Semen characteristics of boars receiving control diet and control diet supplemented with L-carnitine

J. Čeřovský, S. Frydrychová, A. Lustyková, J. Lipenský, M. Rozkot

Institute of Animal Science, Praha-Uhříněves, Kostelec nad Orlicí workplace, Czech Republic

ABSTRACT: The objective of this experiment was to test a hypothesis that L-carnitine supplemented diet would improve semen characteristics in 6 adult crossbred AI boars (Hampshire × Pietrain). The control and the tested diet were identical except the tested period (8 weeks) when the diet was supplemented with 2 g of L-carnitine per boar per day. The semen was collected regularly weekly by a gloved-hand technique. Semen volume, sperm motility and concentration, proportion of alive sperm cells and aspartate aminotransferase (AspAT) activity of semen were determined immediately after the semen collection once every two weeks. Sperm survival rate, morphologically abnormal spermatozoa, seminal plasma mineral components and free amino acid concentration, L-carnitine concentration in semen plasma and in sperm cells were determined after the sample storage (–20°C) at a later time. The differences ascertained between the average values of semen characteristics in the control vs. tested period did not prove a true and unambiguous positive effect on boar semen parameters by dietary supplementation of L-carnitine as our data show in our study: volume (239.11 vs. 250.50 ml; *P* 0.518), sperm concentration (301.67 vs. 350.83 × 10^3/mm^3; *P* 0.309), sperm progressive motility (66.94 vs. 70.00%; *P* 0.409), morphologically abnormal spermatozoa (29.00 vs. 27.46%; *P* 0.802), daily sperm cells output (9.86 vs. 11.71 × 10^9; *P* 0.206), proportion of alive sperm cells (72.56 vs. 74.13%; *P* 0.484), survival spermatozoa ability maintenance (43.29 vs. 38.68%; *P* < 0.01), mineral components in the seminal plasma (Na-, K-, Ca-, Mg-, Zn-; P from 0.138 to 0.968), AspAT activity (in semen plasma – 132.50 vs. 128.31 mU/10^9 spermatozoa; *P* 0.830, in sperm cells – 147.37 vs. 119.01 mU/10^9 spermatozoa; *P* 0.146), semen plasma amino acid concentration – a significant positive effect of L-carnitine in lysine only (0.79 vs.1.17 μmol/100 ml; *P* < 0.01), L-carnitine concentration (in semen plasma 255.40 vs. 259.97 mg/l; *P* 0.884, in sperm cells – 1 110.68 vs. 883.58 mg/l; *P* < 0.01). In conclusion, the studied indicators of semen quality were not significantly enhanced by dietary supplementation of L-carnitine in adult AI boars.

Keywords: L-carnitine; semen parameters; boars

The intake of L-carnitine, quaternary ammonium compound (β-hydroxy-4-*N*-trimethylaminobutyric acid, $C_7H_{15}NO_3$), is ensured mainly by nutrition but the organism is also able to supply its need by an endogenous synthesis in liver, kidneys and brain tissue from the conversion of two essential amino acids lysine and methionine (Jeulin et al., 1994; Cibulka, 2005).

In mammals, free L-carnitine is taken from the blood plasma and is concentrated in the epididymal lumen (Jeulin and Lewin, 1996). In the male reproductive tract very high concentrations (mmol/l) of L-carnitine and acetyl-L-carnitine are found in the epididymides, seminal plasma and spermatozoa (Golan et al., 1982).

In mammals, the origin of free L-carnitine in seminal plasma and spermatozoa is mainly epididymal. The epididymal secretion is the sole source of seminal carnitine (Brooks, 1979). Its concentration in the epididymal plasma of boars ranges

Supported by the Ministry of Agriculture of the Czech Republic (Project No. MZE 0002701401).

from 200 to 300 nmol/mg of protein (Jeulin et al., 1987). Free L-carnitine passes through the sperm plasma membrane by passive diffusion (Jeulin et al., 1994).

