ผลของสารสกัดจากเขากวางอ่อนพันธุ์รูซ่าที่มีต่อความล้าของกล้ามเนื้อและความทนทานในการออกกำลังกายในหนูเพศผู้พันธุ์วิสตาร์

นายรัสสะ ศรีภิรมย์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาวิชาชีวเวชศาสตร์

มหาวิทยาลัยเทคโนโลยีสุรนารี

ปีการศึกษา 2558

EFFECTS OF RUSA DEER (*Cervus timorensis*) VELVET ANTLER EXTRACT ON MUSCLE FATIGUE AND EXERCISE ENDURANCE CAPACITY IN MALE WISTAR RATS

Ratsa Sripirom

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biomedical Sciences

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EFFECTS OF RUSA DEER (*Cervus timorensis*) VELVET ANTLER EXTRACT ON MUSCLE FATIGUE AND EXERCISE ENDURANCE CAPACITY IN MALE WISTAR RATS

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree.

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รัสสะ ศรีภิรมย์ : ผลของสารสกัดจากเขากวางอ่อนพันธุ์รูซ่าที่มีต่อความล้าของกล้ามเนื้อและความทนทานในการออกกำลังกายในหนูเพศผู้พันธุ์วิสตาร์ (EFFECTS OF RUSA DEER (*Cervus timorensis*) VELVET ANTLER EXTRACT ON MUSCLE FATIGUE AND EXERCISE ENDURANCE CAPACITY IN MALE WISTAR RATS) อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร.รุ่งฤดี ศรีสวัสดิ์, 128 หน้า.

ในการศึกษาครั้งนี้ศึกษาผลของสารสกัดเขากวางอ่อนพันธุ์รูซ่า (DAV) และ testosterone ที่มีผลต่อความล้าของกล้ามเนื้อและความทนทานในการออกกำลังกายในหนูขาวเพศผู้พันธุ์วิสตาร์ โดยทำการศึกษาสองการทดลองและมีการบังคับให้หนูทดลองว่ายน้ำโดยใส่น้ำหนักที่หางซึ่งใช้ในการบ่งชี้ถึงความสามารถการทำงาน มีการวัดค่าความสัมพันธ์ของอวัยวะและน้ำหนักตัวของตับและกล้ามเนื้อลาย (soleus extensor digitorum longus (EDL) และ gastrocnemius) และปริมาณไกล-โคเจนในตับและในกล้ามเนื้อ gastrocnemius ของหนูทดลองจากทั้งสองการทดลอง

การทดลองที่ 1 ศึกษาผลการต้านอาการล้าจากสารสกัด DAV โดยแบ่งหนูทดลองเป็น 6 กลุ่ม กลุ่มละ 8 ตัว ประกอบด้วยกลุ่ม corn oil (1 ml/kg i.m.) กลุ่ม TP (80 mg/ml/kg testosterone propionate i.m.) กลุ่มควบคุม (double deionized distilled (DDD) water 1 ml/kg p.o.) และกลุ่ม DAV 100 DAV 200 DAV 400 (100 200 400 mg/ml/kg DAV p.o.) โดยให้สารวันละครั้งนาน 9 วัน ผลของสารสกัด DAV และ testosterone ต่อการต้านอาการล้าแสดงให้เห็นจากการเพิ่มขึ้นของเวลาในการว่ายน้ำจนหมดแรงซึ่งพบในหนูกลุ่มที่ได้รับสารสกัด DAV 200 และ DAV 400 เมื่อเทียบกับกลุ่มควบคุมและกลุ่ม DAV 100 และในกลุ่ม TP เมื่อเทียบกับกลุ่ม corn oil การเพิ่มขึ้นอย่างมีนัยสำคัญถูกพบใน EDL ROW (กลุ่ม TP DAV 100 และ DAV 200) และระดับไกลโคเจนในกล้ามเนื้อ gastrocnemius (กลุ่ม DAV 400) เมื่อเทียบกับกลุ่มควบคุมของแต่ละกลุ่ม และยังพบการลดลงอย่างมีนัยสำคัญของปริมาณไกลโคเจนในตับในกลุ่มที่ได้รับสารสกัด DAV ทุกความเข้มข้นและ testosterone เมื่อเทียบกับกลุ่มควบคุมของแต่ละกลุ่ม

การทดลองที่ 2 ศึกษาผลของสารสกัด DAV ที่มีต่อความทนทานในการออกกำลังกาย หนูทดลองถูกแบ่งออกเป็น 12 กลุ่ม กลุ่มละ 8 ตัว โดยหกกลุ่มแรกได้รับสารเช่นเดียวกับการทดลองที่ 1 โดยได้รับการฝึกการออกกำลังกาย โดยการว่ายน้ำที่ไม่ใส่น้ำหนักที่หาง (Ex) และหกกลุ่มหลังไม่ได้รับการฝึกการออกกำลังกาย (Non-Ex) โดยให้สารดังกล่าววันละครั้งเป็นเวลา 16 วัน ในกลุ่มที่ได้รับการฝึกการออกกำลังกาย ผลการเพิ่มความทนทานในการออกกำลังกายของสารสกัด DAV ได้แสดงให้เห็นจากการเพิ่มขึ้นอย่างมีนัยสำคัญของเวลาที่ใช้ในการว่ายน้ำจนเหนื่อยของกลุ่ม DAV 200 + Ex เมื่อเทียบกับกลุ่ม vehicle + Ex ในทางตรงกันข้ามกลุ่ม TP + Ex แสดงให้เห็นถึงการลดลงอย่างมีนัยสำคัญของเวลาที่ใช้ในการว่ายน้ำจนเหนื่อยเมื่อเทียบกับกลุ่ม corn oil + Ex ในกลุ่มที่ได้รับการฝึก

การออกกำลังกาย ปริมาณไกลโคเจนในกล้ามเนื้อ gastrocnemius ของหนูทดลองในทุกกลุ่มของสารสกัด DAV + Ex และปริมาณไกโคเจนในตับของกลุ่ม DAV 200 + Ex มีค่าสูงกว่ากลุ่ม vehicle + Ex อย่างมีนัยสำคัญ ในกลุ่มที่ไม่ได้รับการฝึกการออกกำลังกาย ปริมาณไกลโคเจนในตับของกลุ่มสารสกัด DAV 400 มีค่าสูงกว่าทุกกลุ่มอย่างมีนัยสำคัญ การฝึกการออกกำลังกาย มีผลไปลดระดับของปริมาณไกลโคเจนในตับอย่างมีนัยสำคัญและเพิ่มระดับของปริมาณไกลโคเจนในกล้ามเนื้อ gastrocnemius ในกลุ่ม DAV 400 อย่างมีนัยสำคัญเมื่อเทียบกับการไม่ได้รับการฝึกการออกกำลังกาย

สรุป ผลการทดลองนี้แสดงให้เห็นถึงฤทธิ์ในการต้านการล้าและการเพิ่มความทนทานในการออกกำลังกายจากสารสกัด DAV ซึ่งสนับสนุนการใช้สารสกัดนี้ในการบรรเทาอาการล้าและหมดแรงของกล้ามเนื้อในมนุษย์ สารออกฤทธิ์ที่พบในสารสกัด DAV เช่น โปรตีน และคอลลาเจน อาจมีผลในการเพิ่มสมรรถนะในการออกกำลังกายและออกฤทธิ์ในการต้านอาการล้า อย่างไรก็ตาม ยังคงต้องการการศึกษาเพิ่มเติมเพื่อให้ทราบถึงกลไกการทำงานในการต้านการล้าและการเพิ่มความทนทานของสารสกัด DAV

สาขาวิชาปรีคลินิก ลายมือชื่อนักศึกษา _____

ปีการศึกษา 2558 ลายมือชื่ออาจารย์ที่ปรึกษา Rungruedee Sisawat

RATSA SRIPIROM : EFFECTS OF RUSA DEER (*Cervus timorensis*) VELVET ANTLER EXTRACT ON MUSCLE FATIGUE AND EXERCISE ENDURANCE CAPACITY IN MALE WISTAR RATS. THESIS ADVISOR : ASST. PROF. RUNGRUDEE SRISAWAT, Ph.D. 128 PP.

ANTI-FATIGUE/ VELVET/ ANTLER/ RUSA DEER/ GLYCOGEN CONTENT

The aims of the present study were to investigate the effects of rusa deer antler velvet extract (DAV) and testosterone on muscle fatigue and exercise endurance capacity in male Wistar rats. Two experiments were performed and the tail weight-loaded forced swimming of animals was employed as a criteria for physical work capacity. Relative organ weight (ROW) of liver and skeletal muscles (soleus, extensor digitorum longus (EDL) and gastrocnemius), liver and gastrocnemius muscle glycogen content were determined.

Experiment 1 investigated the anti-fatigue effect of DAV. Rats were divided into 6 groups (n = 8 each): corn oil (1 ml/kg, i.m.), TP (80 mg/ml/kg, testosterone propionate, i.m.), vehicle (DDD water, 1 ml/kg, p.o.), and DAV 100, DAV 200, and DAV 400 (100, 200, 400 mg/ml/kg DAV, p.o.). All drugs were given once daily for 9 days. Anti-fatigue effects of DAV and TP were demonstrated since significant increases in swimming time to exhaustion were found in DAV 200 and DAV 400 groups as compared to vehicle and DAV 100 groups, and in TP group as compared to corn oil group. Significant increases were found in EDL ROW (TP, DAV 100, DAV 200), and gastrocnemius glycogen content (DAV 400), compared to their respective controls. Significant decrease in liver glycogen content was found in all DAV and TP groups, compared to their respective controls.

Experiment 2 investigated the effect of DAV on exercise endurance capacity. Rats were devided into 12 groups (n = 8 each), six groups of which similar to Experiment 1 had undergone exercise training (swimming without load, EX) and six additional groups had undergone non-exercise trainning (Non-Ex). All drugs were given once daily for 16 days. Exercise endurance capacity of DAV was demonstrated since significant increases in swimming time to exhaustion were found in DAV 200 + Ex, compared to vehicle + Ex group. In contrast to TP + Ex group showed significant decrease in swimming time to exhaustion, compared to corn oil + Ex group. In exercise training, gastrocnemius glycogen contents in all DAV groups and liver glycogen content in DAV 200 + Ex group were significant higher than vehicle + Ex group. In non-exercise training, liver glycogen content in DAV 400 + Non-Ex group was significant higher than all other groups. Exercise training significantly decreased liver glycogen content and significantly increased glycogen content in gastrocnemius muscle in DAV 400 group, compared to non-exercise training.

In conclusion, the present results reveal anti-fatigue activity and exercise endurance capacity of DAV, which support of this extract for muscle fatigue and exhaustion relief in human. Bioactive compounds found in the DAV such as proteins and collagens may improve exercise performance and exhibit anti-fatigue action. However, further studies are needed to elucidate the mechanisms underlying these actions of DAV.

School of Preclinic Student's Signature _____

Academic Year 2015 Advisor's Signature Rungsudee Sirisawat

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Ratsa Sripirom

CONTENTS

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

LIST OF TABLES

LIST OF TABLES (Continued)

Table **Page**

LIST OF FIGURES

LIST OF FIGURES (Continued)

Figure **Page**

CHAPTER I

INTRODUCTION

1.1 Rational of the study

Deer velvet antlers are bony cranial appendages that develop on top of permanent frontal protuberances in male deer, and are derived from antlers before they reach a calcification stage (Ran *et al*., 2009). Deer velvet antlers have been used in traditional oriental medicine for thousands of years to treat of various diseases, control blood pressure, increase hemoglobin levels, increase lung efficiency, improve recuperation from exertion, improve muscle tone, promote glandular functions, sharpen mental alertness, relieve the inflammation of arthritis, heal stomach ulcers and act as a tonic to strengthen the body (Zhang *et al*., 2000; Duarte and Abdo, 2011). Deer antler velvet was claimed to their beneficial effects on sexual function (Conaglen *et al*., 2003). Consumption of deer velvet antlers has increased worldwide, people become more interested in the quantity and chemical composition of deer antler velvet (Jeon *et al*., 2011). Many reports and clinical observations convincingly show that deer velvet antlers contains many components such as sphingomyelin, ganglioside, estrone, estradiol, prostaglandins, collagen, amino acid-sugar combinations and growth factors including insulin-like growth factor-1 (IGF-1) and epidermal growth factor (EGF) (Sadighi *et al*., 2001).

There is tremendous amount of focus in the training of athletes today to get bigger, stronger and faster by any and all means possible. Unfortunately, this often

involves the use of synthetic anabolic agents that have devastating long-term ramifications on the human body. Human growth hormone injections, steroids and other harmful agents have destructive effects on muscle tissues, endocrine system, exocrine systems and even DNA (Climstein *et al*., 2003; Tischler *et al*., 1997). Natural IGF-1 is a safe alternative to building muscle mass and is more effective than growth hormone supplementation itself (Sadighi *et al*., 2001). Deer antler velvet supplementations that contain IGF-1 have been used in runner and weight lifting athletic for many years to enhance muscular strength and improve aerobic performance (Broeder *et al*., 2004). The extract from rusa deer (*Cervus timorensis*) antler velvet may have potent enhancing effects on and exercise endurance performance and may be an anti-fatigue supplement candidate. However, there is no report about the underlying mechanisms responsible for these properties of the extract from deer velvet antler. Therefore, the effects of the extract from rusa deer antler velvet on physical fatigue, exercise endurance capacity were investigated.

1.2 Research objectives

The experiments were designed to clarify the followings:

1. To explore the anti-fatigue effect of the rusa deer velvet antler extract in rats.

2. To evaluate the effects of the rusa deer velvet antler extract on blood biochemical variables and glycogen contents in liver and gastrocnemius muscle in forced swimming capacity and exercise endurance capacity in rats.

3. To study the effects of the rusa deer velvet antler extract on exercise endurance capacity in rats.

1.3 Research hypothesis

Supplementation of the rusa deer velvet antler extract possesses anti-fatigue, and physical endurance-enhancing effects in rats.

1.4 References

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CHAPTER II

LITERATURE REVIEWS

2.1 Rusa deer (*Cervus timorensis*)

Rusa, or Sunda sambar, are medium-sized species of deer that are native to South-East Asia. Rusa deer are widespread in the Indonesian archipelago from where they have been introduced into south-east Kalimantan, New Guinea, the Bismarck Archipelago, New Caledonia, Australia and New Zealand. Two subspecies are found in Australia: Javan rusa (*C. timorensis russa*) and Moluccan rusa (*C. timorensis moluccensis*). Javan rusa stags may stand 110 cm at the shoulder and weigh around 120 kg. Hinds are up to 95 cm. at the shoulder and weigh up to 80 kg. Moluccan rusa are slightly smaller (Sookhareea *et al.*, 2001). The rusa coat varies from greyish to yellowish or reddish brown, shading to darker brown on the hindquarters. The body hair is coarse and somewhat sparse when compared with other deer. Stags develop a mane during winter. New calves have a rich red coat. Adult hinds generally give birth to a single calf although twins are not uncommon after a gestation of about 252 days. Most calves are born around March and April (Ashera *et al.*, 2005). Studies of rusa deer in New South Wales suggest that fecundity is high with 75% of hinds giving birth and 50% of all fawns surviving their first year of life. The high level of fecundity and ability to produce three calves in two years means that rusa have the potential for rapid population growth (Moriarty *et al.*, 2001).

2.2 Antler velvet

Antlers are deer cranial appendages, which are cast and regenerate each year from pedicles, permanent protuberances of the frontal bone. Antlers are unique among animal bones in that they grow and are cast every year. They grow very fast and are covered with "velvet" a thick periosteum well supplied with blood vessels (Li, 2003). Antler velvet differentiates rapidly, showing a sequential development from the tip to the base and then becomes hardened because of progressive mineralization and occlusion of blood vessels (Kay *et al.*, 1982). Thus, the chemical composition of antler velvet may vary greatly with both the antler portion and the stage of antler development. The term "velvet" is now used specifically to indicate the antler's state of growth before calcification or ossification (Fraser *et al.*, 2010).

2.3 Antler cycle

When deer approach puberty during their first year of life, they start to develop pedicles. First antler generation takes place spontaneously when the pedicles grow to the species-specific height. The transformation from pedicles to antlers can be readily visualized as growing antlers, unlike pedicles which are covered with the typical scalp skin, are enveloped by a covering with short fine hair, and known as velvet. In late summer prior to the rutting season, growing antlers become fully calcified and the velvet is shed to expose bare hard bone. Hard antlers are cast in the next spring and new antler regeneration follows immediately in the red deer. From then on, antler development enters a well-defined annual cycle (Li *et al.*, 2003).

Antlers are grown only by male deer with the annual antler cycle being closely linked to seasonal variation in circulating androgen levels. During antler growth,

testosterone concentrations in blood are low and although a growth-promoting role of low androgen levels has been proposed (Kierdorf *et al.*, 2003). Growing antlers possess mesenchyme growth zones located at the tips of the main beam and the branches. Regarding the topographic origin of the cells forming the initial growth zone of the early regenerating antler, it was originally believed that the dermis of the pedicle was the source of these cells (Gu *et al.*, 2007). The development of antlers is a modified endochondral ossification process. After casting, antler re-growth is initiated at the distal rim of the pedicle, where cells dedifferentiate into embryonic stem cells. These mesenchymal cells sequentially proliferate and differentiate into chondroblasts and chondrocytes associated with the formation of cartilage. The transition from undifferentiated cells to chondrocytes is gradual. The longitudinal growth proceeds proximal to distal from the pedicle, while the differentiation points to the opposite direction, from the tip to the base. The matrix of the antler cartilage is biochemically similar to that of other hyaline cartilages; however, it is well vascularized, and ossified bony trabeculae are formed on the cartilage scaffold by mineral deposition (Stéger *et al.*, 2010).

2.4 The chemical composition of antler velvet

The chemical composition of antler velvet varies greatly with both the antler portion and the stage of antler development. Antler velvet composes of many biochemical components including lipids, peptides, carbohydrates and inorganic substances (Choi *et al.*, 2006). Many components such as glycosaminoglycans (chondroitin sulphate and glucosamine chondroitin), sphingomyelin, ganglioside, estrone, estradiol, prostaglandins, collagen, amino acid, sugar, growth factors

including Insulin-like growth factor-1 (IGF-1) and epidermal growth factor (EGF) are also found in antler velvet (Sempere *et al.*, 1989; Isai *et al.*, 1994; Kim *et al.*, 1997; Ran *et al.*, 2009; Rucklidge *et al.*, 1997; Sunwoo *et al.*, 1995; Sunwoo *et al.*, 1997).

Sex hormones, estradiol and progesterone, along with IGF-1 can be found in antler velvet extracted by combination of supercritical found extraction with ultrasonic extraction method (Ran *et al.*, 2009). The IGF-1 is a peptide with a tertiary structure that consists of 70 amino acid residues and has a molecular mass of 7649 Da. IGF-1 plays a role in growth by mediating the action of GH, therefore also being called somatomedin C and promotes cell proliferation, as well as, synthesis of proteoglycans by cartilaginous, connective and bone tissue. In addition, plays an anabolic role by stimulating the uptake of amino acids and glucose by the cells. The anabolic action of IGF-1 is compromised by specific physiological states such as diabetes, protein deficiency, physical exercise, and energy restriction, with these factors being considered as regulators of the secretion and biological activity of IGF-1 (de Rezende Gomes *et al.*, 2004). The thirteen antler velvet (C. *nippon* and C. *elaphus*) were observed the simultaneous determination of eighteen sex hormones (estriol, 17β-estradiol, estrone, 17α-ethinylestradiol, 17α-estradiol, 17β-estradiol-benzoate, 17α-hydroxyprogesterone, medroxyprogesterone, megestrol-17-acetate, norethisterone acetate, progesterone, medroxyprogesterone-17-acetate, chlormadinone 17-acetate, 19-nortestosterone, testosterone, androsterone, testosterone 17-propionate and corticosterone) by HPLC–MS/MS (Lu *et al.*, 2012). The results appeared that testosterone was detected in almost all samples, 17α-hydroxy progesterone was observed only in one sample, and progesterone was found in three samples. The researcher also reported a method for the simultaneous

determination of 11 sex hormones in antler velvet (C. *nippon* and C. *elaphus*) by gas chromatography–tandem mass spectrometry (GC–MS/MS) (Lu *et al*., 2011). Moreover, Zhou *et al*., (1999) has been studied the simultaneous and rapid determination of three main biological base components including uracil, hypoxanthine and undine in antler velvets of sika deer (C. *nippon*) by HPLC method (Zong *et al*., 2014).

Moreover, chemical composition of antlers from Wapiti (*Cervus elaphus*) has been reported (Sunwoo *et al*., 1995). Four sections of fresh antler velvet (tip, upper, middle and base, shown in Figure 2.1) were obtained at 65 days after button casting from 4 years old Wapiti and the chemical compositions in each section are shown in Table 2.1, 2.2, and 2.3.

