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# Effect of dietary administration of oil extract from rosemary on reproductive efficiency in boars

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**RIASSUNTO** – Effetti della somministrazione dell'estratto di rosmarino sull'efficienza riproduttiva di verri. *Il ricorso a sostanze naturali ad azione antiossidante può diventare importante quando i meccanismi di difesa dell'organismo non sono sufficienti a neutralizzare l'eccesso di radicali liberi che si produce a seguito di un aumento del metabolismo basale. L'impiego dell'estratto di rosmarino, in razioni per verri in attività riproduttiva, sottoposti a condizioni di stress termico, ha migliorato la qualità dell'eiaculato (+30% per la concentrazione spermatica e +5% per il n. di spermatozoi vivi) e la sua capacità fecondante (-4,08 punti% di ritorni in calore delle scrofe). Nei soggetti trattati, la produzione steroidea, è rimasta soddisfacente anche nelle prime fasi di esposizione alle elevate temperature.*

**Key words:** rosemary oil extract, antioxidant activity, boars, reproductive efficiency.

**INTRODUCTION** – A decrease in reproductive performance in boars during and immediately after hot summer weather has been previously reported (Park and Yi, 2002). High temperature causes germ-cell destruction and results in a temporary decrease in sperm production and fertility. The increase of metabolic activity following thermic stress matches with a higher production of free radicals that impairs cells, such as spermatozoa, particularly rich in polyunsatured fatty acids and poor in antioxidants systems. In relation to length and intensity of stress conditions, a decrease in sperm concentration and motility and an increase in the abnormal spermatozoa has been reported (Cameron and Blackshaw, 1980). Natural polyphenols, extract from many herbs, and in particular from rosemary (*Rosmarinus officinalis L.*) leaves have strong antioxidant activity (Aruoma *et al.*, 1996). Nevertheless, the effects of natural antioxidants of plant origin on male pig reproductive efficiency are not known.

**MATERIAL AND METHODS** – Twelve Large White boars, reared in the Po Valley, were divided into two homogeneous groups for age (26.4±6.1 months) and housing type and kept under the same environmental conditions. During the experimental period (June-October), the boars received a diet (16% CP, 3000 ME kcal/kg, 2.6 ME/lysine) added (Group T) or not (Group C) with rosemary oil extract (*Rosmarinus officinalis L.*), containing 5.5% of rosmarinic acid, at the dose of 12.5 p.p.m.. The meal was given once daily at the amount of 3.5 kg/head. Every day, from June to September, external temperature and relative humidity were detected to determine the Heat Index (HI) (www.srh.noaa.gov). The semen evaluation started after 30 day treatment with rosemary oil extract. Semen was collected twice per week at 9.00 *a.m.* and used fresh on 943 LWxL sows (farrowing order 4.55±2.30). Sows were inseminated at the natural onset of heat, relieved using a boar, and a sec-

ond time after 24 hours, by means of a homospermic insemination. The pregnancy test was performed at 25 d, using a linear ecograph with a 3.5 MHz probe. Semen volume was determined with a graduated cylinder. Sperm concentration was examined by a hemocytometer. The motility of sperm was estimated on a drop of fresh semen, at 37°C by light microscope at 80X, assigning to each reading a 0 to 5 score. Detection of live and abnormal spermatozoa was evaluated by phase contrast microscopy at 1000X (Superchi *et al.*, 1998) on 150 sperm per sample. At 0, 45 and 90 d from the beginning of treatment, blood samples were collected, at 10.00 *a.m.*, via jugular vein venipuncture in tubes without anticoagulant. Samples were centrifuged at 2500 rpm for 20 min and then the serum was stored at –20 °C until analysis. The concentration of testosterone was measured by a validated radioimmunoassay (Grasselli *et al.*, 1995). Data were analysed by ANOVA (SPSS.12.0.1, 2003) using a linear model with the fixed effects of group, month and interaction. Non parametric data were analysed with $\chi^2$ test. For blood data time 0 was used a covariate.

**RESULTS AND CONCLUSIONS** – The Heat Index (table 1), resulted always higher than or equal to 80. The addition of rosemary extract increased the sperm concentration (P=0.01) and live spermatozoa percentage (P<0.05) (resp. + 30% and + 5%); fluid volume, motility and sperm abnormalities, were not significantly affected by treatment (P>0.05).

