

Effect of feed texture, meal frequency and pre-slaughter fasting on carcass and meat quality, and urinary cortisol in pigs

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Abstract

Carcass and meat quality traits, and urinary cortisol variation was studied in 96 barrows assigned to the following treatments: feed texture (FT; mash *vs.* pellets), meal frequency (MF; 2 *vs.* 5 meals per day) and fasting time (F; 4, 14 and 24 h) according to a 2 × 2 × 3 factorial design. Pigs fed mash, receiving feed five times a day and fasted for 24 h before slaughter had lower carcass dressing yield ($P < 0.001$). A higher ($P < 0.05$) bruise score was found on carcasses from pigs fasted for 14 and 24 h and fed either pelleted or mashed feed five times per day. The pH_u value in the Longissimus muscle increased ($P < 0.05$) with increasing fasting time, whereas in the Adductor muscle it was higher ($P < 0.05$) in pigs fed with pellets in two meals per day and fasted for 24 h. Urinary cortisol tended to be higher in pigs fasted for 14 h compared to those fasted for 4 ($P = 0.10$) and 24 h ($P = 0.06$). The results of this study show a significant influence of pellet feeding on carcass yield in fasted pigs, while the effects of pre-slaughter fasting time on meat quality traits were limited.

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1. Introduction

One of the standards included in many codes of practice or legislations requires that pigs should be fasted before slaughter at reasonable intervals and be given water *ad libitum* (Agriculture & Agri-Food Canada, 1993; SCAHAW, 2002). Among others, the advantage of feed withdrawal prior to slaughter is to increase food safety and improve meat quality (Chevillon, 1994; Eikelenboom, Hoving-Bolink, & Sybesma, 1991). Fasting pigs before slaughter reduces the volume of the stomach content at slaughter, preventing the release and spread of bacterial contamination (e.g. *Salmonella*) through the spillage of gut contents

due to inadvertent puncture of the stomach during the carcass dressing process (Berends, Urlings, Snijders, & van Knapen, 1996; Miller, Carr, Bawcom, Ramsey, & Thompson, 1997). Furthermore, pigs being handled on full stomach are more susceptible to transport sickness (Bradshaw, Hall, & Broom, 1995) and are more difficult to handle (Eikelenboom et al., 1991). These effects may result in a stress response, which can affect ante mortem muscle glycogen reserves with clear consequences on pork meat quality (Foury et al., 2005; Gispert et al., 2000).

A feed withdrawal of 16–24 h before slaughter is recommended in practice (Eikelenboom et al., 1991). However, industry reports and some studies have revealed a high variability in stomach weight at slaughter, even among pigs that were subjected to this fasting interval before slaughter (Guise et al., 1995; Turgeon, 2003). According to Guise

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et al. (1995), smaller feed particle size and pelleting may influence gastric emptying rate in pigs. A number of studies also reported lower whole stomach and content weights in pigs fed ad libitum compared to those subjected to restricted feeding (Magras, Delaunay, & Bénéteau, 2000; Turgeon, 2003). This difference can be explained by the intake of smaller feed portions at each meal in pigs fed ad libitum, which favours food digestion and consequently accelerates stomach emptying (Laplace, Corring, Rérat, & Demarne, 1986). Gregory, McFadyen, and Rayner (1990) also showed that feeding finely-ground feed favoured gastric emptying to the same extent as liquid feeding. The lower feed intake associated with pellet feeding, which is generally more finely ground (Fekete, Castaing, Lavorel, & Leuillet, 1983) might have contributed to this effect. However, the combined effects of mash *vs.* pellet feeding and different fasting intervals on the physiological response to pre-slaughter stress and pork carcass and meat quality variation are not known. This experiment was thus conducted to determine the combined effects of different feeding practices prior to transportation to slaughter and of various feed withdrawal times on stress hormones and carcass and meat quality variation of pigs. Another objective of this study was to determine the relationship between the degree of stomach fill, urinary cortisol, and meat quality traits variation.

