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Aerobic Growth of *Listeria monocytogenes* on Beef Lean and Fatty Tissue: Equations Describing the Effects of Temperature and pH

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ABSTRACT

The aerobic growth rate and the duration of the lag period were determined for *Listeria monocytogenes* strain Murray B growing on ground beef lean and on pieces of fatty tissue. The organism grew at 0°C on lean tissue at pH ≥ 6 and on fatty tissue. It failed to grow at 0°C on lean at pH 5.6 but did grow at 2.5°C. The effect of temperature, between 0 and 30°C, on the growth rate on fatty tissue can be described by a modified Arrhenius equation Ln (gen/h) = -205.73 + 1.2939 x 10^5/K -2.0298 x 10^7/K^2, where K = °Kelvin. This equation accounted for 99.7% of the variance. The combined effect of temperature and pH on the growth rate on beef lean was described by Ln (gen/h) = - 232.64 + 1.4041 x 10^5/K - 2.1908 x 10^7/K^2 + 1.1586 x 10^2/pH - 4.0952 x 10^2/pH^2 (variance accounted for 99.5%). For lean at about pH 5.5-5.6, this equation applied between about 2.5 and 35°C; for lean of pH 6-7, it applied between about 0 and 35°C. Though the lag period increased with decrease in temperature and pH, measured lag times were more variable than generation times, and the goodness of fit of modified Arrhenius equations to lag times was relatively poor (variance accounted for 83-92%).

Listeria monocytogenes is frequently found on meats (*15*) and is able to grow on a number of chilled meats, particularly if the storage temperature is near 5°C and the pH of the meat is ≥6 (*5,10,11*). However, no detailed studies have been made of the effects of both temperature and pH on the growth of *L. monocytogenes* on meat.

There is considerable interest in the development of predictive mathematical models to describe the effects of different parameters on the growth of potential pathogens (*2,3,8,9*). By fitting equations to growth data, a considerable amount of information can be relatively easily condensed into a readily usable form. The equations can then be used by the food industry to estimate the possible extent of growth in foods stored under a variety of conditions. Often the models are derived from results obtained with laboratory broths and are then used to predict the extent of growth of the organism in a variety of foods (*3,9*). While the growth rates predicted from studies with broth systems have sometimes agreed with the data obtained in foods (*9*), at other times the predicted rates have been more rapid (*3*). In this paper the aerobic growth responses of *L. monocytogenes* on beef tissue have been determined and modeled.

The aims of this study were (a) to determine the effects of incubation temperature, the type of tissue, and the pH of lean on the aerobic growth of *L. monocytogenes* strain Murray B on beef and (b) to compare the fit of two of the published mathematical models to measured growth rates and lag times. The two models chosen were the square-root (*22*) and the modified, additive Arrhenius (*8*) equations. Growth rates on beef lean tissue were also compared with published growth rates for a number of foods.

MATERIALS AND METHODS

Preparation of inoculum

A culture of *L. monocytogenes* strain Murray B (Food and Drug Administration, Cincinnati) was grown at 10°C for 3 d in buffered broth (glucose, 2.5 g; tryptone, 15 g; yeast extract, 3 g; dipotassium phosphate, 5.3 g; monosodium phosphate dihydrate, 3.05 g; distilled water, 1 L), and 0.005 to 0.01 ml of the culture (40-80 Klett units; Klett-Summerson colorimeter with a No. 66 filter) was diluted into 100 ml of distilled water to provide the inoculum for the meat.

Lean and fatty tissues

Postrigor beef muscles (*M. semitendinosus*) and fatty tissue with some muscle (*M. biceps femoris*) still attached, were obtained from a commercial boning room. Fatty tissue was selected so that the pH of the attached muscle was 5.5-5.7 (combination spear electrode). Lean tissue samples were selected so that the pH of the *M. semitendinosus* ranged from about 5.5-7. Slices of fatty tissue were prepared by removing the contaminated outer regions of fatty tissue and all of the lean. The outside surface of the *M. semitendinosus* was flamed with a Bunsen burner and the cooked outer layer aseptically removed. Both lean and fatty tissues were cut into pieces (ca. 6 x 1 x 1 cm).

