INFLUENCE OF ENVIRONMENTAL TEMPERATURE AND RELATIVE HUMIDITY ON QUANTITATIVE AND QUALITATIVE SEMEN TRAITS OF RABBITS

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ABSTRACT

In rabbits, it has been shown that the comfort zone temperature is around 21ºC and that their productive and reproductive performance could be impaired when temperature-humidity index (THI) is over 27.8, value that implies the beginning of heat stress. However, no information is available about the optimum range of THI and the value of THI which indicate the starting of cool stress.

The present paper investigated the effect of low to moderate THI index on semen traits of rabbits. Three THI classes were defined: THI=16 (ranged from 14.0 to 17.4), THI=20 (ranged from 18.6 to 20.6) and THI=22 (ranged from 20.7 to 23.1); and the following variables were recorded: absence of gel plugs, urine, calcium carbonate deposits and agglutinated and dead spermatozoa in the ejaculate, individual motility, pH, volume and cell sperm concentration. Differences between THI-class have been found for absence in the ejaculate of gel plugs, urine, calcium carbonate and agglutinated and dead spermatozoa in the ejaculate, for ejaculate volume and for ejaculate pH. Differences were relevant and favourable to THI-22 for ejaculate volume and absence of agglutinated spermatozoa, and important and favourable to THI-16 for absence of urine, calcium carbonate and dead spermatozoa.

Key words: Rabbit, Seminal quality and production, THI index.

INTRODUCTION

An objective of artificial insemination centres and commercial farms is to produce a large number of seminal doses with good fertility at the lowest cost. Therefore, males should show a great reproductive efficiency (large number of doses with high fertility rate). Environmental, management, physiological and genetic factors affect normal spermatogenesis, sperm function and male fertility. Concerning environment, thermal stress implies a deregulation in the thermal neutral zone of the animals, which could lead to changes in the biological functions of animals, so a "seasonal sterility" has been described in several mammalian species, in which high ambient temperatures could affect levels of testosterone and reproductive functionality of rabbit males (Alvariño, 2000). Thermal stress is related with a high number of variables (ambient temperature, relative humidity, photoperiod, wind, solar radiation, etc.), but it seems that the relationship between ambient temperature and relative humidity is the most important to assess the impact of heat stress (Marai *et al.*, 2002).

In rabbits, seasonal effects on male reproduction have been usually studied through seminal parameters (Roca *et al.*, 2005; Theau-Clement *et al.*, 1995), morphologic traits of testis and epididymis (García-Tomas *et al.*, 2007) and serum testosterone (Chiericato *et al.*, 1994). The majority of these studies have taken into account the photoperiod with or without regulation of temperature, the effect of environmental temperature and birth season of males, but only Roca *et al.* (2005) used the temperature-humidity index (THI) modified for rabbits by Marai *et al.* (2002). These authors showed that the acute exposure to high THI had immediate negative effects on sperm concentration, total number of spermatozoa per ejaculate, sperm motility, and variables related to viability and sperm normalcy. The values of these traits showed a great drop two weeks later of the highest THI.

The THI has mainly been used to measure heat stress in hot areas worldwide in several species. However, the effect of low THI values on reproductive performance has not been studied. The aim of the present study was to investigate the effect of cold to moderate THI index on semen production and quality traits in rabbits.

MATERIALS AND METHODS

This study was performed from January to October 2007, at the experimental farm of the Institut de Recerca i Tecnologia Agroalimentàries located in the northeast of Spain (Caldes de Montbui).

Animals and experimental design

Data are related to 350 males belonging to a synthetic line selected for increased post weaning daily gain from 1993 (Gómez *et al.*, 1999). Males were raised with a photoperiod of 16 hours light/day and fed restricted with commercial rabbit pellets (16% crude protein, 4.3% fat, 17% fibre).