The main biological effect of L-carnitine is its involvement in the β-oxidation of long-chain fatty acids after their transport across the inner mitochondrial membrane to the mitochondrial matrix where these can be oxidised and generate energy (Bieber, 1988; Comhaire and Mahmoud, 2003; Cibulka, 2005). Inside sperm cells, L-carnitine transports medium and long-chain fatty acids into the mitochondria where they undergo beta-oxidation leading to the generation of metabolic energy needed for the sperm cells for their progressive movement (Jeulin et al., 1987). Intracellular acetyl-L-carnitine may be a metabolic fuel used by the spermatozoa during their short life (Jeulin and Lewin, 1996). An adequate supplementation of L-carnitine in animal nutrition enables to overcome an energy bottleneck with minimal performance losses, improves energy, fatty and amino acid utilisation and decreases nitrogen excretion (Baumgartner and Jacobs, 1999). L-carnitine also has a protective role against reactive oxygen species (ROS) by antioxidant properties. As a result of oxidative stress, the fusogenicity of the sperm plasma membrane is lost due to peroxidative damage of unsaturated fatty acids (Agarwal and Said, 2004). Oxidative stress in the male germ line leads to the induction of damage in the spermatozoa and loss of integrity in the nucleus and mitochondria (Aitken et al., 2003).

Spermatozoa which enter the epididymis are immotile and their free L-carnitine content is very low or undetectable. During their transit through the epididymis, they become capable of initiating flagellar motion and accumulate a very high concentration of free L-carnitine from the luminal fluid. The plasma membrane of epididymal spermatozoa allows passive diffusion of free L-carnitine from the fluid, during the transit time of 1–10 days. The initiation of sperm motility occurs in parallel with an increase in the concentration of free L-carnitine in the epididymal lumen. But progressive sperm motility indicates a more important metabolic function related to flagellar movement. The flagellar movement is probably independent of the carnitine system while the energy properties of L-carnitine can only be relevant in situations of energy crisis. The cytoplasmic free L-carnitine in mature spermatozoa must be a protective form of mitochondrial metabolism, useful to the survival of this isolated cell (Jeulin et al., 1994).

In some species, dietary supplementation of L-carnitine has been reported to increase sperm concentration and sperm motility. In humans, L-carnitine supplementation (3 g per day) increased the sperm concentration and sperm motility of idiopathic asthenozoospermia patients (Vitali et al., 1995).

Boars that received a diet supplemented with 500 mg per day of L-carnitine increased (by one) the number of AI doses produced per ejaculate (Baumgartner, 1998). "Akey" (2000) conducted a field trial with L-carnitine to determine its effect on the performance of boars housed in a commercial pigsty. Boars were fed for a 9-weeks test period three L-carnitine levels: none, low and high. Feeding the high level of L-carnitine to boars increased semen volume, number of viable sperm cells produced and resulted in an extra 2 doses of semen produced per boar per week for artificial insemination ("Akey", 2000). In boars receiving 230 mg of L-carnitine in their daily ration Wähner et al. (2004) demonstrated an increase in ejaculate volume and sperm concentration. The effect of L-carnitine addition on boar semen quality was studied in 5 Pietrain boars at the age 1.5–2.0 years that received 500 mg of L-carnitine per day for five weeks (Jacyno et al., 2007). The total number of spermatozoa per ejaculate increased significantly ($P < 0.05$). L-carnitine also had a significantly positive effect on major and minor morphological changes of spermatozoa ($P < 0.01$) The positive effect of L-carnitine on boar semen quality was observable as early as within one week of its application (Jacyno et al., 2007). Conversely, boars that were randomly selected for L-carnitine treatment and received a feed mixture supplemented with 500 mg per day L-carnitine for 16 weeks did not show any beneficial effects on boar libido, semen quality, sperm production or maintenance of sperm motility during liquid storage (Kozink et al., 2004).

The objective of the present study was to assess the effect of a diet supplemented with a high level of L-carnitine on semen characteristics in AI boars.

MATERIAL AND METHODS

An experiment was conducted using 6 lean type crossbred (Hampshire × Pietrain) adult AI boars. At the start of this study the boars were older than

two years ($\bar{x}$ = 45 months and 27 days). They were kept under the same housing, feeding and breeding conditions. All the boars were housed individually in single pens with a water nipple-drinker system. They were individually fed a commercial complete feed mixture for breeding boars (KA), depending on their body condition.

According to a laboratory analysis, the concrete basic values of KA complete mixture were as follows: 13.61 metabolisable energy (MJ), 156.51 g crude protein, 41.38 g crude fibre and 9.32 g calcium, 5.34 g phosphorus and 5.00 ± 0.28 mg of L-carnitine per kg of the mixture. The control and experimental diets were identical except the tested period when the diet was supplemented with 2 g of pure L-carnitine (Lohman Animal Health, Cuxhaven, Germany) per boar per day. The L-carnitine supplement was administrated to each of the boars in the diet once daily in the morning feeding manually.

