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Antagonistic Effect of Fatty Acids Against *Salmonella* in Meat and Bone Meal

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The purpose of this study was to determine whether fatty acids have an antagonistic effect on the growth and viability of *Salmonella* organisms. Thirty-two different lipid materials were used, utilizing a wide range of short and long free fatty acid chains expressed as per cent oleic acid.

Pathogenic *Salmonella* organisms are widely distributed in nature and may be transmitted via man, animals, and birds (4). Animal by-product meals may become recontaminated after preparation and could, in turn, contaminate mixed feeds. In a recent report covering a survey of inedible rendering plants (5), it was hypothesized that there may be a *Salmonella* antagonist, possibly free fatty acids, in partially decomposed fatty tissues. These *Salmonella* antagonists appear to have a similar effect on the viability and growth of gram-positive and liptospirae microorganisms (3, 6). Van der Schaaf-Bilthoven (8) found that the addition of acetic acid and butyric acid to nutrient media in the same quantities which occur in the caecum and colon of normal mice inhibited completely the growth of *S. enteritidis* at acidities below *p*H 6.2. Tests in vitro showed that nonvolatile acids of the citric acid cycle prevented the growth-inhibiting action of the volatile fatty acids.

The effectiveness of antibacterial action of fatty acids has been acknowledged by Bayliss (1). It has been reported that germination of the spores of *Clostridium botulinum* is inhibited by oleic acid (2). Certain viruses are also reported to be repressed by fatty acids (7).

MATERIALS AND METHODS

Commercially produced solvent-extracted meat and bone meal was used for all experimental work. This material conformed in all respects to the official definition of meat and bone meal as published by the Association of American Feed Control Officials (1968 ed., p. 58). In these experiments, the effectiveness of a variety of fatty acids, singly and as blends (Table 1), were examined for their ability to repress the growth of *Salmonella* in a solvent-extracted commercial meat and bone meal.

The meat and bone meal was degreased completely by extraction with chemical grade *n*-heptane and sterilized in the autoclave for 15 min at 15 psi and 120 C. Different combinations of fats and fatty acids were added to the sterile meal, so that the finished blend contained 5, 10, 15, 20, 25, and 50% free fatty acid levels by adding a known composition to the defatted meat meal. The samples were then inoculated with the *Salmonella* culture and incubated for 24 hr at 37 C. After the initial 24-hr incubation period, the bacterial count was taken at 24, 48, and 72 hr, and at 1 week on Brilliant Green (BG) agar. The average of these four counts was taken and related to the free fatty acid content of the experimental materials (Table 1).

Different species of microorganisms show wide variations in tolerance to antimicrobial agents. The degree of effectiveness may be determined by measuring the diameter of the zone of inhibition. The procedure used in these experiments is a modification of the sensitivity method (9). To perform this test, a 24-hr *Salmonella* culture was prepared at 37 C in selenite cystine broth. The inoculum was spread evenly by cotton swabs on petri plates (100 by 15 mm) consisting of blood-agar BG agar, eosin methylene blue agar, and *Salmonella-Shigella* agar. Paper discs, 12.7 mm in diameter, were soaked for a few seconds in the respective experimental materials. Three discs were suspended by forceps in each petri plate and were pressed firmly to the surface of the medium. Each plate contained 20 ml of medium. The plates were incubated for 24 hr at 37 C and were checked for the degree of inhibition by measuring the diameter of each zone. A pour plate method was also evaluated.

A BG agar was thoroughly mixed with a known amount of *Salmonella* culture and with 5% of each of the experimental materials. The agar containing the *Salmonella* culture and the fatty material was then poured into petri dishes. The plates were incubated for 48 hr at 37 C. The growth was checked by observation only.

The culture used for all of the experiments was a mixture of the following *Salmonella-Shigella* strains: (i) *S. senftenberg* ATCC 8400, (ii) *S. typhi* ATCC 6539, (iii) *S. choleraesuis* ATCC 10708, (iv) *S. typhimurium* ATCC 6994, and (v) *Shigella flexneri* ATCC 9199. For the preparation of inoculum, fresh cultures were used. A 1.0-ml amount of each serotype culture was introduced into 100 ml of selenite cystine broth and was incubated for 48 hr at 37 C. A 10-ml amount of

TABLE 1. *Thirty-two experimental materials tested for their ability to inhibit Salmonella growth*

