

**UNIVERSIDAD POLITÉCNICA DE MADRID**

**ESCUELA TÉCNICA SUPERIOR DE INGENIEROS AGRÓNOMOS**

**OPTIMIZING RABBIT PRODUCTION UNDER HEAT STRESS:  
CAGE DENSITY AND CAGE SIZE DURING FATTENING, AND  
BREEDING SYSTEM AND WATER QUALITY ON RABBIT DOES**

**TESIS DOCTORAL**

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Ingeniero Agrónomo**

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**2012**

***A la vida y al reto evolutivo que en ella implica  
lanzarse a lo desconocido...***

***A los maestros y guías en este reto...***

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## ***ABBREVIATIONS LIST***

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ADF, acid digestible fiber.  
ADL, acid digestible lignin.  
AI, artificial insemination.  
AIC, Akaike information criterion  
AR(1), autoregressive order one  
BCS, body condition score.  
BIA, bio-electrical impedance.  
BIC, bayesian information criterion  
BW, body weight.  
°C, Celsius degree.  
C, control.  
CCW, chilled carcass weight.  
CFU, colony-forming units.  
cm, centimeter.  
cm<sup>2</sup>, square centimeter.  
CP, crude protein.  
CS, covariance structure.  
d, days.  
db°C, bulb temperature in Celsius degree.  
DM, dry matter.  
dS/m, deciSiemens per meter.  
E, extensive.  
e.g., for example.  
*et al.*, and  
collaborators.  
g, grams.  
h, hour.  
HCW, hot carcass weight.  
kg, kilogram.

kJ, kilojoules.  
m, meter.  
m<sup>2</sup>, square meter.  
max., maximum.  
meq, milliequivalents.  
MJ, megajoule.  
ml, milliliter.  
MPN, most probable number.  
n, number of observations.  
NB, rabbit doe that does not deliver.  
NDF, neutral detergent fiber.  
NS, non significant.  
NTU, nephelometric turbidity units.  
NW, rabbit doe that does not weaned.  
P, probability.  
ppm, part per million.  
Q, quadratic.  
RH, relative humidity.  
rsd, relative standard deviation.  
s.d., standard deviation.  
s.e., standard error.  
SCN, suprachiasmatic nuclei.  
SEM, standard error of the mean.  
THI, temperature-humidity index.  
TOEBEC, total body electrical conductivity.  
vs., versus.  
W, rabbit doe that weaned.

***SUMMARY***

## SUMMARY

The aim of this work was to evaluate different management strategies to optimize rabbit production under chronic heat stress. To achieve it, three trials were conducted. In the first trial, to find the optimal cage density in tropical very dry forest condition, were measured growth performance, mortality rate, injured animals and carcass performance over an initial population of 300 cross-breed rabbits of New Zealand, California, Butterfly, Dutch and Satin, weaned at 30 days ( $535 \pm 8$  g, standard error). Treatments evaluated were: 6, 12, 18 and 24 rabbits/m<sup>2</sup> (3, 6, 9 and 12 rabbits/cage, respectively, each cage of 0.5 m<sup>2</sup>). The maximal temperature-humidity index indicated a severe heat stress from weaning to 2.2 kg body weight (experimental time). At the end of experimental period 10, 20, 30 and 30 rabbits from the treatments of 6, 12, 18 and 24 rabbits/m<sup>2</sup>, respectively, were slaughtered and carcass performance recorded. Average daily gain and feed intake decreased by  $0.31 \pm 0.070$  and  $1.20 \pm 0.25$  g, respectively, per each unit that the density increased at the beginning of the experiment ( $P = 0.001$ ). It increased the length of the fattening period by  $0.91 \pm 0.16$  d ( $P = 0.001$ ) per each unit of increment of density. However, rabbit production (kg/m<sup>2</sup>) increased linear and quadratically with the density ( $P < 0.008$ ). Animals housed at the highest density compared to the lower one tended to show a higher incidence of ringworm (68.9 vs 39.4%;  $P = 0.075$ ), injured animals (16.8 vs 3.03%;  $P = 0.12$ ) and mortality (20.5 vs 9.63%;  $P = 0.043$ ). The proportion of scapular fat ( $P = 0.042$ ) increased linearly with increasing levels of density. Increasing density reduced linearly dorsal length ( $P = 0.001$ ), and reduced linear and quadratically drip loss percentage ( $P = 0.097$  and  $0.018$ , respectively). In the second trial, 46 nulliparous rabbit does (23 clipped and 23 unclipped) with a BW of  $3.67 \pm 0.05$  kg (s.e.) were used to evaluate heat stress and circadian rhythms comparing unclipped and clipped rabbit does, and to study if a more extensive breeding system increase litters performance at weaning without impairing rabbit doe performance,. Rectal temperature, feed and water

intake were recorded for 24 h. Rabbit does were mated 7 d after circadian measurements, and randomly assigned to two breeding systems. Control (C): mated at 14 d after parturition + litter weaned at 35 d of age. Extensive (E): mate at 21 after parturition + litter weaned at 42 d of age. The first three cycles were evaluated concerning to rabbit doe and litter performance. Two hundred twenty eight weaned rabbits, were divided into two cage sizes: 0.5 and 0.25 m<sup>2</sup> with same density (16 rabbit/m<sup>2</sup>) and growing performance was recorded. Farm and rectal temperatures were minimal and feed and water intake maximal during the night ( $P < 0.001$ ). Unclipped rabbit does showed higher rectal temperature ( $P = 0.045$ ) and lower feed intake respect to clipped does ( $P = 0.019$ ) which suggest a lower heat stress in the latter. Kits weaned per litter was reduced by 33% ( $P=0.038$ ) in C group. This reduction was more important in the 2<sup>nd</sup> and 3<sup>rd</sup> cycles compared to the first ( $P \leq 0.054$ ). Rabbit doe feed efficiency tended to decrease in E respect C group ( $P = 0.093$ ), whereas it was impaired from the first to the third cycle by 48% ( $P = 0.014$ ). Growing rabbits from the E group were heavier at weaning (by 38%.  $P < 0.001$ ), showed a higher feed intake (+7.4%) and lower feed efficiency (-8.4%) throughout the fattening period ( $P \leq 0.056$ ) respect to C group. Cage size had minor influence in growing performance. In the third trial, forty five non pregnant and non lactating rabbit does (21 nulliparous and 24 multiparous) were assigned randomly to farm water and to potable water to study if a water quality improvement can affect positively rabbit doe response to heat stress during pregnancy and lactation. A transponder was implanted in each animal to record subcutaneous temperature at 07:30 and 14:30 h. Experimental period extended from pregnancy (with no lactation) to the next lactation (until day 28). Body temperature and milk production were recorded daily, and body condition, feed and water intake weekly. Water quality did not affect any trait ( $P \geq 0.15$ ). Pregnant rabbit does were classified as does that weaned (W: 47%), not weaned (NW: 44%) or those pregnant that did not deliver (NB: 9%). Body temperature and feed intake decreased during pregnancy ( $P \leq 0.031$ ), but water intake remained constant. In this period body temperature decreased with metabolic weight ( $P \leq 0.009$ ). In W and NW does,

from mating to birth energy and protein balance impaired ( $P \leq 0.011$ ). Body temperature of W does tended to be the lowest ( $P \leq 0.090$ ). Pregnancy length and total number of kits born tended to be longer and higher in NW than in W does ( $P = 0.10$  and  $0.053$ , respectively). Kit mortality at birth and from birth to 14 d of lactation was high, being worse for NW than for W does (97 *vs.* 40%;  $P < 0.001$ ). Body temperature during lactation was maximal at day 12, and milk production increased it ( $P \leq 0.025$ ). . In conclusion, in our heat stress conditions densities higher than 18 rabbits/m<sup>2</sup> (34 kg/m<sup>2</sup>) at the end of fattening, are not recommended despite cage size, gestation and lactation productivity impaired not only when lactation is extended and along successive reproductive cycles but also due to a reduced embryo/kit survival and finally water quality improvement did not attenuate negative effect of heat stress.

***RESUMEN***

## RESUMEN

El propósito de éste trabajo fue evaluar diferentes estrategias de manejo para optimizar la producción de conejos bajo estrés térmico. Para lo cual se desarrollaron tres experimentos. En el primer experimento, para encontrar el número óptimo de gazapos por m<sup>2</sup> de jaula durante el cebo en condiciones de bosque muy seco tropical, se estudiaron los rendimientos durante el cebo, mortalidad, animales lesionados y rendimiento de la canal sobre una población inicial de 300 conejos mestizos de Nueva Zelanda, California, Mariposa, Holandés y Satin, destetados a los 30 días de edad ( $535 \pm 8\text{g}$ , error estándar). Los tratamientos evaluados fueron: 6, 12, 18 y 24 conejos/m<sup>2</sup> (3, 6, 9 y 12 conejos/jaula, respectivamente, en jaulas de 0.5 m<sup>2</sup>). Durante el período experimental (destete a 2.2 kg de peso vivo), se observaron valores de THI correspondientes con un estrés térmico severo (THI max. De 31 a 35). Al final del período experimental, 10, 20, 30, y 30 conejos de los tratamientos con densidades de 6, 12, 18 y 24 conejos/m<sup>2</sup>, respectivamente, fueron sacrificados y su canal fue valorada. El promedio de la ganancia diaria y el consumo de alimento disminuyeron en  $0.31 \pm 0.070$  y  $1.20 \pm 0.25$  g, respectivamente, por cada unidad de incremento en la densidad al inicio del experimento ( $P=0.001$ ). Esto alargó el período de engorde en  $0.91 \pm 0.16$  d ( $P=0.001$ ) por cada unidad de incremento de la densidad. Sin embargo, la producción de conejos (kg/m<sup>2</sup>) aumentó lineal y cuadráticamente con la densidad ( $P<0.008$ ). Los animales alojados en las mayores densidades en comparación con el resto tendieron a mostrar una mayor incidencia de tiña ( $68.9$  vs  $39.4\%$ ;  $P=0.075$ ), de cantidad de animales heridos ( $16.8$  vs  $3.03\%$ ;  $P=0.12$ ), así como de mortalidad ( $20.5$  vs  $9.63\%$ ;  $P=0.043$ ). El aumento en la densidad aumentó linealmente la proporción de grasa escapular ( $P=0.042$ ) y redujo linealmente la longitud dorsal ( $P=0.001$ ), y lineal y cuadráticamente el porcentaje de pérdida por goteo ( $P=0.018$ ). En el segundo experimento, 46 conejas nulliparas (23 rasuradas y 23 no rasuradas) con un peso vivo de  $3.67 \pm 0.05$  kg (e.e.) fueron usadas para evaluar el estrés

térmico y los ritmos circadianos comparando conejas rasuradas o no, y estudiar si un sistema de crianza más extensivo mejora el desempeño de la camada al destete sin perjudicar la productividad de la coneja. Durante 24 h se midió la temperatura rectal, consumo de alimento y de agua. Las conejas fueron montadas 7 días después, y distribuidas en dos sistemas de crianza. El control (C): monta a 14 días posparto y destete a 35 d de edad. El extensivo (E): monta a 21 días posparto y destete a 42 d de edad. Se controló la productividad de la coneja y la camada durante los tres primeros ciclos. Doscientos veintiocho gazapos fueron distribuidos en dos tamaños de jaulas (0.5 y 0.25 m<sup>2</sup>) con la misma densidad (16 conejos/m<sup>2</sup>) y se controlaron sus rendimientos productivos. Durante la noche se observaron los valores mínimos para la temperatura ambiental y rectal, y los máximos para consumo de alimento y agua ( $P < 0.001$ ). Las conejas no rasuradas mostraron mayor temperatura rectal ( $P = 0.045$ ) y menores valores de consumo de alimento con respecto a las conejas rasuradas ( $P = 0.019$ ), lo que sugiere un menor estrés térmico en las últimas. El número de gazapos destetados por camada se redujo en 33% ( $P = 0.038$ ) en el grupo C. Este comportamiento se acentuó en el 2<sup>do</sup> y 3<sup>er</sup> ciclo en comparación con el primero ( $P \leq 0.054$ ). La eficiencia alimenticia de las conejas tendió a disminuir en el grupo E con respecto al grupo C ( $P = 0.093$ ), dicha tendencia se acentúa del primer al tercer ciclo en un 48% ( $P = 0.014$ ). Los gazapos en fase de crecimiento provenientes del grupo E fueron más pesados al momento del destete (en 38%  $P < 0.001$ ), mostrando un mayor consumo de alimento (+7.4%) y menor eficiencia alimenticia (-8.4%) a lo largo del engorde ( $P \leq 0.056$ ) con respecto al grupo C. El tamaño de la jaula tuvo una mínima influencia en el comportamiento durante el crecimiento de éstos gazapos. En el tercer experimento, cuarenta y cinco conejas no gestantes ni lactantes (21 nulíparas y 24 múltiparas) se les asignó al azar agua dos tipos de agua: común de la granja y agua potable, con el fin de estudiar si una mejora en la calidad del agua puede afectar positivamente la respuesta de la coneja al estrés térmico durante la gestación y la lactancia. Se les implantó un transponder para registrar la temperatura subcutánea a las 7:30 y a las 14:30 h. El período experimental se extendió desde la gestación (sin

lactancia) hasta la lactancia consecutiva (hasta los 28 días). La temperatura corporal y la producción de leche se controlaron diariamente, y la condición corporal, consumo de agua y alimento, semanalmente. La calidad del agua no afectó a ninguna variable ( $P \geq 0.15$ ). Las conejas preñadas fueron clasificadas como conejas que destetaron (W: 47%), que no destetaron (NW:44%) o aquellas que no parieron (NB: 9%). La temperatura corporal y consumo de alimento disminuyeron durante la gestación ( $P \leq 0.031$ ), mientras que el consumo de agua se mantuvo constante. La temperatura corporal descendió con el peso metabólico durante la gestación ( $P \leq 0.009$ ). El balance de energía y proteína disminuyó desde la monta al parto para las conejas W y NW ( $P \leq 0.011$ ). Durante la gestación la temperatura corporal tendió a ser menor en las conejas W ( $P \leq 0.090$ ). La longitud de la gestación y el número total de gazapos nacidos tendieron a ser mayores en conejas NW que en conejas W ( $P = 0.10$  y  $0.053$ , respectivamente). La mortalidad de los gazapos al parto y del parto a los 14 días de lactancia fue alta, siendo peor para las conejas NW que para las W (97 vs 40%;  $P < 0.001$ ). Durante la lactancia la temperatura corporal alcanzó su valor máximo para el día 12, y la producción de leche indujo un incremento en la misma ( $P \leq 0.025$ ). En conclusión, en nuestras condiciones de estrés térmico y sin importar el tamaño de la jaula, no se recomiendan densidades mayores a 18 conejos/m<sup>2</sup> (34 kg/m<sup>2</sup>) al final del engorde. La productividad de la gestación y la lactancia disminuyen cuando la lactancia es mayor y se suceden varios ciclos reproductivos seguidos. Esto se debe al efecto negativo del estrés térmico sobre la vitalidad y supervivencia del embrión/gazapo. La mejora de la calidad del agua atenuó el efecto negativo del estrés térmico. Las conejas más productoras parece que son aquéllas que consiguen manejar mejor el estrés térmico.

# ***CHAPTER 1***

*LITERATURE REVIEW  
AND OBJECTIVES*

## 1. INTRODUCTION

Currently human lifestyle depends on its nutrition that is getting more importance. On the other hand, human population grows exponentially and productive surface is getting smaller. That situation is more remarkable in countries with a young population where born rate is higher. Most of the tropical countries are in this condition, and shows high rates of undernourishment among their population, overall in their daily protein intake. On the base of that, it is mandatory to develop animal production systems with optimal production performance, replicable in different production levels and ecologically friendly. Meat rabbit production in tropical countries could be an alternative instead other types of meat because the productive characteristics of the system itself (Cheeke, 1986; El-Raffa, 2004; Muñoz, 2006 and Nieves, 2006), and the nutritional benefits for human of this meat (Dalle Zotte 2002; Hernández and Gondret, 2006).

Rabbit production demands the development of many labors to create a suitable environment to obtain the best performance in each of its physiological phases, starting with management skills and commitment on a daily basis (Cheeke, 1986). In spite of its quite good adaptation to different weathers, its productive response to them is very different. Metabolic processes imply heat production. Optimal production is reached in the thermoneutral zone where the animal does not need to spend extra energy on regulate its corporal temperature and therefore can express all its productive potential (Cervera and Fernandez-Carmona, 2010).

Rabbits, due to poor skin exposition, low activity of sweat glands and thick fur, could be considered ineffective on dissipate heat (Marai *et al.*, 1991; Cervera and Fernandez-Carmona, 2010). Typical ways to manage corporal–environment temperature relation in rabbits goes from physical postures, breathing rate and

extending ears through reducing metabolic rhythms. Initially the excessive heat is controlled by increasing their breathing rhythms; at the same time, if that doesn't work they will stretch out their body as much as they could and will extend their ears in order to increase the surface of exposition to improve their heat loss by convection (Marai *et al.*, 1991; Marai *et al.*, 2002; Cervera and Fernandez-Carmona, 2010). If the hot condition remains, most of the rabbits reduce significantly their feed intake and increase water intake that leads to a fall in reproductive performance, weight gain, milk production, weaned kits, feed efficiency, and thereafter farm profitability (Vaughn *et al.*, 1978; Maertens and De Groote, 1990; Marai *et al.*, 1991; Cervera *et al.*, 1997; Marai *et al.*, 2001; Marai *et al.*, 2002; Marai *et al.*, 2005; Cervera and Fernandez-Carmona, 2010; Zeferino *et al.*, 2011).

Most of the researches concerning to heat stress in rabbits, have been running down environmental controlled conditions where there are not fluctuation in ambient temperature and humidity between day and night as it occurred in open-air farms, which are very frequent in tropical countries like Venezuela. Furthermore, most work in rabbits have been done under seasonal heat stress conditions. Nieves *et al.* (1996), pointed out that commercial Venezuelan rabbit production, is carried out in opened-air facilities that imply equipments with high economic value where the profitability play an preponderant role. However, making an evaluation of rabbits production systems in Venezuela both Vanderdys *et al.* (1999) and Barrueta and Bautista (2002) agreed about the main problems in the system: few capacitation of the personnel, lack of technical assistance policies, shortage of researches, careless of reproductive management, and diseases (myxomatosis, mange, coryza, and coccidiosis) that diminish rabbit population.

Therefore, in order to optimize the productive performance of rabbits in tropical conditions is necessary to define which productive model is the most adequate regarding to sheds, suitable cages, breeding systems management, balanced food, good quality water, and also hygiene program to minimise heat stress effects.

## 2. HOUSING SYSTEM

During fattening stage labor demand depends on the number of cages and not on the number of animals per cage, due to the fact that the water and feed distribution could be automatic and in multiples cages; this suggest that the investment cost in cages and in labors could change depending on the number of kits per cage. According to Lukerfahr *et al.* (1980) and Prawirodigdo *et al.* (1985), to optimize the profitability, the farmer should offer to the market the highest number of kits. Based on this, the cage density utilized in a farm is determinant over its profitability, because it affects the number of animals raised and its quality. Thus, an increase in the number of animals per cage reduce the cages and equipments investment costs, but make animals production worse (Maertens and De Groote, 1984; Aubret and Duperray, 1992; Nieves *et al.*, 1996; Mbanya *et al.*, 2004).

According to data reviewed by Trocino and Xiccato (2006), in Europe the cage density vary between 14 and 23 kits/m<sup>2</sup>, namely an available surface per kits in between 425 and 720 cm<sup>2</sup>. The European Food and Safety Authority (2005) recommends at least 625 cm<sup>2</sup>/rabbit, and at the end of fattening a maximum of 40 kg/m<sup>2</sup>, with the purpose to allow a normal rabbit behavior. In this sense, Maertens and De Groote (1984) observed that values between 11.6 and 15.4 rabbits/m<sup>2</sup> (862 and 649 cm<sup>2</sup>/rabbit) reported a growth rate higher than those observed for 19.2 and 23.2 rabbits/m<sup>2</sup> (520 and 431 cm<sup>2</sup>/rabbit). Besides, they indicated that, with the exception of the first period after weaning (5-7 weeks), it was observed a lower daily feed intake for higher density values. It is attributed to the probable overpopulation comfort troubles. These researchers also indicated that increasing rabbit density delayed age at slaughter. Likewise, Aubret and Duperray (1992) evaluating densities between 16.9 and 28.2 rabbits/m<sup>2</sup> (592 an 355 cm<sup>2</sup>/rabbit) pointed that below 19.8 rabbits/m<sup>2</sup> or 46 kg/m<sup>2</sup> (that it is equivalent to values higher than 505

cm<sup>2</sup>/rabbit) were obtained the highest growth rate and feed intake, differences that were larger from 42 d of age onwards. They also observed that increasing densities led to a delayed slaughter time and a productivity reduction.

