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Relationship between Serum L-Carnitine Levels and Sperm Parameters in Boars

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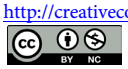

Abstract

This study evaluated the relationship between serum L-carnitine level and sperm parameters in young boars. Serum L-carnitine and semen characteristics were determined for 61 young Duroc boars between the ages of 590 and 630 days. Multiple linear regression analysis was performed to predict total and progressive motility and the total number of spermatozoa based on serum total L-carnitine and free L-carnitine levels. Total number of spermatozoa was not associated with basal serum L-carnitine levels. A regression equation was found in which both total L-carnitine levels and free L-carnitine levels were significant predictors of total and progressive motility ($P < 0.05$). These results suggest that serum L-carnitine level is an important selection parameter for stock boars.

Keywords

Serum L-Carnitine, Sperm Total Motility, Sperm Progressive Motility, Boar

1. Introduction

L-carnitine plays a vital role in cellular energy production by mediating the transport of long-chain fatty acids from the cytosol into the mitochondria and promoting β-oxidation of long-chain fatty acids. Then, L-carnitine transports acyl-group residues away from the mitochondria. L-carnitine also has an important non-enzymatic antioxidant role in the cell and protects both DNA and the membranes of organelles. In particular, L-carnitine plays a role in sperm metabolism by providing energy to spermatozoa, which sustains sperm motility and promotes maturation in the spermatogenic process [1]. L-carnitine is synthesized from lysine and methionine in the liver and kidney epithelia predominantly, though γ-butyrobetaine deoxygenase activity is recognized in the brain tissue

and testicles [2]. L-carnitine is then transported by the blood to skeltal muscles, cardiac muscles, brain tissue, and the testicles, where it is stored. Spermatozoa are exposed to the large amounts of L-carnitine stored in the testicles, and this exposure induces the capacity for progressive motility in the final stage of sperm maturation. In a previous study, we observed the effect of dietary supplementation of L-carnitine on lactation and piglet growth, and observed a possible relationship with genetic differences in the *de novo* synthesis levels of L-carnitine [3]. Another earlier study involving Japanese black steers showed a likely relationship between plasma L-carnitine levels and genetic characteristics [4]. Furthermore, Azzawi *et al.* [5], Mongioi *et al.* [6], Aliabadi *et al.* [7], Garolla *et al.* [8], and Khademi *et al.* [9] reported that men with infertility caused by insufficient L-carnitine synthesis, oral administration of L-carnitine increased sperm motility. In contrast, a low serum L-carnitine level resulting from a genetic deficiency decreased sperm motility. In other words, the serum level of L-carnitine might affect the ability of males to reproduce and might also be an important selection factor for good stock boars. Few studies have examined the relationship between serum L-carnitine level and sperm motility. Therefore, in this study, we aimed to evaluate the relationship between serum L-carnitine level and sperm parameters in young boars for the selection of good stock boars.

2. Materials and Methods

Serum and spermatozoa were collected from 61 healthy young Duroc boars between the ages of 590 and 630 days. The boars were fed a commercial diet twice a day, with no difference in feed intake. Extraction was performed once a week, and the semen was collected by the glove-hand technique with the boar mounted on a dummy sow. After removing the gelatinous fraction by filtering through a gauze, the remaining semen was used for the analysis. Sperm samples were incubated at 37˚C for 15 minutes before analysis of total and progressive motility was performed using a semen analysis system (IVOS II: IMV Technologies, L'Aigle, France) equipped with CASA (computer-assisted sperm analysis) hardware (Hamilton Thorne Co. Ltd., Beverly, MA, USA).

Serum L-carnitine levels were determined using an enzyme cycling method [10] performed by SRL Co., Ltd. (Tokyo, Japan). Total and progressive motility and total number of spermatozoa were predicted by multiple linear regression analysis based on serum total L-carnitine or free L-carnitine level.

