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Dairy cows under experimentally-induced *Escherichia coli* mastitis show negative emotional states assessed through Qualitative Behaviour Assessment

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Abstract

Mastitis and associated pain have been identified as a major health and welfare problem affecting dairy cows, however little is known about how cows emotionally experience this illness. Qualitative behaviour assessment (QBA) is a 'whole animal' methodology for assessing animal emotion, through description and quantification of the expressive qualities of an animal's dynamic style of behaving (eg as relaxed, anxious). The aim of this study was to use QBA to investigate whether emotional expression in dairy cows is affected by an experimental intra-mammary challenge (mastitis) with *Escherichia coli*, and to investigate the relationship of QBA scores with nine other clinical, physiological and behavioural welfare indicators. Six Holstein-Friesian cows were inoculated with *E. coli* in one healthy quarter. Evolution of the disease was assessed using bacteriological growth and somatic cell counts (SCC). The cows' response to the challenge was assessed using QBA, clinical observations, data loggers, rumen temperature sensor, and physiological indicators (inflammation, stress) at ten time-points defining the phase of the disease: before inoculation (Phase 0: 0h), in the pre-clinical Phase (Phase 1: 8h), in the acute phase (Phase 2: 12h, 16h, 24h) and in the remission phase (Phase 3 : 32h, 40h, 56h, 64h and 80h post-inoculation (hpi)). Principal Component Analysis of QBA scores identified two main dimensions of cow expression: PC1, ranging from active/vigorous/happy/bright to suffering/dejected/lethargic, and PC2, ranging from fearful/tense/anxious to confident/calm/relaxed, together explaining 58% of the total variation. QBA PC1 and PC2 scores varied with mastitis phases: QBA PC1 scores decreased by 4.09 in Phase 2, and by 1.98 in Phase 3, reflecting suffering/dejected/lethargic expressivity. QBA PC2 scores decreased by 1.91 in Phase 3, reflecting a confident/calm/relaxed expressivity. Clinical and physiological welfare indicators were associated with QBA. The higher the udder severity score, the body temperature, the concentrations of cortisol, SAA, TNF- α , and IL-1 β , the more the cows were suffering/dejected/lethargic (PC1) (coefficients: -0.51, -0.92, -2.46, 7.52×10^{-5} , -0.72, -1.13 respectively). These findings indicate that dairy cows experienced negative emotional state in the acute phase and positive emotional state in the remission phase of mastitis. This suggests that provision of pain-relief treatment during mastitis may improve animal welfare, and potentially lead to faster disease remission. However the sample size of this study was small, and larger controlled studies are needed to further investigate these findings and hypotheses. The sensitivity of QBA in this small study suggests it could potentially be a useful tool for *E. coli* mastitis detection.

Keywords	Qualitative Behaviour Assessment; Dairy cow; Animal Welfare; Mastitis; Pain; <i>Escherichia coli</i>
Taxonomy	Pain in Animals, Animal Welfare, Veterinary Behavior, Dairy Cattle Behavior
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1 **Dairy cows under experimentally-induced *Escherichia coli* mastitis**

2 **show negative emotional states**

3 **assessed through Qualitative Behaviour Assessment**

4

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25 **KEYWORDS**

26 Qualitative Behaviour Assessment,

27 Dairy cow,

28 Animal Welfare,

29 Mastitis,

30 Pain,

31 *Escherichia coli*

32

33 **HIGHLIGHTS**

- 34 • This study investigated whether the body language (QBA) of dairy cows changed when
35 they developed an udder infection.
- 36 • Dairy cows were less active/vigorous/happy/bright from 12-24 hours, and to a lesser
37 extent from 36-80 hours, after the start of udder infection than before.
- 38 • QBA cow scores significantly correlated to physiological (stress, immune response) and
39 clinical indicators, but not to lying behaviour.
- 40 • QBA could potentially be useful as a tool for *E. coli* mastitis detection.

42 **ABSTRACT**

43 Mastitis and associated pain have been identified as a major health and welfare problem affecting
44 dairy cows, however little is known about how cows emotionally experience this illness.
45 Qualitative behaviour assessment (QBA) is a ‘whole animal’ methodology for assessing animal
46 emotion, through description and quantification of the expressive qualities of an animal’s
47 dynamic style of behaving (eg as relaxed, anxious). The aim of this study was to use QBA to
48 investigate whether emotional expression in dairy cows is affected by an experimental intra-
49 mammary challenge (mastitis) with *Escherichia coli*, and to investigate the relationship of QBA
50 scores with nine other clinical, physiological and behavioural welfare indicators.

51 Six Holstein-Friesian cows were inoculated with *E. coli* in one healthy quarter. Evolution of the
52 disease was assessed using bacteriological growth and somatic cell counts (SCC). The cows’
53 response to the challenge was assessed using QBA, clinical observations, data loggers, rumen
54 temperature sensor, and physiological indicators (inflammation, stress) at ten time-points
55 defining the phase of the disease: before inoculation (Phase 0: 0h), in the pre-clinical Phase
56 (Phase 1: 8h), in the acute phase (Phase 2: 12h, 16h, 24h) and in the remission phase (Phase 3 :
57 32h, 40h, 56h, 64h and 80h post-inoculation (hpi)).

58 Principal Component Analysis of QBA scores identified two main dimensions of cow
59 expression: PC1, ranging from active/vigorous/happy/bright to suffering/dejected/lethargic, and
60 PC2, ranging from fearful/tense/anxious to confident/calm/relaxed, together explaining 58% of
61 the **total variation**. QBA PC1 and PC2 scores varied with mastitis phases: QBA PC1 scores
62 decreased by 4.09 in Phase 2, and by 1.98 in Phase 3, reflecting suffering/dejected/lethargic
63 expressivity. QBA PC2 scores decreased by 1.91 in Phase 3, reflecting a confident/calm/relaxed
64 expressivity. Clinical and physiological welfare indicators were associated with QBA. The higher
65 the udder severity score, the body temperature, the concentrations of cortisol, SAA, TNF- α , and

66 IL-1 β , the more the cows were suffering/dejected/lethargic (PC1) (coefficients: -0.51, -0.92, -
67 2.46, 7.52×10^{-5} , -0.72, -1.13 respectively).

68 These findings indicate that dairy cows experienced negative emotional state in the acute phase
69 and positive emotional state in the remission phase of mastitis. This suggests that provision of
70 pain-relief treatment during mastitis may improve animal welfare, and potentially lead to faster
71 disease remission. However the sample size of this study was small, and larger controlled studies
72 are needed to further investigate these findings and hypotheses. The sensitivity of QBA in this
73 small study suggests it could potentially be a useful tool for *E. coli* mastitis detection.

74

75 **1. INTRODUCTION**

76 Mastitis has been identified as a major health and welfare problem affecting dairy cows
77 (European Food Safety Authority, 2009; Leslie and Petersson-Wolfe, 2012), and is responsible
78 for important economic losses (Fourichon et al., 2001). Mastitis is a source of pain for the cow,
79 due to the inflammation of the udder, and increased intra-mammary and external pressure
80 (Fitzpatrick et al., 2000; Eckersall et al., 2001; Leslie and Petersson-Wolfe, 2012).

81 Sickness is the normal response to infection, characterized by endocrine, autonomic and
82 behavioural changes. It is triggered by soluble mediators that are produced at the site of infection
83 by activated accessory immune cells. These mediators are known as pro-inflammatory cytokines,
84 and include interleukine -1 α and β (IL-1 α and IL-1 β), tumour necrosis factor α (TNF- α) and
85 interleukin 6 (IL-6). They coordinate the local and systemic inflammatory response to microbial
86 pathogens, which acts on the brain to cause behavioural symptoms of sickness (e.g. withdrawal
87 from the physical and social environment, accompanied by anhedonia, fatigue, anorexia, pain,
88 and sleep disorder) (Dantzer et al., 2008). In cows, mastitis is known to induce changes in general
89 activity (Cyples et al., 2012; Veissier et al., 2017), and in **the proportion of** standing and lying

90 (Fogsgaard et al., 2012; Fogsgaard et al., 2015). It also induces a stress response through cortisol
91 release (Hopster et al., 1998) and **a** heart-rate increase (Fitzpatrick et al., 2000). De Boyer des
92 Roches et al. (2017) showed that as the development of mastitis progresses after experimental
93 inoculation of *E. coli* P4, the cows' behavioural and physiological response changes suggested
94 that they experienced discomfort in the preclinical phase (4 to 8 h post inoculation), pain in the
95 acute phase (12 to 24h post inoculation), but neither discomfort nor pain in the remission phase
96 (32 to 80h postinoculation).