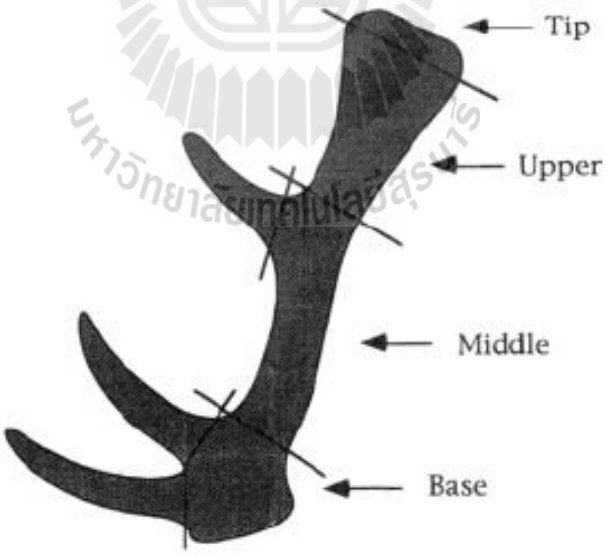

Figure 2.1 Drawing of antlers velvet of Wapiti showing four sections used in analysis of chemical composition (Sunwoo *et al*., 1995).

Table 2.1 Weights and chemical analysis of four sections of antler (Sunwoo *et al*., 1995).

item	antler section			
	tip	upper	middle	base
weight (g)	38.37 ± 3.65^{a}	834.32 ± 43.73^{b}	656.48 ± 27.31^{c}	427.23 ± 11.97^{d}
dry matter (%)	14.36 ± 0.19^{a}	24.95 ± 0.86^{b}	31.35 ± 0.49^{c}	42.05 ± 0.66^{d}
protein (%)	69.08 ± 0.88^{a}	61.50 ± 0.77^{b}	57.13 ± 0.41^{c}	49.27 ± 1.08^{d}
collagen (%)	10.01 ± 0.52^{a}	14.35 ± 1.38^{b}	25.83 ± 0.84^{c}	31.99 ± 1.26^{d}
lipid (%)	18.94 ± 1.00^{a}	2.67 ± 0.07^{b}	1.02 ± 0.05^{c}	0.50 ± 0.04^{cd}
uronic acid (%)	1.24 ± 0.17^{a}	1.36 ± 0.11^{a}	0.16 ± 0.02^{b}	0.11 ± 0.01^{b}
sulfated GAG[b] (%)	3.73 ± 0.47^{a}	4.67 ± 0.27^{b}	0.34 ± 0.03^{c}	0.26 ± 0.03^{c}
sialic acid (%)	0.61 ± 0.01^{a}	0.30 ± 0.06^{b}	0.25 ± 0.03^{b}	0.09 ± 0.02^{c}
ash (%)	9.40 ± 0.27^{a}	34.45 ± 0.61^{b}	39.87 ± 0.60^{c}	48.04 ± 0.40^{d}
calcium (%)	0.42 ± 0.01^{a}	3.32 ± 0.07^{b}	11.77 ± 0.23^{c}	16.50 ± 0.36^{d}
phosphorus (%)	0.39 ± 0.02^{a}	1.84 ± 0.05^{b}	6.95 ± 0.07^{c}	8.59 ± 0.12^{d}
magnesium (%)	0.04 ± 0.00^{a}	0.08 ± 0.00^{b}	0.19 ± 0.01^{c}	0.29 ± 0.01^{d}

[a] All values for chemical analysis are on a dry matter basis. Means in rows with different superscripts are significantly different ($P<0.05$).

[b] GAG, glycosaminoglycan.

Table 2.2 Analysis of amino acid in the four sections of antler (Sunwoo *et al*., 1995).

amino acid	antler section			
	tip	upper	middle	base
aspartic acid	6.64 ± 0.23	7.46 ± 0.37	6.76 ± 0.29	6.78 ± 0.24
glutamic acid	10.52 ± 0.36	9.99 ± 0.39	10.07 ± 0.28	11.20 ± 0.25
serine	3.04 ± 0.20[a]	3.73 ± 0.16[b]	3.29 ± 0.16[ab]	3.51 ± 0.12[ab]
histidine	2.11 ± 0.15[a]	2.84 ± 0.19[b]	1.87 ± 0.20[a]	1.57 ± 0.07[a]
glycine	7.63 ± 0.66[a]	9.70 ± 0.23[b]	12.23 ± 0.68[c]	17.42 ± 0.14[d]
threonine	3.29 ± 0.15[ab]	3.62 ± 0.17[a]	3.07 ± 0.19[ab]	2.90 ± 0.10[b]
arginine	5.15 ± 0.36[a]	5.51 ± 0.12[a]	5.97 ± 0.16[a]	7.26 ± 0.16[b]
alanine	5.74 ± 0.25[a]	6.99 ± 0.21[b]	7.50 ± 0.21[b]	8.87 ± 0.20[c]
tyrosine	2.11 ± 0.10[a]	1.92 ± 0.09[ab]	1.60 ± 0.10[b]	1.62 ± 0.04[b]
valine	4.34 ± 0.15[ab]	4.75 ± 0.26[a]	3.92 ± 0.23[ab]	3.69 ± 0.12[b]
phenylalanine	3.34 ± 0.17	3.90 ± 0.23	3.19 ± 0.21	3.18 ± 0.08
isoleucine	2.37 ± 0.08[a]	1.77 ± 0.08[b]	1.69 ± 0.06[b]	1.76 ± 0.04[b]
leucine	6.42 ± 0.29[ab]	7.22 ± 0.41[a]	5.67 ± 0.38[b]	5.11 ± 0.17[b]
lysine	3.87 ± 0.24	4.33 ± 0.12	3.79 ± 0.21	3.96 ± 0.13

[a] All values represent of dry matter. Means in rows with different superscripts are significantly different ($P<0.05$).

Table 2.3 Fatty acid composition of four sections of antler (Sunwoo *et al.*, 1995).

	antler section			
fatty acid	tip	upper	middle	base
C14:0	1.18 ± 0.03	1.15 ± 0.01	0.87 ± 0.04	1.06 ± 0.23
C16:0	16.27 ± 0.66[a]	15.10 ± 0.12[a]	14.44 ± 0.44[ab]	12.19 ± 0.58[b]
C16:1ω7	4.26 ± 0.35[a]	2.09 ± 0.03[b]	1.19 ± 0.07[bc]	0.83 ± 0.15c
C18:0	7.79 ± 0.21[a]	7.58 ± 0.18[a]	9.28 ± 0.09[b]	9.61 ± 0.54[b]
C18:1ω7 and 9	55.72 ± 1.69	59.31 ± 0.63	54.81 ± 1.12	57.40 ± 2.54
C18:2ω6	2.58 ± 0.09[a]	3.22 ± 0.13[b]	5.20 ± 0.09c	4.61 ± 0.12d
C18:3ω6	0.16 ± 0.00[a]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]
C18:3ω3	0.86 ± 0.20	0.64 ± 0.05	1.02 ± 0.03	0.87 ± 0.05
C20:1ω9	0.19 ± 0.03	0.22 ± 0.05	0.46 ± 0.10	0.18 ± 0.03
C20:2ω6	1.98 ± 0.15[a]	1.28 ± 0.04[b]	0.43 ± 0.2c	0.45 ± 0.02c
C20:3ω6	0.73 ± 0.13	0.79 ± 0.01	0.80 ± 0.07	0.64 ± 0.05
C20:4ω6	2.99 ± 0.19[a]	3.40 ± 0.03[a]	5.28 ± 0.06[b]	5.62 ± 0.52[b]
C22:4ω6	0.39 ± 0.02[a]	0.51 ± 0.12[a]	0.78 ± 0.03[b]	0.82 ± 0.09[b]
C22:6ω3	1.29 ± 0.03	1.30 ± 0.02	1.36 ± 0.03	1.37 ± 0.07
SAFA[b]	27.15 ± 0.89	25.79 ± 0.29	26.82 ± 0.53	25.12 ± 1.29
MUFA[c]	61.89 ± 0.93	63.07 ± 0.23	58.33 ± 0.69	60.50 ± 1.96
ω−3[d]	2.15 ± 0.21	1.94 ± 0.06	2.38 ± 0.05	2.25 ± 0.12
ω−6[e]	8.82 ± 0.24[a]	9.20 ± 0.21[a]	12.48 ± 0.33[b]	12.14 ± 0.58[b]
ω−6:ω−3	4.19 ± 0.47	4.75 ± 0.07	5.25 ± 0.25	5.41 ± 0.04

[a] All values represent of total fat. Means in rows with different superscripts are significantly different ($P<0.05$). [b] Total saturated fatty acids include C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, and C24:O. [c] Total monounsaturated fatty acids include C15:lω5, C16:lω7, C18:lω7, C18:109, and C20:lω9. [d] Total omega-3 fatty acids include C18:303 and C22:6ω3. [e] Total omega-6 fatty acids include C18:2ω6, C18:3ω6, C20:2ω6, C20:3ω6, C20:4ω6, and C22:4ω6.

Furthermore, many kinds of mineral element are observed in deer antler base such as Calcium (Ca), Phosphorus (P), Sodium (Na), Potassium (K), Magnesium (Mg), Iron (Fe), Zinc (Zn), Manganese (Mn), Strontium (Sr), Barium (Ba) and Copper (Cu) (Sui *et al.*, 2014; Hu *et al*., 2015; Wu *et al*., 2013) by atomic absorption spectrometry. In 2005, Zhang reported that the mineral content of deer antler base (Ca, P, K, and Zn) was increased with the deer's age. Fe did not change by the deer's age.

Toxicity studies of antler velvet powder have been assessed in rats, mice and rabbits. Rats received antler velvet powder at a dose of 2 g/kg (p.o.) showed no fatality or adverse events on a short-term (14 days) basis. In 90-day study, 1 g/kg/day regimen also found no detectable significant adverse effects, although a minor change in liver weight was found (Allen *et al.*, 2002). In 2010, the acute toxicity of the protein extracts of deer antler base was investigated by intragastric administration to mice at dose of 40 mg/kg/day for 7 days. No significant differences were found in treated mice for any organ parameter analyzed (including pancreas coefficient and kidney coefficient), and no deaths were observed. These results suggested that the protein extracts of deer antler base has little or no acute toxicity (Ma and Wang, 2010). The long-term toxicity test was studied in rat intraperitoneally injected with deer antler base at doses of 15 and 30 g/kg/day for 28 days. Deer antler base injection had no effect on blood biochemical parameters and the cellular structure of organs and tissues. The stimulated tests were observed in rabbits by subcutaneous injection (s.c.) of 0.1 ml (0.37 g, equivalent to 30 times the clinical application) deer antler base to the left ear or intramuscular injection (i.m.) of 0.25, 0.5, or 1 ml to the quadriceps muscle. No adverse stimulus-response (e.g. edema and hyperemia) was investigated (Chen *et al*., 1987, 1989).

The allergic test was observed by intravenous injection (i.v.) of deer antler base to guinea pigs. Either dyspnea or suffocation was not shown (Kuba, Landete-Castillejos, and Udała, 2015).

2.5 Beneficial effects of antler velvet

Deer antler velvet supposedly belongs to the broad category of substances known as adaptogens. These substances help to restore balance to numerous body processes, especially the endocrine and hormonal processes. Under physical or mental stress, that is either chronic (e.g. because of lifestyle) or short term (e.g. workout or injury) the body processes can be out of balance, thereby causing several minor or serious consequences for a person. Adaptogen preparations represented dry extracts from pantocrin (deer antler velvet extract) of Siberian deer (*Cervus elaphus sibiricus*) (Suslov, 2002). Adaptogen preparations at dose of 100 mg/kg daily possess a weak gonadotropic activity, which favors normalization of the testosterone level in experimental animals (Kropotov *et al.*, 2001). Scientific publication testified on the application of adaptogen preparations to remove chronic fatigue syndrome, reducing nerve and muscle fatigue (Volodin *et al*., 2013). Pantocrin is a great adaptogenic remedy, particularly when combined with other notable adaptogens. In general, pantocrin possesses adaptogenic age-retarding effects and can help support the health of the adrenals, enhance energy metabolism, encourage sexual function, strengthen immune resistance, and more (Yance, 2013).

As a traditional animal based medicine, antler velvet has been used in the East for over 2000 years to prevent or treat various diseases, including cardiovascular disease, gynecological problems, immunological deficiencies, blood cancers, tissue

repair and health promotion (Wu *et al.*, 2013). Health benefits claimed by traditional Chinese medicine for antler velvet include a wide range of effects on systematic exhaustion, depression, cold, lower back pain, weak pulse, impotence, spermatorrhea, low white cell counts, regulate the adrenal cortex, regulate energy metabolism, promote sexual function, promote growth and strengthen resistance. Specific health benefits of antler velvet shown by Western research include anaemia alleviating, anti-aging, anti-cancer, anti-inflammatory, blood pressure control, bone and joint anti-inflammation, growth stimulation, performance enhancement (prevent and repair muscle damage, increase muscular strength and endurance), and immune system stimulating activities, as well as tonic effects (Tuckwell, 2003). Moreover, polypeptide-rich deer antler has been used to treat patients with insulin resistance and help to improve their blood sugar profiles (Wu *et al*., 2013).

Glycosaminoglycans, such as chondroitin sulphate and glucosamine chondroitin, can be found in a great abundance in antler velvet, which have been established as natural remedies to reduce pain, stop joint space narrowing, and even antler velvet the pathology of osteoarthritis, (Sunwoo *et al*., 1997; Faucheux *et al*., 2001). The effects that antler velvet has on arthritic joints and other skeletal system disorders have been demonstrated (Kim *et al*., 2003). Antler velvet supplementation has been shown to reduce or eliminate joint swelling and distortion, inhibit the development of arthritis, significantly increase fracture healing rates, increase bone mineral density, and even increase bone width and osteoblast cells (Zhou *et al*., 1999; Kim *et al*., 2003). Recent research has demonstrated the ability of antler velvet to inhibit microbial and fungal growth by enhancing immunological function and suppressing inflammatory cytokines. Macrophage and splenocyte activity have been

shown to improve with antler velvet supplementation (Dai *et al*., 2011; Kim *et al*., 2004; Min *et al*., 2001).

Explosiveness, muscle recovery time, endurance capacity, strength and body composition can all be improved with the use of antler velvet. Antler velvet supplementation has been shown to reduce body fat and resting creatine kinase levels and increase strength, time to exhaustion, anaerobic performance, and VO_2 max (Suttie and Haines, 2004). Unexpected enhancement in endurance aerobic performance was demonstrated in the study of Suttie and Haines (2004). The enhancing effect on muscular strength during weight training was found in untrained young male students after treating with a water-based extract of deer velvet at a dose of 350 mg/day for 10 weeks. The elevation in blood creatine kinase, a marker of muscle damage, was significantly decreased in the deer velvet powder treated group at a dose of 1500 mg/kg/day (Suttie and Haines, 2004). Gerrard *et al.* (1998) showed that deer velvet pretreatment for 14 days could reduce muscle damage following a simulated downhill run in men who were not trained runners. The anti-fatigue effect of powdered deer antler against immobilized stress was also shown (Shin *et al.*, 1989). Powdered deer antler administered at 1 g/kg for 4 days significantly increased in adrenal weight and ascorbic acid content in the adrenal gland. These findings suggested that the antler's anti-fatigue effect may be related to effect on the adrenal gland.

Protein and collagens, bioactive compounds found in the deer antler base extract, have been suggested to be the major substances responsible for anti-fatigue effect of the deer antler base extract. In the mice model, the pure compound of deer antler collagens could increase swimming time, improve the activity of lactate

dehydrogenase (LDH) and increase hepatic glycogen but decrease serum urea nitrogen (Shi *et al.*, 2011). Intragastric administration of deer antler base extract at a dose of 40 mg/kg/day for 5 days could prolong the swimming time and increase the adrenal coefficients in a forced swimming test. The findings suggested that anti-fatigue effect of the deer antler base proteins may be associated to the enhancement of adrenal function (Su *et al*., 2001). The aqueous extract of deer antler base administered to the mice at a dose of 100 mg/kg/day (i.g.) for 30 days could increase swimming time and decrease the blood level of lactic acid. However, the hepatic glycogen and serum urea nitrogen was not significant difference from control group. The results suggested that fatigue and exercise tolerance may be relieved by the aqueous extract of deer antler (Niu *et al*., 2011).

Potency of the deer antler velvet on improvement of muscle strength and endurance has been demonstrated in human. Sleivert *et al*. (2003) showed that the deer antler velvet powder supplementation could develop muscle strength and endurance in human subjects with 10-week strength program. The increases in isokinetic knee extensor strength and endurance from the begining in the deer antler velvet powder supplementation group were greater than the control group. Broeder *et al*. (2004) studied the effects of testing deer velvet powder and weight training for 10 weeks at a dose of 2700 mg each day. The increase in the strength from the beginning in the group administered the deer velvet powder was significantly higher than the control group. Several growth factors exist in antler velvet including insulin-like growth factor (IGF-1), and epidermal growth factor (EGF), growth factor that promote the development of cartilage cells (Ran *et al.*, 2009).

Growth hormone and IGF-1 have been shown to promote body growth and muscle strength (Vale *et al*., 2009). In fact, the majority of increases in strength and muscle as well as the anti-aging effects of growth hormone is due to its ability to raise IGF-1 levels in the body. IGF-1 also acts as an anti-catabolic and plays a role in preventing muscle atrophy, increasing muscle growth and increasing protein synthesis (Clemmons, 2009; Duan *et al*., 2010; Philippoul *et al*., 2007). In studies involving both established cell lines and primary satellite cell cultures, ligation of the type 1 IGF-1 receptor (IGFR1) has been shown to initiate intracellular signaling cascades involved in key mitogenic and myogenic responses (Rommel *et al.*, 2001). The mitogenic and myogenic effects of IGF-1 are useful for muscle regeneration (Adams *et al.*, 1996). Muscle IGF-1 levels have been shown to induce muscle hypertrophy both *in vitro* and *in vivo* (Adams *et al*., 1998; Coleman *et al*., 1995; Vandenburgh *et al*., 1991). Over expression of IGF-1 in muscle has also been shown to prevent some of the age-related effects on skeletal muscle, such as the decline in muscle mass (Musaro` *et al*., 2001). One of the major issues with losing weight and restricting calorie intake is the accompanying loss in muscle mass. Muscle mass has been directly proven to stimulate metabolism and fat loss. Studies have shown that individuals receiving human growth hormone experience a significant lipolysis effect. GH increases the fat burning mechanism intrinsic to IGF-1; therefore, preserving and increasing lean body mass. IGF-1 also reduces cortisol levels and improves and regulates hormonal levels, which can be affected by calorie-restricted diets (El-Eshmawy *et al*., 2011; Mauras and Haymond, 2005).

Both muscle strength and anabolic hormone levels decline around middle age into old age over a similar time period, and several animal and human studies indicate

that exogenously increasing anabolic hormones (e.g., testosterone and IGF-1) in aged subjects is positively associated with improved muscle strength (Oki *et al.*, 2015).

Testosterone is a hormone expression in males and females. It is correlated with mood regulation, bone formation, sex drive, male sexual development and energy levels. It is also associated with muscle development and improved muscular performance (Bhasin, Woodhouse, and Storer, 2001; Snyder *et al*., 1999). Testosterone, considered to be a powerful anabolic agent, has been administered to elderly individuals to block or retard the muscle loss due to sarcopenia. This is a reason that a few competitors take artificial hormones in adult males for the treatment of hypogonadism, low libido and weakness (Hajjar, Kaiser, and Morley, 1997). Investigations of the effects of testosterone on the cardiovascular system in basic science have been studies. Testosterone has been appeared to display potential antiarrhythmic properties as decreasing action potential duration, shortened QTc interval, and early after depolarizations. (Pham *et al*., 2002; Bai *et al*., 2005; Brouillette *et al*., 2005). Testosterone has additionally been appeared to decrease myocardial infarct size contrasted and subjects not treated with testosterone by modulating the myocardial K_{ATP} channel, (Liu *et al*., 2006; Tsang *et al*., 2008; Liu *et al*., 2012; Er *et al*., 2004) improving lipid metabolism, (Filippi *et al*., 2009; Gupta *et al*., 2008) enhancing vasodilation, (O'Connor, Ivey, and Bowles, 2012) and improving DM (Jackson and Hutson, 1984; Ballester *et al*., 2005), and attenuating atherosclerosis (Bruck *et al*., 1997). The testosterone undecanoate treatment combined with moderate physical training on the estrous cycle, body weight, motor behavior and the morphohistology of the reproductive system, the liver and kidney were studied in rats. The results showed that relative weight of the heart and kidneys

were increased, but not decreased in weight of ovaries in group of trained + testosterone undecanoate. Histopathological assay showed that periportal congestion and isolated foci of hepatic necrosis in rats with testosterone undecanoate. Hence, testosterone undecanoate combined with training promoted ovarian atrophy, liver necrosis, and cardiac hypertrophy in rat (Bento-Silva *et al*., 2010).

