Effect of Laidlomycin Propionate on Bovine Longissimus Muscle Fatty Acid and Cholesterol Content†

S. K. Duckett*,‡ and D. G. Wagner§

Department of Animal Science, Oklahoma State University, Stillwater, Oklahoma 74078

Differences in fatty acid composition and cholesterol content of longissimus muscle (LM) were assessed as a function of ionophore incorporation into a high-concentrate diet. Ribeye steaks (n = 70) were obtained from crossbred steers that had been fed a high-concentrate diet for 121 days with or without the addition of laidlomycin propionate (LP; 11 mg/kg). Total lipid (TL), neutral lipid (NL), polar lipid (PL), and cholesterol contents in the LM were unaffected ($P > 0.05$) by LP incorporation. The concentration of oleic acid increased ($P < 0.05$) in the NL; however, other fatty acids percentages in the TL, NL, and PL were unaffected ($P > 0.05$) with LP addition. The amount (mg/100 mg) of saturated and odd chain fatty acids, and ratio of hypercholesterolemic to hypocholesterolemic fatty acids decreased ($P < 0.05$) with LP addition.

Keywords: *Beef; cholesterol; fatty acids; laidlomycin propionate*

INTRODUCTION

Ionophores are routinely incorporated into high-concentrate diets to enhance rate of gain and feed utilization; however, no information is available as to how lipid composition or cholesterol content in the muscle may be affected by the addition of ionophores to these diets. Monensin addition increased margaric (C17:0) acid concentrations in intramuscular (i.m.) lipid of steers (Marmer et al., 1985) and lambs (Gilka et al., 1989b) consuming a forage-based diet. Lasalocid addition reduced myristic (C14:0) acid content in lamb i.m. lipid. O'Kelly and Spiers (1988) reported monensin addition to alfalfa hay diets increased plasma total cholesterol and cholesteryl ester fatty acid concentrations as a result of increased ruminal lipid synthesis. Together these results suggest that ionophore incorporation, at least on forage-based diets, may reduce ruminal biohydrogenation and alter de novo fatty acid synthesis with an end result of changing the composition of depot fat. Because ruminal bacterial populations are dependent on diet type, this study was designed to evaluate the effects of the ionophore, laidlomycin propionate, on the longissimus muscle (LM) lipid and cholesterol content from steers fed a high-concentrate diet.

MATERIALS AND METHODS

Beef. Angus × Hereford steers (n = 140) were equally allotted to four treatments: control (C), laidlomycin propionate (LP; 11 mg/kg), tylosin (10 mg/kg), or a combination of laidlomycin propionate (11 mg/kg) plus tylosin (10 mg/kg). Laidlomycin propionate (Cattlyst, Syntex Animal Health, West Des Moines, IA) has been approved with claims of improved feed efficiency and increased rate of weight gain of cattle (FDA, 1994). However at the time of this study, LP was still under investigational status which included the stipulation that it had to be withdrawn from the diet 10 days prior to slaughter. All steers were fed the same basal diet (87.8% DM, 1.78 Mcal/kg of NE_m, 1.18 Mcal/kg of NE_g) for 121 days and then commercially slaughtered. Longissimus muscle sections (N = 70), corresponding to the 9−12th ribs, were randomly selected from the C and LP treatments. Steaks were trimmed of all exterior fat and epimysial connective tissue, and then pulverized in liquid nitrogen for storage at −20 °C.

Lipid Extraction. The neutral (NL) and polar (PL) lipid fractions were sequentially separated according to the dry column method of Marmer and Maxwell (1981).

Moisture and Lipid Content. Moisture and crude fat content were determined by drying a 2 g muscle sample at 100 °C for 24 h and then extracting with petroleum ether for 8 h (AOAC, 1984). For NL and PL content, a 5 mL aliquot of the NL and PL extracts was freed of solvent and dried at 95 °C for 24 h (AOAC, 1984). The lipid weight of the NL and PL were summed for each sample to obtain a total (TL) lipid weight. Phospholipid (PhL) content was calculated by determining P content of PL (Vaskovsky et al., 1975) and multiplying by 25.

