EFFECT OF GESTATIONAL AND LACTATIONAL EXPOSURE TO HEAT STRESS ON PERFORMANCE IN RABBITS

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Abstract: Reproductive performance is greatly affected by environmental factors such as temperature. Heat stress (HS) during pregnancy and lactation can influence not only foetal growth but also postnatal development of kits. The aim of this study was to test the effect of HS during gestation and lactation on postnatal growth till Spanish commercial liveweight. To investigate this, 32 primiparous non lactating rabbit does were exposed to 1 of 2 environmental treatments: high temperature (between 25 and 36°C, HS group; n=16) or thermoneutral conditions (between 14 and 20°C, TN group; n=16). Does were allowed to acclimate 30 d before the artificial insemination. At birth, kits were allocated into 4 groups: HS was only applied during gestation (G group; n=54); HS was applied during gestation and lactation period (GL group; n=85); HS was only applied during lactation period (L group; n=60); and TN was applied during gestation and lactation period (C group; n=77). All litters were kept under each experimental environment until weaning at day 30. Then, litters were moved to TN temperatures until slaughter at day 63. Compared with TN does, the HS does presented lower litter size (9.7 and 11.4; P<0.05), litter weight (503.0 vs. 630.5 g; P<0.05) and kit weight at birth (56.6 vs. 61.4 g; P<0.05), as well as a higher stillborn rate (25.4 vs. 9.9%; P<0.05). The kits from does subjected to HS during gestation (G group) had similar postnatal growth compared to offspring from does gestated in TN conditions (C group), whereas kits from does that experienced HS during gestation and lactation (GL group) and during their lactation (L group) presented decreased postnatal growth. Together, these results demonstrate that kits from does that underwent HS during gestation did not alter postnatal growth until Spanish commercial liveweight, whereas HS during lactation resulted in decreased postnatal growth.

Key Words: heat stress, growth performance, gestation, lactation, rabbit.

INTRODUCTION

Heat stress (HS) is known to alter livestock physiology, and reproduction is the first function to be impaired (Hansen, 2009). The most economically important reproductive performance traits of farm animals may be endangered by high environmental temperature (Bloemhof et al., 2008). Specifically, HS reduces growth, alters carcass quality and compromises efficiency, thus undermining the efforts by animal agriculture to produce high-quality protein for human consumption (Baumgard and Rhoads, 2013). The negative effects of HS will likely become more pronounced as climate models predict an increase in extreme summer temperatures for most farming areas (Luber and McGeehin, 2008).

European rabbit meat production is approximately 500 thousand tons, corresponding to a 30% share of world production (Petracci et al., 2009). However, rabbits account for the second highest number of animals slaughtered per year in the European Union-27, with 326,619×10³ head in 2010 (FAOSTAT, 2012). Most production is concentrated in the Mediterranean Region, where Italy is the leading producer, followed by Spain and France (FAOSTAT, 2009).
climate in these countries is warm to hot, with dry summers (in some areas the temperature can easily reach 35°C) and mild to cool, wet winters (de Lima et al., 2013). Nevertheless, the established thermal comfort zone for adult rabbits is from 15 to 21°C (Lebas et al., 1986; Marai et al., 2002; Verga et al., 2007).

