EFFECT OF HIGH TEMPERATURE ON BLOOD LYMPHOCYTE POPULATIONS IN TWO DIFFERENT GENETIC RABBIT LINES.

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ABSTRACT

Stress is generally considered an important factor affecting the productivity of farm animals, as it is able to suppress the immune system and may lead to an increase in the occurrence of diseases in the presence of pathogens. In fact, it has been described that chronic heat stress can affect negatively the immune response in several production animal species, since they are genetically different and they show different ability to deal successfully with environmental challenges. Therefore, rabbits from different genetic lines subjected to heat stress also develop different immune system responses. The present study aimed to determine and compare the ability of rabbit does from two different genetic lines selected by different productive criteria (one selected for productive longevity (LP) and one for litter size at weaning (V, using two generations of the same line: V16, V36), to deal with heat stress in terms of their abovementioned selection criteria. The results pointed out that animals from the LP line showed a higher number of total lymphocytes (in blood). Furthermore, while the animals from line LP were able to modulate their immune response based on the total numbers of lymphocytes throughout the gestation-lactation cycle, animals from lines V16 and V36 did not show such ability to adapt to different situations, since the total cell number remained constant or decreased, reaching very low values in the second parturition. These results may suggest that, regarding immune response to thermal challenges, selecting by prolificacy criteria may have a negative impact on breeding rabbits.

***Key words*:** rabbit; lymphocyte populations; heat stress; genetic origin; litter size, longevity.

INTRODUCTION

The continuous rise in temperatures is clearly affecting in the Mediterranean area, with an extension of the summer season (Coma *et al*., 2009). As well as genetics and management, temperature also plays an important role on the animals welfare and health. The relationship between high temperature and mortality is well known in people living in urban areas, but it has been poorly investigated in livestock (Crescio *et al*., 2010). These changes have a major and obvious impact on animals and their physiological activity; in fact, it has been described that high environmental temperatures produce an adverse effect mainly on reproductive processes (Sirotkin, 2010) and on productive traits (do Amaral *et al*., 2011).

Although much of the genetic variation is caused by differences in ability to minimize hyperthermia (Hammond, 1993), there may also be genetic differences in cellular responses to increased temperature (Kamwanja *et al*., 1994). It has been described in several species that genetically different animals show different responses to environmental heat stress (porcine: Zumbach *et al*., 2008; bovine: Ravagnolo and Misztal, 2002). The effect of heat environmental conditions on rabbits immune system response have been extensively discussed in the last two decades (Marai *et al*., 2002), showing how different selection lines could have different response under stress conditions (Theilgaard *et al*., 2009).
On the other hand in some species, several studies have shown the influential effects of the thermal stress on immune system, showing how the thymus weight decreased and circulating T lymphocyte counts declined (poultry: Guo and Gu, 1988; and sheep: Odongo *et al*., 2006). Others studies, also reported that heat stress directly affects the immune system cells, decreasing the number of viable cells (Kamwanja *et al*., 1994) and

the number of receptors on the immune cells surface (Kappel *et al*., 1991), reducing the proliferative capacity of lymphocyte (do Amaral *et al*., 2010) and the neutrophil function (do Amaral *et al*., 2011); inhibiting the differentiation of B lymphocytes into antibody-secreting cells (Franci *et al*., 1996a) as well as decreasing immunoglobulin and cytokines production (do Amaral *et al*., 2010) and increasing in heat-shock proteins synthesis by lymphocytes (Franci *et al*., 1996b).

The aim of the present study was to determine and compare the ability of rabbit does, from two different lines of breeding rabbits (one selected for longevity productive and one for litter size at weaning), to overcome thermal challenges.