In the course of the whole experimental (monitored) period after the trial preparatory period: the first control collections plus currently the beginning of daily L-carnitine administration (O), tested period 8 weeks (•) and control period 4 weeks after the last L-carnitine application (O), the data on semen was collected for analysis regularly every interval in the course of two weeks at the semen collection frequency once weekly, from all the boars in this experiment. The semen was collected by the gloved-hand technique into a sterilised plastic collector (bag) with the possibility to separate the gelatinous fraction from the liquid part of the ejaculate.

Gel-free volume, percentage of spermatozoa progressive motility, actual estimation of the proportion of viable/dead spermatozoa by eosin/nigrosin staining, sperm concentration (in 1 mm^3) were determined immediately after the semen collection. The sperm cell concentration was determined by a photometric method (Spekol 11, Zeiss, Jenna) and the daily output of sperm cells was calculated from the total sperm output per ejaculate and the length of the previous collection interval. The identification of morphologically abnormal spermatozoa (%) was carried out microscopically (magnification 1 500×) on the stained dry smears of native semen on slides.

To estimate the survival rate of sperm cells (%) semen samples diluted at the ratio 1 part of semen + 8 parts of SUS diluent were used. These samples were stored in a special cooling box at the temperature of 16 ± 1°C for 4 days. Each day of storage 4 ml of diluted semen was sampled and incubated in a water bath at a temperature of 37 ± 1°C for 5 hours and the sperm motility (%) was determined microscopically after one, three and five hours of sample incubation. For the statistical evaluation of this long-term thermoresistance test (TRT) results, the daily average of spermatozoa motility was used.

To determine aspartate aminotransferase (AspAT) activity, semen plasma amino acids, mineral components (Na-, K-, Ca-, Mg-, Zn-) and L-carnitine content the samples of native gel free semen (à 2 ml) were centrifuged (595 g) and samples of semen plasma (supernatants) and sperm cells (sediment) were stored at –20°C until later analysis.

AspAT activity was measured in natural seminal plasma and the extracellular AspAT activity of sperm cells was determined in a supernatant prepared after dilution of sperm sediment with redistilled water, freezing, thawing and centrifugation. AspAT activity was measured by the kinetic method (BIOLATEST set, Lachema Brno, CR) and calculated per 10^9 spermatozoa.

The concentration of fourteen free amino acids in semen plasma was determined to ascertain whether the L-carnitine feed supplementation in breeding boars can have a positive effect in terms of an enhancement of their content.

For the assessment of amino acid concentration the seminal plasma samples (à 1 ml) from all individual boars were deproteinised and after centrifugation 14 free amino acids were determined by means of liquid chromatography and photometrically by means of ninhydrin detection on an automatic analyser of amino acids (AAA, Model 339M). Mineral components in semen plasma were measured on an atomic absorption spectrophotometer (AAS, PYE Unicam, SP9).

L-carnitine in sperm cells, semen plasma and diet was determined by a radiochemical method, which is based on the conversion of carnitine into (^{3}H) acetyl carnitine by carnitine-0-acetyltransferase (Mc Garry and Foster, 1976 cit. Birkenfeld et al., 2006).

L-carnitine concentration was detected only in the first, 3rd, 5th and 7th collection, i.e. in two (1st + 7th) control and two (3rd + 5th) tested collections of semen.

Basic statistical characteristics of the results, arithmetic mean ($\bar{x}$), standard deviation (SD), significance (P) were obtained using the QC Expert

Table 1. Semen characteristics of 6 boars that received a control diet or identical diet supplemented with 2 g L-carnitine per day – tested period

Item	Control		Tested period		*P*-value
	$\bar{x}$	SD	$\bar{x}$	SD	
Ejaculates evaluated	18		24		42
Semen volume (ml)	239.11	46.72	250.50	62.07	0.518
Sperm concentration ($\times 10^3$/mm^3)	301.67	129.35	350.83	168.60	0.309
Progressive motility of spermatozoa (%)	66.94	13.08	70.00	10.63	0.409
Morphologically abnormal spermatozoa (%)	29.00	20.25	27.46	19.10	0.802
Daily sperm cell output ($\times 10^9$)	9.86	3.78	11.71	5.14	0.206
Alive sperm cells (%)	72.56	8.31	74.13	6.09	0.484
Spermatozoon survival ability (%)	43.29	–	38.68	–	0.018

program. Significance was declared at $P < 0.05$ and $P < 0.01$ level.

