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Effects of silkworm and its by-products on muscle mass and exercise performance in ICR mice.

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Abstract

In this study, we investigated the effect of silkworm, and its by-products on exercise endurance, muscle mass, and fatigue recovery using ICR mice model. Powders of silkworm, pupae, dongchunghacho, and silk powder were suspended in water and feed to mice for 2 weeks. The forced swimming time was increased by 4.3, 4.1 and 2.8 seconds after silkworm, pupae, and dongchughacho administration compared to DW fed group. Increases in exercise ability were achieved by difference mechanism according to feeding materials. Pupae increased muscle mass by 129% compared with the control group which may effect on elongation of swimming time. Dongchunghacho improved the plasma concentrations of fatigue markers such as creatinine, blood urea nitrogen (BUN), and D-lactate. Silkworm administration showed dual effect, the muscle mass induction (114% vs. control) and anti-fatigue (plasma creatinine, BUN, and D-lactate were 63, 75, and 78% vs. con) effect which contributed most elongated swimming time. In conclusion, silkworm and its by-products including pupae and dongchunghacho with the predominant protein and bioactive components improved muscle mass and showed anti-fatigue effect which could promote exercise performance ability.

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Introduction

The importance of exercise is being emphasized for the purpose of preventing the increase of metabolic diseases. However, exhausted exercise has been reported to induce stress, fatigue, and tissue damages (Anand *et al*., 2012). In particular, physical fatigue by exercise was caused by lack of energy source (i.e., ATP) and excessive accumulation of metabolite (i.e., lactate), thereby degrading functional performance of the muscle (Hsu *et al*., 2016). Therefore, fatigue could be minimized to maintain the good quality of exercise which affected by creatine, lactate, and glycogen metabolism. Previous studies have shown that improved exercise performance was observed after intake of plant-derived extracts (Anand *et al*., 2012; Hsu *et al*., 2016), creatine (Brenner *et al*., 2000), and lactobacillus (Chen *et al*., 2016). However, anti-fatigue study using animal sources are insufficient such as high protein and lipid based sources.

Silkworms (*Bombyx mori*) that were raised for the silk production are attracting attention as functional materials. Silkworm powder is composed of 55-65% crude protein, 9-14% crude fat with the high ratio of essential amino acids and unsaturated fatty acids, showing a variety of biological activity

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(Cha *et al*., 2009). In addition, the 1-deoxynojirimycin (DNJ), component of silkworms is a piperidine alkaloid compound which has a hypoglycemia effect by inhibiting α-glucosidase (Ryu *et al*., 2013). Silkworm industry produces various by-products such as pupae, dongchunghacho, and silk which showed health-promoting benefits. Silkworm pupae are rich in unsaturated fatty acids that are effective for hyperlipidemia and skin elasticity (Kang *et al*., 2006). Dongchunghacho is produced by inoculation of the mushroom to fifth instar silkworms and then cultivated until fruiting bodies are formed from the silkworm pupae. Blood pressure, blood glucose, and blood lipid lowering effects have been reported using dongchunghacho (Jo *et al*., 2015; Ahn *et al.,* 2007; Choi, *et al.,* 2012). Silk, a product of silkworm cultivation has been used as a natural fiber material, however, these days could be utilized as regenerative biomaterials such as the bio-film, fiber, and hydrogel, etc. (Cao and Wang, 2009; Vepari and Kaplan, 2007). Silkworm feces contain chlorophyll that has a positive effect on alcoholic liver damage (Cha *et al*., 2009). The silkworms and its by-products have high nutritional value; therefore, research on health functionalities has been conducted steadily.

In this study, we compared the silkworm, pupae, dongchunghacho, and silk on endurance exercise effect, muscle mass increasing effect, and fatigue relief effect using ICR mice model. Protein supplementation reported to improve exercise performance, increase nitrogen retention and muscle mass, prevents protein loss, and promotes muscle glycogen synthesis (Williams, 2005). Therefore, intake of high-quality protein derived from silkworms is expected to help improve exercise performance, we analyzed the effects of silkworms and by-products on exercise endurance.

Materials and Methods

Preparation of samples

Silkworm powder and pupae were purchased from sericultural farm UljinNongWon (Uljin, Korea). Dongchunghacho (*Paecilomyces japonica*) was obtained from sericultural farm TaeyangNongJang (Yeongdeok, Korea). Pupae and dongchunghacho samples were prepared as powder using grinder (HM-250, Hyundae Household Appliances cooperation, Incheon, Korea). Silk was hydrolyzed and lyophilized to solid powders for experiment.