Anti-fatigue effect of antler base extracts has been demonstrated by several studies. Administration of deer antler base proteins to mice, at a dose of 40 mg/kg/day (i.g.) for 5 days, markedly extended the swimming time and slightly increased the adrenal coefficients in forced swimming test. These findings suggested that the anti-fatigue effect of deer antler base proteins may be associated to the improvement of adrenal function (Su *et al*., 2001). Moreover, both of deer antler base collagens at dose 800 mg/kg/day (i.g.) and deer antler base proteins at dose 70 mg/kg/day (i.g.) markedly increased the swimming time, improved the activity of lactate dehydrogenase, increased hepatic glycogen and decreased serum urea nitrogen in mice (Wu *et al*., 2013). Furthermore, administration of aqueous extract of deer antler base to mice for 30 days at a dose of 100 mg/kg/day could prolong the swimming time and decrease the level of blood lactic acid, but there was no change in hepatic glycogen and serum urea nitrogen levels. These findings suggested that the aqueous extract of deer antler base efficiently relieved fatigue and enhanced exercise tolerance (Sui *et al*., 2014). In addition, Luo *et al*. (2008) reported that the antler velvet polypeptide (VAP) had anti-fatigue effect on anti-anoxia survival time, pant time after decapitation, burden swimming time and continuously climbing time in mice treated with 30 mg/kg/day of antler velvet polypeptide for 30 days. The water extract of antlers velvet also showed anti-fatigue effects in mice model. The intragastric

administration of water extract of antlers velvet, at dose of 80 mg/kg/day for 30 days, markedly extended the burden swimming time, increased the content of muscle glycogen, liver glycogen and the activity of lactate dehydrogenase, and decreased the content of serum urea nitrogen and blood lactic acid (Chen *et al*., 2014). These findings suggested that the anti-fatigue effect of water extract of antler velvet might be associated to the high ability of expulsion of serum urea nitrogen and blood lactic acid.

In 2003, Sleivert and co-workers reported that the deer antler velvet powder supplementation for 10 weeks could enhance isokinetic knee extensor strength and endurance compared to placebo group in active males who participated in 10-week strength program.

2.6 Muscle fatigue

Intense activation of skeletal muscles generally results in decreased contractile function that is reversed after a period of rest. This activity-induced decline in performance is called fatigue and is in most instances highly dependent on the capacity of the aerobic metabolic system. Therefore, slow oxidative muscle fibers are markedly more fatigue resistant than fast glycolytic fibers under normal conditions (Westerblad *et al*., 2010). Fatigue can be manifest as decreased isometric force production, reduced shortening speed, altered force-velocity relationship, and slowed relaxation (Allen *et al*., 1995; Jones *et al*., 2006). The integration of decreased force production and slowed shortening results in a decreased power output and hence impaired performance in all types of locomotion that depend on muscle shortening.

Slowed relaxation will decrease the frequency at which alternating movements can be performed.

The contraction of skeletal muscle is controlled by a series of events and fatigue can be due to impaired function in any of these events. The series starts in the central nervous system where eventually the α-motorneurones are activated. Each α- motorneuron activates a number of muscle fibers and together they represent the smallest unit in the motor system, the motor unit. All muscle fibers in a motor unit are of the same type and hence metabolically matched to fit the discharge properties of their α-motorneuron. Fatigue may occur as a consequence of impaired α-motorneuron activation and this is called central fatigue (Taylor *et al*., 2006).

There is a complex interplay between the nervous system and skeletal muscles during most types of strenuous exercise, which makes it difficult to design experiments to unequivocally assess the extent of central fatigue. Consequently, the question whether central fatigue is important or not for the decline in performance during various types of physical activity is rather controversial (Hargreaves, 2008).

However, the general picture is that central fatigue is of greater importance during prolonged low intensity activities, where metabolic changes within muscle cells are likely to be limited, whereas intramuscular factors appear to dominate during activities of higher intensity (Place *et al*., 2006). Peripheral fatigue relates to factors within the muscle that cause impaired contractile function during strenuous exercise as well. The mechanisms of peripheral fatigue within muscle are well studied and include impairments in neuromuscular transmission and propagation down the sarcolemma, dysfunction within the sarcoplasmic reticulum involving calcium release

and uptake, availability of metabolic substrates and accumulation of metabolites and actin-myosin cross bridge interactions (Lambert and Flynn, 2002).

Many factors causing peripheral fatigue have been described. These include impairment of excitation-contraction coupling, impairment of energy production, and limitations in fuel supply. The particular processes that result in the production of fatigue are related to the type or intensity of work the muscle is required to perform (Romer *et al*., 2006).

Metabolites are the substances (generally waste products) produced as a result of muscular contraction. They include ADP, Mg^{2+}, reactive oxygen species and inorganic phosphate. Accumulation of metabolites can directly or indirectly produce metabolic fatigue within muscle fibers through interference with the release of calcium from the sarcoplasmic reticulum or reduction of the sensitivity of contractile molecules actin and myosin to calcium (Kent-Braun, 1999).

A high concentration of potassium also causes the muscle cells to decrease in efficiency, causing cramping and fatigue. Potassium builds up in the t-tubule system and around the muscle fiber in general. This has the effect of depolarizing the muscle fiber, preventing the sodium-potassium pump from moving Na^{+} out of the cell. This reduces the amplitude of action potentials, or stops them entirely, resulting in neurological fatigue (Allen *et al*., 2008).

Lactic acid was once believed that to build-up was the cause of muscle fatigue. The assumption was lactic acid had a "pickling" effect on muscles, inhibiting their ability to contract. The impact of lactic acid on performance is now uncertain; it may assist or interrupt muscle fatigue. Produced as a by-product of fermentation, lactic acid can increase intracellular acidity of muscles. This can lower the sensitivity

of contractile apparatus to Ca^{2+} but also has the effect of increasing cytoplasmic Ca^{2+} concentration through an inhibition of the chemical pump that actively transports calcium out of the cell (Hargreaves, 2008). The mechanisms that contribute to muscle fatigue are summarized in Figure 2.2

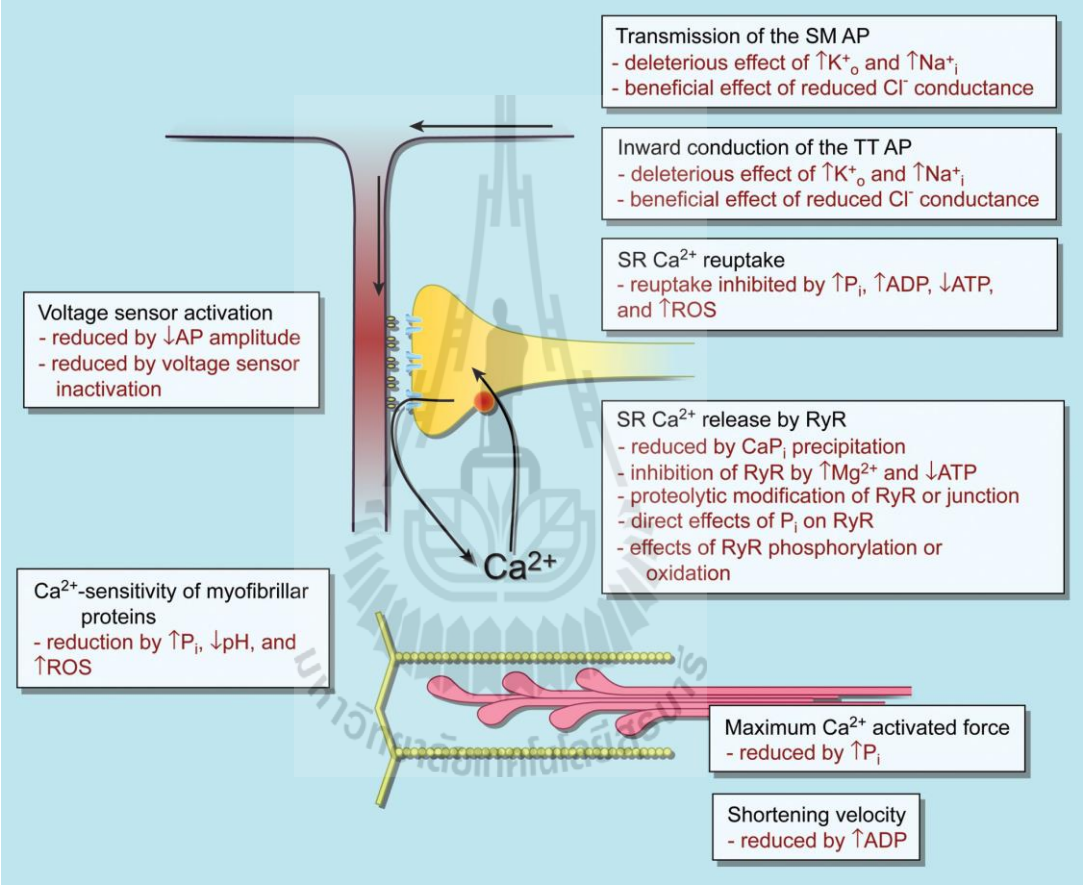

Figure 2.2 Schematic diagram illustrating the mechanisms that contributes to muscle fatigue (Allen, Lam, and Westerblad, 2008). Heading in each box identifies subcellular function, and the subsequent list indicates cellular changes occurring during fatigue that influence the subcellular function. (SM, surface membrane; TT, T-tubule; SR, sarcoplasmic reticulum; AP, action potential)

Muscle fiber type can be measured in three ways: myosin ATPase histochemistry, immunohistochemistry, or (much less commonly) metabolic enzymes. Type II muscle fibers display a faster shortening velocity than type I muscle fibers. A higher proportion of type II muscle fibers may therefore be beneficial for strength and power sports. However, the extent to which type II muscle fibers are displayed may be determined by individual muscles and genetics. Resistance training increases muscle fiber area in all muscle fiber types, in both trained and untrained individuals. Different muscles display different proportions of muscle fiber types. Training for increased muscle size, which is also a key determinant of sports performance, may therefore require a focus on different muscle fiber types for each muscle. Resistance training can cause an increase in type I, type IIA, and type IIX muscle fiber areas, in both trained and untrained individuals (Deschenes *et al*., 2002; Kraemer *et al*., 1995; Fry *et al*., 2004; Scott *et al*., 2001; Wilson *et al*., 2012).

2.7 Exercise endurance capacity

Exercise endurance is an endurance time during prolonged work. Exercise endurance is associated with enhanced lipolysis and sparing of stored glycogen, which results in delaying complete glycogen depletion. The amount of glycogen stored in working muscles is important for the endurance capacity. Glycogen deposited in liver and muscle tissue, which can readily converted to glucose as needed by the body to satisfy its energy needs for exercise endurance (Navarro *et al*., 2002). Increased fat utilization during endurance exercise enables athletes to improve endurance capacity. Therefore, the increase of fatty acid utilization during exercise is supposed to improve endurance capacity (Oh and Ohta, 2003).

2.8 Exercise energy supply

Muscle contraction and exercise are dependent on the breakdown of adenosine triphosphate (ATP) and the concomitant release of free energy (Glaister, 2005). The free energy release is coupled to the energy requirements of cell work. During this very short period, the major driving forces are stored high-energy phosphates and anaerobic glycolysis (Bishop, Girard, and Mendez-Villanueva, 2012). The runners can perform almost without breathing, using energy stored as ATP, creatine phosphate and glycogen (anaerobic metabolism) in the active muscles (Ghosh, 2004; Andersen, 2003). In contrast to long-distance runners, sprinters are often large, very muscular people. Sprinters have a dominance of so-called fast twitch or anaerobic muscle fibers. Those remarkably high speeds can only be maintained while stored high-energy phosphate in the form of phosphocreatine is present (Trappe *et al*., 2015). Almost all studies of phosphocreatine metabolism conclude that stores of phosphocreatine in skeletal muscle are emptied within the first 30 seconds of strenuous activity. After that, the very rapid rate of running must be reduced. The energy supply for those who run from about 60 seconds to three minutes is primarily glycogen stored in muscles and blood glucose (Krustrup *et al*., 2006). These carbohydrates can be rapidly oxidized to pyruvate, lactate and CO_2 to provide the ATP required for muscle activity (Scott, 2005). However, the rate of ATP synthesis rate is far below that seen when using phosphocreatine as the phosphate donor. These differing sources of energy are summarized in the figure. During exercise, intracellular glucose and ATP levels initially fall and AMP levels rise (Winder, 1988). AMP then markedly stimulates glucose transport by activating AMP kinase (Musi and Goodyear, 2003; Richter and Ruderman, 2009). To offset this drain on

extracellular glucose and to maintain a normal plasma glucose level, hepatic glucose production must increase up to fivefold. Initially, this occurs largely from glycogenolysis (Mutel *et al*., 2011). Indeed, endurance can be improved by high carbohydrate ingestion for several days before prolonged exercise (*e.g*., a marathon run) because this increases both liver and muscle glycogen stores (Stellingwerff *et al*., 2007). With exercise of longer duration, however, gluconeogenesis becomes increasingly important as liver glycogen stores become depleted (Zorzano *et al*., 1986). Eventually, fatty acids liberated from triglycerides in adipose tissue form the predominant energy substrate, and they supply two thirds of the needs during sustained exercise (Bahr, Hansson, and Sejersted, 1990). Fatty acids readily enter muscle, where they are degraded by β oxidation to acetyl CoA and then to CO_2. The elevated acetyl CoA level decreases the activity of the pyruvate dehydrogenase complex to block the conversion of pyruvate into acetyl CoA. Hence, fatty acid oxidation decreases the funneling of sugar into the citric acid cycle and oxidative phosphorylation (Costill *et al*., 1979). Glucose is spared so that just enough remains available at the end of the marathon. The simultaneous use of both fuels gives a higher mean velocity than would be attained if glycogen were totally consumed before the start of fatty acid oxidation (Van beek *et al*., 2011). The mechanisms that contribute to energy sources in working muscles are summarized in Figure 2.3

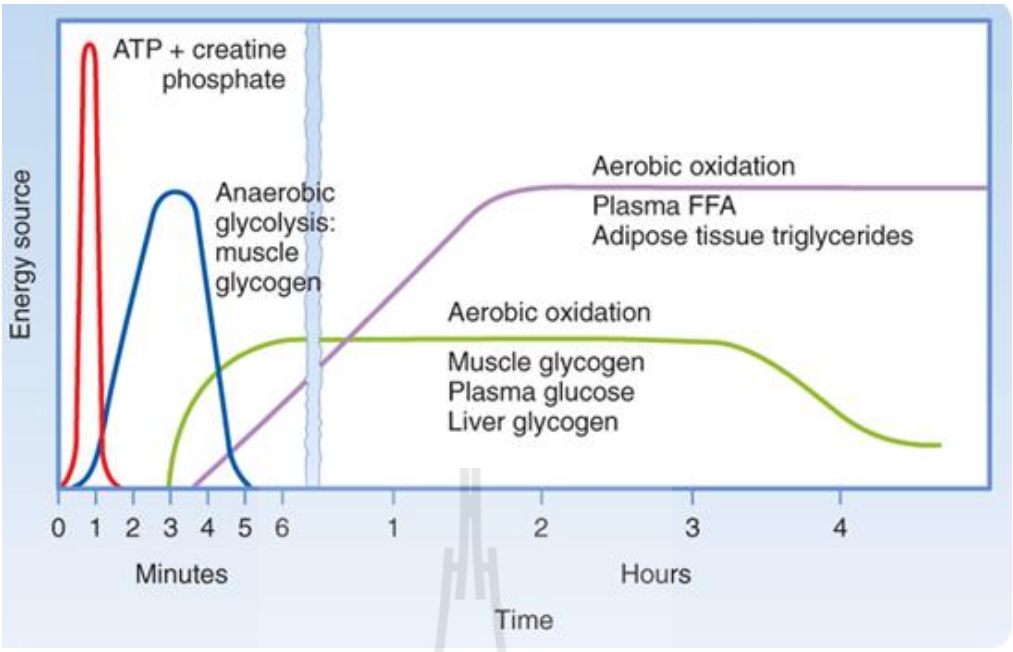

Figure 2.3 Schematic diagram illustrating the energy sources in working muscles (Koeppen and Stanton, 2008). Describe and identifies energy function, and the subsequent list indicates energy source changes occurring during exercise. (ATP, adenosine triphosphate; FFA, free fatty acid).

2.9 References

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CHAPTER III

ANTI-FATIGUE EFFECT OF THE RUSA DEER (*Cervus timorensis*) ANTLER VELVET EXTRACT IN MALE WISTAR RATS

3.1 Abstract

Antler velvet has been used as a traditional animal based medicine to prevent or treat various diseases, promote growth, strengthen body and systematic exhaustion, prevent and repair muscle damage, increase muscular strength and endurance. The present study was designed to investigate the anti-fatigue effects of the rusa deer (*Cervus timorensis*) antler velvet (DAV) in male Wistar rats. The dried milled deer antler velvet (200 g) was extracted with hot water for 5 h. which had a yield of 16.26%. Proteins, saponin, glycosides, cardiac glycosides and mineral elements (P, K, Mg, Fe, and Ca) were detected in DAV. Animal were divided into 6 groups (n = 8 each). Animals received vehicle group received deionized double distilled water (1 ml/kg, p.o.) water (1 ml/kg, p.o.), corn oil (1 ml/kg, i.m.), TP group received testosterone propionate (80 mg/ml/kg, i.m.), and 3 experimental groups (DAV 100, DAV 200, and DAV 400 received DAV at doses of 100, 200, and 400 ml/kg, p.o., respectively) once daily for 9 days. Thirty minutes after last administration, all rats were observed for swimming time to exhaustion in forced swimming capacity test. Body weight gain, relative organ weight (ROW) of liver and hind-limb skeletal muscle (soleus, extensor digitorum longus (EDL) and gastrocnemius), serum and

plasma biochemical parameters, liver glycogen content and gastrocnemius muscle glycogen content.

Anti-fatigue effect of DAV was demonstrated in the present study. DAV at doses of 200 and 400 mg/kg significantly increased swimming time to exhaustion, compared to vehicle and DAV 100 groups. Significant increase in time to exhaustion was also found in TP group, compared to the corn oil (P<0.05). TP significant reduced body weight gain and body weight percentage change as compared to the corn oil group (P<0.05), while there was no change in all DAV groups as compared to the vehicle group. NO significant differences in organ ROWs (liver, soleus, and gastrocnemius), and plasma levels of TG, and ALT were found between all groups. Significant increases in EDL ROW (TP, DAV 100, and DAV 200 groups), blood glucose (DAV 200, and DAV 400 groups), blood AST (DAV 100, DAV 200, and DAV 400 groups), blood creatinine (DAV 200, 400 mg/kg) and gastrocnemius glycogen content (DAV 400 group) were observed, compared to their respective controls (P<0.05). Moreover significant decrease in liver glycogen content were found in all treated groups of DAV and TP group compared to the vehicle group and the corn oil group, respectively (P<0.05).

Over expression of IGF-1 in muscle has also been shown to prevent some of the decline in muscle mass (Musaró *et al.*, 2001). The present findings revealed that could enhance swimming durability in rat model. Hence, the possible cause of this phenomenon might be a role of anti-fatigue substances found in DAV. However, the mechanisms underlying the anti-fatigue effects of DAV are still not fully understood.

3.2 Introduction

Deer antler velvets are bony cranial appendages that develop on top of permanent frontal protuberances in male deer, and are derived from antlers before they reach a calcification stage (Ran *et al*., 2009). Antler velvet has been used as a traditional animal based medicine in the East and in traditional Chinese medicine to prevent or treat various diseases, including cardiovascular disease, gynecological problems, immunological deficiencies and blood cancers, promote tissue repair and also exert health promotion (Wu *et al*., 2013). Antler velvet has been claimed to have a wide range of effects on systematic exhaustion, depression, cold, lower back pain, weak pulse, impotence, spermatorrhea, lowering white blood cell counts, regulating the adrenal cortex, regulating energy metabolism, promoting sexual function, promoting growth and strengthen resistance, anaemia alleviating, anti-aging, anti-cancer, anti-inflammatory, blood pressure control, bone and joint anti-inflammation, growth stimulation, performance enhancement (prevent and repair muscle damage, increase muscular strength and endurance) (Tuckwell *et al*., 2003). Although the mechanism of actions is still unclear, the pharmacological effects could be attributed to bioactive compounds found in the rusa deer antler velvet. Antler velvet composes of many biochemical components including lipids, peptides, carbohydrates and inorganic substances (Choi *et al*., 2006). Many components such as glycosaminoglycans (chondroitin sulphate and glucosamine chondroitin), sphingomylein, ganglioside, estrone, estradiol, prostaglandins, collagen, amino acid-sugar combinations and growth factors including insulin-like growth factor-1 (IGF-1) and epidermal growth factor (EGF) are also found in antler velvet (Ran, 2009). Furthermore, protein component extraction from antler base of sika deer (*Cervus*

nippon) (Niu *et al*., 2011; Shi *et al*., 2011; Su *et al*., 2001), Formosan sambar deer (*Cervus unicolor swinhoei*) tip antler extract (FSDTAE) (Chen *et al*., 2014), pilose antler polypeptide (PAP) (Luo *et al*., 2008), and antler velvet water extract (AVWE) (Zhang *et al*., 2011) could improve fatigue effect through strengthening muscle in skeletal muscle of mice. In human study, deer antler velvet extract could affect muscular strength to knee extension exercise (Sleivert *et al*., 2003; Syrotuik *et al*., 2005). Therefore, the anti-fatigue effects of the rusa deer antler velvet extract were investigated in male rats.

