Effects of Daily Astaxanthin and L-Carnitine Supplementation for Exercise-Induced Muscle Damage in Training Thoroughbred Horses

Fumio Sato\textsuperscript{a,}\textsuperscript{*}, Takaya Omura\textsuperscript{a}, Mutsuki Ishimaru\textsuperscript{a}, Yoshiro Endo\textsuperscript{a}, Harutaka Murase\textsuperscript{a}, Eiji Yamashita\textsuperscript{b}

\textsuperscript{a}Equine Breeding Science Division, Hidaka Training and Research Center, Japan Racing Association, Urakawa, Hokkaido, Japan
\textsuperscript{b}Medical Nutrition Division, Department of Research and Development, AstaReal Co. Ltd., Chuo-ku, Osaka, Japan

\textbf{Abstract}

The effects of dietary supplementation with astaxanthin and L-carnitine on serum markers and clinical incidence rate of exercise-induced muscle damage were studied in training horses. Sixty-three healthy Thoroughbred horses were randomly assigned to two groups and received the same base diet and exercise training throughout this study. The supplement (supp.) group (n = 31) received daily supplementation with astaxanthin (75 mg) and L-carnitine (3,000 mg) for 8 weeks, and the control (cont.) group (n = 32) received no supplementation. Blood samples were collected after high-intensity exercise training at 5 weeks before supplementation, 3 days (3d), and 8 weeks (8w) after the start of the supplementation. The blood samples were analyzed for creatine kinase (CK) activity and lactate dehydrogenase isoenzyme-5 (LDH-5), and a retrospective study was carried out by analyzing medical records for symptoms of exercise-induced muscle damage in both groups. Five horses (two of supp. group and three of cont. group) were excluded from this study for other disease. In the cont. group, CK activity at 8w was significantly increased compared with 3d, whereas the supp. group showed no significant change. After 8w, the CK activity of the supp. group also tended to be lower as compared with that of the cont. group. The incidence rate of exercise-induced muscle damage was significantly lower in the supp. group compared with the cont. group. These results suggest that continuous dietary administration of astaxanthin and L-carnitine attenuates exercise-induced muscle damage in horses.

\textbf{1. Introduction}

During exercise, whole body O\textsubscript{2} consumption increases free radical production of muscle [1]. Exercise-induced oxidative stress is believed to contribute to accelerated muscle fatigue and muscle fiber damage. Reactive oxygen species (ROS) produced during exercise relate to the delayed onset of muscle damage [2]. During the recovery period after exercise, leukocytes and macrophages, which are also potential sources of ROS, invade damaged muscle fibers [3]. An optimal diet and a wide variety of additional antioxidant supplements have been recommended for the management of horses with exercise-induced muscle damage [4–7]. Antioxidant supplementation trials have provided evidence that the exercise-induced muscle damage could be partially prevented in horse [6]. It has also been suggested that dietary vitamin E and selenium might...
protect muscles from ROS [8]. On the other hand, vitamin E and selenium deficiencies have not been identified in horses affected with exertional rhabdomyolysis, and further supplementation with these substances has not been beneficial [7,9]. The administration of ascorbate was supported to be effective in attenuating oxidative stress but did not prevent muscular damage [10]. Therefore, a clinically effective supplementation for preventing exercise-induced muscle damage has not yet been established.

Astaxanthin is a red pigment that belongs to the carotenoid family. Much like β-carotene, astaxanthin has been reported to have strong activity as an inhibitor of lipid peroxidation mediated by ROS [11]. The strong antioxidant effects of astaxanthin have been attracting attention, and astaxanthin is being used as a supplement for promoting human health [12-14]. The singlet oxygen quenching activity of astaxanthin has been shown to be approximately 6,000 times, 800 times, 560 times, and 75 times greater than that of vitamin C, coenzyme Q10, catechin, and alphalipoic acid, respectively, which indicates that astaxanthin is one of the strong antioxidants known [15]. Moreover, astaxanthin does not show any prooxidant activity, and its main site of action is on the cell membrane [16,17]. Astaxanthin was initially regarded simply as a pigment (it was used as a food-coloring agent and color enhancer for aquaculture fish). Now, it is widely used in dietary supplements because its efficacy has been clinically proven, its safety has been established, and its mechanism of action is well understood [18,19].

