Abstract. The aim of this study was to investigate whether Hirsutella sinensis mycelium (HSM) has any antifatigue effect, using a forced swimming model in rats. Forty rats were randomly divided into five groups, each containing eight animals. The control group received 2 ml/kg body weight of distilled water and a positive control group was administered 1.13 ml/kg Quaker Essence of Chicken. The treated swimming groups were administered HSM powder manufactured by Chang Gung Biotechnology Corporation, Ltd., at doses of 63 mg/kg, 189 mg/kg or 378 mg/kg body weight/day, respectively for a period of six weeks. The above experiment was repeated with another 40 rats but for a period of eight weeks. At the end of the experiments, rats were placed in a swimming apparatus and the total swimming time until exhaustion was recorded. Pre-/post-exercise concentrations of serum urea nitrogen (BUN) and lactic acid were also determined. There were no deaths during the study. Physical and behavioral examinations did not reveal any treatment-related adverse effects after dosing. Changes in lactate levels were dose-dependent for the 8- but not the 6-week treatment. BUN levels were more affected by the 8-week treatment of HSM but not significantly altered in the 6-week treatment groups. The 8-week treatment groups showed a significant increase in swimming time to exhaustion compared to the control groups, which was not dose-dependent. For the 6-week treatment, only the middle and high doses increased swimming time to exhaustion. Conjugated diene contents were significantly higher in rats treated at any HSM dose for 8-weeks than the control groups. Swimming did not alter levels of liver glycogen when compared to the control sub-groups. Results of this study demonstrate that HSM improves physical endurance, which may be beneficial in treating conditions where fatigue is a factor and other antifatigue treatments are contraindicated.

Ganoderma lucidum and Agaricus blazei Murrill have been regarded in Asia not only as gourmet cuisine but also as folk medicines and natural health tonics (1, 2). Recently, many mushroom species have been discovered to be miniature pharmaceutical factories producing hundreds of novel compounds with potential biological actions. Mushrooms are a source of compounds having properties of nutraceuticals, anti-oxidants, anticancer, pre-biotic, immunomodulating, anti-inflammatory, cardiovascular, anti-microbial and anti-diabetic actions (3-7). Current research efforts are directed towards identifying the compounds responsible for mediating these biological effects, with polysaccharides and nucleosides appearing to be the major candidates (1, 2).
Cordyceps sinensis grows on the Tibetan Plateau and the Himalayas of Tibet, Qinghai, West-Sichuan, India, Nepal and Bhutan. It is of a genus of fungus that grows on the larva of ghost moths and produces a fruiting body valued as a herbal remedy. The fungus germinates in the living larva in winter, kills and mummifies it, and then the stalk-like fruiting body emerges from the corpse in summer.

In China, C. sinensis is used for treating various diseases, such as fatigue, cough, hyposexuality, asthenia after severe illness, renal dysfunction, and renal failure (8, 9). Oral administration of water extract of C. sinensis mycelia (150 mg/kg/day for 7 days) or ingestion of fruiting bodies of C. militaris (500 mg/kg/day for 4 weeks) significantly prolonged the swimming time of mice by 20 and 24 min, respectively (10, 11). To date, more than 350 Cordyceps-related species have been found worldwide based on fungus which are parasitic in insects and insect hosts. Due to its rarity and outstanding curative effects C. sinensis is more expensive than gold and this has led to some natural substitutes such as C. militaris, C. liangshahensis, C. gunnii, and C. cicadicola being sold in markets (12). In addition, several cultured mycelia of C. sinensis and C. militaris have become the main substitutes of the natural species as commercial health food formulations, and 50 medicines and two dietary supplements related to cultured Cordyceps have been approved by the State Food and Drug Administration of China since 2002 (13).

We investigated whether products from mycelium of another fungus Hirutella sinensis (HSM) could be potential agents or functional foods for maintaining human health for popular healthcare food in Taiwan. The purpose of this study was to determine if HSM would increase the endurance of rats in a forced swimming paradigm. Different concentrations and time periods of HSM administration were used to examine if effects were concentration-and time-dependent.

