

1 **Tear staining in finisher pigs and its relation to age, growth, sex and potential pen**
2 **level stressors**

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14 Short title: Tear staining in finisher pigs

15

16 **Abstract**

17 Tear staining (TS) in the pig has been related to different stressors and may be a useful

18 tool for assessing animal welfare on farm. The aim of the current study was to

19 investigate TS across the finisher period and its possible relation to age, growth, sex

20 and experimentally induced stressors. The study included 80 finisher pens divided

21 between three batches. Within each batch, the pens either included pigs with docked or

22 undocked tails, had straw provided (150 g/pig/day) or not and had a low (1.21 m²/pig,

23 11 pigs) or high stocking density (0.73 m²/pig, 18 pigs). TS (score 1 to 4; from smaller to

24 larger tear stain area, respectively) and tail damage were scored on each individual pig
25 three times per week over the 9-week study period, and the individual maximum TS
26 score within each week was chosen for further analysis. Data were analysed using
27 logistic regression separately for each of the four possible TS score levels. TS scores 1
28 and 2 decreased with weeks into the study period and were negatively related to the
29 average daily gain (ADG) of the pigs, whereas TS score 4 increased with weeks into the
30 study period and was positively related to ADG. None of the TS scores differed between
31 females and castrated males, and neither straw provision nor lowering the stocking
32 density affected the TS scores. However, TS score 1 decreased the last week prior to
33 an event of tail damage (at least one pig in the pen with a bleeding tail wound), whereas
34 TS score 4 increased. The results of the current study advocates for a relation between
35 TS and the factors age, growth and stress in the pig, while no relation was found
36 between TS and the environmental factors straw provision and lowered stocking
37 density. The relations to age and growth are important to take into consideration if using
38 TS as a welfare assessment measure in the pig in the future.

39

40 **Keywords:** Slaughter pigs; Animal welfare; Development; Stress; Tail damage

41

42 **Implications**

43 The degree of tear staining in pigs seems to increase with both age and growth of the
44 pigs. Reasons for this could be: development of the secretory glands, hormonal
45 changes, a larger body size thus filling up the pen, spending more time in a stressful
46 environment with time or experiencing the environment as more stressful when having a

47 higher growth rate. Both age and growth of the pigs are important to take into
48 consideration if using tear staining as a welfare assessment measure in the pig.

49

50 **Introduction**

51 Tear staining (**TS**), i.e. the accumulation of a characteristic dark red-brown stain in the
52 medio-ventral corner of the eye, is used as an indicator of distress and compromised
53 welfare of the laboratory rat (Baumans, 2004) and can easily be assessed without
54 handling the animals. In recent years, it has been hypothesised that because the pig
55 also displays TS, it might be a similarly useful tool for assessing pig welfare in farm
56 conditions (DeBoer *et al.*, 2015, Telkänranta *et al.*, 2016). In the pig, TS has so far been
57 correlated to low social rank (Marchant-Forde and Marchant-Forde, 2014), to a longer
58 latency to approach a novel object (Telkänranta *et al.*, 2016), to social isolation and a
59 barren environment (DeBoer *et al.*, 2015), to individual tail and ear damage scores
60 (Telkänranta *et al.*, 2016) and to measures of HPA and SAM axis activation (DeBoer
61 and Marchant-Forde, 2013, Schmitt *et al.*, 2018); all indicating that TS in the pig could
62 be related to the experience of stressors.

63 In pigs, TS arises from secretions of the lacrimal gland, the superficial gland of the third
64 eyelid and the Harderian gland (**HG**). TS (also termed chromodacryorrhoea or red tears
65 in rodents) arises from the secretions of the HG, and the red colour is created by
66 porphyrins in the secretion (McCafferty and Pinkstaff, 1970, Payne, 1994). The function
67 of fluid secretion from the HG still remains largely hypothetical, and suggestions include
68 a lubrication of the eye, an immune response, a photo protection and reception, and
69 social signalling through pheromone production (Payne, 1994). The anatomy of the HG

70 has been described in detail in the newborn piglet (Munkeby *et al.*, 2006), but the
71 postnatal development of the HG in pigs has so far not been described. However,
72 studies on other species suggest that the HG goes through several morphological
73 changes after its immature structure at birth (López *et al.*, 1992, Elgayar *et al.*, 2015)
74 and that the production of porphyrin increases with age (Chieffi *et al.*, 1996). If the
75 development of the participating glands in pigs affects the production of TS, this is
76 important information to consider if TS should be used for welfare assessment on farm.
77 Furthermore, studies on other species have shown sexual dimorphism in the HG
78 (McCafferty and Pinkstaff, 1970, Buzzell, 1996, Hussein *et al.*, 2015) probably resulting
79 in TS differences between males and females. Studies on the pig have found
80 differences between left and right eye TS with left eye TS relating more to the assumed
81 stressors (Marchant-Forde and Marchant-Forde, 2014, DeBoer *et al.*, 2015, Telkänranta
82 *et al.*, 2016).

