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1 **Growth of *Listeria monocytogenes* is promoted at low temperature when**
2 **exogenous unsaturated fatty acids are incorporated in its membrane**

3

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12

13

14 **Abstract**

15 *Listeria monocytogenes* is a psychrotrophic food-borne pathogen mostly associated with
16 consumption of ready-to eat foods. Due to its high prevalence in raw materials, it is
17 fundamental to control its growth at low temperature. In lipid-rich products, fatty acids can be
18 heterogeneously distributed in the food matrix and can be present in the environment
19 immediately surrounding the pathogen.. In this study, we sought to understand the impact of
20 exogenous fatty acids on the growth and membrane physiology of *L. monocytogenes*
21 according to the temperature and strain. We demonstrate that exogenous unsaturated fatty
22 acids promote the growth of *L. monocytogenes* at 5°C but not at 37°C. The level of growth
23 modifications is dependent upon the strain. At 5°C, there is high incorporation of unsaturated
24 fatty acids, which decreases the weighted-average melting temperature of membrane fatty
25 acids allowing *L. monocytogenes* to compensate for the decrease in fluidity caused by the
26 temperature, thus leading to increased growth. In contrast, the incorporation of saturated fatty
27 acids decreases membrane fluidity and prevents growth at 5°C. This study underlines the
28 absolute necessity to understand better the cold adaptation of *L. monocytogenes* in lipid-rich
29 foods in order to adjust their shelf-life and guarantee their microbiological safety.

30 **Keywords**

31 Exogenous fatty acids, growth promotion, cold adaptation, fatty acid incorporation, shelf-life
32 compromising

33

34 **Abbreviations**

35 FA fatty acids

36 #FA exogenous fatty acids

37 FAME fatty acid methyl esters

38 SFA saturated fatty acids

39 *i*-BFA iso-branched fatty acids

40 *ai*-BFA anteiso-branched fatty acids

41 UFA unsaturated fatty acids

42 SCFA short-chain fatty acids

43 GC-MS gas chromatography coupled with mass spectrometry

44 RTE ready-to-eat

45 WAMT weighted-average melting temperature

46 **1. Introduction**

47 Infectious foodborne diseases remain a real public health issue worldwide. In 2019, more than
48 320,000 human cases due to zoonoses were reported in Europe (EFSA and CDC, 2021).
49 Among them, *L. monocytogenes* caused 2,621 invasive human cases which confirms a stable
50 trend since 2015 after a long period of increasing trend. Invasive listeriosis causes the highest
51 rate of case fatality (17.6%) compared to other zoonoses (EFSA and CDC, 2021). The
52 ubiquitous nature of *L. monocytogenes* leads to its high prevalence in many raw materials and
53 its persistence in the whole food chain. Consequently, its prevalence in ready-to-eat (RTE)
54 foods is also high at the processing stage, in particular for fish production (5.8%) (EFSA and
55 CDC, 2021). Ready-to-eat (RTE) foods are highly concerned because they are typically stored
56 at low temperature which allows the growth of *L. monocytogenes* and they don't receive any
57 subsequent treatment that could inactivate it before consumption. Regulation (EC) No.
58 2073/2005 defines a limit at the retail stage of 100 CFU/g ($n=5$, $c=0$) for RTE foods that are
59 able to support the growth of *L. monocytogenes*. Nevertheless, more than 90% of listeriosis
60 cases were caused by ingestion of RTE foods containing more than 2,000 CFU/g, which is
61 significantly above this regulation limit (Ricci et al., 2018).
62 Therefore, control of product safety regarding listeriosis risk mainly lies in control of
63 pathogen growth at low temperature. When microorganisms are exposed to temperatures out
64 of their "comfort zone" they have to adapt to counteract the effects of temperature on their
65 cellular components (Booth, 2002). The cold adaptation response is a universal bacterial
66 mechanism, which has been particularly studied in psychrotrophic bacteria such as *B. cereus*
67 and *L. monocytogenes* (Alvarez-Ordóñez et al., 2015). Loss of membrane fluidity is the first
68 consequence faced by cells when the temperature decreases. At a given temperature,
69 membrane fluidity and permeability depend on lipid acyl chain composition (Parsons and
70 Rock, 2013). To sustain optimum membrane fluidity and survive in harsh environments such

71 as sub-optimal temperatures or in the presence of toxic compounds, bacterial cells can alter
72 the acyl chain structure of membrane glycerophospholipids depending on their biosynthesis
73 pathway systems. Several adaptations can be made, such as changing the ratios of: 1)
74 saturated (SFA) versus unsaturated fatty acids (UFA), 2) *cis* to *trans* UFA, 3) branched (BFA)
75 versus non-branched FA, changing 4) acyl chain length, and 5) synthesizing cyclopropane FA
76 from UFA (Denich et al., 2003). At low temperature, *L. monocytogenes* adapts its membrane
77 composition by increasing the ratio of *anteiso/iso* branched chain fatty acids (*ai*-BFA / *i*-BFA)
78 and/or by shortening the FA chain length, both of which counterbalance the physical loss of
79 fluidity (Julotok et al., 2010; Mastronicolis et al., 2005, 1998; Neunlist et al., 2005).

80 Beside this intrinsic bacterial ability to adapt to harsh environments, several compounds in the
81 environment could help bacteria to overcome stressful conditions. For instance, osmo-
82 protective compounds present in food products, namely betaine in vegetables and carnitine in
83 meats, are taken up by *L. monocytogenes* and favor its growth at 4°C (Tasara and Stephan,
84 2006). Moreover, several studies have shown that numerous bacteria could use exogenous FA
85 from the environment to adapt their membrane composition (Ando et al., 1992; Brinster et al.,
86 2010, 2009). In particular, *B. cereus* was shown to insert fatty acids from spinach in its
87 membrane at low temperature under anaerobiosis and thus to recover the same maximal
88 population density as at optimal temperature (de Sarrau et al., 2013). Here, we studied the
89 impact of different types of FA (UFA, SFA) available in the environment on the growth of
90 *L. monocytogenes* and on its membrane physiology according to temperature and strain.
91

92 **2. Materials and methods**

93 **2.1. Chemicals**

94 Myristic acid (C14), palmitic acid (C16), oleic acid (C18:1cis9), linoleic acid (C18:2w6) and
95 linolenic acid (C18:3w6) were from Larodan Fine Chemicals (Malmö, Sweden). Solutions
96 were obtained by dispersion of the specific FA in a bovine serum albumin (BSA) solution in
97 phosphate buffer pH 8. The final concentrations in the culture medium were 0.045 mM FA in
98 0.05% BSA.

