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Title: Effects of ventilation system on environmental conditions, performance and rumination in beef cattle during the early fattening period

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Abstract: The aim of this study was to assess the effect of two ventilation systems (ceiling fans vs. axial fans) on environment, performance, rumination and activity of beef cattle during the first period after the arrival to the fattening unit. One group of 106 charolais bulls was raised from August to October in a roofed, loose housing facility with straw bedding. Animals were randomly assigned to pens equipped with one of two types of fans: ceiling fans and axial fans. The trial lasted 70 days, and was divided into 3 periods. In the first and third periods fans were in operation, whereas in the second they were switched off. Animals were evaluated for average daily weight gain, cleanliness, dry matter intake, rumination time, activity level and health condition. The temperature-humidity index was continuously measured within and outside the facility and pens were periodically checked for the dry matter of the bedding.

The average temperature-humidity index was lower in the third period (65.5) compared to the first (74.4) and second (75.3) periods ($P < 0.001$), but was not affected by the ventilation system. Ceiling fans led to higher dry matter of the bedding in the first (30.6 vs. 45.1%; $P < 0.01$), second (29.4 vs. 34.3%; $P < 0.05$) and third periods (22.2 vs. 31.3%; $P < 0.05$) and to cleaner bulls at the end of the trial. Animals raised with ceiling fans showed higher average daily weight gain (1.20 vs. 1.36 kg/day; $P = 0.039$), higher daily rumination time in the first period (431 vs. 475 minutes; $P < 0.01$) and patterns of rumination and activity compatible with a lower level of heat stress. Based on these results, it appears that ceiling fans are promising tools to be used to reduce heat stress and optimize performance of beef cattle.

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Highlights

- A comparison between two ventilation systems for beef cattle farming was proposed
- Ceiling fans led to higher performance and cleaner animals than axial fans
- Ceiling fans led to rumination pattern compatible with a lower level of heat stress

1 **Effects of ventilation system on environmental conditions,**
2 **performance and rumination in beef cattle during the early**
3 **fattening period**

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10

11 **Abstract**

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33

1. Introduction

Heat stress is a condition where the body temperature of an animal exceeds the limits considered physiological, due to a heat load that overcomes its dissipation capacity (Soriani et al., 2013). Among factors contributing to its onset, there are the metabolic rate of the animal and environmental factors such as temperature, relative humidity (RH), air speed and solar radiation (Shiao et al., 2011). Heat stress is a major concern for animal welfare, health and performance both in dairy cows and beef cattle (Morignat et al., 2015; Nardone et al., 2010) because it increases the suffering of animals, which as well as reducing their productions (Nardone et al., 2010), show a higher morbidity and mortality rate (Morignat et al., 2014). This not only causes an increase in health care costs, due to the purchase of medicines, but raises indirect costs caused by lost in production and by the extension of the farming cycle (Thompson et al., 2006). A bovine in heat stress tries to compensate for the increase in body temperature by reducing activity, increasing water intake and through other homeostatic mechanisms which involve sweating, urination and panting (Horowitz et al., 2002, Kazdere et al., 2002). In an attempt to reduce metabolic heat production, animals tend to reduce feed intake and to concentrate it during the coolest hours (Soriani et al., 2013). This change in feeding patterns could impair ruminal fermentations (De Nardi et al., 2016; Salvati et al 2015) and if extended in time, can lead to a ruminal dysfunction, up to the onset of metabolic disorders such as ruminal acidosis (Plaizier et al., 2009).

Among cattle the impact of heat stress is most dramatic in those animals that do not have time to adapt, but suddenly find themselves having to address environmental conditions characterized by temperature and relative humidity significantly higher than those to which they were accustomed to (Soriani et al., 2013, Vitali et al., 2015). This is the case of beef cattle that during the summer are imported from the mountainous regions of France, where they are kept mainly at pasture, and that after a trip by truck, which can last up to 16 h, are housed in roofed facilities typical of the North-

58 eastern Italian regions (Cozzi et al., 2009). Many solutions, which adapt to the different farming
59 systems, were studied to limit the impact of heat stress on animals. Within roofed facilities is
60 sought, where possible, of taking advantage of natural ventilation, favoured by open structures or at
61 least by the presence of doors, windows, roof ridges and other barn design solutions (Shoshani and
62 Hetzroni, 2013). Where these systems are not sufficient, forced ventilation is used by applying fans
63 inside the facilities (Calegari et al., 2014) to improve heat loss by evaporation and reduce the
64 relative humidity. To further reduce temperature, water sprinkling or misting (Mitlohner et al.,
65 2001) are used, although their effectiveness is reduced in the presence of high relative humidity
66 (Shiao et al., 2011). In the case of beef cattle however, the use of showers is not recommended in
67 the presence of deep litter, since they would lead to excessive moisture of the litter, increasing the
68 risk of injuries from slipping (Wechsler et al., 2011), and the extension of the animal surface
69 covered in manure. This last aspect must not be underestimated since, as pointed out by the
70 Guidance on Meat production and hygiene (Food Standards Agency, 2012) animal integuments
71 smeared with manure lead to an increased risk of contamination of the meat, jeopardizing the
72 consumer safety.

