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2 meat quality, of growing-finishing Iberian x Duroc pigs under 3 high environmental temperatures 4 Angela C.F. Oliveira^a, Joel González^b, Salma E. Asmar^b, Norbert P. Batllori^b, Itzel Y. Vera^b, Uriel R. Valencia^b, Rosil Lizardo^b, Tâmara 5 D. Borges^a, Enric Esteve-Garcia^b, Nuria Panella-Riera^b, Leandro B. 6 7 Costa^a and Antoni Dalmau^{b*} 8 ^aSchool of Live Sciences, Pontificia Universidade Católica do 9 Paraná (PUCPR), PO Box 80215-901, Curitiba, Paraná, Brazil; 10 ^bDepartment, of Animal Welfare, Institut de Recerca i Tecnologías 11 Agroalimentàries (IRTA), PO Box 17121, Monells, Girona, Spain 12 13 *Corresponding author at: Telephone: (+34) 636 85 33 33; Fax N°: 14 972630533; E-mail address: antoni.dalmau@irta.cat (A. Dalmau). 15 16 **Abstract** 17 The present study investigated the replacement of 5% of starch (ST) 18 by 5% of sunflower oil (SO), with or without feed restriction, in the 19 diet of growing-finishing Iberian × Duroc pigs exposed to heat stress 20 conditions. The effects of these strategies on the welfare, 21 performance and meat quality of the animals were evaluated. 22 Seventy-two crossbred males $[51.00 \pm 6.29 \text{ kg body weight (BW)}]$ 23 were housed in collective pens and randomly distributed according 24 to their initial BW in climate-controlled rooms under heat stress 25 conditions (30-32 °C; 35%-50% humidity). The experiment was

The effect of feeder system and diet on welfare, performance and

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carried out in a randomized block design, in a 2 × 2 factorial design

composed of two diets (control or oil) and two feed management

28	(ad-libitum and restriction) types. The investigated treatments were:
29	1) control diet (5% ST \times 0% SO) with <i>ad-libitum</i> feed intake, 2) oil
30	diet (replacement of 5% of ST by 5% SO) with ad-libitum feed
31	intake, 3) control diet with restriction feed intake, and 4) oil diet
32	with restriction feed intake. The pigs' behavior and dirtiness score
33	were observed daily, and their performance was assessed weekly.
34	Furthermore, three blood samples were collected from each animal
35	for hemogram analysis during the period of study. The analysis of
36	meat and carcass quality was performed 24 h post-mortem once the
37	animals had reached an average BW of 130-140 kg. Statistical
38	analyses were performed using the PROC MIXED and PROC
39	GENMOD procedures of the Statistical Analysis System (SAS). The
40	diet-regimen interaction produced no effects (P \geq 0.05) on the
41	analyzed variables, except for behavior. Animals on the control diet
42	associated with ad-libitum feed management spent more time in the
43	lateral decubitus position and showed reduced activity. In addition,
44	pigs fed ad-libitum and those that received the control diet displayed
45	the highest level of dirtiness during the experiment. Evaluation of
46	performance revealed that pigs receiving the oil diet showed reduced
47	feed intake (P \leq 0.05) with no difference in average daily gain and
48	consequently presented better feed conversion values compared to
49	animals on the control diet. Furthermore, the oil diet resulted in a
50	higher (P \leq 0.05) carcass yield as well as a higher oleic fat content in
51	the meat. Pigs on restricted feeding management with lower body
52	and carcass weights produced higher values of leanness percentage
53	and carcass compared to animals in the ad-libitum group. In
54	conclusion, the replacement of 5% of starch by 5% of sunflower oil

55 in the pigs' diet during high ambient temperatures improved the 56 animals' welfare, feed efficiency, and carcass characteristics. 57 Keywords: behavior, dirtiness, heat stress, sunflower oil, swine 58 59 1. Introduction 60 With thermal stress impairing efficiency at all stages of 61 production, and with more than 50% of pig production being in 62 tropical and subtropical countries (Renaudeau et al., 2010), the high 63 temperatures turn out to represent one of the major reasons for 64 economic and productive losses (Cottrell et al., 2015) in the pig 65 industry. To reduce body temperature and improve heat dissipation 66 through the ambient environment, heat-stressed pigs exhibit certain 67 behavioral patterns such as increased in respiration rates (Huynh et 68 al., 2005), decreased exploration and social activities (Shi et al., 69 2006), increased water intake (Olczak et al., 2015), wallowing on 70 feces (Bracke, 2011), and a reduced number of visits to the feeder 71 with a consequent reduction in feed intake (Silva et al., 2009). 72 Debrecéni et al. (2014) observed that during periods of high 73 temperature, pigs would avoid physical contact and adopt a lateral lie 74 position at a higher rate than they would a sternal position. Moreover, 75 their wallowing behavior is associated with the difficulty of losing 76 heat to the environment (Bracke, 2011), as almost all of their 77 thermoregulation processes are based on body surface contact with 78 fresh areas (Olczak et al., 2015). Lying in the mud can reduce body 79 temperature by up to 2 °C and, for every degree of increase in the 80 ambient temperature, the frequency of animals lying laterally extends 81

