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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-1β, the more the cows were suffering/dejected/lethargic (PC1) (coefficients: -0.51, -0.92, -2.46, 7.52x10-5, -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; Escherichia coli
Taxonomy	Pain in Animals, Animal Welfare, Veterinary Behavior, Dairy Cattle Behavior

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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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KEYWORDS

Qualitative Behaviour Assessment,

Dairy cow,

Animal Welfare,

Mastitis,

Pain,

Escherichia coli

HIGHLIGHTS

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

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

66 IL-1β, 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 -1α 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

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

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

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

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

2. MATERIALS AND METHODS

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

2.1 Animals, Housing and Feeding

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

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

2.2 Experimental procedures

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

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

2.3 Data Collection

Blood sampling, milk sampling, and clinical observations were performed at 10 time-points just 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-

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

2.3.1 Qualitative Behaviour Assessment

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

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

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 experimented 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-1β (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.

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

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

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

3. RESULTS

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

There was a highly significant effect of time (i.e. mastitis phase) on the bacteriological content (*E. coli* in milk) and milk SCC, clinical (udder severity scores, body temperature), and physiological (cortisol, haptoglobin, SAA, TNF-α, IL-1β) responses following *E. coli* inoculation of the udder (see Table 1 for further details). The *E. coli* concentration at Phase 0 (estimated mean [2.5% – 97.5% Confidence interval]: $1.03x10^{-14}$ [-0.19-0.98] cfu/ml) significantly 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 and by 2.04 [1.00-3.09] at Phase 3 (Table 1). Milk Somatic cell counts at Phase 0 (17.6 [7.55-32.4] $x10^6$), were 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 (Table 1). The udder severity score at Phase 0 ($3.75x10^{-15}$ [-1.26-1.26]) significantly increased by 5.00 [3.66-6.33] at Phase 2, and by 2.93 [1.66-4.19] at Phase 3 (Table 1). Compared to Phase 0, the body temperature at Phase 0 (38.7 [38.2-39.3]°C) significantly increased by 0.81°C [0.19-1.42] at Phase 2 (Table 1). Cortisol levels at Phase 0 (3.38 [2.09-5.62] ng/mL) were significantly multiplied by 3.24 [1.69-12.6] at Phase 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 levels at Phase 0 (8.51×10^{-16} [-0.11-0.11] mg/mL) significantly increased by 0.58 [0.46-0.69] at Phase 3 (Table 1). SAA levels at Phase 0 (7.5 [-57.9 – 73.0] µg/mL) significantly increased by 93.5 [18.8-168.3] at Phase 2, and by 233.4 [162.5-304.4] at Phase 3 (Table1). TNF-α levels at Phase 0 (1.00 [0.21-4.87] pg/mL) were significantly multiplied by 7,585 [1,479-38,904] at Phase 2 (Table 1). IL-1β levels at Phase 0 (3.98 [1.48-10.47]) were significantly multiplied by 52.5 [19.9-141.3] at Phase 2, and by 3.98 [1.55-10.0] at Phase 3 (Table 1).

3.2. Outcomes of the Qualitative Behaviour Assessment

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

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

3.3. Variation of QBA Components in time

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

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

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

4. Discussion

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

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

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

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

4.2 Outcomes of Qualitative Behaviour Assessment and variation in time

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

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

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

4.4. Methodological considerations.

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

4.5. Conclusions

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

characterized by cows being more calm/confident/relaxed. By associating the QBA outcomes to various quantitative data, our results confirm that QBA can be conveniently used as a tool to interpret animals' emotional experience in the context of sickness and associated pain in cattle. The use of QBA to monitor cows responses to mastitis could be used as a means to train farmers and veterinarian students to early and better detect mastitis in dairy cows. Additional studies addressing the inter-observer reliability of QBA terms in the context of sickness behaviour in cattle or performed in larger case studies would be needed to further validate the use of QBA in 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					
E.coli in milk (10^3 cfu/ml)	Fixed effects : coefficients of the model				
	Intercept (Phase 0)	1.03×10^{-14}	-0.98	0.98	2.03×10^{-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	2.85×10^{-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)	3.75×10^{-15}	-1.26	1.26	5.63×10^{-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)	8.51×10^{-16}	-0.11	0.11	2.85×10^{-14}
	Phase 1	-7.64×10^{-16}	-0.15	0.15	2.85×10^{-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 10^3 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 component[1].