3.3 Materials and methods

3.3.1 Rusa deer antler velvet

The rusa deer (*Cervus timorensis*) antler velvet was purchased from Apichart farm (Ratchaburi Province, Thailand) in November 2013. The rusa deer antler velvets were cut off 70 to 75 days after casting from 3 years old rusa stags.

3.3.2 Preparation of the rusa deer antler velvet extract

The rusa deer antler velvet was cut into small thin pieces, dried at 60°C, milled and sieved. The dried milled deer antler velvet (50 g) was extracted four times with 1 L of deionized double distilled (DDD) water in a hot water bath for 5 h and then filtrated through No.1 Whatman filter paper (Whatman Internation Ltd., Maidstone, England). The filtrate was collected, concentrated using a rotary evaporator (Rotavapor model R-205, Bushi, Switzerland) and then converted into crude extract by freeze-drying (Labconco Corporation Ltd., Missouri, USA). The obtained crude extract was stored at -20°C until further used and the percentage yield was determined. This stock extract was used in all experiments performed in

this thesis. On the day of each experiment, rusa deer antler velvet extract was freshly prepared by dissolving in DDD water at the desired concentrations.

3.3.3 Animals

The eight weeks old male Wistar rats (weighting 250-300 g) were housed under standard laboratory conditions (12:12 h dark-light cycle, ambient temperature 25±1°C) with free access to food and water. The experiments were performed following the animal care and use committee guidelines of Suranaree University of Technology (SUT). All studies were conducted under permit of the SUT Animal Care and Use Committee.

3.3.4 Drugs and chemicals

0.9% Normal saline solution

0.9% Normal saline solution was prepared by adding 9 g of sodium chloride (NaCl; Sigma Chemical Co., St. Louis, MO, USA) to 900 ml of deionized distilled (DI) water. This solution was slightly adjusted volume to 1000 ml with DI water in a volumetric flask.

Testosterone propionate solution (TP)

Testosterone propionate (250 mg/ml, Testoviron® Depot, Thailand) was diluted with corn oil into 80 mg/ml/kg BW as needed and injected intramuscular daily dose for 9 days. 250 mg/ml (1 vial) of testosterone propionate was prepared by adding 2.125 ml of corn oil (Corn oil, Sigma Chemical Co., St. Louis, MO, USA) and kept in amber bottle.

80 mg/ml/kg TP was prepared by mixing 250 mg/ml (1 vial) of testosterone propionate with 2.125 ml of corn oil (Corn oil, Sigma Chemical Co., St. Louis, MO, USA) and kept in amber bottle.

95% EtOH solution

95% ethanol solution (v/v) (C_2H_5OH, Analytical Grade ≥ 99.8%, Carlo Erba Reagents, France) was prepared by adding 95 ml of ethanol to 5 ml of DI water.

5% Phenol solution (w/v)

5% Phenol solution was prepared by adding 50 g of phenol (C_6H_5OH; Sigma Chemical Co., St. Louis, MO, USA) to 900 ml of DI water. This solution was slightly adjusted volume to 1000 ml with DI water in a volumetric flask.

30% KOH/Na_2SO_4 solution

30% KOH/Na_2SO_4 solution was prepared by adding 180 g of potassium hydroxide (KOH; Sigma Chemical Co., St. Louis, MO, USA) to 500 ml of DI water. This solution was slightly adjusted volume to 600 ml with DI water and saturated with sodium sulfate (Na_2SO_4; Sigma Chemical Co., St. Louis, MO, USA)

3.3.5 Preparation of glycogen standard solutions

Stock glycogen standard solution 5 mg/ml (w/v) (10 ml) was prepared by adding 50 mg glycogen powder from bovine (Sigma, St. Louis, MO, USA) to 9 ml of DDD water. This solution was slightly adjusted volume to 10 ml with DDD water.

200 µg/ml standard glycogen solution (20 ml):

Preparation: mixed 0.8 ml of stock glycogen standard solution 5 mg/ml with 10 ml of DDD water and then adjusted the final volume to 20 ml.

175 µg/ml standard glycogen solution (20 ml):

Preparation: mixed 17.5 ml of 200 µg/ml standard glycogen solution with 1 ml of DDD water and then adjusted the final volume to 20 ml.

150 µg/ml standard glycogen solution (20 ml):

Preparation: mixed 17.143 ml of 175 µg/ml standard glycogen solution with 1.5 ml of double deionized distilled (DDD) water and then adjusted the final volume to 20 ml.

125 µg/ml standard glycogen solution (20 ml):

Preparation: mixed 16.667 ml of 150 µg/ml standard glycogen solution with 2 ml of DDD water and then adjusted the final volume to 20 ml.

100 µg/ml standard glycogen solution (20 ml):

Preparation: mixed 16 ml of 125 µg/ml standard glycogen solution with 3 ml of DDD water and then adjusted the final volume to 20 ml.

75 µg/ml standard glycogen solution (20 ml):

Preparation: mixed 15 ml of 100 µg/ml standard glycogen solution with 4 ml of DDD water and then adjusted the final volume to 20 ml.

50 µg/ml standard glycogen solution (20 ml):

Preparation: mixed 13.333 ml of 75 µg/ml standard glycogen solution with 5 ml of DDD water and then adjusted the final volume to 20 ml.

25 µg/ml standard glycogen solution (20 ml):

Preparation: mixed 10 ml of 50 µg/ml standard glycogen with 8 ml of DDD water and then adjusted the final volume to 20 ml.

3.3.6 Preparation of the rusa deer antler velvet extract (DAV) solutions

400 mg/ml rusa deer antler velvet extract (DAV 400) (20 ml):

Preparation: dissolved 8 g of rusa deer antler velvet extract (DAV) in DDD water and then adjusted the final volume to 20 ml.

200 mg/ml rusa deer antler velvet extract (DAV 200) (20 ml):

Preparation: mixed 10 ml of DAV 400 with 8 ml of DDD water and then adjusted the final volume to 20 ml.

100 mg/ml rusa deer antler velvet extract (DAV 100) (20 ml):

Preparation: mixed 10 ml of DAV 200 with 10 ml of DDD water and then adjusted the final volume to 20 ml.

The antler velvet extract was prepared every 3 days and stored at 4°C.

3.3.7 Determination of rusa deer antler velvet extract yield

The yield of evaporated dried rusa deer antler velvet extract based on dry weight basis was calculated from the following equation (Stanojevic *et al*., 2009):

$$\% \text{ Yield (g/100 g of antler velvet material)} = \frac{(W1 \times 100)}{W2}$$

where W1 was the weight of the rusa deer antler velvet extract after the solvent evaporation and W2 was the weight of the dry antler velvet material.

3.3.8 Chemical analyses of the rusa deer antler velvet extract

The rusa deer antler velvet extract samples was analyzed for energy, moisture, protein, total fat, ash, calcium, phosphorus, sodium, potassium, magnesium, iron, copper, zinc, and chloride. The methods were conducted by Institute of Nutrition, Mahidol University.

3.3.9 Biochemical screening of the rusa deer antler velvet extract

Qualitative biochemical analysis – The deer antler velvet extract was tested for the presence of bioactive compounds by using following standard procedures (Harborne, 1973; Trease and Evans, 1989; Sofowora, 1993).

3.3.9.1 Test for proteins

Ninhydrin test – The deer antler velvet extract (0.5 g) was boiled with 2 ml of 0.2% solution of Ninhydrin, violet color appeared suggesting the presence or absence of amino acids and proteins.

3.3.9.2 Test for carbohydrates

Iodine test – DAV (0.5 g) was mixed with 2 ml of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate.

3.3.9.3 Test for saponins

DAV (0.5 g) was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

3.3.9.4 Test for glycosides

Salkowski's test – DAV (0.5 g) was mixed with 2 ml of chloroform. Then 2 ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown color indicated the presence of steroidal ring, i.e. glycone portion of the glycoside.

3.3.9.5 Test for cardiac glycosides

Keller-Killiani's test – DAV (0.5 g) diluted to 5 ml water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

3.3.9.6 Test for steroids

DAV (0.5 g) was mixed with 2 ml of chloroform and concentrated H_2SO_4 was added sidewise. A red color produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2 ml of chloroform. Then 2 ml of each of concentrated H_2SO_4 and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

3.3.10 Experimental designs

After an adaptation period for one week, rats (n = 48) were randomly divided into six groups: vehicle group (1 ml/kg DDD water, p.o.), corn oil group (1 ml/kg, corn oil, i.m.), positive group (80 mg/ml/kg, testosterone propionate, i.m.) (Sadowska-Krępa *et al*., 2011) and three treatment groups.

The rats in the treatment groups were gavaged with three different doses of the rusa deer antler velvet extract (DAV): 100 mg/ml/kg for the low-dose group (DAV 100), 200 mg/ml/kg for the middle-dose group (DAV 200), and 400 mg/ml/kg for the high-dose group (DAV 400), respectively. These doses are selected according to the study of Shi *et al*. (2010) which were calculated by converting human dose used in normal folklore to rodent dose based on the following criteria:

$$\text{Rat dose (mg/kg)} = \frac{\text{human dose (mg/kg)} \times 70\ \text{kg} \times 0.018}{200}$$

Testosterone propionate was used as a positive control since it has been greatly associated with muscle development and it could improve muscular performance in forced swimming time to exhaustion study (Kanayama, Hudson, and Pope, 2008).

3.3.11 Forced swimming capacity test

The weight-loaded swimming test was employed in this study to evaluate the effects of the rusa deer antler velvet extract on exercise durability of rats. To avoid circadian variations in physical activity, the test was performed between 10.00 and 17.00 (Mousel, Stroup, and Nielsen, 2001). The procedure was used as previously described (Huang *et al*., 2011) with some modifications. Briefly, DDD water, DAV 100, DAV 200, and DAV 400 were orally administered while corn oil and TP was intramuscularly administered, once daily (9:00 h) for 9 days. In order to make the animals to accustom to swim, swimming were carried out on day 1, 3, 5, and 7 for 10 min, distinguished from day 9, in which rats were loaded nothing on their tails. During these periods, those who were unable to learn to swim were excluded from the experiment. Thirty minutes after the last administration on day 9, the overnight fasted rats were dropped individually into an acrylic plastic pool (90 cm × 45 cm × 45 cm) filled with fresh water maintained at 30±1°C, approximately 60 cm deep so that rats could not support themselves by touching the bottom with their tails. A lead block (5% of body weight) was loaded on the tail root of the rats. The swimming time to exhaustion was used as the index of the forced swimming capacity. The swimming period was considered the time spend floating, struggling and making necessary moments until exhaustion and possible drowning. The rats were assessed to be exhausted when they fail to rise to the surface of water to breath within a 7-s period.

3.3.12 Determination of blood biochemical variables

Immediately after exhaustion, the rats were anesthetized with pentobarbital sodium (Nembutal, Ceva Sante Amimale, Libourne, France) at a dose of

50 mg/kg (i.p.). Blood samples (3 ml) were collected by cardiac puncture. Hind-limb skeletal muscles (soleus, extensor digitorum longus (EDL) and gastrocnemius) from both sides and liver were quickly excised, dried with filter paper and weighed. Tissue samples were then frozen on dry ice and stored at -20°C until further used.

Whole blood obtained by cardiac puncture was collected into the tube with and without anticoagulant. Blood samples in the tube without anticoagulant was cooled for about 3.5 h at 4°C, the serum was prepared by centrifugation (Labconco Corporation Ltd., Missouri, USA) at a speed of 1000×g at 4°C for 20 minutes and the levels of serum lactate dehydrogenase (LDH) were determined by an automatic analyzer (Wang *et al*., 2012). Blood samples in the tube with anticoagulant were centrifuged at 2000×g at 4°C for 5 minutes (Hemmings and Song, 2004), the plasma levels of blood urea nitrogen (BUN), triglyceride (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, and creatinine and serum lactate dehydrogenase (LDH) were determined by an automatic analyzer (Wang *et al*., 2012).

3.3.13 Determination of glycogen levels in tissue samples

After blood collection, the rats were perfused through the left ventricle of the heart with 200-250 ml of ice-cold heparinized saline at a flow rate of 40 ml/min using peristaltic pump (model SP 311, VELP Scientifica, Europe). Immediately after starting the pump, the right atrium was cut to allow an escape route for the blood and perfusion fluid. After the atrium effluent was clear, the rats were perfused with 300-350 ml of 0.9% normal saline solution. The procedure for determination of glycogen levels in liver and gastrocnemius muscle samples was used as previously described (Lo *et al*., 1970) with some modifications. Briefly, the tissue samples were cut and weighted tissues 25 mg into an Eppendorf tube and kept on ice. The solution

of 750 µl of 30% KOH saturated with Na_2SO_4 was added to samples, making sure that the tissue was absolutely immersed in the solution. The tubes were put in a boiling water bath (model WB-22, WiseBath, Korea) for 30 minutes until homogeneous solutions was obtained. After that, tubes were removed from the boiling water bath, swirled and cooled in ice. The 95% EtOH (900 µl) was added to precipitate the glycogen from the alkaline digestate, swirled and stood samples in ice for 30 minutes. The sample tubes were centrifuged (model Z 233M-2, Hermle Labortechnik GmbH., Germany) at 840×g or 3873 rpm for 30 minutes. The supernatant was carefully aspirated and dried tubes upside down (5 minutes). The obtained of glycogen precipitate was dissolved with 1.5 ml of DDD water (vortex until went into solution) and appropriate aliquot of the glycogen solution was pipetted into three 16 × 100 mm test tubes (0.5 ml each). The 0.5 ml of 5% phenol solution was added to the above. Immediately after added phenol solution, rapidly added 2.5 ml of 96-98% H_2SO_4 (within 10-20 sec), the stream of acid being directed against the liquid surface rather than against the side of the test tube (15 ml size), to ensure good mixing. The tubes were then allowed to stand for 10 minutes. They were then shaken and placed for 10-20 minutes in a water bath at 25-30°C, before readings were taken. Have the glycogen standard solutions ready (0, 25, 50, 75, 100, 125, 150, 175, and 200 µg/ml) and prepared blank with 500 µl of DDD water instead of glycogen standard solutions. The absorbance of mixtures was measured by using a spectrophotometer (CECIL 1011, England) at 490 nm. All tests were performed in a triplicate to minimize errors resulting from accidental contamination and read within 30 min.

The equation use to calculate the tissue content of glycogen was assessed by following formula:

$$\text{Grams of glycogen/100 g tissue} = \frac{A_{490}}{k} \times \frac{V}{v} \times \frac{10^{-4}}{W}$$

Where: V = total volume of glycogen solution; v = volume of aliquot used in the color reaction; A_{490} = absorbance at 490 mμ; W = weight of tissue sample in gram; *k* = slope of standard cure; units = 1 per microgram glycogen.

3.3.14 Relative organ weight

Each organ to body weight ratio (relative organ weight: ROW) was calculated as (Aniagu *et al*., 2005; Abdullab *et al*., 2009)

(weight of organ (g) ÷ body weight of rat (g) on the day of sacrifice) × 100%

3.3.15 Statistical analysis

Data were expressed as mean ± SEM. Statistical analysis were assessed by analysis of variance (ANOVA) using SPSS 18.0 (SPSS, Inc., Chicago, IL). Post hoc testing was performed for inter-group comparisons (Duncan's). *P*-Value less than 0.05 ($P<0.05$) was considered statistically significant. All graphs were created by SigmaPlot software (version 10, Systat Software Inc., USA).

3.4 Results

3.4.1 Determination of rusa deer antler velvet extract yield

For the extraction of bioactive compounds in the rusa deer antler velvet, the boiling water extraction method was used in the present study. The percentage yield of the rusa deer antler velvet extract (DAV) was shown in Table 3.1.

Table 3.1 The percent yield of the hot water extract of the rusa deer (*Cervus timorensis*) antler velvet extract.

Parameter	**Percentage** (W/W)
Yield (%) dried antler velvet	16.26 ± 0.59 %

Numbers are the mean percentage ± SEM values of five independent determinations.

3.4.2 Chemical constituents in the of rusa deer antler velvet extract

The amounts of chemical constituents (protein and mineral elements) presented in the rusa deer antler velvet extract were shown in Table 3.2.

Table 3.2 The amount of chemical constituents presented in 1 gram of the rusa deer (*Cervus timorensis*) antler velvet extract.

Chemical constituents	**Mean**
Energy (kcal)	4.00
Moisture (g)	99.18
Protein (N×6.25) (g)	0.94
Total Fat (g)	Not detected
Ash (g)	0.03
Calcium (mg)	Not detected
Phosphorus (mg)	3.36
Sodium (mg)	8.14
Potassium (mg)	10.17
Magnesium (mg)	0.15
Iron (mg)	0.01
Copper (mg)	Not detected
Zinc (mg)	Not detected
Chloride (mg)	0.20

Numbers are the mean values of two independent.

DAV ratio: 1g of DAV dissolved in 100 ml distilled water.

3.4.3 Biochemical screening of rusa deer antler velvet extract

Biochemical screening of DAV showed the presence of proteins, carbohydrates, saponins, glycosides, and cardiac glycosides. Steroids were not found in DAV (Table 3.3).

Table 3.3 Biochemical screening of the rusa deer antler velvet extract.

Tests	**DDD-H_2O**	**Antler velvet (dry)**	**Antler velvet (crude)**	**DAV 400 mg/kg**
Proteins	–	+	+	+
Carbohydrates	–	+	+	+
Saponins	–	+	+	+
Glycosides	–	+	+	+
Cardiac glycosides	–	+	+	+
Steroids	–	–	–	–

+ and – symbols represented the presence and absence of biochemical compounds in the rusa deer antler velvet extract, respectively.

3.4.4 Swimming time to exhaustion study

The effects of 9 days administration of DAV and TP on swimming time to exhaustion in male Wistar rats were shown in Figure 3.1. In the present study, the rusa deer antler velvet extract at doses of 200 mg/kg (DAV 200), 400 mg/kg (DAV 400), and testosterone propionate (TP), significantly increased swimming time to exhaustion when compared to their controls (P<0.05, one way ANOVA, Figure 3.1). Administration of DAV at dose of 100 mg/kg (DAV 100) did not cause change in swimming time to exhaustion when compared with vehicle group. Swimming time to

exhaustion of DAV 200 and DAV 400 groups was significantly higher than DAV 100 groups (P<0.05, one way ANOVA, Figure 3.1).

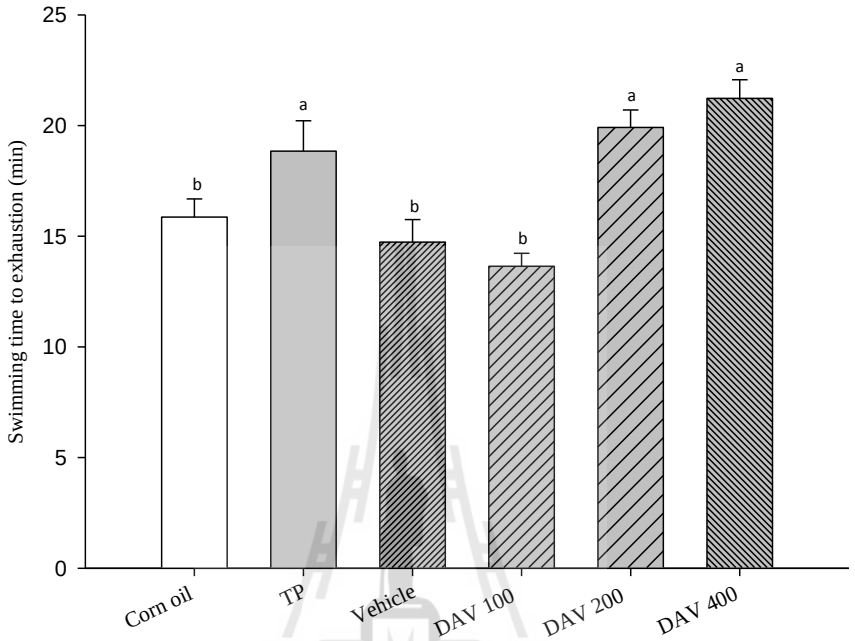

Figure 3.1 Effects of 9 days administration of the rusa deer antler velvet extract (DAV) and testosterone propionate (TP) on swimming time to exhaustion in male Wistar rats. Values are expressed as mean ± SEM; n = 8 per group. Significant difference compared to Corn oil, TP, Vehicle, DAV 100, DAV 200, and DAV 400 group, respectively. TP = testosterone propionate, and DAV = the rusa deer antler extract. Means sharing the same superscript are not significantly different from each other (One-way ANOVA; Duncan's Method; P<0.05).

3.4.5 Body weight gain

The body weight gain and body weight percentage change of male Wistar rats from day 1 to day 9 after administration of testosterone propionate, vehicle

and all doses of DAV were significantly decreased when compared with Corn oil group (P<0.05, one way ANOVA, Figure 3.1, 3.2). Administration of all doses of DAV did not cause change in body weight gain and body weight percentage change when compared with vehicle group (Figure 3.2, 3.3). Body weight percentage change from day 1 to day 9 of all DAV groups were significantly lower than corn oil group (P<0.05, one way ANOVA, Figure 3.3). Moreover, body weight percentage change from day 1 to day 9 in DAV 100 group, but not DAV 200 and DAV 400 groups, was significantly higher than TP group (P<0.05, one way ANOVA, Figure 3.2).