At 20 weeks of age, bucks started the training period with home-made artificial vaginas (containing water at 50ºC) and one ejaculate was collected per male and per week during two weeks. In the intensive meat rabbit production males belonging to a sire genetic line, usually, start their reproductive life between 16 to 20 weeks of age and at 33 weeks they are considered adults. However, the evolution of ejaculated volume and individual motility of the spermatozoa with age (IRTA, unpublished data) shows an important increase between 20 to 33 weeks of age for both variables. Moreover, at 20 weeks of age testis size has only reached about 70% of its adult value, and percentage of seminiferous tubules with presence of spermatozoa was only about 70% of their value at 33 weeks of age (García-Tomas *et al.*, 2007). The ejaculates used for this study were collected at three moments of the buck's productive life to take into account changes in rabbit development, which could affect semen production and quality traits: around 25 weeks old (ranged from 23 to 26 weeks of age), 30 weeks old (ranged from 28 to 32 weeks of age) and 35 weeks old (ranged from 33 to 38 weeks of age). Two ejaculates per male per week were collected, with an interval of 30 minutes between collections.

Semen evaluation

All ejaculates were stored at 35ºC in a dry bath for no more than 15 minutes after collection. Visual features of qualitative semen traits were recorded as binary traits: 0 - absence, 1 - presence of gel plugs (G), urine (U) and calcium carbonate deposits (CC). Ejaculates containing urine and calcium carbonate deposits were discarded, and gel plugs were removed. After that, the variables agglutinated (Ag) and dead (Dead) spermatozoa in the ejaculate, defined as binaries traits: 0 - absence, 1 - presence, and individual motility (IM) of ejaculate were measured in aliquots (25 μl) under a light microscope (Nikon) at x100. Ejaculates were considered with presence of dead spermatozoa when they showed 5-10% of dead spermatozoa. Individual motility was evaluated according to a subjective scale from 0 to 5 (Roca *et al.*, 2000) where 0 meant 0-10% and 5 meant 90-100% of the motile spermatozoa showing progressive movement. The pH (pH) was determined in raw semen by using a 507 Crison pH-metre and volume (V) of the ejaculate was measured by using micropipettes. After this initial evaluation, ejaculates from one buck were pooled and diluted (1:2) in a commercial saline extender for rabbit semen (CUNIGEL, Zaragoza, Spain) and the cell sperm concentration (SCC) was measured by using a Nucleocounter SP-100.

Climate variables

Climate variables (temperature and relative humidity) were recorded each 5 minutes using a hydrothermograph (Tinytag) located into the males room. The mean daily air temperature and the relative humidity were calculated. Temperature-humidity index (THI) was estimated from the following equation reported by Marai *et al.* (2002) for rabbits:

$$\text{THI: db}^{\circ}\text{C} - [(0.31 - 0.31(\text{RH})) (\text{db}^{\circ}\text{C} - 14.4)]$$

where db°C = the temperature and RH = relative humidity percentage / 100

In rabbit, it has been showed that the comfort zone temperature is around 21°C. Therefore to investigate the effect of cold to moderate THI index on semen production and quality traits in rabbits classes of THI index were defined as follow: THI=16 (ranged from 14.02 to 17.41), THI=20 (ranged from 18.6 to 20.6) and THI=22 (ranged from 20.7 to 23.12).

Statistical Analysis

Binary traits were analysed using the procedure GENMOD of the SAS v.8. package with a mixed model including the fixed effects of age (three levels) and the THI index (three levels) and the permanent random male effect to which the observation corresponded. Percentage of incidence of qualitative traits was obtained directly from the following equation:

$$\text{Incidence of qualitative traits (\%)} = \exp^{\text{LSmeans}} / 1 + \exp^{\text{Lsmeans}} \text{ (Hosmer and Lemeshow, 1989)}$$

Quantitative traits were analysed with the procedure MIXED of the SAS v.8. package using a mixed model including the same factors as those considered in the analysis of binary traits.

RESULTS AND DISCUSSION

Table 1 shows Ls means (standard error) for production and qualitative semen traits according to THI-classes. Table 2 shows qualitative binary traits as frequencies to help to an easier and more immediate interpretation of the results. Our general results showed differences between low to moderate THI-classes in most of the studied variables.