Inoculation and incubation

Pieces of lean and fatty tissues were dipped (1-2 s) into the inoculum and allowed to drain for 1-2 min on sterile towels. Each piece of fatty tissue was placed in a test tube (2.5 cm diameter x 15.3 cm). For each storage trial, 12 to 15 test tubes containing the fatty tissue samples were incubated in a temperature-controlled (± 0.05°C), circulating water bath.

Pieces of inoculated lean tissue were comminuted in a blender bowl (Sunbeam Corp. Ltd, Sydney, Australia). Approximate 4-g quantities of the finely minced lean tissue were dispensed into

polyethylene bags (low-density polyethylene, 0.33 mm thick; oxygen transmission rate, ca. 4700 ml/m²/24 h/101KPa at 25°C) and the bags heat sealed. The lean tissue mince was spread as uniformly as possible within each bag (8.7 x 13 cm) to give a thickness of mince of ca. 0.5 mm. The bags (12-15) were placed on wire-mesh racks in air in a sealed container which was submerged in a temperature-controlled (± 0.05°C), circulating water bath.

At least 15 measurements of the bath temperature were taken during each storage experiment, and the mean temperature used as the experimental storage temperature (Tables 1 and 2).

TABLE 1. *Aerobic growth of* L. monocytogenes *on beef fat.*

Exp	Temp (°C)	Meas growth (Gen/h)	Calc[a] growth (Gen/h)	Calc[b] growth (Gen/h)	Meas lag (h)	Calc[c] lag (h)	Calc[d] lag (h)
1	0.0	0.0159	0.0168	0.0057	111.52	55.56	37.38
2	0.0	0.0148	0.0168	0.0057	73.79	55.56	37.38
3	2.5	0.0334	0.0308	0.0257	9.43	24.13	13.67
4	4.7	0.0584	0.0514	0.0567	25.60	12.21	7.36
5	4.8	0.0542	0.0525	0.0584	3.24	11.87	7.19
6	7.5	0.0976	0.0909	0.112	5.78	5.88	4.17
7	10.1	0.154	0.146	0.179	4.28	3.29	2.76
8	10.1	0.150	0.146	0.179	5.99	3.29	2.76
9	14.9	0.329	0.312	0.349	0.38	1.42	1.51
10	15.0	0.309	0.314	0.352	2.23	1.40	1.50
11	19.8	0.539	0.566	0.575	1.59	0.82	0.95
12	19.9	0.481	0.572	0.581	0.48	0.81	0.95
13	22.0	0.659	0.705	0.696	1.00	0.70	0.80
14	24.8	0.956	0.897	0.867	0.99	0.61	0.65
15	25.0	0.872	0.910	0.880	0.48	0.61	0.64
16	27.4	1.14	1.07	1.04	1.03	0.58	0.55
17	30.6	1.45	1.28	1.28	0.54	0.60	0.45
18	30.6	1.23	1.28	1.28	0.33	0.60	0.45

[a] Growth rate calculated from equation 1 (Table 3).
[b] Growth rate calculated from equation 7 (Table 4).
[c] Lag time calculated from equation 2 (Table 3).
[d] Lag time calculated from equation 8 (Table 4).

Viable count

The bags containing the mince were opened and the layer of lean tissue scraped into a tared stomacher bag. The lean tissue was blended for 1 min with nine times its mass of distilled water using a Colworth Stomacher, Model 400 (A. J. Seward and Co. Ltd, London, England). The piece of fatty tissue in each test tube was transferred to a tared blender bowl, and the tube rinsed with a volume of 0.1% peptone water equal to nine times the mass of tissue. The tissue and rinsings were then blended (Sunbeam blender).

Appropriate dilutions were made in 0.1% peptone water. Portions (0.1 or 0.2 ml) were spread on the surface of tryptone soy agar (Oxoid, Basingstoke, England) supplemented with 0.2% yeast extract (Oxoid) and 0.2% glucose (TYSG), and on the selective agar of Lee and McClain (*17*) modified by the addition of 0.05% esculin and 0.05% ferric citrate (ELPM). One set of TYSG plates was incubated at 25°C for 3 d, a second set at 37°C for 24 h, and the ELMP plates were incubated at 37°C for 24 h. The set of TYSG incubated at 25°C enabled psychrotrophic contaminating flora to be assessed. Experiments in which contaminating flora exceeded 10% of the *Listeria* count were discarded. Suspect *Listeria* colonies were counted on all plates. On TYSG, colonies of *Listeria* were bluish in color when viewed by 45° incident light; on ELPM, colonies were surrounded by a black halo. Three to five suspect *Listeria* colonies were isolated and confirmed as *L. monocytogenes* (*12*).

