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Full Length Research Paper

Effect of different levels of royal jelly on biochemical parameters of swimmers

Nazmi Saritaş[1]*, Kadir Yildiz[1], Serdar Büyükipekci[1] and Betül Coşkun[2]

[1]Physical Education and Sports College, Erciyes University, Kayseri/Turkey.
[2]Physical Education and Sports College, Niğde University, Niğde/Turkey.

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This study aims to investigate the effects of different levels of royal jelly supplementation on biochemical parameters in swimmers. Randomly selected 40 male swimmers aged 18 to 25 years attending the same trainings were recruited. Swimmers were assigned to 4 groups each with 10 subjects. Varying amounts of royal jelly (2, 1 g and 500 mg) were given to the 1st, 2nd and 3rd groups and placebo (corn starch) to the 4th group. Participants were trained by swimming totally 20 km in 2 h on 5 days a week for 4 weeks. Resting blood samples were taken before royal jelly administration and after 30 days of application. Then biochemical analyses were performed. Different levels of royal jelly were found to be ineffective on glucose, total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, lactate dehydrogenase (LDH), creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT) levels of the swimmers. Blood urea nitrogen (BUN) and creatinine levels increased after the training program, and BUN level was higher in the group receiving 500 mg royal jelly than those in the other groups. The increment in creatinine levels was higher in those groups receiving higher amounts of royal jelly after the training. A supplementation of 500 mg, 1 and 2 g/day of royal jelly throughout the 30 day-exercise program was not significantly effective in the swimmers. Also, due to its high amino acid content, BUN and creatinine levels tended to increase.

Key words: Royal jelly, swimming, exercise, biochemical parameters, ergogenic aids.

INTRODUCTION

Ergogenic aids regarding nutrition besides training and personal talent are known to be important in athletic success (Zorba et al., 2000). As balanced nutrition improves athletic performance, unbalanced nutrition may affect it negatively. Consumption of commonly and generally used food supplements by athletes is increasing in sports (Şemşek et al., 2001). These food supplements are aiding substances used in order to promote athletic performance besides natural talent and training (Calfee and Fadale, 2006) and they are used to improve endurance, meet energy requirements, increase muscle mass and strength, prevent harmful effects of free O_2 radicals and lactic acid like substances appearing exercise by the athletes (Şemşek et al., 2001). Royal jelly is one of the aids which is rich in vitamins; especially B vitamins, also containing vitamins C, D and E (Genç, 1993) and is abundant in minerals, potassium in the first place (Yıldız and Umudum, 2000).

Royal jelly is secreted by worker-bees from the intracranial and pharyngeal glands for the growth and development of young larvae (Yıldız and Umudum, 2000). It is nutritious (Kanbur et al., 2009) and contains protein, sugar, lipid, vitamins and free amino acids (Silici et al., 2010). Several researches have been conducted to determine the chemical composition of royal jelly (Lercker, 1981; Takenaka, 1982; Boselli et al., 2003). The proteins in royal jelly have antioxidant effects and it is being used as an anti-aging agent and as part of the treatment of cancer, atherosclerosis, hypertension, infertility, asthma, depression and diabetes which are caused by oxidative stress induced by the imbalance

Corresponding author. E-mail: nsaritas@erciyes.edu.tr. Tel: +90-352-4380214. Fax: +90-352-4379379.

between reactive oxygen species.

In addition, royal jelly is regarded as an excellent food for energy requirement, maintaining the physiological equilibrium and body chemistry, strengthening immune system, treating influenza-like diseases, maintaining regular functioning of the kidney and liver, maintaining healthy blood low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol levels (Karabağ et al., 2010).

Regular exercise programs may result in decreases in some of the blood parameters while causing increase in some others. This kind of a difference can be seen in athletes who have high levels of blood parameters in the beginning of training (Zorba et al., 2000). Several studies have been performed to investigate whether supplements has positive effects on athletic performance or not.

In this study, the effect of different levels of royal jelly which has been rather less investigated than other supplements on some blood parameters of training swimmers was investigated.

