

Effects of *Eubacterium coprostanoligenes* and *Lactobacillus* on pH, Lipid Content, and Cholesterol of Fermented Pork and Mutton Sausage-Type Mixes

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ABSTRACT

Pork and mutton sausage-type mixes produced using *Eubacterium coprostanoligenes* with or without *Lactobacillus* as starter culture showed changes in pH, cholesterol and lipid content. Viability of *E. coprostanoligenes* was assessed by culturing samples from each treatment and analyzing for coprostanol, a cholesterol metabolite. The pH and/or cholesterol decreased ($P < 0.05$) in the sausage-type mixes inoculated with *E. coprostanoligenes* or *Lactobacillus* or their combinations. Variability in lipid content was observed among and within treatments. Very low (6.2 $\mu\text{g/g}$ dry matter) concentrations of coprostanol were produced in sausage-mixes and cultures except when inoculated with 2.0 g of *E. coprostanoligenes* (from 8.2 to 130.6 $\mu\text{g/g}$ dry matter).

Key Words: pork, mutton, sausages, *Eubacterium*, *Lactobacillus*

INTRODUCTION

CHEMICAL, PHYSICAL, AND BIOLOGICAL METHODS HAVE BEEN employed to decrease or eliminate fat and cholesterol in foods (Chavari et al., 1988). Physical and chemical methods tend to be nonselective and usually remove nutritional components as well (Deeth, 1983). Use of organic solvents has resulted in residues in the final products. Supercritical fluid extraction, vacuum steam distillation, short path distillation, and complexing with cyclodextrins and saponins have been used also (Deeth, 1983; Smith et al., 1991). Bacterial and/or enzyme methods could provide potential cost and selectivity advantages (Deeth, 1983).

Escherichia coli in human feces was found to degrade cholesterol under aerobic and anaerobic conditions to form neutral products (Owen et al., 1978). Microbial hydrogenation of cholesterol to coprostanol may occur by the intermediates 4-cholesten-3-one and coprostanone (Owen et al., 1983). Microbial transformation of cholesterol by cecal contents from rats converted 50% or more of the cholesterol to coprostanol via the intermediate 4-cholestenone (Bjorkhem and Gustafsson, 1971). *Eubacterium* can anaerobically reduce cholesterol to coprostanol, which is absorbed poorly by the human gastrointestinal tract. *Eubacterium* 21,408 reduced the 4,5 double band of allocholesterol to coprostanol (Gilliland et al., 1984).

Freier (1991) isolated *Eubacterium coprostanoligenes* and characterized it as a small, gram positive, nonspore-forming, coccobacillus, cholesterol-reducing anaerobe (Freier, 1991; Freier et al., 1994). Fermentation of radiolabeled [4-³H,4-¹⁴C]-cholesterol with *E. coprostanoligenes* resulted in 90% conversion of cholesterol to

coprostanol in 5 days involving transfer of a hydrogen from C4 to C5 (Ren, 1991).

Oral administration of *E. coprostanoligenes* to New Zealand White rabbits fed a 0.05% cholesterol-enriched diet resulted in lower ($P < 0.001$) plasma cholesterol concentration. Coprostanol/cholesterol ratios in the digestive tract of rabbits fed the live bacteria were greater than those of rabbits fed boiled bacteria (Li et al., 1995).

Our hypothesis was that *E. coprostanoligenes* could grow in meats during sausage production and decrease cholesterol content of the processed meats. The studies were undertaken to investigate the feasibility of using a cholesterol-reducing bacteria in production of pork and mutton sausage-type mixes. The viability of *E. coprostanoligenes* was evaluated in fermented pork and mutton sausage-type mixes. The pH, total lipids, and concentrations of cholesterol and coprostanol were determined at different stages of production and storage of the sausage-type mixes.

MATERIALS & METHODS

Preparation of starter culture

E. coprostanoligenes ATCC 51222 converts cholesterol to coprostanol, which is poorly absorbed from the human intestinal tract. The bacterial stock was anaerobically maintained in 10 mL liquid media composed of 0.1% lecithin (Sigma Chemical Co. St. Louis, Mo., U.S.A.), 1% casitone, 1% yeast extract (Difco Laboratories, Detroit, Mich., U.S.A.), 0.5% sodium thioglycollate, and 0.1% calcium chloride (Fisher Scientific, Fair Lawn, N.J., U.S.A.). Resazurin was added (0.4%) as an indicator of presence of oxygen. Purity of cultures was monitored using Bacto plates prepared from media containing 1% Bacto-Tryptone, 0.5% Bacto-Yeast, 1% sodium chloride, and 1.5% Bacto-Agar (Difco Laboratories, Detroit, Mich., U.S.A.). Aliquots (4 mL) of *E. coprostanoligenes* stock culture were used to inoculate 400 mL flasks of media similar to the maintenance media by using the Hungate technique (Holdeman et al., 1977). The flasks were incubated (37 °C) for 48 h. *Lactobacillus* commercial starter culture was used (Lactacel® 115, MicroLife Technics, Sarasota, Fla., U.S.A.).