RESULTS

Tables 1–2 and Figures summarise the mean basic biological, chemical and biochemical semen characteristics determined during the whole experimental period in the 7 semen collections: 3 control and 4 trial collections. Supplementing the diet of boars with 2 g L-carnitine did not have a significant beneficial positive effect on the majority of the semen characteristics analysed in this experiment.

The volume of semen (gel free fraction) in the boars that received the supplement increased from 239.11 to 250.50 ml – by 10.47%. However, the difference in this trait was not significant ($P > 0.05$, Table 1). The highest increase in this trait was observed after two and four weeks from the start of feeding the supplemented diet (267.00 ml and 262.33 ml).

In spite of the substantially higher average value of sperm concentration in the treatment period in comparison with the control period – 350.83 vs. 301.67 × 10^3/mm^3, Table 1, the difference was not significant ($P > 0.05$). A substantial decrease in this trait was determined in the two control collections after the end of feeding the supplemented L-carnitine feed mixture to the boars (241.67 and 291.67 × 10^3/mm^3, $P > 0.05$).

Sperm progressive motility and morphologically abnormal spermatozoa means data are nearly similar between the control and treatment periods (66.94 vs. 70.00%, 29.00 vs. 27.46%, $P > 0.05$, Table 1).

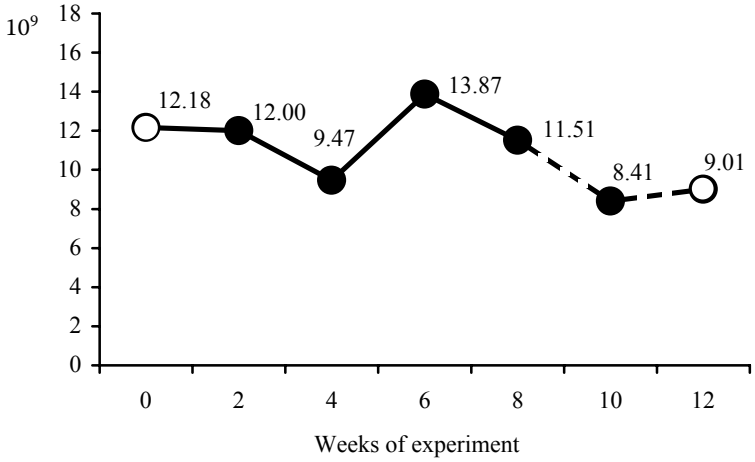

Figure 1. Daily sperm output (10^9) development in the semen of 6 boars in the control and tested period

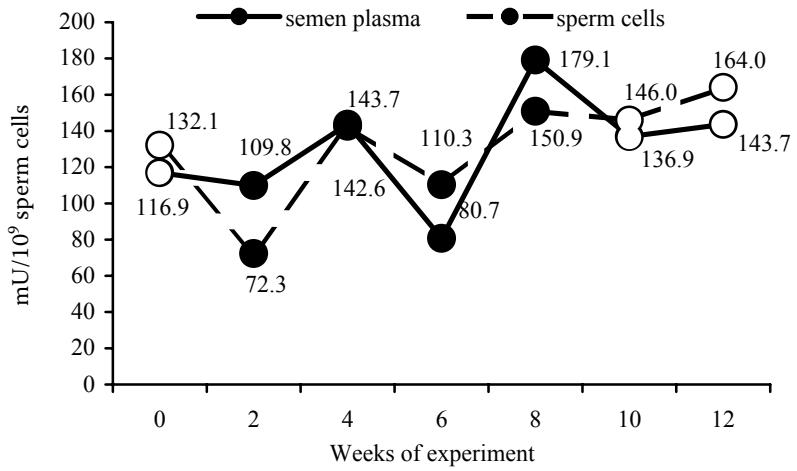

Figure 2. AspAT activity (mU/10^9 sperm cells) in the semen of 6 boars in the control and tested period

The lowest average occurrence of morphologically abnormal sperm cells (18.92%) was found in semen collections after 56 days (8 weeks) of the L-carnitine application period and the maximum in the last two control semen collections (29.00% and 37.00%).