However, density preferred by rabbits depends on its age. Matics *et al.* (2004), observed that early weaned kits (21 d) housed in a four connected-cages system preferred to be in group during the first and the second weeks after weaning (with densities of 33-61 and 31-34 kits/m<sup>2</sup>, respectively), and at 45 d of age they get distributed in each cage of the system with similar densities by themselves. Relation between age and performance kits also has been studied by Morisse and Maurice (1997). They did not observed differences in performance at 6 weeks of age in densities between 15.3 and 23 kits/m<sup>2</sup>, although, at 10 weeks of age animals housed at densities higher than 20.4 rabbits/m<sup>2</sup> stay more time rested and invested less time eating and drinking.

In tropical conditions with high temperature and humidity, cage density during fattening could have more influence on kit performance than in Europeans conditions. Unfortunately most of the works do not indicate climatic conditions (temperature and humidity). Nieves *et al.* (1996), with an annual temperature average of 26°C and relative humidity of 74% (described according to Holdrige (1978) as tropical dry forest), observed that above of 16 rabbits/m<sup>2</sup> (625 cm<sup>2</sup>/animal) feed intake and growth were reduced without affect feed efficiency. On the contrary, these authors detected that densities lower than 14 rabbits/m<sup>2</sup> (714 cm<sup>2</sup>/animal) tended to reduce the animal growth, fact that is explained by the major loss of food energy as consequence of movement. Besides, both Andrea *et al.* (2004) and Mbanya *et al.* (2004) observed a negative effect on growth rate when density raised from 4.8 to 14.3 and from 5 to 10 rabbits/m<sup>2</sup>, respectively.

However, there are authors that do not report differences with different densities. Oliveira and Almeida (2002) did not detect any effect over productive performance studying densities between 11.7 and 16.7 rabbits/m<sup>2</sup> (600 cm<sup>2</sup>/rabbit or higher), and the production increased when density changed from 26.9 to 38.1 kg/m<sup>2</sup>. Neither Prawirodigdo *et al.* (1985) nor Camacho *et al.*

(2003), observed differences in the studied interval (8.6 – 14.5 and 12.2-19.6 kits/m<sup>2</sup>, respectively), for what they recommend the highest densities, because that imply less equipment, housing surface and investment joined to an increase of meat production.

Another interesting aspect that outlines the literature makes reference to the effect that could have farm facilities over carcass quality. Trocino *et al.* (2004) evaluated two densities 12.1 and 16 rabbits/m<sup>2</sup> (826 and 625 cm<sup>2</sup>/rabbit) without significant differences over cold carcass performance (58.8% on average), that is a good result in spite of the early rabbits age (71 days) at slaughtering time. These authors have made the carcass commercial evaluation on conformation, color and fat content, without observing differences between animals reared in groups and in bicellular cages. Regarding the influence of density over the characteristics of carcass there are not previous information in tropical conditions.

### **3. BREEDING SYSTEM**

According to Castellini *et al.* (2003 and 2010), economic efficiency of rabbit production basically depends on the reproductive rabbit does behavior, that is affected by its fertility, prolificacy, weight gain and kit mortality. Furthermore, González (2007), pointed out that rabbit farmers do not usually worry about kit mortality during lactation because these kits do not have initial economic importance, moreover the great difficulty of reducing kit mortality at this time; however this aspect is getting more importance because they are the future commercial farm product. On the other hand, if it is desired to increase rabbitry yield, it is very important to determine the best mating time and the ideal weaning age, because those are parameters that determines length in both reproductive and productive cycles, and could generate an effective increment of the amount of rabbits sold per year. These decisions may also influence rabbit doe longevity and mortality during fattening.

The most common mating day is at 11 days post kindling. Gestation and lactation could occur simultaneously; however, energetically each one represents a very expensive cost for the animal. The longer is the overlapping of these physiological phases the worst is the doe performance throughout its lifespan, that also could get shorter (Feugier and Fortun-Lamothe, 2006; Rommers *et al.*, 2006; Castellini, 2010; Pascual, 2010).

Several works have evaluated the doe productive performance under different intensity levels of breeding system, considering in all cases that intensity in the system is defined by the overlapping days between gestation and lactation. Thus, while more overlapping exists between gestation and lactation more intensive is the system. Xiccato *et al.* (2005) testing three mating times (2, 11 and 26 days post partum) with weaning ages of 21 and 25 days found that number of kits born per litter was lower in mating at 11 days than at 26 days ( $P < 0,01$ ). Besides, as reproductive rhythm became extensive, body protein, fat and energy balances changed from negative values to approach equilibrium (energy balance: -0.14; -0.02; and +0.01 of initial body content in mating at 2, 11 and 26 days respectively). When weaning age changed from 21 to 25 days it increased both kits born alive per litter (7.4 to 9.6), and body water concentration (683 to 693 g/kg). An inverse response was observed for body energy that tended to decrease (7.88 to 7.46 MJ/kg). Xiccato *et al.* (2004), also evaluated mating at 11 d post partum and combined with different weaning ages (21, 26 and 32 day). They observed that despite body energy deficit varied from -8 to -19.4%, when weaning age was delayed from 21 to 32 days, there were no effect of treatments over litter size at weaning and at 32 days. Neither weaning age affected reproductive performance at final kindling (pregnancy length, number and weight of kits born and born alive per litter and fertility rate). Similar results were reported by Feugier and Fortun-Lamonte (2006). They worked with 11 and 25 days for artificial insemination (AI) after birth, and 23 and 35 days for weaning, and indicated that does inseminated at 25 days energy balance was positive, whereas in those at 11 days was negative (+847 kJ/kg<sup>0.75</sup> vs. -729

kJ/kg<sup>0.75</sup>, respectively). In addition, does inseminated at 25 days had higher sexual receptivity, pregnancy rate and fetal viability (+24, +16 and +5.4 percentage units; respectively), although number of kits born alive and weight of litter at birth were not affected by reproductive rhythm. Early weaning age neither affect the reproductive performance of the does. Moreover, Brecchia *et al.* (2008) with a weaning aged fixed at 26 days and comparing AI at 11 days after birth and AI conditioned to 15-30g of perirenal fat depot, pointed out that for conditioned AI (longest period for AI) showed higher number of kits born alive, higher sexual receptivity and fertility rate as well as better body condition (live body weight, estimated perirenal fat and cumulative BCS) and lower levels of progesterone, remarking the negative effect of lactation on reproduction function.

In this way, Szendrő *et al.* (2008) evaluated during 336 days two extensive reproductive rhythms (42 and 56 days). The longer one showed the worst results despite the number of survivals were higher than the shorter one. However, its production in terms of efficiency is lower as it led to a reduction in the number of annual parturitions/doe (7.8 vs. 6.1) and annual kits born alive/doe (69.2 vs. 51.9). They suggested that although this reproductive rhythm was better regarding to the animal welfare, a reduction of 22% in parturitions/year and 25% in kits born/year make it non attractive to the breeders.

In tropical areas all this situation might be studied more carefully because it may be accompanied by heat stress that affects production. In that sense, Marai *et al.* (1991 and 1996), indicate that heat stress limits rabbits rearing, affecting both weight and size of embryos, fattening weight gain, feed intake reduction, dehydration, tissue catabolism, reduction of energetic metabolism and increment of frequency breathing, as was mentioned above. Nevertheless, these troubles are accompanied by other situations affected by heat stress that make a lot of drastic changes in biological functions, and that also affect sexual maturity in males and females, conception index, fertility, gestation length, mortality, between others. In this sense, Marai *et al.* (2001) evaluated seasonal heat stress

over reproduction performance and found a severe heat stress was reflected in rabbit does that declined their conception rate, live body weight, daily weight gain, preweaning weight gain of pups, and feed intake. Similar response was obtained in the litter size and litter weight at weaning.

On the other hand, absence of an detailed and consistent research in this productive system, like is mentioned by Barrueta and Bautista (2002), is one of the significant weakness that tropical countries like Venezuela have in this matter, joined to poor local and regional information that doesn't allow to make productive parameters comparisons that could affect positively productivity of this promissory animal production.

#### **4. WATER QUALITY**

Water quality depends on its mineral concentration and microbial population. Water used in animal production in most industrialized countries, is the same that is used for human consumption. Humans are less tolerant to high levels of minerals and microbes in water than most of the animals including rabbits. Indeed this could explain why there is very little information regarding to water quality used for optimal rabbit production (Gidenne *et al.*, 2010)

Water intake is determined not only by the animal thermoregulatory capacity but also by its quality. Tropical conditions associated with high ambient temperature enhance the importance of both intake and quality water management, especially in animal species less effective in losing heat as rabbits. Table 1, includes chemical composition of water considered "drinkable" for rabbits (Gidenne *et al.*, 2010).

Information on bacteriological limits of water used in rabbit production is scarce. It is not recommended to use water contaminated with bacteria, because rabbit health and performance could be affected and therefore its meat quality. Based on prophylaxis measures, is highly recommended to use one of the low-

cost systems for water disinfection disposable in the market as chlorine addition into the water.

**Table 1.** Chemical composition of drinkable water for rabbits.

	Official recommendations for human consumption <sup>a</sup>	Maximum experimented on rabbits without problems	
	Maximum tolerable	Value	Refrence
<b>pH</b>	6.5-9.2	3.5-9.0	Porter <i>et al.</i> (1988)
<b>Chemical parameters, ppm</b>			
<b>Total soluble salts</b>	1500	3000	Abdel-Samee and El-Masry (1992)
<b>Sodium</b>	150	900	Ayyat <i>et al.</i> (1991)
<b>Potassium</b>	12	140	Ayyat <i>et al.</i> (1991)
<b>Phosphorus</b>	5	---	---
<b>Calcium</b>	200	400	Porter <i>et al.</i> (1988)
<b>Magnesium</b>	150	---	---
<b>Iron</b>	1.0	---	---
<b>Copper</b>	1.5	60	Abo-El-Ezz (1996)
<b>Manganese</b>	0.5	12	Abdel-Samee and El-Masry (1992)
<b>Zinc</b>	15	55	Abdel-Samee and El-Masry (1992)
<b>Aluminium</b>	---	250	Rémois and Rouillière (1998)
<b>Antimony</b>	---	---	---
<b>Arsenic</b>	0.20	---	---
<b>Cadmium</b>	0.05	---	---
<b>Chromium</b>	1.0	---	---
<b>Cobalt</b>	1.0	---	---
<b>Fluoride</b>	2.0	---	---
<b>Lead</b>	0.10	0.40	Habeeb <i>et al.</i> (1997)
<b>Mercury</b>	0.01	---	---
<b>Nickel</b>	1.00	---	---
<b>Vanadium</b>	0.10	---	---
<b>Chloride (Cl)</b>	600	1100	Habeeb <i>et al.</i> (1997)
<b>Sulfate (SO<sub>4</sub>)</b>	400	1340	Rémois and Rouillière (1998)
<b>Nitrate (NO<sub>3</sub>)</b>	50	600	Kammerer and Pinault (1998)
<b>Nitrite (NO<sub>2</sub>)</b>	0.10	11	Morisse <i>et al.</i> (1989)
<b>Ammonium (NH<sub>4</sub>)</b>	0.50	---	---
<b>H<sub>2</sub>S</b>	0.10	---	---
<b>Bicarbonate</b>	---	400	Ayyat <i>et al.</i> (1991)

<sup>a</sup>Official Journal of the European Communities, 1998; Council Directive 98/83/EC of 3 November, 1998, on the quality water intended for human consumption.

## **5. BODY TEMPERATURE, CIRCADIAN RITHMS, BODY COMPOSITION AND PHYSIOLOGICAL STATUS**

Rabbit body temperature is the result of the heat produced in each metabolic process and its interaction with the environment. The thermoregulatory efficacy is based on the ability to keep body temperature between tolerable limits. Both ambient temperature and relative humidity must be considered when heat stress is studied in rabbit production, because they directly influence the energy equilibrium of the animal, changing the flow of heat between the animal and the environment (Cervera and Fernández-Carmona, 2010). The temperature-humidity index (THI) considers together both environment aspects, and is used to estimate heat stress in rabbits (Marai *et al.*, 2002).

Several authors pointed out that in general, rabbit temperature ranged between 38.6° and 40.1°C (Sutherland *et al.*, 1958; Vaughn *et al.* 1978; Hellmann and Claussen, 1980; Trammell *et al.*, 1989; Abdel-Samee, 1997; Jilge *et al.* 2001; Marai *et al.*, 2001; Zeferino *et al.* 2011). Body temperature into the normal limits for rabbits is a response of a good adaptation to environment. According to Cervera and Fernández-Carmona (2010), the environment could be defined not only by the season but also by the production system. Likewise, these authors pointed out that thermoneutral zone could be considered as the ambient temperature range where metabolic rates allow to achieve temperature regulation by non-evaporative physical processes in productive animals. In rabbits it is located between 10° and 30°C, and it is expressed with normal feed intake, normal body temperature and no shivering, sweating or panting. High ambient temperature affects negatively the rabbit homeostasis, increasing in most of the cases body temperature to levels that could compromise the health of the animal (Finzi *et al.*, 1986; Lebas *et al.*, 1986 ; Abdel-Samee, 1997; Finzi *et al.*, 1988a; Finzi *et al.*, 1988b; Marai *et al.* 2002; Zeferino *et al.*, 2011;). It is considered that above 35°C rabbits have serious difficulties to regulate their internal temperature, at 40°C excessive salivation and painted is observed (Lebas

*et al.* 1986), and temperatures close to 42.8°C are considered lethal for rabbit (Marai *et al.* 2004). As body temperature increase, metabolic rate is reduced to keep controlled heat load generated by metabolic activity, leading to a poor productive response. In this sense, Ulberg and Burfening (1967) found that increasing 1° to 2°C in rabbits does temperature, conception rate was reduced. Lubling and Wolfenson (1996) studying induced thermal stress of 1°C upper body temperature in comfort conditions over blood flow to mammary and reproductive systems, observed that after stress induction it was a 30% reduction in blood flow to the ovarian corpora lutea, from 14.1 to 9.9ml/min.g, that could diminish the progesterone outflow into the circulation and thereafter compromise optimal function of this organ.

Ambient temperature, digestive habits (feed intake and faeces excretion) and many blood parameters are used to estimate the nutritional status of animals and follow circadian rhythms (Rosi *et al.*, 1984; Prud'hon, 1973; Carabaño and Merino, 1996). Mammals' circadian rhythms are generated endogenously and show a 24 h rhythm for almost each physiological function. Natural stimulus, like light-dark time, could set each rhythm, but if there is any change the suprachiasmatic nuclei (SCN) will try to maintain it (Jilge *et al.*, 2001). Regarding to body temperature Rosi *et al.* (1984) pointed out that it follows the feeding schedule but also it shows its highest values during the activity period of the day. Indeed, both Finzi *et al.* (1992a) observed the highest values early in the morning, then this value descend gradually until mid morning and finally it increase again until reach its highest values again late afternoon. This behavior could be due to higher metabolic activity during the night related with higher levels of feed intake, which is much reduced during the day (Simplicio *et al.*, 1988; Chiericato *et al.*, 1992; Duperray, 1993). There are few researches about the circadian rhythms of body temperature in rabbits does during pregnancy and lactation that could be important to achieve a better understanding of this physiological phase. However, Jilge *et al.* (2001) working with rabbit does and their kits at  $22 \pm 0.3^{\circ}\text{C}$  and  $55 \pm 10\%$  relative humidity, have reported that core temperature of rabbit does during gestation and

lactation shows a persistent circadian temperature rhythm. Thus, the mean temperature of does during the last 2/3 of gestation described a gradually decrease just until parturition and then it rose significantly one or two days after. Also, rabbit does core temperatures were higher during lactation and showed their highest levels during suckling. Furthermore, they explained that core temperature decreasing prior to parturition could be to induce a reduction of kits metabolic rate, which allows them to increase their survival chance after the oxygen absence they will have during the course of parturition. On the other hand, in rabbit kits circadian rhythm of core temperature is quickly settled by their mother at the nursing time, showing the highest temperatures few hours before and after suckling (Jilge *et al.*, 2001). This temperature increment in kits could help them to profit better the short daily time that their mother spend into the nest nursing them. Ambient temperature can perturb circadian rhythms of rabbit body temperature (Finzi *et al.*, 1994). Likewise, high ambient temperatures could induce changes into the circadian rhythms of digestive habits in order to reduce heat stress.

The productive rabbit lifespan is determinant for the long-term economic success of a rabbitry. An optimal productive rabbit lifespan is frequently achieved if animals are raised into a welfare environment without diminish the system performance. Nutritional, reproductive and sanitary management programs on each phase of rabbit production had been designed to optimize the metabolic activity of each physiological phase. This metabolic activity is reflected in body composition of the rabbit, which will be different not only between phases but also in each phase itself. According to the metabolic activity on each physiological phase, nutritional requirement will be different. Thus, during fattening the animal is growing and making all the structural body changes to prepare all its body for the two next steps: fecundation-gestation and lactation. There are some researches that prove that an undernutrition even in the fetal stage could affect the reproductive performance in rabbits (Rommers *et al.*, 2004; Xiccato *et al.*, 1999). Fecundation-gestation implies energy for oogenesis, spermatogenesis, intercourse (sexual receptivity), fecundation, implantation and

all the processes involved in the development of viable embryos that will change into fetus which will continuously demand nutrients until birth. Also hormonal dynamic is involved. On the other hand, lactation implies all the metabolic processes to make a food good enough (quality and quantity) to guarantee to the offspring not only the fastest growth rate in their lifespan but also the body conditions to survive the transition between liquid and solid diets. Healthy and independent kits eating solid food, increase the probability of perpetuity of the species. As expected, these two last phases are the most demanding and its dynamic changes depending on if rabbit does are nuliparous, primiparous or multiparous (Castellini *et al.*, 2010; Parigi Bini and Xiccato, 1998; Rommers *et al.*, 2004; Pascual *et al.*, 2006; Pascual, 2010), because it could imply the overlapping between them. The overlapping supposes a competition for glucose, long-chain fatty acids and free fatty acids, between mammary glands and fetoplacental units (Fraga *et al.*, 1989; Stephenson *et al.*, 1990). Previous studies (Xiccato *et al.*, 1999; Feugier and Fortun-Lamote, 2006; Arias-Alvarez *et al.*, 2009a), have proved that during overlapping the greater rabbit does reserve mobilizations are to favor lactation, perhaps due to the preservation specie instinct. Pascual (2010), suggested that the recovery of the reserves in rabbit does shows a different pattern than other species. They mobilize fat reserves before birth, that are recovered just after birth, reaching a maximum level of body reserves at day 10 in lactation. It is due probably to prioritize their offspring. In fact, Xiccato *et al.* (2005), conclude that an earlier weaning (21 days of age) allowed rabbit does to better keep their body equilibrium by reducing the body energy utilized for milk yield, than those weaned at 25 days (+4 days of lactation). However Pascual (2010), and Xiccato *et al.* (2005), pointed out that lactation-pregnancy concurrence could affect negatively rabbit does milk production.

Therefore, knowing the rabbit body composition and its dynamic on each physiological phase could provide the information necessary to determine, with more precision, which are the requirements and strategies to improve nutritional and reproductive programs (Pereda, 2010; Taghouti, 2011; Pascual, 2010;

Castellini *et al.*, 2010). Several methods have been developed to set rabbit body composition, especially for rabbit does. Within the most used are, slaughter comparative method (Parigi Bini *et al.*, 1992; Xiccato *et al.*, 2004; Xiccato *et al.*, 2005; Feugier and Fortun-Lamote., 2006), the body condition score (BCS) (Bonanno *et al.*, 2008; Rosell *et al.*, 2008; Brecchia *et al.*, 2008; Sánchez *et al.*, 2012), ultrasounds measurements of perirenal fat thickness (Pascual *et al.*, 2000; Pascual *et al.*, 2002; Brecchia *et al.*, 2008), total body electrical conductivity (TOBEC) (Fortun-Lamothe *et al.*, 2002; Szendrő *et al.*, 2008) and more recently the bio electrical impedance (BIA) (Nicodemus *et al.*, 2009; Pereda, 2010; Romero *et al.*, 2011). Table 2, listed the parameters determined, advantages and disadvantages of each method.

In tropical countries with high temperatures, body composition and its dynamic in each physiological phase could vary from that reported in temperate countries. Characterize them could be determinant in survival of rabbits down heat stress due to the negative effect that fatness, high proteins levels and extra heat during body reserves mobilization have over the rabbit thermoregulatory capacity, especially during gestation and lactation under a intensive or semi-intensive breeding system. However, actual information is scarce and confuse Marai *et al.* (1991).