Animal cares and diets

Mice care and experimental procedures were performed according to protocols approved by Daegu Haany University IACUC (Protocol No. DHU2018-074). ICR mice (6 week old, male) were purchased (Hanabio, Seoul, Korea) and adapted for 1 wk prior to feeding. Mice facilities were maintained at 23±2°C and 50±10% humidity with 12 hr light/dark light controls. Fed and water were *provided ad libitum*. Test materials, including silkworm, pupae, dongchunghacho, and silk were prepared in powders and suspended in DW. Samples were orally administered at 500 mg/kg concentration, once a day for 2 wk. Control groups fed DW.

Forced swimming capacity test

Transparent pool was filled with DW to a depth of 25 cm, and mice were individually dropped for swimming test. Tests were started after 1 hr of the feeding, and the mice were loaded with a lead weighing 5% of their body weight which was tied to mice tail. Tests were terminated when the mice were failed to raise their nose over the DW surface over 5 s.

Analysis of physical activity markers in plasma

Mice were sacrificed using isoflurane, and blood samples were collected from ventral aorta. Plasma was isolated after centrifugation at 3,000 rpm for 20 min. Aspartate amino transferase (AST, Wako Pure Chemical Industries, Osaka, Japan), alanine aminotransferase (ALT, Wako Pure Chemical Industries), blood urea nitrogen (BUN, Asan Pharmaceutical, Seoul, Korea), D-lactate (Bio Vision, CA, USA), creatinine (Sigma Aldrich, MO, USA) were measured from the plasma samples as the manufactures' instruction.

Analysis of glycogen contents in muscle and liver tissues

Mice livers and soleus muscles were isolated and stored at -84°C until further analysis. Glycogen concentrations were quantified using muscle and liver using ELISA kits according manufactures' instruction (Bio Vision).

Statistical analysis

The data are expressed as the means ± SEM. The student's *t*-test was performed for comparisons between the control and test groups. The statistical significance level was set at $P<0.05$.

Results and Discussion

Forced swimming time and muscle weight

Mice were fed silkworm, pupae, dongchunghacho, and silk for 2 wk, then the swimming time was compared (Fig. 1). Silkworm, pupae, and dongchunghacho administration significantly prolonged swimming time compared with the DW group ($P<0.001$). In addition, silkworm, pupae, and silk groups showed increase of muscle/body weight ratio (Table 1). However, liver/body weight was non-significantly changed compared with the control. Silkworm and pupae are high nutritional food materials with high protein and fat content (Yeo *et al*., 2013). The intake of high quality protein has a positive correlation with the increase in muscle mass (Houston *et al*., 2008). Enzymatically hydrolyzed egg which increased absorption of protein elongated the forced swimming time by antioxidant and anti-fatigue effect (Matsuoka *et al*., 2018). In this study, the intake of high-quality protein from silkworm and pupae also influenced the swimming time with increasing muscle mass. Dongchunghacho were estimated to have increased swimming time through other mechanisms than muscle mass changes, therefore, anti-fatigue biomarkers were further measured.

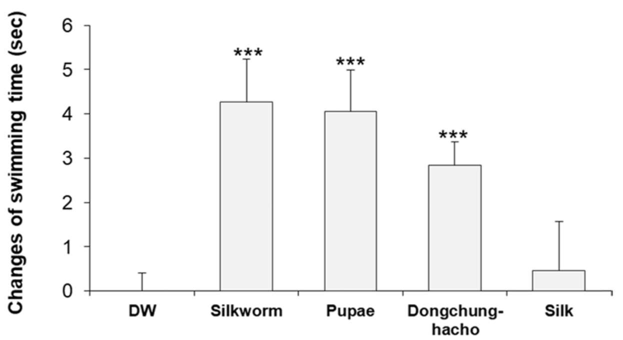

Fig. 1. Forced swimming time after silkworm and its by-products administration. Swimming time was measured at 0 and 2 week of silkworm and its by-products feeding. Data were expressed as $[(\text{Time}_{2week} - \text{Time}_{0week})_{treatment\ group} - (\text{Time}_{2week} - \text{Time}_{0week})_{control\ group}]$. Data indicate Means ± SEM. Significantly difference were analyzed by student's *t*-test, and marked as *$P<0.05$, **$P<0.01$, ***$P<0.001$.

Table 1. Mice liver and muscle weight per body weight after silkworm and its by-products feeding.