83 The overall goal of the current study was to increase the knowledge of TS as a potential
84 indicator of stress by assessing whether pig and environmental factors influence TS in
85 pigs. The more specific aims were: (1) to investigate the development in pen level TS
86 over the finisher production period, (2) to investigate whether pig level TS is sex-
87 dependent and whether it relates to the growth of the pig, (3) to investigate whether pen
88 level TS is affected by a set of environmental conditions representing potential pen level
89 stressors, (4) to investigate whether pen level TS develops differently for pens scored
90 with tail damage and pens not scored with tail damage to assess whether TS has the
91 potential to be an early detector of tail biting.

92

93 **Material and methods**

94 The present study was conducted from 2015 to 2016 in accordance with a protocol
95 approved by the Danish Animal Experiments Inspectorate (Journal no. 2015-15-0201-
96 00593). Further information about the study can be found in Larsen *et al.* (2018).

97

98 *Animal, housing and management*

99 The study was conducted in the experimental stables at Department of Animal Science,
100 Aarhus University, Denmark, including two finisher sections with 16 identical pens in
101 each. The study included 80 finisher pens divided between three batches (batch 1, 3: 32
102 pens each; batch 2: 16 pens) and with a total of 1160 finisher pigs. At assignment, the
103 pigs weighed on average 31.9 ± 6.6 kg and included 595 females and 565 castrated
104 males. Each pen included both males and females with an average sex ratio within pens
105 of 1.08 (number of males divided by number of females).

106 The design and dimensions of the pens can be seen in Figure 1. As part of a larger study
107 design (Larsen *et al.*, 2018) and to test whether TS in pigs depends on different potential
108 environmental pen level stressors, the pens were randomly divided within each batch
109 between one level of each of three factors in a $2 \times 2 \times 2$ factorial design: 1) **TAIL**: pigs
110 with undocked (n=36) or docked tails (n=44), (2) **STRAW**: not provided with straw (n=40)
111 or provided with 150 g of straw per pig per day on the solid floor (n=40), (3) **STOCK**:
112 stocking density of 0.73 m²/pig (n=40, 18 pigs per pen, high) or 1.21 m²/pig (n=40, 11
113 pigs per pen, low). Fewer pens with undocked pigs compared to pens with docked pigs
114 were included due to many of the undocked pigs arriving from a private herd shortly after
115 weaning with bleeding tails in batch 2 and thus not included in the study. Pigs were tail

116 docked according to Danish legislation to half of the tail's original length within the first 4
117 days after birth. Also, the amount of feeding space per pig was kept approximately equal
118 between the two stocking densities.

119 The pigs were fed *ad libitum* with a commercial dry feed, and the feeders were filled three
120 times per day at 0300, 1000 and 1830 h. The room temperature was gradually decreased
121 from 21 to 17 °C over the 9 weeks of the finisher period (SKOV A/S, Roslev, DK).
122 Furthermore, each pen included a room-level, automatically controlled shower system
123 (SKOV A/S, Roslev, DK) above the slatted floor. This was intended for cooling and was
124 activated during all batches from 0800 to 2000 h except if the outdoor temperature fell
125 below 5 °C. The system followed a linear curve going from 1% at a 0.5-°C increase from
126 the temperature curve to 100% at a 4-°C increase. At 1%, the sprinklers were turned on
127 with 45 minutes' intervals for 1 minute and at 100% with 20 minutes' intervals for 3
128 minutes. In the current study, the minimum was 14%.

129

130 *Scoring of tear staining*

131 TS was scored every Monday, Wednesday and Friday each week of the study period (9
132 weeks). During scoring, the observers entered the pen and looked at each individual
133 pig's eyes. TS was scored on a scale from 0 to 5, as presented in Table 1, and for both
134 the left and right eye of each pig. As seen in the description of the scoring protocol in
135 Table 1, the different TS scores accounted for the size of the pig by comparing the TS
136 area to the total eye area. Two observers per day performed the scoring. Batch 1
137 included five different observers who were all trained according to a scoring protocol
138 with pictures and text, both by group-discussions and practical scorings in the stable.

139 Batch 2 included four observers, all of whom were also included in batch 1. Batch 3
140 included five observers of whom one was new and trained by the others. Unfortunately,
141 neither inter nor intra observer reliability was calculated.