99

100 **2.2. Bacterial strains and storage**

101 The strains of *Listeria monocytogenes* used in this study were EGD-e (Murray et al., 1926),
102 CNL895805 (Van Langendonck et al., 1998) and seven other strains isolated from foods
103 (Table 1). The full names of the strains (e.g. Lm_b3d_208) were shorten in the text (e.g.
104 Lm208) to simplify the reading. Stock cultures of *L. monocytogenes* were kept in Tryptone
105 Soya Broth (TSB) (BioMérieux, Marcy l’Etoile, France) supplemented with 20% (v/v)
106 glycerol at –80 °C.

107 Table 1: *L. monocytogenes* strains used in this study

Strain code	Origin	Serotype	CC
EGDe	Guinea pig	1/2a	CC9
Lm_b3d_208 (CNL 895805)	Sheep’s brain	1/2a	CC7
Lm_b3d_226	Milk	4	CC1
Lm_b3d_540	Ham sandwich	1/2a	-
Lm_b3d_541	Eggs & tuna sandwich	1/2a	CC121
Lm_b3d_543	Raw salmon	1/2a	CC121
Lm_b3d_551	Duck foie gras	1/2c	CC9
Lm_b3d_553	Smoked salmon	3a	CC121
Lm_b3d_554	Slices of bacon	3a	CC121
Lm_b3d_562	Raw sheep’s milk	-	CC19

108

109 **2.3. Culture conditions**

110 The strains were inoculated in TSB at 1% v/v with a standardized inoculum ($\sim 10^8$ CFU.mL⁻¹)
111 obtained after two subcultures at 30°C in the same broth. Cultures were then grown in 100-
112 well microplates (Bioscreen C, Labsystems, Helsinki, Finland) or 20 mL flasks. When
113 indicated, the medium was supplemented by a specific exogenous FA (#FA) solution.
114 Bacterial growth was followed for 48 h at 37°C, 6 days at 8°C and 15 days at 5°C by
115 measuring the optical density (OD) at 600 nm in microplates in an automatic
116 spectrophotometer (Bioscreen C, Labsystems, Helsinki, Finland) or discontinuously in 20 mL
117 flasks (spectrophotometer Genesis 30, Thermo Fisher Scientific, Waltham, USA). The
118 addition of 0.05% BSA in TSB was shown to have no significant effect on the growth rate of
119 *L. monocytogenes* at 5°C (0.045 ± 0.004 h⁻¹ versus 0.042 ± 0.01 h⁻¹).

120

121 **2.4. Determination of the growth rate**

122 Maximum specific growth rates (μ_{\max}) of *L. monocytogenes* were estimated from the OD
123 growth kinetics by fitting the modified Gompertz model (Guillier et al., 2007; Pernin et al.,
124 2018) using the complementary macro SolverAid (de Levie, 2012).

125

126 **2.5. Shelf-life alteration in the presence of exogenous FA**

127 Growth simulations were performed to evaluate the impact of the presence of exogenous FA
128 on shelf-life, which is here considered to be the time when the population reaches 100
129 *L. monocytogenes*/g. Five thousand values of growth rates were calculated with the statistical
130 function NORMINV(RAND(),M,SD) which was used to return the inverse of the normal
131 distribution from the experimentally determined mean (M) and standard deviation (SD) of
132 μ_{\max} for each condition (see 2.4). The lag time was calculated from each of the 5,000 growth

133 rate values with the following relation by assuming that the physiological state (K) is at the
134 average value of 2.5 (Augustin et al., 2011):

$$135 \quad K = \mu_{\max} \cdot lag \quad (1)$$

136 Five thousand simulations of growth curves were then performed for each condition using the
137 Gompertz growth model with the following parameters: the growth rate (μ_{\max}), the lag time
138 (*lag*), the initial contamination (N_0) and the final population (N_f). The initial and final
139 populations were respectively set at 1 and 10^9 CFU/g. The mean and standard deviation of the
140 shelf-life were calculated from the 5,000 growth simulations in each condition.

141

142 **2.6. Membrane fatty acid analysis**

143 Bacterial cultures grown as described above were harvested by centrifugation (7,000 g, 20°C)
144 in the early stationary phase according to OD growth curves. Pellets were washed twice with
145 0.1% Triton X-100 (Sigma-Aldrich, St. Louis, USA) (Brinster et al, 2009). Extraction and
146 methylation of FA were carried out directly on bacterial pellets as previously described
147 (Dubois-Brissonnet et al., 2016; Méchin et al., 1999). FA of whole cells were first saponified
148 and esterified by methanolic NaOH and methanolic HCl (1st step: 1 mL NaOH 3.75 M in 50%
149 v/v methanol solution for 30 min at 100°C; 2nd step addition of 2 mL HCl 3.25 M in 45% v/v
150 methanol solution for 10 min at 80°C). Fatty acid methyl esters (FAME) were extracted with
151 a diethyl ether / cyclohexane solution (1:1 v/v). The organic phase was then washed with a
152 dilute base (NaOH 0.3 M). Analytical gas chromatography of FAME was carried out on a
153 GC-MS Trace 1300 / ISQ 7000 (Thermo Fisher Scientific, Waltham, USA) equipped with a
154 BPX70 capillary column (25m, 0.22 mm id) (SGE, Victoria, Australia). Column temperature
155 was set at 100°C for 1 min and then increased to 170°C at the rate of 2°C/min.
156 FAME were expressed as a percentage of the total area and grouped in classes: saturated fatty
157 acids (SFA), unsaturated fatty acids (UFA), *iso* and *anteiso* branched-chain fatty acids (*i*-BFA

158 and *ai*-BFA). Additional indicators of membrane adaptation were also calculated, namely
159 short-chain fatty acid (< C15) contents (SCFA) and weighted-average melting temperature
160 (WAMT). The latter is calculated from the melting temperature of each individual FA and its
161 ratio in the membrane composition (Seel et al., 2018).

$$162 \text{ WAMT } (^{\circ}\text{C}) = \sum_n^1 T_i \cdot \text{FA } (\%)_i$$

163 (2)

164 where T_i ($^{\circ}\text{C}$) is the melting temperature of FA_i , $\text{FA}(\%)_i$ is the percentage of FA_i , and FA_1 to
165 FA_n are each of the FA present in the membrane composition. Melting temperatures of FA
166 ($^{\circ}\text{C}$) were obtained from CAS Common Chemistry (<https://commonchemistry.cas.org/>).