The effect of high temperatures on rabbit doe performance has been studied in experiments carried out in summer (Méndez et al., 1986; Marai et al., 2002), using contemporaneous animals submitted to identical environmental and management conditions in a climatic chamber (Fernández-Carmona et al., 1995, 2003; Marco-Jiménez et al., 2013, 2014) or artificially under in vitro culture of oocytes and embryos (Makarevich et al., 2007). Rabbits are very sensitive to high temperatures because they have few functional sweat glands, limiting their ability to eliminate excess body heat (Maya-Soriano et al., 2015). However, they can adapt to adverse situations after exposure to HS conditions (de Lima et al., 2013). Nevertheless, overcoming the problems resulting from exposure to elevated ambient temperatures during pregnancy is more complicated (Hamid et al., 2012). Nonetheless, stimulus or insult acting during critical periods of foetal growth and development may result in developmental adaptations that permanently change the structure, physiology and metabolism of the offspring (Barker, 1995; Lau and Rogers, 2004; McMillen et al., 2004; Guilloteau and Waterland, 2005; Langley-Evans et al., 2005). This concept, named “foetal origins hypothesis”, has recently been the subject of several research works (Symeon et al., 2015). Previous studies show that upsetting factors (non-thermal) can permanently change growth (Foxcroft et al., 2006, 2009), post-absorptive metabolism (Chen et al., 2010; Pinney and Simmons, 2010) and body composition (Barker et al., 1993; Roseboom et al., 2006). However, the effects of maternal HS on postnatal growth in rabbit are unknown. In consequence, the extent to which in utero hyperthermia affects future animal performance is unknown (Johnson et al., 2015). Therefore, our aim was to determine the growth performance of rabbits exposed to differing in utero and postnatal environments, using contemporaneous animals in a climatic chamber in a realistic scenario resembling that to which populations at the Mediterranean coast are exposed in summer.

**MATERIALS AND METHODS**

Animals protocols followed the Ethical Principles of Animal Care published by Spanish Royal Decree 53/2013 and were approved by the Ethic Committees of the Universitat Politècnica de València. Animals were treated humanely and with regard for alleviation of suffering.

**Animals and experimental design**

The experiment was conducted at the Animal Science Department, Polytechnic University of Valencia (Valencia, Spain). Rabbits from a synthetic line selected by litter size at weaning (Estany et al., 1989) for 36 generations were used. Primiparous rabbit does (n=32) were housed in individual cages (700×500×320 mm) provided with a nest for litters from gestation day 28 in a conventional housing with a light-alternating cycle of 16 h of light and 8 h of darkness under controlled environmental conditions (average daily minimum and maximum temperatures of 14 and 20°C, respectively).

Females were randomly distributed into 2 different experimental groups: some under high environmental temperatures (HS group, ranging from 25 to 36°C, n=16) and the others under thermoneutral conditions (TN group, ranging from 14 to 20°C, n=16). Does were allowed to acclimate for 30 d before being artificially inseminated. The climatic chamber was equipped with a heating/cooling system which scheduled a sine function for the daily environmental temperature, with a minimum temperature of 25°C early in the morning and a maximum of 36°C in the afternoon, with uncontrolled humidity (details see García-Diego et al., 2011). This system ensured environmental stress based on temperature humidity index (THI). THI was calculated using the following formula: \( \text{THI} = T^\circ F - \left[0.55 - 0.55 \times \frac{\text{RH}}{100}\right] \times \left(\text{db}^\circ F - 58\right) \) (LPHSI, 1990), where T: temperature (°F), RH: Relative humidity and db the dry bulb temperature (Figure 1). The average THI was calculated from the maximum and minimum daily THI during the experimental period for TN and HS conditions.

In addition, infrared temperature measurements were recorded at day 12 post-insemination at 12:00 a.m, using the Flir i7 thermal imaging scanner with calibrated temperature interval from −20 up to 250°C. This camera registers the radiation temperature of the object from minimum focus distances of 0.6 m at a sensitivity of 0.1°C.
thermal image is reproduced on a 2.8" display. The sensing system of the camera is based on 140×140 Focal Plane Array uncooled microbolometer. Females were photographed individually inside the cages from a distance of 75 cm. The skin area was manually marked on the image and the software measured temperatures in the selected area (Figure 2).

At birth (considered Day 0), kits were weighed, sexed and microchipped for individual identification. Four groups were then formed: G (n=54): kits gestated under HS temperatures (25 to 36°C) then underwent lactation at TN temperatures (14 to 20°C). L (n=60): kits gestated under TN temperatures (14 to 20°C) then underwent lactation at HS temperatures (25 to 36°C). GL (n=85): kits underwent both gestation (30 d) and lactation (30 d) in HS (25 to 36°C). C (n=77): kits maintained in TN temperatures (14 to 20°C) throughout gestation and lactation (Figure 3).