142

143 *Recording of tail damage pens*

144 Tail damage was recorded by scoring each individual tail simultaneously with the TS
145 scoring. However, tail damage was also recorded on all other days of the week from
146 outside the pen by the stock personnel. If at least one pig in the pen was scored with a
147 bleeding tail wound, then this pen would be characterised as a tail damage pen, and
148 hereafter this day was termed day0 for the respective pen. Afterwards, the pen was no
149 longer included in the study and was not scored for either TS or tail damage. In the
150 current study, the tail scoring data were merely used to identify tail damage pens.

151

152 *Statistical analysis*

153 Prior to analysis, data were investigated descriptively. First, the data only included 332
154 TS score 0 and 131 TS score 5 out of the 26814 individual TS score observations.
155 Thus, TS score 0 was combined with TS score 1 and TS score 5 with TS score 4,
156 referred to as TS score 1 and TS score 4. Second, it was noted that the individual TS
157 scores could from one observation day drop from a high TS score to a low TS and on
158 the next observation day increase to a high TS score again. This may be explained by
159 the TS being washed off a pig due to the activation of the shower system, due to the
160 pigs rubbing themselves against pen mates or inventory or due to an observer
161 difference. Thus, data were aggregated to only include the maximum individual TS

162 score within each week of the study (except for the tail damage data). Third, to study
163 each TS score separately from the other scores, the TS scores were transformed to
164 binomial variables either occurring or not for the single pig in each week of the study.
165 All statistical analyses were performed in R Version 3.4.3 (R Core Team, 2017) using
166 the package "lme4" (Bates *et al.*, 2015) for generalised linear mixed models. All models
167 were logistic regression using the function "glmer" with family set to binomial and were
168 reduced according to a 5-% significance level ($P < 0.05$). Results are presented as the
169 probability of each TS score and differences as odds ratios (**OR**) with connected 95-%
170 confidence intervals (**CI**).

171
172 *Effect of week, eye, TAIL, STRAW and STOCK.* To test the effect of week (1 to 9,
173 continuous), eye (left v. right), TAIL (docked v. undocked), STRAW (yes v. no) and
174 STOCK (low v. high) on the probability of each TS score, the data presented above
175 were further aggregated to pen level by taking the sum of the number of pigs having
176 each TS score as their maximum within each week. Also, only the pens that were never
177 scored as tail damage pens were included to have data where all pens were
178 represented in all weeks of the study. Thus, these data included 315 observations of
179 each TS score for both the left and right eye (35 pens × 9 weeks). Divided between the
180 three factors, the data included observations from 10 pens with undocked pigs, 25 pens
181 with docked pigs, 11 pens with no straw provided, 24 pens with straw provided, 15 pens
182 with the high stocking density and 20 pens with the low stocking density. Each
183 observation in the data contained information on the total number of pigs in the pen and
184 the number of pigs with each TS score as their maximum within each week. Four

185 models were created, one for each TS score, and the response was the proportion of
186 pigs within a pen having the TS score as their maximum within each week. All models
187 included the same main effects: week, eye, TAIL, STRAW and STOCK and the
188 interactions between week and the other main effects. Further, the model specified a
189 random intercept and slope (for the main effects week and eye) for each pen nested
190 within batch number (1-3).

191

192 *Effect of sex and average daily gain.* To test the effect of sex and average daily gain
193 (ADG) from assignment to the end of the study on the probability of each TS score, the
194 data on pig level were further aggregated to include only one observation per individual
195 pig for the entire study period. Again, only the pens that were never scored as a tail
196 damage pen were included to have data where all pens were represented in all weeks
197 of the study. These data included 490 observations of each TS score for both the left
198 and right eye: 490 pigs (252 females and 238 males) divided between the 35 pens.
199 Each observation included the number of weeks for each TS score where it was the
200 pig's maximum score within the week. The models were created separately for the left
201 and right eye. In total, eight models were created, all including the main effects sex and
202 ADG, the interaction between the two and the individual assignment weight as a
203 covariate (average: 31.79 kg; range: 15.45-54.25 kg). Further, the model specified a
204 random intercept for each pen nested within batch number (1-3).