167

168 **2.7. Statistics**

169 The number of replicates is specified in the legend of each figure. All experiments were
170 conducted at least in triplicate with independent subcultures. Statistical analyses were
171 performed using GraphPad Software 9.2.0 (Prism, USA). Two-tailed paired Student's t-tests
172 were performed between #C18:1 and control for each strain in Figure 1. One-way ANOVA
173 with Dunnett's multiple comparison tests (95% confidence interval) were performed between
174 the different conditions and the control for each strain and temperature in Figures 2, 4, 5 and
175 6. According to the p -value, results are reported as non-significant (ns) or significantly
176 different * if $p < 0.0332$; ** if $p < 0.0021$; *** if $p < 0.0002$; **** if $p < 0.0001$.

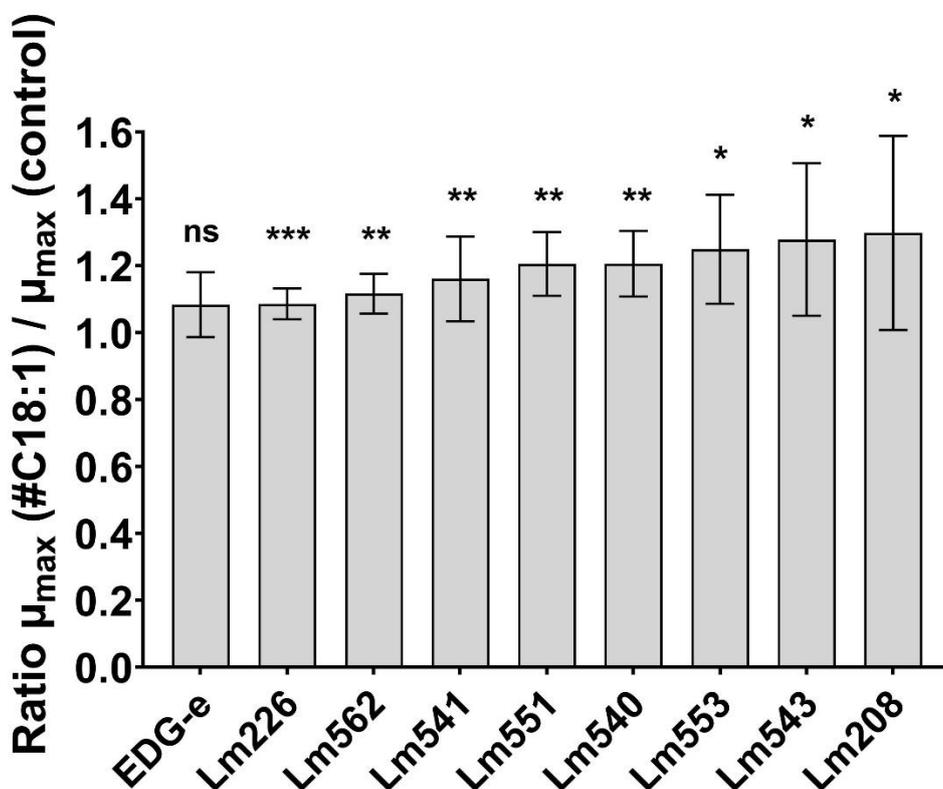
177

178 **3. Results**

179

180 **3.1. *The medium supplementation with oleic acid promotes the growth of several strains***
181 ***of *L. monocytogenes* at 8°C but not at the same level***

182 We first aimed to study the growth of nine different strains of *L. monocytogenes* at 8°C in
 183 TSB supplemented or not with oleic acid (#C18:1). The growth rates at 8°C without
 184 supplementation (control) varied among the different strains, ranging from 0.060±0.03 h⁻¹ to
 185 0.088±0.005 h⁻¹. Except for EGD-e, the growth rate of *L. monocytogenes* in #C18:1 medium
 186 was always significantly higher than that of the control. The ratio $\mu_{\max} (\#C18:1) / \mu_{\max}$
 187 (control) was ranging from 1.1± 0.09 h⁻¹ for EGD-e to 1.3±0.29 h⁻¹ for Lm208 (Figure 1).
 188 EGD-e and Lm208, which respectively showed the lowest and highest increase of growth rate
 189 in #C18:1 medium, were chosen for further experiments.



190
 191 Figure 1: Ratios of growth rates at 8°C of nine different strains of *L. monocytogenes* in TSB
 192 with C18:1 supplementation or without [Ratio = $\mu_{\max} (\#C18:1) / \mu_{\max} (\text{control})$]. Mean and
 193 standard deviation (n=6) are represented. Growth rates in both conditions were compared for
 194 each strain by two-tailed paired Student's t-tests (95% confidence interval).

195

196 **3.2. *The level of growth promotion depends on the type of exogenous fatty acid and on***
197 ***temperature***

198 The growth of strains EGD-e and Lm208 was followed at 5°C or at 37°C in medium
199 supplemented with different SFA, namely myristic acid (#C14) or palmitic acid (#C16) or
200 different UFA, namely oleic acid (#C18:1), linoleic acid (#C18:2) or linolenic acid (#C18:3).
201 Stearic acid (C18) was not used in this study because of the lack of efficiency in dispersing
202 this FA in BSA solution. At 5°C, the growth rates in control conditions for EGD-e and Lm208
203 were similar, being respectively $0.044 \pm 0.013 \text{ h}^{-1}$ and $0.044 \pm 0.008 \text{ h}^{-1}$ (Figures 2A and 2C).
204 With UFA supplementation, the growth rate of Lm208 significantly increased and the ratio
205 $\mu_{\max} (\#UFA) / \mu_{\max} (\text{control})$ was above 1.4 (Figure 2A). Supplementation with #C18:1
206 increased EGD-e growth rate slightly, but not significantly, as previously observed at 8°C
207 (see 3.1). Nevertheless, #C18:2 or #C18:3 supplementations led to a significant increase of
208 EGD-e growth rate at 5°C (Figure 2C).