Furthermore, it was reported that astaxanthin prevents lipid peroxidation in solutions and in various biologic membrane systems [20]. On the mitochondrial membrane, astaxanthin suppresses the oxidative modification of carnitine palmitoyltransferase I (CPT1), which is involved in the membrane transport of long-chain fatty acids into the intramitochondrial site, and improves the efficiency of energy production [21,22]. And, L-carnitine (L-3-hydroxytrimethylaminobutanoate) is required for fatty acid transport by CPT1 [23]. A series of human studies were reported on the preventive effects of orally ingested L-carnitine for post-exercise muscle damage and/or delayed onset of muscle soreness [24]. Sufficient adenosine triphosphate (ATP) supply through L-carnitine function could reduce metabolic stress. Therefore, L-carnitine supplementation has been hypothesized to improve exercise performance through enhanced muscle fatty acid oxidation, altered glucose homeostasis, enhanced acylcarnitine production, and altered muscle fatigue resistance [22,25,26].

Here, we focused on both the strong antioxidant effect of astaxanthin and the enhancement of muscle fatty acid oxidation of L-carnitine for muscle fatigue resistance. We investigated the effects of dietary administration of astaxanthin and L-carnitine supplement on serum markers and the onset of clinical symptoms of exercise-induced muscle damage in training Thoroughbred horses.

2. Materials and Methods

This study was performed with the approval and under the guidelines of the Institutional Animal Welfare and Experiment Management Committee of the JRA Hidaka Training and Research Center.

2.1. Horses

In January, when the study was initiated, 63 clinically healthy and sexually intact young Thoroughbred horses (mean age ± standard deviation [SD]; 20.6 ± 1.1 month, mean body weight ± SD; 452.1 ± 23.8 kg) were 2 months into the start of riding exercises in a training stable. The horses were randomly assigned to two groups while taking gender into account: 31 in the supplement (supp.) group (19 colts and 12 fillies) and 32 in the control (cont.) group (19 colts and 13 fillies). All horses were received the same base diet and training exercise throughout the study.

2.2. Diets

The horses were fed four times daily with hay comprised (approximately 2 kg/d), oats (approximately 2 kg/d) and complete industrial feed (approximately 5 kg/d). The nutritive value and chemical compositions of the complete industrial feed (JRA original 10, NOSAN Co, Kanagawa, Japan) are summarized in Table 1. Other grass hay and water were available for free-feeding in each individual stall.

2.3. Exercise

The exercise regimen was according to a traditional training schedule for young horses: two high-intensity training sessions per week followed by three or four low-intensity sessions. Through the end of January, the horses warmed up on a walking machine. Two days a week after the warm-up, the horses cantered 1600 m on a flat-track course at medium speed with the last 500 m completed at 9.0 m/s. Next, they galloped 1,000 m on a slope course (peak incline of 5.5°) at an average speed of 10 m/s twice a week. On the other 3 days after the warm-up, the horses cantered 2,400 m at a speed of between 7.7 and 9.0 m/s on a

<table>
<thead>
<tr>
<th>Component</th>
<th>DM Content</th>
</tr>
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<tbody>
<tr>
<td>DE</td>
<td>&lt;2.8 Mcal/kg</td>
</tr>
<tr>
<td>Crude fat</td>
<td>&lt;6.0%</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>&gt;9.0%</td>
</tr>
<tr>
<td>Crude ash</td>
<td>&lt;10.0%</td>
</tr>
<tr>
<td>Ca</td>
<td>&lt;1.5%</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.5%</td>
</tr>
<tr>
<td>Mg</td>
<td>&lt;0.4%</td>
</tr>
<tr>
<td>Na</td>
<td>&lt;0.2%</td>
</tr>
<tr>
<td>Cu</td>
<td>&lt;85 mg/kg</td>
</tr>
<tr>
<td>Fe</td>
<td>&lt;290 mg/kg</td>
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<tr>
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<td>&lt;290 mg/kg</td>
</tr>
<tr>
<td>Mg</td>
<td>&lt;80 mg/kg</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>&lt;12,600 IU/kg</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>&lt;575 mg/kg</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>&lt;2,000 IU/kg</td>
</tr>
<tr>
<td>Lysine</td>
<td>&lt;1.0%</td>
</tr>
</tbody>
</table>

Abbreviations: DE, digestible energy; DM, dry matter.
flat course. In February and March, the supplementation was started, and the exercise intensity was gradually increased. The horses warmed up by trotting 800 m, then galloped for two rounds of 1,000 m on a slope course at an average speed ranging between 10.0 and 12.5 m/s, twice a week. On the other 4 days, the horses cantered 2,400 m at speed ranging from 7.7 to 9.0 m/s on a flat-track course. The horses cooled down by walking approximately 2,400 m every training day. Other than the training sessions, the horses were in small, individual paddocks for about 4 hours a day and were housed in a stall the rest of the time.