2. Materials and methods

2.1. Animals and treatment

A total of 96 Duroc × (Landrace × Large White) cross-bred barrows weighing around 63 kg (approximately 16 weeks of age) were randomly divided into four groups (or replicates) of 24 pigs each, and kept in pens of six animals on a commercial swine facility. Over the last six weeks of the finishing period, pens of pigs were either fed pellets or mash (feed texture factor: FT), and were fed either twice or five times per day (meal frequency factor: MF). Animals fed twice a day received their meals at 08h00 and 16h00, whereas the others received five meals every 3 h, starting at 8h00. Pigs were fed a corn-soybean based finisher ration containing 16% CP and 3300 MJ ME/kg. At 110 (±2.79) kg body weight (BW), pigs were shipped in groups of 24 pigs per replicate (six pigs per type combination of feed and number of meals) to the experimental swine unit of Agriculture and Agri-Food Canada (AAFC) in Lennoxville (QC). This transfer was made to ensure better control of the fasting intervals to be applied before slaughter. At the AAFC swine unit, animals within each feeding treatment were kept in small pens in groups of two pigs per pen for a period of five days in order to allow them to recover from transportation stress. The four feeding treatment combinations continued to be applied at the AAFC finishing site until the day of shipment to slaughter. Pigs were weighed two days prior to shipment to the abattoir in order to determine their BW. Prior to shipping, the three

pens within each feeding treatment were randomly assigned to one of three fasting treatments (fasting factor: F). Feed was either not withdrawn (0 h) or withdrawn at 10 or 20 h prior to transportation. Animals were transported for 30 min in a single-decked trailer and were given 3.5 h rest at the AAFC experimental abattoir before slaughter. Total fasting time (from last feed to slaughter) was 4, 14 or 24 h, but water was available all the time. At slaughter, pigs were electrically stunned (head-only; 300 V for 4 s) and exsanguinated in a prone position. All animals were slaughtered in the middle of the day (10:30–13:30 h, 11:00–14:00 h and 14:30–16:00 h for the 14 h, 24 h and 4 h fasting time groups, respectively). Animals were cared for according to the recommended code of practice (Agriculture & Agri-Food Canada, 1993) and following the guidelines of the Canadian Council on Animal Care (1993).

2.2. Carcass weight, skin bruises and stomach weight

Following slaughter, carcasses were split, eviscerated and chilled (1 °C and air velocity of 1 m s⁻¹) according to standard commercial practices. Hot carcass weight was recorded and used to calculate the dressing yield. Skin bruises were assessed on the slaughter line with a photographic scale from 0 (none) to 5 (severe) (MLC, 1985).

Stomachs were removed directly from the dressing line during evisceration. They were identified and stored at 4 °C until they were weighed, full and emptied of their content. Content weights are expressed on a wet weight basis. The proportion of empty stomach weight (ESW) and stomach content weight (SCW) over the body weight (ESW/BW and SCW/BW) was also calculated.

2.3. Meat quality measurements

Muscle pH was assessed at 45 min (pH₄₅) and 24 h (pH₂₄) post-mortem with a pH meter (Oakton Instruments Model pH 100 Series, Nilis, IL) fitted with a spear type electrode (Cole Palmer Instrument Company, Vernon Hills, IL) and an automatic temperature compensation probe by insertion in the *Longissimus* (L) muscle at the 3rd/4th last rib (Canadian grading site) and in the *Adductor* (AD) muscles. At 24 h post mortem the following measurements were conducted in the L muscle in the carcass grading site region: electrical conductivity (EC) by insertion of the Pork Quality Meat probe (PQM-I-INTEK, GmbH, Aichach, Germany); subjective color score using the Japanese Color Standards (JCS) ranging from 1 = pale to 6 = dark color (Nakai, Saito, Ikeda, Ando, & Komatsu, 1975); light reflectance using a Minolta Chromameter CR 300 (D65 light source with 0° viewing angle geometry) according to the reflectance coordinates (CIE L*, a*, b*) after exposing the muscle surface to 1 h blooming time. Drip loss was assessed on an adjacent chop according to a modified “juice container” procedure (Christensen, 2003). Briefly, three cylindrical muscle cores were taken with a cork borer (25 mm diameter) in each slice and

weighed. The cores were then placed into a drip loss plastic container (Christensen Aps Industrivaengetand, Hilleroed, Denmark) and stored at 4 °C. Forty-eight hours after sampling, the sample was removed from the container, carefully dabbed and weighed. Drip loss was finally estimated by calculating the difference between the initial and the final weight of the muscle sample.