205

206 *Changes in tear staining scores prior to tail damage.* To test whether the probability of
207 each TS score changed prior to the scoring of tail damage on day0, and whether this

208 was different for pens not scored with tail damage, each tail damage pen (n=21) was
209 paired with control pens (n=28) from the same batch with the same treatment level of
210 TAIL, STRAW and STOCK and which were never scored as tail damage pens
211 throughout the study period. The initial data were aggregated to only include the last
212 three observation days (1 week) prior to day0 for each respective pair of tail damage
213 and control pens. In this process, a day category variable relative to day0 with three
214 levels were created: day1-3, day4-5 and day6-7. These data included 192 observations
215 of each TS score for both the left and right eye. The models were created separately for
216 the left and right eye. All models included the main effects pen type (tail damage v.
217 control), day category (day1-3 v. day4-5 v. day6-7) and period (1: week 1-3; 2: week 4-
218 6; 3: week 7-9) and the interactions between pen type and the remaining main effects.
219 Further, the model specified a random intercept for each pen nested within pair number
220 (1-21) and batch number (1-3). The model on TS score 1 further included the main
221 effect TAIL and the interaction between pen type and TAIL, as TAIL was shown in a
222 previous model (results presented in a later section) to affect the probability of TS score
223 1.

224

225 **Results**

226 *Descriptive development and variation*

227 The individual max TS scores ranged from 1 to 4 in all weeks of the study period. The
228 means of the individual max TS scores for each week seemed to increase with weeks
229 into the study period for both the left and right eye. However, the deviation in the mean

230 TS scores seemed rather stable both overall and within-pen. Detailed results can be
231 seen in Table 2.

232

233 *Effect of week, eye, TAIL, STRAW and STOCK*

234 The probability of TS score 1 ($P < 0.01$) and 2 ($P < 0.001$) decreased with weeks into
235 the study period, whereas the probability of TS score 4 increased ($P < 0.01$); TS score 3
236 neither decreased nor increased with weeks into the study period. The results are
237 illustrated in Figure 2. A higher probability of TS score 2 was found on the left eye
238 compared to the right eye (OR = 1.14, 95% CI [1.04, 1.26]; $P < 0.05$), whereas a higher
239 probability of TS score 4 was found on the right eye compared to the left eye (OR =
240 1.22, 95% CI [1.09, 1.35]; $P < 0.05$); no difference was found between the left and right
241 eye for TS scores 1 and 3. A higher probability of TS score 1 was found in pens with
242 docked pigs compared to pens with undocked pigs (OR = 1.79, 95% CI [1.02, 3.12]; $P <$
243 0.05), whereas this was not found for the other TS scores. No effect of STRAW or
244 STOCK was found for any of the TS scores.

245

246 *Effect of sex and average daily gain*

247 No differences were found between males and females in the probability of the TS
248 scores. The probability of TS scores 1 ($P < 0.001$) and 2 ($P < 0.001$) decreased with an
249 increase in ADG, whereas the probability of TS score 4 increased ($P < 0.001$); no
250 relationship was found between TS score 3 and ADG. The results for the left eye are
251 illustrated in Figure 3.

252

253 *Changes in tear staining scores prior to tail damage*

254 Of the 80 pens included in the study, 42 of these were scored as tail damage pens of
255 which 62% were scored within the first 3 weeks of the study. The probability of TS score
256 1 was lower in the tail damage pens compared to the control pens (OR = 0.56, 95% CI
257 [0.41, 0.75]; $P < 0.01$) on day1-3 compared to day4-5 (OR = 0.70, 95% CI [0.52, 0.94])
258 and day6-7 (OR = 0.65, 95% CI [0.48, 0.87]; $P < 0.01$), indicating a decrease prior to
259 day0. The probability of TS score 4 was lower on day6-7 compared to day4-5 (OR =
260 0.72, 95% CI [0.53, 0.99]; $P < 0.01$) and day1-3 (OR = 0.64, 95% CI [0.45, 0.89]; $P <$
261 0.05), indicating an increase prior to day0; however, this was only found on the left eye.
262 No effect of pen type or day category was found for either TS scores 2 or 3.

263

264 **Discussion**

265 *The relation to age*

266 The degree of TS increased with weeks into the study period with a decrease in the
267 probability of the lower scores and an increase in the probability of the higher scores as
268 well as a numerical increase in the mean TS. For all weeks, the within-pen deviation in
269 mean TS scores was almost similar to the overall deviation for all pens. This agrees
270 with the results of Telkänranta *et al.* (2016), who also found almost equal overall and
271 within-pen deviations across production systems, and indicates that individual
272 differences might be as important as pen-level environmental factors for the
273 development of TS. In the current study, one possible source of individual variation in
274 stress level might be of social nature caused by the rather competitive feeding system.
275 Also a previous study linked TS to differences in social rank (Marchant-Forde and

276 Marchant-Forde, 2014, Telkänrranta *et al.*, 2016). However, it could also be a result of
277 the within-pen variation in growth rate as supported by the current results.

278 The positive relationship found between the degree of TS and age of the pigs fits well
279 with the findings of Telkänrranta *et al.* (2016) with low TS scores in suckling piglets and
280 an increase with age when compared to scores from finishers and breeder gilts in the
281 same study. This relationship may occur due to the HG getting larger as the pigs grow,
282 resulting in a greater relative secretion, or due to an accumulation effect over time
283 where the older secretions do not wear off, thus the appearance seems more severe.