	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	1.43×10^{-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	7.52×10^{-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.

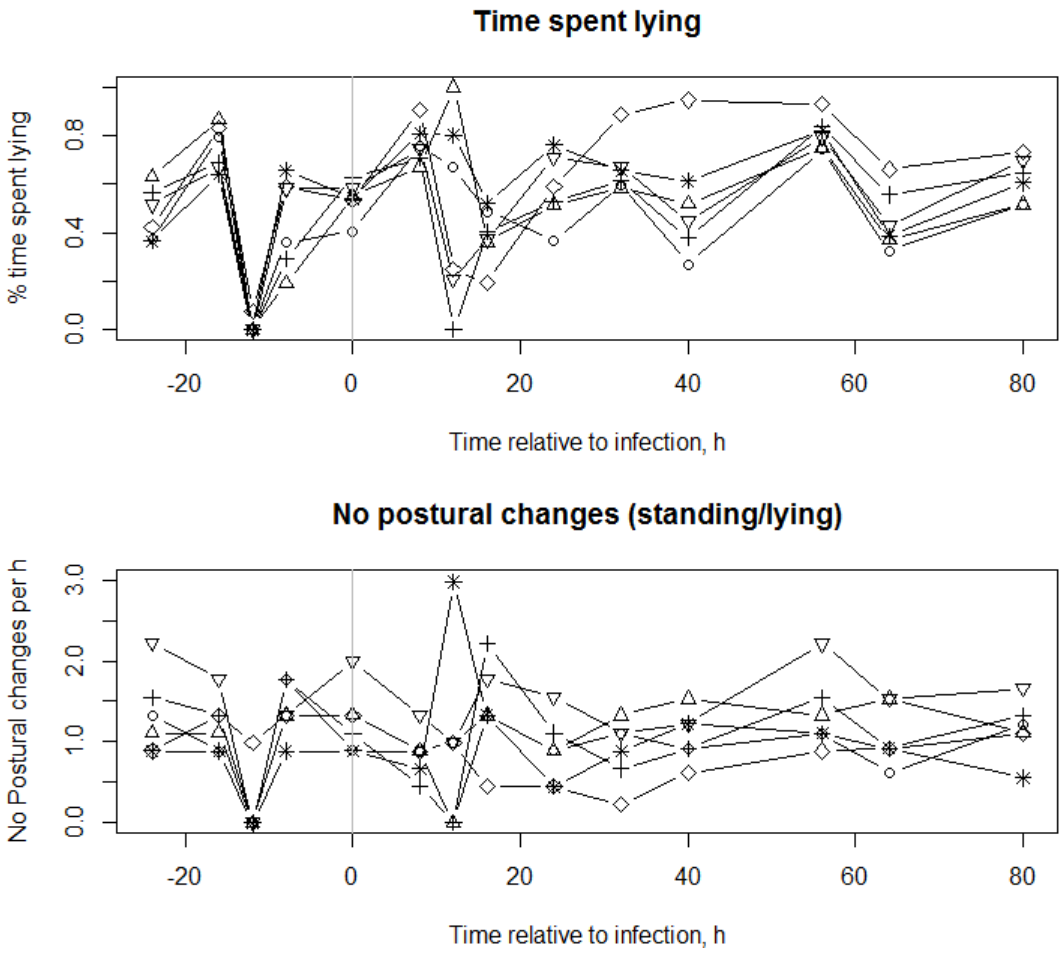

Figure 2. Individual changes in *Escherichia coli* count in milk (log10 x 10^3 cfu/mL), SCC in milk (10^6 cells / mL), udder severity score, body temperature (°C), plasma cortisol (ng / mL), plasma haptoglobin (mg / ml), plasma serum amyloid A (µg/mL), TNF-α in milk (pg / mL), IL-1β (pm / mL), variation 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.