2.4. Astaxanthin and L-Carnitine Supplementation

The supplement contained 1,500 mg of microalgae *Haematococcus pluvialis* containing 2.5% w/w astaxanthin (AstaReal; AstaReal AB, Gustavsberg, Sweden). Its nutritional values are crude fat, 30%; crude protein, 8%; carbohydrates, 50% (including malt dextrin); ash, 4%; crude fiber, 1.5%; and astaxanthin, 2.5%. And it also contains the following minerals: Ca, 12,000 ppm; K, 3,500 ppm; P, 2,600 ppm; S, 1,400 ppm; Na, 700 ppm; Mg, 600 ppm; Fe, 240 ppm; Mn, 80 ppm; Zn, 20 ppm; and Cu, 10 ppm. The daily amounts of 3,000 mg of the product containing the aforementioned components except for astaxanthin are inadequate to have any effects on exercise-induced muscle damage. The supplement also contained 3,000 mg of 50.0% w/w L-carnitine (Carniking; Lonza Japan Ltd, Tokyo, Japan). The product includes another 50.0% w/w of silica. The supplement applied to a free-flowing, non-hygroscopic, and readily miscible inert carrier. Each supplement contained 37.5 mg of astaxanthin and 1,500 mg of L-carnitine and was mixed in fodder for the supp. group. The supp. group received daily administration of the supplement twice a day for 8 weeks from February to March, and the cont. group received no supplement.

2.5. Blood Sampling and Serum Analysis

Blood samples were collected from the jugular vein 4 hours after high-intensity exercise training at 5 weeks before supplementation (−5w), 3 days (3d), and 8 weeks (8w) after the start of the supplementation. Serum samples were stored frozen at −80°C until assay. Creatine kinase (CK) activity was measured by spectrophotometric assays kit (1-type Wako CK, LABOSPECT; Wako Pure Chemical Industries, Ltd, Osaka, Japan) using an automatic biochemistry analyzer system (7020 Clinical Analyzer; Hitachi High-Technologies Co, Tokyo, Japan) according to the manufacturer’s instructions. Lactate dehydrogenase isoenzyme-5 (LDH-5) activity was measured by agarose electrophoresis with the formation of a colored formazan dye using a fully automated electrophoretic analyzer system (Epalyzer-2-plus; Helena Laboratories Japan Co, Saitama, Japan) according to the manufacturer’s instructions.

2.6. Incidence of Clinical Signs of Exercise-Induced Muscle Damage

A retrospective study was performed by analyzing the medical records for clinical symptoms of exercise-induced muscle damage or fatigue in both groups. The clinical signs of lameness, for example stiffness, reluctance to move or muscle pain were evaluated every day after exercise training by four veterinarians. The clinical incidences were compared in each group for every 2-week period from the start to the end of the supplementation.

2.7. Statistical Analysis

All variables were analyzed using a commercially available software program (JMP9; SAS Institute Japan Co, Tokyo, Japan). We examined the mean changes in serum CK and LDH-5 activity in each group using nonparametric Wilcoxon rank sum test (Kruskal–Wallis test) for determination of the significance of the differences between sampling times and groups. A value of *P* < .001 was considered to indicate statistical significance for sampling time, *P* < .05 for between group differences. Furthermore, a chi-square test was used to determine differences between the incidence rates of each group; *P* < .05 indicated significance.

3. Results

Five horses (two fillies of supp. group; two colts and one filly of cont. group) were excluded from this study due to lameness (three) and poor appetite (two) due to an increase in exercise. Therefore, there were 29 in the supp. group (19 colts and 10 fillies) and 29 in the cont. group (18 colts and 11 fillies) included in the study analyses.