Preparation of ground pork and mutton

Pork used in experiments 1 and 2 and pork and mutton for experiment 3 were similar to meats that can be purchased in the market place and were obtained from the Meat Science Laboratory at Iowa State University. The pork and mutton were ground through a 0.32 cm plate and mixed to homogenize. Triplicate samples were taken for analysis of pH, total lipids, cholesterol, and coprostanol. The meats were seasoned (3% sodium chloride, 0.75% dextrose, 0.015% sodium nitrate, 0.38% pepper) and vacuum-mixed. Duplicate or triplicate samples were taken for analysis of pH, total lipids, cholesterol, and coprostanol after seasoning, after fermentation, after cooking, and after 1 mo of storage after cooking.

Meat-mix studies

Triplicate 100 g samples of meat-mix were weighed into autoclaved 400-mL or 600-mL beakers and randomly assigned to each treatment (Table 1). Triplicate 100-g ground pork samples were in-

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Table 1—Assignment of meat samples in the 3 studies^a

Treatments	Study	
	Mixed meat, pork	Pork, mutton
	Total (100 g) meat samples used	
1. Uninoculated (control)		
Pork	3	3
2. <i>Lactobacillus</i>		
Pork		
pellet 0.05 g	3	3
3. <i>Lactobacillus</i> (0.05 g)+ <i>E. coprostanoligenes</i>		
Pork		
pellet 0.05 g	3	— ^b
pellet 0.10 g, 0.25 g, 0.5 g	—	9
4. <i>E. coprostanoligenes</i>		
Pork		
liquid media, 1, 2, 10 mL	9	—
pellet 0.05, 0.10, 0.2 g or 0.5 g	8	—
pellet 0.25 g, 0.5 g, 2.0 g	—	8
5. <i>E. coprostanoligenes</i>		
Mutton		
pellet 0.5 g, 1.0 g	—	6

^aIn the mixed meat and pork studies, liquid media and bacterial pellets were used to inoculate duplicate or triplicate 100-g pork samples; in the other study, only bacterial pellets were used to inoculate duplicate or triplicate pork or triplicate mutton samples.
^bNo sample assigned.

oculated with 1-mL, 2-mL, or 10-mL aliquots of a 48-h *E. coprostanoligenes* culture. Remaining bacteria were harvested in a refrigerated Sorvall® RC2-B centrifuge at 750 rpm for 20 min. Bacterial pellets were weighed (wet) in triplicate (0.05 g and 0.1 g) and duplicate (0.2 g). Each bacterial pellet was suspended in 1 mL of sterile phosphate-buffered saline [0.01 M Na₂HPO₄·7H₂O + 0.15 M NaCl, pH 7.5 (PBS)] and used to inoculate a 100-g sample of ground pork. Triplicate ground pork samples were inoculated also with 0.05 g of *Lactobacillus* pellet or a combination 0.05 g of *Lactobacillus* + 0.05 g of *E. coprostanoligenes* pellets; uninoculated pork mixes were used as controls (Table 1). All samples were incubated (37 °C) in jars for 12 h. Treatments 3 and 4 were incubated under anaerobic conditions to facilitate growth of *E. coprostanoligenes* by using gas jars charged with BBL® GasPak Plus™ system (Becton Dickinson, Md., U.S.A.).

After fermentation, duplicate 0.5-g samples from each sausage-type mix were suspended in 1 mL of sterile PBS and used to inoculate a 9 mL culture media with cholesterol (0.2%) for evaluation of the viability of *E. coprostanoligenes* (Fig. 1). A 1-mL aliquot of each culture was taken for analysis of pH or for analysis of cholesterol and coprostanol. A 1-g sample was taken from each pork sausage-type mix to determine pH or moisture content and a 0.5-g sample for total lipids, cholesterol, and coprostanol.

Pork studies

Triplicate 100-g ground pork samples were inoculated with liquid culture as in the meat-mix studies, and bacterial pellets weighed in triplicate (0.05 g and 0.1 g) and duplicate (0.5 g) were used to inoculate the pork samples. All samples were incubated (37 °C) for 15 h. Treatments 3 and 4 were incubated under anaerobic conditions as described.

Viability of *E. coprostanoligenes*, culture pH, meat pH, total lipids, cholesterol, and coprostanol were determined as described. All sausage-type mixes were cooked (68 °C) for 25 min then stored in a freezer. Cooked sausage-type mix pH, total lipids, cholesterol and coprostanol were determined as described. All sausage-type mixes were vacuum-packaged and held in a typical retail display case at an appropriate temperature (≈25 °C to simulate storage of shelf stable fermented products) and sampled after 1 mo after cooking for analysis of pH, total lipids, cholesterol, and coprostanol.

