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ARTICLE

Effects of Egg Yolk from Hens Fed with *Acanthopanax senticosus* Extract on Physical Endurance and Reproductive Parameters in Rats

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Abstract

Acanthopanax senticosus, a member of the Araliaceae used as an invigorant in traditional Korean medicine, is known to relieve physical fatigue. The principal objective of this study was to evaluate the effects of eggs laid by hens fed on *Acanthopanax senticosus* extracts on physical endurance and reproductive parameters. Male Sprague-Dawley rats at 4 wk of age were divided into 3 groups of 15 rats each: group A (14% egg yolk powder from hens fed with *Acanthopanax senticosus* extracts+86% AIN-93G), group B (14% plain egg yolk powder + 86% AIN-93G) and group C (7% fat source + 93% AIN-93G), and studied for 5 wk. The rats' physical endurance was measured via forced swimming tests. According to the results, 53.3% rats of group A swam for longer than 20 min, in group B and C, only 48.9% and 46.7% of the rats achieved this. In terms of reproductive parameters, sperm motility was significantly higher in group A than in groups B and C ($p<0.05$). However, no differences in sperm count were detected among the groups. Additionally, the serum testosterone levels of groups A and B were higher than that of group C. Our findings suggest that rats fed with egg yolk powder from hens fed on *Acanthopanax senticosus* extracts may improve athletic endurance and reproductive parameters in rats.

Key words: *Acanthopanax senticosus*, egg yolk, athletic endurance, reproductive parameter, rat

Introduction

Recently, several scientific studies have reported that men under severe stress evidence lowered reproductive capacity, accompanied by lower sperm concentration and motility. According to a meta-analysis of 61 papers, Carlsen *et al.* (1992) reported that sperm counts have decreased by 50% over the past 50 years. Semen quality in healthy men has been evaluated in many countries owing to concerns that endocrine-disrupting chemicals, in particular weak estrogenic chemicals that contaminate food, plant, and industrial materials, might be a cause of the impairment of male reproductive function (Sharpe and Skakkebaek, 1993).

The egg as a reference food has been the beneficiary of technology in Korea since 1990, and many efforts have been made to diversify and improve the egg's functional properties. Currently, a variety of functionally altered eggs are on the market including vitamin-fortified eggs, mineral-fortified eggs, nutrition eggs, natural eggs, and herb-plant eggs. Recently, a medicinal plant, *Acanthopanax senticosus,* was used as a supplement in layer feed in an effort to produce a new type of functionally-improved eggs. *Acanthopanax senticosus* is a member of the Araliaceae family and is distributed throughout Korea, Japan, and China. It has been traditionally employed as a tonic (Deyama *et al*., 2001; Yook, 1990). Therefore, the principal objective of this study was to evaluate the effects of the yolks of eggs laid by hens fed with *Acanthopanax senticosus* extract on the endurance performance and reproductive parameters of rats.

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Materials and Methods

Materials

Dried *Acanthopanax senticosus* was purchased from the Daegu medicinal plant market in 2007. A voucher specimen was deposited with MiLim Egg Land Co., Korea. The *Acanthopanax senticosus* was crushed into 80 mesh powder and used in this study. *Acanthopanax senticosus* egg yolks were produced by laying hens fed on a supplement and probiotic-containing diet at a MiLim Egg Land Company farm. The composition of the diet was as

follows: 83.6% rice bran, 1.9% sesame dregs, 9.5% zeolite, and 5.0% *Acanthopanax senticosus* powder, and the administered probiotic contained *Bacillus subtillis* (1.0×10^7 CFU/g), *Lactobacillus acidophilus* (1.0×10^7 CFU/g), and *Saccharomyces cerevisiae* (1.0×10^7 CFU/g). The obtained *Acanthopanax senticosus* egg yolks were lyophilized with a freeze-dryer.

Experimental animals

Forty-five male Sprague-Dawley rats (4 wk-old) were used in this study. The rats were allowed to acclimatize for 5 d in the facility prior to the start of the experiment. The rats were provided *ad libitum* access to tap water and rodent laboratory chow (AIN-diet, 1977). The animal facility was maintained under a 12-h light/dark cycle, and at a temperature of 21-23ºC with a relative humidity of 60%. Body weights and diet intake were recorded at weekly intervals.

Experimental diet

Rats were divided randomly into three groups and administered either an *Acanthopanax senticosus* diet, a plain yolk diet, or a high fat diet (Table 1). The groups were fed for 5 wk on one of these diets. The high fat diet was prepared via the AIN-diet plan, supplemented with 7% fat and oil (lard/corn oil/cholesterol=10:2:1%). The approximate compositions of the experimental diets are provided in Table 2.

Analytical procedures

At the end of the 5 wk, blood samples were also collected from the orbital plexus of the fasting rats and placed in tubes without an anticoagulant. After the samples were allowed to stand for 2 h at room temperature, sera were prepared via 10 min of centrifugation at 3,000 g. The rats were then sacrificed via decapitation and the abdomen of each rat was opened. The livers, kidneys, and spleens were quickly removed, washed in cold saline, blotted dry on filter paper, and weighed. The testes were removed, decapsulated, and immersed immediately in liquid nitrogen and stored at -80ºC.