73 Until a few years ago the forced ventilation in the roofed facilities was achieved through the use of
74 axial fans (Calegari et al., 2014), whereas at present, there are also ceiling fans, characterised by a
75 vertical air flow, that are spreading both in dairy and beef cattle farms (Magrin et al., 2016). The
76 aim of this study was to compare the two ventilation systems (ceiling fans vs. axial fans) to verify
77 their impact on environmental conditions, health and performance in beef cattle during the
78 transition period, that is the phase during which animals have to adapt to the new environment and
79 the new diet.

80

81 **2. Material and methods**

82 *2.1. Experimental groups, housing and feeding*

83 The OPBA Committee (Organismo Preposto al Benessere Animale) approved this study,
84 which was marked with the approval number 13318, on the 28th of January 2016. A group of
85 Charolais bulls (n = 106 animals), which were on average 346 ± 80 days old and weighed (429 ± 24
86 kg), was imported from France and was raised in a beef cattle farm located in the province of
87 Rovigo (RO), in North-Eastern Italy, from the beginning of August until October 9th. Animals were
88 bought at the same cattle collection centre in France and selected based on weight, age, and physical
89 conformation. Upon arrival bulls were housed in a roofed, loose housing facility with an open front
90 and straw bedding. The animals were weighed and stratified into two balanced groups according to
91 age and weight. The bulls were reared in a barn, in 10 contiguous pens (70 m² each), with two
92 different ventilation systems. The animals of group 1 (n = 54) were housed in five pens which were
93 equipped with two ceiling fans (CF), whereas the bulls of group 2 (n = 52) were housed in five pens
94 which had two axial fans (AF). The pens with different ventilation were separated by an empty pen
95 with a windbreak (Fig. 1).

96 The CF and AF were placed at 4 and 2.5 m from the ground, respectively, above the 2nd and
97 the 4th pens of each group by a specialist company, in order to make ventilation as effective as
98 possible in every pen (Fig. 1). The trial lasted 70 days, was divided into 3 periods (P1, P2 and P3)
99 and was characterised by a high THI from the beginning of August to mid-September (P1 and P2)
100 and by a dramatic drop of temperature from mid-September to the end of the trial (P3). In P1 both
101 CF and AF were in operation to see the effect of two ventilation systems in hot conditions, in P2
102 they were switched off to see the effect of the lack of ventilation during the hot period and in P3 all
103 the fans were switched on again to see the effect of two ventilation systems in cooler conditions.

104

105 The CF are characterised by a power consumption of 0.4 kW and have five aluminium
106 blades 1.85 m long and are capable of moving from 36000 m³/h of air at the minimum speed,
107 corresponding to a temperature-humidity index (THI) higher than 60, gradually increasing up to 180

108 000 m³/h at the maximum speed, when THI is higher than 74. The activation of the CF and their
109 speed were automatically adjusted according to temperature and humidity values measured by a
110 sensor positioned inside the facility. The AF are characterised by a power consumption of 1 kW,
111 have 5 blades with a diameter of 1.4 m, and were switched on (THI > 60) and off (THI ≤ 60)
112 according temperature and relative humidity values measured inside the facility. Air speed in both
113 groups was measured using a handheld thermo-anemometer (Metrel HandyMAN TEK1313 Digital
114 Thermo-Anemometer, Testermans, Wisbech, UK). Average air speed was around 1.0 m/s without
115 ventilation, whereas was 4.5 ± 1 m/s with AF and from 1.4 to 6.9 m/s with CF at minimum and
116 maximum speed.

117 In the first three days from arrival the bulls were fed a ration based on corn silage and hay, whereas
118 throughout the rest of the trial a total mixed ration (TMR), typically used in the backgrounding and
119 transition phases in commercial farms was fed. The TMR (on dry matter, DM basis) included on
120 average: corn silage (2.5 kg), pressed sugar beet pulp (2.6 kg), wheat bran (1.3 kg), corn meal (1.1
121 kg), soybean meal (0.5 kg), straw (0.6 kg), vitamins and minerals (0.58 kg). The TMR has been
122 gradually changed from week to week during the trial to meet the growth needs of the animals
123 moving from an initial content of NDF, starch and crude protein of 37.0, 25.0 and 14.9% on DM
124 basis, respectively to a final content of 35.0, 30.0 and 13.7%. The animals were fed ad libitum and
125 the feed amount has been adjusted to have leftovers equal to 5% of the distributed ration. The TMR
126 has been distributed once a day in the morning and drinking water was supplied ad libitum through
127 2 pressure bowls per pen.