by 1.2% (Huynh et al., 2005). In intensive production systems, where

82 pigs do not have access to the mud or lakes, their own feces perform 83 this cooling function (Spoolder et al., 2012). Regarding performance 84 impairment, the reduction and redirection of feed intake during the 85 cooler hours of the day occur to decrease the body heat produced 86 from the digestion, absorption and metabolization of the diet (i.e., 87 heat increment) (Silva et al., 2009). Proteins have the highest values 88 of caloric increase among the ingested nutrients, followed by 89 carbohydrates and oils (Verstegen et al., 1982). Thus, the 90 concentrations of amino acids in the diet, as well as carbohydrates, 91 oils, and fats will directly affect the feed intake of animals subjected 92 to heat stress conditions, and will alter their total body heat 93 production (Li and Patience, 2016). For this reason, it was 94 determined that supplementing the diet with fat can provide pigs with 95 their energy requirements with a minimum level of heat increment 96 (Patience et al., 2015). 97 The Iberian pig (Sus mediterraneus) is a breed with distinct 98 marbling characteristics that originates from the southwest region of 99 Spain. Its production is highly valued due to the myoglobin and iron 100 concentrations, as well as the oleic acid content, in their tissues 101 (Ayuso et al., 2014). These specificities result in high quality 102 products, characterized by a meat with a differentiated texture and fat 103 infiltration highly sought after by consumers (Ayuso et al., 2014), 104 especially for Jamón Ibérico. To retain this differentiated meat while 105 improving reproductive performance, Iberian pigs have been crossed 106 with other breeds (Viguera et al., 2012). Iberian pigs crossed with the 107 Duroc breed produced offspring with an increased growth rate, leaner 108 performance and greater carcass length as well as ham length and

109 perimeter, and a higher percentage of all the noble pieces, compared 110 to purebred animals. 111 The objective of this work was to study the replacement of 5% of 112 starch (ST) by 5% of sunflower oil (SO), with or without feed 113 restriction, in growing-finishing Iberian × Duroc pigs that were kept 114 under heat stress conditions, and to evaluate the effect of these 115 treatment strategies on the animals' welfare (behavior and dirtiness), 116 performance and meat quality. 117 118 2. Materials and methods 119 2.1. Ethical note 120 The experiment was conducted at the Institut de Recerca i 121 Tecnología Agroalimentàries (IRTA) located in Monells, 122 province of Girona, Catalunya, Spain. All experimental protocols 123 used in this study were approved by the Comisión de 124 Experimentación Animal de La Generalitat de Catalunya 125 (protocol number - DAAM 8348). 126 127 2.2. Animals and experimental design 128 Seventy-two crossbred males (50% Iberian × 50% Duroc), 129 with an initial BW of 51.00 ± 6.29 kg, were housed in three 130 climate-controlled rooms with collective pens [four pens per room 131 and six animals per pen (5 m × 2.6 m)] and a 12:12 h 132 light/darkness cycle. Dalmau et al., (2019) determined from two 133 studies (one carried out in the same facilities as the present work 134 and the other performed at another research institute) that pigs 135 exposed to temperatures of approximately 30 °C for 5 h per day,

with a relative humidity of 40%-60% may suffer from heat stress, compared to control animals subjected to lower temperatures. The three rooms used in the present study were subjected to the same high temperature conditions as defined by Dalmau et al., (2019) to trigger a stress response in the pigs. The temperature and humidity in each room were continuously monitored using a Rotronic® HygroLog datalogger (Hygromer C94, sensor Pt100 RTD, 1/3 DIN, Switzerland). Figure 1 shows the mean values per hour of temperature and humidity, for the duration of the study. The calculated THI was 26-27 from 19 00 h until 08 00 h and 30-32 from 10 00 h to 15 00 h. At 09 00 h and 16 00 h, as well as 17 00 h and 18 00 h, THI was 28-29. All animals were individually identified during the entire study with different colored ear tags. The experiment lasted 90 d, from October to January, and was carried out in a randomized block design, in a 2 × 2 factorial scheme composed of two diets (starch or sunflower oil (with or without) and two feed intake management types (ad-libitum or restricted), each with three replicates. The studied treatments were a control diet of 5% ST × 0% SO with ad-libitum feeding, an oil diet with the replacement of 5% ST by 5% of SO and ad-libitum feeding, a control diet of 5% ST \times 0% SO with restricted feeding, and an oil diet with the replacement of 5% of ST by 5% of SO and restricted feeding. Feed were provided in two phases at 50-100 kg BW for the growing and 100-140 kg BW for the finishing periods. The diets were calculated to be isonutritive, except in the case of net energy, due to the replacement of starch by sunflower oil. The composition and the determined nutrient values are

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shown in Table 1, with the diet formulated according to the nutritive requirements of the growing and finishing pigs (NRC, 2012).