284 The postnatal development of the HG in the pig remains to be described. However,
285 studies on rats, mice, guinea pigs and Syrian hamsters found that the HG went through
286 several changes after birth (López *et al.*, 1992, Chieffi *et al.*, 1996, Elgayar *et al.*, 2015).

287 As the HG structure of a newborn pig is typical among mammals (Munkeby *et al.*,
288 2006), the HG of the pig may go through similar morphological and secretory changes.

289 Whether this HG development still occurs as late as in the finisher period remains
290 unknown. However, finishers are relatively immature and may not have reached puberty
291 before being slaughtered. Thus, the HG, and perhaps also other glands involved in the
292 TS secretion in pigs, could still be undergoing such developmental changes.

293

294 *The relation to pen-level stressors*

295 Another explanation for the relation found between TS and age of the pigs could be that
296 the pigs, with proceeding weeks into the study period, spent more and more time in a
297 possibly constantly stressful environment, causing an accumulation of stress over time.

298 If so, this could suggest that TS increases in response to stress experienced by the pig

299 as also suggested by other studies (Marchant-Forde and Marchant-Forde, 2014,
300 DeBoer *et al.*, 2015, Telkänranta *et al.*, 2016). However, this explanation could not be
301 confirmed by the pen-level stressors induced experimentally in the current study. It
302 could be expected that the high stocking density would become more stressful as the
303 pigs grow older, but no difference in development of the TS scores was seen between
304 the two stocking density treatments. Telkänranta *et al.* (2016) found lower TS scores
305 when the pigs were provided with more interesting or different enrichment, and DeBoer
306 *et al.* (2015) found a tendency for a smaller TS area on pigs housed with enrichment
307 compared to in a barren environment. Thus, it could also be expected that pens with
308 straw provided in the current study would have a lower degree of TS. Perhaps the
309 variation in the induced stressors was not large enough relative to the overall stress
310 level in the pens to show an effect on TS, although they did increase the risk of tail
311 damage (Larsen *et al.*, 2018). On the other hand, both provision of straw and a higher
312 stocking density may affect the dirtiness of the pen and pigs, especially towards the end
313 of the finisher period (Larsen *et al.*, 2017). This could decrease the reliability of the TS
314 scoring, as was a concern of the observers during the study, which may have hidden
315 the effect of the stressors on TS in the current study. A higher stocking density may also
316 increase accidental “grooming” in the pigs, which has been related to a decrease in TS
317 in the rat (Baumans, 2004) or leads to an increased risk of heat stress and thereby
318 more wallowing-type behaviour, also decreasing the amount of TS. The experimentally
319 induced stressors could also have been overridden by other stressors common to all
320 pens in the study such as the air quality (Drummond *et al.*, 1980) or competition at the
321 feeder, which was a rather competitive one in the current study.

322

323 *The relation to growth*

324 The degree of TS also increased with increasing ADG, again with a decrease in the
325 probability of the lower scores and an increase in the probability of the higher scores.
326 This relation could confirm the positive relationship between TS and development of the
327 HG. It could also cause the relationship between TS and stress to be less obvious, as
328 there is a negative relation between growth rate and stress (e.g. Hyun *et al.*, 1998,
329 Sutherland *et al.*, 2006). However, pigs with a higher ADG may experience stressors
330 such a metabolic stress or higher competition at the feeder due to a higher motivation to
331 feed. The growth of pigs is not only controlled by growth hormones but also by the
332 thyroid hormones (Cabello and Wrutniak, 1989), and it has been shown in the rat and
333 the hamster that changes in the level of the thyroid hormones may change TS as well
334 (Hoffman *et al.*, 1990, Baccari *et al.*, 2004, Monteforte *et al.*, 2008). Thus, the positive
335 relation between ADG and TS may simply be found due to a hormonal difference
336 between pigs with different growth rates. This is an important relation to consider if
337 using TS scores as a welfare indicator of pigs in the future.

338

339 *The effect of sex*

340 In the current study, no differences were found between barrows and females. In some
341 species, such as the guinea pig, Syrian hamster and miniature pig, the HG has been
342 found to exhibit sexual dimorphism (McCafferty and Pinkstaff, 1970, Buzzell, 1996,
343 Hussein *et al.*, 2015). This in turn suggests that the gland, at least in some species, is
344 regulated by sex steroid hormones. Thus, it was expected to also find a difference in TS

345 scores between sexes in the current study. Why this was not seen could possibly be
346 explained by the fact that all male pigs were castrated shortly after birth. This was also
347 suggested by Buzzell (1996) who found a feminisation in relation to TS when castrating
348 Syrian male hamsters.