Fatty Acid Composition. Fatty acid methyl esters (FAME) were prepared from an aliquot of the NL (Slover and Lanza, 1979) and PL (Maxwell and Marmer, 1983) extracts. The FAME were separated on a SP2340 capillary column (60 m, 0.25 mm i.d., and 0.2 μm film thickness; Supelco, Bellefonte, PA) under the conditions previously described by Duckett et al. (1993). Briefly, column oven temperature was programmed at 155−165 °C at 0.5 °C/min, 165−167 °C at 0.2 °C/min, 167−200 °C at 1.5 °C/min, and held at 200 °C for 18 min. The injector and detector were maintained at 280 °C. Helium was the carrier gas at a flow rate of 1 mL/min. and a split ratio of 1:100. Peaks were identified by comparisons to reference standards from Alltech and Matreya (Matreya, Pleasant Gap, PA). Fatty acids were quantified by incorporating an internal standard, methyl heneicosanoic (C21:0) acid, into each sample. Total fatty acid profiles were calculated by multiplying the percentage of NL and PL in the TL by each fatty acid.

Cholesterol Content. Duplicate 1 mL aliquots of NL were freed of solvent and then redissolved in dichloromethane containing 50 μg/mL of internal standard, stigmasterol, for cholesterol analysis. Samples were separated using a Drug Three Megabore column (10 m, 0.53 mm i.d.; Alltech Assoc., Deerfield, IL) maintained at a temperature of 285 °C with the injector and detector maintained at 300 and 320 °C, respectively. Helium was the carrier gas at the flow rate of 12 mL/min. Cholesterol content was quantified by regression equa-

† Approved for publication by the director, Oklahoma Agric. Exp. Sta.

* Corresponding author.

‡ Current address: Department of Animal and Veterinary Science, University of Idaho, 216 Agriculture Science Building, Moscow, ID 83844-2330 (e-mail sduckett@uidaho.edu; phone (208) 885-7390; fax (208) 885-6420).

§ To whom request for reprints should be addressed.

S0021-8561(96)00576-6 CCC: $14.00

Table 1. Effect of Laidlomycin Propionate on Longissimus Muscle Lipids and Cholesterol Content

	control	laidlomycin propionate	SEM
moisture, %	73.11	73.20	0.22
crude fat, %	4.49	4.09	0.24
total lipid, g/100 g	5.25	4.74	0.27
neutral lipid, g/100 g	4.56	4.06	0.26
polar lipid, g/100 g	0.69	0.68	0.01
phospholipid, g/100 g	0.49	0.56	0.01
cholesterol, mg/100 mg			
wet basis	40.53	38.67	1.23
dry basis	150.93	144.40	4.55
fat free, dry matter basis	188.07	175.58	6.00

tions obtained from known cholesterol standards and then corrected according to the response of the internal standard.

Statistics. Differences in lipid content, cholesterol content, fatty acid percentage, and amount between treatments (LP vs C) were determined using the *t*-test procedure to test the hypothesis that treatment means were equal ($\alpha = 0.05$; SAS, 1985).

RESULTS AND DISCUSSION

Muscle Lipids. Moisture, crude fat, and TL values (Table 1) of the LM were unchanged ($P > 0.05$) by LP addition. Similarly, Gilka et al. (1989a) and Potter et al. (1976) found that ionophore addition did not alter longissimus muscle lipid or moisture content. The proportion of lipid present in the storage (NL) versus the structural component (PL and PhL) was also unchanged ($P > 0.05$) by LP addition. These findings are comparable to Marmer et al. (1985) in that the distribution of lipid in the NL, PL, or PhL fractions was unaffected by ionophore addition.