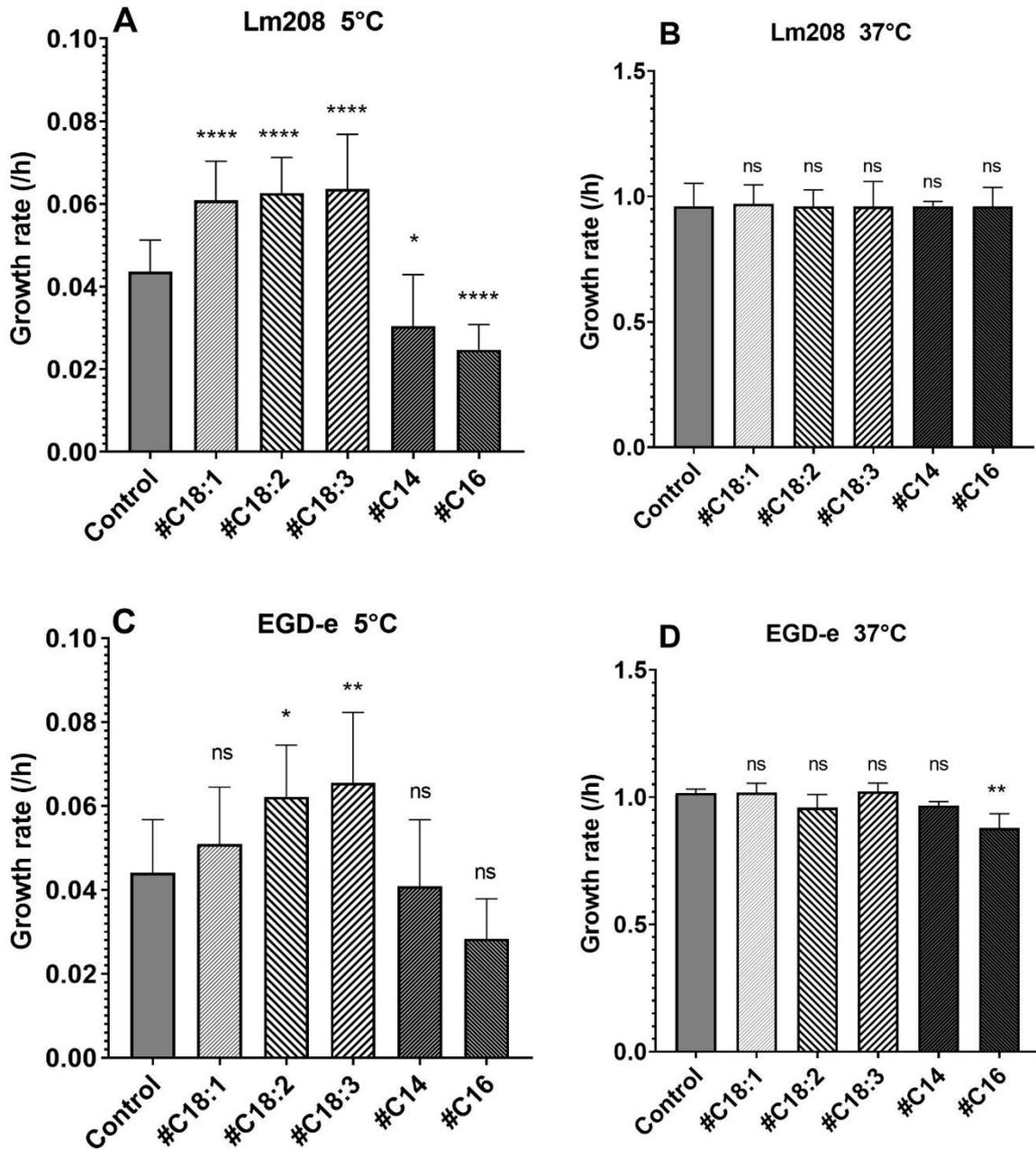
209 Supplementation with SFA led to a significant decrease of the growth rate at 5°C for Lm208
210 and the ratio $\mu_{\max} (\#UFA) / \mu_{\max} (\text{control})$ was 0.7 for #C14 and 0.6 for #C16 (Figure 2A). A
211 slight but non-significant decrease was also observed in EGD-e growth rate with these
212 supplementations (Figure 2C).

213 We analyzed the impact of temperature by following the growth of both strains in the same
214 supplemented media at 37°C. The growth rates in control conditions for Lm208 and EGD-e
215 were respectively $0.961 \pm 0.091 \text{ h}^{-1}$ and $1.015 \pm 0.016 \text{ h}^{-1}$ (Figures 2B and 2D). Whatever the
216 supplementation and strain, no significant differences were recorded in growth rates at 37°C,
217 except for a significant decrease observed for the #C16 EGD-e culture (Figure 2D).