349

350 *Tear staining as an early detector of tail biting*

351 Tail biting in pigs is considered an animal welfare problem as well as an economical
352 problem for the farmer. One negative consequence of tail biting is the development of
353 serious damage on the tail of the pigs that has been related to the experience of pain
354 (Di Giminiani *et al.*, 2017) and an increased risk of infections in the pig (e.g. Valros *et*
355 *al.*, 2004). One strategy to prevent tail biting and the resulting tail damage could be the
356 early detection strategy. The purpose of this strategy is to detect when pigs are going
357 through a period of increased stress. This increased stress could potentially lead to tail
358 biting, resulting in tail damage, and thus it may be possible to detect pens in risk of
359 future tail damage before tail damage occurs. The first step in this strategy is to identify
360 possible early detectors. As both tail biting and TS may be related to stress in the pig, it
361 was hypothesised that TS may also work as an early detector of tail biting.

362 To work as an early detector of tail biting, the TS score needed to either decrease or
363 increase prior to day0. In the current study, TS score 1 decreased and TS score 4
364 increased prior to day0. However, this was seen for both the pens scored with and not
365 scored with tail damage on day0. Thus, TS does not seem a promising early detector of
366 tail biting, at least not when defined as relatively mild as in the current study. However,
367 through changes in TS it may be possible to detect the initiation of an unknown stressor

368 on room or farm level, perhaps leading to tail damage in pigs or pens not able to cope
369 with this stressor. It is well-known that tail biting occurs sporadically (D'Eath *et al.*, 2014)
370 and unevenly between individuals and pens, even when these are exposed to the same
371 environment (Zonderland *et al.*, 2011). This might be due to tail biting being influenced
372 by both internal (such as genetics and health of the pigs) and environmental factors (for
373 a review, see Valros, 2018). Again, this relates TS to stress in the pig. Day0 was
374 observed in all weeks of the study period, and mostly in the first 3 weeks. Thus, this
375 relation is not confounded with pig age or weight. Further, the relation between TS and
376 tail damage was only found on the left eye, which fits well with other studies on the
377 relation between TS and stress in the pig (Marchant-Forde and Marchant-Forde, 2014,
378 DeBoer *et al.*, 2015, Telkänranta *et al.*, 2016). This may be due to cerebral
379 lateralisation, as it has been found that the right hemisphere, which is connected to the
380 left eye, dominates in the processing of negatively correlated emotions (Leliveld *et al.*,
381 2013). Other challenges with the scoring system were also identified in the current
382 study. First, TS scores of the single pig were seen to change from high to low and back
383 to high values within a week. Second, TS scores 2 and 4 and TS scores 1 and 3 seem
384 to be each others' complement. Third, some TS scores seem to depend on whether it
385 being scored on the left or right eye of the pig. Thus, the TS scoring system seems to
386 still need investigation and validation in on-farm situations.

387

388 **Conclusion**

389 Overall, the degree of TS increased with weeks into the study, suggesting a relationship
390 between TS and age of the pigs. This could be due to morphological changes in the

391 participating glands with age or a prolonged experience of a stressful environment. TS
392 was also positively related to the growth rate of the pigs, arguing for both of the above
393 suggested hypotheses but which could also be due to hormonal differences. Lastly, TS
394 did not seem promising as an early detector of tail biting on pen level, and also the
395 application of the scoring system in on-farm situations needs further validation.

396

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404

405 **Declaration of interest**

406 The authors declare no conflict of interest.

407

408 **Ethics statement**

409 The present study was conducted from 2015 to 2016 in accordance with a protocol
410 approved by the Danish Animal Experiments Inspectorate (Journal no. 2015-15-0201-
411 00593).

412

413 **Software and data repository resources**

414 Data and models are not deposited in an official repository.

415

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501

502 **Table 1** *The protocol used for scoring of tear staining (TS; DeBoer-Marchant-Forde*
503 *Scale) in the finisher pigs.*

TS score	Description
0	No sign of tear staining
1	Staining is barely detectable and does not extend below the eyelid
2	Staining is obvious and covers <50% of total eye area
3	Staining is obvious and covers 50-100% of total eye area
4	Staining is severe, covers \geq 100% of total eye area and does not extend below the mouth line
5	Staining is severe, covers >100% of total eye area and extends below the mouth line

