Effects of Dietary Carnitine Supplementation on Plasma Carnitine and Some Serum Biochemical Parameters in Lambs

Introduction

Carnitine is an aminoacid derivative which is synthesized from lysine and methionine in vivo, and widely distributed to various tissues. It is essential in fatty acid oxidation as a cofactor to the transfer of fatty acids across the inner mitochondrial membrane. Carnitine is also important in regulating some other biological processes including urea cycle, gluconeogenesis, stimulation of fatty acid synthesis and metabolism of ketones, branched amino acids, triglycerides and cholesterol [3, 6, 7, 12].

Carnitine plays important metabolic roles in ruminants as well as in other species [11, 23]. In ruminants, a large amount of carbohydrates received via feed is metabolized in the rumen. The metabolites which are actually absorbed, are primarily volatile fatty acids. The energy metabolism of these animals is particularly oriented towards utilization of lipids [11]. In a study conducted on Freisian calves, which were given milk replacer with or without supplements of L-carnitine or DL-carnitine, it has been reported that the carcass quality tended to be better, and that the utilization of dietary fat and glucose metabolism was the best in the calves given supplements of L-carnitine based on the levels of blood parameters [23].
LACOUNT et al. [17] have shown that the increase at the levels of carnitine in milk, plasma, urine and liver was similar, either carnitine was provided in rumen or in abomasum, and that carnitine supplementation improved lipid digestibility. GREENWOOD et al. [15] reported that, with some levels of carnitine supplementation, plasma insulin, glucagon, cholesterol, triglyceride, and amino acid levels were not affected, whereas plasma concentrations of glucose, glycerol, urea and ß-hydroxybutyrate were increased.

This study was performed to investigate the effect of different amounts of dietary carnitine supplementation used to increase fattening and carcass performance in lambs on plasma carnitine levels and some serum biochemical blood parameters. To our knowledge, this has never been investigated in lambs, although certain biochemical blood parameters in some animal species given carnitine supplementation in their feeds for various purposes have been studied.

Materials and Methods

A) ANIMALS AND FEEDING

Thirty male Merino x Kivircik crossbred (F1) lambs aged about five months and weighing 29 kg on average were used in this experiment. The lambs were divided into three groups as control, treatment 1 and 2, containing 10 lambs in each group. All animals were fed for 45 days with alfalfa hay, and compound feed which was prepared according to the N.R.C. [19] feedstuff composition tables. In addition, the treatment groups 1 and 2 received L-carnitine (Carniking, 100 and 200 mg, respectively) mixed into their compound feeds. The nutrient contents of the compound feed and alfalfa hay were determined by the methods described in the A.O.A.C. [1], and the analysis results are shown in Table I.

B) SAMPLE COLLECTION AND ANALYSES

Blood samples were taken from the jugular vein into tubes containing disodium ethylenediamine tetraacetate (Na-EDTA) and also into tubes without anticoagulant on the days 0, 25 and 45 of the experiment between 9.00-10.00 a.m. Blood samples were centrifuged at 1500 x g for 5 min, and the plasma were separated and stored at -20 °C for the carnitine analysis. The blood samples without anticoagulant were allowed to stand 2h at room temperature for proper clotting. The sera obtained after the centrifugation were stored at 4°C overnight, and were analyzed the following morning. The serum glucose (Biocon 4341), total cholesterol (Technicon SM4-1139M90), triglyceride (Technicon SM4-1148M90) and total protein (Biotrol A01394U) concentrations were measured with Technicon DAX 72 Autoanalyser (Miles Inc., Tarrytown, NY, USA). Quality control of DAX Technicon autoanalyser is being performed according to the «Clinical Chemistry Quality Assessment Program» in BiRad Laboratories (9500 Jeronimo Road, Irvine CA).

The plasma carnitine, serum glucose, total cholesterol, triglyceride and total protein concentrations of lambs in each group on the days 0, 25 and 45 of the experiment are shown in Table II. On the 25th and 45th days of the experiment, carnitine values were higher in the treatment 2 than the control group (p < 0.001). Glucose levels were higher in the treatment groups than the control group on the 25th and 45th days of the experiment (p < 0.01 and p < 0.05), respectively. Total cholesterol values were found significantly different at the levels of p < 0.05 only on the 25th day of the experiment between control and treatment groups. The differences in triglyceride values between control and treatment groups were statistically significant at the levels of p < 0.01 and p < 0.05 on the 25th and 45th days of the experiment, respectively. No differences were found for total protein concentrations among the groups on the days 25 and 45 of the experiment.

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C) PLASMA L-CARNITINE ANALYSIS

L-carnitine concentrations were assayed with L-carnitine enzymatic UV kit (Roche Cat. No. 1 242 008) in deproteinized plasma spectrophotometrically (Shimadzu,UV-1601) at 340 nm wavelength. For deproteinization, 1 ml perchloric acid solution (0.6 mol/l) was added to 1 ml ice-cooled plasma, mixed properly, kept in ice-bath for 10 min and centrifuged at 3000 x g for 10 min. Then 1 ml supernatant was pipetted into a fresh centrifuge tube and 0.2 ml potassium carbonate solution (1.2 M) was added onto it. The mixture was mixed properly, kept in ice-bath for 20 min and centrifuged at 3000xg for 5 min. One ml of the separated supernatant was used to determine the L-carnitine level with the enzymatic kit.