	Liver/Body weight	Muscle/Body weight
DW	0.051±0.001	0.007±0.001
Silkworm	0.049±0.002	0.008±0.000**
Pupae	0.050±0.002	0.009±0.000***
Dongchunghacho	0.049±0.001	0.007±0.001
Silk	0.048±0.000	0.010±0.000***

Data indicate Means ± SEM. Significantly difference were analyzed by student's *t*-test, and marked as *$P<0.05$, **$P<0.01$, ***$P<0.001$.

Exercise related markers in plasma

Consumption of silkworm and its by-products changed swimming time as well as the fatigue biochemical markers in plasma. The plasma AST and ALT, which were hepatic injury markers (Huang *et al*., 2015) were measured after forced swimming test (Fig. 2A and 2B). AST was 1.15 to 1.17 mmol/min/mL without significant, while the ALT was increased silk fed mice ($P<0.05$). Creatine is stored in skeletal muscle in the form of free creatine or phosphorylated creatine which produces muscle fiber energy, ATP. Decomposition of muscle tissue is released creatinine into the blood, an anhydrous form of creatine, therefore, plasma levels are increased (Snow and Murphy, 2001). Silkworm and dongchunghacho reduced plasma creatinine by 63 and 71%, respectively ($P<0.05$ vs. DW, Fig. 2C). The BUN is the nitrogen content in the plasma urea, the final metabolite of the protein, and indicator of fatigue. Decomposition of protein increase BUN levels in blood which results in muscle contraction and fatigue (Chen *et al*., 2016). Administration of silkworm and dongchunghacho powders altered BUN level by 75 and 77% in respectively ($P<0.001$ vs. DW, Fig. 2D). Muscular work is an aerobic reaction that provides muscle contraction energy through the breakdown of fat. However, oxygen deficiency promotes anaerobic processes and produces lactic acid as a product (Jung *et al*., 2004). The accumulation of lactic acid in the plasma after swimming was a result of ineffective muscle metabolism, and silkworm and dongchunghacho supplementation relieved these undesirable effects (78 and 77% vs. DW, P<0.05, Fig. 2E). In previous study, intake of *Cordyceps militaris* and *Paecilomyces*