Pork and mutton studies

Triplicate (0.25 g and 0.5 g) and duplicate (2.0 g) samples of

bacterial pellets were weighed for pork samples and triplicate (0.5 g and 1 g) of bacterial pellets for mutton samples. Each amount of bacterial pellet was suspended in 1.5 mL of sterile PBS and used to inoculate a 100-g sample of ground pork or mutton. Triplicate ground pork samples were inoculated with 0.05 g of *Lactobacillus* pellet or the combinations of 0.1 g, 0.25 g or 0.5 g of *E. coprostanoligenes* + 0.05-g *Lactobacillus* pellets; uninoculated pork and mutton mixes were used as controls (Table 1). All samples were incubated (37 °C) for 15 h. Treatments 3, 4, and 5 were incubated under anaerobic conditions as described. After fermentation, cooking, and storage, samples were taken for determination of viability of *E. coprostanoligenes*, analysis of pH, moisture content, total lipids, cholesterol, and coprostanol.

Sample preparation and analytical procedures

Duplicate or triplicate 1-g samples were taken from each raw pork or mutton or fermented sausage-type mix for analysis of pH or moisture content and a 0.5-g sample for total lipids, cholesterol, and coprostanol at each stage of sausage production. Duplicate or triplicate 1-mL samples were taken for pH and cholesterol and coprostanol concentrations.

All pH values were determined in duplicate or triplicate using a 701A digital ionalyzer pH meter (Orion Research®). Each 1-g sample of meat or sausage-mix was suspended in 10 mL of deionized water using a vortex-mixer. Samples (1 mL) were taken for determination of pH of the cultures from fermented sausage-mixes (AOAC, 1980). Duplicates or triplicates (1 g) of raw pork or mutton or fermented sausage-mix were analyzed for moisture by a method described by AOAC (1980). Cholesterol and coprostanol were extracted in duplicate or triplicate (1 mL) samples from cultures from fermented pork or mutton sausage-mixes using 2:1 chloroform:methanol (AOAC, 1980).

Total lipids were extracted from duplicate or triplicate (0.5 g) samples using 18.5 mL of 2.5:1 methanol water + 6.5 mL chloroform as described by Bligh and Dyer (1959). Lipid extracts were saponified with 7.5 mL chloroform and 7.5 mL of aqueous 0.37%

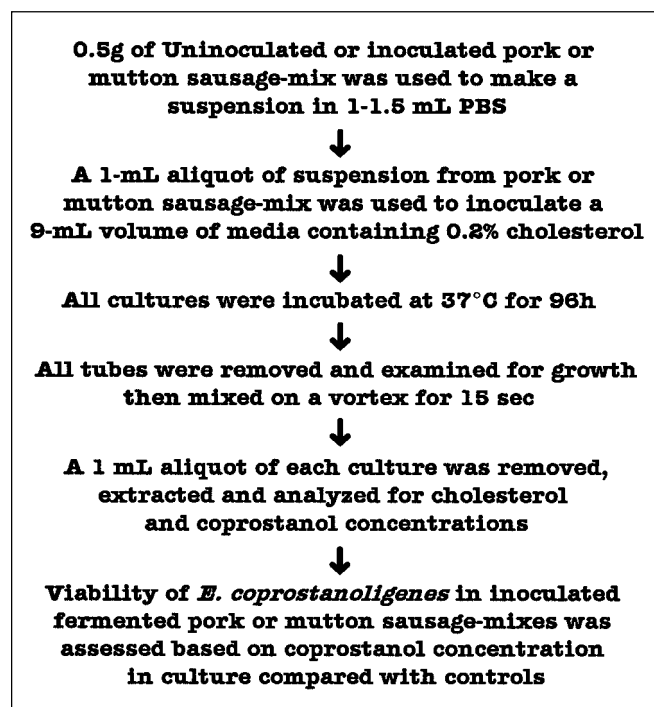


Fig. 1—Procedures used for assessment of the viability of *E. coprostanoligenes* in fermented meat-mix samples.

potassium chloride (KCl) and centrifuged (Intl. Equipment Co., Neeham, Mass., U.S.A.) at 2000 rpm for 25 min. After saponification, the top layer was removed and discarded. Aqueous 0.37% KCl (10 mL) was added to each sample, centrifuged, and the procedures repeated. Lipids were extracted twice with 4 mL of chloroform. All samples were concentrated to dryness under nitrogen gas and total lipids determined.

