THE EFFECT OF L-CARNITINE DAILY SUPPLEMENTATION ON QUALITY OF EJACULATE OF DUROC BOARS

MAGDALENA PRIBILOVA¹, PAVEL HORKY¹, LENKA URBANKOVA¹, MILAN VECERA²
¹Department of Animal Nutrition and Forage Production
²Department of Animal Breeding
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC
magdalena.pribilova@mendelu.cz

Abstract: The objective of this study was to find out whether the daily supplementation of l-carnitine had an effect on the quality of the ejaculate of duroc boars. L-carnitine was supplemented for 60 days, which were divided into 3 periods (n = 30 days). For the experiment were selected 12 duroc boars and were divided into two groups. Control group (n = 6) was fed by basic feed mixture only. Experimental group (n = 6) was fed by basic feed mixture with the addition of 500 mg of L-carnitine/kg of the basic mixture. The monitored ejaculate parameters included volume of ejaculate, sperm concentration, total rate of sperm, motility and percentage of morphologically abnormal sperm. Amount of L-carnitine in ejaculate was monitored as well.

By the results we confirmed the hypothesis, that L-carnitine has a positive effect on quality of ejaculate. Statistically significant effect was determined in sperm motility and in amount of morphologically abnormal sperm. In sperm motility there was insignificant increase in experimental group, but there was statistically significant difference between groups (P < 0.05). In the amount of morphologically abnormal sperm, there was statistically significant increase in experimental group (P < 0.05) and as well statistically significant difference between groups (P < 0.05).

Keywords: L-carnitine, semen, boar, antioxidant

INTRODUCTION

In literature, there is a lot of information about the positive effect of L-carnitine on spermatogenesis (Jacyno et al. 2007). L-carnitine is the vitamin-like amino acid synthesized from lysine and methionine in liver, kidney and brain (Vaz et al. 2002, Jeulin et al. 1994). L-carnitine plays a very important role in lipid metabolism and cellular energy metabolism (Hoppel 2003). It brings long-chain fatty acids into the mitochondria for beta-oxidation, thus producing the energy (ATP) necessary for proper sperm functioning (Hoppel 2003, Horky et al. 2012). It is also very important for detoxification of the organism, because it eliminates acetyl-CoA from mitochondria, excess of which has a toxic effect and it protects the cell membranes from the oxidative damage caused by peroxidation of polyunsaturated fatty acids (Arrigoni-Martelli and Caso 2001, Kalaiselvi and Panneerselvam 1998, Horky et al. 2014). L-carnitine is absorbed during sperm maturation in the epididymis and its concentration varies in the range of 200–300 nmol × mg⁻¹ of protein (Agarwal and Sait 2004, Jeulin et al. 1987). While sperm is passing through the epididymis (1–10 days), they accumulate a high concentration of L-carnitine from the epididymal plasma, thus conferring motility upon the flagellum (Jeulin et al. 1994). L-carnitine improves qualitative parameters of ejaculate, especially an increase of concentration of sperm and motility (Vitali et al. 1995). High concentration, motility and viability of sperm is the key to create more AI doses form one ejaculate.

Artificial insemination clearly augments the rate of genetic improvement, but could be further enhanced by increasing the total number of spermatozoa per ejaculate produced to increase the distribution of genetic material from superior boars. Currently, a single 80–100 ml dose of extended semen contains 2–3 × 10⁹ spermatozoa (Krueger et al. 1999). Total volume of an ejaculate ranges from 255 | P a g e
75–400 ml containing 20–100 × 10^9 spermatozoa (Leman and Rodeffer 1976). Therefore, a single boar ejaculation yields approximately 6–33 AI doses. It would be beneficial to the swine industry to maximize the number of AI doses produced by boars. The increase in usage of artificial insemination will fuel the demand for quality semen from boars trained to mount artificial sows for semen collection (Kozink et al. 2004).

MATERIAL AND METHODS

The experiment was running in the boar insemination station in Velké Meziříčí (N 49° 23.46667', E 15°52.70135'). The experiment lasted for 60 days, which were divided into 3 periods (period 1 = day 0, period 2 = day 30, period 3 = day 60). 12 boars of the Duroc breed, weighing 255 ± 20 kg and 2 ± 0.3 years old, were selected for the experiment. The boars were housed individually in pens (2.5 × 2.5 m). The feed mixture (MEp 12.6 MJ/kg) was fed at a dose of 3.5 kg; the boars had ad libitum access to water. The boars were divided into two groups, where the control group (n = 6) was fed by the basic feed mixture only and the experimental group (n = 6) was fed by the basic feed mixture with addition of 500 mg of L-carnitine per kg of the feed ration.

The ejaculate was taken once a week by using a jump phantom. Methodology of ejaculate analysis was determined by Lovercamp et al. (2013).

Determination of ejaculate volume – volume of the ejaculate was determined by weighing each ejaculate, with 1 g to 1 ml conversion.

Determination of sperm concentration – concentration of the sperms was determined using a self-calibrating photometer (SpermaCueTM, Minitube of America, Verona, WI).

Determination of sperm motility – motility was determined using the Sperm Vision™ software (Minitube of America, Verona, WI) with digital camera connection to a contrast microscope (Olympus microscope IX 71 S8F-3; Tokyo, Japan).

Prior to analysing, 500 μl of each sample was diluted with 500 μl of Androhep diluent and incubated for 30 minutes at 37 °C.

Determination of morphologically abnormal sperms – the phase contrast microscope (Zeiss, Germany) was used for determination of the morphologically abnormal sperms).

Subjective assessment was performed by a single qualified person.