Ten kits were randomised between different litters, matched for sex and weight if possible. All experimental manipulations involving the allocation of kits into their respective experimental groups were performed on Day 0.

**Figure 1:** Daily temperature humidity index under thermoneutral conditions (ranging from 14 to 20°C) and under heat stress conditions (ranging from 25 to 36°C).

**Figure 2:** Thermographic image of a pregnant female in the cage. (A) Females raised under thermoneutral conditions (ranging from 14 to 20°C). (B) Females raised under heat stress conditions (ranging from 25 to 36°C).
After weaning (30 d) independently in the previous experimental conditions, the rabbits were kept under TN temperatures (14 to 20°C) with a photoperiod of 16 h of light and fed a commercial diet ad libitum (15.5% crude protein, 2.3% ether extract, 17.2% crude fibre; dry matter basis) until day 63 (Spanish slaughter liveweight). Fresh water was always available.

Kindling and growth performance

At parturition, litter weight and size (live and stillborn kits) as well as the individual weight of the kits and sex were recorded. Body weights (g) were measured weekly thereafter, up to 63 d of age. In growing rabbits, the body weights (g) were measured weekly in 2 periods: lactation (0 to 30 d) and growth period (30 to 63 d). In addition, body weight gain (g/d) was calculated for both periods. Likewise, mortality rates during the lactation and growing period were recorded.

Statistical analysis

Litter size, litter weight and kit birth weight were analysed using a mixed model with ambient temperature treatment as a fixed effect. For litter weight analysis, litter size was also included in the model as a covariate. Mortality was analysed using a generalised linear model. The error was designated as having a binomial distribution and the Probit Link procedure was used. Binomial data were assigned a 1 if the kit died or a 0 if the kit was alive. The percentages of stillborn kits among groups were analysed with a chi-square test. The body weights of the offspring were analysed using a mixed model for repeated measures with treatment, time, sex and their interactions as fixed factors, as well as the doe as a random factor. The effect of offspring sex was not significant and was therefore excluded from the model. Multiple comparisons were performed using Bonferroni’s multiple range test and significance was set at 0.05. All results are presented as least square means (LSM) and standard error of the means (SEM). All statistical analyses were carried out using a commercially available software program (SPSS, 2002).
RESULTS

THI

The average daily maximum and minimum THI throughout the experimental period was 58.5 to 62.1 for TN and 72.8 to 86.7 for HS conditions.

Body temperatures

Rabbit does were exposed to daily variation from 14 to 20°C (TN conditions) and daily variation from 25 to 36°C (HS conditions), using a climatic chamber that was designed to produce a daily sinusoidal temperature curve (between 12 a.m. and 12 p.m.; from 25 to 36°C); detailed specifications may be found in García-Diego et al. (2011). Average skin temperatures under TN and HS temperatures at day 12 post-insemination were 20.3±0.8 and 32.7±0.9°C, for TN and HS conditions, respectively.

Reproductive traits

Litter size and stillborn kit rate were different between HS and TN conditions, with lower values in does submitted to HS conditions (P<0.05; Table 1). Regarding the stillborn kit rate, the value was higher in HS.

Growth performance

Prenatal HS reduced litter weight and kit birth weight (P<0.05. Table 1). At birth, kits were randomised by body weight (Table 1). No differences in HS and TN conditions during gestation were detected for liveweight and average daily gain in lactation and growing periods (Table 2).

However, regardless of conditions during gestation, liveweight and average daily gain decreased when kits were exposed to HS during lactation (L and GL groups, P<0.05. Table 2). Cumulative mortality during observed periods was not significant among groups (Table 3).

The growth curves of the four groups are shown in Figure 4. No significant differences were observed between C and G groups in the growth patterns. However, the growth curves of the L and GL groups were significantly different from those of the C and G groups (P<0.05).

Table 1: Effect of gestational exposure to heat stress temperatures on reproductive performance.