Cholesterol and coprostanol were extracted from each meat or fermented sausage-mix extract using 4:1 methanol:benzene and 200 μ L of acetyl chloride (100 ± 3 °C) for 1 h. After hydrolysis 5 mL 6% K_2CO_3 were added, extracted with 2 mL benzene, and dried at 65 °C under nitrogen gas. Cholesterol and coprostanol ethers were prepared using silylation grade acetonitrile and 1,3 bis(trimethylsilyl) trifluoroacetamide with 1% trimethyl chlorosilane (Pierce, Rockford, Ill., U.S.A.) (Martin et al., 1972; Subbiah et al., 1972; Overturf et al., 1990). In the meat-mix and pork studies, cholesterol and coprostanol trimethylsilyl ethers were analyzed with a Hewlett-Packard 5830A gas chromatograph, equipped with a flame ionization detector, an integrator, and a glass column (0.9 M, 0.64 mm i.d.), packed with 3% SP-2250 (100/200 Supelcoport) (Supelco®, Bellefonte, Pa., U.S.A.). Column was at 280 °C, with nitrogen carrier gas at 60 mL/min, air at 240 mL/min, and hydrogen at 60 mL/min. Injector was set at 290 °C, detector at 290 °C, and maximum oven temperature at 300 °C. Slope sensitivity was set at 1.5, with chart paper speed of 0.7 cm/min.

In the pork and mutton studies, cholesterol and coprostanol were analyzed with a Tracor 540 gas chromatograph, equipped with a flame ionization detector, split injection system, and capillary column of DB™-5, (30M \times 0.25 μ m i.d. \times 0.25 μ m film thickness) nonpolar bonded phase (5% phenyl)-diphenyl-dimethylpolysiloxane (J & W Scientific, Folsom, Calif., U.S.A.). The column was set at 290 °C with helium as carrier gas at 60 mL/min, air at 400 mL/min and hydrogen at 30 mL/min. Injector was set at 290 °C and detector at 300 °C and a Shimadzu Chromatopac® C-R3A integrator was used with slope sensitivity at 0 and chart paper speed of 0.7 cm/min.

For quantitation, cholestane was used as an internal standard. Trimethylsilyl ethers were identified by comparing retention times with purified standards. Peak areas were determined by a Shimadzu Chromatopac® C-R3A integrator. Concentrations of sterols in each sample extract were obtained by using each sterol standard in each control extract and cholestane as internal standard: the μ g/g of each sterol in each sample = $(A_i/A_x) \times (B_i/B_x) \times (C_i/C_x)$ (AOAC, 1990). Relative amounts of each sterol in each meat sample were expressed as μ g/g (dry matter basis), whereas each sterol in each culture from each meat sample was expressed as μ g/mL of culture.

Statistical analysis

Data were analyzed using the SAS software system package utilizing the Analysis of Variance and Least Significant Differences Procedures. Differences between means were tested at the $P < 0.05$ level.

RESULTS & DISCUSSION

NO DIFFERENCES ($P > 0.05$) WERE OBSERVED IN PH OF RAW (5.57) and seasoned (5.60) pork (data not shown). Thus, addition of spices did not affect initial sausage-type mix pH. The pH values of fermented pork sausage-mixes treated (Treatment 2) with *Lactobacillus* alone (5.18) and combinations (Treatment 3) of 0.5 g each of *Lactobacillus* and *E. coprostanoligenes* pellets (4.44) were different ($P < 0.05$) and lower ($P < 0.05$) than those of the other treatments, which were not different ($P > 0.05$) from the control. Sausage-mixes in Treatments 2 (pH 4.96) and 3 (pH 4.66) of the pork study were different ($P < 0.05$) and lower ($P < 0.05$) than pH values of the other treatments and control. The pH values of cultures from sausage-mixes in the meat-mix study and sausage-mixes after cooking in the pork study, in Treatments 2 and 3, were different ($P < 0.05$) and lower ($P < 0.05$) than those of the other treatments. No differences ($P > 0.05$) were observed in pH values of cultures in the pork study (data not

shown).

In the pork and mutton, differences ($P < 0.05$) were observed in pH values of raw and seasoned pork and mutton (Fig. 2). After fermentation, after cooking, and after storage after cooking, pH values of pork sausage-mixes of Treatments 2 and 3 were different ($P < 0.05$) and lower ($P < 0.05$) than those of the other treatments (Fig. 2). After fermentation, pH values of mutton sausage-mixes of Treatment 5 were lower ($P < 0.05$) than that of the control. No differences ($P > 0.05$) were observed in pH values of mutton sausage-mixes after cooking but pH decreased ($P < 0.05$) after storage. The pH values of cultures from treated pork and mutton sausage-mixes were higher ($P < 0.05$) than those of controls (Fig. 2).

In all three studies, *E. coprostanoligenes* or *Lactobacillus* or combinations of *E. coprostanoligenes* and *Lactobacillus* lowered ($P < 0.05$) pH values of fermented pork or mutton sausage-mixes when compared (Fig. 2) to controls. Combinations of both bacteria caused greater decrease ($P < 0.05$) in pH. Wu et al. (1991) reported no differences ($P > 0.05$) in pH of sausages during production when a specific pH endpoint was used. Differences ($P < 0.05$) observed in pH in our results occurred because no specific pH endpoint was used. Freier (1991) and Freier et al. (1994) found that decreased media pH was caused by acids produced by *E. coprostanoligenes*. Wu et al. (1991) reported that pH of fresh mutton sausages stored for 120 days at 2 to 4 °C did not differ ($P > 0.001$) but pH increased ($P < 0.05$) in sausages stored at 20 to 22 °C.