**Table 2.** Parameters estimated, advantages and disadvantages of the most common methods used to evaluate body composition in rabbits

<b>Method</b>	<b>Body components</b>	<b>Advantages</b>	<b>Disadvantages</b>
<b>Slaughter comparative</b>	Chemical composition measured by the AOAC procedures, nitrogen.	Highly accurate	Expensive, time consuming, destructive and does not allow subsequent evaluations in same animals
<b>Body condition score (BSC)</b>	Estimated by 5 point scale through palpation of the loin region.	Non destructive, allows subsequent evaluations in same animals and economic.	Subjective.
<b>Ultrasounds measurements of perirenal fat thickness</b>	Perirenal fat and estimation of total fat and carcass energy.	Accurate, non destructive and allows subsequent evaluations in same animals.	Limited number of body components estimated.
<b>Total body electrical conductivity (TOBEC)</b>	Estimation of water, lipids, energy, protein and ash content.	Accurate, non destructive, allows subsequent evaluations in same animals and high number of body component estimated.	Difficult management of the equipment and it does not consider digestive content variability.
<b>Bio electrical impedance (BIA)</b>	Estimation of water, lipids, energy, protein and ash.	Accurate, non destructive, allows subsequent evaluations in same animals and high number of body component estimated.	Does not consider digestive content variability.

## **6. OBJECTIVES**

The general aim of this work was to study several factors that can condition rabbit production under chronic heat stress. The following specific objectives were formulated:

- To find the optimal cage density in tropical very dry forest condition by measuring growth performance, mortality rate, injured animals and carcass performance.
- To determine if cage size maintaining stock density influences growth performance.
- To evaluate heat stress and circadian rhythms comparing unclipped and clipped rabbit does.
- To study if a more extensive breeding system (delay of mating and weaning) may increase weight of litters at weaning without impairing rabbit doe performance.
- To study if a water quality improvement can affect positively rabbit doe response to heat stress.
- To characterize the rabbit doe response to heat stress during pregnancy and lactation.

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## ***CHAPTER 2***

*EFFECT OF CAGE DENSITY ON GROWTH AND  
CARCASS PERFORMANCE OF FATTENING RABBITS  
UNDER TROPICAL HEAT STRESS CONDITIONS*

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## ABSTRACT

Three hundred cross-breed rabbits of New Zealand, California, Butterfly, Dutch and Satin, weaned at 30 days and weighing  $535 \pm 8$  g (standard error) were assigned at random to four treatments: 6, 12, 18 and 24 rabbits/m<sup>2</sup> (3, 6, 9 and 12 rabbits/cage, respectively, each cage of 0.5 m<sup>2</sup>) resulting 10 cages/treatment. During the experimental period (from weaning to 2.2 kg body weight) weekly individual live weight, cage feed intake, the incidence of diarrhoea, ringworm and injured rabbits were recorded. The maximal temperature-humidity index ranged from 31 to 35 indicating a temporal severe heat stress. At the end of the experimental period 10, 20, 30 and 30 rabbits from the treatments with densities of 6, 12, 18 and 24 rabbits/m<sup>2</sup>, respectively, were slaughtered and carcass performance recorded. Average daily gain and feed intake from weaning to the end of experimental period decreased by  $0.31 \pm 0.070$  and  $1.20 \pm 0.25$  g, respectively, per each unit that the density increased at the beginning of the experiment ( $P = 0.001$ ). It increased the length of the fattening period by  $0.91 \pm 0.16$  d ( $P = 0.001$ ) per each unit of increment of density. However, rabbit production (expressed in kg/m<sup>2</sup>) increased linear and quadratically with the density ( $P < 0.008$ ). Cage density did not affect feeding efficiency, that was on average 0.214 g/g ( $P = 0.37$ ). Animals housed at the highest density compared to the average of those caged at lower density tended to show a higher incidence of ringworm (68.9 vs 39.4%;  $P = 0.075$ ), a higher injured animals (16.8 vs 3.03%;  $P = 0.12$ ) and a higher mortality (20.5 vs 9.63%;  $P = 0.043$ ). Density did not modify dressing out percentage and chilled carcass weight. The proportion of scapular fat ( $P = 0.042$ ) increased linearly with increasing levels of density, but perirenal fat was not affected ( $P = 0.22$ ). Increasing density reduced linearly dorsal length ( $P = 0.001$ ), and reduced linear and quadratically drip loss percentage ( $P = 0.097$  and  $0.018$ , respectively). Based on these results, under our heat stress conditions it is

recommended to avoid densities higher than 18 rabbits/m<sup>2</sup>, or 34 kg/m<sup>2</sup> at the end of fattening.

**Key words:** Cage density, growing performance, carcass performance, heat stress.

## INTRODUCTION

The cage density used during rabbit fattening is an important factor that influence labour, investment cost, performance and accordingly profitability. In Europe cage density varies, in commercial farms, from 14 to 23 rabbits/m<sup>2</sup> (or from 720 to 425 cm<sup>2</sup>/rabbit) (Trocino and Xiccato, 2006). Besides, densities higher than 19 rabbit/m<sup>2</sup> reduced feed intake and growth rate, with no effect on feed efficiency and mortality (Maertens and De Groote, 1984; Aubret and Duperray, 1992). The European Food and Safety Authority (2005) recommended a minimum surface of 625 cm<sup>2</sup>/rabbit and not more than 40 kg/m<sup>2</sup> at the end of fattening, in order to avoid disturbances in rabbit behaviour. However, the behaviour of rabbits depends on their age. Rabbits just after weaning (at 21 d) tended to huddled together, increasing the stocking density up to 31-61 rabbits/m<sup>2</sup> the first two weeks after weaning, whereas older animals preferred lower densities and when caged at high densities spent less time for eating (Morisse and Maurice, 1997; Matics *et al.*, 2004).

The results of these studies can not be extrapolated to tropical conditions, where it is difficult to have an adequate environmental control, and consequently the increase of cage density can affect more negatively rabbit growth performance. In these conditions, the increase of cage density reduced also feed intake and impaired growth rate, but it also seems to increase mortality rate (Nieves *et al.*, 1996; Mbanya *et al.*, 2004). The cage density recommended by these authors ranged between 5 and 16 rabbits/m<sup>2</sup>, but they do not give any information about the environment temperature and relative humidity.

The aim of this work was to study the effect of cage density under the heat stress environmental conditions of Maracaibo, Venezuela (average temperature 28°C and relative humidity 76%; Peters *et al.*, 1983), by measuring growth performance, mortality rate, injured animals and carcass performance.

## **MATERIAL AND METHODS**

### **Animals and housing**

Three hundred cross-breed rabbits of New Zealand, California, Butterfly, Dutch and Satin, weaned at 30 d were transported just after weaning from a commercial farm (placed in Barinas: 26°C and 74% relative humidity, as average) to our facilities (7 hours long) in Maracaibo (Venezuela). Animals were housed in flat-deck cages of 500 × 100 × 500 mm (0.5 m<sup>2</sup>) equipped with one nipple drinker and one hopper feeder (30 cm available) each one. Water was filtered before stored in the farm water-tank. The farm is an open-air building equipped with a ventilator to favour air recycling and a mesh (80% shade) in the windows to avoid animals were exposed to the sun. Our region climate (Maracaibo) is characterized as tropical very dry forest (Holdrige, 1978). The temperature-humidity index (THI) was calculated according to Marai *et al.* (2001):

$$\text{THI} = \text{db}^{\circ}\text{C} - [(0.31 - 0.031 \text{ RH}) \times (\text{db}^{\circ}\text{C} - 14.4)],$$

where db<sup>°</sup>C is dry bulb temperature in Celsius degrees, and RH is the relative humidity as percentage. According to Marai *et al.* (2002) there is heat stress when THI is higher than 28.9, and under 27.8 there is no heat stress.

### **Experimental procedure**

Rabbits were caged at 6, 12, 18 and 24 rabbits/m<sup>2</sup> (or 3, 6, 9 and 12 rabbits/cage) and they were assigned randomly to one of these four treatments (10 cages/treatment). The average weaning weight was 535 ± 8.0 g (standard error) and rabbits were identified by a number written in their ears. A commercial diet was offered *ad libitum* to the animals containing (g/kg DM): 918 dry matter, 97.8 ash, 164 crude protein, 36.2 ether extract, 124 crude fibre, 361 neutral detergent fibre, 186 acid detergent fibre and 44.6 acid detergent lignin, and an estimated digestible energy value of 11.1 MJ/kg (De Blas *et al.*, 1992).

Individual weight of animals, cage feed intake and mortality were recorded weekly. Dead animals were not substituted. The average number of rabbits per cage and week were used to calculate growth rate and feed intake per cage and per animal. Mortality, diarrhoea incidence and injured animals were expressed in percentage per cage. During the experiment an outbreak of diarrhoea appeared just after weaning. An intramuscular injection with Diarrex H (Aldor C.A., Venezuela) for 3-4 days was executed to control the infection. It contained dimetridazol, sulphamethacine, trimetoprin and tetracycline. Besides, animals diagnosed with ringworm (*Trichophyton* spp.) were treated orally with ketazol (Laboratorios Vargas, Venezuela) that contained ketoconazol. The experiment finished when the average weight of the rabbits in the cage reached 2.2 kg/rabbit. Then 10, 20, 30 and 30 rabbits corresponding to rabbits caged at 6, 12, 18 and 24 rabbits/m<sup>2</sup>, respectively, were slaughtered between 9.30 and 11.00 h. Rabbits were stunned by a neck hit and then bled. Afterwards, they were dissected according to Blasco *et al.* (1993).

### **Chemical analysis**

Procedures of the AOAC (2000) were used to determine the concentrations of DM (934.01), ash (967.05), CP (968.06), ether extract (including acid hydrolysis, 920.39), and crude fibre (932.09). Dietary NDF, ADF and ADL were determined sequentially using the fibertec system (Foss, Denmark) according to the methods of Mertens (2002), the AOAC (2000; procedure 973.187) and Van Soest *et al.* (1991), respectively.

### **Statistical analysis**

The results obtained in this study for growth traits performance (expressed per cage) were analyzed as a completely randomized design with the average weaning weight per cage as a linear covariate and cage density was included as a linear and quadratic covariate, by using the General Linear Model procedure of SAS (SAS Inst. Inc., Cary, NC). In the animals that finished the

experiment we studied for their individual growth rate the interaction between cage density and fattening period (considering four fattening periods: weaning (30 d)-44 d, 44-58 d, 58-85 d, 85 d-2.2 kg BW). This interaction was analysed as a repeated measurement analysis by using a mixed model (CS covariance structure) that included weaning weight as linear covariate, cage density as linear and quadratic covariate, and fattening period (time effect) as classified effect. Interactions among cage density and fattening period were also included. In this model the cage was considered as a random effect. The model used to study carcass traits included the sex as a classified effect, slaughter weight as a linear covariate and cage density was included as a linear and quadratic covariate, by using the General Linear Model procedure of SAS. When quadratic effects were significant the maximum/minimum was calculated and commented, except if it laid out of the range studied. All data are presented as least-squares means.

## **RESULTS AND DISCUSSION**

Inside the farm the relative humidity ranged from 67 to 94% and the minimum temperature varied from 21 to 29°C (that would correspond to the night period, when animals eat most of the feed). According to the review of Cervera and Fernández-Carmona (1998), these temperatures would be lower than the upper critical temperature of weaned rabbits (30°C), but higher than the value for adult rabbits (25°C). The calculated THI ranged between 21 and 28, and according to Marai *et al.* (2002) this would be not heat stress. At the maximum temperatures (recorded around 15:00 h, and varied from 24 to 35°C) THI ranged from 31 to 35, and it implied a very severe heat stress which would impair growing performance response.

Feed intake and growth rate (both expressed per day and rabbit) from weaning to the end of fattening impaired by  $1.20 \pm 0.25$  and  $0.31 \pm 0.070$  g, respectively, per each unit that cage density (rabbits/m<sup>2</sup>) increased at the

beginning of the experiment ( $P < 0.001$ ) (Table 1). This negative effect was recorded in all the fattening stages. However, cage density had no effect on feed efficiency that was on average 0.214 g/g ( $P = 0.37$ ). Accordingly, the reduction of growth rate when cage density increased is directly related to the reduction of feed intake as observed previously (Maertens and de Groote, 1984; Aubret and Duperray, 1992; Nieves *et al.*, 1996; Mbanya *et al.*, 2004). As a consequence, the length of fattening period increased by  $0.91 \pm 0.16$  d ( $P = 0.001$ ) per each unit that increased cage density. Single growth rate of rabbits that finished the fattening period decreased by  $0.25 \pm 0.035$  g/d per each unit of increase of cage density ( $P < 0.001$ ) (Table 2). In these animals the effect of cage density varied depending on the fattening period ( $P = 0.022$ ). During the first two weeks after weaning (from 30 to 44 d of age) the effect of cage density was worse ( $-0.30 \pm 0.080$  g/unit of density increment;  $P < 0.001$ ) than those of 44-58 and 58-85 d period ( $-0.13 \pm 0.057$  and  $-0.12 \pm 0.047$  g/unit of density increment, respectively;  $P \leq 0.024$ ). These results differ from those reported by Maertens and de Groote (1984) and Aubret and Duperray (1992) who did not observe any effect of density during the 10-14 d after weaning, or even with the results of Matics *et al.* (2004) that observed a natural preference of rabbits to increase density around weaning. These negative results just after weaning might be explained by the long transport duration between farms just after weaning and the time required to adapt to the new housing conditions, which might have favoured the outbreak of diarrhoea in this period. In the final fattening period (from 85 d of age to 2.2 kg BW) the negative effect of density impaired again ( $-0.20 \pm 0.069$  g/unit of density increment;  $P = 0.004$ ), which would be related to the reduction of the available surface as observed either in optimal climatic conditions (Maertens and de Groote, 1984; Aubret and Duperray, 1992; Morisse y Maurice, 1997) or in hotter conditions (Nieves *et al.*, 1996; Andréa *et al.*, 2004).

The incidence of ringworm and the animals injured were not affected linear or quadratically by cage density (Table 1). However, rabbits caged at the highest density compared to the average of the three lower densities tended to

be more sensible to ringworm (68.9 vs 39.4%;  $P = 0.075$ ), and to show a greater aggressiveness (reflected in the higher percentage of injured animals, especially in the ears and tail; 16.8 vs 3.03%;  $P = 0.12$ ). The aggressions begun on average at  $68.8 \pm 4.8$  d after weaning (recording the first one 48 d after weaning, but detecting the two thirds from 72 d after weaning onwards). This result indicates the negative impact of high densities on rabbit behaviour due to the lack of comfort, and it is in agreement with the impairment of growth performance in the last fattening period. The highest density also increased mortality rate in the whole fattening period compared to the average of the three lower densities (20.5 vs 9.63;  $P = 0.043$ ). This result differs from previous studies that did not find any relation between cage density and mortality (Maertens and de Groote, 1984; Aubret and Duperray, 1992), but it is in agreement with the trend observed in tropical conditions (Nieves *et al.*, 1996; Mbanya *et al.*, 2004). In this study, mortality affected more severely to animals with lower weaning weight (Figure 1 and 2), which probably had a lower milk intake during lactation and were more sensitive to diarrhoea. This result might suggest the interest to delay the age at weaning.

The negative effect of density on growth performance did not avoid that the final rabbit production ( $\text{kg}/\text{m}^2$ ) increased linearly with density ( $P = 0.001$ ) (Table 3). In this case, a quadratic effect was also observed ( $P = 0.008$ ), in a way that rabbit production did not increase directly proportional to the number of rabbits, that is due to their lower growth rate and higher mortality at the highest cage density. Accordingly, in order to reduce mortality, ringworm and injured animals it is required to be below  $41 \text{ kg}/\text{m}^2$ , which is the highest value recommended in Europe (Trocino and Xiccato, 2006). In our conditions of heat stress it could be suggested a cage density around 16-18 rabbits/ $\text{m}^2$  in order to produce around  $34 \text{ kg}/\text{m}^2$ , and reduce one week the length of fattening compared to animals caged at the highest density. A lower density ( $12 \text{ kg}/\text{m}^2$ ) improves growth rate and fattening duration (by 16 and 4%, respectively) but also reduces final production per  $\text{m}^2$  around 29%. The only possibility to use in

our conditions a high cage density would be to shorten the length of fattening and accordingly reduce significantly the slaughter weight.

Although rabbits were slaughtered when the average weight of the cage was 2.2 kg/rabbit, a negative effect of cage density is observed on slaughter weight ( $P = 0.041$ ), and it has been used as a covariate when carcass traits has been analysed (Table 4). Cage density had minor influence on carcass compared to growth traits, and this is in agreement with previous works (Aubret and Duperray, 1992; Xiccato *et al.*, 1999; Combes and Lebas, 2003). Proportion of scapular fat increased with cage density ( $P = 0.042$ ). The older age at slaughter when cage density increased might explain it (Dalle Zotte *et al.*, 2002). However, this result should be confirmed as no effect was detected on the proportion of perirenal fat ( $P = 0.22$ ), and scapular fat might be removed with the skin. Cage density reduced linearly dorsal length ( $P < 0.001$ ) which is another signal of the lack of comfort in animals caged at high densities.

Drip loss percentage increased linear and quadratically ( $P = 0.097$  and  $0.018$ , respectively) with decreasing densities, showing a minimum value for a density of 18 rabbits/m<sup>2</sup>. The slaughter of younger animals (with almost the same weight: 2,2 kg) when cage density decreased might account for this result as other authors have detected an increase in drip loss percentage when age at slaughter decreased (Xiccato *et al.*, 1993; Bernardini *et al.*, 1995). This result is related to the quadratic trend ( $P = 0.12$ ) of cage density on dressing out percentage which showed a maximum for 17.1 rabbits/m<sup>2</sup>. Cage density also affected quadratically to the lumbar circumference obtaining a maximum value for 14.3 rabbits/m<sup>2</sup>. The sex had minor influence on carcass traits. Females were heavier at slaughter compared to males ( $P = 0.022$ ) and had a lower skin weight proportion ( $P < 0.001$ ). Besides, they tended to have a higher digestive tract weight ( $P = 0.078$ ) and a longer lumbar circumference ( $P = 0.11$ ). Neither cage density nor sex had effect on hot, chilled and reference carcass weight, viscera weight, as well as on proportion of dissectible fat weight (scapular and perirenal) and thigh length.

## **CONCLUSIONS**

A cage density above 6 rabbits/m<sup>2</sup> impaired growth performance during fattening, with minor effects on carcass traits, but improved rabbit production (expressed in kg/m<sup>2</sup>). Besides, a high density (24 rabbits/m<sup>2</sup>) increased mortality, ringworm and injured animals respect to animals caged at lower densities. Accordingly, in our heat stress conditions, it is recommended to use a maximal density of 18 rabbits/m<sup>2</sup> (or 34 kg/m<sup>2</sup> at the end of fattening) to avoid these problems and to maximise rabbit production (kg/m<sup>2</sup>).

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**Table 1.** Effect of cage density on growth performance

Initial density, rabbits/m <sup>2</sup>	6	12	18	24	SEM <sup>1</sup>	L <sup>2</sup>	P <sub>cov</sub> <sup>3</sup>
Initial density, cm <sup>2</sup> /rabbit	1667	833	555	417			
<i>30 (weaning)-44 d</i>							
Body weight at 44 d, g/rabbit	922	886	865	831	12.2	0.001	0.001
Daily gain, g/rabbit·d	22.1	20.3	18.8	14.3	2.08	0.025	0.001
Feed intake, g/rabbit·d	58.6	53.3	50.3	46.6	2.43	0.003	0.001
Feed efficiency <sup>†</sup> , g/g	0.361	0.363	0.365	0.295	0.033	0.26	0.011
Mortality, %	8.79	8.84	7.70	12.2	3.52	0.63	0.015
<i>44-58 d</i>							
Body weight at 58 d, g/rabbit	1262	1198	1176	1129	20.1	0.001	0.001
Daily gain, g/rabbit·d	24.6	21.5	21.4	18.3	1.19	0.003	0.014
Feed intake, g/rabbit·d	83.4	75.7	74.7	73.0	2.83	0.032	0.001
Feed efficiency <sup>†</sup> , g/g	0.297	0.294	0.289	0.243	0.020	0.11	0.75
Mortality <sup>†</sup> , %	0.18	1.63	1.00	4.64	1.41	0.090	0.18
<i>58-85 d</i>							
Body weight at 85 d, g/rabbit	1848	1747	1700	1643	27.9	0.001	0.001
Daily gain, g/rabbit·d	20.9	19.5	18.6	17.4	0.76	0.005	0.079
Feed intake, g/rabbit·d	109	105	93.1	86.6	4.79	0.002	0.16
Feed efficiency, g/g	0.196	0.188	0.200	0.199	0.009 <sup>8</sup>	0.67	0.81
Mortality, %	0.072	0.12	0.13	1.85	0.57	0.077	0.24
<i>85 d-2.2 kg</i>							
Final body weight, g/rabbit	2178	2233	2100	2170	37.8	0.35	0.35
Daily gain, g/rabbit·d	17.3	20.2	12.8	14.4	1.71	0.057	0.46
Feed intake, g/rabbit·d	114	113	93.7	80	4.93	0.001	0.81
Feed efficiency, g/g	0.150	0.183	0.138	0.172	0.016	0.88	0.52
Mortality, %	0.37	0.00	0.44	1.78	1.27	0.44	0.016
<i>30 d (destete)-final</i>							
Length, d	73.1	79.0	82.5	90.3	1.94	0.001	0.001
Daily gain, g/rabbit·d	21.3	20.4	17.5	16.0	0.83	0.001	0.001
Feed intake, g/rabbit·d	97.4	94.1	83.7	76.9	2.96	0.001	0.055
Feed efficiency, g/g	0.219	0.218	0.210	0.208	0.009 <sup>5</sup>	0.37	0.041
Mortality <sup>†</sup> , %	9.42	10.2	9.27	20.5	4.10	0.14	0.002
Ringworm <sup>‡</sup> , %	37.3	44.0	36.6	68.9	12.8	0.20	0.083
Diarrhoea, %	13.3	10.2	5.80	12.0	3.81	0.64	0.10
Injured <sup>‡</sup> , %	0.0	8.00	1.10	16.8	6.07	0.26	0.74

<sup>1</sup> n = 10 cages/treatment. <sup>2</sup> Linear effect of density. Quadratic effect of density was always P > 0.30. <sup>3</sup> Effect of average weaning weight per cage. <sup>†</sup> Significant effect of contrast 24 vs (18, 12, 6) rabbits/m<sup>2</sup> (P < 0.050). <sup>‡</sup> Tendency effect for contrast 24 vs (18, 12, 6) rabbits/m<sup>2</sup> (0.050 < P < 0.15).