There is no doubt about the positive trend of daily sperm cell output in favour of the treatment period (9.86 vs. 11.71 × 10^9, $P = 0.206$; Table 1; Figure 1).

The survival ability of sperm cells (percent motility) in diluted semen samples of experimental boars, stored for 4 days and tested daily by means of a long-term thermoresistance test, decreased gradually during the storage time in both control and trial samples but significantly in the samples of the L-carnitine feeding period (43.29% vs. 38.68%, $P < 0.01$, Table 1). These results indicate that the higher level of L-carnitine intake during the treatment period did not improve the percentage of mobile sperm cells. The samples from the control period exhibited a significantly higher percentage of motile spermatozoa during the storage of diluted semen.

The data presented in Table 2 show the mean content of selected mineral components in semen plasma in the control and treatment periods. Obviously, the differences between control and treatment data are not significant. The data presented here demonstrate that the content of selected minerals in semen plasma remained relatively unchanged during the whole experimental period.

Increased leakage of AspAT from sperm cells to the seminal plasma is a symptom of sperm cell

Table 2. Chemical and biochemical semen characteristics of 6 boars in the control and tested period

Item			Control		Tested period		*P*-value
			$\bar{x}$	SD	$\bar{x}$	SD	
Ejaculates evaluated			18		24		42
Mineral components of semen plasma (mmol/l)		Na	94.22	18.15	102.83	25.99	0.237
		K	21.32	7.59	18.26	4.32	0.138
		Ca	2.08	1.40	2.09	1.22	0.968
		Mg	16.55	7.41	20.95	17.70	0.281
		Zn	0.82	0.24	0.73	0.30	0.321
AspAT activity (mU/10^9 sperm cells)	semen plasma		132.50	50.66	128.31	69.43	0.830
	sperm cells		147.37	51.35	119.01	67.70	0.146
L-carnitine concentration (mg/l)	ejaculates (*n*)		12		12		24
	semen plasma		255.40	68.70	259.97	82.30	0.884
	sperm cells		1 110.68	166.25	883.58	195.72	0.006

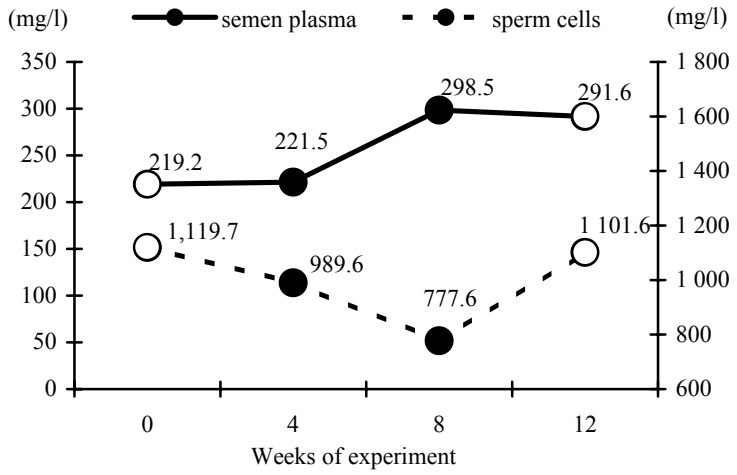

Figure 3. L-carnitine concentration (mg/l) in the semen of 6 boars in the control and tested period

membrane damage. This leads to a deteriorated biological value of the sperm. The development of AspAT activity in the native semen plasma was unexpected in our experiment conducted with carnitine tested boars as well as in sperm cells as residual AspAT activity measured in medium (supernatant) after spermatozoa damage. The lowest (positive) data on AspAT activity in the semen plasma was observed after 6 weeks (42 days) of L-carnitine supplement application (80.7 mU/10^9 sperm cells) and AspAT (negative) activity of sperm cells (72.3 mU/10^9) after 2 weeks from the beginning of the consumption of supplemented diet (Figure 2). The highest (negative) data on AspAT activity in the semen plasma was observed after 8 weeks (56 days) of the L-carnitine application period and positive AspAT activity of the sperm cells at the end of the whole experimental period after 12 weeks in the last (control) collections of semen (179.1 and 164.0 mU/10^9, Figure 2). The differences in AspAT activity between the control and treatment periods were not statistically significant