In the meat-mix study, pH values of cultures from sausage-mixes in Treatments 2 and 3 were lowered ($P < 0.05$) when compared to controls and those of the other treatments. In the pork study, pH

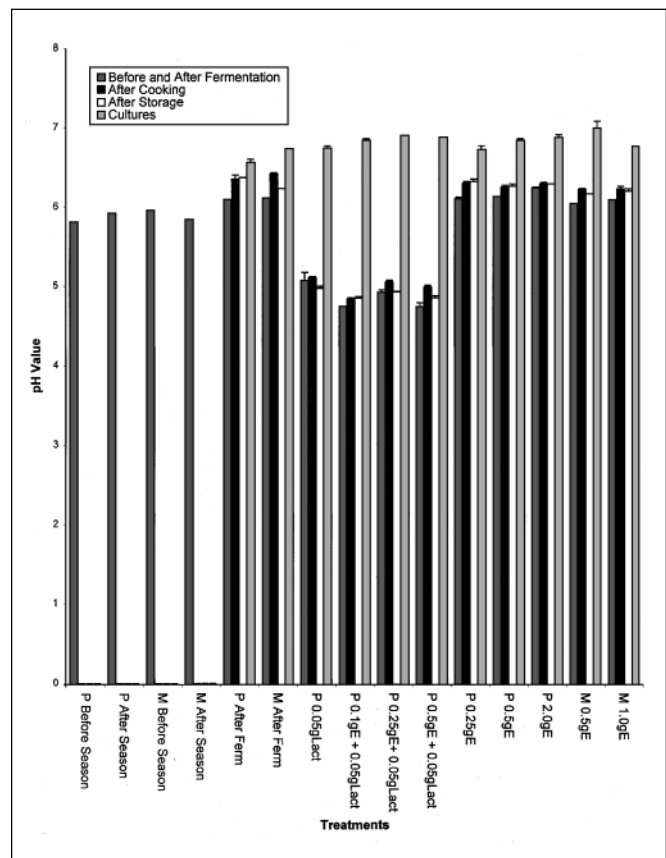


Fig. 2—*Eubacterium* (E) and *Lactabacillus* (Lact) effects on pH of pork (P) and mutton (M) sausage-mixes before and after 15-h fermentation (Fern) (37 °C), after cooking, and after 1 mo storage and in cultures. Season=seasoning. Values represent treatment means \pm SEM of triplicate samples except treated with 2.0 g E in duplicate. Treatments were not replicated.

values of cultures did not differ ($P > 0.05$) (data not shown). In the pork and mutton study, pH values of cultures from treated sausage-mixes increased ($P < 0.05$) when compared (Fig. 2) to those of controls. The variable increases in pH observed among cultures may be attributed to the amounts of basic amino acids in the meat-suspensions (PBS, pH 7.5) added to each culture tube. Wu et al. (1991) indicated that increased pH observed in sausages stored at 20 to 22 °C for 120 days was associated with proteolytic release of basic amino acids.

Lipid content. Lipid percentages observed in the meat-mix study after fermentation (dry matter basis) in treated sausage-type mixes were higher ($P < 0.05$) than those of controls. However, differences ($P < 0.05$) were among and within treatments. In the pork study, no differences ($P > 0.05$) were observed in lipid percentages of sausage-mixes except in those inoculated with 0.1 g of bacterial pellet or 1.0 mL or 2.0 mL of bacterial culture (data not shown).

In the pork and mutton study, lipid percentages on a dry matter basis in pork sausage-type mixes in Treatment 2 and in Treatment 3 inoculated with 0.1 g or 0.5 g of bacterial pellet were higher ($P < 0.05$) than those of controls and others (Fig. 3) that were not different ($P < 0.05$). Lipid percentages of treated mutton sausage-mixes were lower ($P < 0.05$) than controls. After cooking, lipid percentages of treated pork sausage-mixes were higher ($P < 0.05$) than the control except (Fig. 3) for those inoculated with 0.1 g of bacterial pellet in Treatment 3 that were not different ($P > 0.05$) from the control. Treated mutton sausage-mixes had higher ($P < 0.05$) lipid percentages than the control (Fig. 3). After 1 mo storage after cooking, lipid percentages of treated pork sausage-mixes were higher ($P < 0.05$) (dry matter basis) than those of controls, except (Fig. 3) those in Treatment 2 and in Treatment 3 or 4 inoculated with 0.1 g or 0.25 g of *E. coprostanoligenes* that were lower ($P < 0.05$). Lipid percentages of mutton sausage-mixes inoculated with 1.0 g *E. coprostanoligenes* were higher ($P < 0.05$) than the control (Fig. 3).