TABLE 2. *Aerobic growth rate of* L. monocytogenes *on beef lean.*

Exp	pH	Temp (°C)	Meas[a] growth (Gen/h)	Calc[b] growth (Gen/h)	Calc[c] growth (Gen/h)	Meas[a] lag (h)	Calc[d] lag (h)	Calc[e] lag (h)
1	5.61	0.0	N.G.	0.0102	0.0018	N.G.	301.6	316.6
2	5.6	2.4	0.0171	0.0187	0.0124	338.4	135.3	81.60
3	5.55	2.5	0.0172	0.0181	0.0117	298.7	153.7	110.6
4	5.56	5.1	0.0378	0.0336	0.0333	96.40	67.23	41.07
5	5.51	5.5	0.0313	0.0347	0.0340	106.7	71.03	52.09
6	5.46	10.0	0.0885	0.0799	0.0849	20.61	27.86	28.64
7	5.46	10.1	0.0931	0.0813	0.0864	23.25	27.27	28.12
8	5.54	10.1	0.0937	0.0887	0.101	20.60	20.71	15.78
9	5.61	10.1	0.111	0.0952	0.112	15.94	16.62	11.35
10	5.46	15.0	0.180	0.177	0.178	9.62	11.15	13.34
11	5.49	15.0	0.179	0.183	0.189	4.63	10.03	10.64
12	5.55	15.5	0.200	0.208	0.220	8.98	7.67	7.15
13	5.46	20.0	0.333	0.327	0.305	6.96	6.06	7.68
14	5.46	20.0	0.316	0.327	0.305	5.28	6.06	7.68
15	5.56	22.3	0.431	0.458	0.444	2.54	3.57	3.51
16	5.59	22.6	0.450	0.484	0.473	2.56	3.18	3.04
17	5.55	24.9	0.536	0.559	0.537	2.31	3.20	3.00
18	5.59	25.0	0.545	0.586	0.570	2.24	2.81	2.53
19	5.6	25.4	0.597	0.609	0.595	9.72	2.68	2.37
20	5.48	26.5	0.589	0.579	0.541	-	3.84	3.88
21	5.56	27.3	0.659	0.664	0.649	2.09	2.88	2.42
22	5.59	27.4	0.643	0.687	0.678	2.00	2.62	2.14
23	5.57	29.8	0.774	0.766	0.778	2.18	2.74	1.97
24	5.6	29.8	0.769	0.790	0.808	2.04	2.50	1.76
25	5.47	35.0	0.811	0.813	N.D.	4.88	4.43	N.D.
26	5.48	35.0	0.822	0.822	N.D.	6.96	4.27	N.D.
27	5.46	43.2	N.G.	0.799	N.D.	N.G.	8.94	N.D.
28	5.73	14.8	0.231	0.224	0.250	2.71	5.01	4.22
29	5.73	35.0	1.24	1.05	N.D.	1.29	2.00	N.D.
30	6.06	0.0	0.0123	0.0139	0.0038	85.47	110.1	65.00
31	6.09	2.4	0.0293	0.0265	0.0207	30.34	45.18	22.59
32	6.09	4.8	0.0543	0.0467	0.0505	12.39	21.40	11.60
33	6.09	10.0	0.149	0.132	0.158	4.63	5.83	4.52
34	6.1	14.4	0.278	0.271	0.298	2.95	2.50	2.56
35	6.11	14.9	0.292	0.289	0.315	2.59	2.31	2.42
36	6.08	15.7	0.321	0.322	0.345	1.80	2.12	2.27
37	6.11	19.8	0.515	0.530	0.526	1.31	1.26	1.52
38	6.11	19.9	0.527	0.539	0.533	1.62	1.24	1.50
39	6.08	25.1	0.822	0.844	0.807	0.87	0.92	1.03
40	6.11	25.4	0.894	0.875	0.835	1.14	0.87	0.99
41	6.08	30.0	1.18	1.13	1.13	0.87	0.85	0.76
42	6.11	30.1	1.27	1.14	1.14	1.09	0.81	0.73
43	6.08	35.0	1.50	1.32	N.D.	0.92	0.97	N.D.
44	6.08	35.0	1.50	1.32	N.D.	0.74	0.97	N.D.
45	6.1	39.8	1.32	1.38	N.D.	1.10	1.31	N.D.
46	6.09	40.0	1.24	1.37	N.D.	1.27	1.36	N.D.
47	6.34	5.0	0.0406	0.0533	0.0581	0.00	15.01	9.08
48	6.32	15.5	0.315	0.344	0.363	2.76	1.61	1.94
49	6.34	25.1	0.974	0.941	0.877	1.56	0.66	0.86
50	6.68	0.0	0.0150	0.0172	0.0056	60.47	59.23	36.21
51	6.66	2.4	0.0350	0.0319	0.0260	22.54	25.88	14.63
52	6.7	4.9	0.0631	0.0576	0.0632	7.21	11.80	7.56
53	6.98	5.2	0.0738	0.0626	0.0716	7.86	10.28	6.47
54	6.71	10.0	0.170	0.159	0.188	3.73	3.29	3.11
55	6.68	14.8	0.333	0.340	0.363	1.72	1.38	1.76
56	6.98	15.0	0.358	0.360	0.390	1.77	1.24	1.57
57	6.71	20.0	0.559	0.650	0.625	0.28	0.72	1.07
58	6.68	26.0	1.04	1.09	1.01	0.88	0.50	0.69