In the cont. group, serum CK activity was significantly (*P* < .001) increased from −5w (mean ± standard error, 347.6 ± 64.0 IU/L) to both the 3d (395.5 ± 51.2 IU/L) and 8w (987.1 ± 234.0 IU/L) measurements. In the supp. group, serum CK activity was also significantly (*P* < .001) increased between −5w (273.3 ± 19.5 IU/L) and 8w (657.8 ± 354.6 IU/L), whereas no significant change was shown between −5w and 3d (341.2 ± 31.2 IU/L) and between 3d and 8w, respectively. At 8w, the serum CK activity of the supp. group was significantly (*P* < .05) lower than that of the cont. group (Fig. 1A). Serum LDH-5 activity was significantly increased (*P* < .001) in both groups between −5w (supp., 19.7 ± 2.3 IU/L; cont., 22.6 ± 3.7 IU/L) and 8w (supp., 44.4 ± 7.6 IU/L; cont., 92.6 ± 28.2 IU/L), whereas no significant change was shown between −5w and 3d (25.7 ± 3.0 IU/L; cont., 40.0 ± 11.3 IU/L), and between 3d and 8w, respectively. At 8w, serum LDH-5 activity in the supp. group tended to be lower than the cont. group (Fig. 1B).

At 7 to 8 weeks after supplementation was started, the number of horses of clinical signs of exercise-induced muscle damage in the cont. group was increased (Table 2). Furthermore, at 7 to 8 week period, the incidence rate of clinical signs of exercise-induced muscle damage was significantly (*P* < .05) lower in the supp. group compared with the cont. group. In the cont. group, four of six horses had a clinical recurrence, whereas none in the supp. group did.

4. Discussion

In the present study, the continuous dietary supplementation of astaxanthin and L-carnitine decreases the serum...
marker levels of exercise-induced muscle damage in training Thoroughbred horses. Furthermore, it was also showed that the incidence rate of clinical signs of exercise-induced muscle damage was significantly decreased by daily administrating astaxanthin and L-carnitine supplement.

In high-intensity exercise training horse and growing young horse, maintaining adequate carbohydrate availability is important for exercise performance, particularly. Daily nutrient requirement of 24-month heavy exercise horse (429 kg) is known as 27.9 Mcal [27]. In this study, both cont. group and supp. group horses were fed the same amount of hay comprised (approximately 2 kg/d), oats (approximately 2 kg/d) and complete industrial feed (approximately 5 kg/d). Then, total digestible energy of these feeds reach to 25 Mcal. But, this is not so high amount of calories, and this industrial feed used in this study is commonly used for training horses in Japan. Furthermore, it also contains high amount of vitamin E (575 mg/kg). Vitamin E’s widely accepted function is that of a biological antioxidant. It was reported that the vitamin E supplementation of 5,000 IU/d decreases equine white blood cell apoptosis and plasma CK activity during and after endurance race [28]. The natural-source RRR-a-tocopheryl acetate contains 1.36 IU/mg, whereas the synthetic all-rac-
tocopheryl acetate contains 1 IU/mg [27]. Although, in this study, it was considered that daily 2,575 mg of vitamin E intake might have some prevention effects on exercise-induced muscle damage, we used this industrial feed to both cont. group and supp. group, knowingly. Even in such a situation, it was meaningful that the effect of the astaxanthin and L-carnitine supplementation was appeared.

In the supp. group, exercise-induced muscle damage was attenuated by daily administration of astaxanthin (75 mg) and L-carnitine (3,000 mg). A Thoroughbred horse is approximately 10 times heavier than a human. Therefore, many kinds of drugs or supplements for Thoroughbred horses are prescribed at 10 times the volume of humans. In humans, daily intake of astaxanthin (4-6 mg) decreases muscle fatigue and blood rheology [12,13], prevents exercise-induced free radical production, and improves activities performance [29,30]. In healthy elite soccer players, given 4 mg of astaxanthin daily for 90 days was unable to change baseline oxidative biomarkers and changes in oxidation associated with exercise but significantly reduced the exercise-induced increases in biomarkers of muscle damage (CK and alanine transferase) [29]. In preliminary study, we studied the effect of daily administration of 75 mg/d and 750 mg/d astaxanthin extracted from the microalgae for 2 months on serum CK activity in exercise-trained Thoroughbred horses [31]. Then, a significant increase of the serum CK activity was found in the cont. group, whereas it was not found in the both astaxanthin-administrated group. There was no significant difference among 75 mg/d and 750 mg/d administration of astaxanthin extracted from the microalgae. Although, the amounts of 3,000 mg of microalgae contain other components besides 75 mg of astaxanthin, the other components were inadequate to have any effects on exercise-induced muscle damage. The similar effects were found in the preliminary study using astaxanthin extracted from the microalgae [29]. Therefore, 75 mg of astaxanthin content in this study seems to be suitable.