97 In addition to inducing sickness and pain, activation of the immune system can lead to long
98 lasting emotional change in rodents (Low et al., 2012) and humans (Pincus et al., 1996; Dantzer
99 et al., 2008), characterized by depression-like behaviours indicative of anhedonic and negative
100 mental states (Fureix and Meagher, 2015). While this phenomenon is well described in rodents
101 and humans (Dantzer et al., 2008), few data are available in farm species. Recent studies in cattle
102 produced evidence of negative judgement bias ('pessimistic bias') in calves following the painful
103 procedure of hot-iron disbudding (Neave et al., 2013) and maternal separation (Daros et al.,
104 2014). To our knowledge, there is a lack of information on the **emotional consequences of**
105 **mastitis-induced sickness and pain in cows**.

106 The assessment of animal emotion is a critical component of animal welfare research (Désiré et
107 al., 2002; Mendl et al., 2010), and has been implemented through a variety of methods and
108 frameworks (Desire et al., 2002; Boissy et al., 2007; Mendl et al., 2010). Qualitative Behaviour
109 Assessment (QBA) is a 'whole animal' methodology that has been developed to describe and
110 quantify the expressive qualities of an animal's dynamic style of behaving (i.e. body language),
111 using descriptors (e.g. relaxed, anxious) that reflect the animal's affective state (Wemelsfelder,
112 1997; Wemelsfelder et al., 2001). A growing number of studies support the reliability and validity
113 of QBA, both under experimental and on-farm conditions (Rousing and Wemelsfelder, 2006;
114 Rutherford et al., 2012; Serrapica et al., 2016; **Ebinghaus et al., 2017; Hintze et al., 2017**). Some

115 studies of dairy cows on farm however did not find good inter-observer reliability (Bokkers et
116 al., 2012). QBA has been included in both the EU Welfare Quality® and AWIN® protocols as
117 an indicator of ‘positive emotional state’, which has generated further studies of its reliability
118 and validity at group/whole farm level (Wemelsfelder & Mullan, 2014). The aim of this study
119 was to use QBA i) to investigate whether and how experimentally induced intra-mammary
120 challenge (mastitis) with *Escherichia coli* affects emotional expression in dairy cows, and ii) to
121 investigate the relationship of QBA scores with nine other clinical, physiological and behavioural
122 welfare indicators.

123

124 2. MATERIALS AND METHODS

125 This experiment was carried out with the approval of the Val de Loire Ethics Committee for
126 Experiments on Animals (France), DGRI’s agreement APAFIS#813-2015061109103810v2.
127 Animal studies were compliant with all applicable provisions established by the European
128 Directive 2010/63/EU. All methods were performed by approved staff members in accordance
129 with the relevant standard operating procedures approved by the above mentioned ethics
130 committee. All animals used in this study were handled in strict accordance with good clinical
131 practices and all efforts were made to minimize suffering. Limit points for intervening were
132 defined before the start of the experiment but were never reached. All cows recovered completely
133 a few days after the inoculation.

134 2.1 Animals, Housing and Feeding

135 The study was conducted at the INRA animal facility (PFIE, Nouzilly, France). Six Holstein-
136 Friesian cows in their first parity were used. They were part of a larger study on the effect of
137 local immunization on the response of dairy cows to *Escherichia coli* mastitis (Herry et al., 2017).
138 The six cows used in the current study were involved as a control group in the above-mentioned

139 study, which used 18 animals in total. Detailed information on the protocol can be found in Herry
140 et al. (2017). The six cows were housed in a loose housing deep bedded barn (space allowance
141 per cow, 20m²: 15m² of bedded area and 5m² of walking area) at INRA PFIE. They were fed
142 once a day at 10:00 a diet based on corn silage, hay, soybean meal and concentrate, which met
143 the dietary requirements for the transition dairy cow and early lactation (Agabriel, 2010). The
144 mixed ration was regularly pushed back towards the cows during the day and refusal was always
145 above 5%. They were allowed water ad libitum. The cows were milked twice a day (at 0800 and
146 1600) by experienced stockmen in a milking parlour adjacent to the barn.

147 **2.2 Experimental procedures**

148 The experimental procedures imposed on the cows were published in Herry et al (2017) and in
149 de Boyer des Roches et al. (2017). In brief, the experiment was a longitudinal study, with the
150 individual dairy cow being her own control, examining the effects of experimental *E. coli*
151 infection on cows' immunologic response. *E. coli* strain P4 classified as O32:H37, ECOR
152 Phylogenetic group A, and multilocus sequence type ST10 (Blum et al., 2012) was used for
153 intramammary challenge as previously indicated.

154 Cows were challenged at 44 to 56 (average 49) days in milk. Before challenge, all quarters were
155 checked for the absence of intra-mammary infections (i.e. less than 50,000 cells per mL and
156 exempt of viable bacteria). One quarter of each cow was challenged by infusion of 1 mL of the
157 bacterial suspension (1000 cfu/mL). Inoculation was performed at midnight on day 0. Inoculation
158 was performed just after complete milking of the gland, and 8h before the next milking. Complete
159 milkings subsequent to inoculation took place twice a day, and milk samples were collected at 8,
160 16, 32, 40, 56, 64, 80 h post infection (hpi).

161 **2.3 Data Collection**

162 Blood sampling, milk sampling, and clinical observations were performed at 10 time-points just
163 before *E. coli* inoculation (T0), then at 8h, 12 h, 16 h, 24 h, 32 h, 40 h, 56 h, 64 h and 80 h post-

164 inoculation by one experienced veterinarian (de Boyer des Roches et al., 2017). Another
165 observer, a veterinarian who was QBA-trained performed the QBA observations at the same time
166 points, 15 minutes before blood and milk samples were taken. This QBA-trained observer was
167 unaware of the cows being inoculated with *E.coli*.

168 **2.3.1 Qualitative Behaviour Assessment**

169 QBA was assessed using 17 QBA descriptors adapted from the list provided in the Welfare
170 Quality® Assessment Protocol for dairy cattle (Welfare Quality®, 2009) to reflect the diversity
171 of body expressivity in the context of disease: active, vigorous, happy, bright, vigilant,
172 inquisitive, relaxed, fearful, agitated, anxious, confident, calm, tense, sad, suffering, dejected,
173 lethargic, fearful. The instructions provided in the Welfare Quality® Assessment Protocol for
174 dairy cattle protocol were carefully followed in the present study. The QBA data used in the
175 present study were recorded on the six cows just before the other physiological and clinical
176 measurements, which were taken at the 10 time points. To allow the cows to become accustomed
177 to the presence of the observer for QBA recording, and to avoid confusion between time effect
178 and inflammatory challenge effects, the cows were subjected to QBA assessments three times
179 before the challenge, at T-40h, T-32h, and T-16h. Therefore, cows were accustomed to human
180 presence at the time they underwent the experimental treatment.

181 At each time point, the observer quietly approached the cow and performed an individual
182 assessment from a distance by standing at the boundary of the pen, at 4-5m meters from the
183 animal, in order not to disturb it. The observer spent 5 mins observing the cow. When observation
184 was completed, the behavioural expression of the animal was scored on each of the QBA terms
185 along a visual analog scale (VAS) of 125 mm length, labelled from zero to maximum expression.
186 This entire process of QBA assessment of six cows took on average 40 min per time point.