Table 2. Proximate analysis of experimental diet

Diet	Proximate analysis (g/100 g)			
	Moisture	C. fat	C. protein	C. ash
Acanthopanax senticosus	6.87	11.96	23.37	5.77
Control yolk	6.88	11.98	24.09	6.10
High fat diet	7.18	6.01	20.85	6.13

Chemical analysis

The hepatic function values, creatinine and Inorganic phosphate in serum were determined enzymatically using commercially available reagent kits (BM, Germany). Total-cholesterol, triglyceride and creatine-kinase activity were determined enzymatically using commercially available reagent kits (assay kits for R208; Young-Dong Pharm. Co. Ltd., Korea). Testosterone levels were determined via solid-phase ^{125}I radioimmunoassays using Coat-A-Count.

Swimming endurance

Swimming tests were conducted in a cylindrical tank with a diameter of 120 cm and a depth of 50 cm, containing water at 21-23ºC. As the rats in groups exercised more vigorously than the rats that were allowed to swim alone, group swimming was preferred. Exercise endurance capacity was estimated as defined by Jacob and Michaud (1961). Swimming points were recorded as follows: 3 points to those rats with sufficient ability to swim for more than 20 min, 2 points to those rats that could swim for 20 min, and 1 point to those rats that could not swim for 20 min.

Sperm parameters

Sperm counts was determined using a hematocytometer (Neubauer, Germany) and sperm motility was determined via the alive-dead staining method (Hafez, 1993). The DNA contents of the dispersed testicular cells were measured with an FCM (Coulter Epics XL, Coulter Corp., USA) equipped with a 2-W argon laser and operated at a wavelength of 488 nm. Propidium iodide fluorescent emissions were monitored with a 620 nm band-pass filter, along with a dichroic long-pass filter, 645 DL.

Table 1. Experimental design

Group (n=15)	Treatment	Remark
A	*AS* yolk powder (14%) + AIN-diet powder (86%)	*Acanthopanax senticosus*
B	Plain yolk powder (14%) + AIN-diet powder (86%)	Control yolk[1)]
C	*Fat & oil source* (7%) + AIN-diet powder (93%)	High fat diet[2)]

[1)]Plain egg yolk

[2)]Fat & oil source, lard:corn oil:cholesterol (10:2:1% w/w)

Table 3. Effect of diet supplemented with *Acanthopanax senticosus* egg yolk on daily gain and FER in rats during the 5 wk of the experimental period

Group	Initial wt. (g)	Final wt. (g)	Daily gain (g)	Diet intake (g/day)	FER
A	99.6±4.4	371±27.2^{ns}	7.67±2.08^{ns}	20.68±2.97^{ns}	0.38±0.08^{ab}
B	98.3±3.9	378±16.5	8.05±2.18	20.51±3.01	0.40±0.08^{a}
C	100.5±3.4	367±23.7	7.61±2.39	23.09±3.43	0.34±0.07^{b}

Values are expressed as means±SD (n=15).
Means with different letters with a row are significantly different from each other according to Duncan's multiple range test ($p<0.05$).
^{ns}not significant
FER, feed efficiency rate (Weight gain/Feed intake)

Statistical analysis

Data are expressed as means±SD. The significance of differences among treatment groups was determined via ANOVA with Duncan's multiple range test (SAS Institute, Cary, NC, USA). Differences were regarded as significant at $p<0.05$.

Results and Discussion

Growth & organ weight

There were no significant differences in initial weights, daily gain, or diet intake among the treatment groups (Table 3). Feed efficiency ratios were significantly higher in the plain egg yolk group (B) than in the other two groups ($p<0.05$). With regard to organ weight, liver weights in group (A) were the highest among the treatment groups, and there were no significant differences in kidney, spleen, or testes weights (Table 4).

Table 4. Effect of diet supplemented with *Acanthopanax senticosus* egg yolk on organ weight in rats during the 5 wk of the experimental period

Group	Organ weight[1)]			
	Liver	Kidney	Spleen	Testis
A	4.54±0.36^{a}	0.39±0.03^{ns}	0.26±0.03^{ns}	0.40±0.03^{ns}
B	4.40±0.25^{ab}	0.38±0.03	0.27±0.05	0.40±0.05
C	3.30±0.66^{c}	0.37±0.03	0.24±0.05	0.40±0.06

Values are mean±SD (n=15).
^{ns}not significant, [1)]g/100g of body weight

Hematochemicals

SGOT and SGPT activities among the egg yolk treatment groups were high compared to the controls, but not significantly different (Table 5). Lactate dehydrogenase (LDH) and creatine kinase (CK) activities were highest in group A ($p<0.05$). Creatinine contents were not significantly different among the groups. Concentrations of serum total-cholesterol and triglycerides were the highest in group B ($p<0.05$). Inorganic phosphorus concentrations did not differ significantly among the groups.