[a] Measured growth rate and lag time.
[b] Growth rate calculated by equation 3 (Table 3).
[c] Growth rate calculated by equation 9 (Table 4).
[d] Lag time calculated by equation 5 (Table 3).
[e] Lag time calculated by equation 10 (Table 4).
N.G. = No growth.
N.D. = Not done; temperature range not in square-root equation.

pH measurement

The pH was measured (Radiometer TTT2; Radiometer A/S, Copenhagen, Denmark) for each of the blended samples of lean mince used for viable counts, and the mean value of the 12-15 samples in each experiment taken as the measured pH (Table 2).

Growth rate and lag period

The growth rate (gen/h) was estimated from linear regression analysis of log CFU/g on time (h) where 8-12 determinations of viable count appeared to be in the log phase of growth (log 3.5-7.5 CFU/g). The lag time (h) was estimated from the regression equation as the time corresponding to the initial count of the inoculum on the tissue samples.

Curve fitting

The relationship between growth rate and temperature can be described by the modified Arrhenius equation (*8*):

$$\text{Ln Gen/h} = A_0 + A_1/K + A_2/K^2 \quad \text{Equation 1}$$

where K is temperature in degrees Kelvin and A_0, A_1, and A_2 are constants. After the lag period is converted to a rate (i.e., 1/Lag), a similar equation:

$$\text{Ln (1/Lag in hours)} = A_0 + A_1/K + A_2/K^2 \quad \text{Equation 2}$$

can be used to express the influence of temperature on the lag. The constants for both these equations were calculated by multiple regression analysis (Minitab Statistical Software; Minitab Inc., State College, PA) using the experimental data in Table 1.

The combined effects of temperature and pH on growth rate can be described by a modified and additive Arrhenius equation (*8*) of the form:

$$\text{Ln Gen/h} = A_0 + A_1/K + A_2/K^2 + A_3/\text{pH} + A_4/\text{pH}^2 \quad \text{Equation 3}$$

or

$$\text{Ln Gen/h} = A_0 + A_1/K + A_2/K^2 + A_3\text{pH} + A_4\text{pH}^2 \quad \text{Equation 4}$$

where A_0 to A_4 are constants. Similarly, the relationship of the lag time to the temperature of incubation and tissue pH can be described by either:

$$\text{Ln (1/Lag)} = A_0 + A_1/K + A_2/K^2 + A_3/\text{pH} + A_4/\text{pH}^2 \quad \text{Equation 5}$$

or

$$\text{Ln (1/Lag)} = A_0 + A_1/K + A_2/K^2 + A_3\text{pH} + A_4\text{pH}^2 \quad \text{Equation 6.}$$

The constants for these equations were calculated by multiple regression analysis using experimental data in Table 2.