187 **2.3.2. Monitoring of lying behaviour.**

188 Lying behaviour was monitored with data loggers (Hobo Pendant G Data Logger, Onset
189 Computer Corp., Pocasset, MA). Details are given in de Boyer des Roches et al. (2017). Data
190 were selected to keep lying/standing positions of the cows from 0h to 80h post challenge only
191 when these were undisturbed by humans before *E. coli* inoculation (recordings from -11h to -
192 9h), at 8h (recording from 1 to 3 h and from 5 to 6h), 12h (recording from 11 to 12 h), 16 h
193 (recording from 13 to 15 h), 24h (recording from 19 to 24 h), 32h (recording from 29 to 32h),
194 40h (recording from 35 to 38h), 56 h (recording from 49 to 55h), 64 h (recording from 59 to 62h),
195 and 80 h (recording from 67 to 78 h) post-inoculation. A **Microsoft Excel** macro was
196 subsequently used to calculate the percentage of undisturbed time spent lying and the number of
197 postural changes per hour.

198 **2.3.3. Milk Leukocytes and Bacterial Count**

199 Bacteriological examinations were conducted at the 10 time points, just before and after *E. coli*
200 inoculation. Procedures of milk sampling, bacterial population in milk, and the SCC in milk
201 observed in the six cows are described elsewhere (Herry et al., 2017).

202 **2.3.4. Clinical examination**

203 Clinical signs were recorded at the 10 time points. Local mammary signs were assessed by one
204 experienced veterinarian unaware of the cows being inoculated with *E.coli*. The mammary
205 gland was observed and palpated for swelling, firmness, pain and milk appearance to be scored
206 (local severity score) on a 7-point scale: 0-2 mild or no disease, 3-4 moderate disease and 5-7
207 severe disease (Wenz et al., 2006). **Core** body temperature was monitored every 30 minutes using
208 a ruminal sensor (Thermobolus, Medria Elevage, France), validated to monitor cows' health in
209 commercial farms (Bareille et al., 2014).

210 **2.3.4. Physiological Measurements: Hypothalamo-Pituitary-Adrenal Axis and** 211 **Inflammation**

212 Blood samples were collected by venipuncture from the jugular vein to determine physiological
213 parameters at the 10 time points. Blood samples were collected into vacutainer tubes containing
214 Na₂-EDTA (2 tubes of 10 mL) or lithium heparin (2 tubes of 10 mL). Plasma cortisol
215 concentration was determined by radioimmunoassay (Boissy and Bouissou, 1994). Haptoglobin
216 and serum amyloid A (SAA) plasma concentration were determined by immunoprecipitation
217 (Auboiron et al., 1990) and by enzyme linked immuno sorbent assay (ELISA) kit (catalogue No.
218 TP 802, Tridelta Development Limited). TNF- α in milk was determined by ELISA, and
219 commercial available kits were used for IL-1 β (Thermo Scientific, Rockford, IL, USA) as
220 described in Herry et al. (2017).

221

222 **2.4. Statistical analyses**

223 To eliminate the circadian rhythm effect on the cows' lying behaviour (Veissier et al., 1989), we
224 subtracted the data relating to lying behaviour (i.e. time spent lying and no of postural changes
225 per hour) by the observations at the same hour in the day before challenge. We then used this
226 variation (= data from hour Hi to hour Hj observed post challenge – data at hour Hi to hour Hj
227 recorded 24h before challenge) for further analyses. This allowed us to assess the variation in
228 post-inoculation lying indicators from baseline levels.

229 QBA data for each cow at each time point were recorded by measuring the distance in millimeters
230 between the zero point of the VAS scale and the mark on the line made on the scale for each
231 term, to provide a value between 0 and 125. QBA data recorded for all cows over the ten time-
232 points were analyzed together using Principal Component Analysis (PCA, correlation matrix, no
233 rotation). We focused on two main Components (PC1 and PC2) that had an eigenvalue higher
234 than 1. To interpret these Components, we took into account terms that correlated to the
235 Components at values higher than 0.6.

236 From the cows' response to *E. coli* mammary inoculation (i.e., counts of *E. coli* and SCC in milk),
237 we identified four main phases of mastitis: Phase 0 corresponded to times before inoculation (i.e.

238 T0 here), the pre-clinical phase of mastitis (Phase 1) included 4 to 8 h post inoculation; the acute
239 phase of mastitis (Phase 2) included 12 to 24 h post inoculation; and the remission phase of
240 mastitis (Phase 3) corresponded to 32 to 80 h post inoculation (de Boyer des Roches et al., 2017).
241 The variation of QBA PC1, QBA PC2 scores, clinical (Systemic severity score, udder severity
242 score, milk SCC, *E. coli* in milk, Temperature), physiological (Cortisol, Haptoglobin, SAA), and
243 behavioural (variation in time lying and in No of postural changes per h) indicators between the
244 four phases were modelled by linear mixed effect models with 'Phase' as fixed factor and 'cow'
245 as random factor.

246 The association between QBA PC1 and PC2 cow scores, and clinical (udder severity score, body
247 temperature), physiological (Cortisol, Haptoglobin, SAA concentrations) and behavioural
248 (variation in % undisturbed time spent lying; variation in no of postural changes / h)) indicators
249 was modelled by linear mixed effect models with clinical, physiological and behavioural
250 indicators as fixed factor and 'cow' as random factor.

251 To satisfy assumptions of normality of distribution, Cortisol, SCC, TNF- α , and IL-1 β were log-
252 transformed. Analyses were performed using the software R 2.15.2 (R Development Core Team,
253 2009). Package *ade4* was used for PCA, and *lmer* function from the *lme4* package (Bates et al.,
254 2015) for linear mixed effect models. The *lme4* packages does not provide P-values, therefore
255 significance of fixed effect of a parameter was set when the 95% confidence interval of this
256 parameter did not contain 0 (Bates et al., 2015). Normality of residuals and of random effect
257 distribution were visually verified using plots of residuals and quantile-quantile plots of residuals
258 and random effects.

259

260 **3. RESULTS**

261 **3.1. Bacteriological, clinical, physiological and behavioural responses to *E. coli* inoculation**

262 There was a highly significant effect of time (i.e. mastitis phase) on the bacteriological content
263 (*E. coli* in milk) and milk SCC, clinical (udder severity scores, body temperature), and
264 physiological (cortisol, haptoglobin, SAA, TNF- α , IL-1 β) responses following *E. coli* inoculation
265 of the udder (see Table 1 for further details). The *E. coli* concentration at Phase 0 (estimated
266 mean [2.5% – 97.5% Confidence interval]: 1.03x10⁻¹⁴ [-0.19-0.98] cfu/ml) significantly
267 increased by 4.85 [3.50-6.20] $\times 10^3$ cfu/ml at Phase 1, by 3.88 [2.77-4.95] $\times 10^3$ cfu/ml at Phase 2
268 and by 2.04 [1.00-3.09] at Phase 3 (Table 1). Milk Somatic cell counts at Phase 0 (17.6 [7.55-
269 32.4] $\times 10^6$), were significantly multiplied by 3.98 [1.44-11.22] at Phase 1, by 2,290 [977-5,248]
270 at Phase 2 and by 977 [446-2,187] at Phase 3 (Table 1). The udder severity score at Phase 0
271 (3.75x10⁻¹⁵ [-1.26-1.26]) significantly increased by 5.00 [3.66-6.33] at Phase 2, and by 2.93
272 [1.66-4.19] at Phase 3 (Table 1). Compared to Phase 0, the body temperature at Phase 0 (38.7
273 [38.2-39.3]°C) significantly increased by 0.81°C [0.19-1.42] at Phase 2 (Table 1). Cortisol levels
274 at Phase 0 (3.38 [2.09-5.62] ng/mL) were significantly multiplied by 3.24 [1.69-12.6] at Phase
275 1, by 7.41 [4.36-12.6] at Phase 2, and by 2.51 [1.51-4.16] at Phase 3 (Table 1). Haptoglobin
276 levels at Phase 0 (8.51 $\times 10^{-16}$ [-0.11-0.11] mg/mL) significantly increased by 0.58 [0.46-0.69] at
277 Phase 3 (Table 1). SAA levels at Phase 0 (7.5 [-57.9 – 73.0] μ g/mL) significantly increased by
278 93.5 [18.8-168.3] at Phase 2, and by 233.4 [162.5-304.4] at Phase 3 (Table 1). TNF- α levels at
279 Phase 0 (1.00 [0.21-4.87] pg/mL) were significantly multiplied by 7,585 [1,479-38,904] at Phase
280 2 (Table 1). IL-1 β levels at Phase 0 (3.98 [1.48-10.47]) were significantly multiplied by 52.5
281 [19.9-141.3] at Phase 2, and by 3.98 [1.55-10.0] at Phase 3 (Table 1).