MATERIALS AND METHODS

Subjects and protocol

Forty (40) healthy male swimmers aged 18 to 25 years old performing the same training program voluntarily participated in the study. The volunteers were randomly assigned to 4 groups composed of 10 subjects per group and 2, 1 g and 500 mg of royal jelly were given to the subjects in the 1st, 2nd, and 3rd group respectively, while the 4th group received placebo (corn starch). Royal jelly was obtained from Civan Bee farm, Bursa, Turkey. Royal jelly capsules were administered 20 to 30 min before breakfast once a day for 4 weeks except for placebo taking group. Participants underwent a 4-week training in which they swam a total of 20 km in 2 h on 5 days a week. For two months before the study began and during the study period, the swimmers did not use any other supplements such as vitamins or medication.

Ethical committee permission were obtained from Erciyes University Deanery of Medical Faculty as well as written, informed consent from the volunteers.

Physical measurements of the volunteers

Height (via seca tape measure) and body weight were measured and body composition was determined by Tanita BC 418 MA.

Royal jelly

Royal jelly used in the study was obtained from Civan Bee-Keeping Firm (Bursa, Turkey) and kept at -20°C till the study began. Royal jelly and placebo (corn starch) were filled into 500 mg capsules to be stored in the freezer at -20°C.

Determination of amino acids content of royal jelly

The composition of the royal jelly used in the study was determined by the manufacturing company and reported here.

Collecting samples and biochemical analyses

5 ml of resting blood samples of pre-study and post-four weeks training program from each voluntary participant were taken twice in vacuumed jelled serum tubes. Blood samples were centrifuged at 3000 rpm for 10 min and sent to the central laboratory of Erciyes University Hospital, without storing, in order to determine (Siemens Advia 1800 Chemistry System) LDH, CK, HDL and LDL cholesterols, creatinine, triglyceride, ALT, AST, BUN, total cholesterol and glucose levels.

Statistical analysis

Statistical package program (SPSS version 13.0) was used for statistical analysis of the data. Normality was detected by Shapiro-Wilk test. Mean and standard error of mean were used to show brief statistics of the variables. Changes in biochemical parameters of before and after supplementation of the groups were analyzed with two-way anova with repeated measures. Statistical significance was set at p value of < 0.05.

RESULTS

The comparison of the pre- and post-exercise parameters revealed that there was no significant change in body weight, body mass index and body fat percentage between the placebo and experimental groups pre- and post-exercise and in group-time interactions ($p > 0.05$) were shown in Table 1.

Glucose levels of the groups did not differ with time ($p > 0.05$). Changes of BUN and creatinine levels over time were significant. The common effect of group and time on pre- and post-supplementation changes in glucose and BUN levels were insignificant ($p > 0.05$) while the common effects of group and group-time was significant for creatinine levels ($p < 0.05$). No significant difference in inter-groups comparisons of glucose and creatinine levels were detected ($p > 0.05$). BUN levels, however, were higher in 500 mg royal jelly group compared with others ($p < 0.05$) (Table 2).

There was no change in triglyceride, total cholesterol, HDL and LDL cholesterol levels of all groups in time ($p > 0.05$). It was detected that the groups and time did not have a joint effect on triglyceride, total cholesterol, HDL and LDL cholesterols changes of pre- and post-supplementation ($p > 0.05$). There was no significant difference between the groups either ($p > 0.05$), (Table 3).

Interactions of LDH, CK, AST, ALT levels with the time of placebo, 500 mg, 1 and 2 g of royal jelly taking groups were not significantly different ($p > 0.05$). Also, pre- and post-supplementation changes in LDH, CK, AST, ALT levels of the groups did not differ based on the joint effect of group and time ($p > 0.05$), while being insignificant for the same parameters between the groups, either ($p >$

Table 1. The comparisons of physical characteristics between groups before and after exercise with intra-groups before and after exercise in swimmers in placebo and experimental groups.