218

219

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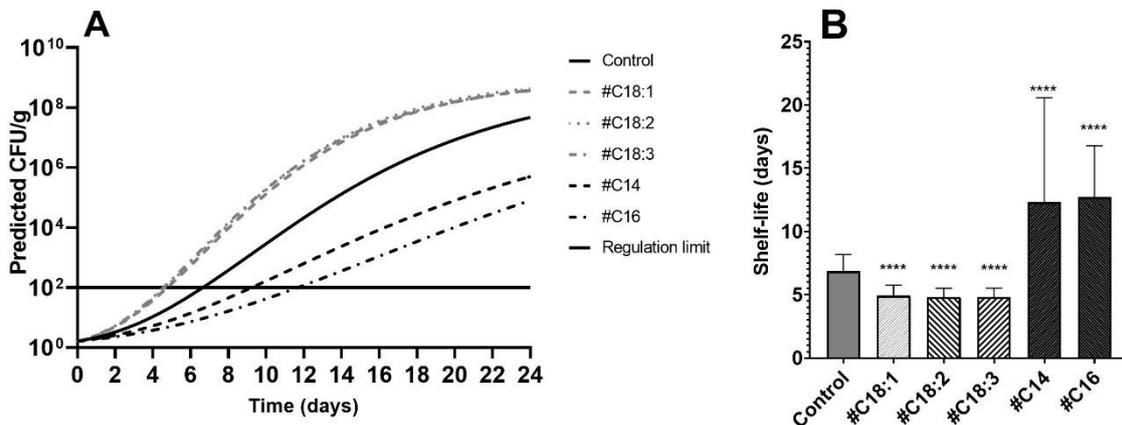
221

222 Figure 2: Growth rates at 5°C (A and C) and 37°C (B and D) of *L. monocytogenes* Lm208 (A
 223 and B) and EGD-e (C and D) with # C18:1, #C18:2, #C18:3, #C14, or #C16 supplementation
 224 or without (control). Mean and standard deviation (n=6 to 18) are represented. Growth rates
 225 were analyzed in comparison with the control by Dunnett's one-way ANOVA (95%
 226 confidence interval).

227

228 3.3. *Shelf-life predictions under FA supplementations*

229 The mean of the 5,000 growth simulations performed from the experimental values of growth
230 rates in cultures without supplementation (Control) or with supplementations are plotted in
231 Figure 3A. The mean and standard deviation of the shelf-life, considered as the time when the
232 population reaches 100 *L. monocytogenes*/g, is represented in Figure 3B. Without
233 supplementation, the population reached 100 CFU/g in 6.9 ± 1.3 days. The shelf-life is
234 significantly lower in #UFA (4.9 ± 0.8 days in #C18:1, 4.8 ± 0.7 days #C18:2 and #C18:3)
235 and higher in #SFA (12.3 ± 8.2 days in #C14 and 12.7 ± 4.1 days in #C16) than in control
236 cultures (Figure 3B).

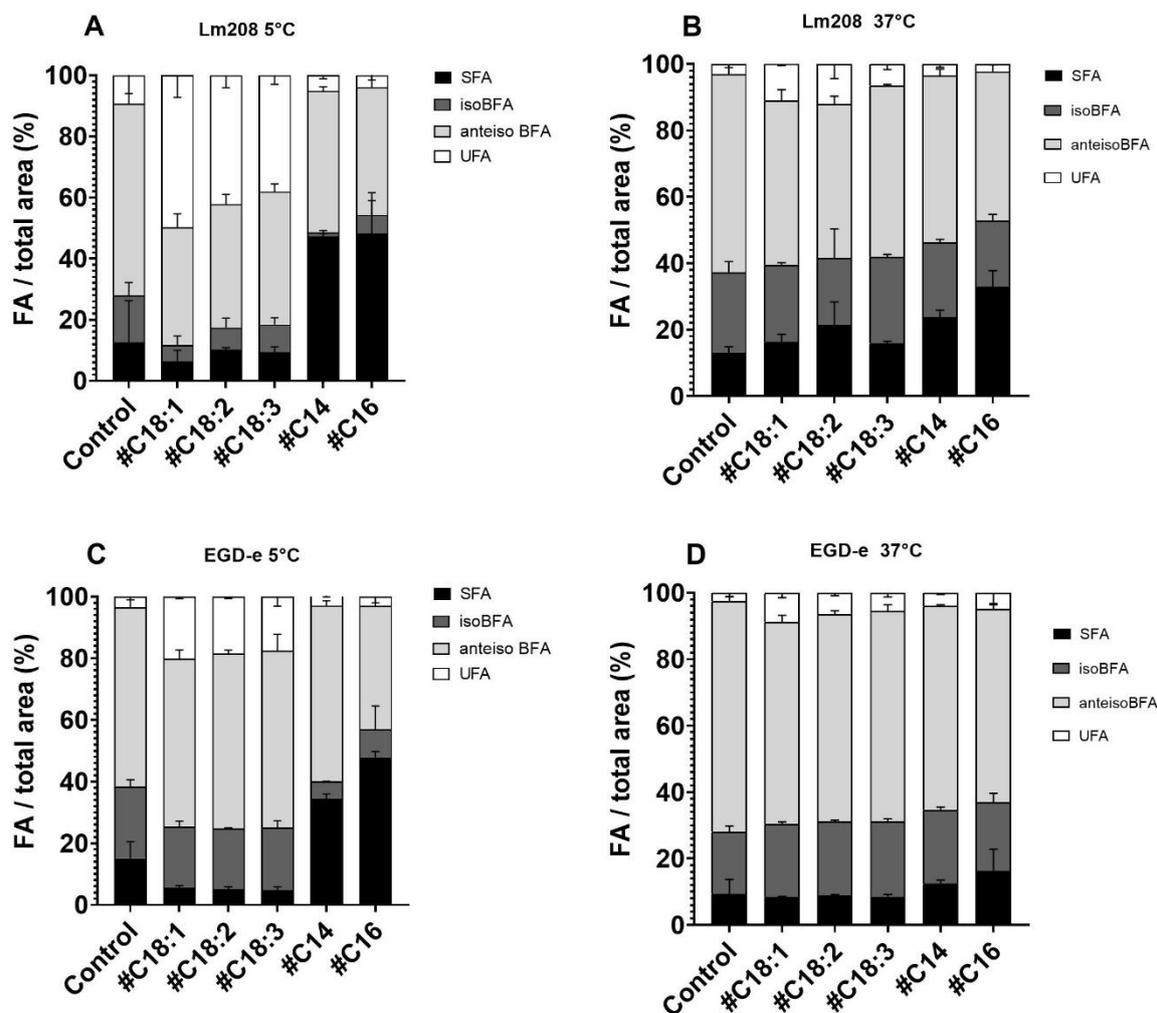


237
238 Figure 3: Growth simulations of CFU/g over time (A) and shelf-life (B) in TSB at 5°C with
239 supplementations or without (Control). Mean of growth curves and mean and standard
240 deviation of shelf-life are presented. 5,000 simulations were processed considering that N_0
241 was 1 CFU/g, K was 2.5 and the growth rate follows a normal distribution around the
242 experimentally determined mean and standard deviation of μ_{max} for each condition. Shelf-lives
243 were analyzed in comparison with the control by Dunnett's one-way ANOVA (95%
244 confidence interval).