Following initiation to the environmental treatments, the average daily feed intake (ADFI) was calculated weekly by weighing the feed provided, and individual body weights were recorded at four different times: when the animals arrived, after 7 days of adaptation (i.e., at the start of the environmental treatment), at the time of feed transition (i.e., from the growing to the finishing period) and at the end of the experiment. The amount of feed offered to pigs in the feed-restricted group was based on the consumption of *ad-libitum* animals and calculated to provide 90% of their feed intake. Collected data were used to determine average daily gain (ADG), and feed conversion ratio (F: G) for each animal and treatment group.

2.3. Blood samples

Blood samples were collected for each animal from the jugular vein after 7 days of adaptation, at the time of feed transition, and one day preceding slaughter. The blood (8 mL per sample) was aseptically stored in tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA, Becton Dickinson, USA) for further hemogram analysis.

2.4. Dirtiness score

The evaluation of dirtiness was based on the classification $\label{eq:classification} described in the Welfare Quality^{\circledR} \ protocol \ (Welfare Quality,$

2009) and was assessed once, every day, in the morning period (08 00 h). The classification used was a score of 0 for animals with feces in less than 20% of the body, 1 for animals with feces in more than 20% but less than 50% of the body, and 2 for animals with feces in more than 50% of the body. Additionally, once a month, a complementary welfare evaluation was carried out once a month, which included other healthy parameters detailed in the Welfare Quality® protocol. The dirtiness evaluation was carried out at five different periods of time to progressively evaluate animal response to the experimental treatments. Each period corresponded to one week of analysis according to the date on which they were performed.

2.5. Total daily activity

The pen was divided in eight equal zones to record the number of activities performed, as well as each animal's distribution and occupation of the pen. The general activity of all animals in each treatment was assessed, for the total duration of the experiment, using a scan sampling method three times per day for 1 min. According to a previously established schedule, the first assessment was carried out at 09 00 h, the second at 16 00 h, and the third randomly selected between 08 00 h and 18 00 h, so that by the end of the study, the data collected were representative of the animal's total daily activity. As the experiment lasted 90 d, the animals were evaluated a total of 270 times. The evaluation of daily activity was divided into three time periods according to the hour of the day when recording was performed, i.e., morning (08

00 h to 10 00 h), noon (11 00 h to 15 00 h) and afternoon (16 00 h to 18 00 h). The assessed behaviors were eating, exploring the environment, interaction with the enrichment material (chains on the side of the pens), lying positions (sternal or lateral), drinking, positive interactions [defined as sniffing, nosing, licking, and moving gently away from the animal without aggressive or flight reaction from this individual, Welfare Quality (2009)], negative interactions [defined as an aggressive behavior, including biting, or aggressive social behavior with a response from the disturbed animal, Welfare Quality, (2009)], and others (any behavior that did not fit into the activities described above).

2.6. Measurement of carcass and fatty acid profile

At the end of the experiment, after the animals had reached an average BW of 130–140 kg, all pigs were weighed, individually identified, and transported to a local slaughterhouse, where they were slaughtered using the carbon dioxide (CO₂) stunning method. Within 20 min *post-mortem*, carcass weight, percentage of leanness, and fat content for each animal were measured at the last rib and recorded using a FoodScan near-infrared spectrophotometer (FOSS, Denmark).

At 24 h *post-mortem*, subcutaneous fat samples over the gluteus medius were collected from the left side of each carcass. Tissue lipids were extracted following the chloroform-methanol procedure described by Folch et al. (1956) and analysis results were expressed as percentage of total fatty acids.

244 2.7. Statistical analysis 245 Statistical analyses were performed using the Statistical Analysis 246 System (SAS) (SAS 9.1 software, SAS Institute INC., Cary, NC). 247 BW, feed intake, ADG, feed conversion, hematocrit, leukocyte 248 content, neutrophil/lymphocyte ratio, carcass weight, fat thickness, 249 leanness percentage, loin thickness, pH, electrical conductivity, 250 intramuscular fat and fatty acid composition were the variables 251 subjected to ANOVA analysis using the SAS PROC MIXED 252 procedure. Body weight was used as a covariate when necessary 253 (e.g., to evaluate performance and meat quality variables). In the case 254 of pen dirtiness, animal dirtiness, drinking, positive and negative 255 interactions and exploratory behaviors, PROC GENMOD with a 256 binomial distribution was used to investigate the interactions between 257 diet (control or oil diet) and regimen (ad-libitum and restricted). Each 258 pen was considered as one unit. The residual maximum likelihood 259 was used as a method of estimation, while the least square means of 260 fixed effects (LSMEANS) adjusted to Tukey's honestly significant 261 differences was used to carry out multiple comparisons. The p-value 262 for significance was fixed at P < 0.05. 263 264 3. Results 265 3.1. Hemogram analysis 266 Hemogram analysis showed no interaction between diet and 267 regimen (Table 2). However, comparisons between the basal and

Comentat [A1]: The original sentence read 'In the case of pen dirtiness, animal dirtiness, drinking, positive and negative interactions and exploratory behaviours, a Proc Genmond with a binomial distribution were used, being the diet (control or oil diet), regimen (ad-libitum and restricted) and interactions effects studied.' I have made modifications to this section to improve the flow of ideas. Please review these changes carefully.