Alternately, the relationship between temperature and growth rate can be expressed by the square-root function (*22*):

$$\sqrt{\text{gen/h}} = C_0 + C_1K \quad \text{Equation 7}$$

where K is temperature in degrees Kelvin and C_0 and C_1 are constants, and the lag time by:

$$\sqrt{1/\text{Lag}} = C_0 + C_1K \quad \text{Equation 8.}$$

The constants for these equations were calculated from the data in Table 1.

Although the square-root relationship between growth rate and temperature has been adapted to include a pH or water activity term (*1,20*), here only three possible pH ranges (Table 2) could be used to estimate the effect of pH on the constants needed for this form of equation. Therefore, another form of the square-root equation was obtained by transforming pH (i.e., pH-X) and by assuming that pH could affect both the slope and intercept of plots of $\sqrt{\text{gen/h}}$ against K. This gave the equation:

$$\sqrt{\text{Gen/h}} = C_0 + C_1K + C_2(1/\text{pH-X}) + C_3(K/\text{pH-X}) \quad \text{Equation 9}$$

where C_0 to C_3 are constants. The value of X was found by optimizing the fit from a regression analysis of the data in Table 2 (for incubation temperatures of 30°C and below) by varying X in steps of 0.1 between 4.0 and 5.5. The value of X in the transformation of pH was taken as that giving the best fit to the data (X found to be 4.8). A similar equation:

$$\sqrt{1/\text{Lag}} = C_0 + C_1K + C_2(1/\text{pH-4.8}) + C_3(K/\text{pH-4.8}) \quad \text{Equation 10}$$

was used to describe the relationship between lag time and pH and temperature. The constants were calculated from the data in Table 2 (for temperatures of 30°C and below).

Agreement between measured and predicted growth rates and lags

Measures of the goodness of fit of the transformed (Ln and square-root) data are given by the residual standard deviation and the coefficient of determination (R^2), or the adjusted coefficient of multiple determination (degrees of freedom adjusted for the added variables) from the regression analysis. The ability of equations 1 to 10 to describe the untransformed data (growth rate in gen/h and lag time) was examined by comparing the difference between calculated and experimental values as a percentage of the experimental value. The equations used for these comparisons were:

$$100 \times (\text{Calculated gen/h} - \text{Experimental gen/h})/\text{Experimental gen/h} \quad \text{Equation 11}$$

$$100 \times (\text{Calculated Lag} - \text{Experimental Lag})/\text{Experimental Lag} \quad \text{Equation 12}$$

RESULTS

Growth on fatty tissue

The effect of incubation temperatures on the measured aerobic growth rates (gen/h) and lag periods (h) for *L. monocytogenes* strain Murray B growing on beef fatty tissue are shown in Table 1.

The coefficients for equations 1 and 2, describing the effect of temperature on the growth rate and lag time, are listed in Table 3 (No. 1 and 2), and those for equations 7 and 8 are listed in Table 4 (No. 7 and 8). The last two equations can be converted to the more familiar forms of the square-root relationships $\sqrt{\text{gen/h}} = 0.03450(K-270.97)$ and $\sqrt{(1/\text{lag})} = 0.04346(K-269.39)$. All these equations are valid only for the temperature range examined, i.e., from 0 to 30.6°C. To enable comparisons to be made with the measured values, the growth rates and lags calculated from the four equations (No. 1, 2, 7, and 8) are listed in Table 1.

Equation 1 accounted for 99.66% of the variance (Table 3, No. 1), and equation 7 for 98.85% (Table 4, No. 7), indicating the generally good fit of both equations. Inspection of the data in Table 1 shows that equation 1 appeared to give better estimates of the experimental growth rates than were given by the square-root equation (equation 7). The ability of the equations to predict growth rates was examined by comparing the differences between calculated and measured growth rates as a percentage of the measured rate (equation 11). When this was done for data from equation 1, only four of the 18 calculated rates differed by

TABLE 3. *Ln equations[a] for the growth rate and lag period of* L monocytogenes.