MATERIALS AND METHODS

Animals and experimental procedure

A total of 72 female rabbits of 3 genetic types were used in the experimental. All rabbit females were housed in a conventional housing (with light alternating cycle of 16 light hours and 8 dark hours, and under controlled environmental conditions: average daily minimum and maximum temperature of 14 and 20ºC, respectively), using individual cages (700×500×320 mm) provided with a nest for litter from 28th day of gestation. Until first parturition all the rabbit does received a rearing diet ad libitum (9 MJ of digestible energy (DE) and 133 g of digestible protein (DP) per kg dry matter (DM)). From this moment, females and their litter were fed the same diet for lactating rabbit does (11.5 MJ DE and 120 g DP per kg DM) provided *ad libitum* until the end of the experiment (2nd parturition).

The LP line was newly founded by selection of females with longevity and productive characteristics (Sánchez *et al*., 2008), while the V line was selected for litter size at weaning. Animals from two generations of V line (16th and 36th) were used in this study. At first partum day, animals from the 3 groups (LP, V16 and V36) were randomly distributed in two different experimental housing systems: CH (where 12 females of each group were maintained in the conventional housing at average daily minimum and maximum of 14 and 20ºC, respectively) and CC (12 females of each group were housed in a climatic chamber, where animals were maintained with a sinusoidal daily curve from 25 to 36ºC). The climatic chamber was equipped with a heating/cooling system, which allowed scheduling a sine function for daily environmental temperature of the sale, with minimum of 25ºC at early morning and maximum of 36ºC at afternoon. This system allowed ensuring an environmental stress as temperature was up 28ºC during the 65% of the day (see technical details at García-Diego *et al*., 2011).

Does were artificially inseminated (AI) at 11 days post-partum (dpp) and successive inseminations were carried out every 21 days, when necessary. Litters were standardized at birth to 9-10 kits and weaned at 28 dpp.

The Committee of Ethics and Animal Welfare of the Universidad Politécnica de Valencia approved this study. All the animals were handled according to the principles of animal care published by Spanish Royal Decree 1201/2005 (BOE, 2005; BOE = Official Spanish State Gazette).

Blood samples of the females were taken at first parturition (start of the environmental challenge), 4 dpp (after a short-exposure to the environmental challenge), 10 dpp (close AI and maximum body condition during lactation), and second parturition (end of the experiment). All the blood samples were drawn from the median artery of the ear using vacuum tubes with EDTA. Diurnal variations in haematological parameters were minimised by collecting blood at approximately the same time (9:00–11:00 h).

Flow cytometry analysis

Blood samples were processed 1 hour after sampling. Before performing the flow cytometry studies, a white blood cells (WBC) count was determined using a haematology analyzer (MEK-6410, Nihon Kohden, Japan). To obtain the leukocyte formula, blood films were made from each sample and stained with May-Grünwald stain-Giemsa.

Flow cytometry analysis was carried out as previously described (Guerrero *et al*., 2011). Primary monoclonal antibodies used are shown in the table 1. As secondary antibodies has been used the Goat anti-mouse IgM: R-Phycoerythrin –human adsorbed- [AbD Serotec]).

Table 1. Monoclonal antibodies used in this study.

Monoclonal antibodies	Isotype	Specificity	Cell labeling	Clone	Company
Mouse anti-human CD14: FITC	IgG2a	CD14	Monocytes and granulocytes	TÜK 4	AbD Serotec
Mouse anti-rabbit α-CD45	IgM	CD45	All leucocytes	ISC76A	VMRD, Inc.

Statistical analysis

To analyse the evolution of lymphocyte populations in the blood of rabbit does, a mixed model (PROC MIXED; Statistical Analysis System, 2002) was used, according to a repeated measures design that takes into account the variation between animals and covariation within them. The model included the genetic type (LP, V16 and V36), the housing (CC or CH), the control day (1st parturition, 4 dpp, 10 dpp and 2nd partum), and their interactions as fixed effects.

RESULTS AND DISCUSSION

The immune cells of different animal species are affected by high temperatures (mice: Won and Lin, 1995, bovine: Kamwanja *et al*., 1994). Franci *et al*. (1996a) reported how thermal stress treatments diminished the capacity of rabbits' peripheral blood mononuclear cells to proliferate and inhibit the differentiation of B-lymphocytes in antibody-secreting cells.