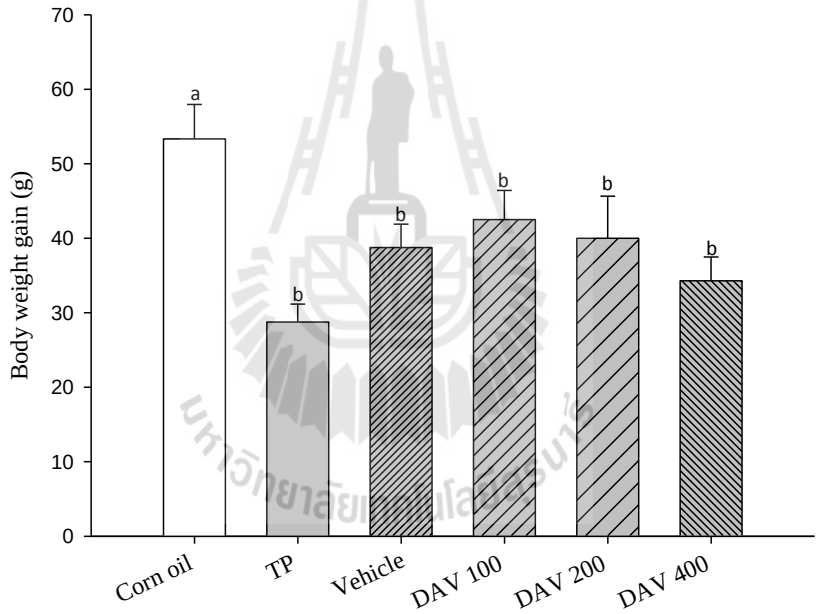

Figure 3.2 Effects of 9 days administration of the rusa deer antler velvet extract (DAV) and testosterone propionate (TP) on body weight gain. Values are expressed as mean ± SEM; n = 8 per group. Significant difference compared to Corn oil, TP, Vehicle, DAV 100, DAV 200 and DAV 400 group, respectively. TP = testosterone propionate, and DAV = the rusa deer antler extract. Means sharing the same superscript are not significantly different from each other (One-way ANOVA; Duncan's Method; P<0.05).

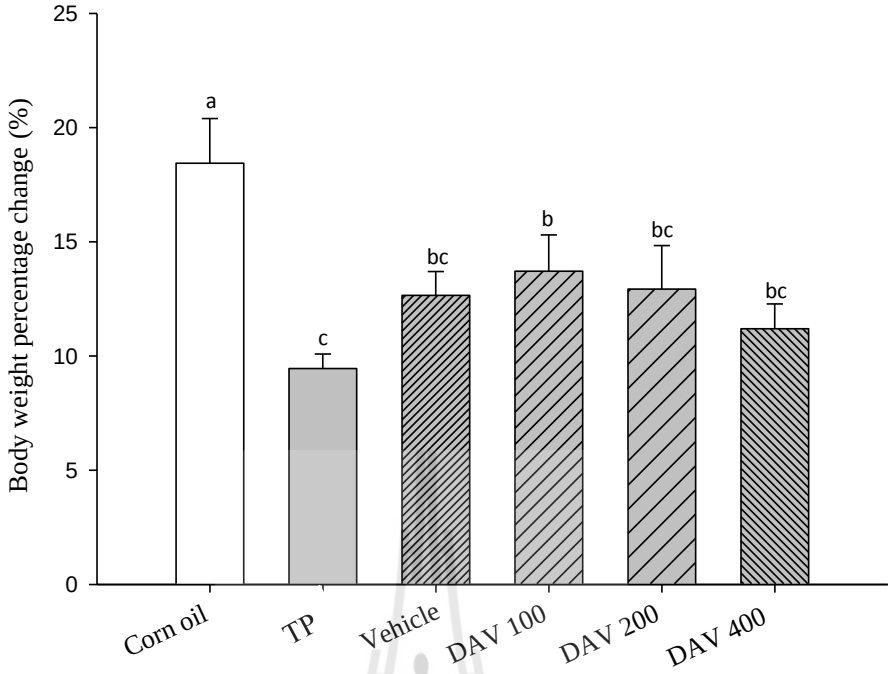

Figure 3.3 Effects of 9 days administration of the rusa deer antler velvet extract (DAV) and testosterone propionate (TP) on body weight percentage change. Values are expressed as mean ± SEM; n=8 per group. Significant difference compared to Corn oil, TP, Vehicle, DAV 100, DAV 200 and DAV 400 group, respectively. TP = testosterone propionate, and DAV = the rusa deer antler extract. Means sharing the same superscript are not significantly different from each other (One-way ANOVA; Duncan's Method; $P<0.05$).

3.4.6 Relative organ weight

Relative organ weights of liver, soleus muscle and gastrocnemius muscle of all treated groups were not different from their control groups. Relative organ weight of extensor digitolum longus (EDL) muscle in TP, DAV 100, and DAV 200 groups were significantly higher than corn oil group ($P<0.05$, one way ANOVA). Relative organ weights of EDL muscle in TP group and DAV 200 group were

significantly higher than corn oil group and the vehicle groups, respectively (P<0.05, one way ANOVA, Table 3.4).

3.4.7 Blood biochemical parameter levels

The blood biochemical parameter levels of triglyceride (TG) and alanine aminotransferase (ALT) in all groups were not different from their control group (Table 3.5). Plasma levels of creatinine in DAV 200 and DAV 400 groups were significantly higher than corn oil, TP, vehicle, and DAV 100 groups. Blood urea nitrogen (BUN) levels in corn oil group, DAV 200 and DAV 400 groups were significantly higher than TP, vehicle and DAV 100 groups (P<0.05, one way ANOVA, Table 3.5). Serum levels of lactate dehydrogenase (LDH) in all groups were significantly higher than corn oil group (P<0.05, one way ANOVA, Table 3.5). Rats treated with TP showed significantly higher serum LDH level than vehicle treated rats (P<0.05, one way ANOVA). The plasma level of aspartate aminotransferase (AST) was significantly higher in all DAV groups than vehicle group (P<0.05, one way ANOVA). Plasma AST level in TP group was significantly lower than corn oil group (P<0.05, one way ANOVA). Significant decreases were found in plasma glucose TP group compared to corn oil group and DAV 100 group compared to vehicle groups (P<0.05, one way ANOVA). Rats treated with the rusa deer antler velvet extract at doses of 200 and 400 mg/kg showed significantly higher plasma glucose level than DAV 100 treated rats (P<0.05, one way ANOVA).

Table 3.4 Effects of 9 days administration of the the rusa deer antler velvet extract and testosterone propionate on relative organ weight (ROW) of liver and hind-limb skeletal muscles (soleus, extensor digitorum longus (EDL) and gastrocnemius) in male Wistar rats.

Groups	ROW (g per 100 g body weight)			
	Liver	**EDL**	**Soleus**	**Gastrocnemius**
Corn oil	3.09 ± 0.10	0.07 ± 0.00 [b]	0.08 ± 0.00	1.08 ± 0.03
TP	2.96 ± 0.14	0.10 ± 0.01 [a]	0.08 ± 0.00	1.11 ± 0.03
Vehicle	2.97 ± 0.07	0.07 ± 0.01 [b]	0.08 ± 0.00	1.07 ± 0.03
DAV 100	2.95 ± 0.07	0.09 ± 0.01 [ab]	0.08 ± 0.00	1.09 ± 0.02
DAV 200	2.85 ± 0.09	0.09 ± 0.00 [a]	0.09 ± 0.01	1.12 ± 0.04
DAV 400	2.94 ± 0.13	0.08 ± 0.00 [ab]	0.09 ± 0.01	1.10 ± 0.05

Values are expressed as mean ± S.E.M; n = 8 per group. Significant difference compared to Corn oil, TP, Vehicle, DAV 100, DAV 200 and DAV 400 group, respectively. TP = testosterone propionate, and DAV = the rusa deer antler extract. Means sharing the same superscript are not significantly different from each other (One-way ANOVA; Duncan's Method; $P<0.05$).

Table 3.5 Effects of 9 days administration of the the rusa deer antler velvet extract (DAV) and testosterone propionate (TP) on blood biochemical parameters of male Wistar rats.

Parameters	Groups					
	Corn oil	**TP**	**Vehicle**	**DAV 100**	**DAV 200**	**DAV 400**
Glucose (mg %)	175 ± 10.29 [a]	67.14 ± 4.07 [d]	94.67 ± 8.32 [c]	76.5 ± 4.41 [d]	112 ± 8.42 [bc]	122.83 ± 8.42 [b]
TG (mg %)	66.5 ± 6.14	60.25 ± 5.95	63.17 ± 5.65	63.5 ± 4.91	58.83 ± 5.76	74.17 ± 6.39
LDH (U/L)	3141.29 ± 229.53 [c]	5521.50 ± 440.19 [a]	4506.38 ± 227.61 [b]	5050.50 ± 373.97 [ab]	5023.88 ± 443.25 [ab]	4619.50 ± 193.13 [ab]
AST (U/L)	227.5 ± 10.73 [a]	182.71 ± 8.25 [bc]	157.67 ± 6.36 [c]	206.83 ± 11.13 [ab]	207 ± 17.54 [ab]	214.57 ± 11.82 [ab]
ALT (U/L)	37.33 ± 2.03	35.29 ± 2.48	35.63 ± 2.75	34.14 ± 2.03	35.43 ± 1.05	40.00 ± 1.67
Creatinine (mg %)	0.63 ± 0.02 [b]	0.62 ± 0.03 [b]	0.65 ± 0.02 [b]	0.63 ± 0.03 [b]	0.88 ± 0.05 [a]	0.95 ± 0.05 [a]
BUN (mg/dl)	26.38 ± 1.03 [a]	22.25 ± 0.52 [b]	21.00 ± 0.95 [b]	22.63 ± 0.70 [b]	25.63 ± 0.92 [a]	27.75 ± 0.94 [a]

Values are expressed as mean ± S.E.M; n = 8 per group. Significant difference compared to Corn oil, TP, Vehicle, DAV 100, DAV 200, and DAV 400 group, respectively. TP = testosterone propionate, and DAV = the rusa deer antler extract. Means sharing the same superscript are not significantly different from each other (One-way ANOVA; Duncan's Method; $P<0.05$).

3.4.8 Glycogen tissue contents

3.4.8.1 Liver glycogen level

Nine days administration of the rusa deer antler velvet extract at doses of 100, 200 and 400 mg/kg, and testosterone propionate, significantly reduced liver glycogen levels in male Wistar rats when compared to their controls (P<0.05, one way ANOVA, Figure 3.4). Liver glycogen level in corn oil group was significantly higher than DAV 100 group and in vehicle group was significantly higher than corn oil and TP group. (P<0.05, one way ANOVA, Figure 3.4).

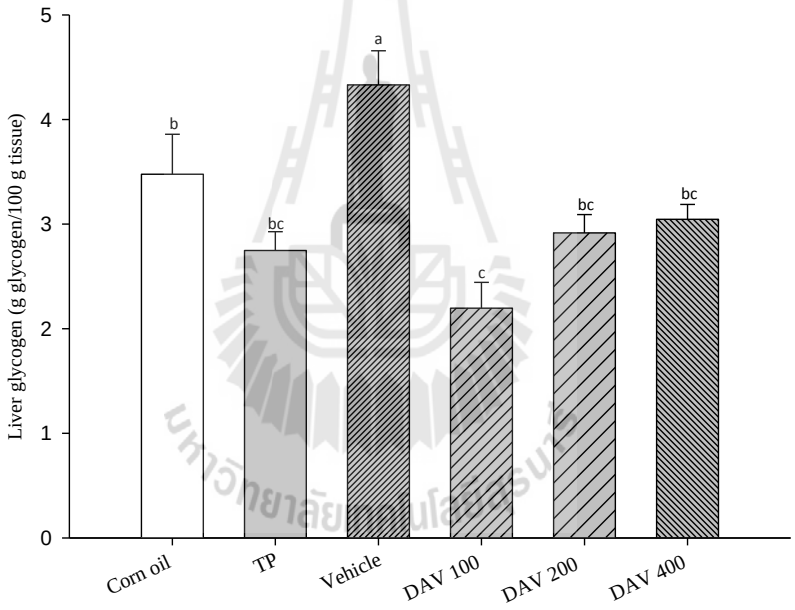

Figure 3.4 Effects of 9 days oral administration of the rusa deer antler velvet extract (DAV) and testosterone propionate (TP) on liver glycogen level in male Wistar rats. Values are expressed as mean ± S.E.M; n = 8 per group. Significant difference compared to Corn oil, TP, Vehicle, DAV 100, DAV 200 and DAV 400 group, respectively. TP = testosterone propionate, and DAV = the rusa deer antler extract. Means sharing the same superscript are not significantly different from each other (One-way ANOVA; Duncan's Method; P<0.05).

3.4.8.2 Gastrocnemius muscle glycogen level

Nine days administration of the rusa deer antler velvet extract at a dose of 400 mg/kg showed significantly higher in gastrocnemius muscle glycogen level than all other groups (P<0.05, one way ANOVA, Figure 3.5).

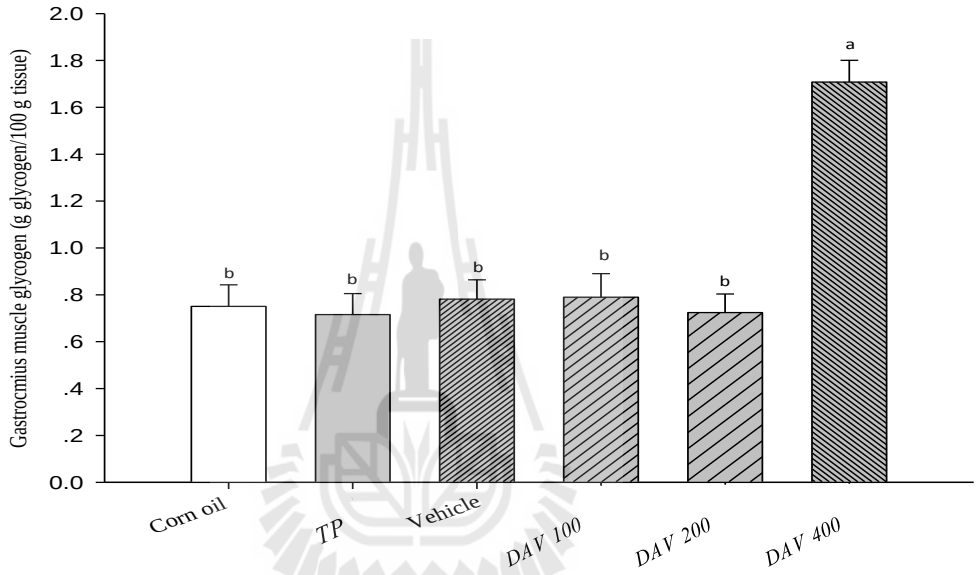

Figure 3.5 Effects of 9 days administration of the rusa deer antler velvet extract (DAV) and testosterone propionate (TP) on gastrocnemius muscle glycogen level (g glycogen/100 g tissue) in male Wistar rats. Values are expressed as mean ± S.E.M; n = 8 per group. Significant difference compared to Corn oil, TP, Vehicle, DAV 100, DAV 200 and DAV 400 group, respectively. TP = testosterone propionate, and DAV = the rusa deer antler extract. Means sharing the same superscript are not significantly different from each other (One-way ANOVA; Duncan's Method; P<0.05).

3.5 Discussion and conclusion

The percentage yield of the hot water extract of the rusa deer antler velvet in this study was 16.26 ± 0.59% (W/W) which is similar to previous study of Shi and co-workers (2010). Screening for biochemical composition of the rusa deer antler velvet extract, saponin, cardiac glycoside, glycosides, proteins, and carbohydrates were found accordingly with the study of Choi and co-workers (2006) which demonstarted that antler velvet composed of many biochemical components including lipids, peptides, carbohydrates and inorganic substances. Moreover, protein component extraction from antler base of sika deer (*Cervus nippon*) (Niu *et al*., 2011; Shi *et al*., 2011; Su *et al*., 2001), Formosan sambar deer (*Cervus unicolor swinhoei*) tip antler extract (FSDTAE) (Chen *et al*., 2014), pilose antler polypeptide (PAP) (Luo *et al*., 2008), and antler velvet water extract (AVWE) (Zhang *et al*., 2011) could improve fatigue effect through strengthening muscle in skeletal muscle of mice. In human study, deer antler velvet extract could affect muscular strength to knee extension exercise (Sleivert *et al*., 2003; Syrotuik *et al*., 2005). However, there was no study, on the anti-fatigue effect of the rusa deer antler velvet extract. The present study revealed the rusa deer antler velvet as an anti-fatigue agent for the first time. Significant increases in swimming time to exhaustion were shown in male Wistar rats daily oral administered the rusa deer antler velvet extract at doses of 200 and 400 mg/kg for 9 days. The present results were in line with previous studies. These results were in line with the study of Su and co-workers in 2001. They demonstrated that deer antler base proteins may be related to the enhancement of adrenal function on anti-fatigue effect in mice. Luo and co-workers (2008) demonstrated that the mice treated with 30 mg/kg/day (p.o.) of antler velvet polypeptide (VAP) for 30 days could

improve the ability of anti-anoxia and anti-fatigue *in vivo*. The pure compound of deer antler collagens could increase swimming time, improve the activity of lactate dehydrogenase and increase hepatic glycogen but decrease serum urea nitrogen in mice (Shi *et al*., 2011). Deer antler base proteins may be related to the enhancement of adrenal function on anti-fatigue effect. Anti-fatigue effect of pilose antler polypeptide was demonstrated in mice (Luo *et al*., 2008). Continuously climbing time and loaded swimming time were significantly increased and serum lactate content was significantly decreased in mice orally administered with 30 mg/kg/day of pilose antler polypeptide for 30 days compared with control groups. The present study also showed significant increase in swimming time to exhaustion after 9 days administration of testosterone propionate, suggesting the improving of testosterone in stimulated/exercised muscles (Boissonneault *et al*., 1989; Nnodim, 2001). Accordingly, testosterone enanthate (3 mg/kg/week for 12 weeks) in normal male subjects increased creatinine excretion reflecting muscle protein synthesis and muscle mass (Griggs *et al*., 1985). Many studies have confirmed that testosterone increases muscle mass (Sinha-Hikim *et al*., 2002), strength and endurance (Urban, 1999), a fact that has led elite athletes to use it in their physical training regimens, although in a questionable manner (Hoberman *et al*., 1995).

Cardiac glycoside has been used medicinally to increase the force of contraction of heart muscle and to regulate heartbeats (Gheorghiade, Adams, and Colucci, 2004). In present study, found that cardiac glycoside in the rusa deer antler velvet extract (DAV). Moreover, Gheorghiade and co-workers (1989) reported that in patients with reduced systolic function and abnormal central hemodynamics who are in sinus rhythm, digoxin can be improves left ventricular ejection fraction (LVEF)

and reduces pulmonary capillary wedge pressure while increasing cardiac output both at rest and during exercise.

Indeed, endurance cloud be improved by high carbohydrate ingestion for several days before prolonged exercise because this increases both liver and muscle glycogen stores (Stellingwerff *et al*., 2007). With exercise of longer duration, however, gluconeogenesis becomes increasingly important as liver glycogen stores become depleted. Glucose is spared so that just enough remains available at the end of the marathon. The simultaneous use of both fuels gives a higher mean velocity than would be attained if glycogen were totally consumed before the start of fatty acid oxidation. In addition, protein and carbohydrate supplement has been reported to potentiate the plasma insulin response of the supplement following a fast or prolonged aerobic exercise (Spiller *et al*., 1987; Zawadzki, Yaspelkis, and Ivy, 1992; van Loon *et al*., 2000;). Thus, it is possible that a carbohydrate and protein supplement may be more effective than a carbohydrate supplement for the sparing of muscle and possibly liver glycogen during variable intensity exercise (Bowtell *et al*., 1999).

Dietary protein ingestion after exercise increases post-exercise muscle protein synthesis rates, stimulates net muscle protein accretion, and facilitates the skeletal muscle adaptive response to prolonged exercise training. In present study have shown that protein ingestion before and during exercise stimulates muscle protein synthesis during exercise. Therefore, protein ingestion before and/or during prolonged exercise training sessions may inhibit muscle protein breakdown, stimulate muscle protein synthesis, and further augment the skeletal muscle adaptive response to exercise training (van Loon, 2014).

Testosterone significantly increased the cross-sectional area of type I fibers in the SOL muscle, and type I and IIA fibers in the EDL muscle of young male Wistar rats. In testosterone-treated group, the cross-sectional area had fiber type-specific changes being beneficial for muscle power and strength. Accordingly, the testosterone cypionate (5 mg/kg, s.c.) reduced cross sectional area percentage of type I fibers in the EDL muscle of old male Wistar rats (Isayama *et al*., 2006). The decreases in body weight gain and body weight percentage change were found in rats treated with testosterone propionate, but not all three studies doses of the rusa deer antler velvet extract did not cause changes in body weight gain. These results suggested that body weight gain and body weight percentage change effects of testosterone may be a result of direct and indirect suppression of the activity of adipocyte lipoprotein lipase, as well as other enzymes involved in triacylglycerol synthesis and storage. In addition, enhancement of growth hormone-dependent lipolysis by testosterone might indirectly partition energy away from fat and to muscle through increased physical activity (Engelson, Pi-Sunyer, and Kotler, 1999).