Cholesterol. Cholesterol content reported on either a wet, dry matter, or fat-free dry matter basis was unchanged ($P > 0.05$) by LP addition. Changes in plasma cholesterol concentrations with monensin addition to a forage-based diet have been reported (O'Kelly and Spiers, 1988). However, Lalman et al. (1993) and Duff et al. (1994) both reported plasma cholesterol concentrations in heifers and steers to be unaffected by monensin or lasalocid addition to a high-concentrate

Table 2. Effect of Laidlomycin Propionate on Fatty Acid Content of the Total Lipid

	percent			mg/100 mg		
	control	laidlomycin propionate	SEM	control	laidlomycin propionate	SEM
C14:0	3.14	2.98	0.06	148.7[a]	122.3[b]	8.6
C14:1	0.65	0.64	0.03	31.8	26.5	2.6
C15:0	0.55	0.52	0.02	25.9[a]	21.1[b]	1.5
C16:0	26.19	25.81	0.17	1227.9[a]	1057.6[b]	59.0
C16:1	3.75	3.54	0.09	180.5[a]	147.1[b]	11.4
C17:0	1.63	1.56	0.04	76.0[a]	64.1[b]	4.0
C18:0	13.34	13.26	0.21	619.0	542.6	28.6
C18:1	42.59	42.98	0.30	2005.5	1765.3	102.0
C18:2	4.47	4.89	0.23	201.2	199.4	7.3
C18:3	0.13	0.11	0.01	6.8	5.3	0.8
C20:4	1.29	1.46	0.08	57.4	59.0	1.9
C22:5	0.13	0.16	0.01	6.0	6.3	0.3
C22:6	0.18	0.20	0.01	8.3	8.3	0.3
U[c]	1.90	1.81	0.05	122.5	109.3	5.7
SFA[d]	42.66	42.04	0.26	1995.7[a]	1722.5[b]	95.0
OCFA[d]	2.17	2.08	0.06	101.9[a]	85.3[b]	5.5
MUFA[d]	47.00	47.15	0.35	2217.9	1938.9	115.0
PUFA[d]	6.21	6.82	0.31	279.8	278.4	8.6
ratio[d]	0.552[a]	0.533[b]	0.006			

[a,b]Control and laidlomycin propionate means differ ($P < 0.05$). [c] U, unidentified fatty acids. [d] SFA, saturated fatty acids; OCFA, odd-chain fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ratio, ratio of hypercholesterolemic (C14:0 + C16:0) to hypocholesterolemic fatty acids (MUFA + PUFA).

Table 3. Effect of Laidlomycin Propionate on Fatty Acid Content of the Neutral Lipid

	percent			mg/100 mg		
	control	laidlomycin propionate	SEM	control	laidlomycin propionate	SEM
C14:0	3.53	3.39	0.07	143.0[a]	117.6[b]	8.3
C14:1	0.76	0.75	0.03	31.3	26.3	2.5
C15:0	0.63	0.60	0.02	25.3[a]	20.9[b]	1.5
C16:0	27.31	26.99	0.18	1098.8[a]	934.1[b]	58.0
C16:1	4.08	3.91	0.09	168.3[a]	137.1[b]	10.9
C17:0	1.78	1.73	0.05	71.0[a]	59.6[b]	4.0
C18:0	13.55	13.50	0.25	540.5	466.5	28.6
C18:1	45.13[a]	45.93[b]	0.28	1822.4	1592.0	100.1
C18:2	1.60	1.63	0.09	64.3	57.2	4.8
C18:3	0.07	0.07	0.01	3.8	3.1	0.8
U[c]	1.54	1.42	0.06	65.9	50.3	5.7
SFA[d]	44.38	43.88	0.28	1782.2[a]	1518.2[b]	93.7
OCFA[d]	2.40	2.33	0.06	96.3[a]	80.5[b]	5.4
MUFA[d]	49.97	50.59	0.30	2022.0	1755.5	112.6
PUFA[d]	1.67	1.70	0.09	68.1	60.3	5.3
ratio[d]	0.598	0.582	0.006			

[a,b]Control and laidlomycin propionate means differ ($P < 0.05$). [c] U, unidentified fatty acids. [d] SFA, saturated fatty acids; OCFA, odd-chain fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ratio, ratio of hypercholesterolemic (C14:0 + C16:0) to hypocholesterolemic fatty acids (MUFA + PUFA).