**Table 2.** Effect of cage density on individual growth performance of rabbits that reached 2.2 kg

Initial density, rabbits/m <sup>2</sup>	6	12	18	24	Rsd	L <sup>1</sup>	P <sub>cov</sub> <sup>2</sup>
Initial density, cm <sup>2</sup> /rabbit	1667	833	555	417			
<i>N</i>	28	54	80	91			
<i>30 (weaning)-44 d</i>							
Body weight at 44 d, g/rabbit	914	892	873	842	102	0.001	0.001
Daily gain, g/rabbit· d	26.3	24.7	23.1	20.9	7.62	0.001	0.12
<i>44-58 d</i>							
Body weight at 58 d, g/rabbit	1248	1199	1176	1137	143	0.001	0.001
Daily gain, g/rabbit· d	24.4	22.4	22.1	21.5	5.43	0.024	0.001
<i>58-85 d</i>							
Body weight at 85 d, g/rabbit	1831	1742	1701	1652	207	0.001	0.001
Daily gain, g/rabbit· d	20.8	19.4	18.7	18.4	4.45	0.014	0.39
<i>85 d-2.2 kg</i>							
Body weight d, g/rabbit	2151	2241	2127	2206	258	0.81	0.001
Daily gain, g/rabbit· d	17.3	20.1	14.9	15.7	6.63	0.004	0.011
<i>30 d (weaning)-2.2 kg</i>							
Daily gain, g/rabbit· d	22.2	21.3	18.9	18.1	3.34	0.001	0.31

<sup>1</sup> Linear effect of density. Quadratic effect of density showed always P > 0.30. <sup>3</sup> Effect of weaning weight.

**Table 3.** Effect of cage density on final rabbit production (kg live body weight/m<sup>2</sup>)

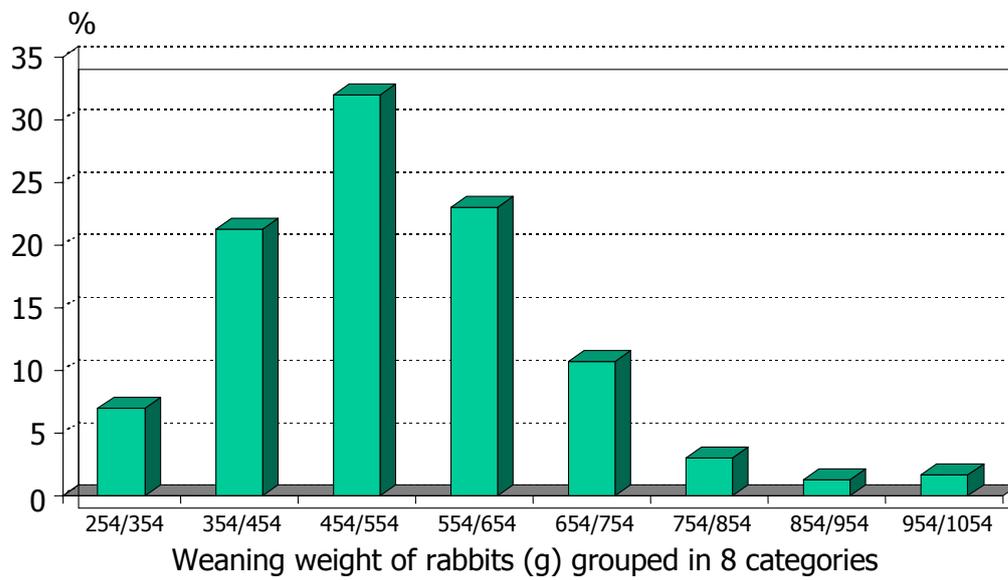
Initial density, rabbits/m <sup>2</sup>	6	12	18	24	EEM <sup>1</sup>	L <sup>2</sup>	Q <sup>3</sup>	P <sub>cov</sub> <sup>4</sup>
Initial density, cm <sup>2</sup> /rabbit	1667	833	555	417				
<i>44 d</i>								
Live body weight, kg/m <sup>2</sup>	4.74	9.76	14.4	17.0	0.52	0.001	0.018	0.11
No rabbits/m <sup>2</sup>	5.31	11.0	16.7	21.0	0.26	0.001	0.20	0.001
<i>58 d</i>								
Live body weight, kg/m <sup>2</sup>	6.51	12.9	19.4	22.1	0.75	0.001	0.066	0.15
No rabbits/m <sup>2</sup>	5.29	10.8	16.5	19.8	0.53	0.001	0.066	0.001
<i>85 d</i>								
Live body weight, kg/m <sup>2</sup>	9.59	18.7	27.8	31.6	1.04	0.001	0.014	0.062
No rabbits/m <sup>2</sup>	5.27	10.8	16.5	19.4	0.59	0.001	0.021	0.001
<i>2.2 kg</i>								
Live body weight, kg/m <sup>2</sup>	11.3	24.2	34.3	41.1	1.54	0.001	0.008	0.008
No rabbits/m <sup>2</sup>	5.19	10.8	16.4	18.9	0.56	0.001	0.024	0.001

<sup>1</sup> n = 10 cages/treatment. <sup>2</sup> Linear effect of density. <sup>3</sup> Quadratic effect of density. <sup>4</sup> Effect of average weaning weight per cage.

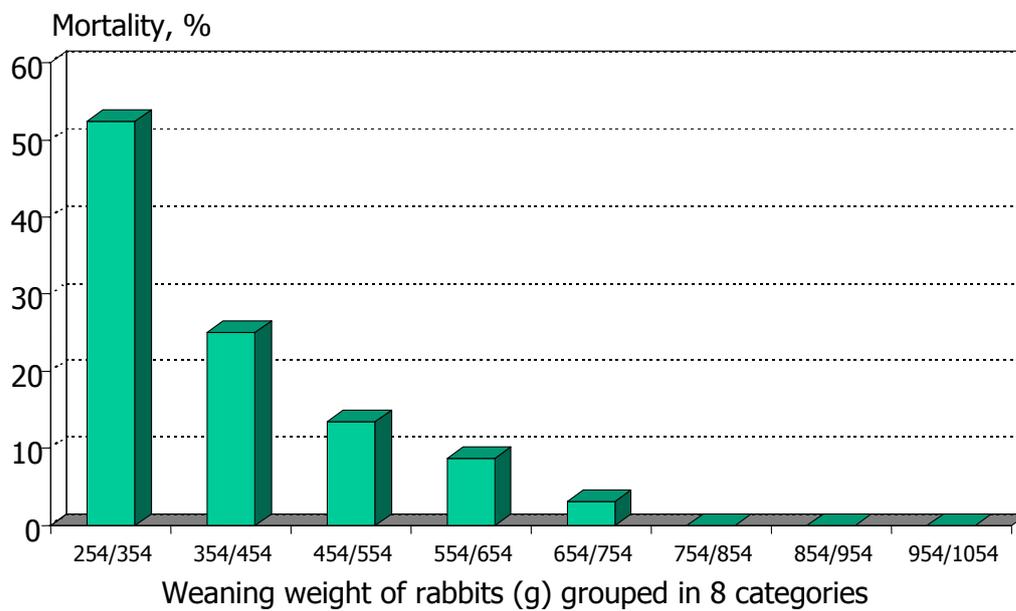
**Table 4.** Effect of cage density and sex on carcass performance

Initial density, rabbits/m <sup>2</sup>	6	12	18	24	Sex		rsd	L <sup>1</sup>	P <sub>sex</sub> <sup>2</sup>	P <sub>cov</sub> <sup>3</sup>
Initial density, cm <sup>2</sup> /rabbit	1667	833	555	417	Males	Females				
N <sub>♀</sub>	4	10	14	14						
N <sub>♂</sub>	6	10	15	16						
Live body weight at slaughter (BW), g	2261	2265	2159	2198	2188	2253	130	0.041	0.022	≠
Skin weight, %BW	16.1	16.8	16.9	16.8	17.3	16.0	1.19	0.27	0.001	0.48
Digestive tract weight, %BW	16.5	15.9	15.5	15.9	15.5	16.3	1.75	0.47	0.078	0.14
Hot carcass weight, %BW	62.9	62.9	62.5	62.5	62.7	62.7	2.05	0.52	0.92	0.20
Hot carcass weight (HCW), g	1386	1388	1380	1379	1384	1383	45	0.52	0.83	0.001
Chilled carcass weight (CCW), g	1230	1256	1262	1250	1252	1248	53	0.61	0.83	0.001
Dressing out percentage <sup>‡</sup> , % BW	55.7	56.9	57.2	56.6	56.7	56.5	2.48	0.56	0.88	0.014
Drip loss percentage <sup>‡</sup> , % HCW	11.3	9.62	8.52	9.41	9.54	9.85	2.50	0.097	0.88	0.016
Head weight, % CCW	10.0	9.96	10.2	10.3	10.3	9.97	0.83	0.22	0.14	0.001
Liver weight, % CCW	4.79	4.89	5.19	5.01	5.00	4.93	0.97	0.50	0.84	0.72
Kidney weight, % CCW	0.95	0.92	0.92	0.90	0.93	0.91	0.15	0.34	0.56	0.12
Other viscera weight <sup>4</sup> , % CCW	1.97	2.08	2.07	2.03	2.07	2.01	0.32	0.98	0.44	0.17
All viscera weight, % CCW	7.71	7.90	8.18	7.94	8.00	7.86	1.17	0.65	0.66	0.38
Perirenal fat weight, % CCW	1.63	1.81	1.54	1.54	1.63	1.63	0.53	0.22	0.97	0.001
Scapular fat weight, % CCW	0.67	0.88	0.89	0.99	0.86	0.86	0.45	0.042	0.88	0.13
Whole fat weight, % CCW	2.29	2.73	2.44	2.54	2.51	2.50	0.87	0.75	0.95	0.003
Reference carcass weight, g	1014	1033	1031	1022	1024	1026	55	0.97	0.83	0.001
Reference carcass weight, % BW	45.9	46.8	46.6	46.3	46.4	46.5	2.56	0.96	0.77	0.002
Dorsal length, cm.	26.9	27.3	26.1	25.4	26.2	26.6	1.61	0.001	0.37	0.039
Thigh length, cm.	7.21	7.13	7.52	7.20	7.25	7.28	0.76	0.86	0.76	0.17
Lumbar circumference <sup>‡</sup> , cm.	16.3	16.7	16.5	16.1	16.2	16.5	0.86	0.13	0.11	0.001

<sup>1</sup> Linear effect of cage density. <sup>2</sup> Effect of sex. <sup>3</sup> Effect of live body weight at slaughter. <sup>4</sup> Thymus, trachea, oesophagus, lungs and heart. <sup>†</sup> Quadratic effect of cage density (P < 0.05). <sup>‡</sup> Quadratic trend of cage density (0.05 < P < 0.15).



**Figure 1.** Rabbit distribution according to their weaning weight (n = 300)



**Figure 2.** Relation between weaning weight and mortality during fattening (n= 300)

## ***CHAPTER 3***

*EFFECT OF BREEDING SYSTEM, CICLE AND CAGE  
SIZE DURING FATTENING ON RABBIT DOE AND  
GROWING RABBIT PERFORMANCE UNDER HEAT  
STRESS*

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## ABSTRACT

In order to evaluate heat stress and circadian rhythm 46 nulliparous rabbit does with a BW of  $3.67 \pm 0.05$  kg (s.e.) were used. They were clipped once or not and rectal temperature, feed and water intake were recorded for 24 h. From this group, 43 rabbit does were mated 7 d after rectal measurements, and randomly assigned to one out of two breeding systems (including in both systems rabbit does that had been clipped or not). In the control one (C) rabbit does were mated 14 d after parturition and litter weaned at 35 d of age, and in the extensive one (E) they were mated 21 after parturition and weaned at 42 d of age. Rabbit doe and litter performance were recorded for six months (first three cycles). Two hundred twenty eight weaned rabbits were divided into two cage sizes: 0.5 and 0.25 m<sup>2</sup> with 8 and 4 rabbits per cage, respectively, to study growing performance. Farm and rectal temperatures were minimal and feed and water intake maximal during the night ( $P < 0.001$ ). Unclipped rabbit does showed higher rectal temperature ( $P = 0.045$ ) and lower feed intake respect to clipped does ( $P = 0.019$ ), which are symptoms of heat stress. Neither breeding system nor cycle number influenced fertility, total number of kits born, born alive or dead per litter (91.6%, 6.98, 5.80 and 1.19 on average, respectively). Kit mortality during lactation tended to increase in E compared to C group (48.5 vs. 63.4%;  $P = 0.070$ ), reducing the number of kits at weaning per litter by 33% ( $P = 0.038$ ). It also increased in the 2<sup>nd</sup> and 3<sup>rd</sup> cycles compared to the first ( $P \leq 0.054$ ). It resulted that feed efficiency (g weaned kits/g feed intake does+litter) tended to decrease in E respect C group ( $P = 0.093$ ), whereas it was impaired successively from the first to the third cycle by 48% ( $P = 0.014$ ). Growing rabbits from the E group were heavier at weaning (by 38%.  $P < 0.001$ ), showed a higher feed intake (+7.4%) and lower feed efficiency (-8.4%) throughout the fattening period ( $P \leq 0.056$ ) respect to C group. However, age at slaughter was not different respect to C group (77.3 d on average). Cage size had minor influence in growing performance. In conclusion rabbit doe and litter productivity

impaired when lactation is extended from 35 to 42 d and along successive reproductive cycles.

**Keywords:** breeding system, cage size, performance, heat stress, rabbit.

## **IMPLICATIONS**

The longer is the productive life of the rabbit doe the lower productivity is obtained under heat stress conditions, mainly due to the high kit mortality during lactation. This impairment is probably related to the difficult of rabbit does to lose body heat that would lead to a reduction of milk production. Accordingly, it seems necessary to develop additional management (e.g. weaning earlier than at 35 d of age), feeding and/or housing strategies to help rabbit doe to remove heat losses in order to optimize rabbit production under heat stress.

## **INTRODUCTION**

The impairment of rabbit doe performance under seasonal heat stress conditions is well described both in experiments carried out during summer in Spain or Egypt (Méndez *et al.*, 1986; Marai *et al.*, 2002a) or in climatic chambers (Fernández-Carmona *et al.*, 1995; Fernández-Carmona *et al.*, 2003). In these studies it is observed that hot climate increased body temperature and reduced feed and digestible energy intake, milk production, litter size and growth, and rabbit doe weight, and increased kit mortality, but performances improved once the heat stress period is overcome. Little information has been obtained in conditions of chronic exposure to heat stress that might difficult the recovery of the animal in any period of the year, as occurs in Maracaibo that combines high temperature and relative humidity along the year. However, both environmental conditions (temperature and relative humidity) and intake/excretion habits in rabbits (Prud'hon, 1973; Carabaño and Merino, 1996) follow circadian rhythms, being feed intake during night (cooler period), that might make easier their adaptation to these conditions.

Heat stress not only reduced rabbit doe productivity but also growth performance of fattening rabbits (Pla *et al.*, 1994; Chiericato *et al.*, 1995; Ayyat

and Marai, 1997). Moreover, under dry tropical conditions fattening mortality increased in small weaned rabbits (< 450 g, which accounted for 25% of the total number of rabbits). Besides, increasing stock densities reduced growth performance and enhanced aggressiveness (Villalobos *et al.*, 2008). The latter is also stimulated by increasing group size in European conditions, when stock density is constant (Princz *et al.*, 2008), and might be a relevant factor under tropical conditions.

Accordingly, under heat stress conditions reproductive management and age at weaning may play an important role to optimize rabbit doe and growth performance. The objective of this work was i) to evaluate heat stress and circadian rhythms in our conditions comparing unclipped and clipped rabbit does, ii) to study if a more extensive breeding system (delay of mating and weaning) may increase weight of litters at weaning without impairing rabbit doe performance, and iii) to determine if cage size maintaining stock density influences growth performance.

## **MATERIAL AND METHODS**

### **Management and housing**

The experiment was conducted at Ana Maria Campos Experimental Farm facilities from late April to the beginning of November. The climate condition is characterized as tropical very dry forest (Holdridge, 1978). The minimal-maximal temperature recorded along the experiment was 25.5-34.5°C and the minimal-maximal relative humidity 65-96%. The farm is an open-air building equipped with a ventilator to favour air recycling and a mesh (80% shade) in the windows to avoid animals were exposed to the sun. There were no artificial light. Rabbits does were housed in flat-deck cages of 500 × 1000 × 500 mm (0.5 m<sup>2</sup>) equipped with one nipple drinker and one hopper feeder (30 cm available) each one. A

commercial pelleted diet containing (g/kg DM) 163 crude protein, 364 neutral detergent fibre and 43 acid detergent lignin, was offered *ad libitum* to all the animals throughout all the trials described. Water was filtered before stored in the farm water-tank. The temperature-humidity index (THI) was calculated according to Marai *et al.* (2001):  $THI = db^{\circ}C - [(0.31 - 0.031 RH) \times (db^{\circ}C - 14.4)]$ , where  $db^{\circ}C$  is dry bulb temperature in Celsius degrees, and RH is the relative humidity as percentage. According to Marai *et al.*(2002b) there is heat stress when THI is higher than 28.9, and under 27.8 there is no heat stress.

### **Heat stress evaluation and circadian rhythms of rabbit does trial**

Forty six crossbred New Zealand White, Californian, Butterfly, Dutch and Satin nulliparous rabbit does with an average weight of  $3.67 \pm 0.05$  kg (s.e.) were divided randomly in two groups (23 per treatment). The first one was clipped once two days before the beginning of the measurements and the second one remained unclipped to study only their capacity to eliminate heat in our conditions as it is not a usual practice. Clipped area extended from scapula to sacred zone with a 20 cm width. A bovine electrical clipping machine was used. During 24 consecutive hours, rectal temperature, feed and water intake were recorded every 4 hours. Rectal temperature was measured by using a clinical digital thermometer inserted 2 cm into the rectum for 2 minutes. In the same period it was registered the farm temperature and the relative humidity. After this experiment rabbit does were not clipped again.

### **Breeding system trial**

Forty three crossbred New Zealand White, California, Butterfly, Dutch and Satin rabbit does from the latter experiment were mated 7 d after rectal measurements with an average weight of  $3.58 \pm 0.06$  kg, by using twelve male rabbits weighing  $3.42 \pm 0.08$  kg. Each rabbit doe was mated twice using two different males, and males mounted twice per day. Once confirmed pregnancy rabbit does were randomly assigned to the two breeding systems (including in

both systems rabbit does that had been clipped or not and from the different crossbred types). In the control one (C) rabbit does were mated 14 d after parturition and litter was weaned at 35 d of age, and in the extensive one (E) they were mated 21 d after parturition and weaned at 42 d of age. At 21 d of lactation the nest were closed (and only open at 0700 h for 10-15 min daily to allow suckling, that was not measured) and a feeder with the same feed of the mother was placed inside the nest to record litter feed intake from 21 d up to weaning, separately from that of their mothers. The following measurements were recorded for six months (the first three cycles, each cycle considered the period between two consecutive parturitions): eliminated does (culled and dead), doe weight (at birth, at 21 d lactation and at weaning), rabbit doe feed intake (at birth, 21 d lactation and at weaning), fertility (number of mating per doe to get pregnant: 100, 50, 33.3%, etc. means mated at first, second or third mating, respectively), pregnancy length, number of kits and kit mortality (at birth, 21 d lactation and at weaning), litter weight and litter feed intake, and feed efficiency among parturitions. It was also studied the difference between the real and theoretical parturition-effective mating interval, being the theoretical values 14 and 21 d for control and extensive reproductive breeding systems, respectively.