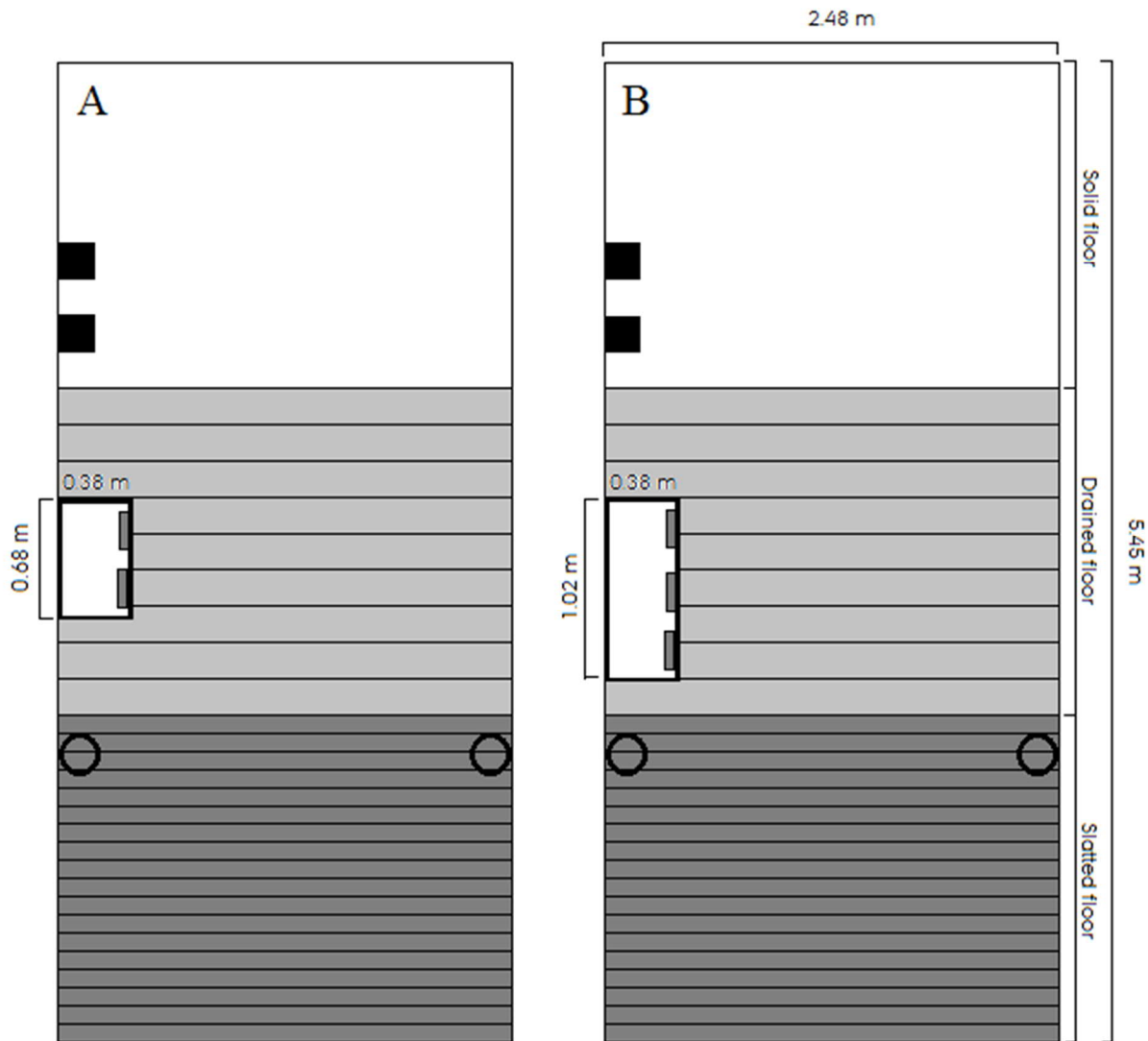
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505 **Table 2** *The descriptive development and deviation in the weekly max individual tear*
 506 *staining (TS) score (pen averages) over the 9 weeks of the study period divided*
 507 *between the left and right eye of the finisher pigs. TS was scored on a scale from 1 to 4*
 508 *(see Table 1).*

Weekly max TS score	Week in the study period								
	1	2	3	4	5	6	7	8	9
<i>Left eye</i>									
Mean	2.23	2.49	2.57	2.81	2.99	3.10	3.23	3.25	3.37
Overall SD	0.70	0.82	0.85	0.86	0.82	0.81	0.76	0.80	0.74
Within-pen SD	0.66	0.79	0.82	0.80	0.76	0.76	0.74	0.77	0.70
<i>Right eye</i>									
Mean	2.31	2.59	2.68	2.84	3.10	3.16	3.28	3.29	3.45
Overall SD	0.73	0.81	0.89	0.87	0.83	0.82	0.77	0.76	0.70
Within-pen SD	0.68	0.78	0.86	0.81	0.77	0.77	0.72	0.73	0.66