The genetic origin of rabbit does may have affected the number of total lymphocytes in blood (P=0,0152). Differences in the number of lymphocytes have been found between genetic lines of chickens (Cheeseman *et al*., 2004) and between breeds of pigs (Clapperton *et al*., 2005). Moreover, it has been proposed that these differences may be implied in resistance to infection by a wide range of pathogens and subsequent disease effects. However, as far as the authors are aware, no such information is available for rabbits.

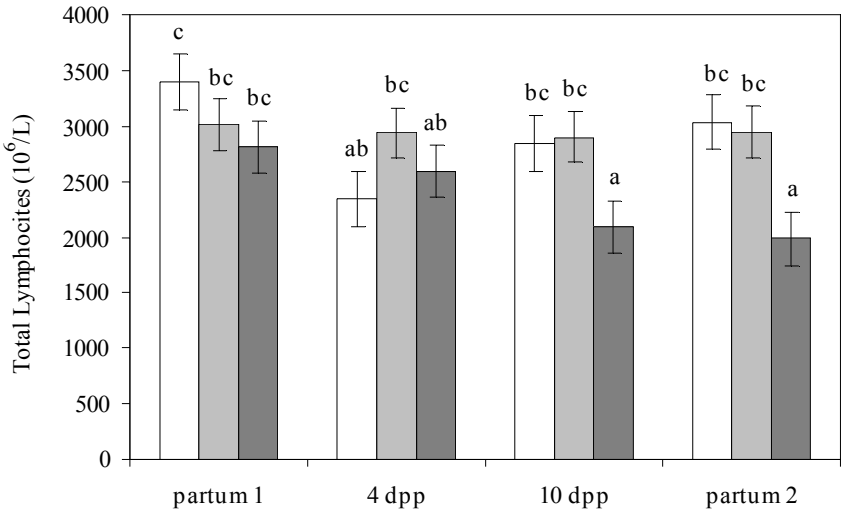

Figure 1. Effect of genetic type (LP □, V16 ■ and V36 ■) on the evolution of total lymphocytes (×10^6/L) in peripheral blood of primiparous rabbit does. [a,b,c] Least square means not sharing the same superscript are significantly different at $P<0.05$.

In this study, the V36 line showed a lower number of total lymphocytes than V16 and LP lines, mainly related with a decrease of this cellular population during the studied period, mainly at 10 dpp and at 2nd parturition (Figure 1). The heat stress also differently affected the total lymphocyte counts of the compared genetic lines as the differences observed under conventional housing between the V36 and V16 lines and as the differences observed at the second partum, so we may hypothesise that selection by litter size from lines V16 to V36 might have some negative effect on the immune function. This difference was observed either in the animals from conventional housing as from climatic chamber (Figure 2). Risk of culling in rabbit does reaches its peak in the two first lactations, especially at the end of pregnancy (Rosell and de la Fuente, 2009); consequently, the possible relationship of the differences found in lymphocyte counts and the culling rate (through illness or death) due to selection by litter size at weaning deserves further research.