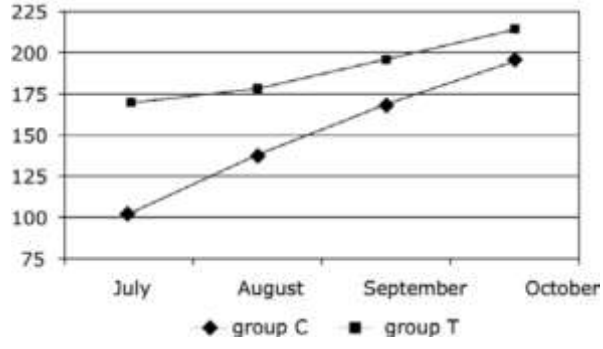
Table 1. LS means for semen characteristics.

| | | Group | | Month | | | | G*M |
|---|---|---|---|---|---|---|---|---|
| | | C | T | July | August | September | October | |
| Mean HI (§) | | - | - | 81.10 | 79.60 | 85.90 | 81.90 | - |
| Fluid volume | ml | 255 | 249 | 254 | 257 | 288 | 299 | n.s. |
| Sperm conc. | x10$^6$/ml | 151.26a | 199.30b | 135.68A | 158.72AB | 182.42B | 214.62C | * |
| Motility | Score 0-5 | 4.66 | 4.42 | 4.48 | 4.56 | 4.51 | 4.61 | n.s. |
| Live sperm | % | 78.59A | 82.33B | 78.35 | 80.28 | 81.36 | 81.86 | n.s. |
| Sperm abnormal. | % | 9.26 | 8.50 | 8.56 | 9.00 | 9.21 | 8.76 | n.s. |

a, b: P=0.01; A, B, C: P<0.05; * P<0.001; n.s.: not significant; §: value found in previous month.

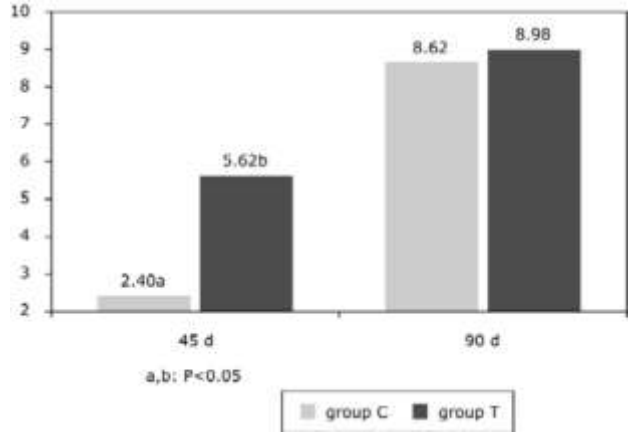
Significant differences for sperm abnormalities percentage have been previously observed after acute exposure to elevated temperature (Cameron and Blackshaw, 1980), but not always if it is prolonged (Park and Yi, 2002), as in the present study. Significant differences among the months of collection (P<0.05) were shown on semen characteristics: sperm concentration was higher in October than in Summer months; moreover the interaction between group and month was significant (P<0.001). T group was less affected by thermic stress than C group (figure 1), with higher values during the trial period.

Figure 1. Sperm concentration (x10$^6$/ml) in relation to period.



Sows inseminated with semen from treated boars showed a significant (P=0.022) reduction of return to oestrum (15.73 *vs.* 19.81%). This could be a consequence of lesser fertilized abilities (Tarocco, 1990) by non-treated boars. In boars, seasonal changes, and particularly temperature and photoperiod, directly affect the steroid production (Trudeau and Sanford, 1986; Levis, 1998; Park and Yi, 2002). Testosterone level was not affected by treatment (C=5.51 ng/ml; T=7.32 ng/ml). Forty five days after the beginning of treatment, serum testosterone concentration was higher in T group (P<0.05) (figure 2). No significant difference between groups was shown at 90 d (P>0.05).

Figure 2. Testosterone concentration (ng/ml) in relation to period.




The results of this study suggest that the antioxidant activity of rosemary extract can limit the negative effects of high temperatures on reproductive efficiency of boars.

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