3. Results

Serum L-carnitine levels as determined by the results of the semen analysis are shown in **Table 1**. Sperm abnormalities were also determined, including bent tail and tail winding, differentially methylated region, rate of proximal protoplasm, and rate of distal protoplasm but their relationship with serum L-carnitine level was not assessed in this study. Multiple linear regression was performed to predict total and progressive motility and total number of spermatozoa based on

serum total L-carnitine or free L-carnitine level. Total number of spermatozoa was not associated with basal serum L-carnitine levels. A significant regression equation was found for the relationship between serum L-carnitine levels and sperm motility (top part of Figure 1): (2, 58) = 3.64303227, P = 0.03233089, with an R^2 = 0.11160214. Predicted motility was equal to 85.6093651 + 0.49513105 (total L-carnitine levels) − 0.0836825 (free L-carnitine levels), where total L-carnitine and free L-carnitine levels were measured in units of μM/L. Both total and free L-carnitine levels were significant predictors of motility. A significant regression

Table 1. Parameters of spermatozoa and serum carnitine levels in this study.

Spermatozoa[1] (×10³)	Total motility (%)	Progressive motility (%)	Total L-carnitine (μM/L)	Free L-carnitine (μM/L)
99,225.7 ± 4485.2	91.5 ± 0.5	63.1 ± 2.1	16.3 ± 0.4	14.8 ± 0.4

Mean ± SE. N = 61. [1]Total counts/ejaculation.