<table>
<thead>
<tr>
<th>Reproductive performance</th>
<th>TN</th>
<th>HS</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Litter size</td>
<td>11.4±0.7</td>
<td>9.6±0.6</td>
</tr>
<tr>
<td>Litter weight (g)</td>
<td>630.5±29.4</td>
<td>503±28.3</td>
</tr>
<tr>
<td>Kit birth weight (g)</td>
<td>61.4±0.9</td>
<td>56.6±1.1</td>
</tr>
<tr>
<td>Stillborn kit rate (%)</td>
<td>9.9</td>
<td>25.4</td>
</tr>
<tr>
<td>Ratio of sex (Male/Female)</td>
<td>0.97</td>
<td>0.87</td>
</tr>
</tbody>
</table>

TN: rabbit does kept under thermoneutral temperatures (between 14 and 20°C) during pregnancy; HS: rabbit does kept under high temperatures (between 25 and 36°C) during pregnancy. n: initial number of litters. *Values within a row with different superscripts differ significantly at P<0.05.

Table 2: Effect of gestational and lactational heat stress on rabbit growth performance.

<table>
<thead>
<tr>
<th>Traits</th>
<th>C</th>
<th>G</th>
<th>L</th>
<th>GL</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>77</td>
<td>54</td>
<td>60</td>
<td>85</td>
</tr>
<tr>
<td>Body weight, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 0 d</td>
<td>57.6±1.3</td>
<td>55.6±1.6</td>
<td>57.4±1.4</td>
<td>59.0±1.2</td>
</tr>
<tr>
<td>at 30 d</td>
<td>454.7±10.2</td>
<td>481.2±12.4</td>
<td>390.6±11.4</td>
<td>422.1±9.6</td>
</tr>
<tr>
<td>at 63 d</td>
<td>1857±28</td>
<td>1924±33</td>
<td>1684±31</td>
<td>1729±26</td>
</tr>
<tr>
<td>Weight gain, g/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-30 d</td>
<td>14.3±0.4</td>
<td>15.6±0.5</td>
<td>12.0±0.4</td>
<td>12.9±0.4</td>
</tr>
<tr>
<td>30-63 d</td>
<td>39.9±0.6</td>
<td>40.9±0.8</td>
<td>36.8±0.7</td>
<td>37.2±0.6</td>
</tr>
</tbody>
</table>

The meaning of C, G, L and GL in Figure 3. n: number of animals.

abc Values within a row with different superscripts differ significantly at P<0.05.
DISCUSSION

HS affects the reproductive traits, male and female gamete formation and function, embryonic development and foetal growth (Hansen, 2009). Taken together with our data, it appears that HS applied during gestation altered kindling performance of does without implications for offspring postnatal growth. Nevertheless, HS applied during lactation negatively affected the postnatal growth until Spanish commercial liveweight (63 d). Essentially, the exposure of does to gestational HS had a negative impact on litter size, litter weight, kit birth weight and stillborn rate, in line with a number of previous studies (Marai et al., 2002; Fernández-Carmona et al., 2003; Hansen et al., 2009; Tusell et al., 2011; Hamid et al., 2012; Marco-Jiménez et al., 2012, 2013). In comparison, far fewer works have focused on HS during gestation and the potential effect on the offspring’s postnatal growth has not been established in rabbit. Studies published to date have reported that stressful environmental experiences during pregnancy contribute to the foetal programming of developmental plasticity and/or enhance adjustment to the postnatal environment (Pluess and Belsky, 2011). Although there is clear evidence of effects of early programming by nutrition and endocrine disruptors on adult reproductive physiology, less is known about thermal stress (Rhind et al., 2001). Specifically, HS experienced during gestation can result in physiological anomalies that extend into post-gestational life (Shiota and Kayamura, 1989; Chen et al., 2010). In this way, Boddicker et al. (2014) suggest that HS during the first half of gestation may have programmed the kits, resulting in an altered postnatal offspring growth and development. In the present study, we demonstrate that kits from does that experienced HS during gestation did not alter postnatal growth until Spanish commercial liveweight (63 d). To the best of our knowledge, the impact of in utero HS on offspring with respect to

Table 3: Effect of gestational and lactational heat stress on mortality rate during growth performance.