245

246 3.4. *The level of incorporation of exogenous FA by L. monocytogenes depends upon the*
 247 *strain, temperature and type of FA*

248 The FA composition of *L. monocytogenes* mainly consists of BFA with *i*-BFA (*i*-C14, *i*-C15,
 249 *i*-C16, *i*-C17) and *ai*-BFA (*ai*-C13, *ai*-C15, *ai*-C17) (Figure 4). The three most abundant BFA
 250 in both strains at both temperatures are *ai*-C15, *i*-C15 and *ai*-C17. At 37°C in control
 251 conditions, BFA represent 84% of the membrane FA of Lm208, SFA (C12, C14, C15, C16,
 252 C18) represent 13% of the composition and UFA (*cis*9 C18:1) 3% (Figure 4B). At 5°C, total
 253 BFA and SFA respectively decreased to 78.1 % and 12.6% and UFA increased to 9.3%
 254 (Figure 4A).

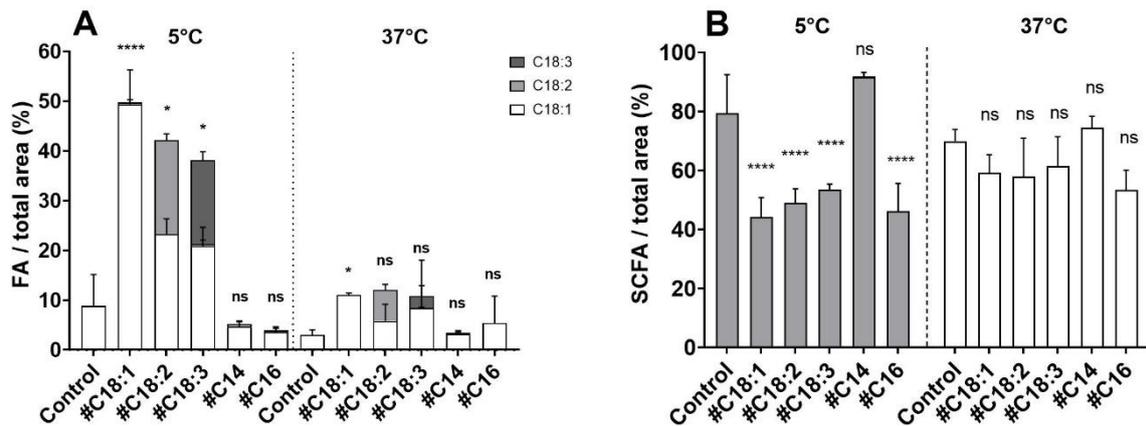


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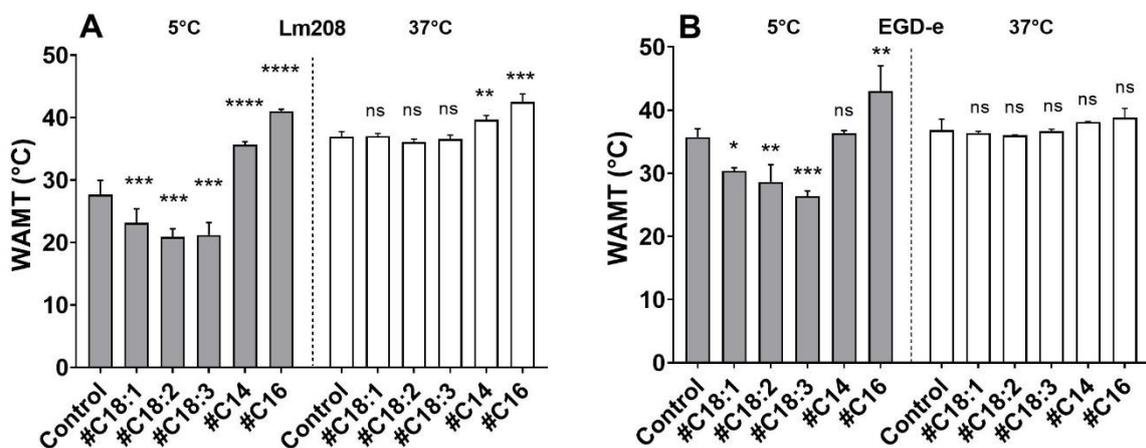
256 Figure 4: Fatty acid composition expressed in 4 FA categories (SFA, *i*-BFA, *ai*-BFA and
257 UFA) of *L. monocytogenes* Lm208 (A and C) and EGD-e (B and D) at 5°C (A and B) and
258 37°C (C and D) with # C18:1, #C18:2, #C18:3, #C14, or #C16 supplementation or without
259 (control). Mean and standard deviation (n=3 to 6) are represented.

260 Medium supplementation with exogenous FA induced modifications in membrane FA
261 composition, but differently according to the strain, temperature and type of FA. In the case of
262 #UFA supplementations at 37°C, UFA in Lm208 membrane composition was respectively
263 11%, 12% and 10.8% for #C18:1, #C18:2 and #C18:3 in comparison with 3% in the control
264 (Figure 4B). At 5°C, levels of UFA were much higher in the membrane, reaching respectively
265 49.7%, 42.1% and 38.1% for #C18:1, #C18:2 and #C18:3 in comparison with 9% in the
266 control (Figure 4A). Moreover, the supplementation with C18:2 or C18:3 induced a
267 significant increase in C18:1 together with the supplemented FA at 5°C (Figure 5A). At both
268 temperatures, Lm208 membrane composition in SFA was not significantly different between
269 #UFA cultures and control (Figure 4 A and B). With #SFA supplementation, the level of SFA
270 in Lm208 membrane significantly increased. In #C14, the membrane contained 17.1% of C14
271 versus 4.6% in the control at 37°C and 44% versus 1% at 5°C (Figure 4A et 4B). Similarly, in
272 #C16 cultures, it contained 30.3% of C16 versus 6.2% in the control at 37°C and 45.9%
273 versus 2.1% at 5°C. In contrast, UFA were not significantly different from the control in
274 #SFA cultures (Figure 5A). Short-chain FA (below C15) of Lm208 were also impacted by
275 these FA supplementations (Figure 5B). In the control, SCFA increased at 5°C compared to
276 37°C. At 5°C in UFA and C16 supplemented cultures, SCFA significantly decreased in
277 comparison to the control. C14 supplementation induced no significant change even though
278 C14 belongs to SCFA, which suggests a decrease in *de novo* synthesized SCFA (Figure 5B).
279 The WAMT of Lm208 membrane FA was lower at 5°C than at 37°C in control cultures
280 (Figure 6A). The WAMT in #UFA cultures was significantly lower to that in the control at