128 *2.2 Animal monitoring*

129 In order to measure the average daily weight gain (ADG) and the effect of ventilation on the
130 cleanliness of animals, at the beginning and at the end of the trial, bulls were individually weighed
131 and evaluated for their body surface covered in manure, using a scale from 1 (< 10%) to 5 (> 75%),
132 as reported by Busby and Strohbahn (2008). The first day after their arrival bulls were vaccinated,

133 treated for external and internal parasites, and fitted with SCR collars (HRLDn Tag; SCR Engineers
134 Ltd., Netanya, Israel) to measure their daily rumination and activity. The SCR collars transmit neck
135 acceleration data to a receiver every two hours. The software (Heatime Pro System /HRLDn Tag,
136 SCR Engineers) interprets these data giving the rumination time and a level of activity. The activity
137 level ranges from 0 to 253 bits (binary digit) per each 2-hour interval and gives an indication of how
138 much the animal has moved, but without specifying the type of action. Data were collected every
139 two hours and summarized on a daily basis (Abeni and Galli, 2017; Moretti et al., 2017).

140 For both rumination and activity, the daily average and some indexes of variation were
141 calculated as reported by Marchesini et al. (2018). Such indexes would indicate the variation of
142 these parameters between consecutive 2-hour intervals within day (DDR is the daily
143 dishomogeneity index in rumination, whereas DDA is the daily dishomogeneity index in activity)
144 or between consecutive days, during the whole trial (DR is the index of dishomogeneity in
145 rumination and DA is the index of dishomogeneity in activity).

146 The values of this indexes range between 0 and 2 and show how the activity and rumination
147 change within a day (DDA or DDR) or throughout the whole trial (DA or DR). A value of 0 means
148 no variation between different intervals, whereas the value 2 means that there are peaks of activity
149 or rumination.

150 Throughout the trial the bulls were visually monitored twice a day (around 09.00 and 18.00)
151 by a veterinarian and by specially trained personnel to verify their health condition and the correct
152 position of the collar. In the presence of clinical signs attributable to fever (e.g., depression) or in
153 the presence of respiratory signs (nasal or ocular discharge, coughing or wheezing) rectal
154 temperature has been measured. The diagnosis of bovine respiratory disease (BRD) and any other
155 pathologies was carried out by a veterinarian on the basis of the typical signs described in the
156 literature (Timsit et al., 2011; Welfare quality, 2009) In case of illness the animals were treated
157 according to the indications given by the veterinarian after the diagnosis.

158 2.3 *Environment and feed monitoring*

159 The temperature and RH were continuously recorded by probes located inside the facility in
160 both the areas with CF and AF. The probes were positioned at about 4 m in height so as not to be
161 influenced by the air flow. The environmental temperature and RH outside the barn were measured
162 by the local meteorological station. From the temperature and RH, THI was calculated (Kelly and
163 Bond, 1971). In concurrence with the recording of temperature and RH, the distribution of animals
164 in the pen was also detected, to see if they were distributed evenly within each pen. To do this, each
165 pen was imaginatively divided into three equal parts (the manger front, the centre and the bottom of
166 the pen) and the distribution of the animals within the 3 parts was recorded. Twice a week,
167 throughout the trial, samples of the bedding material were collected for the analysis of its dry matter
168 (DMB). Three samples (about 500 g) of bedding material (from the front, the centre and the bottom)
169 were collected from each pen, for each ventilation system, refrigerated and analysed for DM within
170 2h from the collection (AOAC, 2003). In order to measure the chemical composition and physical
171 structure of the feed, TMR samples were collected weekly throughout the trial. The samples were
172 immediately sieved using a Penn State Particle Separator for the evaluation of the particle size
173 distribution and then were frozen until the chemical analyses. Feed chemical analyses were
174 performed according to the methods found in literature (ANKOM Technology, 2008; AOAC, 2000;
175 2003; 2005;). Dry matter intake (DMI) of each group was calculated by difference between
176 distributed TMR and 24h leftovers (Magrin et al., 2016).