Table 3. Amino acid concentrations (μmol/100 ml) in semen plasma

Item	Control		Tested period		*P*-value
	$\bar{x}$	SD	$\bar{x}$	SD	
Ejaculates evaluated	18		24		42
Alanine	3.89	1.78	4.47	1.57	0.276
Aspartic acid	2.43	0.90	3.04	2.32	0.248
Glutamic acid	23.83	6.97	25.86	6.27	0.327
Glycine	13.49	4.76	14.07	3.74	0.662
Isoleucine	2.28	1.32	1.63	0.93	0.072
Leucine	2.12	0.95	1.77	0.70	0.179
Lysine	0.79	0.26	1.17	0.59	0.009
Methionine	0.70	0.40	0.71	0.41	0.920
Phenylalanine	1.48	1.09	1.29	0.69	0.520
Serine	1.50	0.59	1.61	0.44	0.473
Taurine	9.28	5.43	10.29	2.92	0.436
Threonine	1.42	0.51	1.63	0.48	0.169
Tyrosine	1.66	1.03	1.50	0.91	0.598
Valine	3.64	1.47	3.19	1.11	0.267

in either case: in semen plasma and in sperm cells (132.50 vs. 128.31 and 147.37 vs. 119.01 mU/10^9, $P > 0.05$, Table 2).

In Table 3, we can see that out of 14 free amino acids monitored an increase was observed in eight cases, a substantial one in lysine ($P < 0.001$) and in isoleucine ($P = 0.072$).

The results of L-carnitine concentration in both semen plasma and sperm cells ejaculated are presented in Table 2 and Figure 3. The maximum concentration of L-carnitine in semen plasma was found in samples taken from boars after the 56-day period of carnitine supplement application (298.5 mg/l, Figure 3). However, the difference between the average values of control and treatment samples was not significant (255.40 vs. 259.97, $P > 0.05$, Table 2). The L-carnitine content in sperm cells had an opposite negative tendency in comparison with the development of carnitine concentration in semen plasma samples during the experimental period. The unexpected lowest content of carnitine in sperm cells was found after 56 days of carnitine administration (777.6 mg/l, Figure 3) in contrast with the maximum in semen plasma samples. The difference between the average values of carnitine concentration in sperm cells of control and treatment samples was highly significant (1110.68 vs. 883.58 mg/l, $P < 0.01$, Table 2) in favour of control samples.

In conclusion, we did not observe any significant increase in L-carnitine concentration in the semen of mature boars, especially in the sperm cells.

DISCUSSION

The results of our experiment with supplementing L-carnitine (2 g per boar per day) to the control diet for a period of 8 weeks (treatment period) did not demonstrate any significant positive effects on the basic semen characteristics in 6 mature breeding boars ($P > 0.05$, Table 1) in spite of the positive higher mean values of semen volume, sperm concentration, daily sperm output and lower abnormal spermatozoa percentage in comparison with the control period. We observed a positive trend in daily sperm output ($P = 0.206$). Wähner et al. (2004) also reported an increase in ejaculate volume and higher sperm count in the ejaculate of boars receiving 230 mg of L-carnitine per day for 6 weeks. The addition of 500 mg L-carnitine per day to boar ration had a positive effect on semen characteristics in 5 Pietrain boars (Jacyno et al., 2007). Similarly, a high level of L-carnitine increased the number of viable sperm cells and the number of insemination doses produced per week (Akey Swine Newsletter, 2000). More sperm cells have important practical consequences for artificial insemination.

On the contrary, according to Kozink et al. (2004), the indicators of semen quality were not improved by dietary supplementation of 500 mg L-carnitine per day per boar. Spermatogenesis in boars requires 34–39 days and epididymal transport involves another 9–12 days (Swierstra, 1968; Almeida et al., 2006). Therefore, if a nutritional supplement such as L-carnitine was to enhance spermatogenesis, no effect would probably be observed until approximately 7–8 weeks after supplementation. In our experiment, it is interesting that the highest significant difference in sperm concentration and daily sperm output was observed in 42 days, i.e. after the spermatogenesis period. It is unlikely that L-carnitine positively affected spermatogenesis in our study according to the subsequent gradual decrease in sperm concentration up to the end of the experiment like in Jacyno's et al. (2007) observation. It is however doubtful that L-carnitine affected directly spermatogenesis manifested through an increase in sperm production.