Comentat [A2R1]: Dr. Dalmau

final values showed increased levels of hematocrit (P < 0.0001),

corpuscular volume (P = 0.002) and lymphocytes (P < 0.000),

but decreased levels of leucocytes (P < 0.0001), neutrophils (P

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<0.000) and neutrophil: lymphocyte ratio (P < 0.0001). Although there was no difference between diets, it was noticed that the neutrophil/lymphocyte ratio differed between the two regimens (P = 0.0302) at the final value, with ad-libitum fed animals (0.667) showing a higher neutrophil/lymphocyte ratio than restricted-fed animals (0.577).

3.2. Dirtiness score

No interaction was found between diet and regimen with respect to the evaluated dirtiness score. However, animal dirtiness was affected by regimen (P = 0.0007), diet (P < 0.0001) and period of the day (P < 0.0001). Animals fed *ad-libitum* were dirtier than those subjected to restricted feeding (Figure 1), and animals fed with the control diet were dirtier than those fed with the oil diet (Figure 2). In addition, the pigs were dirtier during the early periods, especially period 2, than during the later periods (Figure 3).

3.3. Total daily activity

The main results of the scan sampling measurements are presented in Table 3. Three of the evaluated behaviors showed interactions between diet and regimen. The lateral lie position (P = 0.003) occurred mainly in animals that received the control diet with ad-libitum feed management. The same group (control diet + ad-libitum) also showed less activity according to the behavior categories 'pen exploration' (P < 0.000) and 'others' (P = 0.005), when compared with the other treatments. Evaluations carried

out based on the time periods showed that lying laterally occurred mainly at noon (P < 0.0001), while the sternal lie position was mainly observed during the afternoon (P = 0.018). In addition, the animals explored the pen, ate and presented other behaviors mostly in the afternoon, followed by the morning period and lastly at noon (P < 0.0001).

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3.4. Animal growth performance

The results for performance are presented in Table 4. No interaction was observed between diet and regimen in the assessed performance parameters. Separately comparing the effect showed that diet affected feed intake (P < 0.0001) and the feed conversion ratio (P = 0.003) during the growing phase but only affected feed intake (P < 0.0001) in finishing phase. In the growing phase, pigs that received the control diet presented a higher feed intake as well as the lowest feed conversion compared with animals that received the oil diet. Similar observations were recorded in the finishing phase, where the feed intake was higher in the control group compared to the group fed with sunflower oil. When considering all the collected data (for both growing and finishing phases) only an effect on feed intake (P < 0.0001) was observed. The regimen affected parameters in both phases. In the growing phase, the average daily gain (P < 0.001) and feed intake (P < 0.001) were higher in the *ad-libitum* group compared with animals subjected to restricted feeding. All evaluated variables were affected by the regimen in the finishing phase. Animals in the ad-libitum group presented a higher

average daily gain (P < 0.001) and feed intake (P < 0.001), as well as a better feed conversion ratio (P = 0.002) than those under restricted feed management. Assessment of all the collected data (for both growing and finishing phases) showed an effect on average daily gain (P < 0.0001), feed intake (P < 0.0001) and feed conversion ratio (P = 0.0415).

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3.5. Carcass measurement and determination of fatty acid

profile

The effect of diet and feed management on the carcass measurements of carcass and on the fatty acid profile are presented in Table 4. Data obtained showed no interaction between diet and regimen for evaluated parameters. Separately comparing the effects determined that the addition of sunflower oil to the diet only influenced carcass yield, as shown by the higher values (P = 0.019), when compared to the group that were fed the control diet. Also, pigs fed ad-libitum exhibited greater body (P < 0.001), and carcass (P < 0.001) weights than animals which were restricted-fed. However, these animals produced a lower yield of carcass and leanness percentage (P < 0.05). Animals that received oil-supplemented diet presented higher levels of oleic acid (P < 0.0001) compared to animals fed with the control diet. Analysis of the different regimens showed that animals fed *ad-libitum* had a lower linolenic (P = 0.042) acid content than restricted-fed pigs, while no significant difference was observed in the other measured traits.

4. Discussion

4.1. Hemogram analysis

Based on the analysis of hemogram variables, all pigs started the experimental period (day 0) with no statistical differences between the treatments and therefore presented the same "blood parameters".

Comparisons between initial (day 0) and final (day 90) data revealed that the animals presented higher values of hematocrit and corpuscular volume (P < 0.05) at the end of the experimental period. According to published literature, the thermoneutral zone in growing and finishing pigs is 18–20 °C and 12–21 °C, respectively. In the present study, animals were subjected to conditions of moderate thermal stress for 5 h daily, with peak temperatures of 30–32 °C at midday and a plateau of not lower than 25 °C for the remainder of the day. Sreedhar et al. (2013) reported that animals under heat stress can exhibit hemoconcentration due to dehydration, asphyxia or excitement, leading to erythrocyte concentration. Although water was provided *ad-libitum* in the present study, the effect of environmental temperatures on the corpuscular volume and hematocrit parameters were significant.