177 2.4. *Statistical analyses*

178 Statistical analyses were performed using SAS software (2012, release 9.4; SAS Institute Inc., Cary,
179 NC). Data on ADG were subjected to One-way ANOVA to test the fixed effect of ventilation
180 system (CF vs. AF). Data about THI and DMB were subjected to One-way ANOVA using the
181 combined effect of period and ventilation system. Data on individual daily activity and rumination
182 were analysed using PROC MIXED for repeated measures, with animal treated as a random effect

183 and using the autoregressive (AR) covariance structure. The analysis model included the fixed
184 effects of ventilation system (2 levels: CF vs. AF), period (3 levels: P1, P2 and P3) and their
185 interactions. In both ANOVA and mixed procedure, a post-hoc pairwise comparisons between
186 levels of classification variables were performed using Bonferroni correction. Differences in group
187 DMI were tested by using *t*-test within period. For not normally distributed data ($W < 0.90$), non-
188 parametric tests were used. Data on animal cleanliness reduction were subjected to the Mann-
189 Whitney test to assess the effect of ventilation system. Finally, animal distribution within the pen
190 and the number of episodes of BRD were subjected to the calculation of a risk ratio (RR) and 95%
191 confidence intervals (95% CI) to quantitatively describe the association between variables of animal
192 distribution or disease and ventilation system, using a 2×2 contingency table and a chi-squared test
193 with the Yale correlation for continuity.

194 **3. Results**

195 As reported in Fig 2, in P1 and P2 (from the 1st of August until mid-September) the environmental
196 maximum daily THI had values ranging from 73.0 to 88.1, that are considered to be indicative of
197 mild (> 72), high (> 78) and severe (> 88) level of heat stress in cattle (Armstrong, 1994), then THI
198 decreased in P3, ranging from 79 to 58.5, mainly due to the drop of the temperature in late
199 September-October (from 26.3 to 14.7 °C). Inside, the maximum daily THI values were high in the
200 first two periods, showing a range from 70.2 to 80.7 in P1 and from 69.5 to 79.7 in P2. The THI
201 exceeded the value of 72 for 21 and 22 days and the value of 78 for 5 and 6 days in P1 and P2,
202 respectively. In P3 the maximum daily THI inside the facility had a range between 55.7 and 72.6
203 and the THI was higher than 72 only for 3 days.

204 As reported in Table 1, there was no difference in the average daily THI between CF and AF
205 throughout the trial, whereas it is evident that the only significant difference in THI is related to its

206 decline in P3, which corresponds to the drop in temperature that naturally occurs in September-
207 October in North-eastern Italy.

208 The bedding material remained dryer with CF throughout the trial, showing a higher level of DMB
209 (Table 1) compared to AF.

210 Among animals, the ones reared with CF had a less dirty coat at the end of the trial. Immediately
211 after their arrival at the farm, animals were evaluated according to the dirtiness score which was
212 similar for the animals (1.77 ± 0.45). At the end of the trial this score was significantly ($P < 0.001$)
213 affected by the ventilation system, increasing of 2.81 ± 0.74 points in AF animals and 1.16 ± 0.50
214 points in CF ones.

215 As regards the health status of the animals, 23 cases of BRD were distributed throughout the trial
216 (Table 2), mainly in the 2 hottest periods (P1, 12 cases and P2, 8 cases), without significant
217 differences between the two ventilation systems. However, in P2, when the fans were off, there was
218 a tendency to significance ($P = 0.075$), with CF showing a higher number of BRD cases.

219 The use of CF has also led to higher performance of animals that showed higher ADG, both taking
220 into account all the animals (1.36 vs. 1.20 kg/day; $P = 0.039$) and excluding animals with BRD
221 (1.42 vs. 1.29 kg/day, $P = 0.054$), although DMI was not significantly different between CF and AF
222 in any of the periods considered. DMI for CF and AF in P1, P2 and P3 was 11.4 vs. 11.0 kg ($P =$
223 0.992), 10.0 vs. 10.2 kg ($P = 0.430$) and 11.7 vs. 11.6 mg/m³ ($P = 0.593$), respectively.

224 Data on rumination and activity level confirm the effect of the ventilation system on animals,
225 especially during the first period. As reported in Table 3, the animals reared with CF, in P1 have
226 ruminated 44 minutes more daily ($P < 0.01$) and have shown a significantly ($P < 0.05$) lower DR,
227 the index indicating average daily rumination time variations throughout the trial. Unlike
228 rumination, in P1 the daily activity level did not differ significantly between the two ventilation
229 systems and there were no differences in the DA, the index indicating average activity variations

230 between consecutive days (Table 3). What is changing is the DDA that appears higher in CF than in
231 AF, indicating the presence of peaks of activity throughout the day.

232 As expected, there were no differences in rumination and activity level between CF and AF in P2,
233 when the ventilation systems were off. For both CF and AF in P2, rumination and DA were lower
234 and the average daily activity was higher than in P1. No differences in rumination and activity were
235 found between CF and AF during P3. With AF, both average daily rumination and DDA were
236 higher in P3 than in P1, whereas with CF, the only difference between P1 and P3 was found for
237 average daily activity, that was lower in P3.