Variable	Group	Before the supplementation	After the supplementation	F value		
		Mean ± SEM	Mean ± SEM	Time	Time x Group	Group
Weight (kg)	Placebo	61.69 ± 4.86	61.90 ± 4.96	2.80	0.84	0.34
	500 mg	64.34 ± 4.66	64.68 ± 4.52			
	1 g	60.45 ± 3.45	60.75 ± 3.28			
	2 g	65.80 ± 3.73	65.77 ± 3.72			
Body mass Index (kg/m^2)	Placebo	21.36 ± 0.95	21.42 ± 0.97	0.30	0.45	0.13
	500 mg	22.33 ± 1.46	21.69 ± 0.91			
	1 g	21.14 ± 0.95	21.2 ± 0.90			
	2 g	21.53 ± 0.95	21.5 ± 0.93			
Body fat (%)	Placebo	17.15 ± 1.63	16.54 ± 1.46	0.65	0.17	0.95
	500 mg	13.27 ± 1.40	12.61 ± 1.50			
	1 g	13.56 ± 2.20	13.35 ± 2.49			
	2 g	14.8 ± 1.83	14.91 ± 1.85			

Table 2. The comparison of biochemical characteristics between groups before and after exercise with intra-groups before and after exercise in swimmers in placebo and experimental groups.

Variable	Group	Before the supplementation	After the supplementation	F value		
		Mean ± SEM	Mean ± SEM	Time	Time x Group	Group
Glucose (mg/dL)	Placebo	82.80 ± 4.61	86,10 ± 2.89	1.95	2.00	0.27
	500 mg	88.70 ± 4.68	82.80 ± 4.09			
	1 g	82.60 ± 1.42	92.50 ± 3.32			
	2 g	81.60 ± 4.04	87.60 ± 3.07			
BUN (mg/dL)	Placebo	10.20 ± 0.81	11.00 ± 0.68	7,50*	0,19	3.50*
	500 mg	13.00 ± 1.07	14.40 ± 0.93			
	1 g	10.70 ± 0.91	11.50 ± 0.75			
	2 g	11.20 ± 0.51	12.00 ± 0.63			
Creatinine (mg/dL)	Placebo	0.83 ± 0.03	0.91 ± 0.04	69.54**	3.62*	0.42
	500 mg	0.88 ± 0.03	0.94 ± 0.03			
	1 g	0.78 ± 0.02	0.95 ± 0.04			
	2 g	0.80 ± 0.03	0.93 ± 0.05			

*p < 0.05 **p < 0.001.

0.05), (Table 4).

DISCUSSION

Bee products such as pollen, propolis etc. have been used in scientific research area until recently. While some of the products especially royal jelly was studied on athletes, a detailed research on royal jelly supplementation has not been met yet. There was no significant difference in body weight, body mass index and body fat percentage in a one-month supplementation period, which may be ascribed to the voluntary participants being trained and lack of a specific dietary program.

Royal jelly supplementation in different levels did not affect glucose levels similarly with a study in which

Table 3. The comparison of blood lipids between groups before and after exercise with intra-groups before and after exercise in swimmers in placebo and experimental groups.

Variable	Group	Before the supplementation	After the supplementation	F value		
		Mean ± SEM	Mean ± SEM	Time	Time x Group	Group
Triglycerides (mg/dL)	Placebo	117.70 ± 22.26	113.00 ± 17.57	1.17	0.25	0.82
	500 mg	117.20 ± 13.16	103,40 ± 18.30			
	1 g	106.60 ± 14.38	88.50 ± 9.05			
	2 g	133.40 ± 28.89	133.70 ± 17.70			
Total cholesterol (mg/dL)	Placebo	176.50 ± 14.30	172.10 ± 15.71	1.20	1.46	0.65
	500 mg	165.40 ± 10.30	156.30 ± 11.11			
	1 g	153.10 ± 7.73	155.50 ± 6.63			
	2 g	165.40 ± 8.07	166.80 ± 8.75			
HDL cholesterol (mg/dL)	Placebo	47.10 ± 2.84	47.20 ± 3.16	2.50	1.65	0.34
	500 mg	49.70 ± 3.06	50.40 ± 3.78			
	1 g	49.30 ± 4.03	54.70 ± 5.10			
	2 g	50.90 ± 3.75	51.00 ± 3.40			
LDL cholesterol (mg/dL)	Placebo	105.86 ± 10.42	104.22 ± 12.71	1.82	0.82	1.60
	500 mg	92.26 ± 8.05	82.20 ± 8.87			
	1 g	82.48 ± 4.24	83.10 ± 3.42			
	2 g	87.82 ± 7.94	85.06 ± 6.76			