2.4. Urinary cortisol analysis

Urine samples (up to 40 ml) were collected on the slaughter line from the bladder of each animal prior to evisceration. Samples were transferred to tubes containing HCl 6 M (1% of urine volume) as a preservative and immediately frozen (−45 °C) pending cortisol analysis. After extraction, cortisol was measured by high pressure liquid chromatography (HPLC) with UV detection after extraction on reverse phase columns (Hay & Mormède, 1997). Creatinine was measured with the colorimetric quantitative method (Procedure 500, Sigma Diagnostics, Saint-Quentin-Fallavier, France) to correct for urine dilution.

2.5. Statistical analysis

Carcass data were analysed according to a Generalized Randomized Block Design with three factors: feed texture (FT: mash *vs.* pellet), meal frequency (MF: 2 *vs.* 5 meals per day) and fasting time (F: 4, 14 and 24 h). Categorical data were analysed with the Fisher's exact test for each factor separately. All statistical analyses were done using SAS software (SAS, 2002).

3. Results and discussion

3.1. Effect of fasting time, feed texture and meal frequency on carcass and meat quality

The average BW recorded two days prior to slaughter was 116 (±3.5) kg. As shown in Table 1, no effect of the treatments was observed on this variable.

A significant F × MF × FT interaction on carcass weight was found ($P < 0.01$). Carcass weight decreased with feed

withdrawal time but this weight drop was more pronounced in pigs given mash feed over the last six weeks of the finishing phase.

Similarly to Kephart and Mills (2005), the 24 h feed withdrawal slightly decreased (−1%) dressing yield ($P < 0.001$) at a rate of 117 g per hour from 14 to 24 h of fasting. However, these results contrast with those reported by Chevillon (2005) who only found a 30 g weight loss per hour from 18 to 30 h fasting time, with no significant decrease in dressing yield, and by Beattie, Burrows, Moss, and Weatherup (2002) and Ellis (1998) who, instead, observed an increase in dressing yield (from 68.9% to 74.2%) with longer fasting intervals (from 12 to 60 h) as a result of reduction in gut fill and offal weight. Indeed, recent results from Kephart and Mills (2005) showed a 2 kg decrease in viscera (internal organs except kidneys) weight from 16 to 24 h fasting time. The effect of prolonged periods of feed withdrawal on carcass weight loss is still a subject of debate. Warriss and Brown (1983) predicted a weight loss rate of 0.11% per hour between 18 and 48 h of feed withdrawal and Beattie et al. (2002) and Kephart and Mills (2005) reported a 1 kg lower carcass weight in pigs fasted for 20 and 24 h, respectively. However, Chevillon (2005) found that this weight reduction only became significant after 24 h of fasting and a number of other studies failed to find any impact of feed withdrawal time (up to 60 h) on carcass weight (Eikelenboom et al., 1991; Ellis & McKeith, 1999; Turgeon, 2003). Dressing yield was also higher in carcasses from pigs fed with pellets compared to those fed with mash (81.2% *vs.* 79.3%; $P < 0.001$) and in pigs fed five times compared to two times per day (80.7% *vs.* 79.8%; $P < 0.001$). Based on the significant correlation between carcass yield and the ratio ESW/BW ($r = -0.48$; $P < 0.0001$; unshown data), the variation in carcass dressing yield between treatments can be explained by the weight of the stomachs being removed in the dressing procedure. Indeed, the ratios ESW/BW and SCW/BW were lower in pigs fed with pellets ($P < 0.001$ and $P < 0.05$, respectively) and the ratio SCW/BW was lower in pigs fed five times a day and higher in pigs fasted for 24 h before slaughter ($P < 0.05$ and $P < 0.001$, respectively).