238 As regards the distribution of the animals inside the pens, differences between CF and AF were
239 found only in P1. As shown in Table 4, it can be seen that in both cases more than 50% of the
240 observations show the animals distributed evenly. In CF, the bottom part of the pen was avoided in
241 47% of observations whereas that value falls to 29% in AF. The central part of the pen, above
242 which fans are suspended, is almost never avoided in CF (1%), whereas it is avoided in 6% of cases
243 in AF.

244 **4. Discussion**

245 As can be seen, from the 1st of August until mid-September, the levels of THI were compatible with
246 a condition of heat stress (Armstrong,1994) and the THI values measured within the areas with CF
247 and AF were similar and followed the trend of the environmental THI, even though their values
248 were 5.07 ± 3.29 lower within the facility than outside, also during P2, when the fans were off. This
249 difference in THI was likely due to the effect of the facility itself. On the basis of the THI values
250 found within the facility and according to what reported by other authors (Soriani et al., 2013;
251 Armstrong, 1994), it can be estimated that in both the first two periods the animals were in mild and
252 high heat stress, for 62 and 22% of days, respectively, and they have never been in severe heat
253 stress. In P3 the animals experienced only mild heat stress and only for the 12.5% of days.

254 The lack of difference in THI between the two ventilation systems was due to the location of the
255 probes, which were positioned so as not to be influenced by the air flow, and indicates that the
256 animals reared with both CF and AF were subjected to the same environmental condition
257 throughout the trial. Although the THI measured by the probes has not changed, this does not mean
258 that the ventilation systems have not led to differences at the level of the housing environment. A
259 first effect is noticeable in the bedding material, which remained dryer with CF throughout the trial,
260 likely due to the main direction of the air shifted by the fans that in the case of CF is directed down,
261 towards the floor, instead to be almost parallel to the ground as it happens to the AF, allowing the
262 drying of the bedding material. Similar results were found by Magrin and colleagues (2016), who
263 reported a difference of 10.1% in litter moisture between CF and a system based on natural
264 ventilation. These difference in DMB has also led to some direct effects on animals. For example,
265 the increased dryness of the litter has led the animals reared with CF to be less dirty than the ones
266 reared with AF. This result is in line with those found by Magrin et al. (2016) and has an
267 epidemiological significance for public health, because animals with a very dirty coat have a greater
268 risk of bringing pathogens to the slaughterhouse, with a higher risk of carcass contamination
269 (Puyalto et al., 1997). Moreover, the use of CF has also led to a higher ADG, despite DMI was not
270 significantly different between the two groups. This is probably due to the fact that high levels of
271 THI influence energy metabolism (Abeni et al., 2007), increasing cost of thermoregulation,
272 modifying partition of nutrients and decreasing gluconeogenesis (Soriani et al., 2013). In this study
273 the ventilation obtained with CF, compared to that achieved with AF, reduced the impact of THI on
274 animals, leading to higher performance. The effect of CF on animals is confirmed by the differences
275 that have arisen in the rumination and activity level of animals between CF and AF, especially
276 during the first period. In this period in fact, CF animals ruminated for a longer time daily and
277 showed less variation in rumination between consecutive days, if compared to AF animals. Since
278 the ration administered to the two groups was the same, this increase in variability in rumination
279 with AF can be read as a sign of discomfort, due to a less adequate ventilation system, under high

280 THI conditions. Such variability in rumination may be related to an irregular DMI that concentrates
281 on the coolest days and is reduced in the hottest ones, as found by other authors (Shiao et al., 2011)
282 for dairy cows. Reduction in rumination time (Abeni et al., 2017; Moretti et al., 2017) and irregular
283 rumination, (Soriani et al., 2013) are typical signs of heat stress (Moallem et al., 2010), confirming
284 that, during P1, AF cattle suffered a higher heat stress than CF ones. The variation in daily
285 rumination throughout the first period, indicated by the higher DR for AF beef cattle, could have
286 lead also to an impairment of ruminal fermentations which may have further contributed to the drop
287 in ADG. The higher value of DDA found in CF bulls indicates the presence of peaks of activity
288 throughout the day, but it is difficult to attribute a definite meaning to this result without being able
289 to detail the type of activity carried out by animals.