Table 1: Ls means (stderr) for THI index of production and qualitative semen traits

	Production traits		Qualitative traits						
	V (ml)	SCC (x10^6/ml)	G	U	CC	Dead	Ag	pH	IM
THI index									
16	0.62[a]	433.2	2.59[a]	3.74[a]	1.90[a]	-0.55[a]	1.90[a]	7.38[a]	3.07
	(0.04)	(19.2)	(0.21)	(0.37)	(0.17)	(0.15)	(0.23)	(0.04)	(0.08)
20	0.84[b]	393.3	1.97[b]	2.84[b]	1.19[b]	-1.44[b]	2.52[b]	7.54[b]	2.96
	(0.03)	(14.7)	(0.14)	(0.28)	(0.15)	(0.14)	(0.21)	(0.03)	(0.06)
22	0.92[c]	406	2.13[a,b]	2.65[b]	1.20[b]	-1.89[c]	3.49[c]	7.59[b]	2.85
	(0.03)	(16)	(0.17)	(0.22)	(0.13)	(0.17)	(0.29)	(0.03)	(0.07)

Different letters indicate significant different groups at the 5% level
V: volume of ejaculate; SCC: sperm cell concentration; G: ejaculates with absence of gel plugs; U: ejaculates with absence of urine; CC: ejaculates with absence of calcium carbonate deposits; Dead: ejaculates with absence of dead spermatozoa; Ag: ejaculates with absence of agglutinated spermatozoa; IM: individual motility of spermatozoa

Table 2: Frequency of qualitative semen traits absence

	GR	UR	CCR	DeadR	AgR
THI index					
16	93[a]	97.7[a]	87[a]	36.6[a]	87[a]
20	87.8[b]	94.5[b]	76.7[b]	19.1[b]	92.4[b]
22	89.4[a,b]	93.4[b]	76.9[b]	13.1[c]	97[c]

Qualitative traits (%) = $\exp^{\text{LSmeans}} / 1 + \exp^{\text{LSmeans}}$
GR: % of ejaculates with absence of gel plugs; UR: % of ejaculates with absence of urine; CCR: % of ejaculates with absence of calcium carbonate deposits; DeadR: % of ejaculates with absence of dead spermatozoa; AgR: % of ejaculates with absence of agglutinated spermatozoa

Respect to production traits, it has been found an important and significant effect of THI on volume ejaculate. There was a relevant increase on this trait when THI increased from THI-16 to THI-22; the volume ejaculate from THI-16 was 26% and 33% lower than volume ejaculate with THI-20 and THI-22, respectively. Differences in volume ejaculate are not in agreement with results reported by Roca *et al.* (2005) that did not find differences in the variation of volume ejaculate when THI increased.

Cell sperm concentration did not show differences between the THI-class studied in this work. These results were in agreement with those reported by Roca *et al.* (2005). These authors showed, for a period of 15 months, the weekly variation of sperm concentration and total number of spermatozoa per ejaculated. These two sperm production variables fell when THI was around 30, but they remained nearly stables when THI ranged between 15 and 20.

Respect to qualitative traits; we can divide them in two groups: qualitative traits of the ejaculate (G, U, CC, Death, Ag and pH) and qualitative traits of the spermatozoa (IM). Concerning the first group, differences have been detected for all variables. In general, it is evident that for most qualitative traits the THI-16 seemed to imply higher semen quality and lower rate of ejaculate discarding.

Differences were at a low magnitude (2-4%) for G and pH. The rate of ejaculates without gel plugs ranged between 89% and 93%, and values of pH were always neutral (Table 2).