282 3.2. Outcomes of the Qualitative Behaviour Assessment

283 The PCA explained 58 % of the total variation amongst animals, and produced two main
284 components, explaining 35 % (QBA PC1) and 23 % (QBA PC2) of the total variation,
285 respectively (Figure 2). The QBA PC1 (35%) ranged from active/vigorous/happy/bright to

286 suffering/dejected/lethargic (Table 2, Figure 2). QBA PC2 (23%) ranged from
287 fearful/tense/anxious to confident/relaxed/calm (Table 2, Figure 2).

288 3.3. Variation of QBA Components in time

289 There was a significant effect of time (i.e. mastitis phase) on the cows' scores on QBA PC1 and
290 PC2 scores. Cow scores on QBA PC1 at Phase 0 (2.39 [0.69 - 4.1] significantly decreased by
291 4.09 [-6.06 - -2.12] at Phase 2 and by 1.98 [-3.85 - -0.12] at Phase 3 (Table 3, Figure 4), reflecting
292 a stronger suffering/dejected/lethargic expression at Phase 2, and to a lesser extent, at Phase 3.
293 Cow scores on QBA PC2 at Phase 0 (1.51 [-0.04 - 3.06] significantly decreased by 1.91 [-3.31-
294 -0.51] at Phase 3 (Table 3, Figure 4), reflecting a stronger confident/relaxed/calm expression at
295 Phase 3.

296 3.4. Association of QBA PC 1 and 2 scores with clinical, physiological and behavioural 297 measures

298 Several clinical and physiological but not behavioural measures were negatively associated with
299 QBA PC1 and PC2 scores (Table 4). The model analyses suggested the higher the udder severity
300 score, body temperature, Log_{10} (cortisol concentration), SAA concentration, Log_{10} (TNF- α
301 concentration), and Log_{10} (IL-1 β concentration), the lower the values for QBA PC1 scores
302 (coefficients: -0.51, -0.92, -2.46, 7.52×10^{-5} , -0.72, -1.13 respectively), corresponding to cows
303 being described as suffering/dejected/lethargic (see Table 3 for further details). The model
304 analyses suggested the higher the SAA concentration, the lower the values for QBA PC2
305 (coefficient: -5.0×10^{-3}), corresponding to cows being described as confident/relaxed/calm.

306 4. Discussion

307 The scientific literature on the welfare consequences of mastitis is very broad, but is generally
308 only concerned with the cows' behavioural and physiological responses to physical sickness
309 and/or associated pain. To our knowledge, this study is the first to address the impact of mastitis

310 on cows' behavioural expression reflecting their emotional state. Firstly, qualitative behavioural
311 assessment (QBA) indicated that the cows emotional expressivity showed a shift towards greater
312 lethargy, dejection and suffering following inoculation of *E. coli* in the udder, and this decrease
313 in mood was associated with clinical indicators of sickness (udder severity score and body
314 temperature) and with physiological indicators of stress (cortisol) and inflammation (SAA; TNF-
315 α and IL-1 β), but not with quantitative lying behaviour. Secondly, QBA described a shift towards
316 greater confidence and calmness in the cows during recovery from inoculation in phase 3. Despite
317 the small number of dairy cows used in this study, these findings raise new hypotheses for
318 investigating the association between mastitis, pain, and a negative emotional state in dairy cows.

319 **4.1 Cows' clinical and physiological responses to *E. coli* inoculation in the udder**

320 Before inoculation of *E. coli* in the udder, cows' levels of SCC, plasma cortisol, haptoglobin,
321 SAA, TNF- α , IL-1 β , and ruminal body temperature corresponded to the basal values reported in
322 the literature (Hopster et al., 1998; Eckersall et al., 2001; Wenz et al., 2001). The six cows did
323 not express any clinical signs of sickness (Wenz et al., 2006), nor pain (Weary et al., 2006). Lying
324 behaviour recorded for 24h pre-challenge showed they spent 44.0% of their time lying and
325 changed position 1.04 times per hour, slightly higher than reported in previous studies on healthy
326 cattle (Fogsgaard et al., 2012). Together, these results show that cows did not experience any
327 sickness before the mastitic challenge. Following *E. coli* inoculation in the udder, the profiles of
328 *E. coli* development, cellular (SCC) and physiological responses of the six cows fitted the typical
329 pattern already described (Hopster et al., 1998; Eckersall et al., 2001; Bannerman et al., 2004;
330 Schukken et al., 2011). This strategy allows animals to cope with the energetic costs of immune
331 response to fight the disease (Hart, 1988; Dantzer et al., 2008).

332 **4.2 Outcomes of Qualitative Behaviour Assessment and variation in time**

333 Multivariate analysis identified two main dimensions of cow behavioural expression: QBA PC1,
334 ranging from ‘active/vigorous/happy/bright’ to ‘suffering/dejected/lethargic’, and QBA PC2,
335 ranging from ‘fearful/tense/anxious’ to ‘confident/relaxed/calm’. These dimensions concur well
336 with the main dimensions of behavioural expressivity frequently found in previous QBA studies,
337 where components describing variation in mood and/or arousal are often reported (Wemelsfelder
338 et al., 2009; Rutherford et al., 2012; Phythian et al., 2016), forming four quadrants that appear to
339 fit in well with the integrative functional framework of animal emotion and mood proposed by
340 Mendl et al. (2010). This supports the relevance of the dimensions found in the present study for
341 characterizing cow expressions in the context of animal welfare assessment.

342 We analyzed the cows’ behavioural expression in the four phases of mastitis already described
343 by de Boyer des Roches et al. (2017): before challenge (Phase 0), in the preclinical phase (Phase
344 1, 4 to 8 h post inoculation), in the acute phase (Phase 2, 12 to 24h post inoculation), and in the
345 remission phase (Phase 3, 32 to 80h post inoculation). Following inoculation of *E. coli* in the
346 udder, cows’ emotional expressivity showed a shift towards greater lethargy, dejection and
347 suffering (QBA PC1) in Phase 2 and to a lesser extent in Phase 3 of the disease. These results
348 suggest that the acute phase of the disease was associated with a negative emotional state, as
349 previously shown in rodents (Low et al., 2012) and humans (Pincus et al., 1996; Yirmiya, 2000;
350 Dantzer et al., 2008). In addition, the cows’ emotional expressivity shifted towards greater
351 calmness, confidence and relaxation (QBA PC2) in Phase 3 of the disease. This result suggests
352 that the remission phase of the mastitis was associated with a relative increase in positive
353 emotional state; cows were still somewhat lethargic/dejected/suffering as reported above, but at
354 the same time were more calm/confident/relaxed than in phase 2. Such association between
355 recovery from illness and recovery of positive mood has – to our knowledge – not been reported
356 previously for farm animals. Interestingly, Fogsgaard et al. (2012) reported that cows performed
357 more self-grooming behaviour, which has rewarding properties and is associated with positive

358 emotions (Spruijt et al., 1992; Boissy et al., 2007), from 35h following *E.coli* inoculation in the
359 udder (i.e. corresponding to the remission phase of the disease). Further studies are needed to
360 investigate more closely whether and how remission from painful disease might be associated
361 with positive mood in cattle.