281 5°C, whereas it was similar at 37°C. It was significantly higher in #SFA cultures at both
 282 temperatures (Figure 6A).
 283 The EGD-e strain behaved the same way as Lm208 even though #UFA incorporation was less
 284 effective at both temperatures, but especially at 5°C (Figures 4 C and D). The same trends for
 285 WAMT adaptation were observed with EGD-e as with Lm208, but to a lesser extent (Figure
 286 6B).



287
 288 Figure 5: Fatty acid composition in unsaturated FA (C18:1, C18:2 and C18:3) (A) and in
 289 short-chain FA (C<15) (B) at 5°C and 37°C of *L. monocytogenes* Lm208 with # C18:1,
 290 #C18:2, #C18:3, #C14, or #C16 supplementation or without (control). Mean and standard
 291 deviation (n=3 to 6) are represented. FA were analyzed in comparison with the control by
 292 Dunnett's one-way ANOVA (95% confidence interval).



293

294 Figure 6: Weighted-average melting temperature of the membrane fatty acids of
295 *L. monocytogenes* Lm208 (A) and EGD-e (B) at 5°C and 37°C with # C18:1, #C18:2, #C18:3,
296 #C14, or #C16 supplementation or without (control). Mean and standard deviation (n=3 to 6)
297 are represented. WATM were analyzed in comparison with the control by Dunnett's one-way
298 ANOVA (95% confidence interval).

299

300 **4. Discussion**

301

302 In RTE foods, food safety is mainly controlled by preventing *L. monocytogenes* from growing
303 at low temperature and from reaching 100 CFU/g at the end of the shelf-life. Food matrices
304 are heterogeneous systems where microorganisms encounter several types of microstructures
305 and gradients of available resources or inhibitors (Brocklehurst et al., 1995; Malakar et al.,
306 2000). In lipid-rich foods, in particular, available FA could be heterogeneously distributed and
307 unequally available for bacterial contaminants. Thus, we explored in this study the impact of
308 different types of exogenous FA on *L. monocytogenes* growth and membrane adaptation at
309 low temperature.

310 First, nine strains isolated from different food sources were cultivated in the presence or
311 absence of oleic acid at 8°C. This UFA is in high proportion in many foods products
312 including vegetable oils, such as olive (76% of total FA), peanut (48.7% of total FA) and
313 sunflower (15.3% of total FA) oils, which are commonly used in salad sauces or other RTE
314 preparations (Zambiasi et al., 2007). Compared to the other strains, Lm540, Lm553 and
315 Lm208 showed the greatest increase of growth rate in the presence of oleic acid. These strains
316 were respectively isolated from raw or smoked salmons and from sheep brain. We
317 hypothesize that these three strains are habituated to lipid-rich media containing UFA,

318 respectively salmon (about 30.5% of UFA) (Horn et al., 2018) and sheep brain phospholipids
319 (more than 37% UFA) (Palmer et al., 1985).

320 Thereafter, we aimed to evaluate the impact of several UFA and SFA on the growth of
321 Lm208 and EGD-e at 5°C or 37°C. We demonstrated that UFA supplementations generally
322 induced a significant increase of growth rate at 5°C in contrast to SFA supplementations. Free
323 SFA or UFA are both generally known as antimicrobial agents. Straight-chain FA inhibit the
324 growth of *S. aureus* and other Gram-positive bacteria, lauric acid being the most active FA
325 (Kabara et al., 1972; Kelsey et al., 2006). UFA, including C16:1, C18:1, C18: and C18:3, also
326 show antibacterial activity (Kabara et al., 1972), which could be attributed to the ability of
327 these molecules to interact with the membrane, to create pores and to affect the production of
328 energy (Desbois and Smith, 2010). Nevertheless, scarce studies have described a positive
329 impact of FA on bacterial growth, in particular at low temperature. The addition of the apolar
330 phase of spinach to *B. cereus* cultures allows bacterial growth at low temperature without
331 oxygen (de Sarrau et al., 2013). Addition of Tween 80 (containing esterified oleic acid) to a
332 growth medium composed of cheddar cheese extract results in a significant increase in the
333 final cell density of *Lactobacillus casei* at 8°C (Tan et al., 2012). Moreover, a very recent
334 study shows that, similarly to our results, C18:1 and C18 supplementations, by Polysorbate 80
335 and Polysorbate 60, respectively increase and decrease the growth of *L. monocytogenes* at low
336 temperature (6°C) (Flegler et al., 2022). Unlike what occurred at low temperature, we found
337 no significant modifications of bacterial growth rate at 37°C, whatever the supplementation
338 (UFA or SFA). Similarly, growth patterns of *E. coli* in minimal media supplemented with
339 exogenous UFA at 37°C are similar to the control patterns (Herndon et al., 2020). In contrast,
340 supplementation with Polysorbate 80 or Polysorbate 60 results in little increase in the growth
341 rate of *L. monocytogenes* at 37°C (Flegler et al., 2022).