Determination of the total sperm count – determined by calculation (sperm concentration x ejaculate volume).

Biochemical analysis – determination of free L-carnitine in ejaculate: One sample was taken once a month from all boars for the biochemical analysis and frozen for later analyses. 250 μl of pH = 7 phosphate buffer was added to 250 μl of defrosted boar sperm and the solution was poured over by 2 ml of liquid nitrogen. The mixture was homogenized for 2 minutes at 3000 rpm in ice. 1 ml of phosphate buffer was added and the mixture was shaken for 30 minutes at 4 °C and centrifuged at 6000 rpm for 15 minutes afterwards. After centrifugation the supernatant was removed, which 500 μl of 10% TFA was added to. The sample was re-centrifuged and the supernatant taken for analysis using AAA 400.

Statistics – the data were statistically analysed using STATISTICA.CZ version 10.0 (Czech Republic). The results were expressed as the mean ± standard variance. Statistical significance was observed between the groups using ANOVA and Scheffe's test – the two-factor analysis (the first factor was the animal group, the second one – the sampling factor) for parameters of L-carnitine ejaculate volume, sperm concentration and motility, percentage of pathological sperms. The difference (P < 0.05) was considered as significant.

RESULTS AND DISCUSSION

Table 1 shows the effect of L-carnitine supplementation on qualitative ejaculate parameters of each group and in each period of the experiment. From the results it’s obvious, that addition of 500 mg of L-carnitine had insignificant effect on volume of ejaculate, concentration of sperm and total rate of sperm. On the contrary, the control group reached better values of these parameters than the experimental group. The difference between groups reached a peak in the 3. period in concentration of...
sperm (about 131 × 10^6/ml) and total rate of sperm (about 26.06 × 10^9), but these results are not statistically significant. Statistically significant effect of supplementation of L-carnitine has been proven in motility and the amount of morphologically abnormal sperm. Already after 30 days of supplementation there was statistically significant difference between groups in motility of sperm (about 6.46%), (P < 0.05). In the 3. period there were significant differences in motility (8.54%) and the amount of morphologically abnormal sperm (about 9.25%), (P < 0.05).

Table 1 Average values of analysed parameters in each period

<table>
<thead>
<tr>
<th>Period</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control group</td>
<td>Experimental group</td>
<td>Control group</td>
</tr>
<tr>
<td>Volume of ejaculate</td>
<td>168.33 ± 41.76</td>
<td>150 ± 41.44</td>
<td>192.22 ± 29.34</td>
</tr>
<tr>
<td>Concentration of sperm</td>
<td>515.83 ± 67.26</td>
<td>492.5 ± 46.37</td>
<td>514.86 ± 98.36</td>
</tr>
<tr>
<td>Total rate of sperm</td>
<td>85.26 ± 22.21</td>
<td>76.78 ± 24.86</td>
<td>97.52 ± 10.85</td>
</tr>
<tr>
<td>Motility</td>
<td>71.67 ± 4.21</td>
<td>71.67 ± 5.24</td>
<td>67.85* ± 1.82</td>
</tr>
<tr>
<td>Morphologically abnormal sperm</td>
<td>9.83 ± 2.69</td>
<td>7.17 ± 2.70</td>
<td>9.22 ± 2.32</td>
</tr>
</tbody>
</table>

After biochemical analysis, it was found that statistically significant increase of L-carnitine concentration in experimental group was occurred already after 30 days (about 5.02 μg/ml) and after 60 days (about 12.12 μg/ml) of supplementation (P < 0.05). Statistically significant differences between groups were occurred after 30 days (about 6.78 μg/ml) and 60 days (about 18.55 μg/ml) of supplementation (P < 0.05) (Figure 1).

Cerovsky et al. (2009) in his research states that the addition of L-carnitine has no positive effect on the quality of the ejaculate of boars. On the other hand, Jacyno et al. (2007) recorded improvement of qualitative markers of ejaculate after supplementation of 500 mg of L-carnitine in Pietrain boars, especially in decrease of the amount of morphologically abnormal sperm and in increase of the total
sperm count in the ejaculate. In our experiment, we have recorded a decrease of amount morphologically abnormal sperm as well, the difference between groups was about 9.25% ($P < 0.05$). Vitali et al. (1995) in their experiment argue that addition of L-carnitine into boars diet positively affected the motility and the sperm concentration. We could not approve the influence of L-carnitine on concentration of sperm, but in motility we have recorded a statistically significant increase (difference between groups about 8.54%), ($P < 0.05$). Baumgartner (1998) claims that after supplementation of L-carnitine it could be produced more insemination doses form one ejaculate. By the increase of volume of ejaculate and total amount of sperm it is possible to prepare more insemination doses (Jacyno et al. 2007). The increase of total number of sperm in ejaculate could be due to the fact, that L-carnitine is protecting the sperm cells and less dead sperm is absorbed in epididymis (Jeulin and Lewin 1996). As we have recorded no significant increase of volume of ejaculate and concentration sperm, we cannot stand that L-carnitine can uplift the total amount of sperm in ejaculate.

CONCLUSION
The aim of the study was to confirm the hypothesis, that daily supplementation of L-carnitine have a positive effect on quality of ejaculate. We confirmed it in observed parameters: motility of sperm, the amount of morphologically abnormal sperm and concentration of L-carnitine in ejaculate. In volume of ejaculate, concentration of sperm and total rate of sperm there weren’t noticed any positive changes during the experiment.

ACKNOWLEDGEMENT
This project was funded from grants IGA IP 038/2017: Effect of supplementation of L-carnitine on qualitative and quantitative parameters of ejaculate of duroc boars.

REFERENCES


