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EFFECTS OF HONEY (*APIS MELLIFERA* AND *APIS CERANA* SPECIES) SUPPLEMENTATION ON REDUCING BLOOD LACTATE CONCENTRATION IN FUTSAL ATHLETES

KUSNAEDI KUSNAEDI, ADNYANA I KETUT, TOMMY APRIANTONO, SYAHRUDDIN SYAHRUDDIN, JUNAIDI JUNAIDI, BAGUS WINATA

Bandung Institute of Technology, Department of Sport Science, Bandung, Indonesia

Mailing address: Bagus Winata, Bandung Institute of Technology, Department of Sport Science, Jalan Jati Raya Rt.002 Rw.008 No 56 Kelurahan Sukamaju, Kecamatan Cilodong, Kota Depok, Jawa Barat, Indonesia 16415.
tel.: +, e-mail: fransiskusasisibaguswinata@gmail.com

Abstract

Introduction. The purpose of this study was to examine whether 6-week daily consumption of honey from *Apis mellifera* and *Apis cerana* species would affect performance and reduce blood lactate in futsal athletes. ***Subjects and Methods.*** In this study, 30 male futsal athletes volunteered to be subjects. A group of 15 futsal athletes volunteered for random blind assignment to either an *Apis mellifera* honey (AM) group or an *Apis cerana* honey (AC) group. Each group completed tests pre- and post-supplementation for 20 m sprint test and agility t-test. Additionally, blood lactate was measured before and immediately after the tests. ***Results.*** Independent t-test revealed significant changes from before to after supplementation in the AC group (p=0.009) for lactate post. Conversely, independent t-test revealed no significant changes in the AM group (p=0.698) for lactate post. Regarding 20 m sprint performance, there were statistically significant differences for time (p=0.036) and group main effects (p=0.009). Specifically, independent t-test showed significant changes from before to after supplementation just in the AC group (p=0.018). For the t-test, independent t-test revealed significant changes from before to after supplementation in the AC group (p=0.013). ***Conclusions.*** We demonstrated that 1.14 g/kg of *Apis cerana* honey given once a day at breakfast for 6 weeks is more effective in reducing blood lactate concentration and enhancing agility t-test performance than 1.14 g/kg of *Apis mellifera* honey in futsal athletes.

Keyword: blood lactate, muscle function, nutrition, performance.

Introduction

Futsal is one of the sports that has reached an increasing popularity in societies around the world [1]. Specifically, futsal (the 5-side version of soccer, i.e. 1 goalkeeper and 4 outfield players) was introduced in 1930. Since 1989, countries from all the continents have been taking part in the Futsal World Cup that is held every 4 years [2]. A few previous studies pointed that futsal is a competitive game that consists of two 20-min periods of high-intensity and intermittent activities requiring substantial physical, tactical and technical efforts from the players [3, 4, 5]. Previous studies also explained that a physiological mechanism of futsal is that more than 75% of all energy is resynthesized by the oxidative phosphorylation pathway during the match and the ratio of activity to rest is about 1:1 [6, 7].

Because of the fact that futsal is a sport that requires quick movements to perform both offensive and defensive tasks constantly at a high pace, futsal athletes must obtain adequate nutrition [8]. In this context, it is common for these athletes and coaches to use a variety of dietary supplements from both natural and organic sources, in order to reduce fatigue, enhance performance and prevent nutritional deficiencies which affect health [9]. This method is possible as it does not violate doping regulations from WADA when it comes to using other non-natural or organic agents.

Recently, several studies have discussed the benefits of honey such as performance enhancement and health maintenance [10, 11]. It is also claimed that chemical compounds in honeybees such as glucose, fructose, flavonoids, polyphenols and organic acids (antioxidants and anti-fatigue elements) can enhance human performance and prevent nutritional deficiencies which affect health [12]. Honey is a sweet liquid processed by the honey bee [13]. There are several types of honey classified based on the origin or type of bees [14].

Nowadays, two types of honey are very interesting to be tested because they are considered to provide benefits to human health, namely honey from honeybees *Apis mellifera* and *Apis cerana* species [15]. Honeybees from the *Apis mellifera* species belong to Apidae family [16]. *Apis mellifera* was originally found in Europe, Asia, and Africa [15]. The species that live in Asia (including the Indonesian Archipelago) is called *Apis mellifera indica* [17]. *Apis mellifera* honey bees are very social insects as each colony (or each nest) consists of 50,000-80,000 bees [18]. Its ability to produce honey that is very high makes this bee widely introduced in new areas that were previously the areas of the spread of *Apis cerana* [18]. The queen bee is about 1.9 cm long, the male is 1.65 cm and worker bees are around 1.35 cm long, and their body color varies from dark brown to black yellow. These bees are patient and always keep the hive clean [19].