290 When the ventilation systems were off, the fact that the animals reduced their rumination time and
291 DA, and increased their daily activity compared to P1 is in line with what reported by other authors
292 (Abeni et al., 2017; Moretti et al., 2017) who found a reduction of rumination and an increase in the
293 activity level in dairy cows under heat stress, confirming that both ventilation systems contributed
294 to mitigate heat stress during high THI conditions. However, as stated before, CF appeared to be
295 more effective in reducing heat stress than AF, in fact CF in P1 led to a lower DR and a higher
296 DDA compared to P2, whereas no differences between P1 and P2 were found for DR and DDA
297 using AF. The low values of THI detected in P3 did not lead to differences between CF and AF,
298 because the animals were not in heat stress. With AF, the higher value of average rumination time
299 in P3 compared to P1 was due to the lower heat stress suffered by animals during the last period.
300 With CF this difference was not significant, confirming the better capacity of CF in mitigating heat
301 stress during P1.

302 As regards the health status, the ventilation system seemed to have a marginal role, in fact the only
303 difference shown in the BRD cases was found after the fans were switched off. The higher number
304 of BRD cases in CF during P2, would probably mean that the animals in P1 had become

305 accustomed to a more effective level of ventilation with CF and that its sudden interruption led to
306 an increase in the number of BRD cases in P2. This result confirms what has been reported by
307 Vitali et al. (2015) which points out that the lack of gradual adaptation to hot stress can adversely
308 affect animal health.

309 During the trial, we tested the distribution of the animals inside the pens to find out if the ventilation
310 was homogeneous inside the pen, if it could create annoying airflows for animals and if there were
311 differences between the two ventilation systems. Although there were some differences in
312 distribution during P1 between CF and AF, overall, it seems that the ventilation was uniformly
313 distributed within the pen in both systems.

314 **5. Conclusions**

315 Bulls used in this study were subjected to levels of THI compatible with mild to high heat stress,
316 during the first two periods of the trial. Under these conditions, the use of ceiling fans, compared
317 with axial fans, has brought benefits both from the environmental and the animal point of view. CF
318 have led to lower litter moisture. Moreover, they led to cleaner animal coats at the end of the trial,
319 significant improvement in ADG, increased rumination time and variations in patterns of
320 rumination and activity compatible with a lower level of heat stress. Based on these results, it
321 appears that ceiling fans represent promising tools to reduce heat stress conditions and optimize the
322 performance and cleanliness of beef cattle during high THI conditions.

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414 **Fig 1. Location of fans in the barn, from the top (above) and the manger front (bottom).**

415 Ceiling fans (CF), axial fans (AF). Black and thick continuous lines indicate: concrete walls with
416 windows, floor and roof. Dotted lines indicate open sides. Thin continuous black lines indicate
417 pens. Symbol indicates the position of: CF (*) and AF (O).

418

419 **Fig 2. Trend of maximal daily environmental temperature and THI.**

420 THI was measured by the local weather station (WS) and by the probes inside the barn positioned in
421 the areas with ceiling fans (CF) and axial fans (AF), throughout the three periods (P1, P2 and P3) of
422 the trial.