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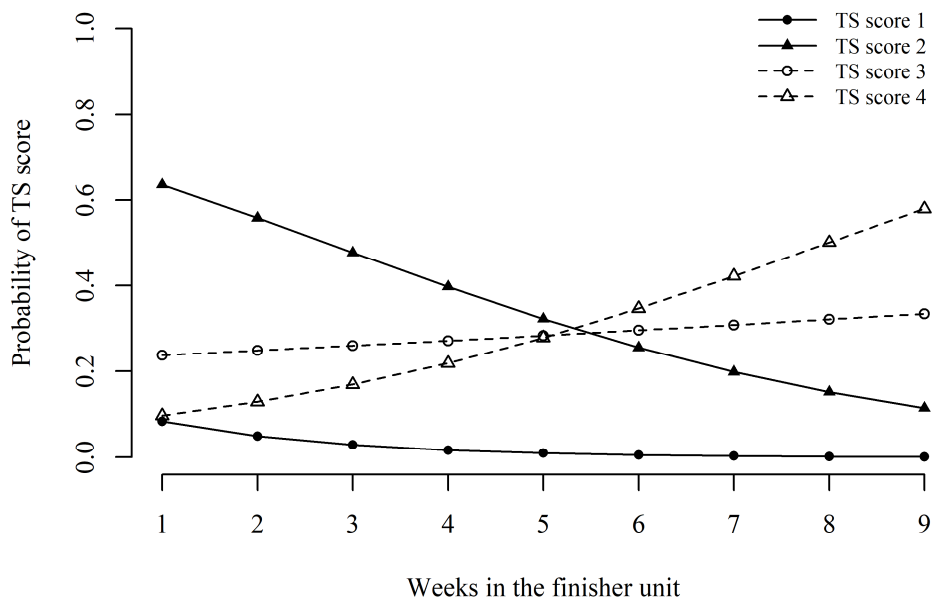
510 **Figures**



511
512 **Figure 1** Drawing of pen dimension and design for (A) pens with a stocking density of
513 1.21 m²/pig (11 pigs) and (B) pens with a stocking density of 0.73 m²/pig (18 pigs). The
514 white rectangle represents the feeder, the hollow black circles represent drinking cups
515 and the solid black squares represent two wooden beams in separate vertical racks. All
516 pens had the same dimensions.

517

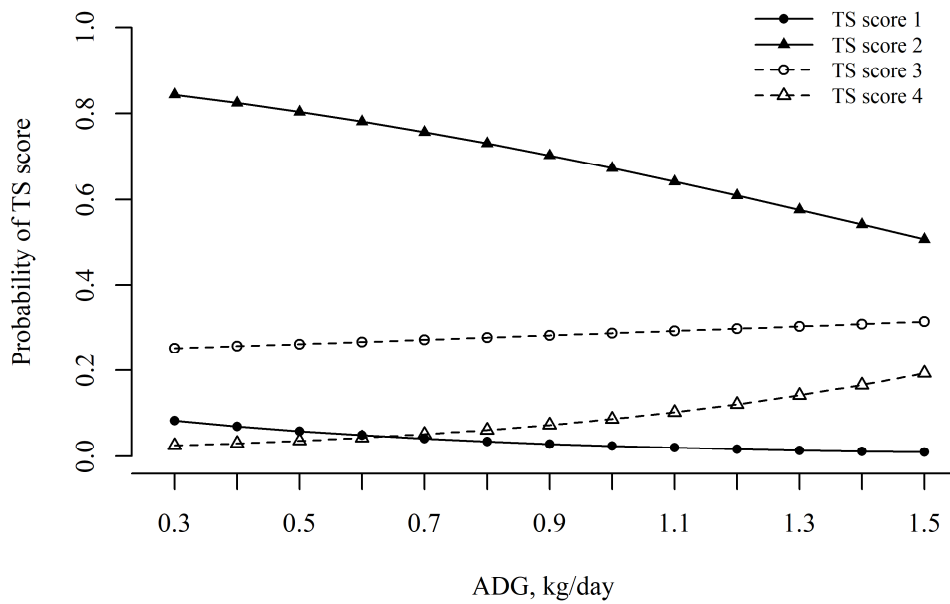
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519

520 **Figure 2** The development in probability of the four tear staining (TS) scores (see Table

521 1) in finisher pigs with weeks into the study period.



522

523 **Figure 3** The development in probability of the four tear staining (TS) scores (see Table
524 1) on the left eye in finisher pigs with increasing average daily gain (ADG, kg/day).

525