The leukocyte count, although decreased from the beginning to the end of the study, was higher than the normal range for the porcine species during the whole experimental period. These values indicated that the animals were still coping with certain stress factors, such as their transportation (Goumon and Luigi, 2017), mixing with other animals, and arrival to a new

environment (Peden et al., 2018) even a few days following their arrival to the experimental facilities.

According to Gaughan (2012), animals adopt different types of mechanisms that help them adapt to climatic changes, including acclimatization, as well as biological and genetic adaptation. The thermoregulatory response during acclimatization is biphasic and time-dependent, characterized by a short-term (STHA) and a long-term (LTHA) phase, which could explain the differences between initial and final blood parameters of the present study. The short-term heat acclimation phase is characterized by bland and fast physiological adaptations, with the objective being to cope with heat stress before more permanent adjustments become necessary (Horowitz, 2001). The long-term heat acclimation phase includes a continuum process of adaptative mechanisms, which results in an increased ability to cope with the hot environmental conditions, thereby making the swine better adapted to the surrounding temperature (Gaughan, 2012). The mechanisms involved in the phases of heat acclimation vary according to the time of exposure and heat intensity (Renaudeau et al., 2010), suggesting that the time period (5 h daily) and intensity of temperature (30-32 °C) applied in the present study were not enough to allow the pigs to adapt by producing drastic changes in their physiological parameters.

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4.2. Behavioral analysis

Dalmau et al. (2019) described how pigs became dirtiest under high environmental temperatures rather than under thermoneutral conditions, and concluded that changes in behavior (i.e., lying on the feces) and their consequences (dirtiness) may be the first to occur before any other change in physiological parameters or performance. In the present study, pigs were dirtier during the early periods than in the final ones, confirming that the animals had more difficulty leading with the high temperatures at the beginning of the experiment. Furthermore, the reduction in lying and wallowing in the excretion area during the final stages of the study could indicate that the pigs had undergone an adaptation process. In a study by Renaudeau (2010) on pigs that were exposed to high temperatures long term, the decrease in heat production indicated that animals had developed adaptative changes to assist with the acclimatization process, such as decreasing their physical activity in order to favor heat exchange between their bodies and the floor. Therefore, the elevated score of dirtiness detected at the initial stages of the experiment could be because the pigs had not yet adapted to the fixed ambient temperature (corresponding to the beginning of the thermal treatment). The dirtiness decreased in the later stages of the experiment, indicating that the pigs had adapted in order to cope with the heat stress conditions, thereby reducing their wallowing behavior. Pigs fed with the control diet were dirtier than those fed with the oil-supplemented diet, while pigs fed ad-libitum were dirtier

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than those subjected to restricted feeding. Therefore, strategies

involving diet supplementation with oil or restriction of feed consumption could be potentially applied in hot environments in order to reduce feed intake and, consequently, metabolic heat production (Brooke et al., 2015). It is important to note that thermoregulation in pigs is dependent on the external temperature as well as on the animal's internal heat, which can be decreased if digestive heat is lowered (e.g., by reducing the amount of food or changing the diet to include more digestible ingredients).

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According to the scan sampling data, animals that received the control diet with ad-libitum feed management were less active compared with animals subjected to the other treatments $(P \le 0.05)$. These results were based on the time spent in the lateral lie position (which was higher for this group), on the reduced exploratory activity, as well as on the 'other behaviors' category. According to Olczak et al. (2015), due to the pig's physiological particularities (such as reduced functional sweat glands and fat layer) some behavioral patterns can be observed, including reduced general activity as well as predominant resting and lying behavior. The resting behavior, particularly the lateral lie position, increases the surface area of the animal's body in contact with the floor, and provides a more effective heat exchange compared to the sternal lie position (Olczak et al., 2015). The occurrence of a high percentage of animals lying laterally is considered a sign of thermal stress in pig farms, usually manifesting as the first indicator, even before other physiological or performance behaviors (Dalmau et al., 2019).

The amount of heat produced by pigs is dependent on several factors, including the metabolic rate of muscle activity (Cottrell et al., 2015), as well as metabolic heat production due to digestion, absorption and metabolization of feed nutrients (Silva et al., 2009), which in turn explains the differences between feed management and diet composition on the reduced activity observed in the present study.

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Evaluations carried out at different stages of the day determined that the pigs lay down laterally mainly at noon, when the temperature was increased, but adopted the sternal lie position most frequently during the morning and afternoon, when the temperatures were lower. As previously mentioned, the lateral lie position is a strategy adopted by pigs in order to dissipate excess body heat caused by increased ambient temperatures, thus explaining their necessity to express this behavior during the hottest period of the day. Furthermore, the animals fed and became more active (by exploring the pen and performing other activities) during the afternoon. While the porcine species is highly motivated to explore their surroundings and forage for food, such investigations and manipulation activities lead to excess heat production due to muscle activity (Cottrell et al., 2015). Under the present heat stress conditions, the pigs searched for areas which provided minimum contact with other individuals in order to lie down, explaining the predominance of the exploratory activity during the fresh hours of the day (afternoon), and the lowest values during the hottest hours of the day (noon and morning).