### **Cage size trial**

Two hundred twenty eight weaned rabbits from the first two cycles of the latter experiment were divided into two cage sizes for fattening. Half of the animals of each treatment were caged in wire-mesh cages of 500 × 1000 × 500 mm (0.5 m<sup>2</sup>) at a rate of 8 rabbits per cage. The remaining group was housed in cages of 500 × 500 × 500 mm (0.25 m<sup>2</sup>) at a rate of 4 rabbits per cage. Both groups maintained the same stock density (16 rabbit/m<sup>2</sup>), that is lower than the maximal recommended by Villalobos *et al.* (2008) in our conditions (18 rabbit/m<sup>2</sup>). The large cages were equipped with two nipple drinkers and two hopper feeders, whereas the small cages had one nipple drinker and one hopper feeder. Feed intake, growth rate, feed efficiency, length of fattening,

aggressiveness (number of rabbits injured) and mortality was recorded until rabbits reached 2.1 kg BW.

### **Statistical analysis**

Results obtained from doe rabbits to evaluate heat stress and rabbit doe and litter performances were analyzed by a repeated measures analysis using MIXED procedure of SAS (Littell *et al.*, 1996). The model for evaluating heat stress included treatment (clipped or not), period of the day (2200 to 0200, 0200 to 0600, 0600 to 1000, 1000 to 1400, 1400 to 1800, and 1800 to 2200 h) and its interaction. In the case of rectal temperature metabolic weight was also included as a covariate. Variance and covariance matrix structure were modelled according to an auto-regressive structure for the measurements repeated in each rabbit doe, which was considered as a random effect (Littell *et al.*, 1998). The model for rabbit doe and litter performances included breeding system (control *vs.* extensive), cycle number (1, 2, and 3) and its interaction. Variance and covariance matrix structure was modelled according to a compound symmetry structure and rabbit doe was also considered a random effect. Means comparison was made by t-test when cycle effect was significant ( $P < 0.050$ ). Data from total removed, dead and culled does were analysed using logistic regression (GENMOD procedure of SAS, considering a binomial distribution) and results were transformed from the logit scale. Finally, data from the cage size trial was analyzed as a factorial structure 2 (cage sizes)  $\times$  2 (breeding systems)  $\times$  2 (cycles) by using GLM procedure of SAS, including in the model as main factors cage size (large/0.5 *vs.* small/0.25 m<sup>2</sup>), breeding system (control *vs.* extensive) and cycle number (1 *vs.* 2) and their interactions. All variables in tables are least squared corrected means. Statistical analysis was made using the Statistical Analysis System, SAS (version 8.2).

## RESULTS

### Heat stress evaluation and circadian rhythms of rabbit does trial

In our farm rabbits does were under heat stress from 0800 to 1900 h (Figures 1 and 2), being exposed to very severe stressed from 1000 to 1800 h. It is in agreement with the influence of day time on rectal temperature ( $P < 0.001$ ). Maximal rectal temperatures match up with the sun period were farm temperature and THI increased, and relative humidity decreased (Figure 3). The maximum farm temperature (and THI value) is reached around 1400 h, that is the same time in which rabbits showed their highest rectal temperature. Day time also affected feed and water intake ( $P < 0.001$ , Figures 4 and 5), decreasing both during the sun light period. From 1800 h onwards farm temperature/THI, and rectal temperature decreased and rabbit does begun their feed and water intake period. The lowest farm temperature/THI and rectal temperature and highest feed and water intake were observed during night that is the natural period for rabbit meal.

Rabbit does that remained unclipped increased rectal temperature (39.4 vs. 39.3°C,  $P = 0.045$ ) and decreased feed intake (7.87 vs. 10.0 g/kg<sup>0.75</sup>/4 h,  $P = 0.019$ ) respect to those clipped. An interaction clipping × time was observed for feed intake due to the especially high feed intake of clipped rabbits at the first hours after sunset ( $P < 0.043$ , Figure 4). Water intake varied in parallel to feed intake and was not influenced by clipping (21.6 ml/kg<sup>0.75</sup>/4h, on average). The ratio water/feed intake in this study was 2.5 ml water/g feed.

### Breeding system trial

The proportion of total eliminated, dead and culled does was not affected either by breeding system or by cycle number being on average 21, 14.1 and 6.8%, respectively (Table 1). Fertility and weight of rabbit does at birth, 21 d lactation and weaning was not affected by breeding system or cycle number,

being on average 92.2% and 3.66, 3.71 and 3.79 kg respectively (Table 2). Pregnancy length tends to increase by 0.6 d with the extensive breeding system compared to the control one ( $P= 0.10$ ) with no effect of cycle number. The difference between the real and theoretical parturition-effective mating interval was not affected by breeding systems, but it increased in the third compared the first two cycles ( $P = 0.046$ ). Feed intake of rabbit does from birth to weaning tended to decrease with the extensive breeding system compared to the control one ( $P = 0.11$ ). However, feed intake from weaning to next parturition and among parturitions were not affected by breeding system (86.8, 44.0 and 72.0 g/BW<sup>0.75</sup> × d, respectively). Feed intake during lactation was higher in the 2<sup>nd</sup> and 3<sup>rd</sup> cycles compared to the first one ( $P \leq 0.081$ ), but cycle number did not affect feed intake after weaning and among parturitions. Neither breeding system nor cycle number influenced the total number of kits born per litter and those born alive or dead (6.98, 5.80 and 1.19, respectively), but kit mortality at birth tended to be lower in the first cycle compared the next two cycles ( $P = 0.12$ ). The number of kits at 21 d per litter tended to decrease ( $P = 0.10$ ), whereas at weaning decreased by 33% ( $P = 0.038$ ) with the extensive breeding system compared the control one. Cycle number did not affect these variables. Accordingly, extensive breeding system tended to increase kit mortality during lactation ( $P = 0.070$ ). It also increased in the 2<sup>nd</sup> and 3<sup>rd</sup> cycles compared to the first one ( $P \leq 0.054$ ). The extensive breeding system tended to reduce feed efficiency (g weaned kits/g feed intake does+litter) ( $P = 0.093$ ), whereas cycle number impaired it from the first to the third cycle by 48% ( $P = 0.014$ ). No effect was observed from the previous clipping on any trait of rabbit does.

Data of Table 2 is conditioned by the relatively high number of parturitions/lactations that finished with no weaned kits (34.5%). In order to show what are the real performances of litters in this study, additional information has been included considering only the litters with at least one kit at weaning (Table 3). In these litters, the extensive system did not affect the number of kits at birth, but tended to increased their weight ( $P = 0.11$ ) which is probably related to the trend to increase pregnancy length. The extensive system

reduced the number of kits at 21 d and at weaning (by 28%.  $P \leq 0.008$ ) and litter weight at 21 d (by 26%.  $P = 0.038$ ). However, kits from the extensive group tended to be heavier at 21 d ( $P = 0.086$ ) and at weaning (by 39%.  $P = 0.001$ ). Litter feed intake were similar from 21 to 35 d of lactation for both breeding systems (23.0 g/d and kit as average). However, it increased in the extensive compared to the control group from 21d to weaning (by 62%.  $P < 0.001$ ). The weight of litter at 21 d tended to be maximal in the first cycle compared 2<sup>nd</sup> and 3<sup>rd</sup> cycles ( $P = 0.084$ ), whereas litter feed intake was maximized at the 3<sup>rd</sup> cycle ( $P = 0.018$ ).

### **Cage size trial**

Growing rabbits from the extensive breeding system were heavier at weaning (by 38%.  $P < 0.001$ ), and showed a higher feed intake (+7.4%) and lower feed efficiency (-8.4%) throughout the fattening period ( $P \leq 0.056$ ) respect to rabbits from control breeding system (Table 4). Their fattening period was 9 days shorter than those of control group ( $P < 0.001$ ), but the age at the end of the fattening period was not different ( $77.3 \pm 1.70$  d). Growing rabbits caged in large cages increased feed intake by 5.6% throughout the fattening trial ( $P \leq 0.050$ ), but no influence was observed in growth rate and feed efficiency. Weaned weight of rabbits from the first cycle tended to be higher than those of the second cycle (1016 *vs.* 957.  $P = 0.10$ ), and tended to show a higher growth just after weaning (35.5 *vs.* 33.4.  $P = 0.15$ ) that was compensated later (25.7 *vs.* 27.8.  $P = 0.099$ ) obtaining similar final weight at the end of fattening. Neither breeding system nor cage size influence the uniformity of weight of rabbits at the end of fattening (average s.d.: 0.22 kg). There were no animals with injuries, and no mortality was observed in this experiment.

## **DISCUSSION**

### **Heat stress evaluation and circadian rhythms of rabbit does trial**

Most of the eating/drinking activity of nulliparous rabbit does was done during the night period that is the normal circadian rhythm of rabbits (Carabaño and Merino, 1996), and which is regulated by the time of sunrise/sunset (or light on/off). According to Marai *et al.* (2001) the night would be the only period with no heat stress in our farm and in which rabbit does showed minimal rectal temperature. However, it did not mean that rabbit does were completely free of heat stress, as when they remain unclipped they increased rectal temperature not only during the day but also during the night, decreasing their feed intake. In fact, when minimal temperature was higher than 24°C, as occurs in our study, Pascual *et al.* (1996) also observed an important reduction of milk production as a consequence of heat stress. Furthermore, data reviewed by Cervera *et al.* (1998) indicated that the upper critical temperature for rabbits would be close to 24-25°C. The ratio water/feed intake was higher than that recorded at 20°C in adult rabbits (Prud'hon, 1976), but lower than that observed by Finzi *et al.* (1992) in a climatic chamber (2.5 vs. 3.5 and 8.3 ml/g at 26 and 32°C, respectively) or by Marai *et al.* (2005) in Egyptian hot conditions (4.4 ml/g), and similar to Egyptian mild conditions (2.8 ml/g). It might be explained by the differences in both ambient temperature and water temperature and/or water quality among studies (Abdel-Samee, 1997; Marai *et al.*, 2005). These results suggest that feed intake is limited by heat stress, even during the night, and that clipping favours body heat dissipation enhancing feed intake during the night, as also observed by Finzi *et al.* (1994), probably due to the difficulty of increase heat loss during the day.

### **Breeding system trial**

The effects of heat stress can be observed when comparing our performances with recent European studies (Quevedo *et al.*, 2006a and 2006b;

Nicodemus *et al.*, 2010). In our conditions (that differed in environment, but also in genetic) we observed a reduction of the total number of kits born alive per litter (-4.1), that is accounted for the lower prolificacy (-3.2) and higher number of kits born dead (+0.9). We also recorded a higher mortality at birth (16.0 *vs.* 7.5%) and during lactation (56.0 *vs.* 10.0%) respect to these European studies, but similar to data obtained in hot conditions that might be partially explained by the reduction of milk production (not determined in this study) (Marai *et al.*, 2002a; Fernández-Carmona *et al.*, 2003). Besides, the high temperature might have also make difficult the search of the mammary area by young suckling kits, as according to Jeddi (1971) this search would be absent when temperature is higher than 36°C. However, minor differences were observed for fertility (Nicodemus *et al.*, 2002) suggesting that problems related to doe receptivity and/or male fertility were negligible.

The use of the extensive breeding system compared to the control one tended to increase kit mortality from birth to weaning, reducing the number of kits at 21d and at weaning per litter. These results might be accounted for a reduction of milk production in the extensive rabbit does, as their litters showed a lower weight at 21 d compared to the control group. Litter weight at 21 d is the variable more related to milk production as until to this time kits practically do not eat feed (Lebas, 1968; Fernández-Carmona *et al.*, 2006). Accordingly, the reduction of milk production would have impaired kit viability. In fact, most of mortality of suckling rabbits (> 90%) occurred during the first 21 d of lactation. The reduction of milk production might be explained by the trend to reduce feed intake during lactation in rabbit does from the extensive group. The longer lactation in the extensive system might have increased heat load, resulting in a reduction of feed intake in this period and milk production in order to reduce heat production. These results and the lack of effect of breeding system on feed intake of rabbit does among parturitions lead to a trend to reduce feed efficiency of rabbit does from the extensive group. When feed efficiency is calculated for the global productive period (breeding + fattening periods), the differences between control and extensive breeding system still remain (0.289 *vs.* 0.225 g

rabbit at the end of fattening/g total feed consumed (lactation + fattening). The numerical productivity (number of weaned kits per rabbit doe and year) decreased in the extensive group (23.3 vs. 13.0, respectively), showing values much lower than in European conditions ( $\geq 62$  kits weaned/cage and year. Nicodemus *et al.*, 2002). However, breeding system did not affect weight of rabbit does, the difference between the real and the theoretical parturition-effective mating interval, prolificacy, or fertility, that remained high compared to previous studies performed under hot conditions (Marai *et al.*, 2002a).

Rabbit does and litter performances were impaired along the successive reproductive cycles, from the 1<sup>st</sup> to the 3<sup>rd</sup> one (delay between the real and theoretical parturition-effective interval, increase of kit mortality during lactation, and decrease of litter weight at 21 d and at weaning and feed efficiency). Besides, rabbit does were not able to growth from the first parturition onwards, in spite of the increase of lactation feed intake along cycles, which apparently would have avoided to reach their adult weight (Fernández-Carmona *et al.*, 2003). These results show that rabbit doe productivity decreased along successive reproductive cycles or when lactation was extended, possibly due to its progressive weakening, and its inability to recover properly under our chronic heat stress conditions.

### **Cage size trial**

The limited milk production would have been the responsible of the high mortality rate during lactation. It would have produced a natural selection, and that the survival kits were those stronger kits of each litter, that might explain the absence of mortality during fattening. Growing rabbits weaned at 42 d of age were heavier at weaning, and showed a higher feed intake and lower feed efficiency throughout the fattening period than those weaned at 35 d. Garrido *et al.* (2009) also recorded a higher weaning weight and lower feed efficiency in the growing rabbits weaned later (35 vs. 25 d of age).

Large cages enhanced feed intake compared to small cages, probably due to feed waste. In general, rabbits housed in larger groups reduced the time of resting and increased their activity in European conditions (Princz *et al.*, 2008), which would have favoured feed waste. However type of cage did not influence growth rate, feed efficiency, uniformity of final weight or aggressiveness as observed under European conditions by Rommers and Meijerhof (1998).

In conclusion, these results indicate that our rabbits are under heat stress. In this situation, as longer is the production period (either extended lactation from 35 to 42 d or cycle number) the lower productivity is obtained, mainly due to the high kit mortality during lactation. It might be probably related to the difficult of rabbit does to lose body heat that would lead to a reduction of milk production. Accordingly it seems necessary to develop additional management (e.g. weaning earlier than at 35 d of age), feeding and/or housing strategies to help rabbit doe to remove heat losses.

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**Table 1.** Effect of breeding system (remating interval/weaning age: 14/35 vs. 21/42 d post partum) and cycle on total eliminated, mortality and culled rabbit does

	Breeding system		Cycle <sup>‡</sup>		
	Control 14/35	Extensive 21/42	1	2	3
Remating interval/Weaning age, d					
Number of rabbit does	24	19	43	40	36
Total eliminated, %	20.8	21.1	6.98	10.0	5.55
Mortality, %	12.5	15.8	2.32	7.50	5.55
Culling, %	8.33	5.26	4.65	2.50	0.00

<sup>‡</sup>No significant effect of breeding system, cycle and breeding system × cycle were detected.

**Table 2.** Effect of breeding system (remating interval/weaning age: 14/35 vs. 21/42 d post partum) and cycle on rabbit doe performance (mean  $\pm$  s.e.)

	Breeding system		Cycle			Significance <sup>1</sup>	
	Control 14/35	Extensive 21/42	1	2	3	Breeding system	Cycle
Remating interval/Weaning age, d							
Number of rabbit does	22	18	40	36	34		
Doe weight, kg							
At birth	3.73 $\pm$ 0.075	3.59 $\pm$ 0.084	3.65 $\pm$ 0.060	3.64 $\pm$ 0.061	3.69 $\pm$ 0.062	ns	ns
At 21 d lactation	3.73 $\pm$ 0.088	3.69 $\pm$ 0.096	3.76 $\pm$ 0.072	3.73 $\pm$ 0.077	3.64 $\pm$ 0.081	ns	ns
At weaning	3.81 $\pm$ 0.088	3.77 $\pm$ 0.10	3.78 $\pm$ 0.069	3.84 $\pm$ 0.072	3.75 $\pm$ 0.083	ns	ns
Fertility, %	92.4 $\pm$ 2.69	91.1 $\pm$ 2.92	91.3 $\pm$ 3.26	90.3 $\pm$ 3.34	93.6 $\pm$ 3.61	ns	ns
Pregnancy length, d	32.1 $\pm$ 0.24	32.7 $\pm$ 0.26	32.4 $\pm$ 0.22	32.4 $\pm$ 0.22	32.5 $\pm$ 0.24	0.10	ns
Difference between real and theoretical parturition-effective mating interval, d	4.88 $\pm$ 2.32	7.15 $\pm$ 3.22	1.69 <sup>a</sup> $\pm$ 2.19	2.55 <sup>a</sup> $\pm$ 2.31	13.8 <sup>b</sup> $\pm$ 4.44	ns	0.046
Feed intake of rabbit does, g/(BW <sup>0.75</sup> $\times$ d)							
Birth- weaning	86.6 $\pm$ 2.76	79.9 $\pm$ 3.03	77.2 <sup>a</sup> $\pm$ 2.55	84.9 <sup>b</sup> $\pm$ 2.87	87.7 <sup>b</sup> $\pm$ 3.73	0.11	0.026
Weaning- next parturition	45.8 $\pm$ 4.27	42.3 $\pm$ 5.77	44.5 $\pm$ 4.18	41.3 $\pm$ 4.99	46.2 $\pm$ 7.60	ns	ns
Among parturitions	74.1 $\pm$ 2.62	70.0 $\pm$ 3.45	70.2 $\pm$ 2.46	74.9 $\pm$ 2.92	71.1 $\pm$ 4.46	ns	Ns
Total number kits born per litter	7.26 $\pm$ 0.41	6.71 $\pm$ 0.46	6.48 $\pm$ 0.41	7.32 $\pm$ 0.42	7.17 $\pm$ 0.45	ns	Ns
Number kits born alive	5.82 $\pm$ 0.38	5.78 $\pm$ 0.42	5.61 $\pm$ 0.40	5.90 $\pm$ 0.40	5.88 $\pm$ 0.43	ns	Ns
Number kits born dead	1.45 $\pm$ 0.28	0.94 $\pm$ 0.31	0.87 $\pm$ 0.28	1.41 $\pm$ 0.29	1.31 $\pm$ 0.31	ns	Ns
Number kits at 21 d per litter	3.39 $\pm$ 0.37	2.47 $\pm$ 0.41	3.23 $\pm$ 0.38	2.80 $\pm$ 0.39	2.77 $\pm$ 0.43	0.10	Ns
Number kits at weaning per litter	3.25 $\pm$ 0.34	2.16 $\pm$ 0.37	3.12 $\pm$ 0.37	2.56 $\pm$ 0.38	2.43 $\pm$ 0.40	0.038	Ns
Kit mortality, %							
At birth <sup>2</sup>	19.8 $\pm$ 3.82	12.1 $\pm$ 4.24	11.2 $\pm$ 3.73	20.1 $\pm$ 3.76	16.7 $\pm$ 4.01	ns	0.12
Birth-21 d lactation <sup>3</sup>	47.8 $\pm$ 5.68	57.8 $\pm$ 6.21	41.7 $\pm$ 5.94	57.5 $\pm$ 6.07	59.2 $\pm$ 6.70	ns	0.054
Birth-weaning <sup>3</sup>	48.5 $\pm$ 5.38	63.4 $\pm$ 5.96	43.1 <sup>a</sup> $\pm$ 5.86	60.3 <sup>b</sup> $\pm$ 5.99	64.4 <sup>b</sup> $\pm$ 7.02	0.070	0.029
Litter weight at weaning, kg	2.52 $\pm$ 0.29	2.32 $\pm$ 0.32	3.05 $\pm$ 0.31	2.19 $\pm$ 0.32	2.02 $\pm$ 0.38	ns	0.054
Feed efficiency, g weaned kits/g feed intake rabbit doe + litter	0.234 $\pm$ 0.022	0.175 $\pm$ 0.026	0.274 <sup>a</sup> $\pm$ 0.026	0.197 <sup>ab</sup> $\pm$ 0.029	0.142 <sup>b</sup> $\pm$ 0.036	0.093	0.014

The interaction breeding system  $\times$  cycle was not significant ( $P > 0.15$ ) for all traits. <sup>2</sup> Expressed over total born. <sup>3</sup> Expressed over number of born alive

**Table 3** Effect of breeding system (remating interval/weaning age: 14/35 vs. 21/42 d post partum) and cycle on litter performance during lactation (mean  $\pm$  s.e.) in litters that at least weaned one kit per parturition<sup>1</sup>