In insemination centres, the most frequent reasons for discarding ejaculates are the presence of urine, deposits of calcium carbonate, dead and/or agglutinated spermatozoa, and a low individual motility. In our study, the rejection was undertaken in two steps; before semen dilution the ejaculates containing residual amounts of urine or calcium carbonate were eliminated, after that an evaluation of individual motility and the absence/presence of dead and agglutinated spermatozoa was performed and semen was eliminated if the scores for individual motility were less than 2 on a subjective scale ranging 0-5, if there was more than 50% of death spermatozoa and if there was presence of agglutinated spermatozoa. Every THI-class presented all reasons for ejaculate refusal. There were significant differences between THI-16 and the other two THI-class for U and CC (estimated odds ratio THI-16 *versus* THI-20 = 2.45 and estimated odds ratio THI-16 vs. THI-22 = 2.98). These differences were moderate in magnitude (7% more ejaculates without U and CC) and favourable to THI-16, therefore the rejection of ejaculates due to U and CC was smaller for THI-16 than for THI-20 and THI-22. Differences in Dead between THI-class were very important; THI-16 had 18% and 24% more ejaculates without presence of dead spermatozoa than THI-20 and THI-22, respectively. For Dead variable, it seemed that refusal of ejaculates was lower for THI-16, but it is important to know that real percentages of ejaculate elimination were less than expected percentages (18% and 24%) since ejaculates were considered with presence of dead spermatozoa when they showed 5-10% of dead spermatozoa while their elimination started, as we said before, when ejaculates had 50% of dead spermatozoa. Finally, differences in Ag were found between THI-class. These differences were moderate and unfavourable to THI-16 (estimated odds ratio THI-16 vs. THI-20 = 0.54 and estimated odds ratio THI-16 vs. THI-22 = 0.2). For this variable THI-22 had 10% more ejaculates without presence of agglutinated spermatozoa. There are not previous studies concerning the effect of environmental temperatures and relative humidity on the ejaculate qualitative traits in rabbits.

With reference to semen quality traits of spermatozoa, there were no differences in IM between studied THI-class. Roca *et al.* (2005) showed a significant and negative effect of high THI index on sperm motility index, but this variable remained practically constant when THI ranged between 15 and 20. This result could be in agreement with that reported by us in this study.

CONCLUSIONS

Ejaculate parameters studied in this study were affected by cold to moderate Temperature-Humidity index (THI), but there was not a clear picture concerning differences between THI-class. For ejaculate volume and absence of agglutinated spermatozoa differences were relevant and favourable to THI-22. For variables absence of urine, calcium carbonate and dead spermatozoa, THI-16 seemed to present bigger quality semen than other THI-class. Additional variables related to quality of spermatozoa, as viability and morphology, could be considered in further studies.

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REFERENCES

Alvariño J.M.R. 2000. Reproductive performance of male rabbit. *In: Proc. 7th World Rabbit Congress, Valencia, Spain, Vol. A, 13-35.*

Chiericato G.M., Boiti C., Canali C., Rizzi C., Rostellato V. 1994. Effets de l'age et de la température ambiante sur les concentrations circulantes hormonales chez le lapin. *In: Proc. 6èmes Journées de la Recherche cunicole, La Rochelle, France, Vol. 1, 137-144.*

García-Tomás M., Sánchez J., Rafel O., Ramon J., Piles M. 2007. Post-natal sexual developement of testis and epididymis in the rabbit: growth and maturity patterns of macroscopic and microscopic markers. *Theriogenology* (in press).

Gómez E.A., Baselga M., Rabel O., Ramon J. 1999. Selection, diffusion and performances of six Spanish lines of meat rabbit. *Options Méditerranéennes, series cahiers, 41, 289-292.*

Hosmer D.W., Lemeshow S. 1989. Applied logistic regression. *Wiley Interscience Publication, USA.*

Marai I.F.M., Habeeb A.A.M., Gad A.E. 2002. Rabbits' productive, reproductive and physiological performance traits as affected by heat stress: a review. *Livestock Production Science, 78, 71-90.*

Roca J., Martínez S., Orengo J., Parrilla I., Vázquez J.M., Martínez E.A. 2005. Influence of constant long days on ejaculate parameters of rabbits reared under natural environment conditions of Mediterranean area. *Livestock Production Science, 94, 169-177.*

Theau-Clément M., Michel N., Esparbié J., Bolet G. 1995. Effects of artificial photoperiods on sexual behaviour and sperm output in the rabbit. *Anim. Science, 60, 143-149.*