Individual muscles tend to be a mixture of various fiber types, but their proportions vary depending on the actions of that muscle and the species. For instance, in humans, the soleus muscles contain type I fibers, the EDL muscles contain type II fibers, while the gastrocnemius muscles contain mixed type fibers. In the present study, relative organ weights of the liver, soleus muscle and gastrocnemius muscle of testosterone propionate treated rats and all three studied doses of the rusa deer antler velvet extract treated groups were not change. Relative organ weight of EDL muscle was increased in testosterone propionate treated rats and the rusa deer antler velvet extract at a dose of 100 mg/kg and 200 mg/kg treated rats.

The increases in muscle mass by testosterone propionate and the rusa deer antler velvet extract may be associated with the increase in muscle protein synthetics since testosterone enanthate could increase muscle mass by increasing muscle protein synthesis (Griggs *et al*., 1985). Moreover, these changes may be associated with an increase fiber diameter, suggested that testosterone may induce muscle cell hypertrophy (Hughes *et al*., 2015).

In consistent with the increases in swimming time to exhaustion, increases in plasma glucose levels were also found in rats treated with the rusa deer antler velvet extract at a dose of 200 and 400 mg/kg. These changes may be a result of the increased demand for glucose by contracting muscle causes an increased glucose uptake to working skeletal muscle (blood glucose) during prolonged exercise *(*Wu *et al*., 2013). The results of blood sugar profile may be related with the result of liver glycogen content (Sanchez *et al*., 1971). The increases in plasma glucose levels were correlated with the reduction in liver glycogen contents in rats treated with the rusa deer antler velvet extract. Hence, the rusa deer antler velvet extract may promote increased breakdown of liver glycogen to glucose.

ALT and AST measurements are important for the assessment of liver damage (Jang *et al*., 2012). There was no change in plasma ALT levels in all treatment groups. The increases in plasma AST levels were found in all studies doses of the rusa deer antler velvet extract. These findings highlight the importance of imposing relevant restrictions on strenuous exercise prior to and during clinical studies, and exhibit the need to consider of intense muscular activity as possible causes of asymptomatic elevations of liver function tests in daily clinical practice (Pettersson *et al*., 2008).

LDH is known to be an authentic index of muscle damage as it catalyzes the inter-conversion of pyruvate and lactate (Kim *et al*., 2003). Significant increase in the levels of serum LDH released by damage muscle tissue was observed (Stenner *et al*., 2005). Rats in all treated groups of the rusa deer antler velvet extract showed a slightly higher degree, but not significantly difference, in serum LDH levels after exhaustive exercise than those in the control group. These findings indicated that the rusa deer antler velvet extract might induce glycogen degradation *via* lactate dehydrogenase pathway without any muscle tissue damage (Stenner *et al*., 2006).

In tissues and cells that consume ATP rapidly, especially skeletal muscle, phosphocreatine (PCr) serves as an energy reservoir for the rapid buffering and regeneration of ATP. Thus creatinine, a breakdown product of PCr, is an important indicator for rapid consumption of ATP (Wallimann *et al*., 1992). The present study showed the rusa deer antler velvet extract at doses of 200 and 400 mg/ml/kg significantly increased creatinine levels indicating rapid consumption of ATP.

The rusa deer anter velvet extract at doses of 200 and 400 mg/ml/kg for 9 days significantly increased blood urea nitrogen levels. A subject during exercise inhibits hepatic protein catabolism or increases renal urea excretion. This phenomenon might be associated with the role of IGF-1 in the rusa deer antler velvet extract on muscle hypertrophy via testosterone (Hughes *et al*., 2015). To study of Sokal *et al*. (2013). suggested that in term of during exercise affects protein metabolism, resulting in a positive nitrogen balance. This phenomenon has fundamental importance in understanding human metabolic processes and may have indications in training programs for athletes. Hence, the possible causes of high BUN was increased production (muscle and other protein breakdown, increased

consumption of proteins in the diet). Moreover, the possible causes of high BUN was increased muscle damage and other protein breakdown after exhaustive exercise in associated with the early stage of muscle hypertrophy process in the group received IGF-1 (Adams *et al*., 1998; Coleman *et al*., 1995; Vandenburgh *et al*., 1991) in the rusa deer antler velvet extract. In contrast to the present finding, Chen and colleague (2014) found that the levels of lactic acid and blood urea nitrogen were slightly reduced and only glucose level was no change in Formosan sambar deer tip antler extract (FSDTAE) group after forced-swimming capacity tests in mice.

Energy storage and supply is an important factor related to exercise performance. In terms of energy expenditure with exercise, rapid ATP consumption and energy deficiency is a critical cause of physical fatigue. Glycogen is the predominant source of glycolysis for ATP production. Therefore, glycogen storage directly affects exercise ability. Previous study found that the more glucose is metabolized, the more liver glycogen stores are reduced. The increases in muscle glycogen content following the administration of the rusa deer antler velvet at a dose of 400 mg/kg were found in the present study. This finding suggested the capability of the rusa antler velvet extract in maintenance muscle during exhaustive exercise causing muscle fatigue. Raising muscle glycogen increases the capacity for prolonged exhausting exercise, while a low initial glycogen concentration is associated with more rapid development of fatigue (Hermansen, Hultman, and Saltin, 1967). The major factor in determining the steady-state glycogen concentration in resting muscle appears to be the rate of muscle glucose uptake (Ren *et al*., 1993). Ren and co-workers (1994) demonstrated that exercise could induce the adaptive increase in glucose transporter 4 (GLUT4) that potentiating insulin-stimulated glucose transport capacity. This effect of exercise might function to increase the rate of muscle

glycogen repletion. The rapid adaptive increase in GLUT4 expression could provide a survival advantage by making possible more rapid replenishment of muscle glycogen stores when carbohydrate is eaten between exercise bouts or even when exercise is continued at a lower intensity. The rapid adaptive increase in GLUT4 may also be help to explain the data that a few days of exercise training can result in a significant enhancement of insulin action on glucose disposal (Rogers *et al*., 1988). There is considerable evidence that depletion of muscle glycogen results in development of fatigue and that raising muscle glycogen concentration delays onset of exhaustion during prolonged strenuous exercise (Ahlborg *et al*., 1967; Bergström *et al*., 1967).

In conclusion, this is the first report of the anti-fatigue effects on swimming time to exhaustion studies of the rusa deer antler velvet extract in male Wistar rats. The rusa deer antler velvet can enhance swimming durability. However, the underlying mechanisms are still not fully understood.

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CHAPTER IV

EFFECTS OF THE RUSA DEER (*Cervus timorensis*) ANTLER VELVET EXTRACT ON EXERCISE ENDURANCE CAPACITY IN MALE WISTAR RATS

4.1 Abstract

Physical fatigue is the failure to maintain working at the level of one's normal capacities. Antler velvet has been claimed to have a wide range of effects on systematic exhaustion and performance enhancement (prevent and repair muscle damage, increase muscular strength and endurance). The exercise endurance capacity effect of the rusa deer antler velvet extract (DAV) was investigated in male Wistar rats. Ninety six rats were devided into exercise trainning (swimming without load) and non-exercise trainning groups that received corn oil (1 ml/kg, i.m.), testosterone propionate (TP, 80 mg/ml/kg, i.m.), vehicle (1 ml/kg double deionized distilled (DDD) water, p.o.), and DAV (100, 200, and 400 mg/ml/kg) orally once daily for 16 days (n = 8, each). On day 16, all rats were observed for time to exhaustion after the weight-loaded forced swimming. Body weight gain, relative organ weight (ROW) of liver and hind-limb skeletal muscles (soleus, extensor digitorum longus (EDL) and gastrocnemius), biochemical parameters [glucose, triglyceride (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, blood urea nitrogen (BUN), and lactate dehydrogenase (LDH)], liver glycogen content and

gastrocnemius muscle glycogen content were determined. In non-exercise training, there was no significant difference in time to exhaustion in all groups, DAV significantly increased liver glycogen content, and TP, DAV 100 and DAV 200 significantly increased glycogen content in gastrocnemius muscle as compared to their respective controls (P<0.05). In exercise training, TP significantly decreased while DAV 100 and DAV 200 significantly increased time to exhaustion to their respectively control (P<0.05), DAV 200 significantly increased liver glycogen content, and DAV 100, DAV 200, and DAV 400 significantly increased glycogen content in gastrocnemius as muscled compare to their respective control (P<0.05). In comparison to non-exercise training, exercise training group that received TP showed significant reduction while exercise training groups that received DAV 200 and DAV 400 showed significant enhancement in time to exhaustion (P<0.05). Moreover, significant decreases in body weight gain and body weight percentage change (corn oil, TP, DAV 200, and vehicle groups), plasma glucose levels (corn oil, DAV 100 and DAV 400 groups), plasma triglyceride levels (TP group), and liver glycogen content (DAV 400 group) were found in exercise training compared to non-exercise training (P<0.05). In addition, significant increases in serum LDH levels (corn oil, vehicle, TP and DAV 100 groups), plasma creatinine levels (corn oil and vehicle groups), plasma AST level (vehicle, DAV 100, DAV 200 and DAV 400 groups), plasma creatinine levels (vehicle, corn oil, DAV 100 and DAV 400 groups), and the glycogen content in gastrocnemius muscle (DAV 400 group) were found in exercise training compared to non-exercise training (P<0.05). The present findings suggested that DAV appeared to have potential to support exercise endurance capacity and DAV may be useful for development of physical strength. DAV may increase the glycogen contents in liver

and gastrocnemius muscle in both non-exercise and exercise training by improving glycogen reserve and glycogen-sparing effect. Bioactive compounds found in the deer antler base extract such as protein, collagens and insulin-like growth factor 1 (IGF-1) may play a key role in endurance capacity effect of DAV. However, further studies are needed to elucidate effect of DAV on exercise endurance capacity.

4.2 Introduction

Physical fatigue is the failure to maintain working at the level of one's normal capacities. It is common in everyday life but usually becomes particularly appreciable during heavy exercise. Fatigue is the most general form of sub-health state, and approximately 75% of people in the world are in a sub-health state (Hagberg *et al*., 1981). Antler velvet has been claimed to have a wide range of effects on systematic exhaustion, depression, cold, lower back pain, weak pulse, impotence, spermatorrhea, low white cell counts, regulate the adrenal cortex, regulate energy metabolism, promote sexual function, promote growth and strengthen resistance, anaemia alleviating, anti-aging, anti-cancer, anti-inflammatory, blood pressure control, bone and joint anti-inflammation, growth stimulation, performance enhancement (prevent and repair muscle damage, increase muscular strength and endurance) (Tuckwell *et al*., 2003). Antler velvet composes of many biochemical components including lipids, peptides, carbohydrates and inorganic substances (Choi *et al*., 2006). Consumption of deer antler velvet has increased worldwide, with consumers interested in the quantity and chemical composition of deer antler velvet (Jeon *et al*., 2011). Many reports and clinical observations convincingly showed that deer antler velvet contains many components such as sphingomylein, ganglioside, estrone,

estradiol, prostaglandins, collagen, amino acid-sugar combinations and growth factors including insulin-like growth factor-1 (IGF-1) and epidermal growth factor (EGF) (Ran *et al*., 2009). Deer antler has also been shown to increase strength, endurance, anaerobic performance and VO_2 max in subject participants (Broeder *et al*., 2004). Moreover, the fermented deer antler extract could enhance swimming to exhaustion in mice (Jang *et al*., 2014). Thus, the extract from rusa deer antler velvet may have potent enhancing effects on exercise endurance performance. Therefore, the effects of the extract from rusa deer antler velvet on physical fatigue and exercise endurance capacity were investigated.

4.3 Materials and methods

All materials and methods were similar to those previously described in chapter III, except as otherwise the specified methods and materials were mentioned in this chapter.

4.3.1 Experimental designs

After an adaptation period for one week, 8-week-old male Wistar rats (n = 96) were randomly divided into twelve groups (6 groups for exercise training (swimming without load) and 6 groups for non-exercise training):

- Corn oil + Ex group: Corn oil (vehicle of positive group, 1 ml/kg, i.m.) + exercise training group,

- Corn oil + Non-Ex group: Corn oil (vehicle of positive group, 1 ml/kg, i.m.) + non-exercise training,

- TP + Ex group: Testosterone propionate or TP (positive control, 80 mg/ml, i.m.) + exercise training,

- TP + Non-Ex group: Testosterone propionate or TP (positive control, 80 mg/ml, i.m.) + Non-Exercise training,

- Vehicle + Ex group: Vehicle (1 ml/mg DDD water, p.o.) + exercise training,

- Vehicle + Non-Ex group: (1 ml/mg DDD water, p.o.) + Non-Exercise training,

- DAV 100 mg/kg + Ex group: (100 mg/kg the rusa deer antler velvet extract, p.o.) + exercise training,

- DAV 100 mg/kg + Non-Ex group: (100 mg/kg the rusa deer antler velvet extract, p.o.) + non-exercise training group,

- DAV 200 mg/kg + Ex group: (100 mg/kg the rusa deer antler velvet extract, p.o.) + exercise training,

- DAV 200 mg/kg + Non-Ex group: (100 mg/kg the rusa deer antler velvet extract, p.o.) + non-exercise training,

- DAV 400 mg/kg + Ex group: (100 mg/kg the rusa deer antler velvet extract, p.o.) + exercise training,

- DAV 400 mg/kg + Non-Ex group: (100 mg/kg the rusa deer antler velvet extract, p.o.) + non-exercise training.

The doses of the rusa deer antler velvet extract were selected according to the study of Shi and co-workers (2010) which were calculated by converting human dose used in normal folklore to rodent dose based on the following criteria: rat dose (mg/kg) = [human dose (mg/kg) × 70 kg × 0.018] / 200.

4.3.2 Exercise endurance capacity

The exercise training groups (received corn oil, TP, vehicle, DAV at doses of 100, 200, and 400 mg/ml) were subjected to swimming training (without any load) for 16 days (6 days a week). Exercise endurance capacity was observed by the weight loaded forced swimming test as previously described by Kumar *et al*. (2011). Briefly, the rats were trained for 30 min/day with subsequent increase of 10 min/day till it reaches 1 h. Swimming was performed in groups of three in an acrylic plastic pool (90 cm × 45 cm × 45 cm) filled with water up to 60 cm and maintained at a temperature between 34°C and 36°C. During the exercise protocol, the rats in non-exercise groups (received corn oil, TP, vehicle, DAV at dose of 100, 200, and 400 mg/ml) were kept in a plastic cage containing about 3 cm of water maintained at same temperature to exclude potential stress and other potential confounding effects. On day 16, after administered 30 minute all rats were allowed to swim till exhaustion with a load of 3% body weight attached to the tails. The uncoordinated movements and staying under the water for 10 s without swimming at the surface were accepted as the exhaustion criteria of the rats. At this point, the rats were rescued and swimming time was recorded for each rat.

4.3.3 Determination of blood biochemical variables

Immediately after swimming exercise, the rats were anaesthetized with pentobarbital sodium (50 mg/kg, i.p.). Whole blood obtained by cardiac puncture was collected into the tube with and without anticoagulant. The serum level of LDH and the plasma levels of BUN, TG, AST, ALT, glucose and creatinine were determined by an automatic analyzer as mentioned in chapter III.

4.3.4 Determination of glycogen levels in tissue samples

Hind-limb skeletal muscles (soleus, extensor digitorum longus and gastrocnemius) from both sides and liver were quickly excised, dried with filter paper and weighed. The liver and muscles were frozen on dry ice. The obtained frozen tissues were stored at -20°C until further used. The procedure for determination of glycogen levels in liver and gastrocnemius muscle was similar to those previously described in chapter III.

4.3.5 Relative organ weight

Organ to body weight ratio (relative organ weight: ROW) of liver, soleus, EDL and gastrocnemius was calculated as (weight of organ (g) ÷ body weight of rat (g) on the day of sacrifice) × 100% (Abdullab *et al*., 2009; Aniagu *et al*., 2005).

4.3.6 Statistical analysis

Results were expressed as mean ± S.E.M. Statistical analysis were assessed using two-way ANOVA followed by Duncan's post hoc test (SPSS 18.0, SPSS, Inc., Chicago, IL). Post hoc testing was performed for inter-group comparisons. *P*-values less than 0.05 ($P<0.05$) were considered statistically significant. All graphs were created by SigmaPlot software (version 10, Systat Software Inc., USA).

4.4 Results

4.4.1 Exercise endurance capacity study

The effects of 16 days administration of the rusa deer antler velvet extract and testosterone propionate with and without exercise training on exercise endurance capacity in male Wistar rats were shown in Figure 4.1. There was no

significant difference in swimming time to exhaustion in all non-exercise training groups. Swimming time to exhaustion of all exercise training groups except TP group were significantly increased when compared to their respective non-exercise treatment groups (P<0.05 two-way ANOVA). There was no significant difference between exercise training and non-exercise training in testosterone propionate treatment. In the exercise training, swimming time to exhaustion of the rats treated with the rusa deer antler velvet extract at a dose of 200 mg/ml was significantly higher than all other treated groups, except corn oil group (P<0.05, two-way ANOVA).

4.4.2 Body weight gain

The body weight gain and body weight percentage change of male Wistar rats on day 16 from day 1 after administration of testosterone propionate and the rusa deer antler velvet extract with and without exercise training was shown in Figure 4.2 and 4.3, respectively. There was no significant difference in body weight gain between exercise training and non-exercise training in rats treated with the rusa deer extract at a dose of 100 mg/kg. Body weight gain of rats received corn oil, testosterone propionate, vehicle, DAV 200 and DAV 400 in exercise training was markedly lower than non-exercise training (P<0.05 two-way ANOVA). There was no significant difference in body weight percentage change in all exercise training groups. In non-exercise training, the rusa deer antler velvet extract at a dose of 100 mg/kg (DAV 100) significantly reduced body weight percentage change when compared with vehicle treatment (P<0.05 two-way ANOVA). In comparison to exercise to non-exercise training, exercise training groups that received corn oil, testosterone propionate, DAV 200, DAV 400 and vehicle showed significant reduction of body weight percentage change (P<0.05 two-way ANOVA), while there

was no significant difference in a groups treated with the rusa deer antler velvet extract at a dose of 100 mg/kg.

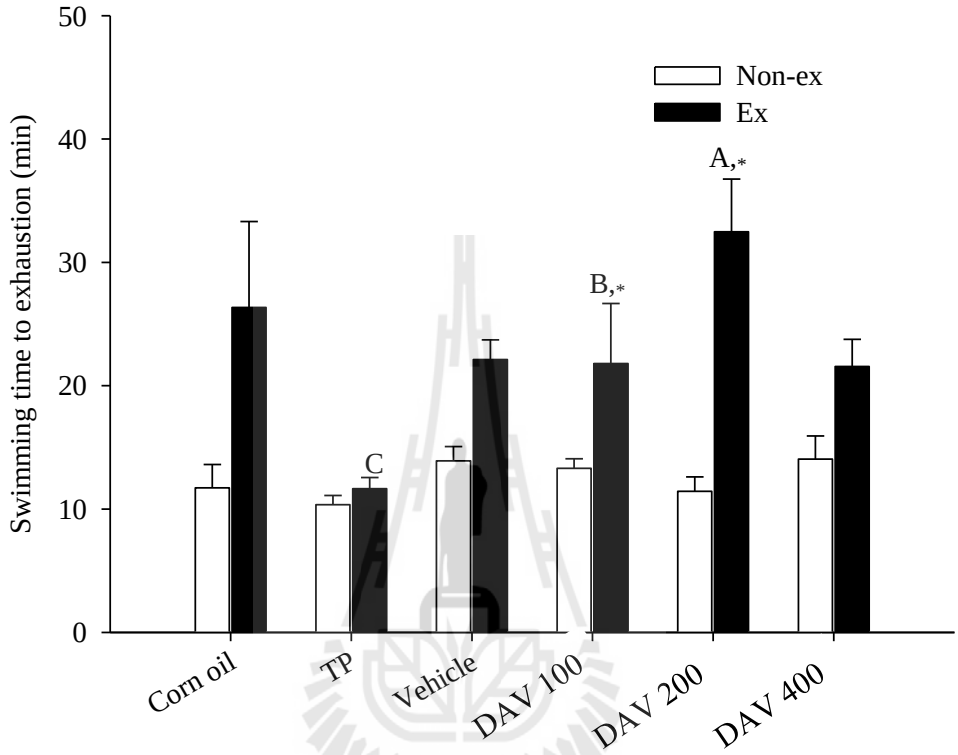

Figure 4.1 Effect of 16 days administration of the rusa deer antler velvet extract (DAV) and testosterone propionate (TP) on exercise endurance capacity. Values are expressed as mean ± SEM; n = 8 per group. * indicates significant difference compared between Non-Ex and Ex with same treatment. Small letters indicate significant differences within Non-Ex. Capital letters indicate significant differences within Ex. Means sharing the same superscript are not significantly different from each other (P<0.05, Two-way ANOVA; Duncan's Method). TP = testosterone propionate, DAV = the rusa deer antler velvet extract, Ex = Exercise training, Non-Ex = Non-Exercise training.