The variability observed in lipid percentages of sausage-type mixes may have resulted from conditions under which the mixes were processed, size of inoculum used, and interactions of bacteria with the mixes. Bacus (1984) reported that lipolytic microorganisms could degrade meat fats by hydrolysis via a lipase and/or oxidation by oxidases liberating fatty acids. Freier (1991) and Freier et al. (1994) found that *E. coprostanoligenes* had phospholipase activity and required lecithin for growth. Lecithin was shown to hydrolyze because free fatty acids were detected in the media, but lecithin was not detected. *E. coprostanoligenes* may have caused hydrolysis of cholesterol esters in the meats liberating cholesterol and free fatty acids, resulting in variability of lipids extracted.

Cholesterol and coprostanol. Cholesterol concentrations of raw ground pork (dry matter basis) before fermentation in the meat-mix study were lower ($P < 0.05$) than those of fermented sausage-type mixes, but were higher ($P < 0.05$) in the pork study. Differences ($P < 0.05$) in cholesterol concentration were observed among and within treatments in both studies (data not shown). Cultures from control sausage-mixes in the meat-mix study had higher ($P < 0.05$) cholesterol concentrations than those of treated sausage-mixes. In the pork study, cholesterol concentrations in cultures from sausage-mixes in Treatment 3 and in Treatment 4 inoculated with 0.1-g or 0.5-g pellet or 1 mL of *E. coprostanoligenes* culture were different ($P < 0.05$) from controls and other treatments that were not different ($P > 0.05$). No coprostanol was detected in any sausage-mix or culture except in cultures from sausage-mix inoculated with 0.5 g of *E. coprostanoligenes* pellet (data not shown).

In the pork and mutton study, cholesterol concentrations observed (dry matter basis) in raw ground pork control were not different ($P > 0.05$) before and after fermentation. However, differences ($P < 0.05$) were found among and within treated pork sausage-type mixes (Fig. 4). Sausage-mixes in Treatment 3 had lower ($P < 0.05$) cholesterol concentrations than all treatments and controls. Treated mutton sausage-mixes had lower ($P < 0.05$) cholesterol concentrations when

compared (Fig. 4) to controls. Small amounts of coprostanol were detected in control pork and mutton before fermentation, in mutton controls after fermentation, and in treated pork and mutton sausage-mixes (Fig. 5). However, in pork sausage-mixes in Treatment 4 inoculated with 2.0 g *E. coprostanoligenes*, amounts were higher ($P < 0.05$) than those of all other sausage-mixes. The small amounts of coprostanol detected in sausage-mixes were not different ($P > 0.05$).

After cooking, no differences ($P > 0.05$) in cholesterol or coprostanol concentrations were observed in pork sausage-mixes (Fig. 4 and 5). Treated mutton sausage-mixes had higher ($P > 0.05$) cholesterol concentrations (Fig. 4) than controls and coprostanol concentrations were not different ($P < 0.05$) (Fig. 5). After 1 mo storage following cooking, cholesterol was detected in pork and mutton sausage-mixes, but no coprostanol was detected in any sausage-mix (data not shown). Cholesterol concentrations in cultures from control pork sausage-mixes were lower ($P > 0.05$) than all treatments except (Fig. 4) Treatment 3 inoculated with 0.5 g *E. coprostanoligenes* pellet that were not different ($P > 0.05$). Cultures from mutton sausage-mixes inoculated with 0.5 g of *E. coprostanoligenes* pellet had higher ($P < 0.05$) cholesterol concentrations than the other mutton treatments (Fig. 4). Small amounts or no coprostanol were detected in cultures from pork or mutton sausage-mixes except (Fig. 5) in Treatment 4 inoculated with 2.0 g of *E. coprostanoligenes* that were higher ($P < 0.05$) than the other treatments.

Variations observed in cholesterol concentrations in sausage-type mixes and cultures could have resulted from several factors: size of ground meats used, amount of cholesterol in the meats, type of starter culture used, interactions of bacteria with condiments and meats, and conditions under which the sausage-mixes were processed. Wu