Asian honey bees *Apis cerana* are honeybees originating from south and southeast Asia, with sub-species variations found in China, India, Indonesia, Japan, Malaysia, Nepal, Bangladesh, Solomon Islands and Papua New Guinea [20]. Morphologically, the size of *Apis cerana* is the smallest among honey bee species that form a nest in a closed place. However, among *Apis cerana* themselves, their body sizes also differ from one location to another [21]. There are not as many *Apis cerana* colonies as there are *Apis mellifera* colonies. Therefore, the amount

of honey produced by them is not as large as in the case of *Apis mellifera*. However, *Apis cerana* is more resistant to parasitic attacks than *Apis mellifera* [22].

On the other hand, a number of studies observed that other types of natural and organic supplementation (as antioxidants and anti-fatigue elements) from fruit and vegetables are relatively well documented [23, 24], while data on natural honey supplementation to enhance performance and health maintenance in athletes are still limited. To the authors' best knowledge, no available studies have reported the effects of honey on performance enhancement in athletes, especially in futsal athletes. Therefore, the purpose of this study was to examine whether 6-week daily consumption of honey from *Apis mellifera* and *Apis cerana* species) would affect performance and reduce blood lactate in futsal athletes. We hypothesized that a 6-week supplementation period of honey from *Apis mellifera* and *Apis cerana* species would significantly increase performance and reduce blood lactate in futsal athletes.

Material and Methods

Participants

In this study, 30 male futsal athletes (20-22 years of age) who study in State University of Jakarta were recruited based on the following criteria: national or international level tournament participation, 15 or more hours of training per week, current and past non-smokers, participants with no concomitant diseases and no current use of any antioxidants or anti-fatigue drugs during the experimental period and one month before. All volunteers provided informed consent to participate in this study. All the procedures were approved by the ethics committee of the State University of Jakarta.

Training routines were evaluated by a training team (n =2) prior to pre-testing and it was ensured that only individuals following a suitable resistance-training program were recruited. To assess the adequacy of nutrient intake, a nutrition team (n =2) also participated in monitoring the participants who completed a consecutive dietary record over 7 days prior to the study. Anthropometric characteristics of the participants are shown in Table 1.

Table 1. Anthropometric characteristics of participants (AM and AC groups)

Variables	AC group $\bar{x}$ (SD)	AM group $\bar{x}$ (SD)
Age (years)	21.67±0.72	21.80±1.01
Weight (kg)	69.66±6.21	68.50±4.22
Height (cm)	176.47±6.03	175.93±4.77
BMI (kg/m2)	22.37±1.62	22.14±1.19

Experimental overview

A double-blind, randomized, parallel group comparison was performed to examine whether *Apis mellifera* honey and *Apis cerana* honey would enhance sprint and agility T-test performance and reduce blood lactate in futsal athletes. A group of 15 futsal athletes volunteered for random, blind assignment to either an *Apis mellifera* honey (AM) group or an *Apis cerana* honey (AC) group. Each group completed tests (20 m sprint test and agility t-test) pre- and post-supplementation. Additionally, blood lactate was measured before and immediately after the tests. All the subjects followed the same training schedule during the 6 weeks of supplementation and also they were familiarized with each exercise testing protocol separately.

Experimental design

After the enrolment, the participants were randomized into two groups to receive the following treatments once a day for 6 weeks. *Apis mellifera* honey (1.14 g/kg) was given to the AM group in the morning (for breakfast) during the 6 weeks of the study, and *Apis cerana* honey (1.14 g/kg) was given to the AC group at the same time of day. Before undergoing the pre-test, the participants were asked to have a light meal (before 9:00 p.m.) on the previous day and not to eat food or drink caffeine beverages on the test day. The next morning at 8:00 a.m., anthropometric measurements were made. Body weight and body fat percentage were measured on OMRON HBF-375 Karada Scan Body Composition Scale, with the participants wearing minimal clothes and being barefoot. Body height was measured with a stadiometer with 0.1cm readability (Seca 214 Portable Stadiometer, Cardinal Health, Ohio, USA) according to the described standardized procedures. The body mass index was calculated as the ratio of body mass (kilograms) divided by body height (metres) squared.