Table 1

Effects of fasting interval (F), meal frequency (MF) and feed texture (FT) on carcass quality traits and empty stomach and content weight/body weight ratios

Fasting interval (h)	4				14				24				SEM	Significance ^a			Interaction
	2		5		2		5		2		5			F	MF	FT	
Meal frequency	P	M	P	M	P	M	P	M	P	M	P	M					
Feed texture	P	M	P	M	P	M	P	M	P	M	P	M					
Body weight (kg)	114.1	116.6	116.7	116.6	118.8	115.7	115.1	116.6	117.1	117.1	115.5	114.1	1.0	NS	NS	NS	NS
Carcass weight (kg)	93.1	92.3	94.9	93.4	96.8	90.5	94.6	93.7	93.3	91.8	93.0	90.4	0.7	**	NS	***	**
Dressing yield (%)	81.7	79.2	81.4	80.2	81.5	78.2	82.2	80.4	79.7	78.5	80.5	79.3	0.4	***	***	***	NS
Skin bruise score	1.2	1.1	1.2	1.2	1.2	1.2	1.2	1.4	1.1	1.2	1.4	1.2	0.1	NS	*	NS	*
ESW/BW (%) ^b	0.41	0.43	0.41	0.42	0.38	0.42	0.40	0.47	0.48	0.47	0.47	0.42	0.0	NS	NS	***	NS
SCW/BW (%) ^c	0.87	0.32	0.40	0.67	0.25	0.42	1.11	0.69	0.29	0.90	0.55	0.18	0.1	***	*	*	NS

FT, feed texture; MF, meal frequency; F, fasting time; P, pellet; M, mash.

^a NS, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

^b ESW/BW: empty stomach weight/body weight.

^c SCW/BW: stomach content weight/body weight.

Feed restriction prior to slaughter has been reported to increase aggressiveness, especially after mixing unfamiliar groups of pigs (Brown, Knowles, Edwards, & Warriss, 1999; Murray, Robertson, Nattress, & Fortin, 2001; Turgeon, 2003). There is also evidence that fasted pigs not only fight more violently but also for longer periods (Fernandez, Meunier-Salaun, Ecolan, & Mormède, 1995) resulting in higher incidence of severe bruises on the carcass at slaughter (Brown et al., 1999; Kelley, McGlone, & Gaskins, 1980). Since pigs in the present study were not mixed and were handled under controlled conditions before slaughter, bruise scores were very low (maximum score of 1.4 corresponding to very slight bruises in the MLC chart). However, a $F \times MF \times FT$ interaction on this variable was found ($P < 0.05$). More bruised carcasses were found in pigs fasted for 14 and 24 h and fed either pelleted or mash feed five times per day. Besides confirming the effect of fasting on pig fighting during transport and lairage, this result means this effect is more pronounced in pigs that are not used to long intervals without food, as is the case with pigs fed five times a day. Increased aggressivity can be seen as a sign of frustration caused by the lack of food or hunger (Arnore & Dantzer, 1980). However, the low skin bruise score and the little difference in this variable between treatments (0.1–0.2 score difference) would indicate that the level of frustration and subsequent aggressiveness in the pigs at the pre-slaughter conditions applied in this study (fasting but not mixing) was limited.

As no single effect of MF and FT have been found, only results originating from the effects of F and of the interaction of $F \times MF \times FT$ are presented and discussed (Table 2). Except for pH_u , no significant effects of the feeding treatments were observed on meat quality traits both in the L and in the AD muscles in this study. The pH_u of the L muscle of pigs fasted for 4 and 14 h were

lower ($P < 0.05$) by 0.07 and 0.03 U, respectively, compared to that from pigs fasted for 24 h. Maribo (1994) reported increased muscle acidification in the loin muscle of pigs which were not fasted and were slaughtered immediately after the arrival at the abattoir. A higher risk of PSE (pale, soft, exudative) pork condition is achieved for fasting time below 18 h (Guàrdia et al., 2004). On the other hand, long fasting periods, when associated to cold stress, long transport or lairage and mixing, would tend to decrease the incidence of PSE meat and increase the prevalence of DFD (dark, firm, dry) pork due to muscle glycogen exhaustion (Eikelenboom et al., 1991; Gispert et al., 2000; Leheska, Wulf, & Maddock, 2003). According to Guàrdia et al. (2005), the risk of DFD pork increases with fasting times longer than 22 h. However, a number of other studies (Beattie et al., 2002; De Smet et al., 1996; Morrow, See, Eisemann, Davies, & Zering, 2002; Murray et al., 2001) reported no or very little impact of feed withdrawal on meat quality. It has been observed that only 20% of muscle glycogen is lost over 24 h of fasting (Jones, Rompala, & Haworth, 1985; Warriss, 1989), but this reduction can be enough to influence pH_u in those muscles supporting the animal's posture and weight, such as the AD muscle (Fernandez & Tornberg, 1991; Warriss, 1982). In this study, a significant $F \times FT \times MF$ interaction was present for the pH_u value in the AD muscle, this value being higher in the AD muscle of 24 h fasted pigs and fed pellets in two meals per day over the last six weeks of finishing ($P < 0.05$). It may be hypothesized that the reduction in the number of meals per day and the associated lower energy intake (not recorded) may have decreased the glycogen stores in this muscle. Low endogenous glycogen stores in the muscle increase the predisposition to DFD pork, especially in oxidative muscle types like the AD muscle having a lower