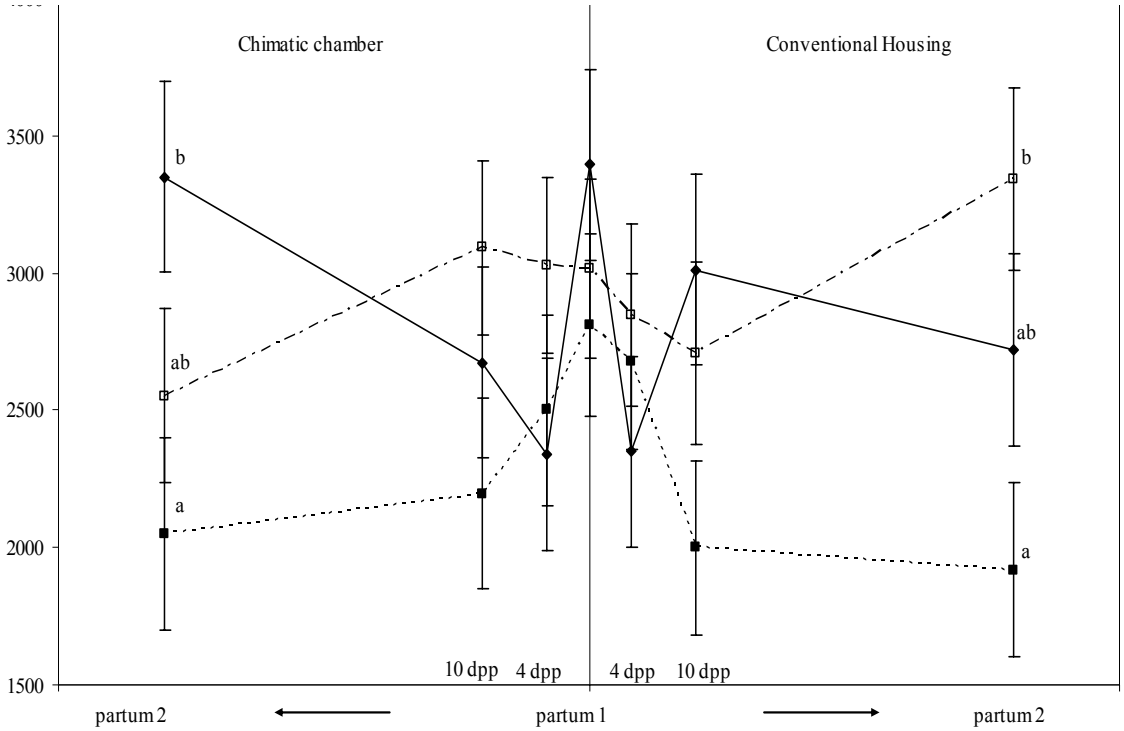

Figure 2. Effect of genetic type (□ LP, □·□V16 and ···V36) on the evolution of total lymphocytes ($\times 10^6$/L) in peripheral blood of primiparous rabbit does in different housing (climatic chamber and conventional housing). [a,b,c] Least square means not sharing the same superscript at the same housing and control are significantly different at P<0.05. dpp: days post 1st partum significantly different at P<0.05. dpp: days post 1st partum.

The animals from line LP modulated their response, diminishing the number of lymphocytes at 4 dpp and being increased later, whereas the line V16 is kept constant (Fig. 1). It has been reported that only the rabbit does with less wear during the gestation-lactation cycle were able to adjust their number of lymphocytes. Previous studies have demonstrated a major robustness of the LP rabbits in comparison with animals from V line, showing a higher efficiency in the utilization of their natural reserves for to recover successfully from environmental and productive challenges (Theilgaard *et al*., 2007). Guerrero *et al*. (2011) reported that rabbit does with less physiological wear because of shorter lactations may be more capable of modifying their number of lymphocytes throughout the productive cycle in a less body condition-dependent way; thus, the differences observed in the current study may relate with the immune system being more capable to adapt to the productive cycle in LP than in V36 rabbit does under normal favourable conditions. In fact, the evolution of the number of lymphocytes between LP and V16 lines was inverse in both chambers, mainly at 2nd parturition. While in the conventional housing the line V16 had the highest counts of total lymphocytes, after the challenge, it was the line LP the one that showed the highest counts of lymphocytes, especially evident if compared with the line V36 (Fig. 2). So these results could be related with a major capacity of adaptation of the immune system in the LP rabbit does during the productive cycle than V rabbits.

CONCLUSIONS

In conclusion, these results might indicate that selection for litter size at weaning (V lines) during so many generations might have affected the capacity of response and adaptation of the immune system. The utilization of LP rabbits in commercial rabbitries would increase the robustness of the animals, allowing to improve the productivity without affect the immune system, probably linked to a better capacity of resource management.

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