D) STATISTICAL ANALYSES

The statistical analyses to determine the differences among the groups were performed by the statistical software programme SPSS running «One-way ANOVA and Tukey-Multiple Comparisons Test». The analysis of data according to the time course within the groups was performed by the statistical software programme GraphPad Instat V2.02 running «Repeated measures ANOVA and Tukey-Multiple Comparisons Test».

Results

The plasma carnitine, serum glucose, total cholesterol, triglyceride and total protein concentrations of lambs in each group on the days 0, 25 and 45 of the experiment are shown in Table II. On the 25th and 45th days of the experiment, carnitine values were higher in the treatment 2 than the control group (p < 0.001). Glucose levels were higher in the treatment groups than the control group on the 25th and 45th days of the experiment (p < 0.01 and p < 0.05), respectively. Total cholesterol values were found significantly different at the levels of p < 0.05 only on the 25th day of the experiment between control and treatment groups. The differences in triglyceride values between control and treatment groups were statistically significant at the levels of p < 0.01 and p < 0.05 on the 25th and 45th days of the experiment, respectively. No differences were found for total protein concentrations among the groups on the days 25 and 45 of the experiment.

Table I. — The nutrient content of compound feed and alfalfa hay.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Analysis</th>
<th>Compound Feed</th>
<th>Alfalfa Hay</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM¹</td>
<td>%</td>
<td>87.69</td>
<td>89.93</td>
</tr>
<tr>
<td>CP²</td>
<td>%</td>
<td>16.05</td>
<td>17.35</td>
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<tr>
<td>CF³</td>
<td>%</td>
<td>9.13</td>
<td>25.11</td>
</tr>
<tr>
<td>EE⁴</td>
<td>%</td>
<td>1.52</td>
<td>1.46</td>
</tr>
<tr>
<td>A⁵</td>
<td>%</td>
<td>7.22</td>
<td>8.85</td>
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<tr>
<td>NFE⁶</td>
<td>%</td>
<td>53.82</td>
<td>37.16</td>
</tr>
</tbody>
</table>

¹ DM : Dry matter, ² CP: Crude protein, ³ CF: Crude fiber, ⁴ EE: Ether extract, ⁵ A: Ash, ⁶ NFE: Nitrogen free extract. The results of analysis were given in natural dry matter content of feed.
The results of the analysis of data according to the time course showed that none of the groups exhibited statistical significance for the serum glucose and triglyceride levels among the experiment days (Table III). The total protein concentrations were different between the 0 and 45th days, and 25th and 45th days of the experiment in the control group (p < 0.05). In the treatment group 2, the total protein was found statistically significant among all the days of experiment at the p<0.05 level and, cholesterol values were found statistically significant between the 0 and 45th, and 25th and 45th days of the experiment at the p < 0.05 and p < 0.01 levels, respectively. While carnitine levels were found statistically significant at the level of p < 0.05 between the 0 and 25th days, and the 0 and 45th days of the experiment in the treatment group 1, statistically significant differences at the levels of p < 0.01 were determined among all the days of the experiment in the treatment group 2.

**Discussion**

In the present study, the serum glucose (3.48-3.92 µmol/l), total cholesterol (1.16-1.51 mmol/l), total protein (60.00-67.00 g/l) values were found consistent with the normal values in the literature [2, 5, 10, 14], while the triglyceride (0.22-0.31mmol/l) levels were slightly higher than the values reported by Altintas and Fidanci [2].

CHAPA et al. [9] investigated the effect of intravenous administration of L-carnitine on selected metabolites in Suffolk ewes and reported that plasma carnitine concentration increased after the administration, and that plasma glucose levels of the groups administered carnitine were higher than those of the control group. Carnitine concentrations were reported as 51.9 µmol/l and 102.3 µmol/l, 96.4 µmol/l in control group and groups administered carnitine at doses of 6.36 and 12.72 mmol/kg body weight [9,17, respectively].

LACOUNT et al. [17] showed that L-carnitine supplementation increased the concentrations of carnitine in plasma and liver, and improved lipid digestibility. They reported that carnitine concentrations in plasma increased linearly with carnitine supplementation, but concentrations of glucose, non-esterified fatty acids and urea were unaffected by the amount of dietary carnitine. A linear decrease in the plasma total cholesterol concentrations parallel to the increase at the carnitine levels was seen in another study conducted by the same authors [18]. The authors suggested that the dietary carnitine could be resistant to ruminal degradation.

Glucose levels were higher in the treatment groups than in the control group in this study (Table II). While this finding is compatible with those of GREENWOOD et al. [15] and CHAPA et al. [9], it is different from those of LACOUNT et al. [18] who did not observe any changes at the glucose levels after carnitine supplementation.