Table 2
Effects of fasting interval (F), meal frequency (MF) and feed texture (FT) on meat quality traits of the L and AD muscles

Fasting interval (h)	4				14				24				SEM	Significance ^a	Interaction
	Meal frequency		Meal frequency		Meal frequency		Meal frequency		Meal frequency		Meal frequency				
Feed texture	P	M	P	M	P	M	P	M	P	M	P	M	F	$F \times MF \times FT$	
<i>Longissimus</i>															
pH_{45}	6.49	6.50	6.34	6.58	6.47	6.37	6.57	6.50	6.53	6.31	6.47	6.40	0.10	NS	NS
pH_u	5.50	5.48	5.50	5.54	5.53	5.54	5.57	5.56	5.62	5.58	5.53	5.58	0.03	*	NS
L^*	54.37	53.97	53.70	52.62	54.35	52.00	53.06	53.84	52.10	54.09	54.43	53.88	1.10	NS	NS
a^*	8.45	8.37	8.14	8.72	7.69	8.52	8.28	8.67	7.83	9.06	9.00	8.33	0.35	NS	NS
b^*	6.32	6.19	5.97	5.90	6.19	5.83	5.78	6.37	5.84	6.64	6.98	6.24	0.36	NS	NS
JCS ^b	2.06	2.13	2.22	2.47	2.13	2.53	2.34	2.19	2.47	2.19	2.25	2.31	0.21	NS	NS
PQM (μ s) ^c	4.12	3.64	4.75	3.63	4.18	4.69	3.54	4.99	3.40	5.10	5.28	4.77	0.76	NS	NS
Drip loss (%)	5.72	3.89	5.88	4.39	4.88	4.36	4.34	5.17	3.20	4.14	5.19	4.73	0.70	NS	NS
<i>Adductor</i>															
pH_{45}	6.24	6.21	6.20	6.39	6.37	6.37	6.31	6.18	6.34	6.19	6.29	6.31	0.09	NS	NS
pH_u	5.54	5.59	5.51	5.62	5.60	5.64	5.64	5.55	5.74	5.65	5.60	5.67	0.04	***	*

FT, feed texture; MF, meal frequency; F, fasting time; P, pellet; M, mash.

^a NS, not significant; * $P < 0.05$; *** $P < 0.001$.

^b Based on Japanese Color Scales (from 1 = pale to 6 = dark; Nakai et al., 1975).

^c Electrical conductivity measured by Pork Quality Meter (PQM-I-INTEK, GmbH, Aichach, Germany).

glycolytic potential (Hambrech et al., 2005; Monin, Mejenes-Quijano, Talmant, & Sellier, 1987).

3.2. Effects of fasting time on urinary cortisol concentration at slaughter

The urinary cortisol concentration was only affected by the fasting time ($P < 0.05$; unshown data). However, when contrasts between fasting intervals were made, cortisol in the urine collected at slaughter only tended to be higher in pigs fasted for 14 h (33.3 ng/g creatinine) compared to those fasted for 4 h (22.5 ng/g creatinine; $P = 0.10$) and 24 h (21.2 ng/g creatinine; $P = 0.06$). This difference between 14 and 24 h fasted groups in cortisol levels cannot be attributed to the variation in the diurnal cycle as both groups were slaughtered around midday which is the time at which cortisol levels achieve their peak (Hay, Meunier-Salaun, Brulaud, Monnier, & Mormède, 2000). In a recent study Koopmans, van der Meulen, Dekker, Corbijn, and Mroz (2005) showed that the diurnal rhythm in cortisol is independent of feeding. Hence, the urinary cortisol variation recorded in 14 and 24 fasted pigs in this study is hard to explain. Surprisingly, no variation in the cortisol levels was found between 4 and 24 fasting intervals. The literature results on the effects of fasting on cortisol levels are somewhat contradictory. Gispert et al. (2000) found lowest blood levels of cortisol in pigs fasted from 12 to 18 h. Whereas other studies reported an increase in blood or salivary cortisol levels starting from 15 h of fasting (Haupt, Baldwin, Haupt, & Hills, 1983; Parrott & Misson, 1989).