No.	Type[b]	Substrate	A_0	$A_1 \times 10^{-5}$	$A_2 \times 10^{-7}$	$A_3 \times 10^{-2}$	$A_4 \times 10^{-2}$	SEE	R^2
1	Ln(g/h)	Fat	-205.73	1.2939	-2.0298	-	-	0.0883	0.9966
2	Ln(1/h)	Fat	-434.31	2.6188	-3.9428	-	-	0.7247	0.8315
3	Ln(g/h)	Lean	-232.64	1.4041	-2.1908	1.1586	-4.0952	0.0933	0.9952
5	Ln(1/h)	Lean	-445.45	2.5376	-3.8374	3.7573	-13.195	0.4337	0.9243

[a] $\text{Ln(gen/h) or Ln(1/h)} = A_0 + A_1/K + A_2/K^2 + A_3/pH + A_4/pH^2$.
[b] Growth rate (gen/h) or lag period (h).
SEE = Standard error of the Ln estimate, or residual standard deviation.
R^2 = Adjusted coefficient of multiple determination.

TABLE 4. *Square-root equations[a] for the growth rate and lag period of* L monocytogenes.

No.	Type[b]	Substrate	C_0	$C_1 \times 10^2$	C_2	$C_3 \times 10^3$	SEE	R^2
7	√Gen/h	Fat	-9.3483	3.4500	-	-	0.0400	0.9885
8	√1/h	Fat	-11.707	4.3458	-	-	0.2903	0.7221
9	√Gen/h	Lean	-11.063	4.0865	2.6125	-9.7315	0.0254	0.9925
10	√1/h	Lean	-13.917	5.1862	6.0580	-22.749	0.1718	0.7631

[a] $\text{√Gen/h or √1/h} = C_0 + C_1K + C_2\{1/(pH-4.8)\} + C_3\{K/(pH-4.8)\}$.
[b] Growth rate (gen/h) or lag period (h).
SEE = Standard error of the square-root estimate, or residual standard deviation.
R^2 = Coefficient of determination (adjusted coefficient of multiple determination for lean data).

more than 10% from the measured rates, and none differed by more than 20%. For data calculated from the square-root model (equation 7), nine of the 18 calculated generation times differed by more than 10% from the measured values, and two (Exp. 1 and 2) differed by more than 50%.

Although increasing incubation temperature decreased the measured lag times for *L. monocytogenes* growing on fatty tissue, there appeared to be other factors contributing to variability which were not controlled in the experimental design. Inspection of the lag times for experiments 1-5 (Table 1) illustrates this variability. It is not surprising then that for both of the equations for lag times, the goodness of fit was relatively poor. Equation 2 accounted for 83% of the variance (Table 3, No. 2) and equation 8 for only 72% (Table 4, No. 8).

Growth on lean tissue

When *L. monocytogenes* strain Murray B was inoculated onto beef lean tissue, both pH and incubation temperature influenced the growth rate and lag time (Table 2).

The coefficients for the logarithmic equations 3 and 4, describing the combined effects of temperature and pH on growth rate, are given in Table 3 (No. 3) and Table 5 (No. 4), respectively. The goodness of fit of equation 3 was only marginally better (standard error of the estimate = 0.0933; adjusted coefficient of multiple determination = 0.9952; Table 3, No. 3) than that given by equation 4 (standard error of the estimate = 0.0948; adjusted coefficient of multiple determination = 0.9951; Table 5, No. 4).

The coefficients for the logarithmic equations 5 and 6, describing the relationship between lag period on lean and pH and incubation temperature, are given in Table 3 (No. 5) and Table 5 (No. 6), respectively. Again the goodness of fit of equation 5 was only marginally better than that given by equation 6.

To enable comparisons to be made with the measured values, the growth rates and lag times calculated from equations 3 and 5 are listed in Table 2.

The coefficients for the modified and transformed square-root equations (equation 9 and 10) describing the relationship of growth rate and lag to temperature (approximate range 0 to 30°C) and pH are listed in Table 4 (No. 9 and 10). The growth rates and lag times calculated from these two square-root equations are listed in Table 2.