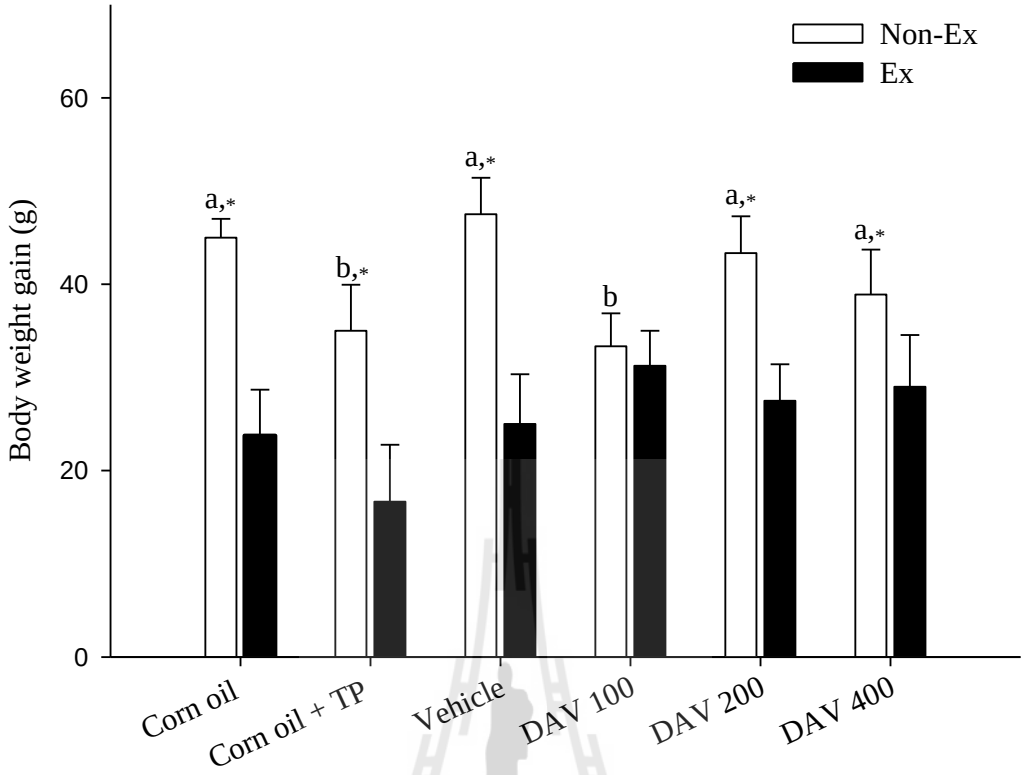

Figure 4.2 Effects of 16 days administration of the rusa deer antler velvet extract (DAV) and testosterone propionate (TP) on body weight gain. Values are expressed as mean ± SEM; n = 8 per group. * indicates significant difference compared between Non-Ex and Ex with same treatment. Small letters indicate significant differences within Non-Ex. Capital letters indicate significant differences within Ex. Means sharing the same superscript are not significantly different from each other (P<0.05, Two-way ANOVA; Duncan's Method). TP = testosterone propionate, DAV = the rusa deer antler velvet extract, Ex = Exercise training, Non-Ex = Non-Exercise training.

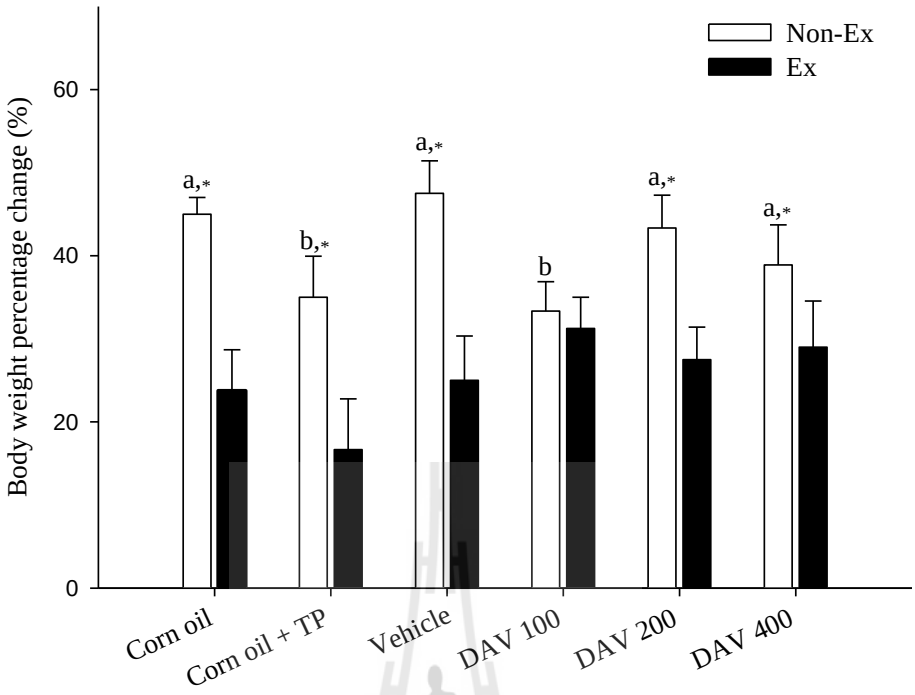

Figure 4.3 Effect of 16 days administration of the rusa deer antler velvet extract (DAV) and testosterone propionate (TP) on body weight percentage change. Values are expressed as mean ± SEM; n = 8 per group. * indicates significant difference compared between Non-Ex and Ex with same treatment. Small letters indicate significant differences within Non-Ex. Capital letters indicate significant differences within Ex. Means sharing the same superscript are not significantly different from each other (P<0.05, Two-way ANOVA; Duncan's Method). TP = testosterone propionate, DAV = the rusa deer antler velvet extract, Ex = Exercise training, Non-Ex = Non-Exercise training.

4.4.3 Relative organ weight

There was no significant difference in relative organ weights (ROW) of the liver, extensor digitolum longus muscle, soleus muscle and gastrocnemius muscle of all groups in both exercise and non-exercise training (Table 4.1).

4.4.4 Blood biochemical parameter levels

The blood biochemical parameter levels (Glucose, TG, LDH, AST, ALT, Creatinine, and BUN) of male Wistar rats after administration of testosterone propionate and the rusa deer antler velvet extract with and without exercise training were shown in Table 4.2. Alanine aminotransferase (ALT) in rats administered with all treatments in exercise training and non-exercise training were not statistically different (Table 4.2).

In non-exercise training, plasma glucose levels in rats received the rusa deer antler velvet extract at a dose of 100 mg/kg (DAV 100) was significantly higher than vehicle treated rats ($P<0.05$ two-way, ANOVA), while all other groups were not significant difference. In exercise training, there was no significant difference in plasma glucose levels in all groups. In comparison to non-exercise training, exercise training groups that received corn oil and all doses of the rusa deer antler velvet extract showed significant reduction of plasma glucose level ($P<0.05$, two-way ANOVA), while there was no significant difference in testosterone propionate and vehicle treated groups.

In non-exercise training, plasma TG levels in rats received corn oil was significantly higher than rats received the rusa deer antler velvet extract at dose of 100 mg/kg (DAV 100) ($P<0.05$ two-way, ANOVA), while all other groups were not significant different. In exercise training, plasma TG levels in rat received testosterone propionate were significantly lower than that of corn oil and vehicle treatment, in rats received the rusa deer antler velvet extract the dosed of 100 and 400 mg/kg were significantly lower than vehicle treated rats and in rats received the rusa deer antler velvet extract at the dosed of 400 mg/kg was significantly lower than

corn oil treated rats ($P<0.05$, two-way ANOVA). In comparison to non-exercise training, exercise training groups that received testosterone propionate showed significant reduction while vehicle treated group showed significant increase in plasma TG levels ($P<0.05$, two-way ANOVA), all other groups were not significant different.

In non-exercise training, there was no significant difference in serum LDH levels in all groups. In exercise training, serum LDH level corn oil group was significantly higher than all other groups, DAV 200 and DAV 400 treated groups were significantly lower than vehicle group and in DAV 100 treated group significantly lower than corn oil group, but significantly higher than DAV 200 and DAV 400 groups ($P<0.05$ two-way, ANOVA). In comparison to non-exercise training, exercise training group was received corn oil, TP, vehicle and DAV 100 showed significant enhancement in serum LDH level ($P<0.05$).

In non-exercise training, plasma AST level in corn oil group was significantly higher than vehicle group ($P<0.05$, two-way ANOVA), there was no significant difference in all other groups. In exercise training, plasma AST levels in corn oil treated rats was significantly lower than DAV 200 group, in TP group was significantly lower than vehicle, DAV 200 and DAV 400 groups, and in DAV 100 group was significantly lower than DAV 200 group ($P<0.05$, two-way ANOVA). In comparison to non-exercise training, exercise training groups than received vehicle, DAV 100, DAV 200, and DAV 400 showed significant enhancement in plasma AST ($P<0.05$).

In non-exercise training, there was no significant difference in plasma creatinine levels in all groups. In exercise training, plasma creatinine level in corn oil

group was significantly higher than DAV 200 group ($P<0.05$), while there was no significant difference in all other groups. In comparison to non-exercise training, exercise training groups that received corn oil, vehicle, DAV 100, and DAV 400 showed significant enhancement in plasma creatinine levels ($P<0.05$, two-way ANOVA).

Table 4.1 Effects of 16 days administration of the rusa deer antler velvet extract (DAV) and testosterone propionate on relative organ weight (ROW) of liver, extensor digitorum longus (EDL), soleus and gastrocnemius.

Groups		ROW (g per 100 g body weight)			
		Liver	**EDL**	**Soleus**	**Gastrocnemius**
Corn oil	Non- Ex	2.61 ± 0.10	0.09 ± 0.00	0.09 ± 0.00	1.15 ± 0.03
	Ex	2.42 ± 0.07	0.09 ± 0.00	0.08 ± 0.00	1.03 ± 0.07
TP	Non- Ex	2.40 ± 0.11	0.10 ± 0.01	0.09 ± 0.01	1.17 ± 0.03
	Ex	2.42 ± 0.11	0.09 ± 0.00	0.08 ± 0.00	1.09 ± 0.03
Vehicle	Non- Ex	2.65 ± 0.17	0.09 ± 0.00	0.08 ± 0.01	1.10 ± 0.03
	Ex	2.52 ± 0.06	0.09 ± 0.00	0.08 ± 0.01	1.13 ± 0.04
DAV 100 mg/kg	Non- Ex	2.60 ± 0.10	0.09 ± 0.00	0.08 ± 0.00	1.12 ± 0.03
	Ex	2.62 ± 0.12	0.10 ± 0.00	0.08 ± 0.00	1.16 ± 0.02
DAV 200 mg/kg	Non- Ex	2.44 ± 0.05	0.09 ± 0.00	0.08 ± 0.00	1.13 ± 0.02
	Ex	2.42 ± 0.08	0.09 ± 0.00	0.09 ± 0.00	1.13 ± 0.02
DAV 400 mg/kg	Non- Ex	2.50 ± 0.05	0.09 ± 0.01	0.08 ± 0.01	1.14 ± 0.03
	Ex	2.49 ± 0.07	0.09 ± 0.00	0.08 ± 0.00	1.17 ± 0.03

Values are expressed as mean ± S.E.M. DAV = the rusa deer antler velvet extract, TP = testosterone propionate, Ex = Exercise training, Non-Ex = Non-Exercise training.

Table 4.2 Effects of 16 days administration of the rusa deer antler velvet extract (DAV) and testosterone propionate (TP) on blood biochemical parameters of male Wistar rats.

Groups		Parameters						
		Glucose (mg%)	**TG (mg/dl)**	**LDH (U/L)**	**AST (U/l)**	**ALT (U/l)**	**Creatinin (mg%)**	**BUN (mg/dl)**
Corn oil	Non-Ex	115.67± 3.88(ab,*)	100.71 ± 8.89(a)	5402 ± 1154.13	245± 30.39(a)	41 ± 2.18	0.91 ± 0.11	21± 0.88
	Ex	87.33 ± 6.10	103.83 ± 8.88(A)	14503.83 ± 1328.20(A,*)	250.40 ± 14.99(CB)	44.88 ± 3.28	1.18 ± 0.04(A,*)	22.71 ± 1.10(AB)
TP	Non-Ex	109.86± 10.79(ab)	91.43 ± 6.73(ab)	5882.86± 1073.04	186.20 ± 27.74(ab)	41.29 ± 4.49	0.79 ± 0.10	21.63 ± 1.37
	Ex	102 ± 9.56	68.50 ± 5.65(B,*)	9042 ± 1427.84(BC)	203.67 ± 21.74(C)	44.63 ± 3.03	0.99 ± 0.12(AB)	23.25 ± 1.72(AB)
Vehicle	Non-Ex	105.83 ± 6.83(b)	83 ± 6.62(ab)	4992 ± 674.31	152.57± 11.03(b)	37.38 ± 2.14	0.88 ± 0.10 (e)	20.50 ± 0.88
	Ex	106.67 ± 9.01	107 ± 11.32(A,*)	11254.17± 806.98(BC,*)	290.20 ± 27.21(AB,*)	40.40 ± 5.19	1.16 ± 0.09(A,*)	21.83 ± 1.45(B)
DAV 100	Non-Ex	140.17±7.83(a,*)	70.83 ± 7.35(b)	4870.86 ± 419.79	193.50 ± 30.48(ab)	37.89 ± 2.90	0.73 ± 0.08 (g)	20.67 ± 1.00
	Ex	85.14 ±5.11	85.17 ± 11.48(B)	9572.50 ± 1010.48(B,*)	267 ± 15.70(B,*)	43 ± 2.15	1.13 ± 0.13(A,*)	23.50± 2.01(AB)
DAV 200	Non-Ex	131.6±12.78(ab,*)	81 ± 6.12(ab)	6627.11 ± 708.33	162.83 ± 9.51(ab)	41.88 ± 5.35	0.73 ± 0.10	20.67 ± 0.61
	Ex	99 ±11.63	80.17 ± 8.80(B)	6592.17 ± 1443.28(BC)	352.80 ± 38.11(A,*)	45 ± 5.18	0.90 ± 0.15(B)	25.88 ±2.0(A,*)
DAV 400	Non-Ex	128.83±11.57(ab,*)	79.67 ± 7.45(ab)	5864.13 ± 449.12	185.13 ± 27.57(ab)	38.13 ± 2.53	0.67 ± 0.02	22.11 ± 0.48
	Ex	94.57 ± 11.27	79.33 ± 4.58(B)	6640.88 ± 816.58(C)	305 ± 39.68(AB,*)	50 ± 3.04	1.05 ± 0.08(A,*)	22.63 ± 1.09(AB)

Values are expressed as mean ± SEM; n = 8 per group. * indicates significant difference compared between Non-Ex and Ex with same treatment. Small letters indicate significant differences within Non-Ex. Capital letters indicate significant differences within Ex. Means sharing the same superscript are not significantly different from each other (P<0.05, Two-way ANOVA; Duncan's Method). TP = testosterone propionate, DAV = the rusa deer antler velvet extract, Ex = Exercise training, Non-Ex = Non-Exercise training.

4.4.5 Glycogen tissue contents

4.4.5.1 Liver glycogen levels

The effects of 16 days administration of testosterone propionate and the rusa deer antler velvet extract on liver glycogen levels in male Wistar rats with and without exercise training were shown in Figure 4.4. In non-exercise training, liver glycogen level in DAV 400 group was significantly higher than all other groups (P<0.05, two-way ANOVA). In exercise training, liver glycogen level in DAV 200 group was significantly higher than vehicle group (P<0.05, two-way ANOVA), while there was no significant difference in all other groups. In comparison to non-exercise training, exercise training groups that received vehicle and DAV 400 showed significant reduction in liver glycogen levels (P<0.05, two-way ANOVA).

4.4.5.2 Gastrocnemius muscle glycogen level

The effects of 16 days administration of testosterone propionate and the rusa deer antler velvet extract on gastrocnemius muscle glycogen level in male Wistar rats with and without exercise training were shown in Figure 4.5. In non-exercise training, gastrocnemius muscle glycogen level in TP, DAV 100 and DAV 200 group were significantly higher than corn oil, vehicle and DAV 400 groups (P<0.05, two-way ANOVA). In exercise training, gastrocnemius muscle glycogen levels in all DAV groups (DAV 100, 200, 400) were significantly higher than corn oil, TP, and vehicle groups (P<0.05). In comparison to non-exercise training, exercise training group that received DAV 400 showed significant enhancement in gastrocnemius muscle glycogen levels (P<0.05).

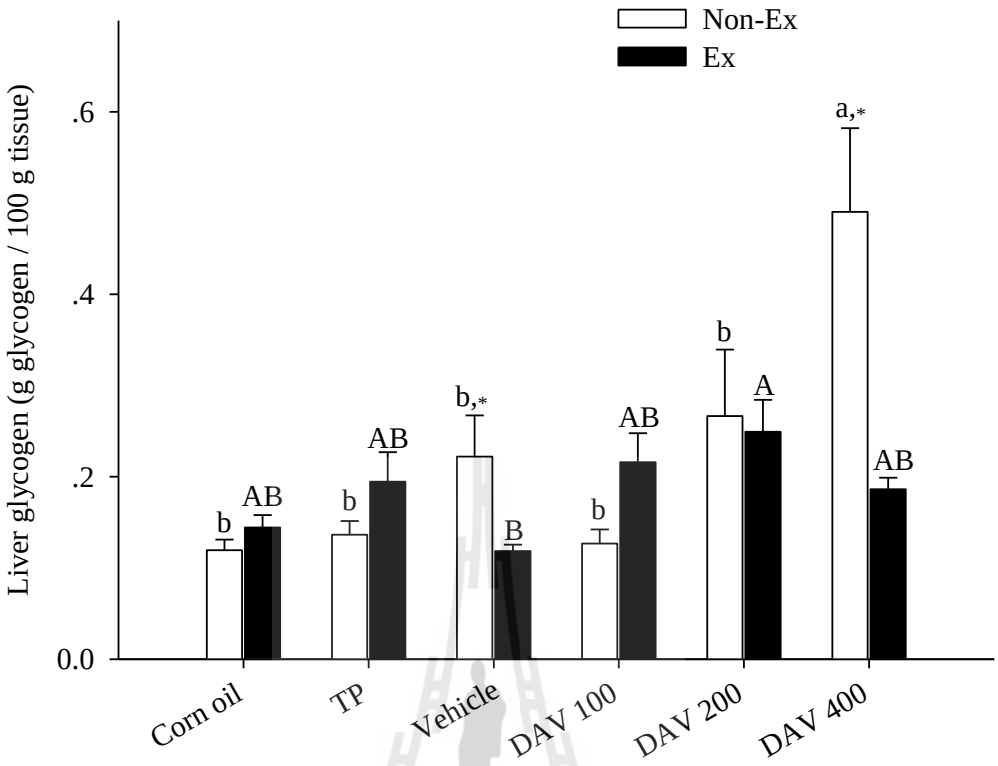

Figure 4.4 Effect of 16 days administration of the rusa deer antler velvet extract (DAV) and testosterone propionate (TP) on liver glycogen contents. Values are expressed as mean ± SEM; n = 8 per group. * indicates significant difference compared between Non-Ex and Ex with same treatment. Small letters indicate significant differences within Non-Ex. Capital letters indicate significant differences within Ex. Means sharing the same superscript are not significantly different from each other (P<0.05, Two-way ANOVA; Duncan's Method). TP = testosterone propionate, DAV = the rusa deer antler velvet extract, Ex = Exercise training, Non-Ex = Non-Exercise training.