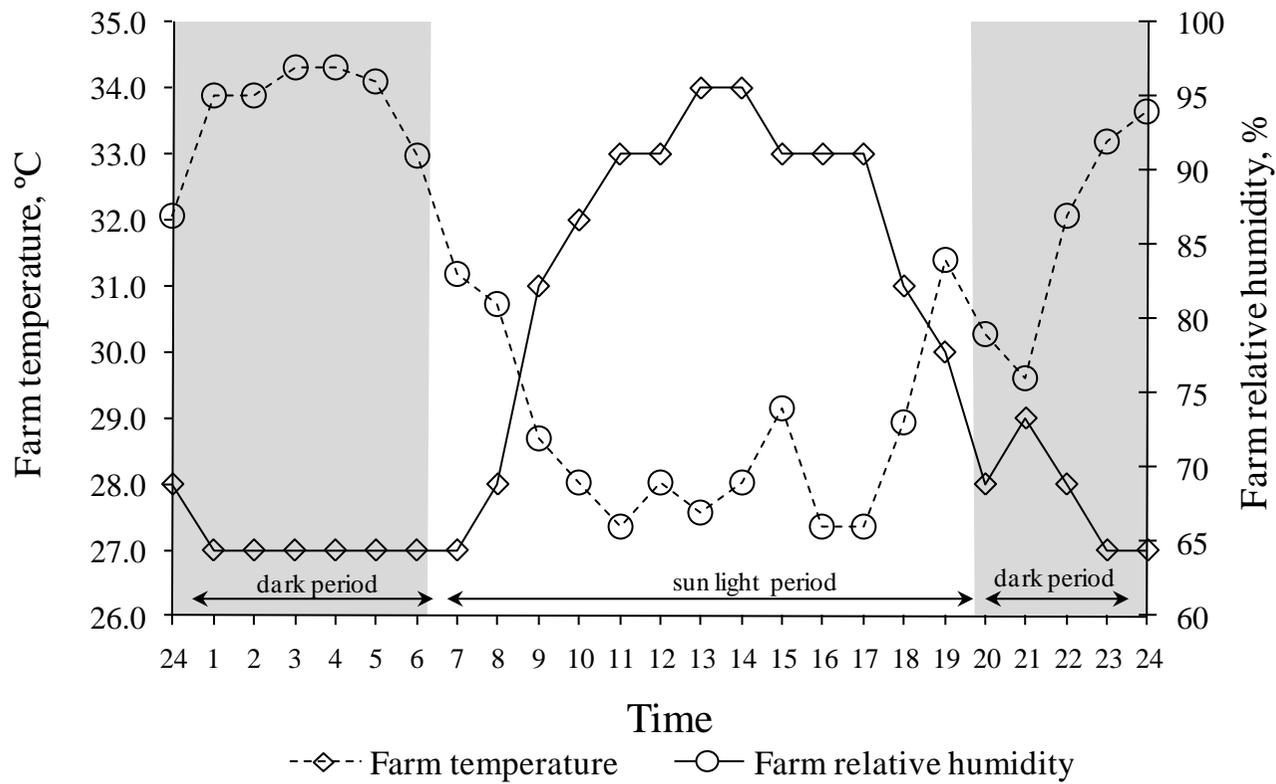
	Breeding system		Cycle			Significance <sup>2</sup>	
	Control 14/35	Extensive 21/42	1	2	3	Breeding system	Cycle
Remating interval/Weaning age, d							
Number of rabbit does							
For birth data	22	18	39	35	33		
For weaning data	20	17	31	24	15		
Number of kits at birth	6.23 $\pm$ 0.33	5.78 $\pm$ 0.35	5.75 $\pm$ 0.37	6.16 $\pm$ 0.37	6.12 $\pm$ 0.40	ns	ns
Number of kits at 21 d	4.71 $\pm$ 0.29	3.49 $\pm$ 0.32	4.25 $\pm$ 0.30	4.23 $\pm$ 0.33	3.82 $\pm$ 0.35	0.008	ns
Number of kits at weaning	4.67 $\pm$ 0.29	3.30 $\pm$ 0.32	4.13 $\pm$ 0.28	3.97 $\pm$ 0.32	3.87 $\pm$ 0.34	0.004	ns
Weight of kits at:							
Birth, g	56.6 $\pm$ 1.91	58.2 $\pm$ 2.08	55.5 $\pm$ 1.98	55.3 $\pm$ 2.02	57.0 $\pm$ 2.13	0.11	ns
21 d, g	352 $\pm$ 15.1	392 $\pm$ 16.7	386 $\pm$ 15.1	367 $\pm$ 16.6	363 $\pm$ 17.6	0.086	ns
Weaning, g	803 $\pm$ 28.9	1115 $\pm$ 32.6	1007 $\pm$ 28.6	931 $\pm$ 32.3	938 $\pm$ 41.3	0.001	0.14
Litter weight at 21 d lactation, kg	1.76 $\pm$ 0.087	1.30 $\pm$ 0.095	1.58 $\pm$ 0.086	1.43 $\pm$ 0.094	1.31 $\pm$ 0.10	0.038	0.084
Litter feed intake 21d-weaning, g/d $\times$ kit	22.4 $\pm$ 1.03	36.2 $\pm$ 1.26	29.6 <sup>ab</sup> $\pm$ 1.35	25.3 <sup>a</sup> $\pm$ 1.50	33.0 <sup>b</sup> $\pm$ 1.93	0.001	0.018

<sup>1</sup> Litters with no kit alive were excluded from these calculations. <sup>2</sup> The interaction breeding system  $\times$  cycle was not significant ( $P > 0.15$ ) for all traits.

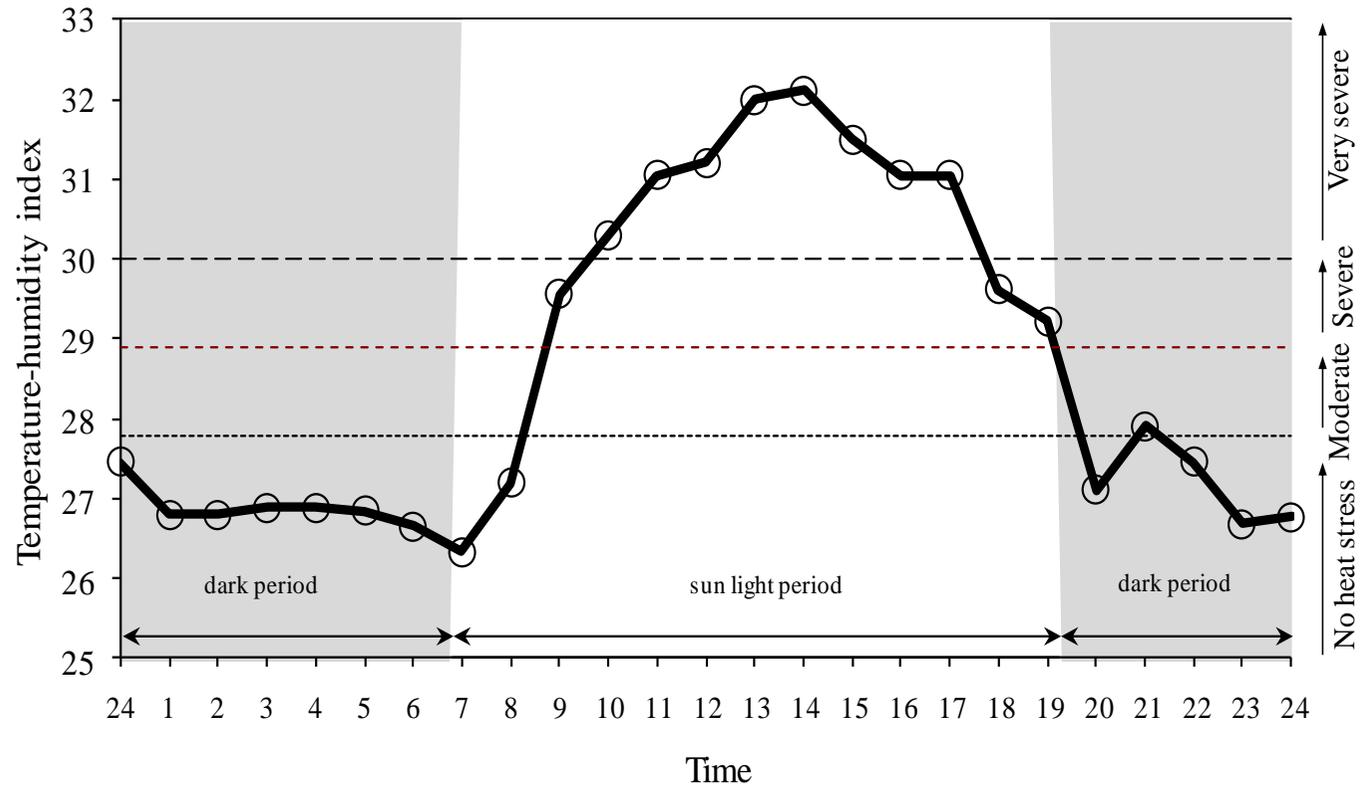
**Table 4** Effect of breeding system (remating interval/weaning age: 14/35 vs. 21/42 d post partum), cycle (1 and 2) and cage size (large vs. small) on growth performance from weaning to 2.1 kg body weight (results expressed per cage)

Breeding system Remating interval/Weaning age, d	Control 14/35		Extensive 21/42		rsd	Significance <sup>‡</sup>		
	Large	Small	Large	Small		Breeding system	Cage size	Cycle
Cage size								
No. cages	10	13	7	10				
Weaning weight, g/rabbit	795	860	1158	1132	108	0.001	ns	0.10
Weaning-7 d								
Feed intake, g/(rabbit × d)	61.3	70.0	75.2	88.9	15.7	0.003	0.034	ns
Growth rate, g/(rabbit × d)	35.1	37.4	35.4	37.2	5.70	ns	ns	ns
Feed efficiency, g gain/g intake	0.595	0.551	0.499	0.429	0.125	0.015	ns	ns
Weaning-14 d								
Feed intake, g/(rabbit × d)	72.0	78.4	85.2	93.5	10.9	0.001	0.046	ns
Growth rate, g/(rabbit × d)	35.0	35.6	32.9	34.5	4.42	ns	ns	0.15
Feed efficiency, g gain/g intake	0.490	0.467	0.389	0.378	0.084	0.001	ns	ns
14 d-2.1 kg								
Feed intake, g/(rabbit × d)	97.0	100.1	100.6	106.8	7.14	0.032	0.050	0.12
Growth rate, g/(rabbit × d)	27.3	26.9	25.2	27.4	3.53	ns	ns	0.099
Feed efficiency, g gain/g intake	0.281	0.269	0.250	0.258	0.032	0.056	ns	ns
Weaning-2.1 kg								
Feed intake, g/(rabbit × d)	89.1	92.9	94.5	101.0	5.73	0.001	0.008	ns
Growth rate, g/(rabbit × d)	29.6	29.9	28.3	30.2	2.66	ns	ns	ns
Feed efficiency, g gain/g intake	0.333	0.322	0.300	0.300	0.027	0.003	ns	ns
Fattening length, d	44.9	41.7	35.1	33.6	5.37	0.001	ns	ns

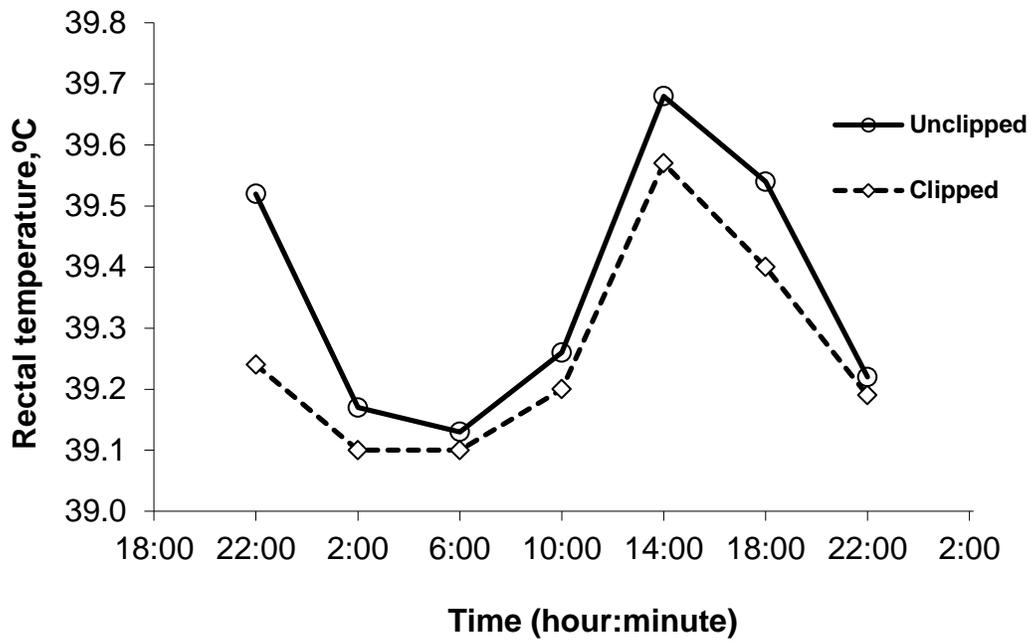
<sup>‡</sup> The interactions breeding system × cage size, breeding system × cycle, cage size × cycle, and breeding system × cage size × cycle were not significant for any trait.



**Figure 1.** Circadian evolution of temperature and relative humidity inside the farm during the 24 h experimental period to evaluate heat stress and circadian rhythms of rabbit does

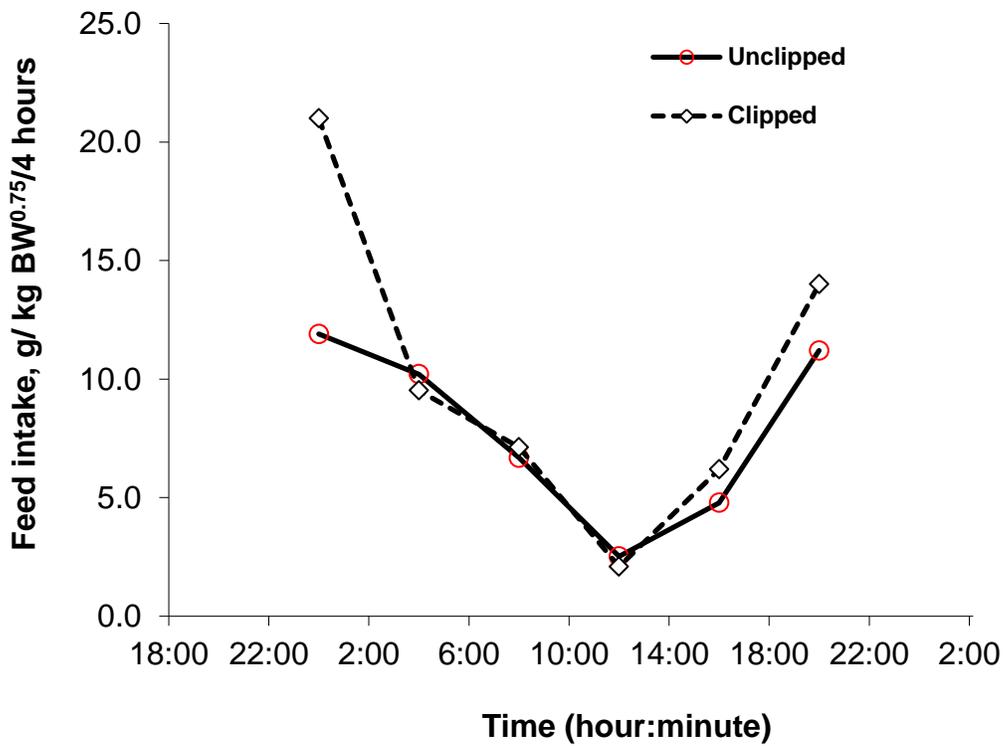


**Figure 2.** Circadian evolution of temperature-humidity index (THI) inside the farm calculated according to Marai *et al.* (2001) during the 24 h experimental period to evaluate heat stress and circadian rhythms of rabbit does



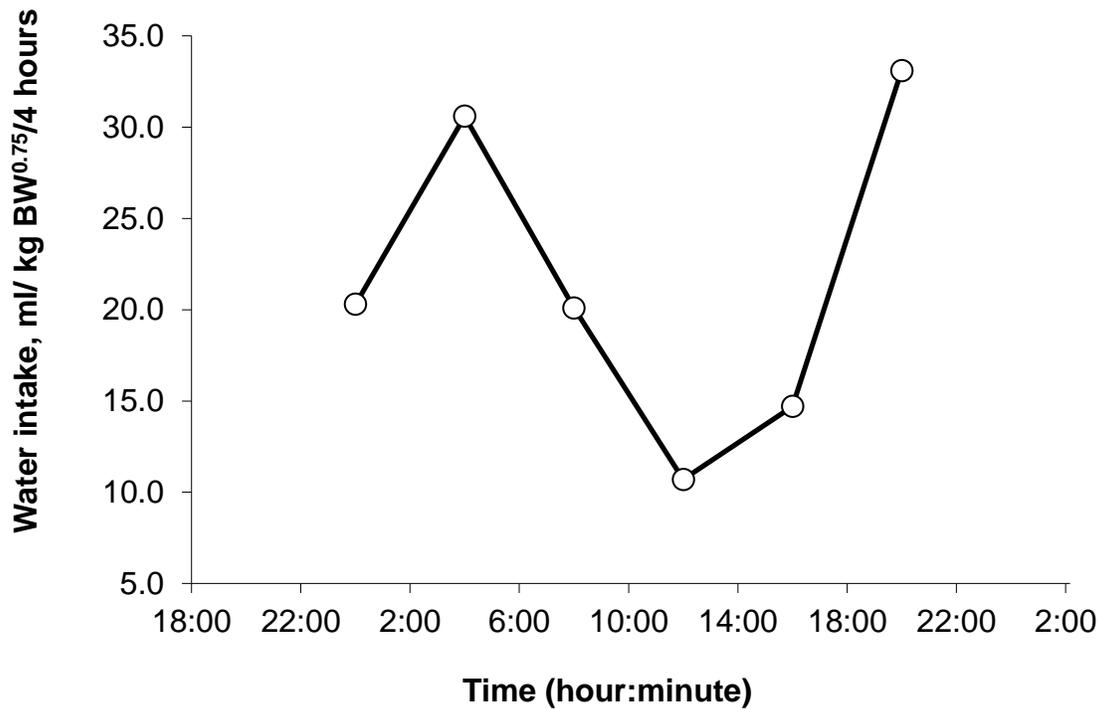
**Figure 3.** Circadian evolution of rectal temperature of clipped or unclipped rabbit does (n = 23/treatment).

( $P_{\text{clipping}} = 0.045$  and  $\text{s.e.}_{\text{clipping}} = 0.036$ ;  $P_{\text{time}} < 0.001$  and  $\text{s.e.}_{\text{time}} = 0.042$ ;  $P_{\text{clipping} \times \text{time}} = 0.22$  and  $\text{s.e.}_{\text{clipping} \times \text{time}} = 0.060$ ;  $P_{\text{metabolic weight}} = 0.021$ )



**Figure 4.** Circadian evolution of feed intake (g/kg BW<sup>0.75</sup>/4 hours) of clipped or unclipped rabbit does (n = 10/treatment)

( $P_{\text{clipping}} = 0.019$  and  $s.e._{\text{clipping}} = 0.61$ ;  $P_{\text{time}} < 0.001$  and  $s.e._{\text{time}} = 1.15$ ;  $P_{\text{clipping} \times \text{time}} = 0.043$  and  $s.e._{\text{clipping} \times \text{time}} = 1.62$ )



**Figure 5.** Circadian evolution of water intake (g/kg BW<sup>0.75</sup>/4 hours) of rabbit does (n= 10/treatment)

( $P_{\text{clipping}} = 0.75$  and  $s.e._{\text{clipping}} = 2.01$ ;  $P_{\text{time}} < 0.001$  and  $s.e._{\text{time}} = 3.95$ ;  $P_{\text{clipping} \times \text{time}} = 0.73$  and  $s.e._{\text{clipping} \times \text{time}} = 5.57$ )

## ***CHAPTER 4***

*EFFECT OF PHYSIOLOGICAL STATUS AND WATER  
QUALITY ON BODY TEMPERATURE, BODY CONDITION  
AND PRODUCTIVITY OF RABBIT DOES UNDER  
CHRONIC HEAT STRESS*

## **ABSTRACT**

The aim of this work was to characterize the rabbit doe response under chronic heat stress during pregnancy and the consecutive lactation and the effect of water quality. Forty five non pregnant and non lactating rabbit does (21 nulliparous and 24 multiparous) were assigned randomly to farm water (turbidity: 110 NTU) and to potable water (1.8 NTU). Farm temperature ranged from 23 to 37°C and relative humidity from 99 to 35%. Animals were implanted a transponder to record subcutaneous temperature at 07:30 and 14:30 h. Experimental period extended for one pregnancy and the next lactation (28 d) and it was recorded daily body temperature and milk production, and weekly body condition, feed and water intake. Water quality did not affect any trait throughout the experiment ( $P \geq 0.15$ ). All rabbit does became pregnant and according to their reproductive success they were classified as does that weaned their litters (W: 47%), does which litters died during lactation (NW: 44%) and pregnant does that did not deliver (NB: 9%). During pregnancy body temperature and feed intake decreased ( $P \leq 0.031$ ), water intake remained constant and energy and protein balance impaired in W and NW does from mating to birth ( $P \leq 0.011$ ). Body temperature of W does tended to be lower and feed intake higher ( $P \leq 0.090$ ) than those of NW and NB does. Body temperature during lactation showed a linear and quadratic effect of day of lactation, being maximal at day 12 of lactation. Body temperature decreased with metabolic weight during pregnancy ( $P \leq 0.009$ ) with no effect during lactation, whereas milk production increased it ( $P \leq 0.025$ ). Pregnancy length and total number of kits born tended to be longer and lower in NW than in W does ( $P = 0.10$  and  $0.053$ , respectively.). Kit mortality at birth and from birth to 14 d of lactation was high, being worse for NW than for W does (97 vs. 40%.  $P < 0.001$ ) related to the low kit vitality at birth rather than to a lack of milk production. In conclusion, in

our conditions heat stress limit productivity due to a reduced embryo/kit survival and the improvement of water quality did not attenuate negative effect of heat stress. More productive rabbit does seem to be those that manage better heat load.