Equation 3 gave a slightly better fit to the transformed growth rate data (adjusted coefficient of multiple determination = 0.9952; Table 3) than was given by equation 9 (adjusted coefficient of multiple determination = 0.9925; Table 4). However, the high percentage of variance accounted for by both equations indicated the generally good fit of both equations. The ability of the equations to predict growth rate (gen/h) was examined by comparing the differences between calculated and measured rates using equation 11. Forty-one (71%) of the 58 rates (Table 2) calculated by equation 3 differed by less than 10% from the measured rates. There were only three examples where the difference was more than 20% and these included two experiments where no growth was observed. Thirty-seven (74%) of the 50 growth rates calculated by equation 9 differed by less than 10% from the measured rates; eight values differed by more than 20%.

Both equations predicted growth on lean tissue under some circumstances where no growth was detected (Table 2). On lean tissue with a mean pH 5.61, *L. monocytogenes* failed to grow during 13 weeks storage at 0°C (Table 2, Exp. No. 1). Similarly, no growth of *L. monocytogenes* was observed on lean tissue with a mean pH 5.46 incubated for 48 h at 43.2°C (Table 2, Exp. No. 27), though equation 3 predicted growth. It appears that for lean tissue with pH about 5.5-5.6, equation 3 was valid only between about 2.5

TABLE 5. *Alternate Ln equations[a] for the growth rate and lag period of* L. monocytogenes *on beef lean tissue.*

No.	Type[b]	Substrate	A_0	$A_1 \times 10^{-5}$	$A_2 \times 10^{-7}$	A_3	$A_4 \times 10^{1}$	SEE	R^2
4	Ln(g/h)	Lean	-240.66	1.4011	-2.1865	4.8856	-3.5628	0.0948	0.9951
6	Ln(1/h)	Lean	-471.26	2.5302	-3.8269	15.787	-11.572	0.4356	0.9236

[a] Ln(gen/h) or Ln(1/h) = $A_0 + A_1/K + A_2/K^2 + A_3pH + A_4pH^2$.
[b] Growth rate (gen/h) or lag period (h).
SEE = Standard error of the Ln estimate, or residual standard deviation.
R^2 = Adjusted coefficient of multiple determination.

and 35°C, and equation 9 only for temperatures between about 2.5 and 30°C.

For both equations 5 and 10, describing the relationship of lag time to pH and temperature, the goodness of fit to the measured data was relatively poor. This was particularly noticeable when the observed lag times exceeded a few days (e.g., Exp 1-5, Table 2). Only 76% of the variance was accounted for by equation 10 (Table 4, No. 10). Equation 5 accounted for considerably more of the variance (92.4%, Table 3). When the differences between calculated and measured lag times were compared (equation 12), 12 (21%) of the 57 values (Table 2) calculated by equation 5 differed by more than 50% from the measured lags, and 10 (20%) of the 49 values calculated by equation 10 differed by more than 50%.

DISCUSSION

Both the logarithmic, or modified Arrhenius (equation 1), and the square-root (equation 7) models effectively described the effect of temperature on the aerobic growth rate of *L. monocytogenes* strain Murray B growing in pure culture on beef fatty tissue. Similarly, the combined effects of temperature and pH on the growth rate on beef lean tissue could be modeled using modified forms of these equations (equations 3 and 9). The high percentage of variance (about 99%) that was accounted for by both types of models indicated their generally good fit to the data. However, for both lean and fatty tissues, the modified Arrhenius model gave a somewhat better fit and better estimates of the experimentally measured growth rates than were given by the square-root model. Equation 1 with the coefficients listed in Table 3 is, therefore, preferred for predicting the growth rate of *L. monocytogenes* on beef fatty tissue, and equation 3, with the coefficients in Table 3, for predicting growth rates on beef lean tissue.

In spite of this, calculation of growth rates can be simplified, with some loss in precision, by using equation 3 to estimate growth on fatty tissue as well. If a notional pH of 6.35 was assumed and this value inserted in equation 3, calculated growth rates on fatty tissue agreed reasonably well with measured rates (standard error of Ln gen/h estimate = 0.101 compared with 0.088 given by equation 1, Table 3). Such an approach (i.e., assuming pH = 6.35) could probably be used to estimate the maximum possible growth rate of *L. monocytogenes* on sides of beef. Much of the surface tissue of sides is fatty tissue and exposed lean has a pH of about 6.4-6.5 (*6*). Real growth rates, however, are likely to be slower than calculated as often the water activity of surface tissue is <0.99.