L-carnitine may increase sperm viability, so perhaps fewer dead spermatozoa would be reabsorbed and as a consequence, the number of sperm cells in ejaculate increases (Jeulin and Lewin, 1996). L-carnitine is also implicated in buffering the cell against high concentrations of mitochondrial acetyl-CoA converting it into acyl carnitine. Surplus acetyl-CoA inhibits the activity of pyruvate dehydrogenase, a key enzyme in mitochondrial energy metabolism in men. This function of L-carnitine may further improve the survival rate of ejaculated spermatozoa and increase the total number of ejaculated spermatozoa (Matalliotakis et al., 2000) and logically the daily sperm output at the same semen collection frequency. But in our study the spermatozoa survival rate in TRT test decreased in time significantly, to a larger extent in tested than in control samples ($P < 0.01$, Table 1). This effect can be explained by the higher proportion of less valuable spermatozoon recovered vitality in epididymis due to the L-carnitine function.

Data on mineral components ascertained in semen plasma samples remained relatively unchanged in both tested and control samples without significant differences between the control and

tested period. An insignificantly higher concentration of Na^+ component in the treatment period ($P = 0.237$) can be explained by Na^+ ions needful as a transmitter of L-carnitine inside the cells in the case of L-carnitine uptake in boars above its normal concentration. Recently, a high affinity of Na^+ driven organic cation transporter was shown to transport L-carnitine into the cells of the epididymal epithelium (Rodrigues et al., 2002; cit. Agarval and Said, 2004).

The important significant difference in lysine concentration between the treatment and control mean data in favour of treatment samples was presented in Table 3 (1.17 vs 0.79 μm/100 ml; $P < 0.01$). The extra L-carnitine intake of the boars in this experiment (2 g per day) would be used for an explanation of lysine saving in a situation when the L-carnitine concentration within the lumen (fluid) of epididymis reached a sufficient normal level. We did not observe the same situation in the second amino acid methionine as a potential substrate of carnitine formation.

According to Jacyno et al. (2007) the addition of 500 mg of L-carnitine per day for 5 weeks had a positive effect on the activity of AspAT in the boar seminal plasma. AspAT activity decreased by 135 mU/10^9 spermatozoa ($P < 0.01$). A slightly positive insignificant fall of AspAT in seminal plasma in the treatment period in comparison with the control value was observed in our experiment (128.31 vs. 132.50 mU/10^9 spermatozoa, $P > 0.05$, Table 2) but so-called residual AspAT activity was, on the contrary, negative for spermatozoa from the treatment period, lower than in control samples (119.01 vs. 147.37 mU/10^9 spermatozoa, Table 2). The differences between the control and treatment samples of the boar seminal plasma AspAT activity are not significant ($P > 0.05$) in contrast to Jacyno's et al. (2007) results. In general, the AspAT activity in semen plasma was substantially lower in our experiment in comparison with Jacyno's et al. (2007) results probably owing to other boars used.

Carnitine itself is accumulated by spermatozoa during maturation in the epididymis (Agarwal and Sait, 2004). The relatively high daily dose of L-carnitine supplementation (2 g/day/boar) during the duration of spermatogenesis employed in our experiment could have the effect on the carnitine concentration increase in epididymis and so in both plasma and sperm cells ejaculated. However, the L-carnitine content in semen plasma and in sperm cells was not significantly increased in our study.

CONCLUSION

The study presents a report on the effects of L-carnitine supplementation on boar semen characteristics. However, the differences between parameters of semen were not statistically significant in favour of the carnitine treatment period. In our experiment, none of the boars was predetermined to have an unusually low sperm quality. Perhaps, there must probably be pre-existing subfertility conditions of animals for L-carnitine to consistently affect sperm characteristics as it is in subfertile men. The authors, however, cannot rule out the possibility that optimal levels of L-carnitine, higher number of experimental animals and length of experimental period could have a positive effect on the boar semen parameters.

Acknowledgements

The authors are grateful to Chovservis a.s. (Czech Republic), to Lohmann Animal Health, Cuxhaven (Germany) and Dr. Kluge from Martin Luther University, Halle-Wittenberg (Germany), for help in the accomplishment of this trial.

REFERENCES

Agarwal A., Said T.M. (2004): Carnitines and male infertility. Reproductive Bio Medicine Online, 8, 376–384.