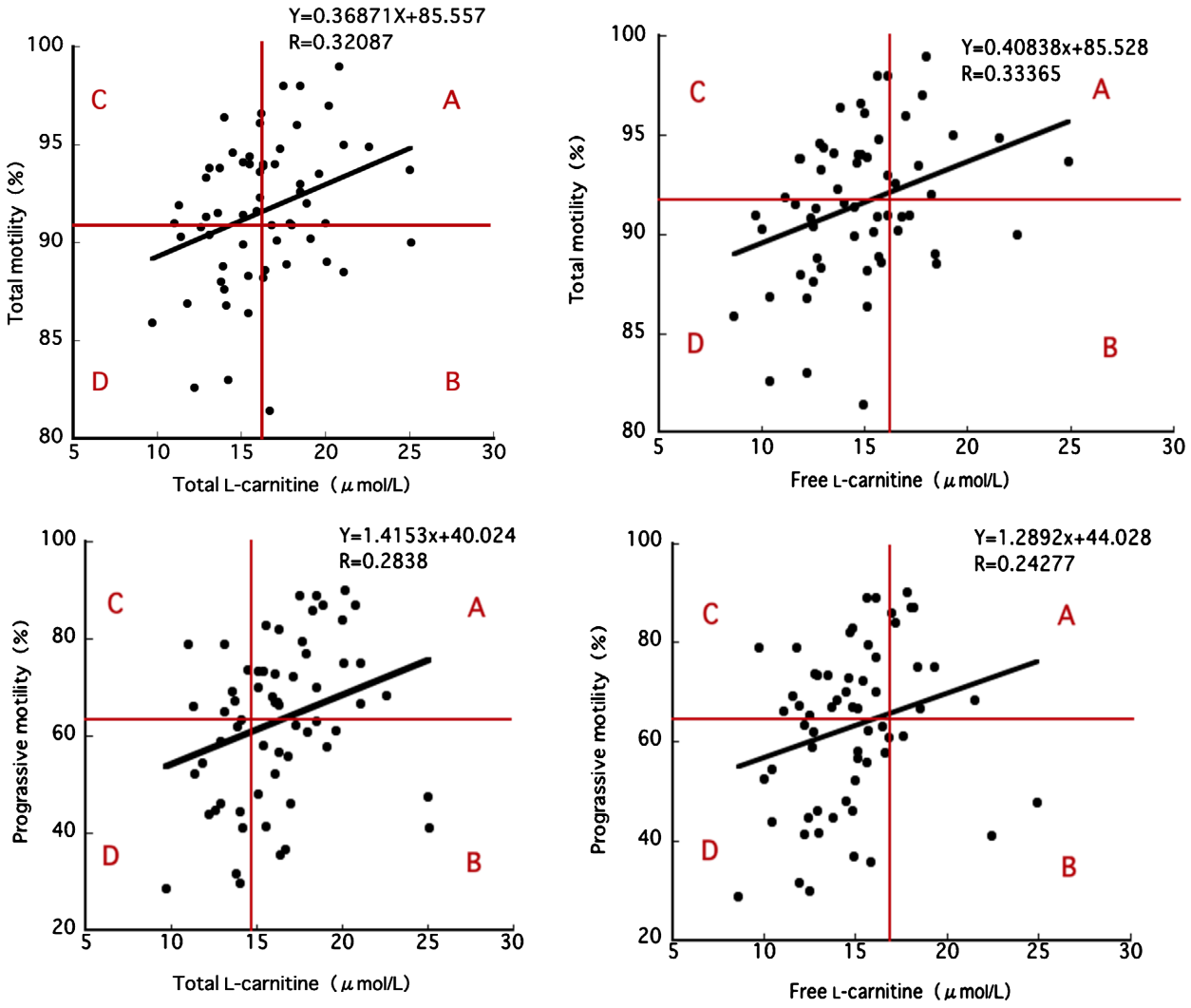

Figure 1. Significant (P < 0.05) linear regression recognized between serum total or free L-carnitine and total or progressive motility.

equation was found for the relationship between serum L-carnitine levels and sperm progressive motility (bottom part of **Figure 1**): (2, 58) = 3.28891357, P = 0.04436009, with an R^2 = 0.10185891. Predicted progressive motility was equal to 39.679129 − 3.3706251 (total L-carnitine level) + 4.49488128 (free L-carnitine level), where total L-carnitine and free L-carnitine levels were measured in units of μM/L. Both total L-carnitine levels and free L-carnitine levels were significant predictors of total and progressive motility.

The top part of each plot shows relationship between serum total or free L-carnitine levels and total motility and bottom part shows the relationship between serum total or free L-carnitine levels and progressive motility. In this study, the threshold values of total and progressive motility and the relationship with serum L-carnitine levels were according to their mean values, but these will be further investigated in the future.

4. Discussion

The amount of dietary nutrient supplementation, especially that of a de novo synthesizable nutrient, in the body has a large effect on the physiological reactions it induces. Earlier studies on L-proline employed the deletion method to remove L-proline from the medium used to culture cells [11] [12]. The biochemical function of L-carnitine differs from that of L-proline, and thus L-carnitine is not a common component of proteins, especially in plants. Because L-carnitine is provided only through de novo synthesis, it is difficult to use the deletion method to evaluate its nutritional roles.

Reports on the impact of dietary supplementation with L-carnitine on semen characteristics are inconsistent [3] [13] [14] [15] [16], *i.e.* dietary L-carnitine is effective in some reports and ineffective in some reports. The reason for this inconsistency seems to be the results of differences in the amount of L-carnitine synthesized de novo in the body, which is influenced by the amount administered. The relationship between L-carnitine and male infertility has been studied extensively [5] [6] [7] [8] [9] and treatment with L-carnitine has been shown to improve total and progressive motility. These reports showed the male infertility can be caused by L-carnitine deficiency, particularly in the epididymal plasma.

In the present study, a positive relationship between serum L-carnitine and total number of spermatozoa was not found, in disagreement with previous reports [5] [6] [15]. The present results were separated into four groups as shown in **Figure 1**. One group (area A) showed an obvious positive regression of serum L-carnitine level with total and progressive motility. Another group (area D) had low serum L-carnitine levels, which are caused by low de novo synthesis of L-carnitine, which might be improved through dietary administration of L-carnitine. The other two groups (areas B and C) were unrelated to the serum L-carnitine level. One of them (area B) had a high serum L-carnitine level and low motility, whereas the other (area C) had a low serum L-carnitine and high motility. We were unable to clarify the reason, although there may be another genetic factor that relates to

sperm motility. The thresholds of motility and progressive motility and their relationship with the serum L-carnitine level will be further investigated in future work, but we use the mean values in the present study. It is possible that the low total and progressive motility observed in area D might be recovered through the dietary administration of L-carnitine, similar to the male infertility treatments in human patients [5] [6] [7] [8] [9].

In this study, we demonstrated that serum L-carnitine level is an important selection parameter for stock boars and that sperm characteristics might be improved by the high serum L-carnitine level following dietary administration of L-carnitine in boars with a genetic deficiency.

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Animal Welfare Statement

The animal protocol for this study was approved by the Animal Care and Use Committee of Shizuoka Professional University of Agriculture.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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