362 **4.3 Association between QBA and clinical, physiological and behavioural indicators**

363 The cows' perceived levels of lethargy, dejection and suffering were associated with high udder
364 severity score, high body temperature, high levels of physiological indicators of stress (i.e.
365 cortisol) and high levels of physiological indicators of inflammation (i.e. SAA, TNF- α and IL-
366 1 β). However, we did not find any association between QBA and lying behaviour (variation in
367 time spent lying; number of postural changes per hour). These findings are in line with recent
368 research in cattle (Stockman et al., 2011; Stockman et al., 2012), and sheep (Wickham et al.,
369 2012) demonstrating a significant correlation between QBA and physiological indicators relevant
370 to welfare. Such findings support that QBA addressed important aspects of cow health and
371 welfare, and is able to provide complementary information to help interpret the wider impact of
372 mastitis on dairy cows' mood and welfare state.

373 **Considering** these findings in somewhat more detail, it was found that when blood cortisol levels
374 increased after inoculation in phase 2 by a factor of 7.4 compared to phase 1, (reflecting
375 Hypothalamus-pituitary-adrenal (HPA) axis activation), the cows' mood decreased towards an
376 expressivity perceived as dejected, lethargic and suffering. Transient activation of the HPA axis
377 is known to coincide with emotional activation, but measurement of HPA activity cannot by itself
378 tell whether this is a shift towards negative or positive emotion (Boissy et al., 2007). Thus the
379 addition of QBA to studies of **animal emotion** can add key information about the meaning of
380 physiological activation in terms of the animal's welfare (Rutherford et al., 2012). A shift in the
381 cows' mood towards lethargic/dejected/suffering was also associated with high **levels** of
382 cytokines IL-1 β and TNF- α , a finding that is consistent with reports on the effects of cytokines

383 release in the literature. In laboratory animals, the release of cytokine was found to mediate
384 behavioural responses such as a decrease in general activity and exploratory behaviour, a
385 decrease in social interactions and food intake, an increase in anhedonic behaviour, and an
386 impairment of learning (Yirmiya, 2000). In humans, cytokine release during experimental
387 endotoxemia correlates with anxiety, depressed mood and decrease of memory performances
388 (Reichenberg et al., 2001).

389 However, QBA of cows described as lethargic/dejected/suffering was not found to be associated
390 with higher levels in the cows' lying behaviour. This result is perhaps surprising in that i) many
391 previous studies report associations between QBA and quantitative measures of behaviour
392 (Rousing and Wemelsfelder, 2006; Napolitano et al., 2008; Minero et al., 2009; Rutherford et
393 al., 2012; Sant'Anna and Paranhos da Costa, 2013), and ii) one might expect that physical
394 sickness could lead to reduced activity and higher levels of rest in association with negative mood
395 (Yirmiya, 2000). However, first of all, none of the previous QBA studies investigated QBA's
396 association with behaviour in the context of sickness or pain. Moreover, the lack of association
397 between lethargy/dejection and lying behaviour might reflect that QBA is essentially a 'whole
398 animal' measure and does not depend on the level of particular physical behaviours such as lying
399 or resting. QBA is designed to integrate differences in an animal's overall demeanour, specifying
400 not *what* it does, but *how* it moves around in whatever it does (Wemelsfelder et al., 2001; Fleming
401 et al., 2016). In this way, lethargy and dejection could be perceived generally as expressive
402 qualities of all the animals' movements while they were sick, whether they were walking, eating,
403 standing or lying. Through this capacity to discern subtle expressive qualities of demeanour
404 generally, QBA can provide information which conventional ethograms would fail to notice, and
405 which, as the findings reported here indicate, can help to interpret the impact of sickness and
406 associated physical changes on the animals' welfare.

407 Finally, we observed an association between cows' emotional expressivity and SAA : the cows
408 were perceived calmer, more confident and relaxed when levels of SAA were high. This result

409 might be perceived as counterintuitive: we would have expected to see more relaxed cows when
410 the indicators of inflammation were low. In fact, this association does not reflect a biological
411 link, because it is explained by the kinetics of SAA secretion through the inflammatory process.
412 SAA peak corresponds to the remission phase, rather than to the acute phase of inflammation
413 (characterized by an increase in the heart rate, respiratory rate and body temperature). These
414 kinetics have been shown in other mastitis studies (e.g. Herry et al., 2017; Fogsgaard et al., 2012;
415 Vels et al., 2009). This result therefore supports the association between recovery from illness
416 and recovery of positive mood in cattle.

417 **4.4. Methodological considerations.**

418 For reasons of feasibility, QBA was performed by a single assessor through direct observation
419 of the cows. It could be argued that it is difficult under such circumstances to discern whether
420 the observed fluctuations in QBA scores over time were based on genuine shifts in the animals'
421 state, or were simply due to random variation in the observer's scoring patterns over time
422 (Temple et al., 2013; Phythian et al., 2016). However, the QBA outcomes were not random; QBA
423 dimensions were similar to those identified in previous studies (Rutherford et al., 2012; Minero
424 et al., 2016), and, in the present study, were significantly and meaningfully correlated to clinical
425 and physiological indicators of stress and inflammation, which supports their relevance. There is
426 nevertheless a need to further address the inter-observer reliability of using QBA fixed list terms
427 to describe the expressive behavioural repertoire of cattle with mastitis.

428 **4.5. Conclusions**

429 Our results show that dairy cows' behavioural expression, assessed through qualitative
430 behavioural assessment (QBA), was assessed to be more lethargic/dejected/suffering in the acute
431 sickness phase (12-24 hpi), and to a lesser extent in the remission phase, of mastitis (32-80 hpi)
432 induced by inoculation of *E. coli* in the udder. The remission phase (32-80 hpi) was also

433 characterized by cows being more calm/confident/relaxed. By associating the QBA outcomes to
434 various quantitative data, our results confirm **that QBA** can be conveniently used as a tool to
435 interpret animals' emotional experience in the context of sickness and associated pain in cattle.
436 **The use of QBA to monitor cows responses to mastitis** could be used as a means to train farmers
437 and veterinarian students to early and better detect mastitis in dairy cows. Additional studies
438 addressing the inter-observer reliability of QBA terms in the context of sickness behaviour in
439 cattle or performed in larger case studies would be needed to further validate the use of QBA in
440 such context.

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Table 1. Coefficients^{1,2,3,4} of linear mixed effect models of bacteriological, clinical, physiological and behavioural measures between Phases of mastitis from 6 Holstein-Friesian cows inoculated with *E. coli*: phase 0 corresponded to times before inoculation, phase 1 included 4 to 8 h post inoculation; phase 2, 12 to 24 h post inoculation; and phase 3, 32 to 80 h post inoculation.