SHIMURA and HASEGAWA [21] reported that the administration of carnitine reduced the concentrations of triglycerides and total cholesterol in both liver and serum in rats administered with a high-fat diet, and that the addition of carnitine to a high-cholesterol diet decreased the levels of cholesterol and lipids in serum. They suggested that the addition

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Days of experiment</th>
<th>Control X ± SEM</th>
<th>Treatment 1 X ± SEM</th>
<th>Treatment 2 X ± SEM</th>
</tr>
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<tbody>
<tr>
<td>Carnitine (µmol/l)</td>
<td>(0)</td>
<td>43.67 ± 3.85a</td>
<td>46.84 ± 3.10a</td>
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<td></td>
<td>(25)</td>
<td>38.90 ± 2.48a***</td>
<td>86.54 ± 5.40b</td>
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<td>(45)</td>
<td>42.18 ± 2.92a***</td>
<td>86.91 ± 3.35b</td>
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<td>Glucose (mmol/l)</td>
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<td>3.53 ± 0.12a</td>
<td>3.66 ± 0.09a</td>
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<td></td>
<td>(25)</td>
<td>3.53 ± 0.10a**</td>
<td>3.80 ± 0.07ab</td>
<td>3.92 ± 0.06b</td>
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<tr>
<td></td>
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<td>3.48 ± 0.13a</td>
<td>3.81 ± 0.10ab</td>
<td>3.87 ± 0.09b</td>
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<tr>
<td>Total</td>
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<td>1.18 ± 0.05a</td>
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<tr>
<td>Cholesterol (mmol/l)</td>
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<td>1.40 ± 0.07a**</td>
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<td>1.16 ± 0.03b</td>
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<tr>
<td></td>
<td>(45)</td>
<td>1.51 ± 0.09a</td>
<td>1.37 ± 0.07a</td>
<td>1.36 ± 0.03a</td>
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<tr>
<td>Triglyceride (mmol/l)</td>
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<td>0.28 ± 0.02a</td>
<td>0.23 ± 0.01a</td>
<td>0.25 ± 0.01a</td>
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<tr>
<td></td>
<td>(25)</td>
<td>0.30 ± 0.01a**</td>
<td>0.23 ± 0.02b</td>
<td>0.22 ± 0.01b</td>
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<tr>
<td></td>
<td>(45)</td>
<td>0.31 ± 0.02a</td>
<td>0.23 ± 0.01b</td>
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<tr>
<td>Total protein (g/l)</td>
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<td>61.50 ± 1.30a</td>
<td>60.90 ± 0.70a</td>
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<tr>
<td></td>
<td>(25)</td>
<td>62.00 ± 1.00a</td>
<td>59.90 ± 1.30a</td>
<td>61.50 ± 1.00a</td>
</tr>
<tr>
<td></td>
<td>(45)</td>
<td>66.30 ± 0.70a</td>
<td>63.90 ± 2.00a</td>
<td>67.10 ± 1.50a</td>
</tr>
</tbody>
</table>

a,b,c Means within a row with the different letters are significantly different ,
*p < 0.05, **p < 0.01, *** p < 0.001, X = Mean, SEM = Standard error of mean.

**Table II.** — The plasma carnitine concentrations and some biochemical serum parameters of control, treatment 1 and treatment 2 groups (n = 10).
of carnitine might improve the lipid metabolism in obesity. In our study, total cholesterol levels on the 25th day of experiment and triglyceride levels on the 25th and 45th days of the experiment were lower in the treatment groups than those in the control (Table II). These results are consistent with SHIMURA and HASEGAWA [21] and LACOUNT et al. [18]. A central role for carnitine in the lipid metabolism has been proposed [7, 22]. The results of the present study support the hypothesis that the carnitine improves lipid digestibility and increases lipid utilization reported previously [7, 17, 21].

Plasma carnitine concentrations were determined as 38.90-109.37 µmol/l in the control group. The treatment groups 1 and 2 had higher carnitine concentrations than the control group on the 25th and 45th days of the experiment (Table II). The values determined in this study are compatible with the values reported by CHAPA et al. [9] (51.9 µmol/l). The results of the analysis of data according to the time course showed that the carnitine concentrations were statistically significant at the levels of p < 0.01 among all the days of experiment in treatment group 2, while there were differences at the levels of p < 0.05 between the 0 and 25th days, and 0 and 45th days of the experiment in the treatment group 1 (Table III). Plasma carnitine concentrations increased due to the amount of dietary carnitine in the present study. This finding is consistent with the results of the studies performed by LACOUNT et al. [18], CHAPA et al. [9] and GREENWOOD et al. [15].

In this study, an increase in plasma carnitine and serum glucose concentrations was seen, whereas a decrease in triglyceride and total cholesterol concentrations were observed after the carnitine supplementation. According to the results of this study, it might be concluded that the carnitine supplementation enhances lipid utilization in lambs. Also, the increased plasma carnitine concentrations shows that at least a portion of the dietary carnitine might be absorbed without degradation in the rumen.

References