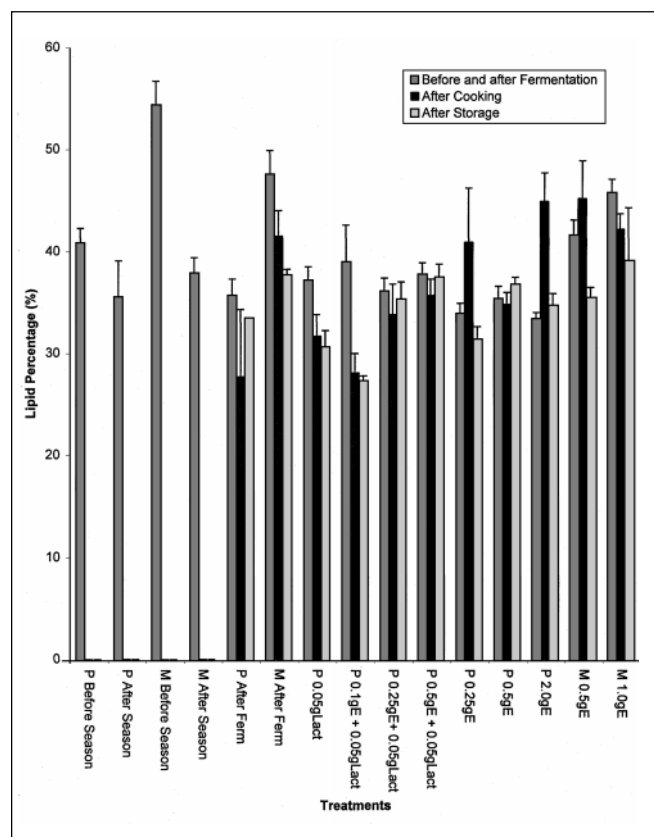


Fig. 3—*Eubacterium* (E) and *Lactabacillus* (Lact) effects on lipid content of pork (P) and mutton (M) sausage-mixes before and after 15-h fermentation (Ferm) (37 °C), after cooking, and after 1 mo storage. Season = seasoning. Values represent treatment means ± SEM of triplicate samples except treated with 2.0 g E in duplicate. Treatments were not replicated.

et al. (1991) reported greater ($P > 0.05$) cholesterol in high-temperature-stored products, but no differences ($P < 0.05$) for fresh-cooked and 120-day sausages at 2 to 4 °C or 20 to 22 °C. Bacus (1984) reported that lipolytic microorganisms degraded meat fats by hydrolysis via lipase and/or oxidation by oxidases, liberating fatty acids. Freier (1991) and Freier et al. (1994) reported that *E. coprostanoligenes* had phospholipase activity and may have caused hydrolysis of cholesteryl esters in the sausage-mixes, liberating cholesterol and free fatty acids during fermentation and resulting in variations in extraction and detection of cholesterol. Wu et al. (1991) reported that mutton sausages made with *Pediococcus acidilactici* H had a lower ($P < 0.05$) percent retention of cholesterol during processing and storage than those made with Lactacel 75 and with *Lactobacillus plantarum* 27.

Small amounts or no coprostanol detected in sausage-mixes and cultures, except (Fig. 5) when inoculated with 2.0 g of *E. coprostanoligenes*, may be dependent on the dose of bacteria, availability of cholesterol; viability of the bacteria; and conditions suitable for bacterial growth, for production of cholesterol reductase, and for reduction of cholesterol to coprostanol. Bacus (1984) reported that fatty acids liberated by interactions of bacteria with meats may be inhibitory to microorganisms. *E. coprostanoligenes* may have caused hydrolysis of cholesteryl esters in the meats, liberating free fatty acids, which may have affected their ability in some meat-mixes to reduce cholesterol to coprostanol. Freier (1991) and Freier et al. (1994) found that *E. coprostanoligenes* did not grow or convert cholesterol to coprostanol when fatty acids and cholesterol were added to basal media. The greatest amounts of coprostanol were in media with initial pH

7.2, 7.5, and 7.0, and final media pH 6.4, 6.3, and 6.2 after incubation (Fig. 2 and 5). No coprostanol was in cultures from sausage-mixes with initial meat pH 4.74 to 4.92 in our study. This confirms the results of Freier (1991) and Freier et al. (1994) that no coprostanol was in any media with initial pH of 5.55. Small amounts or no coprostanol were in sausage-mixes or cultures with pH 4.74 to 6.12 or pH 6.72 to 6.89, except (Fig. 2 and 5) when inoculated with 2.0 g of *E. coprostanoligenes* with pH 6.24 and 6.87.

For practical efficacy of *E. coprostanoligenes* application to decrease cholesterol content of meats, our results have identified several areas of specific research needs: determination of (1) maximal growth phase of bacteria; (2) optimal pH and temperature; (3) optimum size of ground meat particles; (4) adequate amount of inoculum; (5) consistency of the ground meat; (6) optimal protein, fat, and moisture of the meat; and (7) optimal time for adequate bacterial growth to reduce cholesterol to coprostanol.