Materials and Methods

Experimental animals. Male Sprague-Dawley rats, specific pathogen-free, eight weeks old and weighing 250-300 g, were obtained from the BioLASCO Taiwan Co., Ltd. and were used for the forced swimming model in all experiments. Forty rats were randomly divided into five groups, each containing eight animals. The control group received 2 ml/kg body weight of distilled water. The positive control group was fed 1.13 ml/kg body weight of Quaker Essence of Chicken (Quaker Co.; Taipei, Taiwan). The treated swimming groups were treated with HSM powder (Chang Gung Biotechnology Corporation, Ltd., Taipei, Taiwan, R.O.C.), dissolved in 2 ml distilled water, and administered by oral gavage at doses of 63 mg/kg, 189 mg/kg or 378 mg/kg body weight/day, respectively for a period of six weeks. Another 40 rats were treated under the same conditions but for a period of eight weeks. Animals were maintained in a filtered laminar air flow-controlled room under a 12-h light/dark cycle at a temperature of 20±2˚C with humidity 75±15%, at the animal facility of the Animal Medicine Center, College of Medicine, National Taiwan University, Taipei, Taiwan. Animals received autoclaved water and were fed ad libitum with laboratory pelot chow following the guidelines of the National Science Council of the Republic of China. Experiments were performed according to the regulations and guidelines for animal experiments in Taiwan, which are in agreement with the Helsinki declaration (14).

Forced swimming model. Swimming was performed in an adjustable-current water pool to evaluate effects of HSM on physical fatigue. We used an acrylic plastic pool (100 cm × 50 cm × 80 cm) filled with water (to a depth of 60 cm) maintained at a temperature of 29-31˚C with an electric heater. Animals could not support themselves by touching the bottom of the pool with their feet and were therefore forced to swim. In order to accustom the animals to swim, at the last week of the administration, animals were fasted for 30 min and then were made to swim for 30 min on seven consecutive days before swimming session. At the first day of the swimming session, rats were fasted for 30 min and the first sample blood was collected from the tail vein to establish the background data of lactate. And then animals were subjected to an acute bout of swimming to 10 min with current velocity set at 1.5 m/s and then a second blood sample was collected from the tail vein. The serum was prepared by centrifugation at a speed of 3000 x g for 10 min. Lactate levels were determined from the first and second samples using an autoanalyzer (Model 7070, Hitachi, Tokyo, Japan) and commercial kits (Hitachi, Tokyo, Japan). The rate of increase of lactate was calculated by comparison of these two samples.

At the second day of the swimming session, thirty minutes after the final oral administration of HSM, a third blood sample was collected from the tail vein to build up the background of BUN and then animals were placed in the swimming pool for 30 min with current velocity set at 1.5 m/s. At the end of the swimming test, animals were dried with paper towels and returned to their cages for 30 min, after which the fourth blood sample was taken. Serum urea nitrogen (BUN) levels in these samples was determined by a BUN kit (Beckman DxC 800, USA). The rate of increase of BUN was found by comparison of these two samples.

On the third day, rats were fasted for 30 min and placed in the swimming pool. They were allowed to swim until they failed to rise to the surface of the water to breathe for 7 s. They were rescued and that time was defined as the maximum swimming exhaustion time. After the endurance test, a fifth blood sample was collected from the tail vein to determine conjugated diene (CD) levels.

Rats were then sacrificed and their livers were dissected immediately, washed with 0.9% saline, and blotted dry with filter papers. Liver samples (100 mg) were accurately weighed, followed by the addition of 8 ml of homogenization buffer from an Anthracene Ketone reagent kit (NetSun Co., Zhejiang, China). Each aliquot of sample was sufficiently homogenized with a vortex mixer and then transferred to a centrifuge tube followed by centrifugation (3000xg, 15 min). A 1 ml aliquot of the supernatant fluid was transferred to another centrifuge tube followed by the addition of 4 ml ethanol (95%). All sample tubes were stoppered and deposited overnight. The sample homogenate was then centrifuged for about 15 min at a speed of 3000xg for complete protein precipitation. The supernatant was removed and the glycogen was dissolved in 2 ml water. The hepatic glycogen level was determined by anthrone colorimetry. To the brief, 10 ml of anthrone reagent are delivered
into each tube with glycogen, vigorously. The stream of anthrone reagent is directed into the center of the tube and should be sufficient to insure good mixing. As each tube receives anthrone reagent, it is tightly capped with an air condenser and placed in a boiling water bath to a depth a little above the level of the liquid in the tubes for 15 min and then removed to a cold water bath and cooled to room temperature. The contents of each tube are transferred to a calorimeter tube and read at 20 mμ.