423

Conflict of interest statement

The authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Giorgio Marchesini

Figure
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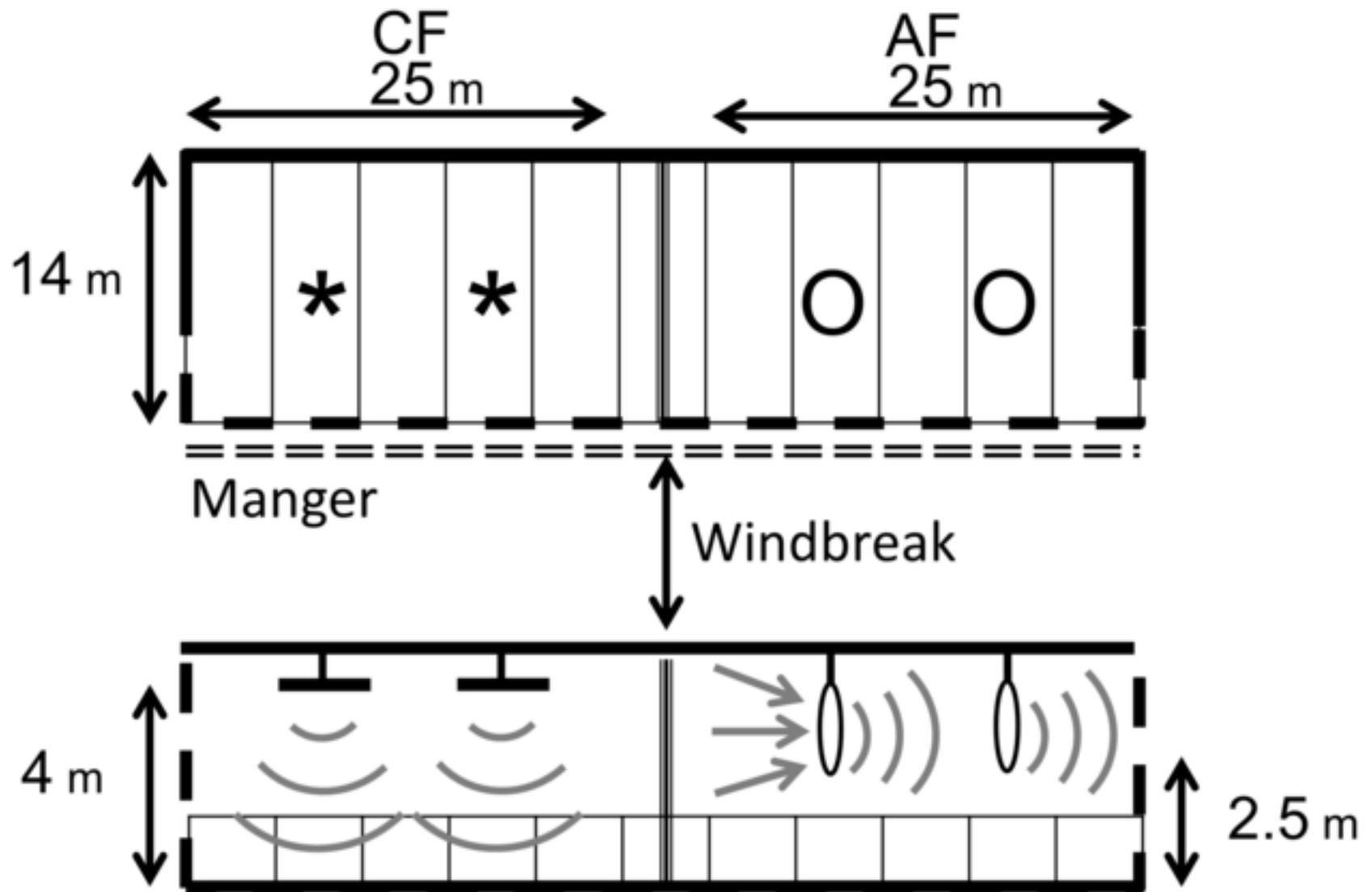


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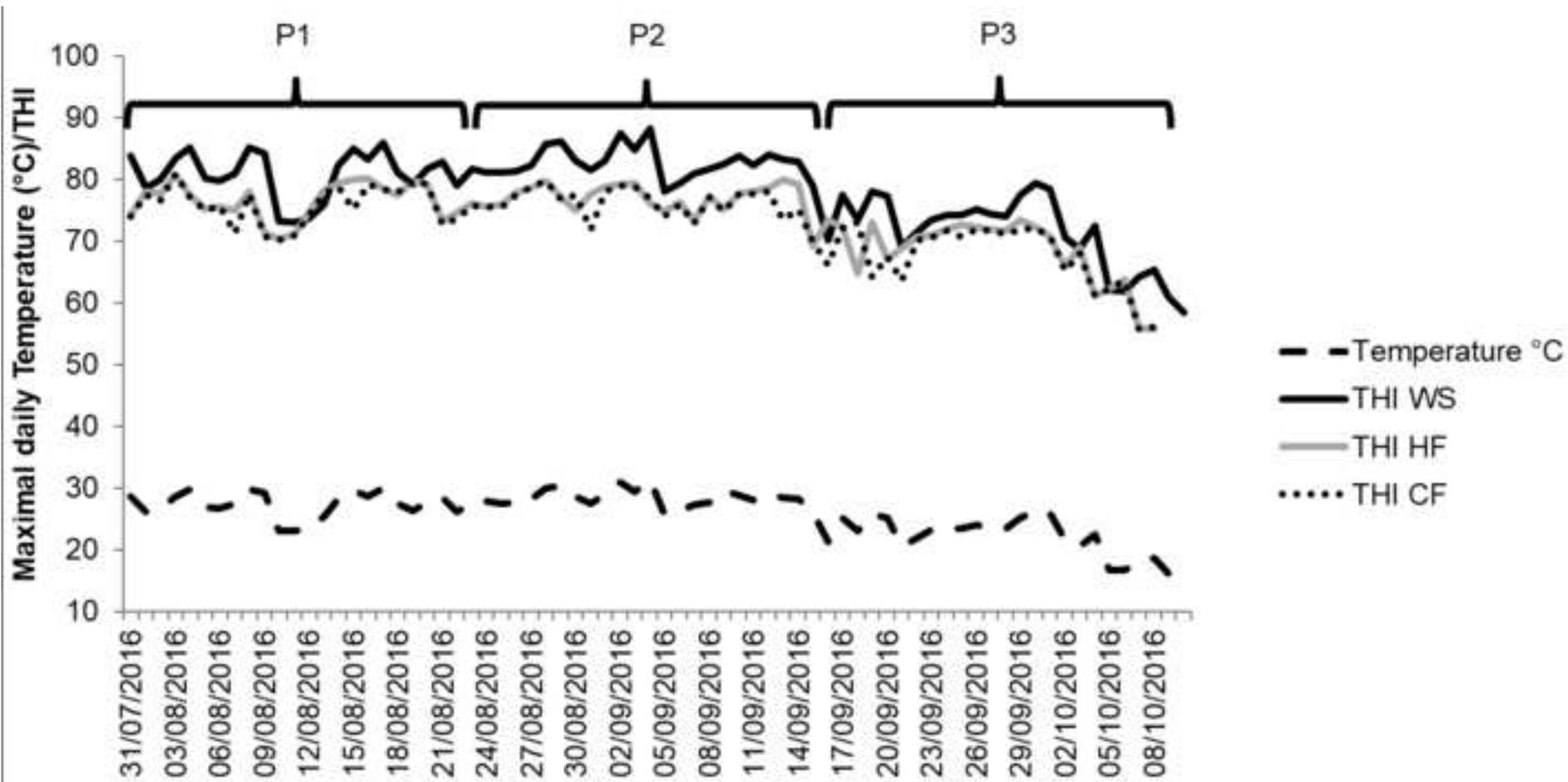