Aitken R., Baker M., Sawyer D. (2003): Oxidative stress in the male germ line and its role in the aetiology of male in fertility and genetic disease. Reproductive Bio Medicine Online, 7, 65–70.

Akey Swine Newsletter (2000): Akey's field trial experience with L-carnitine supplementation of sow diets. Lewisburg, OH: Akey Inc., Germany, 1–2.

Almeida F.F.L., Leal M.C., Franca L.R. (2006): Testis morphometry, Duration of spermatogenesis and spermatogenic efficiency on the wild boar (*Sus scrofa*). Biology of Reproduction, 75, 792–799.

Baumgartner M. (1998): Boars react positively to L-carnitine supplements. Pig Topics, 13, 22 pp.

Baumgartner M., Jacobs St. (1999): L-carnitine: importance for pig breeding. Lohman Information, 22, 15 pp.

Bieber L.L. (1988): Carnitine. Annual Review of Biochemistry, 57, 261–283.

Birkenfeld C., Kluge H., Eder K. (2006): L-carnitine supplementation of sows during pregnancy improves the

suckling behaviour of their offspring. The British Journal of Nutrition, 96, 334–342.

Brooks D.E. (1979): Carnitine, acetylcarnitine and the activity of carnitine acyltransferases in seminal plasma and spermatozoa of men, rams and rats. Journal of Reproduction and Fertility, 56, 667–673.

Cibulka R. (2005): Metabolic effects of carnitine and its role in medicine. Clinics in Endocrinology and Metabolism, 13, 24–28.

Comhaire F.H., Mahmoud A. (2003): The role of food supplements in the treatment of the infertile man. Reproductive BioMedicine Online, 7, 385–391.

Golan R., Setchell B.P., Burrow P.V., Lewin L.M. (1982): A comparative study of carnitine and acylcarnitine concentration in semen and male reproductive tract fluids. Comparative Biochemistry and Physiology B., 72, 457–460.

Jacyno E., Kolodziej A., Kamyczek M., Kawecka M., Dziadek K., Pietruszka A. (2007): Effect of L-carnitine supplementation on boar semen quality. Acta Veterinaria Brno, 76, 595–600.

Jeulin C., Lewin L.M. (1996): Role of free L-carnitine and acetyl – L-carnitine in post gonadal maturation of mammalian spermatozoa. Journal of Reproduction Update, 2, 87–102.

Jeulin C., Soufir J.C., Marson J., Paquignon M., Dacheux J.L. (1987): The distribution of carnitine and acetylcarnitine in the epididymis and epididymal spermatozoa of the boar. Journal of Reproduction and Fertility, 79, 523–529.

Jeulin C., Dacheux J.L., Soufir J.C. (1994): Uptake and release of free L-carnitine by boar epididymal spermatozoa *in vitro* and subsequent acetylation rate. Journal of Reproduction and Fertility, 100, 263–271.

Kozink D.M., Estienne M.J., Harper A.F., Knight J.W. (2004): Effects of dietary L-carnitine supplementation on semen characteristics in boars. Theriogenology, 61, 1247–1258.

Matalliotakis I., Koumantakis Y., Evageliou A., Matalliotakis G., Goumenou A., Koumantakis E. (2000): L-carnitine levels in the seminal plasma of fertile and infertile men: correlation with sperm quality. International Journal of Fertility and Women's Medicine, 45, 236–240.

Swierstra E.E. (1968): Cytology and duration of the cycle of seminiferous epithelium of the boar duration of spermatozoa transit through the epididymis. The Anatomical Record, 161, 171–186.

Vitali G., Parente R., Melotti C. (1995): Carnitine supplementation in human idiopathic asthenospermia: clinical results. Drugs under Experimental and Clinical Research, 21, 157–159.

Wähner M., Geyer M., Hallfarth G., Hühn V. (2004): Der Einfluss von Zulagen einer Vitaminemulsion mit L-carnitine auf die Spermaeigenschaften von Besamungsebern. Züchtungskunde, 76, 196–207.

Received: 2008–08–14
Accepted after corrections: 2009–03–26

Corresponding Author

Doc. Ing. Josef Čeřovský, DrSc., Institute of Animal Science, Přátelství 815, 104 00 Prague-Uhříněves, Czech Republic
Tel. +420 494 323 291, fax +420 494 323 384, e-mail: vuzvkostelec@tiscali.cz