Exptl materials	Free fatty acids in order of per cent oelic acid	No. of viable *Salmonella* in meat meal after treatment[a]	Inhibitory effect of fatty acids against *Salmonella* on BG agar (zone of inhibition, mm)
Edible tallow (stabilized)	0.56	600	15.0
Edible tallow (unstabilized)	0.81	700	14.7
Top white tallow	1.90	500	14.0
Bleachable fancy tallow	3.9	300	15.0
Special tallow	7.0	300	14.7
Yellow grease	10.0	800	14.3
No. 2 tallow	25.0	1,500	14.7
Top white (nonanoic acid blend)	28.0	700	15.0
A-white grease (butyric acid blend)	28.5	0	40.0
Yellow grease (nonanoic acid blend)	30.0	600	14.0
Edible oil (butyric acid blend)	30.7	0	30.0
Fancy tallow (nonanoic acid blend)	31.0	600	15.0
Edible oil (nonanoic acid blend)	32.0	600	14.7
Choice white (butyric acid blend)	35.2	0	30.7
Special tallow (nonanoic acid blend)	37.0	600	13.7
Fancy tallow (butyric acid blend)	41.0	0	38.3
Hi-acid fancy tallow (butyric acid blend)	41.4	0	32.3
Yellow grease (butyric acid blend)	42.3	0	40.0
Special tallow (butyric acid blend)	43.0	0	34.7
Oleic acid (BFT blend)[c]	50.0	1,300	15.0
Oleic acid (yellow blend)	71.0	1,000	14.7
Palmitic acid	101.0	600	13.3
Stearic acid	102.5	500	13.7
Commercial oleic acid	103.6	800	15.7
Linoleic acid	104.0	500	15.3
Red oil (nonanoic acid blend)	114.0	500	15.7
Undecanoic acid	158.0	80	15.3
Nonanoic acid	177.0	100	16.0
Caprylic acid	200.0	70	17.3
Caproic acid	246.0	0	37.3
Valeric acid	277.0	0	TPI[b]
Butyric acid	328.0	0	TPI

[a] Treatment was with a 5% concentration of experimental materials. Control samples; counts ranged from 1,100 to 2,400 per g.

[b] Total plate inhibition.

[c] BFT, bleachable fancy tallow.

this culture added to 100 g of meat meal was used as a standard inoculum.

RESULTS AND DISCUSSION

The sensitivity and pour plate techniques were used to determine the effect of 32 fatty materials, containing various amounts of free fatty acids, on *Salmonella*. The results of these tests are presented in Table 1. Fatty materials of relatively high molecular weight, including both triglycerides and free fatty acids containing nine or more carbon atoms (Table 1), inhibited *Salmonella* growth to some extent when evaluated by the pour plate and sensitivity methods. When these triglycerides and high molecular weight fatty acids were added to meat and bone meal inoculated with *Salmonella*, the initial bacterial count was immediately reduced and no additional change occurred when the samples were incubated for 1 week at 37 C.

TABLE 2. *Zone of inhibition of antimicrobial agents on Salmonella*

Antimicrobial agents	Zone of inhibition (mm)[a]				
	1%	3%	5%	7%	10%
Acetic acid	18.3	24.6	28.7	32.3	35.7
Propionic acid	19.1	25.2	27.3	29.3	31.4
Lactic acid	15.4	19.6	21.9	23.7	26.1
Citric acid	15.3	19.4	21.8	23.3	25.8
Phosphoric acid	16.4	22.4	25.1	30.5	33.2

[a] At 5%, long chain fatty acids had an average zone of inhibition of 15 mm.

Free fatty acids of low molecular weight (butyric, valeric, caproic) inhibited the growth of *Salmonella* almost completely in the culture media when either the sensitivity or pour plate method

was used. When these fatty acids were added to meat and bone meal inoculated with *Salmonella*, no viable organisms could be recovered from the meat and bone meal samples. Blends of butyric acid with fats of different grades (Table 1) exhibited the same inhibitory and antagonistic effect against *Salmonella* organisms in culture media and meat and bone meal.

In view of these results and those obtained by other investigators, it appeared likely that shorter chain fatty acids could exert a bacteriostatic or a bactericidal or both types of effect on *Salmonella*. It also appeared that other organic and inorganic acids should either exert an antagonistic effect or enhance the activity of these volatile fatty acids through their acidity. It was shown that this is indeed true (Table 2). Citric, lactic, phosphoric, acetic, and propionic acids all exhibited inhibitory effects on *Salmonella*.

Thus, fatty materials, particularly the low molecular weight volatile fatty acids, exert inhibitory and, in some cases, bactericidal effects against *Salmonella* not only in culture media but also in high protein meal. These findings may form the basis for the development of formulations containing these nontoxic, naturally occurring compounds to control *Salmonella* in products produced by the rendering and allied industries.

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