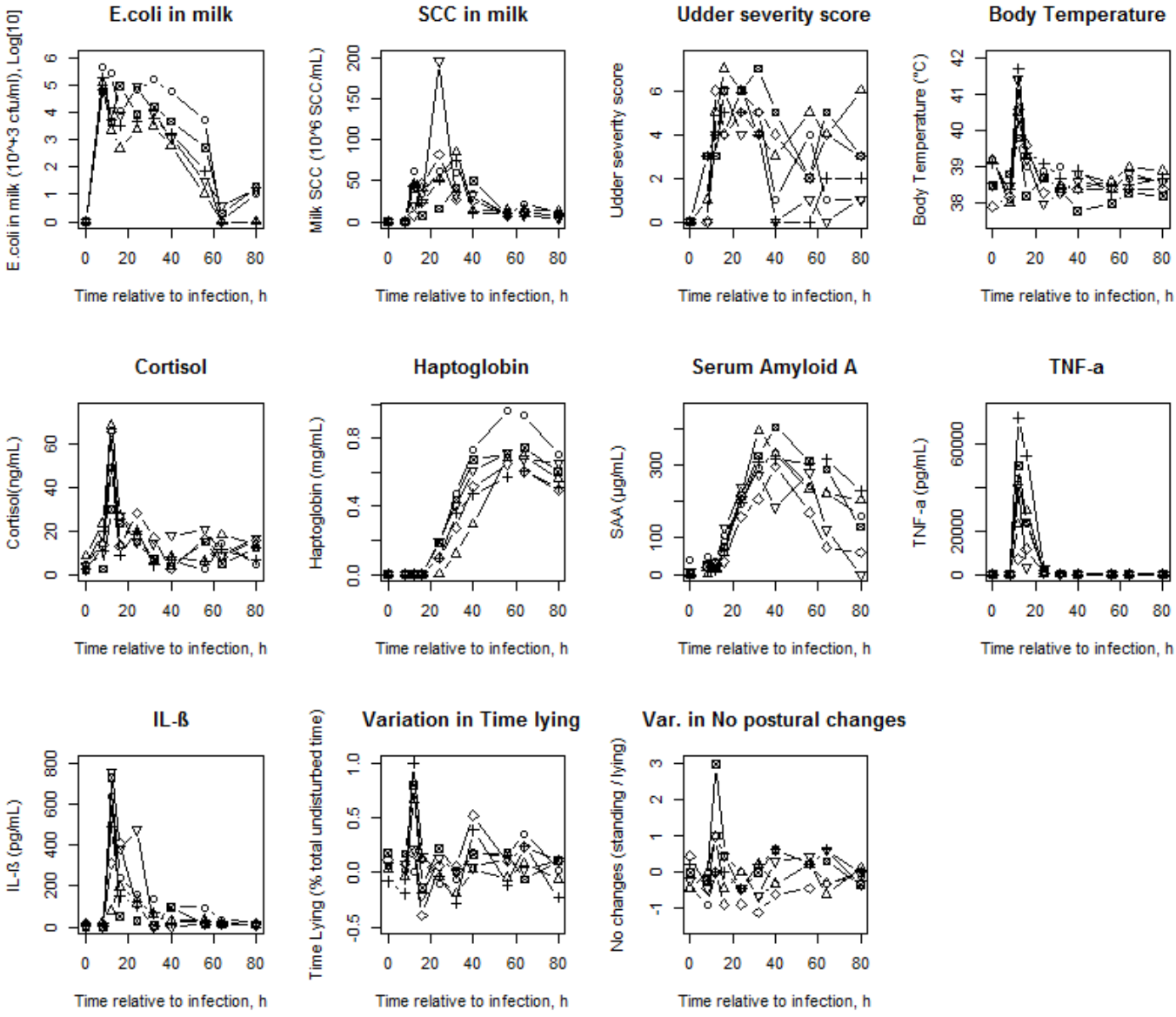

Figure 3. Loadings of the 17 QBA terms on QBA – Component 1 and QBA – Component 2 analysis from 6 Holstein-Friesian cows inoculated with *E. coli*, at 0h. Axes reflect arbitrary scaling values.