**Key words:** physiological status, water quality, body temperature, heat stress, rabbit

## **INTRODUCTION**

Rabbit doe productivity is compromised under chronic heat stress conditions, especially due to the high kit mortality at birth and during the first weeks of lactation both in climatic chambers with controlled climatic conditions (Fernández-Carmona *et al.*, 1995) or under our chronic heat stress conditions (Villalobos *et al.*, 2010). In the latter work we observed that an extensification of the breeding system (longer lactation period and delayed mating after birth with the same weaning-birth interval) even impaired rabbit productivity, that could be related to its difficulty to manage properly heat load. Under heat stress conditions, water intake and water/feed intake ratio increases to favor heat loss through water respiratory vaporization as reviewed by Marai *et al.* (2002). In these circumstances, water quality is very relevant. The reduction of salinity in the water led to a decrease of rectal temperature and water intake improving growth rate (Marai *et al.*, 2005). The reduction of water temperature showed also a positive effect on rabbit does reducing kit mortality during lactation (Marai *et al.*, 2001), although this is more difficult to obtain in practical conditions.

The aim of this work was to characterize the rabbit doe response under chronic heat stress during pregnancy and the consecutive lactation and the effect of water quality by recording body temperature, body condition, feed and water intake and productivity.

## **MATERIAL AND METHODS**

### **Management and housing**

The experiment was conducted at Ana Maria Campos Experimental Farm facilities from late July to the end of November. The climate condition is

characterized as tropical very dry forest (Holdridge, 1978). The farm is an open-air building equipped with a ventilator to favour air recycling and a mesh (80% shade) in the windows to avoid animals were exposed to the sun. There were no artificial light. Temperature and relative humidity inside the farm was recorded daily every hour. The minimal-maximal temperature recorded along the experiment was 22.7-37.2°C and the minimal-maximal relative humidity 35-99% (Figures 1a, 1b). The temperature-humidity index (THI) was calculated according to Marai *et al.* (2001):  $THI = db^{\circ}C - [(0.31 - 0.031 RH) \times (db^{\circ}C - 14.4)]$ , where  $db^{\circ}C$  is dry bulb temperature in Celsius degrees, and RH is the relative humidity as percentage (Figure 1c). According to Marai *et al.* (2002) there is heat stress when THI is higher than 28.9, and under 27.8 there is no heat stress. Rabbits does were housed in flat-deck cages of 500 × 1000 × 500 mm (0.5 m<sup>2</sup>) equipped with one nipple drinker and one hopper feeder (30 cm available) each one. The nipple drinker was connected to a 5 L plastic container. A commercial pelleted diet containing (g/kg DM) 192 crude protein, and 120 crude fibre, was offered *ad libitum* to all the animals throughout all the trial.

### **Heat stress evaluation and water quality**

Forty five crossbred New Zealand White, Californian, Butterfly, Dutch and Satin non pregnant and non lactating rabbit does were divided randomly in two groups: 23 does drank treated farm water (well water filtered and chlorinated. Included 10 nulliparous does) and 22 potable water (sold for human consumption. Included 11 nulliparous does) (Table 1). Both types of water were fit for rabbit consumption (Gidenne *et al.*, 2010), but potable water had lower pH, turbidity, total salinity and conductivity and aerobic heterotrophic bacteria than farm water. Both types of water had an average temperature of 26°C (max-min: 28-23°C) at 7:30 h and 30°C (max-min: 32-27°C) at 14.30 h. In order to monitor subcutaneous rabbit doe temperature a IPTT-300 transponder (BioMedic Data Systems Inc., USA) was

implanted according to manufacturer instructions in each rabbit doe and body temperature recorded twice a day with a BMDS 1122 IPTT reader system (07:30 and 14:30 h, the moments of minimal and maximal body temperature in our conditions according to Villalobos *et al.*, 2010). Two weeks later rabbit does were mated. They were mated again 11 d after birth and experimental period ended at weaning (28 d of lactation). Each rabbit doe was mated twice using two different males that mounted twice per day. Does were separated from their litters after parturition. Milk production was estimated daily from weight loss of does during suckling (10 min, once a day). Feed and water intake of rabbit does was measured periodically. Growth and feed intake of young rabbits from 21 days of age until weaning at 28 days was measured. Number of kits, their weight and mortality were recorded during lactation. It was also estimated the body composition of does by the bioelectric impedance analysis (BIA, Pereda, 2010) at mating, during gestation (weekly), at birth and during lactation (7, 11, 19 d and at weaning). The first day of the experimental period rabbit does assigned to both types of water showed similar live body weight, body temperature and body energy content ( $3525 \pm 75$  g,  $39.4 \pm 0.1$  °C and  $35.0 \pm 1.7$  MJ, respectively. Mean  $\pm$  standard deviation).

AOAC (2000) procedures were used to determine the concentrations of DM (934.01), crude protein (968.06) and crude fibre (932.09). The water analyse were made following the 20th edition of Standard Methods for the Examination of Water and Wastewater (1998). It was determined aerobic heterotrophic bacteria (Pour plate method 9215C), total coliforms (Multiple-tube fermentation technique 9221B), fecal coliforms (Multiple-tube fermentation technique 9221E), pH (Electrometric method 4500-H<sup>+</sup> B), and turbidity (Nephelometric method 2130B).

### **Statistical analysis**

Body temperature (at 07:30 and 14:30 h, during gestation and lactation) was analyzed as repeated measurements along time using a mixed model that included

as fixed effects water quality, age and type of rabbit doe (age: nulliparous vs. multiparous; type: pregnant that weaned its litter, W, vs. pregnant which did not weaned its litter, NW, vs. pregnant with no birth, NB), polynomial contrasts of day from mating, the interactions between the previous factors, and linear effects of farm temperature, doe metabolic weight (expressed in kg) and daily milk production. Daily milk production was analysed similarly including in the model as fixed effects water quality, linear and quadratic effect of day of lactation, and the linear effect of number of suckling rabbits. Variance and covariance matrix structure were selected according to the AIC and BIC values, and modelled according to an auto-regressive structure, AR(1), for the measurements recorded daily (temperature and milk production) in each rabbit doe, which was considered a random effect (Di Rienzo *et al.*, 2011). In these cases, variability was reported with the residual standard deviation, *rsd*, and the autoregressive correlation coefficient, *arc*. Feed and water intake was also analyzed as repeated measurements along time for gestation and lactation using a mixed model that included as fixed effects water quality, age and type of rabbit, period of gestation or lactation, the interactions between the previous factors, and linear effects of farm temperature, doe metabolic weight and daily milk production. Variance and covariance matrix structure were modelled according to a compound symmetry structure and rabbit doe was also considered a random effect. Live body weight and body condition (moisture, fat, protein, ash and energy content and live weight) of rabbit does were analysed in the same way but farm temperature and milk production were not considered. Means comparison was made by t-test when the effect of type of rabbit doe was significant ( $P < 0.050$ ). Data from proportions of rabbit does according to their weaning success or their drinking behaviour, and kit mortality were analysed using logistic regression (considering a binomial distribution) and results were transformed from the logit scale. An analysis of variance was performed for energy and chemical composition balance, length of pregnancy, number of kits per litter and its weights including in the model water quality, age and type of rabbit doe. Only W rabbit does were

considered for the traits measured during lactation (temperature, body condition, milk production, water and feed intake). All variables in tables are least squared corrected means. Statistical analysis was performed using InfoStat.

## RESULTS

In spite of the differences on water quality it did not affect any trait throughout the experiment ( $P \geq 0.15$ ). Rabbit does were classified according to their reproductive success in order to correlate it with any of the measurements performed, resulting 47, 44 and 9% of W, NW and NB does, respectively. Body temperature at 07:30 was higher than at 14:30 h, both during pregnancy and lactation, mainly related to the higher farm temperature at midday ( $P < 0.001$ ; Figures 1a, 2a, 2b). Body temperature (at 07:30 and 14:30 h) decreased during pregnancy following a 5<sup>th</sup> order polynomial ( $P \leq 0.031$ , Figure 2a, 2b). When it was analyzed weekly it was observed a weekly linear effect in the second and third week and in the last days of pregnancy, but no change during the first and fourth week as shown in Figure 2. Body temperature of W does tended to be lower than those of NW and NB does, both at 07.30 (39.07 *vs.* 39.23  $\pm$  0.059 °C, respectively) or at 14.30 h (39.50 *vs.* 39.76 $\pm$  0.060 °C, respectively.  $P \leq 0.090$ ). Body temperature during lactation showed a linear and quadratic effect of day of lactation, being maximal at day 12 of lactation (44 d from mating). Body temperature increased with farm temperature at 07:30 and 14:30 h either in pregnancy (+0.020 and 0.11  $\pm$  0.0063 °C/°C, respectively.  $P < 0.001$ ) or in lactation (+0.071 and 0.11  $\pm$  0.010 °C/°C, respectively.  $P < 0.001$ ). Body temperature at 07:30 and 14:30 h decreased with metabolic weight during pregnancy (-0.42 and -0.57 $\pm$ 0.16 °C/kg BW<sup>0.75</sup>, respectively.  $P \leq 0.009$ ) with no effect during lactation, whereas milk production increased them (0.0015 and 0.0024 $\pm$ 0.00067 °C/g milk, respectively.  $P \leq 0.025$ ).

Nulliparous does tended to be warmer during pregnancy than multiparous does (+0.1°C,  $P = 0.10$ ), although during lactation their body temperature were similar (data not shown). Difference between body temperature at 07:30 and 14:30 h was higher during lactation than during pregnancy (0.56 vs.  $0.37 \pm 0.041$  °C,  $P < 0.001$ ).

Live body weight of rabbit does increased linearly during pregnancy ( $13.5 \pm 1.08$  g/d,  $P < 0.001$ , Figure 4a), and was higher in NB compared to W and NW rabbit does ( $P = 0.007$ ). It was accounted for the cubic increase of estimated body moisture content ( $P \leq 0.025$ , Figure 4b) in W and NW does, whereas in NB does for the linear increase in estimated body fat content ( $4.72 \pm 2.04$  g/d,  $P = 0.035$ , Figure 4c). The latter produced the parallel trend to increase estimated body energy in NB does ( $0.30 \pm 0.14$  MJ/d,  $P = 0.054$ , Figure 4d). Furthermore, in all does estimated body protein and ash content increased linearly during pregnancy ( $0.58 \pm 0.17$  and  $0.18 \pm 0.026$  g/d, respectively,  $P < 0.001$ , Figures 4e, 4f). In contrast, estimated body fat and energy content in W and NW does evolved cubically ( $P \leq 0.015$ ). Live body weight of rabbit does during lactation increased linear and quadratically reaching a maximal value at 22 d of lactation (54 d from mating,  $P \leq 0.048$ ). It was accounted partially for the linear increase of estimated body moisture content ( $7.04 \pm 1.50$  g/d,  $P < 0.001$ ), and for the quadratic increases of estimated protein and ash content (maximal values at 25 and 26 d of lactation,  $P \leq 0.048$ ). Furthermore, estimated body fat and energy content evolved quadratically ( $P < 0.001$ ) showing a maximal value around 13 d of lactation (45 d from mating). According to these results W and NW rabbit does showed a negative energy, and protein balance from mating to birth (-1.6 and -12.4%, -3.1 and -3.8%, respectively, expressed in percentage of the energy and protein content at mating) whereas it was higher ( $P \leq 0.011$ ) and positive for NB does (21.2 and 3.1%, respectively). At the end of lactation there were no significant variation in energy and chemical content in W does.

During pregnancy, nulliparous were lighter than multiparous does and accordingly contained less water, fat, protein and ash ( $P \leq 0.012$ , Data not shown).

Although they grew faster and retained more protein than nulliparous does during pregnancy (3.8 vs. -8.3%.  $P < 0.001$ ), but their size remained smaller during lactation compared to multiparous does ( $P \leq 0.003$ ).

Feed intake during pregnancy decreased linear and quadratically from mating to birth, mainly in the late third that decreased by 40% ( $P < 0.001$ , Table 2), and increased in heavier ( $33.8 \pm 15.1$  g/kg  $BW^{0.75}$ ,  $P = 0.026$ ) and in W compared NW and B rabbit does (by 17%,  $P = 0.064$ ). Water intake remained constant during pregnancy, being lower in nulliparous than in multiparous rabbit does (by 26%,  $P = 0.049$ ). These results led to an increase in the water/feed intake ratio during the second half of pregnancy ( $P < 0.001$ ) and lower ratios in nulliparous and W rabbit does (Table 2). Feed intake during lactation was only affected by milk production ( $0.55 \pm 0.12$  g/g,  $P < 0.001$ ). Water intake increased in the last third of lactation ( $P < 0.001$ ) resulting in an increase of water/feed intake in this period ( $P = 0.005$ ). Lactation water intake increased with milk production ( $1.30 \pm 0.48$  g/g,  $P = 0.009$ ). Daily milk production evolved linear and quadratically along lactation ( $P < 0.001$ , Figure 3) showing a maximal value at day 17 of lactation (49 d from mating) and increased with the number of suckling rabbits ( $13.6 \pm 1.51$  g/d per additional suckling rabbit,  $P < 0.001$ ).

Pregnancy length and total number of kits born tended to be longer and higher in NW than in W does ( $P = 0.10$  and  $0.053$ , respectively, Table 3), but weight of kits at birth was not affected. Kit mortality at birth and from birth to 14 d of lactation was high, being worse for NW than for W does (62.6 vs. 47.8% at birth and 97.3 vs. 40.4% from birth to 14 d, respectively,  $P < 0.001$ ).

## DISCUSSION

This experiment confirmed that under severe chronic heat stress conditions rabbit doe productivity is seriously compromised, even if the pregnancy is not overlapped with the lactation period. The improvement of water quality (especially its microbial content, turbidity and salinity) did not affect rabbit does, and it might confirm the wide tolerance limits of rabbits compared to humans (Gidenne *et al.*, 2010), especially to relatively high saline waters (Marai *et al.*, 2005). However, the strong negative effects of heat stress on other physiological traits might have hindered the beneficial effects of a better water quality. This result might be also influenced by the high temperature of both types of water that would have not helped does to lose heat.

In this study all rabbit does were successfully mated, but they showed a poor performance either before birth (9% pregnant does did not deliver probably due to embryo reabsorption) as after birth (high perinatal and postnatal kit mortality probably related to a low litter vitality) in agreement with our previous study in the same conditions (Villalobos *et al.*, 2010) or those obtained in a climatic chamber (Fernández-Carmona *et al.*, 1995; Fernández-Carmona *et al.*, 2003). This was probably accounted for the negative impact of heat stress on embryo quality and its survival in the post-implantation period, rather than to a low milk production as commented previously (Rich and Alliston, 1970; Villalobos *et al.*, 2010). A short incubation of spermatozoa, or of zygotes soon after fertilization, at 40° respect to 38°C in rabbit does maintained at 21°C was detrimental to embryo survival, although fertilizing semen capacity was not altered and these effects might not become apparent until the late stages of embryonic development (Alliston *et al.*, 1965; Burfening and Ulberg, 1968). It was also observed a higher embryo reabsorption rate and foetus malformation in rabbit does exposed for 2 h from 25 to 45°C the 9<sup>th</sup> day of pregnancy (Hellman and Claussen, 1980). These effects were

also observed in farms with a temporal and less severe heat stress, where the farm temperature at the insemination (for males) and during the embryos' implantational period (for females) were especially important (Tusell *et al.*, 2011) as observed previously in more extreme heat stress conditions (Ulberg and Burfening, 1967). It might be related to the reduction of ovarian blood flow observed in heat-stress induced pregnant rabbit does (Lublin and Woldfenson, 1996). In our work, rabbit does able to wean at least one kit (W) tended to show a lower body temperature and higher feed intake during pregnancy compared to NW and NB does (-0.21°C). It could explain the higher number of total kits born and born alive of W does, although their number and viability was not good either. These differences in performance and body temperature among rabbit does might be due to the individual differences in the adaptation to manage heat load in these conditions.

The linear reduction of body temperature during pregnancy observed in our study was similar to that reported by Jilge *et al.* (2001) for rabbit does maintained at 22°C or rats between 14 and 36°C (Fewell, 1995; Eliason and Fewell, 1997). In these studies body temperature remained constant the first days of pregnancy before the gradual decrease until birth, but there was not available an explanation for this variation. In this sense, the last week of pregnancy it has been reported that the deep body temperature on fetuses exceeded the maternal aortic temperature (Hart and Faber, 1965). Accordingly, reduction of body temperature of rabbit does might favor fetuses heat loss that is done via the fetal body surface or the umbilical circulation and it would help to ensure survival during the perinatal period (Eliason and Fewell, 1997), although it may be altered under heat stress (Lublin and Wolfenson, 1996). In our study, the reduction in body temperature during pregnancy can be partially related to the reduction of feed intake observed in this study, especially in the second half of pregnancy. Furthermore, we observed during pregnancy that body temperature decreased with metabolic weight, and it could be related to the effort of heavier does to help their fetuses to dissipate heat (even if does with higher metabolic weight increased feed intake in this period).

It is relevant that under our heat-stress conditions rabbit doe was able to control and reduce their body temperature during pregnancy in a similar way than at 22°C (Jilge *et al.*, 2001), although it was not enough to warrant kit survival. It would suggest that body temperature exceeded the maximal acceptable for an adequate embryo development, in spite of the capacity showed by rabbit doe to modulate it. In fact, just after birth body temperature increased around 0.4°C, similar to the result of Jilge *et al.* (2001).

In the last third of pregnancy rabbit does seem to mobilize a major proportion of fat and energy reserves (but no protein or ash) to meet the high energy requirements for fetuses development (Pascual *et al.*, 2006), leading to a negative energy balance, in agreement with data reported by Pascual (2010). After birth feed intake increased leading to a recovery of fat, energy and most protein reserves in the first 10 d of lactation as indicated also by Pascual (2010). It probably explains the increase of body temperature of rabbit does, even if the milk production was maximized 5 d later (close to that observed previously. Maertens *et al.*, 2006). The small litter size in this experiment allowed rabbit does to maintain body condition during the peak of milk production in contrast to that reported by Pascual (2010). However, body condition impaired once lactation and pregnancy were overlapped.

The small litter size and the low viability of newborns do not allow us to discuss if the negative effects of heat stress on lactation traits would be as important as in pregnancy. In fact, in our work weaned rabbits suckled daily more milk than in other works using modern rabbit hybrids with better environmental conditions (Nicodemus *et al.*, 2010) and rabbit does did not change the body condition during lactation. However, in our previous work we observed that rabbit doe productivity decreased along the first three cycles that could be related to the progressive depletion of body reserves.

In conclusion, in our conditions heat stress limit productivity due to a reduced embryo/kit survival and the improvement of water quality did not attenuate negative effect of heat stress. More productive rabbit does seem to manage better heat load but they cannot maintain body condition in spite of their low productivity. It would be required to identify the maximal body temperature for an optimal reproductive success and look for the management strategies to obtain it.

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**Table 1.** Chemical and bacteriological characterization of water offered to rabbit does.