CD levels were determined according to the method of Ward et al. (15). CDs were extracted from 50 μl plasma using a 2:1 (vol/vol) mixture of chloroform and methanol; 2 ml of the chloroform-methanol mixture, preheated to 45˚C, were added to 50 μl of serum. The mixture was then vigorously mixed (with a vortex machine) for 2 min, then mixed with 1 ml of distilled water acidified with 0.1 M HCl to a pH of 2.5. After vortexing, the material was subjected to centrifugation (2,000 × g for 5 min), and 0.75 ml of the lower layer was aspirated, transferred to a test tube, and dried under a nitrogen flow. The residue was reconstituted with 0.5 ml of heptane and the absorbance was measured spectrophotometrically at 233 nm. The results were reported as absorbance units (ABSU). The substance presented stronger antioxidant capacity at the lower absorbance values.

Statistical analysis. All values are presented as means±SE. Differences between the groups were analyzed by Student’s t-test. p-Values of less than 0.05 were considered significant.

### Results

**Effects of HSM on food intake and body weight.** No animals died during the study. Physical and behavioral monitoring did not reveal any treatment-related adverse effects after dosing. Daily food intake and final body weight were not significantly different among positive control and treatment sub-groups compared to their respective control subgroups.
Overall food consumption of animals receiving HSM was similar to that of the control subgroups and it was not statistically significant (Table I).

**Effects of HSM on lactate and BUN levels.** The results in Table II show that six weeks of HSM treatment did not alter blood lactate levels after swimming for 10 min (p>0.05). In contrast, 8-week HSM treatment significantly reduced blood lactate levels when measured after 10 min swimming compared to the negative control (Table II). Levels of BUN were significantly lower in the sub-groups under 8-week HSM treatment, except for the low dose (Table II). Differences in serum urea nitrogen were not significantly different in the groups treated for the 6-week HSM treatment (Table II).

**Exhaustive swimming test.** It can be seen from Table III that the 8-week HSM treatment of all groups significantly increased swimming time to exhaustion in comparison to control sub-groups. Among all administration sub-groups, only medium dose in 8-week treatment present more endurance than positive control. An enhancing effect of HSM on swim time after six weeks of treatment was only observed for the medium- and high-dose groups (Table III).

**Liver glycogen and blood CD levels after exhaustive swimming.** As shown in Table IV, after swimming, CD levels of the medium- and high-dose 6-week HSM treatment groups and all three doses of the 8-week treatment groups were all significantly lower than those of the control groups (p<0.05). It meant that HSM presented stronger antioxidant capacity. HSM treatment for six or eight weeks did not alter liver glycogen levels when compared to control sub-groups (p>0.05) (Table IV).

**Discussion**

It is well-established that blood lactate is the product of carbohydrate glycolysis under anaerobic conditions, and that glycolysis is the main energy source for intense exercise over short time periods. The accumulation of blood lactate is a major cause of fatigue during physical exercise (16-20). In the present study, we showed that HSM effectively delayed a rise in blood lactate levels and slowed effects of physical fatigue.

BUN is an important biochemical blood parameter associated with fatigue. Urea is formed in the liver as the end-product of protein metabolism. There is a positive correlation between the urea nitrogen level in vivo and exercise tolerance. In the present study, HSM at high or medium doses for eight weeks inhibited production of urea nitrogen after exercise. The 6-week HSM treatment was ineffective in reducing exercise-induced accumulation of urea nitrogen.

Energy for exercise is derived initially from the breakdown of glycogen. Strenuous exercise will deplete muscle glycogen, and later, energy will be produced from circulating glucose released by the liver (21). Glycogen levels are a sensitive indicator associated with fatigue. We found that HSM treatment did not increase liver glycogen following forced swimming.

In conclusion, the data suggest that HSM can extend swimming time to exhaustion in rats. HSM treatment also reduced blood lactate and BUN levels but did not have an effect on tissue glycogen. HSM has anti-fatigue effects and could be a supplement to improve endurance in medical conditions where fatigue is a debilitating factor. Further studies are needed to identify mechanisms of the antifatigue actions of HSM.

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**References**


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