4.3. Animal growth performance and meat quality

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In the present study, pigs kept in ad-libitum feed management during the growing and finishing periods had a higher average daily gain and feed intake than those in the restricted group. The supply of feed is one of the factors influencing feed intake in growing-finishing pigs (Patience et al., 2015). As the feed intake of animals in the restricted group was calculated to provide only 90% of their intake capacity, this variable would be reduced. When comparing the effect of diet types (control vs oil) in the growing and finishing phases, it was observed that the feed intake was reduced with the addition of oil in the diet. Generally, pigs' feed intake is related to their daily requirement of energy. When the dietary energy content is higher, the requirement is reached more easily and pigs therefore reduce their feed consumption (Kil et al. 2014), as observed in the present study. Animals that received 5% of sunflower oil in their diet presented better feed conversion than animals in the control group. This could be because the inclusion of oil in the diet increases the availability of amino acids from crude protein content and, consequently, increases the efficiency and use of these nutrients, thereby improving average daily gain and feed conversion (Brooke et al., 2015). Some researchers have found that a diet rich in oleic acid resulted in higher oleic acid levels in the pigs' fatty acid profile,

some researchers have found that a diet rich in oleic acid resulted in higher oleic acid levels in the pigs' fatty acid profile, in agreement with the results of the present study. These changes in the fatty acid profile represent an added benefit in terms of quality for the consumer market. According to Csapó and

513	Salomon (2013), the fatty acid composition of food products is of
514	great importance with respect to healthy human nutrition.
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516	5. Conclusion
517	In conclusion, pigs adapt to heat stress conditions by modifying
518	their behavior accordingly as well as by being dirtier and lying
519	laterally with a higher frequency. Regarding performance parameters,
520	the replacement of 5% of starch by 5% of sunflower oil during high
521	ambient temperature conditions improved the feed efficiency, meat
522	characteristics (although fat firmness, stability and shelf-life should
523	be further studied) as well as the animal's welfare, and is therefore an
524	efficient nutritional strategy for pigs kept under high temperatures or
525	heat stress conditions.
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531	
532	Declaration of interest
533	All authors declare that there are no conflicts of interest
534	concerning to the information provided in this paper.
535	
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538	animal welfare, nutrition and meat quality, from four different
539	research institutions (ETSIA, ITACVL, PUCPR and IRTA), in a

540	multidisciplinary project that aimed to test practical strategies to
541	reduce the impact of weather on productivity of pigs in commercial
542	production systems. The authors acknowledge all the colleagues in
543	the project, slaughterhouse management and personnel for their
544	valuable input and collaboration.
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Tables Table 1. Composition and calculated nutrients of the experimental diets¹

	50 - 100 Kg 100 - 140 Kg				
	Control diet	Oil diet	Control diet	Oil diet	
Ingredients %					
Barley	33.57	33.57	45.00	45.00	
Maize	0.00	0.00	4.59	4.59	
Wheat	13.41	13.41	25.00	25.00	
Corn starch	5.00	0.00	5.00	0.00	
High oleic sunflower oil	0.98	5.98	0.91	6.00	
Triticale	20.00	20.00	0.00	0.00	
Integral sunflower	8.00	8.00	8.00	8.00	
Bran	5.00	5.00	5.00	5.00	
Sepiolite	1.97	1.97	1.97	1.87	
Soy 47%	8.79	8.79	1.85	1.84	
Molasses beet	0.48	0.48	0.48	0.48	
Calcium carbonate	1.74	1.74	1.08	1.10	
Lysine sulfate ²	0.15	0.15	0.25	0.25	
Salt	0.33	0.33	0.40	0.40	
NP – 316 – Fi	0.30	0.30	0.30	0.30	
Monacal phosphate	0.24	0.24	0.11	0.11	
Resdox-N	0.02	0.02	0.02	0.02	
Liquid colin 75%	0.02	0.02	0.02	0.02	
L-threonine	0.00	0.00	0.03	0.03	
Calculated nutrient composition %					
Dry matter (%)	90.30	90.96	90.46	91.17	
Crude protein (%)	14.50	14.47	11.00	10.97	
Calcium (%)	0.98	0.98	0.69	0.70	
Crude fat (%)	2.61	7.52	2.54	7.55	
Crude fiber (%)	5.60	5.59	5.98	5.97	
Starch (%)	43.90	38.90	48.31	44.00	
Carbohydrates (%)	2.96	2.96	2.41	2.41	
Phosphorus (%)	0.44	0.44	0.37	0.37	
Chloride (%)	0.30	0.30	0.35	0.35	
Sodium (%)	0.16	0.16	0.18	0.18	
Lysine (%)	0.72	0.72	0.55	0.55	
Methionine (%)	0.24	0.24	0.18	0.18	
Methionine +Cystine (%)	0.54	0.54	0.42	0.42	
Threonine (%)	0.51	0.51	0.40	0.40	
Tryptophan (%)	0.19	0.19	0.14	0.14	
Oleic C18 1%	1.00	4.83	1.00	5.09	
Linoleic C18 2%	0.92	1.35	0.92	1.14	
T_unsaturated_%	2.04	6.52	2.00	6.36	
T saturated %	0.37	0.76	0.39	0.82	
Unsaturated:Saturated	5.47	8.55	5.07	7.79	
Net energy Kcal/Kg	2250.00	2524.00	2293.00	2575.00	
Metabolizable energy Kcal/Kg	3066.00	3340.00	3066.00	3348.00	
Total	100.00	100.00	100.00	100.00	

Total of 4161.09 Kcal of net energy; 2000.00 UI of vitamin A; 800.00 UI of vitamin D; 100.00

mg of vitamin E. ¹ Composition of basal diet for control and oil experimental diets, for growing (50 - 100 Kg) and finishing pig's (100-140 Kg) periods.