	<b>Water</b>		
	Farm well <sup>1</sup>	Farm <sup>2</sup>	Potable <sup>3</sup>
Ph	6.84	7.68	6.82
Electric conductivity at 25°C, dS/m	0.65	0.60	0.38
Cations, mg/L			
Ca <sup>++</sup>	22.2	23.2	8.00
Mg <sup>++</sup>	14.5	13.7	8.75
Na <sup>+</sup>	80.5	80.5	53.4
K <sup>+</sup>	12.5	10.5	7.41
Total cations	129.6	128.0	77.5
Anions, mg/L			
HCO <sub>3</sub> <sup>-</sup>	118.4	85.4	90.9
Cl <sup>-</sup>	151.5	159.3	54.5
SO <sub>4</sub> <sup>=</sup>	13.4	20.2	37.9
Total anions	283.3	264.9	183.3
Total salinity, mg/L	412.9	392.8	260.9
Turbidity, NTU <sup>4</sup>	140	110	1.8
Aerobic heterotrophic bacteria, CFU/ml	244	28	6
Total coliforms, MPN/100ml	>1600	<2	<2
Fecal coliforms, MPN/100ml	65	<2	<2

<sup>1</sup> Nor filtered nor chlorinated. <sup>2</sup> Farm well water filtered and chlorinated. <sup>3</sup> Water sold for human consumption. <sup>4</sup> NTU: Nephelometric Turbidity Units. <sup>5</sup> CFU: Colony-forming units. <sup>6</sup> MPN: Most probable number.

**Table 2.** Effect of period, water quality, age and type of rabbit doe, metabolic weight and milk production on feed and water intake and their ration during pregnancy and lactation.

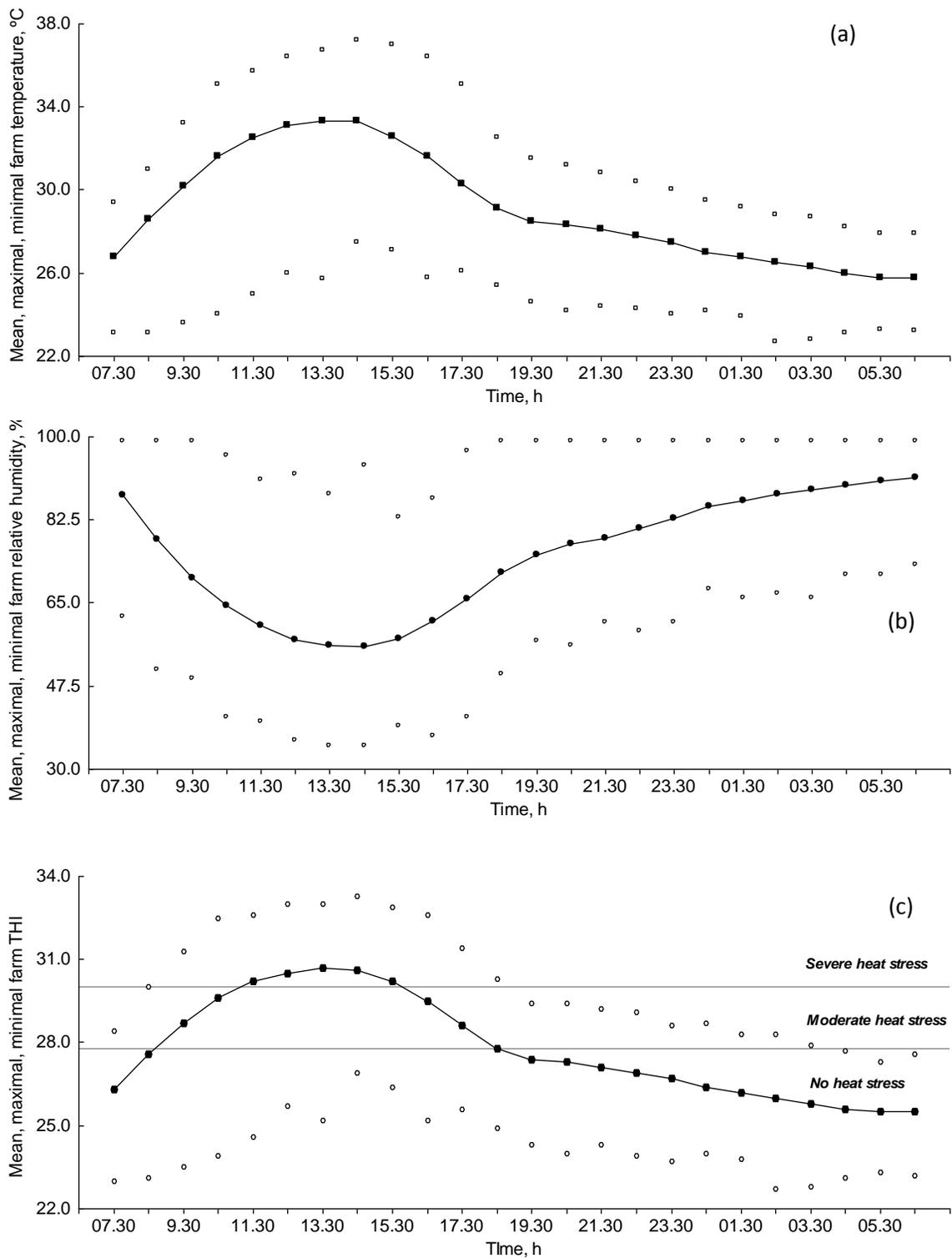
	Feed intake, g/d	Water intake, g/d <sup>1</sup>	Water/feed intake,
<b>Pregnancy</b>			
Period, d			
0-7	99.8 <sup>a</sup>	343	3.74 <sup>a</sup>
7-14	99.2 <sup>a</sup>	335	3.48 <sup>a</sup>
14-21	90.0 <sup>ab</sup>	377	4.58 <sup>ab</sup>
21-29	84.5 <sup>b</sup>	381	5.81 <sup>bc</sup>
29-birth	59.2 <sup>c</sup>	351	7.21 <sup>c</sup>
SEM	4.75	31	0.72
P <sub>pregnancy period</sub> <sup>2</sup>	< 0.001	NS	< 0.001
<b>Water quality</b>			
Potable	84.2	358	4.68
Farm	90.2	357	4.35
SEM	4.22	33	0.57
P <sub>water quality</sub>	NS	NS <sup>3</sup>	NS
<b>Age of does</b>			
Nuliparous	90.1	307	3.86
Multiparous	82.2	418	6.29
SEM	4.45	35	0.61
P <sub>age of does</sub>	NS	0.049	0.016
<b>Type of does</b>			
Weaned, W	95.1	356	3.82 <sup>a</sup>
Not weaned,	81.9	396	6.37 <sup>b</sup>
Not birth, NB	81.2	308	4.61 <sup>ab</sup>
SEM	3.84	31.2	0.92
P <sub>type of does</sub>	0.064	NS	0.005
P <sub>metabolic weight</sub>	0.026	NS	NS
<b>Lactation<sup>2,4</sup></b>			
Period, d			
0-7	168	396 <sup>a</sup>	2.41 <sup>ab</sup>
7-11	167	371 <sup>a</sup>	2.27 <sup>a</sup>
11-19	155	401 <sup>a</sup>	2.70 <sup>bc</sup>
19-28	163	456 <sup>b</sup>	2.87 <sup>c</sup>
SEM	7.51	38	0.23
P <sub>lactation period</sub>	NS	< 0.001	0.005
<b>Water quality</b>			
Potable	158	382	2.48
Farm	168	430	2.64
SEM	7.14	48	0.28
P <sub>water quality</sub>	NS	NS	NS
<b>Age of does</b>			
Nuliparous	165	357	2.32
Multiparous	162	455	2.80
SEM	7.54	50	0.30
P <sub>age of does</sub>	NS	NS	NS
P <sub>metabolic weight</sub>	NS	NS	NS
P <sub>milk production</sub>	< 0.001	0.009	NS

<sup>1</sup> Records of water intake of periods in which rabbit doe wasted water were removed. <sup>2</sup> Linear and quadratic effect of period (P < 0.001). <sup>3</sup> NS: P > 0.15. All interactions were NS. <sup>4</sup> Only for does that weaned at least one kit (W).

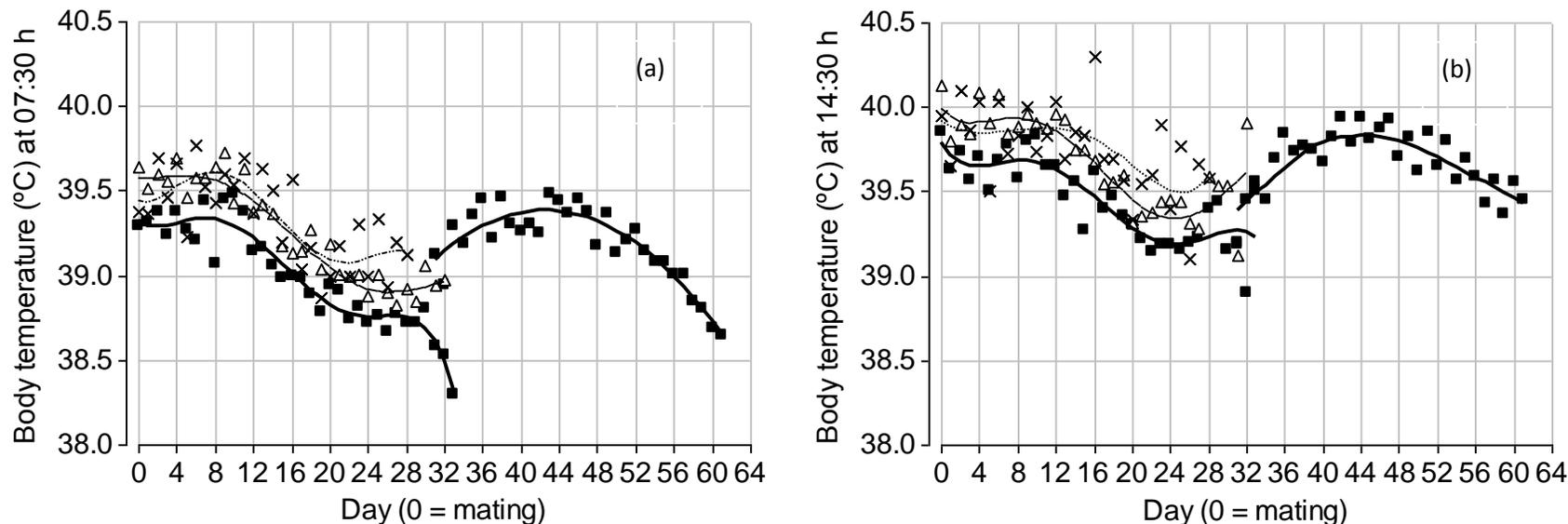
**Table 3.** Rabbit doe productivity success during lactation.

	<b>Rabbit does that weaned litter (W)</b>	<b>Rabbit does that did not wean litter (NW)</b>	<b>SEM</b>	<b>P</b>
N	21	20		
Pregnancy length, d	32.0	32.5	0.24	0.10
Total number kits born	6.38	4.95	0.51	0.053
Number kits born alive	4.71	1.85	0.42	< 0.001
Number kits alive at				
14 d	2.81	0.050	0.25	< 0.001
Weaning (28 d)	2.52	0	0.31	< 0.001
Weight of kits (g) at				
Birth	59.1	59.7	3.37	NS <sup>1</sup>
Weaning (28 d))	559	–	25.5	–
Total milk production, kg	2.27	–	0.21	–

<sup>1</sup> NS: P > 0.15. No effect of age of does (nulliparous vs. multiparous) was observed (P > 0.15).

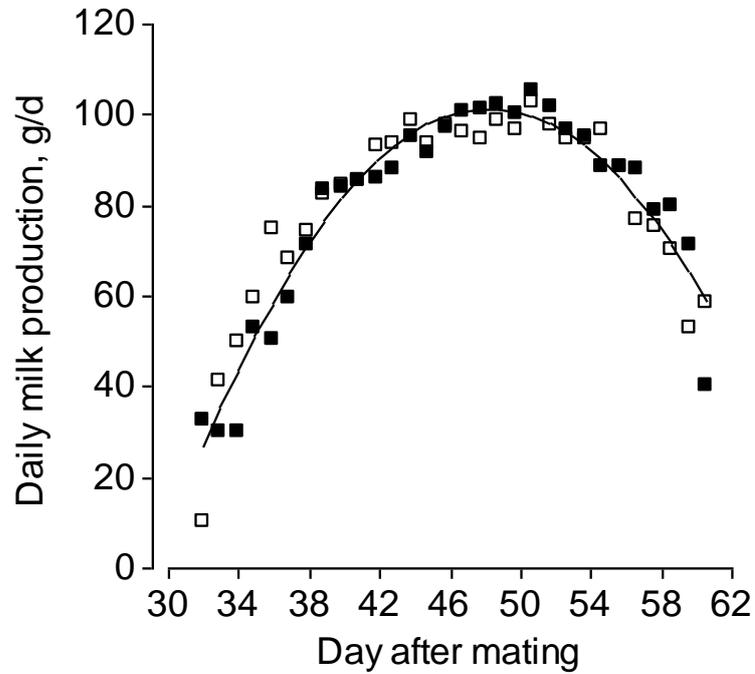


**Figure 1.** Circadian evolution of temperature, relative humidity and temperature-humidity index (THI, calculated according to Marai *et al.*, 2001) inside the farm during the experimental period (Mean, maximal and minimal values for each hour).

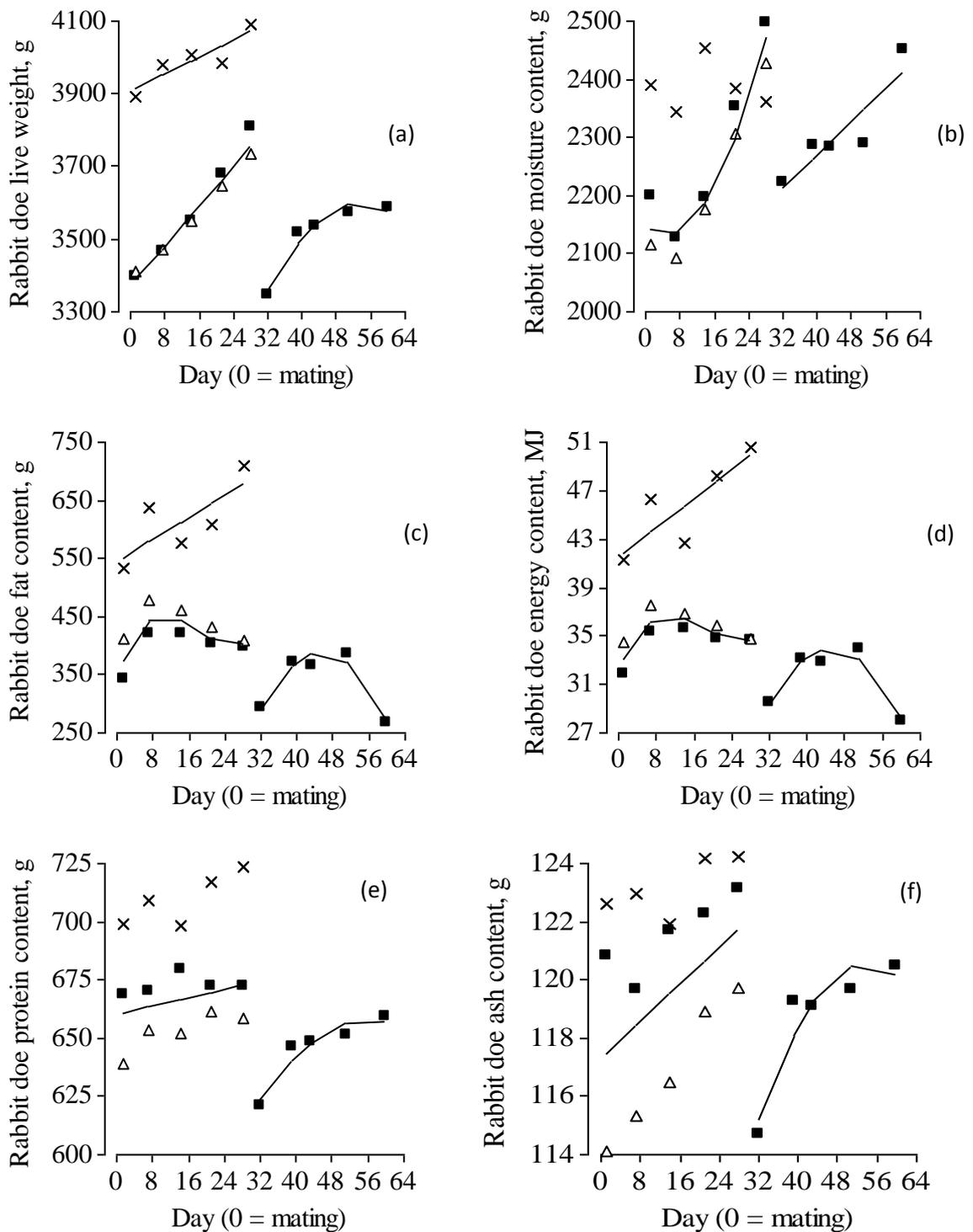


**Figure 2.** Evolution of body temperature at 07:30 and 14:30 h from mating (day 0) to weaning (day 61) of rabbit does during gestation and lactation (0 to 32 d pregnant-non lactating does, 32 to 43 d lactating does, 43 to 62 lactating-pregnant does). ■ Pregnant does that weaned their litters (W, n = 21). △ Pregnant does which litter died during the first two weeks after birth (NW, n = 20. Lactation data not shown). × Pregnant does 12 d after mating with no birth (NB, n = 4).

Effects on body temperature during pregnancy at 07:30 and 14:30 h: polynomial effect of 5<sup>th</sup> order of day after mating ( $P \leq 0.031$ .  $rsd = 0.29$ ,  $arc = 0.21$ ), linear effect of farm temperature (0.020 and  $0.11 \pm 0.0063$ , respectively.  $P \leq 0.002$ ), linear effect of metabolic weight (-0.42 and  $-0.57 \pm 0.16$ .  $P \leq 0.009$ ) and trend of type of doe (W, NW, NB.  $P \leq 0.090$ ). Effects during lactation at 07:30 and 14:30 h: Quadratic effect of day after mating ( $P \leq 0.005$ .  $rsd = 0.27$ .  $arc = 0.14$ ), linear effect of farm temperature (0.071 and  $0.11 \pm 0.010$ , respectively.  $P < 0.001$ ), linear effect of daily milk production (0.071 and  $0.0024 \pm 0.0063$ .  $P < 0.001$ ). No other effects were significant ( $P > 0.15$ ).



**Figure 3.** Daily milk production of W rabbit does (weaned at least one kit) from birth to weaning (28 d of lactation). ■ Rabbit does offered potable water (n = 11) □ Rabbit does offered farm water (n = 10). Quadratic effect of day after mating on milk production ( $P < 0.001$ ,  $rsd = 20.8$ ,  $arc = 0.69$ ) and linear effect of number of suckling kits ( $13.6 \pm 1.5$ ,  $P < 0.001$ ). No other effects were significant ( $P > 0.15$ ).



**Figure 4.** Evolution of live weight (g), chemical (g) and energy (MJ) content of rabbit does along pregnancy and lactation. ■ Pregnant does that weaned their litters (W, n = 21). △ Pregnant does which litter died during the first two weeks after birth (NW, n = 20). Lactation data not shown). × Pregnant does 12 d after mating with no birth (NB, n = 4). Residual standard deviation in pregnancy/lactation for live weight (96/195 g), moisture (113/156 g), fat (83/94 g), energy (3.9/4.5 MJ), protein (19/25 g) and ash (3.1/4.4 g). All the linear, quadratic and cubic effects of day after mating represented in these figures were significant ( $P < 0.05$ ).

## ***CHAPTER 5***

### *GENERAL CONCLUSIONS AND IMPLICATIONS*

## GENERAL CONCLUSIONS

According to the objectives of this thesis the general conclusions are:

- In very dry forest conditions the cage density should be lower than 18 rabbits/m<sup>2</sup> (34 kg/m<sup>2</sup>), and cage size does not exert a significant influence on growth performance of rabbits during fattening.
- In our conditions regardless clipped or not, rabbits does were under heat stress, and it is confirmed that in this condition rabbit does performance is severely affected.
- A more extensive breeding system (delay of mating and weaning) and successive production cycles impaired rabbit doe performance, especially kit mortality during the first days of lactation.
- Water quality improvement did not affect rabbit doe response to heat stress.
- Rabbit does able to maintain a lower body temperature during pregnancy had a higher productivity.
- The high body temperature during pregnancy may affect the viability of embryos, and kits development after birth leading to a high mortality the first days of lactation.
- Energy and protein balance was negative during pregnancy in non-lactating does, whereas no variation occurred during lactation in spite of the low productivity recorded.

## **IMPLICATIONS**

At present it is possible to fatten growing rabbits under our extreme heat stress conditions. However, it is not possible to develop a complete productive system to produce rabbit meat due to the severe effects on kit mortality. It would be necessary to:

- Identify the maximal body temperature that allows rabbit does (and males) a normal embryo development.
- Select a breeding system with no or minimal overlapping between pregnancy and lactation.
- Select the environmental and nutritional strategies that help the rabbit does and males to manage better the heat load.