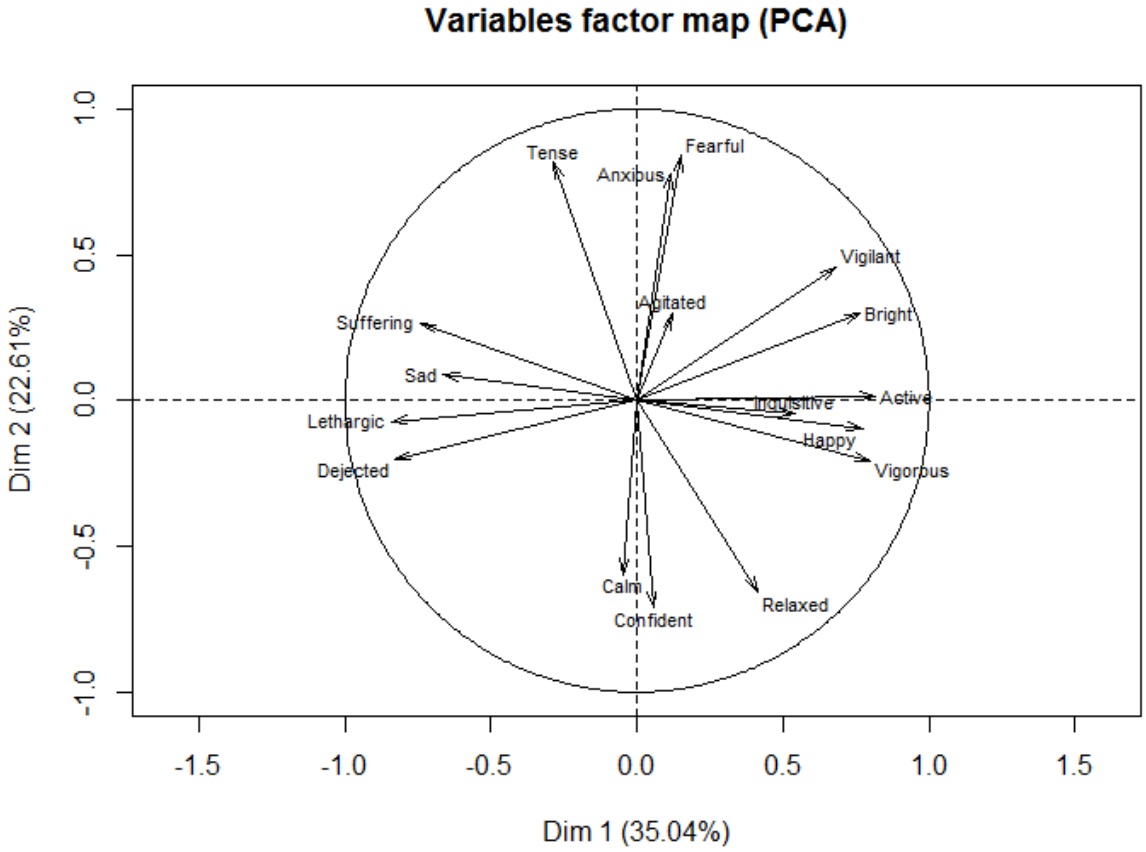

Figure 4. Changes in QBA PC 1 and in QBA PC 2 in 6 Holstein-Friesian cows inoculated with *E. coli*, at 0h.