3.3. Relationship between stomach content at slaughter, meat quality traits and urinary cortisol

The effects of the dietary treatments on stomach content weights at slaughter will be presented elsewhere (Saucier et al., in preparation). Stomach content weight (SCW) data were analyzed to study the relationship between the degree of stomach fill at slaughter, meat quality and urinary cortisol variation. Fifty-one per cent of the stomachs harvested in this study had contents weighing more than 500 g (670 g on average; range 69–2687 g) which is the recommended threshold to prevent carcass contamination at slaughter (Chevillon, 1994). This content was also higher than that reported in several surveys conducted in different countries over the last decade (Chevillon, 1994; Guise et al., 1995; Lynch et al., 1998).

Among all pork quality variables evaluated, only pH_u was significantly correlated ($r = -0.214$; $P < 0.05$) with SCW at slaughter in this study, which means that each weight increase of the stomach content may contribute to higher muscle acidification (Table 3). This result would support the hypothesis advanced by Eikelenboom et al. (1991) that the increased PSE pork incidence in short-term fasted pigs can be related to the higher physical effort made by pigs being handled on a full stomach. However, given the small magnitude of this correlation, it can be concluded

Table 3

Correlation coefficients between meat quality traits in the L and AD muscles and stomach content weight (SCW) at slaughter

	SCW (g) ^a
<i>Longissimus</i>	
pH_{45}	0.134
pH_u	-0.214*
L^*	-0.090
a^*	-0.065
b^*	-0.147
JCS ^b	0.047
PQM (μs) ^c	-0.130
Drip loss (%)	-0.029
<i>Adductor</i>	
pH_{45}	0.066
pH_u	-0.180

^a SCW: stomach content weight.

^b Based on Japanese Color Scales (from 1 = pale to 6 = dark; Nakai et al., 1975).

^c Electrical conductivity measured by Pork Quality Meter (PQM-I-INTEK, GmbH, Aichach, Germany).

* $P < 0.05$.

that handling pigs on a full stomach under the experimental pre-slaughter conditions of this study had a limited impact on pork quality variation.

Furthermore, the higher stress susceptibility of unfasted pigs hypothesized by Eikelenboom et al. (1991) cannot be supported by the measurement of cortisol levels in the urine. Indeed, no significant correlation between stomach content and urinary cortisol was found in the present study (data not shown). The reason for this lack of relationship may be twofold. Firstly, the stress level imposed on the pigs might not have been sufficiently great or different between treatments to change the level of cortisol significantly. Indeed, as observed by Hambrech, Eissen, and Versteegen (2003), small scale studies conducted under experimental conditions, like this one, often fail to simulate the stress experienced by pigs in practical conditions. Secondly, the diurnal cycle of cortisol production might have masked differences in the cortisol level (Hay et al., 2000) as urine was collected from pigs slaughtered over a 6 h time period (from 10h30 until 16h30).

No significant correlation between urinary cortisol and pork quality was found either (data not shown). This result suggests that urinary cortisol might not be the best indicator of pork quality as already found by Foury et al. (2005) who reported that meat quality traits, such as pH and color, were better correlated with urinary catecholamine levels (mainly adrenaline) than with cortisol.

4. Conclusions

The results of this study show a positive influence of pellet feeding on carcass yield in fasted pigs. Fasting had limited influence on carcass yield, but slightly increased the incidence of bruises on the carcass, especially in pigs fed ad libitum. When fasting is not complicated by other pre-slaughter practices (i.e. mixing, long transport or lairage

time) muscle glycogen does not deplete to such an extent that pork quality is influenced negatively. Handling pigs on a full stomach in the experimental conditions applied in this study did not result in a higher physiological response to stress.

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