The duration of the lag period of *L. monocytogenes* in both laboratory media and foods varies with the growth temperature of the inoculum and the subsequent incubation temperature (*13,25,26*). Since it is likely that at least some of the contamination of foods with *Listeria* will come from chilled environments, inocula here were grown at 10°C. However, even though the inocula were from mid- to late-log-phase cultures, there was considerable scatter in the measured lag periods on lean and fatty tissues. Considerably more scatter of lag times than for generation times has been observed also by others (*2,18*). It is not surprising that the goodness of fit of both types of model to such scattered data was poor.

It was difficult to compare aerobic growth rates of *L. monocytogenes* on lean with data published for growth in laboratory broths. In some cases growth rates were slower (*21,26*), and in other cases faster (*3,4*), than observed or predicted for lean tissue.

Growth rates predicted by equation 3 for growth on lean tissue were compared with data for aerobic growth on a number of foods (Table 6). Reported growth rates on vegetable products such as corn and clarified cabbage juice were significantly slower than rates predicted for lean at the same pH and temperature. Although growth rates in milk and in 2% milk tended to be less than on beef lean, growth rates in ultra-high temperature milk appeared very close to those expected for lean. Similarly, growth rates in chicken broth, raw chicken, cooked chicken, and cooked ground meat were also close to those predicted from equation 3. It seems likely that, at least for some foods in the pH range of about 6 to 7 and with a high water activity and containing no microbial inhibitors, equation 3 will give a reasonable estimate of the growth rate of *Listeria*. Below pH 6, equation 3 may be applicable only to meats since the natural acidulant of lean is lactic acid.

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TABLE 6. *Aerobic growth rate of* L. monocytogenes *on some foods compared with rate predicted for beef lean tissue.*

Food	No. of strains	Temp (°C)	pH	Growth rate (Gen/h)	Calc[a] rate (Gen/h)	Ref
Cabbage juice[b]	2	30	6.1	0.63-0.66[c]	1.135	7
Corn	1	5	6.6-6.7	0.039	0.057-0.058	14
Whole milk[b]	4	4	6.6[d]	0.027-0.033	0.046	23
Whole milk[b]	4	8	6.6[d]	0.069-0.093	0.107	23
Whole milk[b]	1	10	6.6	0.106[c]	0.156	19
Skim milk[b]	1	10	6.6	0.125[c]	0.156	19
Whole milk[b]	4	13	6.6[d]	0.143-0.192	0.257	23
2% Milk[b]	2	13	6.6[d]	0.216-0.223	0.257	24
UHT milk	1	0	6.6	0.013-0.016	0.017	25
UHT milk	1	2.5	6.6	0.030-0.042	0.032	25
UHT milk	1	5	6.6	0.050-0.053	0.057	25
UHT milk	1	7.5	6.6	0.063-0.105	0.097	25
UHT milk	1	9.3	6.6	0.111-0.182	0.137	25
Chicken broth[b]	3	0	6.4	0.008-0.013	0.016	25
Chicken broth[b]	3	2.5	6.4	0.022-0.033	0.031	25
Chicken broth[b]	3	5	6.4	0.040-0.077	0.055	25
Chicken broth[b]	1	7.5	6.4	0.077-0.111	0.093	25
Chicken broth[b]	1	9.3	6.4	0.143-0.200	0.131	25
Chicken breast[e]	1	6	5.8	0.038-0.045	0.050	13
Chicken breast[f]	1	10	5.8-6.0[d]	ca. 0.10[c]	0.110-0.125	5
Ground meat[b]	4	4	5.8-6.0	0.036[c]	0.032-0.037	16
Ground meat[b]	4	8	5.8-6.0	0.111[c]	0.075-0.086	16
Ground meat[b]	4	12	5.8-6.0	0.156[c]	0.154-0.176	16

[a] Gen/h for beef calculated from equation 3.
[b] Autoclaved.
[c] Estimated from data in reference.
[d] Estimated pH value.
[e] Raw, skinless chicken breasts with normal "other" flora.
[f] Cooked, boneless chicken breasts with surviving "other" flora.

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