Table 4. Effect of Laidlomycin Propionate on Fatty Acid Content of the Polar Lipid

	percent			mg/100 mg		
	control	laidlomycin propionate	SEM	control	laidlomycin propionate	SEM
C14:0	0.84	0.70	0.07	5.8	4.7	0.6
C14:1	0.06	0.02	0.02	0.5	0.2	0.2
C15:0	0.55	0.52	0.02	0.6	0.3	0.2
C16:0	19.68	19.36	0.22	129.2	123.5	4.4
C16:1	1.80	1.53	0.10	12.2	9.9	0.9
C17:0	0.74	0.68	0.04	5.0	4.5	0.3
C18:0	12.04	11.95	0.11	78.5	76.1	2.4
C18:1	27.84	26.84	0.55	183.2	173.2	7.7
C18:2	21.16	22.69	0.64	136.9	142.3	4.5
C18:3	0.46	0.33	0.06	3.1	2.3	0.4
C20:4	8.90	9.40	0.26	57.4	59.0	1.9
C22:5	0.92	1.01	0.04	6.0	6.3	0.3
C22:6	1.29	1.31	0.04	8.3	8.3	0.3
U[a]	4.14	4.10	0.10	26.8	26.2	1.0
SFA[b]	32.56	32.02	0.30	213.5	204.2	7.2
OCFA[b]	0.82	0.72	0.05	5.6	4.8	0.4
MUFA[b]	29.74	28.40	0.62	195.9	183.3	8.6
PUFA[b]	32.73	34.74	0.82	211.7	218.2	6.2
ratio[b]	0.329	0.319	0.006			

[a] U, unidentified fatty acids. [b] SFA, saturated fatty acids; OCFA, odd-chain fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ratio, ratio of hypercholesterolemic (C14:0 + C16:0) to hypocholesterolemic fatty acids (MUFA + PUFA).

diet. The association between plasma and tissue cholesterol levels are low ($r = 0.22$; Wheeler et al., 1987) indicating that a change in plasma cholesterol is not indicative of a change in tissue cholesterol levels.

Fatty Acids. Addition of LP to the diet did not alter ($P > 0.05$) the percentage of myristic (C14:0), palmitic (C16:0), or stearic (C18:0) acid in the TL (Table 2), NL (Table 3), or PL (Table 4). The amount (mg/100 mg) of myristic (C14:0) acid, palmitic (C16:0) acid, and saturated fatty acids in the TL and NL was lower ($P < 0.05$) due to small numerical changes ($P > 0.05$) in lipid content and saturated fatty acid percentages with LP addition. In the PL, none ($P > 0.05$) of the saturated fatty acids amounts (mg/100 mg) was affected by LP addition. Myristic acid has been shown to be reduced with lasalocid addition but not monensin in lambs (Gilka et al., 1989b). Addition of monensin or lasalocid to forage-based diets did not change palmitic acid, stearic acid, or total saturated fatty acid content (Gilka et al., 1989b; Marmer et al., 1985).

Pentadecyclic (C15:0) and margaric (C17:0) acids and the total odd-chain fatty acid (OCFA) amounts (mg/100 mg) were reduced ($P < 0.05$) in the TL and NL with LP addition; however, the percentage of these fatty acids in the LM was unaffected ($P > 0.05$). Both Marmer et al. (1985) and Gilka et al. (1989b) reported increased maragaric acid concentration in the lipid of steers and lambs fed monensin on high-roughage diets. These authors attributed the increase in margaric acid content to the greater quantities of propionate relative to other VFA available for de novo fatty acid synthesis. Research with LP in high-concentrate diets has not shown a definite influence of this ionophore on ruminal VFA concentrations (Zinn and Spires, 1987; Galyean et al., 1992). Thus, the lack of change in percent of the OCFA would support the lack of a major shift in volatile fatty acid proportions by LP addition to a high-concentrate diet.