Endurance performances

The effects of the diet supplemented with *A. senticosus* egg yolk on endurance performance in the rats is shown in Table 6. Swimming tests were conducted a total of three times during the 4th week. Swimming tests were conducted for 20 min each. Exhaustion as a marker for exercise endurance capacity was recorded at the time at which a rat remained submerged under the water surface for 10 sec (Dawson and Horvath, 1970). The total num-

Table 5. Effect of diet supplemented with *Acanthopanax senticosus* egg yolk on hematochemicals in rats during the 5 wk of the experimental period

Group	Hematochemicals							
	SGOT	SGPT	LDH	CK	CRT	TC	TG	Pi
A	104.7±11.5^{ns}	46.7±8.43^{ns}	477.2±49.0^{a}	1296 ±256.2^{a}	0.50±0.00^{ns}	77.29±13.63^{b}	121.58±25.75^{b}	9.75±0.83^{ns}
B	90.1±14.8	45.4±6.80	387.2±39.9^{b}	681.9±113.6^{b}	0.50±0.04	90.14±10.77^{a}	162.37±23.94^{a}	9.50±0.75
C	86.8±9.40	42.5±3.41	368.0±21.5^{b}	598.6±83.9^{b}	0.50±0.04	71.29±7.72^{b}	123.49±19.62^{b}	9.32±1.00

Values are expressed as means±SD (n=15).
Means with different letters with a row are significantly different from each other according to Duncan's multiple range test ($p<0.05$).
^{ns}not significant.
SGOT, serum glutamic oxaloacetic transaminase (IU/L)
SGPT, serum glutamic pyruvic transaminase (IU/L)
LDH, lactate dehydrogenase (IU/L); CK, creatine kinase (IU/L); CRT, creatinine (mg/dL); TC, total-cholesterol (mg/dL); TG, triglyceride (mg/dL); Pi, inorganic phosphate (mg/dL)

Table 6. Effect of diet supplemented with *Acanthopanax senticosus* egg yolk on endurance performances in rats during the 5 wk of the experimental period

Group (n=15)	Swimming			
	1st	2nd	3rd	Total number[2)]
A	8[1)]	7	9	24/45
B	7	7	8	22/45
C	9	7	5	21/45

[1)]Number of individual has sufficiently swimming ability after 20 min (point: +++ 3)
[2)]Total number of individual has sufficiently swimming ability (point: +++ 3) after 20 min for measuring three times.

bers of rats exhibiting sufficient swimming ability to stay afloat for 20 min over three measurements were 24, 22, and 21 among the treatment groups, respectively, and thus were highest in the *A. senticosus* egg yolk group (53.3% rats of group A). The high swimming ability of group (A) may be due to the anti-fatigue property of *Acanthopanax senticosus* (Huang *et al*., 2011).

Sperm parameters

Testicular cells obtained from rats fed on a diet supplemented with *A. senticosus* egg yolk for 5 wk were assessed for sperm count and motility, and placed in suspension, stained with PI, and measured via flow cytometry (Table 7). The egg yolk feeding groups (A, B) evidenced significantly higher serum testosterone concentrations than the controls (4.63 and 4.80 ng/mL *vs* 1.90 ng/mL) ($p<0.05$). Sperm motility was highest in the *A. senticosus* egg yolk group (90%) relative to the other two groups ($p<0.05$). The sperm counts of the *A. senticosus* egg yolk group (35.76×10^7/mL) were slightly higher, but not significant. Similarly, Mkrtchyan *et al*. (2005) has been reported a positive effect of *Acanthopanax senticosus* on the spermatozoids in the whole ejaculate, the percentage of active forms of spermatozoids, and fertility indexs.

Spermatogenesis is a process that begins at puberty. In the fetus, the stem cells migrate from the intermediate mesoderm to the genital ridge. The stem cell in spermatogenesis in adult mammals is a subset of the type A spermatogonia (Meistrich, 1993). Spermatogenesis was facilitated by testosterone from Leydig's cells. We noted no changes in the proportion of 1n (mature and immature haploid), 2n (diploid), and 4n (tetraploid) cells in the rat testes.

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Table 7. Effect of diet supplemented with *Acanthopanax senticosus* egg yolk on testosterone level and sperm parameters in rats during the 5 wk of the experimental period

Group	testosterone (ng/mL)	Sperm		Spermatogenesis* (5000 cell count)		
		motility (%)	count ($\times10^7$/mL)	haploid (N)	diploid (2N)	tetraploid (4N)
A	4.63±2.38[a]	90.0±7.10[a]	35.76±2.04[ns]	3340±400[ns]	803±265[ns]	857±318[ns]
B	4.80±2.00[a]	75.7±13.1[b]	32.36±2.76	3270±566	909±489	821±137
C	1.90±1.38[b]	76.5±15.1[b]	33.66±1.93	3265±276	838±196	897±225

Values are expressed as means±SD (n=15).
Means with different letters with a row are significantly different from each other as determined by Duncan's multiple range test ($p<0.05$).
[ns]not significant
*N, haploid; 2N, diploid; 4N, tetraploid

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