Table 1. Combined effect of the ventilation system and of the period (P1, P2, P3) on the average daily THI and on the dry matter of the bedding material (DMB).

	AF			CF			SEM	P-value
	P1	P2	P3	P1	P2	P3		
Average Daily THI	74.6 ^a	75.3 ^a	65.8 ^b	74.3 ^a	74.9 ^a	65.3 ^b	0.565	< 0.001
DMB (%)	30.6 ^c	29.4 ^c	22.2 ^d	45.1 ^a	34.3 ^b	31.3 ^{bc}	1.79	< 0.001

^{a, b, c, d,} means with different superscript letters within a row differ significantly (P < 0.05).

Table 2. Effect of ceiling fan (CF) and axial fan (AF) on the onset of BRD throughout the three periods (P1, P2, P3).

	Number of BRD cases			P-value	RR	95% CI
	CF	AF	Total cases			
P1	5	7	12	0.707	1.454	0.49-4.29
P2	7	1	8	0.075	0.148	0.019-1.16
P3	2	1	3	0.974	0.519	0.048-5.55

RR, relative risk; CI, Confidence interval.

Table 3. Effect of ventilation system, period (P1, P2, P3) and their interaction (V × P) on the individual activity and rumination parameters.

	Axial fans			Ceiling fans			SEM	P-Value		
	P1	P2	P3	P1	P2	P3		Period (P)	Ventilation (V)	(V x P)
Rumination										
Average daily rumination (min)	431 ^b	410 ^c	456 ^a	475 ^a	407 ^c	439 ^{ab}	7.72	0.001	0.374	< 0.001
DDR	0.533	0.511	0.504	0.511	0.509	0.506	0.010	0.155	0.540	0.310
DR	0.193 ^a	0.164 ^{ab}	0.168 ^{ab}	0.145 ^b	0.190 ^a	0.170 ^{ab}	0.007	0.478	0.378	< 0.001
Activity										
Average daily activity (bits)	474 ^b	511 ^a	460 ^{bc}	464 ^b	479 ^{ab}	438 ^c	5.91	< 0.001	0.007	< 0.001
DDA	0.179 ^b	0.182 ^b	0.200 ^a	0.214 ^a	0.173 ^b	0.213 ^a	0.005	< 0.001	0.055	< 0.001
DA	0.070 ^a	0.058 ^b	0.059 ^{ab}	0.067 ^a	0.054 ^b	0.059 ^{ab}	0.003	< 0.001	0.511	0.768

DR, dishomogeneity index of rumination; DDR, daily dishomogeneity index of rumination; DR, dishomogeneity index of activity; DDA, daily dishomogeneity index of activity.

^{a, b, c,} means with different superscript letters within a row, differ significantly ($P < 0.01$).

Table 4. Animal distribution along the pen during the first period (P1).

Animal distribution within the pen			AF	CF	P-value	RR	95% CI
Back	Centre	Manger					
Evenly distributed							
	O		59%	51%	0.046	1.2	1.0-1.3
Distributed at the ends							
	O		6%	1%	< 0.001	9.0	2.1-38.4
Distributed in the centre and nearby the manger							
	O		35%	48%	< 0.001	0.7	0.6-0.9

Grey cells = presence of animals; White cells = lack of animals; RR, relative risk; CI, Confidence interval; O = fan location within the pen