glucose levels of subjects in all groups did not significantly change after a 4 week daily royal jelly supplementation of 6 g (Guo et al., 2007). In a human trial of royal jelly supplementation, glucose levels was found to decrease significantly 2 h after the application (Münstedt et al., 2009) whereas different levels (200, 600, 800 mg/kg) of royal jelly administration on rabbits for 6 weeks significantly increased glucose levels (Elnagar, 2010). The conflicts may result from duration of exercise, blood sampling when resting or immediately after the exercise or the differences in participants.

Blood urea nitrogen levels of all groups similarly and significantly increased at the end of a month's training period. Royal jelly supplementation in 100 mg/kg on mice did not show significant difference on BUN levels in a study (Yapar et al., 2009). BUN levels of royal jelly (50 vs. 100 mg) supplemented groups composed of rats had been found to decrease compared with control group (Silici et al., 2010). The protein and amino acid content of royal jelly may have led to BUN increase. Significant increments occured in creatinine levels of groups before and after royal jelly supplementation in this study – having much more increased especially in groups taking higher levels of royal jelly. On the contrary, in a study, 100 mg/kg royal jelly supplementation was not found to affect creatinine levels in mice (Yapar et al., 2009).

The study result, triglyceride, HDL, LDL and total cholesterols levels of the swimmers were not affected by royal jelly supplementation. Similarly, no significant changes was determined in triglyceride levels of subjects in a study conducted for 4 weeks including 6 g of royal jelly (Guo et al., 2007), whereas in another human trial, a daily dosage of 100 mg royal jelly given to the participants caused decreases in serum triglyceride levels (Yıldız and Umudum, 2000). Additionally, other studies on rats led to decreases in triglyceride (El-Nekeety et al., 2007; Silici et al., 2010), being supported by a rabbit study in which different amounts of (200, 600, 800 mg/kg) royal jelly was supplemented for 6 weeks and resulted in decrease in triglyceride (Elnagar, 2010). The reason for the difference may be the amount of the royal jelly supplementation and changes induced by duration of exercise.

The cholesterol levels were not found to be similar in both control and royal jelly supplemented (50 mg/kg) groups composed of mice (Kanbur et al., 2009). In a 4-week royal jelly supplementation with 6 g/day, there were significant decreases found in total and LDL cholesterols values (Guo et al., 2007). The rats receiving 100 mg/day royal jelly had significantly decreasing levels of total cholesterol compared with cisplatin group (Silici et al., 2010).

HDL cholesterol levels were found to increase induced by fumonisin administration to rats alone while 100 to 150 mg/kg royal jelly with fumonisin decreased (El-Nekeety et al., 2007). HDL cholesterol levels were higher compared

Table 4. The comparisons of enzyme characteristics between groups before and after exercise with intra-groups before and after exercise in swimmers in placebo and experimental groups.