Table 2

<table>
<thead>
<tr>
<th>Administration Periods</th>
<th>0–2 wk</th>
<th>3–4 wk</th>
<th>5–6 wk</th>
<th>7–8 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supp. group (n)</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Incidence rate (%)</td>
<td>6.9</td>
<td>3.4</td>
<td>3.4</td>
<td>3.4</td>
</tr>
<tr>
<td>Cont. group (n)</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>6 (4)</td>
</tr>
<tr>
<td>Incidence rate (%)</td>
<td>3.4</td>
<td>0.0</td>
<td>13.8</td>
<td>20.7</td>
</tr>
</tbody>
</table>

The number in parentheses of the last 7–8-wk administration period in cont. group indicates the number of horses that had a recurrence of clinical signs.

* Indicates a significant difference between the values (P < .05).
Furthermore, a series of human studies were reported on the preventive effects of orally ingested L-carnitine for post-exercise muscle damage [24]. The experimental data were obtained as serum analysis such as lactate response; purine catabolism: plasma hypoxanthine and uric acid concentration; free radical generation: xanthine oxidase, malondialdehyde responses; cytosolic leak out proteins: serum myoglobin, CK, fatty acid-binding protein. L-carnitine supplementation at 3 weeks made those parameters being alleviated in a short time frame, 180 minutes, and/or delayed onset in several days after squat exercise. Muscle disruption assessed from magnetic resonance imaging besides perceived muscle soreness showed better results in L-carnitine received group than those in placebo group. Although the possible mechanisms underlying is not clear enough, some working hypothesis was presented. High glycolytic rates by training brings in accumulation of adenosine diphosphate (ADP) and $H^+$, which activates the adenylate kinase reaction followed by ATP and adenosine monophosphate (AMP) formation from two ADP molecules. Subsequently, AMP is oxidized to hypoxanthine. In addition, ATP shortage inhibits calcium ATPase pumps to increase intracellular calcium. And then calcium-dependent proteases were activated, which leads to cleave a portion of xanthine dehydrogenase, converting it into xanthine oxidase. The produced xanthine oxidase uses $O_2$ as an electron acceptor, which generates superoxide radicals followed by sequential chain reaction of harmful lipid peroxidation. Sufficient ATP supply through L-carnitine function could reduce metabolic stress. Astaxanthin may act to quench ROS in the course of events. A recent understanding of L-carnitine function implies another possible mechanism on muscle cell protection. When energy demand increases, free fatty acid molecules are released actively to be delivered into mitochondria. Free fatty acid molecules *per se* play physicochemically harmful role against mitochondrial membrane by inducing membrane permeability transition. Cytochrome c is released from the damaged mitochondria, which forms apoptosisomes in the cytoplasm to induce caspase cascades resulting in apoptosis [32]. Coexisting sufficient amount of L-carnitine molecule attenuates the impact of the membrane toxicity [33]. Muscle cell damage may be initiated, in a part, from this manner. L-carnitine and astaxanthin, the ROS quencher, could concert beneficially. However, it was reported that significant beneficial effects could not be achieved by L-carnitine (2,000–6,000 mg) supplementation alone [22]. we used daily 3,000 mg of L-carnitine as the experimental dosage in this study. It is reported that total L-carnitine in equine gluteal muscle increased by 46% after 5 weeks of its supplementation and training; moreover, the supplementation combined with regular exercise training induces muscle modification toward a muscle type composed of more IIa fibers and capillaries, together with sparing effect of glycogen [34]. On other species, long-term ingestion of L-carnitine resulted in increment of the muscle content in trained rat [35] and human athlete [36]. In those, physical performances were improved besides the muscle content increment. It is also noteworthy that the L-carnitine concentration changes were shown only in the case accompanied by trainings, which are commonly observed over various mammalians. Furthermore, astaxanthin has been reported to suppress the oxidation of CPT1, which is involved in the membrane transport of long-chain fatty acids into the intramitochondrial site, and to improve the efficiency of energy production [21]. And L-carnitine is required for fatty acid transport by CPT1 [21]. Increased fatty acid utilization and decreased carbohydrate utilization are thought to be important for improving exercise performance. Then, the combined administration of astaxanthin and L-carnitine could cause an increase in utilization of fatty acids as an energy source, which spared the using of the glycogen, and performed efficient energy production [23,37]. When needed, L-carnitine is synthesized from raw L-lysine and L-methionine in the liver, but this production may be insufficient in the case of athletes who perform strenuous exercise [22]. In these instances, it is necessary to supplement L-carnitine. Astaxanthin and L-carnitine supplementation has been hypothesized to have a synergistic effect for preventing exercise-induced muscle damage in training Thoroughbred horses. A further study must be needed to investigate optimum doses between astaxanthin and L-carnitine to reduce the exercise-induced muscle damage.