342 The increased growth rate we have demonstrated with UFA supplementation can compromise
343 shelf-life. According to growth simulations, two days of shelf-life are lost when UFA are
344 available in the growth environment. UFA are present in numerous food matrices, in
345 particular fish, such as salmon, and sauces containing vegetable oils. Moreover, the FA profile
346 of food products tends to be modified nowadays in favor of UFA at the expense of SFA, so as
347 to respect nutritional recommendations (INRA and Anses, 2013). FA in complex foods
348 generally occur in esterified forms such as surfactants (Polysorbates), phospholipids, or
349 triglycerides but plant, animal or bacterial lipases can partially break them down into free FA.
350 Polysorbates, phospholipids or free FA can be dispersed in the aqueous phase, but not
351 triglycerides which are trapped in emulsions. According to their localization and to their
352 ability to get in contact with bacteria, lipids and FA can potentially have different levels of
353 impact on *L. monocytogenes* growth. Studies investigating the influence of lipid content on
354 the growth parameters of *L. monocytogenes* in complex foods are scarce. Most have been
355 conducted in model emulsified systems, in which oil droplets structure the medium from
356 liquid to solid according to the percentage of fat and where most of the lipids are inside
357 droplets and thus are not in contact with the bacteria. In this type of emulsion, the presence of
358 fat droplets was shown to increase the μ_{\max} of *L. monocytogenes* at 4°C, though the authors
359 could not explain why (Verheyen et al., 2018). We can hypothesize that some free UFA could
360 be dispersed in the aqueous phase during the emulsifying process and could impact the
361 growth of *L. monocytogenes*.

362 In order to further understand the impact of UFA on the growth of *L. monocytogenes*, we
363 explored bacterial membrane FA composition. The membrane of the two strains of
364 *L. monocytogenes* is composed of SFA, BFA and UFA, as already shown in several studies
365 (Annous et al., 1997; Rogiers et al., 2017). Despite UFA synthesis is not described in
366 *L. monocytogenes*, many studies found them in small proportions in its membrane when

367 grown without UFA supplementation (Bisbiroulas et al., 2011; Hingston et al., 2017;
368 Mastronicolis et al., 2010; Püttmann et al., 1993). We could hypothesize that C18:1 could be
369 incorporated from trace C18:1 present in the culture medium, even though we could not detect
370 any UFA in the medium alone (data not shown). Another hypothesis is that the membrane
371 UFA could be converted from corresponding SFA by a desaturase. *B. cereus* produces two
372 acyl-lipid desaturases, DesA and DesB, creating double bonds in the FA chain in positions 5
373 and 10, respectively (Alvarez-Ordóñez et al., 2015). Hingston et al. (2017) suggest that a
374 desaturase-like system exists in *L. monocytogenes* that could convert C16:0 to C16:1 and
375 C18:0 to C18:1. They made this assertion based on the fact that UFA levels in the membrane
376 decreased when *L. monocytogenes* was treated with a desaturase inhibitor (Vadyvaloo et al.,
377 2002).

378 Low temperature induces modifications in FA composition of *L. monocytogenes* such as the
379 shortening of FA length, the switching of branching from *i*- to *ai*-BFA and the increase in
380 UFA. These well-known membrane modifications tend to counterbalance the physical
381 reduction of membrane fluidity due to low temperature by synthesizing FA with lower
382 melting point temperatures (Annous et al., 1997; Hingston et al., 2017).

383 When both studied strains of *L. monocytogenes* were cultivated in FA-supplemented medium,
384 their membrane composition was impacted by the incorporation of exogenous FA. Consistent
385 with our study, it was shown that exogenous FA are incorporated in the membrane
386 phospholipids of *L. monocytogenes* (Flegler et al., 2022). This FA incorporation is
387 concomitant with a decrease of SFA and BFA synthesis which could save bacterial energy.
388 FA incorporation was here shown to be strain- and temperature-dependent (higher in Lm208
389 than in EGD-e and higher at 5°C than at 37°C), but not selective regarding the type of FA.
390 Indeed, every exogenous FA that we made available in the medium was incorporated.
391 Nevertheless, the consequences of this incorporation are different according to the type of FA.

392 When UFA are available in the medium at low temperature, their great uptake dramatically
393 decreases the WAMT of the membrane FA. Although the melting temperature of the
394 cytoplasmic membrane also depends on the total polar lipid structure, this parameter can be
395 considered as an indicator for membrane fluidity (Seel et al., 2018). The decrease of WAMT
396 due to UFA incorporation probably allows *L. monocytogenes* to preserve its membrane
397 fluidity despite the low temperature and thus to grow faster than the control. Similarly, it was
398 hypothesized that the incorporation of environmental FA into the membrane of *L. casei* favors
399 bacterial growth by optimizing membrane fluidity while saving energy (Tan et al., 2012). Due
400 to their high melting temperature, exogenous SFA increase the WAMT of the membrane FA
401 when incorporated. Despite the energy saving of FA synthesis, the higher WAMT of the
402 membrane leads to a more rigid membrane which prevents an optimal exchange of
403 metabolites and thus decreases the growth rate. The mechanisms of FA incorporation in the
404 membrane phospholipids of *L. monocytogenes* should be explored. In Gram-positive bacteria,
405 exogenous FA are bound by the FA binding protein, FakB, phosphorylated by the FA kinase,
406 FakA, and the resulting acyl-phosphate is converted into acyl-ACP by an acyltransferase PlsX
407 (Yao and Rock, 2017). Further investigation at genomic and transcriptomic levels is needed to
408 understand why EGD-e have lower rates of incorporation of UFA which are concomitant with
409 lower growth increase compared to Lm208, and to decipher the whole mechanisms of FA
410 incorporation in *L. monocytogenes* at a molecular level.

411

412 **5. Conclusion**

413

414 In this study, we show that various FA are highly incorporated by the membrane of
415 *L. monocytogenes* at low temperature, probably because this is a way to save energy used in
416 FA synthesis. Nevertheless, UFA and SFA incorporations have opposite effects on

417 *L. monocytogenes* growth at low temperature. UFA supplementation induces a decrease in
418 phospholipid melting point temperature and promotes growth of the pathogen. In contrast,
419 SFA supplementation inhibits bacterial growth. These behaviors could have fundamental
420 consequences in terms of food safety and shelf-life definition. In lipid-rich food products,
421 where the distribution of FA is not homogeneous, the composition of the microenvironment
422 immediately surrounding the bacteria should be considered to predict pathogen growth. More
423 generally, models of predictive microbiology should consider the heterogeneity of bacterial
424 behavior in relationship to food composition heterogeneity.

425

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429

430 **6. References**

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