the corresponding controls but not significantly so. The wethers fed 5 or 10% jojoba meal showed numerically lower weight gain than the control wethers, but the differences were not significant. Compared to the control animals, the sheep fed the jojoba meal rations attempted to sort out the jojoba meal particles, some of which were left unconsumed. Jojoba meal was reported to contain a bitter flavor (Booth et al., 1974).

Verbiscar et al. (1981) reported that treatment with *Lactobacillus* not only renders jojoba meal nontoxic to mice, poultry, sheep, and cattle but also increases the palatability of the deoiled meal that would otherwise be poorly accepted in animal rations. These findings were also confirmed by Swingle et al. (1985) with beef cattle. Various methods of detoxification of jojoba meal have also been reported to improve its acceptability to lambs (Nelson et al., 1979). In feeding trials with lambs, deoiled but nondetoxified jojoba meal in the ration reduced feed intake (Trei et al., 1979). Verbiscar et al. (1981) suggest that treatment of jojoba meal with *Lactobacillus* resembles an ensiling process. On the basis of this suggestion, 0 (control), 5, 10 and 20 wt % of jojoba meal was mixed with freshly harvested and chopped corn plants, and the mixtures were ensiled in evacuated plastic containers holding approximately 100 lbs (45.45 kg) of the plant material. Thirty days later, the plastic containers were opened and five Dorset ewe lambs were assigned to each ration for a period of 10 days. The animals readily consumed the control and 5 and 10% jojoba meal rations but largely rejected the 20% ration. This preliminary study indicates that ensiling crop mixtures containing low percentages of deoiled jojoba meal may indeed improve its palatability.

Carcass characteristics of lambs were not significantly affected by addition of jojoba meal to diet (Table IX). Slaughter weights of lambs were significantly less ($p < 0.05$) with both levels of jojoba meal. Therefore, several carcass traits were affected. Measures of fat thickness at the 12th rib, percent kidney and pelvic fat, and leg conformation are closely related to live weight or carcass weight within groups of lambs of similar genetic background (Drew and Reid, 1975). Lighter lambs contain less fat as a percentage of empty body weight or carcass weight. Nutritional manipulation of growth rate does not alter body composition in lambs when comparisons are made on a constant-weight basis (Firth, 1984; Jacobs et al., 1972; Kemp et al., 1970). Quality grade, an indicator of palatability, and yield grade, an indicator of expected yield of closely trimmed retail cuts, were also unaffected by jojoba meal addition to the diet.

Table IX. Least-Squares Means for Effects of Jojoba Meal on Lamb Carcass Characteristics and USDA Quality and Yield Grades

characteristics	control	jojoba meal 5%	jojoba meal 10%	S^a
live weight, kg	48.2	42.3	39.5	2.58
carcass wt, kg	24.9	23.3	24.3	0.84
dressing,[b] %	54.7	51.3	53.5	0.85
quality grade[c]	12.1	11.8	11.0	0.40
12th rib fat thickness,[d] cm	1.13	0.69	0.66	0.09
leg conformn[c]	9.4	10.0	11.2	0.51
kidney and pelvic fat, %	3.6	3.4	2.8	0.41
yield grade[e]	4.5	3.7	3.6	0.30

[a] S = standard error of the mean. [b] Carcass weight divided by live weight. [c] 13 = prime plus; 9 = choice average; 2 = utility minus. [d] The average of four measures: two over the longissimus and two over the lower rib. [e] 1.0 = highest yield trimmed retail cuts; 5.9 = lowest yield of trimmed retail cuts.

On the basis of results of this feeding trial, jojoba meal might serve as a satisfactory high-protein feed supplement in lamb rations. Its palatability to farm animals may be improved, however, by ensiling, but more extensive feeding trials must be conducted to firmly establish this observation. The fact that jojoba is grown mainly in the Western United States could limit its availability, but the jojoba plant is presently being introduced in other locations such as South America, Africa, and Australia.

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Registry No. GGTP, 9046-27-9; aminopyrene *N*-demethylase, 9037-69-8; urea, 57-13-6.

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Determination of Sodium Salinomycin in Chicken Skin/Fat by High-Performance Liquid Chromatography Utilizing Column Switching and UV Detection

Gary P. Dimenna,* James A. Creegan, Lennox B. Turnbull, and George J. Wright

A high-performance liquid chromatographic (HPLC) method has been developed for the quantitative determination of salinomycin in chicken skin/fat. For this procedure skin/fat homogenate (10 mL, equivalent to 2 g of tissue) was extracted with methanol. The extract was partitioned with carbon tetrachloride, applied to a silica gel column, and, in turn, run through a C_{18} column. The purified salinomycin solution was then oxidized with pyridinium dichromate and washed with sodium bicarbonate. The derivatized product was purified by running it through a silica gel column. The HPLC system utilized an automated on-line column-switching system with UV detection at 225 nm; the minimum limit of detection was 100 ppb. Peak height ratios of salinomycin to internal standard were used for quantitation.

INTRODUCTION

Sodium salinomycin (Figure 1) is a polyether antibiotic possessing anticocidial and growth-promoting activity (McClure et al., 1980). Its chemistry and biological activity have been described by Miyazaki et al. (1974).

Until now, a thin-layer, bioautographic technique (Heil et al., 1984) has generally been used for detecting salinomycin and similar compounds such as monensin (Donoho and Kline, 1967) in animal tissues. Although thin-layer bioautographic techniques are inherently selective and very sensitive, they lack precision and are time consuming. HPLC methods, on the other hand are generally precise, accurate, selective, sensitive, and amenable to automation and lend themselves to repetitive analyses. Goras and Lacourse (1984) and Blanchflower et al. (1985) have developed HPLC methods for salinomycin in feeds that utilize postcolumn reaction with vanillin and show potential for detecting low levels of salinomycin in tissue.

Department of Drug Metabolism, A. H. Robins Company, Richmond, Virginia 23261-6609.

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