After the anthropometric measurements, a 100-µl sample of fingertip capillary blood was obtained to measure lactate pre. Participants performed a warm-up (6-min jogging at 6.8 km h-1) prior to pre-testing. The pre-test began at 9:30 a.m. We measured anaerobic capacity (20 m sprint test and agility t-test). There was a 10-sec interval between each test. Immediately after the last task, lactate post was obtained. The participants were allowed to drink water ad libitum; however, they were encouraged to drink enough to maintain hydration. All the participants were also required to sleep from 9:00 p.m. to 6:00 a.m.

During the experiment period of 6 weeks, the subjects lived in the athletes' dormitories and followed the same training sessions and nutrition guidelines provided by the training and nutrition teams. Furthermore, the intensity of exercises was similar within the two groups. The training program included specific tactical and technical training sessions, technique development training sessions and free futsal sparring. The training program was adopted and based on a previous training program method [25]. The heart rate intensity was monitored with a test employing a Polar RS400 Finland.

All the participants received their assigned honey supplementation during breakfast for a period of 6 weeks. A blinded research team member was responsible for providing honey supplementation to the participants according to their group assignment, and logs were also recorded to ensure their compliance with juice supplementation consumption. Post-testing measures were identical to baseline testing and initiated 24 h after the final day of supplementation.

Sprint test

A 20 m "all-out" running sprint was performed in the SARAGA Athletic Stadium. Instructions to start running as fast as possible were given upon test initiation. At the beginning of the test, the participant got ready in a "standing start position" at one end of the 20 m sprint track (i.e. cone A). The first test administrator counted down to the start of the test (3 – 2 – 1 – GO). On the "GO" signal, the participant sprinted at maximal effort to the end of the 20 m track (i.e. cone B). As soon as the subject crossed the 20 m line, the second test administrator (standing on the finish line) shouted "CLEAR". The running time of the sprints was recorded using the beam photocell system (Microgate, Bolzano, Italy)

Agility t-test

The agility t-test, administered using a version standardized on the basis of previous literature [26], was performed to assess speed and agility, including forward, lateral, and backward running in futsal athletes. Four cones were set out (5 meters apart). The participant began the test at cone A. The administrator counted down to the start of the test (3 – 2 – 1 – GO). On the "GO" signal, the test administrator pressed the start button on the stopwatch and the participant sprinted to cone B and touched the base of the cone with his right hand. Then, he turned left, shuffled sideways to cone C and touched its base, this time with his left hand. Afterwards, the participant shuffled sideways to the right to cone D and touched the base with his right hand. Then, he shuffled back to cone B, touched it with his left hand and ran backwards to cone A. The stopwatch was stopped as he passed cone A. The trial was not taken into consideration if the participant put one foot in front of the other while shuffling, failed to touch the base of the cones or failed to face forward throughout the test. The best time of two successful trials was recorded with an accuracy of 0.1 s. All the times were recorded using a stopwatch (Seiko Stopwatch S23601P).

Statistical Analysis

The values are presented as mean ± SD. Normal distribution of the sample was checked using the Shapiro-Wilk test. A 2 (group: AM and AC) × 2 (time: pre, post) repeated measures analysis of variance (ANOVA) was performed for each parameter. Independent t-test was used to determine any differences between pre- and post-supplementation in each group. 95% confidence interval (CI) and percent changes were calculated. Statistical significance was set at the level of $p < 0.05$. The SPSS software (V.21.0) was employed in the study.

Results

Table 2 displays mean and SD values as well as percent changes for 20 m sprint, agility t-test and blood lactate concentration before and after supplementation in groups AC and AM.

Blood lactate concentration

Independent t-test revealed no significant differences from before to after supplementation in the AC group (p = 0.933) and the AM group (p = 0.599) for the lactate pre. Additionally, ANOVA showed no significant time × group interactions (p = 0.637). The statistical analysis revealed no significant main effect for group (p = 0.176) and time (p = 0.710). Furthermore, independent t-test showed significant changes from before to after supplementation in the AC group (p = 0.009) for the lactate post. Conversely, independent t-test revealed no significant changes in the AM group (p = 0.698) for the lactate post. Specifically, for the lactate post there was no significant main effect for group (p = 0.112), time (p = 0.288) and significant time × group interactions (p = 0.397).