<table>
<thead>
<tr>
<th>Period</th>
<th>Experimental groups</th>
<th>C</th>
<th>G</th>
<th>L</th>
<th>GL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactation</td>
<td></td>
<td>16.0±3.6</td>
<td>18.0±4.5</td>
<td>20.0±4.3</td>
<td>12.0±3.1</td>
</tr>
<tr>
<td>Growing</td>
<td></td>
<td>11.0±3.4</td>
<td>8.0±3.6</td>
<td>16.0±4.3</td>
<td>13.0±3.4</td>
</tr>
<tr>
<td>Global</td>
<td></td>
<td>25.0±4.2</td>
<td>25.0±5.1</td>
<td>30.0±4.9</td>
<td>24.0±4.0</td>
</tr>
</tbody>
</table>

The meaning of C, G, L and GL see in Figure 3.

Figure 4: Effect of gestational and lactation exposure to heat stress on body weight (g) of rabbit offspring from 0 to 63 d of age. The meaning of C, G, L and GL see in Figure 3. C: ●; G: ○; L: △; GL: ▲. Values at a same age with asterisk differ significantly at P<0.05.
growth has not been previously reported in rabbits. Nevertheless, HS applied during lactation resulted in decreased growth and final liveweight with the consequent impact on production, which was consistent with earlier studies in this field (Renaudeau et al., 2012; Baumgard and Rhoads, 2013; Zeferino et al., 2013). Studies published to date have considered that rabbit growth is mainly dependent on two factors, birth weight and litter size, as the kit spends half of its life with the mother (gestation and lactation, Poigner et al., 2000). On this subject, in the present work litter size was artificially equalised to 10 kits per doe at parturition day, matched for body weight. In fact, there was no significant difference between the 4 experimental groups in the mean initial body weight after cross-fostering (Table 2). Considering this evidence, we observed that HS during lactation affected postnatal growth, regardless of HS experienced by the does during gestation. In this respect, gestational and lactational HS does had low feed intake, depressed metabolic rate and low milk yield (Maertens and De Groote, 1990; Fernández-Carmona et al., 1995, 2003; Pascual et al., 1999; Marai et al., 2002), resulting in less feed for the growing kits (El Saidy et al., 2016). In rabbit, it has been established that growth performance of kits up to 21 d of age mainly depends on their mother’s milk production and composition (Maertens et al., 2006), especially during the 3rd wk of lactation (Szendró et al., 1998). Moreover, HS also decreased feed intake and depressed the performance of growing rabbits (Marai et al., 2002; Zeferino et al., 2011, 2013). Consequently, HS depressed slaughter weight at a fixed market age as well as the commercial and reference carcass weights (Zeferino et al., 2011). However, in the present work, all the animals were exposed to TN conditions during the growing period (30 to 63 d). In spite of this, we obtained the same results; kits that underwent HS during lactation resulted in decreased growth and final liveweight, regardless of HS experienced by their does during gestation. In this regard, it is worth mentioning that a small reduction would have been offset by compensatory growth, a phenomenon well documented in rabbits when ad libitum feeding is applied during the growing period (Ledin, 1984; Dalle Zotte et al., 2005; Gidenne et al., 2009). However, during growth period the expectable compensatory growth was not observed for kits that experienced lactational HS, although at the end of the period, the liveweight of the kits was within the compensating limit (less than 20%, Ledin, 1984), suggesting the detrimental effect of lactational HS on slaughtered liveweight and the economic return in rabbit meat production.

CONCLUSIONS

In summary, our results demonstrated that does exposure to HS during gestation had a negative impact on litter size, litter weight, kit birth weight and stillborn rate. Nevertheless, kits from does that experienced HS during gestation did not have any implications on postnatal growth till Spanish commercial liveweight. Instead, regardless of HS during gestation, HS during lactation resulted in decreased Spanish commercial liveweight. Additional research into how HS affects carcass and meat quality traits will be done. Therefore, producers choosing to alleviate HS should focus their environmental measures during lactation to promote optimum growth performance of rabbits.

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