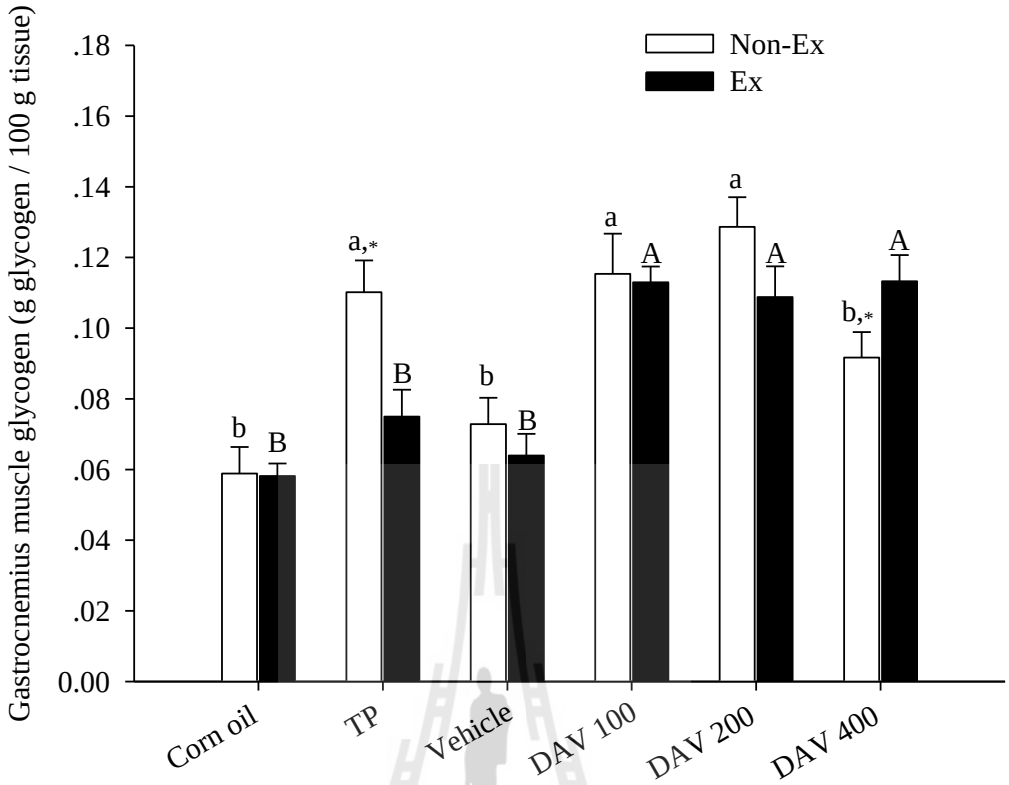

Figure 4.5 Effect of 16 days administration of the rusa deer antler velvet extract (DAV) and testosterone propionate (TP) on gastrocnemius muscle glycogen contents. Values are expressed as mean ± SEM; n = 8 per group. * indicates significant difference compared between Non-Ex and Ex with same treatment. Small letters indicate significant differences within Non-Ex. Capital letters indicate significant differences within Ex. Means sharing the same superscript are not significantly different from each other (P<0.05, Two-way ANOVA; Duncan's Method). TP = testosterone propionate, DAV = the rusa deer antler velvet extract, Ex = Exercise training, Non-Ex = Non-Exercise training.

4.5 Discussion and conclusion

The present study revealed for the first time that of the hot water extract of the rusa deer antler velvet possessed swimming endurance capacity and anti-fatigue effects on male rats. Fifteen days supplementation of DAV 100 and DAV 200 once a day selectively enhanced time to exhaustion in exercise training rats rather than non-exercise training. The endurance capacity effect of DAV may be not related to testosterone available in DAV since TP significant reduced time to exhaustion in exercise training. The previous study showed that New Zealand deer antler velvet powder (freeze drying) supplementation for 10 weeks appeared to enhance in isokinetic knee extensor, muscular strength program and endurance training response of active males on 10-week strength program while antler velvet extract supplement had no effect (Sleivert *et al*., 2003). Many anti-fatigue substances found in deer antler could enhance endurance performance such as glycoside proteins that attach to sugar molecules and the primary site of action appeared to be in the pituitary gland, the growth hormone secreted from the pituitary gland which in turn could generate the liver to produced IGF-1, IGF-1 could repair cartilage damage, and increase strength and muscle mass (Hemmings and Song, 2004; Suttie and Harris, 2000). The brain has been shown to be upregulated on IGF-1 by anti-fatigue treatment and to increase the transport of amino acids into muscle cells throughout the body, therefore, regenerating tissues after vigorous exercise (Carro *et al*., 2000). Furthermore, Shin and co-workers (2011) suggested that the anti-fatigue effect of deer antler was associated to its effect on the adrenal gland and its components. They found that deer antler extract, when administered orally in rats at 1 g/kg for 4 days consecutively, could significantly increase the weight and ascorbic acid content in the adrenal gland,

also increase swimming capacity compared to the control group. It was thought that deer antler extract could improve exercise endurance according to these mechanisms. Moreover, it was suggested that the bioactive compounds found in the deer antler base extract such as protein and collagens have been shown to have positive effects on swimming time to exhaustion (Shi *et al*., 2011).

The present study showed that exercise training caused significant reduction in body weight gain and body weight percentage change in male Wistar rats from day 1 to day 16 after corn oil, vehicle, DAV 200 and TP treatments compared to non-exercise training, while DAV 100 and DAV 400 treatments, did not caused any difference. Reduction of body weight gain and body weight percentage change by testosterone and DAV 200 may be a result of direct and indirect suppression of the activity of adipocyte lipoprotein lipase, as well as other enzymes involved in triacylglycerol synthesis and storage and enhancement of growth hormone-dependent lipolysis. In addition, testosterone might indirectly partition energy away from fat and to muscle through increased physical activity (Engelson, Pi-Sunyer, and Kotler, 1999).

A significant increase in plasma glucose levels in non-exercise training was found in DAV 100 treatment compared with control group. All doses of DAV in exercise training could enhance significant reduction in plasma glucose levels compared with non-exercise training. These results indicated the increased demand for glucose by contracting muscle causes an increased glucose uptake to working skeletal muscle (blood glucose) during prolonged exercise (Wu *et al*., 2013).

ALT and AST measurements are important for the assessment of liver damage (Jang *et al*., 2012). In present study, of rat treated with DAV 200 mg/kg was

significantly increased blood AST in a group of exercise treatment when compared to. Corn oil and TP group, but not vehicle group. These findings highlight the importance of imposing relevant restrictions on strenuous exercise prior to and during clinical studies, and exhibit the need to consider of intense muscular activity as possible causes of asymptomatic elevations of liver function tests in daily clinical practice (Pettersson *et al*., 2008).

LDH is known as an accurate indicator of muscle damage (Clarkson and Hubal, 2002). The rise of LDH indicates that muscle damage has occurred or is occurring. In non-exercise training, serum LDH levels in all treatment groups were similar. In exercise training, TP, DAV 200 and DAV 400 treatment significantly suppressed serum LDH levels as compared to their respective control groups. Exercise training caused markedly elevated serum levels of LDH in corn oil, TP, vehicle, and DAV 100 groups when compared with non-exercise training. Exercise-induced damage caused elevated serum LDH levels could be suppressed by treatment with the rusa deer antler extract at doses of 200 and 400 mg/kg. The results suggested that the enhancement effect of deer antler extract on exercise endurance likely through the protection of the corpuscular membrane by modifying several enzyme activities. Song-huan and co-workers (2009) reported that mice given deer antler by intragastric administration for 30 days were markedly improved in regard to enzyme activities related to exercise endurance compared with the control group. Jang and co-workers (2014) also reported that fermented elk deer antler (*Cervus canadensis*) extract could increase swimming time to exhaustion and could suppress exercise-induced serum levels of LDH in BALB/c mice, suggesting exercise endurance capacity of the deer antler.

Corn oil, vehicle, DAV 100, and DAV 400 groups in exercise training showed significant increases in plasma creatinine levels compared to non-exercise training. These effects may be due to the increase of creatine kinase levels, responsible for the breaking down of creatine phosphate into creatinine in muscle, in the exercise group than in the sham groups (Samra and Abcar, 2012). Moreover, Ehlers and co-workers (2002) reported significant serum creatine kinase (CK) elevations after participation in 2 day football practices, which remained elevated for at least 7 days. Participants who had higher peak power had smaller increases in CK. Furthermore, intense exercise can increase creatinine by increasing muscle break down (Hamilton *et al*., 1972).

Significant reduction in plasma TG levels were found in TP, DAV 100 and DAV 400 groups in exercise training compared to their respective controls. Testosterone significantly reduced while vehicle significant increased plasma TG levels in exercise training compared to non-exercise training. All doses of DAV could suppress the elevated levels of TG. It has been known that triglyceride levels were reduced at the beginning of the exercise and thereafter they returned to the control level despite the exercise being continued. The triglyceride level was reduced again during the exhaustive exercise (Stankiewicz-Choroszucha and Górski, 1980). Moreover, Dall and co-workers (1983) suggested the ability of exercise training to attenuate diabetic hypertriglyceridemia, in chow-fed and sucrose-fed rats that were allowed to run spontaneously in exercise wheel cages, was appeared to be related to lower plasma free fatty acid concentrations, and decreased in plasma triglyceride secretion.

Liver glycogen levels were significantly increased in DAV 400 group of non-exercise training, and in DAV 200 group of exercise training, as compared with vehicle groups. Glycogen levels in gastrocnemius muscle were significantly increased in TP, DAV 100, and DAV 200 groups of non-exercise training, and in all DAV groups of exercise training as compared with their control groups. DAV 400 significant enhanced glycogen content in liver, but not in gastrocnemius muscle, in exercise training compared to non-exercise training. The pure compound of deer antler collagens from deer antler base extract have been suggested to have a multitude of beneficial effects on improvement of the activity of blood lactate dehydrogenase and increase hepatic glycogen enhancement (Zhang *et al*., 2011).

DAV may increase the glycogen content in both liver and gastrocnemius muscle in both exercise and non-exercise training by improving glycogen reserve and glycogen-sparing effects. Glycogen-sparing effect can improve time to exhaustion because glycogen depletion is associated with physical exhaustion. Delayed utilization of glycogen resulted in endurance exercise capacity improvement (Holloszy *et al*., 1998; Oh and Ohta, 2003). Evaluating muscle glycogen cause increase the capacity for prolonged, exhausting exercise, while a low initial glycogen concentration is associated with more rapid development of fatigue (Hermansen, Hultman, and Saltin, 1967). The major factor in determining the steady-state glycogen concentration in resting muscle appears to be the rate of muscle glucose uptake (Ren *et al*., 1993). The study of Ren and co-workers (1994) demonstrated that the adaptive increase in glucose transporter 4 (GLUT4), by potentiating insulin-stimulated glucose transport, might function to increase the rate of muscle glycogen repletion. The rapid adaptive increase in GLUT4 expression could provide a survival advantage by

making possible more rapid replenishment of muscle glycogen stores when carbohydrate is eaten between exercise bouts or even when exercise is continued at a lower intensity. The rapid adaptive increase in GLUT4 may also be help to explain the data that a few days of exercise training can result in a significant enhancement of insulin action on glucose disposal (Rogers *et al*., 1988). There is considerable evidence that depletion of muscle glycogen results in development of fatigue and that raising muscle glycogen concentration delays onset of exhaustion during prolonged strenuous exercise (Ahlborg *et al*., 1967; Bergström *et al*., 1967). It is well documented that the adaptations induced by endurance exercise training, primarily the increase in muscle mitochondria, have a potent glycogen-sparing effect during exercise that plays a major role in improving endurance (Holloszy and Coyle, 1984)

In conclusion, the present study provided evidence to support that of the rusa deer antler velvet could be promoted anti-fatigue and endurance-improving supplement since the hot water extract of the rusa deer antler velvet appears to have anti-fatigue activity and have a potential to elevate exercise endurance capacity in rat model. Although the extract bioactive compounds and mechanisms of DAV on anti-fatigue and exercise endurance capacity remain to be elucidated.

4.6 References

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CHAPTER V

CONCLUSION

5.1 Conclusion

The present study, demonstrated for the first time the potent anti-fatigue activity and exercise endurance capacity of the rusa deer antler velvet extract *in vivo*. Adult male Wistar rats were used to evaluate these properties using forced swimming test. The summary of data obtained from the study of anti-fatigue effects of the rusa deer antler velvet and testosterone are shown in Table 5.1 and from the study of exercise endurance capacity of the rusa deer antler velvet and testosterone are shown in Table 5.2 and 5.3.

Rusa deer (*Cervus timorensis*) antler velvet contains many components such as proteins, collagen, amino acids, lipids, and insulin-like growth factor-1 (IGF-1), which possess potent anti-fatigue activity and exercise endurance capacity (Jeon *et al*., 2011). Deer antler velvet extract known to be a superior source of IGF-1 that contributes to anti-fatigue and the exercise endurance capacity, as well as muscle hypertrophy which appears to be met *via* the cell proliferation and differentiation. IGF-1 especially has been shown to be upregulated in the brain by anti-fatigue treatment and to increase the transport of amino acids into muscle cells throughout the body, thus regenerating these tissues after exercise. Furthermore, Shi *et al*. (1989) suggested that the anti-fatigue effect of deer antler was related to its effect on the adrenal gland. The GH-IGF-1 axis plays an important role for the IGF-1-dependent

production of testosterone from human adrenal gland (Mesiano *et al.*, 1997). IGF-1 induces the expression and activity of key enzymes of adrenal androgen biosynthesis (Guercio *et al*., 2003). Indeed, endurance cloud be improved by high carbohydrate ingestion for several days before prolonged exercise because this increases both liver and muscle glycogen stores (Stellingwerff *et al*., 2007). With exercise of longer duration, however, gluconeogenesis becomes increasingly important as liver glycogen stores become depleted. Glucose is spared so that just enough remains available at the end of the marathon. The simultaneous use of both fuels gives a higher mean velocity than would be attained if glycogen were totally consumed before the start of fatty acid oxidation. In addition, protein and carbohydrate supplement has been reported to potentiate the plasma insulin response of the supplement following a fast or prolonged aerobic exercise (Spiller *et al*., 1987; Zawadzki, Yaspelkis, and Ivy, 1992; van Loon *et al*., 2000). Thus, it is possible that a carbohydrate and protein supplement may be more effective than a carbohydrate supplement for the sparing of muscle and possibly liver glycogen during variable intensity exercise (Bowtell *et al*., 1999).

Dietary protein ingestion after exercise increases post-exercise muscle protein synthesis rates, stimulates net muscle protein accretion, and facilitates the skeletal muscle adaptive response to prolonged exercise training. In present study have shown that protein ingestion before and during exercise stimulates muscle protein synthesis during exercise. Therefore, protein ingestion before and/or during prolonged exercise training sessions may inhibit muscle protein breakdown, stimulate muscle protein synthesis, and further augment the skeletal muscle adaptive response to exercise training (van Loon, 2014). Additionally, effects of the rusa deer antler velvet extract was demonstrated (Figure 5.1). The rusa deer antler velvet extract has a modulatory

effect on anti-fatigue, promote energy supply, muscle hypertrophy and muscle cell poliferation. Thus, the rusa deer antler velvet extract may involve in the regulation of prolong exercise.

Table 5.1 Effects of 9 days administration of the rusa deer antler velvet extract and testosterone on observation parameters.

Parameters	DAV 100	DAV 200	DAV 400	TP
Swimming time to exhaustion	↔	↑	↑	↑
Body weight gain	↔	↔	↔	↓
Body weight percentage change	↔	↔	↔	↓
Liver ROW	↔	↔	↔	↔
EDL ROW	↔	↑	↔	↑
Soleus ROW	↔	↔	↔	↔
Gastrocnemius ROW	↔	↔	↔	↔
Liver glycogen	↓	↓	↓	↓
Gastrocnemius glycogen	↔	↔	↑	↔
Glucose	↓	↔	↔	↓
TG	↔	↔	↔	↔
LDH	↔	↔	↔	↑
AST	↑	↑	↑	↓
ALT	↔	↔	↔	↔
Creatinine	↔	↑	↑	↔
BUN	↔	↑	↑	↔

DAV 100, DAV 200, DAV 400 compared with vehicle group. TP group compared with corn oil group.

(↔ no change, ↑ increase, and ↓ decrease).

Table 5.2 Effects of 16 days administration of the rusa deer antler velvet extract and testosterone on observation parameters in exercise training and non-exercise training.

Parameters	Exercise training				Non-Exercise training			
	DAV 100	DAV 200	DAV 400	TP	DAV 100	DAV 200	DAV 400	TP
Swimming time to exhaustion	↔	↑	↔	↔	↔	↔	↔	↔
Body weight gain	↔	↔	↔	↓	↔	↔	↔	↔
Body weight percentage change	↔	↔	↔	↔	↓	↔	↔	↔
Liver ROW	↔	↔	↔	↔	↔	↔	↔	↔
EDL ROW	↔	↔	↔	↔	↔	↔	↔	↔
Soleus ROW	↔	↔	↔	↔	↔	↔	↔	↔
Gastrocnemius ROW	↔	↔	↔	↔	↔	↔	↔	↔
Liver glycogen	↔	↑	↔	↔	↔	↔	↑	↔
Gastrocnemius glycogen	↑	↑	↑	↔	↑	↑	↔	↑
Glucose	↓	↔	↓	↓	↑	↔	↔	↔
TG	↔	↓	↓	↓	↔	↔	↔	↔
LDH	↔	↔	↔	↔	↔	↔	↔	↔
AST	↔	↔	↔	↔	↔	↔	↔	↔
ALT	↔	↔	↔	↔	↔	↔	↔	↔
Creatinine	↔	↔	↔	↔	↔	↔	↔	↔
BUN	↔	↔	↔	↔	↔	↔	↔	↔

DAV 100 + Ex, DAV 200 + Ex, DAV 400 + Ex compared with vehicle group. TP + Ex compared with corn oil + Ex.

DAV 100 + Non Ex, DAV 200 + Non Ex, DAV 400 + Non Ex, compared with vehicle + Non Ex group. TP + Non-Ex compared with corn oil + Non-Ex. (↔ no change, ↑ increase, and ↓ decrease).

Table 5.3 Comparative effects of 16 days administration of the rusa deer antler velvet extract and testosterone between exercise training and non-exercise training on observation parameters.

Parameters	Exercise training compared with Non-Exercise training			
	DAV 100	DAV 200	DAV 400	TP
Swimming time to exhaustion	>	>	>	↔
Body weight gain	↔	<	↔	<
Body weight percentage change	↔	<	↔	<
Liver ROW	↔	↔	↔	↔
EDL ROW	↔	↔	↔	↔
Soleus ROW	↔	↔	↔	↔
Gastrocnemius ROW	↔	↔	↔	↔
Liver glycogen	↔	↔	↔	↔
Gastrocnemius glycogen	↔	↔	<	↔
Glucose	<	<	<	↔
TG	↔	↔	↔	<
LDH	>	↔	↔	>
AST	>	>	>	↔
ALT	↔	↔	↔	↔
Creatinine	>	↔	>	↔
BUN	↔	↔	↔	↔

(↔ no difference, > more than, and < less than).

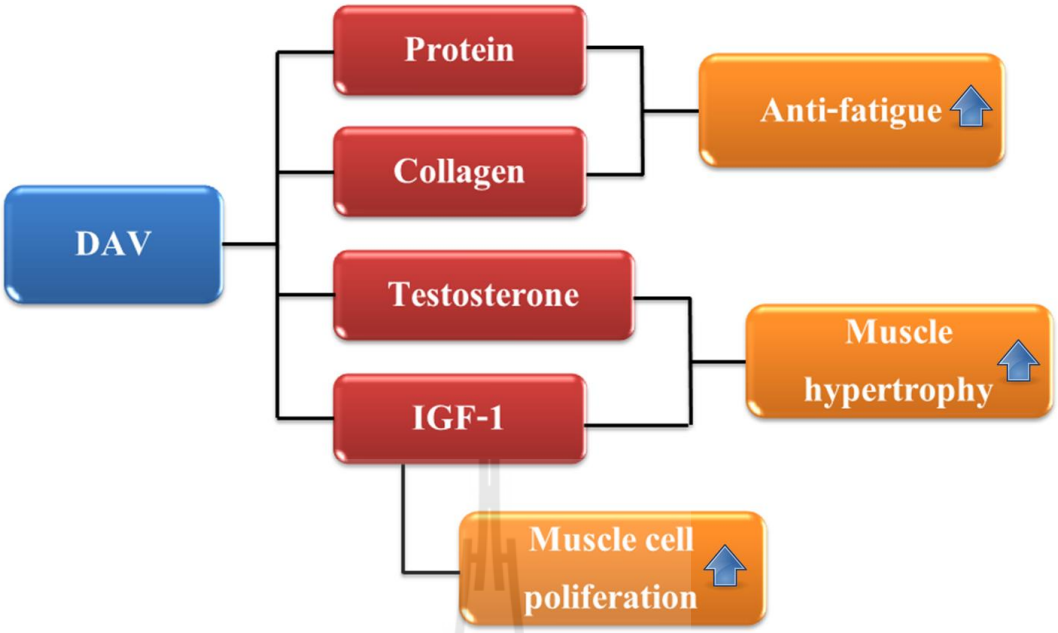

Figure 5.1 Mechanism of anti-fatigue and endurance capacity actions of the deer antler velvet extract. (DAV: deer antlr velvet extract; IGF-1: Insulin like growth factor-1).

In conclusion, this study demonstrated potent anti-fatigue activity and enhancing effect on exercise endurance capacity of rusa deer antler velvet extract *in vivo*. Its bioactive compounds, notably protein, collagen, testosterone and IGF-1 may be responsible for the potent anti-fatigue activity and enhancing effect on exercise endurance capacity of rusa deer antler velvet extract. Further studies are needed to clarify the understanding mechanisms underlying potent anti-fatigue activity and enhancing effect on exercise endurance capacity of rusa deer antler velvet extract.

5.2 References

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APPENDIX

APPENDIX

LIST OF PRESENTATIONS

List of presentations:

1. Ratsa Sripirom and Rungrudee Srisawat. (29-30 July, 2014). The anti-fatigue effect of the extract from rusa deer (*Cervus timorensis*) velvet antler in male Wistar rats (Oral presentation). **Proceedings of 2014 International Conference on Food and Nutrition Technology (ICFNT 2014)**, Hong Kong.
2. Ratsa Sripirom and Rungrudee Srisawat. (6-8 November 2015). The exercise endurance capacity effect of rusa deer (*Cervus timorensis*) velvet antler extract in male Wistar rat. **The 41st Congress on Science and Technology of Thailand**, Nakhon Ratchasima, Thailand.

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