20 m sprint

Regarding 20-m sprint performance, there were statistically significant differences for time (p = 0.036) and group main effects (p = 0.009) but no significant differences for time × group interaction effects (p = 0.951). Specifically, independent t-test revealed significant changes from before to after supplementation just in the AC group (p = 0.018).

Agility t-test

For the t-test, there was no significant two-way interaction for time × group (p = 0.520), main effect for group (p = 0.843) and time (p = 0.408). However, independent t-test revealed significant changes from before to after supplementation in the AC group (p = 0.013) for the t-test.

Discussion

To the best of our knowledge, this is the first study to compare the effects of *Apis cerana* honey with *Apis mellifera* honey congestion based on anaerobic performance. The current results demonstrate that 1.14 g/kg of *Apis cerana* honey supplementation improved average speed in the 20 m sprint test. Additionally, blood lactate concentration decreased after six weeks of supplementation. Increases in agility t-test were also observed. These results were no different when 1.14 g/kg of *Apis mellifera* honey was co-ingested. The present results suggest that all supplementation methods will lead to similar performance outcomes after six weeks of supplementation.

While we found it difficult to find previous investigations that would support the findings of this study, we may speculate that honey which has a 1:1 ratio of fructose to glucose may help to promote better blood sugar levels [12]. Furthermore, this phenomenon has a relationship between the availability of glucose in blood and glycogen reserves [27]. Glycogen acts as a reserve of energy that is stored in the liver. On the other

Table 2. Effects of *Apis cerana* honey and *Apis mellifera* honey supplementation on blood lactate concentration, 20 m sprint and agility t-test

Variables	AC group				AM group				Anova p values (b)		
	Before	After	Δ%	p(a)	Before	After	Δ %	p(a)	Group	Time	Group x Time
Lactate pre (mmol/l)	2.16±0.43	2.15±0.51	-0.046	0.933	2.28±0.37	2.39±0.69	0.048	0.599	0.176	0.710	0.637
20 m sprint (s)	3.68±0.52	3.45±0.45	-0.625	0.018*	3.98±0.20	3.74±0.43	-0.060	0.017*	0.009*	0.036*	0.951
Agility t-test (s)	11.53±0.83	11.15±0.91	-0.032	0.013*	11.41±1.04	11.37±1.10	-0.003	0.363	0.843	0.408	0.520
Lactate post (mmol/l)	10.45±1.94	9.69±1.48	-0.072	0.009*	10.75±1.25	10.66±1.36	-0.008	0.698	0.112	0.288	0.397

Abbreviations: AC group was given 1.14 g/kg of *Apis cerana* honey; AM group was given 1.14 g/kg of *Apis mellifera* honey; lactate pre – blood lactate concentration measured before the tests; lactate post – blood lactate concentration measured after the tests. The values are presented as mean ± SD. (a) Superscript: Independent t-test. * – values are significantly different between groups AC and AM (p<0.05). (b) Superscript: A 2 × 2 repeated measures analysis of variance (ANOVA). * – values are significantly different between groups AC and AM (p<0.05).

hand, glycogen is an important energy source in activity [28]. Increased glycogen as an energy reserve in the liver can be related to increased physical activity. Furthermore, the release of liver glycogen occurs when the source of energy in the form of glucose in blood decreases. It is intended to maintain glucose homeostasis in blood [29].

Our study has some potential limitations. Firstly, physical, role-related and emotional functioning as well as the participants' lifestyle differences may have had an impact on the obtained results. Secondly, the lack of any repeated efforts may have attenuated the effects of *Apis cerana* honey and *Apis mellifer*a honey supplementation on anaerobic performance. To address these limitations, long-term studies involving a large cohort and the control of the diet and exercise are needed in order to confirm the potential effects of *Apis cerana* honey and *Apis mellifer*a honey on athletes' performance.

Conclusion

We demonstrated that 1.14 g/kg of *Apis cerana* honey given once a day at breakfast for 6 weeks is more effective in reducing blood lactate concentration and enhancing agility t-test performance than 1.14 g/kg of *Apis mellifera* honey in futsal athletes. Additional research is needed to study mechanisms behind these improvements and to rectify discrepancies found in the results of some studies.

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