Oleic (C18:1) acid was increased ($P < 0.05$) in concentration 2% in the NL but unchanged ($P > 0.05$) in the TL and PL with LP addition. Kobayashi et al. (1992) reported digesta from lambs fed salinomycin to be higher in unsaturated fatty acids, particularly oleic acid. Palmitoleic (C16:1) acid content (mg/100 mg) was reduced ($P < 0.05$) in the TL and NL by LP addition. The percentage and amount of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids were unchanged ($P > 0.05$) in the TL, NL, and PL. Similarly, Marmer et al. (1985) and Gilka et al. (1989b) both reported MUFA and PUFA were unaffected by ionophore addition to forage-based diets (Marmer et al., 1985; Gilka et al., 1989b).

The ratio of hypercholesterolemic (C14:0 + C16:0) to hypocholesterolemic (MUFA + PUFA) fatty acids in the TL was reduced 3% with LP addition. The lowering of this ratio appeared to result in small, nonsignificant changes in myrisitic and palmitic acids and in unsaturated fatty acids with LP addition. In this ratio, stearic (C18:0) acid is omitted from the equation because it exerts neither a negative nor a positive effect on plasma cholesterol (Hegsted et al., 1965; Keys et al., 1965; Bonanome and Grundy, 1987). Both myristic and palmitic acids have been associated with increased plasma cholesterol levels in humans and thus are labeled hypercholesterolemic (Hegsted et al., 1965; Keys et al., 1965). The MUFA and PUFA are considered to be hypocholesterolemic as both are essentially equivalent in lowering plasma LDL cholesterol (Mattson and Grundy, 1985).

ABBREVIATIONS USED

C, control, no ionophore; i.m., intramuscular lipid; LM, longissimus muscle; LP, laidlomycin propionate; MUFA, monounsaturated fatty acids; NL, neutral lipid fraction; OCFA, odd-chain fatty acids; PhL, phospholipid; PL, polar lipid fraction; ratio, ratio of hypercholesterolemic (C14:0 + C16:0) to hypocholesterolemic (MUFA + PUFA) fatty acids; SFA, saturated fatty acids; TL, total lipid.

LITERATURE CITED

AOAC. *Official Methods of Analysis*, 14th ed.; Association of Official Analytical Chemists: Washington, DC, 1984.

Bonanome, A.; Grundy, S. M. Effect of stearic acid on plasma cholesterol and lipoprotein levels. *N. Engl. J. Med.* **1988**, *318*, 1244.

Duckett, S. K.; Wagner, D. G.; Yates, L. D.; Dolezal, H. G.; May, S. G. Effects of time on feed on beef nutrient composition. *J. Anim. Sci.* **1993**, *71*, 2079.

Duff, G. C.; Galyean, M. L.; Branine, M. E.; Hallford, D. M. Effects of lasalocid and monensin plus tylosin on serum metabolic hormones and clinical chemistry profiles of beef steers fed a 90% concentrate diet. *J. Anim. Sci.* **1994**, *72*, 1049.

FDA. New animal drugs for use in animal feeds; Laidlomycin propionate potassium. *Fed. Reg.* **1994**, *59*, 18296.

Galyean, M. L.; Malcolm, K. J.; Duff, G. C. Performance of feedlot steers fed diets containing laidlomycin propionate or monensin plus tylosin, and effects of laidlomycin propionate concentration on intake patterns and ruminal fermentation in beef steers during adaptation to a high-concentrate diet. *J. Anim. Sci.* **1992**, *70*, 2950.