Table 2. Basal and final values of hemogram analysis of growing and finishing pigs, kept under heat-stress conditions (30°C - 32°C) according to the diets (control x oil) and regimen (*ad-libitum* x restriction) applied.

Tuestments	Items ¹							
Treatments	HT (%)	VB (fL)	LE, (µL)	LI (%)	NE (%)	N:L (µL)		
Basal values								
Control diet + ad-libitum	33.035	44.523	22694	49.353	47.647	1.002		
Oil diet + ad-libitum	33.082	44.311	24024	49.471	47.529	1.002		
Control diet + restriction	34.323	46.743	22924	46.412	50.235	1.168		
Oil diet + restriction	33.659	46.429	22624	48.412	48.471	1.021		
SEM	0.640	1.156	1090.470	1.733	1.701	0.084		
P-Value	0.580	0.965	0.458	0.589	0.630	0.385		
Final values								
Control diet + ad-libitum	38.892	46.601	17692	56.083	38.750	0.702		
Oil diet + ad-libitum	37.192	45.815	17817	59.000	36.583	0.633		
Control diet + restriction	37.746	52.430	17392	60.385	35.077	0.589		
Oil diet + restriction	37.912	50.672	19363	62.062	34.187	0.565		
SEM	0.842	1.319	896.760	1.346	1.353	0.036		
P-Value	0.321	0.741	0.357	0.679	0.671	0.590		
Basal vs Final values								
Basal	33.555^{b}	44.156 ^b	23949 ^a	48.412 ^b	48.471 ^a	1.049^{a}		
Final	37.784 ^a	47.509 ^a	19151 ^b	59.383 ^a	36.149 ^b	0.617^{b}		
SEM	0.437	1.106	864.860	0.778	0.761	0.038		
P-Value	0.000	0.002	0.000	0.000	0.000	0.000		

¹HT: hematocrit; VB: mean corpuscular volume; LE: leukocytes; LI: lymphocytes, NE: neutrophyls; N:L: neutrophil/lymphocyte ratio.

 $^{^2}$ Lysine sulphate supplied per Kg of the nucleus: 54.6% of L-lysine; 80% of crude protein; 0.11% of phosphorus.

^{ab}Different letters in the same column indicate statistical difference (P < 0.05);

SEM: The standard error of the mean;

Table 3. Frequency of observed behaviours (total number of 7080 observations made) in growing pigs kept under heat-stress conditions according to the period, diet (control x oil) and regimen (ad-libitum x restriction) applied.

Tuestments	Items¹								
Treatments	LL	LS	EM	EX	PΙ	NI	E	D	О
Control diet + ad-libitum	729ª	553	23	87 ^d	55	6	164	63	102°
Oil diet + <i>ad-libitum</i>	626^{b}	545	23	152°	49	12	156	62	$140^{\rm b}$
Control diet + restriction	315°	601	17	281 ^b	43	6	153	128	220 ^a
Oil diet + restriction	353°	636	24	286ª	30	11	119	112	198ª
P-Value	0.003	0.465	0.458	0.000	0.122	0.891	0.099	0.402	0.005
Period ²									
Morning	683 ^y	817 ^{xy}	36	256 ^y	76	15	204 ^y	144	211 ^y
Noon	898 ^x	687 ^y	13	128 ^z	42	8	56 ^z	107	179 ^y
Afternoon	442 ^z	831 ^x	38	422 ^x	59	12	332 ^x	114	270 ^x
P-Value	0.000	0.018	0.506	0.000	0.367	0.594	0.000	0.207	0.000

¹LL: lying laterally; LS: lying sternly; EM: Exploring enrichment material; EX: exploring; E: eating; D: drinking; IP: positive interaction; IN: negative interaction; O: others.

²Morning (08:00 h to 10:00 h); Noon (11:00 h to 15:00 h); Afternoon (16:00 h to 18:00 h). ^{ab}Different letters in the same column indicate statistical difference (P < 0.05) to the interaction diet and regimen applied.

^{xy}Different letters in the same column indicate statistical difference (P < 0.05) between periods.

Table 4. Performance, measurements of carcass and fatty acid profile of growing and finishing pigs kept under heat-stress conditions (30°C - 32°C) according to the diets (control x oil) and regimen (ad-libitum x restriction) applied.