		Estimate	Confidence interval		t-value
			2.50%	97.5%	
Bacteriological indicators and Somatic cell counts					
<i>E. coli</i> in milk (10 ³ cfu/ml)	Fixed effects : coefficients of the model				
	Intercept (Phase 0)	1.03x10 ⁻¹⁴	-0.98	0.98	2.03x10 ⁻¹⁴
	Phase 1	4.85	3.50	6.20	6.97
	Phase 2	3.88	2.77	4.98	6.82
	Phase 3	2.04	1.00	3.09	3.80
	Random effects : standard deviations				
	Animal	0.34	0.0	0.85	-
Residuals	1.21	0.98	1.43	-	
Log ₁₀ [Milk SCC (10 ³ SCC/mL)]	Fixed effects : coefficients of the model				
	Intercept (Phase 0)	4.19	3.88	4.51	25.4
	Phase 1	0.6	0.16	1.05	2.59
	Phase 2	3.36	2.99	3.72	17.6
	Phase 3	2.99	2.65	3.34	16.6
	Random effects : standard deviations				
	Animal	2.85x10 ⁻¹⁰	0.00	0.19	-
Residuals	0.41	0.33	0.47	-	
Clinical Indicators					
Udder severity score	Fixed effects : coefficients of the model				
	Intercept (Phase 0)	3.75x10 ⁻¹⁵	-1.26	1.26	5.63x10 ⁻¹⁵
	Phase 1	1.67	0.03	3.3	1.98
	Phase 2	5.00	3.66	6.33	7.26
	Phase 3	2.93	1.66	4.19	4.49
	Random effects : standard deviations				
	Animal	0.69	0.07	1.45	-
Residuals	1.46	1.19	1.73	-	
Body Temperature (°C)	Fixed effects : coefficients of the model				
	Intercept (Phase 0)	38.7	38.2	39.3	139.2
	Phase 1	-0.4	-1.15	0.35	-1.03
	Phase 2	0.81	0.19	1.42	2.54
	Phase 3	-0.21	-0.8	0.36	-0.71
	Random effects : standard deviations				
	Animal	0.09	0.00	0.37	-
Residuals	0.67	0.55	0.79	-	
Physiological Indicators					
Log ₁₀ [Cortisol (ng/mL)]	Fixed effects : coefficients of the model				
	Intercept (Phase 0)	0.53	0.32	0.75	4.82
	Phase 1	0.51	0.23	0.8	3.49
	Phase 2	0.87	0.64	1.1	7.24
	Phase 3	0.4	0.18	0.62	3.51
	Random effects : standard deviations				
	Animal	0.09	0.00	0.21	-
Residuals	0.26	0.21	0.31	-	
Haptoglobin (mg/mL)	Fixed effects : coefficients of the model				
	Intercept (Phase 0)	8.51x10 ⁻¹⁶	-0.11	0.11	2.85x10 ⁻¹⁴
	Phase 1	-7.64x10 ⁻¹⁶	-0.15	0.15	2.85x10 ⁻¹⁴
	Phase 2	0.04	-0.07	0.16	0.68
	Phase 3	0.58	0.46	0.69	10.00
	Random effects : standard deviations				
	Animal	0.04	0.00	0.10	-
Residuals	0.13	0.11	0.15	-	
SAA (µg / mL)	Fixed effects : coefficients of the model				
	Intercept (Phase 0)	7.5	-57.9	73.0	0.22
	Phase 1	14.3	-77.2	106.3	0.3
	Phase 2	93.5	18.8	168.3	2.42
	Phase 3	233.4	162.5	304.4	6.36
	Random effects : standard deviations				
	Animal	15.30	0.00	49.2	-
Residuals	82.1	66.8	97.2	-	

Log ₁₀ [TNF-α (pg / mL)]	Fixed effects : coefficients of the model				
	Intercept (Phase 0)	-4.15x10 ⁻¹⁵	-0.68	0.69	-1.14x10 ⁻¹⁴
	Phase 1	0.84	-0.02	1.7	1.88
	Phase 2	3.88	3.17	4.59	10.6
	Phase 3	0.63	-0.04	1.29	1.81
	Random effects : standard deviations				
	Animal	0.42	0.12	0.86	-
Residuals	0.77	0.63	0.91	-	
Log ₁₀ [IL -1β (pg/mL)]	Fixed effects : coefficients of the model				
	Intercept (Phase 0)	0.6	0.17	1.02	2.69
	Phase 1	0.04	-0.48	0.57	0.17
	Phase 2	1.72	1.3	2.15	7.79
	Phase 3	0.6	0.19	1.00	2.86
	Random effects : standard deviations				
	Animal	0.28	0.10	0.56	-
Residuals	0.46	0.38	0.56	-	
Behavioural indicators					
Variation in Time Lying from baseline (% total undisturbed time)	Fixed effects : coefficients of the model				
	Intercept (Phase 0)	0.06	-0.12	0.24	0.63
	Phase 1	-0.04	-0.31	0.22	-0.32
	Phase 2	0.09	-0.12	0.30	0.82
	Phase 3	0.01	-0.19	0.21	0.09
	Random effects : standard deviations				
	Animal	3.7x10 ⁻⁹	0.00	0.08	-
Residuals	0.24	0.19	0.28	-	
Variation in No postural changes/24h from baseline (undisturbed time)	Fixed effects : coefficients of the model				
	Intercept (Phase 0)	-0.07	-0.54	0.39	-0.29
	Phase 1	-0.29	-0.93	0.34	-0.89
	Phase 2	0.23	-0.29	0.75	0.84
	Phase 3	0.04	-0.45	0.53	0.13
	Random effects : standard deviations				
	Animal	0.20	0.00	0.46	-
Residuals	0.57	0.46	0.68	-	

¹ Example of R Formula for *E.coli* in milk: lmer (*E.coli* in milk ~ Phase + (1 | animal))

² Example for Body temperature: mean score at Phase 0 was (mean [2.5%-97.5% confidence interval]): 38.7 [38.2-39.3]°C. Compared to Phase 0, body temperature increased significantly by 0.81 [0.19-1.42] at Phase 2: the mean at Phase 2 is 39.51 and the confidence interval for this increase is [0.19-1.42].

³ Example for SCC: mean SCC at Phase 0 was exp (4.19) = 15.4 x 10³ SCC/mL. Compared to Phase 0, Log Milk Somatic cell count (4.19 [3.88-4.51]) is significantly multiplied by 3.98 [1.44-11.22] at Phase 1, by 2,290 [977-5,248] at Phase 2 and by 977 [446-2,187] at Phase 3.

⁴ 95% confidence interval which does not contain zero indicates the parameter is significant (Bates et al., 2015).

Table 2. Principal component analysis of the 17 QBA terms applied to 6 Holstein-Friesian cows at 10 time points: eigenvalue, proportion of variance explained by the component and correlation of each variable to the component¹.

	Component 1	Component 2
Eigenvalue	5.95	3.84
Proportion of total variance explained by component (%)	35.04	22.61
Correlation of each variable to component:		
Active	0.82	0.01
Vigorous	0.80	-0.20
Happy	0.77	-0.09
Bright	0.76	0.29
Vigilant	0.68	0.46
Inquisitive	0.54	-0.04
Relaxed	0.42	-0.66
Fearful	0.15	0.84
Agitated	0.12	0.29
Anxious	0.12	0.78
Confident	0.05	-0.71
Calm	-0.5	-0.60
Tense	-0.29	0.82
Sad	-0.66	0.09
Suffering	-0.74	0.27
Dejected	-0.83	-0.20
Lethargic	-0.84	-0.07

1. Bold represent variables that contribute most to the component (P< 0.05).

Table 3. Coefficients ^{1,2} of linear mixed effect models of BQA PC1 and PC2 between Phases of mastitis from 6 Holstein-Friesian cows inoculated with *E. coli*: phase 0 corresponded to times before inoculation, phase 1 included 4 to 8 h post inoculation; phase 2, 12 to 24 h post inoculation; and phase 3, 32 to 80 h post inoculation.

		Estimate	Confidence interval		t-value
			2.50%	97.5%	
Qualitative Behaviour PC					
QBA PC1	Fixed effects : coefficients of the model				
	Intercept (Phase 0)	2.39	0.69	4.1	2.70
	Phase 1	-1.73	-4.14	0.67	-1.38
	Phase 2	-4.09	-6.06	-2.12	-4.00
	Phase 3	-1.98	-3.85	-0.12	-2.05
	Random effects : standard deviations				
	Animal	0.00	0.00	0.87	-
	Residuals	2.17	1.77	2.53	-
QBA PC2	Fixed effects : coefficients of the model				
	Intercept (Phase 0)	1.51	-0.04	3.06	1.87
	Phase 1	-1.01	-2.81	0.79	-1.09
	Phase 2	-1.51	-2.98	0.00	-1.98
	Phase 3	-1.91	-3.31	-0.51	-2.66
	Random effects : standard deviations				
	Animal	1.14	0.49	2.24	-
	Residuals	1.61	1.31	1.91	-

¹ R Formula for QBA PC1: lmer(QBAPC1 ~ Phase + (1 | animal))

² 95% confidence interval which does not contain zero indicates the parameter is significant (Bates et al., 2015).

Table 4. Coefficients^{1,2} of linear mixed effect models between QBA PC1, PC2, and the clinical, physiological and behavioural measures.