Variable	Group	Before the supplementation	After the supplementation	F value		
		Mean ± SEM	Mean ± SEM	Time	Time x Group	Group
LDH (µ/L)	Placebo	173.00 ± 6.45	184.60 ± 8.50	1.82	0.72	1.35
	500 mg	178.60 ± 10.06	172.20 ± 6.43			
	1 g	177.60 ± 6.65	187.80 ± 10.75			
	2 g	184.70 ± 10.48	200.30 ± 7.78			
CK (µ/L)	Placebo	116.60 ± 19.15	113.40 ± 16.46	0.28	0.25	0.63
	500 mg	112.30 ± 17.88	110.20 ± 18.71			
	1 g	117.00 ± 20.53	127.10 ± 26.27			
	2 g	141.70 ± 24.67	154.10 ± 31.44			
AST (µ/L)	Placebo	18.30 ± 0.90	18.60 ± 1.42	0.23	0.88	1.61
	500 mg	19.40 ± 1.19	19.90 ± 1.45			
	1 g	19.40 ± 0.96	19.50 ± 1.51			
	2 g	23.30 ± 1.80	21.10 ± 1.86			
ALT (µ/L)	Placebo	14.10 ± 2.07	13.10 ± 2.42	2.97	1.33	0.83
	500 mg	19.50 ± 3.08	18.10 ± 2.77			
	1 g	17.40 ± 1.41	18.00 ± 2.04			
	2 g	19.80 ± 3.84	15.50 ± 2.96			

with pre-supplementation of royal jelly in an animal and human with atherosclerosis (Vittek, 1995). Despite not being easily changed by discontinuous exercises and trainings, while increasing levels of HDL cholesterol depending on endurance exercises were detected, HDL cholesterol levels, also, could be influenced by age differences of exercising subjects.

LDL cholesterol levels were found to decrease by 6 g/day royal jelly supplementation for 4 weeks in humans (Guo et al., 2007). Conversely, LDL cholesterol levels increa-sed in subjects with atherosclerosis and animals by royal jelly supplementation (Vittek, 1995). Lipid levels which cannot be changed by mild and discontinuous exercises were thought to decrease based on the differences in training periods and in sedentary subjects who perform any sports activity.

The propolis which is a bee product has been found to cause a significant rise in LDH enzyme levels compared with alcohol administered rats (Kolankaya et al., 2002). The post-exercise muscle damage results in transition of muscle-specific components from membrane torn into bloodstream in which CK is mostly present (Şendil, 2008). CK and some other enzymes also cause pain in muscles as a result of severe exercise. The increase in this kind of enzymes indicates muscle damage (Alibeyoğlu, 2008). In this study, royal jelly supple-mentation did not lead to significant changes in CK and LDH levels which show muscle damage. After long term exercise, serum CK and LDH were investigated in order to determine the effects of branched chain amino acid supplementation on the relevant enzymes which showed that CK levels of post-exercise were significantly higher while being statistically insignificant compared with pre-test levels (Coombee and McNaughton, 2000).

Changes on AST and ALT enzymes of groups were not significant in this study. However, royal jelly (100 to 150 mg/kg) and fumonisin supplemented rats had decreased levels of AST (El-Nekeety et al., 2007). In other rat studies, AST levels had been shown to significantly decrease (Kanbur et al., 2009; Silici et al., 2010; Elnagar, 2010) which could be arisen from difference in experi-mental groups. In a mouse study, ALT enzyme levels did not differ significantly (Kanbur et al., 2009). A 6 week royal jelly supplementation in different dosages (200, 600, 800 mg/kg) in rabbits (Elnagar, 2010) and in another study with rats, ALT levels decreased (Silici et al., 2010). Statistically insignificant as it is, in our study, there have been similar falls in ALT levels in the group receiving higher amounts of royal jelly. Severity and duration of exercise, age, nutrition and performance may have caused such differences between the studies.

This study has some limitations: Firstly, the participants were informed about dietary measures but were not controlled, and diet was not recorded the day before the endurance exercise tests. Secondly, the exercise protocols were performed on highly physically trained individuals. Therefore, the responses observed may not be representative of sedentary individuals.

Consequently, different levels of royal jelly supplementation for one month were not found to cause a difference in some of the biochemical parameters. However, the increase in BUN levels is thought to have resulted from the amino acid content of royal jelly. We think that future studies should be directed to whether or not longer term royal jelly supplementation affects the biochemical parameters of athletes.

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