Items ¹		Diet		Regimen ³				
Items	CD	OD	SEM	P-Value	AD	RE	SEM	P-Value
Growing phase								
BW (Kg)	51.01	50.75	1.56	0.869	51.13	50.63	1.56	0.747
ADG (Kg)	0.85	0.87	0.01	0.568	0.93^{x}	0.79^{y}	0.01	0.001
ADFI (Kg)	2.97^{a}	2.79^{b}	0.01	0.000	3.08^{x}	2.68^{y}	0.01	0.001
F:G	3.52^{a}	3.25^{b}	0.06	0.003	3.35	3.42	0.06	0.379
Finishing phase								
BW (Kg)	103.01	103.69	1.99	0.738	106.79 ^x	99.91 ^y	1.99	0.001
ADG (Kg)	0.61	0.57	0.02	0.163	0.67^{x}	0.52^{y}	0.02	0.001
ADFI (Kg)	3.58^{a}	3.21^{b}	0.04	0.000	3.58^{x}	3.22^{y}	0.04	0.001
F:G	6.19	5.88	0.24	0.367	5.48 ^y	6.60^{x}	0.24	0.002
Final BW (Kg)	130.90	129.69	1.84	0.640	136.92^{x}	123.68 ^y	1.84	0.001
Growing - Finishing								
ADG (Kg)	0.73	0.72	0.02	0.631	0.80^{x}	0.65^{y}	0.02	0.000
ADFI (Kg)	3.27^{a}	3.01^{b}	0.04	0.000	3.33^{x}	2.95^{y}	0.04	0.000
F:G	4.86	4.57	0.20	0.319	4.41 ^x	5.01 ^y	0.20	0.041
Carcass traits								
CW (Kg)	96.46	96.60	1.44	0.944	100.92^{x}	92.13 ^y	1.44	0.001
YC (%)	73.69^{b}	74.51a	0.27	0.019	73.72^{y}	74.48^{x}	0.27	0.029
LP (%)	28.33	28.60	1.16	0.870	23.85^{y}	33.09^{x}	1.16	0.001
Fatty acid profile								
C16:0 (%)	19.33 ^a	17.46 ^b	2.23	0.001	18.54	18.25	2.23	0.615
C17:0 (%)	0.28^{a}	0.18^{b}	0.14	0.045	0.18	0.28	0.14	0.053
C18:0 (%)	11.82a	9.59^{b}	1.41	0.000	10.83	10.59	1.41	0.498
C18:1n9 (%)	46.47^{b}	53.36 ^a	1.72	0.000	50.16	49.66	1.72	0.259
C18:2n6 (%)	11.83 ^a	11.32 ^b	1.00	0.050	11.35	11.80	1.00	0.082
C18:3n3(%)	0.84 ^a	0.66^{b}	0.56	0.000	0.73 ^y	0.77 ^x	0.03	0.042

¹ADG of average daily gain; ADFI average daily feed intake; F:G feed:gain ratio.

BW: body weight; CW: carcass weight; YC: Yield of carcass; LP: Leanness percentage;

C16:0: palmitic; C17:0: margaric; C18:0: stearic; C18:1n9: oleic; C18:2n6: linoleic; C18:3n3: linolenic; ²Diet: CD (diet containing 5% corn starch and 0% sunflower oil); OD (diet containing 0% corn starch and 5% sunflower oil).

³Regimen: AD (free access to feed); RE (feed management with a 10% restriction).

abDifferent letters in the same line indicate statistical difference (*P*<0.05) between diets; xyDifferent letters in the same line indicate statistical difference (*P*<0.05) between regimens;

SEM: The standard error of the mean;

Figure 1. Mean values of temperature (°C-black line) and humidity (%-grey line) found in the three rooms along the study per hour

(from 01:00 to 24:00h).



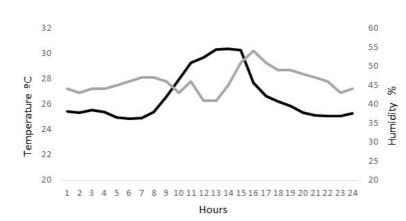


Figure 2. Percentage of pigs' dirtiness in the two regimens applied (ad-libitum and restricted) along the study. The mean values are composed of the analysis of 2376 observations during the experimental period. *The means differ by Chi-Square test (P < 0.0007) between regimens applied.

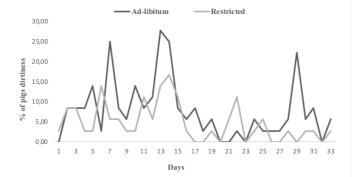


Figure 3. Percentage of pigs' dirtiness according to the diet fed

(control and oil diet) along the study. The mean values are composed

of the analysis of 2376 observations during the experimental period.

*The means differ by Chi-Square test (*P* < 0.0001) between diets

applied.

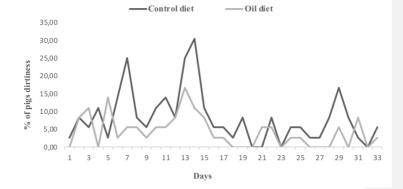


Figure 4. Percentage of pigs' dirtiness during the different periods of
 the study. The mean values are composed of the analysis of 2376
 observations during the experimental period. *The means differ by
 Chi-Square test (P < 0.0001).