Gilka, J.; Jelinek, P.; Jankova, B.; Krejci, P.; Habrda, J. Carcass traits and meat quality of male lambs fed monensin or lasalocid. *Meat Sci.* **1989a**, *25*, 265.

Gilka, J.; Jelinek, P.; Jankova, B.; Knesel, P.; Krejci, P.; Masek, J.; Docekalova, H. Amino acid composition of meat, fatty acid composition of fat and content of some chemical elements in the tissues of male lambs fed monensin or lasalocid. *Meat Sci.* **1989b**, *25*, 273.

Hegsted, D. M.; McGrandy, R. B.; Myers, M. L.; Stare, F. J. Quantitative effects of dietary fat on serum cholesterol in man. *Am. J. Clin. Nutr.* **1965**, *17*, 281.

Keys, A.; Andreson, J. T.; Grande, F. Serum cholesterol response to changes in diet. IV. Particular saturated fatty acids in the diet. *Metab.* **1965**, *14*, 776.

Kobayashi, Y.; Wakita, M.; Hoshino. S. Effects of the ionophore salinomycin on nitrogen and long-chain fatty acid profiles of digesta in the rumen and duodenum of sheep. *Anim. Feed Sci. Technol.* **1992**, *36*, 67−76.

Lalman, D. L.; Petersen, M. K.; Ansotegui, R. P.; Tess, M. W.; Clark, C. K.; Wiley, J. S. The effects of ruminally undegradable protein, propionic acid, and monensin on puberty and pregnancy in beef heifers. *J. Anim. Sci.* **1993**, *71*, 2843.

Marmer, W. N.; Maxwell, R. J. Dry column method for the quantitative extraction and simultaneous class separation of lipids from muscle tissue. *Lipids* **1981**, *16*, 365.

Marmer, W. N.; Maxwell, R. J.; Wagner, D. G. Effects of dietary monensin on bovine fatty acid profiles. *J. Agric. Food Chem.* **1985**, *33*, 67.

Mattson, F. H.; Grundy, S. M. Comparison of effects of dietary saturated, monounsaturated and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. *J. Lipid Res.* **1985**, *26*, 194.

Maxwell, R. J.; Marmer, W. N. Systematic protocol for the accumulation of fatty acid data from multiple tissue samples: tissue handling, lipid extraction and class separation, and capillary gas chromatographic analysis. *Lipids* **1983**, *18*, 453.

O'Kelly, J. C.; Spiers, W. G. Monensin induced metabolic changes in cattle fed a restricted intake of lucerne hay. *J. Agric. Sci.* **1988**, *111*, 403.

Potter, E. L.; Raun, A. P.; Cooley, C. O.; Rathmacher, R. P.; Richardson, L. F. Effect of monensin on carcass characteristics, carcass composition and efficiency of converting feed to carcass. *J. Anim. Sci.* **1976**, *43*, 678.

SAS. *SAS User's Guide for PC Computers*; SAS Inst. Inc.: Cary, NC, 1985.

Slover, H. T.; Lanza, E. Quantitative analysis of food fatty acids by capillary gas chromatography. *J. Am. Oil Chem. Soc.* **1979**, *56*, 933.

Vaskovsky, V. E.; Kostetsky, E. Y.; Vasensin, I. M. A universal reagent for phospholipid analysis. *J. Chromatogr.* **1975**, *114*, 129.

Wheeler, T. L.; Davis, G. W.; Clark, J. R.; Ramsey, C. B.; Rourke, T. J. Cholesterol content of longissimus muscle, subcutaneous fat and serum of two beef cattle breeds. *J. Anim. Sci.* **1987**, *65*, 1531.

Zinn, R. A.; Spires, H. R. Influence of laidlomycin propionate on feedlot performance and digestive function in steers. *Proc. West. Sect. Am. Soc. Anim. Sci.* **1987**, *38*, 325.

Received for review August 5, 1996. Accepted January 28, 1997.[⊗]

JF9605761

[⊗] Abstract published in *Advance ACS Abstracts,* April 1, 1997.