In generally, CK is intracellular enzyme present in greatest amounts in skeletal muscle, myocardium, and brain. Disruptions of these cell membranes release CK from the cellular cytosol into the systemic circulation. On this basis, in healthy horses, elevated serum level of CK activity after intense exercise is able to be used as a marker of exercise-induced muscle damage. Horses may have CK activity levels that are significantly to greatly increased, depending on muscle damage severity. If the horses have rhabdomyolysis, CK levels increase more than 100 times of normal levels with great deviation in the cases. Furthermore, it is also generally known that lactate dehydrogenase (LDH) comprises four subunits and forms five kinds of isozymes. Isoenzyme patterns showed a selective concentration of LDH-5 in skeletal muscle, whereas in the heart, LDH-1 and -2 were predominant [38], and LDH-5 activity is correlated to muscle fatigue. Although the great deviation was identified after 8-week supplementation in both CK and LDH-5 activities, it was a matter of course for those biomarkers that the normal distributions could not be expected. At 8w, the serum CK activity of the supp. group was significantly ($P < .05$) lower than that of the cont. group using nonparametric statistical analysis method. The LDH-5 activity in the supp. group also tended to be lower than the cont. group. Then, it was also considered that the dietary continuous supplementation of astaxanthin and L-carnitine might attenuate exercise-induced muscle damage in training Thoroughbred horses.

Exertional rhabdomyolysis is probably the most common muscle disorder occurring in horses either during or immediately after exercising [39]. The onset of exertional rhabdomyolysis is potentially caused by factors such as overexertion, extreme tension, electrolyte imbalance, dietary issues (high-grain diets), or genetic disorders [40–42]. The clinical signs of this disease are variable, ranging from slight stiffness to immobility, signs of pain, and reluctance to move [40,43]. If such clinical signs are observed in a training horse, the trainer must reduce the
exercise intensity or change the feeding and potentially change the race schedule. These matters limit and inhibit the performance of the horse. Exertional rhabdomyolysis occurs in approximately 5.0% of all Thoroughbred race horses in the United Kingdom and United States [44,45]. Hence, the onset of the exertional rhabdomyolysis is responsible for substantial economic loss and is considered to be a serious problem by the racing community. Although we analyzed only medical records for clinical symptoms of exercise-induced muscle damage, retrospectively, in actuality, almost all horses were measured serum CK activity and serum amyloid A, but not all horses. And, the time points of the blood sampling for the clinical examination were not uniform. Therefore, in some cases, the clinical symptoms of exercise-induced muscle damage were not matched with such serum biological markers. Then, the horses, which were shown clinical symptoms of exercise-induced muscle damage, might be not equal with the onset horses of exertional rhabdomyolysis. However, the incidence rate for clinical signs of lameness related to exercise-induced muscle damage was significantly lower in the supp. group compared with the cont. group at 7 to 8-week administration period. Furthermore, no horse had a recurrence of clinical symptoms in the supp. group compared with four horses in the cont. group. In supp. group, the clinical symptoms of each onset horses were observed only one time. Then, it was considered that the dietary continuous supplementation of astaxanthin and L-carnitine might attenuate exercise-induced muscle damage in training Thoroughbred horses.

5. Conclusions

The present study showed that continuous dietary administration of astaxanthin and L-carnitine supplements reduced serum CK and LDH-5 activity levels and prevented the onset of clinical signs of exertional rhabdomyolysis in training Thoroughbred horses. Thus, administering astaxanthin and L-carnitine supplement might be helpful in maintaining condition and performance of exercising Thoroughbred horses.

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