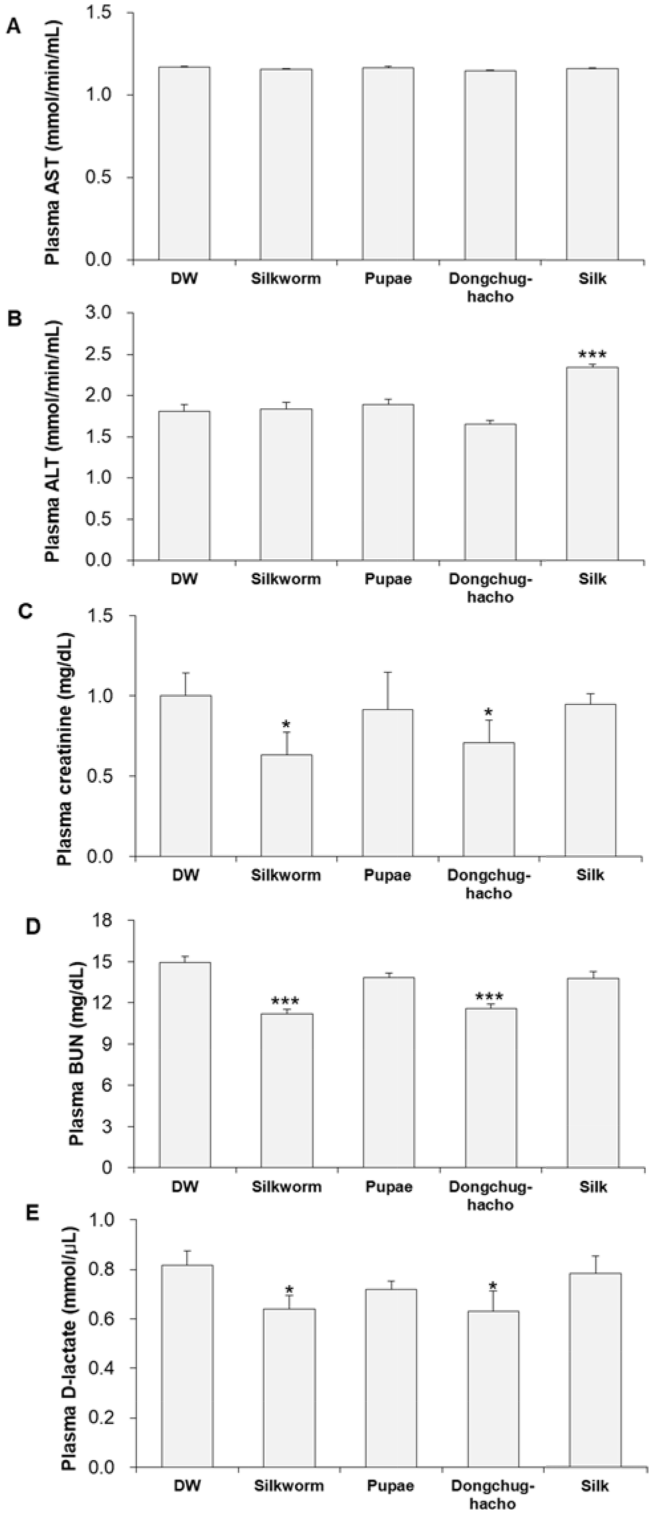

Fig. 2. Anti-fatigue markers in plasma after silkworm and its by-products administration.
(A) AST, (B) ALT, (C) Creatine, (D) BUN, (E) D-lactate level. Analysis was performed using plasma samples after 2 weeks of feeding. Data indicate Means ± SEM. Significantly difference were analyzed by student's *t*-test, and marked as **P*< 0.05, ***P*<0.01, ****P*<0.001.

japonica which are mycelium of the dongchunghacho were also increased swimming time by the anti-fatigue effect regardless of muscle mass (Jung *et al*., 2004). In the present study, there was no change in the muscle mass of dongchunghacho, but fatigue relief effect was improved. In addition, the silkworm increased swimming time by dual effect, anti-fatigue effect and increase of muscle mass.

Muscle and liver glycogen contents

Muscle glycogen level was increased after silkworm and dongchunghacho administration by 122% and 132%, respectively (*P*<0.05, Fig.3). In addition, silkworm and dongchunghacho administration as well as pupae were induced liver glycogen levels with the significance (134, 136, and 122% vs. control, *P*<0.05). Glycogen is the storage form of glucose which found in the liver and muscle. During exercise, glycogen is converted to plasma glucose, and depletion of glycogen is a major cause of fatigue (Roach, 2002). Therefore, maintaining appropriate of glycogen storage levels in the muscle and liver may delay fatigue and improve exercise endurance. The intake of silkworms, pupae, and dongchunghacho was increased glycogen content in muscle or liver, which positively affected swimming time.

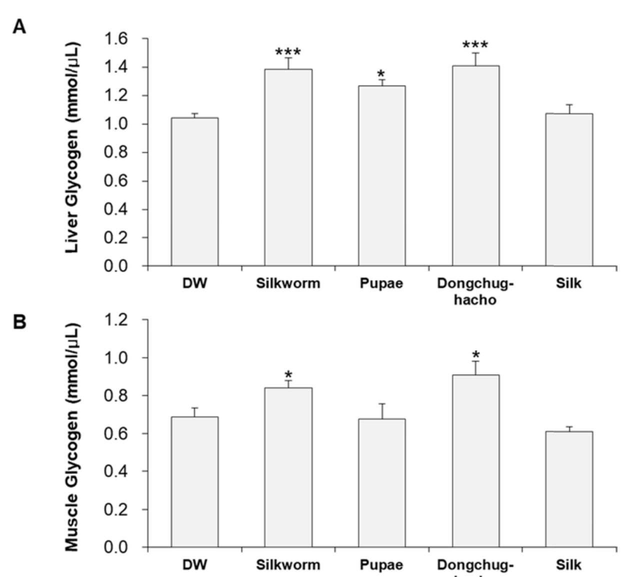

Fig. 3. Glycogen contents in liver and muscle after silkworm and its by-products administration. (A) Liver glycogen, (B) Muscle glycogen level. Analysis was performed using liver and muscle tissue extracts after 2 weeks of feeding. Data indicate Means ± SEM. Significantly difference were analyzed by student's *t*-test, and marked as **P*< 0.05, ***P*<0.01, ****P*<0.001.

Conclusion

The intake of silkworm, pupae, and dongchunghacho was increased swimming time; however, there was a difference in mechanism. Silkworms showed the greatest increase in swimming time due to dual mechanism, the increased muscle mass and reduced fatigue. On the other hand, swimming time was elongated by increase of muscle mass in pupae and decrease of fatigue in dongchunghacho, in respective. Silk showed the effect of increasing muscle mass, but there was no effect on exercise efficiency. This is presumed to be due to other offsetting factors. The results of this study are expected to be used as basic data for related industries such as improvement of energy metabolism using silkworm and its by-products.

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