		QBA - PC1				QBA - PC 2			
		Estimate	Confidence interval		t-value	Estimate	Confidence interval		t-value
			2.50%	97.5%			2.50%	97.5%	
Clinical Indicators									
Udder severity score	Fixed effects : coefficients of the model								
	Intercept	1.62	0.59	2.68	3.12	0.42	-0.80	1.65	0.69
	Udder Severity Score	-0.51	-0.76	-0.24	-3.89	-0.13	-0.34	0.07	-1.28
	Random effects : standard deviations								
	Animal	0.31	0.00	1.23	-	1.14	0.47	2.23	-
	Residuals	2.2	1.82	2.64	-	1.66	1.38	2.02	-
Body Temperature (°C)	Fixed effects : coefficients of the model								
	Intercept	35.7	7.17	64.3	2.45	-10.5	-32.6	10.2	-0.99
	Body temperature	-0.92	-1.65	-0.18	-2.45	0.27	-0.26	0.84	0.98
	Random effects : standard deviations								
	Animal	0.00	0.00	0.86	-	1.08	0.39	2.14	-
	Residuals	2.36	1.96	2.81	-	1.69	1.4	2.04	-
Physiological Indicators									
Log ₁₀ [Cortisol (ng/mL)]	Fixed effects : coefficients of the model								
	Intercept	2.58	0.85	4.31	2.92	0.53	-1.05	2.12	0.64
	Log ₁₀ (Cortisol)	-2.46	-4.02	-0.91	-3.11	-0.51	-0.69	0.69	-0.83
	Random effects : standard deviations								
	Animal	1.43 x 10 ⁻¹⁰	0.00	0.96	-	1.14	0.46	2.25	-
	Residuals	2.29	1.90	2.73	-	1.68	1.39	2.03	-
Haptoglobin (mg/mL)	Fixed effects : coefficients of the model								
	Intercept	-0.53	-1.39	0.31	-1.22	0.29	-0.82	1.42	0.55
	Haptoglobine	1.77	-0.21	3.75	1.75	-0.99	-2.39	0.4	-1.39
	Random effects : standard deviations								
	Animal	0.00	0.00	0.88	-	1.109	0.44	2.19	-
	Residuals	2.41	2.01	2.88	-	1.67	1.39	2.02	-
SAA (µg / mL)	Fixed effects : coefficients of the model								
	Intercept	0.02	-1.01	0.99	-0.02	0.84	-0.38	2.07	1.4
	SAA	7.52x10 ⁻⁵	2.06	2.95	0.03	-0.005	-0.008	-0.002	-3.28
	Random effects : standard deviations								
	Animal	0.00	0.00	0.92	-	1.24	0.57	2.39	-
	Residuals	2.48	2.06	2.95	-	1.54	1.28	1.86	-
Log ₁₀ [TNF-α (pg / mL)]	Fixed effects : coefficients of the model								
	Intercept	1.13	0.41	1.85	3.07	-0.03	-1.15	1.11	-0.05
	Log ₁₀ (TNF-α)	-0.72	-1.02	-0.41	-4.60	0.02	-0.23	0.27	0.13
	Random effects : standard deviations								
	Animal	0.00	0.00	0.98	-	1.12	0.44	2.22	-
	Residuals	2.12	1.76	2.53	-	1.69	1.41	2.05	-

Log ₁₀ [IL -1β (pg/mL)]	Fixed effects : coefficients of the model								
	Intercept	1.61	0.45	2.78	2.71	0.19	-1.09	1.54	0.29
	Log ₁₀ (IL -1β)	-1.13	-1.84	-0.42	-3.13	-0.14	-0.68	0.44	-0.49
	Random effects : standard deviations								
	Animal	6.81x10 ⁻¹⁰	0.00	0.96	-	1.17	0.47	2.29	-
	Residuals	2.29	1.91	2.73	-	1.69	1.40	2.05	-
Behavioural indicators									
Variation in Time Lying from baseline (% total undisturbed time)	Fixed effects : coefficients of the model								
	Intercept	-0.02	-0.70	0.64	-0.08	-0.01	-1.11	1.07	-0.03
	Time Lying	0.32	-2.38	3.02	0.23	0.19	-1.72	2.05	0.20
	Random effects : standard deviations								
	Animal	0.00	0.00	0.92	-	1.13	0.46	2.23	-
	Residuals	2.48	2.06	2.95	-	1.69	1.41	2.05	-
Variation in No postural changes/24h from baseline (undisturbed time)	Fixed effects : coefficients of the model								
	Intercept	0.003	-0.62	0.64	0.01	0.001	-1.08	1.08	0.002
	No postural changes	0.19	-0.85	1.23	0.36	0.06	-0.71	0.83	0.154
	Random effects : standard deviations								
	Animal	0.00	0.00	0.90	-	1.13	0.45	2.23	-
	Residuals	2.47	2.05	2.94	-	1.69	1.40	2.05	-

¹ R Formula for QBA PC1 and systemic severity score: lmer(QBAPC1 ~ Systemic severity score + (1 | animal))

² 95% confidence interval which does not contain zero indicates the parameter is significant (Bates et al., 2015).

Figure 1. Changes in the percentage of time spent lying (A) and in the Number of postural changes per h (B) in 6 Holstein-Friesian cows inoculated with *E. coli*, at 0h. Grey line correspond to the inoculation of *E. coli* in the udder.

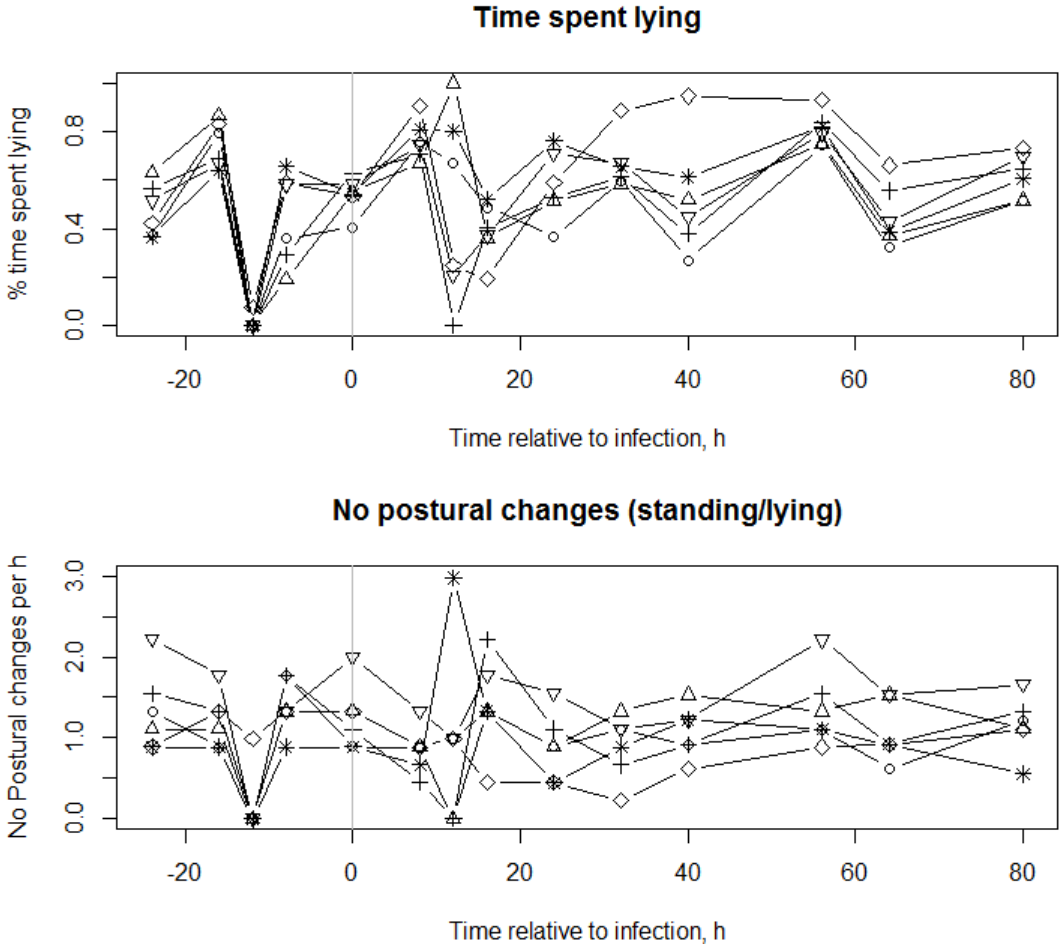


Figure 2. Individual changes in *Escherichia coli* count in milk ($\log_{10} \times 10^3$ cfu/mL), SCC in milk (10^6 cells / mL), udder severity score, body temperature ($^{\circ}\text{C}$), plasma cortisol (ng / mL), plasma haptoglobin (mg / ml), plasma serum amyloid A ($\mu\text{g}/\text{mL}$), TNF- α in milk (pg / mL), IL-1 β (pm / mL), variation in the percentage of time lying and in the number of postural (standing/lying) changes from baseline in 6 Holstein-Friesian cows inoculated with *E. coli*, at 0h.