CONCLUSIONS

E. COPROSTANOLIGENES OR *LACTOBACILLUS* OR COMBINATIONS of *E. coprostanoligenes* and *Lactobacillus* lowered pH values in sausage-type meat mixes. Variations in lipid content and cholesterol concentrations may be attributed to the lipolytic characteristic of *E. coprostanoligenes*. Cholesterol concentrations decreased in pork sausage-mixes treated with the combinations and in mutton sausage-mixes treated with *coprostanoligenes*. Coprostanol in pork and mutton sausage-mixes and cultures confirmed that *E. coprostanoligenes*

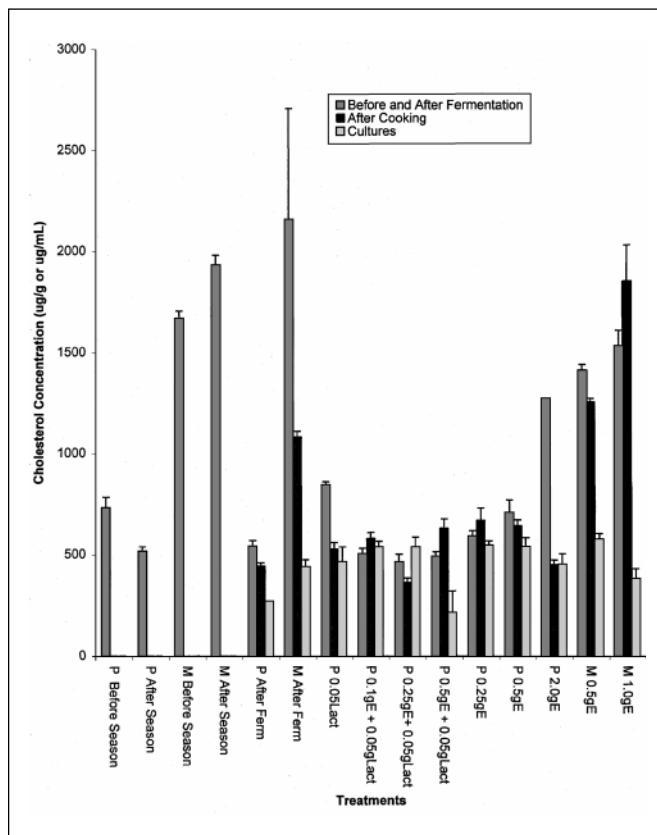


Fig. 4—*Eubacterium* (E) and *Lactabacillus* (Lact) effects on cholesterol concentrations of pork (P) and mutton (M) sausage-mixes before and after 15-h fermentation (Ferm) (37 °C), after cooking, and in cultures. Season=seasoning. Values represent treatment means \pm SEM of triplicate samples except treated with 2.0 g E in duplicate. Treatments were not replicated.

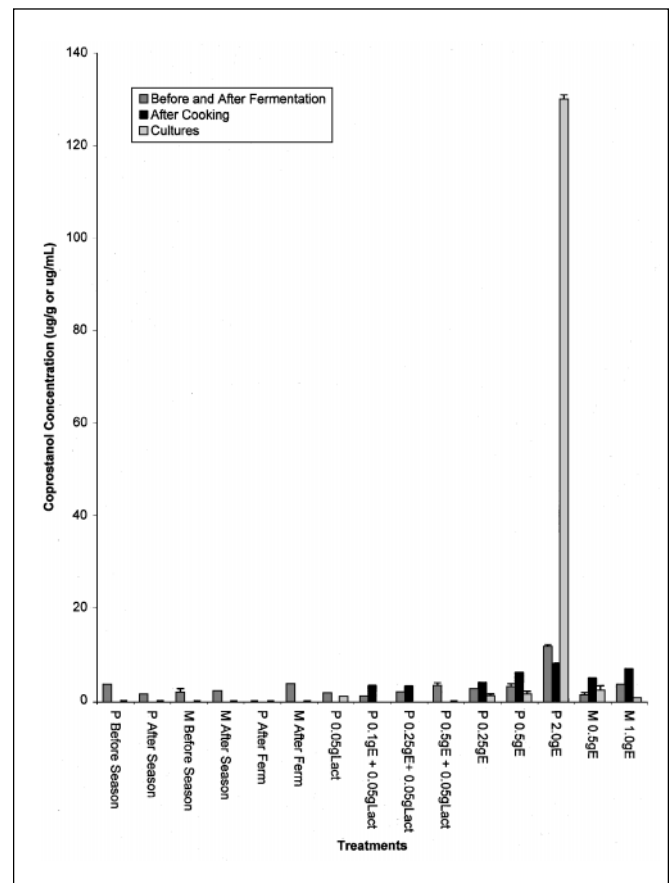


Fig. 5—*Eubacterium* (E) and *Lactabacillus* (Lact) effects on coprostanol concentrations of pork (P) and mutton (M) sausage-mixes before and after 15-h fermentation (Ferm) (37 °C), after cooking, and in cultures. Season=seasoning. Values represent treatment means \pm SEM of triplicate samples except treated with 2.0 g E in duplicate. Treatments were not replicated.

would remain viable during fermentation while converting cholesterol to coprostanol and would maintain its ability to reduce cholesterol to coprostanol after fermentation.

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