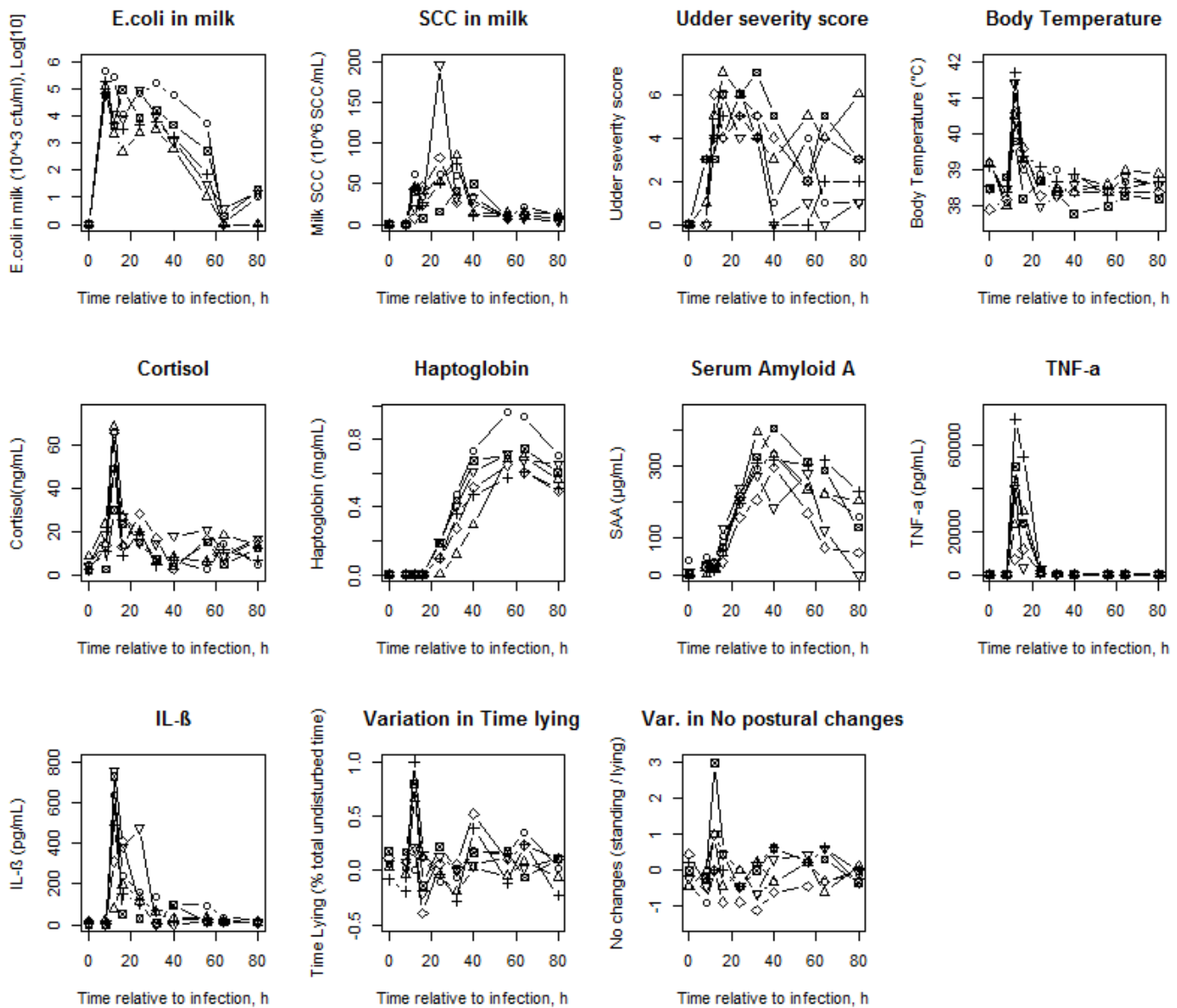


Figure 3. Loadings of the 17 QBA terms on QBA – Component 1 and QBA – Component 2 analysis from 6 Holstein-Friesian cows inoculated with *E. coli*, at 0h. Axes reflect arbitrary scaling values.

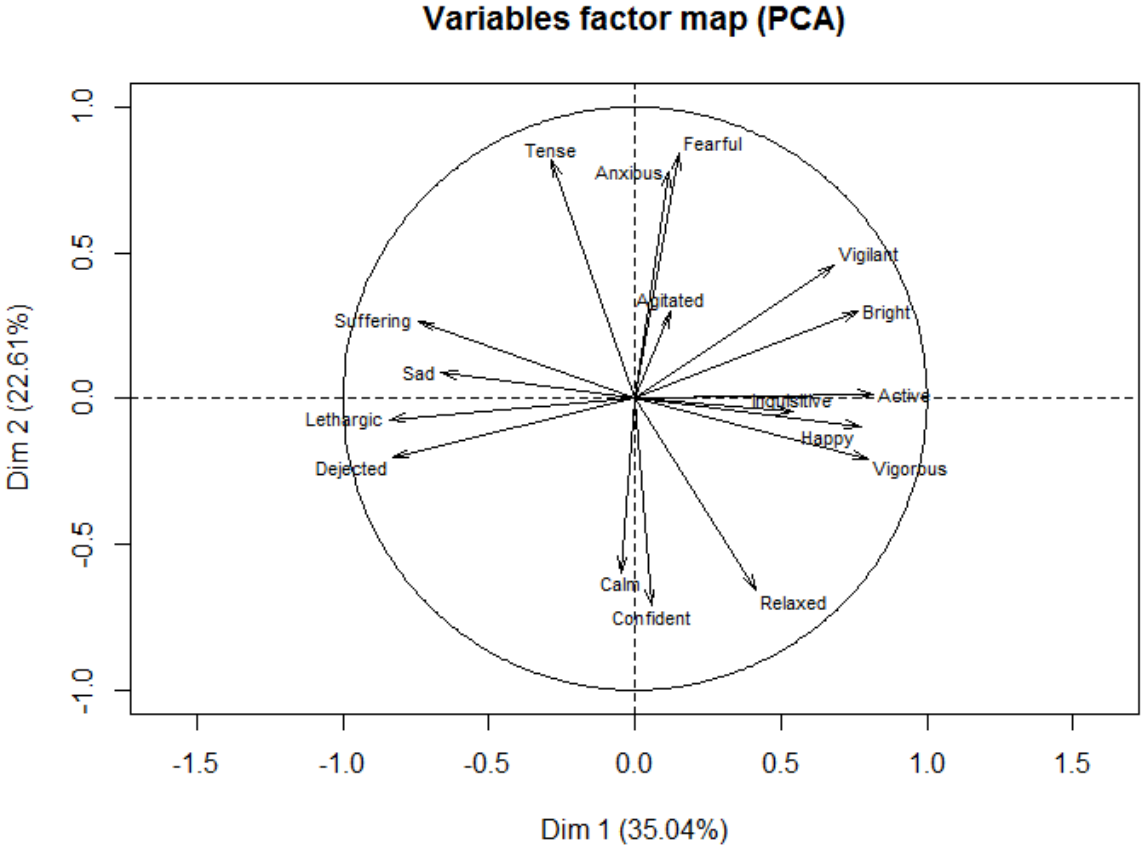


Figure 4. Changes in QBA PC 1 and in QBA PC 2 in 6 Holstein-Friesian cows inoculated with *E. coli*, at 0h.

